APPLICATION OF INTERNUCLEAR, DOUBLE-RESONANCE TECHNIQUES TO CARBOHYDRATES. DETECTION OF SMALL COUPLING-CONSTANTS

B. COXON
Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234 (U.S.A.)

(Received November 12th, 1970; accepted in revised form January 14th, 1971)

ABSTRACT

6-Deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-d_{12} (**1**-d_{12}) has been synthesized by an exchange reaction of its non-deuterated analog (**1**) with acetone-d_6. The p.m.r. spectrum of **1**-d_{12} at 90 MHz has been analyzed iteratively by means of a computer program for magnetic-equivalence factoring. Confirmation of the assignments of lines in the analysis has been investigated by proton–proton, internuclear, double-resonance (indor) techniques, which served also for the detection of a small long-range coupling-constant. Examples of indor spectra containing general Overhauser effects, spin-tickling effects, or transient nutations are shown, and are discussed in relation to the experimental power-levels of the observing and double-resonance frequencies, and their sweep-rates. The mass spectra of **1** and **1**-d_{12} are analyzed and compared.

INTRODUCTION

Although first-order analysis of the nuclear magnetic resonance spectra of carbohydrates[1] is used extensively in structural and conformational investigations, it is not often clear what errors have been introduced into the reported coupling-constants and chemical shifts by the use of an inexact analysis. These errors are undoubtedly small for weakly coupled systems of nuclei, but become more significant when strong coupling (a large J/δ ratio) is present. For example, first-order values of coupling constants may differ by at least 13% from those obtained by a more-refined analysis[2]. The measurement of more-accurate parameters may be important for several reasons: (*a*) more-vigorous testing is possible of the many theoretical and semi-empirical equations[3] that relate vicinal and long-range coupling-constants to interatomic dihedral angles and to atomic electronegativities; (*b*) determination of the populations of conformers at different temperatures may depend on the measurement of quite small differences of coupling constants or of chemical shifts, and it is advisable, therefore, to eliminate any large systematic errors in these parameters, and

---

*Presented at the joint meeting of the Chemical Institute of Canada and the American Chemical Society, Toronto, Ontario, Canada, on May 27, 1970.

(*c*) the availability of more-accurate parameters should permit more-precise correlations with molecular structure and conformation.

In recent years, the accurate analysis of n.m.r. spectra has been considerably simplified by the introduction[4,5] of iterative computer-programs that initially calculate a trial theoretical spectrum or a set of nuclear-spin energy-levels from assumed chemical-shifts and coupling constants, and then repetitively calculate corrections to these initial parameters, so that calculated spectral lines or energy levels are brought into coincidence with observed lines[5] or energy levels[4], respectively, according to a criterion of least squares. Fortunately, there have been parallel developments in the graphical and cathode-ray-tube display of digital data that facilitate comparison of the theoretical and experimental spectra.

Iterative analyses of carbohydrate systems containing up to seven nuclei having spin values of 1/2 have been reported[2,6–10]. In the present work, it was desired to test the applicability, to carbohydrate systems containing magnetically equivalent nuclei, of the iterative program[11] UEAITR; this program is an extended version of an earlier program[5], LAOCN3, that uses for magnetic equivalence[12] a model based on a composite particle having spin > 1/2 for factoring the n.m.r., Hamiltonian matrix into smaller submatrices that can be processed more rapidly by the computer than can the full matrix. In the UEAITR program, diagonalization of the matrices is accomplished by the Householder method, which is more efficient[11] than the Jacobi procedure used in the earlier programs.

One problem in using programs of the LAOCN3 or UEAITR type is the correct assignment of theoretical transitions to the peaks in the experimental spectrum. However, modern versions of these programs can, in their output, optionally include tables of energy levels of simple product spin-functions and lists of progressively and regressively connected transitions (see Fig. 1) that can be correlated with experiment[11]. A variety of double-resonance techniques is available for determining whether two transitions have an energy level in common and if they have a progressive or regressive connection. Among the methods involving sweeping* of the observing frequency (f_1) are the following. (*a*) Irradiation of a transition may be made with a very weak, but constant, double-resonance frequency (f_2)†; a general, nuclear

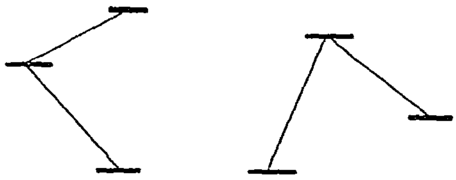

(a) (b)

Fig. 1. (*a*) Progressively connected transitions; (*b*) regressively connected transitions.

---

*Only frequency-sweep methods at constant magnetic-fields are considered here.

†With $\gamma^2 H^2{}_2 T_1 T_2 \sim 1$, where [13], $2H_2$ is the amplitude of f_2.

Overhauser effect can be observed[13] in which the relative intensities of connected lines change, because of a redistribution of the populations of their energy levels. An increase in the intensity of the observed line is noted if it is progressively connected to the irradiated line; conversely, a decrease is observed if the connection is regressive[13]. This technique has been but little used, because, experimentally, it is difficult to differentiate the small changes of intensities of lines from those due to momentary degradations of field homogeneity. (*b*) "Spin-tickling", in which a nondegenerate transition is subjected to a moderately weak double-resonance frequency (f_2)*, may be performed. Owing to the enforced mixing of spin states, connected transitions are split[16,17] into doublets and, if line-widths are determined mainly by the inhomogeneity of the magnetic field, regressively connected transitions are split[17] into sharp, well-resolved doublets**, whereas progressively connected transitions are split into broadened, poorly resolved doublets**. The magnitude of the splitting is proportional to the power of f_2 and to the square root of the intensity of the irradiated line[17]. (*c*) Selective spin-decoupling[16,18,19], in which two or more transitions of a sub-multiplet are irradiated strongly by a constant f_2, may be employed†. Pairs of connected transitions are split into four components, two of which are almost degenerate, and two have almost zero intenisty, the net result being collapse of doublets to singlets‡. An indication of common energy-levels is given, but not of the nature of the connection. (Methods (*b*) and (*c*) are obviously extremes of the same, double-resonance phenomenon.) (*d*) Double-quantum transitions[21], for which the power of f_1 is increased well above the level required for nonsaturation of single-quantum lines, but f_2 is not used, may be employed. Under these conditions, one or more pairs of progressively connected transitions can be excited simultaneously, with the absorption of two quanta[21] ($\Delta F_z = 2$). The frequency of the double-quantum transition is given (except for a small error due to Bloch–Siegert shift) by the mean of the frequencies of the progressively connected lines, and, therefore, the latter lines can be identified if they are found to be symmetrically spaced on either side of a double-quantum transition[21]. Such transitions are sharp (as compared with saturation-broadened, single-quantum lines), and the dependence of their intensities on the cube of the strength of f_1 when this is below the saturation level indicates why they are normally "forbidden". It has been pointed out[21] that, in some aspects, double-quantum transitions are a special case of double resonance, and, in fact, similar computational methods have been used for each.

