608. *Gibberellic Acid. Part XV.* The Nuclear Magnetic Resonance Spectra and Structures of Gibberellic Acid Derivatives.*

By N. SHEPPARD.

The hydrogen (proton) nuclear magnetic resonance spectra of methyl gibberellate and its acetyl derivative give detailed evidence for the structure of ring A of gibberellic acid.

THE structure (I; R = R′ = H) has recently [1,2] been proposed for gibberellic acid on evidence from chemical and spectroscopic measurements. In this paper the evidence that hydrogen (proton) nuclear magnetic resonance spectroscopy provides for this structure is considered in more detail.

Much of the previous discussion in the literature on the structure of gibberellic acid has

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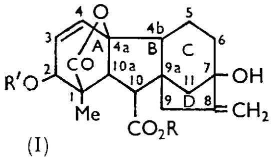
* Part XIV, preceding paper.

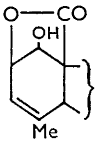
[1] Cross, Grove, MacMillan, Moffatt, Mulholland, Seaton, and Sheppard, *Proc. Chem. Soc.*, 1959, 302.

[2] Cross, Grove, McClosky, Mulholland, and Klyne, *Chem. and Ind.*, 1959, 1345.

been concerned with ring A. It will be shown that the nuclear magnetic resonance spectrum provides strong support for the structure of this ring shown in (I) and rules out all but one of the alternatives (II—V) [3,4,5] that have been considered. The remaining structure, (V), would be very difficult to distinguish from (I) on the basis of its nuclear resonance spectrum and all the spectroscopic arguments of sections (1)—(3) below can be applied equally well to it. However, there appear to be sound chemical grounds for preferring (I) to (V),[1,6] so that the latter will be disregarded here. The nuclear resonance spectrum also provides some evidence for the stereochemical relation of substituents on ring B and, by implication, for the relative arrangement of rings A and B.



(I)



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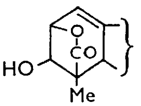
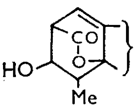
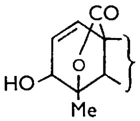


(III)



(IV)



(V)

The first magnetic resonance measurements were made on methyl gibberellate [7] (I; R = Me, R′ = H) and its dihydro- [8] (VI) and tetrahydro-derivative [3] (VII; R = H), all dissolved in dioxan. The spectra of the first and the last of these are illustrated in Figs. *a* and *c*; much of the spectrum of the solute cannot be observed because of the strong solvent bands but, nevertheless, useful information was obtained from these spectra. Subsequently the acetyl derivatives [6,7,9] of these three compounds, as well as methyl benzoylgibberellate, became available. These are soluble in chloroform and, because the main band of this solvent lies at low chemical shift values, virtually complete spectra (Figs. *b, d, e*) were obtained for them.

In the Figure the positions of the resonances are plotted on a chemical shift (σ) scale based on cyclohexane ($\sigma = +3{\cdot}9$) as an internal reference. The individual peaks were actually measured relative to the strong solvent resonances (chloroform or dioxan) in dilute solutions, these solvent resonances having been calibrated against cyclohexane either in separate experiments or, occasionally, *in situ*. The σ scale (which corresponds approximately to a zero based on water as a reference) [10,11] can be converted to the alternative τ scale, based on tetramethylsilane as an internal reference,[12] by adding 4·67 to each of the σ values.

(1) *Methyl Resonances.*—The resonances of methyl groups always give rise to prominent features in the spectrum, as all three hydrogen atoms contribute to the same band. Further, if there are no immediately adjacent hydrogen atoms to cause splitting of the resonance by spin–spin interaction, such bands are very sharp and strong. In the spectrum of methyl acetylgibberellate (I; R = Me, R′ = Ac) in chloroform solution (Fig. *b*) there are three such sharp and outstandingly strong bands at σ values of 1·6, 3·22, and 4·2. The positions of the first two are such that they, and analogous pairs of bands in spectra *d* and *e*, are clearly to be assigned to the methyl groups of the ester, CH_3–[O], and of the acetyl group, CH_3–[C=O], respectively. As expected, the acetyl band near 3·2 is missing from the dioxan solution spectra of methyl gibberellate (Fig. *a*) and of its tetrahydro-derivative (Fig. *c*).

