ANNUAL REPORTS ON NMR SPECTROSCOPY

Volume 11A

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NMR SPECTROSCOPY

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ANNUAL REPORTS ON
NMR SPECTROSCOPY

Edited by

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PREFACE

Largely on account of a continued expansion in the applications of nitrogen NMR, Volume 11 of Annual Reports is divided into two parts. An indication of the range of areas of molecular science which are critically dependent upon NMR investigations is provided by the diverse choice of topics covered in Volume 11A. The review on amino acids, peptides and proteins by Dr H. W. E. Rattle provides a current account of an area previously covered in Volume 6B of this series. Biologically important areas of research are also taken into account in the chapter by Professor S. Forsén and Dr B. Lindman on ^{25}Mg and ^{43}Ca NMR. The remaining three reports in Volume 11A deal with material which, although previously dealt with *inter alia*, is specifically covered for the first time in this series, the topics in question being ^{13}C–^{13}C couplings by Dr's P. E. Hansen and V. Wray and the ^{13}C NMR of Group VIII organometallic compounds by Dr P. S. Pregosin.

Volume 11B is devoted to a comprehensive and up-to-date account of nitrogen NMR by Professor M. Witanowski and his coworkers. This review serves to expand upon those provided previously, in Volumes 2, 5A and 7, on this important topic.

It gives me great pleasure to express my gratitude to all of the contributors to Volume 11 for their diligence and willing cooperation which has provided the basis for this volume.

University of Surrey,
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England

G. A. WEBB
December 1980

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Nitrogen NMR

M. WITANOWSKI, L. STEFANIAK AND G. A. WEBB

NMR of Amino Acids, Peptides, and Proteins (1977–1979)

H. W. E. RATTLE

Biophysics Group, Portsmouth Polytechnic, Portsmouth, U.K.

I. INTRODUCTION

A few years ago, biological NMR was described by a cynical observer as "the technique with the eternally rosy future". In some respects he was right. The technique is a very powerful one, and has demonstrated its ability to elucidate the structures of smaller molecules to the extent that its use is essential for most organic chemists, but when applied to biological macromolecules it becomes subject to three major disadvantages. The first of these, the inherent insensitivity of the technique brought about by the very small energy gap between nuclear orientations in a magnetic field, has been largely overcome by the use of Fourier transform methods for rapid data acquisition and by high-technology detection and amplification circuits in the probe which make for maximum detection efficiency. The second disadvantage, that of the poor resolution consequent upon the large number of adjacent resonances and the increased linewidths found for the spectra of macromolecules, is being eroded by the development of spectrometers operating at very high frequencies, but progress in this direction is slow, very expensive, and in any case can offer only an improvement rather than a complete solution, at least in the forseeable future. The third disadvantage, that of the assignment of individual resonances once they have been resolved, will remain a problem to be overcome by ingenuity and a great deal of hard work. Overcoming the problems of resolution and assignment is thus a necessary feature of any NMR approach to macromolecules, and it is true to say that many papers which reach the literature are reports of work which never really succeeded in overcoming both. In so far as such papers may describe interesting technical advances they are of value, but ultimately judgement must be on the basis of biochemical significance regardless of the technical barriers overcome on the way. This is a major criterion used in selecting papers for this review, apart from the early section on techniques.

Before embarking on this section, it may be of value to some readers to summarise the chief methods used in overcoming the resolution problem. This problem is most marked in proton spectroscopy; the much larger chemical shift range found, for example, in ^{13}C spectra shifts the main problem from resolution to assignment and signal-to-noise ratio. Faced with a protein proton spectrum in which the majority of the resonances coalesce into featureless humps, most experimenters resort to one or more of the following methods.

(a) The use of resonances which are naturally clear of the rest. In particular, methyl groups shifted to low frequency by ring-current effects and the high frequency aromatic and histidine resonances are frequently used.

(b) Labelling with a resonant probe nucleus. Examples here include the introduction of fluorine or ^{13}C nuclei into selected parts of the molecule,

usually to monitor changes of environment of the probe on changing, say, pH or ionic strength, or substrate or inhibitor binding.

(c) "Negative labelling" with a non-resonant nucleus. In particular this method has been applied in producing protein molecules which are per-deuterated except for a few selected amino acids.

(d) Use of magnetically perturbing probes, and in particular the substitution of paramagnetic metal ions for diamagnetic metals at the active sites of enzymes. Manganese is frequently used, as are the lanthanide series of elements which have the advantage of providing a variety of magnetic perturbing effects while being chemically identical. Perturbing probes are most powerful when used in conjunction with difference spectroscopy.

(e) Difference spectroscopy. It is perfectly possible to subtract one NMR spectrum from another which differs from it in some small way—a chemically modified amino acid sidechain, a different paramagnetic probe, a spin-decoupling irradiation—and thus to observe only the effects of the perturbation. With the reasonably sharp resonances found in high-field spectrometers, however, exact registration of the two spectra is needed for a successful subtraction; a shift of less than 1 Hz can produce quite large distortions.

(f) Line narrowing by convolution or multipulse methods. Several related methods of data modification are available for post-accumulation improvement of linewidths, at some cost in signal-to-noise ratio. Resolution may also be improved by using the spin-echo method of Hahn normally used for measuring T_2. The various methods for line narrowing have been critically reviewed and compared (see Fig. 1).

(g) Chemical modification. This may vary in scale from the nitration of a single tyrosine to the cleavage of the whole molecule into fragments.

Most, if not all, of the work described in succeeding sections relies heavily on one or more of these methods to cope with resolution difficulties, and frequently with assignment as well. Chemical modification and spin decoupling are, of course, particularly valuable in assignment. For readers requiring a fuller introduction to the application of NMR to biomolecules, a few introductory texts are available. The most elementary of these gives a non-mathematical treatment and covers ESR as well as NMR.[2] More comprehensive treatment of applications to proteins and peptides is given by Wüthrich,[3] while the treatment by Dwek[4] is still valuable, particularly in its treatment of enzymes and as an introduction to magnetic-probe and relaxation methods. Major reviews which may also prove valuable cover the application of high-resolution NMR to biological systems,[5] and of ^{13}C resonance to peptides and proteins.[6] Volumes of review articles are also beginning to make their appearance, notably the first of a series *Biological Magnetic Resonance*[7] and a volume entitled *Biological Applications of*

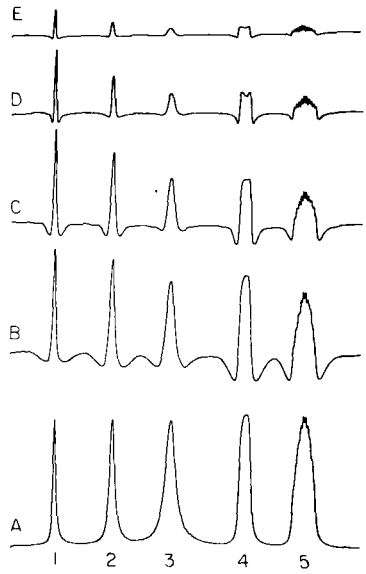

FIG. 1. Some simulated spectral lines given by Gassner *et al.* to illustrate the effects of various post-accumulation line-narrowing procedures. The lines are, from left to right: (1) single Lorentzian line, $\Delta_\gamma = 5$ Hz; (2) single Lorentzian line, $\Delta_\gamma = 10$ Hz; (3) single Lorentzian line, $\Delta_\gamma = 20$ Hz; (4) superposition of ten single Lorentzian lines, LB = 5 Hz, separated by 2 Hz, all with the same intensity; (5) superposition of ten single Lorentzian lines, LB = 5 Hz, separated by 4 Hz, with the intensities obeying a Gaussian distribution. (A) Normal spectrum; (B) trapezoidal convolution, TM = 100; (C) trapezoidal convolution, TM = 200; (D) trapezoidal convolution, TM = 500; (E) sine bell convolution (8K of data memory was used, in QP; spectral width ±2500 Hz).[1]

Magnetic Resonance.[8] Individual reviews from these are referred to under appropriate headings later in this article.

II. EXPERIMENTAL METHODS

A. Instrumental techniques

Measurement of spin–spin couplings in macromolecules. The pulse sequence 90°–ζ–180°–ζ data collection has long been used in spin-echo experiments to measure the transverse relaxation time T_2. In the rotating frame of reference, the 90° pulse rotates the nuclear magnetisation vector into the xy plane, where transverse relaxation mechanisms dephase the spins in a random manner, this being superimposed on a systematic dephasing caused by field inhomogeneities and the different Larmor frequencies of the

nuclei. This continues for time ζ, when the 180° pulse produces a reversal of the systematic dephasing, and results in a spin-echo reaching its maximum at time ζ later. It is pointed out[9] that a doublet of coupling constant J modulates with a frequency J Hz; in other words the systematic dephasing takes place at $2\pi J$ radians s^{-1} in the rotating frame, and if the doublet is collapsed to a singlet during data accumulation by time-shared irradiation at the resonance to which the doublet is coupled, the observed singlet has the same intensity as the doublet, and because of the modulation passes through zero when $\zeta = J/4$. By this means, accurate J values for resonances may be measured even when the splitting itself cannot be resolved. Figure 2 shows

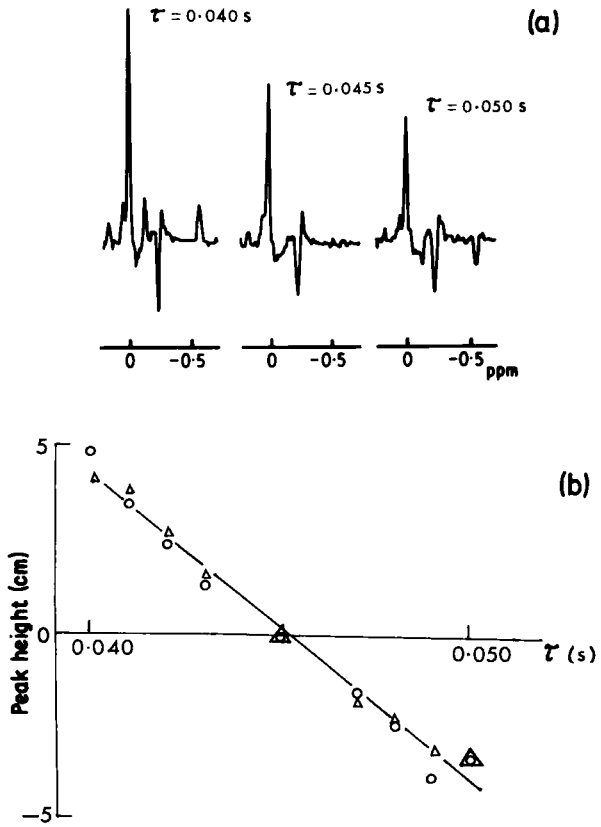

FIG. 2. (a) Convolution difference spectra of a 5mM lysozyme solution in D_2O at pH 4·0 and 340 K for three values of the delay time τ in the 180°–τ–90°–τ sequence. The two leucine–17 methyl doublets at −0·14 and −0·57 ppm, decoupled to singlets by time-shared irradiation at 0·68 ppm, are shown before, at, and after the first null.[9]

(b) Plot of peak height against τ for the resonances at −0·14 ppm (○) and −0·57 ppm (△), showing $\tau_0 = 0{\cdot}045$ s, which corresponds to $J = 5{\cdot}55$ Hz.[9]

an example[9] and demonstrates the effect for leucine–17 methyl doublets, giving their J value as $5{\cdot}55 \pm 0{\cdot}1$ Hz.

Another method for elucidating spin-coupling patterns which has received recent attention is that of 2-dimensional J-resolved spectroscopy.[10] This technique has been addressed to the problem of homonuclear broad-band decoupling in NMR spectra. The simplifying effects on a spectrum of heteronuclear broad-band decoupling are already well used, particularly in the decoupling of ^{13}C resonances from coupled ^{1}H nuclei, but the elimination of all spin couplings in any spectrum would be a valuable aid in interpretation. The effect is achieved indirectly in the 2-dimensional method, which is also able to give approximate values for J. Instrumentation and methodology have been simply described,[11,395] and applications to the basic pancreatic trypsin inhibitor given.[12,13] In the latter of these two papers an extension of the technique to allow selective spin-decoupling is described. Very considerable data-handling capabilities are required, since a 2-dimensional matrix of data has to be built up. In outline, the technique again consists of a basic $90°$–ζ–$180°$–ζ pulse sequence, with accumulation of the resultant spin-echo. The experiment is repeated for a large number N of different ζ values; the contribution of the spin multiplets to the echo is modulated by their splittings and so varies from one ζ value to another. If each echo is accumulated as M data points, the data are now in the form of an $M \times N$ matrix (N rows, M columns). Fourier transformation of the rows separates out the contributions of the different resonances in the usual way; these are displayed along the W_2 axis. A second Fourier transformation is then made of the columns of data and the result displayed on the W_1 axis. Any resonance which is not modulated by spin-coupling will make the same contribution to each element of the column, and the Fourier transform of the column will simply be that of a constant; such resonances appear on the $W_1 = 0$ line. Any multiplet, making regularly varying contributions to the elements of a column, will appear as a peak in values down the column at a position governed by its J value. When suitably displayed, both chemical shift and spin coupling information are readily accessible; the example in Fig. 3 shows the result on part of the spectrum of the basic pancreatic trypsin inhibitor. The 2-dimensional method is quite difficult, and presents problems in phase correction and ensuing distortions, but will undoubtedly make a useful contribution.

B. Relaxation methods

The ability of NMR to make dynamic measurements is one of its major strengths. Jardetzky[14] has, for example, reviewed measurements of mobility in different regions of proteins with a view to discovering more about molecular switching mechanisms. Relaxation measurements on proteins are

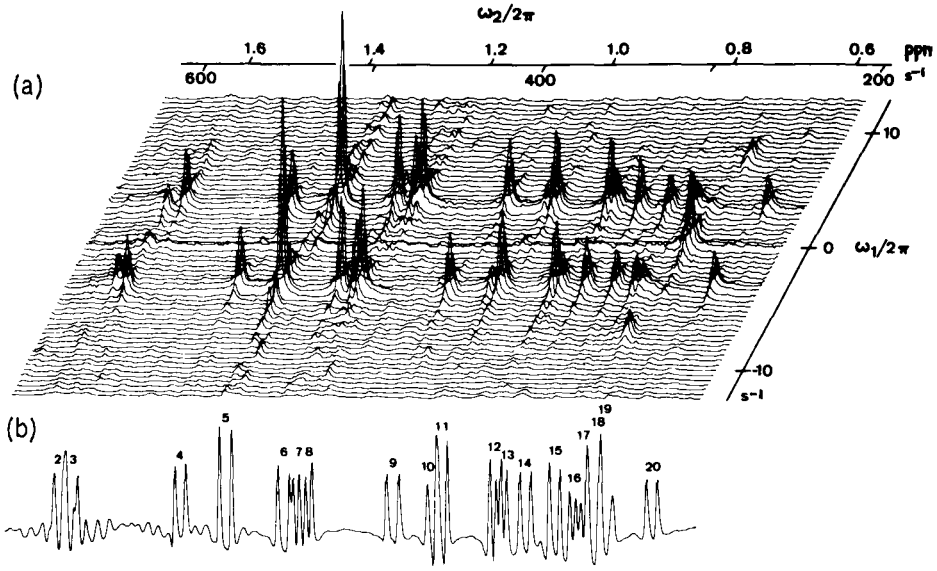

FIG. 3. (a) Two-dimensional *J*-resolved 360 MHz ^{1}H NMR spectrum of a 0·01 M solution of the basic pancreatic trypsin inhibitor in D_2O, pD = 4·5, 60 °C. The figure shows the expanded region from 0·5 to 1·7 ppm of a spectrum computed from 64 × 8192 data points. Prior to the Fourier transformations the FIDs were multiplied with an increasing exponential to reduce the linewidths.[12]

(b) One-dimensional ^{1}H NMR spectrum obtained after digital filtering with the sine bell routine. The individual methyl resonances are indicated by the numbers 2–20.[12]

not entirely straightforward, however, and in particular results are likely to be affected by internal cross-relaxation and internal librational motions. A theory for the influence of cross-relaxation between protons has been given.[15] It is pointed out that the T_1 values of individual protons tend to become equal through spin diffusion and are dominated by the relaxation of rotating methyl groups which act as relaxation sinks. The theory has been tested[16] and it is concluded that cross-relaxation dominates the T_1 measurements for larger proteins. Andree[17] provides both a theoretical and an experimental treatment of cross-relaxation between macromolecule and small bound ligands. The relaxation times of solvent water protons are shortened by cross-relaxation effects involving solute protein protons; the effect has been considered in some detail.[18,19] A review and critical survey of the use of proton relaxation enhancement in biochemistry is given by Burton *et al.*[20]

The other effect to be taken into account when measuring relaxation rates is that of internal librational motions of the protein chain. These are pursued in some detail by Howarth[21] for ^{13}C measurements of proteins and peptides.

In later papers[22,23] a more complete theory is developed, covering field, temperature, and concentration dependence of the motions and their effect on relaxation times.

The nuclear Overhauser effect (NOE) has been made accessible for use on macromolecules by the development of NOE difference methods. Chapman *et al.*[24] describe a difference technique (see Fig. 4) in which eight free induction decays are added while irradiating at a chosen frequency, then eight are subtracted with no irradiation. When applied to lysozyme with irradiation at one of the ring-current shifted methyl resonances, selective NOE are observed not only from protons in the same amino acid sidechain

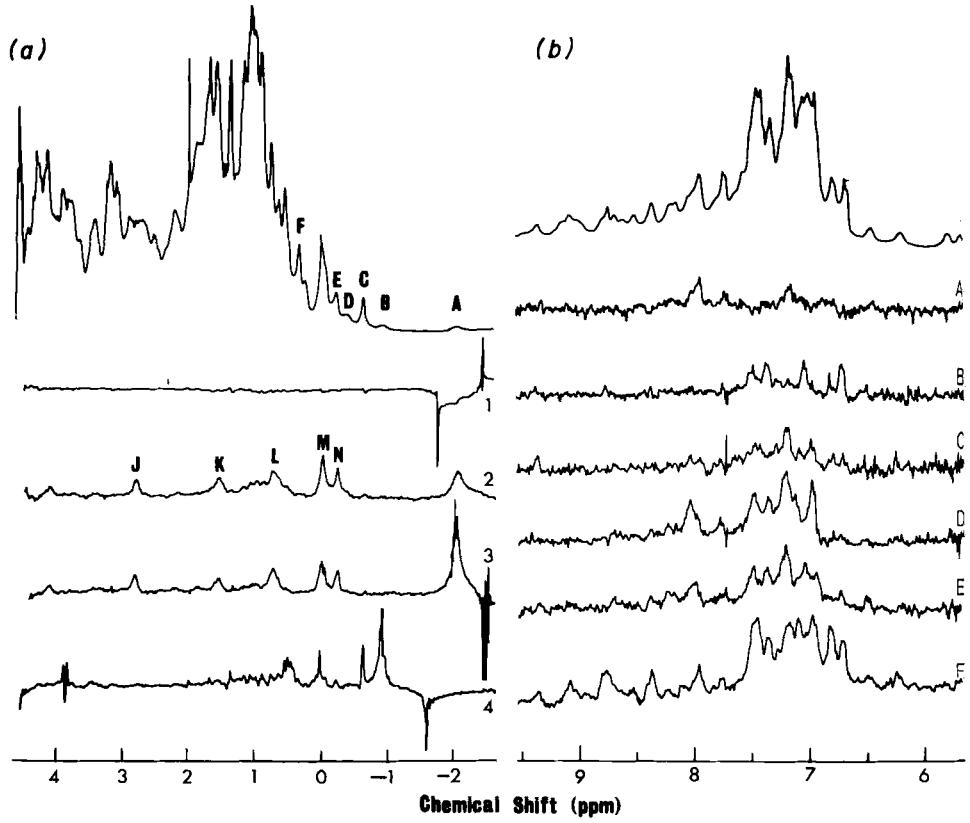

FIG. 4. (a) Low frequency region of the 270 MHz NMR spectrum of 5 mM hen egg white lysozyme in 50 mM $LaCl_3$–D_2O, pD 5, 37 °C. The traces 1–4 are difference spectra (all × 10 gain): (1) time-shared irradiation of resonance; (2) gated irradiation of peak A; (3) time-shared irradiation of peak A; (4) time-shared irradiation of peak B.[24]

(b) High frequency region of the 270 MHz NMR spectrum of 5 mM hen egg white lysozyme in 50 mM $LaCl_3$–D_2O, pD 5, 37 °C. The traces A–F are difference spectra—time-shared irradiation of the corresponding peaks in (a).[24]

but also from the resonances of the aromatic amino acids whose proximity to the methyl groups causes the ring-current shift. A similar technique has been described by Richarz and Wüthrich[25] who simultaneously spin-decoupled the NOE difference spectra. The NOE has been used to study the conformation of small molecules bound to proteins; irradiation at the frequency of a proton in the bound ligand results in an intensity change in the signal from a different proton in the bound excess ligand via an Overhauser effect between the two protons in the bound state. Since the effect is shown to depend on close spatial proximity (within 4 Å) of the protons in the bound form, it may provide useful conformational information.[26]

An aid to the assignment of aromatic amino acids, and in distinguishing between surface and buried residues, has been developed in photochemically induced dynamic nuclear polarisation. Data are accumulated in the presence of a dye molecule (fluorescein or 3-*N*-carboxymethyl-lumiflavin) at low concentration, with alternate FIDs being accumulated additively while the sample is illuminated with laser light and subtractively with no irradiation. The resultant difference spectra contain only the resonances of residues accessible to the dye and thus on the surface of the protein. Reports have been made on the application of this technique to the bovine pancreatic trypsin inhibitor,[27] to porcine pancreatic phospholipase A_2,[28] and to luliberin.[29]

C. Labelling

Many of the papers discussed later in this article employ labelling techniques, but it may be apposite to mention a few specifically under the heading of experimental methods. An isotope effect has been found in the chemical shift of ^{31}P bound to oxygen: $^{31}P^{16}O_4$ and $^{31}P^{18}O_4$ resonances are separated by 12 Hz at 145 MHz;[30] in phosphates with various distributions of ^{18}O and ^{16}O, all species can be resolved (Fig. 5). This means, for example, that each phosphate of ATP could be differently labelled, or that transfer rates of ^{18}O from non-phosphate compounds to phosphates can be followed, and offers interesting possibilities in the study of enzyme-catalysed phosphate exchange reactions. Deuterium labelling is a rather more widely used technique; Griffiths *et al.*[31] describe in detail the preparation of selectively deuterated amino acids, with assays and expected yields, and a deuterium-labelling method for the assignment of histidine resonances in proteins is outlined by Arata *et al.*[32] Chemical modification of free NH_2 groups of proteins can be achieved by interaction with H_2CO in the presence of sodium cyanoborohydride;[33] the resulting *N,N*-dimethyl derivatives can, of course, contain ^{13}C, ^{2}H, or radioactive ^{14}C and ^{3}H atoms.

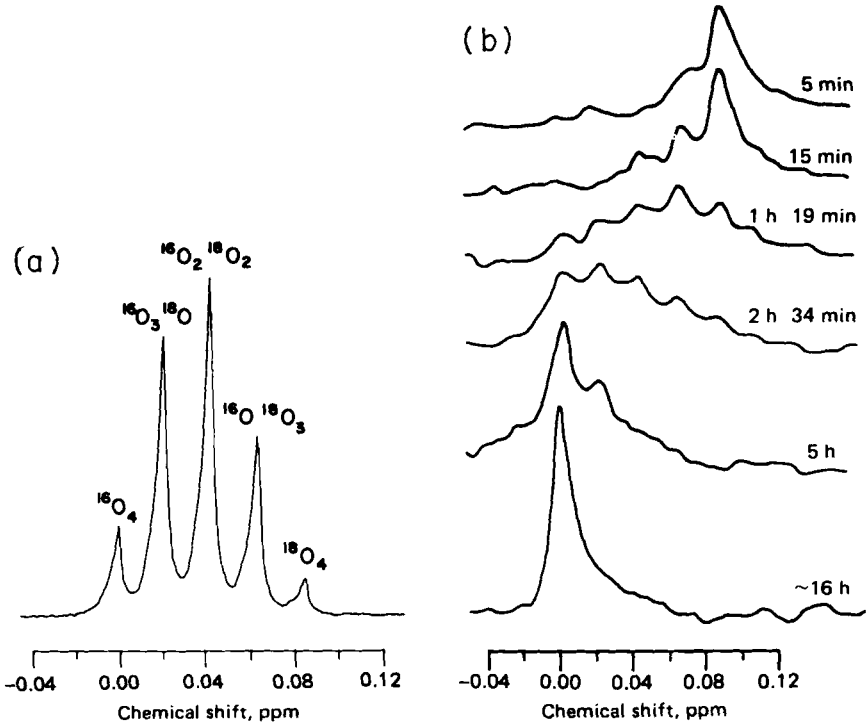

FIG. 5. (a) ^{31}P NMR spectrum of a randomised sample of inorganic phosphate with mixed ^{16}O and ^{18}O isotopes, taken at 145·7 MHz, acquisition time 8·2 seconds, pulse angle 45°.[30] (b) Time course of $P_i(^{18}O)$–water exchange catalysed by yeast inorganic pyrophosphatase. Each spectrum was accumulated for 8 minutes at 24·3 MHz with 40 scans, an acquisition time of 6 seconds, and a pulse angle of 60°.[30]

D. Useful data

A number of recently published papers present useful data for peptide and protein NMR. In particular, a systematic study of proton NMR parameters of amino acids incorporated in the synthetic tetrapeptide H-Gly-Gly-X-Ala-OH will prove very valuable in proton NMR studies of proteins. Part of these data is presented as Table I.[34] The same tetrapeptides have been used to investigate amide proton titration shifts in H_2O solution. Only a very limited number of amide protons show sizeable intrinsic titration shifts; since these are to low frequency and have now been measured, they can be distinguished from conformation-dependent shifts, particularly those related to H-bonding to carboxylate groups, which thus may be used as sensitive probes of conformation changes.[35] Some ^{13}C chemical shift data are presented in Tables II and III.

III. AMINO ACIDS AND SYNTHETIC PEPTIDES

As investigation of natural peptides and proteins becomes more and more practicable, interest in amino acid studies is predictably waning. Nevertheless some interesting results still emerge; for example, Mayer *et al.*[36] report an intramolecular hydrogen bond between the COO^- of a glutamic acid residue in a peptide and its NH group, and from the same laboratory comes a paper[37] which demonstrates the formation of a complex with two hydrogen bonds between the sidechains of arginyl and glutamyl residues positioned adjacent to each other in a dipeptide. Addition of guanine disrupts the complex, the guanine forming a complex with the carboxylate group; no strong interaction was found with any other bases, or between any bases and the arginyl residue. Hydrogen bonding is also found to influence the rotamer distribution of the histidine sidechain in di- and tri-peptides containing histidine and glycine;[38] changes in the rotamer population correlate with ionisation of the carboxylate group of glycine. The tautomeric equilibrium of the imidazole ring of isolated histidine has been investigated using ^{15}N, ^{13}C, and ^{1}H heteronuclear coupling constants in samples 95% enriched with ^{15}N in the imidazole nitrogens; Blomberg *et al.*[39] report an interaction of the α-amino group with the lone pair of the imidazole πN at higher pH values, and deduce the conformation of the whole molecule as well as indications of probable dimerisation at a pH around the imidazole pK value.

Not surprisingly, the amino acid which has attracted most investigations into its conformation is proline, which can exert major conformational influences in a peptide. Solution studies on proline using lanthanide shift reagents show that in chloroform solution a planar ring conformation does not occur; rather, the ring exists as approximately 60% "conformer A" which is a half-chain with C_γ up and C_β down, and 40% "conformer B" which is an envelope with C_γ down.[40] Once incorporated in linear peptides, proline peptide bond *cis–trans* isomerism is susceptible to NMR investigation. Studies of Ser-Pro-Pro and Arg-Pro-Pro tripeptides show that the two peptide bonds are *trans-trans*, *cis-cis*, *cis-trans*, and *trans-cis* in order of decreasing stability.[41] A number of proline-containing tripeptides of the type X^1-Pro-X^2 were synthesised by Deslauriers *et al.*[42] who also found *cis–trans* isomerism in the X^1–Pro bond, with larger X^2 residues favouring an increase in the *cis* content. The same laboratory[43] also synthesised a number of tripeptide diastereoisomers, including Pro-Leu-Gly-NH_2 and Pro-D-Leu-Gly-NH_2, and found that isomer- and pH-dependent ^{13}C chemical shifts are very small, in contrast to the earlier findings[44] that cyclo-(Pro-Leu) and cyclo-(Pro-D-Leu) exhibit major chemical shift differences. A detailed study of the conformation of cyclo-([85% ^{13}C]Asp-Pro) using ^{1}H–^{1}H, ^{13}C–^{1}H, and ^{13}C–^{13}C coupling constants shows that the puckering of the pyrrolidone ring is similar to that found in cyclo-(Leu-Pro)

TABLE I

^{1}H NMR parameters[a] of the 20 common L-amino acid residues in the linear tetrapeptides H-Gly-Gly-X-L-Ala-OH.[34]

Amino acid residue	Chemical shifts, δ (from TSP ± 0·002 ppm)					Spin–spin coupling constants J (±0·5 Hz)			
	αNH	αCH	βCH	others		$^3J_{H_\alpha NH}$	$^3J_{\alpha\beta}$	others	
Gly	8·391	3·972				5·6			
Ala	8·249	4·349	1·395			6·5	7·0		
Val	8·436	4·184	2·130	γCH_3	0·969	7·0	6·9	$^3J_{\beta\gamma}$	6·9
					0·942				
Ile	8·195	4·224	1·894	γCH_2	1·478	7·0	7·6	$^3J_{\beta\gamma CH_2}$	9·5
					1·190				3·5
				γCH_3	0·943			$^3J_{\beta\gamma CH_3}$	6·9
				δCH_3	0·885			$^3J_{\gamma\delta}$	7·0
Leu	8·423	4·385	(1·649)	γCH	(1·649)	6·5	7·2	$^3J_{\gamma\delta}$	6·3
			(1·649)	δCH_3	0·943		7·2		
					0·899				
Ser[b]	8·380	4·498	3·885			6·5	5·1		
			3·885				5·1		
Thr	8·236	4·346	4·220	γCH_3	1·232	6·9	5·0	$^3J_{\beta\gamma}$	6·3
Asp[b]	8·410	4·765	2·837			7·0	5·7	$^2J_{\beta\beta}$	16·3
			2·753				8·3		
Glu	8·368	4·295	2·092	γCH_2	2·314	7·0	4·6	$^2J_{\beta\beta}$	14·7
			1·969		2·283		9·5	$^3J_{\beta\gamma}$	7·3
								$^2J_{\gamma\gamma}$	14·6
Lys	8·408	4·358	1·870	γCH_2	(1·471)	6·5	5·6	$^2J_{\beta\beta}$	14·0
			1·747	δCH_2	(1·708)		7·8	$^3J_{\delta\epsilon}$	7·7
				ϵCH_2	3·023				
					3·023				
				ϵNH_3^+	7·519[a]				
Arg	8·274	4·396	1·915	γCH_2	(1·719)	6·9	5·5	$^2J_{\beta\beta}$	16·0
			1·796	δCH_2	3·312		7·6		
				NH	6·622[a]				
					7·166[a]				

Asn[b]	8·747	4·755	2·831	γNH_2	6·912[a]	7·5	5·8	$^2J_{\beta\beta}$	16·0
			2·755		7·591[a]		8·3		
Gln	8·411	4·373	2·131	γCH_2	2·379	6·0	5·0	$^2J_{\beta\beta}$	15·0
			2·010	δNH_2	6·875[a]		8·8	$^3J_{\beta\gamma}$	7·5
					7·594[a]				
Met	8·418	4·513	(2·164)	γCH_2	(2·633)		5·7		
			(2·000)		(2·633)		8·6		
				εCH_3	2·128				
Cys[c]	8·312	4·686	3·278			7·7	4·0	$^2J_{\beta\beta}$	13·9
			2·958				9·6		
				ring protons					
Trp	8·094	4·702	3·322	C2H	7·244		6·0	$^2J_{\beta\beta}$	14·5
			3·195	C4H	7·649		7·8		
				C5H	7·167			$^3J_{ring}$	8·0
				C6H	7·244				
				C7H	7·504				
				NH	10·220[a]				
Phe	8·228	4·663	3·223		(7·339)	9·4	5·6	$^2J_{\beta\beta}$	14·0
			2·991				10·3		
Tyr	8·183	4·604	3·127	C3, 5H	6·857	6·8	5·6	$^3J_{\beta\beta}$	14·0
			2·922	C2, 6H	7·149		9·0	$^3J_{ortho}$	8·2
His	8·415	4·630	3·263	C2H	8·120	8·0	6·0	$^2J_{\beta\beta}$	15·4
			3·198	C4H	7·140		6·9		
Pro[b,d]		4·471	(2·295)	γCH_2	(2·030)		8·8	$^2J_{\beta\beta}$	15·0
			(1·981)	δCH_2	3·653		5·0	$(^3J_{\beta\gamma} = {}^3J_{\gamma\delta} = 6·3)$	

[a] Conditions: solvent, D_2O; pD7·0; 35 °C. Numbers in parentheses are approximate values which could not be quantitatively confirmed by spectrum simulation because of the high complexity of the spin system. The labile protons were studied in H_2O solution, at pH values between 2·2 and 5·0. For most residues, the amide proton chemical shifts thus obtained are, to a good approximation, valid also at pH 7·0; for Asp and Glu, sizeable pH variations may occur between acidic and neutral pH.

[b] Data obtained from the protected peptides CF_3CO-Gly-Gly-X-Ala-OCH_3.

[c] Measured in Z-Gly-Gly-Cys-L-Ala-OH where Z = benzyloxycarbonyl protecting group.

Z-Gly-Gly-Cys-L-Ala-OH

[d] Only the parameters for *trans* Pro are given.

TABLE II

^{13}C chemical shifts[a] of selected amino carboxylic acids.[394]

Amino carboxylic acid	pH	CO_2	αC	βC	γC	δC	εC
Glycine	0·45	171·21	41·46				
	4·53	173·58	42·81				
	12·01	182·66	45·99				
α-Alanine	0·43	173·97	50·09	16·53			
	4·96	176·98	51·87	17·45			
	12·52	185·67	52·73	21·66			
2-Aminobutyric acid	0·40	173·39	55·32	24·46	9·63		
	5·11	176·06	57·21	25·00	9·74		
	12·81	184·75	58·67	28·89	10·44		
2-Aminovaleric acid	0·51	173·63	54·06	33·04	18·91	14·05	
	4·97	176·32	56·02	33·80	18·96	14·11	
	12·97	185·06	57·10	38·17	19·56	14·54	
2-Aminocaproic acid	0·55	173·63	54·30	30·67	27·38	22·17	14·58
	—[b]						
	13·34	185·01	57·32	35·50	28·40	23·69	15·48
β-Alanine	0·49	175·72	36·55	32·23			
	5·03	179·36	37·95	34·82			
	12·56	182·66	39·25	41·57			
4-Aminobutyric acid	0·45	178·15	40·22	23·33	31·80		
	5·12	182·22	40·70	24·79	35·20		
	12·75	184·46	41·89	30·18	36·28		

5-Aminovaleric acid	0·37	179·26	40·65	27·43	22·25	34·28	
	5·04	183·49	40·59	27·70	23·44	37·25	
	13·04	184·84	41·67	32·88	24·36	38·49	
6-Aminocaproic acid	0·27	179·90	40·86	27·70	26·24	24·90	34·77
	5·13	183·82	40·81	27·76	26·51	26·08	37·68
	12·93	185·12	41·78	32·72	27·16	26·84	38·71
Valine	0·30	172·86	59·77	30·31	17·98, 18·47[c]		
	5·64	175·43	61·76	30·31	17·84, 19·15		
	12·60	184·07	63·17	32·93	17.88, 20·26		
Leucine	0·37	173·97	52·79	40·11	25·12	22·06, 22·74[c]	
	—[b]						
	13·00	185·43	55·89	45·50	25·55	22·54, 23·66	
Isoleucine	0·28	172·81	58·70	37·06	25·94(CH_2), 15·26(CH_3)	12·06	
	6·04	175·33	60·89	37·10	25·60(CH_2), 15·85(CH_3)	12·30	
	12·84	184·12	62·34	39·82	25·21(CH_2), 16·72(CH_3)	12·25	

[a] In ppm vs. external TMS; positive shifts indicate deshielding relative to TMS. 0·3 M amino carboxylic acid in H_2O; 25 °C. Carbon atoms are numbered from the nitrogen.

[b] Zwitterion form of 2-aminocaproic acid and leucine not sufficiently soluble.

[c] Non-equivalent geminal methyl groups.

TABLE III

^{13}C chemical shifts[a] of some amino carboxylic acids.[394]

	pH	$CO_{2(alpha)}$	CO_2	αC	βC	γC	δC	εC
			Amino dicarboxylic acids					
Aspartic acid[b]	0·41	172·03	174·36	50·60	34·96			
	6·73	175·48	178·73	53·46	37·78			
	12·73	183·39	181·26	55·26	44·48			
Glutamic acid[b]	0·32	172·62	177·42	53·41	26·13	30·70		
	6·95	175·77	182·42	55·98	28·22	34·68		
	12·51	183·88	184·02	57·20	33·03	35·31		
			α,ω-Diamino carboxylic acids					
Ornithine[c]	0·46		172·76	53·65	28·08	23·98	40·22	
	5·02		175·28	55·48	28·67	24·03	40·32	
	13·53		184·60	57·16	33·31	29·43	41·94	
Lysine[c]	0·50		173·20	53·97	30·51	22·58	27·59	40·54
	6·03		175·78	55·86	31·15	22·58	27·65	40·54
	13·85		184·79	57·26	35·74	23·55	32·99	41·78

[a] In ppm vs. external TMS; positive shifts indicate deshielding relative to TMS.
[b] 0·3 M in H_2O, 25 °C; carbon atoms numbered from the nitrogen atom.
[c] 0·2–0·3 M in H_2O, 25 °C; carbon atoms numbered from the α-amino group.

in the solid state.[45] The work requires very long accumulation of the signals from unenriched carbon atoms coupled to the ^{13}C-enriched ones.

The pentapeptide cyclo-(Gly-Pro-Gly-D-Ala-Pro) has been shown by Pease *et al.*[46] to contain predominantly all-*trans* peptide bonds with intramolecular hydrogen bonds forming one β turn (1 ← 4 H bond) and one γ turn (1 ← 3 H bond). The peptide has been used as a model system for ^{15}N NMR analysis.[47] The presence of a second conformation has been confirmed, as had been suggested for peptide–cation complexes in the earlier study. The similar cyclo-(Gly-Pro-Ser-D-Ala-Pro) has been shown by Pease *et al.*[48] to exhibit the β and γ hydrogen-bonded turns. Cyclic proline-containing hexapeptides have been subjected to ^{13}C T_1 measurements[49] which show that the mobility of the proline rings is dependent on the conformation of the peptide, as might perhaps have been expected. Rather less predictable is the discovery that a small proportion of residues in poly-(L-proline) and poly-(γ-hydroxy-L-proline) are to be found in the *cis* form in aqueous solution.[50,51]

Synthetic polypeptides as a whole arouse less interest than they once did when a controversy raged over helix–coil interconversion rates in solution. It now seems clear that, in molecules which are genuinely partly helical, helix–coil interconversion occurs rapidly; theoretical calculations bear this

out.[52] The two separate α-CH and backbone NH resonance peaks observed in proton NMR are in fact due to polydispersity in the molecules, so that in a partly helical polydisperse sample the larger molecules are predominantly helical and the smaller ones predominantly random coil, with only a few molecules of intermediate size actually undergoing fast exchange. Gearhart and Sawyer[53] demonstrate a relationship between degree of polymerisation in polyglutamic acid and chemical shift of the α-CH in pH conditions favouring random coil formation; the relationship disappears in the presence of high salt concentrations. The dynamic properties of poly-(γ-benzyl-L-glutamate) and poly-(β-benzyl-L-aspartate) are reported by Pivcova *et al.*,[54] yielding correlation times of the order of 2×10^{-8} s and 1×10^{-9} s for backbone carbon atoms in the helical and random coil forms respectively. Aggregation behaviour of random copoly-(Lys^{50} Tyr^{50}) and copoly-(Lys^{50} Phe^{50}) is reported by Saito *et al.*;[55] unlike polylysine, which simply forms helices at high salt concentrations, hydrophobic interactions between the aromatic sidechains of these polypeptides lead to aggregation with a corresponding loss of resonance area as linewidths increase beyond detectability.

Just as synthetic polypeptides formed valuable model compounds for the original development of ^{1}H and ^{13}C resonance for use with proteins, they are now being used to help in the development of ^{15}N spectroscopy. A long series of papers is typified by those of Kricheldorf and Hull[56,57] in which neighbouring residue effects in glycine-containing polypeptides are elucidated, and in which the stereospecificity of the polymerisation of D,L-alanine and of D,L-valine under various polymerisation conditions is shown. ^{15}N NMR found an early application in an attempt to detect deviations from planarity of peptide bonds necessary for the formation of a γ turn in a repeat peptide of tropoelastin.[58] A systematic variation of $^{1}J(^{15}\text{N}-^{1}\text{H})$ with planarity is observed. This work forms part of a long-developed series of experiments on repeat peptides of tropoelastin, the progress of which may be followed from references given in one of the latest of the series.[59] It is clear that β and γ turns are major and recurrent features of the peptides, which are of the form Ala-Pro-Gly-Val-Gly-Val. Another series of peptides which show β turn conformations are the fibrinogen-like peptides investigated by Von Dreele, Rae, and Scheraga.[60,61] In the latter paper, peptides with fibrinogen-like activity are studied by ^{1}H and ^{13}C NMR, and the discovery that the ring of D-Phe may be folded back over an adjacent valine or pipecolic acid residue is used to support a suggestion that this particular conformational feature may be advantageous for the binding of fibrinogen itself to thrombin. The binding of calcium and magnesium ions to model peptides and to fragment 1 of bovine prothrombin is reported by Robertson *et al.*[62] and Marsh *et al.*[63] following ^{25}Mg and ^{43}Ca resonance studies; both Ca^{2+} and Mg^{2+} bind to prothrombin fragment 1, and in both cases this

binding shows dependence on protein pK_a values in the region of 4 and 7. The change in the region of pH 4 is attributed to deprotonation of one of the unusual γ-carboxy glutamate residues. In addition, the work shows that ion binding sites accessible to Mg^{2+} exist which are not competitive with Ca^{2+}. Both divalent cations induce helical structure in fragment 1.

Finally in this section we may mention a comprehensive structure determination of a small chemotactic peptide, formyl-L-methionyl-L-leucyl-L-phenylalanine. The basic backbone conformation of this molecule closely approximates a small β antiparallel pleated sheet, suggesting a possible mode of receptor–chemotactic peptide interaction.[64]

IV. HORMONES

250 MHz ^{1}H NMR of the pituitary hormone corticotropin (ACTH) and a number of fragments of the molecule is reported by Toma *et al.*[65] The use of peptide fragments permits the assignment of signals from tyrosine-23 and valine-22, and the sensitivity of these resonances to the *cis–trans* isomerism of proline-24 is used to show the ratio of the two conformers, providing an interesting instance of sidechain resonances reporting on backbone conformation. Histidine titrations of the two-subunit glycoprotein pituitary hormone lutropin (LH) from pig reveal the intriguing fact that a histidine, α-87, which has an abnormally low pK_a of 3·3 in the isolated α-subunit, regains a normal pK_a in the intact hormone. This implies a major conformational change on combining the two subunits, sufficient to move this histidine from a solvent-inaccessible apolar environment to a more solvent-accessible environment. Maghuin-Rogister *et al.*[66,67] discuss the significance of this in terms of the generation of biological activity by subunit association. Similar general conclusions, but different specific ones, are reported by Brown *et al.*[68] who worked on the related bovine and equine lutropin hormones. By comparing the two they conclude that bovine histidine α-87 titrates at pH ~5·4 while histidine α-83 does not titrate over the range pH 4·0–8·0. However, two non-titrating histidines are still found in the intact hormone (α and β subunits), although again it is concluded that histidine environments change on association of the subunits.

Perhaps because their size makes them accessible to NMR studies, two hormones secreted by the posterior portion (neurohypophysis) of the pituitary gland, oxytocin and vasopressin, have for a long time been usefully examined by NMR.[69] Nitrogen-15 NMR has been applied to oxytocin, with values reported for the chemical shifts of all nitrogen nuclei and for T_1, NOE enhancements, and one-bond NH coupling constants. Live *et al.*[70] also point out the problems posed for ^{15}N measurements by the presence of paramag-

netic ions in the solution, and describe a method for their removal. Other studies on oxytocin now largely centre on the conformational effects of making substitutions in the sequence. Walter *et al.*[71] report on the synthesis of two analogues, with the substitution of D-alanine for residues Ile-3 and Gln-4 respectively. These two positions are half-way round the ring structure, linked by a disulphide bridge between Cys-1 and Cys-6, of the hormone. In theory the native molecule allows the formation of either a type I or a type II β turn; of the two analogues, the first (D-Ala^3) allows only a type II′ β turn, while the second (D-Ala^4) allows only type II. Similarities between the 220 MHz ^{1}H spectra of native oxytocin and the second analogue indicate that a type II β turn is in fact present in the native form. These conclusions are supported by a related study in which proline was substituted as residue 3 and glycine as residue 4.[72] The same oxytocin analogue has also been used for ^{13}C studies,[73,396] and comparisons have been made with other analogues which also contain proline as residue 3, resulting in some conformational heterogeneity of the ring moiety. Results from this work are largely concerned with the *cis–trans* isomerism of the Tyr–Pro bond and longitudinal relaxation rates of different regions of the molecule; it is claimed by Wyssbrod *et al.*[74] that in order to make further progress in both ^{13}C and ^{1}H NMR, notably in terms of assignment and the measurement of spin coupling constants, it is necessary to make still further chemical modifications. Both Wyssbrod *et al.*[74] and Hruby *et al.*[75] report on a series of selectively deuterated hormones including (8-lysine)- and (8-arginine)-vasopressin, oxytocin, and the closely related naturally occurring hormones mesotocin, isotocin, aspartocin, and glumitocin. In all these quite rigorous studies it appears that the conformation of the cyclic moiety is very similar. It does not, however, appear to be rigid since measurements of NH exchange rates, by the transfer of solvent saturation method,[76] show rates consistent with a rapid dynamic equilibrium between folded and highly solvated forms. It is suggested that the structure of oxytocin contains two hydrogen bonds, one between Tyr-2 CO and Asn-5 NH, the other between Cys-1 CO and Cys-6 NH, forming a small region of parallel β structure.[76]

Oxytocin binds strongly to the much larger proteins, also found in the posterior pituitary, the neurophysins. Reports of NMR spectroscopy of isolated neurophysins are few, but Lord and Breslow,[77] using spin-labelled peptides which are known to bind at the hormone-binding site, find a second, weaker, binding site near Tyr-49 in the neurophysin. They follow this by some work on the resonances from N-terminal residues of neurophysin, including a spin-label study which shows that the N-terminus is ~14 Å from residue 3 of peptides bound to the strong hormone-binding site.[78] In contrast to experiments on isolated neurophysins, those on the binding of oxytocin to neurophysins are much more numerous. Carbon-13 labelling appears to be the method of choice, and Griffin *et al.*,[79,80] using ^{13}C

labels in both the cyclic moiety and the acyclic C-terminal tripeptide, report that the latter region of the oxytocin molecule is apparently unaffected by binding to neurophysin. This conclusion is strongly supported by evidence from Blumenstein *et al.*[81] following a study in which ^{13}C labelling of Cys-1 was used to follow the titration of the N-terminal NH_2 of the molecule. This has a pK_a of 6·3 in free hormone, but the value is more than 9·5 in the bound form, indicating a strong interaction with a negatively charged group or region on the neurophysin. This idea was extended[82] to show that, while at neutral pH the hormone is tightly bound (1–5 s^{-1}) to neurophysin, at low pH the dissociation rate is ~100-fold faster. This is attributed to the breaking of a salt link between the N-terminal amino group and a neurophysin sidechain carboxyl group. At high pH values a decrease in the binding constant with no apparent change in the dissociation rate constant is said to be due to deprotonation of the α-amino group. Meanwhile, Deslauriers *et al.*,[73] working on similarly N-terminal cystine ^{13}C enriched oxytocin, claim that T_1 measurements provide more accurate monitors of peptide–protein interaction than do the ^{13}C chemical shifts used by Blumenstein *et al.*, although their general conclusions regarding the pH dependence of oxytocin–neurophysin binding are very similar.

The peptide hormone glucagon, secreted by the pancreas, acts by stimulating the production of cAMP which in turn activates lipases which cleave, and thus mobilise, stored fats in adipose tissue. Complex formation between glucagon and dimyristoylphosphatidylcholine was investigated by Jones and Epand.[83] Early studies of glucagon using NMR had been beset by solubility and aggregation problems, but Rothgeb *et al.*[84] describe a method of improving solubility via the methylation of methionine-27 followed by carbamate formation with $^{13}CO_2$ which they claim retains the normal physiological conformation of the molecule. A more straightforward way of overcoming the problem is described by Bösch *et al.*[85] who made use of the very high sensitivity of their 360 MHz ^1H NMR spectrometer to look at glucagon at very high dilutions. The molecule in its monomeric form proves to be in a predominantly flexible state, with a small structured region from residues 22 to 25 which is identical to that of fragment 20–23 of human parathyroid hormone. The backbone conformation in the C-terminal part of the glucagon molecule in solution is not the α-helical structure observed in crystalline glucagon.

While glucagon stimulates the mobilisation of stored fat, its storage is stimulated by another pancreatic hormone, insulin. In its native form, insulin is hexameric and contains two Zn^{2+} ions. The 270 MHz ^1H spectra of zinc-free insulin as Zn^{2+} is added show no signs of change after the addition of 1 equivalent of Zn^{2+} per hexamer, indicating that the conformation of the hexamer is fixed by this point. A conformational change is induced by the addition of anions high on the Hofmeister series to native insulin.[86]

In a study[87] of angiotensin II a pH-induced *trans* to *cis* isomerisation of the His-6-Pro-7 peptide bond, detected by ^{13}C NMR, correlates with a 10-fold increase in biological activity. Hydrogen–deuterium exchange studies[88] indicate that the hormone is tightly folded, since more than three peptide NH hydrogens are non-exchangeable. This view is supported by the nearly isotropic overall tumbling motion of angiotensin II revealed by ^{13}C longitudinal relaxation measurements.[89] Mono- and bi-selective excitation proton T_1 measurements of sidechain motion in a peptide of angiotensin show that the motion of the peptide does not satisfy the extreme narrowing conditions.[90] It has been shown[91] that the histidine C-2 and C-4 protons and carbons can be used to report on the *cis–trans* isomerism of a His-Pro peptide bond. The effect was used to follow the isomerisation of angiotensin and thyroliberin analogues with change in pH. According to theoretical calculations both angiotensin II and enkephalin should favour folded conformations at neutral pH and more extended ones at acid pH values.[92]

The existence of two folded forms, dependent on pH, for the opioid pentapeptide methionine-enkephalin is responsible for some early discrepancies between published spectra.[93,94] More detailed studies at 270 MHz (see Fig. 6) suggest the existence of three conformations in the pH region 1–10.[95] A first conformational transition occurs corresponding to titration of the N-terminus, and a second concomitantly with the phenolic group titration. Rotamer populations of Phe-4 are found to vary considerably with solvent in organic and aqueous solutions,[96] and significant concentration dependence of C_α proton chemical shifts in deuterated dimethyl sulphoxide solution of Met-enkephalin indicates molecular aggregation of a type which allows intermolecular ring-current effects.[97] Dimethyl sulphoxide was used as solvent by Stimson *et al.*[98] in their ^{13}C NMR study of leucine-enkephalin in which selective ^{13}C enrichment removes some ambiguities in ϕ and χ measurements; they concluded that the molecule contains a type I β bend at residues Gly-3-Phe-4 and that the sidechains of Tyr-1, Phe-4, and Leu-5 exist predominantly in one conformation (tg^-) in this solvent. Enkephalin has the sequence of residues 1–5 of β-endorphin, which in turn forms residues 61–91 of lipotropin. An outline structure for β-endorphin has been obtained by Levine *et al.*[99] using a Gd^{3+} relaxation probe. Although Tancrede *et al.*[100] report α-endorphin to be a flexible polymer, the ^{13}C spin–lattice relaxation time measurements indicate that in aqueous solutions the molecule may even be a random coil, with the enkephalin segment varying little in motional properties from the rest of the peptide. The same laboratory has contributed a relaxation study of the binding of enkephalin to phosphatidylserine. Binding of the enkephalin is much weaker than that of morphine derivatives, and no evidence of binding of Met-enkephalin to either egg lecithin or cerebroside sulphate is found.[101]

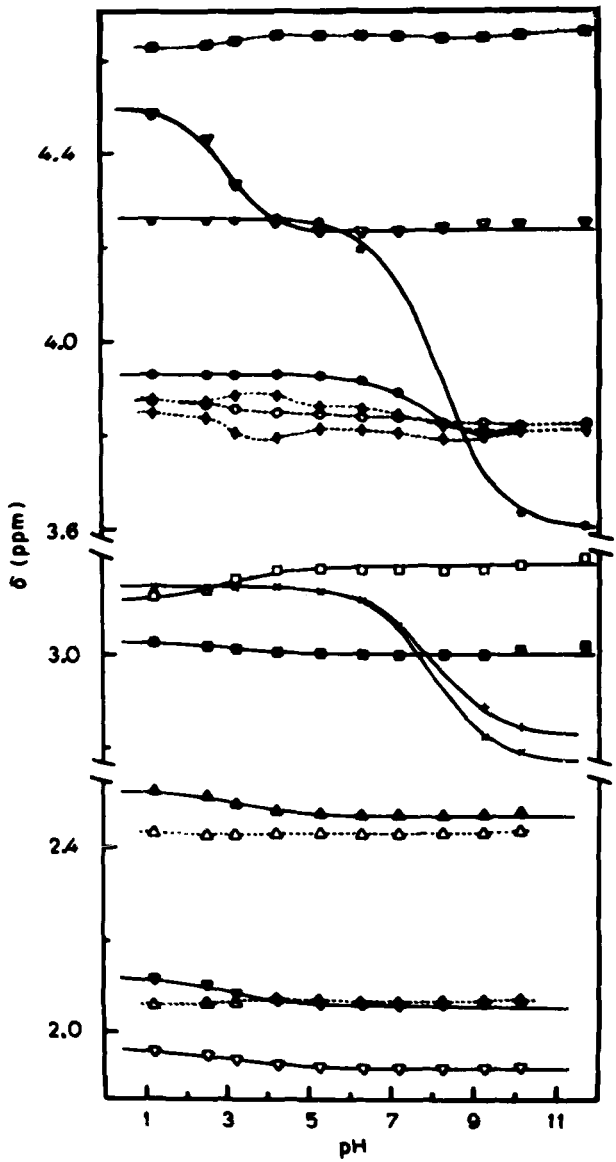

FIG. 6. Plot of chemical shifts versus pH for aliphatic protons of Met-enkephalin.

Tyr-1: H^{α} (*) H^{β} (×) $H^{\beta'}$ (+)
Gly-2: H^{α} (○) H^{β} (●)
Gly-3: H^{α} (◇) $H^{\alpha'}$ (◆)
Phe-4: H^{α} (⊡) $H^{\beta 2}$ (■) $H^{\beta 3}$ (□)
Met-5: H^{α} (▽) $H^{\beta 2}$ (▽) $H^{\beta 3}$ (▼)
$H^{\gamma 2}$ (△) $H^{\gamma 3}$ (▲) H_3^{ε} (△)

The solid lines correspond to the function $pH = pK_{\alpha} + S^{-1} \log[(\delta_{HA} - \delta)/(\delta - \delta_{A^-})]$. The broken lines are a simple interpolation between the experimental points.[95]

The nonapeptide hormone bradykinin has been studied by Lintner *et al.*[102] who are able to assign all the α-CH ^{1}H signals and most others at 270 MHz and thus to follow pH-dependent conformational changes in some detail. Ionisation of the terminal carboxyl group of the peptide affected not only residues 8 and 9 at the C-terminus, but also the α-CH protons of glycine-4, an effect attributed to cooperative effects along the protein backbone. Phenylalanine-8 appears to be an important residue in determining both the *cis–trans* behaviour of the proline residue 7 and the chemical shifts in the NMR spectrum. Replacement of serine-6 by glycine leads to a dramatic change in *cis/trans* ratio, and this was accompanied by a decrease in activity of the molecule.[103] Measurements of the ^{13}C T_1 values indicate rapid motion for all peptide chains, supporting the interpretation that bradykinin exists in solution primarily in a disordered state.[104]

V. SMALL PEPTIDE TOXINS AND ANTIBIOTICS

Like many other small peptides, the relatively accessible NMR parameters of gramicidin-S, a cyclic decapeptide, encourage the application of sophisticated techniques. Kuo *et al.*[105,106] followed the biosynthesis of specifically deuterated gramicidin-S by a series of difference scalar decoupling experiments in which all C_α–C_β systems were analysed. Their results confirm earlier conclusions that there is rotation about the C_α–C_β bond in the sidechains of residues 1–4 of the peptide, and argue strongly for the presence of only one conformer for the proline residue. The same group has already concluded[107] from proton–proton NOE data that its conformation is a II′ β turn/antiparallel β pleated sheet. The ^{15}N assignments for all nitrogen atoms in gramicidin-S were made by Abu Khaled *et al.*[108]

The propensity of the linear polypeptide antibiotic gramicidin-A to form ion-conductive channels through membranes has been investigated by several laboratories. Perdeuteration of the hydrocarbon chains of dimyristoylphosphatidylcholine enabled Feigenson *et al.*[109] to observe a linewidth of some 80 Hz for tryptophan proton resonances from the peptide after sonication into bilayer vesicles, thus indicating significant local mobility for the residues, while addition of lanthanide ions causes chemical shifts indicative of solvent accessibility and thus a probable interfacial location of the tryptophan residues. More detailed studies using ^{13}C and ^{19}F labelling of the termini of the peptide led Weinstein *et al.*[110] to the conclusion that the C-terminus of the peptide is near the surface of the bilayer, while the N-terminus is buried deep within the bilayer, and thus to the suggestion of a helical N-terminal to N-terminal dimer as the major conformation for the gramicidin channel. In solution in deuterated dimethyl sulphoxide,

however, the peptide is suggested[111] to have an LD-ribbon structure, following comparison with the known conformations of alternating L- and D-residues in synthetic polypeptides.

Formation of ion channels by gramicidin-A has been investigated by Urry *et al.*[112] who used ^{23}Na NMR as a probe. Having first shown[113] that a full interaction of the antibiotic with the hydrophobic core of the phospholipid micelles is only obtained after heating the preparation to 68 °C for a short time, it was demonstrated that the interaction of Na^+, characterised by changes in ^{23}Na longitudinal relaxation times, only occurs after this high-temperature incubation has taken place. The temperature dependence of the ^{23}Na NMR linewidth indicates an exchange process with an interaction energy of 6·8 kcal mol^{-1}, which is essentially the same as the activation energy reported for transport through the channel in lecithin bilayer studies. Other ^{23}Na NMR studies[114] show that in ethanol–water mixtures the gramicidin-A dimer binds Na^+ with a binding constant $K = 4\ M^{-1}$.

A single molecule of the cyclic depsipeptide ionophore valinomycin can transport 10^4 K^+ ions per second across a membrane. The solution conformation of valinomycin was studied by Bystrov *et al.*[115] who extensively assigned ^{13}C residues and determined ^{1}H–^{13}C coupling constants, with the conclusion that in media of weak polarity the molecule forms a bracelet structure formed by six fused β turns, with axial amino acid carbonyls. On complexation with a cation, the carbonyl groups bend towards the centre of the molecular cavity. In solvents of medium polarity, the molecule is found to be in a propeller configuration with three type II β turns. Cyclic peptide analogues of valinomycin have also been investigated, cyclo-(Pro-Val-D-Ala-D-Val)$_3$ by Davis and Gisin[116] and cyclo-(Val-Gly-Gly-Pro)$_3$ by Easwaran *et al.*[117] Both bind potassium strongly, though not necessarily in the same way as valinomycin.

A comprehensive study of another cyclic antibiotic, tyrocidine A, is presented by Kuo and Gibbons.[105] They apply two distinct forms of difference double resonance to provide scalar decoupling and proton–proton NOE data. In addition to confirming the sequence of the decapeptide, they provide a model for the structure of the molecule involving a β-I turn/β-II′ turn/antiparallel β pleated sheet conformation. The β sheet is identified in part by the ^{1}H–^{1}H NOE between two α-CH protons of the central residues, in this case Phe-7 and Orn-2. Space precludes the inclusion of details of other peptide antibiotics, but leading references are given by Callens and Anteunis[118,119] (virginiamycin S), Higashijima *et al.*[97,397] (toxin II from *Alternaria mali*), Perkins *et al.*[120] (polymyxin B), Kitagawa *et al.*[121] and Hawkes *et al.*[122] (viomycin), and Garbay-Jaureguiberry *et al.*[123] (iturin A).

VI. TRYPSIN INHIBITORS

One of the most intensively studied molecules in biochemical NMR is the bovine basic pancreatic trypsin inhibitor. This small protein (mol. wt. 6500) binds at the active site of trypsin at alkaline pH so tightly that, although it is a substrate for the enzyme activity, no water molecule is able to enter to complete the catalytic step. In addition to investigations on the molecule for itself, it has been used as a convenient test-bed for the development of NMR techniques including decoupling, NOE, and the 2-dimensional *J*-resolved spectroscopy. The papers mentioned below are far from a complete bibliography, but the others are extensively cross-referenced. Starting from extensive assignments of the ^{1}H spectra[124,125] and ^{13}C spectra[25,126] of the inhibitor at high field (360 MHz for protons and 90·5 MHz for carbon) the work has developed into an investigation of the effect of a salt link between N- and C-terminals of the protein on the overall stability of the structure, to which it contributes 4·2 kJ mol^{-1}.[127] This salt bridge is competitively inhibited by the binding of lanthanide shift reagents[128] which also bind to carboxylate sidechains within the molecule, enhancing the resolution of the spectrum and in particular enabling the dynamic properties of aromatic residues to be investigated. A study using highly refined atomic coordinates for the molecule was then undertaken to determine the mechanism of conformation-dependent chemical shifts in BPTI.[129] The dominant contribution to the observed conformation-dependent shifts of aliphatic sidechain resonances is made by the ring-currents of aromatic residues, but these do not make dominant contributions to the conformation-dependent shifts of either backbone protons or aromatic sidechain protons. Further studies involved chemical modifications such as cleavage of the disulphide bond 14–38 and cleavage of the bond Lys-15–Ala-16 which is the site which is cleaved by trypsin.[130,131] In each case the molecule is somewhat destabilised and denatured at a lower temperature, but maintains essentially the conformation of the unmodified protein. Attention was then turned to the assignment, hydrogen–deuterium exchange rates, and exchange kinetics of the labile amide protons in the inhibitor molecule, reported in a series of papers[11,130,132] in which all the known proton assignments for the basic pancreatic trypsin inhibitor are presented along with a new multi-state model for globular proteins. The exchange rates of interior amide groups appear to be governed by global rather than local fluctuations in the protein[131] although this last point would be argued by Hilton and Woodward[133] who draw the opposite conclusion from their study of the pD dependence of exchange rates of eight amide protons in BPTI. In a later paper Hilton and Woodward[134] elaborate their theory to postulate two separate mechanisms, with differing temperature dependences, for the

accessibility to solvent which leads to exchange; one of these is related to thermal unfolding, with a high activation energy of ~60 kcal mol^{-1}, while the other is a dynamic process of the folded conformation with an activation energy of 20–35 kcal mol^{-1}. We must await further evidence to decide between the two models. Other, related trypsin inhibitors which can profitably be compared with the BPTI include the glycoprotein cow-colostrum trypsin inhibitor,[135] the isoinhibitor K from the snail *Helix pomatia*,[136] the soybean trypsin inhibitor,[137] and the porcine pancreatic trypsin inhibitor.[138]

A number of individual amino acid residues within the *Streptomyces* subtilisin inhibitor were subjected to detailed NMR analysis by Akasaka *et al.*[139] who find a very mobile segment between residues 52 and 75 in the hydrophobic core of the protein. The mobility is inhibited by the binding of the inhibitor to subtilisin.

VII. ENZYMES

A. Oxidoreductases

One of the attractions of enzymes such as liver alcohol dehydrogenase is that one or both of the Zn^{2+} cations can be substituted by a paramagnetic cation. Boccalon *et al.*[140] substituted one of the zinc atoms with cobalt, measuring the resultant effects on the relaxation rates and chemical shifts of substrate ethanol. The mean distances of the CH_2 and CH_3 protons from the active site Co are found to be 3·8 and 5·3 Å respectively, agreeing well with the ethanol being directly coordinated with the active site metal in an approximately *anti* conformation. Chlorine-35 NMR quadrupole relaxation measurements[141] on both native and carboxy-methylated horse liver alcohol dehydrogenase indicate that anions in solution do not bind to the active site metal ions, but rather to basic sidechains in the protein, probably arginines 47 and 271. The tetrameric glyceraldehyde-3-phosphate dehydrogenase, on binding to substrate glyceraldehyde-3-phosphate, causes shifts in the substrate ^{31}P resonance. With enzyme bound to the inner face of a membrane vesicle, alterations in the Na^+/K^+ ratio at the outer face cause changes in these shifts. Changes in potassium level, but not of sodium, thus cause conformational changes through the membrane. Fossel and Solomon[142] claim this as evidence for a functional link between the phosphate dehydrogenase and $(Na^+ + K^+)$-ATPase, since the half-values for the conformation changes are the same as those for the activation of the ATPase. Specific histidine resonances, assigned to the exterior histidines 162 and 327, are resolved in glyceraldehyde-3-phosphate dehydrogenase by Scheek *et al.*[143] using photo-CIDNP, and found to have pK_a values of 6·90 and 6·60 respectively.

Glutamate dehydrogenase is a large hexameric protein whose subunits have a molecular weight of about 56 000. When the binding of dicarboxylic acid substrate analogues to the enzyme is monitored by ^{1}H NMR, it shows very little discrimination between analogues of different length, charge, bulkiness, and conformational rigidity.[144] Further studies of substrate and coenzyme binding[145–147] using stable nitroxide spin-labels linked to the activator, ADP, for the enzyme show that NADP binds in an open conformation while the activator binds in the neighbourhood of the active centre but with very little overlap with the coenzyme site. Binding of coenzyme to dehydrogenases may be monitored using ^{15}N NMR.[148] A series of experiments in which ^{15}N labelled NAD chemical shifts are shown to be highly sensitive to the redox state of the enzyme are reported. The kinetics of binding of the coenzymes to lactate dehydrogenase isoenzymes H_4 and M_4 from chicken are monitored using linewidth and chemical shift data with detailed computerised analysis.[149] At room temperatures the lifetimes for the complex of enzyme with NAD and NADH are of the order of 1 ms and 10 ms respectively.

