N.m.r. Assignments of Ring A Hydrogens in Gibberellin A_4 Methyl Esters and some Derivatives

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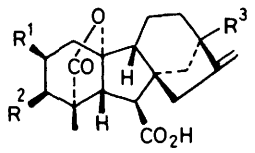
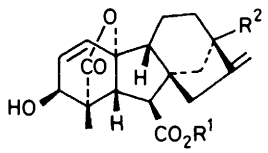
Full assignments of the ^1H n.m.r. chemical shifts of the ring A protons in gibberellin A_4, 3-*epi*-gibberellin A_4 and 3-oxogibberellin A_4 methyl esters have been made on the basis of ^1H and ^2H n.m.r data of [2,2,6-$^2H_3$]-, [1β,3-$^2H_2$]-, and [1β,2,2,3,6-$^2H_5$]-labelled derivatives These assignments have been used to determine the stereochemistry of the deuterium atoms in [1β,2β-$^2H_2$]gibberellin A_4 methyl ester, prepared *via* catalytic deuteriogenation of gibberellin A_7 16,17-epoxide methyl ester.

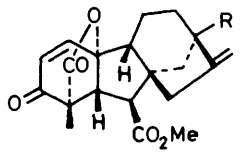
Functionalisation of ring A of the gibberellins (GAs) plays an important role in the biosynthesis and metabolism of this family of plant hormones. For example in the fungus, *Gibberella fujikuroi*, GA_4 (1) is metabolised to GA_7 (4) by 1,2-didehydrogenation.[1] In higher plants, 2β-hydroxylation is a widespread metabolic process.[2,3] Isotopically labelled GAs are important in the mechanistic study of such bio-conversions. Thus the preparation of isotopically labelled substrates of defined stereochemistry is required. This paper describes, firstly the preparation of a series of deuteriated derivatives of 3-*epi*-GA_4 methyl ester (9), GA_4 methyl ester 3-ketone (17), and GA_4 methyl ester (22) and secondly the assignment of the chemical shifts of the signals due to the ring A protons and deuterium atoms in the ^1H and ^2H n.m.r. spectra and hence the stereochemistry of isotopic label. These results are applied to the determination of the position and stereochemistry of deuterium atoms introduced into GA_4 (1) by catalytic deuteriogenation.



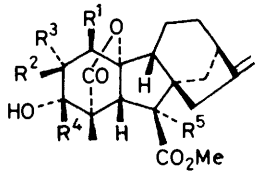
	R^1	R^2	R^3
(1)	H	OH	H
(2)	H	OH	OH
(3)	OH	OH	OH



(4) $R^1 = R^2 = H$
(5) R^1 = Me, R^2 = H
(6) R^1 = H, R^2 = OH



(7) R = H
(8) R = OH



	R^1	R^2	R^3	R^4	R^5
(9)	H	H	H	H	H
(10)	H	^2H	^2H	H	^2H
(11)	^2H	H	H	^2H	H
(12)	^2H	^2H	^2H	^2H	^2H
(13)	H	^2H	H	H	H
(14)	H	H	^2H	H	H
(15)	^2H	^2H	H	H	H
(16)	^2H	H	H	H	H

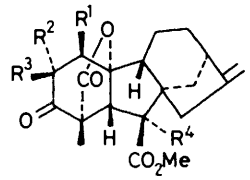
Results and Discussion

Preparation of Labelled Compounds.—The required [^2H]-gibberellins (GAs) were prepared from mixtures of GA_4 (1) and GA_7 (4) which are available in reasonable quantities from cultures of the fungus, *Gibberella fujikuroi*. Oxidation of mixtures of GA_4 and GA_7 methyl esters (22) and (5) with manganese dioxide gave a separable mixture of GA_4 methyl ester (22) and the enone (7). Conjugate reduction of the enone [4-6] with sodium borodeuteride in methanol in the presence of copper(I) chloride gave [1β,3β-$^2H_2$]-3-*epi*-GA_4 methyl ester (11), containing 1.74 deuterium atoms per molecule, and a minor amount of [1β,3α-$^2H_2$]-GA_4 methyl ester (24), containing 1.64 atoms deuterium per molecule.

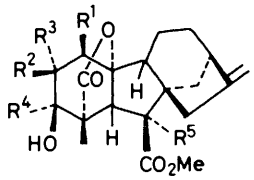
Gibberellin A_4 (1) is known [7] to epimerise at C-3 in basic medium by a retro-aldol reaction *via* the aldehyde (31). Isotopic exchange of the enolate of the aldehyde provides a method of inserting 2 deuterium atoms at C-2. Thus treatment of GA_4 methyl ester (22) with sodium methoxide in methan-[^2H]ol gave [2,2,6-$^2H_3$]-3-*epi*-GA_4 methyl ester (10) containing 2.72 deuterium atoms per molecule, a minor product was [2,2,6-$^2H_3$]-GA_4 methyl ester (23) containing 2.68 deuterium atoms per molecule. In a similar fashion treatment of [1β,3β-$^2H_2$]-3-*epi*-GA_4 methyl ester (11) with sodium methoxide in methan[^2H]ol gave [1β,2,2,3β,6α-$^2H_5$]-3-*epi*-GA_4 methyl ester (12) (M^+, 351) and [1β,2,2,3α,6α-$^2H_5$]-GA_4 methyl ester (25) (M^+, 351).