[3] Cross, Grove, MacMillan, and Mulholland, *Chem. and Ind.*, 1956, 954.

[4] Cross, Grove, MacMillan, Mulholland, and Sheppard, *Proc. Chem. Soc.*, 1958, 221.

[5] Takahashi, Seta, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1958, **22**, 432.

[6] Cross, *J.*, 1960, 3038.

[7] Cross, *J.*, 1954, 4670.

[8] Grove, Jeffs, and Mulholland, *J.*, 1958, 1236.

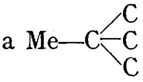
[9] Grove, unpublished work.

[10] Wertz, *Chem. Rev.*, 1955, **55**, 829.

[11] Pople, Schneider, and Bernstein, " High Resolution Nuclear Magnetic Resonance," McGraw-Hill, London, 1959.

[12] Tiers, *J. Phys. Chem.*, 1958, **62**, 1151.

From its intensity the third peak at $\sigma = 4{\cdot}2$ must also correspond to a methyl group without immediately adjacent hydrogen atoms, and this rules out structure (IV) which has a Me·CH group. From its position the resonance may be attributed to a

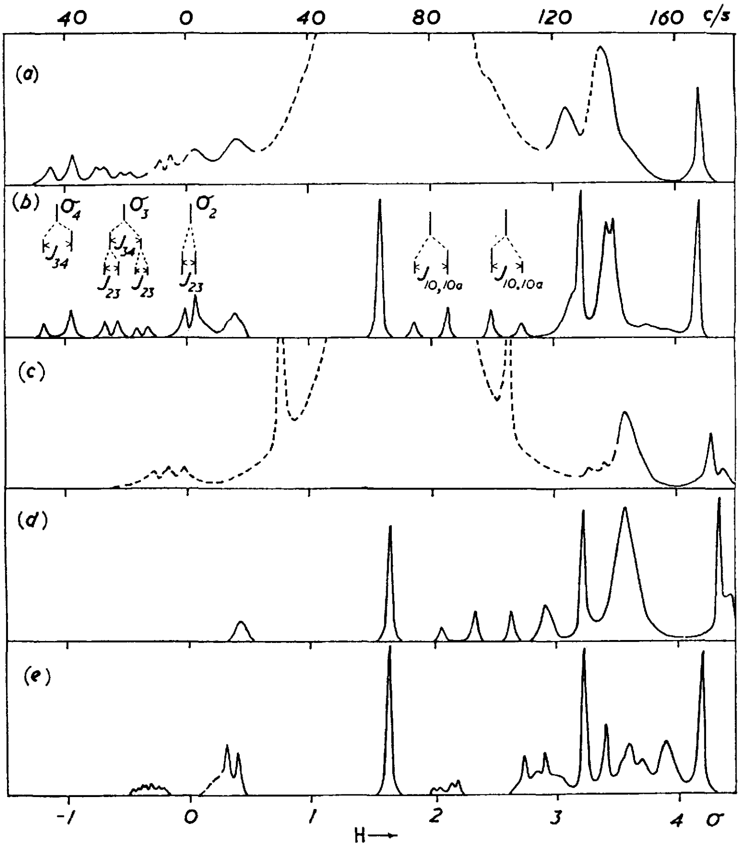


$\mathrm{Me{-}C}\begin{smallmatrix}\diagup C\\ -C\\ \diagdown C\end{smallmatrix}$

group as in (I) or (III). A methyl group attached to a double bond, as in (II), would be

Hydrogen (proton) nuclear magnetic resonance spectra of (a) methyl gibberellate (I; R = Me, R′ = H) *in dioxan, (b) methyl acetylgibberellate* (I; R = Me, R′ = Ac) *in chloroform, (c) methyl tetrahydrogibberellate* (VII; R = H) *in dioxan, (d) methyl acetyltetrahydrogibberellate* (VII; R = Ac) *in chloroform, and (e) the acetyl derivative* (VIII) *in chloroform.*

Bands in the spectra wholly or partly caused by resonances and side-bands of the solvents are indicated by broken lines. The chemical shift, σ, scale is based on cyclohexane ($\sigma = +3{\cdot}9$) *as internal standard. The zero corresponds approximately to the resonance of* H_2O.