The fact that dihydrofolate reductase is the target for the folic acid analogue inhibitors aminopterin and methotrexate which have strong anti-cancer activity gives it a more than intrinsic interest. The 3-dimensional structure of the dihydrofolate reductase–NADPH–methotrexate complex is available, and this has been used to assist assignments of the NMR spectra,[150] for example of two tryptophan residues which ^{19}F labelling shows to be in close proximity in the folded molecule.[151,152] It is also possible to explain the large low frequency shift of ^{13}C labelled NADP on binding to the reductase in terms of desolvation effects. It is possible to provide consistent assignments of the five histidine residues in the molecule using the crystal data, which suggests that the structures of the molecule in crystalline form and in solution are closely similar, and to titrate the histidine residues (His-124, pK' 7·9–8·2; 141, 7·2–7·4; 149, 6·5–6·7; 114, 5·7–6·3; 45, 5·2–5·9).[153] A model for the binding of trimethoprim, another anti-cancer agent, to dihydrofolate reductase from *Lactobacillus casei* is presented by Cayley *et al.* (Fig. 7)[154] in which the normally unobservable aromatic protons of trimethoprim are detected by transfer of saturation experiments and difference spectroscopy following selective deuteration of the drug. Chemical shift changes are interpreted in terms of the ring-current shift contributions from the aromatic rings of trimethoprim and that of phenylalanine-30. Selective deuteration of all aromatic residues except the 2,6-protons of tyrosine is used[155] to demonstrate that the inhibitors of dihydrofolate reductase bind differently from the substrate. A series of selective ^{13}C labelling experiments on the same enzyme from *Streptococcus faecium* yields a large amount of data, among which we may note a reinforcement of the argument for different substrate and inhibitor

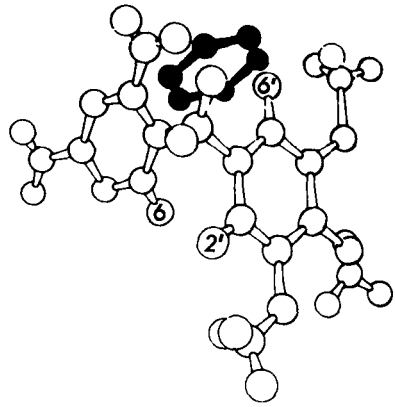

FIG. 7. One of the two possible conformations of trimethoprim in its complex with dihydrofolate reductase, showing its relationship to the aromatic ring of Phe-30 (shaded). This conformation has $\theta_1 = 205°$ and $\theta_2 = 100°$.[154]

binding,[156] the division of arginine residues into a "fixed" and a "mobile" group, with some of each involved in ligand binding,[157,158] slow exchange of tryptophan residues between two stable states,[159] and a suggestion that NADP binding results in charge transfer between the nicotinamide ring and the S atom of methionine.[158]

Bovine superoxide dismutase (erythrocuprein) is a dimer of molecular weight 31 300, each subunit containing one atom of copper and one of zinc. Measurement of proton relaxation rates as a function of frequency, temperature, pH, and CN^- concentration[160] shows that, at pH 9 or less, only one water molecule is bound to each Cu^{2+} ion, but that at higher pH a second site becomes available, possibly owing to cleavage of the bond between Cu^{2+} and the histidine residue that bridges to Zn^{2+}. Complexation of fluorine to the copper ion was found by ^{19}F resonance studies to have a ΔH of ~ 6 kcal mol^{-1}.[161] The two most buried histidine residues, 44 and 69, give proton resonances at 15·35 and 13·4 ppm, which are slowly exchanging with solvent ($\sim 2 \times 10^{-5}$ s^{-1} at 37 °C); NOE data[162] have been used to establish that the broad resonances at 12·1 and 13·6 ppm originate from histidine-41, which has a very abnormal pK_a of about 10·4. This residue is claimed[163,164] to be very labile, with a hydrogen–deuterium exchange half-life of ~9 hours at 40 °C and pH greater than 8. Further studies reveal that, even over very long periods of 12 days or more, histidines bonded to the metal ions do not exchange with solvent, while the non-bonded histidines 19 and 41 exchange completely in this period; in the apoenzyme, all histidines exchange completely within this time.[165] Two zinc ions can be made to bind to each subunit of the apoenzyme, though the second is shown to have a binding constant at least an order of magnitude less than the first.[166] Changes in the

whole subunit structure appear to accompany binding of the first Zn^{2+}, while binding of the second produces only local effects.

B. Transferases

The transferases, which form class 2 in the enzyme catalogue, provide a number of examples of investigations based on observation of the magnetic resonance of nuclei, often fluorine or phosphorus, which are incorporated in substrates or inhibitors. A first example may be found in the thymidylate synthetase–dUMP complex; Byrd *et al.*[167] give early details of a study using 5-fluoro-2′-deoxyuridylate to provide ^{19}F resonance signals in a ternary complex with the enzyme and 5,10-methylenetetrahydrofolate. Evidence is found for covalent linkage of the pyrimidine ring to both the enzyme and the methylenetetrahydrofolate. Much greater detail of the suggested ternary structure is presented by Byrd *et al.*[168] while Lewis *et al.*[169] conclude from similar further work that in the binary complex, lacking the folate cofactor, no covalent bond is formed between the enzyme and the nucleotide.

The chemical shift of the ^{31}P resonance from pyridoxal phosphate bound to rabbit muscle glycogen phosphorylase (b or a) is not affected by succinylation of the enzyme, which causes it to partially dissociate. This is not consistent with a location of the cofactor near the subunit interface. However, a cofactor analogue does report the binding of glucose to the enzyme, indicating a location near the catalytic site. Further details of the charge state of the cofactor and of the effects of the allosteric effector adenosine thiomonophosphate are given.[170] Selective carbamylation of lysine-258 of aspartate transaminase using KCNO or of the N-terminus using NCO results in the enzyme being able to accept phospho-pyridoxyl trifluoroethylamine instead of the normal pyridoxal phosphate.[171] The fluorinated cofactor analogue is then able to report via its ^{19}F chemical shifts on the titration of the active-site lysine residue, permitting the determination of its pK_a as 8·2. No such shifts are observed for the modified apoenzyme.

The interaction of chromium(III)ADP, an exchange-inert paramagnetic analogue of MgADP, with yeast hexokinase was studied by measuring the effects of CrADP on the longitudinal relaxation rate of the protons of water and the protons and ^{31}P atom of enzyme-bound glucose-6-phosphate.[172,173] The results indicate the formation of an enzyme–CrADP–glucose-6-phosphate complex, with a Cr^{3+}–P distance of 6·6 Å implying the absence of a direct coordination of the glucose phosphoryl group by metal. Because after phosphorylation of ADP the metal ion is coordinated to the transferred phosphoryl group, the phosphoryl group must migrate some 3·6 Å during the transfer reaction. A somewhat similar series of studies, on the conformation of tetra-ammine-cobalt(III)ATP bound to bovine heart protein

kinase, are reported by Granot *et al.*[174] (and references therein). The binding of metal nucleotide substrates and substrate analogues to the catalytic subunit of this cAMP-dependent kinase induces the appearance of an additional tight inhibitory divalent cation binding site, and this effect was used by binding Mn^{2+} at this site and measuring a number of Mn^{2+}–^{31}P and Mn^{2+}–^{1}H distances from the longitudinal relaxation enhancement brought about by the paramagnetic ion. The results are consistent with either bidentate α,γ or tridentate α,β,γ coordination of the triphosphate chain of both stereoisomers of the ATP derivative by the manganese ion.

Muscle pyruvate kinase catalyses the reaction

$$\text{ATP} + \text{pyruvate} \rightleftharpoons \text{ADP} + \text{phosphoenolpyruvate}.$$

^{31}P NMR of the reaction mixture of such a reaction, under conditions chosen so that all components are present, yields six separate ^{31}P signals corresponding to the six phosphate groups in the reaction mixture for this and other phosphoryl transfer enzymes. It is thus possible, from linewidths and the presence or absence of exchange, to determine lifetimes and exchange rates.[175] For example, at catalytic concentrations of pyruvate kinase, pH 8·0, and 15 °C, the equilibrium constant is $\sim 3 \times 10^{-4}$ with MgATP as reactant and MgADP as product, whereas the equilibrium constant for enzyme-bound substrates and products is of the order of 1.[176] Earlier studies of the same enzyme include ^{7}Li NMR measurements of its lithium-binding propensities[177] and paramagnetic-probe investigations of the conformation of substrates,[178] of the distance between enzyme and nucleotide-bound metal ions,[179] and of the structures of enzyme-bound nucleotide complexes at high concentrations of enzyme and substrate.[180]

The phosphoenolpyruvate-dependent phosphotransferase system of bacteria relies on a phosphocarrier protein called HPr. This shows a sharp, reversible denaturation behaviour at alkaline pH, and contains three tyrosines whose titration may be monitored by ^{1}H NMR.[181] Sequential nitration of these tyrosines permits assignment of their resonances, leading to the conclusion that tyrosines 56 and 37, with pK values of 10·5 and 11·5, are at most only slightly buried, while tyrosine-6, which does not titrate before alkaline denaturation of the protein, is deeply buried.[182] A link between the conformation of the phosphocarrier protein, the protonation state of its active histidine, and the phosphoryl group transfer step is established by the ^{1}H and ^{31}P NMR studies of Dooijewaard *et al.*[183] Other kinase enzymes which have been studied recently include adenylate kinase,[184] carbamate kinase,[185] 3-phosphoglycerate kinase,[186] and phosphoglucomutase.[187] In the last of these, clear evidence is adduced of the close proximity of the bound metal ion and the active site phosphoserine residue.

C. Hydrolases

The photo-CIDNP spectra of tryptophan-3 of bovine pancreatic phospholipase A_2 were obtained, as a function of pH, by Jansen *et al.*[188] The aromatic protons undergo a pH-dependent chemical shift change with a pK of 8·9, corresponding to the deprotonation of the NH_3^+ of the N-terminal alanine residue. This is interpreted as the result of a conformational change consequent on the breaking of a salt link which had been holding the N-terminus in the interior of the protein. Histidine titrations of pancreatic phospholipase A_2 are reported by Aguiar *et al.*[189] The activities of cobra venom phopholipases A_2 and C towards different phospholipids in mixed micelles with Triton X-100 are reported by Roberts *et al.*[190] and of α-phospholipase A_2 from the rattlesnake *Crotalus adamanteus* venom towards human blood serum high-density lipoprotein-3 by Brasure *et al.*[191]

1. Alkaline phosphatase

The alkaline phosphatase of *Escherichia coli* is a dimeric zinc metalloenzyme of two identical subunits. Varying the metal content while following the tight phosphate binding at the active site by its ^{31}P chemical shift shows that in all cases only one phosphate is tightly bound per dimer, although variations do occur in the structure of the non-covalent phosphate complex.[192] Further information on the rate constants which characterise the formation and breakdown of the non-covalent and covalent enzyme–phosphate intermediates is provided through ^{31}P saturation transfer experiments, by which Otvos *et al.*[193] are able to show unequivocal evidence for direct metal–phosphate interaction. Phosphorus-31 NMR also shows that, while only 1 mol of non-covalent complex is formed at pH 8 by the 2-zinc enzyme, and 1 mol of covalent intermediate at pH 6·5 by the 2-cadmium enzyme which is also active, enzymes containing an extra pair of Zn^{2+} or Cd^{2+} ions plus two Mg^{2+} ions are able to form two such complexes. This varying effect of metal ion content may account for earlier conflicting reports of the stoicheiometry of the enzyme[194] and suggests a possible role for metal ions in the environment as allosteric activators of the enzyme, quite separate from the direct catalytic role of the bound Zn^{2+} ions. Some weight is lent to this argument by Norne *et al.*[195,196] whose ^{35}Cl NMR measurements of anion binding to alkaline phosphatase suggest the existence of two forms of the enzyme with different Zn^{2+} binding properties, and a marked interdependence of Mg^{2+} and Zn^{2+} binding. An interesting series of experiments by Bock and Cohn[197] follows the progress of the phosphate(oxygen)–water exchange process in alkaline phosphatase by using the ^{18}O-induced shift in ^{31}P NMR, which enables signals from the various P_i isotopic hybrids from $P^{16}O_4$ to $P^{18}O_4$ to be distinguished. The kinetic scheme appears to involve the non-covalent enzyme–P_i complex dissociat-

ing more rapidly than the covalent complex is formed. The isotope shift method was also used to characterise the water–phosphate oxygen exchange process in human prostatic acid phosphatase.[198] Inorganic phosphate binding to yeast inorganic pyrophosphatase was investigated using the fact that the enzyme binds manganese;[199] the Mn^{2+}–^{31}P distance, at 6·2 Å, is consistent with outer sphere binding.

2. *Ribonucleases*

There are a number of reasons for the continuing international popularity of ribonuclease A among NMR spectroscopists, quite apart from its small size. One of these is its reversible denaturation. The reason for this ready reversibility may well be that it never really unfolds properly; Howarth[22] reports that even at pH 1·1 and 70 °C there is still considerable motional restriction of cysteine and proline residues, and suggests that the denatured state is still semistructured, although Lenstra *et al.*[200] state that the deviations from a random coil are small. The presence of a structural intermediate at a very early stage of refolding at 10°, a structure including the residue histidine-12, may link with these conclusions.[201] Das *et al.*[202] report that the presence of inhibitors helps stabilise RNase A against unfolding. A novel NMR technique, that of rotating frame spin–lattice relaxation in the presence of an off-resonance radiofrequency field, was applied by James and Sawan[203] to the unfolding of RNase A by successive addition of guanidinium chloride, with the result that a sequential motional increase for histidines 12, 105, 119, and finally 48 was revealed; previous standard T_1 measurements had not revealed the differences.[204]

The propensity of ribonuclease A to reassociate as the active ribonuclease S^1 after cleavage of the S-peptide (residues 1–20) from the S-protein (21–124) is another reason for its popularity with experimenters. Good use of this property was made by Niu *et al.*[49] who synthesised the peptide 1–15 using solid-phase methods with selective ^{13}C enrichment and used the resultant ^{13}C NMR spectrum to characterise the environment of each site in the complex. They show that aspartate-14 is a hydrogen-bond acceptor, further supporting the conclusive evidence of Santoro *et al.*[205] for an interaction between aspartate-14 and histidine-48. The aromatic residues of RNase A were tentatively assigned to their ^{13}C resonances by Egan *et al.*[206] and their accessibility to solvent was further elucidated by Bolscher *et al.* (see Fig. 8)[207] who used photo-CIDNP difference spectra in the presence of 3-*N*-carboxymethyl-lumiflavin. Tyrosines 76 and 115 are the only ones to show emission in the spectrum, and in addition cross-relaxation effects result in positive enhancements for the C_2 and C_4 protons of the active site histidine-119. This histidine also provides a strong binding site for Co^{2+} at pH 5·5, with His-105 providing a weak secondary site which becomes

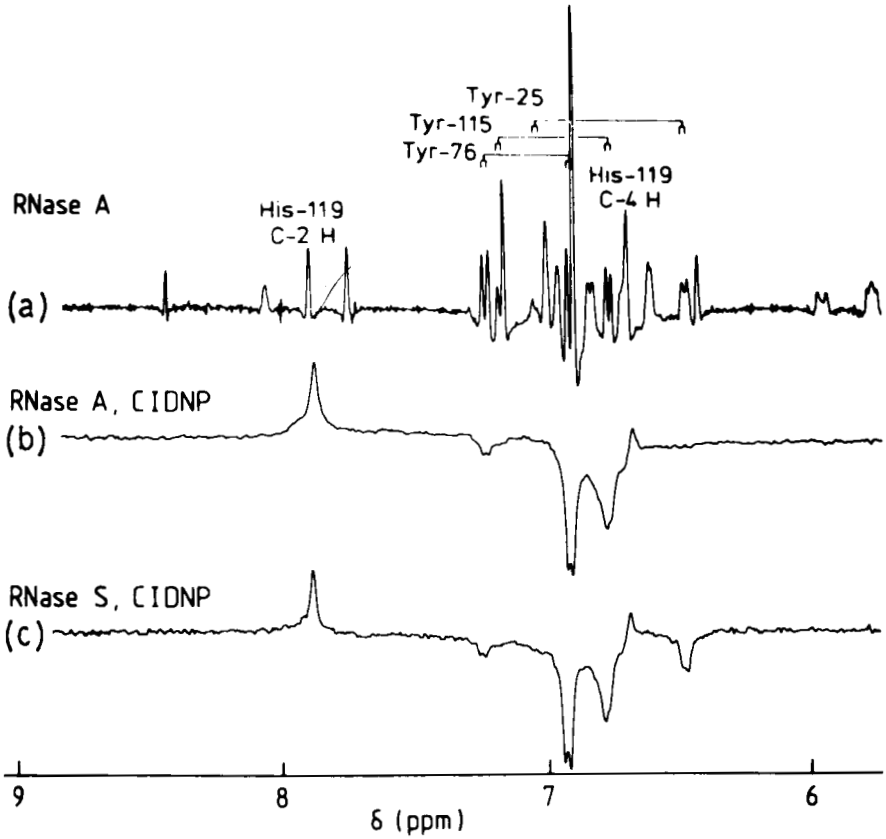

FIG. 8. (a) The aromatic region of the 360 MHz ^{1}H NMR spectrum of $3{\cdot}0\times10^{-3}$ M bovine ribonuclease in D_2O, pH 7·0 (direct meter reading), 38 °C. The samples were deuterated by incubating three times in D_2O at 60 °C for 10 min, followed by lyophilisation. The resolution was enhanced by multiplication of the free-induction decay by a sine bell. Chemical shifts are relative to DSS.

(b) Photo-CIDNP difference spectrum (light minus dark) of $1{\cdot}5\times10^{-3}$ M RNase A and 2×10^{-4} M 3-*N*-carboxymethyl-lumiflavin after accumulation of 25 pulses (without resolution enhancement); conditions the same as under (a). Negative peaks indicate emission, positive peaks enhanced absorption.

(c) Photo-CIDNP difference spectrum of RNase S; same conditions as under (b).[207]

equally strong by pH 7.[208] His-119 is also implicated in the binding of 2′-deoxy-2′-fluororibose substrate analogues[209] and hexacyanochromate anions.[210]

Porcine pancreatic ribonuclease is a glycoprotein; the titration of its histidine residues is very insensitive to the removal of 80% of the carbohydrate from the protein, suggesting that the heterosaccharides project

away from the surface of the protein into the solution environment.[211] Ribonuclease T_1, a quite different protein produced by microorganisms, has been studied with ^1H and ^{31}P NMR by Arata *et al.*[398] They find that the catalytic activity of the enzyme apparently depends on histidine-40 and a carboxyl group, in contrast to the RNase A case in which two histidines act as a general acid and a general base to conduct the catalytic action. A scheme of the active site and of the interaction of the enzyme with 3′-GMP is presented.

3. Lysozyme

Hen egg-white lysozyme, as well as being the first protein to yield an atomic-resolution crystal structure, was a very early test-bed for lanthanide shift reagents. In general it has been assumed that the magnetic susceptibility tensor of these metals is axially symmetric, an assumption which is rejected by Agresti *et al.*[212] for Nd^{3+} and Ce^{3+} and by Lenkinski *et al.*[213] for cobalt. Since the shift perturbations produced by Co^{2+} and the broadenings induced by Gd^{3+} obey different geometrical relations, the assignments for the signals from the indole NH protons of the tryptophan residues in lysozyme may be cross-checked by using both metals, in association of course with the crystallographic data for the enzyme.[214] The free availability and good solubility of lysozyme make it also a good subject for natural-abundance ^{13}C NMR spectroscopy; this was used by Dill and Allerhand[215] to investigate the effect of chemical modifications of Trp-108 on the binding of lanthanide cations (the binding is weakened by a factor of more than 20) and by Goux and Allerhand[216] to study the carboxymethylation of histidine residues. The titration behaviour of the ^{13}C resonances of carboxyl groups in RNase and lysozyme is reported by Shindo *et al.*[217] and the mobility and self-association of lysozyme by deuterium resonance by Wooten and Cohen;[218] apparently lysozyme is predominantly dimerised at pH 7·5.

4. Proteases

The high frequency histidine resonances of proteins are often obscured by slowly exchanging backbone NH signals, Markley and Ibanez[219] overcame this problem by reversibly unfolding bovine chymotrypsinogen in ^2H_2O to ensure complete NH exchange, and were then able to study the titration behaviour of the histidine resonances in detail. As well as inflections in the titration curves caused by interactions with other titrable sidechains, they were able to show significant differences in the structure of the catalytic centre between the zymogen and the activated enzyme; the abnormal chemical shift of active site histidine-57 in the zymogen becomes normal when the molecule is activated. Markley[220] also describes a technique for observing protein proton spectra in undeuterated water solution, which

enables the observation of hydrogen-bonding protons which would normally have exchanged in D_2O solution. The behaviour of a peak at 18 ppm, assigned to the histidine-57–aspartate-102 hydrogen bond, is consistent with the presence of two structural states of the enzyme in dynamic equilibrium with lifetimes in the 0·5–2 ms range. Binding of an inhibitor abolishes the equilibrium and leads to the formation of a number of new hydrogen bonds. Further treatment of the titration of histidine-57 is described by Porubcan *et al*;[221,222] in the latter report the titration of His-57 is followed by an interesting technique involving following the ^{31}P signal from the neighbouring residue serine-195, which has been modified by diisopropylphosphorylation. This work is carried out on a range of seven serine proteases or their zymogens; formation of the serine derivative has a greater effect on His-57 titration in the enzymes than in the zymogens.

A series of papers from the laboratory of Gerig[223–225] describes the use of fluorine as a probe for chymotrypsin structure, both through the formation of complexes of the enzyme with fluorocinnamate and by the formation of trifluoromethylbenzenesulphonyl modifications. *N*-Acetyl-L-phenylalaninal, in its aldehyde rather than hydrated form, is an effective inhibitor of chymotrypsin. Cross-saturation NMR reveals that it binds to the enzyme with the formation of a hemiacetal between the aldehyde and the active site serine residue.[226] This study is based on earlier work[227] in which evidence for hemithioacetal formation in the inhibition of papain by *N*-acylaminoacetaldehydes and *N*-acylaminopropanones is claimed to be the first direct evidence for the formation of a tetrahedral adduct with papain, supporting the suggested involvement of such intermediates in papain-catalysed hydrolysis.

5. ATPases

An interesting labelling agent for the active site of ATPases is described by Granot *et al.*[228] The complex Co(III)–phenanthroline–ATP is paramagnetic, resulting from the incorporation of superoxide anion into the coordination sphere of the Co(III) complex. Another interesting approach to ATPase is via $^7Li^+$ binding; there is a Li^+ site 7·2 Å from the single catalytically active Mn^{2+} site on sodium-potassium ion dependent ATPase[229] which appears from competition studies to be a K^+-type site. More wide-ranging studies from the same group, this time on sarcoplasmic reticulum calcium-ion transport ATPase, reveal that Gd^{3+} binds to the two Ca^{2+} binding sites of the enzyme. Lithium activates the enzyme in place of potassium, and relaxation measurements show that the two Gd^{3+} ions are 7·0 and 9·1 Å from the lithium-binding site. The separation of the two calcium-binding sites is thus 16·1 Å or less.[230] Earlier studies of the same protein show that its motion in the membrane is quite rapid, especially when

compared with the motion of other membrane-bound proteins such as rhodopsin.[231]

D. Lyases, isomerases, and ligases

The only carbon–carbon lyase in this report is the ribulose phosphate carboxylase from *Rhodospirillum rubrum*. O'Leary *et al.*[232] report that activation of this enzyme by Mg^{2+} and CO_2 appears to involve formation of a carbamate between the CO_2 and an enzyme NH_2 group; they base this conclusion on the appearance of a broad resonance at 164·9 ppm in addition to the expected resonance for $H^{13}CO_3^-/CO_3^-$ at 161·8 ppm.

Much more interest has been shown in the carbon–oxygen lyase carbonic anhydrase. A number of reports centring on the use of paramagnetic probes at the active site of bovine carbonic anhydrase have appeared, starting with the effect of Ca^{2+} on water relaxation in solutions of the protein[233] and of Cu^{2+} and Co^{2+} on ^{31}P resonance shifts in phosphate ion[234] and CO_2/HCO_3^- interactions with the copper-substituted enzyme. ^{13}C longitudinal relaxation measurements appear to show that whereas HCO_3^- binds at the metal ion, substituting for a water molecule in the process, CO_2 does not, although it does still bind in the protein part of the active-site cavity.[235] Carbon-13 NMR of carbonic anhydrase carboxymethylated at histidine-200 is reported by two separate groups. Jeffers *et al.*[236] state that the chemical shift and relaxation behaviour of the label ^{13}C are consistent with the immobilisation of the carboxylate at pH 8 and its approach or coordination to Zn, a coordination which is disrupted on lowering the pH to 5·5. Internal motion of the carboxylate is eliminated by inhibitor binding; the catalytic consequences of carboxymethylation may be due to the coordination to Zn rather than any influence that the modification has on the role of histidine-200 in the catalytic mechanism. Coordination of the carboxylate to Zn is also favoured by the detailed ^{13}C-label pH-dependence studies conducted by Uiterkamp *et al.*[237] Aniline and methanol are specific, active-site directed inhibitors of bovine carbonic anhydrase B; evidence is presented by Westerik *et al.*[238] that aniline is coordinated to the metal, either at a fifth coordination site or by replacing one of the histidine sidechains which normally coordinate the metal, while methanol does not bind directly to the metal ion but rather to a hydrophobic region within the catalytic site.

Porcine pancreatic colipase has a short region containing three tyrosine residues (53, 56, 57 in the sequence). Two groups of workers have studied the titration behaviour of these, finding pK values in the region of 10·3 for residues 56 and 57 and more than 11 for Tyr-53. In addition, Wieloch *et al.*[239] report that the binding of taurodeoxycholate affects the resonances of all three tyrosines and of one histidine. Canioni and Cozzone[240] extend this by noting that it is histidine-86 which is interacting with the two adjacent

tyrosines, and that therefore presumably the peptide chain is folded such that regions around residues 56 and 86 come together to form a hydrophobic-aromatic binding site which may be involved in the binding of bile salt micelles to the protein.

The ^{31}P NMR spectrum of dihydroxyacetone phosphate in the presence of triose phosphate isomerase shows two resonances; that of the free keto form is broadened, but not that of the hydrate, showing that the hydrate does not bind non-productively to the enzyme and need not be taken into account when calculating steady state kinetic parameters.[241] In addition[242] ^{13}C and ^{31}P NMR of 2-phosphoglycollate in the presence of triose phosphate isomerase at different pH values seems to show that it binds as the trianion, indicating two modes of ligand binding. Further studies on the system are reported by Campbell *et al.*[243] and a model is suggested to explain the pH dependence of enzyme reactions.

Overactivity of phosphoribosylpyrophosphate synthetase causes gout; the molecule also binds CrATP in competition with MgATP but not in competition with ribose-5-phosphate, indicating that MgATP and ribose-5-phosphate bind at separate sites. From the paramagnetic effects of bound CrATP on T_1 values, Li *et al.*[244] could determine three distances from Cr^{3+} to ribose-5-phosphate, ranging from 6·7 to 8·0 Å. Both the α- and the β-anomer of ribose-5-phosphate bind close to the Cr^{3+}, and with the given distances the 1-OH of α-ribose-5-phosphate could be in van der Waals contact with the β-P of the CrATP, which is consistent with a direct nucleophilic displacement of AMP from ATP by α-ribose-5-phosphate; the β-anomer appears to be incorrectly oriented for such an attack. Another application of paramagnetic perturbations to enzyme–substrate interactions is reported by Chock *et al.*[245,246] By combining fluorescence energy transfer with ^{13}C and ^{31}P NMR from ATP-2-^{13}C-adenylylated glutamine synthetase from *E. coli* with Mn^{2+} and Co^{2+} at the metal binding sites, they determine that the covalent modification site is very close to the catalytic centre of the enzyme, and in addition that the adenylyl site is on the surface of the enzyme.

VIII. HAEM PROTEINS

A. Cytochromes

Variations in the chemical shift of methionine NMR resonances for different cytochromes indicate that there is a change in Fe–S bond length with variation in redox potential, with ~0·1 Å shortening associated with a decrease in redox potential of ~400 mV. Such a correlation can be directly related to the donor power of the methionine S and ultimately to the protein conformation.[247] Model systems for the study of haem proteins are

discussed at some length by La Mar;[248] model systems are also discussed by Budd *et al.*[249] and Huang and Kassner.[250]

Cytochrome-c oxidase is a member of the cytochrome-a group in which the haem prosthetic group contains a formyl sidechain. It is found in association with lipids, and Falk and Karlsson[251] report that the resonances from phosphatidylcholine vesicles containing cytochrome-c oxidase show intensity reductions which vary with the amount of protein present, indicating that some lipid molecules are immobilised in the vicinity of the protein molecule. About 0·7 mg of lipid is immobilised per mg of oxidase. Application of a quadrupole-echo Fourier transform method to deuterium resonance of specifically deuterated phospholipids enabled Kang *et al.*,[252] reasoning from linear plots of quadrupole splitting vs. protein/lipid ratio, to infer that a simple 2-site exchange model may suffice for the protein–lipid interaction; in a later study[253,254] the addition of ^{31}P resonance enabled the observation of the effect of cholate on the interaction.

Proteins of the cytochrome-b group are defined as containing a protohaem or haem group not covalently bound to protein; the group includes cytochrome P-450. Philson *et al.*[255] report that the presence of this protein in aqueous solution significantly affects the transverse and longitudinal relaxation times of the water protons in a way which is consistent with one or two protons exchanging between a site on a haem ligand and bulk water, with a residence time on the ligand of the order of 1 μs. The difference between T_1 and T_2 observed in the fast exchange limit is then used to deduce a Fe–proton distance of 2·9 Å, and the results are used as a basis for a discussion of the axial haem ligand. Accessibility of the iron atom to solvent protons in P-450 and P-448 has also been measured, in a similar manner, by Maricic *et al.*[256] both in microsomes and for solubilised samples. The haem-accessibility league table runs: rat P-448 < rabbit P-448 < rat P-450 < rabbit P-450 in microsomes; rabbit P-448 < *Pseudomonas putida* P-450 < rat P-448 < rat P-450 < rabbit P-450 in solubilised samples. For the latter, it appears that increased haem specificity is associated with a closing of the haem cleft.

The cytochromes c, in which the prosthetic group is covalently linked to the protein, have been studied using several approaches, and by a large number of different laboratories. Starting with NMR assignments, we find the two tryptophan residues and four of the tyrosine residues assigned in cytochrome-c from *Euglena gracilis* and the axial haem ligands identified as histidine-14 and methionine-56.[259,258] The intermolecular exchange of electrons has a rate constant of 5×10^6 mol^{-1} s^{-1}, about three orders of magnitude faster than that of a mammalian cytochrome-c. Assignments have been reported for the haem-c proton resonances for both horse heart cytochrome-c[259,260] and the cytochrome c-557 from *Crithidia oncopelti*.[261]

Comparison of the two sets of results shows that both the haem crevice and the haem electronic structure are almost identical between the c-557 and the mammalian cytochrome-c. A comparison with mammalian proteins is also made by Smith[262] following assignment of haem-c resonances of the cytochrome-c_2 of *Rhodospirillum rubrum*. The comparison process is taken much further by Cookson *et al.*[263]—all mitochondrial cytochromes c are very similar to the cytochromes c_2 from Rhodospirillaceae. In the smaller bacterial cytochromes, *Pseudomonas aeruginosa* cytochrome c-551 and *Euglena gracilis* c-552, the orientation of groups near the haem is similar, but the folding of the polypeptide chain is different. The haem environment of these last two is similar to that of the larger bacterial and mitochondrial cytochromes, but two low-potential cytochromes investigated have unusual haem environments. Such comparisons doubtless have evolutionary significance; similar ones are discussed from the evolutionary point of view by Keller and Wüthrich.[260] Alongside structural studies we inevitably find measurements of pH dependence.[264] Among other interesting data it is concluded that the 6th ligand (methionine S) is more weakly bound than the 5th (histidine imidazole) and that an isomerisation at pH 9 is associated with the displacement of the bonded methionine-80 by lysine-79. Also to be expected in the literature are studies of conformational mobility; Burns and La Mar[265] show evidence from the linewidth of a single hyperfine shifted methyl resonance that an amino acid sidechain in contact with the haem group in horse heart cytochrome-c is in dynamic equilibrium between two conformations. Binding of small molecules or ions to the cytochrome molecule has been followed by Morishima *et al.*[266] who noted the effects of the presence of nitrogenous bases on the structure of the haem environment, and Andersson *et al.*[267] whose ^{35}Cl transverse relaxation rate measurements lead them to conclude that the binding site for Cl^- ion is near the exposed haem edge. It is possible to form a 1 : 1 cation–cytochrome-c complex using Cr^{3+}; the binding site is located on or near the protein surface, ~17 Å from the ligand methionine. It is not coordinated with tyrosine-67, but rather lies near histidine-26.[172,173]

Chemical modifications of cytochromes c have also been used to help in understanding its mechanism. Schejter *et al.*[268] chemically enriched the two methionine groups with ^{13}C, showing that, while the Met-80 ^{13}C resonance exhibits the expected behaviour [different chemical shifts in the reduced Fe(II) and cyano-Fe(III) forms, and no observable signal in the oxidised Fe(III) form due to relaxation effects], the unbonded Met-65 is also sensitive to the oxidation state of the iron. They are able to confirm the displacement of Met-80 from the iron atom at alkaline pH. pH-dependent ^{15}N shifts from ^{15}N-labelled cyanide ion bound to cytochrome-c and myoglobins are associated with a number of sidechain titrations by Morishima and Inubushi.[269]

B. Myoglobins

An early application of natural-abundance ^{13}C NMR was to horse and red kangaroo ferri- and cyanoferri-myoglobins.[270] All the non-protonated sidechain carbon atoms of aromatic amino acids give detectable resonances in the cyano forms; most of those from histidine sidechains show normal titration behaviour, but three do not in each case. Two of these are assigned to uncoordinated histidines, probably histidines 24 and 36, either in the imidazolium or in the $N^{\delta 1}$–H imidazole state, and the third to the co-ordinated histidine-93. A comparative study of sixteen myoglobins permits Botelho and Gurd[271] to assign a total of eleven histidine resonances and to compare[272] their pK values with an extension of the Tanford–Kirkwood theory, with good agreement. Further assignments, of histidine residues which only give visible resonances in the deoxy and oxy forms of myoglobins but not in the met form, and thus are thought to be near the haem group, were made by Ohms *et al.*[273] Of course, such studies of pH dependence are only of use in so far as they cast light on mechanism; the acid Bohr effect of deoxymyoglobin was studied by La Mar *et al.*,[274,399] revealing a single, well defined, pH-induced structural change, modulated by a single titrable group with a pK of ~5·7. The Bohr effect, however, does not appear to depend on such a change, since the oxygen affinity changes smoothly over a range of several pH units. Two interestingly different experimental approaches to myoglobin have appeared. La Mar *et al.*[275] have made a carbonyl-myoglobin enriched with ^{13}C and ^{57}Fe in order to detect ^{13}C–^{57}Fe coupling constants which show, from comparison with model compounds, that the carbonyl is not tilted with respect to the haem plane, while Boxer and Wright[276] claim the first well defined complex between the chlorophyll chromophore and apomyoglobin, using NMR to provide information on the location and orientation of the chromophore.

C. Haemoglobins

Not unnaturally, the chief experimental interest in haemoglobin is in the source of its cooperativity and in the binding of various ligands to it. Both of these aims are assisted by the existence of a large number of mutant haemoglobins, differing only in one or a few residues. For example, Viggiano *et al.*[277] have compared the proton spectra of haemoglobins Osler (β145HC2Tyr→Asp) and McKees Rocks (β145HC2Tyr→Term). On the addition of inositol hexaphosphate to a solution in which these molecules are predominantly in the oxy quaternary structure, their spectra become comparable to those of haemoglobins in the deoxy quaternary structure, but without a characteristic resonance at −6·4 ppm which can therefore be assigned to the proton involved in a hydrogen bond between tyrosine-145

and valine-98 of the subunit. Once assigned, this resonance becomes an important tertiary structure probe. Similar comparative studies from the same laboratory may be found.[278,279]

One of the most intriguing approaches to the relation between quaternary structure and oxygen affinity in haemoglobins is that using mixed spin derivatives. A recent example is provided by Perutz *et al.*[280] who have studied the effect of inositol hexaphosphate (IHP) on derivatives of haemoglobin from a number of sources. Ultraviolet spectra are used as a reliable monitor of changes between the quaternary oxy (T) and quaternary deoxy (R) states; NMR measurements of paramagnetic susceptibility show that the derivatives which undergo an R→T structure change on addition of IHP exhibit a change in spin equilibrium corresponding to a free energy change of -1 kcal mol^{-1} haem, while in those which remain in the R state on addition of IHP the free energy change is an order of magnitude smaller. The attractive 2-state concerted models for haemoglobin oxygenation are challenged, however, by Viggiano *et al.*[281,282] In the first of these papers the mechanism of oxygenation is shown to be unaltered in D_2O, and evidence against the simple model is presented; in the second paper the following observations are reported, based on following oxygenation through two hyperfine shifted and two exchangeable protons at 360 MHz: (a) in the absence of organic phosphates there is no preferential O binding to the α or β chains; (b) in the presence of organic phosphate the α chains have the higher oxygen affinity; (c) the ligand-induced structural changes are not concerted; (d) some cooperativity must be present within the deoxy quaternary state during oxygenation. Further, the variations in exchangeable resonances strongly suggest that the breaking of one or more inter- or intra-subunit linkages of a ligated subunit can affect similar linkages in unligated subunits within tetrameric haemoglobin. The different haem environments in methaemoglobin and metmyoglobin have been studied by Morishima *et al.*[266] who conclude that the strength of the histidine–Fe interactions varies as: MetMb $<\alpha$ subunits of MetHb $\leqslant\beta$ subunits of MetHb. Neya and Morishima[283] add information based on the ^{1}H resonances of a number of high-spin and low-spin complexes of intact Hb and separate subunits; in particular they note apparent structural changes in the β subunit which are separate from the hemichrome formation in the β haem pocket. Nonequivalence of the subunits is also reported by Ikeda-Saito *et al.*[284] following ^{1}H NMR of haemoglobins in which the iron is partially replaced by cobalt. A linkage of the electronic structure of the prosthetic groups with subunit cooperativity also appears.

The greatly enhanced propensity of reduced haemoglobin-S (β6Glu→Val) to associate, with consequent "sickling" of the red corpuscles, has been studied by a ^{13}C–^{1}H double resonance method which enables quantitative estimates of the amount of polymerised Hb-S present in

cells—as much as 80% of the total present—to be made.[285,286] Rotating-frame spin–lattice relaxation in the presence of an off-resonance radio-frequency field indicates that, even in the oxygenated state, there remains a tendency to aggregate, although it is much smaller.[287]

A great variety of small probe molecules and ions can be attached to haemoglobin; the groundwork for studies using $C^{15}N^-$, in terms of chemical shift measurements for a number of haemoproteins, has been laid by Morishima *et al.*[264,288–290] and for ^{13}C-labelled isocyanides by Dill *et al.*[291] 2,3-Diphosphoglycerate binds tightly to human deoxyHb and weakly to oxyHb. To locate the binding sites, haemoglobin was spin-labelled using a nitroxide radical at each of the two β-93 methionine sulphur atoms. An appreciable effect on ^{31}P resonance of bound diphosphoglycerate is observed, consistent with a distance of about 15 Å between the nitroxide radical and either of the two phosphorus nuclei.[292,293] The effect of altering chemical substituents on the haem periphery in haemoglobin and myoglobin was investigated by Moon *et al.*;[294] the ^{13}C resonance of bound ^{13}CO shows effects which are transmitted electronically from substituent to ligand, but other major differences between resonances from ^{13}CO bound to myoglobin and the haemoglobin subunits are imposed by differences in protein structure rather than by the haem modifications.

Apparently contradictory results are reported for the binding of chloride ion to haemoglobin. Norne *et al.*[295] measured the $^{35}Cl^-$ linewidths of Hb solutions in the presence and absence of 2,3-diphosphoglycerate, in 0·2 or 0·5 M NaCl, and at pH 6·5 or 7·2. The change in linewidth is linear with oxygen saturation, and reaches a maximum at the third oxygenation step. Linewidth broadening is interpreted as resulting from release of Cl^- from haemoglobin, and this takes place on oxygenation even when all chlorine has been displaced from the diphosphoglycerate binding site. The shape of the curve, which shows change of linewidth as a function of oxygen saturation, is taken as a measure of the fraction of haemoglobin molecules in the R state. In contrast, Bruman *et al.*[296] report that more Cl^- is bound to oxygenated than to deoxygenated haemoglobin, basing their conclusions on quadrupole-relaxation studies on various mutant and chemically modified haemoglobins. Other reports of the binding of small molecules to haemoglobin include a detailed study of benzene and toluene binding, showing a definite binding at or in proximity to the haem Fe atom,[297] and a study of the binding of ^{13}C-labelled isocyanoethane ($C_2H_5N^{13}C$) which shows it to be a sensitive probe for the modifications of the distal environment of the haem because of its steric interactions with the amino acids of the haem pocket.[298]

Monomeric insect haemoglobin, produced from larvae of the insect *Chironimus thummi thummi*, provides what may prove to be useful probes into haemoglobin structure. Ribbing *et al.*[299] have succeeded in obtaining components III and IV of this haemoglobin separate from each other; by

comparing ^{1}H and ^{13}C NMR data they are able to see that there are two different arrangements possible for the haem in the haem pocket, while La Mar *et al.*[275] observed the cyanomet forms of three of the monomeric Hb, with hyperfine shifted resonances indicating again that all these are found in two forms, with pH-dependent shift changes closely parallel to the Bohr effect curves.

The haemoglobin produced symbiotically in the root nodules of leguminous plants, leghaemoglobin, has been subjected to some preliminary studies by NMR. Johnson *et al.*[300] observed the behaviour of the proximal histidine C-2 proton, which gives an extremely low frequency signal in the unprotonated form of the molecule owing to its placement within 5 Å of the face of the haem ring, while Trewhella *et al.*[301,302] follow the anion binding of the molecule, suggesting a model for interaction with anions in which a haem propionate acts as an electrostatic gate which restricts the access of ions to the haem pocket.

D. Horseradish peroxidase

Horseradish peroxidase has a single haem group in a protein of molecular weight 40 000. The slow hydrogen–deuterium exchange of its proximal histidine is interpreted by La Mar and De Ropp[303] as indicating a buried haem pocket, and the insensitivity of its chemical shift to substrate binding as an argument against direct interaction between the imidazole and substrate. The molecule associates with aromatic donor molecules (indeed it may be donor-bound to a large extent *in vivo*), and Morishima and Ogawa[304] followed this binding by its effects on the 220 MHz spectrum of protons on and near the haem. The donors bind to a CN^{-} complex of the molecule in which the sixth coordination position of the Fe is occupied, indicating that they bind to a sterically specific site near, but not at, the iron atom. The larger benzhydroxamic acid is able to bind to this site and to the iron, indicating the close proximity of the aromatic binding site to the haem. Morishima and Ogawa[304] also give references to a number of earlier studies of the same protein by themselves and other workers.

IX. OTHER PROTEINS

A. Small copper proteins

The brilliant blue protein azurin from *Pseudomonas aeruginosa* shows a number of sharp lines in its reduced form in both the ^{1}H NMR[305] and natural-abundance ^{13}C NMR[306] spectra, some of which broaden or disappear upon change to the paramagnetic oxidised form of the copper atom. The ^{13}C NMR spectra imply that tryptophan-48, one tyrosine, one histidine,

and three or four phenylalanine residues are near the Cu moiety. A more detailed ^{1}H NMR study is reported by Hill and Smith[307] in which redox titration is used to correlate signals from the two forms, and the paramagnetic effects are used to deduce distances of assigned proton groups from the copper atom. The same workers[308] previously concluded that for both azurin and plastocyanin the spectra are consistent with two histidines, one cystine, and one methionine as ligands to Cu^{2+}. Carbon-13 NMR spectra confirm that histidines 38 and 91 are coordinated, and that the coordination is by the $N^{\delta 1}$ of the imidazole ring in both cases.[309] A high degree of conservation of residues near the copper site in plastocyanins from a wide range of higher plants is noted by Freeman *et al.*[310] who observe broadenings in the resonances of tyrosine, phenylalanine, and aliphatic residues, as well as the expected two histidines and one methionine. Stellacyanins (from *Rhus vernicifera*) lack any methionine, although two histidine residues coordinate the copper ion; the lack of methionine is a possible cause of differences between stellacyanins and other small copper proteins.[311,312] The binding of sodium and calcium to haemocyanin has been studied by ^{23}Na NMR by Norne *et al.*[195] Exchange between bound sodium ion and bulk solution is rapid, and a non-exponential decay observed for the transverse magnetisation indicates that non-extreme narrowing conditions apply. There are at least two Na^+ binding sites on haemocyanin, the stronger of which has a binding constant of 100 M^{-1}; only these sodium ions are displaced in the presence of calcium ions, needing 3–5 Ca^{2+} ions per binding site to complete the displacement. A linkage between sodium and oxygen binding is also noted.

B. Snake venom toxins

The peptide toxins of snake venom fall into two classes of about 62 and 72 residues, and are quite stable so they are eminently suitable for NMR investigations, although the acquisition of samples is not without its hazards. The sea-snake toxin erabutoxin has been analysed by X-ray crystallographic methods, so the 270 MHz ^{1}H NMR experiments of Inagaki *et al.*[313] are firmly founded; the refusal of histidine-7 to titrate until the protein denatures at pH 2·85 confirms that this residue is tightly buried in solution, while the tryptophan-29, which is invariant throughout the snake venom toxins, is apparently exposed and mobile. For comparison, temperature and pH variation of solutions of α-toxin from the South African cobra *Naja nigricollis* was used by Arsen'ev *et al.*[314] to draw the conclusions that histidines 4 and 31 titrate normally, that the invariant tryptophan has only limited accessibility to solvent and is in close proximity to histidine-31 and a carboxyl group, and that there is restricted motion of the invariant tyrosine-24. Comparison with the neurotoxin II of *Naja naja oxiana* seems to

indicate a common spatial structure; the same workers have more recently studied the lysyl sidechains of this toxin by acetylation and trifluoroacetylation.[315] The results obtained in all the above studies are broadly comparable to those of Fung *et al.*[316] from cobrotoxin, who also assign several slow-exchanging (below pH 9) non-titrating resonances to amide protons of the β sheet which X-ray analysis has shown to exist in cobrotoxin. Comparisons of the exchange rates of labile protons in two related toxin components, a neurotoxin and a cardiotoxin from *Naja mossambica*,[317] seem to show that although there are many structural similarities between these proteins the cardiotoxin must have a much more flexible conformation in solution, so the different functional properties of these toxins may be related to their different molecular dynamics.

C. Lipid–protein interactions

Melittin associates with lysophosphatidylcholine vesicles regardless of whether it is in its monomeric or tetrameric state, giving well resolved superimposed ^{1}H NMR spectra from the two components, with local mobility and fast exchange within the complexes.[318] Problems arising from the overlapping of protein and lipid spectra can be overcome by the use of fully deuterated micelles as described by Brown.[319] Preliminary studies show that the hydrophobic and hydrophilic regions of the melittin amino acid sequences indicate different conformational and dynamic changes on interacting with the micelle. Deuteration of lipids, this time at specific sites, was employed by Rice *et al.*[253] in their study of a variety of protein–lipid systems using deuterium NMR at 34 MHz. Proteins either disorder or have little effect on hydrocarbon chains above the gel to liquid-crystal transition temperature, but prevent chain crystallisation below this temperature; no evidence for any ordered lipid near the protein is found in the liquid crystalline state. Immobilisation of 20% of the phospholipid in human low-density lipoprotein, revealed by ^{31}P NMR,[320] disappears upon trypsin treatment and is therefore attributed to interactions between the phospholipid and B-peptide. The same effect is not noted in human high-density lipoprotein,[321] consistent with the occurrence of smaller peptides in this system. Interactions of the myelin basic protein incorporated in liposomes are indicated by the fact that ^{13}C-enriched methionine S-CH_3 resonance exhibits linewidths of the order of 15 Hz as compared with the 1 Hz or so for the protein in free solution;[322] preliminary structural NMR studies of myelin basic protein are reported by Littlemore.[323] The relevance of the very mobile carbohydrate chains found associated with the small membrane glycoprotein glycophorin for its interactions at the cell surface is discussed by Egmond *et al.*[324]

D. Proteins associated with muscle; calcium-binding proteins

Myosin ^{1}H NMR reveals signals from amino acids moving too fast to be accounted for by simple rotations of groups on a rigid backbone. The mobile region is found by comparison of the spectra of fragments of myosin to be located almost entirely in the myosin subfragment 1 (the "head" of the myosin molecule), and to constitute nearly a quarter of its structure. The binding of actin quenches the mobility. A quantitative treatment of the broad signals from myosin and its subfragments indicates that the highly mobile region may be in the "swivel" between subfragment 1 and the rest of the molecule, or in the actin binding site, or both.[325] The subfragment 1 of myosin is also responsible for its ATPase activity; the time course of this has been followed, using ^{31}P NMR, by Levitskii *et al.*[326] using purified S1. Subfragment 1 also catalyses oxygen exchange between inorganic phosphate and water; this has been followed using the ^{18}O-induced shift in ^{31}P resonances by Webb *et al.*[327] Exchange probably occurs by formation of myosin-ATP from a myosin–ADP–inorganic phosphate complex, thus reversing the ATPase reaction.

Tropomyosin is a muscle regulatory protein associated with the thin filaments, and Mak *et al.*[328] have been able to separate it into two components, α and β. Phosphorus-31 NMR indicates that only the α-component of 284 residues is phosphorylated. Radioactive labelling confirms that a single phosphorylation site, serine-283, is involved. Titration of the two histidines, 153 and 286, was followed by Edwards and Sykes[329] who conclude from the distribution of resonances in the middle of the titration that more than two interconverting forms of the protein are present. The muscle regulatory complex containing tropomyosin is completed by the oligomeric protein troponin, of which the C subunit is of greatest interest since it is to this that the regulatory calcium ions bind. Levine *et al.*[330] report very substantial conformational changes in apotroponin-C upon calcium binding to the two high-affinity sites on the molecule, and in a later paper compare these with the changes induced by magnesium.[331] Calcium produces its full structural effects even in the presence of excess magnesium, but the distinction between high- and low-affinity sites is blurred under these solution conditions, which correlates with the observation that the Ca^{2+} threshold for the activation of tension in some myofibrils is higher in the presence of large amounts of Mg^{2+}. Binding of Cd^{2+} to troponin-C, monitored by ^{113}Cd NMR, is reported by Forsen *et al.*[332] A fragment of troponin-C, the largest produced by cyanogen bromide treatment, which binds calcium strongly, has been the subject of 270 MHz ^{1}H studies[333] from which it is found that Ca^{2+} causes major structural changes in the fragment, though not quite the same ones as in troponin-C. When Pr^{3+} is used as a shift reagent in place of Ca^{2+}, the induced shifts in the

resonances of ligands indicate that the ligands are some 25% further away from the metal than the equivalent ligands in parvalbumin.[334]

Considerable sequence homologies exist between troponin-C and the multifunctional brain regulator protein calmodulin. NMR spectral changes on binding Ca^{2+} and Mg^{2+} are qualitatively similar to those of troponin-C, indicating that the structural changes too may be similar.[335] More detailed studies[336] make the similarities even more apparent.

A small storm blew up over the binding of Na^{+} to parvalbumins. Grandjean *et al.*[337] claim that the monovalent ion binds to decalcified parvalbumin, albeit much more weakly than Ca^{2+}, on the basis of ^{23}Na NMR measurements. The contention was argued by Parello *et al.*[338] whose studies fail to reveal such binding except in the presence of EGTA, the complexing agent used in the decalcification process. Gerday *et al.*[339] replied to the effect that, since the parvalbumin used is only singly decalcified and there is no good evidence for any binding of EDTA to such parvalbumins, it is not possible for EGTA to provide a sodium binding site as suggested. Meanwhile, parvalbumins are being studied using a remarkable variety of techniques; ^{113}Cd has been used as a calcium analogue[340] indicating that the two Ca^{2+} ion sites have different structures, while Cave *et al.*[341] have used ^{25}Mg NMR to establish the presence of magnesium-specific sites which differ from the Ca^{2+} sites. Application of the relaxation enhancement method using Gd^{3+} and Mn^{2+} indicates that Gd^{3+} appears to be a specific probe of the calcium sites, losing almost all contact with solvent water on binding, while Mn^{2+} probes a secondary site near the EF calcium-binding site.[342] Lanthanide substitution has been used by Nelson *et al.*[343] to assign a number of ^{13}C resonances. Nelson[344] notes a pH dependence of chemical shift and spin–lattice relaxation time of ^{19}F in parvalbumin labelled at cystine-18 which is indicative of protein expansion and increased motional freedom as the charge on the lysine sidechains changes.

E. Ribosomal proteins and histones

Earlier NMR and other structural studies of ribosomal proteins were performed on molecules extracted by an acid–urea process. Morrison *et al.*[345] point out that, with the exception of S20, all molecules extracted in this way exhibit less specific tertiary structure, as monitored by ring-current shifted resonances, than the same proteins extracted using salt. Salt-extracted S4 may be cleaved at residue 46, leaving a C-terminal fragment which retains its rRNA binding properties and shows most of the spectral perturbations found in the intact protein, so the N-terminal region may be inferred to be in a random coil in free solution, though not in the ribosome.[346] S15, on the other hand, appears to be entirely globular and compact[347] while S1

reveals considerable tertiary structure in physiological buffers, but more structural flexibility than is normal for globular proteins.[348] In addition it has a spectrum which is independent of the method of preparation. Like S4, L7/L12 appears to have a globular C-terminal region, although Gudkov *et al.*[349] report that its N-terminal region forms a long α-helix.

With the discovery that, like ribosomal proteins, histones H2A, H2B, H3, and H4 only take up their final structures in a multimeric complex, in this case the protein core of nucleosomes, studies of isolated core histone fractions are of less interest than they once were. The location of significant folded regions remains important, however, and Crane-Robinson *et al.*[350] report on the preparation of a number of cleaved fragments of H4 which show clearly that it is the basic N-terminal regions of H4 which remain free upon self-aggregation of the molecule. Self-aggregation of histones H2A and H4 has been studied by means of ^{13}C NMR.[351,352] It appears that this method can monitor self-aggregation more accurately than other physico-chemical methods. Specific dimeric and tetrameric histone complexes were subjected to hydrogen–deuterium exchange studies by Nicola *et al.*[353] in which it was shown that highly structured regions of the molecules are rich in hydrophobic amino acids, arginine and some acidic sidechains, while most of the lysine and some alanine remains in a mobile state. The suggestion is made that arginine may be important in inter- or intra-subunit interactions in histone complexes; work on the exchange dynamics of the arginine sidechain, leading to the suggestion of a non-symmetrical arginine–ligand interaction,[354] may eventually help to cast light on this.

Unlike the other histones, H1 is located outside the chromatin core particle, presumably being engaged in the formation of higher-order chromatin structures. Like them, it is ubiquitous in eukaryotes, except that in certain organisms it is partly replaced by a rather similar protein (H5 in avian erythrocytes, ϕ1 in sea urchin sperm, etc). Both H1 and H5 show[355,356] clear NMR evidence in the form of ring-current shifted resonances for a tight globular structure, and in both cases this structure is retained after limited digestion has removed peptides from both the N- and the C-terminal regions of the molecule, implying a three-domain structure. More detailed study[357,358] of the globular region of H1 shows that guanidylation of its lysine sidechains does not affect its ability to fold, implying that they are on the outside; modification of the single tyrosine, however, does affect the folding and permits a number of assignments of ring-current shifted resonances. Interproton Overhauser effects are demonstrated as an indication of the tight packing of the hydrophobic core. Similar studies on the globular region of H5 reveal a somewhat different structure, although there are extensive sequence homologies between the globular domains of the two molecules. Carbon-13 NMR seems to show a globular region for H1 which, at residues 41–89, is rather smaller than that found from proton

resonance and digestion studies.[351,352] The cell-cycle dependence of histone H1 phosphorylation, which is probably an important factor in the control of chromosome folding, has come under some initial investigation.[359,360] It appears that phosphorylation at serine-37 makes the removal of H1 from DNA easier under some solution conditions.

High-resolution NMR of complete chromatin core particles[361] shows the freedom of the basic N- and C-terminal regions of histones H2A and H2B, although H3 and H4 are fully immobilised in this large complex with DNA. Arginine residues, which are largely in the globular regions of these molecules, are implicated in the binding to DNA.

The bacteriophage gene 5 protein interacts strongly with DNA, causing unwinding. A series of experiments involving d(pC-G-C-G) tetranucleotide show that the unwinding appears to be determined by fluctuations in the double-helical structure of the DNA, with aromatic residues from the protein, whose resonances are displaced by the interaction, intercalating with the bases and preventing re-formation of the double helix.[362] Selective deuteration of the tyrosine residues of gene 5 protein[363] shows that only the 3,5-protons of the three surface tyrosines (two others are buried) interact with the bases. The other resonance to be perturbed is that of Phe-13, so a model of DNA binding involving Tyr-26–base–Phe-13 intercalation is suggested.

F. Recognition proteins

General dimensions, polarity and asymmetry features, and the assignment and arrangement of the dinitrophenyl contact residues in the combining site of the mouse immunoglobulin A dinitrophenyl-binding protein MOPC 315, were assembled from a combination of NMR, ESR, model building, and chemical modification studies by Dwek *et al.* (Fig. 9).[364] On this basis they were able[365] to investigate the binding of 2,4,6-trinitrophenyl derivatives to the Fc fragment of the immunoglobulin, finding that the phenyl ring stacks with tryptophan-93 on the light chain, and is contained in a "box" made by Trp-93 and Tyr-34 on the light chain, and Ala-34 on the heavy chain. The depth and charge distribution of the binding site are measured by ^{31}P NMR[366] of phosphorus-containing dinitrophenyl derivatives; the depth is about 1 nanometre. A new development in these studies[367] is the attachment of a metal chelating group on to the DNP group, so that a paramagnetic ion can be introduced into the binding site and measurements made on metal–sidechain distances using difference spectroscopy. The same immunoglobulin, MOPC 315, was also studied by Kooistra and Richards[368] using ^{19}F NMR. The ^{19}F signal on binding remains constant regardless of the amount of constant region attached to the binding site, implying that the constant region does not affect binding site inter-

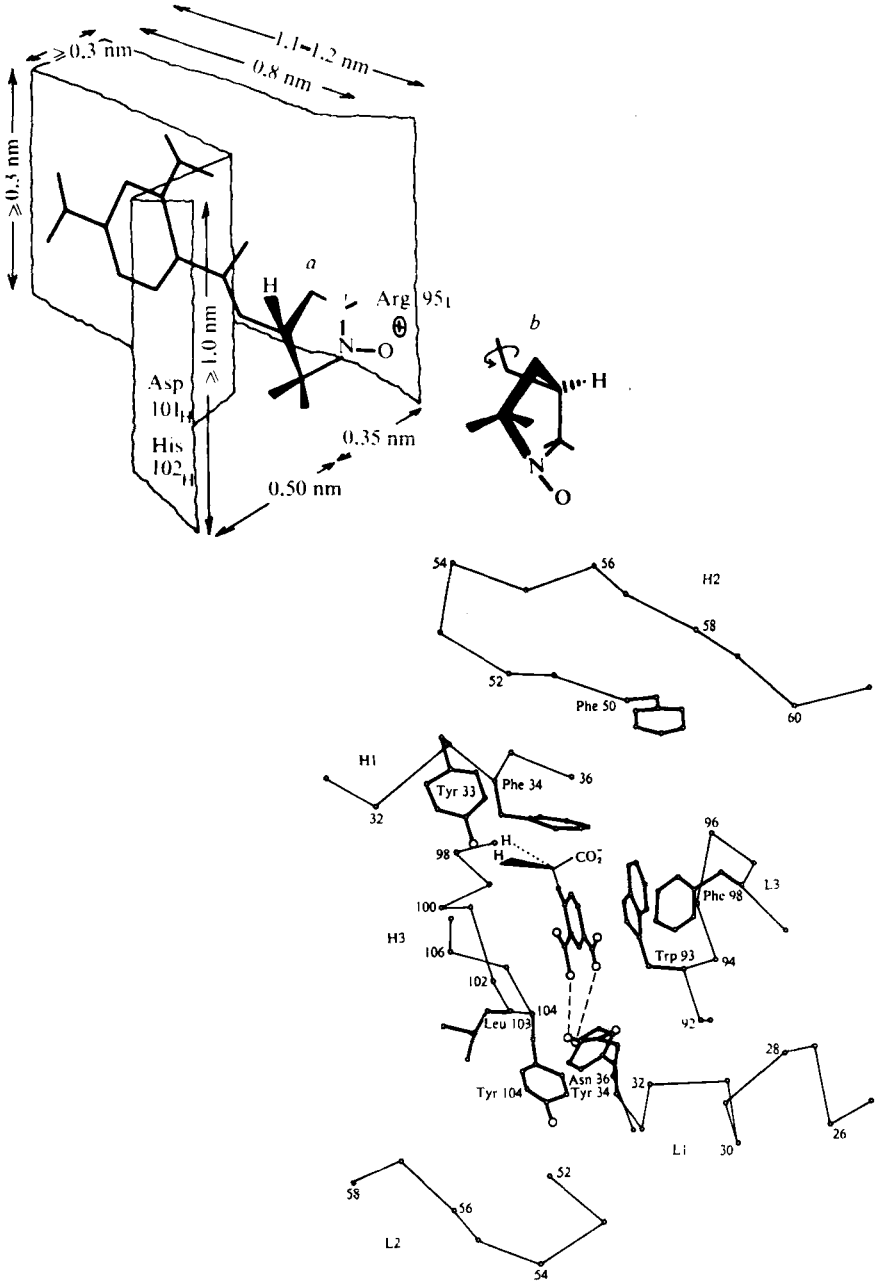

FIG. 9. Details of the antibody binding site of the mouse Ig A protein MOPC 315 in solution as refined by a combination of NMR, ESR, model building, and chemical modification methods by Dwek *et al.*[364]

actions. Comparisons between the binding sites of a number of different mouse myeloma immunoglobulins were made using ^{13}C and ^{31}P NMR[369,400] and ^{19}F NMR[370] techniques on labelled haptens; substantial differences are noted and are detailed in the references given. An interesting lead on hapten-induced structural changes of immunoglobulins is provided by Vuk-Pavlovic *et al.*[371] By introducing a mercuric ion between the cysteinyl sulphurs of the single inter-heavy-chain disulphide bond, and then observing the width of a $^{35}Cl^-$ resonance from chloride ions exchanging between the mercury coordination sphere and solvent, they are able to see an effect of hapten binding which implies hapten-induced alteration of steric relations in the hinge region.

The light chains of immunoglobulin are secreted by some tumour patients and are known as Bence-Jones proteins. Arata and Shimizu[372] were able to study 15 λ-type and 3 κ-type Bence-Jones proteins, and to show a number of differences, particularly in the titration of histidine-198, which always has a pK below 4·5. In the κ-type this histidine does not begin to titrate until denaturation has started, implying that the constant domain which contains it is more compact in the κ-type than in the λ-type proteins.

Moving on to other types of recognition proteins, we find studies of the binding of mono- and oligo-saccharides to concanavalin-A, a tetrameric lectin which agglutinates cells by binding to cell-surface sugars. By replacing some of the normal calcium by magnesium and observing solvent proton relaxation dispersion, Brewer and Brown[373,374] were able to compare the binding of different saccharides, since saccharide binding appears to cause a conformational change which alters the residence time of solvent water molecules at the paramagnetic metal. Apparently all the mono- and oligo-saccharides which bind cause the same changes, which can be explained by the presence of a single binding site; the greater affinity of some oligo-saccharides for con-A can then be explained in terms of there being more than one binding residue in the oligomer chain rather than the presence of an extended binding site.

A periplasmic protein from *E. coli* recognises branched-chain amino acids. Chauvet-Monges *et al.*[375] have studied the T_2 behaviour of leucine, isoleucine, valine, and threonine in the presence of this protein and conclude that two types of bonds are formed, one in the amino acid region and the other on the hydrocarbon chain. Also involved in an active transport system is the histidine-binding protein J of *Salmonella typhimurium*, which undergoes substantial conformational changes on binding L-histidine.[376] Later, Manuck and Ho[377] compared the off-rate constant of histidine binding by protein J from various strains of *S. typhimurium* by observing chemical shifts as a function of substrate concentration.

The lac repressor molecule is a tetramer, each of whose subunits has a molecular weight of 37 300. It is thus somewhat more than twice as large as

the haemoglobin molecule, but from the NMR point of view lacks the perturbing factors of porphyrin ring and iron atom; thus any NMR investigation of the lac repressor is an ambitious and long-term project. Such a project began with the preparation of selectively deuterated analogues of the lac repressor and the presentation of its ^{1}H and ^{2}H NMR spectra in the denatured form,[378] and spectral changes in the inducer isopropyl-β-D-thiogalactoside on binding.[379] It continues more recently with some theoretical considerations[380] which provide for the possibility that residues 7–22 may constitute a left-handed δ-helix ($4{\cdot}3_{14}$) in solution, a structure which is consistent with the observed NMR spectrum and would allow stacking of tyrosines 7, 12, and 17. The N-terminal "headpiece" of the lac repressor also appears to contain the majority of the relatively mobile residues in the protein.[381] No doubt much more will be heard of this project.

Far from being specific recognition proteins, the serum albumins often act as general-purpose carriers. Recent papers on the binding of small molecules to albumins include one of Malik and Sadler[382] on the attachment of gold-phosphine drugs, another by Lubas *et al.*[383] on binding of a series of straight- and branched-chain alcohols, and a pair of $^{35}Cl^-$ relaxation studies in which the high-affinity Cl^- binding sites on human plasma albumin are found to be doubly cationic at neutral pH. The Cl^- sites are shown to have a great deal of freedom to move about relative to the protein as a whole.[384,385] Ovalbumin is the model chosen for a set of T_1 measurements of the denaturation of protein.[386] Below 45 °C, $\log k$ is proportional to T^{-1}, regardless of the direction of temperature change, while in the denaturation region the relation depends on the direction of change, reflecting the irreversibility of the denaturation. From the slope of $\log k$ vs. T^{-1} activation energies are calculated.

The metal-binding protein metallothionein, present in all animal tissues, has a molecular weight of 6600 of which one third consists of cysteine residues. Its apoprotein appears to be randomly coiled in solution, though the metalloprotein shows a well defined tertiary structure with some part of the molecule not readily accessible to solvent.[387,388] Cadmium-113 NMR studies indicate that each Cd^{2+} is coordinated to at least three cysteinyl S ligands, and the removal of proton–cadmium spin coupling reveals a considerable amount of Cd^{2+}–Cd^{2+} coupling, showing that there must be a metal cluster arrangement.[389]

G. Structural proteins

Collagen has shown itself amenable to study by cross-polarisation ^{13}C NMR in its native structures. Schaefer *et al.*[390] have run comparisons between samples of skin, tendon, cartilage, and ivory in the solid state, the last with magic-angle spinning which enhances the resolution to something

approaching that of solution ^{13}C NMR spectra. Molecular dynamics of collagen have been studied by Jelinski and Torchia[391] using ^{13}C-labelled collagen from chick calvaria tissue culture. The relaxation and NOE measurements show that collagen must be undergoing rapid torsional and rod-like reorientation in the fibril, so formation of a fibrillar structure does not require the existence of a unique set of intermolecular interactions at the helical surfaces.

The coat protein of tobacco mosaic virus self-assembles into disc- and rod-like oligomers. Jardetzky *et al.*[392] show by the ^{1}H NMR spectrum of the 4S particle that a region probably from Asp-88 to Arg-112 is unusually mobile. This conclusion is almost precisely borne out by De Wit *et al.*[393] who infer, from measurements on specifically ^{13}C-labelled TMV protein oligomers, that about 17% of the carbon atoms in the complex are still mobile.