The [^2H]-labelled GA_4 3-ketones (18)—(21) were prepared by oxidation of the corresponding alcohols with Jones reagent. The [2,2,6-$^2H_3$]alcohol (10), containing 2.72 atoms deuterium per molecule, gave the ketone (18) without loss of deuterium. The [1β,3β-$^2H_2$]alcohol (11), containing 1.74 deuterium atoms per molecule gave the ketone (19) containing 0.82 deuterium atoms per molecule.

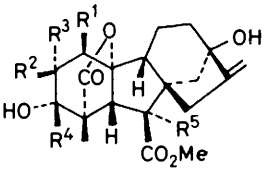
[1β,2β-$^2H_2$]Gibberellin A_4 methyl ester (26) was prepared from GA_7 methyl ester (5) as follows. Treatment of the ester (5) with 1 molar equivalent of *m*-chloroperbenzoic acid in chloroform at 0 °C for 24 h gave the 16,17-epoxide (32). Selective epoxidation of the 16-ene of 13-hydroxygibberellins had previously been achieved [8,9] with 2.5 molar equivalents of *m*-chloroperbenzoic acid in dioxane–benzene at 5 °C for 48 h; under these conditions, GA_7-methyl ester (5) gave an inseparable mixture of mono- and di-epoxides (32) and (33). Reduction of the mono-epoxide (32) with deuterium gas and a palladium–calcium carbonate catalyst gave [1β,2β-$^2H_2$]-GA_4-16,17-epoxide (34) containing 1.58 deuterium atoms per molecule; a higher incorporation of deuterium, 1.82 atoms per molecule, was obtained when the catalyst was pre-washed with deuterium oxide. A minor product of these reductions was the hydrogenolysis product (35). Regeneration of the 16-ene from the 16,17-epoxide (34) by the method of Cornforth



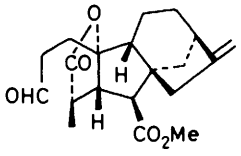
	R^1	R^2	R^3	R^4
(17)	H	H	H	H
(18)	H	^2H	^2H	^2H
(19)	^2H	H	H	H
(20)	^2H	^2H	^2H	^2H
(21)	^2H	H	^2H	H



	R^1	R^2	R^3	R^4	R^5
(22)	H	H	H	H	H
(23)	H	^2H	^2H	H	^2H
(24)	^2H	H	H	^2H	H
(25)	^2H	^2H	^2H	^2H	^2H
(26)	^2H	^2H	H	H	H



	R^1	R^2	R^3	R^4	R^5
(27)	H	H	H	H	H
(28)	H	^2H	^2H	H	^2H
(29)	H	^2H	H	H	H
(30)	^2H	^2H	H	^2H	H



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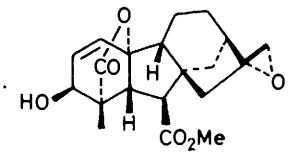

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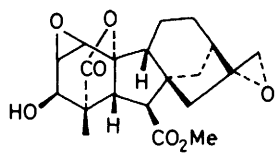

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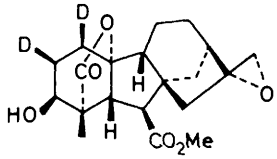

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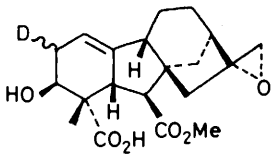

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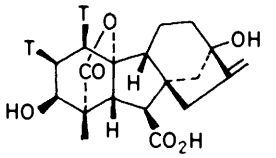

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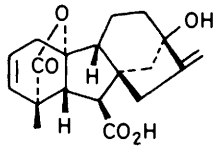

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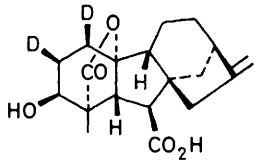

(38)

et al.[10] gave [1β,2β-^2H]$_2$-GA$_4$ methyl ester (26) containing 1.82 deuterium atoms per molecule.

Oxidation of [1β,2β-$^2H_2$]-GA$_4$ methyl ester (26) with Jones reagent gave the corresponding ketone (21) containing 1.78 deuterium atoms per molecule. Reduction of this ketone with sodium borohydride in methanol gave [1β,2β-$^2H_2$]-3-*epi*-GA$_4$ methyl ester (15) containing 1.69 deuterium atoms per molecule. Exchange of the deuterium at C-2 by a proton by treatment of [1β,2β-$^2H_2$]-3-*epi*-GA$_4$ methyl ester (15) with sodium methoxide in methanol gave [1β-^2H]-3-*epi*-GA$_4$ methyl ester (16) containing 0.90 deuterium atoms per molecule.

Stereochemical Assignments.—For 3-*epi*-GA$_4$ methyl ester (9), the proton assignments, shown in Table 1, were determined by comparison of the ^1H n.m.r. spectrum of the unlabelled compound with the ^1H and ^2H n.m.r. spectra of the [^2H]-labelled compounds (10)—(12).

