924. *Gibberellic Acid. Part XXXI.*[1] *The Nuclear Magnetic Resonance Spectra of Some Gibberellin Derivatives*

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The nuclear magnetic resonance spectra of some gibberellin derivatives have been measured in pyridine and deuterochloroform solution and some differences correlated with structural features.

The diamagnetic anisotropy of pyridine and its consequent effect on the nuclear magnetic resonance spectra of solute molecules renders it unsuitable as a normal solvent for this form of spectroscopy.[2] However, examples have appeared of its use in the assignment of steroidal methyl resonances [3] and in a study of β-lumicolchicine.[4] Nuclear magnetic resonance spectroscopy has played an important part [5-7] in the study of gibberellin

[1] Part XXX, B. E. Cross, J. R. Hanson, and R. N. Speake, *J.*, 1965, 3555.
[2] N. F. Chamberlain, *Analyt. Chem.*, 1959, **31**, 56.
[3] G. Slomp and F. MacKellar, *J. Amer. Chem. Soc.*, 1960, **82**, 999.
[4] O. L. Chapman, H. G. Smith, and R. W. King, *J. Amer. Chem. Soc.*, 1963, **85**, 803.
[5] N. Sheppard, *J.*, 1960, 3040.
[6] D. C. Aldridge, J. F. Grove, R. N. Speake, B. K. Tidd, and W. Klyne, *J.*, 1963, 143.
[7] P. M. Bourn, J. F. Grove, T. P. C. Mulholland, B. K. Tidd, and W. Klyne, *J.*, 1963, 154.

chemistry. Because of their low solubility in deuterochloroform the spectra of a number of derivatives were determined in pyridine solution. To assist in the assignment of resonances a number of gibberellin derivatives were then examined in both solvents. As a result it has been possible to associate the difference in chemical shift between the two solvents with certain structural features and thus to appreciate the value of comparing spectra determined in the two solvent systems. The spectra were determined at 60 Mc./sec. with tetramethylsilane as an internal standard and are tabulated.

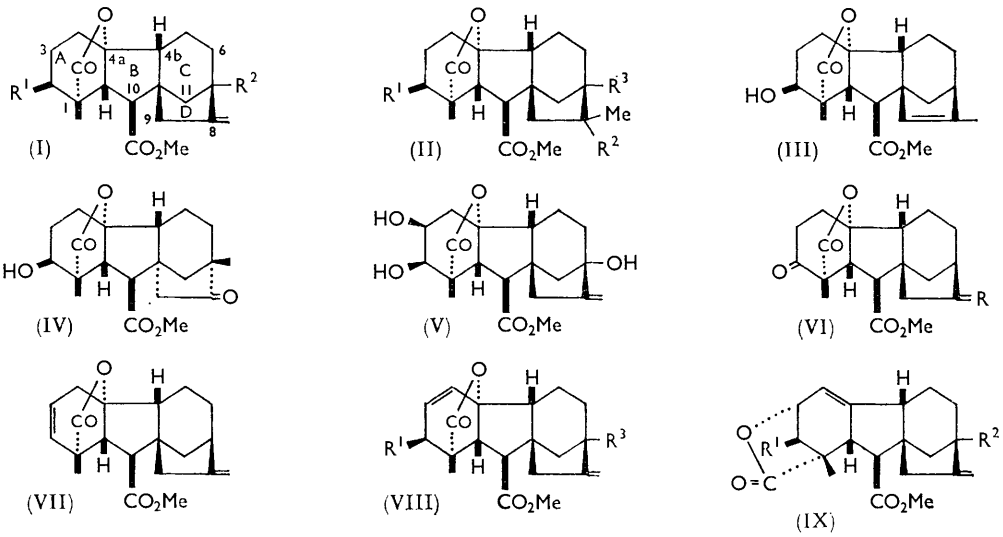
Chemical shifts (τ values) of protons in some gibberellin derivatives of structures (I)—(IX)
(i) in deuterochloroform; (ii) in pyridine solution; (iii) in deuteroacetone