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Carbon–Carbon Coupling Constants: Discussion

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I. INTRODUCTION

The field of carbon–carbon coupling constants is in an expansive period with most of the new information coming from biosynthetic studies. The present review continues where Wasylishen[1] finished and covers the period 1976–1979 with a few papers from 1980; it is followed by tables containing data appearing in papers published during 1978–1980, according to the scheme used elsewhere by Wray.[2] References to data from the period 1976–1978 are made to his previous tabulation.[2] The reason for such an extensive data collection is to avoid undue length of the text and to make available, in a readily accessible form, all of the relevant data published by April 1980.*

Interest in carbon–carbon coupling constants has increased dramatically in recent years. The use of carbon–carbon couplings in biosynthetic studies has provided a wealth of data, mostly for one-bond coupling constants and usually of limited accuracy.

Long-range couplings have been recently reviewed.[1–3] Carbon–carbon coupling constants are also covered in the specialist periodical reports dealing with NMR[4–6] and with biosynthetic studies.[7,8] Theoretical aspects have been treated by Kowalewski[9] and by Ellis and Ditchfield.[10] Accounts of carbon–carbon coupling constants are included in two recent textbooks.[11,12] Of other more specialized reviews may be mentioned those dealing with carbohydrates,[13,14] biosynthesis,[15–18] amino acids and peptides,[19] polycyclic aromatics,[20] and conformational aspects.[21]

* Numbers in parentheses throughout this review refer to entries in the tables that follow it, and to those in ref. 2.

The present review concentrates upon the factors controlling carbon–carbon couplings and their use in stereochemical studies. Most recent studies have shown that not only three-bond coupling constants but also one- and two-bond couplings may have a large number of applications in the future. Much information about coupling constants is gained from biosynthetic studies. However, it is not intended in this review to concentrate upon the biosynthetic significance of carbon–carbon couplings. Such data may be useful for assignment purposes and, with increased knowledge about typical ranges for the various types of coupling constants, this type of use is probably going to increase. New techniques to obtain carbon–carbon coupling constants have emerged, though they are still only promising.* Theoretical calculations of coupling constants are only included in cases in which they support, or are closely linked to, experimental findings, since such calculations are the subject of a chapter to appear in Volume 12 of this series.

II. ONE-BOND COUPLING CONSTANTS

The NMR spectra of ^{13}C-enriched molecules used in biosynthetic studies are among the richest sources of one-bond couplings. The accuracy of measurement of these coupling constants is usually low. Most of the substituents contain nitrogen, or sulphur, or oxygen as the first atom. Studies of substituted benzenes have, in addition, delivered a wealth of material.

A. Small ring systems

Small ring systems have attracted much interest. The theoretical prediction by Newton *et al.*[22,23] that $^1J(C_1–C_3)$ in bicyclo[1.1.0]butane is negative has led to an experimental proof of this prediction.[24,25] In diethyl 1-methyl-3-phenylbicyclo[1.1.0]butane-*exo*,*exo*-2,4-dicarboxylate the value of $^1J(C_1–C_3)$ is $-5{\cdot}4$ Hz,[24] and in 2,2,4,4-tetramethylbicyclo[1.1.0]butane the corresponding coupling is $-17{\cdot}49$ Hz.[25] A numerical value of 16 Hz observed in 1-cyanobicyclo[1.1.0]butane[26] is most likely also negative in sign. Use of the equation suggested by Newton *et al.*[22] leads to a negative s character, which is without meaning.[25] The possible contribution of a negative two-bond coupling seems not to have been considered. As seen in Section IIIB, the value of ^2J in cyclopropanes may amount to a value in the region of -12 to -15 Hz. In order to use expressions relating ^1J(C–C) to s character of the intervening bond, the two-bond contribution should first be eliminated.

One-bond coupling constants in three-membered rings vary from $10{\cdot}0$ to $21{\cdot}0$ Hz. The variation, in the three-membered ring hydrocarbons, seems to be related to the size of the neighbouring rings. One-bond couplings in epoxides have been reported in a few cases.[27,28] The values are quite

* See paragraph in smaller type on page 93.

different, 28·1 Hz in styrene epoxide and tetraphenylethylene oxide, whereas values of 32–33 Hz are reported for a pentalenolactone (818).[28] If we compare ^1J in tetraphenyl-1,2-dihydroxyethane and ethane, we find a substituent effect of 6·7 Hz. Comparison of ^1J in cyclopropanes (~15 Hz) with ^1J in epoxides (~30 Hz) shows that the difference cannot merely be explained by the simple substituent effect of an oxygen. Assuming that the geometries are similar in both ring types, the difference could stem from different two-bond couplings. Further studies of heterocyclic three-membered rings can probably shed more light upon the influence of ^2J. Reich and Trend[29] argue, based upon a ^1J(C–C) value of 37·25 Hz, that a postulated episelenurane is an open-chain compound. A comparison of $^1J(C_1–C_2)$ in methylcyclopropane with that in *cis*- and *trans*-1-bromo-2-methylcyclopropane shows no change in the *cis* case, but an increase of 1 Hz in the *trans* case.[30] Whether this effect is of more general application remains to be seen.

One-bond couplings in four-membered rings were studied by Stöcker and Klessinger.[31] Since all substituents have carbon as the first atom, the variation in value of the couplings is small (27·3–29·1 Hz). Jokisari[32] has determined $^1J(C_2–C_3)$ in oxetane (21) and thietane (24). The coupling constant in the former is quite similar to that observed in methylcyclobutane (753). The values of ^1J in cyclobutanone and bromocyclobutane[33] were redetermined (31, 35) and those of chlorocyclobutane (37) added. The effects of halogen substitution are very modest, ~2 Hz.

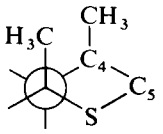
Coupling constants in five- and six-membered rings (108, 109) are similar to those observed in acyclic compounds (26, 710).

B. Stereochemical dependence

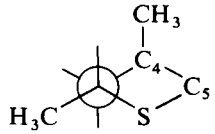
Barna and Robinson[34] found for 2-methylpiperidines a considerable difference between the values of ^1J(C–C) when the methyl group is equatorial or axial, whereas only a small effect is found for the corresponding onium salts (802, 793). The differences are ascribed to lone-pair effects like those observed on ^1J(C–H) [35] or ^1J(C–N).[1] They can probably also be rationalized in terms of overlap of the lone-pair electrons with the anti-bonding orbitals of the C–C bond.[36,37] The differences observed for carbon–carbon couplings are much smaller than for ^1J(C–H). In 2-methyl-4-t-butylcyclohexanone a similar difference is observed. The low value found in *trans*-2-methyl-4-t-butylcyclohexanone compared with $^1J(C–CH_3)$ in methylcyclohexane[38,39] (800, 108) is attributed to hyperconjugative withdrawal of electrons from the C–Me bond by the C=O group. The higher value in the *cis* derivative, in which the C–Me bond is in the same plane as the C=O bond, is not explained but could be caused by electric field effects.

Berger[40] has observed different $^1J(C_2–C_3)$ couplings in *endo*- and *exo*-6-chlorobicyclo[2.2.2]octan-2-one (358, 359). In the latter ^1J is smaller than in the unsubstituted molecule (362). A similar result is obtained in *endo*-4-chlorobicyclo[3.2.1]octan-6-one compared with the unsubstituted molecule (360, 363). It is noticed that in both of these cases the C=O and the C–Cl bonds are in a transoid arrangement.

Differences between $^1J(C–CH_3)$ in acetone oxime have been reported (203).[2] In *cis*- and *trans*-2,4-dimethylthiazolidine Llinares *et al.*[41] have reported two different values for the $J(C_4–C_5)$ coupling constant (757). If the preferred configurations are [1] and [2] it is difficult to explain this difference.



[1]



[2]

Also in carbohydrates $^1J(C_1–C_2)$ is different in some α- and β-anomers. Different couplings are observed in the anomeric pairs of mannose (85),[42] mannosamine hydrochloride (89),[43] glucose pentaacetate (176),[44] *erythro*- and *threo*-furanose (726, 727).[45]

The empirical material is small as yet, but it looks as though $^1J(C–C)$ may become a useful parameter for stereochemical studies around sp^3 hybridized carbons.

C. Isotope effects

An unusual finding has been reported by Bengsch and Ptak.[46] In uniformly enriched threonine they found different values of $^1J(C_\alpha–C_\beta)$ when the γ-atom is ^{12}C rather than ^{13}C. No similar isotope effect has been reported in doubly enriched compounds,[27,47,48] nor have any such effects been observed with carbon–hydrogen[35] or hydrogen–hydrogen coupling constants.[49]

D. Couplings involving carbonyl carbons

Coupling constants in aromatic acids, esters, ketones, and aldehydes fall into the following ranges: acid derivatives 71·7–75·8 Hz, aldehydes and ketones 50·5–54·8 Hz, while amides fall in between these two areas.[50] Furthermore, in sterically hindered acids, esters, and ketones the $^1J(CO–C)$ values are insensitive to the degree of conjugation. A small but significant difference is noticed[48] between dimethyl phthalate (1124), phthalic anhydride (596), and methyl benzoate (602).

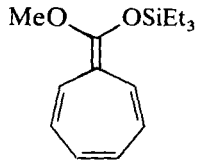
Coupling constants to sp^3 hybridized carbons lie typically in the following ranges: acids and esters 55–59 Hz, ketones 40–41 Hz, and aldehydes ~55 Hz.

In amino acids, such as aspartic and glutamic acids, ^1J(C–C), involving the carboxyl carbon, decreases upon deprotonation of the carboxyl group. The long-range dependence of the $^1J(CO–C_\alpha)$ couplings on titration of the remote carboxyl group is in the same direction. On the other hand, $^1J(C_2–C_3)$ in glutamate increases by 0·7 Hz as the carboxyl group is deprotonated. $^1J(C_2–C_3)$ in aspartate, however, decreases by 1·2 Hz but this decrease is almost entirely due to deprotonation of the sidechain carboxyl group. ^1J values in amino acids are relatively insensitive to deprotonation of the amino group.[51] Some of the trends observed in glutamic and aspartic acids confirm previous results in simpler amino acids.[52] Coupling constants in other α-substituted acids show small substituent effects.[53] Deprotonation of aromatic acids also leads to a small decrease in ^1J(C–C).[54]

E. Coupling constants across other double bonds

The coupling constants across $CH_2{=}C$ in *exo*-methylenebenzocycloalkanes[55] are very similar (866, 877, 883, 889) to those observed in methylenecyclohexanes.[56] Using the Weigert and Roberts equation,[33] taking the % s character from the SCF density matrix, excellent agreement between the observed and calculated ^1J(C–C) values is found.[55]

Rapp *et al.*[57,58] have observed very large one-bond couplings in heptafulvenes (461, 471, 475); the largest coupling constant reported across a double bond is 104·5 Hz in [3].



[3]

In cyclobutene $^1J(C_1–C_4) = 32·14$ Hz (850)[59] which is somewhat larger than the corresponding coupling in the cyclobutanes. In fomannosin,[60,61] in which both carbons are sp^2 hybridized, $^1J(C_4–C_8) = 44$ Hz (463).

Iron tricarbonyl complexes of butadiene (452), penta-1,3-diene (456), and cyclohexa-1,3-diene (458) show very similar $^1J(C_1–C_2)$ values.[62] $^1J(C_2–C_3)$ has only been measured in the complex of the non-symmetrical pentadiene, but is assumed to be similar in all three of the complexes concerned. $^1J(C_1–C_2)$ and $^1J(C_2–C_3)$ are furthermore alike in the complexed pentadiene. The couplings in *cis*-butadiene are not known but

the *trans* compound showed quite different couplings.[63] Since the coupling constants may be related to bond length, the two bonds were surmised to be equally long in the complex.[62]

F. Anions

One-bond coupling constants have been used to estimate the hybridization state of carbanions. Bywater *et al.*[64] found coupling constants in accordance with sp^2 bybridization in 2,5-diphenyl-2,5-dipotassiohexane (903, 1080) and in 2-lithio-4,4-dimethyl-2-phenylpentane (893, 1067). In 1-lithio-2,5,5-trimethylhex-2-ene the coupling constants in tetrahydrofuran at −20 °C also indicate that C_1 is sp^2 hybridized but at room temperature the coupling is smaller (865). Small coupling constants are also observed in isopropyl- and t-butyl-lithium (707, 732).[65] The effects of association upon ^1J(C–C) are briefly discussed.[64] A very small coupling constant is observed in 1,1-dibromoethyllithium (683).[66] The rather large ^1J(C–Li) and small ^1J(C–C) couplings are taken as evidence for an unusual geometry around the lithiated carbon, probably with an increased p character of the C–C bond.

Albright and Schweizer[67] have performed theoretical CNDO/2 and INDO calculations on ethyllithium to estimate the charge distribution and energies. A conformation of ethyllithium with a C–C–Li bond angle of 90° and a C_α–C_β–H bond angle of 109·47° gives a much smaller energy than that obtained for a conformation with a C–C–Li bond angle of 109·47° or with the angle C–C–H 120° or 90°. In the former case $^1J(C_1–C_2)$ is calculated to be 6·5 Hz while in the latter the value obtained for ^1J is 40·3 Hz. The very small value bears resemblance to that observed by Siegel *et al.*[66]

G. Both carbons aromatic

1. *Benzene derivatives*

One-bond couplings have been measured for about thirty monosubstituted benzenes.[68] A redetermination of $^1J(C_1–C_2)$ in nitrobenzene[33] gives a much larger result (510, 511). A redetermination of ^1J in benzene results in a slightly smaller value (55·95 Hz [68] or 55·3 ± 0·5 Hz [69]) than previously reported.[33] $^1J(C_1–C_2)$ in substituted benzenes have a range of 21·3 Hz, whereas the ranges for $^1J(C_2–C_3)$ and $^1J(C_3–C_4)$ are 4·7 Hz and 1·9 Hz. $^1J(C_1–C_2)$ values are correlated with the Huggins electronegativity of the first atom of the substituent (correlation coefficient = 0·943). This type of relationship does not explain the variation observed for nitrogen- or carbon-containing substituents. Correlations with two-parameter equations show no improvement. A new parameter, group electron withdrawing power (ΔQ_X), is defined as the difference between the total charge density of a CH_3

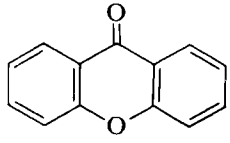
group in CH_3X and the total charge density of the CH_3 group in CH_4 (the total charge density being calculated using the INDO procedure). Correlation of $^1J(C_1–C_2)$ with ΔQ_X for twenty-one first-row elements shows an improved correlation coefficient of 0·97 and a correct differentiation for those couplings in which the first atom of the substituent is nitrogen or carbon. For $^1J(C_2–C_3)$ and $^1J(C_3–C_4)$ the variations are much smaller and not very regular although $^1J(C_2–C_3)$ for halogen substituents decreases slightly with decreasing electronegativity.[68]

Coupling constants in benzene moieties have also been reported for biomolecules (see below).

2. *Polycyclic aromatic hydrocarbons*

Coupling constants in polycyclic aromatic hydrocarbons (PAH) have been investigated in naphthalenes (546–552),[70,71] phenanthrenes (560–562, 566–568),[72,73] pyrenes,[70] and anthracenes (559).[74] One-bond coupling constants in PAH vary between 53 and 63 Hz.[72] A rough correlation between π-bond order and magnitude has been reported.[72] If one-bond couplings are plotted against either π-bond order or bond length, they fall upon one of two parallel lines. On the upper one fall couplings for which both carbons are hydrogen-bearing, while on the lower line are those appropriate to one of the coupled atoms being a quaternary carbon at a junction. No examples are known in which both carbons are of the junction type such as the coupling between C_{4a} and C_{8a} in naphthalene. It may also be revealing to plot these couplings against the product of s character as suggested by Frei and Bernstein.[75]

Two basic structures appear quite often in biosynthetic materials, the xanthone [4] and the anthraquinone [5] skeletons. As these skeletons are



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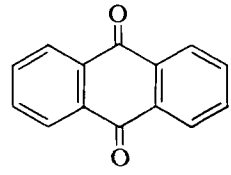


[5]

often substituted, they constitute a source for testing additivity of carbon–carbon coupling constants. A comparison of these data also makes possible an assessment of the transferability of results from simpler systems like substituted benzenes, naphthalenes, etc. A few of the assignments are considered to be questionable (see below) as judged from their carbon–carbon coupling constants, but this should not be taken as an indication that all published data have been scrutinized critically.

The anthraquinone skeleton is represented in several compounds. $^1J(C_1–C_9)$ has been measured in anthraquinone itself (638).[50] The coupling constants obtained for diacetylmacrosporin (1086)[76] are in very good agreement with those observed in hydroxybenzenes. The values for phenyl acetate have not been published but from 1-acetoxynaphthalene (556)[73] it is known that $^1J(C_1–C_2)$ is somewhat larger than in the hydroxy compound. The anthraquinoid structure is also present in averufin (1087),[77,78] versicolorin (1076),[79] versiconal acetate (1093, 1094),[80] versiconol acetate (1090),[80] daunomycinone and islandicin triacetate (1092, 1097),[81] and an aza derivative phomazarin (1091).[82] $^1J(C_4–C_{14})$ seems too large in averufin, versicolorin, versiconol acetate, and versiconal acetate, whereas $^1J(C_8–C_{8a})$ in phomazarin is on the low side. The xanthone skeleton is found in ravenelin (1068)[83] and in bikaverin (1085)[84]. In the latter $^1J(C_4–C_{4a})$ is 8·6 Hz larger than in hydroxybenzene and $^1J(C_{12}–C_{12a})$ is 14·1 Hz larger than in anthraquinone.

Among other derivatives containing benzene or naphthalene moieties can be mentioned secalonic acid (584),[85] 2-hexyl-5-propylresorcinol (572),[86] demethoxyviridin (1082),[87] scylatone (553, 554),[86,88] chartreusin (585),[89] deoxyherqueinone and herqueichrysin triacetate (1095, 1096).[90] A very unusual coupling constant value is reported for xanthomegnin (1098).[91] $^1J(C_{11'}–C_{12'})$ is 12·6 Hz larger than the corresponding coupling in 1-hydroxynaphthalene (551). Other examples are pisantin (1073),[92] altersolanol (1070),[76] flavoglausin (1083),[93] and ochratoxin A (1089).[94]

3. *Substituent effects*

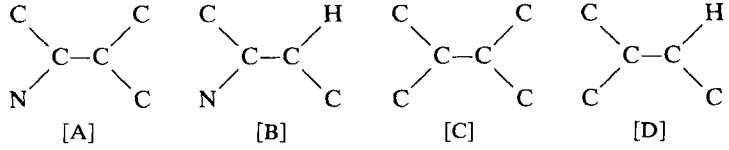
The large number of benzene derivatives investigated have given a broad experience of substituent effects.[68] Some other substituent effects may be obtained from the substituted PAHs.[20,72–74] In general, substituent effects observed in benzenes and PAHs are similar.[68,72] Whether substituent effects are additive or not has not been investigated in detail. From the biomolecules studied they seem, within the accuracy of these types of data, to be additive. However, a substitution pattern often encountered in biomolecules is two oxygen-containing substituents *meta* to one another. The coupling constants quoted are often slightly larger than expected from addition of the substituent effects.

4. *Heteroaromatics*

Among the classes of heteroaromatics investigated are substituted pyridines, quinolines, and pyrroles. The pyridine moiety is part of nicotinic acid (1028), anabasine (1054), nicotine (1055), nornicotine (1043), and anatabine (1050).[95] $^1J(C_5–C_6)$ varies from 55·0 to 59·1 Hz and is thus not very different from that in benzene. $^1J(C_4–C_5)$ is 54 Hz in nicotinic acid, again

quite similar to benzene. $^1J(C_5–C_6)$ is much larger in the 5-fluoronicotinic acid sodium salt (1021) and 5-fluoroanabasine (1052) than in the parent compounds owing to the fluorine substituent effect.[96] $^1J(C_5–C_6)$ in α,β-dipyridyl is ~55 Hz[97,98] but is considerably higher in acidic solution.[97] The coupling constants in quinoline (1039) are close to those of naphthalene (548, 549). An unusual feature is observed in 3-amino- and 3-nitro-quinoline (1042, 1038).[95] $^1J(C_2–C_3)$ is increased upon substitution but not nearly as much as in the corresponding benzene derivatives, whereas $^1J(C_3–C_4)$ is unchanged.

Coupling constants for pyrrole derivatives are found in some simple di- and tri-substituted pyrroles which are used as model compounds in the study of porphyrins, bilirubins, biliverdins, and vitamin B_{12}. $^1J(C_2–C_3)$ varies from 60·6 to 64·6 Hz and $^1J(C_3–C_4)$ from 54·1 to 55·2 Hz in the simple pyrroles studied.[99,100] Much of the work has been done on specifically enriched materials so only a few coupling constants have been observed. $^1J(C_4–C_5)$ has been reported in protoporphyrin (498)[101,102] and in sirohydrochlorin octamethyl ester (1017). The values of $^1J(C_4–C_5)$, $^1J(C_{14}–C_{15})$, and $^1J(C_{15}–C_{16})$ are different from $^1J(C_9–C_{10})$.[103] A similar difference is reported in 20-methylsirochlorin (1018).[104] In prodiginines most types of couplings are represented (483).[105] Wray *et al.*[99] have studied ^1J(C–C) in ^{13}C-enriched mesobiliverdin-IXα dimethyl ester (494) and mesobilirubin-IXα dimethyl ester (495) as well as in some model compounds (477–480). ^1J(C–C) values of the fragments [A] and [B] are related to the bond lengths, and a smooth non-linear dependence is found. This dependence is used to estimate the degree of conjugation in the two systems of interest, the biliverdin and the bilirubin systems. Similar results were obtained for the [C] and [D] fragments. The ^1J(C–C) couplings observed in vitamin B_{12} (499)[106]



were correlated with typical bond lengths for the corrin system. The coupling constants in vitamin B_{12} seem to be unusually large;[106] they are probably influenced by the presence of the cobalt atom.

5. *Other aromatic compounds*

In dimethylferrocene $^1J(C_1–C_2) \approx {}^1J(C_2–C_3) \approx 47$ Hz.[107] This is only slightly larger than that observed in the iron tricarbonyl compounds. The low value probably originates from the electropositive iron atom and the resulting negative charge density on the cyclopentadienyl carbons. The

authors predict $^1J(C_1–C_2)$ in phenyllithium to be at the low extreme. The magnitude may also be related to ring size. $^1J(C_1–C_2)$ in cyclopentadiene itself is 38·5 Hz.[108] $^1J(C_2–C_3)$ is not yet known in the diene but in 1-methylcyclopent-1-ene $^1J(C_1–C_2) = 72·0$ Hz.[109]

H. Couplings involving sp hybridized carbons

Only a few couplings of this category have been reported. Wasylishen and Pettitt[110] have investigated benzyl cyanide (982). A few acetylenes have also been studied.[111,112]

III. TWO-BOND COUPLING CONSTANTS

Two-bond coupling constants are divided into two categories, those in which the coupling path contains only carbons and those in which it contains carbons and heteroatoms. The former type is treated first.

Two-bond couplings may be either positive or negative in sign. Couplings involving triple bonds and those across carbonyl carbons have long been known to be positive[113] but for most other kinds of two-bond coupling constants substituent effects may lead to either positive or negative values. It is essential to determine the signs of two-bond coupling constants and to understand the effects of substituents.

A. Couplings across carbonyl carbons

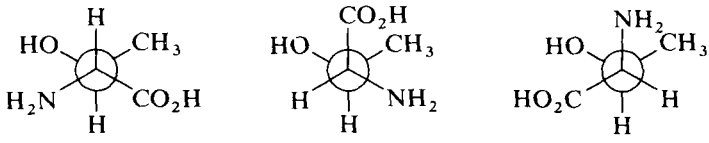
The couplings are positive and large. They have been observed in many biomolecules; some are tabulated.[3] ^2J(C–CO–C) in acetone and butanone is 15–16 Hz.[113] Similar values are observed in five-membered bicyclic compounds (779, 780).[114] In compounds in which one carbon is part of a double bond, ^2J is close to 12 Hz as observed in malonomicin (890),[115] colletrotrichin (1015),[116] griseofulvin (901),[117] and acetophenone (857).[118] In acenaphthenone (881)[118] and in aflatoxin B_1 ^2J is 18–21 Hz.[119] Much smaller values (~8–9 Hz) are found in scylatone (868)[120] and tenelin (1011).[121,122] The small coupling in the last two compounds may be caused by hydrogen-bonding. A similar reduction has been observed in ^2J(C–CO–H) couplings.[123]

A single case of coupling across a C=N bond is reported and the carbon–carbon couplings obtained are compared with similar carbon–hydrogen and hydrogen–hydrogen coupling constants.[47]

B. C–C–C fragments (all three carbons sp^3 hybridized)

In hydrocarbons ^2J is probably negative but very few signs are known. In the sidechains of amino acids the signs of two-bond couplings have been

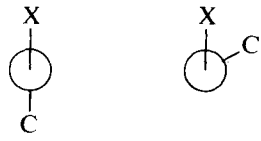
determined.[46] The values of $^2J(C_\alpha-C_\gamma)$ are +0·3 and 0·4 Hz in threonine and glutamic acid respectively. These couplings should reflect the rotamer distribution since an electronegative substituent in the *trans* position as in [6](I) will give a positive contribution, and a *gauche* substituent as in [6](II) and [6](III) a negative contribution (see below). The more positive value for threonine is probably due to the hydroxy group on the β carbon.



(I) (II) (III)

[6]

Berger[40] has observed large couplings in bicyclo[3.2.1]octanes substituted at C_6 (123). The coupling consists of two contributions, a two- and a three-bond coupling as in other five-membered rings. The three-bond coupling is positive and the large magnitude of the total coupling points to either a very small or a positive two-bond coupling. In 2-substituted adamantanes Berger and Zeller[124] have observed that $^2J(C_{\gamma syn}-C_2) > {}^2J(C_{\gamma anti}-C_2)$ (141, 143). Both of the above mentioned observations may be explained if an electronegative substituent in a *trans* position makes a positive contribution to the coupling; see [7a].



[7a] [7b]

Examples of polysubstituted two-bond coupling pathways are found in carbohydrates. Walker *et al.*[42] found in glucose, methyl glucopyranoside, mannose, methyl mannopyranoside, galactose, and fucose a numerical value for $^2J(C_1-C_3)$ of ~3·4–4·3 Hz in the β derivatives and no observable coupling in the corresponding α compound. Similar but slightly smaller couplings are found in glucose and mannose amines (88, 89).[43] Gagnaire *et al.*[44] observed comparable values in glucose, in methylglucose tetra- and penta-acetate. In some disaccharides couplings close to those of monosaccharides are observed.[44,128] In β-hexanolamine glucoside (836) a ^2J value similar to that observed in β-galactopyranose was observed. Nunez and Barker[125] conclude that there appears to be no distortion of the galactopyranosyl ring. A similar argument was used in the case of α-D-galactopyranosyl phosphate.[45a] In 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylideneglucofuranose $^2J(C_2-C_4)$ is determined to be +1·5 Hz.[126] This coupling is

the sum of two contributions, ^3J(C–C–O–C) and ^2J(C–C–C), of which the former is most likely to have a positive sign while the latter is, on this evidence, not very negative. The principles developed for two-bond carbon–hydrogen coupling constants have been discussed in connection with carbon–carbon couplings,[42] but the discussion is hampered by the absence of some knowledge of signs. However, the general principle that a *trans* substituent gives a positive and a *gauche* a negative, but not necessarily equally large, contribution will probably be very useful. Substituent effects at both terminal carbons will have to be considered.[42]

Two-bond couplings in four-membered rings were observed in the early period of carbon–carbon coupling constant investigations because of their large magnitude.[33] The coupling in cyclobutanone has been confirmed (308, 309) by Jokisaari[32] who reported no couplings in bromo- and chloro-cyclobutane, neither in oxetane nor in thietane. Stöcker and Klessinger[31] reported two-bond couplings in methyl and carboxylic acid derivatives of cyclobutane. The values fall between 7·3 and 8·3 Hz. Cyclobutene (850)[59] gives a $^2J(C_1–C_4)$ result very similar to those found in cyclobutane. The sign of two-bond couplings in four-membered rings has not been determined but from analogy with proton–proton coupling constants the small bond angles in this ring system may suggest a negative coupling constant.[33] Substituent effects are, so far, not well documented but their magnitude is moderate and they do not follow the scheme suggested (see below). ^2J in a heterocyclic four-membered ring, clavulanic acid, shows a coupling of the same magnitude (942)[127] as in the above-mentioned homonuclear compounds.

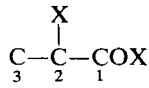
In five-membered rings smaller two-bond couplings than in four-membered rings are usually found,[118,119] and in six-membered rings, other than carbohydrates, the two-bond couplings are usually small.[38,39,129] Some typical values of ^2J in the series cyclohexanes, cyclopentanes, and cyclobutanes are −3, 0, and (−)8 Hz respectively. The coupling in cyclopentanes consists of two contributions, one across two bonds and one across three bonds; the latter is positive whereas the former is negative. Since ^3J values are typically about 5 Hz in cyclic systems (Section IVA), an observed value close to zero indicates a two-bond coupling close to −5 Hz in cyclopentane. An increasingly negative value with decreasing ring size could thus lead to an estimated two-bond coupling in cyclopropanes of −12 to −15 Hz (see Section IIA).

C. Couplings involving a C–C–COX skeleton

1. *Single bonds*

Two-bond couplings are usually small.[3] Only the signs of $^2J(CO–C_\beta)$ in amino acids have been determined.[46] They are negative in serine, threonine,

aspartic acid, and glutamic acid. The variation is small, −0·6 to −1·3 Hz. The negative sign is predicted from a comparison with carbon–proton coupling constants and supported by the substituent effects found in 2-substituted carboxylic acids.[3] This agreement shows that electronegative substituents at C_2 as in [8] lead to a more positive coupling; this finding is parallel to that for

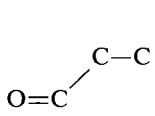


[8]

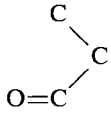
carbon–proton coupling constants.[35,130] Negative couplings to the sidechain carboxylic acid groups in aspartic and glutamic acids were also reported (933).[46] Among the carboxylic acid derivatives aspyrone is a special case having $^2J(CO–C) = 6·2$ Hz (375).[131,132]

Effects of ionization of carbocyclic acids may be judged from cyclo-(Asp-Pro).[133] The value of $^2J(COOH–C_4)$ changes from 2·0 to 1·2 Hz upon ionization.

Couplings involving carbonyl carbons of ketones are also small; examples are adamantan-2-one (381),[124] t-butyl ketones (402),[50] tetralone,[54] and phenalanone,[3] in which the couplings lie between 0 and 2·1 Hz. As already mentioned, cyclobutanone falls clearly outside this range (308). Couplings with a geometry as shown in [9] are most likely to be positive, whereas couplings for a structure like [10] may well be negative, as judged from the small coupling observed in t-butyl ketones.



[9]



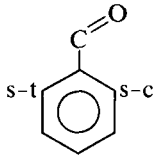
[10]

2. *Double bonds*

Two-bond couplings are reported in *cis*- and *trans*-cinnamic acid (1117)[53] and their methyl and ethyl esters (1123),[53,134] *trans*-crotonic acid (588),[53] 3-methylbut-2-enoic acid (589),[53] and *N,N*-dimethyl-*trans*-crotonamide (1109).[53] The couplings are in all cases small, 0·50–2·17 Hz. The dependence upon the orientation of the carbonyl group is uncertain and the small magnitude of the coupling makes it difficult to use this parameter for stereochemical studies.

3. *Aromatic bonds*

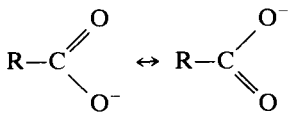
In aromatic ketones (see [11]) a difference between $^2J(CO–C)_{s-c}$ and $^2J(CO–C)_{s-t}$ was observed.[50] A preferred orientation is obtained either by substitution in the *ortho* position [methyl groups (607, 631) or in the form of adjacent rings such as in naphthalenes (646) and pyrenes (659)] or by building the carbonyl function into a fixed structure.[3,50] $^2J(CO–C)_{s-t} > {}^2J(CO–C)_{s-c}$ and couplings in compounds with free rotation fall in between the values for these two couplings. In compounds in which sterically space-filling groups prevent coplanarity of the C=O group and the aromatic ring, the magnitudes of the coupling constants decrease. Aldehydes behave like ketones, whereas acids and acid derivatives show $^2J(CO–C)_{s-c} > {}^2J(CO–C)_{s-t}$. Signs have been determined to be positive in naphthalene-*N*,*N*-dimethylcarboxamide[50] and are believed to be generally so. These findings may be used to determine the preferred orientation of carbonyl groups. Other examples of differentiation between ^2J(s-c) and ^2J(s-t) are reported.



[11]

Different $^2J(CO–C_1)$ and $^2J(CO–C_3)$ values were found in 2-acetylparacyclophane (656)[135] but no signs of a transannular effect are apparent. The couplings are very much like those observed in 2-methylacetophenone (607).

In vanillin (3-hydroxy-2-methoxybenzaldehyde) different couplings to C_2 and C_6 are observed (1115).[134] In nicotinamide the different two-bond couplings are taken as evidence for a preferred orientation of the carbonyl group towards the nitrogen (1107).[27] In nicotinic acid sodium salt (1106) very similar couplings are found.[97] Whether this observation may be taken as evidence of a fixation of the double bond in the carboxylate anion is not clear. An even more astonishing finding is the different values for $^2J(CO–C_2)$ and $^2J(CO–C_9)$ in naphthalenecarboxylic acid potassium salt (1127).[54] In this compound the COO^- group is supposed to be a hybrid of the two forms [12a] and [12b].



[12a] [12b]

The differences between ketones and aldehydes on the one hand and acid derivatives on the other may possibly be explained by regarding a carbonyl group merely as a substituted carbon. Carbonyl groups are thus behaving like sp^3 hybridized carbons as already described. A difference between singly and doubly bonded oxygen has to be inferred in order to explain the differences between ketones and acids. Support for an approach relating the difference in coupling constants to the nature of the substituents is found in thioamides (949) which give less pronounced differences than amides as sulphur is less electronegative than oxygen.[54]

D. Couplings across olefinic double bonds

Two-bond couplings across double bonds are given in *exo*-methylenebenzocycloalkanes (866, 877, 883, 889)[55] between the exocyclic carbon and the hydrogen substituted carbons. An increase is observed in going from five- to eight-membered rings. No couplings are found to the quaternary carbons. Calculated coupling constants are five times as large as those observed. This may indicate that the Fermi contact mechanism is not dominant.

E. C–C=C (double bond aromatic)

Couplings between aromatic carbons and α-carbons (sp^2 hybridized) have been reported both in benzene (858, 860, 871, 887)[136] and in naphthalene (874, 882)[118] derivatives. A comparison of ^2J in toluene (230) and t-butylbenzene (871)[68] shows a large difference, whereas the data for toluene (230), benzyl chloride (224), and dichlorodiphenylmethane (887) show an irregular trend. $^2J(C_9–C_1)$ in 9,10-dihydroanthracene (895)[72] compares well with the analogous coupling in dibenzyl (160).[27] $^2J(C_9–C_{11})$ on the contrary is very small and negative (895). A very small coupling is also found in acenaphthenone (881). In fluorene the value of $^2J(C_9–C_1)$ is similar to that of toluene (886). ^2J in 1-naphthylmethanol and 1-naphthylacetic acid reveals slightly different coupling constants to the two *ortho* carbons (874, 882).[118] A possible explanation for the large differences between similar compounds may be that the two-bond coupling depends upon the number and orientation of the C–H bonds or the substituent at the C_α carbons. For a discussion of similar effects in three-bond couplings see Section IVA.

F. Couplings across aromatic bonds

The discussion of two-bond coupling constants in aromatic systems and especially benzenes has been restricted by the lack of knowledge of signs of coupling constants and the value for benzene (to be used as a reference).

Signs of two-bond couplings have now been determined in PAHs. Hansen *et al.*[72,74] found that couplings between carbons belonging to two different rings are positive, whereas couplings between carbons within the same ring are negative, being the more so the higher the bond-order of the intervening bonds. These generalizations are based on few determined signs but are further supported by substituent effects. Substituents *trans* to a bond (see [7a]) lead to a positive contribution and *gauche* substituents to a negative one. Based upon these assumptions the coupling constants in naphthalene, phenanthrene, pyrene, and anthracene can be rationalized[72] and lead to a prediction of a ^2J value for benzene of $-2{\cdot}5$ Hz. The absolute magnitude in benzene has recently been confirmed.[137] The study of substituted benzenes (non-enriched) is limited to coupling constants larger than ~2 Hz. Very few have been reported[68] except in compounds with substituents, such as I, CH_3, Bu^t, $P(C_6H_5)_2$. The fact that most couplings are smaller than 2 Hz supports the finding that ^2J in benzene is negative and that electronegative substituents lead to an increase (compared with more positive values). The two-bond coupling $^2J(C_1–C_3)$ was redetermined in toluene[138] and the new value (1032) shows a much smaller substituent effect. $^2J(C_2–C_4)$ values in substituted benzenes are close to that of benzene itself (2·46–2·89 Hz). A slight increase is observed in the series I, Br, Cl, F.[68] Two-bond couplings across a substituted carbon are not well documented. In 1-hydroxynaphthalene $|^2J(C_2–C_{8a})| = 0{\cdot}50$ Hz compared with $(-)2{\cdot}5$ Hz in naphthalene.[72] 3-Fluoropyridine (3·5 Hz)[96] may be compared with nicotinic acid (2·8 Hz).[95] In contrast to these small substituent effects a very large two-bond coupling (9 Hz) is reported for 3-nitroquinoline. $^2J(C_2–C_4)$ in quinoline is likely to be of the order of 3 Hz as judged from nicotinic acid (1028).

Large two-bond couplings are observed for 1,1′-dimethylferrocene (558).[107]

G. Couplings involving sp hybridized carbons

Two-bond couplings involving cyanide carbons have been subject to some confusion due to a misquotation by the original author.[139] This mistake has unfortunately been repeated by myself[3] and others.[140] $^2J(C–C–(C{\equiv}N))$ results have been reported for benzonitrile (520),[141] 2-methylbenzonitrile (1168),[136] and benzyl cyanide (1169).[140] In all cases the couplings are around 3 Hz which is comparable to similar types of coupling constants.

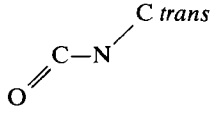
Couplings across triple bonds are invariably large and positive in sign (671–673).[4,111,112]

H. Couplings through heteroatoms

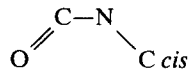
The couplings most thoroughly studied are those through nitrogen, but also some involving oxygen, sulphur, boron, and ruthenium.

1. *Nitrogen*

Two-bond couplings in amides have been determined.[142,143] Couplings in which the C=O and the other carbon are *trans* (see [13a]) are large and positive, whereas those with the carbons *cis* (see [13b]) are small (915, 918, 919, 927).



[13a]



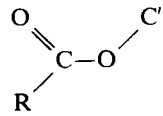
[13b]

The large couplings noted in phthalimide (597),[74] pyrrolin-2-ones (346, 347),[99] 5(1*H*)-pyrromethanones,[99] and cyclo(tri-L-prolyl) (819)[144] are thus the result of an addition of two positive contributions. No $^2J(C_2–C_4)$ coupling has been detected in 2,4-dimethylthiazolidine,[41] underlining the importance of an electronegative atom in a *trans* position.

Couplings across nitrogens are observed in heteroaromatic compounds, such as pyrroles. A large $^2J(C_1–C_4)$ is observed in a substituted pyrrole (654, 655),[99] whereas the two-bond coupling between C_2 and the quaternary carbons in benzimidazoles has not always been observed.[145]

2. *Oxygen*

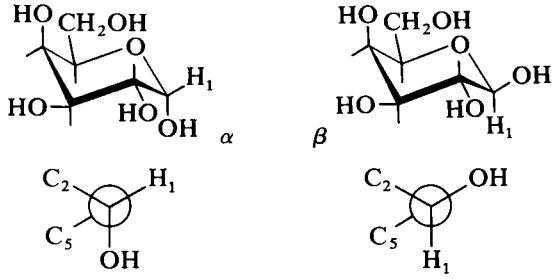
The finding of a positive $^2J(CO–N–C)_{trans}$ and the fact that $J(CO–X–CO)$ data are of similar magnitude for X = N, O, and S in phthalimide, phthalic anhydride, and phthalic thioanhydride are taken as evidence for positive values of $^2J(CO–O–CO)$ and $^2J(CO–S–CO)$.[74] A negative two-bond coupling has been determined in esters in which the geometry is most likely *cis* [14].



[14]

In carbohydrates $^2J(C_1–O–C_5)$ and $^2J(C_1–C_3)$ show a marked difference between α- and β-anomers.[42,45] In the α-anomer [15] the couplings are $\sim$1·5–2·0 Hz whereas they are not observable in the β-anomer [16]. In the β compounds a positive contribution is expected because of the α-OH group, whereas in the α compounds a negative contribution adds to the basically negative coupling of an unsubstituted C–O–C fragment. $^2J(C–O–C)$ in

methyl ether is −2·4 Hz.[113] Small two-bond couplings (~1·5 Hz) are observed between C_1 and C′–X in disaccharides.[44]



[15] [16]

3. Boron and ruthenium

A two-bond coupling through boron was reported for the *closo*-carborane 1,5-$C_2B_3H_5$ (686).[146] The large magnitude (15·0 Hz) could be general for boron, or possibly the result of the special geometry and bonding situation of the cage structure. A coupling through ruthenium is found in *cis*-$Ru(CO)_4I_2$ (1103).[147]

I. Summary

Two-bond couplings through carbons and heteroatoms seem to behave in the same way. The orientation of substituents relative to the two bonds plays a major role in determining the sign and magnitude of two-bond couplings. The experience gained up to now is summarized in Fig. 1.

The relationship between the direction of the substituents and two-bond couplings may make them a very useful tool in stereochemical studies. Three-bond couplings have, so far, been leading in interest because of their dihedral dependence. However, as discussed in the next section, the difficulty of assessing the importance of the many possible substituent effects may make this relationship less useful in complex molecules. The advantage of using two-bond coupling constants is that substituents at both C_1 and C_3 may provide stereochemical information, and that correction is easily made for substituents at C_2.

IV. THREE-BOND COUPLING CONSTANTS

Three-bond coupling constants and Karplus curves have almost become synonyms although this was most likely not the original intention of Karplus.[148] Much effort has been put into attempts to produce correlations

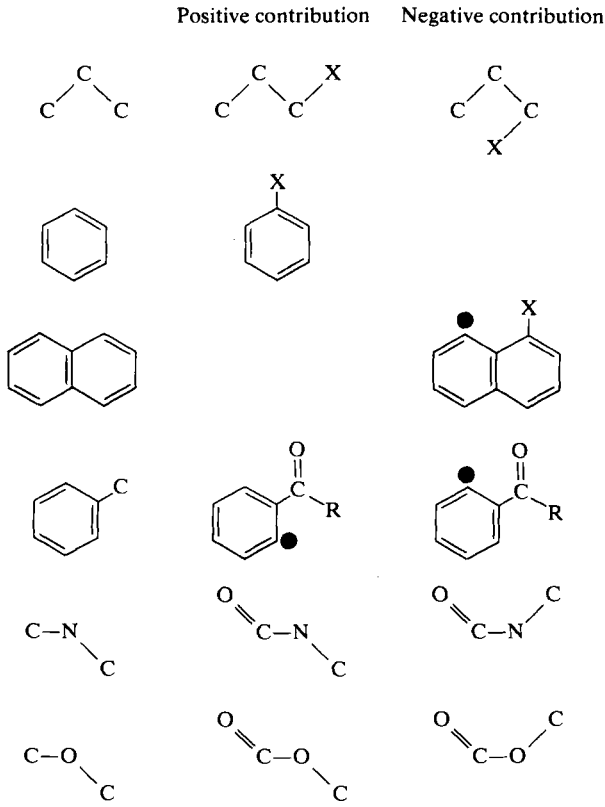
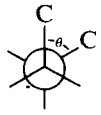


FIG. 1. A comparison of how substituent effects perturb two-bond coupling constants.

between ^3J and the dihedral angle θ as in [17]. The understanding of substituent effects on ^3J is of vital importance.



[17]

A. Couplings through aliphatic bonds

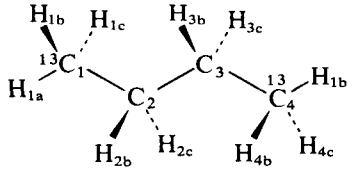
Berger[114] has selected a set of molecules with well known geometries and fulfilling the following criteria: no bond and angle strain, and no through-space interaction between the labelled centre and the reporter group. Assuming additivity of the spin coupling if transmitted over more than one

coupling pathway, a Karplus curve was constructed and the following equation obtained:

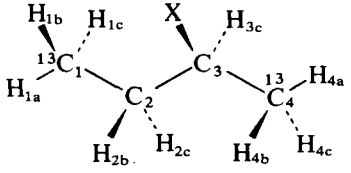
$$^3J = 1{\cdot}67 + 0{\cdot}176\cos\theta + 2{\cdot}24\cos 2\theta \quad (1)$$

The experimental curve was compared with a theoretical one for butane.[149] The two curves look similar except for an offset. Calculation of coupling constants in the actual compounds results in better agreement and the author warns against the use of data based upon model compounds. One of the features in the compounds considered, bridgehead–bridgehead interactions, is not present in butane.

The number of restrictions it is necessary to impose upon the model compounds make it questionable if "ordinary" compounds can be analysed using a Karplus equation. Barfield[150] has investigated the importance of non-bonded interactions using INDO-FPT calculations. To estimate the contributions of various pathways, elements of the Fock matrices associated with orbitals centred on various non-bonded atom pairs are set equal to zero. Dimethyl substitution at C_2 of the butane fragment produces a 20% decrease of $^3J(180°) = {}^3J(trans)$, but very little change for other dihedral angles. In contrast, for a 1,1-dimethyl substitution, the values for dihedral angles of 0° and 60° are substantially reduced. For 1,1-difluoro substitution, couplings for dihedral angles smaller than 90° decrease, but increase for angles larger than 90°. For 2,2-difluoro substitution all the calculated values are less than for butane. On the basis of these calculations Barfield concluded that the non-bonded contributions dominate this type of coupling, so it is not possible to obtain a satisfactory representation by a simple trigonometric relationship such as that shown in equation (1). γ-Substituent effects upon $^3J(180°)$ have been further studied.[151] It is shown that substituents at C_3 lead to a decrease in magnitude of $^3J(180°)$. The same procedure demonstrates that non-bonded interactions between C_1 and hydrogen atoms at the γ-carbon make positive contributions (see [18]), whereas substituents such as methyl, fluorine, etc. give negative contributions. The effect of substitution at C_γ is thus enhanced as the positive contribution from the γ-hydrogens is removed, and a negative contribution from a substituent at C_γ is added on. The negative non-bonded contribution in the case of a methyl group stems from interactions between the hydrogens at C_1 and at the methyl group substituted at C_γ (see [19]). However, the



[18]



[19]

presence of a hydrogen atom on the γ-substituent is shown not to be a prerequisite of a negative γ-effect.[151]

A comparison of ^3J values in a butane fragment, disubstituted at C_3 with either two methyl or two ethyl groups, points towards a positive δ-effect as ^3J is larger in the latter compound. An alternation of effects is generally predicted. It is emphasized that the impinging rear lobe effect is not responsible for the substituent effects observed in methylcyclohexanes and methyladamantanes.[151] The IRL approach has been criticized in previous papers.[152,153]

Both theoretical and experimental three-bond coupling constant values of butan-1-ol (50) have been reported as well as experimental data for other primary alcohols.[129] It appears that the effect of terminal substitution for eclipsed fragments is less than expected but, in general the effects of substitution are small. Three-bond couplings are observed in bicyclo-[2.2.2]octane and bicyclo[3.2.1]octane.[40] The very small couplings in the latter indicate a flattening of the six-membered ring.[40] A three-bond coupling in bio-enriched cholesterol is in good agreement with that predicted from the actual geometry (191).[154] In 2-substituted adamantanes $^3J(C_2\text{–}C_{\delta syn}) > {}^3J(C_2\text{–}C_{\delta anti})$. Substituent effects are small.[124]

B. ^3J (CO–C)

1. Amino acids

One of the fields in which a Karplus equation may be used is in the analysis of sidechain conformations of amino acids. However, as Karplus curves have not been well defined, the usual approach is to use $^3J(180°)$, also called ^3J_t, and $^3J(60°)$, known as ^3J_g. Carbon–carbon coupling constants may provide additional evidence and confirm estimates made from hydrogen–hydrogen and carbon–hydrogen couplings. Ptak *et al.*[155] have estimated $^3J_g \approx 0$ Hz and $^3J_t \approx 3$ Hz for threonine, and $^3J_g = 0{\cdot}5$ Hz and $^3J_t \approx 5$ Hz for aspartic acid. Fermandjian *et al.*[156] arrived at similar couplings for aspartic acid. For amino acid residues $^3J_g = 0{\cdot}6$ Hz and $^3J_t = 3{\cdot}9$ Hz. For valine, $^3J_g \approx 0$ and $^3J_t \approx 5{\cdot}0$ Hz give the best fit with ^1H–^1H and ^{13}C–^1H coupling constant data.[53] London *et al.*[51] have adopted this approach and calculate $^3J_g = 1{\cdot}2$ Hz and $^3J_t = 4{\cdot}5$ Hz for aspartate and $^3J_g = 0{\cdot}6$ Hz and $^3J_t = 3{\cdot}0$ Hz for glutamate. The values for glutamate are quite close to those of leucine, which is as expected. Very poor results are obtained for all calculated coupling constants of the diprotonated species.

The ^3J_g and ^3J_t data obtained so far indicate that several sets of ^3J_g and ^3J_t results may have to be determined. Amino acids with fixed geometries may tell how the different ionization stages influence ^3J values.

Changes in pH have a dramatic effect upon the rotamer distribution of aspartic acid which is also reflected in the observed coupling constants.[51]

Two approaches to conformational studies may be taken, a static and a dynamic one. Vičar *et al.*[157] have calculated both rotamer populations and the corresponding θ angle of a fixed conformation in *cis*-cyclo-(Val-Val) (957, 958) and *cis*- and *trans*-cyclo-(Leu-Leu) (964, 965). No distinction between the two cases has been made.

^3J(CO–C_γ) coupling constants have been observed[156] in valine residues of [Val^5]angiotensin II at pH 1·2 and used in the thyrotropine releasing hormone[158] and in cyclo-(Asp-Pro) to estimate the population of rotamer I, both in the COOH and in the COO^- ionization state of the β-carboxyl group (1120, 1121).[133] A ^3J(CO–C_γ) value of 0 is used to predict a puckered conformation of cyclo-(L-prolyl-L-prolyl-L-prolyl).[144]

Toma *et al.*[159] have estimated the sidechain conformation of leucine in uniformly labelled Gly-Pro-Leu-Gly and the conformation of proline in Gly-Pro-Asn-Gly on the basis of ^3J(H–H) and ^3J(C–C) results. The measurement of these couplings was made easier by selective decoupling of the C_β atom. In general, it appears that in uniformly labelled materials it is advantageous to measure $^3J(^{13}C–^{12}C–^{12}C–^{13}C)$ as line-broadening induced by $^{13}C–^{13}C$ dipole interactions may alter the shape of the ^{13}C multiplet of the three spin system ^{13}C, ^{12}C, ^{13}C, ^{13}C and lead to an erroneous value of the coupling constant.

2. *Carboxylic acids*

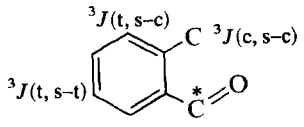
Three-bond couplings involving carboxyl groups are found in malonymycin (1140)[115]. The theoretical calculations of London *et al.*[51] reveal that $^3J(C_2–C_5)_t > {}^3J(C_1–C_4)_t$ in glutamate. In other words, the value of ^3J_t is larger in carboxylic acids than in amino acids or, expressed differently, the amino group at the α-carbons has a negative substituent effect. The analysis of the NMR data for 3-methylbutanoic acid supports this suggestion.[54]

C. Aromatic compounds

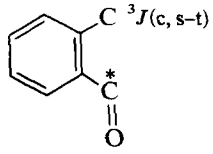
1. *Couplings between* α-carbons

The very small values of ^3J(CO–CH_3) in acetophenone (607) and *ortho*-substituted phenones and carboxylic acids, as well as those of ^3J(CO–C_8) and ^3J(CO–C_{10}) in 1-naphthalene and pyrene carboxylic acids and esters (618, 657), are ascribed to a negative contribution from overlap between orbitals of the C=O and methyl groups.[50] In these compounds the carbonyl group points towards the methyl group or the *peri* carbon. The couplings are denoted ^3J(c, s-c) [20]. In 2-methylbenzaldehyde the conformer ratio between [20] and [21] is 1 : 1 and a somewhat larger coupling constant is observed (939).[136] The existence of non-bonded contributions is underlined by the increase of ^3J when the C=O group is twisted away from the methyl

group.[50] This has been confirmed by Marshall *et al.*[136] by means of calculations of ^3J (CO–CH_3) in *trans*-crotonaldehyde. The dependence of the coupling upon the orientation of the carbonyl group is clearly demonstrated. Furthermore, the importance of the position of the methyl hydrogens is noted.



[20]



[21]

Couplings between α-carbons, either sp^3 or sp hybridized, are generally larger than those involving a carbonyl group. A non-bonded contribution in the case of tolunitrile is also demonstrated.[136]

Marshall *et al.*[160] have studied three-bond coupling constants in carboxylic acids of the type 1,4-dihydrobenzoic acid, 1,4-dihydro-1-naphthoic acid, and 9,10-dihydro-9-anthroic acid. No dihedral angle dependence is reported. A contribution to ^3J is suggested in which hyperconjugative interactions between the C–COOH bond and the p_π orbital of the C_1 ring carbon occur.

2. *Couplings between aromatic carbons*

Three-bond couplings in aromatic compounds have been correlated with π-bond order.[72] The coupling constants divide into classes according to the number of coupling paths and their geometries. Couplings with a *cis* geometry show poor correlation and the value for benzene (1020, 1024) does not fit the equation well. The dependence on π-bond order does not necessarily reflect the participation of the π-electrons, as π-bond order is also related to many other properties of aromatic systems.

Theoretical calculations by London[161] on tryptophan and by Hansen *et al.*[72] on naphthalene reveal that the orbital and the dipolar contributions to the coupling are small, but not negligible.

Couplings similar to $^3J(C_1–C_7)$ in naphthalene are reported in tryptophan (1061)[161] and in nobomycin (576).[162]

Three-bond coupling constants in substituted benzenes fall within the following ranges: $^3J(C_1–C_4)$ 8·9–10·66 Hz and $^3J(C_2–C_5)$ 6·6–11·1 Hz. The former show no correlation with electronegativity, whereas the latter depend linearly upon the electronegativity of the first atom of the substituent. The less electronegative the substituent, the larger the coupling constant. Theoretical calculations do not reproduce this trend.[68] However, they demonstrate that intermolecular effects may be ignored in such cal-

culations. Substituted phenanthrenes have been analysed[73] but the lack of knowledge of $^3J(C_1–C_X)$ in phenanthrene makes comparisons difficult. The only type of substituent investigated in some detail in other systems is one with oxygen as first atom. A hydroxy or a methoxy substituent in benzene leads to a reduction of $^3J(C_1–C_4)$. A decrease in the value of ^3J for the same type of coupling pathway is noticed in 1-hydroxypyrene whereas in 9-methoxyanthracene an increase is reported.[74] For other geometries both increases and decreases are observed, but in all cases the substituent effect is smaller than 1 Hz.

3. Couplings between C_α and aromatic carbons

Coupling constants from sp, sp^2, and sp^3 hybridized α-carbons have been investigated. For sp hybridized carbon only a single example has been reported, that of *o*-tolunitrile (1168).[136] Of sp^2 hybridized carbons both olefinic and carbonyl carbons have been investigated. The latter are of special interest since the value of ^3J(c, s–c) indicates that non-bonded interactions are important. ^3J(t, s–c) and ^3J(t, s–t) are not clearly distinguished in the same way as ^2J(s–c) and ^2J(s–t).[50] ^3J_t depends upon the orientation of the carbonyl group, the bond order of the intervening aromatic bonds, the bond angles, and the degree of twist of the carbonyl group.[54]

In the case of sp^3 hybridized carbons the investigations are less detailed.[136]

4. Couplings between C_β and aromatic carbons

A very large three-bond coupling has been reported, $^3J(CH_3–C_2)$ in acetophenone (857).[118] Couplings between C_β and C_2 in stilbenes and tetraphenylethylene were shown to reflect the degree of twist of the phenyl ring out of the plane of the double bond (564, 582).[72] In this way the twist angles of 9,10-diphenylphenanthrene (581),[72] 1,2,3,4-tetraphenylnaphthalene (582), and 2,3,4,5-tetraphenylcyclopentadienone (583) have been estimated. A similar situation is encountered in 5(1*H*)-pyrromethanones (477, 478) and in mesobiliverdin-IXα dimethyl ester (494). $^3J(C_4–C_7)$ and $^3J(C_{13}–C_{16})$ in the latter reflect the change in bond length and in the interplanar angle.[99]

The three-bond couplings between *exo*-methylene carbons and the aromatic ring carbons of *exo*-methylenebenzocycloalkanes are also in this category. A dihedral angle dependence is obtained. However, deviations of coupling constants in the compound with a five-membered ring (1048) suggest that valence angles also influence the value of ^3J.[55]

D. Couplings through heteroatoms

1. *Nitrogen*

London[163] has observed a $^3J(C_\beta–C_2)$ in histidine (218a); its magnitude depends upon the protonation of the imidazole ring. A value for $^3J(CH_3–N–C–C)$ was observed in 2-methylthiazoline (721).[164] An unusually large three-bond coupling $^3J(C_2–C_4)$ was found in 1,3-diazaazulene (1034).[145] In benzimidazoles, $^3J(C_2–C_4)$ values are of the order of 5–6 Hz.[145] $^3J(C_2–C_5)$ interactions in pyridine have long been known to be around 12 Hz in magnitude (502).[33] Similar couplings are observed in nicotinic acid (1028)[95] and a pyrazine (1019).[165] This large coupling could be the result of the addition of two different coupling paths but this suggestion is contradicted by the azaazulene results.

In nitroquinoline several three-bond couplings are found:[95] $^3J(C_2–C_{4a})$, $^3J(C_2–C_8)$, and $^3J(C_3–C_{8a})$ (1038). $^3J(C_2–C_8)$ is unusually small compared with that of benzimidazole. $^3J(C_2–C_{4a})$ and $^3J(C_3–C_{8a})$ are both smaller than the 12 Hz found in pyridine. This may be due to a substituent effect.

Coupling through nitrogen is also encountered in couplings between carbonyl acid carbons and the γ-ring carbons of proline residues.[133,144,156,166] ^3J provides useful information about the puckering of the ring. Slight differences between *cis*- and *trans*-proline are observed in Asp-Pro (952).[156] ^3J varies dramatically with pH in [S]-proline which reflects a change in ring puckering (312).[166] In cyclo-(Asp-Pro) two different couplings are observed (951).[133] Stimson *et al.*[167] have reported small three-bond coupling constants of ~1 Hz (1164) between the carbonyl carbons of the peptide backbone of Tyr-Gly-Gly-Phe-Val.

2. *Oxygen*

Very few three-bond couplings through oxygen have been reported. A $^3J(CN–C–O–CO)$ interaction of 1·5 Hz is observed in ethyl 4-acetoxy-4-cyanobutyrate.[168] The geometry of glycosidic bonds is of great interest. Nunez and Barker observed $^3J(C_1–O–C_{n'})$ in β-hexanolamine glycoside (836)[125] and Gagnaire *et al.*[44] observed similar values in β-nigerose octaacetate, β-laminaribiose octaacetate, α-maltose octaacetate, and α-cellobiose octaacetate (192, 193).

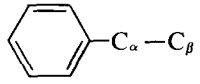
E. Summary

The finding that non-bonded interactions play a dominant role in three-bond couplings has curbed any optimism that $^3J(C–C)$ might become a major parameter in stereochemical studies. It will, of course, still be possible

to extract Karplus equations but only for limited groups of similar compounds.

V. COUPLINGS OVER FOUR OR MORE BONDS

Coupling constants over four or more bonds have mostly been reported in highly conjugated molecules. Four-bond couplings from α-carbonyl carbons to C_4 atoms in benzene rings are negative in sign,[48,74] as are couplings from sp and sp^2 hybridized α-carbons in diphenylacetylene (679) and *trans*-stilbene (564) [22]. The magnitudes of those couplings for which signs have been determined are very much like other four-bond couplings of the same category. In the case of an sp^3 hybridized α-carbon a negative sign has been obtained in 9,10-dihydroanthracene (895).[74] The magnitude of this coupling is, however, smaller than most others of this type.[118,136] Five-bond coupling constants, $^5J(C_\beta–C_4)$, are most likely to be positive in diphenylacetylene and *trans*-stilbene.[72] A negative $^4J(C_{2'}–C_4)$ interaction has been observed in 2,3,4,5-tetraphenylcyclopentadienone.[72]



[22]

In polycyclic aromatic hydrocarbons negative four-bond couplings are observed.[72,74] The signs of five-bond couplings have not been determined but they appear likely to be positive.[72]

The observation of long-range couplings in 1,4-dihydrobenzoic acid, 1,4-dihydro-1-naphthoic acid, and 9,10-dihydro-9-anthroic acid is explained by a π-contribution from overlap between the C–COOH and the aromatic p_π orbitals.[160] π-Orbitals are thus involved in all the reported coupling constants over four or more bonds. Substituent effects on four-bond couplings are variable.[50,72]

VI. GENERAL CONSIDERATIONS

A. Coupling mechanisms

The calculations by Barfield *et al.*[150,151] have revealed that interactions involving atoms not in the coupling path play a major role in determining the magnitude of three-bond coupling constants. Studies of the effects of substitution show that they are mediated through atoms on the substituents. In consequence of such findings two-bond couplings may depend upon substituents at the terminal carbons in a rather complicated manner. The

dependence of ^2J values upon the spatial orientation of substituents has been amply shown.[42,43,50,136] Three-bond couplings having a terminal carbonyl carbon are also shown to be abnormally small.[50,136] More direct evidence that non-bonded interactions, through H–H and H–F coupling paths, contribute to C–H and C–F coupling constants is found in some 9-methylphenanthrenes.[169]

B. Additivity

The addition of couplings along several coupling paths has been studied by Marshall *et al.*[118] They have compared coupling constants in cyclic systems with multiple coupling paths with those across one coupling path, as observed in some model compounds; reasonable agreement is obtained. In the light of the many possible factors influencing coupling constants, only a gross relation may be expected in most cases.

Berger[114] has assumed additivity in a study of the dihedral dependence of three-bond couplings.

C. Comparison with other coupling constants

Marshall *et al.* have correlated J (C–C) in methyl tetrolate with J (C–H) in propyne and found a ^nJ(C–C)/^nJ(C–H) ratio of 0·4–0·7 Hz. The signs of two-bond carbon–carbon, carbon–hydrogen, and hydrogen–hydrogen couplings, including sp^3, sp^2, and sp hybridized carbons have been compared.[27] Runge and Firl[170] have compared the ^1K (C–C) with ^1K (N–C) interactions of structurally related compounds and arrived at the following equation.

$$^1K(^{15}N\text{–}^{13}C) = 0{\cdot}51\ ^1K(^{13}C\text{–}^{13}C) - 3{\cdot}19\ (10^{20}\ cm^{-3}) \qquad (2)$$

The relationship is taken as evidence that the two coupling mechanisms are similar. The importance of nonbonded contributions to $^3J(CO\text{–}C)_{cis}$ is likely to prevent a comparison with other types of couplings.[136]

VII. MISCELLANEOUS

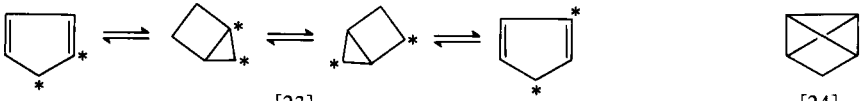
A. Direct dipolar couplings

Direct dipolar carbon–carbon coupling constants have been obtained from benzene oriented in a liquid crystal (ZLI 1167). It appears that the indirect contributions are significant and prevent the use of D (C–C) coupling values in the structural determination of molecules.[69]

B. Rearrangements

Labelled illudin M was isomerized to isoilludin M by Bradshaw *et al.*[171] Two pathways are possible: a double pinacolic shift or a simple 1,2-methyl group migration. The one-bond coupling constant points clearly towards the

latter possibility. Photochemical rearrangement of [1, 2-$^{13}C_2$]cyclopentadiene has been monitored by observation of carbon–carbon couplings.[108] The skeletal rearrangement [23] is observed and a new product, tricyclo[2.1.0.0^{2,5}]pentane [24], has been isolated. Coupling constants have



[23] [24]

been employed in a study of the acid-catalysed rearrangement of 2,2,4-trimethylpentan-3-one.[172] Rearrangements during biosynthetic studies have been monitored by means of carbon–carbon couplings in many instances. An elegant example of this is to be found in the observation of a three-bond coupling in vulgamycin.[173]

Recent publications include discussions of the following topics: a new universal technique combining double quantum coherence[174] with 2-dimensional spectroscopy for obtaining J(C–C) from non-enriched compounds;[175] use of ^3J in conformational studies of diamantane;[176] stereochemical dependence revealed by ^2J of azlactones and the acids and esters obtained by hydrolysis;[177] MO theory applied to azulene couplings;[178] relation of ^1J to empirical structure-determined factors[179] and to stretching force constants;[180] study of the Kiliani reaction using [^{13}C]cyanide;[181] use of ^{13}C NMR in the study of biosynthesis,[182] and biosynthetic formation of vindoline,[183] nicotine,[184] virginiamycin M,[185] dihydrofusarubin,[186] uroporphyrinogens,[187] kaurene,[188] and 2-(buta-1,3-dienyl)-3-hydro-4-(penta-1,3-dienyl)tetrahydrofuran;[189] coupling constants of diacetylenes[190,191] and of lithium compounds.[192]

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Carbon–Carbon Coupling Constants: Data

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I. INTRODUCTION

The tables of data presented here are intended as an update of previous work[1] and are arranged accordingly. Data have been compiled from the authors' own records and from the literature found by *CA Selects: Nuclear Magnetic Resonance (Chemical Aspects)*, to June 1979, and *Carbon and Heteroatom NMR*, from July 1979. Most of the available data from the end

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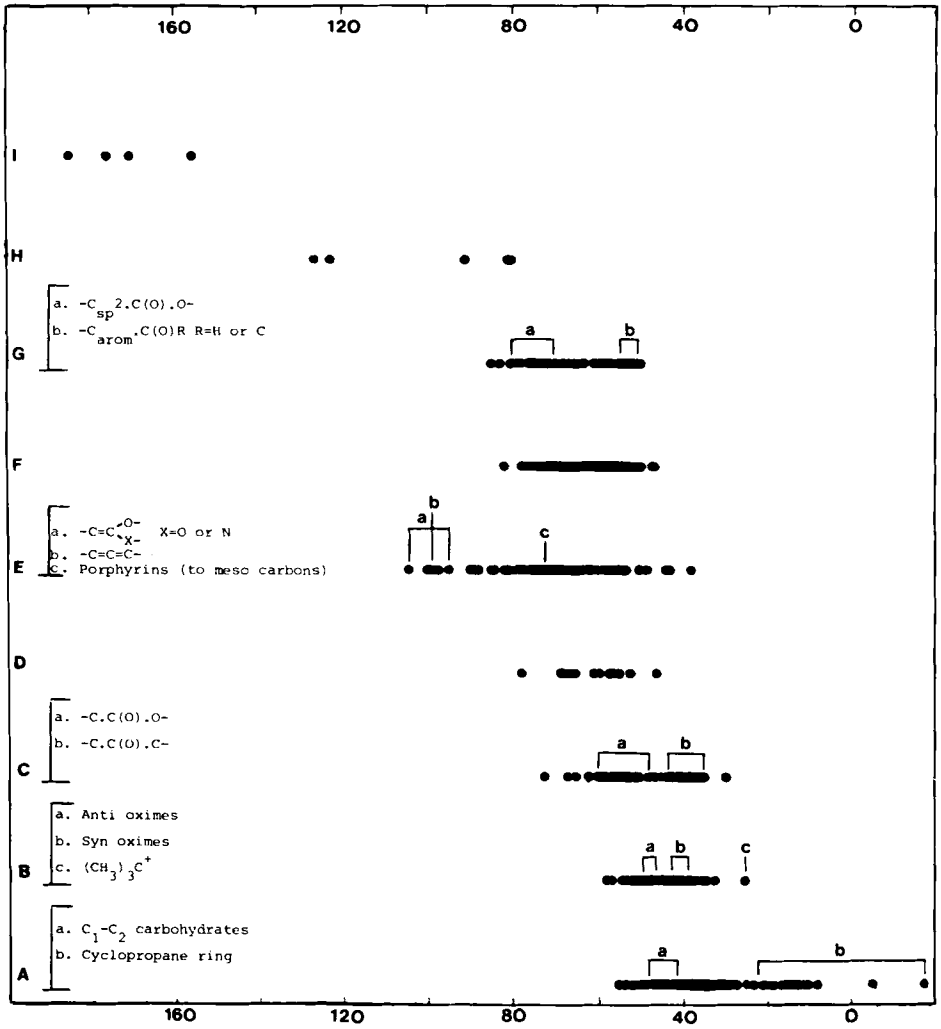

FIG. 1. One-bond coupling constants (~1800) plotted according to the categories A–I. Typical ranges are given and are commented upon elsewhere.[107]

of 1978 to April 1980, together with those overlooked in the original compilation, are tabulated.

