The ^{13}C Nuclear Magnetic Resonance Spectra of Some Gibberellins

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The ^{13}C n.m.r. spectra of some C_{19} gibberellins have been assigned and the labelling pattern of gibberellic acid from [2-^{13}C]mevalonic acid has been confirmed.

PROTON n.m.r. spectroscopy has played an important role in the determination of the structure of the gibberellin plant hormones.[1,2] These diterpenoids possess a common carbon skeleton (1) but differ in their sites and levels of oxidation. ^{13}C N.m.r. spectroscopy has a clear part to play in locating these sites.[3] We record here our assignments for the C_{19} gibberellins. The spectra were determined at 25.15 MHz on a Fourier transform instrument operating in both proton noise-decoupled and off-resonance decoupled modes. The results are tabulated for a group of gibberellins and appropriate derivatives which were selected to facilitate the analysis. The resonances were assigned by using the simplest of the C_{19} gibberellins, gibberellin A_9 (1) [4] as a basis.

The olefinic resonances at 107 (triplet, C-17) and 156 p.p.m. (singlet, C-16) were readily recognized. The presence of an adjacent acetoxy-group at C-13 has a significant (3 p.p.m.) shielding effect on C-16 although a hydroxy-group has little effect. The ring A methyl group (C-18) was also easily recognized since it appears

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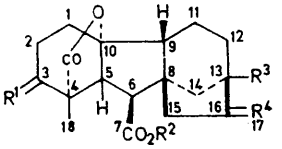
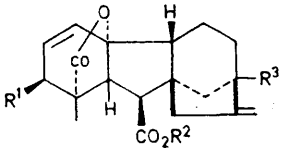
as a high-field quartet in the off-resonance spectrum. It is markedly shielded by an adjacent C-3 carbonyl group [*cf.* (1) and (5)] and to a lesser extent by an adjacent hydroxy-group [*cf.* (3)].



(1) $R^1 = H_2$, $R^2 = R^3 = H$, $R^4 = CH_2$
(2) $R^1 = H_2$, $R^3 = H$, $R^2 = Me$, $R^4 = O$
(3) $R^1 = H,\beta\text{-OH}$, $R^2 = Me$, $R^3 = H$, $R^4 = CH_2$
(4) $R^1 = H,\beta\text{-OH}$, $R^2 = Me$, $R^3 = H$, $R^4 = O$
(5) $R^1 = R^4 = O$, $R^2 = Me$, $R^3 = H$
(10) $R^1 = H,\beta\text{-OAc}$, $R^2 = Me$, $R^3 = OAc$, $R^4 = CH_2$



(6) $R^1 = OH$, $R^2 = R^3 = H$
(7) $R^1 = OH$, $R^2 = Me$, $R^3 = H$
(8) $R^1 = OAc$, $R^2 = Me$, $R^3 = OH$
(9) $R^1 = R^3 = OAc$, $R^2 = Me$
(11) $R^1 = R^3 = OH$, $R^2 = Me$

The remaining resonances were grouped in terms of their structure in the off-resonance spectra. The resonances associated with C-1, C-2, C-3, C-11, C-12, C-14, and C-15 in gibberellin A_9 (1) appeared as triplets. Two of these, at 30.7 and 19.4 p.p.m., were assigned to C-1 and C-2 respectively, since these signals were absent from those compounds which possessed a 1,2-double bond [(6)—(9)]. Furthermore in di-*O*-acetyl[1,2-$^2H_2$]gibberellin A_1 methyl ester [as (10)], prepared by selective catalytic deuteriation of methyl gibberellate,[5] resonances at 25.5 and 27.7 p.p.m. had collapsed. The resonance at 19.4 p.p.m. was assigned to C-2 since it shifts on hydroxylation [*cf.* (3)], and oxidation of C-3 to a carbonyl group [*cf.* (5)], whilst the resonance associated with C-1 shows, in gibberellin A_4 methyl ester (3), the characteristic shielding of a carbon atom β to an axial hydroxy-group.[6,7] The resonance at 34.3 p.p.m. was assigned to C-3 since it shifts to 70.2 p.p.m. when a hydroxy-group is introduced at this centre [*cf.* (3)]. The high-field triplet at 16.1 p.p.m. was assigned to C-11 by analogy with the steroids [7] and the kaurenolides.[8] In the gibberellins C-11 is shielded by 1,3-diaxial interaction with the ring D bridge. The triplet at 31.4 p.p.m. was assigned to C-12. This resonance moves upfield to 25.0 p.p.m. on the introduction of a carbonyl group at C-16 [*cf.* (4) and (5)]. A comparable effect has been noted in the steroid series from a C-17 carbonyl group (see ref. 7; *cf.* also ref. 8). This resonance moves to lower field on introduction of a hydroxy-group at C-13 [*cf.* (8)]. The resonance at 44 p.p.m. was assigned to C-15 since it shifts to 50.4 p.p.m. on introduction of a carbonyl group at C-16 (*cf.* (2) and (4)]. This signal disappeared when both the oxo-esters (2) and (4) were treated with sodium methoxide in methan[^{2}H]ol. The signal at 36.8 p.p.m. was then assigned to C-14. It moved upfield on introduction of a carbonyl group at C-16 and downfield when a hydroxy-group was introduced at C-13.

