

¹³C AND PROTON NMR SPECTRA OF 2(1H)PYRAZINONES†

J. C. MACDONALD*, G. G. BISHOP and M. MAZUREK

National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada S7N OW9

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Abstract—¹³C and proton NMR spectral data are given for eleven 2(1H)pyrazinones. Assignments of chemical shifts were made by methods which included: deuterium exchange with certain protons of 3-alkyl substituents; change of chemical shifts of certain carbon atoms with change in pH; the use of long-range coupling constants for ¹³C to protons; and various correlations among assigned spectra.

INTRODUCTION

Various 3,6-substituted 2(1H)pyrazinones are secondary metabolites produced by certain species of *Aspergillus*¹ and *Streptomyces*.^{2,3} Determination of the structure of these by PMR spectra is not necessarily straightforward. For example, assignment of the structure of arglecin,² primarily from such spectra, was initially incorrect.^{4,5} Even the initial assignment⁶ of the PMR spectrum for the simpler compound 3,6-dimethyl 2(1H)pyrazinone had to be later corrected.^{7,8} PMR spectra and natural-abundance ¹³C NMR (CMR) spectra would probably complement each other and eliminate uncertainties in structural determinations and in assignment of spectra. The present work describes and develops methods for assigning spectra of 2(1H)pyrazinones.

RESULTS

Rather than discussing the assignment of NMR spectra for each compound of Fig. 1 separately and in detail, the interpretative methods are enumerated below, and their application to the assignment of particular compounds is mentioned. As many of the methods as possible were used with each compound, and the various methods gave an internally consistent set of results. Methods 4–8 were developed in the present work, and include the conclusions made from assigned spectra.

Method 1. *Off-resonance, continuous-wave, proton spin decoupling.*^{9,10} This method was used to show the number of protons attached to a particular C atom in a CMR spectrum, and to correlate CMR and PMR spectra. With such correlations, if a chemical shift assignment could be made for a particular methyl, methylene, or methine group of a compound in either a CMR or PMR spectrum, assignments could be made for the group in both spectra.

Method 2. *Coupling between ring protons.* Observation of such couplings was used to assign the PMR spectra of compound 1, and in conjunction with method 1, the CMR spectra. The method is based on the finding¹¹ that the coupling in 2-substituted pyrazines was 2.4–3.9 Hz for *ortho*-coupling, 1.3–1.5 Hz for *para*-coupling, and less than 0.5 Hz for *meta*-coupling. Our spectra in CDCl₃ of compounds 2 and 3 were in accord with this finding, giving values of 4.0 and 1.5 Hz for *ortho*- and *para*-coupling, respectively. *Meta*-coupling (compound 4) was too low to be evaluated.

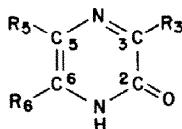
Method 3. Coupling between ring protons and the protons of methyl substituents. Observation of such couplings was used to assign the PMR spectra of compounds 2 and 3, and in conjunction with method 1, much of the CMR spectra for these compounds. The long-range coupling of Me protons to ring protons in methyl pyrazines has been shown⁸ to be about 0.6–0.7 Hz for *ortho*- and *para*-coupling, and 0.3 Hz for *meta*-coupling. Our spectra in CDCl₃ of compound 2 (*para*-coupling 0.8 Hz, *meta*-coupling 0.25 Hz) and compound 3 (*ortho*-coupling 0.9 Hz, *meta*-coupling 0.3 Hz) were in general agreement with this finding.

Method 4. Exchange of protons with D₂O. This method was useful in assigning a 3-Me substituent, or the methylene group in a 3-isobutyl substituent, for compounds 5, 6, 7, 8 and 10, in either PMR or CMR spectra. When 2(1H)pyrazinones with a Me substituent in the 3-position were heated in D₂O for 1/2 hr at 98° and pH 2, the protons of the 3-Me substituent exchanged with deuterium (compounds 2, 5, 6 and 7). Me substituents in other positions in compounds 5, 6 and 7, did not exchange, but the 6-H in compound 5 did exchange. The above method was developed from the finding² that the methylene protons of the 3-isobutyl substituent of arglecin, 8, exchanged with D₂O, and our finding that the same protons of compound 10 exchanged with the procedure just outlined. The exchange of protons with deuterium caused obvious decreases in intensity of appropriate signals in either CMR or PMR spectra.