The tables have been divided according to the formal hybridization of the coupled carbons. Each table is arranged according to an index of molecular formulae; the conventional priority of elements has been adopted. Each table consists of (1) the entry serial number, with cross reference serial numbers in parentheses, (2) a literature reference number, (3) the molecular formula of the compound, (4) the structure of the compound, and (5) a series of columns corresponding to the coupling constants classified as to ^{1}J (directly bonded), ^{2}J (geminal), and ^{n}J (vicinal and longer range). If geminal, vicinal, or longer range coupling occur through a heteroatom the nature of the heteroatom is indicated in parentheses after the coupling. The serial numbers begin where the previous compilation terminated.

No attempt has been made to indicate the experimental error in the couplings although this may be taken as better than ±0·2 Hz for investigations specifically concerned with J(C–C) and ±1 Hz for most biosynthetic studies.

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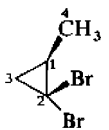
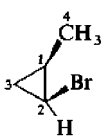
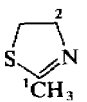
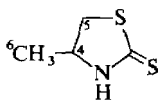
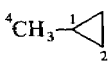
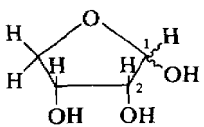
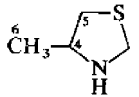
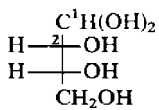
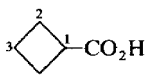
II. TABLES OF CARBON–CARBON COUPLING CONSTANTS

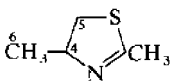
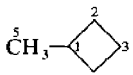
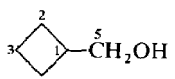
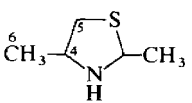
A. Both coupled carbons formally with sp^3 hybridization

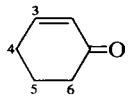
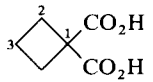
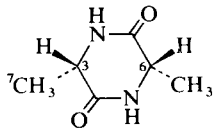
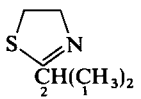
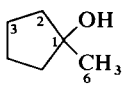
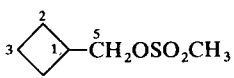
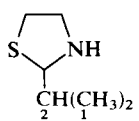
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
683	2	$C_2H_3Br_2Li$	$CH_3{\cdot}CBr_2Li$	8		
684	2	$C_2H_3Br_3$	$CH_3{\cdot}CBr_3$	38		
685	2	$C_2H_4Br_2$	$CH_3{\cdot}CHBr_2$	37		
686	97	$C_2H_5B_3$	1,5-$C_2B_3H_5$		15·0 (B)	
687	3	C_2H_5Br	$CH_3{\cdot}CH_2Br$	36·0		
688	3	C_2H_5Cl	$CH_3{\cdot}CH_2Cl$	36·1		
689	4	C_2H_5FO	$CH_2F{\cdot}CH_2OH$	40·8		
690	3	C_2H_5I	$CH_3{\cdot}CH_2I$	35·8		
691	3	$C_2H_5NO_2$	$CH_3{\cdot}CH_2NO_2$	35·7		
692	3	C_2H_6	$CH_3{\cdot}CH_3$	34·6		
693	3	C_2H_6O	$CH_3{\cdot}CH_2OH$	37·7		
694	4	$C_2H_6O_3S$	$CH_3{\cdot}CH_2{\cdot}SO_3H$	33·4		
695	3	C_2H_7N	$CH_3{\cdot}CH_2NH_2$	35·8		
696	3	C_3H_5N	$CH_3{\cdot}CH_2CN$	33·0		
697 (916)	4	C_3H_6O	$CH_3{\cdot}CH_2CHO$	35·4		
698	3	$C_3H_6O_2$	$CH_3{\cdot}CH_2{\cdot}CO_2H$	34·8		
699	3	C_3H_7Br	$(CH_3)_2CHBr$	37·1		
700	4	C_3H_7Br	$(CH_3)_2CHBr$	36·8		
701	3	C_3H_7Cl	$(CH_3)_2CHCl$	37·3		
702	4	C_3H_7Cl	$(CH_3)_2CHCl$	36·9		
703	5	C_3H_7ClS	$CH_2Cl{\cdot}CH_2{\cdot}SCH_3$	37		

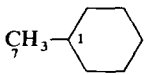
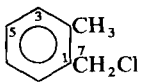
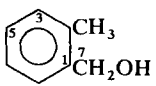
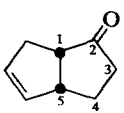
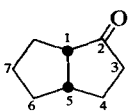
704	6	C_3H_7F	$(CH_3)_2CHF$	39·1
705	3	C_3H_7I	$(CH_3)_2CHI$	36·7
706	4	C_3H_7I	$(CH_3)_2CHI$	36·3
707	6	C_3H_7Li	$(CH_3)_2CHLi$	22·9
708	3	$C_3H_7NO_2$	$(CH_3)_2CHNO_2$	36·3
709 (920)	7	$C_3H_7NO_3$	(L)-$CH_2OH{\cdot}CH(NH_2)CO_2H$	37·3
710	3	C_3H_8	$CH_3{\cdot}CH_2{\cdot}CH_3$	33
711	3	C_3H_8O	$CH_3{\cdot}CH_2{\cdot}OCH_3$	39·0
712	3	C_3H_8O	$(CH_3)_2CHOH$	38·6
713	6	C_3H_8O	$(CH_3)_2CHOH$	38·1
714	3	C_3H_9N	$(CH_3)_2CHNH_2$	37·3
715	6	C_3H_9N	$(CH_3)_2CHNH_2$	37·1
716	8	$C_4H_6Br_2$		(1-2) 14·4 (1-3) 12·3 (1-4) 43·2
717	9	$C_4H_6O_4$	$HO_2CCH_2{\cdot}CH_2{\cdot}CO_2H$	54·9
718	8	C_4H_7Br		(1-2) 13·4 (1-3) 12·8 (1-4) 45·1
719	8	C_4H_7Br	As above with Br and H interchanged	(1-2) 14·7 (1-3) 12·2 (1-4) 45·2

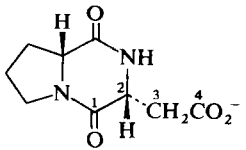
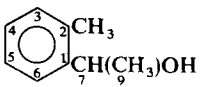
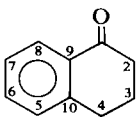
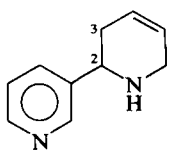
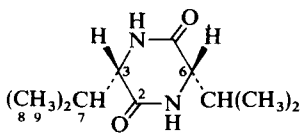
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
720 (924) (1101)	7	$C_4H_7NO_4$	(L)-$HO_2CCH_2{\cdot}CH(NH_2)CO_2H$	36·7		
721 (852)	10	C_4H_7NS				(1-2) 7 (N)
722	11	$C_4H_7NS_2$		(4-5) 33·5 (4-6) 37·1		
723	8	C_4H_8		(1-2) 13·3 (1-4) 43·4		
724	3	C_4H_8O	$CH_3{\cdot}CH_2{\cdot}CO{\cdot}CH_3$	35·5		
725	3	$C_4H_8O_2$	$(CH_3)_2CH{\cdot}CO_2H$	34·4		
726	12	$C_4H_8O_4$		α (1-2) 43·3 β (1-2) 46·9		
727	12	$C_4H_8O_4$	As above with C(2) substituents interchanged	α (1-2) 45·9 β (1-2) 42·3		
728	3	C_4H_9Br	$(CH_3)_3CBr$	37·8		
729	3	C_4H_9Cl	$(CH_3)_3CCl$	38·0		
730	6	C_4H_9F	$(CH_3)_3CF$	40·3		
731	3	C_4H_9I	$(CH_3)_3CI$	36·6		
732	6	C_4H_9Li	$(CH_3)_3CLi$	28·4		

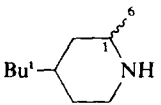
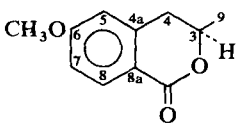
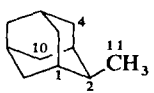
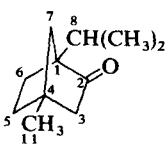
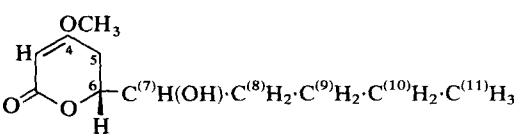
733	3	$C_4H_9NO_2$	$(CH_3)_3CNO_2$	37·6	
734	4	$C_4H_9NO_2$	$(CH_3)_3CNO_2$	37·8	
735 (928)	7	$C_4H_9NO_3$	(L)-$C^{(1)}H_3{\cdot}C^{(2)}HOH{\cdot}C^{(3)}H(NH_2)CO_2H$	(1-2) 38·4 (2-3) 37·2	(1-3) +0·3
736	11	C_4H_9NS		(4-5) 30·0 (4-6) 37·6	
737	6	C_4H_{10}	$(CH_3)_3CH$	35·0	
738	3	$C_4H_{10}O$	$(CH_3)_2CH{\cdot}OCH_3$	39·9	
739	3	$C_4H_{10}O$	$(CH_3)_3COH$	39·6	
740	6	$C_4H_{10}O$	$(CH_3)_3COH$	39·0	
741	12	$C_4H_{10}O_5$		(1-2) 48·4	
742	12	$C_4H_{10}O_5$	As above with C(2) substituents interchanged	(1-2) 49·0	
743	3	$C_4H_{11}N$	$(CH_3)_3CNH_2$	37·6	
744	6	$C_4H_{11}N$	$(CH_3)_3CNH_2$	37·3	
745	13	C_5H_6		(1-2) 19·0 (2-3) 34·0	(1-3) 3·5
746 (931)	14	$C_5H_8O_2$		(1-2) 27·5	(1-3) 8·3
747	3	C_5H_9N	$(CH_3)_3CCN$	33·6	
748 (933) (1105)	7	$C_5H_9NO_2$	(L)-$HO_2C{\cdot}C^{(3)}H_2{\cdot}C^{(2)}H_2{\cdot}C^{(1)}H(NH_2)CO_2H$	(1-2) 34·7 (2-3) 35·2	(1-3) −0·4

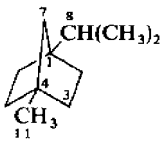
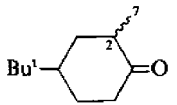
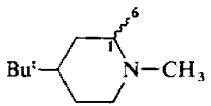
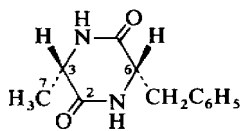
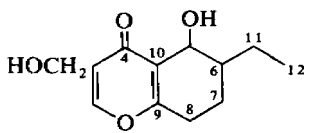
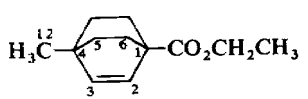
No.	Ref.	Mol. formula	Structure		^1J	^2J	^nJ
749	10	$C_5H_9NO_2$	$(C^{(1)}H_3)_2C^{(2)}H{\cdot}CHOH{\cdot}CN$		(1-2) 35		
750	4	$C_5H_9NO_2$	$(CH_3)_3C{\cdot}NCO$		38·5		
751	11	C_5H_9NS			(4-5) 29·9 (4-6) 38·6		
752	11	$C_5H_9NS_2$	As above with CH_3 replaced by SCH_3		(4-5) 30·2 (4-6) 38·4		
753	14	C_5H_{10}			(1-2) 29·1 (1-5) 36·1 (2-3) 28·4	(1-3) 8·1	
754	3	$C_5H_{10}O$	$(CH_3)_2CH{\cdot}CO{\cdot}CH_3$		34·7		
755	14	$C_5H_{10}O$			(1-2) 28·8 (1-5) 39·3	(1-3) 8·1	
756	3	$C_5H_{10}O_2$	$(CH_3)_3CCO_2H$		35·3		
757	11	$C_5H_{11}NS$		*trans* *cis*	(4-5) 29·5 (4-6) 37·9 (4-5) 39·5 (4-6) 40·1		
758	3	C_5H_{12}	$(CH_3)_3C{\cdot}CH_3$		33·7		
759	10	$C_5H_{12}ClNO_2$	$(C^{(1)}H_3)_2C^{(2)}HCH(NH_3Cl)CO_2H$		(1-2) 34		
760	3	$C_5H_{12}O$	$(CH_3)_3C{\cdot}OCH_3$		40·3		
761	15	$C_5H_{12}Si$	$(CH_3)_2SiCH_2C^{(1)}H_2C^{(2)}H_2$		(1-2) 24·6		

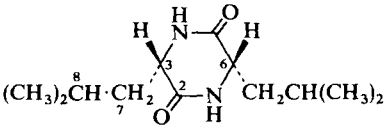
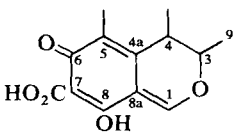
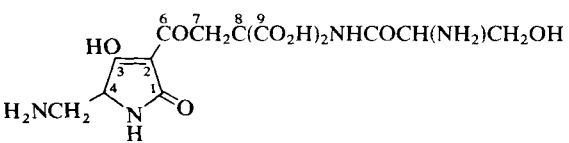
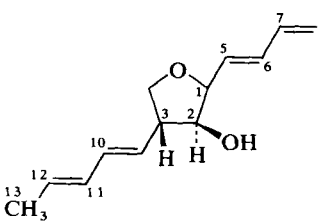
762 (854) (935) (986) (1108)	4	C_6H_8O		(4-5) 33·6 (5-6) 31·5	
763 (936)	14	$C_6H_8O_4$		(1-2) 27·3	(1-3) 7·3
764 (937)	16	$C_6H_{10}N_2O_2$		(3-7) 37·4	
765 (938)	16	$C_6H_{10}N_2O_2$	As above with C(6) substituents interchanged	(3-7) 36·4	
766	10	$C_6H_{11}NS$		(1-2) 36	
767	3	$C_6H_{12}O$	$(CH_3)_3CCO{\cdot}CH_3$	34·9	
768	17	$C_6H_{12}O$		(1-2) 37·1 (1-6) 39·8	(1-3) 1·22
769	4	$C_6H_{12}O_2$	$(CH_3)_3C{\cdot}CO_2CH_3$	35·1	
770	14	$C_6H_{12}O_3S$		(1-2) 28·4 (1-5) 39·6	(1-3) 8·3
771	10	$C_6H_{13}NS$		(1-2) 35	

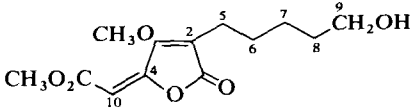
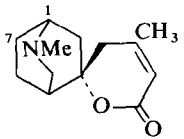
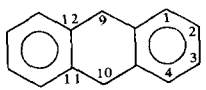
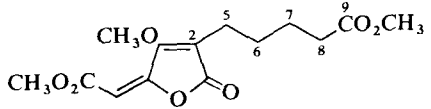
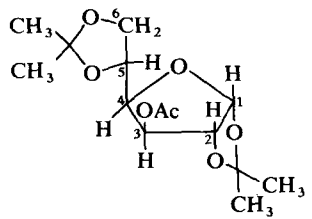
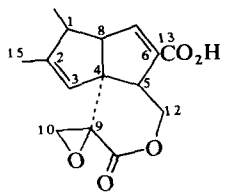
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
772	4	$C_6H_{15}N$	$(CH_3{\cdot}CH_2)_3N$	38·1		
773	4	$C_6H_{16}Si$	$(CH_3{\cdot}CH_2)_3SiH$	31·7		
774	18	C_7H_{14}		(1-7) 35·6		
775 (858)	19	C_8H_9Cl				3·05
776	3	C_8H_{10}	$CH_3{\cdot}CH_2{\cdot}C_6H_5$	34·2		
777 (860)	19	$C_8H_{10}O$				3·15
778 (943)	20	$C_8H_{10}O$		(3-4) 35·2	(1-3) 15·5 (3-5) 0·8	
779 (944)	20	$C_8H_{10}O$	As above with C=O at C(3)	(1-2) 34·7	(2-4) 17·0	
780 (945)	20, 21	$C_8H_{12}O$		(3-4) 34·7	(1-3) 15·9 (3-5) 0·6	(3-6) 0·8
781 (946)	20	$C_8H_{12}O$	As above with C=O at C(3)	(1-2) 36·0		(2-7) 1·8
782	99	$C_9H_{11}Cl_3Se$	$p CH_3{\cdot}C_6H_4{\cdot}SeCl_2{\cdot}CH_2{\cdot}CH_2Cl$	37·25		

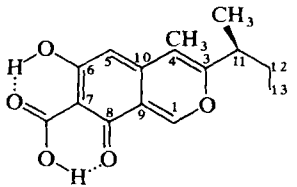
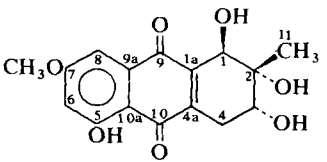
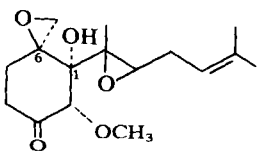
783 (950) (1120)	22	$C_9H_{11}N_2O_4^-$		(2-3) 40·0
784	3	C_9H_{12}	$(CH_3)_2CH{\cdot}C_6H_5$	34·8
785 (951) (1121)	22	$C_9H_{12}N_2O_4$	As No. 783 with CO_2^- replaced by CO_2H	(2-3) 40·3
786 (864)	19	$C_9H_{12}O$		(7-9) 38·15
787 (867) (953) (1049) (1122)	4	$C_{10}H_{10}O$		(2-3) 31·5
788	23	$C_{10}H_{12}N_2$		(2-3) 37
789	3	$C_{10}H_{14}$	$(CH_3)_3C{\cdot}C_6H_5$	35·7
790 (957)	16	$C_{10}H_{18}N_2O_2$		(3-7) 35·5 (7-8) 34·9

No.	Ref.	Mol. formula	Structure		^1J	^2J	^nJ
791 (958)	16	$C_{10}H_{18}N_2O_2$	As above with C(6) substituents interchanged		(3-7) 34·9 (7-8) 35·1		
792	18	$C_{10}H_{21}N$		*cis* *trans*	(1-6) 38·4 (1-6) 36·5		
793	18	$C_{10}H_{22}N^+$	As above with $\succ NH$ replaced by $\succ NH_2^+$	*cis* *trans*	(1-6) 36·4 (1-6) 36·6		
794 (879) (1062) (1132)	25	$C_{11}H_{12}O_4$			(3-4) 36 (3-9) 41		
795	24, 26	$C_{11}H_{18}$			(2-11) 35·7	(1-11) 1·1	(4-11) 0·4 (10-11) 3·44
796	24	$C_{11}H_{18}O$	As above with CH_3 replaced by CH_2OH		(2-11) 38·2	(1-11) 0·5	(10-11) 3·54
797 (959)	20	$C_{11}H_{18}O$			(3-4) 33·0 (4-5) 31·9 (4-7) 30·8 (4-11) 39·7	(4-1) 2·4	(4-8) 3·4
798 (880) (1133)	27	$C_{11}H_{18}O_4$			(6-7) 42 (8-9) 35 (10-11) 35		

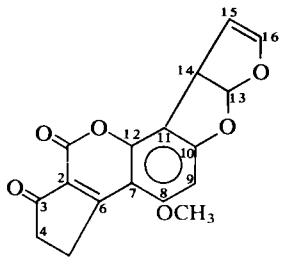
799	20	$C_{11}H_{20}$			(4-3) 32·4 (4-7) 32·3 (4-11) 39·2	(4-1) 6·6	(4-8) 3·9
800	18	$C_{11}H_{20}O$		*cis* *trans*	(2-7) 37·6 (2-7) 33·8		
801	18	$C_{11}H_{23}N$		*cis* *trans*	(1-6) 39·3 (1-6) 35·3		
802	18	$C_{11}H_{24}N^+$	As above with $\geq NCH_3$ replaced by $\geq NHCH_3^+$	*cis* *trans*	(1-6) 36·8 (1-6) 37·3		
803 (962)	16	$C_{12}H_{14}N_2O_2$			(3-7) 36·3		
804 (963)	16	$C_{12}H_{14}N_2O_2$	As above with C(6) substituents interchanged		(3-7) 37·5		
805 (884) (1136)	28	$C_{12}H_{16}O_4$			(6-7) 33·1 (11-12) 35·0		
806 (885)	20	$C_{12}H_{18}O_2$			(4-5) 31·8 (4-12) 39·6	(4-6) 2·2	(1-4) 12·7

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
807	20	$C_{12}H_{20}O_2$	As above with reduced double bond	(4-3) 33·4	(4-2) 1·7	(1-4) 12·2
808 (964)	16	$C_{12}H_{22}N_2O_2$		(3-7) 35·2		
809 (965)	16	$C_{12}H_{22}N_2O_2$	As above with C(6) substituents interchanged	(3-7) 35·5		
810 (888) (998) (1139)	29	$C_{13}H_{14}O_5$		(3-9) 37·8		
811 (890) (967) (1000) (1140)	30	$C_{13}H_{18}N_4O_9$		(7-8) 42·5		
812 (891) (1001)	31	$C_{13}H_{18}O_2$		(2-3) 36		

813 (892) (1002)	32	$C_{13}H_{18}O_6$		(6-7) 35 (8-9) 35		
814	34, 35	$C_{13}H_{19}NO_2$		(1-7) 33		
815 (895)	36	$C_{14}H_{12}$				(9-10) 3·4
816 (896) (968)	32	$C_{14}H_{18}O_7$		(6-7) 35		
817	37	$C_{14}H_{24}O_7$		(1-2) 33·6 (2-3) 44·1 (3-4) 39·1 (4-5) 48·0 (5-6) 34·2	(2-4) 1·6	(1-5) 4·3 (O) (2-5) 1·5 (3-6) 1·5
818 (897) (1141)	41	$C_{15}H_{15}O_5$		(1-8) 36 (9-10) 33·5 (5-12) 35·5		

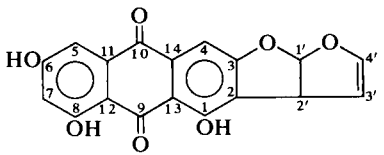
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
819 (898) (1003) (1142)	38	$C_{15}H_{16}O_5$		(12-13) 35·0		
820 (969)	39	$C_{15}H_{21}O_3$	$(-CO\cdot\overline{C^{(1)}H\cdot C^{(2)}H_2\cdot C^{(3)}H_2\cdot C^{(4)}H_2\cdot N}-)_3$	(1-2) 35·6 (2-3) 34·0	(1-4) 5·3 (N)	
821 (899) (1070) (1143)	40	$C_{16}H_{16}O_7$		(2-11) 41		
822 (900) (1071) (1144)	40	$C_{16}H_{16}O_8$	As above with OH at C(4) *trans* to OH at C(3)	(2-11) 40		
823 (1005)	42	$C_{16}H_{23}O_5$		(1-6) 53		

824 (970) (1006) (1072) 43 $C_{17}H_{12}O_6$



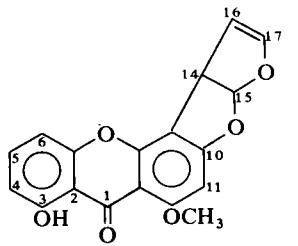
(13-14) 33

825 (1007) (1076) (1145) 44 $C_{18}H_{10}O_7$



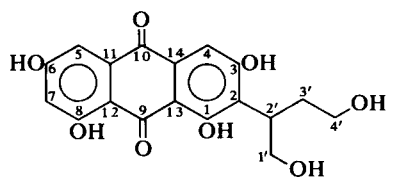
(1′-2′) 33·1

826 (1008) (1077) (1146) 43, 45 $C_{18}H_{12}O_6$

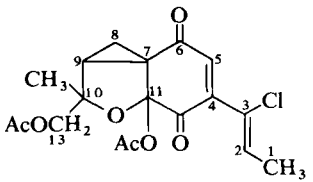
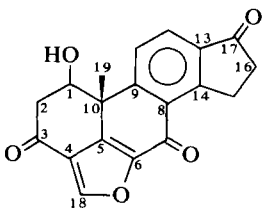
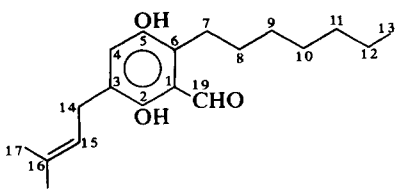
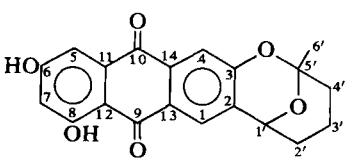


(14-15) 34

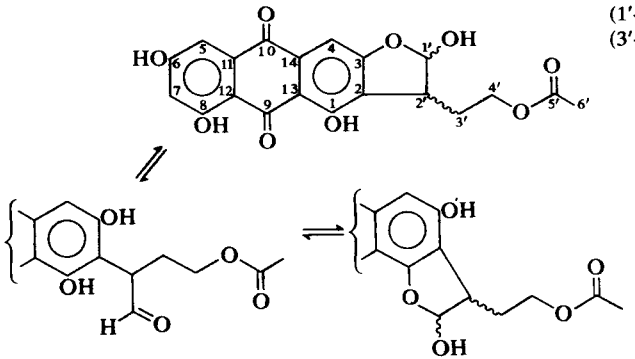
827 (1079) (1147) 46 $C_{18}H_{16}O_8$



(1′-2′) 37·8
(3′-4′) 37·7

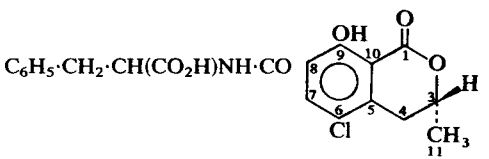
No.	Ref.	Mol. formula	Structure		^1J	^2J	^nJ
828 (902) (1009) (1148)	47	$C_{18}H_{19}ClO_7$		Ac = CH_3CO	(7-11) 47 (8-9) 16 (10-13) 44		
829 (971) (1010) (1082)	48	$C_{19}H_{14}O_5$			(10-19) 34		
830 (904) (1083) (1149)	49	$C_{19}H_{28}O_3$			(8-9) 29 (10-11) 35 (12-13) 35		
831 (1087) (1152)	50	$C_{20}H_{16}O_7$			(1'-2') 35 (3'-4') 32 (5'-6') 49		

832 (972) (1088) (1153) 46 $C_{20}H_{16}O_9$



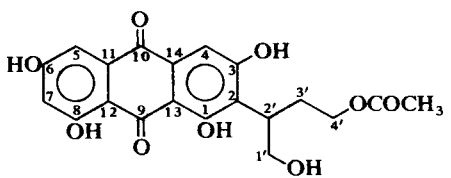
(1′-2′) ~41
(3′-4′) 38·7

833 (907) (1089) (1154) 51 $C_{20}H_{18}ClNO_6$



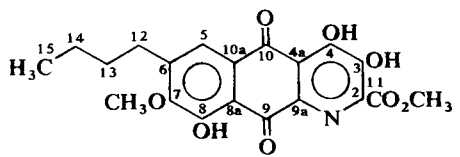
(3-11) 39·9

834 (1090) (1155) 46 $C_{20}H_{18}O_9$

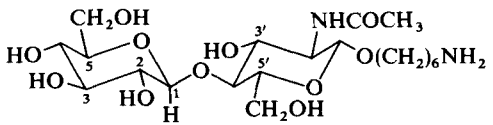
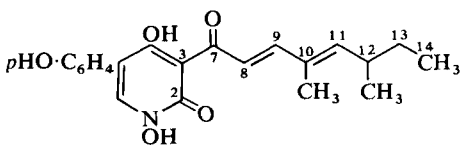
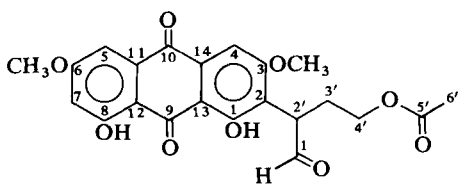
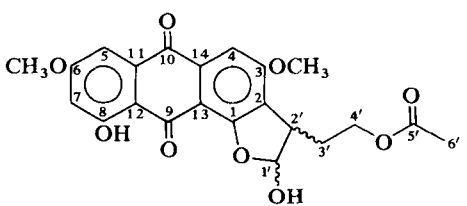


(1′-2′) 37·7
(3′-4′) 38·3

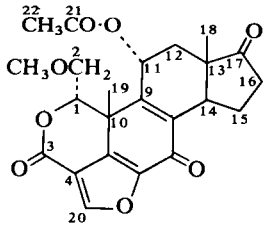
835 (1091) (1156) 52 $C_{20}H_{19}NO_8$



(12-13) 31
(14-15) 36·5

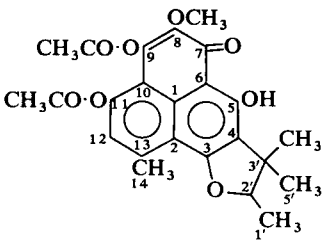
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
836	53	$C_{20}H_{38}N_2O_{11}$		(1-2) 46	(1-3) 3·7 (1-5) 0 (O)	(1-3′) ~0 (O) (1-5′) ≤1·5 (O)
837 (909) (1011) (1158)	54, 55	$C_{21}H_{23}NO_5$		(13-14) 34·4		
838 (973) (1093) (1159)	46	$C_{22}H_{20}O_9$		(3′-4′) 39·0		
839 (974) (1094) (1160)	46	$C_{22}H_{20}O_9$		(1′-2′) 41 (3′-4′) 38·5		

840 (910) (975) (1012) (1161) 56 $C_{23}H_{24}O_8$



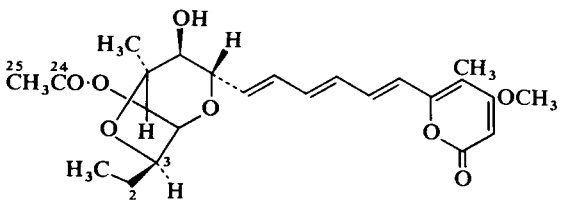
(1-2) 42
(1-10) 35
(11-12) 35
(12-13) 35
(13-18) 30
(14-15) 30
(10-19) 33

841 (911) (1013) (1095) (1162) 57 $C_{24}H_{24}O_8$



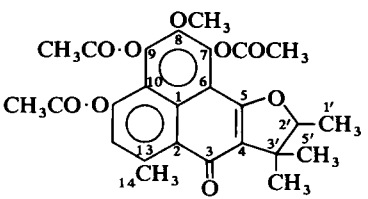
(1′-2′) 40
(3′-5′) 37

842 (976) 58 $C_{25}H_{32}O_8$

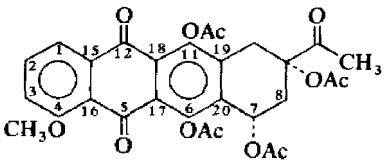
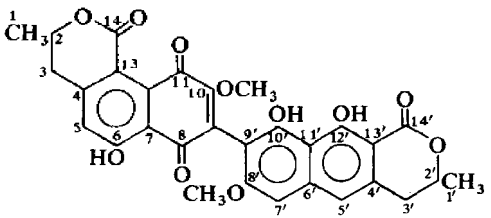
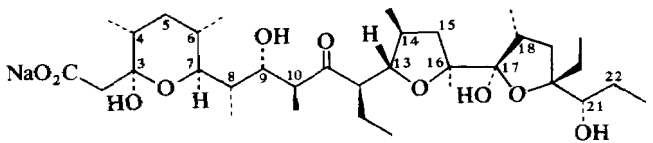


(2-3) 40·1

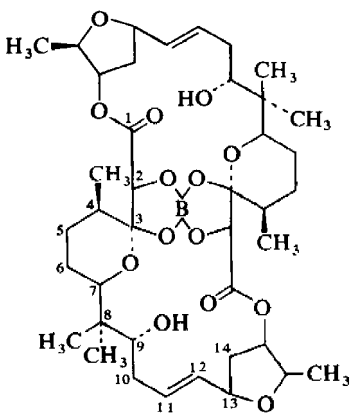
843 (912) (1014) (1096) (1163) 57 $C_{26}H_{26}O_9$



(1′-2′) 40
(3′-5′) 37

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
844	5	$C_{28}H_{34}N_6S_3$	$C_6H_5{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}N(CS{\cdot}NHC_6H_5)$ $C_6H_5{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}CH_2{\cdot}C^{(3)}H_2{\cdot}C^{(4)}H_2$	(3-4) 35		
845 (1097) (1165)	59	$C_{29}H_{26}O_{12}$	 Ac = CH_3CO	(7-8) 37·5		
846 (913) (1098) (1166)	60	$C_{30}H_{24}O_{11}$		(1-2) 40 (1'-2') 40		
847	61	$C_{34}H_{59}NaO_{10}$		(3-4) 45·7 (5-6) 33·3 (7-8) 40·0 (9-10) 41·5 (13-14) 34·9 (15-16) 36·9 (17-18) 39·3 (21-22) 38·4		

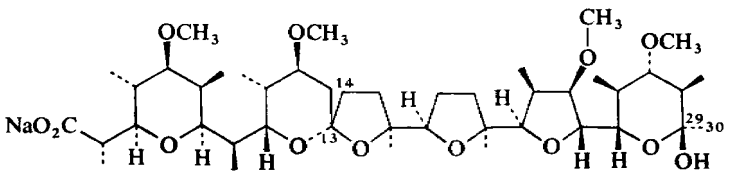
848 62 $C_{40}H_{60}BNaO_{14}$



(977)
(1016)

(3-4) 47·6
(5-6) 31·7
(7-8) 39·1
(9-10) 39·1
(13-14) 34·7

849 63 $C_{44}H_{75}NaO_{14}$



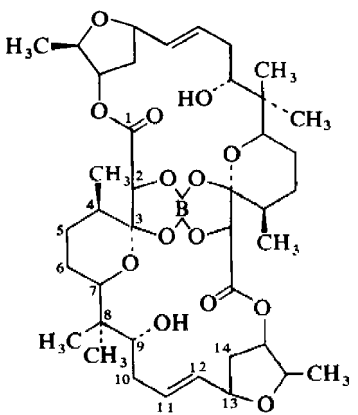
(13-14) 42
(29-30) 47

B. One coupled carbon formally with sp^3 hybridization and the other with sp^2 hybridization

850 64 C_4H_6

(1-4) 32·13 (1-3) 8·93

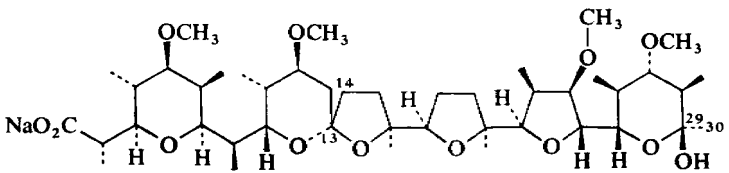
848 62 $C_{40}H_{60}BNaO_{14}$



(977)
(1016)

(3-4) 47·6
(5-6) 31·7
(7-8) 39·1
(9-10) 39·1
(13-14) 34·7

849 63 $C_{44}H_{75}NaO_{14}$

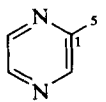
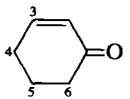
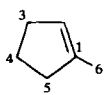
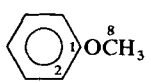
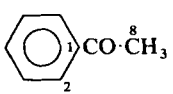


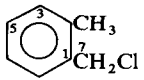
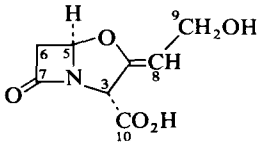
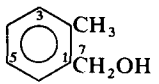
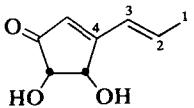
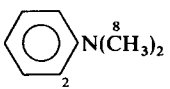
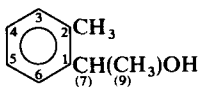
(13-14) 42
(29-30) 47

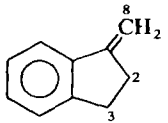
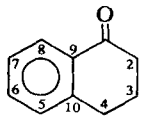
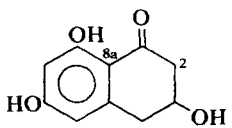
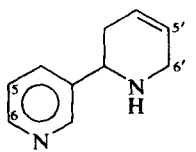
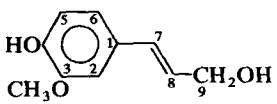
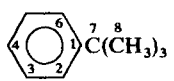
B. One coupled carbon formally with sp^3 hybridization and the other with sp^2 hybridization

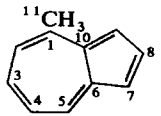
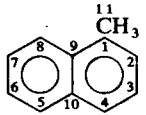
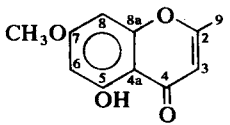
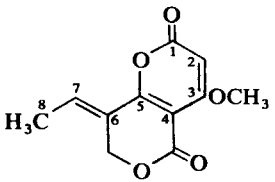
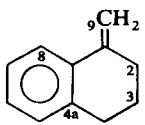
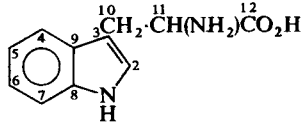
850 64 C_4H_6

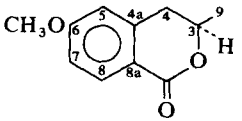
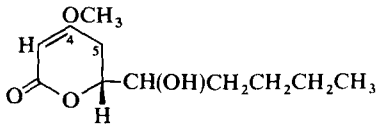
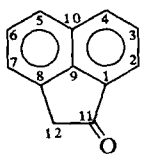
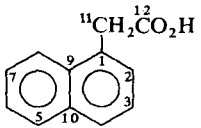
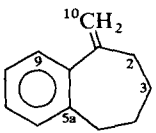
(1-4) 32·13 (1-3) 8·93

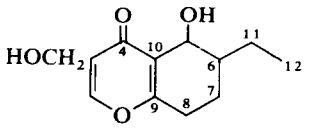
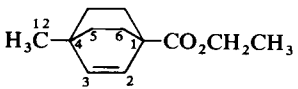
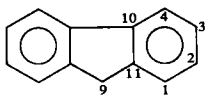
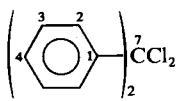
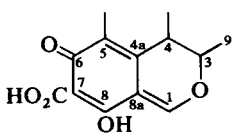
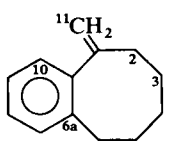
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
851 (921) (984) (1099)	4	C_4H_6O	$C^{(1)}H_2{=}C^{(2)}H{\cdot}C^{(3)}O{\cdot}C^{(4)}H_3$		(2-4) 15·3	
852 (721)	10	C_4H_7NS		53		
853	4	$C_5H_6N_2$		(1-5) 51·2		
854 (762) (935) (986) (1108)	4	C_6H_8O		(3-4) 38·2		
855 (987)	17	C_6H_{10}		(1-5) 38·1 (1-6) 44·7	(1-3) 4·88 (1-4) 1·71	
856 (533) (1033)	69	C_7H_8O			(1-8) 2.33 (O)	(2-8) 4.08 (O)
857 (940)	17	C_8H_8O			(1-8) 13·7	(2-8) 8·4

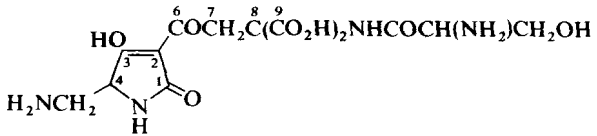
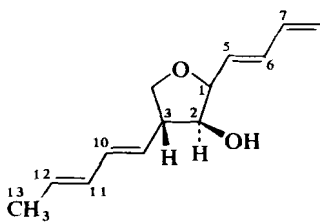
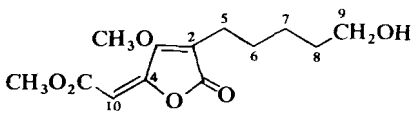
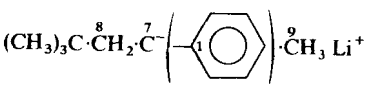
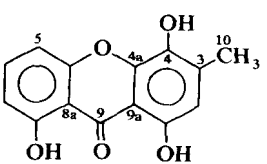
858 (775)	19	C_8H_9Cl				(7-2) 2·81 (7-6) 2·15	(7-3) 4·39 (7-5) 4·39 (7-4) 0·61
859 (942)	65	$C_8H_9NO_5$			(8-9) 47·3		
860 (777)	19	$C_8H_{10}O$			(1-7) 47·50	(7-2) 3·22 (7-6) 2·54	(7-3) 3·08 (7-5) 3·66 (7-4) 0·73
861 (989) (1116)	66	$C_8H_{10}O_3$			(1-2) 42		
862 (541) (1037)	69	$C_8H_{11}N$					(2-8) 2·89 (N)
863	33	C_9H_{10}	$CH_2{=}C^{(7)}(C_6H_5)C^{(9)}H_3$		(7-9) 41·8		
864 (786)	19	$C_9H_{12}O$			(7-1) 47·31	(7-2) 3·66 (7-6) 1·34	(7-3) 2·92 (7-5) 3·05 (7-4) <0·5
865	33	$C_9H_{17}Li$	$(CH_3)_3C{\cdot}CH_2{\cdot}CH{=}C^{(2)}(CH_3)C^{(1)}H_2Li$	*cis* (C_6D_6, r.t.) (THF, −20 °C) *trans* (C_6D_6, r.t.) (THF, −80 °C)	 (1-2) 36·3 46·5 (1-2) 35·1 46·3		

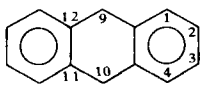
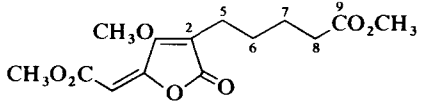
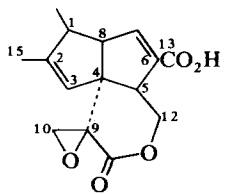
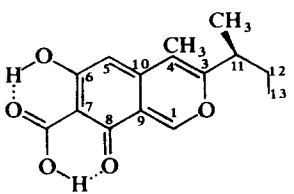
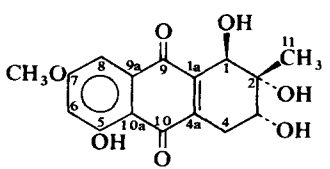
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
866 (991) (1048)	67	$C_{10}H_{10}$		(2-8) <0·6		(3-8) 2·1
867 (787) (953) (1049) (1122)	4	$C_{10}H_{10}O$		(4-10) 41·0		
868	108	$C_{10}H_{10}O_4$			(2-8a) 9	
869 (1050)	82	$C_{10}H_{12}N_2$		(5′-6′) 42·0		
870 (992) (1051)	68	$C_{10}H_{12}O_3$		(8-9) 47·2		
871 (251) (554) (1053)	69	$C_{10}H_{14}$		(1-7) 43·21	(2-7) 1·95	(3-7) 3·18 (2-8) 2·23

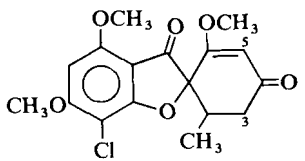
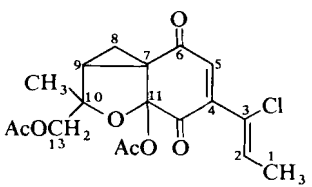
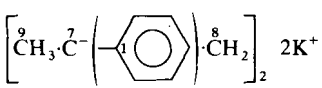
872 (1056)	70	$C_{11}H_{10}$			(1-11) 41·4		
873 (1057)	98	$C_{11}H_{10}$			44·6		
874	17	$C_{11}H_{10}O$	As above with CH_3 replaced by CH_2OH		(11-1) 47·36	(11-2) 2·93 (11-9) 2·69	(11-3) 3·42 (11-8) 1·34 (11-10) 2·69
875 (993) (1059) (1129)	25	$C_{11}H_{10}O_4$			(2-9) 51		
876 (994) (1130)	71	$C_{11}H_{10}O_5$			(7-8) 42		
877 (995) (1060)	67	$C_{11}H_{12}$				(9-2) 1·5	(9-3) 2·8
878 (996) (1061) (1131)	72	$C_{11}H_{12}N_2O_2$		pH 1·1 6·7 11·8	(3-10) 49·9 48·9 49·2	(3-11) 2·1 2·4 1·9	

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
879 (794) (1062) (1132)	25	$C_{11}H_{12}O_4$		(4-4a) 42		
880 (798) (1133)	27	$C_{11}H_{18}O_4$		(4-5) 48		
881 (960) (1134)	17	$C_{12}H_8O$		(8-12) 45·12	(1-12) 17·58 (9-12) 1·56	(6-12) 3·52 (10-12) 1·17
882 (961) (1135)	17	$C_{12}H_{10}O_2$		(1-11) 45·1	(2-11) 2·83 (9-11) 2·15	(3-11) 4·30 (8-11) 2·93 (10-11) 2·54
883 (997) (1065)	67	$C_{12}H_{14}$			(2-10) 2·0	(3-10) 1·9

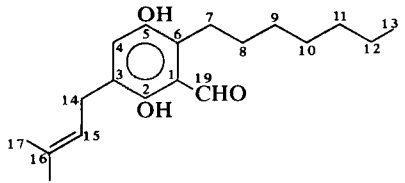
884 (805) (1136)	28	$C_{12}H_{16}O_4$		(8-9) 48·0		
885 (806)	20	$C_{12}H_{18}O_2$		(4-3) 39·5	(4-2) 1·3	
886	17	$C_{13}H_{10}$		(9-11) 42·25	(9-1) 3·12 (9-10) 4·50	(9-2) 3·75 (9-4) 2·50 (9-3) 0·50
887	73	$C_{13}H_{10}Cl_2$		(1-7) 51·6	(2-7) 3·21	(3-7) 4·52 (4-7) 1·02
888 (810) (998) (1139)	29	$C_{13}H_{14}O_5$		(4-4a) 40·9		
889 (999) (1066)	67	$C_{13}H_{16}$			(2-11) 2·2	(3-11) 2·9

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
890 (811) (967) (1000) (1140)	30	$C_{13}H_{18}N_4O_9$		(3-4) 40·0	(2-7) 11·7	
891 (812) (1001)	31	$C_{13}H_{18}O_2$		(1-5) 50 (12-13) 43		
892 (813) (1002)	32	$C_{13}H_{18}O_6$		(2-5) 48		
893 (1067)	33	$C_{13}H_{19}Li$		(7-8) 50·1 (7-9) 51·2		
894 (1068)	102	$C_{14}H_{10}O_5$		(3-10) 42·1		

895 (815)	36	$C_{14}H_{12}$		(9-12) 42·0	(9-1) +2·35 (9-11) −0·20	(9-2) +3·35 (9-4) +3·30 (9-3) −0·35
896 (816) (968)	32	$C_{14}H_{18}O_7$		(2-5) 45		
897 (818) (1141)	41	$C_{15}H_{15}O_5$		(2-15) 45 (3-4) 43·5		
898 (819) (1003) (1142)	38	$C_{15}H_{16}O_5$		(3-11) 52·2		
899 (821) (1070) (1143)	40	$C_{16}H_{16}O_7$		(1-1a) 47 (4-4a) 40		

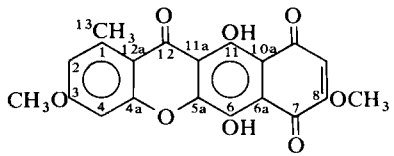
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
900 (822) (1071) (1144)	40	$C_{16}H_{16}O_8$	As above with OH at C(4) *trans* to OH at C(3)	(1-1a) 47 (4-4a) 44		
901 (177)	103	$C_{17}H_{14}ClO_6$			(3-5) 12·2	
902 (828) (1009) (1148)	47	$C_{18}H_{19}ClO_7$		(1-2) 44		
903 (1080)	33	$C_{18}H_{20}K_2$		(7-8) 49·4 (7-9) 47·7		

904 49 $C_{19}H_{28}O_3$ (830) (1083) (1149)



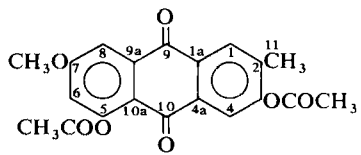
(6-7) 43
(14-15) 43
(16-17) 42

905 74 $C_{20}H_{14}O_8$ (1085) (1150)



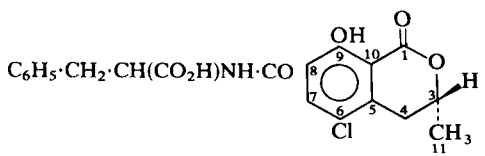
(1-13) 41·8

906 40 $C_{20}H_{16}O_7$ (1086) (1151)



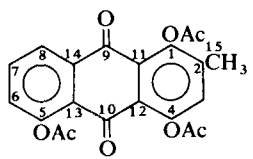
(2-11) 44

907 51 $C_{20}H_{18}ClNO_6$ (833) (1089) (1154)



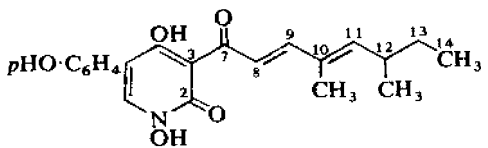
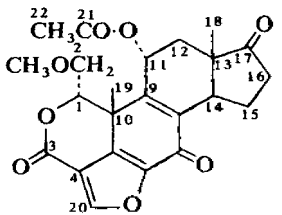
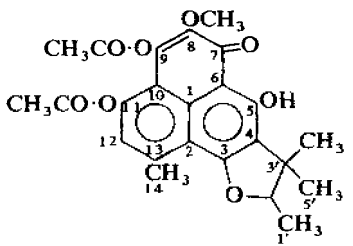
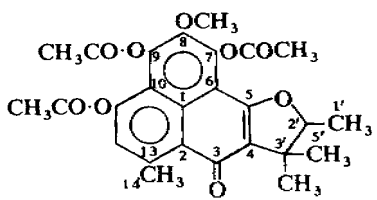
(4-5) 42·3

908 59 $C_{21}H_{16}O_8$ (1092) (1157)



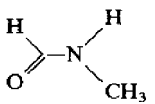
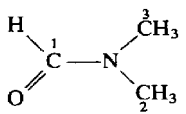
Ac = $COCH_3$

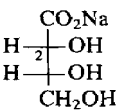
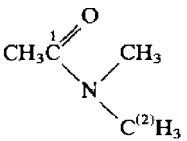
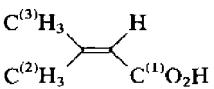
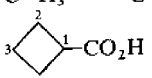
(2-15) 44·4

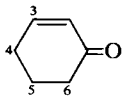
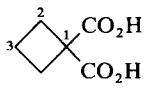
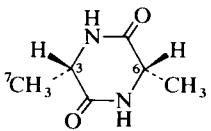
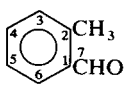
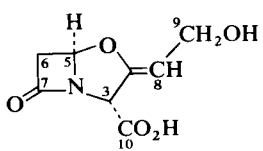
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
909 (837) (1011) (1158)	54, 55	$C_{21}H_{23}NO_5$		(11-12) 43·2		
910 (840) (975) (1012) (1161)	56	$C_{23}H_{24}O_8$		(9-11) 48		
911 (841) (1013) (1095) (1162)	57	$C_{24}H_{24}O_8$		(13-14) 42		
912 (843) (1014) (1096) (1163)	57	$C_{26}H_{26}O_9$		(13-14) 42		

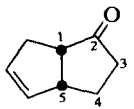
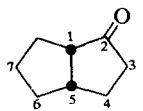
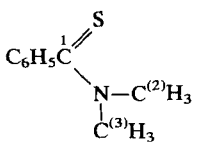
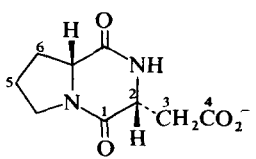
913 (846) (1098) (1166)	60	$C_{30}H_{24}O_{11}$	See No. 846 for structure		(3-4) 41 (3′-4′) 41	
914 (1018)	75	$C_{51}H_{64}N_4O_{16}$	See No. 1018 for structure		(20-20′) 44·2	

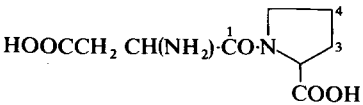
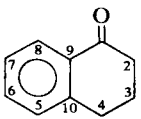
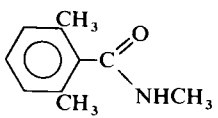
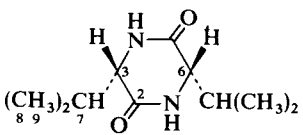
C. One coupled carbon formally with sp^3 hybridization and the other is a carbon of a carbonyl function

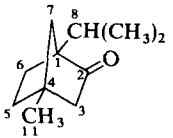
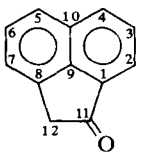
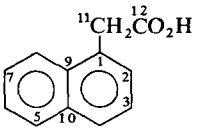
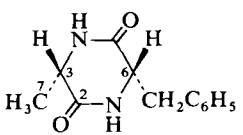
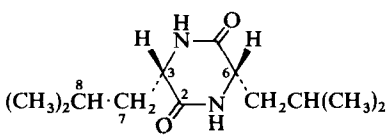
915	76	C_2H_5NO				1·43 (N)
916 (697)	4	C_3H_6O	$CH_3{\cdot}CH_2{\cdot}CHO$		55·3	
917	12	$C_3H_6O_4$	$HOCH_2{\cdot}CH(OH)CO_2H$		59·4	
918	77	C_3H_7NO	$CH_3{\cdot}CO{\cdot}NHCH_3$	*syn*		1·0 (N)
919	77	C_3H_7NO				(1-3) +4·93 (N) (1-2) +0·47 (N)
920 (709)	7	$C_3H_7NO_3$	(L)-$CH_2OH{\cdot}CH(NH_2)CO_2H$		53·1	−0·6
921 (851) (984) (1099)	4	C_4H_6O	$CH_2{=}CH{\cdot}COCH_3$		42·2	
922 (1100)	19	$C_4H_6O_2$	$CH_3{\cdot}CH{=}CH{\cdot}CO_2H$	*cis* *trans*		2·38 7.50
923	78	$C_4H_6O_2$	$CH_3{\cdot}CH{=}CH{\cdot}CO_2H$	*trans*		7.10

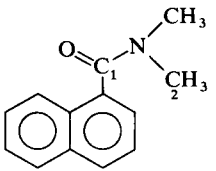
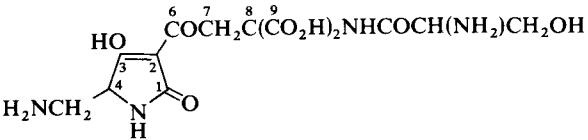
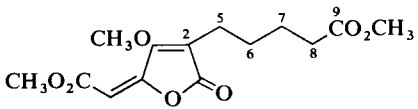
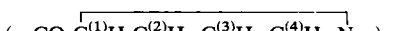
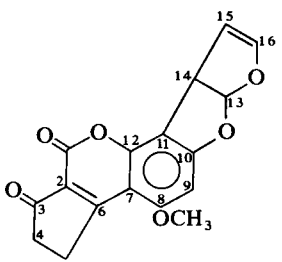
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
924 (720) (1101)	7	$C_4H_7NO_4$	(L)-$HO_2C^{(4)}\cdot C^{(3)}H_2\cdot C^{(2)}H(NH_2)C^{(1)}O_2H$	(1-2) 53·1 (3-4) 50·7	(1-3) −1·2 (2-4) −1.7	
925	12	$C_4H_7NaO_5$		52·8		
926	12	$C_4H_7NaO_5$	As above with C(2) H and OH interchanged	54·2		3·7
927	77	C_4H_9NO			(1-2) (+)2·6 (N)	
928 (735)	7	$C_4H_9NO_3$	(L)-$CH_3\cdot CHOH\cdot CH(NH_2)\cdot CO_2H$	53·0	−0·7	1·7
929	79	$C_4H_9NO_3$	(L)-$CH_3\cdot CHOH\cdot CH(NH_3^+)\cdot CO_2^-$			1·7
930 (1104)	78	$C_5H_8O_2$				(1-2) 1·54 (1-3) 7·50
931 (746)	14	$C_5H_8O_2$		58.1		
932	78	$C_5H_9BrO_2$	$C^{(4)}H_3)_2C^{(3)}H\cdot C^{(2)}H\ Br\cdot C^{(1)}O_2H$	(1-2) 61·88	(1-3) 0·5	(1-4) 2·85, 1·92
933 (748) (1105)	7	$C_5H_9NO_2$	(L)-$HO_2C^{(5)}\cdot C^{(4)}H_2\cdot C^{(3)}H_2\cdot C^{(2)}H(NH_2)C^{(1)}O_2H$	(1-2) 53·3 (4-5) 51·7	(1-3) −1·3 (3-5) −1·0	(1-4) 2·0 (2-5) 3·2
934	9	$C_5H_9NO_4$	$HO_2C^{(5)}\cdot C^{(4)}H_2\cdot CH_2\cdot CH(NH_2)CO_2H$	(4-5) 53·7		

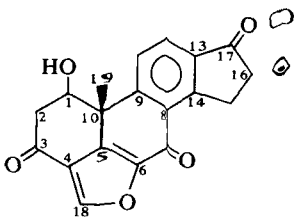
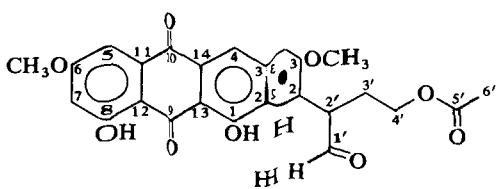
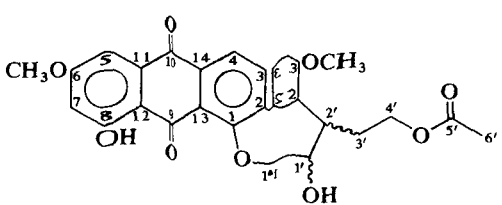
935 (762) (854) (986) (1108)	4	C_6H_8O		40·1		
936 (763)	14	$C_6H_8O_4$		58·7		
937 (764)	16	$C_6H_{10}N_2O_2$		(2-3) 50·5	(2-7) 0·5–1·0	
938 (765)	16	$C_6H_{10}N_2O_2$	As above with C(6) substituents interchanged	(2-3) 50·6	(2-7) 0.5–1·0	
939 (1112)	19	C_8H_8O				1·88
940 (857)	17	C_8H_8O	$C_6H_5{\cdot}COCH_3$	42·7		
941 (1113)	19	$C_8H_8O_2$	$o CH_3{\cdot}C_6H_4{\cdot}CO_2H$			0·66
942 (859)	65	$C_8H_9NO_5$		(6-7) 38·4 (3-10) 67·0	(5-7) 8·6	

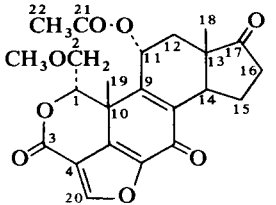
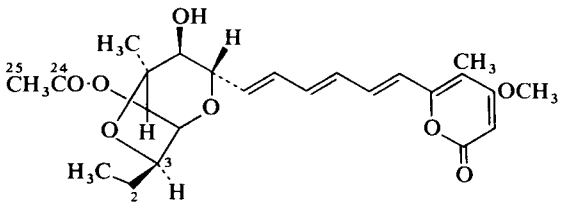
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
943 (778)	20	$C_8H_{10}O$		(2-3) 36·8		
944 (779)	20	$C_8H_{10}O$	As above but with C=O at C(3)	(2-3) 36·8		
945 (780)	20	$C_8H_{12}O$		(2-3) 35·9		
946 (781)	20	$C_8H_{12}O$	As above but with C=O at C(3)	(2-3) 36·6		
947	80	$C_8H_{16}O$	$C^{(1)}H_3{\cdot}C^{(2)}O{\cdot}C^{(3)}(CH_3)_2{\cdot}CH(CH_3)_2$	(1-2) 40 (2-3) 40		
948 (1118)	19	$C_9H_{10}O$	o-$CH_3{\cdot}C_6H_4{\cdot}COCH_3$	40·6		0·78
949	77	$C_9H_{11}NS$			(1-2) +0·80 (1-3) +1·73	
950 (783) (1120)	22	$C_9H_{11}N_2O_4^-$		(1-2) 52·0 (3-4) 51·0	(2-4) 1·2 (1-3) 1·7	

951 (785) (1121)	22	$C_9H_{12}N_2O_4$	As above with CO_2^- replaced by CO_2H		(1-2) 52·0 (3-4) 56·0	(2-4) 2·0 (1-3) 1·8	(1-5) 2·9 (1-6) 1·95
952	106	$C_9H_{16}N_2O_5$		*cis* *trans*			(1-3) 2·7 (1-4) 2·7 (1-3) 2·7 (1-4) 2·9
953 (787) (867) (1049) (1122)	4	$C_{10}H_{10}O$			40·5		
954 (1123)	78	$C_{10}H_{10}O_2$	$C_6H_5{\cdot}CH{=}CH{\cdot}CO_2CH_3$	*trans* *cis*		2·50 (O) 2·50 (O)	
955	96	$C_{10}H_{10}O_4$	$HO_2C^{(1)}{\cdot}C^{(2)}H(C_6H_5)CH_2{\cdot}CO_2H$		(1-2) 55		
956	77	$C_{10}H_{13}NO$		*syn* *anti*		~0·5 ~3·1	
957 (790)	16	$C_{10}H_{18}N_2O_2$			(2-3) 50·3	(2-7) 0·5–1·0	(2-8) 2·9 (2-9) 0·5
958 (791)	16	$C_{10}H_{18}N_2O_2$	As above with C(6) substituents interchanged		(2-3) 50·6	(2-7) 0·5–1·0	(2-8) 2·9 (2-9) 0·5

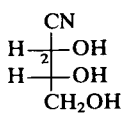
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
959 (797)	20	$C_{11}H_{18}O$			(2-4) 1·0	
960 (881) (1134)	17	$C_{12}H_8O$		42·72		
961 (882) (1135)	17	$C_{12}H_{10}O_2$		57·04 or 54·93		
962 (803)	16	$C_{12}H_{14}N_2O_2$		(2-3) 51·0	(2-7) 1·2	
963 (804)	16	$C_{12}H_{14}N_2O_2$	As above with C(6) substituents interchanged	(2-3) 50·8	(2-7) 1·7	
964 (808)	16	$C_{12}H_{22}N_2O_2$		(2-3) 50·3	(2-7) 0·5–1·0	(2-8) 0·8

965 (809)	16	$C_{12}H_{22}N_2O_2$	As above with C(6) substituents interchanged	(2-3) 50·2	(2-7) 0·5–1·0	(2-8) 0·8
966	77	$C_{13}H_{13}NO$		$CHCl_3$ Me_2CO	(1-2) +2·96 (N) +2·79	
967 (811) (890) (1000) (1140)	30	$C_{13}H_{18}N_4O_9$		(6-7) 41·1 (8-9) 50·1		
968 (816) (896)	32	$C_{14}H_{18}O_7$		(8-9) 55		
969 (820)	39	$C_{15}H_{21}O_3$		55·0	0	0
970 (824) (1006) (1072)	43	$C_{17}H_{12}O_6$		(3-4) 40		

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
971 (829) (1010) (1082)	48	$C_{19}H_{14}O_5$		(2-3) 39 (16-17) 39		
972 (832) (1088) (1153)	46	$C_{20}H_{16}O_9$	See No. 832 for structure	(5'-6') 59·4		
973 (838) (1093) (1159)	46	$C_{22}H_{20}O_9$		(1'-2') 38·6 (5'-6') 59·8		
974 (839) (1094) (1160)	46	$C_{22}H_{20}O_9$		(5'-6') 59·0		

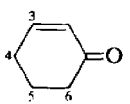
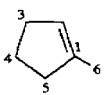
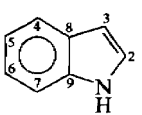
975 (840) (910) (1012) (1161)	56	$C_{23}H_{24}O_8$		(16-17) 37 (21-22) 59	
976 (842)	58	$C_{25}H_{32}O_8$		(24-25) 60·0	
977 (848) (1016)	62	$C_{40}H_{60}BNaO_{14}$	See No. 848 for structure	(1-2) 65·0	

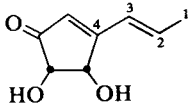
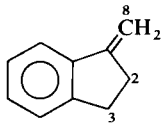
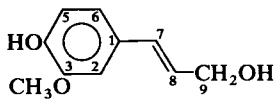
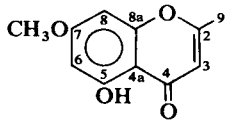
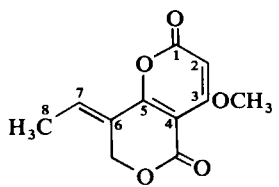
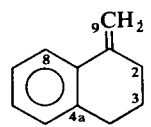
D. One coupled carbon formally with sp^3 hybridization and the other with sp hybridization

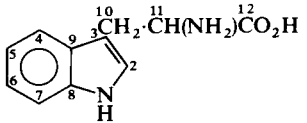
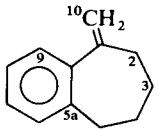
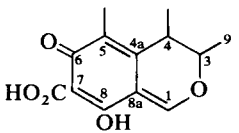
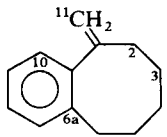
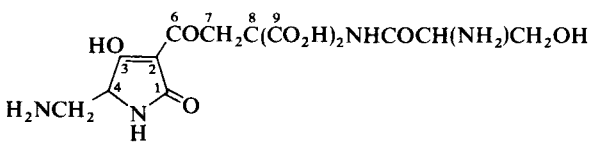
978	12	$C_3H_5NO_2$	$HOCH_2{\cdot}CH(OH)CN$	59·4	
979	12	$C_4H_7NO_3$		60·8	
980	12	$C_4H_7NO_3$	As above with C(2) H and OH interchanged	60·8	4·0