Several of the foregoing methods have found extensive use in determination of the relative signs of coupling constants[2,6,14,17–19,21–25].

---

*Where[15], $2\pi J > \gamma H_2 > 1/T_2$. For a Lorentzian line of width $\Delta\nu$, the latter inequality can be derived from an alternative statement[14] of conditions for spin-tickling, namely, that $\gamma H_2/2\pi \sim \Delta\nu$, since, for such a line[15], $\Delta\nu = 1/\pi T_2$.

**Multiplets can be produced if the irradiated transition is degenerate[14].

†Where[13,16] $2\pi J \lesssim \gamma H_2$

‡A residual splitting may remain in some cases where a group of nuclei of spin > ½ is observed, or if f_2 is not strong enough, or is off resonance [16,20].

Alternative methods have been developed[13,26] in which f_1 is held constant at the peak of a resonance while f_2 is swept through the resonances of the other chemically shifted nuclei in a sample. A spectrum of the variation in intensity of the observed line is obtained[13,22,26] as a function of f_2. Such techniques have been described as internuclear, double resonance[26] (indor), and at least three different types may be distinguished. Two of these correspond, in principle, and, in power levels of f_2, to the f_1 sweeps already described in (*a*) and (*b*). Thus, for very weak values of the swept frequency f_2 (case *a*) only the general, nuclear Overhauser effect is observed[13], due to changes in the populations of energy levels as f_2 sweeps through a transition connected with that monitored by f_1. Experimentally, these changes can be manifested as both positive and negative excursions of the recorder pen from the constant baseline that represents the signal intensity of the monitored line in the absence of any perturbation[13]. Under suitable conditions, the positive and negative peaks indicate the perturbation of progressively, and regressively, connected transitions, respectively[13,27,28]. It will be seen that, as the power of f_2 is increased, population changes are gradually swamped by the spin-tickling effect (case *b*), so that only negative peaks are observed as the recorder pen sinks into the trough between the doublet components produced by tickling of a connected transition.

A third type of indor experiment based on observation of transient nutations of the net, macroscopic, magnetization vector has been reported[29]. Under the somewhat special conditions of adiabatic, rapid passage, combined with saturating values of f_1 and f_2, an initial positive or negative peak due to perturbation of progressively or regressively related transitions is followed by a damped oscillation (of constant frequency[29]) of a type reported earlier by Torrey[30] as resulting from a sudden application of f_1 (only), on resonance. This behavior has been attributed[29] to an abrupt transfer of spins to the common energy-level as the result of inversion of the populations of the levels of the irradiated transition by adiabatic passage of f_2 through resonance. Precession of the magnetization vector about the direction of f_1 in the rotating frame of reference then decays exponentially, with a time-constant given[19,29] by the harmonic mean of the relaxation times, *i.e.*, $2T_1T_2/(T_1+T_2)$.

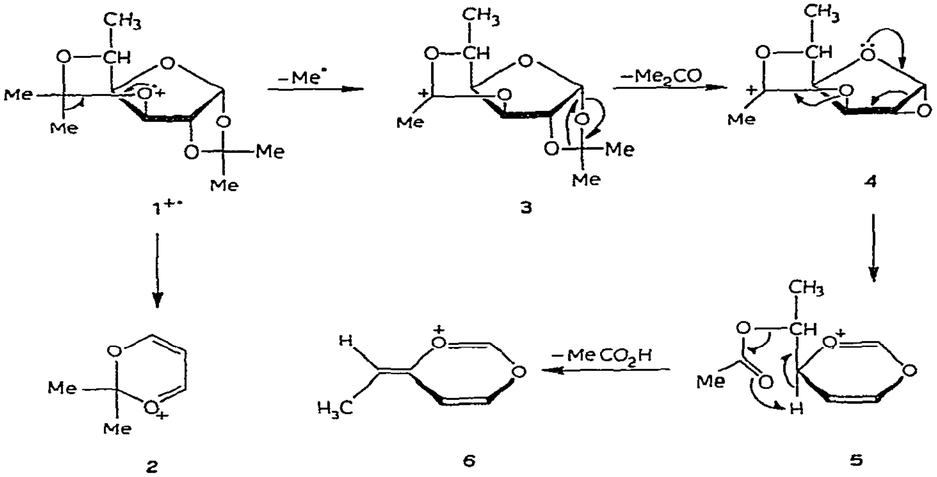
Indor methods are particularly advantageous for studies of the intensity changes due to population rearrangements, because the homogeneity of the magnetic field is monitored continuously by the height of the baseline of the spectrum[13]. It has also been claimed[13] that these intensity changes are less susceptible to interference from partially overlapping lines in the indor mode than in the normal, spin-tickling mode (which has f_1 swept and f_2 constant).

In the present work, 6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-d_{12} (**1**-d_{12}) was selected for study by homonuclear, indor techniques as a convenient system containing a group of magnetically equivalent protons.

RESULTS AND DISCUSSION

Initially, 6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (**1**) was investigated, but, as its isopropylidene methyl proton signals at 90 MHz were found to overlap those of the protons attached to C-6, the isopropylidene groups were replaced by isopropylidene-d_6, by direct exchange of **1** with acetone-d_6, catalyzed by sulfuric acid. Studies of this reaction by p.m.r. spectroscopy showed that the 3,5-isopropylidene group was exchanged much more rapidly than the 1,2-, as would be expected. The structure of **1**-d_{12} was confirmed by elemental analysis, and by i.r., p.m.r., and mass spectroscopy.