Method 5. Change of chemical shifts with increase in pH. This method was used to assign ring C atoms, and any 6- α C atom, in CMR spectra for water-soluble 2(1H)pyrazinones. Where such C atoms bore protons, the assignment was extended to PMR spectra by Method 1. The chemical shifts of these C atoms changed in a regular fashion with increase in pH, and plots of pH vs chemical shift resembled titration curves for a weak acid. Values for pK obtained from such plots were close to the pK_a of the molecule (Table 1). For the nine compounds investigated, the total shifts, Δ in ppm, were: 2-C, 7.0 to 8.5; 3-C, -7.4 to -10.2; 5-C, 2.5 to 3.0; 6-C, 11.0 to 15.4; 6- α C, 3.5 to 4.5; and thus were typical either in magnitude or sign for each carbon.

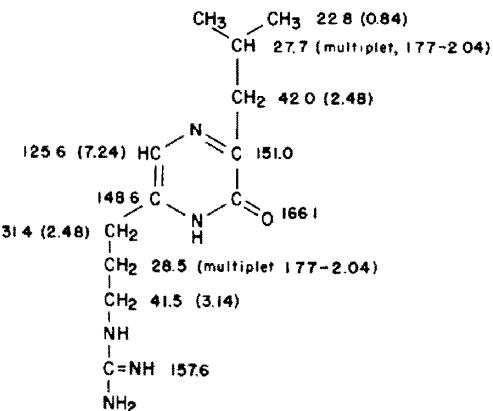
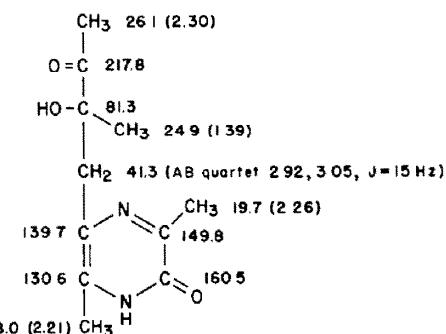
In PMR spectra, the chemical shifts of ring protons 3-H and 6-H also changed in a regular fashion with pH (Table 1), but the total shifts were small. Small shifts were also observed for some other C atoms and protons in CMR and PMR spectra, and selection of an appropriate pH value

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COMPOUND

- 1 $R_3 = R_5 = R_6 = H$
- 2 $R_5 = R_6 = H, R_3 = CH_3$
- 3 $R_3 = R_6 = H, R_5 = CH_3$
- 4 $R_3 = R_5 = H, R_6 = CH_3$
- 5 $R_6 = H, R_3 = R_5 = CH_3$
- 6 $R_5 = H, R_3 = R_6 = CH_3$
- 7 $R_3 = R_5 = R_6 = CH_3$
- 8 ARGLECIN, see fig 2
- 9 see fig 3
- 10 $R_5 = H, R_3 = R_6 = (CH_3)_2 CH CH_2 -$ (FL AVACOL)
- 11 $R_5 = H, R_3 = (CH_3)_2 CH CH_2 -, R_6 = CH_3 CH_2 CH (CH_3) -$ (DESOXYASPERGILLIC ACID)

Fig. 1.

Fig. 2. $\delta_{13C}(\delta_H)$ for arglecin, pH 10.4 in H_2O Fig. 3. $\delta_{13C}(\delta_H)$ for compound 9, pH 9.5 in H_2O .Table 1. Change in either ^{13}C or proton chemical shift, Δ , on making neutral 2(1H)pyrazinones alkaline, and pK for this shift.

