Application of ^{13}C Nuclear Magnetic Resonance to the Study of Gibberellins

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The ^{13}C n.m.r. spectra of a series of gibberellins have been measured and analysed almost completely. The spectrum of ^{13}C-enriched gibberellins (mixture of A_1 and A_3) produced by *Gibberella fujikuroi* fed with sodium [1-^{13}C]acetate is also reported.

RECENT advances in instruments and techniques have made it possible to apply ^{13}C n.m.r. to the study of naturally occurring substances. In order to establish a method both for structural elucidation and for biosynthetic studies, we have measured and analysed the ^{13}C n.m.r. spectra of a series of gibberellins (1)—(19). Assignments are shown in Tables 1 and 2.

Assignments were made by analysing the proton noise-decoupled and off-resonance decoupled spectra. Comparisons amongst gibberellin homologues with

TABLE 1

Assignment of ^{13}C n.m.r. spectra of C_{19} gibberellins*

Carbon	A_1	A_3	A_4	A_5	A_7	A_9	A_{16}	A_{30} †	A_{35}
1	28·1 (t)	132·3 (d)	28·2 (t)	35·6 (t)	132·3 (d)	30·9 (t)	71·8 (d)	132·4	29·4 (t) [a]
2	29·2 (t)	134·3 (d)	29·2 (t)	128·1 (d) [a]	134·1 (d)	19·8 (t)	39·7 (t)	134·3	29·9 (t) [a]
3	70·0 (d)	70·0 (d)	70·0 (d)	133·1 (d) [a]	70·0 (d)	34·6 (t)	70·4 (d)	70·0	69·8 (d)
4	55·5 (s)	54·5 (s)	55·5 (s)	48·5 (s)	54·4 (s)	49·1 (s)	54·8 (s)	54·7	55·6 (s)
5	52·5 (d) [a]	51·6 (d)	51·9 (d)	56·1 (d)	52·1 (d)	58·2 (d)	51·1 (d)	52·0	52·2 (d)
6	52·7 (d) [a]	52·1 (d)	52·8 (d)	52·2 (d)	52·4 (d)	52·8 (d)	53·0 (d)	53·4	52·9 (d)
7	175·2 (s)	174·8 (s)	175·2 (s)	N.o. ‡	174·8 (s)	174·8 (s)	174·9 (s)	174·9	175·2 (s)
8	49·8 (s)	50·6 (s)	51·5 (s)	50·7 (s)	52·1 (s)	51·3 (s)	52·1 (s)	52·9	51·9 (s)
9	53·5 (d)	53·5 (d)	54·0 (d)	53·5 (d)	52·9 (d)	54·1 (d)	54·0 (d)	50·8	61·0 (d)
10	93·9 (s)	91·1 (s)	94·1 (s)	91·9 (s)	91·4 (s)	93·0 (s)	96·2 (s)	91·3	94·6 (s)
11	18·0 (t)	17·6 (t)	16·5 (t)	18·0 (t)	16·3 (t)	16·4 (t)	18·7 (t)	27·8	64·0 (d)
12	39·9 (t)	39·9 (t)	31·8 (t)	39·8 (t)	31·8 (t)	31·7 (t)	32·3 (t)	75·4	43·0 (t)
13	77·9 (s)	77·7 (s)	39·4 (d)	77·8 (s)	39·2 (d)	39·3 (d)	39·1 (d)	49·0	39·4 (d)
14	46·3 (t)	45·6 (t)	37·4 (t)	46·6 (t)	36·9 (t)	37·2 (t)	37·0 (t)	34·7	37·4 (t)
15	44·0 (t)	44·0 (t)	45·0 (t)	43·7 (t)	45·0 (t)	44·8 (t)	45·2 (t)	45·6	45·6 (t)
16	159·2 (s)	159·1 (s)	157·7 (s)	158·7 (s)	157·7 (s)	157·6 (s)	158·2 (s)	153·4	158·0 (s)
17	106·5 (t)	106·7 (t)	107·2 (t)	106·4 (t)	107·4 (t)	107·1 (t)	107·4 (t)	109·3	107·4 (t)
18	15·6 (q)	15·5 (q)	15·5 (q)	15·8 (q)	15·5 (q)	17·7 (q)	14·9 (q)	15·7	15·8 (q)
19	179·0 (s)	179·6 (s)	179·0 (s)	N.o.‡	179·6 (s)	178·8 (s)	179·3 (s)	N.o.‡	179·5 (s)

* Shifts in p.p.m. downfield from internal Me_4Si; multiplicities given are those in the off-resonance proton-decoupled spectrum.
† Off-resonance proton-decoupled spectrum not measured. ‡ Not observed owing to the short pulse interval.
[a] Shifts thus indicated in any vertical column may be reversed.