Compound		C-1	C-8 substituents		C-10	C-10a	C-2	C-3	C-4
I; $R^1 = R^2 = H$ (gibberellin A_9 methyl ester) [a]	(i)	8·92	5·15	5·05	7·28	7·51			
	(ii)	8·88	5·13	5·03	7·14	7·39			
I; $R^1 = OH$, $R^2 = H$ (gibberellin A_4 methyl ester) [b,c]	(i)	8·85	5·15	5·05	7·29	6·78	6·15		
	(ii)	8·56	5·14	5·04	7·07	6·32	5·94		
I; $R^1 = R^2 = OH$ (gibberellin A_1 methyl ester) [d]	(i)	8·85	4·96	5·05	7·33	6·78	6·15		
	(ii)	8·56	4·41	4·95	7·03	6·27	5·92		
II; $R^1 = OH$, $R^2 = R^3 = OH$	(i)	8·87	9·01	(J 7 c./sec.)	7·29	6·86	6·17		
	(ii)	8·56	9·10	(J 7 c./sec.)	7·05	6·32	5·95		
III [e]	(i)	8·85	8·32	(J 1·5 c./sec.)	7·42	6·89	6·18		
	(ii)	8·55	8·38	(J 1·5 c./sec.)	7·15	6·34	5·95		
IV [f]	(i)	8·78	8·96		7·36	6·78	6·14		
	(ii)	8·53	8·98		6·96	6·28	5·95		
II; $R^1 = R^2 = OH$, $R^3 = H$ (gibberellin A_2 methyl ester) [g]	(iii)	8·97	8·72		7·30	6·78	6·15		
	(ii)	8·55	8·55		6·98	6·21	5·92		
I; $R^1 = OAc$, $R^2 = H$ [b]	(i)	8·94	5·15	5·05	7·36	6·83	5·15		
	(ii)	8·79	5·12	5·02	7·15	6·61	4·86		
V; (gibberellin A_8 methyl ester) [h]	(ii)	8·56	5·09	4·45	7·05	6·20	5·85	5·21	
VI; $R = CH_2$ [e]	(i)	8·83	5·15	5·05	7·22	6·92			
	(ii)	8·66	5·12	5·02	7·02	6·63			
VI; $R = CH_3$, H [e,i]	(i)	8·85	9·05		7·23	6·96			
	(ii)	8·62	9·08		7·03	6·61			
II; $R^1 = \alpha$-OH, $R^2 = R^3 = H$ [e]	(i)	8·85	9·08		7·28	7·51	6·40		
	(ii)	8·53	8·82		6·96	7·28	6·15		
VII	(i)	8·78	5·15	5·05	7·21	7·42	4·28	4·28	
	(ii)	8·72	5·12	5·02	7·05	7·21	4·31	4·31	
VIII; $R^1 = OH$, $R^2 = H$ (gibberellin A_7 methyl ester) [a]	(i)	8·77	5·15	5·05	7·29	6·78	5·87	4·14	3·70
	(ii)	8·48	5·15	5·03	7·03	6·38	5·58	3·96	3·63
VIII; $R^1 = R^2 = OH$ (methyl gibgerellate) [j]	(ii)	8·46	4·55	4·98	6·95	6·31	5·52	3·89	3·59
VIII; $R^1 = OAc$, $R^2 = H$ [f]	(i)	8·86	5·15	5·05	7·27	6·70	4·70	4·18	3·62
	(ii)	8·70	5·13	5·03	7·06	6·52	4·42	4·14	3·52
VIII; $R^1 = R^2 = OAc$ [i]	(i)	8·86	4·84	5·03	7·25	6·67	4·68	4·16	3·62
	(ii)	8·69	4·41	4·95	6·99	6·46	4·42	4·13	3·52
IX; $R^1 = OH$, $R^2 = H$ [e]	(i)	8·80	5·09		7·44	6·72	5·77	5·28	4·15
	(ii)	8·62	5·10		7·22	6·22	5·49	5·12	4·15
IX; $R^1 = R^2 = OH$ [k]	(i)	8·82	4·91	5·06	7·46	6·74	5·79	5·30	4·22
	(ii)	8·64	4·56	4·96	7·12	6·20	5·49	5·12	4·12
IX; $R^1 = OAc$, $R^2 = OH$ [k]	(i)	8·80	4·96		7·44	6·70	4·96	4·96	4·25
	(ii)	8·64	4·96	4·55	7·17	6·37	4·89	4·67	4·15
IX; $R^1 = R^2 = OAc$ [l]	(i)	8·81	4·98		7·43	6·70	4·98	4·98	4·25
	(ii)	8·65	4·95	4·65	7·15	6·38	4·65	4·82	4·20