compound	1	2	3	4	5	6	7	8	9
Δ for:									
2-C	7.4	8.7	8.5		7.9	7.6	7.0	7.5	7.0
3-C	-7.4	-9.5	-10.0	-8.9	-10.2	-9.1	-9.2	-9.2	-8.9
5-C	2.6	3.3	2.3	3.0	2.5	3.0	3.0	2.7	2.3
6-C	11.0	13.9	15.4		15.0	11.7	12.6	11.6	11.7
3- α C		0.5			0.4	0.4	0.4	0.4	0.3
5- α C			0.2		0.2		1.2		0.6
6- α C				4.6		3.5	4.5	3.9	4.3
3-H	-0.30		-0.35	-0.31					
5-H	-0.04	0.09		0.02		0.13		-0.01	
6-H	0.36	0.35	0.33		0.33				
pK for:									
molecule	8.2	9.1	8.4	8.6	9.4	9.4	9.8	8.9	9.7
2-C	8.5	9.3	8.5		9.6	9.7	10.0	9.1	9.7
3-C	8.5	9.3	8.4	8.7	9.6	9.7	9.9	9.0	9.7
5-C	8.5	9.4	8.5	8.8	9.6	9.8	10.1	9.1	9.8
6-C	8.5	9.3	8.5		9.6	9.7	10.0	9.1	9.7
6- α C				8.7		9.7	10.0	9.1	9.7
3-H	8.5		8.2	8.6					
6-H	8.2	9.5	8.7		10.1				
Δ = $(\delta$ at pH 11 to 12) - $(\delta$ for neutral 2(1H)pyrazinone)									

sometimes prevented overlapping signals. For example, at pH 5.4, signals for three different C atoms of the ring substituents of arglecin, 8, coincided, but were separated at pH 10.4, so that a better correlation of CMR and PMR spectra could be made (Fig. 2). Also, certain proton Me signals of compound 9 coincided for both pH 5.6 and 12, but were separated at pH 9.5 (Fig. 3).

Method 6. Correlation of chemical shifts for closely related compounds. The ^{13}C chemical shifts for the 3-isobutyl substituent of compound 10 were assigned by comparison with the CMR spectrum of compound 11, which also has a 3-isobutyl substituent. Other data useful in making correlations are shown in Table 2, and were

used to assign the CMR signals for ring carbon atoms of compounds 4, 6, 10 and 11, in $CDCl_3$.

For neutral 2(1H)pyrazinones in either H_2O or $CDCl_3$ (Table 2) the following correlations can be made. The shifts for 2-C and 3-C were either within 0.2 ppm of each other, or else δ for 2-C was greater than that for 3-C. Shifts for 5-C and 6-C has lower δ values. If only one of 5-C or 6-C had an alkyl substituent, then the one bearing the substituent had the higher δ value. If 5-C and 6-C had the same substituent (either H or CH_3), the assignment had to be made by another method. Where $CDCl_3$ was the solvent, and method 5 could not be used, methods 3 and 1 were used to assign 5-C and 6-C for compound 2, but are obviously not useful for compound 7. Unambiguous assignment of 5-C and 6-C for compound 7 in $CDCl_3$ was therefore not possible.

The chemical shifts for Me C atoms in dimethyl- and trimethyl-substituted 2(1H)pyrazinones (compounds 5-7) were in the order: 3-Me > 5-Me > 6-Me.

In PMR spectra of neutral 2(1H)pyrazinones (Table 2), the chemical shift of 3-H was greater than that of 5-H and/or 6-H in the same molecule (compounds 1, 3 and 4), and that of 3-Me protons was either similar to (compound 9) or greater than (compounds 5, 6 and 7) that of 5-Me and/or 6-Me protons in the same molecule.

Method 7. ^{13}C -proton coupling between ring C atoms and substituents. Observation of such coupling was useful in distinguishing between 2-C and 3-C in compounds 2 and 5 (Experimental), and in determining the number of α H atoms of a substituent on 3-C (e.g. compound 9) from the $^3J_{CH}$ coupling of 2-C.

The coupling of ^{13}C in ring C atoms of 2(1H)pyrazinones to ring protons was 177-189 Hz for