The most-intense ion-currents observed in the mass spectrum (for selected data, see Table I) of **1** were those at *m/e* 43 and 113. In the spectrum of **1**-d_{12}, these were shifted to *m/e* 46 and 119, corresponding to the retention of one and two isopropylidene methyl groups, respectively, and, therefore, to the structures of the acetylium cation and the highly stable 2,2-dimethyl-*m*-dioxenium ion (**2**) formed by scission of the C-2–C-3 and C-4–O-4 bonds.



Comparatively strong peaks at *m/e* 229 (M − 15) and *m/e* 238 (M − 18) were observed in the spectra of **1** and **1**-d_{12}, respectively, corresponding to loss of a methyl group from *one or the other* of the isopropylidene groups, to give, for example, the cation (**3**) which, on elimination of a molecule of acetone, affords the 1,2-epoxide ion (**4**), which can undergo rearrangement to the 5-*O*-acetyl cation (**5**, *m/e* 171). Loss of a molecule of acetic acid from **5** then gives the stable, cross-conjugated, substituted, *m*-dioxenium ion (**6**, *m/e* 111) from both **1** and **1**-d_{12}.

An alternative degradation of **1** involves scission of the C-3–C-4 bond to give **7**, followed by fragmentation to the propenyl epoxide cation (**8**, *m/e* 57), a protonated dioxolene aldehyde (**9**, *m/e* 129), and acetone (*m/e* 58). Elision of the aldehyde group can then give the parent 2,2-dimethyldioxolene cation (**10**, *m/e* 100), which suffers loss of a methyl group to give the 2-methyldioxolenium ion (**11**, *m/e* 85).

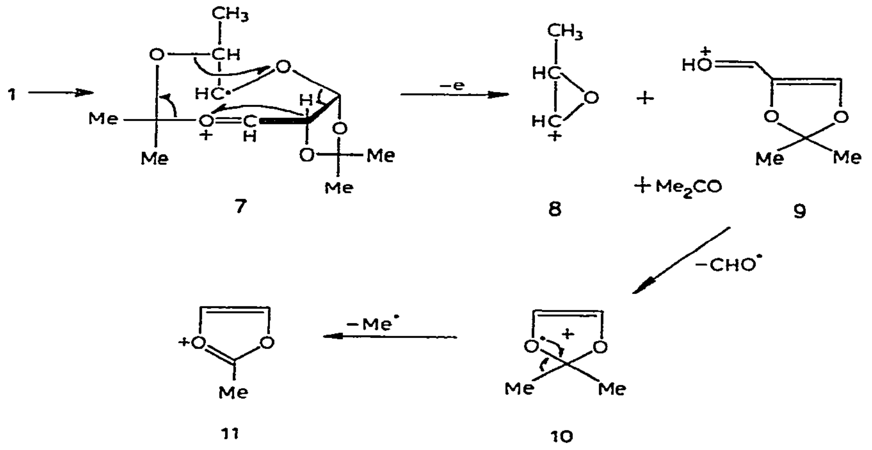
The foregoing mechanisms of degradation are similar to those suggested[31] for other di-*O*-isopropylidenealdohexoses, including the closely related 1,2:3,5-di-*O*-isopropylidene-α-D-xylofuranose. However, for **1**, the appearance of an additional

TABLE 1

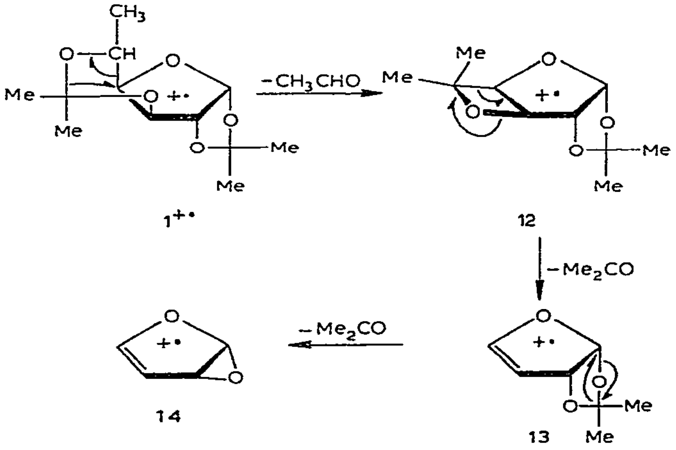
MASS-SPECTRAL DATA FOR 6-DEOXY-1,2:3,5-DI-*O*-ISOPROPYLIDENE-α-D-GLUCOFURANOSE

R.i. [a]*for*	m/e	258	257	256	245	238	229	212	200	187	186	183	174	171
1					0.39[b]		6.49		7.38	0.44	0.89			4.86
1-d_{12}		1.15	2.21	0.72		9.86		7.93				1.78	2.38	
R.i. [a]*for*	m/e	148	142	135	129	120	119	118	114	113	112	111	106	100
1			14.35		14.82				10.89	100.00	2.38	10.36		11.61
1-d_{12}		17.12		12.38		16.47	100.00	14.06				6.92	8.41	
R.i. [a]*for*	m/e	88	85	84	83	71	65	64	59	58	57	55	46	43
1			12.50	8.51	10.95	9.05			49.40	10.89	8.51	10.54		100.00
1-d_{12}		6.44		7.33	11.06		31.61	8.34					81.00	

[a] R.i. = relative intensity (% of base peak). [b] Corresponding fragment-ions from the deuterium-labeled and unlabeled compounds are indicated by arrows between the intensity values.



series of ions is evidently initiated by elimination of a molecule of acetaldehyde to give an oxetane ion (**12**, *m/e* 200) which loses successive molecules of acetone, thereby giving the 1,2-*O*-isopropylidene-D-*glycero*-tetro-3-enofuranose ion (**13**, *m/e* 142) and the furan 2,3-oxide cation (**14**, *m/e* 84).



A pressure-sensitive M+1 ion (*m/e* 245) was also produced from **1**, and both M+1 and M+2 ions (*m/e* 257 and 258) were formed from **1**-d_{12}. The shifts of *m/e* observed in the mass spectra of the latter compound are entirely consistent with the structure proposed and with the foregoing mechanisms of degradation.