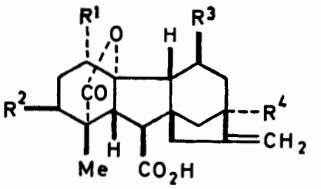
TABLE 2

Assignment of ^{13}C n.m.r. spectra of C_{20} gibberellins *

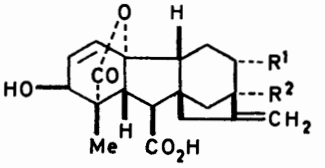
Carbon	A_{13}	A_{14}	A_{17} †	A_{18} †	A_{23} †	A_{28} †	A_{36}	A_{37}	A_{38} †	A_{39} †
1	30·8 (t)	28·4 (t)	37·7	28·5	27·6	31·0	27·5 (t)	29·7 (t)	29·7	31·3
2	32·2 (t)	34·6 (t)	22·6	34·6	30·2	31·9	30·1 (t)	33·6 (t)	33·7	32·2 [a]
3	71·2 (d)	71·0 (d)	39·0	70·9	72·9	71·1	73·0 (d)	73·5 (d)	73·5	71·6
4	50·6 (s)	49·5 (s) [a]	46·4	49·6	49·7	51·3	50·2 (s)	49·0 (s)	49·1	51·2 [b]
5	50·4 (d)	50·2 (d)	56·5	51·0	48·2	50·7	47·7 (d)	46·8 (d)	45·8	50·6 [c]
6	52·1 (d)	51·9 (d)	52·2	51·9	51·8	52·1	51·7 (d)	52·7 (d)	52·7	52·2
7	177·1 (s)	177·9 (s) [b]	177·0 [a]	177·9 [a]	176·4 [a]	177·5 [a]	176·4 (s) [a]	174·9 (s) [a]	174·9 [a]	177·2 [d]
8	50·6 (s)	50·0 (s) [a]	48·7	48·6	52·8	48·9	50·2 (s)	49·9 (s)	47·8	51·0 [c]
9	57·0 (d)	57·6 (d)	56·9	57·2	56·7	56·6	56·7 (d)	56·1 (d)	55·9	54·1
10	57·6 (s)	44·6 (s)	57·7	44·7	48·4	57·3	49·5 (s)	41·9 (s)	41·6	58·4
11	19·4 (t)	17·3 (t)	20·4	18·9	19·7	20·6	18·6 (t)	16·1 (t)	17·2	31·8 [a]
12	32·2 (t)	32·6 (t)	40·1	40·8	40·2	40·3	32·2 (t)	31·9 (t)	40·0	75·7
13	40·2 (d)	40·6 (d)	78·3	78·3	78·2	78·4	39·9 (d)	39·9 (d)	78·3	51·9 [b]
14	36·9 (t)	39·8 (t)	46·0	49·1	45·9	46·4	37·2 (t)	36·7 (t)	45·8	33·4
15	47·3 (t)	47·1 (t)	45·7	46·2	45·9	46·4	46·9 (t)	45·4 (t)	45·1	48·4
16	157·9 (s)	157·3 (s)	158·9	158·7	159·1	159·3	157·6 (s)	157·9 (s)	159·6	154·7
17	105·9 (t)	105·7 (t)	105·1	105·3	105·4	105·0	106·2 (t)	106·4 (t)	105·4	107·7
18	24·8 (q)	25·4 (q)	29·9	25·1	22·6	24·9	22·6 (q)	21·1 (q)	21·2	25·3
19	178·0 (s) [a]	180·6 (s) [b]	177·9 [a]	180·7 [a]	177·0 [a]	177·9	N.o.‡ [a]	175·8 (s) [a]	175·6 [a]	178·0 [d]
20	178·5 (s) [a]	15·5 (q)	178·4 [a]	15·3	N.o.‡	178·6 [a]	N.o.‡	74·4 (t)	74·4	178·7 [d]