Table 2. ¹³C and proton chemical shifts of neutral 2(1H)pyrazinones

Compound,	2-C	3-C	5-C	6-C	3- α C	5- α C	6- α C	3-H	5-H	6-H	3- α H	5- α H	6- α H
1, H ₂ O*	160.3	146.8	126.5	131.2				8.09	7.55	7.44			
2, H ₂ O	157.9	158.1	124.3	125.9	20.1				7.27	7.31	2.31		
2, CDCl ₃	157.9	157.7	124.0	123.5	20.2				7.36	7.14	2.47		
3, H ₂ O	157.3	147.2	134.9	125.3		19.0		7.99		7.25		2.24	
3, CDCl ₃	157.1	147.0	134.1	123.3		19.3		8.15		7.07		2.31	
4, H ₂ O	+	144.4	125.0	+			16.3	7.85	7.30			2.26	
4, CDCl ₃	+	145.1	124.3	136.7			16.3	8.05	7.31			2.32	
5, H ₂ O	156.6	156.8	133.1	122.7	20.1	19.1			7.05	2.28	2.16		
5, CDCl ₃	156.8	156.2	132.2	120.5	20.1	19.4			6.94	2.44	2.26		
6, H ₂ O*	158.4	153.7	123.2	137.3	19.6		16.0		7.08		2.26		2.22
6, CDCl ₃	158.5	153.6	122.7	134.5	19.6		15.9		7.14		2.41		2.29
7, H ₂ O	157.5	152.4	130.7	133.3	19.3	18.0	16.0			2.20	2.10	2.15	
7, CDCl ₃	157.9	152.4	(129.5	130.7)	19.6	18.6	16.0			2.40	2.24	2.27	
8, H ₂ O*	150.7	157.4	123.0	139.7	41.7		27.7		7.23		2.51		2.65
9, H ₂ O	157.9	153.1	129.8	135.3	19.5	41.0	16.4			2.27	2.92	3.05	2.27
9, CDCl ₃	157.5	152.0	129.0	133.1	19.2	39.2	15.8			2.3	2.75	3.07	2.3
10, CDCl ₃	158.5	156.5	123.1	137.7	41.7		39.5		7.17		2.65		2.39
11, CDCl ₃	158.5	156.5	121.3	142.5	41.5		37.3		7.19		2.65		2.6

3- α H, 5- α H, and 6- α H refer to protons attached to 3- α C, 5- α C, and 6- α C, respectively. Where exact assignment was not possible, shifts for CMR spectra are enclosed in parentheses, and those for PMR spectra are given to two significant figures.

*In these three cases, PMR spectra were run in D₂O. Otherwise, CMR and PMR spectra were run in the same solvent at the same concentration.

*Values not observable (see Experimental).

one-bond coupling, 6–12 Hz for two-bond and three-bond coupling, and 1.5 Hz or less for four-bond coupling (Table 3). This agrees with the finding¹² that the coupling to ring protons in methyl pyrazines was greater for two- and three-bond couplings (9–10 Hz) than for four-bond couplings (about 1 Hz).

The two-bond coupling of a ring C atom to the protons of its Me substituent was 6–7 Hz. For a ring carbon atom one bond further removed from a methyl or isobutyl substituent, the coupling to the α protons was 2.5–5 Hz, and gave a triplet (isobutyl substituent) or quartet (Me substituent) for this coupling, ³J₀ (Table 3). No longer range couplings to alkyl substituents, such as ⁴J_m or ⁵J_p, were observed.

Method 8. Prediction of ¹³C chemical shifts for 3- and 6-substituents. Many of the naturally-occurring 2(1H)pyrazinones have 3- and 6-substituents of such structure as to suggest a biogenetic derivation of these compounds from amino acids. The following method with its empirical correction values should be useful in at least the preliminary assignment of the CMR spectra of such compounds.

The ¹³C chemical shifts of the carbon atoms in substituents of three naturally-occurring 3,6-disubstituted 2(1H)pyrazinones (compounds 8, 10 and 11) were grossly similar (within 3.1 ppm) to those for corresponding C atoms of amino acids from which the pyrazinones may be synthesized biologically (e.g. desoxyaspergillic acid, 11, may be biosynthesized from one molecule of leucine and one of isoleucine). In addition, when empirical corrections were made according to whether the substituent was in the 3- or 6-position, and whether the carbon atom was α ,

β , or γ to the ring, a better fit (within 0.9 ppm) between observed and calculated data was obtained. This is shown in Table 4 for compounds 10 and 11 in CDCl₃, and compound 8 in its neutral form (pH 5.3) in water.

Compound 9 was an unexpected by-product in a synthesis, and determination of its structure illustrates some of the above methods. CMR spectra, and off-resonance decoupling (method 1) showed that the compound contained four Me groups, one methylene group, one quaternary carbon plus four ring quaternary carbons, and one ketonic carbon. Shifts of signals in CMR and PMR spectra with change in pH (method 5) were used to assign the ring quaternary carbons and a 6-methyl carbon in CMR spectra, and to select pH 9.5 as suitable for separating Me signals in the PMR spectrum. Coupled CMR spectra (method 7) showed that the 2-C signal was a quartet (¹J₀ = 3 Hz), as was the 3-C signal (²J = 7 Hz). Therefore, the 3-substituent was a Me group, whose ¹³C chemical shift was assigned by comparison to other 3-Me substituted 2(1H)pyrazinones (method 6). Coupled CMR spectra also showed that the 6-C signal was complex enough to indicate that the 5- α C atom was not a quaternary carbon, but more likely the methylene group.