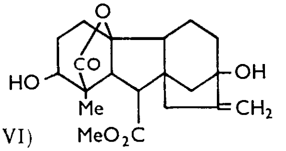


expected to give a much lower σ value (near 3), and this structure can therefore also be eliminated.[4]

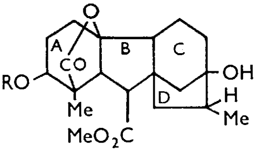
The spectra of methyl tetrahydrogibberellate (VII; R = H) and its acetyl derivative (VII; R = Ac) should show two resonances caused by methyl groups attached to saturated carbon atoms, a very sharp one from the methyl substituent on ring A, and a doublet caused by the methyl group on ring D. In the spectra observed (Figs. *c* and *d*) it appears that these two resonances overlap, so that one component of the doublet coincides with the sharp methyl band at $\sigma \sim 4{\cdot}3$.

(2) *Resonances in the Olefinic* CH *Region.*—The complex of weak bands between $\sigma = -1{\cdot}5$ and $+0{\cdot}5$ in the spectrum of methyl gibberellate (Fig. *a*) and of its acetyl derivative

(Fig. *b*), will next be considered. Structure (III) for gibberellic acid should give a spectrum considerably different from that of (I), of a type to be discussed in section (4) below.



(VI)



(VII)

For structure (I), the four olefinic CH bonds (two in ring A, and two in ring D) and the single CH bond of type [R′O]–CH–[C=C] in ring A are expected to give bands in this region. The spectra of this ester and its acetyl derivative are, as expected, very similar. However, it is particularly clear from the spectrum of methyl gibberellate itself (and also from that of methyl benzoylgibberellate, not illustrated) that there is a pair of broad resonances of similar shape and area at $\sigma = 0{\cdot}05$ and $0{\cdot}4$, one of which overlaps a sharp doublet at $0{\cdot}02$ in the spectrum of the acetyl compound. The pair of broad bands can be attributed to the two olefinic hydrogen atoms of the $C{=}CH_2$ group in ring D, as shown by the fact that they occur also in the spectra of methyl allogibberate[2] and methyl acetyldihydrogibberellate (in the latter case with some detailed modification of the spectrum by an overlapping band), in both of which the structure of ring D is retained as in gibberellic acid. The single band that remains near $0{\cdot}4$ in the spectrum of methyl acetyltetrahydrogibberellate despite the reduction of the $C{=}CH_2$ group of ring D (Fig. *d*) is probably to be attributed to the CH–[O] group in ring A. The bands in this region of the spectrum of the dilute dioxan solution of methyl tetrahydrogibberellate are all caused by the solvent.

The remaining pattern of eight lines between $\sigma = +0{\cdot}25$ and $-1{\cdot}25$ in the spectra of methyl gibberellate and its derivatives can readily be assigned to the 3-hydrogen system [O]–$\overset{2}{\text{C}}$H–$\overset{3}{\text{C}}$H=$\overset{4}{\text{C}}$H postulated for ring A of structure (I) and provides strong confirmation for the presence of this grouping. In particular, if the two doublets between $\sigma = -0{\cdot}25$ and $-0{\cdot}75$ are imagined to be collapsed into singlets, the typical 4-line pattern is found, as expected for the two chemically non-equivalent olefinic CH bonds.[11]

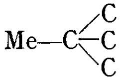
The analysis of the pattern is illustrated diagrammatically in Fig. *b*; for the acetyl derivative the chemical shift and coupling constants for these three hydrogen atoms have the values $\sigma_2 = 0{\cdot}025$, $\sigma_3 = -0{\cdot}5$, $\sigma_4 = -1{\cdot}05$, $J_{23} \sim 3{\cdot}8$ c./sec. (observed separations 3·9, 4·1, and ~3·3 c./sec.), $J_{34} = 9{\cdot}3$ c./sec. (observed separations 9·3, 9·2, 9·4 c./sec.), and $J_{24} \sim 0$. These values are all consistent with data obtained from olefinic systems of analogous structure.[11] Although the number of hydrogen atoms contributing to this general region of the spectrum could be the same for structure (III) of ring A, the lone C=CH group on this ring would then be expected to give a broad resonance with many fine-structure components of the type found at $-0{\cdot}36$ in Fig. *e*. The absence of this feature weighs heavily against the assignment of structure (III) to ring A of gibberellic acid itself (cf. ref. 1).