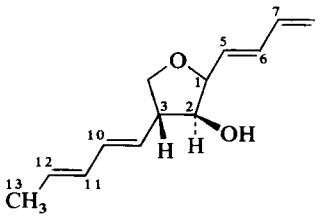
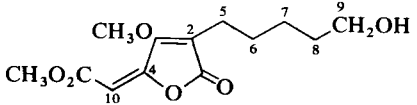
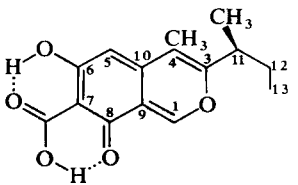
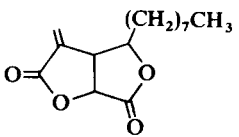
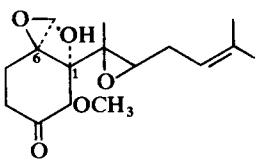
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
981 (1168)	19	C_8H_7N	o-$CH_3{\cdot}C_6H_4{\cdot}CN$			1·95
982 (1169)	81	C_8H_7N	$C_6H_5{\cdot}CH_2CN$	57·2		

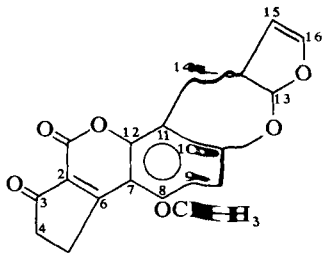
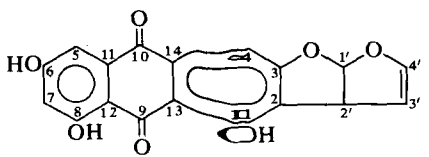
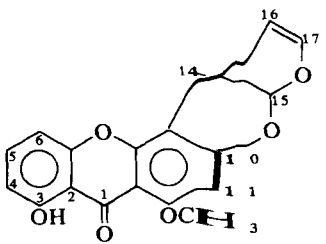
E. Both coupled carbons formally with sp^2 hybridization (other than aromatic and carbonyl carbons)

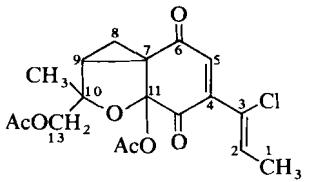
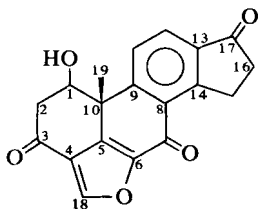
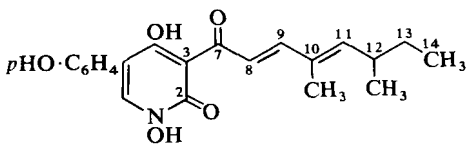
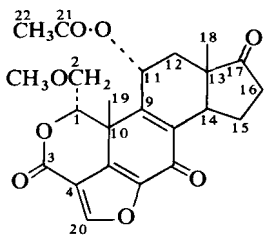
983	9	$C_4H_4O_4$	$HO_2CCH{=}CHCO_2H$	*trans*	70·2	
984 (921) (1099)	4	C_4H_6O	$CH_2{=}CH{\cdot}COCH_3$		66·1	
985	33	C_5H_8	$CH_2{=}CH{\cdot}C^{(2)}(CH_3){=}C^{(1)}H_2$		(1-2) 70·9	
986 (762) (854) (935) (1108)	4	C_6H_8O			62·2	
987 (855)	17	C_6H_{10}			72·0	
988 (1035)	4	C_8H_7N			(2-3) 68·4	

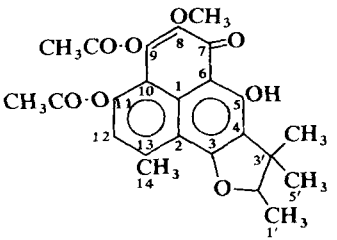
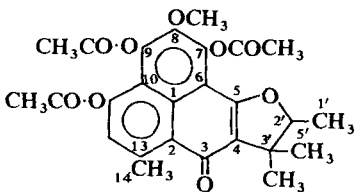
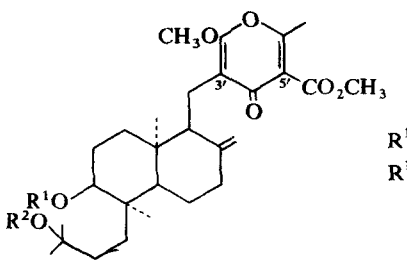
989 (861) (1116)	66	$C_8H_{10}O_3$		(3-4) 56
990	33	C_9H_{10}	$CH_3{\cdot}C(C_6H_5){=}CH_2$	72·4
991 (866) (1048)	67	$C_{10}H_{10}$		73·9
992 (870) (1051)	68	$C_{10}H_{12}O_3$		72
993 (875) (1059) (1129)	25	$C_{11}H_{10}O_4$		71
994 (876) (1130)	71	$C_{11}H_{10}O_5$		(3-4) 67 (5-6) 63·5
995 (877) (1060)	67	$C_{11}H_{12}$		72·6

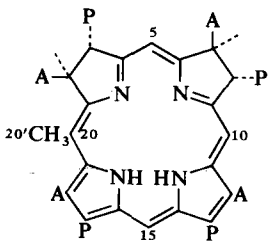
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
996 (878) (1061) (1131)	72	$C_{11}H_{12}N_2O_2$		pH 1·1 (2-3) 6·7 11·8	70·2 70·1 70·0	
997 (883) (1065)	.67	$C_{12}H_{14}$			71·0	
998 (810) (888) (1139)	29	$C_{13}H_{14}O_5$			(1–8a) 69·6 (7-8) 56·9	
999 (889) (1066)	67	$C_{13}H_{16}$			71·1	
1000 (811) (890) (967) (1140)	30	$C_{13}H_{18}N_4O_9$			65·0	

1001 (812) (891)	31	$C_{13}H_{18}O_2$		(6-7) 54 (10-11) 56
1002 (813) (892)	32	$C_{13}H_{18}O_6$		(4-10) 90
1003 (819) (898) (1142)	38	$C_{15}H_{16}O_5$		(4-10) 43·1 (5-6) 65·1 (1-9) 71·2
1004	95	$C_{15}H_{22}O_4$		75
1005 (823)	42	$C_{16}H_{23}O_5$		74

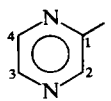
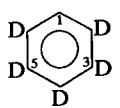
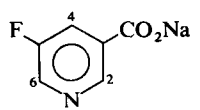
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1006 (824) (970) (1072)	43	$C_{17}H_{12}O_6$		(2-6) 60 (15-16) 75		
1007 (825) (1076) (1145)	44	$C_{18}H_{10}O_7$		(3′-4′) 75·0		
1008 (826) (1077) (1146)	43, 45	$C_{18}H_{12}O_6$		(16-17) 76		

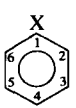
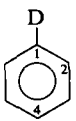
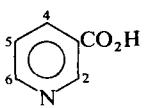
1009 (828) (902) (1148)	47	$C_{18}H_{19}ClO_7$		(3-4) 65	
1010 (829) (971) (1082)	48	$C_{19}H_{14}O_5$		(5-6) 38 (4-18) 66	
1011 (837) (909) (1158)	54, 55	$C_{21}H_{23}NO_5$		(9-10) 53·6	(3-8) 7·7
1012 (840) (910) (975) (1161)	56	$C_{23}H_{24}O_8$		(4-20) 75	

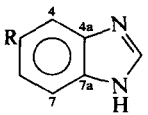
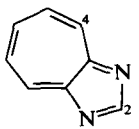
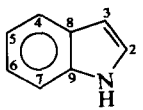
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1013 (841) (911) (1095) (1162)	57	$C_{24}H_{24}O_8$		(8-9) 85		
1014 (843) (912) (1096) (1163)	57	$C_{26}H_{26}O_9$		(4-5) 66		
1015 (195)	104	$C_{29}H_{42}O_8$	 $R^1 = CHO, R^2 = H$ / $R^1 = H, R^2 = CHO$ } Same value for both		(3'-5') 12	
1016 (848) (977)	62	$C_{40}H_{60}BNaO_{14}$	See No. 848 for structure	(11-12) 72·1		

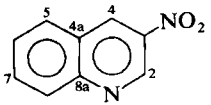
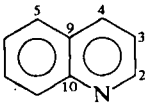
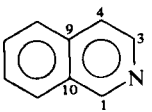
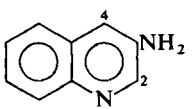
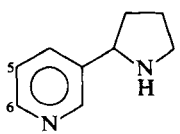
1017	83	$C_{50}H_{62}N_4O_{16}$	As below with C(20) CH_3 replaced by H		(4-5) 71	(11-13) 5·4 or 5·0	(4-20) 6 (N)
					(9-10) 77·5	(17-19) 5·0 or 5·4	(5-9) 5·7 (N)
					(14-15) 71		(16-20) 6 (N)
					(15-16) 71		(10-14) 4·4 (N)
1018 (914)	75	$C_{51}H_{64}N_4O_{16}$		P = $CH_2CH_2CO_2CH_3$ A = $CH_2CO_2CH_3$	(4-5) 73·3 (9-10) 77·0 (14-15) 72·3 (15-16) 72·3		(5-9) 7·3

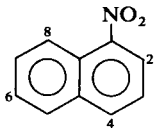
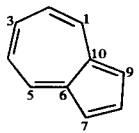
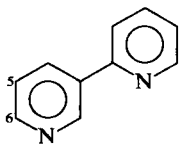
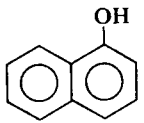
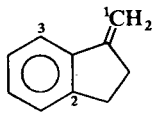
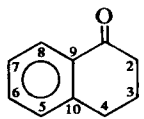
F. Both coupled carbons formally with sp^2 hybridization (both aromatic or aromatic and olefinic carbons)

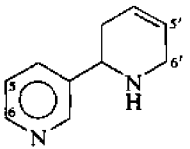
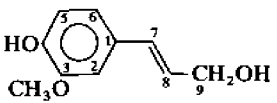
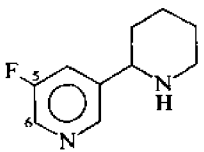
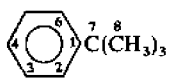
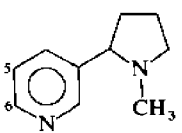
1019	4	$C_5H_6N_2$		(1-2) 54·6 (3-4) 53·6		(1-3) 17·3 (N) (2-4) 16·4 (N)
1020	84	C_6HD_5		(1-2) +55·95	(1-3) 2·46	(1-4) +10·01
1021	85	$C_6H_3FNNaO_2$		(4-5) 70 (5-6) 71·7	(4-6) 3·5	

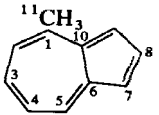
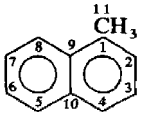
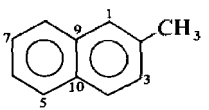
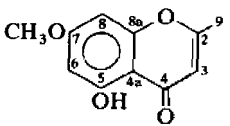
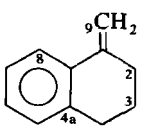
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1022 (504)	69	C_6H_5Br	 X = Br		(2-4) 2·75	
1023 (505)	69	C_6H_5Cl	PhCl (as No. 1022 with X = Cl)		(2-4) 2·81	
1024	101	C_6H_5D		(1-2) 55·3		(1-4) 10·08
1025 (507)	69	C_6H_5F	PhF (as No. 1022 with X = F)		(2-4) 2·89	
1026 (509)	69	C_6H_5I	PhI (as No. 1022 with X = I)		(2-4) 2·63 (1-3) 2·53	
1027 (511)	69	$C_6H_5NO_2$	$PhNO_2$ (as No. 1022 with $X = NO_2$)		(2-4) 2·63	
1028	82	$C_6H_5NO_2$		(4-5) 54 (5-6) 55·0	(3-5) 2·4 (4-6) 2·8	(3-6) 12·5 (N)
1029	69	$C_6H_8N_2$	$PhNH{\cdot}NH_2$ (as No. 1022 with $X = NH{\cdot}NH_2$)	(1-2) 62·7 (2-3) 58·6 (3-4) 55·9		
1030 (520) (1167)	69	C_7H_5N	PhCN (as No. 1022 with X = CN)		(2-4) 2·61	(1-4) 10·95 (2-5) 9·05

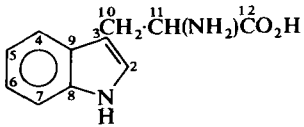
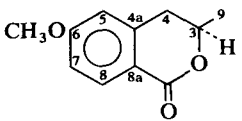
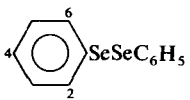
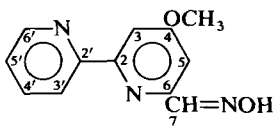
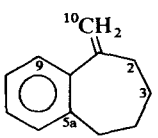
1031	86	$C_7H_6N_2$	 R = H			
			Other compounds with R = CH_3, Cl, CH_3CO, and NO_2 have the same J(C–C) values		(2-4a) and (2-7a) 1–2 (N)	(2-4) and (2-7) 5–6 (N) (2-5) and (2-6) ~0 (N)
1032 (530)	69	C_7H_8	$PhCH_3$ (as No. 1022 with X = CH_3)		(1-3) 2·05 (2-4) 2·57	
1033 (533) (856)	69	C_7H_8O	$PhOCH_3$ (as No. 1022 with X = OCH_3)		(2-4) 2·81	
1034	86	$C_8H_6N_2$				(2-4) 12·2 (N)
1035 (988)	4	C_8H_7N		(3-8) 54·5 (6-7) 60·0		
1036 (589)	4	C_8H_9NO	$PhC(CH_3)$=NOH (as No. 1022 with X = $C^{(7)}(CH_3)$=NOH)	(1-7) 61·7		
1037 (541) (862)	69	$C_8H_{11}N$	$PhN(CH_3)_2$ (as No. 1022 with X = $N(CH_3)_2$)		(2-4) 2·74	

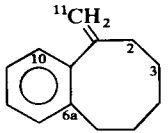
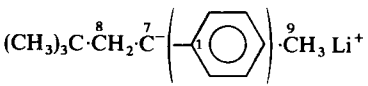
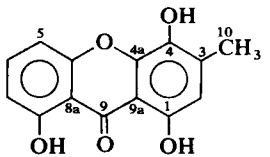
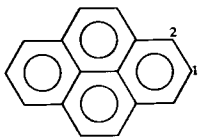
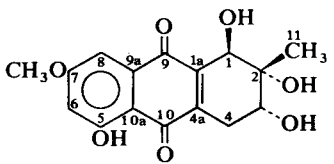
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1038	82	$C_9H_6N_2O_2$		(2-3) 62·4 (3-4) 60	(2-4) 9	(2-8) 3 (N) (2-4a) 7·5 (N) (3-8a) 10 (N)
1039	82	C_9H_7N		(2-3) 51·1 (3-4) 58		
1040	4	C_9H_7N	As above	(2-3) 50·7 (3-4) 57·9 (4-9) 49·8 (9-10) 53·1 (8-10) 64·4		
1041	4	C_9H_7N		(3-4) 59·8 (4-9) 54·2 (1-10) 53·7 (9-10) 51·3		
1042	82	$C_9H_8N_2$		(2-3) 56·3 (3-4) 58		
1043	82	$C_9H_{12}N_2$		(5-6) 56·1		

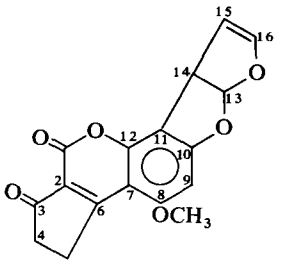
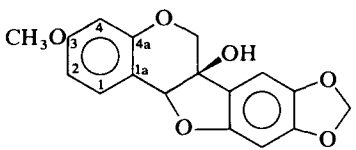
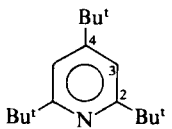
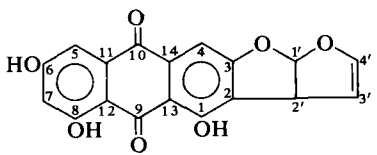
1044	87	$C_{10}H_7NO_2$			(1-2) 71·60 (1-8a) 67·05 (3-4) 59·26 (5-4a) 56·34 (5-6) 59·27 (7-8) 60·01 (8-8a) 57·37		
1045	70	$C_{10}H_8$			(1-2) 59·3 (1-10) 61·3	(1-3) 1·5 (1-6) 5·5	(1-4) 3·7 (1-7) 2·0
1046	23	$C_{10}H_8N_2$		$CDCl_3$ H^+	(5-6) 54·9 (5-6) 58·7		
1047 (552)	1	$C_{10}H_8O$	The structure of No. 552 of ref. 1 should be 				
1048 (866) (991)	67	$C_{10}H_{10}$					(1-2) 4·8 (1-3) 1·7
1049 (787) (867) (953) (1122)	4	$C_{10}H_{10}O$			(5-6) 56·6 (6-7) 54·6 (8-9) 59·1 (9-10) 55·2 (5-10) 56·2		

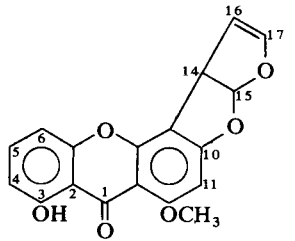
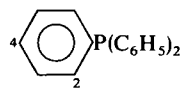
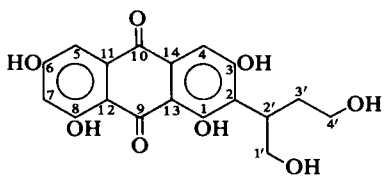
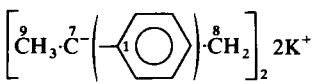
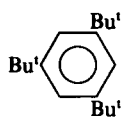
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1050 (869)	82	$C_{10}H_{12}N_2$		(5-6) 59·1		
1051 (870) (992)	68	$C_{10}H_{12}O_3$			(2-7) 2·9 (6-7) 1·8	(3-7) 4·9 (5-7) 5·1 (2-8) 4·4 (6-8) 5
1052	85	$C_{10}H_{13}FN_2$		(5-6) 76		
1053 (251) (554) (871)	69	$C_{10}H_{14}$			(1-3) 2·11 (2-4) 2·58	
1054	82	$C_{10}H_{14}N_2$	As No. 1052 with F replaced by H	(5-6) 55·1		
1055	82	$C_{10}H_{14}N_2$		(5-6) 54·8		

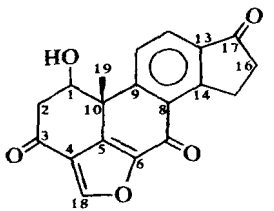
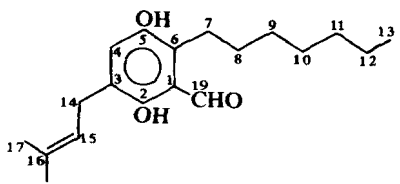
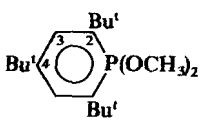
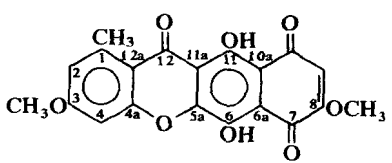
1056 (872)	70	$C_{11}H_{10}$		(1-10) 61·0	(1-3) 0·8 (1-6) 6·0 (1-9) 0·95	(1-4) 3·4 (1-5) 2·0 (1-7) 2·0 (1-8) 7·5
1057 (873)	98	$C_{11}H_{10}$		(1-2) 61·8 (1-9) 58 (3-4) 61·0 (4-10) 55·7	(1-8) 1·5 (4-5) 2·2 (5-9) <1	(1-4) 7·7 (1-5) 2·7 (1-7) 5·5 (4-6) 5·2 (5-7) 1·0 (5-8) 2·7
1058	98	$C_{11}H_{10}$		(1-2) 61·8 (1-9) 56·2 (4-10) 55·6 (7-8) 61 (8-9) 55·5	(1-3) ~1 (1-10) ~1 (1-8) 2·2 (2-4) <1 (4-9) <1	(1-4) 7·0 (1-5) 2·9 (1-6) 1·2 (1-7) 5·5 (4-6) 5·4 (4-7) 1·3 (4-8) 2·9
1059 (875) (993) (1129)	25	$C_{11}H_{10}O_4$		(4a-5) 64 (5-6) 73 (6-7) 70 (7-8) 71 (8-8a) 73 (4a-8a) 64		
1060 (877) (995)	67	$C_{11}H_{12}$				(9-4a) 3·6 (9-8) 4·2

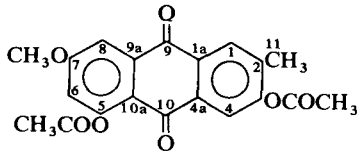
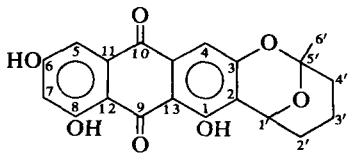
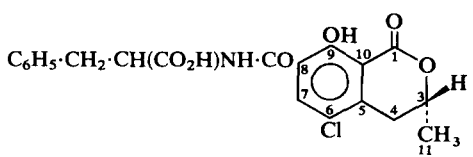
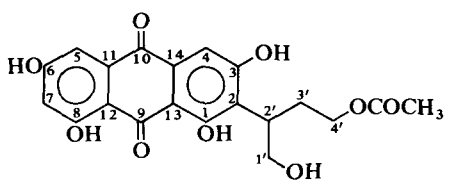
No.	Ref.	Mol. formula	Structure		^1J	^2J	^nJ
1061 (878) (996) (1131)	72	$C_{11}H_{12}N_2O_2$		pH 1·1 6·7 11·8	(3-9) 54·9 (3-9) 54·7 (3-9) 54·5	(3-4) 1·4 (3-8) 5·0 (3-4) 1·8 (3-8) 4·8 (3-4) 1·0 (3-8) 4·9	(3-5) 4·1 (3-7) 2·0 (3-5) 3·8 (3-7) 1·5 (3-5) 3·7
1062 (794) (879) (1132)	25	$C_{11}H_{12}O_4$			(4a-5) 67 (5-6) 67 (6-7) 72 (7-8) 73 (8-8a) 67		
1063	69	$C_{12}H_{10}Se_2$			(1-2) 59·29 (2-3) 55·27 (3-4) 56·47	(2-4) 2·59	(1-4) 9·84 (2-5) 8·80
1064	88	$C_{12}H_{11}N_3O_2$			(2-3) 63·7 (3-4) 65·9 (4-5) 66·5 (2-2') 71·7 (2'-3') 64 (3'-4') 54·2 (4'-5') 53·9 (5'-6') 54·0		
1065 (883) (997)	67	$C_{12}H_{14}$					(10-5a) 2·4 (10-9) 2·4

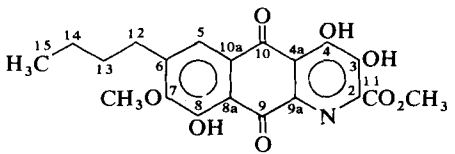
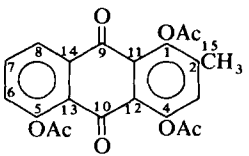
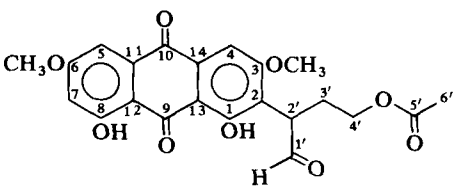
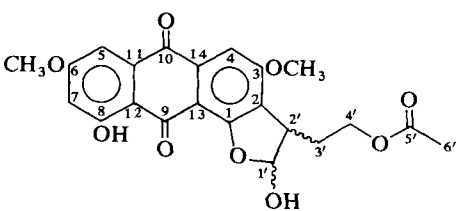
1066 (889) (999)	67	$C_{13}H_{16}$		(11-6a) 2·2 (11-10) 2·2
1067 (893)	33	$C_{13}H_{19}Li$		(1-7) 71·7
1068 (894)	102	$C_{14}H_{10}O_5$		(1-9a) 64·1 (4-4a) 77·7 (9-8a) 54·3
1069 (573)	87	$C_{16}H_{10}$		(1-2) 57·30
1070 (821) (899) (1143)	40	$C_{16}H_{16}O_7$		(5-6) 69 (7-8) 65
1071 (822) (900) (1144)	40	$C_{16}H_{16}O_8$	As above with OH at C(4) *trans* to OH at C(3)	(5-6) 69 (7-8) 65

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1072 (824) (970) (1006)	43	$C_{17}H_{12}O_6$		(7-12) 64 (8-9) 71 (10-11) 61		
1073	89	$C_{17}H_{14}O_6$		(1-1a) 60 (1-2) 60 (2-3) 66 (3-4) 70 (4-4a) 72		
1074	90	$C_{17}H_{29}N$		(2-3) 58·48 (3-4) 56·94		
1075	90	$C_{17}H_{29}P$	As above with N replaced by P	(2-3) 55·81 (3-4) 61·23		
1076 (825) (1007) (1145)	44	$C_{18}H_{10}O_7$		(1-13) 62·7 (2-3) 62·6 (4-14) 63·5 (5-6) 62·6 (7-8) 69·7		

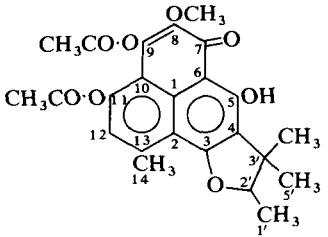
1077 (826) (1008) (1146)	45, 43	$C_{18}H_{12}O_6$		(3-4) 70 (5-6) 58·5 (10-11) 72
1078	91	$C_{18}H_{15}P$		(1-2) 55·01 (2-3) 55·32
1079 (827) (1147)	46	$C_{18}H_{16}O_8$		(1-13) 62·1 (2-3) 68·6 (4-14) 63·5 (5-6) 62·7 (7-8) 69·9
1080 (903)	33	$C_{18}H_{20}K_2$		(1-7) 73·4
1081	90	$C_{18}H_{30}$		58·90

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1082 (829) (971) (1010)	48	$C_{19}H_{14}O_5$		(9-11) 60 (11-12) 60 (12-13) 56 (8-14) 55		
1083 (830) (904) (1149)	49	$C_{19}H_{28}O_3$		(2-3) 65 (4-5) 65		
1084	90	$C_{19}H_{35}O_2P$		(2-3) 59·32 (3-4) 62·11		
1085 (905) (1150)	74	$C_{20}H_{14}O_8$		(2-3) 63·4 (4-4a) 74·2 (5a-11a) 68·4 (6-6a) ~63 (10a-11) 65·5		

1086 (906) (1151)	40	$C_{20}H_{16}O_7$		(1-1a) 60 (4-4a) 62 (5-6) 73 (7-8) 67
1087 (831) (1152)	50	$C_{20}H_{16}O_7$		(1-13) 64 (2-3) 65 (4-14) 65 (5-6) 62·5 (7-8) 70
1088 (832) (972) (1153)	46	$C_{20}H_{16}O_9$	See No. 832 for structure	(1-13) ~63 (2-3) ~62 (4-14) ~63·5 (5-6) 63 (7-8) 70·1
1089 (833) (907) (1154)	51	$C_{20}H_{18}ClNO_6$		(6-7) 67·4 (8-9) 68·8
1090 (834) (1155)	46	$C_{20}H_{18}O_9$		(1-13) 61·8 (2-3) 68·8 (4-14) ~63.6 (5-6) 63·0 (7-8) 70·0

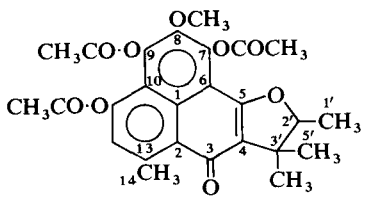
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1091 (835) (1156)	52	$C_{20}H_{19}NO_8$		(3-4) 72 (6-7) 68 (8-8a) 63 (9-9a) 63 (5-10a) 60		
1092 (908) (1157)	59	$C_{21}H_{16}O_8$	 Ac = $COCH_3$	(1-11) 73·6 (4-12) 71·7 (5-6) 70·3 (7-8) 56·0		
1093 (838) (973) (1159)	46	$C_{22}H_{20}O_9$		(1-13) 62·6 (2-3) 70·9 (4-14) 64·1 (5-6) 65·7 (7-8) 70·3		
1094 (839) (974) (1160)	46	$C_{22}H_{20}O_9$		(1-13) 70·0 (2-3) 76·3 (4-14) ~67 (5-6) 66·2 (7-8) 66·9		

1095 57 $C_{24}H_{24}O_8$
(841)
(911)
(1013)
(1162)



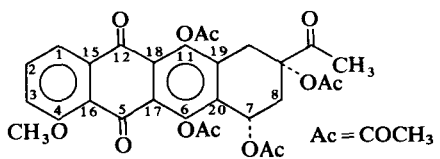
(1-10) 56
(2-3) 64·5
(4-5) 68
(11-12) 68

1096 57 $C_{26}H_{26}O_9$
(912)
(1014)
(1163)



(1-10) 57
(6-7) 75
(8-9) 82

1097 59 $C_{29}H_{26}O_{12}$
(845)
(1165)



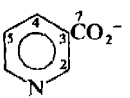
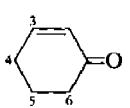
(1-15) 53·1
(2-3) 62·4
(4-16) 70·2
(6-20) ~70
(11-19) ~63

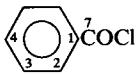
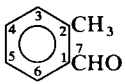
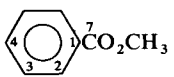
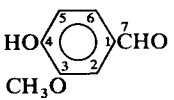
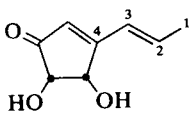
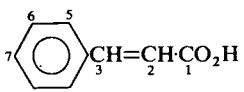
1098 60 $C_{30}H_{24}O_{11}$
(846)
(913)
(1166)

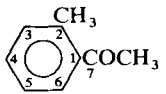
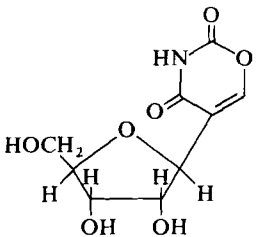
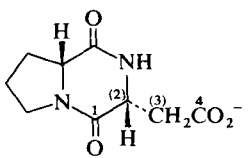
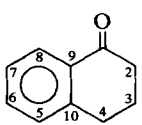
See No. 846 for structure

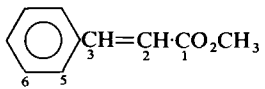
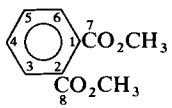
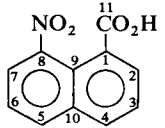
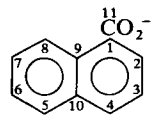
(5-6) 64
(5'-6') 56·5
(7'-8') 71
(9'-10') 70
(11'-12') 78

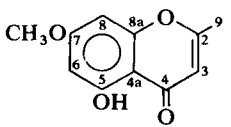
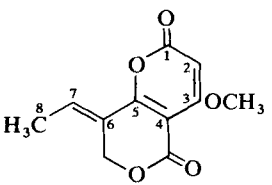
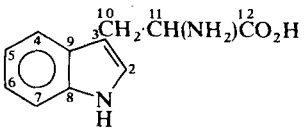
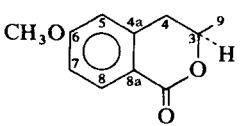
G. Both coupled carbons formally with sp^2 hybridization, one of which is a carbonyl function

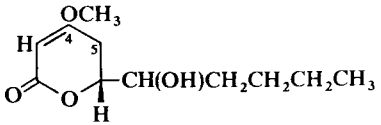
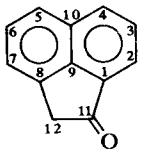
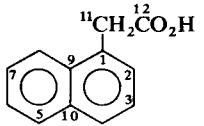
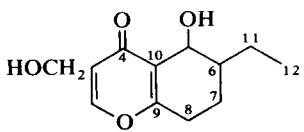
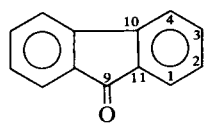
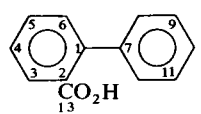
No.	Ref.	Mol. formula	Structure		^1J	^2J	^nJ
1099 (851) (921) (984)	4	C_4H_6O	$CH_2{=}CH{\cdot}COCH_3$		51·3		
1100 (922)	19	$C_4H_6O_2$	$CH_3{\cdot}CH{=}CH{\cdot}CO_2H$	*cis* *trans*		<0·5 <0·5	
1101 (720) (924)	7	$C_4H_7NO_4$	(L)-$HO_2C^{(4)}{\cdot}CH_2{\cdot}CH(NH_2)C^{(1)}O_2H$				(1-4) 3·0
1102	79	$C_4H_7NO_4$	As above				(1-4) 3·3
1103	92	$C_4I_2O_4Ru$	*cis*-$Ru(CO)_4I_2$			4	
1104 (930)	78	$C_5H_8O_2$	$(CH_3)_2C{=}CH{\cdot}CO_2H$		73·25	2·17	
1105 (748) (933)	7	$C_5H_9NO_2$	(L)-$HO_2C{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2)CO_2H$				≤0
1106	23	$C_6H_4NO_2^-$			(3-7) 65·7	(2-7) 3·7 (4-7) 2·4	(5-7) 3·6
1107	100	$C_6H_6N_2O$	As above with CO_2^- replaced by $CONH_2$			(2-7) 3·5 (4-7) 1·8	
1108 (762) (854) (935) (986)	4	C_6H_8O			50·7		

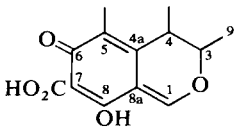
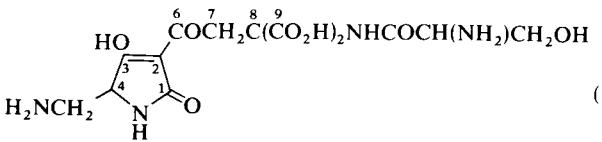
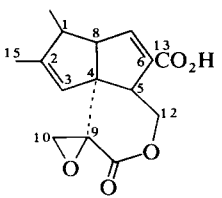
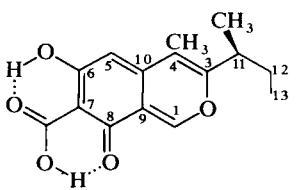
1109	78	$C_6H_{11}NO$	$CH_3{\cdot}CH{=}CH{\cdot}CO{\cdot}N(CH_3)_2$	*trans*		(1-3) 0·58	
1110	68	C_7H_5ClO			(7-1) 73·35	(7-2) 3·53	(7-3) 5·46 (7-4) 1·18
1111	68	$C_7H_6O_2$	As above with COCl replaced by CO_2H		(7-1) 71·8	(7-2) 2·54	(7-3) 4·53 (7-4) 0·9
1112 (939)	19	C_8H_8O				(7-2) 3·57 (7-6) 3·14	(7-3) 4·35 (7-5) 4·68 (7-4) 1·03
1113 (941)	19	$C_8H_8O_2$	As above with CHO replaced by CO_2H			(7-2) 3·08 (7-6) 2·27	(7-3) 4·67 (7-4) 1·08 (7-5) 4·40
1114 (538)	69	$C_8H_8O_2$			(7-1) 74·9	(7-2) +2·49	(7-3) +4·59 (7-4) −1·04
1115	68	$C_8H_8O_3$			(7-1) 55	(7-2) 3·8 (7-6) 5·2	(7-3) 5·4 (7-5) 5·7 (7-4) 1
1116 (861) (989)	66	$C_8H_{10}O_3$			52		
1117	78	C_9H_8O		*cis* *trans*	(1-2) 73·50 (1-2) 73·86	(1-3) 0·50 (1-3) 1·66	(1-4) 2·60 (1-5) 0·45 (1-4) 7·00 (1-5) ~0·2 (1-6) ~0·25 (1-7) 0

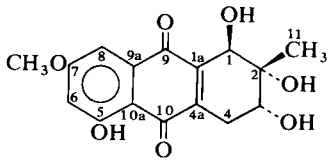
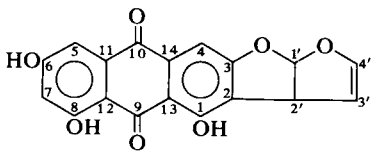
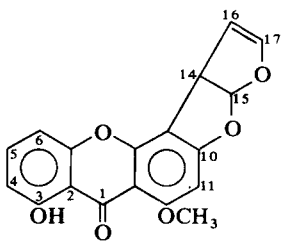
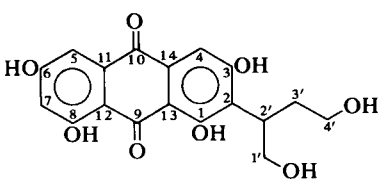
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1118 (948)	19	$C_9H_{10}O$			(7-2) 1·73 (7-6) 3·66	(7-3) 3·84 (7-5) 4·21 (7-4) 0·98
1119	9	$C_9H_{11}NO_7$		64·5		
1120 (783) (950)	22	$C_9H_{11}N_2O_4^-$				(1-4) 2·5
1121 (785) (951)	22	$C_9H_{12}N_2O_4$	As above with CO_2^- replaced by CO_2H		D_2O DMSO	(1-4) 2·0 (1-4) 1·75
1122 (787) (867) (953) (1049)	4	$C_{10}H_{10}O$		50·5		

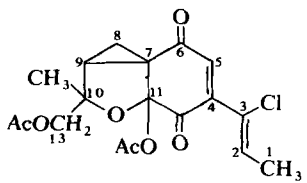
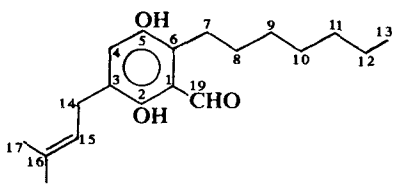
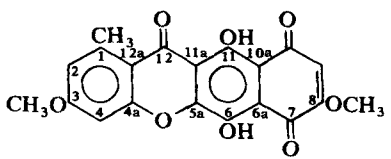
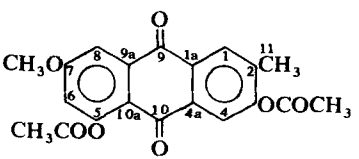
1123 (954)	78	$C_{10}H_{10}O_2$		*cis*	(1-2) 75·50	(1-3) 0·53	(1-4) 2·57 (1-6) ~0
				trans	(1-2) 76·50	(1-3) 1·56	(1-4) 7·06 (1-5) 0·2 (1-6) 0·25
1124	105	$C_{10}H_{10}O_4$			(1-7) 72·6	(7-2) 2·0 (7-6) +1·6	(7-3) +4·1 (7-5) 4·5 (7-4) −1·0 (7-8) 1·4
1125	87	$C_{11}H_7NO_4$			(11-1) 73·50	(11-2) 1·48 (11-9) 2·45	(11-3) 4·67 (11-10) 3·64 (11-4) 1·0 (11-5) ~0 (11-6) ~0 (11-7) ~0
1126	87	$C_{11}H_7NO_4$	As above with NO_2 at C(5)		(11-1) 72·12	(11-2) 1·62 (11-9) 3·82	(11-3) 4·70 (11-10) 4·23 (11-8) ~0·75 (11-4) 0·76 (11-5) 0·71 (11-6) ~0 (11-7) ~0
1127	87	$C_{11}H_7O_2^-$				(11-2) 1·84 (11-9) 2·70	(11-3) 4·35 (11-10) 3·27 (11-8) 1·14 (11-4) ~0 (11-5) ~0 (11-6) ~0 (11-7) ~0

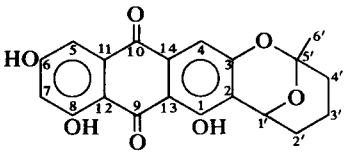
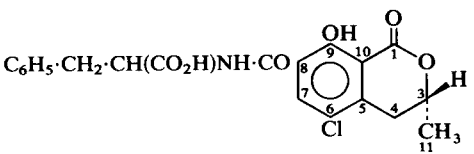
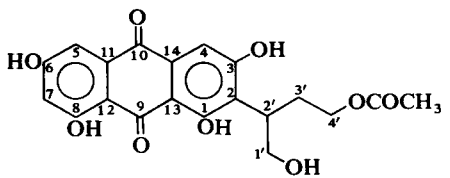
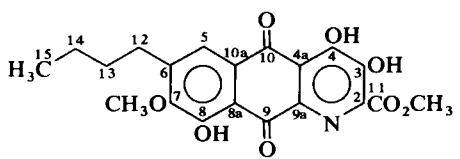
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1128	87	$C_{11}H_8O_2$	As above with CO_2^- replaced by CO_2H		(11-2) 1·88 (11-9) 3·53	(11-3) 4·78 (11-10) 4·01 (11-8) 0·76 (11-4) 1·11 (11-5) 0·43 (11-6) ~0 (11-7) ~0·3
1129 (875) (993) (1059)	25	$C_{11}H_{10}O_4$		(3-4) 57 (4-4a) 57		
1130 (876) (994)	71	$C_{11}H_{10}O_5$		(1-2) 80·5		
1131 (878) (996) (1061)	72	$C_{11}H_{12}N_2O_2$		pH 1·1 6·7 11·8		(3-12) 2·5 2·4 2·6
1132 (794) (879) (1062)	25	$C_{11}H_{12}O_4$		72		

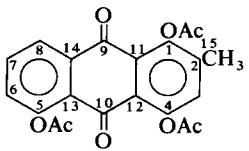
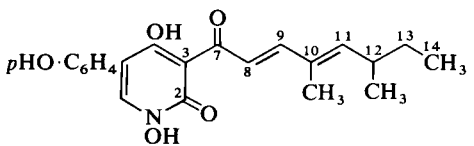
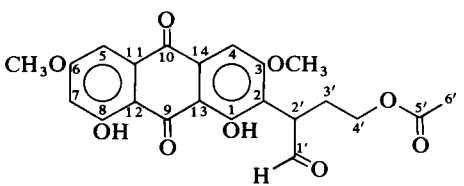
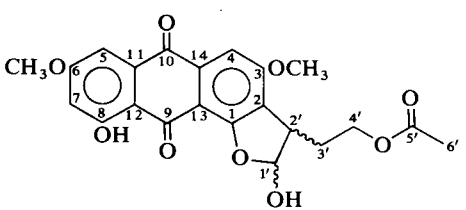
1133 (798) (880)	27	$C_{11}H_{18}O_4$		74		
1134 (881) (960)	17	$C_{12}H_8O$		(11-1) 54·81	(11-2) 1·83 (11-8) 3·66 (11-9) 9·80	(11-3) 4·03 (11-7) 4·15 (11-10) <2·0 (11-4) 0·98
1135 (882) (961)	17	$C_{12}H_{10}O_2$			(12-1) 1·37	(12-2) 2·56 (12-9) 3·17 (12-10) 0·77
1136 (805) (884)	28	$C_{12}H_{16}O_4$		(4-10) 55·1		
1137	17	$C_{13}H_8O$			(9-1) 2·62 (9-10) 7·88	(9-2) 3·75 (9-4) 3·75 (9-3) 0·88
1138	17	$C_{13}H_8O_2$			(13-1) 2·68 (13-3) 1·70	(13-4) 4·70 (13-6) 2·68 (13-7) 1·10 (13-5) 1·0

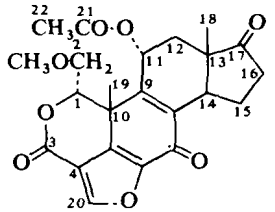
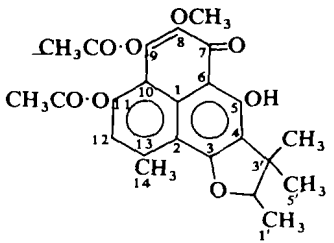
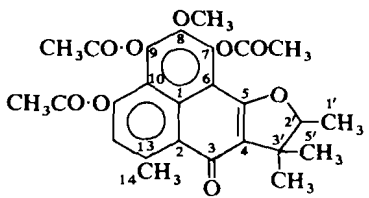
No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1139 (810) (888) (998)	29	$C_{13}H_{14}O_5$		(5-6) 63·6		
1140 (811) (890) (967) (1000)	30	$C_{13}H_{18}N_4O_9$		(1-2) 68·8		(6-9) 2·6
1141 (818) (897)	41	$C_{15}H_{15}O_5$		(6-13) 75·5		
1142 (819) (898) (1003)	38	$C_{15}H_{16}O_5$		(7-8) 63·2		

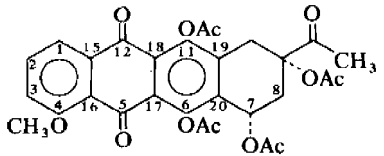
1143 (821) (899) (1070)	40	$C_{16}H_{16}O_7$		(9-9a) 54 (10-10a) 54
1144 (822) (900) (1071)	40	$C_{16}H_{16}O_8$	As above with OH at C(4) *trans* to OH at C(3)	(9-9a) 54 (10-10a) 57
1145 (825) (1007) (1076)	44	$C_{18}H_{10}O_7$		(9-12) 58·7 (10-11) 54·2
1146 (826) (1008) (1077)	45, 43	$C_{18}H_{12}O_6$		(1-2) 58
1147 (827) (1079)	46	$C_{18}H_{16}O_8$		(9-12) 58·3 (10-11) 53·3

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1148 (828) (902) (1009)	47	$C_{18}H_{19}ClO_7$	 Ac = CH_3CO	(5-6) 54		
1149 (830) (904) (1083)	49	$C_{19}H_{28}O_3$		(1-19) 55		
1150 (905) (1085)	74	$C_{20}H_{14}O_8$		(12-12a) 68·0 (7-8) ~65		
1151 (906) (1086)	40	$C_{20}H_{16}O_7$		(9-9a) 54 (10-10a) 54		

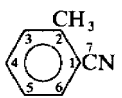
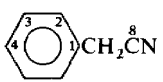
1152 (831) (1087)	50	$C_{20}H_{16}O_7$		(9-12) 58 (10-11) 54
1153 (832) (972) (1088)	46	$C_{20}H_{16}O_9$	See No. 832 for structure	(9-12) 58·9 (10-11) 53·7
1154 (833) (907) (1089)	51	$C_{20}H_{18}ClNO_6$		(1-10) 67·1
1155 (834) (1090)	46	$C_{20}H_{18}O_9$		(9-12) 58·6 (10-11) 53·5
1156 (835) (1091)	52	$C_{20}H_{19}NO_8$		(2-11) 83 (4a-10) 53

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1157 (908) (1092)	59	$C_{21}H_{16}O_8$	 Ac = $COCH_3$	(9-14) 54·2 (10-13) 56·4		
1158 (837) (909) (1011)	54, 55	$C_{21}H_{23}NO_5$		(7-8) 55·9	(2-7) 4·6	
1159 (838) (973) (1093)	46	$C_{22}H_{20}O_9$		(9-12) 57·7 (10-11) 54·6		
1160 (839) (974) (1094)	46	$C_{22}H_{20}O_9$		(9-12) 57·2 (10-11) 53·9		

1161 (840) (910) (975) (1012)	56	$C_{23}H_{24}O_8$		(3-4) 85
1162 (841) (911) (1013) (1095)	57	$C_{24}H_{24}O_8$		(6-7) 59·5
1163 (843) (912) (1014) (1096)	57	$C_{26}H_{26}O_9$		(2-3) 53
1164	93	$C_{28}H_{37}N_5O_7$	$H_2NCH(pHOC_6H_4)C^{(2)}ONH{\cdot}C^{(3)}H_2{\cdot}C^{(4)}ONH{\cdot}C^{(5)}H_2{\cdot}C^{(6)}ONH$ $\cdot C^{(7)}H(CH_2C_6H_5)C^{(8)}ONH{\cdot}C^{(9)}H(CH_2CH(CH_3)_2)C^{(10)}O_2H$	(2-4) 0·9 (N) (4-6) 1·0 (N) (6-8) 1·0 (N) (8-10) 1·0 (N)

No.	Ref.	Mol. formula	Structure	^1J	^2J	^nJ
1165 (845) (1097)	59	$C_{29}H_{26}O_{12}$	 Ac = $COCH_3$	(5-17) 54·8 (12-18) ~55		
1166 (846) (913) (1098)	60	$C_{30}H_{24}O_{11}$	See No. 846 for structure	(7-8) 58 (10-11) 61 (13-14) 68 (13′-14′) 68		

H. One coupled carbon formally with sp^2 hybridization and the other with sp hybridization

1167 (520) (1030)	69	C_7H_5N	$C_6H_5{\cdot}CN$			5·51
1168 (981)	19	C_8H_7N			(7-2) 2·34 (7-6) 2·59	(7-3) 4·88 (7-5) 5·34 (7-4) 1·22
1169 (982)	81	C_8H_7N			(8-1) 3·53	(8-2) 3·37 (8-3) <0·3 (8-4) <0·3

I. Both coupled carbons formally with sp hybridization

1170	94	$C_{14}H_{10}$	$C_6H_5{\cdot}C{\equiv}C{\cdot}C_6H_5$	185·0		

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Calcium and Magnesium NMR in Chemistry and Biology

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I. INTRODUCTION

NMR has become a very useful tool in the study of several metal ions[1] but the preferential interest in studies of ions such as Li^+,[2] Na^+,[2-4] Cd^{2+},[4-6] Co^{2+},[7] and Mn^{2+} [7] (the latter two using the paramagnetic properties in indirect studies) indicates that one has been extensively limited by sensitivity problems. Application of the NMR spectra of some of the key metal ions like Mg^{2+}, Ca^{2+}, and Zn^{2+} to biological structure and function problems was virtually non-existent up to one or two years ago. As we hope to demonstrate in this treatment, which reviews and analyses some chemical and

biological applications of calcium and magnesium NMR, there has been too much pessimism in the case of ^{25}Mg and ^{43}Ca NMR. Since these ions (because of their closed electron shells), in contrast to most biologically and chemically interesting transition metal ions, lack other useful spectroscopic properties such as ESR spectra, ultraviolet/visible spectra, or luminescence properties, progress in the understanding of their biological function has been much retarded.

Some fundamental properties of the alkaline earths are given in Table I. For magnesium and calcium (and also strontium and barium which because of their chemical similarity to Mg and Ca are potential substitutes in experimental studies) only the divalent ionic state needs to be considered in aqueous systems. Magnesium forms many covalent compounds (e.g. the Grignard reagents), stable only under anhydrous conditions, while the importance of such compounds is insignificant for calcium. Both magnesium and calcium ions are strongly hydrated in aqueous solution and in many interionic interactions the primary hydration sheath is retained. For studies of aqueous Mg^{2+} solutions it is important to recall that the insoluble hydroxide may be easily formed.

Both magnesium and calcium occur widely in biological as well as in non-biological systems as indicated in Table II. The relation of these elements to biological structure and function is a subject of intense research.[8-12] Calcium is found in bone and in the biological fluids as free ion as well as bound to a large number of proteins where it can serve a structural role or as an activator of enzymatic processes. Among important calcium-requiring physiological processes may be mentioned blood clotting, visual excitation, and muscle contraction. The calcium ion is a good cross-linking agent known to stabilize cell–cell interactions and subunit interactions in multi-subunit proteins. The binding of some proteins, like prothrombin, to phospholipid bilayers appears to be mediated by calcium ions. About 70 different calcium binding proteins[10] have been described and for about 6 of these the three-dimensional structure has been determined. The calcium ion seems to be liganded exclusively by oxygens from carboxylate groups, peptide carbonyls, hydroxyl groups, or water molecules.

While calcium has a high intercellular concentration and a low intracellular one, the converse is true for magnesium. Magnesium ions are known to activate a number of enzymes involved in the transfer of phosphate groups; calcium acts as an inhibitor of many magnesium-activated enzymes. Magnesium is found to be coordinated to both oxygen and nitrogen ligands in biological systems. An example of nitrogen coordination is chlorophyll. The interaction of both Ca^{2+} and Mg^{2+} with biological polyelectrolytes, like nucleic acids, mucopolysaccharides in connective tissue, and other polysaccharides like alginate, is well known. As an example, magnesium ions are

TABLE I

Atomic and nuclear properties of the alkaline earths.

	^{9}Be	^{25}Mg	^{43}Ca	^{87}Sr	^{137}Ba
Spin (I)	3/2	5/2	7/2	9/2	3/2
Natural abundance (%)	100	10·1	0·145	7·02	11·32
NMR frequency at 6 T (MHz)[a]	35·90	15·64	17·19	11·07	28·39
Electric quadrupole moment (10^{-28} m^2)	0·05[a]	0·22[a]	−0·05[b]	0·36[c]	0·28[c]
Relative sensitivity of equal number of nuclei at constant field ($^{1}H = 1·00$)	$1·39\times10^{-2}$	$2·68\times10^{-3}$	$6·39\times10^{-3}$	$2·69\times10^{-3}$	$6·86\times10^{-3}$
Relative sensitivity ($^{13}C = 1·00$)	0·874	0·168	0·402	0·169	0·431
Relative receptivity at natural isotopic abundance ($^{13}C = 1·00$)	79	1·54	0·053	1·07	4·41
Sternheimer antishielding factor of M^{2+} ion[d]	0·81	4·32	13·12	41	123
Electronic configuration	(He)$2s^2$	(Ne)$3s^2$	(Ar)$4s^2$	(Kr)$5s^2$	(Xe)$6s^2$
Ionization potentials (kJ mol^{-1})[8] 1st	895	734	587	541	500
2nd	1746	1443	1140	1059	959
3rd	14000	9700	4930	4170	3580
"Absolute" standard enthalpy of hydration of cation at 298 K (kJ mol^{-1})[e]	−2487	−1922	−1592	−1445	−1304
"Absolute" standard entropy of hydration of cation at 298 K (J mol^{-1} K^{-1})[e]	—	−268	−209	−205	−159

[a] G. H. Fuller, *J. Phys. Chem. Ref. Data*, 1976, **5**, 835.

[b] Ref. 15; A. Rosén, private communication, 1980; see also discussion in the present article.

[c] Ref. 21 in Chapter 1 of Ref. 1.

[d] E. A. C. Lucken, *Nuclear Quadrupolar Coupling Constants*, Academic Press, London, 1969; K.D. Sen and P. T. Narasimhan, in *Advances in Nuclear Quadrupole Resonance*, Vol. 1 (ed. J. A. S. Smith), Heyden, London, 1974, p. 277.

[e] Data discussed in Chapter 7 of Ref. 62. On the scale chosen here the standard enthalpy of hydration of H^+ is −1090·8 kJ mol^{-1} and the standard molal entropy of H^+(aq.) is zero.

required for the biological activity of transfer RNA. The strong interaction of calcium ions with surfactants has been observed by everybody (lime soap!). It is an obvious, but unexplored, fact that there is a biologically significant interaction of both Mg^{2+} and Ca^{2+} with biological lipids.

TABLE II

Concentrations (ppm) of magnesium and calcium in rocks, water, and living materials.[8]

	Mg^{2+}	Ca^{2+}	Ratio[a]
Igneous rocks[b]	25000	40000	1:1
Sedimentary rocks[b]	10000	30000	1:1·8
Limestone	3000	300000	1:182
Sea-water	1400	400	1:0·2
Salmon (whole blood)	24	240	1:6·0
Myxine (whole blood)	440	240	1:0·28
Man (whole blood)	36	60	1:1
(plasma)	36	120	1:2
(interstitial fluid)	50	120	1:1·44
(cell fluid)	2640	4	1:0·0009
(muscle)[c]	75	1400	1:0·09
(nerve)[c]	750	280	1:0·23

[a] Calculated as relative numbers of atoms (corrected for relative atomic masses).
[b] Average values expressed as ppm of dry weight.
[c] ppm of whole tissue.

II. NMR PROPERTIES

A summary of some properties of the alkaline earths of relevance in the present context is given in Table I. The ratio between the Larmor frequencies of ^{25}Mg and ^{43}Ca on one hand and the Larmor frequency of ^{37}Cl on the other has been accurately determined in aqueous solutions of simple salts by Lutz and coworkers.[13] The Larmor frequencies given in Table I refer to the values for dilute aqueous solutions.

The NMR sensitivity of ^{25}Mg and ^{43}Ca, for equal numbers of nuclei, is certainly low but not exceedingly low, in particular not for ^{43}Ca. The receptivity at natural isotopic abundance of ^{43}Ca is, however, one of the lowest in the Periodic Table; only ^{57}Fe, ^{15}N, and a few other isotopes have lower values.[1] These circumstances can be greatly improved through the use of isotope enriched material, by a factor of about 10 for ^{25}Mg and by 500–600 for ^{43}Ca, in the latter case at a non-negligible cost.

The values of the electric quadrupole moments given in Table I deserve a few brief comments. It is not generally realized that accurate values of nuclear quadrupole moments may be exceedingly difficult to obtain. Their determination usually involves the measurement of a hyperfine splitting in the atomic spectrum of a particular isotope—a hyperfine splitting caused by nuclear electric quadrupole–electron interactions. The experimental parameter obtained is then the electric quadrupole interaction constant with the dimensions of frequency. In order to deduce the nuclear quadrupole

moment from this it is necessary to know the electric field gradient at the nucleus in the particular electronic state studied. Whereas the interaction constant in the case of ^{43}Ca is known to four significant figures, the electric field gradient (a quantity that must be calculated theoretically) is known with considerably less accuracy. A Hartree–Fock type calculation yields a value of $-0{\cdot}09 \times 10^{-28}$ m^2 for the electric quadrupole moment, but this is probably too large. The most refined theoretical calculations performed so far, using either multiconfigurational Hartree–Fock[14] or many-body perturbation theory,[15] indicate that the true value is approximately $-0{\cdot}050 \times 10^{-28}$ m^2.

It may be mentioned that this is not too far from a value whose modulus is $0{\cdot}06 \times 10^{-28}$ m^2, which was derived by us[16,17] using an entirely different approach, i.e. by comparing the experimental relaxation with that predicted by the theory of Hertz[18] and treating the nuclear quadrupole moment as an unknown parameter to be determined.

III. EXPERIMENTAL ASPECTS

The first observations of the magnetic resonances of ^{25}Mg,[19] ^{43}Ca,[20] ^{87}Sr,[21] ^{135}Ba,[22] and ^{137}Ba [22] were reported in the 1950s. In recent years, very precise NMR determinations of the magnetic moments—which are of great importance for establishing absolute shielding scales—have been performed by Lutz, Schwenk, *et al.* for ^{25}Mg,[13] ^{43}Ca,[13,23] ^{87}Sr,[24,25] ^{135}Ba,[26] and ^{137}Ba.[26] As a curiosity it may also be noted that the NMR signal of the radioactive ^{41}Ca isotope (half-life $1{\cdot}1 \times 10^5$ years) has been observed for a saturated aqueous solution of $Ca(NO_3)_2$.[27]

As is evident from Table I, the NMR frequencies of ^{25}Mg and ^{43}Ca, at B_0 fields that can be reached with iron core magnets, are very low. Few spectrometers with iron core magnets have in reality been satisfactorily equipped for the study of resonances at 6–7 MHz or lower frequencies. Partly for this reason, but certainly also because of the poor receptivity of ^{25}Mg and ^{43}Ca, few applications of NMR studies of these nuclei were reported before 1977.

With the use of cryomagnets, the NMR frequencies may be brought up to quite respectable values; on a spectrometer operating at a field of 11·7 T (500 MHz ^1H NMR frequency) the signals of ^{25}Mg and ^{43}Ca appear at 30·5 and 33·5 MHz respectively, and the detectability is significantly improved.

In their pioneering studies of ^{25}Mg NMR, Magnusson and Bothner-By,[28] using a frequency swept spectrometer operating at 3·67 MHz ($B_0 = 1{\cdot}41$ T), were able to obtain a satisfactory signal-to-noise ratio on 2 M $MgCl_2$ solutions (^{25}Mg in natural abundance). In 1969, Bryant[29] using similar equipment obtained good ^{43}Ca NMR spectra on 0·91 M aqueous solutions using 31·7% enriched ^{43}Ca.

Using a FT NMR spectrometer operating at a B_0 field of 1·8 T, Lutz *et al.*[13] in 1975 obtained a ^{25}Mg NMR signal with a signal-to-noise ratio of 7 : 1 on a ~8 mM solution of $Mg(ClO_4)_2$ after 57 000 transients (total time 190 min). In this context it should perhaps be emphasized that the concept of "detectability" is somewhat vague. If a true representation of the NMR lineshape is *not* desired then it is advantageous to pulse very rapidly,[59] resulting in an exaggerated linewidth. This technique may however be employed when for example only chemical shift changes are of interest. Whenever a true representation of the linewidth or lineshape is of importance, as for example in most biological applications of ^{25}Mg and ^{43}Ca NMR, more rigorous experimental requirements must be met. In order to avoid artificial broadening of an NMR signal in a FT NMR experiment it is necessary to sample the free induction decay (FID) for a time T_S that satisfies the condition $T_S > \pi T_2$ where T_2 is the time constant characterizing the exponential decay of the FID. If the decay of the FID is the sum of several exponentials it is the longest time constant that determines T_S.

Since NMR studies of physicochemical and biophysical problems usually involve a lot more than the mere detection of a signal, the use of ^{25}Mg and ^{43}Ca enriched samples offers enormous advantages. In fact few meaningful biophysical studies could be made without the use of enriched ^{43}Ca, and our investigations described below have been made with isotope enriched samples.

A further improvement of sensitivity in FT NMR studies arises from the use of cryomagnet spectrometers. Most commercial NMR spectrometers of this type are equipped with saddle-shaped Helmholtz type transmitter/receiver RF coils around the cylindrical sample tube, placed on the symmetry axis of the B_0 field. As pointed out by Hoult in particular,[30] the Helmholtz coils are inferior to solenoids in terms of achievable signal-to-noise ratio by a factor of about 2·5, all other things being equal. In order to pick up the rotating magnetization a solenoid must have its cylindrical axis perpendicular to the B_0 field, which means that the sample must be inserted sideways into the probe in a cryomagnet of the common type. This in turn usually implies that the probe must be taken out of the magnet every time a new sample is to be inserted. This may seem impractical but it is our experience in Lund—where for several years we have used a solenoid type probe in our ^{25}Mg and ^{43}Ca studies—that the time spent on sample exchange generally is a small fraction of the total time of an NMR run, particularly in biological studies.

In many applications of ^{25}Mg and ^{43}Ca NMR, the experimental linewidths (or the static quadrupolar splittings observed) may become very large—of the order of kHz. The accurate recording of spectra covering a broad spectral range with a pulse spectrometer is difficult since much of the information is contained in the very early part of the FID. The dead time of

the spectrometer must accordingly be very short, preferably of the order of 10 μs. If the rapidly decaying FID is caused by static interactions rather than by rapid transverse relaxation, many of the problems of a finite dead time can be overcome by the use of the "quadrupolar spin echo" technique.[31]

We give below some examples of studies performed on a home-built FT NMR spectrometer,[6,32] which well illustrate the sensitivity problems that occur practically. It is possible to obtain good signal-to-noise ratios after about 100 transients for simple aqueous solutions with 1 mM ^{43}Ca (61% enriched) and 5 mM ^{25}Mg (90% enriched). The instrument used employs a wide-bore 6 T superconducting magnet made by Oxford Instruments. In order to achieve the highest possible sensitivity the probes are of the solenoid type and sample tubes (usually containing about 3 ml of sample) are inserted perpendicularly to the cylindrical axis of the probe body.

It may be mentioned also that the two abundant isotopes of barium (^{135}Ba and ^{137}Ba) have very large quadrupole moments and extremely large quadrupole relaxation broadening even for the free aqueous ion. Thus barium NMR will have few chemical and certainly no biological applications. $^{87}Sr^{2+}$ also shows considerable line-broadening (although much less than Ba), and since its receptivity is very low it should be of little biological use. In a careful study[24] the aqueous Sr^{2+} ion has been investigated down to about 0·1 M, but in order to reasonably monitor line-broadening, accompanying binding to macromolecules, markedly higher concentrations are required.

IV. NMR PARAMETERS

Among the common NMR parameters of potential use for solutions, i.e. chemical shift, spin–spin coupling, relaxation times, and diffusion coefficient, one can directly rule out spin–spin coupling and diffusion coefficient for sensitivity and relaxation reasons. For problems of ion binding, self-diffusion studies are very attractive but spin-echo NMR is here ruled out by the low magnetogyric ratios of ^{25}Mg and ^{43}Ca, and quadrupole relaxation effects are also unfavourable for such studies.

The chemical shift range of ^{25}Mg is very small, and chemical shift effects on binding in aqueous systems are, especially for macromolecular systems, probably always unobservably small compared with quadrupole relaxation effects (paramagnetic systems may be an exception under certain conditions); for non-aqueous systems, marked chemical shift changes between different solvents and on ion-pairing have been observed (see below). For ^{43}Ca the balance is more in favour of chemical shift effects because of the larger number of electrons extending the shift range as well as a smaller quadrupole moment. Chemical shift changes on binding to compounds with

small volume are often observable while the interaction with macromolecules is much more difficult to monitor in this way.

Relaxation mechanisms possible for ions in diamagnetic systems are mainly magnetic dipole–dipole relaxation, quadrupole relaxation, chemical screening anisotropy relaxation, spin–rotation relaxation, and scalar relaxation. It is generally assumed, on the basis of the sizeable quadrupole moments and the small magnetic moments, that only quadrupole interactions contribute significantly to relaxation, but this has not been examined systematically.

For ^{25}Mg (and even more so for the Sr and Ba isotopes) the sizeable quadrupole moment leaves little doubt that quadrupole relaxation is strongly predominant, and this is supported by the more rapid relaxation in D_2O than in H_2O observed by Simeral and Maciel.[33] For ^{43}Ca, possible contributions from other relaxation mechanisms may be worthy of examination.

For anisotropic solutions, like liquid crystalline phases, the use of quadrupole splittings has developed into a powerful method in the study of ion binding mainly for alkali and halide ions.[34] Similar experiments have not been reported for $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ although it would seem relatively straightforward to obtain the quadrupole splittings provided that systems can be found which can stand the presence of divalent ions; studies of this type should be of interest in surfactant science as well as in membrane science. The general theory directly applicable to Mg^{2+} and Ca^{2+} has been reviewed.[34]

V. QUADRUPOLAR EFFECTS FOR SPIN 5/2 AND 7/2 NUCLEI

The appearance of an NMR spectrum of a quadrupolar nucleus will depend on whether the nucleus moves in an isotropic or anisotropic system. In anisotropic systems, for example a solid or a liquid crystalline phase, the spectrum may consist of several peaks. The separation of the peaks—the quadrupole splitting—gives information on orientation effects, at both a microscopic and a macroscopic level, and in the case of a quadrupolar ion, on the mode of ion binding. In isotropic systems only a single resonance signal will be observed. The shape of the signal may, however, in certain circumstances deviate from the simple Lorentzian lineshape, and the decay of both the transverse and the longitudinal magnetization may be multiexponential. In such cases detailed lineshape and/or relaxation studies provide separate information on the quadrupole coupling constant and the correlation time characterizing the fluctuation of the electric field gradient.

Quadrupole relaxation is caused by the interaction of the nuclear electric quadrupole moment, eQ, with fluctuating electric field gradients produced

by the environment. The field gradients may be either intramolecular or intermolecular in origin, and the efficiency of the relaxation will depend on both the magnitude of the field gradients and the rate at which they fluctuate. The fluctuations are usually characterized by the ensemble average correlation between the field gradient at various times. When this correlation function is simple and decays exponentially with the time interval, the time constant for the decay, τ_c, may be used to characterize the fluctuation. When τ_c is much smaller than the inverse Larmor frequency, ω, i.e. $\omega\tau_c \ll 1$, the decays of the longitudinal and transverse magnetizations of a quadrupolar nucleus are equal and both exponential. It is thus possible to define relaxation rates $R_1 = 1/T_1$ and $R_2 = 1/T_2$ which can be shown to be given by[35,36]

$$R_1 = R_2 = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi^2(1+\eta^2/3)\tau_c \tag{1}$$

where χ is the nuclear quadrupole coupling constant (in Hz) defined by

$$\chi = e^2 q_{zz} Q/h \tag{2}$$

where q is the electric field gradient at the nucleus with q_{zz} as the biggest component, e is the charge of the electron, and Q is the nuclear quadrupole moment; η is an asymmetry parameter for the field gradient and lies in the range $0 < \eta < 1$. In molecules, the asymmetry parameter rarely exceeds 0·5—in which case $(1+\eta^2/3) \approx 1{\cdot}08$—and very likely the situation in ionic Mg^{2+} and Ca^{2+} complexes is not much different. This means that the asymmetry parameter in equation (1) may be neglected for all practical purposes.