In the PMR spectrum at pH 9.4, four Me singlets were found, two of which could be assigned to 3-Me and 6-Me substituents by method 1, because the ¹³C shifts for these groups had been established. An AB quartet was also observed, which was assigned to the methylene group. This group would probably be adjacent to an asymmetric quaternary C atom to cause such non-equivalent chemical shifts for its protons. A PMR spectrum in perdeuterated

Table 3. Coupling of ring carbon atoms of 2(1H)pyrazinones to protons, Hz

Compound	1	2	3	4	5	6	7	10
pH, if in H_2O	8.9	8.8	9.6	12	8.8	11	12	(CDCl_3)
<u>Coupling to:</u>								
<u>Ring Protons:</u>								
$^1\text{J}_p$ for 3-C	183		180	180				
5-C	186	189		183		182		187
6-C	180	184	177		182			
$^2\text{J}_{\text{H}}$ for 2-C	8		9*	8				
5-C	8*	6	8*		6			
6-C	11	12		10		10		12
$^3\text{J}_{\text{H}}$ for 2-C	8	7	7*		7			
3-C	9	9		9		9		10
5-C	10*		10*	10				
<u>Alkyl Protons:</u>								
$^2\text{J}_p$ for 3-C		7		7	6	7	+	
5-C			7		6		6	
6-C				6	6	6	+	
$^3\text{J}_{\text{H}}$ for 2-C		3		3	3	2.5	3	
5-C				5	4	4	5	
6-C		4		5		4		

* $^1\text{J}_p$ coupling to ring protons was 1.5 Hz for 3-C of compounds 1 and 5, and 1 Hz or less in other cases.

*In these cases unambiguous assignment was difficult. Possibly the values 7 and 9 Hz for 2-C should be interchanged, and also the values 8 and 10 Hz for 5-C.

*The multiplet to be interpreted would show coupling not only to αH , but also to βH of the isobutyl substituent, and was too ill-defined to obtain coupling values.

Table 4. Empirical calculation of ^{13}C chemical shifts for 3- and 6-substituents of naturally occurring 2(1H)pyrazinones

Compound	10		11		8		
Correction*	Calc.	Δ	Calc.	Δ	Calc.	Δ	
3- αC	0.9	41.6	0.1	41.6	-0.1	41.6	0.1
3- βC	2.1	27.2	-0.2	27.2	-0.2	27.2	0.5
3- γC	0.2	22.6	0	22.6	0	22.6	0
6- αC	-0.4	40.3	-0.8	36.4	0.9	27.8	-0.1
6- βC	3.0	28.1	-0.1	28.4	0.1	27.6	0.1
6- $\beta\text{C}(\text{H}_3)$	3.0			18.6	-0.1		
6- γC	-0.1	22.3	-0.2	11.9	-0.1	41.1	0.2

*Values to which the correction was added are for C-3, C-4, etc. for the amino acids leucine, isoleucine, and arginine.²⁰ The values for the terminal methyl groups of leucine were averaged, since they are chemical shift equivalent in the isobutyl substituents of pyrazinones. Δ = observed value—calculated value.

dimethylsulfoxide showed a tertiary OH (singlet, δ 5.12) and a broad NH signal (δ about 12.3).

At this stage, the substituent on 5-C could be reasonably formulated only as $\text{CH}_3\text{C}(=\text{O})\text{C}(\text{OH})(\text{CH}_3)\text{CH}_2\text{--}$. For the two Me signals of this substituent as yet unassigned in the PMR spectrum, that of $\text{CH}_3\text{C}(=\text{O})$ was assigned a chemical shift of 2.30, and the other a chemical shift of 1.39, by comparison with catalogued spectra.¹³ Method 1 was used to assign the ^{13}C signals

corresponding to these groups. The results were uniquely in accord with the structure in Fig. 3.