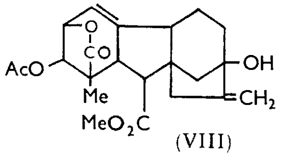
(3) *Other Resonances.*—In the spectrum of methyl acetylgibberellate there is a symmetrical quartet of lines between $\sigma = 1{\cdot}75$ and 3 which corresponds to an isolated pair of interacting (and therefore chemically adjacent) CH bonds with similar chemical shift ($\sigma \sim 2{\cdot}0$ and $2{\cdot}6$, $J = 11{\cdot}0$ c./sec.) (observed separations 10·8, 11·2 c./sec.). The only reasonable assignments for these two pairs of lines are that they correspond either to CH bonds at positions 10 and 10*a* on ring B, or to those of the 8-methylene group attached to ring D. The latter hydrogen nuclei are chemically non-equivalent because of the non-planarity of ring D. The saturated nature of the surroundings of the other isolated CH_2 group, at position 11, common to rings C and D would cause its resonances to be at higher chemical shifts.

The fact that this quartet of lines persists in the spectra of methyl acetyldihydro- and acetyltetrahydro-gibberellate (in Fig. *d* the fourth component of the quartet is overlapped by another band) shows that it cannot be assigned to the methylene group of ring D, for reduction of the $\mathrm{C{:}CH_2}$ group should then have caused marked changes in the position and fine-structure of the resonances. Further, the band at $\sigma = 3{\cdot}1$ in the spectrum of Fig. *a* which has disappeared in Fig. *c* can most probably be assigned to the 9-methylene group of ring D.

It being established that this quartet of lines is probably associated with the CH–CH group of ring B, the magnitude of 11·0 c./sec. for the coupling constant between the 10- and the 10a-hydrogen atom becomes of interest. From analyses of the spectra of other ring compounds[13] it has been shown that the size of this type of coupling constant is very sensitive to the mutual orientation of the adjacent C–H bonds, and hence to the relative orientations of the other substituents on the two carbon atoms. In six-membered rings axial-equatorial and equatorial-equatorial (*i.e., gauche*) pairs of C–H bonds usually have $J < 3{\cdot}5$ c./sec., whereas axial-axial (*i.e., trans*) pairs have $J \sim 5$—10 c./sec. One possibility is, therefore, that the two C–H bonds are coplanar and *trans* with respect to each other. However, Karplus[14] has shown by theoretical calculation that a *cis*-configuration of adjacent C–H bonds is also expected to give rise to a large coupling constant. All that can be concluded is that the two C–H bonds are probably nearly coplanar. However, this restriction carries with it implications for the relative orientations of the ester group on ring B, and the adjacent C–C bond of ring A.

A final point concerns the chemical shifts of the two hydrogen atoms giving rise to this quartet. Whereas one of these is adjacent to the ester-carbonyl group and its resonance is hence expected to be in this region, the other C–H bond is immediately attached only to saturated carbon atoms and would normally be expected to give rise to a resonance above $\sigma = 3$. However, no other spectroscopic assignment appears reasonable, and this C–H bond has a rather strained and unusual location.

(4) *Spectrum of the Acetyl Derivative* (VIII).—The structure of the acetyl derivative (VIII) *vis-à-vis* methyl acetylgibberellate seems to be firmly established on chemical grounds, and to be identical with the latter except that ring A has the alternative structure (III). In agreement with this formulation there is strong resonance at 4·2, consistently with the presence of the  $\mathrm{Me{-}C}\!\begin{array}{l}\diagup\mathrm{C}\\ {-}\mathrm{C}\\ \diagdown\mathrm{C}\end{array}$ group of ring A, and broad resonance with much semiresolved substructure at —0·35, of the type expected for the lone olefinic C–H bond on the same ring. The resonances of such CH groups are split into multiplets, not only by immediately adjacent CH bonds, but also by long-range interaction with C–H bonds in allylic positions at the other end of the C=C linkage.[11] It is possible that the complex —0·35 band comprises also the resonance of the CH–[O] group on ring A adjacent to the olefinic CH.