The ^2H n.m.r. spectrum of [2,2,6-$^2H_3$]-3-*epi*-GA$_4$ methyl ester (10) in chloroform contained three signals at δ 1.45, 2.25, and 2.70, assigned to deuterons at C-2, C-2, and C-6 respectively. The ^1H n.m.r. spectrum of (10) in [^2H]chloroform, compared with the ^1H spectrum of the non-deuteriated compound (9), contained an AB-system with J_{gem} 14 Hz for the C-1 protons at δ 1.52 and 2.10, a doublet at δ 2.70 was absent and a doublet at δ 2.52 was simplified to a singlet, indicating that these resonances were attributable to the 6- and 5-protons respectively. The 3-H signal in (10) was obscured by the methoxy singlet at δ 3.68 but, in [$^2H_5$]-pyridine, it was observed as a separate singlet at δ 3.90, showing that there were two deuterons at C-2.

The ^2H n.m.r. spectrum of [1β,3β-$^2H_2$]-3-*epi*-GA$_4$ methyl ester (11) in chloroform contained two signals at δ 1.52 and 3.68. As shown earlier, the C-1 protons occur at δ 1.52 and 2.10 and the C-3 proton at δ 3.68. Thus conjugate reduction of the enone (7) with sodium borodeuteride had introduced deuterium stereospecifically at C-1. The ^1H n.m.r. spectrum of (11) in [^2H]chloroform at 360 MHz contained a broad doublet at δ 2.22 with J_{gem} 14 Hz, the broadening being attributable to equatorial–equatorial coupling. From the absence of any other large coupling, the doublet at δ 2.22 was tentatively assigned to the 2β-H from which it follows that compound (11) contains a 1β-deuterium.

Table 1. Assignments of chemical shifts in ^1H n.m.r. spectra of 3-*epi*-GA$_4$ methyl ester (9)

	$CDCl_3$	C_5D_5N
1α-H	2.15	—
1β-H	1.52	—
2α-H	1.45	—
2β-H	2.22	—
3β-H	3.68	3.96
5-H	2.52	2.82
6-H	2.73	3.08

The ^1H n.m.r. spectrum of [1β,2,2,3,6-$^2H_5$]-3-*epi*-GA$_4$ methyl ester (12), compared with that of the non-deuteriated parent (9), showed the absence of signals at δ 1.50 and 2.20 due to the 1β-, 2α-, and 2β-protons. A multiplet at δ 2.10, assigned to 1α-H, in the parent (9) had simplified to a singlet in (12).

From these data, it may be concluded that reduction of the enone (7) with sodium borodeuteride gave [1β,3β-$^2H_2$]-3-*epi*-GA$_4$ methyl ester (11). This conclusion, which agrees with previous results,[4,5] was directly confirmed by comparison of the ^1H n.m.r. spectra of the GA$_4$ methyl ester 3-ketone (17) and of the deuteriated ketones (18)—(20).

In [$^2H_5$]pyridine the ^1H n.m.r. spectrum of the undeuteriated ketone (17) contained an 8-line multiplet at δ 2.30 with J_{gem} 14 Hz, $J_{ax,eq}$ 7 Hz and $J_{eq,eq}$ 4 Hz. In the ^1H n.m.r. spectrum of [2,2,6-$^2H_3$]-GA$_4$ methyl ester 3-ketone (18) the signal at δ 2.30 was simplified to a doublet with J_{gem} 14 Hz; it is

Table 2. Assignments of chemical shifts in ^1H n.m.r. spectra of GA_4 methyl ester 3-ketone (17)

	$CDCl_3$	C_5D_5N	C_6D_6
1α-H	2.50	2.30	1.60
1β-H	1.72	1.88	0.95
2α-H	2.62	2.60	2.02
2β-H	2.62	2.60	2.02
5-H	3.08	3.42	2.84
6-H	2.79	3.09	2.75

Table 3. Assignments of chemical shifts in the ^1H n.m.r. spectra of GA_4 methyl ester (22)

	$CDCl_3$	C_6D_6
1α-H	1.70	1.63
1β-H	1.72	1.47
2α-H	1.76	1.67
2β-H	1.73	1.43
3α-H	3.81	3.53
5-H	3.17	3.30
6-H	2.67	2.82

therefore assigned to the 1α-proton. A doublet at δ 1.88 with J_{gem} 14 Hz was assigned to the 1β-H and the absence of multiplets at δ 2.58 for the 2α-H and 2β-H exposed a broad triplet, assignable to 13-H. In the ^1H spectrum of [1β-^2H]-GA_4 methyl ester 3-ketone (19) the multiplets at δ 2.58 (2α-H and 2β-H) and δ 2.30 (1α-H), present in the spectrum of the undeuteriated ketone (17), were simplified and the signal at δ 1.88 due to the 1β-H was absent. In the ^1H n.m.r. spectrum of [1β,2,2,6-$^2H_4$]-GA_4 methyl ester 3-ketone (20), the absence of signals at δ 1.88 (1β-H) and 2.58 (2α-H and 2β-H), and the presence of the singlet at δ 2.30, earlier assigned to 1α-H, were in accord with deuterium being specifically located at 1β.