[a] B. E. Cross R. H. B. Galt and J. R. Hanson, *Tetrahedron*, 1962, **18**, 451. [b] N. Takahashi, Y. Seta, H. Kitamura, and Y. Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 396. [c] J. F. Grove, J. MacMillan, T. P. C. Mulholland, and W. B. Turner, *J.*, 1960, 3049. [d] J. F. Grove, P. W. Jeffs, and T. P. C. Mulholland, *J.*, 1958, 1236. [e] D. C. Aldridge, J. R. Hanson, and T. P. C. Mulholland, *J.*, 1965, 3539. [f] B. E. Cross, *J.*, 1960, 3022. [g] J. F. Grove, *J.*, 1961, 3545. [h] J. MacMillan, J. C. Seaton, and P. J. Suter, *Tetrahedron*, 1962, **18**, 349. [i] B. E. Cross, J. R. Hanson, and R. N. Speake, *J.*, 1965, 3555. [j] B E. Cross, *J.*, 1954, 4670; J. S. Moffatt and M. Radley, *J. Sci. Food Agric.*, 1960, **7**, 386. [k] B. E. Cross, J. F. Grove, and A. Morrison, *J.*, 1961, 2498. [l] D. F. Jones, J. F. Grove, and J. MacMillan, *J.*, 1964, 1835.

A methyl group at position 1 is a characteristic structural feature of all the known gibberellins. Although it is an equatorial substituent, its chemical shift in the nuclear

magnetic resonance spectrum is sensitive to the presence of other ring A substituents. Thus the deshielding by a 2β-hydroxyl group has been noted (Table, ref. *l*) (cf. I; R′ = R^2 = H; I, R^1 = OH, R^2 = H and I; R^1 = R^2 = OH). Deshielding by a 2α-hydroxyl (II; R^1 = α-OH, R^2 = R^3 = H) by a 2-ketone (VI), by a Δ^2-olefin (VII), and from a Δ^3-olefin (cf. I; R^1 = OH, R^2 = H and VIII; R^1 = OH, R^2 = H; I; R^1 = OAc, R^2 = H; and VIII; R^1 = OAc, R^2 = H) is also apparent. On the other hand acetylation apparently removes this deshielding (cf. I; R^1 = OH, R^2 = OH and I; R^1 = OAc, R^2 = H). However, when the spectra determined in deuterochloroform and pyridine solution were compared, it was possible to distinguish between effects on this methyl group due to olefinic unsaturation and those due to oxygen substituents. Whereas pyridine

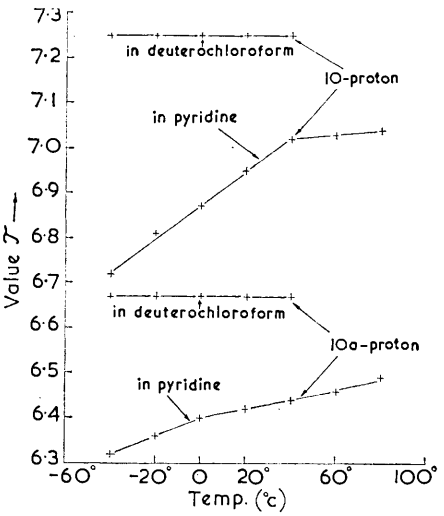


increased the deshielding due to oxygen substituents (from $\Delta\tau$ = 0·07 to 0·29), it left unchanged the deshielding due to olefinic unsaturation. Thus even the acetyl derivative (I; R_1 = OAc, R_2 = H) shows a difference in the chemical shift of the methyl group between the two solvent systems. Hence the use of the two solvent systems served to distinguish in this case between effects due to oxygen and those due to olefinic unsaturation. It is interesting to note that gibberellin A_8 methyl ester (V) with an additional hydroxyl group at position 3 has the 1-methyl resonance at τ 8·56 in pyridine solution identical to that of gibberellin A_1 methyl ester (I; R^1 = R^2 = OH).

The 10,10a AB quartet is a characteristic and important feature of the nuclear magnetic resonance spectra of the gibbane skeleton.[5] The 10a proton is a β-axial substituent on ring A and hence 1,3 diaxial transannular effects might be expected from the 2-position.[8] On the other hand the 10-proton is an α-substituent on ring B. Thus the position of the 10-proton resonance remained fairly constant within the range τ 7·22—7·36 in deuterochloroform and at lower field (τ 6·96—7·15) in pyridine. This is in agreement with some previous assignments made [6] by Aldridge *et al.* by comparison with methyl cyclopentanecarboxylate. However the position of the 10a proton resonance was remarkably susceptible to ring A substitution. Thus in gibberellin A_9 methyl ester (I; R^1 = R^2 = H) the 10a-proton resonance appeared at τ 7·51, not far removed from the predicted value [6] (τ 7·6) for the chemical shift of a hydrogen at a ring junction in a rigid system and β to a methoxycarbonyl group. The shifts from this position in, for example, gibberellin A_4 methyl ester (I; R^1 = OH, R^2 = H) are consistent with transannular 1,3-diaxial interactions. Furthermore the 10a-proton resonance of the 2-epimer (II; R^1 = α-OH, R^2 =

[8] K. Mori, M. Matsui, and Y. Sumiki, *Agric. Biol. Chem.* (*Japan*), 1963, **27**, 530.