The proton assignments shown in the proton spectrum of **1**-d_{12} (see Fig. 2b) were initially confirmed by conventional, spin-decoupling experiments. For example, irradiation at the frequency of the methyl doublet caused the H-5 quintet to collapse to a sharp doublet. By use of the magnetic-equivelence factoring-program[11] UEAITR, the spectrum was analyzed as an ABCDEF$_3$ system comprised by H-1–H-5 and H$_3$C-6, initial chemical-shifts and coupling constants for calculation of a trial theoret-

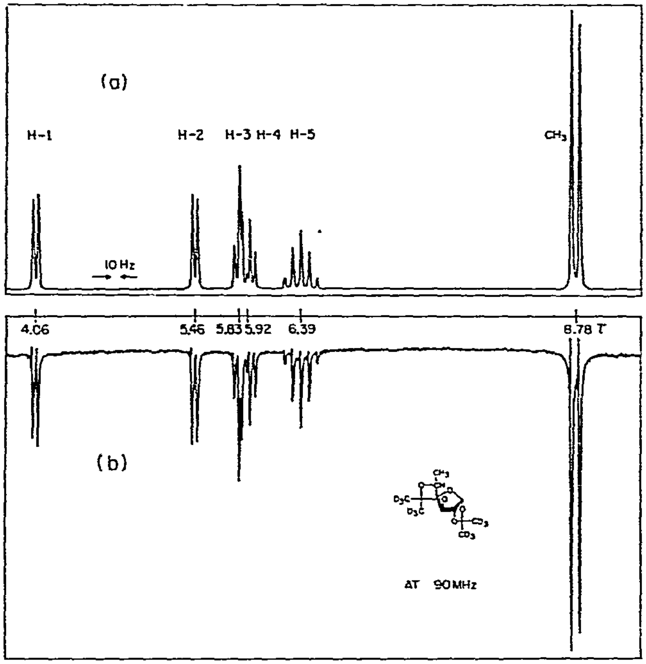


Fig. 2. P.m.r. spectra of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-d_{12} (1-d_{12}). (*a*) Computed spectrum after iterative analysis by magnetic-equivalence factoring; (*b*) observed spectrum of solution in 8:1 (v/v) acetone-d_6–benzene at 90 MHz.

ical spectrum being obtained by first-order analysis of Fig. 2b. Iterative refinement of these parameters (see Experimental section) gave a theoretical spectrum (Fig. 2a) in excellent agreement with the observed spectrum. However, as has been emphasized elsewhere[3], many criteria besides a good visual fit must be satisfied before an iterated, theoretical spectrum can be considered to represent a valid analysis of an observed spectrum. One of the more important of these criteria is that the assignments of observed lines to theoretical transitions in the iterative section of the n.m.r. program should be consistent with the information derived from double-resonance experiments, particularly those of the selective type in which only one transition, or a sub-multiplet of transitions, is perturbed. The validity of the assignments for 1-d_{12} was investigated by means of a series of indor experiments used in conjunction with the lists of progressively and regressively connected transitions provided by the computer program.

In Fig. 3 are shown the results of some indor experiments in which the H-1 line at lower field was monitored by f_1 while f_2 was swept through the resonance frequencies of the other ring-protons; spectra *a*–*d* are from left to right, with f_2 decreasing, and *e*–*h*, from right to left with f_2 increasing. A normal spectrum (*i*) is also shown, for comparison. If the H-1 and H-2 peaks in this spectrum are labeled from left to right (with decreasing frequency) as A1, A2, B1, and B2, respectively, the computer output indicates that each of the 48 transitions that constitute the monitored peak A1 forms a regressively connected pair with one of the 48 transitions that make up

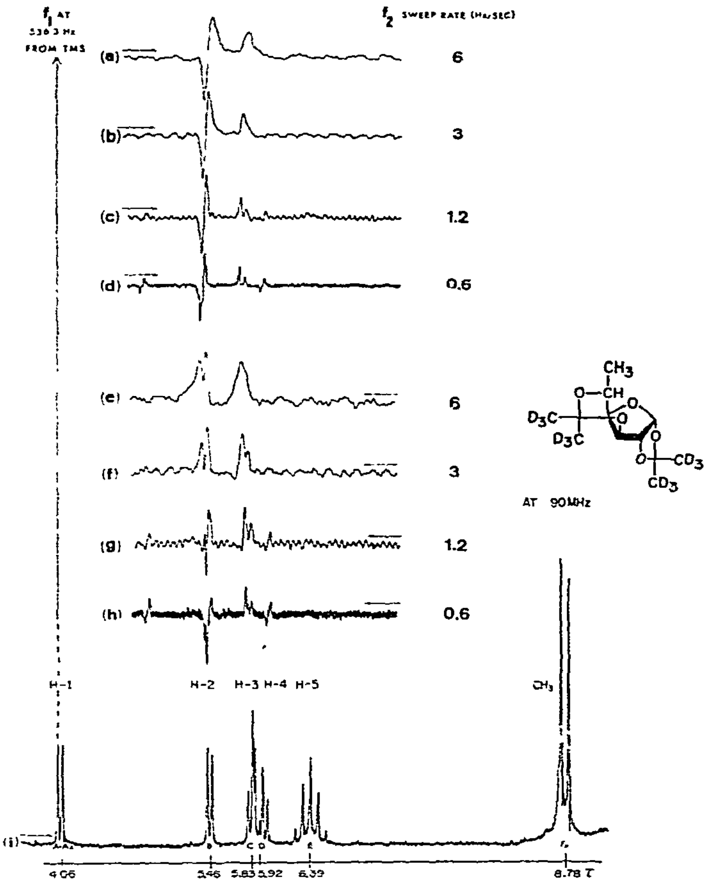


Fig. 3. Proton–proton, internuclear, double-resonance (indor) experiments on 1-d_{12} with f_2 decreasing (spectra *a–d*) or increasing (*e–h*) at various rates (f_1 constant at peak A1 of H-1, except for single-resonance spectrum *i*).