The assignments of the signals for the ring A protons in GA_4-methyl ester 3-ketone (17) are shown in Table 2. They confirm the assignments, shown in Table 1 for 3-*epi*-GA_4 methyl ester (9). The latter assignments differ from those for 3-*epi*-GA_1 methyl ester (27), made by Hanson *et al.*[5,6] Their assignments were made by analyses of the ^1H n.m.r. spectra of the non-deuteriated compound (27) and the [2,2,6-$^2H_3$], [2β-^2H], and [1β,2β,3-$^2H_3$] derivatives (28)—(30) in [^2H]-chloroform. They prepared [2β-^2H]-3-*epi*-GA_1 methyl ester (29) by reduction of the enone (8) with sodium borohydride in methan[^2H]ol in the presence of copper(I) chloride. However, in the absence of ^2H n.m.r. data they did not detect that under these conditions a 6 : 4 ratio of 2β- and 2α-deuteriated products (13) and (14) were obtained from the enone (7) (see following paper).

For GA_4 methyl ester (22) the ring A protons were assigned in an analogous manner to that for 3-*epi*-GA_4 methyl ester (9). The assignments are shown in Table 3. The resonances of the 1- and 2- protons were superimposed in [^2H]chloroform but were resolved in [$^2H_6$]benzene.

These assignments were used to determine the stereochemistry of the isotopic labelling in [1β,2β-$^2H_2$]-GA_4 methyl ester (26), prepared *via* catalytic deuteriogenation of GA_7 methyl ester 16,17-epoxide (32). The ^2H n.m.r. spectrum of the epoxide (34) showed 1 broad singlet at δ 1.50 and the ^1H n.m.r. spectrum contained a doublet with *J* 4 Hz at δ 3.85, assigned to 3-H. In the proton-decoupled ^{13}C n.m.r. spectrum of [1β,2β-$^2H_2$]-GA_4 methyl ester (26), the signals at δ 27.1 (C-1) and 28.1 (C-2) were reduced in intensity showing the presence of deuterium at C-1 and C-2. The [^2H] n.m.r. spectrum of [1β,2β-$^2H_2$]-GA_4 methyl ester (26), in benzene, showed a broad singlet at δ 1.45 and the ^1H n.m.r. spectrum contained a doublet (*J* 4 Hz) at δ 3.85, assigned to 3-H. These data suggested that the deuterium atoms were stereospecifically located at 1β and 2β. This assignment was confirmed by analyses of the n.m.r. spectra of the corresponding ketone (21) and 3-epimers (15) and (16).

The ^1H n.m.r. spectrum of [1β,2β-$^2H_2$]-GA_4 methyl ester 3-ketone (21) in [$^2H_5$]pyridine, compared with that of the undeuteriated ketone (17), showed the absence of a signal at δ 1.88, assigned earlier to 1β-H and a simplification of the multiplet at δ 2.30, assigned to 1α, to a broad doublet (*J* 7 Hz). A broad doublet at δ 2.60 (*J* 7 Hz) was assigned to 2α-H. The broadening may be due to deuterium coupling and also proton coupling in the 20% of the mono-deuteriated compound, shown by mass spectrometry to be present in the sample. The ^2H n.m.r. spectrum of the 3-epimer (15) contained signals at δ 1.5 and 2.20 assigned to 1β-^2H and 2β-^2H respectively. The ^1H n.m.r. spectrum of the 3-epimer (15) in [^2H]pyridine contained a doublet, *J* 11 Hz, at δ 3.95 assigned to 3-H. This axial–axial coupling confirmed the presence of a 2β(*eq*)-deuterium. Some additional coupling of this signal at δ 3.95 from residual material with two protons at C-2 was evident. Finally the ^2H n.m.r. spectrum of [1β-^2H]-3-*epi*-GA_4 methyl ester (16) showed one signal at δ 1.5 (1β-^2H) and the ^1H n.m.r. spectrum, in [$^2H_5$]pyridine, contained a broadened singlet at δ 3.95, assigned to 3-H coupled to both protons on C-2.

These results illustrate the use of ^1H and ^2H n.m.r. and of mass spectrometry in assigning isotopic labelling patterns. They establish that catalytic deuteriogenation of GA_7 methyl ester 16,17-epoxide (32) introduces stereospecifically deuterium at the 1β- and 2β- positions. This result is in accord with previous conclusions [11,12] that catalytic tritiogenation of GA_3 (6) over 5% palladium on calcium carbonate [11–13] gives [1β,2β-$^3H_2$]-GA_1 (36) although evidence was only provided for the 2β-stereochemistry with the reasonable assumptions that enzymatic 2β-hydroxylation of GA_1 (2) to GA_8 (3) occurred with retention of configuration [14] or that dehydration of GA_1 (2) to GA_5 (37) occurred stereospecifically *trans*.[15]

The present direct proof that sodium borodeuteride reduction of the GA_7-enone (7) provides a 1β-H-label, combined with the previous [4] demonstration that the derived [1β-^2H]-GA_4 is metabolised to GA_3 (6), with retention of the 1β-deuterium, in cultures of *Gibberella fujikuroi*, provides confirmation of previous evidence by Evans *et al.*[1] that formation of the 1,2-double bond in GA_3 (6) occurs by loss of the 1α-hydrogen. We are currently investigating the conversion of [1β,2β-$^2H_2$]-GA_4 (38) into GA_7 (4) in both *G. fujikuroi* and in higher plants.