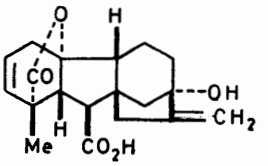
* Shifts in p.p.m. downfield from internal Me_4Si; multiplicities given are those in the off-resonance proton-decoupled spectrum.
† Off-resonance proton-decoupled spectrum not measured. ‡ Not observed owing to the short pulse interval.
[a–d] Shifts thus indicated in any vertical column may be reversed.

various substitution patterns and considerations of relative peak heights, which reflect nuclear Overhauser effects and relaxation times, provided useful tools for analyses. If necessary the spectra of deuteriated and ^{13}C-enriched compounds were also studied.



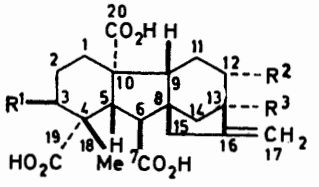
(1) A_1 $R^1=R^3=H$, $R^2=R^4=OH$
(2) A_4 $R^1=R^3=R^4=H$, $R^2=OH$
(3) A_9 $R^1=R^2=R^3=R^4=H$
(4) A_{16} $R^1=R^2=OH$, $R^3=R^4=H$
(5) A_{35} $R^1=R^4=H$, $R^2=R^3=OH$

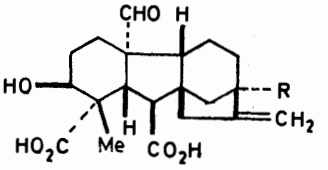




(6) A_3 $R^1=H$, $R^2=OH$
(7) A_7 $R^1=R^2=H$
(8) A_{30} $R^1=OH$, $R^2=H$

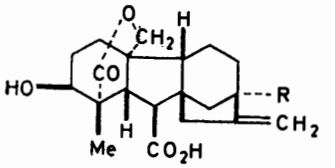
(9) A_5

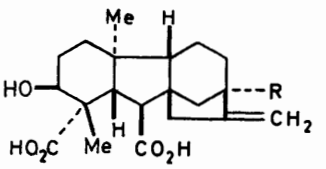




(10) A_{13} $R^1=OH$, $R^2=R^3=H$
(11) A_{17} $R^1=R^2=H$, $R^3=OH$
(12) A_{28} $R^1=R^3=OH$, $R^2=H$
(13) A_{39} $R^1=R^2=OH$, $R^3=H$

(14) A_{23} R = OH
(15) A_{36} R = H





(16) A_{37} R = H
(17) A_{38} R = OH

(18) A_{14} R = H
(19) A_{18} R = OH

For example, the spectrum of gibberellin A_4 was analysed as follows. The signals due to carbons carrying no hydrogen are distinguishable from other signals, which appear as singlets in the proton off-resonance decoupled spectrum. On the basis of the chemical shifts, the singlets at 94·1, 157·7, 175·2, and 179·0 p.p.m. were assigned to C-10, C-16, C-7, and C-19, respectively. Since C-4 is linked to a carbinol and a carbonyl system (C-3 and C-19, respectively), it should be more deshielded than C-8. Thus, the singlet at 55·5 p.p.m. could be assigned to C-4 and that at 51·5 p.p.m. to C-8. The quartet at 15·5 p.p.m. is characteristic of C-18, and the triplet at 107·2 p.p.m. is assignable to C-17 on the basis of its low chemical shift.

A comparison of the spectra of gibberellins A_1 (1), A_4, A_9 (3), and A_{35} (5), which have different hydroxy-substitution patterns, indicated that the doublets at 39·4 and 70·0 p.p.m. in the spectrum of gibberellin A_4 should be due to C-13 and C-3, respectively, and also that the triplet at 16·5 p.p.m. is due to C-11. Since the 11-hydroxy-group of gibberellin A_{35} is expected to cause a downfield shift of the C-9 and C-12 signals but to have little effect on C-14 and C-15,[1] the triplet at 31·8 p.p.m. in the spectrum of gibberellin A_4 could be assigned to C-12; the corresponding signal is shifted to 43·0 p.p.m. in the spectrum of gibberellin A_{35}. The triplet at 37·4 p.p.m. in the spectrum of gibberellin A_4 could be assigned to C-14 and that at 45·0 p.p.m. to C-15, because the former is shifted to 44·0 p.p.m. in the spectrum of gibberellin A_1 (deshielded by the 13-OH) and the position of the latter is hardly affected. The doublet at 52·8 p.p.m. was assigned to C-6; its position is not expected to be affected by functionalities in ring A and/or rings C and D. Two remaining doublets at 51·9 and 54·0 p.p.m. were assigned to C-5 and C-9, respectively, since the former should resonate further downfield in the spectrum of gibberellin A_9 as a result of the removal of the 3-OH in gibberellin A_4, but the latter should not.