B1, but forms a progressively connected pair with one of the 48 transitions that comprise B2. This connectivity is more obvious if it is assumed that H-1 and H-2 are coupled only to each other, as is suggested by the normal spectra shown in Figs. 2 and 3. An energy-level diagram for an isolated AB system is shown in Fig. 4, and, as

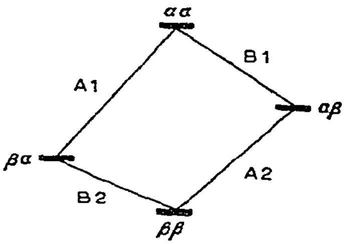


Fig. 4. Nuclear-spin, energy-level diagram for an AB system.

may be seen, transitions A1 and B1 are regressively connected, whereas A1 and B2 are progressively connected. On the assumptions that B1 and B2 are independently perturbed by sweeping f_2, and that the power of f_2 is such that only population rearrangements occur, irradiation of B1 will increase the population of the $\alpha\alpha$ energy-level, thus leading, for transition A1, to a smaller excess of population in the $\beta\alpha$ level. Because the intensity of a transition of a weakly coupled system is given[19] by the product of the transition probability and the population excess in the lower level, the monitored intensity of A1 will decrease, giving a negative peak. Similarly, irradiation of transition B2 will increase the population of level $\beta\alpha$, thereby increasing its excess of population with respect to the $\alpha\alpha$ level, and hence increasing the intensity of A1, to give a positive, indor peak. Inspection of the H-2 regions of Fig. 3 revealed that these predictions are borne out by spectra *a–d*; the monitored transition (A1) responds as expected, for perturbations of both B1 and B2. However, if the progressively connected transition (B2) is perturbed first, as for spectra *e–h*, the response of A1 to a perturbation of B1 depends on the sweep-rate, so that a negative response is obtained for sweep-rates of 0.6 and 1.2 Hz/sec, but a positive response for rates of 3 and 6 Hz/sec. These spectra illustrate the influence of relaxation phenomena on the results of indor experiments, and emphasize that information on the type of connectivity is best obtained under quasi-stationary conditions, that is, by use of low sweep-rates.

From Fig. 3, it may also be seen that perturbation of H-3 (peaks C1 and C2) by sweeping f_2 results in positive increments to the monitored line A1. As the indor spectra indicate only changes of peak height (and not of integrated intensities), it appears that the increased height of A1 could, in principle, be due either to an intramolecular, nuclear, Overhauser effect[8] caused by relaxation of H-1 by magnetic dipole–dipole interaction through space with H-3, or, alternatively, to momentary spin-decoupling of an unresolved coupling between H-1 and H-3 as f_2 sweeps through the resonance frequencies of H-3. Inspection of molecular models of **1**-d_{12} suggests that H-1 and H-3 are spatially remote, and, hence, an intramolecular, nuclear, Overhauser effect through space is unlikely to be operative. However, optimization of the magnetic-field homogeneity and expansion of the single-resonance spectra of H-1–H-4 to a sweep-width of 1 Hz/cm of chart paper revealed a long-range coupling-constant $J_{1,3}$ of 0.45 Hz, and two other small couplings, of $J_{2,3}$ 0.41 Hz and $J_{2,4}$ 0.42 Hz. Thus, indor experiments provide a method for the detection of unresolved coupling-constants.

Results similar to those shown in Fig. 3 were obtained when peaks B1 or B2 were monitored by f_1 while f_2 was swept through the resonant frequencies of H-1.

In other experiments, each of the H-5 peaks (E1, E2, E3, E4, and E5) was monitored in turn by f_1 while f_2 was swept through the resonant frequencies of the coupled nuclei H-4 (peaks D1, D2, D3, and D4) and H_3C-6 (peaks F1 and F2), the peaks being labeled from high to low frequency, as before. By use of the fact that intense lines interact with a double-resonance frequency more strongly than weak lines[17,19], efforts were made to correlate the observed indor spectra with the lists of progressively and regressively connected transitions in the output of the computer program. However, interpretation was complicated by the near-degeneracy of the transitions where the perturbed peaks contained a mixture of transitions progressively and regressively related to the peaks monitored (for example, peaks D1–D4 in relation to E2, E3, and E4). In general, correlation was possible if, according to the program output, the perturbed peak contained a clear majority of *either* progressively *or* regressively, connected transitions; in terms of total computed intensities, this ratio was three, or greater. This was true for monitored peaks E1, E2, E4, and E5 in relation to perturbed peaks D1–D4, and F1 and F2. For example, when peak E2 was monitored, positive indor peaks were observed at the locations of D3, D4, and F2, and negative indor peaks, at D1, D2, and F1. For the last three peaks, the ratios of total computed intensities of transitions, connected to E2 regressively and progressively, were 3.1:1, 3.2:1, and 3.0:1, respectively, and, for D3, D4, and F2, 0.34:1, 0.34:1, and 0.33:1.

When peak E3 was monitored, complex patterns of alternating positive and negative peaks were observed as f_2 was swept through F1 and F2 (see Fig. 5) and through D1–D4. These patterns were not amenable to a simple interpretation, and inspection of the connectivity (to E3) computed for the perturbed peaks indicated that they contained approximately equal intensities of progressive and regressive transitions. The exact appearance of the patterns was markedly dependent on the power of f_2 and on the direction of sweep, even at low sweep-rates (see Fig. 5).

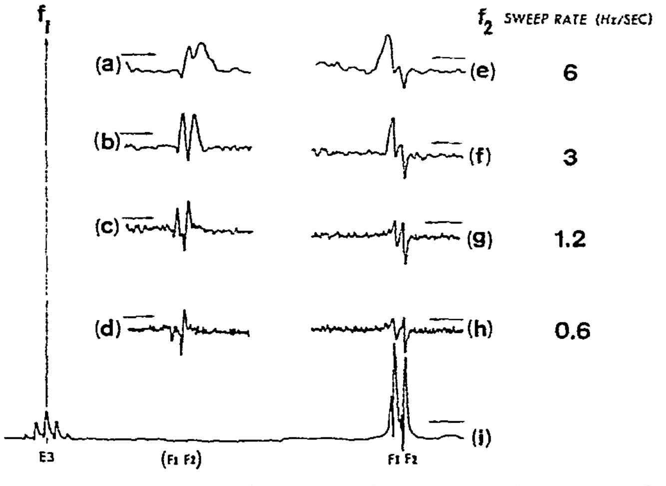


Fig. 5. Proton–proton indor experiments on 1-d_{12}, with peak E3 (H-5) monitored by a constant f_1 and with H_3C-6 (peaks F1 and F2) perturbed by f_2 either decreasing (spectra *a–d*), or increasing (*e–h*), at various rates.