Experimental

General experimental details have been described in a previous paper.[4] Where indicated a 360 MHz Brüker spectrometer was used.

Reduction of ent-10β-*Hydroxy*-3-*oxo*-20-*norgibberell*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (7).—(a) *With sodium borohydride–copper*(I) *chloride*. The enone (7) (250 mg) in methanol (30 ml) was stirred with copper(I) chloride. Sodium borohydride (100 mg) was added. After 1 h, the mixture was poured into water which was acidified with hydrochloric acid and extracted with ethyl acetate. Purification of the extract by flash chromatography gave, from elution with 30% ethyl acetate–light petroleum, GA_4

methyl ester (22) (45 mg), m.p. 172—174 °C (lit.,[16] m.p. 176 °C) δ_H 1.15 (s, 18-H_3), 2.67 (d, *J* 10 Hz, 6-H), 3.17 (d, *J* 10 Hz, 5-H), 3.70 (s, OMe), 3.81 (m, 3-H), 4.85 and 4.95 (2 br s, 17-H_2); *m/z* 346 (M^+, 5%), 328 (7), 314 (100), 284 (63), 268 (20), and 224 (89).

Elution with 40% ethyl acetate–light petroleum gave 3-*epi*-GA_4 methyl ester (9) (160 mg), m.p. 169—171 °C (lit.,[17] m.p. 166—167 °C); δ_H (360 MHz) δ 1.17 (s, 18-H_3), 2.52 (d, *J* 10 Hz, 5-H), 2.73 (d, *J* 10 Hz, 6-H), 3.72 (s, OMe), 4.85 and 4.97 (2 br s, 17-H_2); δ_H (C_5D_5N) 1.55 (s, 18-H_3), 2.82 (d, *J* 10.5 Hz, 5 H), 3.04 (d, *J* 10.5 Hz, 6-H), 3.70 (s, OMe), 3.94 (m, 3-H), 4.92 and 5.00 (2 br s, 17-H_2); *m/z* 346, (M^+, 19%), 328 (79), 314 (100), 300 (62), 286 (74), 268 (30), and 242 (23).

(b) *With sodium borodeuteride–copper*(I) *chloride*. The enone (7) (100 mg) in methanol (15 ml) and copper(I) chloride were stirred with sodium borodeuteride (20 mg) for 1 h and then worked up as described in (a). Double elution p.l.c. with ethyl acetate–light petroleum (1 : 1) gave from the band at R_F 0.6, [1β,3-$^2H_2$]-3-*epi*-GA_4 methyl ester (11) (52 mg) (with 1.74 deuterium atoms per molecule); δ_{1H} (360 MHz; C_5D_5N) 1.55 (s, 18-H_3), 2.82 (d, *J* 10.5 Hz, 5-H), 3.04 (d, *J* 10.5 Hz, 6-H), 3.70 (s, OMe), 4.92 and 5.00 (2 br s, 17-H_2); δ_{2H} ($CHCl_3$) 1.52 (1β-^2H) and 3.68 (3-^2H); *m/z* 348 (M^+, 22%), 330 (93), 316 (100), 302 (57), 288 (78), 270 (33), 243 (38), and 225 (23).

Extraction of the band at R_F 0.6 gave [1β,3-$^2H_2$]-GA_4 methyl ester (24) (12 mg) (containing 1.64 deuterium atoms per molecule); δ 1.15 (s, 18-H_3), 2.67 (d, *J* 10.5 Hz, 6-H), 3.17 (d, *J* 10.5 Hz, 5-H), 3.70 (s, OMe), 4.85 and 4.95 (2 br s, 17-H_2); δ_{2H} (C_6H_6) 1.45 (1β-^2H), 3.5 (3-^2H); *m/z* 348 (M^+, 4%), 342 (42), 316 (100), 282 (55), 226 (67), and 91 (22).

Extraction of the band at R_F 0.8 gave unchanged starting material (7) (20 mg).

Exchange Reactions with Sodium Methoxide in Methan[^{2}H]*ol.*—(a) ent-3β,10β-*Dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (9). 3-*epi*-GA_4 methyl ester (9) (50 mg) in methan[^{2}H]olic sodium methoxide was heated under reflux for 6 h. The solvent was removed under reduced pressure and the residue diluted with water, acidified with hydrochloric acid, and extracted with ethyl acetate. Double elution p.l.c. of the product with ethyl acetate–light petroleum (1 : 1) afforded, from the band at R_F 0.45, [2,2,6-$^2H_3$]-3-*epi*-GA_4 methyl ester (10) (38 mg), m.p. 164—165 °C (lit.,[17] m.p. 166—167 °C) containing 2.72 deuterium atoms per molecule; δ 1.17 (s, 18-H_3), 2.52 (s, 5-H), 3.72 (s, OMe and 3-H), and 4.85 and 4.97 (2 br s, 17-H_2); δ_H (C_5D_5N) 1.55 (s, 18-H_3), 2.82 (s, 5-H), 3.70 (s, OMe), 3.94 (s, 3-H), 4.92 and 5.00 (s br s, 17-H_2); δ_{2H} 1.45 (2α-^2H), 2.22 (2β, ^2H), and 2.70 (6-^2H); *m/z* 349 (M^+, 9%) 331 (62), 317 (100), 303 (58), 289 (69), 271 (19), and 245 (25).