Two triplets at 28·2 and 29·2 p.p.m. are not present in the spectrum of gibberellin A_7 (7), suggesting that these are due to C-1 and C-2. The signal at 28·2 p.p.m. was assigned to C-1, on the basis of an analysis of the spectrum of gibberellin A_1 carrying 20% deuterium on C-1. The *C*D signal is observed as satellites of the signal due to $C(1)H_2$ as a result of C,D coupling.

Since gibberellin A_1 and A_{35} possess the same ring A structure as gibberellin A_4, the signals due to the carbons in ring A of gibberellins A_1 and A_{35} were easily assigned by comparison of the spectra. Introduction of the 13-OH into the gibberellin A_4 structure, which affords gibberellin A_1, was expected to cause a large downfield shift of the C-12 and C-14 signals (*ca.* 10 p.p.m.) as well as of the C-13 signal (*ca.* 40—45 p.p.m.).[1] Thus the signals at 39·9, 77·9, and 46·3 p.p.m. were assigned to C-12, C-13, and C-14, respectively, and other signals were assigned as shown in Table 1. Assignments for gibberellin A_{35} were made similarly (Table 1).

The spectrum of gibberellin A_{16} (4), which possesses the same BCD ring structure as gibberellin A_4, showed

[1] J. D. Roberts, F. J. Weigert, J. I. Krochwitz, and H. J. Reich, *J. Amer. Chem. Soc.*, 1970, **92**, 1338.

a signal pattern very similar to that of gibberellin A_4 except for the signals due to C-1 and C-2, at 71·8 and 39·7 p.p.m.

As expected, the spectrum of gibberellin A_9 (3) showed a signal pattern similar to that of gibberellin A_4 with respect to the carbons in rings C and D. Since removal of the axial hydroxy-group on C-3 in gibberellin A_4 was expected to cause an upfield shift of the C-2 and C-4 signals (*ca.* 5 p.p.m.) as well as of the C-3 signal (*ca.* 35—40 p.p.m.) [1] and a small downfield shift of the C-1 and C-5 signals, the resonances at 30·9, 19·8, 34·6, 49·1, and 58·2 p.p.m. in the spectrum of gibberellin A_9 were assigned to C-1, C-2, C-3, C-4, and C-5, respectively.

The spectrum of gibberellin A_3 (6) was analysed by comparison with that of gibberellin A_1. The assignment was confirmed by measuring the spectrum of ^{13}C-enriched gibberellin A_3 produced by *Gibberella fujikuroi* fed with sodium [1-^{13}C]acetate, which showed clear enhancement of the signals assigned to C-2, C-4, C-10, C-11, C-14, and C-16 in comparison with the spectrum of unlabelled gibberellin A_3; however, enhancement of the signals due to C-6 and C-8 was not clear owing to some contamination by gibberellin A_1. The spectrum of gibberellin A_7 (7) showed a pattern similar to that of gibberellin A_4 (2) except for the signals due to C-1 and C-2, and was easily analysed (Table 1).

The spectrum of gibberellin A_{30} (8) was analysed (Table 1) by comparison with those of gibberellins A_3 and A_7. It is noteworthy that the 12-hydroxy-group causes a larger shift of the C-16 and C-17 signals than the 13-OH; this observation is useful in confirming the presence of the 12-OH.

The spectrum of gibberellin A_5 (9) was analysed on the basis of analyses of the spectra of gibberellins A_1, A_3, and A_9.