The resonances associated with C-5, C-6, C-9, and C-13 appeared as doublets. That at 38.8 p.p.m. was assigned to C-13 since it was replaced by a singlet at 78.0 p.p.m. in the 13-hydroxygibberellins (8)—(11). The difficult distinction amongst C-5, C-6, and C-9 was made on the basis of deuteriation studies. Base-catalysed deuteriation of the oxo-esters (2) and (4) led to exchange at both C-15 and C-6 (disappearance of the δ 2.73 proton

TABLE 1

^{13}C N.m.r. data of gibberellins (p.p.m. from Me_4Si)

Compd.	Carbon atom 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	OCH_3
(1) * *a*	30.7	19.4	34.3	49.1	58.0	51.8	178.0	51.3	53.9	93.6	16.1	31.4	38.8	36.8	44.3	156.5	107.5	17.2	179.6	
b	30.9	19.8	34.6	49.2	58.2	52.9	175.0	51.3	54.1	93.3	16.4	31.7	39.3	37.1	44.9	157.7	107.3	17.8	179.2	
(2) * *a*	30.4	19.2	34.2	49.0	57.8	52.9	172.5	49.7	54.1	92.9	16.7	24.9	44.7	34.5	50.4	220.1		17.1	178.8	52.1
(3) † *a*	27.4	28.1	70.2	54.6	51.6	51.1	173.4	51.6	53.7	94.2	16.1	31.5	38.9	36.8	44.5	156.8	107.5	14.7	178.6	51.9
b	28.0	29.2	69.7	55.3	51.8	51.8	173.5	51.8	53.8	94.0	16.4	31.7	39.2	37.1	44.8	157.3	107.5	15.5	178.8	52.1
(4) † *a*	27.1	28.1	69.9	54.7	51.1	52.7	172.6	49.8	53.9	93.9	16.8	25.0	44.8	34.6	50.5	220.6		14.6	178.6	52.2
(5) *a*	30.6	34.9	199.8	63.1	56.7	52.8	173.6	51.3	53.4	92.5	16.9	24.9	44.5	34.6	50.2	219.3		10.3	171.5	52.5
(6) * *a*	132.1	133.2	69.9	53.4	52.2	50.5	175.8	52.2	51.7	90.9	16.0	31.4	38.6	36.5	44.4	156.4	107.8	14.6	178.5	
b	132.4	134.2	69.9	54.4	52.9	52.0	175.0	52.4	52.1	91.5	16.3	31.8	39.1	36.9	45.0	157.7	107.4	15.6	179.8	
(7) * *a*	132.4	132.9	69.8	53.5	52.2	50.8	173.1	52.4	51.6	91.1	15.9	31.4	38.6	36.4	44.5	156.5	107.7	14.4	178.9	52.2
(8) ‡ *a*	129.1	134.2	70.2	52.1	53.4	50.5	172.3	50.5	50.9	90.2	16.9	38.3	78.0	43.1	44.7	156.7	107.6	14.3	177.0	52.0 (i)
b	129.1	135.1	70.6	52.5	53.6	50.8	172.5	50.5	51.2	90.5	17.4	39.7	77.5	43.5	44.9	158.6	107.2	14.6	177.3	51.8 (ii)
(9) § *a*	129.2	134.2	70.3	52.2	53.4	50.4	172.3	51.0	51.0	90.0	16.9	36.4	84.1	39.8	42.7	153.4	108.2	14.3	176.9	52.2 (iii)
(10) ¶ *a*	25.5	27.7	71.3	53.1	53.0	50.2	172.5	51.0	52.5	92.8	17.0	36.3	84.3	40.2	42.7	153.4	107.9	14.5	176.3	52.1 (iv)

[a] In $CDCl_3$; *b*, in C_5D_5N. (i) Acetate 20.7 and 169.9. (ii) Acetate 170.0 and 20.5. (iii) Acetates, 169.2(2), 20.7, and 22.0. (iv) Acetates 169.7, 170.1 21.1, and 22.0.