Extraction of the band at R_F 0.6 gave [2,2,6-$^2H_3$]-GA_4 methyl ester (23) (7 mg) containing 2.68 deuterium atoms per molecule; m.p. 173—175 °C (lit.,[16] m.p. 176 °C), δ ($CDCl_3$) 1.15 s, (18-H_3), 3.17 (s, 5-H), 3.70 (s, OMe), 3.81 (s, 3-H), 4.85 and 4.95 (2 br s, 17-H_2); δ_{2H} δ (C_6H_6) 1.45, (2β-^2H), 1.65, (2α-^2H), and 3.5 (6-^2H); *m/z* 349 (M^+, 7%), 317 (100), 287 (54), and 227 (67).

(b) ent-1β,3α-*Dideuterio*-3β,10-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*Methyl Ester* 19,10-*Lactone* (11). [1β,3-$^2H_2$]-3-*epi*-GA_4 methyl ester (11) (60 mg) in methan[^{2}H]olic sodium methoxide was heated under reflux for 6 h and then worked up as previously described. Double elution p.l.c. of the extract with ethyl acetate–light petroleum (1 : 1) gave, at R_F 0.5, [1β,2,2,3,6-$^2H_5$]-3-*epi*-GA_4 methyl ester (12) (39 mg) containing 4.12 deuterium atoms per molecule; δ ($CDCl_3$) 1.17 (s, 18-H_3), 2.15 (s, 1α-H), 2.52 (s, 5-H), 3.71 (s, OMe), and 4.85 and 4.95 (2 br s, 17-H_2); δ_{2H} ($CHCl_3$) 1.5 (1β-^2H and 2α-^2H), 2.2 (2β-^2H), 2.70 (6-^2H), and 3.68 (3-^2H); *m/z* 351 (M^+, 13%), 333 (72), 319 (100), 305 (29), 291 (53), 273 (19), and 229 (28).

Extraction of the band R_F 0.65 gave [1β,2,2,3,6-$^2H_5$]-GA_4 methyl ester (25) (10 mg) containing 4.04 deuterium atoms per molecule; δ ($CDCl_3$) 1.15 (s, 18-H_3), 3.17 (s, 5-H), 3.70 (s, OMe), and 4.85 and 4.96 (2 br s, 17-H_2); δ_{2H} (C_6H_6) 1.45 (1β-^2H and 2β-^2H), 1.65 (2α-^2H), 3.5 (3-^2H), and 2.8 (6-^2H); *m/z* 351 (M^+, 8%), 333 (16), 319 (100), 289 (58), and 229 (67).

ent-10β-*Hydroxy*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*Methyl Ester* 19,10-*Lactone* (17).—(a) *From* 3-epi-GA_4 *methyl ester* (9). 3-*epi*-GA_4 methyl ester (5) (50 mg) in acetone (10 ml) was treated dropwise with Jones reagent[18] with stirring. After 10 min methanol was added and the solvent removed under reduced pressure. The residue was diluted with water and recovered in ethyl acetate. P.l.c. of the product with ethyl acetate–light petroleum (2 : 1) gave GA_4 methyl ester 3-ketone (17) (40 mg) as a gum; δ ($CDCl_3$) 1.18 (s, 18-H_3), 2.79 (d, *J* 10.5 Hz, 6-H), 3.08 (d, *J* 10.5 Hz, 5-H), 3.72(s, OMe), and 4.88 and 5.00 (2 br s, 17-H_2); δ (C_5D_5N) 1.41 (s, 18-H_3), 3.09 (d, *J* 10.5 Hz, 6-H), 3.42 (d, *J* 10.5 Hz, 5-H), 3.68 (s, OMe), and 4.90 and 5.00 (2 br s, 17-H_2); δ (C_6D_6), 1.37 (s, 18-H_3), 2.75 (d, *J* 10.5 Hz, 6-H), 2.84 (d, *J* 10.5 Hz, 5-H), 3.18 (s, OMe), and 4.85 and 4.95 (2 br s, 17-H_2); *m/z* 344 (M^+, 52%), 312 (100), 284 (37), 257 (17), 240 (19), 189 (15), 160 (13), and 91 (25).