In further experiments, either of the H_3C-6 peaks (F1 or F2) was monitored while f_2 was swept through the resonance frequencies of H-2–H-5. The indor spectra resulting from perturbation of the coupled nucleus H-5 are shown in Fig. 6 as a function of the power of f_2. At low power-levels (Fig. 6d), both positive and negative excursions may be seen. The positive excursions at peaks E4 and E5 correlate with computed progressive:regressive intensity-ratios of transitions in these peaks of 4.37:1 and 7.6:0, respectively, and the negative excursions at peaks E1 and E2 with progressive:regressive ratios of 0:8.7 and 0.27:1. For peak E3, progressively and regressively connected transitions alternate as the peak is swept, and their total computed intensities are more nearly equal (34.0 and 28.4, respectively); both positive and negative excursions were observed at the location of E3, but these do not reflect the full complexity of the connectivity.

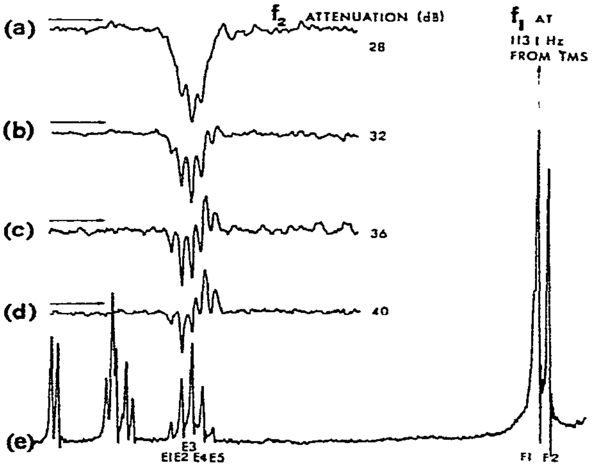


Fig. 6. Proton–proton indor experiments on 1-d_{12} at 90 MHz with peak F1 (H_3C-6) monitored by a constant f_1, and H-2–H-5 perturbed by a decreasing f_2 applied at various attenuations (dB down from 0.5 watt) (*a*) 28, (*b*) 32, (*c*) 36, (*d*) 40; (*e*) f_1 swept, and f_2 off. TMS refers to tetramethylsilane.

From the spectra shown in Fig. 6, it may be seen that, as the power of f_2 is increased (spectra *d* through *a*), all of the pen excursions become negative, thereby demonstrating how the general Overhauser effect (due to population changes only) is swamped by the spin-tickling effect at higher power-levels. For low levels of f_2 that gave *only* the general Overhauser effect, these spectra were affected but little by variation of the power of f_1 within the range normally used for observation of spectra. However, some variation of the relative magnitudes of the excursions (although not of their sense) with sweep-rate was noted.

It has been suggested[17] that indor spectra recorded under spin-tickling conditions will show a sharp dip of the recorder pen for perturbation of regressively connected transitions, but a broad dip for progressively connected transitions. This difference is not apparent in the spectra shown in Fig. 6, and it appears that connectivity is, in this instance, better determined by observation of the general Overhasuer effect by use of very low levels of f_2.

Examples of transient nutations[29,30] were obtained from some of the indor experiments in which either peak A1 or A2 of H-1 was monitored by f_1 while f_2 was swept through H-2. These nutations were observed, for particular combinations of the power levels of these two frequencies, with high sweep-rates. For example, in Fig. 7 are shown some results obtained by monitoring A1 with a saturating value of f_1 while f_2 was either decreased (spectra *a–c*), or increased (spectra *d–f*), at various rates. Inspection of the spectra *a*, *b*, *d*, and *e* reveals that, for the higher sweep-rates of 6 and 3 Hz/sec, the normal negative-positive responses due to excitation of regressively and progressively connected transitions (as shown in Fig. 3) are followed by a damped oscillation. The constant frequency of this oscillation serves to distinguish it from conventional "ringing", the frequency of which depends[32] on the instantaneous difference between the Larmor frequency and the observing frequency (f_1) swept. The nutations were *not* observed at the lowest sweep-rate (1.2 Hz/sec), thus emphasizing their dependence[33] on a time of passage through resonance much shorter than T_1 and T_2.

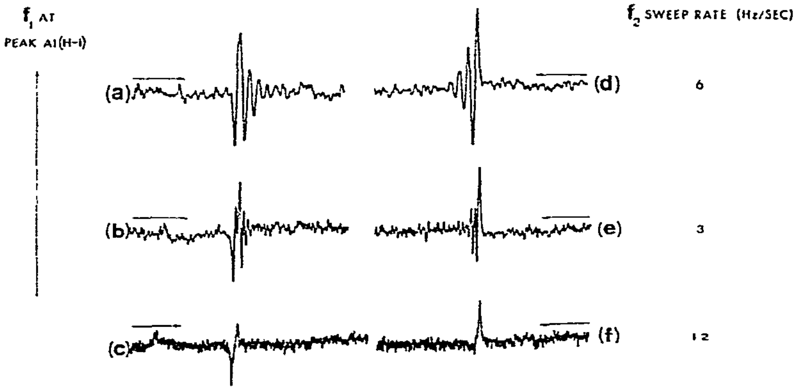


Fig. 7. Indor spectra obtained from 1-d_{12} at 90 MHz by monitoring peak A1 (H-1) with f_1 (attenuation, 38) while H-2 was perturbed by f_2 (attenuation, 44) either decreasing (spectra *a–c*) or increasing (spectra *d–f*) at various rates. Transient nutations are apparent in spectra a, b, d, and *e*.

According to a theory[29,30], the frequency f of transient nutations is given by $f = \gamma H_1 \lambda / 2\pi$ Hz, where λ is the matrix element of the transition monitored. In the present study, the values of f were typically in the range 0.8–1.1 Hz, and the best examples of nutations were obtained from experiments in which f_1 and f_2 (each a nominal 0.5 watt) were attenuated by 38–40 dB and 40–45 dB, respectively. At lower power-levels of f_1, or higher levels of f_2, the transient oscillations disappeared, and only the normal indor spectra were observed, as in Fig. 3.