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resonance). The signals at 52.9 and 52.7 p.p.m. in the ^{13}C spectrum then collapsed. On insertion of a C-3 hydroxy-group [*cf.* (1) and (3)], the resonance which was assigned to C-5 showed a significant upfield shift (*ca.* 6 p.p.m.) while the resonance assigned to C-9 remained constant. The distinction between these resonances in the spectra of gibberellin A_7 methyl ester (7) and methyl mono-*O*-acetylgibberellate (8) was made on the basis of a series of selective proton ' tickling ' experiments. When methyl mono-*O*-acetylgibberellate (8) was irradiated at δ 2.16, the resonance at 50.9 p.p.m. sharpened. Irradiation at δ 2.76, which is the centre of the upper AB doublet (H-6) led to sharpening of the resonance at 50.5 p.p.m., irradiation at δ 3.31 (the centre of the lower AB doublet, H-5) sharpened the resonance at 53.4 p.p.m., and irradiation at δ 3.73 (OMe) sharpened the resonance at 52.0 p.p.m. Similar experiments were carried out with gibberellin A_7 methyl ester (7); thus irradiation at δ 2.35 sharpened the signals at 51.6 (C-9) and 38.6 p.p.m. (C-13), at δ 2.79 sharpened that at 50.8 (C-6), at δ 3.23 sharpened that at 52.2 (C-5), at δ 3.72 sharpened that at 52.2 (OMe), and at δ 4.35 sharpened that at 69.8 p.p.m. (C-3). The lower field olefinic doublet resonances in compounds (6)—(9) were assigned to C-2 because of the effects observed as a result of acetylation at C-3 and on changing the solvent to pyridine.

The resonances associated with C-4, C-8, and C-10 were singlets and were identified in the following manner. That at 93.6 p.p.m. was assigned to C-10 which bears the lactonic oxygen. It showed an upfield shift (*ca.* 3 p.p.m.) on introduction of the 1,2-double bond [*cf.* (3) and (6)]. The position of the C-4 resonance reflected the changes in ring A [*e.g.* the insertion of an adjacent 3-hydroxy- or 3-oxo-group; *cf.* (4) and (5)] while the resonance assigned to C-8 remained relatively constant, only reflecting changes at C-16 [*cf.* (1) and (2)]. The lactone (C-19) and carboxylic (C-7) carbon resonances were distinguished by the effect of methylation on the latter [*cf.* (6) and (7)]. In compound (5) the lactone carbonyl carbon resonance showed a marked upfield shift as it lies within the shielding cone of the C-3 carbonyl group.

A number of the gibberellins are poorly soluble in deuteriochloroform. Deuteriopyridine is a better solvent and hence for comparison purposes a number of spectra were also determined in this solvent. Furthermore selective solvent shifts in the proton n.m.r. spectra have provided useful structural information.[2] Compounds (1), (3), (6), and (8) were selected with the effect of hydroxy-groups in mind. However in this instance solvent shifts appeared to be less useful. Although in general carbon atoms adjacent to hydroxylic functions (*e.g.* C-12 in the presence and absence of a 13-hydroxy-group) showed the larger solvent shifts, this effect was by no means universal. On the other hand in gibberellin A_7, which lacks a 13-hydroxy-function, the adjacent olefinic C-16 resonance showed a marked shift.