Analyses of ^{13}C n.m.r. spectra of tricarboxygibberellins such as gibberellins A_{13} (10), A_{17} (11), A_{28} (12), and A_{39} (13), which have carboxy-groups on C-4, C-6, and C-10 but differ in the position and number of hydroxy-groups, were conducted as follows. On the basis of the chemical shifts and coupling patterns in the proton off-resonance decoupled spectrum of gibberellin A_{13}, the signals due to C-3, C-7, and C-16 to C-20 were easily assigned as shown in Table 2, although the three carbonyl signals were not distinguishable. Since gibberellin A_{13} contains the same CD ring structure as gibberellins A_4, A_7, and A_9, the carbons in rings C and D (C-11 to C-15) of gibberellin A_{13} are expected to show a signal pattern similar to those of gibberellins A_4, A_7, and A_9. In particular, the signals at 32·2 and 40·2 p.p.m. were clearly due to C-12 and C-13, which are located far from the carboxy-groups on C-4, C-6, and C-10. Use of the proton off-resonance decoupling technique supported this assignment and further indicated that the signal at 32·2 p.p.m. overlapped with another signal. The assignment of the signals due to C-11, C-14, and C-15 was confirmed by comparison of the spectra of the tricarboxygibberellins A_{13}, A_{17}, A_{28}, and A_{39}. The methylene signal at 19·4 p.p.m. in the spectrum of gibberellin A_{13} was assigned to C-11, since the signal was absent in this region only in the spectrum of gibberellin A_{39}. The methylene signal at 36·9 p.p.m. could be assigned to C-14; the corresponding signal appears upfield in the spectrum of gibberellin A_{39}, but downfield in those of gibberellins A_{17} and A_{28}. It is reasonable to assign the signal at 47·3 p.p.m. to C-15, since it appears at lowest field of the methylene signals except for that due to C-17, by analogy with the assignments of the C_{19} gibberellins. Thus, the signals due to C-11 to C-15 were all definitely assigned.

The methine signal at 57·0 p.p.m. in the spectrum of gibberellin A_{13} could be assigned to C-9, since the corresponding signal appears at higher field in the spectrum of gibberellin A_{39}, owing to the effect of the 12-OH, as in the case of gibberellin A_{30} (8). Since gibberellins A_{13}, A_{28}, and A_{39} have the same ring A structure, a comparison of their spectra, together with a consideration of the coupling pattern in the proton off-resonance decoupled spectrum of gibberellin A_{13}, gave information on the signals due to the carbons in ring A. Thus, the methylene signals at 30·8 and 32·2 p.p.m., the latter of which overlaps with the signal due to C-12, should be due to C-1 and C-2. Though it was difficult to distinguish the signals due to C-1 and C-2, the signal at 30·8 p.p.m. may be assigned to C-1 on the basis of the results with [1-^{2}H]gibberellin A_1.

The quaternary carbon signal at 57·6 p.p.m. was assigned to C-10, since the corresponding signal appears at higher field in the spectrum of gibberellin A_{36} (15), which possesses an aldehyde group at C-10 instead of a carboxy-group. The methine signal at 52·1 p.p.m. could be assigned to C-6, since this should be the least affected by changes in functionalities in rings A, C, and D, and similar chemical shifts are observed in the spectra of gibberellins A_{17}, A_{28}, A_{39}, *etc.* Thus, the remaining methine signal at 50·4 p.p.m. must be due to C-5. The intensity of the signal at 50·6 p.p.m., which appears as a prominent singlet in the proton off-resonance decoupled spectrum, suggests the presence of two quaternary carbons, namely C-4 and C-8. Thus, the spectrum of gibberellin A_{13} was analysed almost completely, as shown in Table 2.

The spectra of gibberellins A_{17}, A_{28}, and A_{39} were analysed by comparison with each other and with that of gibberellin A_{13}. The 12-hydroxy-group in gibberellin A_{39} had the same effect on the chemical shifts of C-16 and C-17 as in gibberellin A_{30}.

Gibberellin A_{36} (15) has the same structure as gibberellin A_{13} (10) except for the substitution at C-10. The change of substituent from CO_2H (A_{13}) to CHO (A_{36}) is expected to cause a large upfield shift of the C-10 signal and small shifts of the C-1, C-5, and C-9 signals. Thus, the signals at 49·5, 27·5, 47·7, and 56·7 p.p.m. were assigned to C-10, C-1, C-5, and C-9, respectively. Other signals were assigned by comparison of the spectrum with that of gibberellin A_{13}. The structural relationship between gibberellins A_{23}

(14) and A_{28} (12) is the same as that between gibberellins A_{36} and A_{13}. A similar analysis in the case of gibberellin A_{36}, together with a comparison with the spectra of gibberellins A_{23} and A_{36}, made it possible to assign all the carbons of gibberellin A_{23} as shown in Table 2. The absence of the signals of the aldehyde carbonyl group in gibberellin A_{23} and of the two carbonyl groups in gibberellin A_{36} seems to be due to the short pulse interval.