Situations with non-exponential correlation functions are frequently encountered, for example in solutions where an ion interacts with a polyelectrolyte. In such situations it may often be useful to describe relaxation as being determined by two processes, one a rapid local motion that has the effect of partly reducing the quadrupole coupling and the other a slow overall motion (for example the reorientation of the polyelectrolyte) that averages out the remainder of the quadrupole interaction. If the time scales of the two motions are sufficiently different the relaxation rate can be written as the sum of two terms corresponding to the two motions.[37] The slower motion generally gives the main contribution and the relaxation rate may then be written as

$$R_1 = 1/T_1 = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} (\chi S)^2 \tau_{c,\text{slow}} \tag{3}$$

where S is an order parameter characterizing the rapid motion.[37]

In non-extreme narrowing situations (i.e. $\omega\tau_c \geqslant 1$) the concept of relaxation times is no longer valid; we must instead consider explicitly the decays

of the transverse (M_T) and longitudinal (M_L) magnetizations.[38] These decays may be written[38-41]

$$\Delta M_L(t) = \Delta M_L(0) \sum_{i=1}^{N} c_i \, e^{-k_i t} \tag{4}$$

$$M_T(t) = M_T(0) \sum_{i=1}^{N} d_i \, e^{-l_i t} \tag{5}$$

($N = 2$ for $I = 3/2$; $N = 3$ for $I = 5/2$; $N = 4$ for $I = 7/2$)

The general behaviour of spin $I = 5/2$ and $7/2$ nuclei turns out to be much more complicated than that of spin $I = 3/2$ nuclei. In the latter case the preexponential factors in equations (4) and (5) do not depend on the parameters that determine the relaxation (ω, τ_c, etc.) and analytical expressions may be derived for the magnetization decays. By contrast, for spin $I = 5/2$ and $7/2$ the exponential factors as well as the preexponential factors depend on the relaxation parameters. Exact analytical solutions are intractable and one has to rely on numerical solutions.

Bull *et al.*[41] have considered the relaxation of $I = 5/2$ and $7/2$ nuclei engaged in a two-site chemical exchange. Under conditions where the probability of finding the nucleus in one site (B)—which may be considered as a macromolecular binding site—is very much smaller than that of the other site (A) the relaxation behaviour may be described with the same number of exponentials as for non-exchanging spins. Some illustrative results of the calculations[41] are given in Figs. 1 and 2. The curves have been calculated under the assumption that the site A is in the extreme narrowing limit ($\omega\tau_c = 10^{-3}$) while for the nuclei in the less abundant site, B, $\omega\tau_c$ is chosen to be 5·0. The values of the exponential and preexponential factors are shown as a function of $p_B\chi_B^2/p_A\chi_A^2$ where χ_A and χ_B are the quadrupole coupling constants for sites A and B respectively.

It may be inferred from Figs. 1 and 2 that in many regions the relaxation is dominated by a single exponential. Accordingly it may be very difficult to establish experimentally the nonexponentiality. Whereas non-exponential behaviour for spin $I = 3/2$ nuclei is frequently observed (see refs. 4 and 42), so far no clear-cut example of non-exponential relaxation of spin $I = 7/2$ nuclei has been reported. In the $^{43}Ca^{2+}$ experiments in biological systems performed in our laboratory the observed line shapes are virtually Lorentzian. On the other hand, the apparent T_1 of ^{43}Ca, as determined by pulse experiments, is not equal to the apparent T_2 as obtained from the lineshape, and non-extreme narrowing effects are clearly indicated.

The case of spin $I = 5/2$ nuclei is intermediate between the $I = 3/2$ and $7/2$ situations. Non-exponential behaviour has not been observed but differences in apparent T_1 and T_2 are encountered. In view of the latent information content in an established non-exponential relaxation behaviour

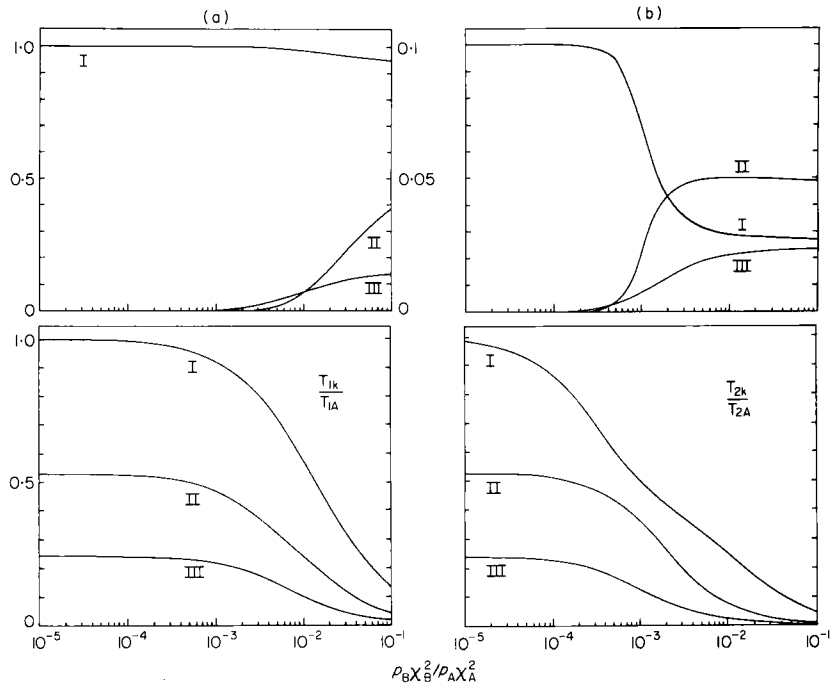


FIG. 1. (a) The *longitudinal* relaxation of a spin $I = 5/2$ nucleus undergoing rapid chemical exchange between two sites A and B, the A site assumed to be in the extreme narrowing limit ($\omega\tau_c = 10^{-3}$) and the B site representing a binding site of a biological macromolecule with a long correlation time ($\omega\tau_c = 5{\cdot}0$). The normalized amplitudes (coefficients c_i in eq. 4) are given in the upper plot and relaxation times (k_i^{-1} in eq. 4) in the lower plot as functions of the ratio $p_B\chi_B^2/p_A\chi_A^2$, the ratio of the fractions of nuclei in each site (p) times the ratio of the squares of the quadrupole coupling constants (χ). The relaxation times are given as a fraction of that for the free ion, i.e. that of the A site in the absence of exchange. Note that the amplitudes of components II and III are plotted on the expanded scale to the right and that the relaxation may be described approximately as a single exponential (I).[41]

(b) The *transverse* relaxation of a spin $I = 5/2$ nucleus undergoing rapid exchange between two sites A and B with the same relaxation characteristics as in (a). The normalized amplitudes (coefficients d_i in eq. 5) are given in the upper plot and the relaxation times (l_i^{-1} in eq. 5), given as a fraction of that for the free ion, are shown in the lower plot. As in (a), the relaxation data are given as functions of the ratio $p_B\chi_B^2/p_A\chi_A^2$.[41]

(it allows the separate determination of the correlation time τ_c and the quadrupole coupling constant χ) further work on the analysis of magnetization decays in $I = 5/2$ and $7/2$ spin systems appears of importance. In the case of spin $I = 3/2$ nuclei a linearization of the magnetization decays has given expressions for effective T_1 and T_2 that are valid—and very useful—in the region $\omega\tau_c \leqslant 1{\cdot}5$.[45,46] A perturbation treatment of $I = 5/2$ and $7/2$ systems with similar objectives in mind is being developed in our laboratories by Halle.[47]

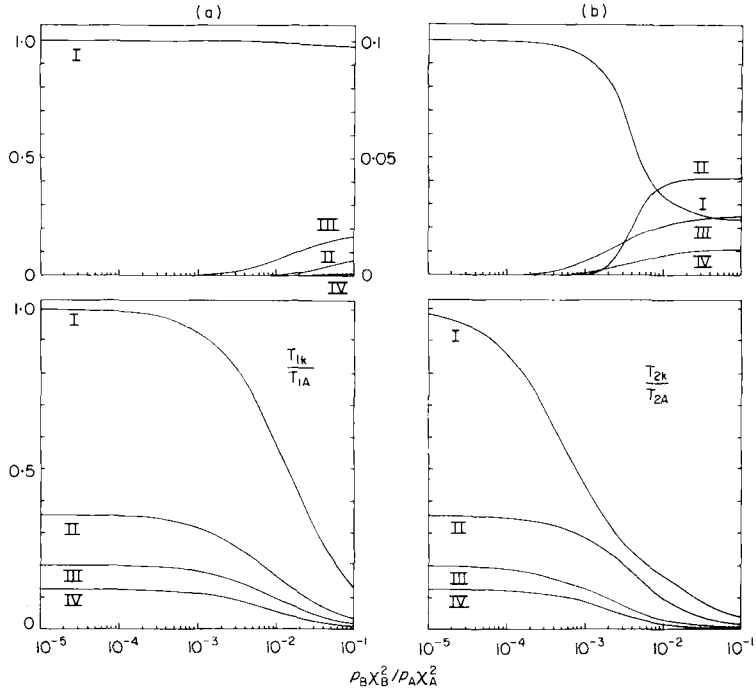


FIG. 2. (a) The *longitudinal* relaxation of a spin $I = 7/2$ nucleus undergoing rapid chemical exchange between two sites A and B with the same relaxation characteristics as in Fig. 1a. The normalized amplitudes (c_i of eq. 4) are given in the upper plot and the relaxation times (k_i^{-1} of eq. 4) are given in the lower plot as functions of $p_B\chi_B{}^2/p_A\chi_A{}^2$ (see the legend of Fig. 1a). Note that the amplitudes of components II–IV are plotted on the expanded scale to the right and that one component (I) dominates the relaxation.[41]

(b) The *transverse* relaxation of a spin $I = 7/2$ nucleus undergoing rapid chemical exchange between two sites A and B with the same relaxation characteristics as in Fig. 1a. The normalized amplitudes of the components (coefficients d_i in eq. 5) are given in the upper plot and the relaxation times (c_i^{-1} in eq. 5) are given in the lower plot as fractions of that for the free ion (the A site in the absence of exchange).[41]

Since for the types of problems of interest to us the ions sample, as a function of time, different environments which are characterized by different values of χ and τ_c and thus of T_1 and T_2, it is appropriate to add a few comments to the special situation for spin 5/2 and 7/2 nuclei described above. The observable NMR signals contain contributions from all the different sites that the ions occupy, and relaxation is dependent on the τ_c and χ values as well as on the distribution of ions over the different sites and on the average lifetimes of the ions in the sites. In refs. 4 and 36 we have rather thoroughly discussed exchange effects for quadrupolar nuclei and methods to identify different exchange conditions, and only a few points are made here.

If relaxation in all the different sites occurs under extreme narrowing conditions and one site (typically the free aqueous ion) is much more populated than the others, relaxation is given by the following equation attributed to Swift and Connick[48] but in fact earlier given by Hertz[49]

$$1/T_1 = 1/T_{10} + \sum p_i/(T_{1i} + \tau_{ex,i}) \quad (6)$$

and an equivalent equation for T_2. Here T_{10} is the relaxation rate of the predominant site while the p_i are the populations of different "bound" sites ($p_i \ll 1$) having intrinsic relaxation times T_{1i}. $\tau_{ex,i} = k_{off,i}^{-1}$ is the average lifetime for the ion in site i. With reference to equation (6) it is convenient to introduce an "excess" relaxation rate (subscript ex) as the difference between the observed relaxation rate and that of the free ion.

For a two-site situation with only bound (B) and free (A) ions, equation (6) reduces to ($R = 1/T_1$ or $1/T_2$)

$$R_{ex} = R_{obs} - R_A = p_B/(R_B^{-1} + k_{off}^{-1}) \quad (7)$$

If B is a state on a macromolecule, R_B is normally so large that the separate observation of the NMR signal from the bound ion is very difficult. In the limit of slow exchange the binding can therefore not be recorded by the NMR method. Exchange has to be sufficiently rapid so that k_{off}^{-1} is non-negligible compared with R_B^{-1}, which is estimated to be 10^3–10^4 s^{-1} for ^{25}Mg and ^{43}Ca, to allow the binding to be observed. k_{on} will be strongly dependent on whether ion dehydration occurs on binding, the maximal diffusion controlled rate being 10^8–10^9 M^{-1} s^{-1}; in the case of dehydration the value will be several orders of magnitude lower. With a required $k_{off} \geqslant 10^3$ s^{-1} one obtains for the binding constant $K \leqslant 10^5$ M^{-1} for relaxation effects to be observable; if k_{on} is reduced the limiting K is also reduced. It can be seen that the method is inapplicable for the strongest type of binding but it may work well for binding of intermediate strengths.

VI. RELAXATION OF AQUEOUS ALKALINE EARTH IONS

A. Infinite dilution relaxation rates

There are a number of studies of alkaline earth relaxation in aqueous solution but owing to the difficulty in reaching low concentrations as well as instrumental broadening in linewidth studies there is still some uncertainty in the exact infinite dilution relaxation rates ($R_1 = R_2$). Several studies of $^{25}Mg^{2+}$ linewidths have been reported but that of Simeral and Maciel[33] is probably the one least affected by instrumental broadening. These authors obtain $1/T_2^0 \approx 8{\cdot}5$ s^{-1} (superscript 0 is used to indicate infinite dilution). Our own studies of T_1 suggest a somewhat lower value, $1/T_1^0 \approx 6$ s^{-1} at 25 °C. Recently, a more detailed study[61] of $^{25}Mg^{2+}$ relaxation as a function

of salt concentration has given an improved value of $1/T_1^0 = 4{\cdot}5 \pm 0{\cdot}2\ s^{-1}$ at 25 °C. For $^{43}Ca^{2+}$, linewidth studies[13,23,33] are not accurate enough to establish T_2^0. We have obtained $1/T_1 = 0{\cdot}75\ s^{-1}$ for 0·2 M $CaCl_2$ and this is probably close to $1/T_1^0$. Banck and Schwenk[24] obtained from linewidth studies $1/T_2^0 = 205 \pm 40\ s^{-1}$ for $^{87}Sr^{2+}$. Lutz and Oehler[57] obtained by extrapolation from the linewidth for barium halide solutions $1/T_2^0 = 1700 \pm 160\ s^{-1}$ for $^{135}Ba^{2+}$.

There are two main theoretical approaches to ion quadrupole relaxation, the "electrostatic" approach of Hertz,[18] considering the field gradients from nearby ions and solvent molecules treated as point charges and dipoles, and the "electronic distortion" model of Deverell,[50] considering field gradients due to distortions from spherical symmetry of the electron cloud which are produced in collisions by short-range overlap repulsive forces. There are difficulties involved in the testing of the latter model while for the infinite dilution case, where only solvent molecules cause relaxation, the relaxation rates may be given by relatively simple expressions for the electrostatic model. An attempt to compare experimental and theoretical data for the alkaline earths was made in ref. 16. For weakly solvated ions, the "fully random distribution model", which assumes random orientation and distribution of solvent dipoles, is most relevant while for strongly solvated ions the "fully oriented solvation model" should apply. With cubic symmetry of the solvate complex the contribution from the first solvation sphere vanishes and the main contributions to the quadrupole relaxation come from the second solvation layer. For the alkaline earths one would expect the truth to lie somewhere in between these two extremes, and the observed relaxation rates do lie in between the values predicted by the two models. The observed relaxation rates are in close agreement with predictions of the electrostatic model and therefore provide good support for it.[16] ($^{43}Ca^{2+}$ is an exception to these conclusions in ref. 16 and falls outside the predicted range, but this is due to an erroneous quadrupole moment, $0{\cdot}2 \times 10^{-28}\ m^2$, then available; using the recently determined value, $-0{\cdot}05 \times 10^{-28}\ m^2$, very good agreement is obtained.) In the recent $^{25}Mg^{2+}$ relaxation study of Holz *et al.*[61] the authors have attempted a more detailed analysis in terms of the electrostatic theory and infer a near cubic symmetry of the inner hydration sphere of Mg^{2+}.

B. Concentration dependence of relaxation

The ion–ion contribution to relaxation is much more difficult to account for theoretically. One reason is that, for strongly solvated ions characterized by a partial or total symmetry quenching of the field gradient due to the solvent, a change of this quenching will be affected by surrounding ions in a

way that is difficult to estimate.[51–53] As regards the direct ion–ion effect on relaxation (where the ionic charge directly produces a field gradient) it is important to consider quenching of the field gradients from the ions due to an ion cloud effect.[51]

Little information is available on the ion–ion contributions to relaxation, and thus we have at present little chance of deducing which ion–ion effects are most important for relaxation. For $^{25}Mg^{2+}$, Lutz *et al.*[13] and Simeral and Maciel[33] have studied the concentration dependence of the linewidth for a few cases. The concentration dependence is found to be weak and the difference between different anions small. According to Simeral and Maciel[33] the sequence of increasing linewidth is $Cl^- < ClO_4^- < Br^- < NO_3^-$, while Lutz *et al.*[13] found ClO_4^- and Br^- to change places. Heubel and Popov[54] mention results in agreement with those of Simeral and Maciel;[33] additionally these authors report that line broadening is much more marked with acetate than with the inorganic anions. Holz *et al.*[61] have recently presented a detailed study of the concentration dependence of $^{25}Mg^{2+}$ relaxation for $MgCl_2$ and $Mg(NO_3)_2$ solutions. The relaxation increases more rapidly with concentration in the latter case; for example, $1/T_1$ for 4 molal solutions is 18 s^{-1} for chloride and 45 s^{-1} for nitrate. For $^{85}Sr^{2+}$ the sequence is $Br^- < Cl^- < NO_3^- < ClO_4^-$ according to Banck and Schwenk.[24] The irregular sequences may indicate that "unquenching" effects in combination with direct ion–ion effects are important. For Ca^{2+} and Ba^{2+} there are as yet no reports on the concentration and anion dependence of relaxation.

VII. RELAXATION IN NON-AQUEOUS SOLVENTS

Only $^{25}Mg^{2+}$ relaxation has been investigated for a few non-aqueous solvents by Heubel and Popov[54,55] These authors found, for certain solvents like tetrahydrofuran, propylene carbonate, acetone, and dimethylformamide, $^{25}Mg^{2+}$ linewidths of $MgCl_2$ or $MgBr_2$ exceeding 100 Hz, indicating strong cation–anion interactions (probably contact ion-pairing); the linewidths are particularly large for poorly solvating solvents. On addition of iodine, trihalide ions are formed and this leads to a marked line-narrowing which may be explained in terms of a looser ion pair with the bulkier anion. ^{25}Mg NMR seems promising for the monitoring of ion pair formation and for differentiating between contact and solvent-separated ion pairs. Heubel and Popov[54] also observed a line-broadening in ^{25}Mg NMR on addition of a cryptand to a solution of $MgCl_2$ in methanol, while addition of a crown ether to Mg^{2+} in dimethylformamide gave no effect, indicating in the latter case that there is no complexation.

VIII. CHEMICAL SHIFTS IN AQUEOUS AND NON-AQUEOUS SOLUTIONS

Four aspects are of interest concerning calcium and magnesium chemical shifts (a low frequency shift is here taken as negative): the absolute shielding of the ions relative to the free atoms; the chemical shift effects exerted by the solvent and by other ions and molecules in the solution; the possibility of using the chemical shift as a mere titration indicator in studies of chemical association phenomena; and, finally, theoretical approaches which permit a rationalization of observed chemical shifts. Unfortunately, as regards all these matters we have as yet only a small amount of information.

The shielding of monoatomic ions in solution arises from short-range interactions and depends on adjacent solvent molecules and ions. It seems reasonable to believe that for both ^{25}Mg and ^{43}Ca the shielding differences are controlled by changes in the paramagnetic shielding term. Kondo and Yamashita[56] have put forward a theory which may qualitatively account for shielding effects of the alkali and halide ions.[2,36,50] For the alkaline earths the available data are insufficient for a meaningful correlation with theory. In the model, shielding is determined by three factors, i.e. an average excitation energy, the expectation value of r^{-3} for an outer p electron, and sums of overlap integrals of outer p orbitals of the ion studied and outer orbitals of other ions or solvent molecules.

For the theoretical interpretation of chemical shift data it is important to know the absolute nuclear screening scale, for example to have the shielding of the aqueous ion at infinite dilution relative to that of the free atom. Such determinations for several nuclei have been made by Lutz and coworkers from a combination of their precise measurements of resonance frequencies with optical pumping data; unfortunately the latter are difficult to obtain with the required precision and this mainly causes the current imprecision in the absolute shielding scales. Lutz *et al.*[13,23] obtained, for $^{43}Ca^{2+}$, $\sigma_{M^{2+},aq} - \sigma_{M,free} = -(2 \pm 5) \times 10^{-4}$, while Sahm and Schwenk[25] obtained, for $^{87}Sr^{2+}$, $-(6{\cdot}3 \pm 6{\cdot}0) \times 10^{-4}$; thus in these cases it is not possible even to obtain the sign with certainty. For Ba^{2+} the situation is better though. Here Lutz and Oehler[57] obtained the values $-(8{\cdot}2 \pm 0{\cdot}2) \times 10^{-4}$ and $-(8{\cdot}0 \pm 0{\cdot}2) \times 10^{-4}$ for ^{135}Ba and ^{137}Ba respectively. As expected there is no significant isotope effect on the shielding. The values for Ca, Sr, and Ba do not prove, but are consistent with, an expected increase in absolute nuclear shielding with increasing atomic number.

The most studied aspect of the chemical shifts concerns their concentration dependence in aqueous solutions, and reports for $^{25}Mg^{2+}$,[13,33,58] $^{43}Ca^{2+}$,[13,23] $^{87}Sr^{2+}$,[24] and $^{135}Ba^{2+}$[57] have been presented. Simeral and Maciel[33] as well as Lutz *et al.*[13] observed small $^{25}Mg^{2+}$ chemical shifts (less than 0·5 ppm up to 4 molal) with concentration but the anion (NO_3^-, ClO_4^-,

Cl^-, Br^-) sequences obtained are somewhat different. For $^{43}Ca^{2+}$, Lutz *et al.*[13,23] find that the chemical shifts follow the anion sequence $NO_3^- < ClO_4^- <$ infinite dilution $< Br^- < Cl^-$ with a shift range surpassing 20 ppm at the highest concentrations. The same anion sequence is found for $^{87}Sr^{2+}$ but here the chemical shifts change more rapidly with concentration.[24] The latter is in agreement with observations for other groups in the Periodic Table, namely that the chemical shift range increases with increasing atomic number. An increased shielding with oxyanions and a decreased one with halide ions agrees with observations for the alkali ions.[2] In terms of the Kondo–Yamashita model[56] there is decreased overlap with oxyanions and increased overlap with halide ions. For $^{135}Ba^{2+}$, Lutz and Oehler[57] were unable to establish significant chemical shift effects with concentration for the halides (up to 1·6 molal). Furthermore, Lutz and coworkers[13,57] in comparing H_2O and D_2O as solvents have been unable to obtain an isotope effect outside the experimental error (2 and 5 ppm, respectively) for $^{43}Ca^{2+}$ and $^{135}Ba^{2+}$.

Magnesium NMR in non-aqueous solvents has been explored by Heubel and Popov[54,55] with some interesting results. Rather large effects of solvent and salt concentration are observed in a number of cases. For example, for $MgBr_2$ in acetonitrile there is (with increasing salt concentration) a marked high frequency shift (up to ~20 ppm) and similar behaviour is found for $MgCl_2$ in propylene carbonate. On addition of I_2 to these solutions there is, as shown in Fig. 3, a strong low frequency shift with a rather well defined stoicheiometry of one I_2 per halide ion. This is ascribed to dissociation of contact ion pairs on formation of trihalide ions; with only solvent molecules as nearest neighbours the concentration dependence of the chemical shift is weak. For low dielectric constant solvents, the chemical shift depends on concentration even with the trihalide ions; here the electrostatic interaction is strong enough to induce some contact ion-pairing even with trihalide ions.

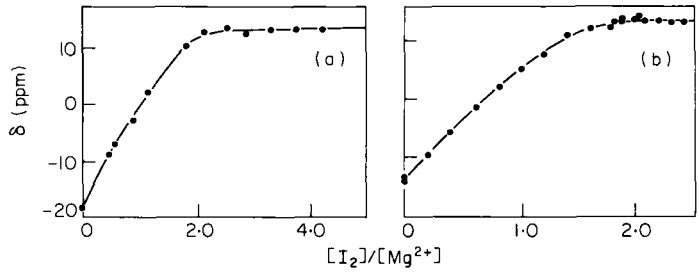


FIG. 3. Effect on the ^{25}Mg chemical shift of addition of I_2 to (a) $MgBr_2$ in acetonitrile and (b) $MgCl_2$ in propylene carbonate. The chemical shift, low frequency taken as positive, is given as a function of the molar ratio of iodine to magnesium.[54]

Attempts were made by Heubel and Popov[54] to study other types of association as well, but addition of either a cryptand in methanol or a crown ether in dimethylformamide does not produce a large ^{25}Mg nuclear screening change. For $^{43}Ca^{2+}$ in non-aqueous solution it has only been reported[13] that methanol gives a 17 ppm higher shift than water; apparently this should open possibilities for studies of solvation (including preferential solvation) phenomena and ion-pairing in non-aqueous media.

IX. COMPLEX FORMATION WITH LOW MOLECULAR WEIGHT COMPOUNDS

Mg^{2+} and Ca^{2+} form complexes with a large variety of ligands,[60,62] for example carboxy and phosphate groups and hydroxy groups of carbohydrates, to mention just a few. Many low molecular weight complexes with such ligands have great biological, pharmaceutical, and technical importance, and their stability and kinetic properties are of interest. NMR studies of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ have as yet been little utilized for complexation studies although such studies should be very useful. Detailed NMR studies of small Mg^{2+} and Ca^{2+} complexes are also important for the interpretation of NMR results for macromolecular systems.

Complexation equilibria of the general type

$$M + C \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} MC \tag{8}$$

where M is a small quadrupolar ion and C is a low molecular weight compound, are usually associated with a change in the nuclear screening and/or the relaxation rate of the M nucleus. In the limiting case when the off-rate, k_{off}, is very much larger than both the nuclear screening difference $\Delta\omega$ in radians s^{-1} between the "free" M nucleus and the M nucleus in the MC complex, and the relaxation rates of the two sites, only one averaged NMR signal is observed. The nuclear screening and relaxation parameters of this signal will then simply be a weighted average of the values of the "free" and complexed nuclei and may be changed by varying the composition of the solution. Such studies often allow a determination of the equilibrium constant(s) as well as the intrinsic NMR parameters of the MC complex. In the other extreme case, when k_{off} is very much smaller than the nuclear screening difference, separate NMR signals from the "free" solvated M and from the MC complex will be observed.

Under conditions intermediate between these two limiting cases the exchange rate will directly influence the observed NMR spectrum, and through a variable temperature study the rate constant may generally be calculated. Such a calculation is by no means trivial since the relaxation rate

of the M nucleus as well as the binding constant, k_{on}/k_{off}, will also change with temperature.

The relaxation of ^{25}Mg and ^{43}Ca in low molecular weight complexes usually occurs under extreme narrowing conditions. Although this may simplify the theoretical treatment of the lineshape in the intermediate exchange region and thus render the calculation of rate constants less cumbersome, it is a distinct disadvantage in other respects. Thus only the product of the square of the quadrupole coupling constant (χ) and the correlation time (τ_c) can be determined from the relaxation rate of the M nucleus in the MC complex. Under conditions of non-extreme narrowing these quantities may often be determined separately (see Section V). To calculate χ for the M nucleus in the MC complex the value of τ_c may either be taken from hydrodynamic theory (for example using the Debye–Stokes–Einstein equation) or determined through an NMR relaxation study of some nucleus (^1H, ^2H, ^{14}N, ^{13}C, etc.) in the ligand C. The degree of rigidity of the MC complex becomes a matter of importance in such types of analysis.

^{25}Mg and ^{43}Ca NMR studies of complex formation in the pre-FT era were mostly of an exploratory nature.[28,29,63,64] The study by Magnusson and Bothner-By[28] however amply demonstrated that the interaction of Mg^{2+} with a large variety of ligands, di- and tri-carboxylic acids, hydroxy-carboxylic acids, amino acids, inorganic and organic phosphates, etc. could be monitored through ^{25}Mg NMR. Similarly, Bryant[29] demonstrated the applicability of ^{43}Ca NMR for investigating complexing of Ca^{2+} with ATP and pointed to its usefulness in studies of calcium-binding proteins—a prediction that has only recently been borne out owing mainly to large experimental difficulties (see Section XI).

In 1972 Bryant[63] made the first detailed ^{25}Mg NMR study of the interaction between ATP and Mg^{2+}. Mg^{2+} concentrations in the molar and ATP concentrations in the millimolar range were used. The ^{25}Mg linewidth was found to decrease with increasing temperature. A substantial chemical exchange contribution to the linewidth at lower temperatures was inferred and the decrease in linewidth attributed to exchange effects. A fitting of the experimental data to a two-site exchange model gives $k_{off} = 2 \times 10^4\ s^{-1}$ and a nuclear screening difference of 260 ppm between "free" and complexed Mg^{2+}. In the light of more recent studies of counterion and ligand effects on $^{25}Mg^{2+}$ chemical shifts in aqueous solutions (Section VIII)—which show the chemical shift to be very insensitive to the formation of complexes—this screening difference is most likely too large by two orders of magnitude. An alternative interpretation of the experimental results must be sought. The temperature dependence of the ^{25}Mg relaxation rate in the Mg–ATP complex (as well as of the complex formation constant) must probably be taken into account. Furthermore ATP slowly decomposes into ADP (and AMP) and inorganic phosphate at elevated temperatures.

Robertson *et al.*[65,66] have, with improved experimental conditions, investigated the binding of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ to γ-carboxyglutamic acid (Gla) containing tripeptides and to a larger polypeptide—fragment 1 of prothrombin (see also Section XI). The recently discovered amino acid Gla has been found in Ca^{2+} binding proteins involved in blood coagulation. The coagulation system is very complex and involves a cascade of biochemical reactions some of which involve protein–membrane interactions. It is generally postulated that these interactions are electrostatic in nature and that Ca^{2+} ions form bridges between the polar region of the phospholipids and Gla residues on the protein.[67]

Robertson *et al.* employed magnesium and calcium enriched in the isotopes ^{25}Mg (98·25%) and ^{43}Ca (79·98%) which allowed the NMR studies to be performed at ion concentrations of about 20 mM. The addition of the di- and tri-peptides—each containing two neighbouring Gla residues—causes considerable increase in the ^{25}Mg linewidth but essentially no chemical shifts.[65,66] From the concentration dependence of the linewidth, the dissociation constants of the 1 : 1 complexes are calculated to be about 0·6 mM at pH 6·5–6·8.

For ^{43}Ca the experimental conditions preclude the observation of linewidth variations but appreciable nuclear screening changes are observed and a dissociation constant of about 0·6 mM is calculated for the complex between Ca^{2+} and the dipeptide Z-D-Gla-D-Gla-OMe (Z = benzyloxycarbonyl).

The pH dependence of the ^{25}Mg linewidth in the presence of the tripeptides was studied by Robertson *et al.*[66] (see Fig. 4). The resulting titration

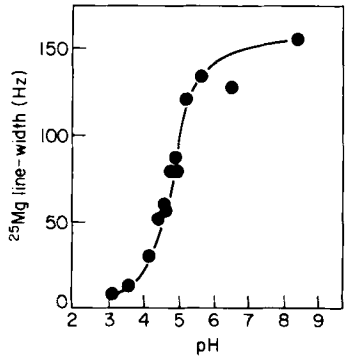
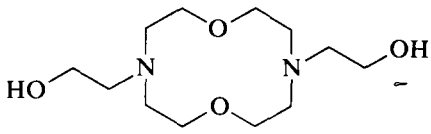


FIG. 4. The pH dependence of the ^{25}Mg linewidth in the presence of the tripeptide L-Arg-D-Gla-D-Gla-OMe (Gla = γ-carboxyglutamic acid). The conditions of the experiment are: Mg^{2+} concentration = 18·1 mM; peptide concentration = 28·9 mM; 98·25% isotope enriched ^{25}Mg was used and the spectra were obtained on 4·0 ml samples on a Varian XL-100 FT NMR spectrometer.[66]

curves show inflection points in the pH range 4·6–4·8. From comparisons with known pK_a values for the four deprotonation steps of other –Gla-Gla– fragments it was concluded that deprotonation of the third sidechain carboxylic acid group of the tripeptides is necessary for significant binding of Mg^{2+} to occur.[66]

In a recent FT NMR study[68] the interaction of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ with diketo ligands (diacetyl, pentane-2,4-dione, hexane-2,5-dione) has been investigated. No isotope enrichment was used and studies were made on 1·0 M aqueous solutions of MgI_2 and CaI_2 containing 0·02 M of the diketone ligands. Under these conditions a ~13 ppm shift of the ^{43}Ca resonance to low frequency was observed for all three ligands. By contrast no shift of the ^{25}Mg signal was observed. Low frequency ^{43}Ca shifts in the presence of several oxyanions, for example formate and lactate, have been observed by Lutz *et al.*[13]



[1]

Separate ^{43}Ca NMR signals from free and liganded Ca^{2+} have been observed[69] in solutions containing an excess of the ligands EDTA, EGTA, and [1] (see Fig. 5). For these ligands the affinity for Ca^{2+} is very high ($\log K = 10{\cdot}6$ for $EDTA^{4-}$ and 10·9 for $EGTA^{4-}$;[70] for the non-ionic complex with [1] $\log K \approx 6{\cdot}9$ [71]) and the off-rate can be estimated to be of

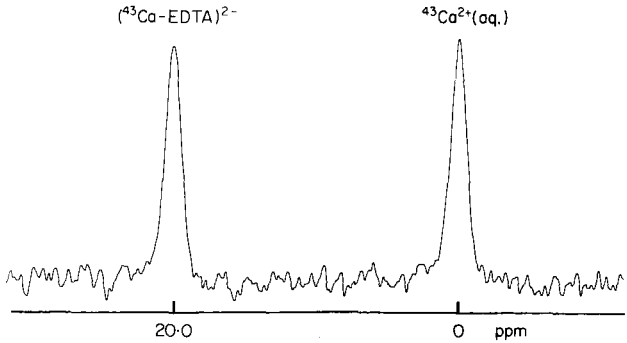


FIG. 5. The room-temperature ^{43}Ca NMR spectrum of an aqueous solution containing 4 mM Ca^{2+} and 2 mM EDTA at pH 7·0. The spectrum was obtained at 17·2 MHz on a FT NMR spectrometer built at Lund University and equipped with a wide-bore 6·0 T cryomagnet. About 4 ml of sample was used and the spectrum was obtained after 1000 transients or 17 min; 61% isotope enriched ^{43}Ca was used.

the order of $10\text{–}10^{-2}\,s^{-1}$. Thus clearly we have here a slow exchange situation. The ^{43}Ca chemical shift differences observed between "free" and liganded Ca^{2+} are given in Table III. In all cases the ^{43}Ca signal from the complex occurs at a higher frequency than the "free" signal.

The T_1 values for the ^{43}Ca signals from the complexes were measured using the inversion recovery method on solutions with Ca^{2+} concentrations of about 4 mM. The results are summarized in the second column of Table III. The T_1 value for the EDTA complex is surprisingly long.

TABLE III

^{43}Ca NMR parameters for some Ca^{2+} complexes at 23 °C.[69] ^{43}Ca chemical shifts are reported relative to the "free" signal and shifts towards higher frequency are taken as positive. The ^{43}Ca data were obtained on aqueous solutions with calcium concentrations in the range 0·5–4 mM. The ^{13}C data (see text) were obtained on 40 mM concentration of the complexes.

Ligand	δ (ppm)	T_1 (ms)	τ_c (s)	χ (MHz)
EDTA	20·0	150±10	$5{\cdot}8\times10^{-11}$	0·5±0·1
EGTA	27·9	8±3	$8{\cdot}0\pm10^{-11}$	2·0±0·4
[1]	4·7	22±7	$5{\cdot}6\pm10^{-11}$	1·4±0·3

In order to obtain the correlation time of the various complexes, the ^{13}C spin–lattice relaxation times for all the carbons, except in the carbonyls, were determined. From these T_1 values the correlation time, τ_c, is calculated using the equation

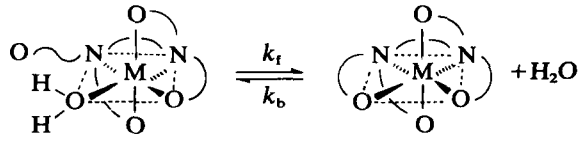
$$1/T_1 = (\gamma_H \gamma_C \hbar)^2 \,.\, \tau_c / r^6 \tag{9}$$

where γ_H and γ_C are the magnetogyric ratios for ^1H and ^{13}C respectively and r is the hydrogen–carbon bond distance. The calculated average correlation times are given in Table III. Using the values of τ_c obtained from the ^{13}C T_1 measurements and the ^{43}Ca T_1 data, the quadrupole coupling constant, χ, of ^{43}Ca in the complexes is calculated from the equation

$$(1/T_1)_{Ca} = (2\pi^2/49)\chi^2 . \tau_c \tag{10}$$

The resulting values of χ are listed in the last column of Table III. The low quadrupole coupling constant for the EDTA complex—which mirrors the surprisingly long value of T_1 for ^{43}Ca in this complex—deserves some comment. The low value may simply be a consequence of an accidental near cancellation of the electric field gradient at the place of the nucleus. On the other hand, it may also be the result of a fast process in the EDTA complex that will partly average the field gradient to a smaller value. Not much is known about the solution structure and dynamics of Ca^{2+}–EDTA complexes. Spectroscopic studies by Higginson *et al.*[72] have however

indicated that transition metal–EDTA complexes in aqueous solutions are labile and may take up two conformations, one "pentacoordinated" and one hexacoordinated. We may illustrate this by the following scheme:



A "walking" of the ligand around the central metal, presumably through nitrogen inversion, has been suggested by Day and Reilley[73] for the Pb^{2+}–EDTA complex to explain the equivalence of the acetate protons in ^1H NMR spectra. Such a process could well be thought of as associated with a water exchange as in the above scheme. A central question is the rate at which such processes occur. Harada *et al.*[74] have made an ultrasonic absorption study of Ca^{2+}–EDTA in aqueous solutions. They observed an absorption maximum near 8 MHz that is ascribed to the reaction in the scheme above. The corresponding exchange rates ($k_f = 3{\cdot}6 \times 10^7\ s^{-1}$ and $k_b = 1{\cdot}5 \times 10^7\ s^{-1}$) are however too small to cause an averaging of the quadrupole coupling constant in a complex with an overall correlation time of about 10^{-11} s.

X. ION BINDING TO POLYELECTROLYTES

Linear polymers where the monomers contain ionized or ionizable groups are usually referred to as polyelectrolytes; we use here this restricted definition although one could properly include other systems under this heading. Many synthetic polyelectrolytes are well known because of their technical applications, and in biology polyelectrolytes such as nucleic acids and polysaccharides have a wide structural and functional role. This role is exerted in combination with the binding of simple cations, in particular Mg^{2+} and Ca^{2+} but also K^+ and Na^+. Since all these ions lack other spectroscopic properties the possibility of using NMR has been considered with great interest for some years. Mainly for sensitivity reasons almost exclusively ^{23}Na NMR has been utilized so far. As can be inferred from a recent review,[4] this has given most valuable and novel information on ion binding equilibria and dynamics. The rough general picture is one of ion binding being a relatively simple function of the linear charge density of the polyelectrolyte. Thus binding is essentially zero below a certain critical charge density, while above this counterion binding occurs to keep this at an approximately constant effective value. The so-called ion condensation model[75] is in approximate agreement with experimental results, but distinct

deviations exist. One further result of the $^{23}Na^{+}$ studies is that the "bound" counterions are quite mobile along the polyion and may have residence times on the polyion as small as 1–10 ns.

$^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ studies of ion binding to several polyions have been performed by Gustavsson[76] in our laboratories. Partly owing to the complex relaxation behaviour of spin 5/2 and 7/2 nuclei, and partly because of a complex exchange situation, these data are difficult to interpret in detail; also the binding process is often not describable in terms of a simple two-site model. In view of these complications we only present some illustrative results and point out some conclusions which necessarily have to be rather preliminary.

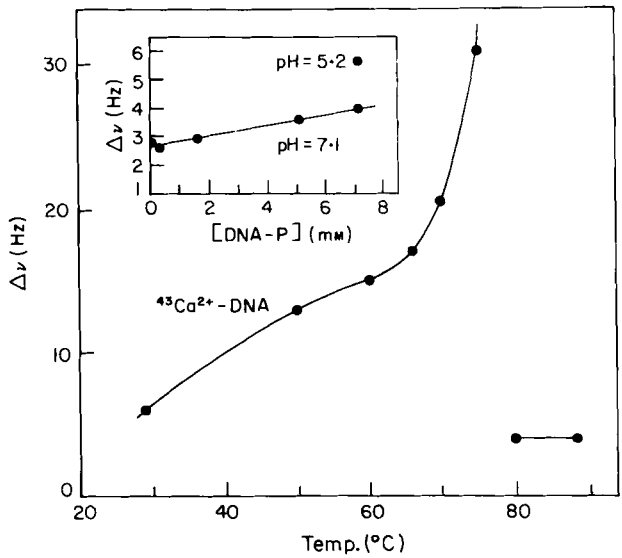


FIG. 6. $^{43}Ca^{2+}$ linewidth in solutions of DNA. Temperature dependence of the linewidth for a solution of 7·1 mM DNA-P and 35 mM Ca^{2+} at pH 5·2. Insert shows the effect of DNA concentration at 27 °C.[77]

The applicability of ^{25}Mg and ^{43}Ca NMR for investigations of ion–polyion interactions may be illustrated by some recent studies of ion binding to DNA.[77] Figure 6 presents the ^{43}Ca excess linewidth as a function of temperature. Significant features are the marked increase in relaxation with increasing temperature over a wide range, and the much lower relaxation rate above the "melting point" which corresponds to a transition from a double-stranded helical DNA to a single-stranded random coil state. This is in qualitative agreement with the ion condensation model[75] according to

which the number of counterions bound is determined by the linear charge density, a quantity which drops considerably at DNA melting. According to Manning's model[75] the Ca^{2+} binding should change from about 88% of polyion charge neutralization to a much lower level at the conformational transition. From the temperature dependence it can be deduced that the exchange of bound Ca^{2+} has a great influence on relaxation for the helix; the average lifetime of Ca^{2+} bound to DNA is estimated to be $\sim 10^{-2}$ s at 25 °C.

The results for $^{25}Mg^{2+}$ relaxation in DNA solution shown in Fig. 7 have the qualitative pattern of a case with exchange control at low temperatures

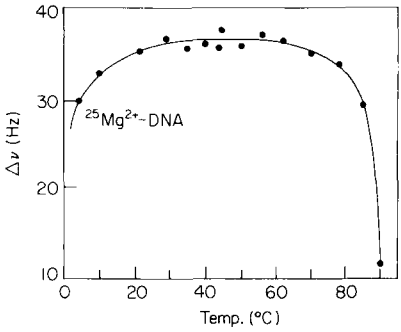


FIG. 7. Temperature dependence of the $^{25}Mg^{2+}$ linewidth for a solution of 7·1 mM DNA-P and 88 mM Mg^{2+} at pH 7·4.[77]

and relaxation control at high concentrations; however, the broad transition may indicate the inapplicability of a simple model with a single type of bound counterion. Complementary studies, which should permit an adequate analysis of these data, are in progress and it can so far only be said that the Mg^{2+} lifetime at DNA seems to be much smaller than that of Ca^{2+}; this indicates different hydration states of the bound ions (see Section V). For the Mg^{2+}–DNA system it can also be mentioned that significant deviations from simple ion condensation behaviour are noted.

In order to solve problems of interpretation it is very important to consider several polyelectrolyte systems in parallel; this distinguishes general polyelectrolyte behaviour from group specific effects. In Fig. 8 are given $^{25}Mg^{2+}$ linewidths for four additional systems:[76,78] one synthetic polymer (polyacrylic acid), two mucopolysaccharides (dermatan sulphate and chondroitin sulphate), and one peptide-bound chondroitin sulphate intended to mimic the proteoglycans found in connective tissue. Apparently there is a range of behaviour possible, with only dermatan sulphate showing indications of a simple change-over from exchange control at low temperatures to relaxation control at high temperatures. There is no doubt that ^{25}Mg

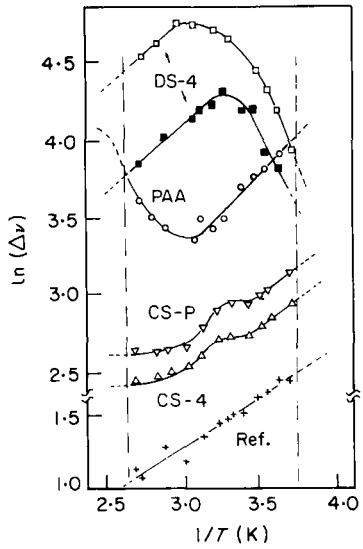


FIG. 8. The logarithm of the $^{25}Mg^{2+}$ NMR linewidth versus inverse temperature for aqueous solutions of dermatan-4-sulphate (DS-4; [DS-4] = 70 mM and $[Mg^{2+}]$ = 181 mM, pH = 4·05, □; [DS-4] = 68 mM and $[Mg^{2+}]$ = 125 mM, pH = 3·17, ■), polyacrylate (PAA; [PAA] = 93 mM and $[Mg^{2+}]$ = 62 mM, pH = 4·19, ○), chondroitin-4-sulphate (CS-4; [CS-4] = 70 mM and $[Mg^{2+}]$ = 125 mM, pH = 5·03, △), a protein–chondroitin sulphate complex (CS-P; [CS-P] = 75 mM and $[Mg^{2+}]$ = 122 mM, pH = 5·17, ▽), and a 100 mM $MgCl_2$ solution (Ref., +). (By courtesy of Hans Gustavsson.)

NMR may give unique and valuable information on ion–polyion interactions but also that much further work is required to interpret the data adequately.

XI. ION BINDING TO PROTEINS

As mentioned in the Introduction, calcium and magnesium ions are necessary for the activity of a large number of enzymes, regulatory proteins, and other biological macromolecules. It is therefore somewhat surprising that ^{25}Mg and ^{43}Ca NMR have only been applied to protein binding studies since 1978–9. Although the total number of studies performed at the time of writing (July 1980) is still rather limited they have considerably increased our knowledge as to what types of problem can be studied and what type of information may be obtained. In the present section we first make a few general comments on the applicability of ^{25}Mg and ^{43}Ca NMR to studies of ion binding to proteins. This is followed by a brief survey of experimental

work and the section is concluded with a summary of the types of information that may be obtained from ^{25}Mg and ^{43}Ca NMR.

A. General comments

Quadrupolar relaxation is very efficient and the direct observation of NMR signals from $^{25}Mg^{2+}$ or $^{43}Ca^{2+}$ firmly bonded to a large slowly tumbling protein molecule is in general very difficult (but not impossible as is shown below). It is however possible to obtain biochemically relevant information through NMR studies of the cations also when there is a sufficiently fast chemical exchange between the protein binding site(s) and the "free", solvated ion.[2-4,17] This approach, which may be termed the *indirect* method, has for a number of years been used with good results in the study of Cl^- (^{35}Cl and/or ^{37}Cl) and Na^+ (^{23}Na) in biological systems.[2-4,17] The basic theory of the indirect method has been extensively discussed elsewhere[2,4] and we here restrict ourselves to a very brief and simplified account.

Under conditions when only a small fraction, p_B, of the cations are bonded to one type of site on the macromolecule, the observed NMR relaxation rates, R_{obs}, are given by (see Section V)

$$R_{obs} = R_{free} + p_B/(R_B^{-1} + k_{off}^{-1}) \tag{11}$$

where k_{off} is the off-rate of the ion from the binding site, R_B is the relaxation rate of the bonded ion, and R_{free} is the relaxation rate of the "free" solvated ion. The difference $R_{obs} - R_{free}$ is referred to as the *excess relaxation rate*, R_{ex}. Equation (11) is a poor approximation when p_B becomes appreciable—more than 0·1 say—and in many of the applications discussed below more complete treatments using the full expressions for chemical exchange in a two-site system are used. Nor is equation (11) strictly valid when non-extreme narrowing conditions (i.e. $\omega\tau_c \geqslant 1$) apply to the macromolecular binding site as discussed in Section V. Equation (11) is however adequate to illustrate the limits of applicability of the indirect method.

Present experimental evidence indicates that R_B values for $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ are often of the order of 10^3–10^4 s^{-1}. The rate of formation of Ca^{2+} complexes with low molecular weight ligands is of the order of 10^8–10^9 M^{-1} s^{-1}, i.e. the process is essentially diffusion controlled.[79,80] If we assume the on-rate, k_{on}, for the formation of a Ca^{2+}–protein complex to be of the same order of magnitude as for smaller complexes, we may infer from equation (11) that proteins with calcium binding constants $K_B = k_{on}/k_{off}$ equal to or smaller than 10^5 M^{-1} are amenable to study by the indirect method. For Mg^{2+}, rates of formation of low molecular weight inner-sphere complexes are usually of the order of 10^5–10^6 M^{-1} s^{-1} depending on the charge of the ligand.[79-82] This implies that proteins with magnesium binding

constants of the order of 10^2–10^3 M^{-1} or smaller should be suitable for study by the indirect method. It should be added that Mg^{2+} has been observed to bind to some biological macromolecules, e.g. tRNA, with its water co-ordination sphere still intact.[81] In such situations the on-rates for the ion binding of Mg^{2+} may approach values similar to those for Ca^{2+}, which means that binding constants larger than 10^3 M^{-1} can be studied by the indirect method.

The binding constant limits of the indirect method should be regarded as rules-of-thumb. In general one should expect that the determination of chemical exchange rates of Mg^{2+} and Ca^{2+} will be limited to systems where the binding constants are close to the upper limits given above.

B. Applications to individual proteins

1. Parvalbumin

Parvalbumins are low molecular weight (~11 500) proteins that are found in the muscles of most vertebrates. They have a strong affinity for Ca^{2+} ($K_B \approx 10^7$ M^{-1})[84,85] and it has been suggested that their physiological role is simply to act as a buffer of intracellular calcium ions.[86] The X-ray structure of parvalbumin from carp has been established; two Ca^{2+} ions are found to be located at specific sites called CD and EF.[87]

In 1978 Parello *et al.* published a study of the temperature and pH dependence of the ^{43}Ca NMR linewidth in the presence of carp parvalbumin.[88] Calcium with 61·63% isotope enrichment in ^{43}Ca was used and the experiments were performed on a Varian XL-100 spectrometer operating at 6·73 MHz. Ca^{2+} concentrations of 85–100 mM and protein concentrations of the order of 1 mM were used. Very small ^{43}Ca excess linewidths were observed at room temperature and neutral pH, indicating slow chemical exchange of Ca^{2+} ions to the high affinity Ca^{2+} sites. Significant line-broadenings are observed above 65 °C and at pH exceeding 10.

In a subsequent paper, Cavé *et al.*[89] studied the interaction of Mg^{2+} with parvalbumins using ^{25}Mg NMR. Magnesium with a 97·9% enrichment in the isotope ^{25}Mg was used. $^{25}Mg^{2+}$ concentrations of the order of 100 mM and protein concentrations between 0·1 and 1 mM were used. Under these conditions ^{25}Mg excess linewidths of the order of 5–25 Hz are observed at room temperature. A variable temperature study of the ^{25}Mg linewidth of a solution containing $(Ca^{2+})_2$-parvalbumin indicates that the linewidth at room temperature is influenced by the chemical exchange rate; the linewidth passes through a maximum at 35–40 °C indicating that fast exchange conditions prevail above 50 °C.

Experiments indicate that Ca^{2+} and Mg^{2+} compete for the parvalbumin sites, causing ^{25}Mg NMR line-broadening with similar affinities ($K_{Mg}/K_{Ca} \approx 2$). This indicates that the Mg^{2+} binding sites probed by ^{25}Mg NMR are not

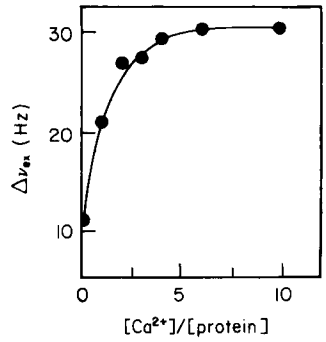


FIG. 9. The ^{25}Mg NMR excess linewidth in hake parvalbumin solutions as a function of Ca^{2+} concentration; 99 mM Mg^{2+}, 97·9% enriched in ^{25}Mg, was used and spectra were obtained at 6·12 MHz.[89]

the CD and EF sites (for these sites the affinity of Ca^{2+} is 10^2–10^3 times that of Mg^{2+}) and points to the existence of secondary cation binding sites. The addition of Ca^{2+} to an apoparvalbumin solution containing Mg^{2+} causes an initial increase in the $^{25}Mg^{2+}$ relaxation rate as shown in Fig. 9. This increase is related to a change of the Mg^{2+} binding characteristics—structural (R_B), thermodynamic (p_B), or kinetic (k_{off})—of secondary sites as a consequence of structural changes of the parvalbumin molecule when Ca^{2+} enters the CD and EF sites.

As a final comment we mention that the Mg^{2+} concentration in cells is of the order of 1–5 mM and thus considerably higher than that of Ca^{2+}. Consequently Mg^{2+} may, under physiological conditions, bind to proteins with binding constants, K_{Mg}, of the order 10^3 M^{-1}; the binding of Ca^{2+} under the same conditions requires binding constants, K_{Ca}, several orders of magnitude higher.

2. *Bovine serum albumin and nitrogenase Fe protein*

A ^{25}Mg NMR study of Mg^{2+} binding to two "test" proteins, bovine serum albumin (BSA) and the iron protein of nitrogenase from the nitrogen-fixing bacterium *Klebsiella pneumoniae* (Kp2), has been presented by Bishop *et al.*[90] Both proteins have approximately the same molecular weight (~67 000). Natural abundance ^{25}Mg (i.e. 10·1%) was used; with a JEOL FX-100 NMR spectrometer operating at 6·10 MHz the practical lower concentration limit is ~15 mM Mg^{2+} (an overnight run or 120 000 transients).

The aim of the NMR study was primarily to determine the Mg^{2+} affinity of the two proteins. To that end the ^{25}Mg linewidth was determined as a function of $^{25}Mg^{2+}$ concentrations at fixed protein concentrations. In

determinations of binding constants using the indirect NMR method it may be convenient to rewrite equation (11) in the following form[91]

$$R_{obs} = R_{free} + \frac{nK_B[P]_0 R_B}{1 + K_B[M]} \tag{12}$$

where $[P]_0$ is the total protein concentration, [M] is the total concentration of the ligand under study, n is the number of equivalent non-interacting binding sites with binding constant K_B. In the derivation of equation (12) we have for simplicity assumed that fast exchange conditions prevail, i.e. $k_{off} \gg R_B$. Equation (12) may be rewritten, using $R_{ex} = R_{obs} - R_{free}$,

$$1/R_{ex} = (1/nR_B[P]_0)(1/K_B + [M]) \tag{13}$$

Plots of $(R_{ex})^{-1}$, or $(\pi\Delta\nu_{ex})^{-1}$, versus [M] should thus give straight lines of slope $(nR_{2B}[P]_0)^{-1}$ with $K_B{}^{-1}$ as the intercept on the negative [M] axis. Bishop *et al.* used equation (13), in approximating $\Delta\nu_{ex}$ with $\Delta\nu_{obs}$, to determine a binding constant $K_B = 50$–67 M^{-1} for BSA. For the nitrogenase protein the observed intercept is not significantly different from zero and the binding constant has not been determined.

Using values of n obtained from previous Mg^{2+} binding studies, Bishop *et al.* have calculated relaxation rates $R_{2B} = (8{\cdot}6 \pm 0{\cdot}8) \times 10^3$ s^{-1} for $^{25}Mg^{2+}$ bound to Kp2 and $(3{\cdot}1 \pm 0{\cdot}3) \times 10^3$ for $^{25}Mg^{2+}$ bound to BSA.[90] No ^{25}Mg nuclear screening changes are observed in the experiment, in agreement with other NMR studies of Mg^{2+} binding to proteins.

3. *Prothrombin fragment 1*

Prothrombin and its γ-carboxyglutamate containing region, which can be cleaved off enzymatically (fragment 1), have been briefly discussed in Section IX. The binding of divalent metal ions to fragment 1 has been studied with different spectroscopic techniques, for example circular dichroism and fluorescence quenching.[92,93] Some of the binding data are in apparent conflict. Whereas both fluorescence quenching and circular dichroism data, for example, show similar effects of Ca^{2+} and Mg^{2+} on the fragment 1 structure, phospholipid binding data clearly demonstrate that only Ca^{2+} is able to promote fragment 1 phospholipid binding.

In an effort to clarify these questions, Marsh *et al.*[94] (see also ref. 66) have used both ^{25}Mg and ^{43}Ca NMR. Enriched material was used (79·98% ^{43}Ca and 98·25% ^{25}Mg) and the NMR spectra were obtained on a modified Varian XL-100 FT NMR spectrometer. Excess linewidths of some 10 Hz were obtained on solutions containing 20 mM $^{25}Mg^{2+}$ or $^{43}Ca^{2+}$ and 30–50 μM of fragment 1. The effect of added Ca^{2+} on the ^{25}Mg linewidth in the presence of fragment 1 is shown in Fig. 10. If the reduction in linewidth is attributed entirely to a reduction in the fraction of $^{25}Mg^{2+}$ bonded to

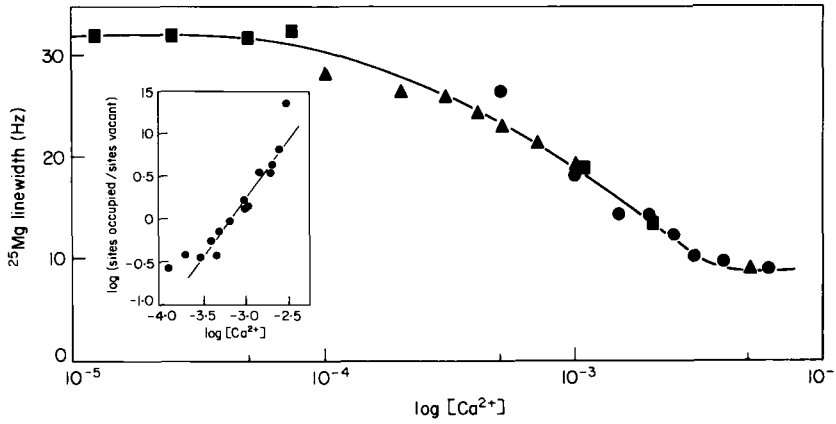


FIG. 10. The ^{25}Mg NMR linewidth as a function of the Ca^{2+} concentration in a solution containing 20 mM Mg^{2+} (98·25% enriched in the isotope ^{25}Mg) and 29 μM of prothrombin fragment 1 at pH 7·0. Inserted in the figure is a Hill plot constructed from the experimental data. The slope of this plot (which is a measure of the cooperativity in the ion binding) was found to be 1·37.[94]

fragment 1 it appears that only about 75% of the originally bound Mg^{2+} ions are replaceable by Ca^{2+} concentrations approaching 10 mM. The reciprocal experiment, observing the ^{43}Ca NMR linewidth on addition of Mg^{2+}, shows no clear-cut levelling off of the $^{43}Ca^{2+}$ linewidth at high Mg^{2+} concentrations. It appears therefore that Ca^{2+} is more strongly bonded to fragment 1 than are Mg^{2+} ions.

The pH dependence of the $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ NMR linewidths was also studied by Marsh *et al.*[94] At pH 3 little Ca^{2+} binding is evident. As pH is increased, however, the $^{43}Ca^{2+}$ linewidth also increases with a pronounced inflection point at pH 3·7. Following a plateau at pH 5–6 a further increase is observed above pH 7. The pH dependence of the $^{25}Mg^{2+}$ linewidth follows the same pattern.[66] The pH dependence of the $^{25}Mg^{2+}$ linewidth in the presence of 20 mM Ca^{2+} is found to be substantially different from that in the absence of Ca^{2+}. No increase in ^{25}Mg linewidth above pH 6–7 is observed, indicating that the high pH binding sites are peculiar to site(s) capable of binding Ca^{2+} with high affinity.

The ^{25}Mg and ^{43}Ca NMR studies thus point to the fact that Mg^{2+} can occupy some sites on fragment 1 for which the affinity for Ca^{2+} is low and that these sites are associated with a pK_a value near 4.

4. Troponin C

The contraction of skeletal muscles is triggered by the release of a pulse of Ca^{2+} ions, and on a molecular level the action of Ca^{2+} is generally assumed

to be mediated by a protein complex called troponin. Troponin consists of three subunits, troponin C (TnC), troponin T (TnT), and troponin I (TnI). Of these TnC is known to bind Ca^{2+} and thereby to undergo conformation changes.[95] TnC has a molecular weight of about 18 000; its primary structure is known but not its tertiary structure. Equilibrium dialysis studies indicate that TnC has two classes of binding site, two sites with a high affinity for Ca^{2+} and a lower affinity for Mg^{2+} ("Ca^{2+}–Mg^{2+} sites"; $K_{Ca} = 2{\cdot}1 \times 10^7\ M^{-1}$ and $K_{Mg} = 5 \times 10^3\ M^{-1}$)[96] and two sites that bind Ca^{2+} more weakly but not Mg^{2+} ("Ca^{2+} specific regulatory sites"; $K_{Ca} = 3{\cdot}2 \times 10^5\ M^{-1}$). The alleged Ca^{2+} specificity of the latter sites may simply be due to experimental difficulties in measuring binding constants of the order of $1–10\ M^{-1}$.[97]

^{25}Mg and ^{43}Ca NMR has recently been used to study the interaction between Mg^{2+} and Ca^{2+} and TnC. In this investigation, as in all other studies presented below, enriched material is used ($^{25}Mg \sim 90\%$; $^{43}Ca \sim 61\%$); the FT NMR spectra are obtained on the 6·0 T spectrometer built at Lund University (see Section III). Solenoid type probes are used in which the sample tubes—usually containing about 3 ml of sample—are inserted from the side.

The ^{43}Ca NMR excess linewidth in the presence of TnC as a function of the Ca^{2+} concentration is shown in Fig. 11. This figure strikingly illustrates the benefits of being able to study Ca^{2+} concentrations in the mM range. The

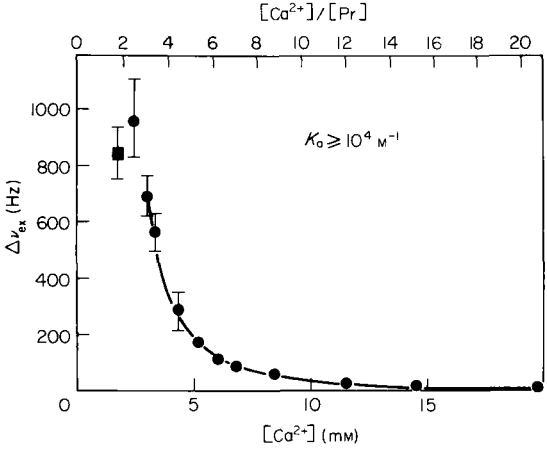


FIG. 11. The ^{43}Ca NMR excess linewidth as a function of the Ca^{2+} concentration in an aqueous solution of troponin C (TnC). The experimental conditions are: (●) 0·94 mM TnC; (■) 1·72 mM TnC, 3·16 mM Ca^{2+}; temp. 23 °C and pH 7·0.[6,98] The full drawn curve is fitted with a calcium binding constant $10^5\ M^{-1}$.

The NMR spectra in this figure and in all the following figures have been obtained at a B_0 field of 6·0 T on the FT NMR spectrometer constructed at Lund University. Isotope enriched material has also been used throughout.

^{43}Ca NMR signal seen at a Ca^{2+}/TnC ratio of ~1·8 may in all likelihood be ascribed to $^{43}Ca^{2+}$ ions bound to the high affinity Ca^{2+}–Mg^{2+} sites. The lineshape is virtually Lorentzian even at this point, as it is at all higher Ca^{2+} concentrations. The linewidth of 900 Hz (corresponding to an effective transverse relaxation rate R_{2B} of $2{\cdot}8\times10^3\ s^{-1}$) is surprisingly small and points to high symmetry of the binding sites. The ^{43}Ca signals observed for Ca^{2+}/TnC ratios greater than 3 are attributed mainly to $^{43}Ca^{2+}$ ions exchanging with the two "Ca^{2+} specific regulatory sites". The observed dependence of $\Delta\nu_{ex}$ on Ca^{2+} concentration in Fig. 11 is consistent with a binding constant $K_{Ca}\geq10^4\ M^{-1}$ (the fully drawn curve in Fig. 11).[6,98] It should be emphasized that in this curve-fitting procedure a total lineshape analysis is needed in order to take chemical exchange effects into account (see Fig. 12). The approximations in equations (11) or (12) may not be used in this case since the fraction of Ca^{2+} ions bonded to TnC under some experimental conditions employed is much larger than 0·1.

The temperature dependence of the ^{43}Ca NMR linewidth in the presence of TnC (Fig. 12a) is almost a textbook example of a change from chemical exchange dominated linewidth at lower temperatures to relaxation rate determined linewidth at higher temperatures. In Fig. 12b an example of the observed ^{43}Ca NMR signal is shown. An analysis of the temperature dependence data of Fig. 12a gives the following preliminary rate parameters for the Ca^{2+}–TnC exchange from the Ca^{2+} specific regulatory sites (300 K):[6,98] $k_{off}=10^3\ s^{-1}$; $\Delta H^{\ddagger}=38\ kJ\ mol^{-1}$; $\Delta S^{\ddagger}=59\ J\ mol^{-1}\ K^{-1}$. The off-rate obtained from the ^{43}Ca NMR is in good agreement with the rate of structural relaxation of TnC upon the sudden removal of Ca^{2+} as monitored by a fluorometric stopped-flow study.

The dependence of the $^{25}Mg^{2+}$ NMR linewidth on the Mg^{2+} concentration in TnC solutions is shown in Fig. 13.[6,98] The data have been analysed in the same way as the ^{43}Ca NMR data in Fig. 11, to give a binding constant K_{Mg} of $4\times10^2\ M^{-1}$. This is an average binding constant for all sites possessing fast or intermediate Mg^{2+} exchange rates. The competition experiments in Fig. 14 (where the ^{25}Mg NMR excess linewidth is followed as a function of the Ca^{2+} concentration at a constant TnC concentration) indicate that it is the Ca^{2+}–Mg^{2+} sites that give rise to the major part of the observed ^{25}Mg NMR line-broadening. An alternative interpretation, considered less likely, is that the ^{25}Mg line-broadenings are due to Mg^{2+} binding to some other site the Mg^{2+} binding characteristics of which are drastically changed as Ca^{2+} enters into the two high-affinity Ca^{2+}–Mg^{2+} sites.