Overall, the results indicate that, if the spectral peaks of carbohydrate derivatives are not too highly degenerate or comprised of complex mixtures of progressively and regressively connected transitions, the various types of homonuclear indor experiment have potential for aiding the assignment of transitions in computerized, iterative analyses of spectra. Detection of unresolved coupling-constants is a useful bonus.

Proton–proton indor studies of simple carbohydrate derivatives by using a modified Varian HA-100 n.m.r. spectrometer* have recently been described[34]. This instrument was used to detect hidden resonances in the proton spectra of sucrose octaacetate and of fully mutarotated solutions of D-ribose and 2-deoxy-α-D-*arabino*-hexopyranose[34].

EXPERIMENTAL

General. — P.m.r. spectra were recorded in the frequency-sweep mode with a Bruker Scientific spectrometer model HFX-11 operating at 90 MHz with internal, field-frequency (f_0) stabilization on the signal either of tetramethylsilane or benzene (for experiments in which the methyl protons were to be strongly perturbed by f_2). Automatic shimming of the *y*-magnetic field-gradient was employed. Independent, calibrated attenuators were used for controling the power levels of f_0, f_1, and f_2, and correct synchronization of f_1 and f_2 sweeps was assured by careful adjustment of the sweep-offset controls in conjunction with a frequency counter. Solutions of **1**-d_{12} (13–73 mg) in 8:1 (v/v) acetone-d_6–benzene (0.45 ml) were filtered while close to a small magnet, and were thoroughly purged with nitrogen before use. Indor spectra were usually recorded at a sweep width (f_2) of 10 Hz/cm by using a variety of sweep-times: 100, 200, 500, and 1,000 sec, corresponding to sweep-rates of 6, 3, 1.2, and 0.6 Hz/sec. Small coupling-constants were measured, and their assignments confirmed as follows, by spin-decoupling at a sweep width (f_1) of 1 Hz/cm by using a sweep-rate of 0.3 Hz/sec. (*a*) On irradiation of H-1 (quartet), unresolved sextets (H-2 and H-3) collapsed to an unresolved triplet and unresolved quartet, respectively (removal of $J_{1,2}$ and $J_{1,3}$). (*b*) On irradiation of H-2, the H-3 multiplet sharpened to give a semi-resolved quartet, a semi-resolved octet (H-4) became a quartet, and the H-1 quartet collapsed to a narrow doublet (removal of $J_{1,2}$, $J_{2,3}$, and $J_{2,4}$). Confirmatory results were obtained by irradiation of H-3 and H-4, and assignment of H-5 was verified by strong irradiation of H_3C-6.

Line positions used in the iterative analysis were mean values of four single-resonance spectra, two of which were obtained with f_1 increasing, and two with f_1 decreasing.

Mass spectra were recorded with a CEC spectrometer Model 21-491, at an ionization potential of 70 eV, and infrared spectra with a Perkin–Elmer grating spectrometer model 257.

*Preparation of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose-*d$_{12}$ (**1**-d$_{12}$). — (*a*) *Monitoring by p.m.r spectroscopy.* A p.m.r. spectrum of **1** (139 mg) in 8:1 (v/v) acetone-d_6–benzene (0.45 ml) at 90 MHz showed methyl signals at τ 8.59, 8.71, 8.72, 8.74, and 8.81 p.p.m.

Five minutes after addition of concentrated sulfuric acid (1 μl) to the solution, its spectrum showed a strong peak for acetone at τ 7.97, and the intensities of the peaks at τ 8.71 and 8.72 had markedly diminished; after a further 30 min, they had

---

*Mention of commercial instrumentation in this publication does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the equipment identified is necessarily the best available for the purpose.

disappeared. Over a period of three days, the peaks at τ 8.59 and 8.74 gradually became less intense, but replacement of the acetone singlet by an intense quintet due to acetone-d_5 indicated that scrambling of the protons of acetone and sulfuric acid had occurred. The pale-yellow solution was, therefore, stirred with a suspension of powdered calcium oxide in benzene, and filtered, and the combined filtrate and washings were evaporated to a syrup that was treated, as before, with the reagents for exchange of the isopropylidene groups. After two days, the most intense methyl-group signals that remained were components of a doublet (H_3C-6, $J \sim 6$ Hz) at τ 8.74 and 8.81. However, other peaks in the spectrum suggested that some hydrolysis had occurred.

A comparison of the changes observed in the methyl resonance with the chemical shifts of the isopropylidene methyl groups of 1,2-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (τ 8.59 and 8.75) and of 1,2:3,5-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (τ 8.59, 8.73, 8.74, and 8.76) measured with solutions in 8:1 (v/v) acetone-d_6–benzene indicated that the 3,5-isopropylidene group of **1** was exchanged rapidly (<35 min), but the 1,2 group more slowly (4–5 days).

(*b*) *Isolation.* A mixture of **1** (242 mg), acetone-d_6 (5 ml), anhydrous copper(II) sulfate (625 mg), Drierite (282 mg), and concentrated sulfuric acid (15 μl) was stirred for 2.7 days at room temperature. The suspension was diluted with benzene, made neutral by stirring with powdered calcium oxide, and filtered through Filter Cel. The solids were washed with benzene, and the filtrate and washings were combined and evaporated to a syrup. P.m.r. spectroscopy (90 MHz) of this material indicated that exchange of the isopropylidene groups was not quite complete, and the reaction was therefore repeated for 4 days. P.m.r. spectroscopy then indicated that exchange was complete, but t.l.c. and p.m.r. showed that the proportion of a by-product had increased. The products were separated by chromatography of a solution of the syrup (194 mg) in benzene (8 ml) on silicic acid (25 g, 100 mesh) with elution with 5:1 (v/v (light petroleum ether–ether containing 0.1% of triethylamine. Evaporation of the first fraction gave **1**-d_{12} as a very pale-yellow syrup (87 mg), $[\alpha]_D^{25}$ +33.4° (*c* 0.66, chloroform). ν_{max}^{film} 2260m cm^{-1} (CD_3 stretch); bands at 2998 (CH_3 stretch), 1387, and 1378 cm^{-1} (CH deformation) less intense than in the spectrum of the unlabeled compound.

Anal. Calc. for $C_{12}H_8D_{12}O_5$:C, 56.2; H, 3.15; D, 9.4. Found: C, 56.2; H, 3.2; D, 9.5 (The two last values were calculated from the found value of 7.9 for H+0.5D, by assuming a H:D ratio of 3:4).