Though gibberellin A_{37} (16) differs from gibberellin A_4 (2) in carrying a δ- instead of a γ-lactone system, it was expected that C-11 to C-17, which are located far from the lactone linkage, would show a signal pattern similar to that of gibberellin A_4. Thus, their chemical shifts were assigned by comparison with each other and from the coupling patterns in the proton off-resonance decoupled spectrum of gibberellin A_{37}, as shown in Table 2. There remained the assignment of the signals due to the carbons in rings A and B. The carbonyl carbon signals at 174·9 and 175·8 p.p.m. were assigned to C-7 and C-19, though they were not clearly differentiated. The carbinol carbon, C-3, was assigned the signal at 73·5 p.p.m., and C-20 that at 74·4 p.p.m., since the former collapses to a doublet and the latter to a triplet in the proton off-resonance decoupled spectrum. The methyl carbon signal at 21·1 p.p.m. was easily assigned to C-18, and the methylene signals at 29·7 and 33·6 p.p.m. to C-1 and C-2, respectively.

Finally, there remained the assignment of C-4 and of the carbons in ring B (C-5, C-6, C-8, C-9, and C-10). On the basis of the observation that the signal due to C-6, which is expected to be least affected by the functionalities in rings A, C, and D, is observed at *ca.* 52 p.p.m. in all spectra of C_{19} and other C_{20} gibberellins, the methine signal at 52·7 p.p.m. was assigned to C-6. Since the signals due to C-4 and C-8 in the tricarboxy- and the formyl-gibberellins appear further downfield than 46 p.p.m., the quartenary carbon signal at 41·9 p.p.m. should be due to C-10. Of the two remaining quaternary carbon signals at 49·0 and 49·9 p.p.m., the former was assigned to C-4 and the latter to C-8, because the C-8 signal suffers an upfield shift due to the 13-OH in gibberellin A_{38} (17) but the C-4 signal does not (*cf.* the spectra of gibberellins A_4 and A_1 and of gibberellins A_7 and A_3). The methine carbons C-5 and C-9 were assigned the signals at 46·8 and 56·1 p.p.m., respectively, on the basis of the assignments of the spectra of gibberellins A_{13} and A_{36}. Thus, the spectrum of gibberellin A_{37} was completely analysed. The spectrum of gibberellin A_{38} was analysed by comparison with those of gibberellins A_{37}, A_{17}, and A_{23}, as shown in Table 2.

The spectra of gibberellins A_{14} (18) and A_{18} (19) were analysed in the following manner. Superimposition of the two spectra differentiated the signals due to the carbons in ring A from those due to carbons in rings C and D. The proton off-resonance decoupled spectrum of gibberellin A_{14} indicated the nature of each signal. Thus, all the carbons of gibberellins A_{14} and A_{18} were correlated with the signals in their spectra, as shown in Table 2. These assignments were confirmed by comparisons of the spectra of gibberellins A_{14}, A_{13}, A_{36}, and A_{37}, and of gibberellins A_{18}, A_{17}, A_{23}, and A_{38}.

DISCUSSION

As described above, nine spectra of C_{19} and ten of C_{20} gibberellins were analysed. C_{19} Gibberellins showed a typical signal pattern different from those of C_{20} gibberellins, characterised by two carbonyl carbon signals and the C-10 signal (Table 1). Some variations are observed in the spectra of C_{20} gibberellins corresponding to variation of the C-10 substituent. Thus, tricarboxygibberellins show three carbonyl carbon signals at *ca.* 177—179 p.p.m., and δ-lactone gibberellins (A_{37} and A_{38}) show a characteristic signal pattern due to C-7, C-19, and C-20 (Table 2). These structural features are easily recognised in ^{13}C n.m.r. spectra. However, it is sometimes difficult to differentiate C-10 aldehyde gibberellins (A_{23} and A_{36}) from C-10 methyl derivatives (A_{14} and A_{18}) simply from the proton noise-decoupled spectrum: the aldehydic carbonyl carbon signal may not be observed owing to its long relaxation time and to aldehyde–lactol interconversion;[2] consequently the aldehyde derivatives tend to show one or two carbonyl carbon signals, like the C-10 methyl gibberellins.