The temperature dependence of the ^{25}Mg linewidth in the presence of TnC shows the same general behaviour as that shown for ^{43}Ca in Fig. 12a. A preliminary calculation of the chemical exchange rate of Mg^{2+} gives $k_{off}=8\times10^2\ s^{-1}$ at 300 K.[6,98]

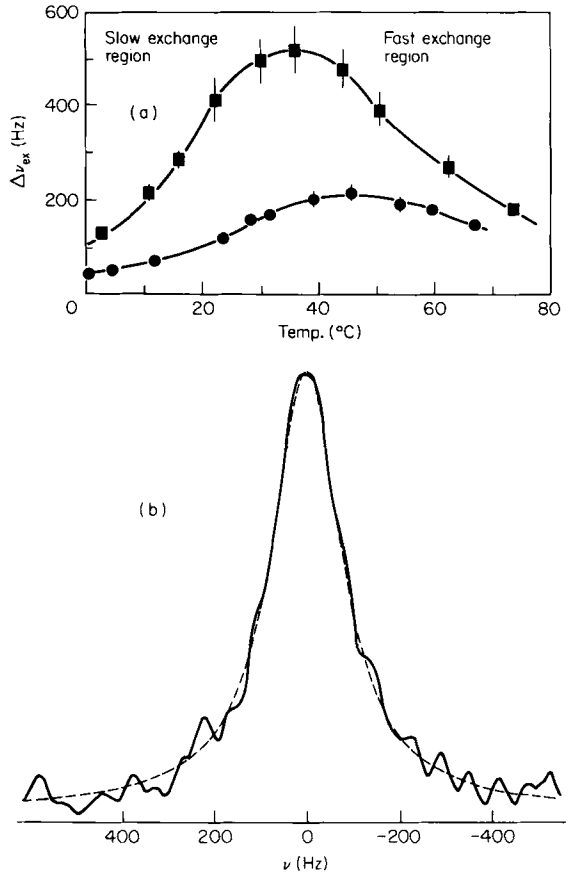


FIG. 12. (a) The temperature dependence of the ^{43}Ca NMR linewidth in the presence of TnC. Conditions are: (■) 0·86 mM TnC, 3·67 mM Ca^{2+}, pH 7·0; (●) 0·75 mM TnC, 5·92 mM Ca^{2+}, pH 7·1.[6,98]

(b) The experimental ^{43}Ca NMR signal at 46 °C obtained under the conditions: 5·9 mM Ca^{2+}, 0·75 mM TnC, pH 7·0, ~30 min. The broken line is a Lorentzian lineshape with the same linewidth as for the observed signal.[6,98]

The ^{25}Mg and ^{43}Ca NMR studies of Mg^{2+} and Ca^{2+} binding to TnC bear out the generalizations made in the introduction to the present section concerning the limits of the indirect method and the possibilities of determining exchange rates.

5. Calmodulin

It has recently been demonstrated that calcium regulation of a large number of enzyme systems and other cellular events is mediated by a low

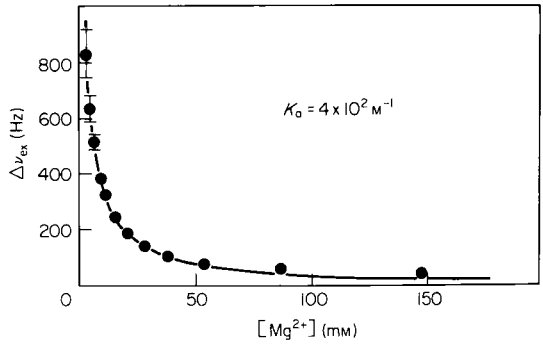


FIG. 13. The ^{25}Mg NMR excess linewidth as function of the Mg^{2+} concentration in the presence of Ca^{2+}-free TnC. Conditions are: (●) 0·93 mM TnC, pH 6·8, 23 °C. The full drawn curve has been calculated using a Mg^{2+} binding constant of 4×10^2 M^{-1}.[6,98]

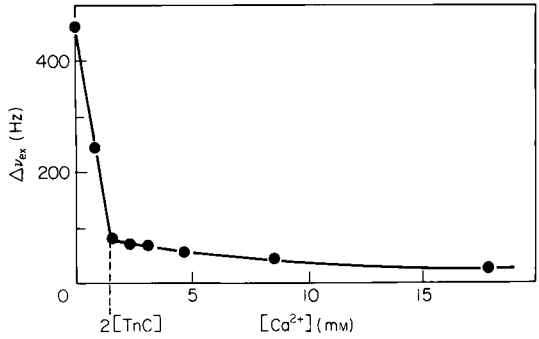
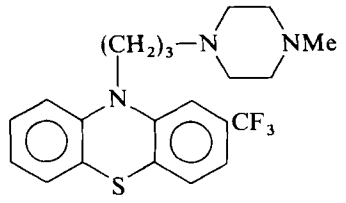


FIG. 14. The effect of added Ca^{2+} on the ^{25}Mg NMR excess linewidth in the presence of TnC. The breaking point occurs at a molar ratio Ca^{2+}/TnC of 2. Conditions: 0·74 mM TnC, 2·9 mM Mg^{2+}, pH 7·1, 24 °C.[6,98]

molecular weight compound named calmodulin.[99] Available evidence indicates that calmodulin is a widespread calcium regulatory protein in all eukaryotic cells. Calmodulin has a molecular weight of 16 700 and its primary sequence—which is highly conserved in Nature—is homologous to that of parvalbumins and troponin C. Its amino acid sequence can be divided into four internally homologous domains, each of which has a potential calcium binding site.[100] Several studies have indicated that calmodulin can bind four moles of Ca^{2+} per mole of protein. There is however some disagreement as regards the relative number of high and low affinity sites although for example ^{113}Cd NMR clearly points to two high affinity and two low affinity sites, very much as in TnC.[101]

The biological activity of calmodulin is strongly inhibited by certain antipsychotic drugs of the phenothiazine type, for example chlorpromazine and trifluoperazine (TFP, stellazine) [2]. TFP has been reported to bind to calmodulin with a binding constant of about 10^6 M^{-1}.[102]



[2]

^{43}Ca NMR figures have been used to study the interactions between TFP and calmodulin.[6,103,104] In the presence of 0·4 mM calmodulin from calf brain the excess linewidth of a 3 mM $^{43}Ca^{2+}$ solution is about 80 Hz. The effect of TFP additions on the ^{43}Ca NMR spectrum is shown in Fig. 15. A

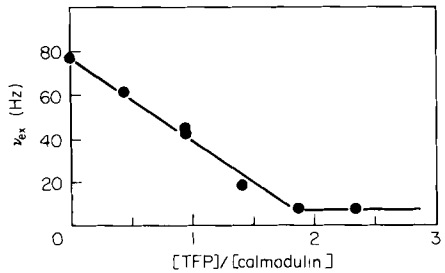


FIG. 15. The effect of added trifluoperazine (TFP) on the ^{43}Ca NMR excess linewidth in the presence of calmodulin. Conditions: 0·41 mM calf brain calmodulin, 2·96 mM Ca^{2+}, pH 7·1, 23 °C.[6,103]

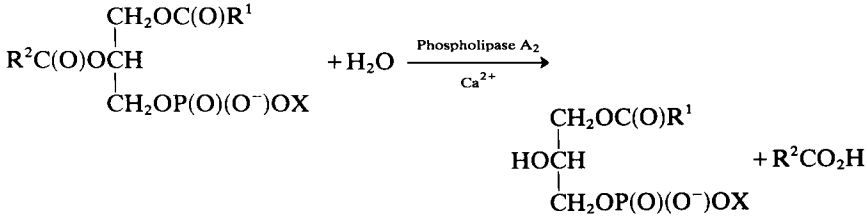
stoicheiometry of 2 moles of TFP bound per mole of calmodulin is indicated, but what is the cause of the reduced linewidth? A study of the temperature dependence of the ^{43}Ca excess linewidth in the absence and presence of TFP indicates that the linewidth at room temperature is determined by the chemical exchange rate and that the binding of TFP to calmodulin causes a decrease in the exchange rate and thus in the linewidth.[104] An analysis of the temperature dependence of the ^{43}Ca NMR linewidth in the absence of TFP gives $k_{off} = 10^3$ s^{-1} for the exchange rate of Ca^{2+}, in all likelihood from the weaker of the two types of Ca^{2+} binding sites of calmodulin. In the presence of TFP the off-rate is reduced by almost an order of magnitude.[6,104] A parallel study of the effect of TFP on the Ca^{2+}

exchange rate in troponin C gives very similar results. In the case of TnC some 5 or 6 moles of TFP are, however, necessary to reduce the $^{43}Ca^{2+}$ excess linewidth to near zero.[104]

The reduction caused by TFP in the off-rate of Ca^{2+} from the regulatory sites of TnC—and presumably also from the regulatory sites of calmodulin—should have important consequences for the physiological function of these proteins. However, few data are available to substantiate this.

6. *Phospholipase A_2*

Phospholipase A_2 is a low molecular weight (~14 000) enzyme that specifically catalyses the hydrolysis of the 2-acyl ester bonds of naturally occurring and synthetic phospholipids according to the scheme:[105]



where X is any of the residues observed in phospholipids, i.e. choline, ethanolamine, glycerol, etc., and R^1 and R^2 are alkyl chains. The primary structure of phospholipase A_2 from different sources is known.[106] The enzyme is secreted by the mammalian pancreas as the zymogen prophospholipase A_2 and trypsin cleavage of an N-terminal heptapeptide from the zymogen produces the active enzyme. The proenzyme as well as the enzyme have been reported to bind 1 mole of Ca^{2+} per mole of enzyme with a binding constant of about 10^3 M^{-1} and an additional mole of Ca^{2+} with a binding constant of the order of 30 M^{-1} or less.[107]

The excess linewidth of the ^{43}Ca NMR signal as a function of the Ca^{2+} concentration in the presence of 2·0 mM prophospholipase A_2 is shown in Fig. 16.[6,108] The experimental data may be fitted with a binding constant $K_{Ca} = 1{\cdot}1 \times 10^3$ M^{-1} (at 297 K). The temperature dependence of the excess linewidth (Fig. 17) shows a transition from exchange rate dominated linewidth at temperatures below room temperature and relaxation rate dominated linewidth at higher temperatures. A preliminary calculation of the rates of Ca^{2+} exchange gives $k_{off} = 1{\cdot}4 \times 10^3$ s^{-1} (at 300 K). Measurement of the effective longitudinal and transverse relaxation rates for $^{43}Ca^{2+}$ in the presence of prophospholipase A_2 indicates that the correlation time for the Ca^{2+} binding site is 12 ns.[108] This value is close to that expected for the rotational diffusion of the whole protein molecule and may be taken to indicate that the structure of the Ca^{2+} binding site is relatively rigid.

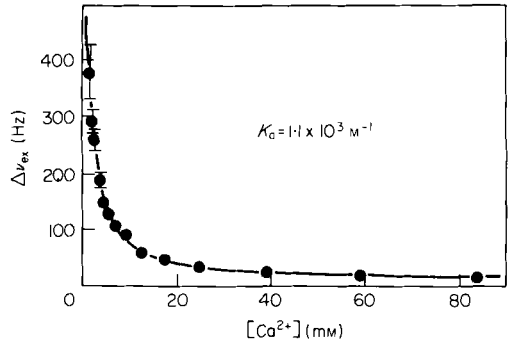


FIG. 16. The ^{43}Ca NMR excess linewidth as a function of the Ca^{2+} concentration in the presence of 2·0 mM prophospholipase A_2—a lipolytic proenzyme (23 °C, pH 7·5). The full drawn curve is calculated with a Ca^{2+} binding constant of $1{\cdot}1 \times 10^3$ M^{-1}.[6,108]

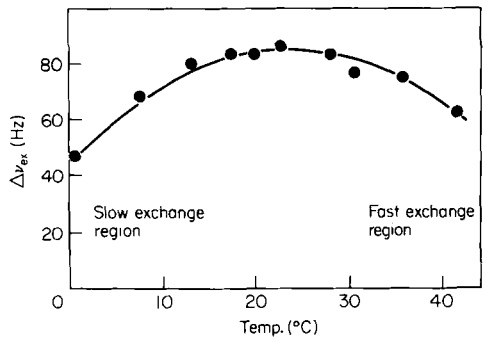


FIG. 17. The temperature dependence of the ^{43}Ca NMR excess linewidth in a solution containing 1·7 mM prophospholipase A_2 and 5·9 mM Ca^{2+} (pH 7·4). A preliminary evaluation of the exchange rate gives $k_{off} = 1{\cdot}4 \times 10^3$ s^{-1} for the Ca^{2+} exchange at 300 K.[6,108]

A comparison of the relation between the value of the Ca^{2+} binding constant and the off-rate obtained for prophospholipase A_2 with the corresponding figures for TnC and calmodulin reveals an interesting difference. The off-rates found for Ca^{2+} binding to TnC and calmodulin are of the order of magnitude that one would expect if the on-rates are essentially diffusion controlled (see the discussion in the introduction to the present section). On the other hand the on-rate of Ca^{2+} binding to prophospholipase A_2 must be *several orders of magnitude slower* than the on-rates to TnC and calmodulin to account for the relation between K_{Ca} and k_{off} in this case. It appears that these differences reflect the different nature of the Ca^{2+} binding sites in prophospholipase A_2 on the one hand and TnC and calmodulin on the other. The latter regulatory proteins probably have flexible sequences with amino

acid sidechains that may easily wrap around an incoming Ca^{2+} ion. By contrast the Ca^{2+} binding site of prophospholipase A_2 is largely a rigid entity that is not accessible to a Ca^{2+} ion upon each encounter in solution (see also the discussion by Williams[86]).

The pH dependence of the ^{43}Ca excess linewidth in a prophospholipase A_2 solution is shown in Fig. 18. The observed curve may be fitted to a single protonation step with a pK_a of 5·2.[6,108]

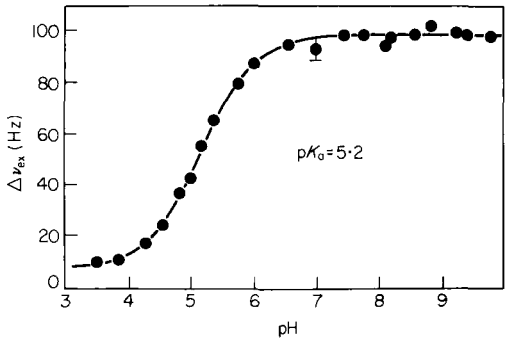


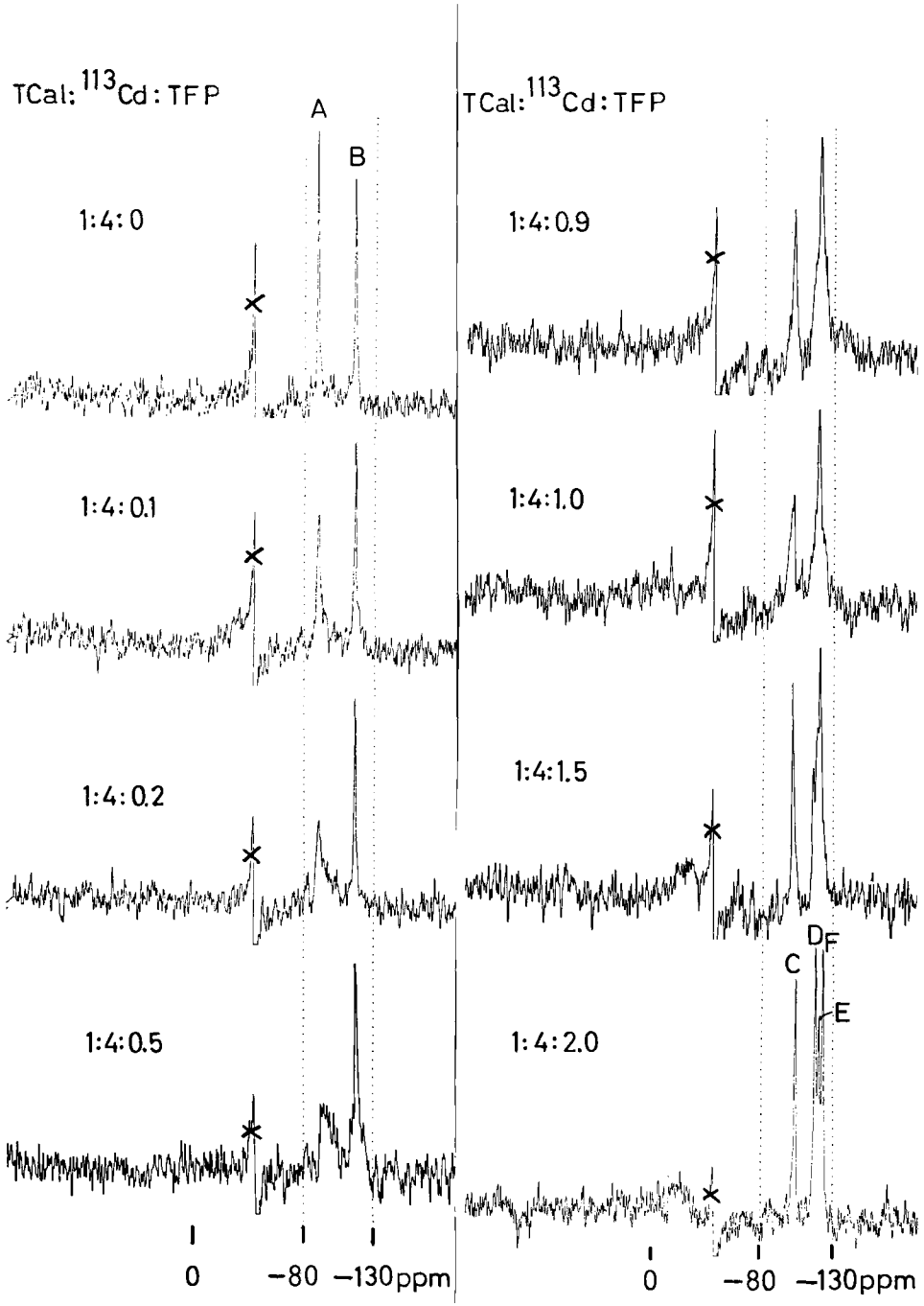
FIG. 18. The pH dependence of the ^{43}Ca NMR excess linewidth in a solution containing 1·7 mM prophospholipase A_2 and 5·9 mM Ca^{2+}. The changes in the linewidth can be fitted to a single protonation step with a pK_a of 5·2.[6,108]

C. Summary

The limited number of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ NMR studies made so far of ion binding to proteins clearly demonstrate the great usefulness of the indirect NMR method. Current FT NMR techniques allow the study of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ in the millimolar (and even submillimolar) concentration range. The type of information that can be obtained is: (1) binding constants in the range $1–10^4\ M^{-1}$; (2) the competition of different cations for the Ca^{2+} or Mg^{2+} binding site(s); (3) the effects of other ligands (drugs etc.) on the protein; (4) pK_a values of groups responsible for the Mg^{2+} or Ca^{2+} binding; (5) the dynamic parameters—exchange rates and correlation time(s)—characterizing the cation binding site.

XII. NMR WITH SUBSTITUTION PROBES OF Ca^{2+} AND Mg^{2+}

The above presentation has hopefully given an insight into both the strengths and limitations of ^{25}Mg and ^{43}Ca NMR for the study of chemical and biological problems. When the NMR properties of the bound metal ion can be studied we possess a very powerful tool for investigating its co-ordination chemistry and dynamics. This possibility is always at hand for the


TCal:113Cd:TFP
A
B
1:4:0
1:4:0.1
1:4:0.2
1:4:0.5
TCal:113Cd:TFP
1:4:0.9
1:4:1.0
1:4:1.5
C
D
F
E
1:4:2.0
0
−80
−130ppm
0
−80
−130ppm

case of sufficiently rapid exchange while in the slow exchange limit we are limited to complexes that are not too large; at least large and medium sized macromolecules are at present excluded if slow exchange pertains. One remedy in such a situation may be to substitute another metal ion, with more favourable NMR properties, for Ca^{2+} or Mg^{2+}. The probe should then have reasonably similar ionic radius and coordination properties; great caution must always be exercised in applying results for the probe system to the parent system of chemical or biological interest. The approach may be illustrated for the case of Ca^{2+}, where $^{23}Na^{+}$, $^{113}Cd^{2+}$ (or rather equivalently $^{111}Cd^{2+}$), and Gd^{3+} have been used as probes. The ionic radii, 0·097 nm for Na^{+}, 0·097 nm for Cd^{2+}, and 0·094 nm for Gd^{3+}, are thus close to the ionic radius of Ca^{2+} which is 0·099 nm. Using $^{23}Na^{+}$ [3,4] instead of $^{43}Ca^{2+}$ increases NMR sensitivity considerably but above all it gives a much higher exchange rate. However, it is the experience of our laboratories that Na^{+}, in its binding to both proteins and polyelectrolytes, may behave very differently from Ca^{2+} and thus may be a bad probe. In the use of Gd^{3+} as a probe one is generally monitoring the proton relaxation enhancement (PRE) of the water. The PRE method may give useful information on the hydration properties of functional and structural metal ions. Other useful probe ions are Co^{2+} and Mn^{2+}. A critical survey illustrating pitfalls and difficulties has recently been presented.[7]

The most interesting approach at present is certainly the use of ^{113}Cd (or ^{111}Cd) NMR. The Cd^{2+} has been shown to replace Ca^{2+} specifically in several Ca-proteins and to give similar coordination properties. The sensitivity of ^{113}Cd NMR is almost twice that of ^{43}Ca NMR (and at natural abundance much more so) but above all ^{113}Cd, being a spin 1/2 nucleus, has rather slow relaxation even when bound to a macromolecule. For several calcium proteins it has therefore been relatively straightforward to obtain ^{113}Cd spectra of high quality. The use of ^{113}Cd NMR to study calcium proteins has recently been described.[6] We conclude by giving an experimental spectrum (Fig. 19) illustrating this very powerful complement to ^{43}Ca NMR in elucidating Ca binding in biology.

FIG. 19. The ^{113}Cd NMR spectra of $(Cd)_4$-calmodulin (bovine testes) as a function of added amounts of the drug TFP (see Fig. 15 and text). The ^{113}Cd NMR chemical shift is characteristic of oxygen ligands. The two ^{113}Cd NMR signals in the absence of TFP (attributed to two high affinity Ca^{2+} binding sites of calmodulin) are seen to be affected already at a molar ratio TFP/$(Cd)_4$-calmodulin of 0·1. At higher molar ratios four ^{113}Cd NMR signals appear; the two additional signals are attributed to two "low affinity" Ca^{2+} binding sites of calmodulin that become observable owing to a reduced chemical exchange rate of $^{113}Cd^{2+}$ to these sites as TFP is bound to calmodulin. In each case the peak marked with a cross is an instrumental artefact due to an improperly balanced quadrature detector.[101]

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^{13}C NMR of Group VIII Metal Complexes

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I. INTRODUCTION

The utility of ^{13}C NMR in the study of transition metal complexes has long been recognized, and several recent review articles have appeared.[1–4] Further, the growth of applications involving this nucleus is so rapid that the reviews are often concerned specifically with one type of complex, e.g. metal–phosphine[1] or metal–carbonyl[2] complexes. As with ^{1}H NMR it is becoming difficult to obtain a reasonable overview of the ^{13}C literature since the method is so common that increasingly one finds the data relegated to the small print of the experimental section! In view of this situation, this reporter has restricted himself to complexes of the Group VIII metals (the iron, cobalt, and nickel triads) with the reports stemming primarily from the period 1976–1978. A more comprehensive report should appear shortly.[5]

A brief survey of more than two hundred reports involving ^{13}C NMR in Group VIII chemistry shows:

(a) Those who most use ^{13}C NMR are to be found in the field of organometallic chemistry (and by this is meant complexes containing either metal–carbon or metal–olefin bonds).

(b) The single largest topic is concerned with metal carbonyl chemistry; within this area dynamic ^{13}C NMR studies have achieved some prominence.

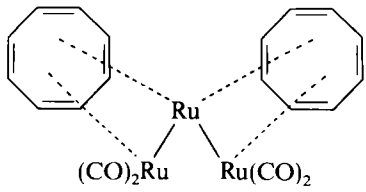
(c) Complexes of the metals iron and platinum have been most intensively studied using this NMR method.

Points (a) and (b) are not surprising since, by definition, organometallic chemistry and the study of metal carbonyls place heavy emphasis on carbon ligands. Point (c) is less obvious and this author feels that the favourable solubility of the iron complexes, and the spin–spin coupling information in the case of platinum (^{195}Pt, $I = 1/2$, natural abundance = 33·7%), may be partly responsible.

Given a mixture of papers covering interests which range from purely synthetic to highly spectroscopic, the organization of the discussion presents a problem; however, the reader is likely to find the desired material by assuming that the iron, cobalt, and nickel triads are discussed sequentially under a given heading.

II. THEORY AND METHODOLOGY

The majority of ^{13}C NMR reports deal with the applications of this technique in the structural chemistry of the complexes; however, some are method oriented. Lyerla *et al.*[6] have measured ^{13}C NMR spectra for tetracarbonylbis(cyclooctatetraene)triruthenium(0) [1] in the solid state. Using magic angle (54°44′) spinning, high power decoupling of the protons, and cross polarization schemes for signal enhancement, they show that the cyclooctatetraene rings are mobile in the solid state at 27 °C (a single resonance at $\delta = 64{\cdot}1$); however, the ^{13}C spectrum at −180°C shows at least six well resolved signals spread over ~50 ppm, indicating lower symmetry.



[1]

There is also a report[7] on the solid state ^{13}C NMR characteristics of several arene chromium tricarbonyl complexes. Interestingly, it is suggested that a substantial part of the shielding resulting on complexation of the arene derives from the external magnetic field having a specific orientation.

These results suggest a promising new application for ^{13}C NMR spectroscopy in inorganic chemistry.

Jordan and Norton[8,9] have reported ^{13}C nuclear relaxation mechanisms in some transition metal–methyl compounds. In the complex [$FeCH_3$(cp)-

$(CO)_2$], relaxation of the usual dipole–dipole variety is dominant; however, for the osmium complex *cis*-[$Os(CH_3)_2(CO)_4$] there is a spin-rotation contribution. The chemical screening anisotropy contribution is considered to be negligible.

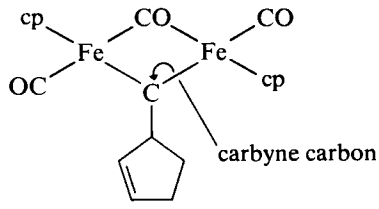
There have been several reports[10,11] concerned with chemical shift theory. The paramagnetic screening contribution, σ_p, is found to be responsible for the *changes* in $\delta^{13}C$ (σ_d can be larger numerically than σ_p). The calculations were performed for metal–carbonyl compounds and, although the agreement between the observed and calculated changes is good, no one individual component of σ_p is exclusively responsible. The high frequency shift in the ^{13}C carbonyl signal "... is a consequence of the product of the three factors in the Pople–Karplus equation, so qualitative attempts to discuss ^{13}C chemical shifts in complexes in terms of any one of these factors are probably doomed from the start".[10] This is probably a good point to remember since, even in simple molecules such as pyridine, σ_p can prove to be more complicated than first thought.[12]

III. ORGANOMETALLIC COMPOUNDS

There have been many reports containing ^{13}C NMR results for organometallic compounds, as this is an area of chemistry currently enjoying increasing popularity. The division of this chapter into sections on σ and π bonding carbons as well as coordinated C≡O is somewhat difficult since, although the topics can be clearly defined, many reports contain data from several headings and a clear separation is not obvious. Typically a research group may concern itself with the metal–olefin interaction, as studied by ^{13}C NMR, in complexes where there are several M–C≡O bonds. This type of molecule is often encountered, for example in the chemistry of iron, ruthenium, and osmium, and the reader must therefore expect considerable overlap between the sections in this chapter.

A. Metal σ-bonded carbon complexes

The *trans* iron carbyne complex [2] is worthy of note in that the carbyne ^{13}C signal appears at $\delta = 448{\cdot}3$. This may well be the highest frequency ^{13}C



[2]

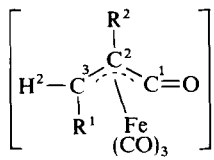
signal observed to date.[13] The corresponding *cis* compound shows δ^{13}C(carbyne) = 443·7. Not quite so dramatic are the high frequency shifts in acyl complexes. The iron acyl complexes [Fe(acyl)(cp)(CO)L] show δ^{13}C values in the region 243–277 ppm,[14] and, interestingly, the acyl carbon ^{13}C shift is more sensitive to changes in L than is the ^{13}C≡O shift.

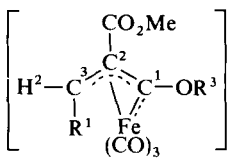
The novel complexes [3] and [4] from $[FeH(CO)_4]^-$ and either H–C≡C–COR or MeO_2C–C≡C–CO_2Me show acyl carbon resonances below 240 ppm; some data are collected in Table I.[15]

Carbon-13 resonances for iron isonitrile complexes[16–18] can also appear at very high frequency when the isonitrile is bridging; for example, $[Fe_2(cp)_2(CO)_3(CNPh)]$ shows δC≡NPh = 257·9 ppm.[18] A similar obser-

TABLE I

^{13}C NMR chemical shifts for some iron carbonyl complexes.[15]





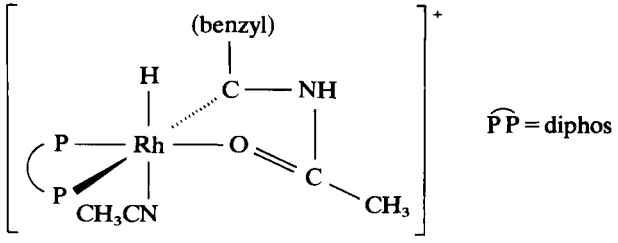
[3a]	R^1 = H	R^2 = CO_2Me	[4a]	R^1 = H	R^3 = Me	
[3b]	CO_2Me	CO_2Me	[4b]	CO_2Me	Me	
[3c]	H	COMe				
[3d]	H	CHO				
[3e]	CO_2Me	H				

Complex	Olefinic carbon	CO_2Me	CO_2Me	Fe–C≡O	Fe–C=O or Fe=COR	Other resonance
[3a]	24·6	50·9	174·2	217·2	242·7	135–125 PPh_3
	23·3					
[3b]	37·1	51·0	177·4	214·7	235·7	134–125 PPh_3
	25·1	50·5	172·9			
[3c]	25·4			216·9	246·1	134–125 PPh_3
	23·2					199·1 *C*OMe
						34·0 CO*Me*
[3d]	35·9			216·3	254·4	134–125 PPh_3
	23·5					183·2 CHO
[3e]	42·4	50·4	178·4	217·8	246·0	133–125 PPh_3
	17·8					
[4a]	52·4	54·1	167·5	210·8	270·0	67·8 CO*Me*
	31·6					
[4b]	51·4	52·6	175·8	208·6	270·9	69·9 CO*Me*
	41·8	52·2	166·3			

vation was made for the cluster $[Pt_3(Bu^tN{\equiv}C)_6]$ which has δCN(bridging) = 231·3 and δCN(terminal) = 163·3.[19]

There are several reports[20–22] containing carbene ^{13}C chemical shifts in many Group VIII metals. Carbene complexes are known to show signals at very high frequencies.[4]

Only one report of ^{13}C data in σ-bonded rhodium complexes has appeared; this concerns the interception of a hydridoalkylrhodium intermediate in a catalytic hydrogenation reaction.[23] The cationic complex [5]



[5]

was measured using a sample enriched in ^{13}C. Coupling to both the non-equivalent phosphorus atoms and the metal is observed.

Moving to the right in the Periodic Table, there are reports for palladium σ-bound carbon complexes derived from phosphonium, arsonium, sulphonium, and pyridinium stabilized ylides,[24] as well as mention of ^{13}C chemical shifts in a 4-pentanonate carbon coordinated complex[25] and nickel–carbon ylide.[26] However, most reports in this area are for platinum complexes.

The early successes of Clark and coworkers[27–30] with ^{13}C NMR of platinum–methyl complexes have spurred further applications such that there now exist ^{13}C data for platinum–methyl hydridotris-(1-pyrazolyl)borates,[31,32] cyclopentadienyl,[33] diethyldiselenocarbamato,[34] dimethyl bisphosphite,[35] and binuclear tetramethyl methyl[36,37] complexes.

The few reports on platinum–aryl complexes contain extensive ^{13}C NMR data. Coulson[38] has measured the spectra of the molecules *trans*-$[PtX(C_6H_5)(PEt_3)_2]$ and has (a) correlated δ^{13}C and $^3J(^{195}Pt–^{13}C)$ (see Table II) with Taft and Swain–Lupton substituent constants and (b) put forward an argument in favour of Pt–C_6H_5 π back-bonding. Together with ^{31}P studies, there are also ^{13}C data for some aryl–platinum complexes of the type [PtX(R)(cod)] in which R is an aryl moiety containing a heteroatom.[39] These latter compounds are the products of the reaction of $[PtCl_2(cod)]$ with aryl tin compounds; data for both the aryl and cod carbons are given in Table III.

TABLE II

^{13}C NMR chemical shifts for some C_6H_5X and phenylplatinum complexes.[38]

	C_6H_5X			*trans*-$(PCH_2CH_3)_2Pt(C_6H_5)X$					
X	*ortho*	*meta*	*para*	*ortho*	*meta*	*para*	P–C	PCH_2–C	Other
H	128·50	128·50	128·50						
CH_3	129·19	128·39	125·47	139·90	126·90	120·36	13·94	8·00	
C_2H_5	127·90	128·38	125·63	140·03	126·81	120·56	13·38	8·04	$\delta PtCH_2 = 4·26$
$CH{=}CH_2$	126·33	128·60	127·90	140·18	126·80	120·69	14·17	7·86	$\delta CH(vinyl) = 140·18$
									$\delta CH_2(vinyl) = 121·86$
C_6H_5	127·36	128·87	127·36	140·00	126·87	120·83	13·81	7·82	
[a]				136·40	126·80	120·60	15·64	8·15	
F_2H				136·48	127·74	121·96	12·79	7·50	
F	115·46	130·11	124·01	137·20	127·32	121·33	12·89	7·46	
Cl	128·76	129·84	126·55	137·35	127·85	121·75	13·54	7·77	δC_1 (aryl) = 139·88
[a]				136·20	127·20	122·40	16·94	8·07	$\delta C_1(aryl) = 157·90$
							15·04	8·31	
Br	131·73	130·17	126·98	137·18	127·90	121·85	14·14	7·77	
I	137·56	130·27	127·52	136·91	128·01	122·07	15·33	7·83	$\delta C_1(aryl) = 144·0$
NCO	124·82	129·62	125·79	~137·8	127·84	121·75	14·02	7·71	
N_3	119·11	129·85	124·94	137·24	127·85	122·03	13·81	7·77	
OCH_3	113·99	129·53	120·73	138·16	127·21	120·89	12·68	7·71	
SC_2H_5	(129·20)[b]	(128·88)[b]	125·86	138·05	127·58	121·33	13·48	7·98	
CN	132·32	129·25	132·86	138·05	127·91	122·30	15·49	8·00	$\delta CN = 35·81$
									$\delta C_1(aryl) = 151·44$
NO_2	123·58	129·41	134·70	137·72	127·68	122·66	13·60	7·61	
$SnCl_3$	134·10	133·24	130·49	135·95	128·93	124·02	16·73	8·27	

[a] *cis*-isomer. [b] Assignment not known because of inability to distinguish *ortho* from *meta* using Scott's method.

TABLE III

^{13}C–{^{1}H} NMR data for some [Pt(cod)(R)X] complexes.[39]

		Aryl carbons[a,b]						cod carbons[b]			
								vinyl		methylene	
R	X	C^1	C^2	C^3	C^4	C^5	C^6	*trans*-aryl	*trans*-Cl	*trans*-aryl	*trans*-Cl
2-C_4H_3S	Cl	137·2	127·3	127·1	129·1			113·3	90·0	28·5	31·9
		(1060)	(61)	(35)	(37)			(50)	(188)	(18)	(19)
2-C_4H_3O	Cl		115·5	110·2	143·8			112·1	89·6	28·6	31·7
								(50)	(188)	(18)	(19)
2-C_8H_5O	Cl		112·1	129·0	120·7	121·9	122·9	113·5	90·4	28·4	31·8
								(50)	(186)	(18)	(19)
3-C_8H_7	Cl	148·8	145·4	137·3	118·6	127·3	131·5	115·8	86·2	27·9	32·2
				(46)		(46)	(12)	(30)	(206)	(24)	(26)
C_6H_4Mep	Cl	140·0	133·5	129·2	133·5	129·2	133·4	115·5	87·1	27·9	32·1
								(27)	(210)	(23)	(24)
Ph	Cl	143·4	133·4	127·8	123·7	127·8	133·4	115·2	87·1	27·5	31·7
			(12)	(50)	(8)	(50)	(12)	(28)	(208)	(24)	(26)
2-C_4H_3S	2-C_4H_3S	145·7	126·9	126·4	130·7			104·4		29·9	
		(1186)	(82)	(56)	(59)			(66)		(<3)	
2-C_4H_3O	2-C_4H_3O	164·1	117·2	109·9	143·5			103·2		29·9	
			(162)	(53)	(59)			(66)		(<3)	
2-C_8H_5O	2-C_8H_5O	168·3	113·9	129·7	119·8	121·5	122·1	105·2		29·9	
								(64)		(<3)	
C_6H_4OMeo	C_6H_4OMeo	141·8	161·0	120·9	123·3	110·4	135·5	102·0		29·9	
		(1077)	(17)	(76)		(37)	(24)	(57)		(<3)	
C_6H_4Clm	C_6H_4Clm	156·7	132·7	133·5	123·1	128·7	133 8	105·3		29·9	
		(1086)	(31)	(97)	(11)	(86)	(37)	(49)		(<3)	
C_6H_4Brp	C_6H_4Brp	153·3	136·0	130·5	117·0	130·5	136·0	105·1		29·9	
			(40)	(81)		(81)	(40)	(49)		(<3)	

[a] Ring numbers are based on Pt at C^1. [b] J (Pt–C)/Hz in parentheses.

TABLE IV

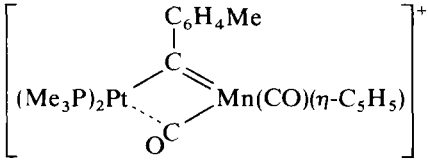
^{13}C NMR data for some isonitrile complexes.[48]

Complex	δ(C)	^1J(Pt–C)	δ(C^1)	CN*R*	P*C*H$_2$	PCH$_2$*C*H$_3$
trans-[PtCl(CNMe)(PEt$_3$)$_3$]$^+$	111·1	1720		31·4 ^3J(Pt–C) 19·2	14·9 $\lvert^1J+{}^3J\rvert$ 35·4 ^2J(Pt–C) 27·5	7·96 ^3J(Pt–C) 19
trans-[PtBr(CNMe)(PEt$_3$)$_2$]$^+$	111·3	1721		31·2 ^3J(Pt–C) 19·1	15·5 $\lvert^1J+{}^3J\rvert$ 35·1 ^2J(Pt–C) 29·0	7·95 ^3J(Pt–C) 19
trans-[PtCl(CNBu^t)(PEt$_3$)$_2$]$^+$	109·6			61·2 (CN*C*Me$_3$) ^3J(Pt–C) 15·1 CNC*Me*$_3$ 29·5	14·9 $\lvert^1J+{}^3J\rvert$ 34·2 ^2J(Pt–C) 28·7	8·06 ^3J(Pt–C) 18
trans-[PtCl(CNC$_6$H$_{11}$)(PEt$_3$)$_2$]$^+$	108·6			56·67 (C^1) ^3J(Pt–C) 15·1 32·03 (C^{2,6}) 24·45 (C^4) 23·60 (C^{3,5})	14·93 $\lvert^1J+{}^3J\rvert$ 35·5 ^2J(Pt–C) 27·5	8·07 ^3J(Pt–C) 18
trans-[PtCl(*p*NO$_2$C$_6$H$_4$NC)(PEt$_3$)$_2$]$^+$	124·8		131·0 ^3J(Pt–C) 27	128·4 (C^{2,6}) 125·6 (C^{3,5}) 148·2 (C^4)	15·1 $\lvert^1J+{}^3J\rvert$ 35·4 ^2J(Pt–C) 29·5	8·05 ^3J(Pt–C) 18
trans-[PtCl(*p*ClC$_6$H$_4$NC)(PEt$_3$)$_2$]$^+$	121 ^2J(P–C) ~ 15		124·4 ^3J(Pt–C) 27	128·1 (C^{2,6}) ^4J(Pt–C) ~ 6 130·6 (C^{3,5}) 137·3 (C^4)	15·1 $\lvert^1J+{}^3J\rvert$ 35·4 ^2J(Pt–C) 30	8·0 ^3J(Pt–C) 18
trans-[PtCl(CNC$_6$H$_5$)(PEt$_3$)$_2$]$^+$	120 ^2J(P–C) ~15		125·9 ^3J(Pt–C) 25	126·5 (C^{2,6}) ^4J(Pt–C) ~ 10 130·4 (C^{3,5}) 131·4 (C^4)	15·17 $\lvert^1J+{}^3J\rvert$35·1 ^2J(Pt–C) 28·6	8·07 ^3J(Pt–C) 16
trans-[PtCl(*p*MeC$_6$H$_4$NC)(PEt$_3$)$_2$]$^+$	119·2 ^2J(P–C) ~15	1730	123·1 ^3J(Pt–C) 27·1	126·2 (C^{2,6}) ^4J(Pt–C) ~ 9 130·9 (C^{3,5}) 142·2 (C^4)	15·1 $\lvert^1J+{}^3J\rvert$ 34·2 ^2J(Pt–C) 29·1	8·0 ^3J(Pt–C) 18

trans-[PtCl(2,6-$Cl_2C_6H_3NC$)(PEt_3)$_2$]$^+$	124 ^2J(P–C) ~15			131·8 ($C^{2,6}$) ^4J(Pt–C) 10·4 129·5 ($C^{3,5}$) 133·7 (C^4)	15·1 $\lvert^1J + {}^3J\rvert$ 35·5 ^2J(Pt–C) 28·3	8·0 ^3J(Pt–C) 18
cis-[$PtCl_2$(CNMe)(PEt_3)]	111·6	1726		31·0 (CNCH_3) ^3J(Pt–C) 22·5	16·4 ^1J(P–C) 40·3 ^2J(Pt–C) 40·3	8·1 ^2J(P–C) 3·7 ^3J(Pt–C) 3·7
cis-[$PtCl_2$($CNBu^t$)(PEt_3)]				59·4 (CNCMe_3) 30·0 (CNCMe_3)	16·5 ^1J(P–C) 40·3 ^2J(Pt–C) ~10	8·1 ^2J(P–C) ~3 ^3J(Pt–C) 3
cis-[$PtCl_2$(CNC_6H_{11})(PEt_3)]	111·4	1742		55·5 (C^1) ^3J(Pt–C) ~18 32·0 ($C^{2,6}$) 22·8 ($C^{3,5}$) 24·6 (C^4)	16·47 ^1J(P–C) 40·3 ^2J(Pt–C) 40·0	8·01 ^2J(P–C) 3 ^3J(Pt–C) 2
cis-[$PtCl_2$(*p*ClC_6H_4NC)(PEt_3)]	122·1 ^2J(P–C) ~15	1740	124·9 ^3J(Pt–C) 25	127·7 ($C^{2,6}$) ^4J(Pt–C) ~8 130·3 ($C^{3,5}$) 136·9 (C^4)	16·7 ^1J(P–C) 39·1 ^2J(Pt–C) 40·5	8·1 ^2J(P–C) 3·3 ^3J(Pt–C) 24
cis-[$PtCl_2$(CNC_6H_5)(PEt_3)]	120·2	1740		126·3 ($C^{2,6}$ + C^1) 130·0 ($C^{3,5}$) 130·8 (C^4)	16·7 ^1J(P–C) 40·3 ^2J(Pt–C) 39·8	8·2 ^2J(P–C) 3·4 ^3J(Pt–C)
cis-[$PtCl_2$(*p*MeC_6H_4NC)(PEt_3)]	120·3	1720	123·9 ^3J(Pt–C) 24	126·0 ($C^{2,6}$) ^4J (Pt–C) ~12 130·5 ($C^{3,5}$) 141·4 (C^4) 21·5 ($CH_3C_6H_4$)	16·6 ^1J(P–C) 40·3 ^2J(Pt–C) 40·2	8·01 ^2J(P–C) ~3 ^3J(Pt–C) 27
cis-[$PtCl_2$(*p*$MeOC_6H_4NC$)(PEt_3)]	114·0		118·7 ^3J(Pt–C) ~24	115·2 ($C^{3,5}$) 127·8 ($C^{2,6}$) 160·8 (C^4) 55·9 (OCH_3)	16·7 ^1J(P–C) 40·3 ^2J(Pt–C) 40·3	8·15 ^2J(*P*–C) ~3 ^3J(Pt–C) ~3
cis-[$PtCl_2$(CNC_6H_{11})$_2$]	105·8			55·82 (C^1) 31·80 ($C^{3,6}$) 22·57 ($C^{3,5}$) 24·70 (C^4)		

There is one report on the platinum cyclopropane complexes $[PtX_2(CH_2)_3(\text{substituted pyridine})_2]$,[40] and four papers on platinum–acyl complexes.[41–44] These latter stress $^1J(^{195}Pt-^{13}C)$ values as well as $\delta^{13}CO$ values. For carbon ligands with multiple bonds to nitrogen there are several studies[45–47] concerned with platinum cyano complexes, and two reports[48,49] containing extensive chemical shift data for isonitrile compounds of the elements of Groups VI, VII, and VIII. Selections of these isonitrile and cyano ^{13}C NMR data are in Tables IV and V respectively.

The complex [6] is interesting in that the bridging carbene carbon resonance appears at 338·1 ppm. The μ-C resonance in



[6]

$[PtRe(\mu CC_6H_4Mep)(CO)_2(PMe_2Ph)(\eta\text{-}C_5H_5)][BF_4]$ is found at 382·6 ppm.[50] Further data are available for bridging ligands of other metals.[51] There is no obvious relationship between [2] and [6] but it is worth noting that the bridging carbon atoms show very high frequency resonances.

TABLE V

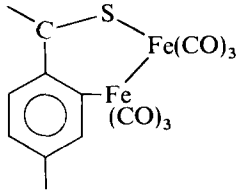
^{13}C chemical shifts and linewidths for some diamagnetic cyano complexes in D_2O.[47]

Complex	δ	$\Delta\nu_{1/2}$(Hz)[a]	Complex	δ	$\Delta\nu_{1/2}$(Hz)[a]
Octahedral hexacyano complexes			*Tetrahedral tetracyano complexes*		
$Fe(CN)_6^{4-}$	177·2	3·2	$Cu(CN)_4^{3-}$	161·6	6·5
$Ru(CN)_6^{4-}$	162·3	3·5	$Zn(CN)_4^{2-}$	147·0	5·5
$Os(CN)_6^{4-}$	142·5	2·8	$Cd(CN)_4^{2-}$	149·8	7·0
$Co(CN)_6^{3-}$	140·1[b]	16	$Hg(CN)_4^{2-}$	153·2	4·0
$Rh(CN)_6^{3-}$	131·9[c]	5·2	*Linear dicyano complexes*		
$Ir(CN)_6^{3-}$	110·9	26	$Ag(CN)_2^-$	150·0	3·0
$Pd(CN)_6^{2-}$	104·2	2·0	$Au(CN)_2^-$	154·2	6·0
$Pt(CN)_6^{2-}$	84·7[d]	17	$Hg(CN)_2$	144·6	[f]
Square-planar tetracyano complexes					
$Ni(CN)_4^{2-}$	136·6	2·5			
$Pd(CN)_4^{2-}$	131·9	2·0			
$Pt(CN)_4^{2-}$	125·7[e]	2·2			
$Au(CN)_4^-$	105·2	5·2			

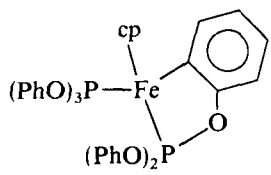
[a] Width at half-height ±5%. [b] Eight-line pattern; $^1J(Co-C) = 126·0 \pm 0·8$ Hz. [c] Doublet; $^1J(Rh-C) = 33·6$ Hz. [d] Three-line pattern (^{195}Pt, 33·7%); $^1J(Pt-C) = 808$ Hz. [e] Three-line pattern; $^1J(Pt-C) = 1034$ Hz. [f] Linewidth is temperature dependent: $\Delta\nu_{1/2} = 62$ Hz (30 °C), 15 Hz (70 °C).

B. Cyclometallation

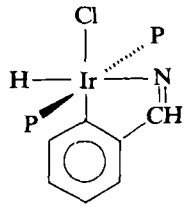
There have been several reports concerned with the ^{13}C NMR characteristics of cyclometallated complexes. Specifically, Alper[51a] and Stewart *et al.*[52] have reported data for iron complexes, whereas others[53,54] have worked with iridium. The latter research has been concerned with mechanistic studies on the activation of C–H bonds by transition metals.[55] There is a report[56] of a metallated diazobutadiene of nickel(II) [12] as well as reports on metallated rhodium ylides[57] and alkenylpyridine[58] complexes. An interesting aspect of these ^{13}C NMR studies is that the metallated aromatic carbon is shifted to high frequency relative to the unmetallated ligand; this observation may well have analytical value for the organometallic chemist. Examples of cyclometallated complexes are [7], [8], [11], and [12]; some data may be found in Table VI.



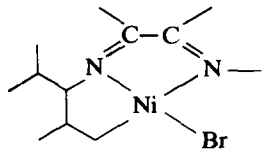
[7][51]



[8][52]



[11][53]



[12][56]

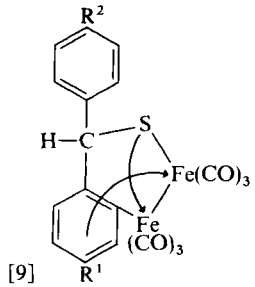
C. Metal carbonyl complexes

The chemistry of metal carbonyls continues to attract many chemists, and an increasing number of reports contain ^{13}C NMR data from complexes enriched in ^{13}CO. Many of the measurements are performed with some relaxation reagent present—Cr(acac)$_3$ is still quite popular—since the ^{13}C≡O T_1 is long. In contrast to other ^{13}C NMR studies, dynamic ^{13}C NMR plays a major role in an effort to obtain a better understanding of C≡O scrambling processes.

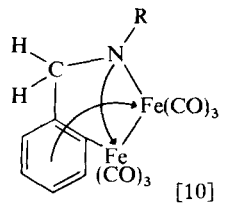
The cluster complex [13] serves as a convenient bridge from the σ-bonded carbon sections. $(Et_4N)_2[Fe_6C(CO)_{16}]$, in which the carbide is at the centre

TABLE VI

^{13}C NMR data for some *ortho*-metallated iron complexes.[51a]



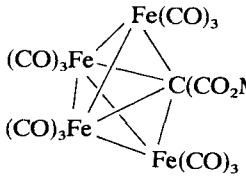
[9]



[10]

δ(C)	Assignment
	[9] $R^1 = R^2 = H$
63·3	Benzylic carbon
123·3, 125·4, 126·4, 127·2, 127·6, 128·2, 128·6, 131·5	Phenyl carbons
155·2	Iron substituted phenyl carbon
	[9] $R^1 = R^2 = CH_3$
20·4	R^1 carbon
21·0	R^2 carbon
62·9	Benzylic carbon
123·9, 124·6, 126·2, 128·0, 128·7, 131·4, 134·9, 137·2, 140·2	Phenyl carbons
153·5	Iron substituted phenyl carbon
209·3, 209·7	Carbonyl carbons
	4,4′-Dimethoxythiobenzophenone
55·5	Methoxy carbons
113·2	Carbons *ortho* to methoxy bearing carbons
132·1	Carbons *meta* to methoxy bearing carbons
140·8	Thiocarbonyl substituted carbons
163·1	Methoxy substituted carbons
233·3	Thiocarbonyl carbon

δ(C)	Assignment
	[9] $R^1 = R^2 = OCH_3$
54·9	$R^1 = OCH_3$
55·4	$R^2 = OCH_3$
62·8	Benzylic carbon
113·7, 121·8, 127·5, 128·8, 135·1, 136·0, 137·0	Phenyl carbons
156·2	Iron substituted carbon
159·1	R^1 substituted carbon
162·1	R^2 substituted carbon
209·8, 210·2	Carbonyl carbons
	[9] $R^1 = R^2 = N(CH_3)_2$
40·0	$R^1 = N(CH_3)_2$
41·2	$R^2 = N(CH_3)_2$
63·3	Benzylic carbon
104·3, 111·8, 117·8, 127·1, 127·6, 133·0, 135·7	Phenyl carbons
147·5	R^1 substituted carbon
151·5	R^2 substituted carbon
161·4	Iron substituted carbon
210·2, 210·7	Carbonyl carbons
	[10] $R = CH_3$
55·0	Methyl carbon
77·2	Methylene carbon
126·8, 128·9, 129·4, 131·5,	Phenyl carbons
152·7	Iron substituted carbon
212·3, 212·7	Carbonyl carbons



[13]

of an octahedron of iron atoms, affords [13] on oxidation.[59] For $[Fe_4H(\eta^2\text{-}CH)(CO)_{12}]$,[59a] which has the methine H bridging C and Fe, $\delta CH = 335$. The cluster complexes $[Co_6(CO)_{15}{}^{13}C]^{2-}$ ($\delta^{13}C = 330{\cdot}5$)[60] and $[Rh_6(CO)_{15}C]^{2-}$ ($\delta^{13}C = 264{\cdot}7$)[61] which have fully encapsulated carbide carbon also show high frequency signals. In the latter case splitting occurs due to coupling to ^{103}Rh, 13·7 Hz, whereas the former affords a broad signal. The structure of the hexarhodium complex is known and shows two eclipsed triangles of rhodium, six terminal CO ligands ($\delta = 198{\cdot}1$), and inequivalent bridging CO ($\delta = 225{\cdot}2$, 236·3). There are carbonyl cluster complexes in which carbon,[62–64] nitrogen,[65] and sulphur[66,67] have been fully or partially encapsulated.

The chemistry of iron carbonyl complexes has taken full advantage of ^{13}C NMR methods. Graham and coworkers[68–70] have reported in detail on the stereochemically non-rigid 6-coordinate complexes *cis*-$[Fe(CO)_4(ER_3)_2]$ which contain SiR_3 and SnR_3 ligands. Using the values of $^1J(^{57}Fe\text{–}^{13}CO)$ and $^2J(^{29}Si\text{–}^{13}CO)$,[71] assignments of the ^{13}CO resonances are made and the intramolecular nature of the rearrangement is proved. Data for analogous Ru and Os complexes are also available and some are shown in Table VII. There are several studies reporting ^{13}C shifts in mononuclear iron carbonyls containing chelating phosphines,[72,73] $[Fe(cp)CH_3(CO_2)]$ and related PPh_3 complexes,[74] and a thiocarbonyl, $[Fe(CO)_4(CS)]$, which is still dynamic on the NMR time scale at −70 °C ($\delta CS = 327{\cdot}5$, $\delta CO = 211{\cdot}2$).[75] The complex cyclooctatetraenetricarbonyliron has been reinvestigated using ^{13}C NMR and found to undergo 1,2 shifts.[76] This new result is in contrast to previous, less successful, studies using ^{1}H NMR and shows the value of ^{13}C NMR.

Turning to dimeric compounds, the continued interest in the isomerization of diiron carbonyl complexes is shown by ^{13}C NMR studies on η^6-(bicyclo[6.2.0]dodeca-2,4,6-triene)hexacarbonyldiiron(Fe–Fe), $[Fe_2(CO)_6(C_{10}H_{12})]$,[77] $[Fe_2(POP)_2(CO)_5]$ and $[Fe_2(POP)(CO)_7]$, where $POP = (EtO)_2POP(OEt)_2$,[78] and $[Fe_2(\eta^5\text{-}C_5H_4CMe_2CMe_2\text{-}\eta C_5H_4)(CO)_4]$. In this last complex the cyclopentadienyl groups have been linked together to restrict specific isomerization pathways.[79] For the linked "chelating" cyclopentadienyl the *cis–trans* isomerization and CO scrambling involves a mechanism that requires a bridged complex to be converted directly to staggered configurations of the non-bridged intermediates, plus internal rotation to account for bridged-terminal carbonyl exchange.[79] All three

TABLE VII

^{13}C chemical shifts[a] for some *cis*-$M(CO)_4(ER_3)_2$ derivatives.[68]

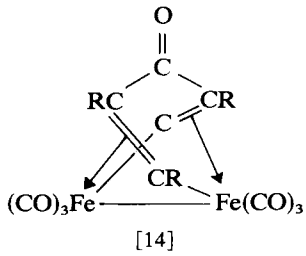
Compound	CO_{ax}		CO_{eq}	Me	Temp. (K)
$Fe(CO)_4(SiMe_3)_2$	208·50		207·64	7·50	183[b]
		208·07		7·61	298[b]
		207·96		7·71	301
$Fe(CO)_4(SiMe_2Cl)_2$	205·70		203·27	12·09	253
		204·53		12·30	303
$Fe(CO)_4(SiMeCl_2)_2$	202·46		200·14	17·16	253
	202·46		200·04	16·89	303
$Fe(CO)_4(SiCl_3)_2$	199·44		197·34		303
	199·71		197·88		308[b]
$Fe(CO)_4(GeMe_3)_2$	208·94		207·15	6·96	223[b]
		208·07		7·34	293[b]
$Fe(CO)_4(SnMe_3)_2$	207·86		208·07	−3·67	163[c]
		207·75		−3·29	273
		207·75		−3·24	293[c]
		208·07		−2·75	300[b]
$Ru(CO)_4(SiMe_3)_2$	198·53		191·62	7·72	253
$Ru(CO)_4(SiMe_2Cl)_2$	194·54		188·12	12·36	303
$Ru(CO)_4(SiMeCl_2)_2$	190·60		185·42	17·37	305
$Ru(CO)_4(SiCl_3)_2$	187·46		183·63		293[b]
$Ru(CO)_4(GeMe_3)_2$	198·04		191·30	7·02	303
$Ru(CO)_4(SnMe_3)_2$	197·39		192·27	−4·74	300
$Ru(CO)_4(PbMe_3)_2$	196·96		192·21	−0·86	303
$Os(CO)_4(SiMe_3)_2$	181·48		172·58	6·74	303
$Os(CO)_4(SiMe_2Cl)_2$	177·54		169·61	11·44	303
$Os(CO)_4(SiMeCl_2)_2$	173·71		167·23	16·51	303
$Os(CO)_4(GeMe_3)_2$	180·40		171·88	5·24	293
$Os(CO)_4(SnMe_3)_2$	179·05		172·26	−6·63	303
	179·21		172·58	−6·69	293[b]
$Os(CO)_4(PbMe_3)_2$	179·32		172·31	−3·18	303

[a] Solvent is [$^2H_8$]toluene except as noted.
[b] CD_2Cl_2 as solvent.
[c] CD_2Cl_2–[$^2H_{14}$]methylcyclohexane (1 : 1) as solvent.

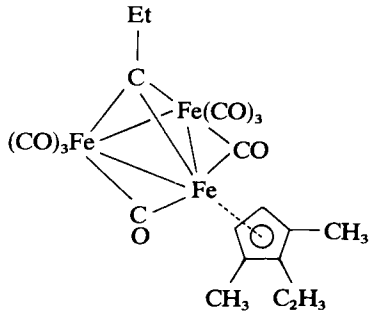
reports[77–79] contain X-ray structural data and extensive variable temperature ^{13}C NMR work. Temperatures below −120 °C are reached using mixtures involving CD_2Cl_2 and $CHFCl_2$.

There are also ^{13}C NMR measurements on diiron complexes of guaiazulene,[80] azulene,[81] and indene,[82] and an interesting new diphenylacetylene nonacarbonyl whose structure is represented by [14].[83]

Further reports include low temperature ^{13}C NMR studies on the diiron complexes $[Fe_2(CR^1{=}CR^2{-}CR^3{=}CR^4)(CO)_6]$ and $[Fe_2(\mu CR^1{=}$



[14]



[15]

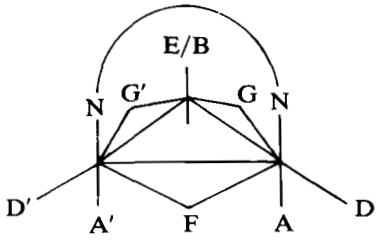
$CR^2COCR^3{=}CR^4)(CO)_6]$ which contain σ and η bonded olefins,[84] as well as the bicyclic nonatriene complex $[Fe_2(C_9H_{10})(CO)_6]$.[85]

Moving from dimers to trimers, the somewhat unusual octacarbonyl (η-1,3-dimethyl-2-vinylcyclopentadienyl)-μ_3-propylidene-triangulo-triiron [15] formed from $[Fe_3(CO)_{12}]$ and methylacetylene has been characterized in the solid state and by ^{13}C NMR in solution;[86] it contains an Fe_3C cluster similar to the Co_3C part of $[Co_3(CY)(CO)_9]$. The apical carbon in the Fe_3C unit has $\delta^{13}C = 345{\cdot}6$, which is more than 100 ppm to high frequency of the resonance from [13]. Iron-57 satellites in $Fe_3(CO)_{12}$ have been observed, and the average value $^1J(^{57}Fe{-}^{13}CO) = 24{\cdot}9$ Hz agrees with the value of 23·4 Hz found for $Fe(CO)_5$.[87]

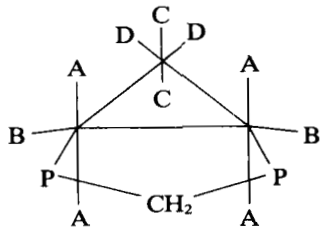
Turning to ruthenium, the ^{13}C NMR spectrum of the diruthenium triene complex η^6-(bicyclo[6.2.0]deca-2,4,6-triene)hexacarbonyldiruthenium,[88] an analogue of the related diiron complex,[85] makes it the first diruthenium complex to be studied by ^{13}C NMR methods. The mechanism of the fluxional process is basically the same as that for the diiron compound.

The difference in the solid state structure of $[Fe_3(CO)_{12}]$ [89] and that of both $Ru_3(CO)_{12}$ [90] and $Os_3(CO)_{12}$ [91] has prompted further solution studies on these triangular compounds. The ^{13}C NMR spectra of these three complexes reveal one signal each for the Fe and Ru, but two for the Os compound,[92] suggesting that the Os complex is the least dynamic on the NMR time scale.

Detailed variable temperature (−156 to +75 °C) studies on (1,2-diazine)decacarbonyl-triangulo-triruthenium show three distinct phases of fluxionality and suggestions capable of accounting for these are put forward.[93] The static structure represented by [16] is consistent with the low temperature ^{13}C NMR and X-ray studies. In structures [16] and [17], A, B, C, D, E, etc. represent the various carbonyl positions and N—N and P the diazine and phosphinomethane ligands respectively. The decacarbonyl $[Ru_3(CO)_{10}(dppm)]$ (dppm = bisdiphenylphosphinomethane), a compound that is suggested to be suitable as a model for CO migration on a metal



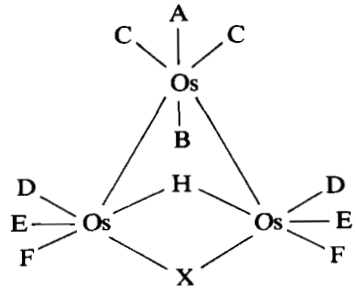
[16]



[17]

surface, has been studied by NMR and found to have structure [17]. A cluster similar to [18] has been found with pyridazine.[94]

Several groups have reported low temperature ^{13}C NMR spectra for the tetranuclear hydride cluster $[H_2FeRu_3(CO)_{13}]$.[95,96] The studies use the high frequency position of a bridging carbonyl relative to a terminal carbonyl, and the relatively large value of $^2J(^{13}CO\text{–}H_{hydride})_{trans}$ as assignment aids; however, there seems to be some disagreement on the mechanism of the fluxionality. These studies have led to further investigations including the molecules $[H_2FeRuOs_2(CO)_{13}]$ [97] and $[H_2Ru_4(CO)_{13}]$.[98]



[18]

As an end to the ruthenium carbonyl clusters there are ^{13}C measurements on $[Ru_3H(C_2C(CH_3)_3)(CO)_9]$ from a study of the reaction of $[Ru_3(CO)_{12}]$ with 3,3-dimethylbut-1-yne,[99] and data on the solid state and solution structures for $[Ru_4(CO)_{12}(C_2Ph_2)]$ [100] and $[Ru_6(CO)_{18}]^{2-}$.[101]

Limiting spectra for the osmium triangular clusters $[Os_3(norbornadiene)(CO)_{10}]$ [102] and $[Os_3(CO)_{12-n}(PEt_3)_n]$ [103] have been obtained. The former complex equilibrates the CO ligands via a triply bridged intermediate, whereas the latter is thought to undergo carbonyl exchange selectively along one edge of the Os_3 triangle at low temperature.

Low temperature ^{13}C NMR spectra for nine complexes of the type $[Os_3H(CO)_{10}X]$ [18] (X = SEt, SPh, NBu^nH, OH, Cl, Br, I, CO_2Me, and CO_2CF_3) have been recorded[104] and these data are shown in Table VIII

TABLE VIII

Limiting ^{13}C NMR data for some osmium and ruthenium carbonyl complexes of type [18].[104]

Complex	δ	Relative intensity	Assignment[a]	Coupling constants[b]
$[Os_3(CO)_{10}H(SEt)]$	180·0	1	A, B	
	179·8	1	A, B	
	176·3	2	D, E, F	
	173·7	2	C	
	170·4	2	D, E, F	
	169·5	2	D, E, F	10·4
$[Os_3(CO)_{10}H(SPh)]$	180·7	1	A, B	
	180·1	1	A, B	
	176·0	2	D. E, F	
	173·8	2	C	
	171·8	2	D, E, F	
	169·3	2	D, E, F	10·5
$[Os_3(CO)_{10}H(NBu^nH)]$	191·3	1	A, B	
	183·4	1	A, B	
	179·4	2	C, D, E, F	
	178·2	2	C, D, E, F	
	174·9	2	C, D, E, F	12·2
	172·8	2	C, D, E, F	
$[Os_3(CO)_{10}H(OH)]$	182·1	1	A, B	
	180·6	1	A, B	
	176·4	4	C, D, E, F	
	172·3	2	C, D, E, F	15·0
	169·6	2	C, D, E, F	
$[Os_3(CO)_{10}H(Cl)]$	183·8	1	A, B	
	183·3	1	A, B	
	177·5	2	D, E, F	
	176·9	2	C	
	172·9	2	D, E, F	
	172·5	2	D, E, F	10·0
$[Os_3(CO)_{10}H(Br)]$	185·4	1	A, B	
	184·8	1	A, B	
	178·1	2	D, E, F	
	177·1	2	C	
	173·3	2	D, E, F	
	172·3	2	D, E, F	11·7
$[Os_3(CO)_{10}H(I)]$	184·9	1	A, B	
	183·8	1	A, B	
	175·3	2	D, E, F	
	173·8	2	C	
	170·5	2	D, E, F	
	168·5	2	D, E, F	9·6
$[Os_3(CO)_{10}H(CO_2Me)]$	186·2	1	A, B	
	184·6	1	A, B	
	178·7	2	C	
	177·0	6	D, E, F	

TABLE VIII—*cont.*

Limiting ^{13}C NMR data for some osmium and ruthenium carbonyl complexes of type [18].[104]

Complex	δ	Relative intensity	Assignment[a]	Coupling constants[b]
$[Os_3(CO)_{10}H(CO_2CF_3)]$	185·0	1	A, B	
	183·4	1	A, B	
	177·1	2	D, E, F	
	176·1	2	C	
	175·8	2	D, E, F	
	175·3	2	D, E, F	
$[Ru_3(CO)_{10}(NO)_2]$	198·4	4		
	196·3	2		
	196·2	2		
	182·2	2		
$[Ru_3(CO)_8(NO)_2(PPh_3)_2]$	206·1	4		
	200·4	2		8·3
	197·7	2		$[J(^{13}C-^{31}P)]$

[a] Based on relative intensity and fluxional behaviour.
[b] $J(^{13}C-H)$ in Hz unless otherwise stated.

where A, B, C, D, E, and F represent the various carbonyl positions. A polytopal rearrangement in an $[Os(CO)_4]$ unit is considered. Continuing in osmium hydride chemistry, the complexes $[M_3H_2(CO)_9S]$ and $[M_3H_3(CO)_9(CCH_3)]$ (M = Ru, Os) have been shown by variable temperature ^{13}C NMR studies in solution to have the solid state geometries,[105] whereas the cluster $[Os_3H(CO)_{11}]^-$, derived from $[Os_3(CO_{12}]$ and KOH, has the same structure as that established for $[Fe_3H(CO)_{11}]^-$ in the solid state.[106] This osmium hydrido anion can be used as starting material to synthesize the new osmium clusters $Os_3H[(CO)_9(CNBu^t)(COR)]$ (R = Me, Et)[107] which contain *O*-alkylated bridging carbonyl groups. The alkylated carbonyl resonance occurs at ~350 ppm to high frequency of TMS; again this is a form of bridging carbon which appears at high frequency. Concluding this paragraph on osmium hydrides, the ^{13}C NMR data of $[Os_3H(CO)_{10}\cdot O_2C\cdot Os_6(CO)_{17}]^-$ [108] and the hexadienyl complex $[Os_2H(CO)_9(C_6H_7)]$ [109] have been reported.