P.m.r. spectral analysis. — Initial coupling-constants and chemical shifts for computation of a trial, theoretical spectrum were obtained by first-order analysis of the averaged, single-resonance data for **1**-d_{12}. By using the UEAITR program[11], this computation, which included listing of progressively and regressively connected transitions, utilized 25 sec of processor time on a Univac 1108 computer. The iterative computations (in which 118 theoretical transitions were assigned to 17 observed peaks) proceeded, for 58 sec, through four cycles that had root-mean-square errors of transition frequencies of 0.240, 0.083, 0.080, and 0.080 Hz. With chemical shifts

(ν, Hz) referred to tetramethylsilane, the parameters obtained were ν_1 534.6 (H-1), ν_2 408.9, ν_3 375.0, ν_4 366.8, ν_5 325.3, ν_6 110.1 (H_3C-6), together with $J_{1,2}$ 3.81, $J_{1,3}$ 0.45, $J_{2,3}$ 0.41, $J_{2,4}$ 0.42, $J_{3,4}$ 4.00, $J_{4,5}$ 6.84, $J_{5,6}$ 6.40 Hz. The probable errors computed for these parameters were <0.03 Hz. The trial and refined theoretical spectra were displayed on a Calcomp, digital, incremental, x–y plotter Model 763. A line-width function based on a Gaussian distribution of Lorentzian components, with half-line widths of 0.85 Hz, was used for the refined spectrum (see Fig. 2).

ACKNOWLEDGMENTS

Thanks are due Dr. F. W. Parrish for a sample, Dr. R. B. Johannesen for his advice, Mr. E. E. Hughes for recording the mass spectra, and Dr. R. Schaffer for his support.

REFERENCES

1 L. D. HALL, *Advan. Carbohyd. Chem.*, 19 (1964) 51.
2 B. COXON, *Carbohyd. Res.*, 14 (1970) 9.
3 For a review, see B. COXON, *Methods Carbohyd. Chem.*, 6, in press.
4 J. D. SWALEN AND C. A. REILLY, *J. Chem. Phys.*, 37 (1962) 21.
5 S. CASTELLANO AND A. A. BOTHNER-BY, *J. Chem. Phys.*, 41 (1964) 3863; *LAOCN3*, a publication of the Mellon Institute, Pittsburgh, Pa. (1966).
6 B. COXON, H. J. JENNINGS AND K. A. MCLAUCHLAN, *Tetrahedron*, 23 (1967) 2395.
7 C. B. BARLOW, E. O. BISHOP, P. R. CAREY AND R. D. GUTHRIE, *Carbohyd. Res.*, 9 (1969) 99.
8 B. COXON, *Carbohyd. Res.*, 12 (1970) 313.
9 L. D. HALL AND P. R. STEINER, *Can. J. Chem.*, 48 (1970) 451.
10 B. COXON, *Carbohyd. Res.*, 13 (1970) 321.
11 R. B. JOHANNESEN, J. A. FERRETTI AND R. K. HARRIS, *J. Mag. Res.*, 3 (1970) 84.
12 R. C. FERGUSON AND D. W. MARQUARDT, *J. Chem. Phys.*, 41 (1964) 2087.
13 R. KAISER, *J. Chem. Phys.*, 39 (1963) 2435.
14 R. FREEMAN AND N. S. BHACCA, *J. Chem. Phys.*, 38 (1963) 1088.
15 J. W. EMSLEY, J. FEENEY AND L. H. SUTCLIFFE, *High Resolution Nuclear Magnetic Resonance Spectroscopy*, Vol. 1, Pergamon, Oxford, 1965, p. 30.
16 W. A. ANDERSON AND R. FREEMAN, *J. Chem. Phys.*, 37 (1962) 85.
17 R. FREEMAN AND W. A. ANDERSON, *J. Chem. Phys.*, 37 (1962) 2053.
18 *Cf.*, R. FREEMAN AND D. H. WHIFFEN, *Mol. Phys.*, 4 (1961) 321.
19 R. A. HOFFMAN AND S. FORSEN, in J. W. EMSLEY, J. FEENEY, AND L. H. SUTCLIFFE (Eds.), *Progress in Nuclear Magnetic Resonance Spectroscopy*, Vol. 1, Pergamon, Oxford 1965, p. 15.
20 W. A. ANDERSON AND F. A. NELSON, *J. Chem. Phys.*, 39 (1963) 183.
21 W. A. ANDERSON, R. FREEMAN AND C. A. REILLY, *J. Chem. Phys.*, 39 (1963) 1518.
22 R. FREEMAN AND W. A. ANDERSON, *J. Chem. Phys.*, 39 (1963) 806.
23 R. FREEMAN, *J. Chem. Phys.*, 40 (1964) 3571.
24 L. D. HALL AND J. F. MANVILLE, *Carbohyd. Res.*, 8 (1968) 295.
25 L. D. HALL AND J. F. MANVILLE, *Carbohyd. Res.*, 9 (1969) 11.
26 E. B. BAKER, *J. Chem. Phys.*, 37 (1962) 911; 45 (1966) 609.
27 V. J. KOWALEWSKI, D. G. DE KOWALEWSKI AND E. C. FERRA, *J. Mol. Spectrosc.*, 20 (1966) 203.
28 V. J. KOWALEWSKI, in J. W. EMSLEY, J. FEENEY AND L. H. SUTCLIFFE (Eds.), *Progress in Nuclear Magnetic Resonance Spectroscopy*, Vol. 5, Pergamon, Oxford, 1969, p. 1.
29 J. A. FERRETTI AND R. FREEMAN, *J. Chem. Phys.*, 44 (1966) 2054.
30 H. C. TORREY, *Phys. Rev.*, 76 (1949) 1059.
31 D. C. DEJONGH AND K. BIEMANN, *J. Amer. Chem. Soc.*, 86 (1964) 67.
32 Ref. 15, p. 41.
33 A. ABRAGAM, *The Principles of Nuclear Magnetism*, Clarendon, Oxford, 1961, p. 65.
34 R. BURTON, L. D. HALL AND P. R. STEINER, *Can. J. Chem.*, 48 (1970) 2679.