Of possible theoretical interest was the finding that the observed CMR shifts for ring C atoms of methyl-substituted 2(1H)pyrazinones (compounds 1-7) as their sodium salts in water (pH 11-12) could be expressed by additive parameters, with agreement within ± 0.4 ppm. The chemical shifts equalled "X", a value close to that obtained for the appropriate carbon atom of the sodium salt of pyrazinone, 1, plus chemical shift changes owing to Me groups either attached directly to the ring carbon (α parameter), or in the *ortho*, *meta* or *para* position from the carbon (*o*, *m* or *p* parameters). The following values were obtained:

For 2-C: $X = 167.7$, $\alpha = -1.3$, $p = -1.8$, $m = -0.3$
 For 3-C: $X = 139.1$, $\alpha = 9.3$, $m = -1.7$, $p = -3.7$
 For 5-C: $X = 129.3$, $\alpha = 7.8$, $\delta = -1.5$, $m = -1.7$
 For 6-C: $X = 142.6$, $\alpha = 8.8$, $\delta = -2.3$, $p = -2.8$

Attempts to extend this type of calculation to neutral Me-substituted 2(1H)pyrazinones (data in Table 2) revealed that "X" values calculated for compounds 2-7 were, in general, not compatible with observed shifts for pyrazinone itself. Also, because X parameters and some other parameters were dependent on the solvent, and also because the calculations were of no useful predictive value when the substituents were other than methyl or H (e.g. compounds 8, 9, 10 and 11), the practical applicability

of such calculations was low, and inferior to the correlations made in method 6.

EXPERIMENTAL

CMR spectra were obtained by the pulse Fourier transform method with a Varian XLFT-100 NMR spectrometer operating at 25.2 MHz. PMR spectra were obtained by the field sweep method with a Varian HA-100 NMR spectrometer operating at 100 MHz. While TMS was used as an internal reference for proton spectra run with CDCl₃ as a solvent, in other cases an internal secondary reference was used to permit correction of data to δ referred to $\delta_{\text{TMS}} = 0$. The references were dioxane for aqueous solutions ($\delta_{\text{diox}} = 67.4$, $\delta_{\text{H}_2\text{O}} = 3.72$), and CDCl₃ itself ($\delta_{\text{CDCl}_3} = 77.0$ for center peak of CDCl₃ triplet).

CMR spectra for Tables 1 and 2. These were generally obtained with an acquisition time of 0.4 sec (2.5 Hz per spectral point), and noise-irradiation decoupling of protons. Neutral 2(1H)pyrazinones were dissolved in H₂O (containing 5% D₂O for the heteronuclear lock signal) at concentrations near saturation (0.25–2.0 M) to obtain initial spectra. The pH of such solns ranged from 3.7–6.0. H₂O was used in preference to D₂O because 2-C of 2(1H)pyrazinones gave stronger signals in H₂O. In most cases, satisfactory PMR spectra could be obtained with the same solns as used for CMR spectra. Further spectra were obtained at various pH values after adding small volumes of sat NaOH aq. pK values for individual atoms were determined and calculated by a method similar to that used for the spectrophotometric determination of pK.¹⁴

$$pK = \text{pH} + \log \frac{(\delta \text{ for sodium salt} - \delta \text{ at given pH})}{(\delta \text{ at given pH} - \delta \text{ for neutral compound})}$$

Each pK value given in Table 1 was the average of at least four values obtained throughout the alkaline titration range of the compound, and standard deviations ranged from 0.03–0.12, with an average standard deviation of 0.06 for all the data. The pK_a values for the whole molecule were determined either by titration, or by a spectrophotometric method¹⁴ at a wavelength of 240 nm.

Where solubility permitted, spectra were obtained in CDCl₃, as well, generally with 0.25–1.0 M solns.

Because of the instability of 5-methyl 2(1H)pyrazinone, 3, in its neutral form, spectra were run immediately after dissolving the compound in H₂O or CDCl₃. At alkaline pH the compound was much more stable, and this precaution was not necessary.

Compound 4, was poorly soluble in water, but more soluble in the presence of 3. The data for Tables 1 and 2 for 4 in water were obtained from spectra for 3 which contained about 15% of 4 (0.15 M). Spectra in CDCl₃ were obtained with pure 4 (0.03 M). In both cases, weaker signals in the CMR spectra could not be located, and the data is, of necessity, incomplete. At pH 9.8–11.7, 4 was much more soluble (2 M) and no difficulty was encountered in locating and assigning signals.