The 13-carbinol carbon showed almost the same chemical shift (*ca.* 78 p.p.m.) in all spectra of C_{19} and C_{20} gibberellins; however, the 3-carbinol carbon shift varied according to the precise structure (*ca.* 70 p.p.m. in C_{19}, *ca.* 71 p.p.m. in tricarboxylic and C-10 methyl, *ca.* 73 p.p.m. in C-10 aldehyde, and *ca.* 74 p.p.m. in δ-lactone gibberellins, with the exception of gibberellins containing an α-glycol system). The effects of the 12- and the 13-OH on the chemical shifts of C-16 and C-17 are interesting. Introduction of a 12-hydroxy-group causes a clear upfield shift of the C-16 signal and a clear downfield shift of that due to C-17; the introduction of a 13-hydroxy-group causes a small downfield shift of the C-16 signal and a small upfield shift of that due to C-17. These observations may be useful for the identification of the presence of a hydroxy-group at C-12 or C-13.

On the basis of the foregoing data, ^{13}C n.m.r. has been applied to the elucidation of the structure of a new gibberellin, gibberellin A_{40} (see following paper).

EXPERIMENTAL

Spectra were determined on a JEOL PS 100 Fourier transform spectrometer operating at 25·15 MHz (pulse width 8—16 μs at pulse intervals of 0·8—8·0 s). Samples were studied as 0·01—0·6M-solutions in [$^2H_5$]pyridine with 0·5% Me_4Si as internal standard; 1000—150,000 accumulations with 4096 or 8192 data points for 6250 Hz were used.

Preparation of Deuteriated Gibberellin A_1.—The hydrogenolysis product[3] (1·5 g) obtained by hydrogenation of gibberellin A_3 methyl ester was treated with hydrochloric

[2] J. R. Bearder and J. MacMillan, *J.C.S. Perkin I*, 1973, 2824.
[3] D. C. Aldridge, J. F. Grove, P. McCloskey, and W. Klyne, *J. Chem. Soc.*, 1963, 2569.

acid in deuterium oxide–acetone (conc. HCl–D_2O–Me_2CO 7 : 22 : 50 v/v) at 70 °C for 4·5 h. Then acetone was removed at pH 7·0 *in vacuo* and the mixture was extracted with ethyl acetate at pH 9·0. Preparative t.l.c. of the organic phase gave gibberellin A_1 methyl ester (75 mg), and hydrolysis with lithium propane-1-thiolate [4] then gave gibberellin A_1 (40 mg). The content of deuteriated gibberellin A_1 was calculated as 20% from the ratio of the intensities of $C(1)D$ and $C(1)H_2$ signals and from the ratio of *m/e* 363 and 362 peaks in the mass spectrum of the methyl ester.

Preparation of ^{13}C-*Enriched Gibberellins.*—*Gibberella fujikuroi* (strain G-2) was subcultured at 25 °C for 1 week in a medium containing glucose (30 g), ammonium tartrate (6 g), KH_2PO_4 (3 g), $MgSO_4,7H_2O$ (1 g), and NH_4Cl (1 g) in water (1 l). Portions (5 ml) of the subcultured mycelium were transferred to new flasks containing the same medium (100 ml per 500 ml flask). Sodium [1-^{13}C]acetate (100 mg) was added to each flask after 3 days incubation, and incubation was continued for 4 days more. The culture broth (1 l) was filtered to remove mycelium and the filtrate was extracted with ethyl acetate at pH 2·5. The organic phase was fractionated into an ethyl acetate-soluble acidic fraction and a neutral fraction in the usual manner. From the ethyl acetate-soluble fraction, ^{13}C-enriched gibberellins (mixture of gibberellins A_1 and A_3) (17 mg) were obtained after preparative t.l.c. G.l.c. analysis indicated that gibberellins A_1 and A_3 were percent in the ratio 1 : 2.

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[4] P. A. Bartlett and W. S. Johnson, *Tetrahedron Letters*, 1970, 4459.