R^3 = H), lacking this interaction, reverts to τ 7·51. Deshielding may also be noted from a 2β-acetoxyl and 2-carbonyl group. The earlier explanation [6] of the position of this resonance did not take into account this interaction. The deshielding due to a 1,3-diaxial interaction with a hydroxyl group is again amplified by pyridine (τ from 0·73 to 1·07), a small change in chemical shift being noted for the 2-epimer. Thus the effect showed some stereochemical specificity. This point can be illustrated more clearly in an examination of the 8-methylene proton resonances. These are readily distinguished at τ 5·15 and 5·05 by comparison with the corresponding dihydro-derivatives (cf. VI; R = CH_2 and VI; R = CH_3, H). An adjacent 7-hydroxyl brought about a significant shift in the position of one of these resonances which was further changed by pyridine (cf. I; R^1 = OH, R^2 = H and I; R^1 = R^2 = OH). Dreiding models show that one of these protons will lie closer to the hydroxyl group than the other. A similar difference in chemical shift between the two solvent systems was apparent in the resonances of the C-2 and C-3 protons.



The 10,10a proton resonances of methyl diacetylgibberelate

Indeed a further application of this effect can be seen in those regions where olefinic and –CH–(O) resonances occur together and it is necessary to distinguish between them. Thus in acetylgibberellin A_4 methyl ester (I; R^1 = OAc, R^2 = H) the 2-proton resonance appeared at τ 5·15 together with the C-8 methylene proton resonances. However on redetermining the spectrum in pyridine solution the former was shifted to τ 4·86 whilst the latter remained constant.

The interpretation of spectra may be confused in this region by overlapping multiplets and again choice of a suitable solvent may serve to separate and clarify these resonances and thus to assist in the determination of coupling patterns. An example of this was furnished by the ester (IX; R^1 = OAc, R^2 = OH). The spectrum was originally recorded [5] in chloroform solution at 40 Mc. The 60 Mc. deuterochloroform spectrum agreed in general with this. In particular the resonances from the C-8-methylene protons and those due to the C-2 and C-3 protons overlapped. However when the spectrum was redetermined in pyridine solution this region was resolved revealing a triplet at τ 4·67 (J = 5 c./sec.) due to the C-3 proton, a doublet at τ 4·89 (J = 5 c./sec.) due to the C-2 proton, and the broader olefinic resonances at τ 4·55 and 4·96.

Since the effect of pyridine apparently involved co-ordination at specific sites in the molecule it should show a temperature dependence. The spectrum of methyl diacetylgibberellate (VIII; R^1 = R^2 = OAc), a compound which was sufficiently soluble in the two solvent systems, was observed over the range −40° to +80° in pyridine and compared with that in deuterochloroform over the range −40° to +40°. The variation in position

of 10 and 10a resonances as examples are shown graphically (see Figure). In deuterochloroform the chemical shift remained constant over the range −40° to +40°. Whilst the position in pyridine solution changed, confirming the part played by solvation, the change in chemical shift shown in pyridine solution was least at the higher temperatures. The effect was also noted for other resonances such as the C-1 Me which varied from τ 8·57 to 8·82 over the range −40° to +80°. [$^2H_6$]Acetone was used as a solvent for the poorly soluble gibberellin A_2 methyl ester (II; $R^1 = R^2 = OH$, $R^3 = H$). Comparison of this spectrum with that of gibberellin A_4 methyl ester (I; $R^1 = OH$, $R^2 = H$) and in particular the position of the 1-methyl proton resonance suggests that this solvent, unlike pyridine, shields certain protons.

Thus in those cases where material is at a premium necessitating the application of as wide a range of physical methods as possible, the comparison of nuclear magnetic resonance spectra in two solvent systems such as pyridine and deuterochloroform may provide useful information.* However since these effects are due to solvation, they are therefore susceptible to the overall shape of the molecule and may vary in magnitude from series to series, caution must be exercised in the choice of model compounds.

Experimental

The spectra were determined on Varian Associates A60 spectrometers with tetramethylsilane as an internal standard. Pyridine was AnalaR grade and distilled from potassium hydroxide before use. The preparation of compounds used has been described previously.

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* Since the completion of this work a study of the effect of benzene on steroidal methyl resonances [9] has been described. However the majority of gibberellin derivatives are not sufficiently soluble in benzene to enable their spectra to be determined at present.

[9] For a recent use of benzene in this way see N. S. Bhacca and D. H. Williams, *Tetrahedron Letters,* 1964, 3127.