CMR spectra for Table 3. These were obtained with an acquisition time of 2 sec (0.5 Hz per spectral point). The solvent and pH were chosen so the solubility of the compound would be high (1–2 M) and signals for ring C atoms would be well separated.

For 1 and 7, the coupled spectrum was interpreted on the basis of overlapping doublets and quartets, respectively (Table 3). For 2–6, interpretation of multiplets of coupled spectra was facilitated as follows. When a continuous-wave decoupling power of intensity of about 500 Hz[‡] was applied near the frequency of the Me protons, coupling to the Me protons was removed, while coupling to aromatic protons remained at about 0.7 times that for a coupled spectrum. Conversely, when this decoupling intensity was applied near the frequency of the aromatic protons, coupling to the aromatic protons was removed, while coupling to Me groups remained at about 0.7 times that in a coupled spectrum. With such selectively decoupled spectra, couplings to aromatic

protons and to Me protons could be observed independently, and the long-range couplings, where necessary, could be evaluated by dividing observed couplings by the appropriate factor (about 0.7). The exact value of the factor was determined by comparing the unambiguous one-bond C–H couplings in coupled and selectively-decoupled spectra.

The technique of selectively removing coupling to aromatic protons was also of use in distinguishing between 2-C and 3-C signals where these were close together (compounds 2 and 5, Table 2). This was possible because coupling of 2-C and 3-C to 3-Me protons was of different magnitude (Table 3), and the two overlapping quartets were easily interpreted.

Compounds 2, 3, 5, 7 and 9. These were prepared by the method of Karmas and Spoerri,¹⁵ modified in that the reaction mixture was titrated with HCl to pH 5–7, to avoid strongly acidic conditions which can decompose the products (especially 3).

5-methyl 2(1H)pyrazinone, 3. The compound was isolated from the reaction mixture as quickly as possible, and was sublimed at 73° and 0.03 torr for several days. The product was analyzed by liquid chromatography on a column, 50 × 0.2 cm diameter, of Corasil II (Waters Associates) with the solvent CHCl₃ : EtOH : H₂O (97:3:0.1, by volume) and detected by UV absorption at 320 nm. The first half of 3 to sublime contained about 7% of 4, while the last half contained about 15% of 4. These products were used without further purification and were stored at –14°.

3,5,6-Trimethyl 2(1H)pyrazinone, 7, and compound 9, 5 - (2 - hydroxy - 2 - methyl - 3 - oxobutyl) - 3,6 - dimethyl 2(1H)pyrazinone. These products were analyzed by chromatography on a Corasil column (as above) with the solvent EtOAc : H₂O : HOAc (99:0.8:0.2). When freshly-distilled diacetyl was used, the reaction mixture¹⁵ contained 7 and 9 in a molar ratio of about 7:1. Compound 7 was obtained free of 9 by crystallization from n-butyl acetate. When an old (partially polymerized) sample of diacetyl was used directly, the reaction mixture contained 7 and 9 in a molar ratio of about 1:1. Compound 7 was sublimed directly from the crude product at 90° and 0.01 torr, and subsequent sublimation at 110° yielded 9, 2.32 g from 8.45 g alanine hydrobromide (21%), recrystallized from EtOAc, m.p. 156–157°, molecular weight 224 (chemical ionization mass spectrum). (Found: C, 59.11; H, 7.12; N, 12.39. C₁₁H₁₆N₂O₃ requires: C, 58.91; H, 7.19; N, 12.49%; mol. wt. 224).

Other compounds. 2-chloro-6-methylpyrazine was prepared⁸ from 2,6-dichloropyrazine and hydrolyzed¹⁶ with KOH to give 4. Flavacol, 10, and 6, were prepared by known methods.^{17,18} Compound 11, was obtained from Dr. J. D. Dutcher¹⁹ and 8, was isolated from cultures of *Streptomyces toxytricini*.²

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[†]Obtained with 2.5 Hz/spectral point. A more accurate value, at 0.7 Hz/spectral point, is 76.88.

[‡]For calibration of decibel power in terms of Hz, see J. C. MacDonald and M. Mazurek, *J. Mag. Resonance* **19**, 51 (1975).

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