(b) *From* [2,2,6-$^2H_3$]-3-epi-GA_4 *methyl ester* (10). [2,2,6-$^2H_3$]-3-*epi*-GA_4 methyl ester (10) was oxidised as described above to give [2,2,6-$^2H_3$]-GA_4 methyl ester 3-ketone (18) containing 2.69 deuterium atoms per molecule; δ_{1H} ($CDCl_3$) 1.18 (s, 18-H_3), 3.08 (s, 5-H), 3.72 (s, OMe), and 4.88 and 5.00 (2 br s, 17-H_2); δ_{2H} ($CHCl_3$) 2.6 (2α-^2H and 2β-^2H) and 3.1 (6-^2H); *m/z* 347 (M^+, 66%), 315 (100), 287 (41), 259 (13), 243 (24), 189 (22), and 161 (18).

(c) *From* [1β,3-$^2H_2$]-GA_4 *methyl ester* (11). [1β,3-$^2H_2$]-3-*epi*-GA_4 methyl ester (11) was oxidised with Jones reagent as previously described to give, after purification by p.l.c., [1β-^2H]-GA_4 methyl ester 3-ketone (19) containing 0.90 deuterium atoms per molecule; *m/z* 345 (M^+, 60%), 313 (100), 285 (46), 258 (19), 241 (23), 189 (25), and 160 (15).

(d) *From* [1β,2,2,3,6-$^2H_5$]-3-epi-GA_4 *methyl ester* (12). [1β,2,2,3,6-$^2H_5$]-3-*epi*-GA_4 methyl ester (12) was oxidised as previously described to give [1β,2,2,6-$^2H_4$]-GA_4 methyl ester 3-ketone (20) containing 3.08 deuterium atoms per molecule; δ_H ($CDCl_3$) 1.18 (s, 18-H_3), 2.50 (s, 1α-H), 3.08 (s, 5-H), 3.70 (s, OMe), and 4.86 and 4.96 (2 br s, 17-H_2); *m/z* 348 (M^+, 58%), 316 (100), 288 (42), 261 (24), 244 (20), 189 (27), and 161 (19).

ent-16β,17-*Epoxy*-16β,17-*dihydro*-3α,10β-*dihydroxy*-20-*norgibberell*-1-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (32).—Gibberellin A_7 methyl ester (5) (150 mg) in chloroform (8 ml) was treated with *m*-chloroperbenzoic acid (70 mg) at 5 °C for 24 h. The mixture was diluted with chloroform, washed with aqueous sodium sulphite then with aqueous sodium hydrogen carbonate and finally with water. Removal of the solvent under reduced pressure to give a white solid which on purification by p.l.c. with ethyl acetate–light petroleum (6 : 4) gave, at R_F 0.4, GA_7 16,17-*epoxide methyl ester* (32) (102 mg), m.p. 167—169 °C (Found: C, 66.4; H, 6.8. $C_{20}H_{24}O_6$ requires C, 66.66; H, 6.66%); δ ($CDCl_3$) 1.25 (s, 18-H_3), 2.83 (d, *J* 11 Hz, 6-H), 2.84 (s, 17-H_2), 3.19 (d, *J* 11 Hz, 5-H), 3.74 (s, OMe), 4.16 (d, *J* 3 Hz, 3-H), 5.91 (d, d *J* 9 Hz, *J* 3 Hz, 2-H), 6.34 (d, *J* 9 Hz, 1-H); *m/z* 360 (M^+, 96%), 329 (50), 300 (58), 282 (37), 267 (27), 255 (39), 238 (100), 221 (51), 179 (79), 155 (78), 135 (82), 105 (35), and 91 (69).

Elution of band R_F 0.7 gave unchanged starting material (5) (40 mg).

ent-1α,2α-*Dideuterio*-3α,10β-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (26).—Gibberellin A_7 16,17-epoxide methyl ester (33) (100 mg) in tetrahydrofuran (5 ml) was stirred under an atmosphere of deuterium gas in the presence of 10% palladium on calcium carbonate (20 mg) for 1 h at room temperature. The mixture was filtered through Celite and the solvent removed under reduced pressure. Purification by p.l.c. with ethyl acetate–light petroleum (6 : 4) gave, at R_F 0.4, [1,2-$^2H_2$]GA_4 16,17-*epoxide methyl ester* (34) (75 mg) containing 1.58 deuterium atoms per molecule, m.p. 187—188 °C (Found: C, 65.85; H, 7.25. $C_{20}H_{24}O_6{}^2H_2$ requires C, 65.93; H, 7.69%); δ_{2H} (C_6H_6), 1.5 (1β- and 2β-^{2}H); δ_{1H} ($CDCl_3$) 1.15 (s, 18-H_3), 2.73 (d, *J* 11 Hz, 6-H), 2.83 (s, 17-H_2), 3.18 (d, *J* 11 Hz, 5-H), 3.71 (s, OMe), and 3.84 (d, *J* 4 Hz, 3-H); *m*/*z* 364 (M^+, 100%), 346 (32), 333 (35), 318 (45), 302 (92), 286 (59), 246 (40), 242 (91), 229 (47), 183 (43), 156 (44), 129 (58), and 91 (50).

The above experiment was repeated and the catalyst pre-washed with D_2O and dried thoroughly to give [1,2-$^2H_2$]-GA_4 16,17-epoxide methyl ester (34) containing 1.82 deuterium atoms per molecule.