The ^{13}C NMR spectra of the complexes $[Os_3(CO)_{10}X_2]$, which have structure [18] with H = X, reveal that stereospecific exchange of labelled CO occurs on the non-unique osmium atoms.[110] There is also a separate short report for $[Os_3(CO)_{10}(NO)_2]$.[111] For osmium there are ^{13}C NMR data for higher molecular weight clusters containing five ($[Os_5H(CO)_{15}]^-$),[112] six ($[Os_6H_2(CO)_{18}]$),[113] and seven ($[Os_7(CO)_{21}]$)[114] metal atoms. The ^{13}C NMR spectrum of the heptaosmium complex shows this molecule to have the same monocapped octahedral arrangement in solution as in the solid state.

The number of ^{13}C NMR studies on the cobalt triad, by comparison with the iron triad, is much smaller. Spectra for the mononuclear 5-coordinate compounds $[Co(EX_3)(CO)_4]$ (E = C, Si, Ge, Sn, or Pb; X = F, Cl, CH_3, C_4H_9, $CH_2C_6H_5$, or C_6H_5) have been recorded.[115] The axial and equatorial carbonyl groups are averaged at high temperatures, but exchange slowly at lower temperatures, with the ligands $SnCl_3^-$ (below −160 °C) and CF_3

TABLE IX

^{13}C NMR parameters and carbonyl stretching frequencies for some rhodium carbonyl complexes.[120]

Compound	Solvent	δ	^1J(Rh–C)	^2J(Rh–C)	$\nu(^{12}CO)$ (cm^{-1})
$Rh(Ph_3P)_2COCl$	$CDCl_3$	187·4	73	16	1980 ($CHCl_3$)
$Rh(Et_2PhP)_2COCl$	$CHCl_3$	179.8	64·9	9.6	1953 (C_6H_6)
$Rh(tol_3Sb)_2COCl$	CH_2Cl_2	185·5	68·3		
$[Rh(CO)_2Cl]_2$	$CDCl_3$	177·5	75		2089, 2035 (C_6H_{14})
$[Rh(CO)_2Cl]_2$	C_6H_6	177·8	75·5		
$Ph_4P^+[Rh(CO)_2Cl_2]^-$	$CDCl_3$	181·3	72		2065, 1980 ($CHCl_3$)
$Pr_4N^+[Rh(CO)_2Cl_2]^-$	$(CD_3)_2CO$	183·1	72		2070, 1994 (CH_2Cl_2)
$Pr_4N^+[Rh(CO)_2Br_2]^-$	$(CD_3)_2CO$	183·4	72		2068, 1993 ($CDCl_3$)
$Rh_2(phtal)(CO)_4$	CH_2Cl_2	181·6	72·7		2094–2001 (solid)
$Rh(py)(CO)_2Cl$	CH_2Cl_2	183·1	64·5		2075, 2010
		181·2	67·3		(solid)
$Rh(py)(CO)_2Cl$	$C_6H_5CH_3$	185·6	67		
		181·3	73		
$Rh(acac)(CO)_2$	C_6H_5D	177·7	73		2086, 2013 ($CHCl_3$)
$Rh(acac)(CO)_2$	CH_2Cl_2	183·8	71·9		
$Rh(oxq)(Co)_2$	C_6H_5D	186·3	68	*trans*-N	2084, 2010
		184·9	71	*trans*-O	($CHCl_3$)
$Rh(acac)(Ph_3P)CO$	C_6H_5D	190·4	76	25	1984 ($CHCl_3$)
$Rh(acac)(Ph_3P)CO$	CH_2Cl_2	189.5	74.8	24.8	
$Rh(acac)(Ph_3As)CO$	CH_2Cl_2	188·4	73·7		1976
$Rh(oxq)(Ph_3P)CO$	C_6H_5D	192·0	71	21	1970 ($CHCl_3$)
$Rh(oxq)(C_8H_{14})CO$	$CDCl_3$	187·7	76		1978 ($CHCl_3$)
$Rh(Ph_3P)_2COCl{\cdot}TCNE$	$CDCl_3$	182·7	61	11	2071 (solid)
$Rh(oxq)(Ph_3P)CO{\cdot}TCNE$	C_6H_5D	183·0	59	13	2075 (solid)
$Rh(Ph_3P)_2COCl_3$	$CDCl_3$	175·5	58	8	2105 (solid)
$Rh(acac)(Ph_3P)COI_2$	C_6H_5D	180·3	55	15	2094 (C_6H_6)
$Rh(\eta^5\text{-}C_5H_5)(CO)_2$	$CHCl_3$	190·9	83		2051, 1987 (C_6H_6)

(−10 °C) representing the extreme cases. The dimeric complexes $[Co_2(CO)_6(RC_2R')]$[116] and the sulphur capped trinuclear $[FeCo_2(CO)_9S]$[117] have also been investigated at lower temperatures; these are shown to undergo CO scrambling. The question of differential scrambling of CO ligands in the tetranuclear clusters $[FeCo_3H(CO)_{12}]$[118] and $[Co_4(CO)_{10}(Ph-C{\equiv}CPh)]$[119] has been looked into. In the former complex the stereochemical non-rigidity is limited to the Co_3 unit, whereas from the ^{13}C NMR spectrum of $[Co_4(CO)_{12}]$ total scrambling is found.[118] For a series of rhodium carbonyl complexes, an approximate correlation of $\delta^{13}CO$ with $\nu(CO)$ has been observed. If the analysis is restricted to square planar complexes, the variations in $\nu(CO)$ parallel the changes in $^1J(^{103}Rh-^{13}C)$ (see Table IX).[120] Evans *et al.*[121] discuss the variable temperature ^{13}C NMR spectra for $[Rh_2(cp)_2(CO)_2(PR_3)]$, $[Rh_4(CO)_{12}]$, $[Rh_4(CO)_{11}(PPh_2R)]$, and $[Rh_6(Co)_{15}]^-$ amongst others. A variety of CO fluxional mechanisms are observed. The anions $[Rh_{13}H_{5-n}(CO)_{24}]^{n-}$ ($n = 2$ and 3) give ^{13}C NMR spectra at room temperature which suggest a certain

TABLE X

NMR parameters[a] (^1H, ^{13}C, and ^{195}Pt) for some complexes of the type $[NPr^n_4][PtCl_2R(CO)]$.[126]

R		δ(H)	J(Pt–H)	δ(C)	J(Pt–C)	δ(C)	J(Pt–CO)	δ(Pt)[b]
Me		1·0	78·8	−15·5	568	162·5	2013	664
Et	(α)	1·97	−85·0	3·0	568	161·9	2155	657
	(β)	1·23	+71·4	18·8	14			
Pr^n	(α)	1·98	85·7	13·3	577	161·8	2141	652
	(β)			26·9	11			
	(γ)			17·8	79			
Pr^i	(α)	2·6	110[c]	20·7	592	162·3	2237	727
	(β)	1·42	66·6	28·8	~4			
Bu^n	(α)	1·99	85·0	10·7	576	161·8	2144	653
	(β)			36·1	12			
	(γ)			26·4	79			
	(δ)			14·0	~0			
Ph	(α)			130·0	853	159·4	2042	878
	(o)	7·44	59·2	137·8	35			
	(m)	~7·0		128·0	60			
	(p)			124·4				

[a] From ^1H and $^1H-\{^{195}Pt\}$ INDOR measurements on CH_2Cl_2 solutions except for R = Ph($CDCl_3$), and $^{13}C-\{^1H\}$ measurements on $CDCl_3$ solutions except for R = Me [$(CD_3)_2CO$] and R = Ph (CH_2Cl_2–CD_2Cl_2).

[b] In ppm to high frequency of 21·4 MHz [ν(TMS) 100 MHz].

[c] From INDOR spectrum.

selection in the carbonyl scrambling process. Interestingly the hydrides migrate rapidly around the inside of the hexagonal close-packed structure.[122] It is useful to be able to decouple ^{108}Rh since this helps in spectral assignment and provides metal chemical shift data.[122a]

Completing this column in the Periodic Table is a report on the ^{13}C NMR characteristics of $[Ir_4(CO)_{12-n}(PPh_2Me)_n]$ $(n = 1–4)$.[123]

Spectra for 116 $[NiL(CO)_3]$ derivatives have been measured.[124] The donor/acceptor ratio of L is discussed and the CO force constant is related to δ^{13}CO. Several studies on the ^{13}C NMR of mononuclear platinum carbonyl complexes have appeared,[125–127] two of these in connection with detailed vibrational spectroscopic studies.[125,126] In these Pt(II) compounds the carbonyl ligand reveals a low *trans* influence, similar to chloride. The value of $^1J(^{195}Pt–^{13}CO)$ can be as large as 2237 Hz, as in *cis*-$[NPr^n_4][PtCl_2(Pr^i)(CO)]$, or as small as 990 Hz, as in *trans*-$[PtH(CO)(PEt_3)_2][BF_4]$, and the Pt–C≡O bond distance reflects this change.[127] A collection of some of these data is shown in Tables X and XI. There is a single report concerning the ^{13}C and ^{195}Pt NMR characteristics of polyhedra of the type $[PT_n(CO)_{2n}]^{2-}$ $(n = 3, 6, 9, 12, 15)$.[128] The spectra of these clusters provide evidence against terminal edge carbonyl exchange within $[Pt_3(CO)_3(\mu CO)_3]$ and for rotation and interexchange of Pt_3 triangles.

TABLE XI

^{13}C NMR data for some platinum complexes.[127]

Complex	δ(CO)[a]	^1J(Pt–C)	^2J(Pt–C)
trans-$[PtH(CO)(PEt_3)_2][BF_4]$	182·8	990	7·5
trans-$[Pt(ClC_6H_4)(CO)(PEt_3)_2][BF_4]$	176·8	978	9
trans-$[Pt(CH_2Ph)(CO)(PEt_3)_2][BF_4]$	175·7	960	10
trans-$[Pt(NCS)(CO)(PPh_3)_2][BF_4]$	164·9[b]	1817	10
trans-$[Pt(NO_3)(CO)(PPh_3)_2][BF_4]$	160·5[b]	1817	10
	158·8	1795	10
trans-$[PtBr(CO)(PPh_3)_2][BF_4]$	159·0	1772	9
trans-$[PtCl(CO)(AsEt_3)_2][BF_4]$	158·8	1740	
trans-$[PtCl(CO)(PPh_3)_2][BF_4]$	158·6	1788	9
trans-$[PtCl(CO)(AsPh_3)_2][BF_4]$	158·2	1724	
trans-$[PtI(CO)(PPh_3)_2][BF_4]$	157·4	1658	6
cis-$[PtCl_2(CO)(AsEt_3)]$	155·8	1725	
[Hpy][$PtCl_3(CO)$]	151·8	1730	
$[PtBr(CO_2Me)(PPh_3)_2]$	169·3	1345	7·5
$[PtCl(CO_2Me)(PPh_3)_2]$	168·5	1346	7·5
$[PtCl_2(CNMe)(AsEt_3)]$	107·2[c]	1723	

[a] For solutions in $CDCl_3$ unless otherwise stated.

[b] For solutions in $(CD_3)_2CO$.

[c] Refers to CH_3NC; $\delta(CH_3NC)$ 31·8 ppm.

D. π Olefinic complexes

The metal–olefin interaction attracts attention from both organic and inorganic research groups and is therefore a popular subject for ^{13}C NMR studies. There has been a marked increase in the number of these investigations on iron carbonyl olefin complexes. Tetracarbonyl mono-olefin iron complexes of *trans* cycloalkenes, *trans* cyclic dienes,[129] and allyl cations[130] have been investigated using ^{13}C NMR spectroscopy, and in the tricarbonyl diolefin iron family there are reports on butadiene,[131] oxa- and aza-[4.4.3]propellanes,[132] bicyclic 2,3-dimethylidene cyclic olefins,[133] cyclonona-triene and -tetraene,[134] and cyclononatrienone.[134a] The butadiene complex $[Fe(C_4H_6)(CO)_3]$ has been investigated in some detail via a complete analysis of all the proton–carbon coupling constants.[135] The data have been interpreted in terms of a non-planar C,H skeleton in which the C–C bond lengths are approximately equal with some sp^3 hybridization at the terminal carbons. Figure 1 shows the experimental and calculated spectra for the two types of butadiene carbon as shown by [19] and [20].

A ^{13}C NMR report on $[Fe_2(CO)_7(C_8H_8O)]$ (C_8H_8O = 5,6-dimethylene-7-oxabicyclo[2.2.1]hept-2-ene) is complemented by structural and theoretical studies. The molecule contains both $Fe(CO)_3$(diene) and $Fe(CO)_4$(monoene) units[136] and thus fits in the "monomer" category. Some binuclear 4*H*-indenediiron pentacarbonyl compounds have also been measured.[137]

The CO ligands have been replaced by $P(OCH_3)_3$ ligands in a report[138] in which ^{13}C NMR data for twenty-four complexes of the type [Fe(η^4-diene)-(phosphorus ligand)$_3$] are given (see Table XII). Interestingly, these complexes may be prepared by metal atom evaporation techniques. Data for similar complexes, prepared from $Fe(1,5\text{-}C_8H_{12})_2$ and $P(OCH_3)_3$, have been given.[139] There is a mention of ^{13}C NMR data for $[Fe(CO)_2(CNEt)$-(cyclohexa-1,3-diene)].[140]

Carbon NMR spectra of iron cyclopentadiene complexes continue to appear.[141–145] In particular, acyl ferrocenes have been protonated, both on iron and oxygen,[142] and studied from the point of view of acyl rotation.[141] The first ^{57}Fe–^{13}C coupling constants in ferrocene (4·70 Hz) and 1,1-dimethylferrocene (4·56 Hz) have been measured and compared with calculated values.[144] The presence of adjacent ferrocene units stabilizes carbonium ions sufficiently to allow their study by ^{13}C NMR methods. Thus the isopropyl,[146] α-cymantrenyl,[147] and 1,1′-di-(1-methyl-1-ethylium)[148] ferrocenyl cations have all been studied. Lastly, there are ^{13}C NMR reports on [1,1′](1,3-cyclopentylene)ferrocenophane, "caged" ferrocenes,[143] $[Fe(cp)Cl(CO)_2][(PF_2)_2NCH_3]_2]$,[145] and polymethylferrocenes.[149]

There are fewer reports for ruthenium olefin complexes,[150,151] with [Ru(butadiene)$(CO)_3$] showing distortions similar to those of the iron analogue.[150]

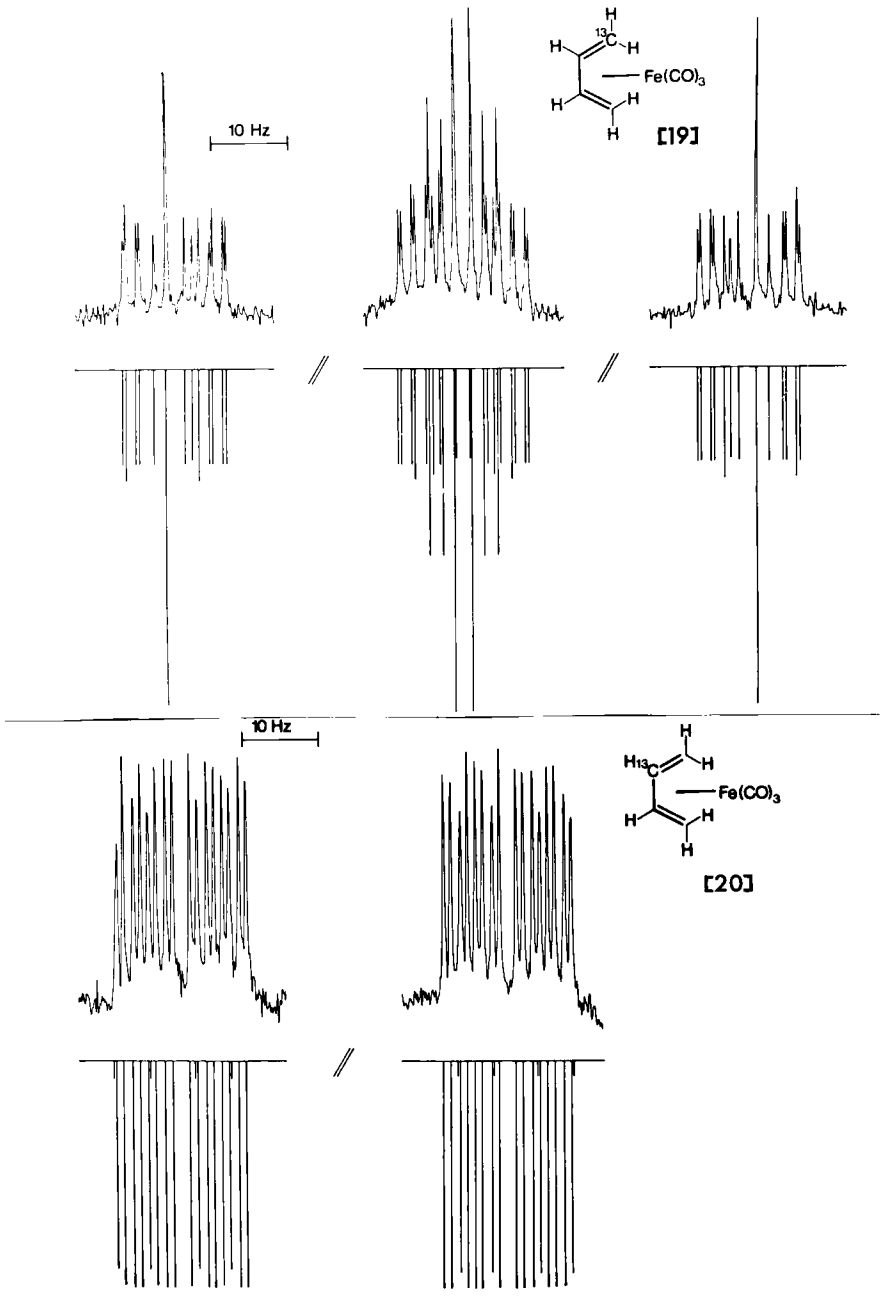
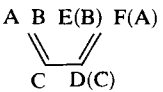


FIG. 1. Comparison of observed (upper) and theoretical (lower) ^{13}C NMR spectra for the two types of carbon in a butadiene carbonyl complex.

TABLE XII

^{1}H and ^{13}C NMR of some metal evaporation species.[138]



Complex[a]	NMR data[b]
$Fe(P(OMe)_3)(butadiene)_2$	C, 4·41 (4, $J = 8$); OMe, 3·53 (9, $J_{PH} = 10$); A, 1·00 (4, $J = 8$); B, −0·70 (4, $J = 8, 16$)
$Fe(P(OMe)_3)_3(butadiene)$	C, 4·98 (2, $J = 6$); OMe, 3·50 (27, $J = 3$); A, 1·40 (2); B, 0·59 (2, $J = 6$)
$Fe(CO)_3(butadiene)$	C, 4·50 (2); A, 1·25 (2); B, 0·23 (2) (AA′BB′CC′)
$Fe(P(OEt)_3)_3(butadiene)$	C, 3·64 (2, 6); OCH_2, 4·25 (18, 7); A, 1·64 (2); OCMe, 1·20 (27, 7); B, −0·46 (2, 6)
$Fe(P(OPr^i)_3)_3(butadiene)$	OCH, 4·77 (9); C, 4·32 (2); A, 2·07 (2); CMe_2, 1·27 (54, $J = 7$); B, −0·73 (2)
$Fe(P(OPh)_3)(butadiene)_2$	*o*-Ph, 7·16 (6, 8); *m*-Ph, 7·02 (6, 8); *p*-Ph, 6·86 (3, 8); C, 4·36 (4, 7); A, 1·52 (4); B, −0·43 (4, 9, $J_{PH} = 17$)
$Fe(P(OPr^i)_3)(isoprene)_2$	OCH, 4·80 (3); C, 3·9 (2); Me-D, 1·9 (6); $OCMe_2$, 1·25 (18); F, 1·0 (2); A, 0·8 (2); B, −0·8 (2); E, −0·9 (2)
trans-$Fe(P(OPh)_3)(isoprene)_2$	*o*-Ph, 7·41 (6, 8); *m*-Ph, 7·06 (6, 8); *p*-Ph, 6·87 (3, 8); C, 3·79 (2, 9); Me-D, 1·70 (6); F, 1·31 (2); A, 1·12 (2, 8, 2); B, −0·40 (2, 9, 2, $J_{PH} = 18$); E, −0·58 (2, 1, $J_{PH} = 18$)
$Fe(P(OMe)_3)$-*trans*-(*t*-pd)$_2$	C, 4·40 (2, $J = 5, 7, 9, J_{PH} = 1$); D, 4·22 (2, $J = 8, 5, J_{PH} = 1$); OMe, 3·61 (9, $J_{PH} = 10$); A, 0·92 (2); Me-F, 0·86 (6, $J = 6, J_{PH} = 2$); E, −0·23 ($J = 6, 8, J_{PH} = 14$); B, −0·72 (2, $J = 9, J_{PH} = 16·5$)
$Fe(P(OMe)_3)(dmbd)_2$	OMe, 3·43 (9, $J_{PH} = 9$); Me-C, 1·73 (12); A, 0·91 (4); B, 0·74 (4, $J_{PH} = 20$)
$Fe(P(OEt)_3)(dmbd)_2$	OCH_2, 4·03 (6, $J = J_{PH} = 7$); Me-B, 1·78 (12); OCMe, 1·18 (9, $J = 7$); A, 0·95 (4, $J = 2$); B, −0·75 (4, $J = 2, J_{PH} = 19·5$)
	$^{13}C\{^{1}H\}$: C, 90·0 ($J_{PC} = 3$); A, 60·2 ($J_{PC} = 6$); OCH_2, 38·5 ($J_{PC} = 18$); Me-C, 19·2; $OCMe_2$, 16·4 ($J_{PC} = 6$)

$Fe(P(OEt)_3)_3$(dmbd)	OCH_2, 4·02 (18); Me-C, 2·27 (6); A, 1·82 (2, 2); OCMe, 1·20 (27, 7); B, −0·59 (2)
$Fe(P(OPr^i)_3)$(dmbd)$_2$	OCH, 4·76 (3, $J = J_{PH} = 6$); Me-C, 1·79 (12); $OCMe_2$, 1·25 (18, $J = 6$); A, 0·95 (4, $J = 2$); B, −0·80 (4, $J = 2$, $J_{PH} = 20$) $^{13}C\{^1H\}$: C, 90·2 ($J_{PC} = 3$); A, 68·4 ($J_{PC} = 9$); OCH, 39·3 ($J_{PC} = 16$); $OCMe_2$, 24·6 ($J_{PC} = 4$); Me-C, 19·3
$Fe(P(OMe)_3)$(*t*, *t*-hd)$_2$	C, 4·42 (4, $J = 8, 2$); OMe, 3·75 ($J_{PH} = 10$); Me-A, 0·91 (12, $J = 6, 2$); B, −0·60 (4, $J = 6, 8$, $J_{PH} = 15$) $^{13}C\{^1H\}$: C, 79·08 ($J_{PC} = 3$); B, 52·48 ($J_{PC} = 7$); OMe, 50·30 ($J_{PC} = 12$); A, 18·65
$Fe(P(OMe)_3)_3$(1,3-cod)	C, 4·89 (2, $J = 7, 2$); OMe, 3·55 (27, $J = 3·3$); A, 2·87 (2); CH_2-B, 2·13 (4); CH_2, 1·56 (4, $J = 3$) $^{13}C\{^1H\}$: C, 86·18, OMe 51·47; A, 50·69; CH_2-B, 27·30; CH_2, 26·91
$Fe(P(OMe)_3)_3$(chpd)	C, 4·89 (2); OMe, 4·02 (27); A, 2·52 (2); CH_2-B, 2·20 (2), 2·00 (2); CH_2, 1·55 (2)
$Fe(P(OMe)_3)_3$(chxd)	C, 4·89 (2); OMe, 3·51 (27); A, 2·66 (2); CH_2-B, 1·90 (2, $J = 9$). 1·58 (2, $J = 9$)
$Fe(CO)_3$(chxd)	C, 4·90 (2); A, 2·91 (2); CH_2-B, 1·55 (2), 1·35 (2)
$Fe(P(OMe)_3)_3$(cot)	room temp. cot, 5·25 (8). OMe (27, $J = 6$)
$Fe(P(OMe)_3)_3$(cotri)	CH-B, 6·10 (1, $J = 10, 2$); CH, 5·96 (1, $J = 3$); C, 5·10 (1); D, 5·02 (1, $J = 6, 2$); A, 4·78 (1, $J = 6$); OMe, 3·54 (27); F, 3·18 (1); CH_2, 3·04 (1), 1·9(2), 1·65 (1, $J = 13$)
$Fe(P(OMe)_3)_2$(1,3-chxd)(1,4-chxd)	CH(1,4-chxd), 6·20 (2); C, 4·09 (2); OMe, 3·33 (18); FeCH(1,4-chxd), 3·24 (2); CH_2(1,4-chxd), 2·8 (4); A, 2·50 (2); CH_2-B, 1·63 (2), 1·37(2)
$Fe(C_6H_6)$(chxd)	C_6H_6, 4·82 (6); C, 4·50 (2, $J = 2, 4$); A, 2·45 (2, $J = 4, 2, 1$); CH_2-B, 1·51 (2, $J = 8, 2$), 1·20 (2, $J = 8, 1$)
$Fe(C_6H_6)(P(OMe)_3)_2$	C_6H_6, 4·94 (6, $J_{PH} = 2·5$); OMe, 3·36 (18, $J_{PC} = 6·0$)
$Fe(P(OMe)_3)_2(C_6H_7)H$	CH, 6·0 (1); CH, 5·5 (2); CH, 3·4 (2); OMe, 3·33 (18); CH_2, 2 (2); hydride, −18·65 (1, $J_{PH} = 80$)

[a] dmbd = 2,3-dimethylbutadiene; cod = cyclooctadiene; chpd = cycloheptadiene; chxd = cyclohexadiene; cot = cyclooctatetraene; cotri = cyclooctatriene; *t*-pd = *trans*-pentadiene; *t,t*-hd = *trans,trans*-hexa-2,4-diene; C_6H_6 = benzene; C_6H_7 = cyclohexadienyl.

[b] (Relative integration, coupling constants in Hz); *J* implies J_{HH} unless indicated otherwise.

There is little material concerning ^{13}C NMR in cobalt olefin chemistry other than an interesting report on paramagnetic cobaltocenes $[Co(C_5H_4R)_2]$ (R = H, Me, Et, Pr^i, Bu^n, Bu^t)[152] and a mention of a quinone complex of cobalt(I).[153] Data for this latter plus rhodium and iridium analogues are shown in Table XIII; however, ^{13}C NMR data for olefin compounds of rhodium are more abundant. There is a report for co-ordinated ethylene in the complexes $[RhCl(C_2H_4)(2,6\text{-lutidine})L]$ (L = CO, C_2H_4, PR_3),[154] although most complexes contain the olefin as a chelating ligand, e.g. as a multidentate olefin, such as cycloocta-1,5-diene (cod),[155,156] a substituted norbornadiene,[157] or as one arm of an unsaturated phosphine or arsine ligand.[158,159] In the latter category there are chemical shift data for rhodium and iridium complexes of *trans*-1,6-bis(diphenylphosphino)hex-3-ene $Ph_2PCH_2CH_2CH{=}CHCH_2CH_2PPh_2$, tribut-3-enylphosphine $P(CH_2CH_2CH{=}CH_2)_3$, and tripent-4-enylphosphine $P(CH_2CH_2CH_2CH{=}CH_2)_3$, which together with the values of $^1J(^{103}Rh\text{–}^{13}C)$ are interpreted in terms of the Dewar–Chatt–Duncanson model of metal–olefin bonding.

TABLE XIII

^{13}C NMR chemical shifts[a] for some quinones and quinone complexes of Ni, Co, Rh, and Ir.[152]

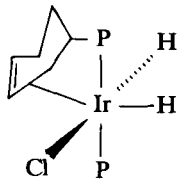
Compound	Solvent	C(1,4)	C(2,3,5,6)	CH_3	C_5H_5
2,3,5,6-Tetramethyl-	$CDCl_3$	$-187{\cdot}3_7$	$-140{\cdot}2_9$	$-12{\cdot}1_6$	
1,4-benzoquinone	D_2SO_4	$-191{\cdot}9_3$	$-146{\cdot}4_5$	$-12{\cdot}8_2$	
1,4-Benzoquinone	$CDCl_3$	$-186{\cdot}9_5$	$-136{\cdot}2_2$		
1,4-Hydroquinone	EtOH–D_2O	$-150{\cdot}6_0$	$-116{\cdot}9_7$		
(dq)Ni(cod)[b]	$CDCl_3$	$-153{\cdot}6_9$	$-112{\cdot}1_8$	$-12{\cdot}0_9$	
(dq)Co(C_5H_5)	$CDCl_3$	$-157{\cdot}2_4$	$-91{\cdot}2_7$	$-14{\cdot}4_9$	$-85{\cdot}4_1$
	D_2SO_4	$-131{\cdot}8_3$	$-97{\cdot}9_5$	$-9{\cdot}8_3$	$-89{\cdot}6_7$
(dq)Rh(C_5H_5)	$CDCl_3$	$-161{\cdot}6_5$	$-93{\cdot}5_1(9)$[c]	$-14{\cdot}7_7$	$-88{\cdot}2$ (1)
	D_2SO_4	$-133{\cdot}6_0(4)$	$-100{\cdot}9_6(6)$	$-9{\cdot}2_2$	$-90{\cdot}8_8(2)$
(dq)Ir(C_5H_5)	$CDCl_3$	$-164{\cdot}8_1$	$-82{\cdot}9_7$	$-14{\cdot}8_6$	$-81{\cdot}8_3$
	EtOH–D_2O	$-161{\cdot}8_8$	$-85{\cdot}8_4$	$-14{\cdot}8_3$	$-83{\cdot}8_4$
	D_2SO_4	$-130{\cdot}0_8$	$-92{\cdot}5_0$	$-8{\cdot}6_9$	$-83{\cdot}4_0$

[a] Chemical shifts with a precision of ±0·06 ppm.
[b] dq = 2,3,5,6-tetramethyl-1,4-benzoquinone, cod = cycloocta-1,5-diene.
[c] $^1J(^{103}Rh\text{–}^{13}C)$ in Hz, ±1 Hz.

Based on X-ray diffraction data for a series of platinum and rhodium olefin complexes[160] it is suggested that the change in the nuclear screening of a carbon on coordination to a metal varies as the reciprocal of the metal–carbon distance cubed. Further, a linear correlation between $\delta^{13}CH_3$ and

δ ^{1}H(hydride) in some methyl platinum and hydrido platinum complexes is reported. Although no quantitative interpretation is offered, these are useful empiricisms.

In a novel paper[160a] ^{13}C NMR data for the complex [21] which contains a dehydrogenated cyclohexane ring are reported.



[21]

There are several reports of rhodium and iridium cyclopentadienyl complexes containing ^{13}C chemical shift data,[161–163] and in some cases the molecules also contain chelating diolefins.[161,162] The dimeric complexes $[Rh_2(cp)_2(CO)_3]$, $[Rh_2(cp)_2(CH_2)(CO)_2]$, and $[Rh_2(cp)_2(NO)_2]$ have been found to have $^{1}J(^{103}Rh–^{103}Rh)$ values of approximately 4 Hz;[163] these represent the first reported data of this kind.

In the nickel triad there are two reports on the ^{13}C NMR of Ni(0) complexes of the type $[Ni(olefin)(PR_3)_2]$. The earlier[164] involves phosphite ligands and considers the question of metal–olefin bonding in terms of π-olefin *vs.* metallocyclopropane. The cyclopropane model is suggested to be deficient and, based on coordination chemical shifts, the order of back-bonding ability is found to be $Ru(CO)_4 > Fe(CO)_4 > Ni[P(O\text{-}o\text{-}tol)_3]_2$. The more recent report[165] concerns tertiary phosphines and correlates the olefin coordination chemical shift with phosphine basicity.

The zerovalent palladium complex of diisopropyldibenzylideneacetone (dipbda) has been suggested to have the composition $[Pd_2(dipbda)_3]$ based on ^{13}C and ^{1}H NMR studies.[166] The analogous platinum compound is reported as well; both have asymmetric structures in solution.

In contrast to palladium, the olefin chemistry of platinum(II) has received much attention during the review period.[167–177] Several of these papers are concerned with metal–olefin bonding and the *trans* influence,[167,168,171] and correlate the ^{13}C NMR data with vibrational spectroscopic results. A lowering of the C=C bond order in ethylene, as indicated by a decrease in ν(C=C), is linearly related to the low frequency shift of the olefin carbons.[167]

A recent report[175] on the molecules *trans*-$[PtCl_2(olefin)(NC_5H_4Me)]$ contains a rationalization of the ^{13}C chemical shifts based on a valence-bond description, and a linear correlation of $\nu(C{\equiv}O)$ with $^{1}J(^{195}Pt–^{13}C{\equiv}O)$ in the complexes *trans*-$[PtCl_2(CO)(NC_5H_4X)]$. A sampling of these data is given in

TABLE XIV

^{13}C NMR data for complexes of the type (olefin)$PtCl_2$($pMeC_5H_4N$) in $CDCl_3$.[175]

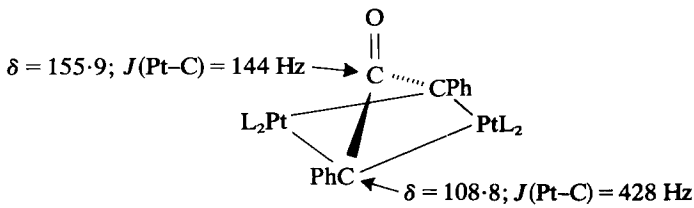
Olefin	Vinylic carbons					
	=CH_2			=CHR		
	$\delta(C^1)$	$\Delta\delta(C^1)^a$	$^1J(^{195}Pt-^{13}C)$	$\delta(C^2)$	$\Delta\delta(C^2)^a$	$^1J(^{195}Pt-^{13}C)$
Ethylene	75·05	47·35	164·4			
Propene	71·54	43·46	158·9	99·20	36·10	151·3
But-1-ene	69·52	42·88	160·0	105·06	34·24	157·9
cis-But-2-ene				90·75	32·95	149·8
trans-But-2-ene				92·69	30·41	152·0
Hex-1-ene	69·61	44·54	159·9	103·40	35·83	157·0
cis-Hex-3-ene				95·71	35·33	155·8
trans-Hex-3-ene				97·11	33·90	161·0
4-Methylpent-1-ene	70·49	45·01	159·6	102·20	35·77	157·4
5-methylhex-1-ene	69·80	44·15	160·1	103·98	35·38	156·4
3,3-Dimethylbut-1-ene	64·18	44·73	146·8	116·62	33·05	152·0
4,4-Dimethylpent-1-ene	71·29	45·07	159·8	100·71	35·40	158·4
Cyclopentene				100·95	29·78	150·0
Cyclohexene				95·04	32·02	146·0
Cycloheptene				95·63	36·59	162·7
Cyclooctene				93·84	36·13	161·8

Olefin	Carbons in olefin substituents (R)					
	C^α		C^β		C^γ etc.	C^β of $pMeC_5H_4N$
	$\delta(C^\alpha)$	$^2J(^{195}Pt-^{13}C)$	$\delta(C^\beta)$	$^3J(^{195}Pt-^{13}C)$	$\delta(C^\gamma)$	$^3J(^{195}Pt-^{13}C)$
Ethylene						37·5
Propene	21·20	23·3				36·6
But-1-ene	28·11	23·2	14·06	36·4		35·9
cis-But-2-ene	15·60	21·6				
trans-But-2-ene	20·42	34·0				
Hex-1-ene	34·23	21·9	31·37	33·7	22·10, 13·74	36·0
cis-Hex-3-ene	22·65	20·1	13·33	32·5		35·8
trans-Hex-3-ene	27·46	28·4	14·39	39·7		35·6
4-Methylpent-1-ene	43·62	22·0	28·52	31·7	23·14, 21·66	36·9
5-Methylhex-1-ene	32·79	23·3	38·66	33·2	23·90, 22·83, 22·25	35·6
3,3-Dimethylbut-1-ene	35·56	17·7	29·59	20·4		37·0
4,4-Dimethylpent-1-ene	48·20	21·2	31·46	30·0	29·41	37·0
Cyclopentene	32·47	17·9	23·07			37·3
Cyclohexene	26·80	14·9	21·32			37·6
Cycloheptene	28·24	16·1	24·64	39·0	32·99	34·0
Cyclooctene	28·0	17·6	29·23	43·8	26·21	34·2

[a] $\Delta\delta$ is the high frequency shift from the free olefin.

Table XIV. There are two reports on olefin complexes of platinum containing optically active ligands,[176,177] both stressing the value of ^{13}C and ^{195}Pt NMR, in addition to relatively short reports containing some ^{13}C NMR data for platinum complexes of vinyl ether,[171] 1,1-bis(dimethylamino)ethylene,[173] cyanoethene,[170] and a 5-coordinate ethylene α-diimine complex.[169] The question of deuterium incorporation in the olefin sidechain of $[Pt_2Cl_4(olefin)_2]$ has been considered using ^{13}C NMR methods.[172] Both the site and degree of deuteration can be determined.

Many ^{13}C NMR data on olefin and acetylene complexes of zerovalent platinum have been reported, although the NMR work is incidental to the chemistry.[178–186] Attention has centred around the reactions of bis(cycloocta-1,5-diene)platinum,[178–182] and a sampling of some of these data is shown in Table XV. The binuclear complex $[Pt_2\mu\text{-}(PhC)_2(CO)(cod)_2]$ [22],



[22]

which originates from diphenylcyclopropenone and cycloocta-1,5-diene-bis(methyl vinyl ketone)platinum, has the cyclopropenone bridging the two metals after ring opening at the carbon–carbon double bond.[181]

There have been additional reports (for early data see ref. 4) on π allyl complexes of palladium,[187,188] and there are extensive data for the fluxional η^3-cycloheptadienylpalladium complexes [23] (see Table XVI). There is also a report for some μ-allylbis(η-cyclopentadienyldicarbonyliron) cations.[189]

IV. COORDINATION COMPLEXES

Of the nine Group VIII metals, ^{13}C NMR has been most widely applied in the coordination chemistry of cobalt(III) and platinum(II). The studies are concerned primarily with chelating nitrogen ligands and often take advantage of the simplicity of the $^{13}C\{^1H\}$ spectra to make structural assignments based on symmetry considerations. For cobalt, the ligands include ethylenediamine(en),[190,191] phenyl-substituted en, cyclic ethylenediaminetetraacetates,[192] glycine,[193] 2,6-bis(aminomethyl)pyridine,[194] alkyl glyoxime derivatives,[195–197] and alkylcorrinoids.[198] A correlation between the log of the observed rate constant for the reaction of bromide ion with $[CoX(DH)_2(P(OCH_3)_3)]$ (DH = monoanion of dimethylglyoxime) and the

^{1}H and ^{13}C NMR data for some platinum complexes.[179]

Complex[a]	Solvent and temperature[b]	^{1}H	^{13}C
[Pt(cod)(dmf)]	A	4·85 [4H, CH, cod, *J* (PtH) 63], 5·96 [2H, CH=CH, *J* (PtH) 82], 6·68 (6H, Me), 8·73 (8H, CH_2)	172 (C=O), 95 [CH, cod, *J* (PtC) 101], 94 [CH, cod, *J* (PtC) 102], 51 (Me), 44 (CH, dmf), 30 (CH_2), 29 (CH_2)
[Pt(cod)(def)]	A	4·92 [4H, CH, cod, *J* (PtH) 63], 5·88 [2H, CH=CH, *J* (PtH) 80], 6·06 [4H, CH_2CH_3, *J* (HH) 7], 8·74 (8H, CH_2), 9·00 [6H, CH_2CH_3, *J* (HH) 7]	172 (C=O), 95 [CH, cod, *J* (PtC) 102], 94 [CH, cod, *J* (PtC) 104], 59 (CH_2, def), 44·5 (CH, def), 31 (CH_2, cod), 29 (CH_2, cod), 15 (Me, def)
[Pt(cod)(ma)]	C	4·40 [4H, CH, cod, *J* (PtH) 38], 6·28 [2H, CH=CH, ma, *J* (PtH) 76], 7·68 (8H, CH_2)	171 (C=O), 100 [CH, cod, *J* (PtC) 104], 97 [CH, cod, *J* (PtC) 102], 42 [CH, ma, *J* (PtC) 337], 30 (CH_2)
[Pt(CNBu^t)(dmf)$_2$]	A	5·36–5·56 [AB system CH=CH, *J* (AB) 10, *J* (PtA) 52, *J* (PtB) 64], 6·82 (12H, Me, dmf), 9·29 (9H, Me, CNBu^t)	170 [C=O, *J* (PtC) 41], 52–55 (br, CH, dmf), 52 (Me), 30 (Me, CNBu^t)
[Pt(CNBu^t)(dmm)$_2$]	B	6·00 [4H, CH=CH, *J* (PtH) 60], 6·56 (12H, Me, dmm), 8·96 (9H, Me, CNBu^t)	169 [C=O, *J* (PtC) 37], 53 [CH, dmm, *J* (PtC) 188], 51 (Me, dmm), 30 (Me, CNBu^t)
[Pt(CNBu^t)(def)$_2$]	A	4·56–4·77 [AB system, CH=CH, *J* (AB) 10, *J* (PtA) 56, *J* (PtB) 68], 6·00 [4H, CH_2, *J* (HH) 7], 6·10 [4H, CH_2, *J* (HH) 7], 8·92 [6H, Me, *J* (HH) 7], 9·05 [6H, Me, *J* (HH) 7], 9·14 (9H, Me, CNBu^t)	170 (C=O), 169 (C=O), 60·5 (CH_2), 60·3 (CH_2), 58 (NC), 55 [CH, def, *J* (PtC) 142], 51 [CH, def, *J* (PtC) 190], 29 (Me, CNBu^t), 14·6, 14·2 (Me, def)
[Pt(CNBu^t)(ma)$_2$]	D	5·16 [4H, CH=CH, *J* (PtH) 62], 8·28 (9H, Me, CNBu^t)	54 [CH, ma, *J* (PtC) 168], 30 (Me)
[Pt(CNBu^t)$_2$(dmf)]	B	5·78 [2H, CH=CH, *J* (PtH) 60], 6·52 (6H, Me, dmf), 9·12 (18H, CNBu^t)	174 [C=O, *J* (PtC)43], 57 (CMe_3), 50 (Me, dmf), 39 [CH, dmf, *J* (PtC) 247], 30 (Me, CNBu^t)

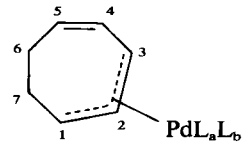
$[Pt(CNBu^t)_2(dmm)]$	B	6·44 (6H, Me, dmm), 6·48 [2H, CH=CH, *J* (PtH) 60], 9·08 (18H, $CNBu^t$)	173 [C=O, *J* (PtC) 43], 57 (CMe_3) 51 (Me, dmm), 38 [CH, dmm, *J* (PtC) 288], 30 (Me, $CNBu^t$)
$[Pt(CNBu^t)_2(def)]$	B	5·76 [2H, CH=CH, *J* (PtH) 64], 5·88 [4H, CH_2, *J* (HH) 7], 8·92 [6H, CH_3CH_2, *J*(HH) 7], 9·1 (18H, $CNBu^t$)	174 [C=O, *J* (PtC) 43], 59 (CH_2), 57 (CMe_3), 40 [CH, def, *J* (PtC) 245], 30 (Me, $CNBu^t$), 15 (Me, def)
$[Pt(CNBu^t)_2(ma)]$	D	6·52 [2H, CH=CH, *J* (PtH) 62], 8·40 (18H, $CNBut^t$)	173 [C=O, *J* (PtC) 43], 59 (CMe_3), 36 [CH, ma, *J*(PtC) 265], 30 (Me, $CNBu^t$)
$[Pt(CNBu^t)(dmf)(P(C_6H_{11})_3)]$	C	6·40 (3H, Me, dmf), 6·50 (3H, Me, dmf), 6·66 and 6·86 [H_A, H_B, ABX system with ^{195}Pt satellites, CH=CH, *J*(AB) 9, *J* (AP) 10, *J* (BP) 2, *J* (PtA) 55, *J* (PtB) 62], 8·20–8·73 [33H, $P(C_6H_{11})_3$], 8·52 (9H, $CNBu^t$)	
$[Pt(C_2H_4)(dmf)_2]$	B	5·12 [4H, CH=CH, dmf, *J* (PtH) 60], 6·12 [$(AB)_2$ system, 4H, C_2H_4], 6·72 (12H, Me, dmf)	168 [C=O, *J* (PtC) 43], 75 [C_2H_4, *J* (PtC) 80], 58 [CH, dmf, *J* (PtC) 163], 51 (Me, dmf)
$[Pt(C_2H_4)(dmm)_2]$	B	5·64 [4H, C_2H_4, *J*(PtH) 52], 5·80 [4H, CH=CH, dmm, *J* (PtH) 66], 6·56 (12H, Me, dmm)	168 (C=O), 80 [C_2H_4, *J* (PtC) 76], 57 [CH, dmm, *J* (PtC) 200], 52 (Me, dmm)
$[Pt(dmf)_3]$	B	4·36 [6H, CH=CH, *J* (PtH) 58], 6·68 (18H, Me, dmf)	166 [C=O, *J* (PtC) 41], 68 [CH, dmf, *J* (PtC) 130], 52 (Me, dmm)
$[Pt(def)_3]$	B	4·28 [6H, CH=CH, *J* (PtH) 58], 6·10 [12H, CH_2, *J* (HH) 7], 9·00 [18H, CH_3, *J* (HH) 7]	165 (C=O), 68 [CH, def, *J* (PtC) 131], 61 (CH_2), 14 (Me)
$[NEt_3(CH_2Ph)][PtCl(dmf)_2]$	C	2·48 (5H, C_6H_5), 5·50 (2H, $C_6H_5CH_2$), 5·00 (CH=CH), 6·42 (12H, Me, dmf), 6·74 (6H, CH_3CH_2), 8·56 (9H, CH_3CH_2)	

[a] dmf = *trans*-CH(CO$_2$Me) = CH(CO$_2$Me); def = *trans*-CH(CO$_2$Et)=CH(CO$_2$Et); dmm = *cis*-CH(CO$_2$Me)=CH(CO$_2$Me); ma = CH=CH—C(O)—O—C=O

[b] A = [$^2H_8$]toluene at −80 °C; B = [$^2H_6$]benzene at room temperature; C = [^{2}H]chloroform at room temperature; D = [$^2H_6$]acetone at room temperature

TABLE XVI

^{13}C NMR chemical shifts of C^3 and $\Delta G^{\ddagger}$ for a series of fluxional η^3-cycloheptadienylpalladium complexes in CH_2Cl_2.[187]



[23]

Complex	$\delta(C^3)$	$\Delta G^{\ddagger}$ (kcal mol^{-1})
$[Pd(C_7H_9)(CH_3COCHCOCH_3)]$	71·7	16·6
$[Pd(C_7H_9)(CF_3COCHCOCF_3)]$	75·2	15·5
$[Pd(C_7H_9)(Me_2NCH_2CH_2NMe_2)]^+$	76·1	14·7
$[Pd(C_7H_9)(S_2CNMe_2)]$	77·1	14·8
$[Pd(C_7H_9)Cl]_2$	78·8	15·1
$[Pd(C_7H_9)(bipyridyl)]^+$	78·9	14·9
$[Pd(C_7H_9)Br]_2$	80·9	14·0
$[Pd(C_7H_9)I]_2$	84·3, 84·8[a]	13·0
$[Pd(C_7H_9)(MeSCH_2CH_2SMe)]^+$	85·7	11·8
$[Pd(C_7H_9)(AsEt_3)_2]^+$	86·5	9·8
$[Pd(C_7H_9)(PEt_3)_2]^+$	87·7	9·5
$[Pd(C_7H_9)(Ph_2AsCH_2CH_2AsPh_2)]^+$	87·9	8·9
$[Pd(C_7H_9)(Ph_2PCH_2CH_2PPh_2)]^+$	88·5	8·8
$[Pd(C_7H_9)(\eta^4$-hexamethylbicyclo-[2.2.0]hexadiene$)]^+$	89·2	8·7
$[Pd(C_7H_9)\{P(OMe)_3\}_2]^+$	91·6	8·2
$[Pd(C_7H_9)(\eta^4$-cycloocta-1,5-diene$)]^+$	93·7	~6·8[b]
$[Pd(C_7H_9)(\eta^4$-cyclooctatetraene$)]^+$	95·7	~7·1[b]

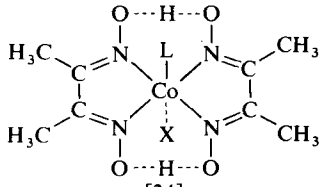
[a] Two isomers are present owing to the asymmetry of the allyl ligands in the dimer.
[b] Estimated values at −89 °C; limiting low temperature spectra could not be achieved.

^{13}C chemical shift of the phosphite carbon has been observed.[196] A sampling of data for some cobaloximes is shown in Table XVII. There is a recent report on the complexes $[Co(DH)_2(5R\text{-tetrazolate})(PBu^n_3)]$ combining ^{13}C NMR with theoretical and structure studies.[199] There seems to be little of interest on rhodium compounds but there are several reports on iridium–phosphine complexes which contain ^{13}C NMR data;[200–202] however, these are also incidental to the chemistry.

There is continuing interest in the carbon NMR properties of quadridentate porphyrin complexes of iron. ^{13}C NMR data can be found for low-spin ferric complexes containing 2-coordinated cyanide ligands,[203] the ^{13}CN in a series of model cyano iron porphyrins, with shifts for $K_3Fe(CN)_6$

TABLE XVII

^{13} NMR data[a] for (Bupy)Co(DH)$_2$X complexes of type [24]; X = common ligands.[197]



[24]

X	Imine C	Oxime Me C	Pyridine carbons		
			C^{α}	C^{β}	C^{γ}
NO_3	153·89	13·22	150·82	123·14	164·33
Br	152·83	13·09	149·85	123·01	163·72
Cl	152·44	13·08	150·28	123·03	163·85
N_3	151·98	12·89	150·49	123·02	163·68
NO_2	152·43	12·79	149·94	122·94	163·81
CN	151·93	12·76	149·02	123·16	163·88
$SO_2C_6H_4CH_3$	152·39	12·57	149·59	122·87	163·28
$P(O)(OCH_3)_2$	151·70	12·40	148·89	122·75[b]	162·75
CH_2Br	150·07	12·32	149·66	122·56	162·37
C_6H_5	150·15	12·20	149·61	122·50	162·08
CH_3	148·84	12·00	149·43	122·36	161·84
Uncoordinated			149·51	120·51	159·66

[a] 0·1 M in $CDCl_3$; ppm from TMS.
[b] $^4J(^{31}P-^{13}C) = 3·6$ Hz.

(−3583 ppm) and $K_4Fe(CN)_6$ (−177·3),[204] a ruthenium tetraphenyl porphyrin,[205] and a model study using aluminium.[206]

Additionally in the quadridentate ligand area there are two reports[207,208] on nickel complexes of a ligand derived from an ethylenediamine and a β-diketone. There are new measurements of thiocyanate complexes and these have been found to be of value in the question of linkage isomerism (NCS *vs.* SCN).[209]

Most of the ^{13}C NMR data for non-organometallic palladium complexes stem from studies of tertiary phosphine derivatives of palladium. Nelson and coworkers, while studying equilibria in [PdX_2(tertiary phosphorus ligand)$_2$] [210–213] and [PdX_2(phosphonate)$_2$] [214] compounds, have made detailed studies of the phosphine ligand ^{13}C chemical shifts and $^nJ(^{31}P-^{13}C)$ coupling constants. Balimann *et al.*,[215,216] with a somewhat more spectroscopic approach, including $^{13}C\{^1H,^{31}P\}$ studies, have also contributed in this area. In the latter work the values of $^1J(^{31}P-^{13}C)$ are suggested to be

dependent on the *trans* influence, and $\delta^{31}P$ correlates linearly with $\delta^{13}C^{\alpha}$ in the complexes *trans*-$[PdCl_2(PBu^n_3)L]$.[215] There are also data for some analogous complexes of Pt(II).[215a] Some $\delta^{13}C$ and $J(^{31}P-^{13}C)$ data are shown in Tables XVIII–XX.

The solution behaviour of the complexes Pd(tertiary phosphine)$_n$ (n = 2–4) has been studied by ^{13}C NMR, with the 2-coordinate complexes favoured by sterically bulky phosphines.[216]

As with Co(III) there have been several ^{13}C NMR studies on chelating nitrogen complexes of platinum. Complexes of methyl-substituted glycines,[217] proline and piperolic acid,[218] and substituted ethylenediamine derivatives[219] have all been investigated with a view to using the various $^nJ(^{195}Pt-^{13}C)$ couplings as conformational probes.

Worthy of note are several reports[217,220] which stress the difference in sign between $^2J(^{195}Pt-^{13}C)$ (negative) and $^3J(^{195}Pt-^{13}C)$ (positive) in the en

TABLE XVIII

Phosphorus–carbon coupling constants[a] in some Pt(II) and Pd(II) compounds.[215]

	Compound	^1J(P–C$^{\alpha}$) (Hz)	^1J(P–C^1) (Hz)
Pt(II):	*trans*-$[PtCl_2(NHEt_2)(PPr^n_3)]$	37·4	
	trans-$[PtCl_2(pCH_3C_6H_4NH_2)(PBu^n_3)]$	38·8	
	trans-$[PtCl_2(PTolyl_3)(PBu^n_2)]$	31·8	53·4
	trans-$[PtCl_2(PBu^n_3)_2]$	32·2[b]	
	trans-$[PtHCl(PPh_3)_2]$		54·2[b]
	sym-trans-$[Pt_2Cl_4(PBu^n_3)_2]$	39·6	
	cis-$[PtCl_2(PBu^n_3)_2]$	38·0[b]	53·6[b]
	cis-$[PtCl_2(PTolyl_3)(PEt_3)]$	38·0	
	cis-$[PtCl_2(AsTolyl_3)(PBu^n_3)]$	37·4	
Pd(II):	$[Bu^n_4N][PdCl_3(PEt_2Ph)]$	33·0	
	trans-$[PdCl_2(NHEt_2)(PBu^n_3)]$	32·2	
	trans-$[PdCl_2(pyridine)(PBu^n_3)]$	32·2	
	trans-$[PdCl_2(AsTolyl_3)(PBu^n_3)]$	27·8	
	trans-$[PdCl_2(NHEt_2)(PTolyl_3)]$		60·1
	trans-$[PdCl_2(pyridine)(PTolyl_3)]$		55·7
	trans-$[PdCl_2(PBu^n_3)_2(PTolyl_3)]$	23·9	43·9
	trans-$[PdCl_2(PBu^n_3)_2]$	26·4[b]	
	trans-$[PdCl_2(AsTolyl_3)(PTolyl_3)]$		51·3
	trans-$[PdCl_2(PTolyl_3)_2]$		51·3[b]
	trans-$[PdCl_2(AsBu^n_3)(PBu^n_3)]$	26·4	
	cis-$[PdCl_2(P(OEt)_3)(PEt_2Ph)]$	32·2	
	sym-trans-$[Pd_2Cl_4(PBu^n_3)_2]$	30·8	
	$PEtPh_3^+Br^-$	52·5	85·4
	$PPr^iPh_3^+Br^-$	46·4	86·1

[a] Coupling constants are ±0·7 Hz.
[b] $|^1J(P-C)+{}^3J(P-C)|$.

TABLE XIX

Phosphorus–carbon coupling constants[a] in some Pd^{2+} complexes.[216]

Compound	^1J(P–C$^\alpha$)	^2J(P–C$^\beta$)	^3J(P–C$^\gamma$)
sym-trans-$[Pd_2Cl_4(PMe_2Ph)_2]$	39·6		
cis-$[PdCl_2(PMe_2Ph)_2]$[b]	36·6		
trans-$[PdCl_2(PMe_2Ph)_2]$[b]	32·2		
sym-trans-$[Pd_2Cl_4(PMePh_2)_2]$[c]	40·3		
cis-$[PdCl_2(PMePh_2)_2]$[b]	37·4		
trans-$[PdCl_2(PMePh_2)_2]$	32·2		
sym-trans-$[Pd_2Cl_4(PEt_2Ph)_2]$	34·4	3·7	
cis-$[PdCl_2(PEt_2Ph)_2]$[b]	33·0	1·4	
trans-$[PdCl_2(PEt_2Ph)_2]$[b]	27·8	n.o.	
trans-$[PdCl_2(PEt_2Ph)(py\rightarrow O)]$	35·9	3·7	
trans-$[PdCl_2(PEt_2Ph)(py)]$	35·2	2·9	
trans-$[PdCl_2(PEt_2Ph)(NHEt_2)]$[c]	33·7	n.o.	
sym-trans-$[Pd_2Cl_4(PBu^n{}_3)_2]$	30·8	2·9	16·1
trans-$[PdCl_2(PBu^n{}_3)_2]$[b]	26·4	n.o.	13·2
trans-$[PdCl_2(PBu^n{}_3)(py)]$	32·2	n.o.	13·2
trans-$[PdCl_2(PBu^n{}_3)(pip)]$[c]	30·9	n.o.	14·0
trans-$[PdCl_2(PBu^n{}_3)(NHEt_2)]$[c]	32·2	n.o.	13·2
trans-$[PdCl_2(PBu^n{}_3)(AsTol_3)]$	27·8	n.o.	14·6
trans-$[PdCl_2(PBu^n{}_3)(PTol_3)]$[d]	23·9	n.o.	13·2
trans-$[PdCl_2(PBu^n{}_3)(AsBu^n{}_3)]$[e]	26·4	n.o.	14·6

n.o. = not observed.

[a] Coupling constants are ±0·7 Hz.

[b] Sum of the couplings ^nJ(P–C) + ^{n+2}J(P–C) given.

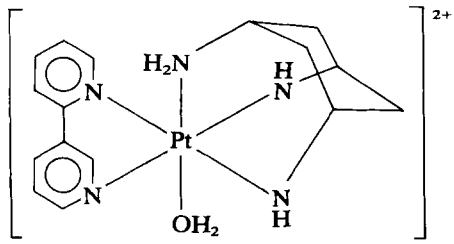
[c] ^3J(P–Pd–N–C) varies from 2·9 to 3·7 Hz in these complexes.

[d] Calculated using ^{13}C and ^{31}P spectra.

[e] ^3J(P–Pd–As–C) = 4·4 Hz.

compounds. A Karplus type relationship for $^3J(^{195}Pt-^{13}C)$ has been found[220] with a value of 52 Hz suggested for a 180° dihedral angle. Some data are shown in Tables XXI and XXII.

The novel Pt(IV) compound [25] formed by oxidation of the cation [Pt(2,2′-bipyridyl)(1,3,5-triaminocyclohexane)]$^{2+}$ has been characterized



[25]

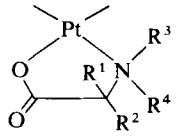
TABLE XX

^{13}C NMR data for some L_2PdCl_2 and other complexes.[210]

L	Benzyls					Phenyls			
	$\delta(CH_2)$	$\delta(C^1)$	$\delta(C^{2,6})$	$\delta(C^{3,5})$	$\delta(C^4)$	$\delta(C^1)$	$\delta(C^{2,6})$	$\delta(C^{3,5})$	$\delta(C^4)$
				L_2PdCl_2					
Bzl_3P	28·74	133·72	130·31	128·61	126·80				
Bzl_2PPh	27·88	132·91	130·47	128·33	126·80	133·15	130·57	128·33	126·80
$BzlPPh_2$	32·29 39·29	131·27	130·56	127·79	126·66	134·03	130·56	128·00	126·66
$(BzlO)_3P$	69·83	135·34	128·57	128·30	128·67				
$(BzlO)_2PPh$	70·85	135·51	128·64	128·39	128·75	131·19	128·58	128·22	132·51
$BzlOPPh_2$	70·76	133·05	128·47	128·18	128·57	132·92	128·50	128·18	131·94
$(Bzl_2N)_3P$	52·49	136·31	130·33	129·14	127·92				
$(Bzl_2N)_2PPh$	53·30	137·70	128·97	128·05	126·95	134·0	134·28	134·00	130·56
Bzl_2NPPh_2	52·90	134·02	129·14	128·28	126·95	134·01	132·20	127·47	130·33
$(BzlS)_3P$	39·46	131·31	130·52	129·44	127·42				
$(BzlS)_2PPh$	39·95	132·68	129·52	128·53	126·79	131·59	130·65	128·97	
$BzlSPPh_2$	39·71	131·83	131·11	128·85	128·21	135·36	131·88	128·83	129·37
				$(Bzl_nPPh_{3-n})PtCl_2$					
Bzl_3P	27·61	133·60	130·34	128·46	126·68				
Bzl_2PPh	26·73	133·08	130·56	128·22	126·71	133·27	130·56	128·22	126·71
$BzlPPh_2$	37·91	133·82	130·81	127·98	127·04	134·02	131·36	127·73	127·52
				$(Bzl_nPPh_{3-n})Pd(N_3)_2$					
Bzl_3P	28·49	133·05	130·03	128·94	127·10				
Bzl_2PPh	31·65 28·76	132·26	130·07	128·46	127·12	132·55	130·44	128·46	126·84
$BzlPPh_2$	32·49 35·97	132·76	130·49	128·63	127·19	133·40	131·10	128·42	126·98

TABLE XXI

^{13}C chemical shifts and ^{195}Pt–^{13}C coupling constants[a] for some glycinate chelates.[217]



Compound	R^1	R^2	R^3	R^4	N–CH_3	C=O	C^α	C–CH_3
$Pt(gly)Cl_2^-$	H	H	H	H		190·59 (46)	48·33 (30)	
$Pt(sarc)Cl_2^-$	H	H	H	CH_3	44·16 (17)	188·60 (30)	59·04 (25)	
$Pt(dmgly)Cl_2^-$	H	H	CH_3	CH_3	55·52 (14)	186·34 (15)	69·16 (22)	
$Pt(ala)Cl_2^-$	H	CH_3	H	H		191·26 (36)	55·71 (25)	19·26 (31)
cis-$Pt(meala)Cl_2^-$	H	CH_3	H	CH_3	37·78 (21)		63·00 (24)	15·12 (29)
trans-$Pt(meala)Cl_2^-$	H	CH_3	CH_3	H	43·72 (22)	190·90 (28)	66·42 (23)	17·89 (11)
$Pt(dmala)Cl_2^-$	H	CH_3	CH_3	CH_3	53·80 (19) 48·68 (19)	188·01	72·77 (21)	13·84 (18)
$Pt(aba)Cl_2^-$	CH_3	CH_3	H	H		192·33 (14)	61·94 (22)	26·98 (21)
$Pt(meaba)Cl_2^-$	CH_3	CH_3	CH_3	H	38·37 (23)	190·77 (20)	67·83 (20)	22·82 (22) 26·52 (10)
$Pt(dmaba)Cl_2^-$	CH_3	CH_3	CH_3	CH_3	48·68 (23)	189·91 (~5)	74·56 (18)	22·25 (10)

[a] Values for J(Pt–C) (Hz) are given in parentheses under the chemical shift of the corresponding carbon.

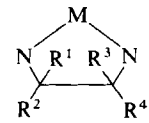
by ^{13}C NMR and X-ray methods and contains relatively stable deprotonated amino groups.[221]

A more extensive set of data for Pt(IV) nitrogen chelate compounds has been reported.[222] Chemical shift data for platinum complexes of the chelates *o,o'*-dihydroxybenzenes[223] and 1,1-dithiolates[224] are also available.

The application of platinum amino complexes in cancer research has prompted further reports on the interaction of platinum complexes with biologically relevant nitrogen ligands. ^{13}C chemical shifts for complexes of cytosine[225] and 1-methylimidazole-2 thiol[226] are available, as are data from

TABLE

^{1}H and ^{13}C NMR chemical shifts[a] and coupling constants



					^{1}H resonances				
					phenyl	diamine ring			methyl
Complex	R^1	R^2	R^3	R^4	C_6H_5	CH	$CH_2(R^3, R^4)$		CH_3
$[Pt(S\text{-pn})_2]^{2+}$	H	CH_3	H	H		3·05	2·8	2·6	1·3
$[Pt(S\text{-pn-}d_2)_2]^{2+}$						3·05 (20·0)			1·3
$[Pt(NH_3)_2(pn\text{-}d_1)]^{2+}$							2·8 (64·0)	2·6 (10·0)	1·3
$[Pt(S,S\text{-bn})_2]^{2+}$	H	CH_3	CH_3	H		2·65 (10·0)			1·4
$[Co(CN)_4(R\text{-phenen})]^-$	Ph	H	H	H	7·5	4·0	3·1	2·9	
$[Pt(en)(R\text{-phenen})]^{2+}$	Ph	H	H	H					
$[Pt(en)((-)_D\text{-stien})]^{2+}$	H	Ph	Ph	H		(10·0)		(38·8)	
$[Pt(en)(ms\text{-stien})]^{2+}$	H	Ph	H	Ph	7·3	4·5 (40·0)		2·8 (38·8)	

[a] Coupling constants Pt–C and Pt–H (Hz) are given in parentheses.

an extensive study of *cis*-$[Pt(NH_3)_2X_2]Y_2$ complexes (X = imidazoles, pyrimidines, 3-substituted pyridines, inosine, guanosine; Y = Cl^-, ClO_4^-).[227]

V. CONCLUDING REMARKS

In this chapter an attempt has been made to cover succinctly some of the more interesting contributions of ^{13}C NMR to Group VIII metal chemistry. This may well have led to "overdigestion" in some cases; moreover, there is no doubt that many valuable contributions have been overlooked, and to these authors I express my apologies. Nevertheless it is hoped that, from the

XXII

(^{195}Pt–^{13}C and ^{195}Pt–H) of some metal amine complexes.[219]

^{13}C resonances						
phenyl				diamine ring		methyl
C^1	C^2	C^3	C^4	CH	CH_2	CH_3
				56·33	53·10 (11·8)	17·44 (36·6)
				59·92		18·65 (48·8)
132·70 (39·1)	126·65	126·65	124·36	59·69	48·04 (14·6)	
137·04 (46·4)	130·51	130·51	128·99	66·72		
134·50 (24·4)	130·60	129·72	128·82	66·47 (11·0)		

papers discussed, the reader should be able to piece together the ways in which this NMR method is currently being applied and perhaps derive some direct personal benefit.

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