(VIII)

If the structure (VIII) for this acetyl derivative is correct there are, however, differences of a considerable degree between its spectrum (*e*) and that of methyl acetylgibberellate (*b*). Thus the strong doublet band caused primarily by the saturated $\mathrm{CH_2}$ groups of ring C at $\sigma = 3{\cdot}45$ in spectrum (*b*) has split into several separate bands (at 3·4, 3·6, and 3·9) in spectrum (*e*). In addition the typical pair of broad bands near +0·1 and +0·4 caused by the $\mathrm{C{=}CH_2}$ group in spectrum (*b*) is not readily discernible in spectrum (*e*) although there is a broad resonance near $\sigma = +0{\cdot}25$ that is overlapped by bands caused by CH–[O] groups which somewhat obscure this region of the spectrum. Finally, the quartet of lines in spectrum (*b*) between $\sigma = 1{\cdot}75$ and 3 is considerably modified in spectrum (*e*).

To some extent the latter result is expected on structural grounds for in (VIII), with structure (III) for ring A, the C–H bond at position 10a has become allylic. Long-range

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[13] Pople, Schneider, and Bernstein, *op. cit.*, Chapter 14.

[14] Karplus, *J. Chem. Phys.*, 1959, **30**, 11.

coupling with the olefinic CH on ring A then causes its resonance, centred near 2·1, to consist of a quartet rather than a doublet of lines. The CH group at position 10 probably gives rise to the sharp doublet centred near $\sigma = 2{\cdot}8$ which is superimposed on another broad band, possibly caused by the CH group at position 4*b*. If the above assignment of the CH resonances for positions 10 and 10a is correct it is found that the coupling constant between them has been reduced from 11·0 c./sec. in methyl acetylgibberellate to 6·3 c./sec. in the isomer (observed separations 6·2 and 6·4 c./sec.). This is probably partly caused by a change in the angle of twist about the intervening C–C bond of ring B. Indeed, the other differences between spectra (*b*) and (*e*) seem only to be accountable if either the removal of the lactone ring from position 4a, or the movement of the double bond in ring A to become adjacent to ring B, has caused a considerable redistribution of steric strain throughout the molecule.

Experimental.—Methyl benzoylgibberellate (By Mr. J. F. GROVE). Methyl gibberellate (160 mg.) in pyridine (1·5 ml.) was treated with benzoyl chloride (0·05 ml., *ca.* 1·5 mol.) during 23 hr. at room temperature in the dark. After removal of volatile constituents at 25°/1 mm., the gummy residue was washed in ether with 2N-hydrochloric acid, sodium hydrogen carbonate solution, and water. Recovery gave *methyl benzoylgibberellate,* needles (51 mg.), m. p. 164—165° [from ether–light petroleum (b. p. 40—60°)] (Found: C, 70·1; H, 6·25. $C_{27}H_{28}O_7$ requires C, 69·8; H, 6·1%), $\nu_{\max.}$ (Nujol mull) 3500, 3420 (OH), 1784, 1741, 1729, 1716 cm.$^{-1}$ (C=O).

Spectra. The nuclear magnetic resonance spectra were obtained with a Varian Associates V-4300 B spectrometer operating at 40 Mc. with sample spinning and flux stabilisation. The quoted separations are normally correct to ±0·3 cycle/sec., and the σ values to ±0·05 in units of 10^{-6} (*i.e.*, ±2 c./sec.).

The author is indebted to Dr. B. E. Cross and Mr. J. F. Grove (Akers Research Laboratory, Imperial Chemical Industries Limited), who suggested this application of nuclear magnetic resonance, provided the samples, and gave much help in discussions. He is also grateful to Mr. E. Liddell for obtaining most of the spectra. The nuclear magnetic resonance spectrometer was purchased with a grant from the Wellcome Foundation.

UNIVERSITY CHEMICAL LABORATORY,
LENSFIELD ROAD, CAMBRIDGE.

[*Received, January 8th,* 1960.