Sodium iodide (400 mg) and sodium acetate (150 mg) in ice-cooled glacial acetic acid (7 ml), acetone (1 ml) and water (0.5 ml) were stirred with freshly activated zinc dust (400 mg). [1β,2β-$^2H_2$]-GA_4 epoxide methyl ester (34) (70 mg) in acetone (2 ml) was added dropwise and the mixture stirred for 2 h. The solution was filtered, diluted with ethyl acetate, and washed with aqueous sodium hydrogen carbonate solution. Purification of the extract by p.l.c. with ethyl acetate–light petroleum (1 : 1) gave, at R_F 0.7, [1β,2β-$^2H_2$]-GA_4 methyl ester (26) (45 mg) containing 1.82 deuterium atoms per molecule, m.p. 176—178 °C (lit.,[16] m.p. 176 °C), δ_{2H} (C_6H_6) 1.45 (1β-^{2}H and 2β-^{2}H); δ_{1H} ($CDCl_3$) 1.14 (s, 18-H_3), 2.68 (d, *J* 11 Hz, 6-H), 3.17 (d, *J* 11 Hz, 5-H), 3.70 (s, OMe), 3.81 (d, *J* 4 Hz, 3-H), and 4.84 and 4.95 (2 br s, 17-H_2); *m*/*z* 348 (M^+, 3%), 316 (92), 286 (70), 270 (23), 226 (100), and 91 (34).

ent-1α-*Deuterio*-3β,10β-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (16).—[1β,2β-$^2H_2$]-GA_4 methyl ester (26) (20 mg) in acetone (5 ml) was treated dropwise with Jones reagent with stirring. After 10 min methanol (0.5 ml) was added and the solvent removed under reduced pressure. The residue was diluted with water and recovered in ethyl acetate. P.l.c. of the product with ethyl acetate–light petroleum (2 : 1) gave [1,2-$^2H_2$]-GA_4 methyl ester 3-ketone (21) (18 mg) as a gum, δ_H ($CDCl_3$) 1.18 (s, 18-H_3), 2.45 (br d, *J* 7 Hz, 1α-H), 2.63 (br d, *J* 7 Hz, 2α-H), 2.80 (d, *J* 10.5 Hz, 6-H), 3.07 (d, *J* 10.5 Hz, 6-H), 3.70 (s, OMe), 4.86 and 4.96 (2 br s, 17-H_2); δ_H (C_6H_5N) 1.42 (s, 18-H_3), 2.30 (br d, *J* 7 Hz, 1α-H), 2.62 (br d, *J* 7 Hz, 2α-H), 3.11 (d, *J* 10.5 Hz, 6-H), 3.46 (d, *J* 10.5 Hz, 5-H), 3.68 (s, OMe), and 4.90 and 5.00 (2 br s, 17-H_2); *m*/*z* 346 (M^+, 55%), 314 (100), and 286 (39).

[1,2-$^2H_2$]-GA_4 methyl ester 3-ketone (21) (16 mg) in methanol (5 ml) was stirred with sodium borohydride (10 mg). After 1 h the mixture was poured into water, acidified with hydrochloric acid, and extracted with ethyl acetate. Purification of the extract by flash chromatography gave, from elution with 40% ethyl acetate–light petroleum [1β,2β-$^2H_2$]-3-*epi*-GA_4 methyl ester (15) (12 mg), m.p. 168—170 °C (lit.,[17] m.p. 166—167 °C) containing 1.69 deuterium atoms per molecule; δ_{2H} ($CHCl_3$) 1.45 (1β-^{2}H) and 2.2 (2β-^{2}H); δ_{1H} (C_5D_5N) 1.55 (s, 18-H_3), 2.83 (d, *J* 10.5 Hz, 6-H), 3.05 (d, *J* 10.5 Hz, 5-H), 3.70 (s, OMe), 3.95 (d, *J* 11 Hz, 3-H), and 4.91 and 5.00 (2 br s, 17-H_2); *m*/*z* 348, (M^+, 70%), 316 (100), 302 (49), 288 (64), 270 (20), 260 (15), 244 (14), and 226 (20).

[1β-2β-$^2H_2$]-3-*epi*-GA_4 methyl ester (11) (11 mg) in methanolic sodium methoxide was heated under reflux for 6 h and then worked up as previously described. Double elution p.l.c. of the extract with ethyl acetate–light petroleum (1 : 1) gave, at R_F 0.55, [1β-^{2}H]-3-*epi*-GA_4 methyl ester (16) (7 mg) containing 0.9 deuterium atoms per molecule; δ_{2H} ($CHCl_3$) 1.45 (1β-^{2}H); δ_{1H} (C_5D_5N), 1.55 (s, 18-H_3), 2.84 (d, *J* 10.5 Hz, 6-H), 3.05 (d, *J* 10.5 Hz, 5-H), 3.70 (s, OMe), 3.95 (br s, 3-H), and 4.91 and 5.00 (2 br s, 17-H_2); *m*/*z* 329 (M^+, 71%), 315 (100), 301 (52), 287 (61), 269 (30), 259 (25), and 225 (19).

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