Gibberellic acid is a diterpenoid and as such incorporates four molecules of mevalonic acid. In the original study with [2-^{14}C]mevalonic acid [9] the sites of labelling were unequivocally established as C-7 and C-18; assignment of the remaining sites (C-1 and C-12) was based on analogy. The tacit assumption has been made in subsequent studies that this carbon-labelling pattern is correct. With the assignment of the ^{13}C spectra complete, a simple method for confirming this labelling pattern was available. The optimum time and dilution for feeding the mevalonic acid to *Gibberella fujikuroi* was established by using [2-^{14}C]mevalonic acid. [2-^{13}C]Mevalonic acid (90% enrichment) containing [2-^{14}C]mevalonic acid (25 μCi) was fed to a three-day-old shake culture of *Gibberella fujikuroi*. The gibberellic acid was isolated after a further 9 days and purified as its methyl ester (11). It showed a ^{14}C incorporation of 3.7%. The resonances at 15.3 (C-18), 39.7 (C-12), 132.0 (C-1), and 172.8 p.p.m. (C-7) clearly showed enrichment at these positions (see Table 2), confirming the accepted labelling pattern. The peak heights were normalized with respect to that of C-19, which has been shown not to be labelled by [2-^{14}C]-mevalonic acid.[9]

TABLE 2

Incorporation of [2-^{13}C]mevalonic acid into methyl gibberellate (11).

		Peak height [a]	
δ_c (C_5D_5N)	Assignment	Unenriched sample	Enriched sample
15.3	C-18	1.3	3.3
17.4	C-11	1.0	1.3
39.7	C-12	1.4	4.4
43.7	C-14	1.2	1.2
45.1	C-15	1.3	1.3
50.7	C-8	1.8	1.9
51.3	C-9	2.4	2.0
51.5	OCH_3	2.4	2.1
51.7	C-6	1.4	1.6
53.2	C-5	1.9	2.1
54.2	C-4	1.3	1.7
69.8	C-3	2.2	2.4
77.6	C-13	1.8	2.2
90.8	C-10	2.0	1.8
107.0	C-17	1.7	1.5
132.0	C-1	2.0	6.5
134.3	C-2	2.4	2.2
158.7	C-16	1.9	1.9
172.8	C-7	1.5	6.1
179.3	C-19	1.0	1.0

[a] Normalized with respect to C-19 signal.

EXPERIMENTAL

General experimental details have been described previously.[8]

ent-10β-*Hydroxy*-3,16-*dioxo*-17,20-*dinorgibberella*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (5).—*ent*-3α,10β-Dihydroxy-16-oxo-17,20-dinorgibberella-7,19-dioic acid 19,-10-lactone 7-methyl ester [10] (4) (300 mg) in acetone (10 ml) was treated with 8N-chromium trioxide reagent (0.3 ml) at room temperature for 2 h. The solution was then treated with methanol and diluted with water. The product was filtered off, washed with water, passed through a short column of alumina in ethyl acetate, and crystallized from aqueous

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methanol to give the *diketone* (5) which crystallized as plates, m.p. 203—204° (Found: C, 65.6; H, 6.2. $C_{19}H_{22}O_6$ requires C, 65.9; H, 6.4%), $\nu_{max.}$ 1785, 1745, and 1710 cm^{-1}, τ 8.80 (3H, s, 18-H), 7.15 (1H, d, J 10 Hz, 6-H), 6.90 (1H, d, J 10 Hz, 5-H), and 6.25 (3H, s, OCH_3).

Deuteriation of the Oxo-esters.—Sodium (100 mg) was dissolved in methan[^{2}H]ol (5 ml). The oxo-ester (2) (130 mg) was added in a small amount of ether and the solution was left at room temperature overnight, acidified with deuteriochloric acid, and diluted with water. The product was recovered in ether; had m.p. 196—198° (lit.,[4] 204—207°), M^+ 335 ($C_{19}H_{21}{}^2H_3O_5$). The oxo-ester (4) was treated similarly.

Incubation of [2-^{13}C,2-^{14}C]*Mevalonic Acid with* Gibberella fujikuroi.—[2-^{13}C]Mevalonic acid [11] (70 mg) in benzene was mixed with a solution in benzene of [2-^{14}C]mevalonic acid (25 μCi) and the solvent was evaporated off under a stream of nitrogen. The residue was taken up in ethanol (3 ml) and distributed between three three-day-old 250 ml cultures of *Gibberella fujikuroi* on shake culture. The metabolites were recovered after a further nine days. The extract (370 mg) was purified by preparative layer chromatography on silica in chloroform–ethyl acetate–acetic acid (5 : 5 : 1). The gibberellic acid was then methylated with diazomethane and further purified by preparative layer chromatography on silica in ethyl acetate. The methyl gibberellate (45 mg; 2 030 892 disint. min^{-1}; 3.7% incorporation) was dissolved in [$^2H_5$]pyridine (0.5 ml) and the ^{13}C n.m.r. spectrum was recorded (see Table 2; 88 056 scans).

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