# Stereoselective Deuteriation at Carbon 2 of the Gibberellins

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Various routes are described for the preparation of 2α- and 2β-deuteriated gibberellins. The stereoselective preparation of gibberellins $A_4$ and $A_9$, containing 0.8 deuterium atoms per molecule of which 90% was located at the 2α-position, was achieved *via* 2β-iodo-3-oxogibberellin $A_4$. An improved incorporation (0.9 deuterium atoms per molecule) was obtained *via* 2β-bromo-3-oxogibberellin $A_4$, the preparation of which, from 2β-iodo-3-oxogibberellin $A_4$, is discussed.

In the preceding paper [1] we reported the synthesis of [1β,2β-$^2H_2$]-gibberellin $A_4$ (1). This paper describes the stereoselective synthesis of [2α-$^2$H]gibberellins $A_4$ and $A_9$ (2) and (3). These compounds were required to study the stereochemistry of the 2β-hydroxylation of gibberellins $A_9$ (4) and $A_{20}$ (5) in higher plants [2,3] and the 2α-hydroxylation of gibberellin $A_9$ in *Gibberella fujikuroi*.[4] The stereochemistry of the isotopic label in the products was determined by $^1$H and $^2$H n.m.r. spectroscopy utilising the assignments described in the preceding paper.[1]

## Results and Discussion

In order to examine the stereospecificity of an isotopic label introduced at C-2 in $GA_9$ methyl ester (20), it was necessary to assign the signals in the $^2$H n.m.r. spectrum due to 2α-$^2$H and 2β-$^2$H. [2,2,6-$^2H_3$]-$GA_9$ methyl ester (21) was prepared from [2,2,6-$^2H_3$]-3-*epi*-$GA_4$ methyl ester (22).[1] Treatment of (22) with phosphorus oxychloride gave [2,2,6-$^2H_3$]-3β-chloro-$GA_9$ methyl ester (23) whose $^1$H n.m.r. spectrum displayed a singlet at δ 4.08 assigned to 3-H. Reduction of the chloro compound (23) with tri-n-butyltin hydride gave [2,2,6-$^2H_3$]-$GA_9$ methyl ester (21) containing 2.58 deuterium atoms per molecule. The $^2$H n.m.r. spectrum of (21) displayed signals at δ 1.55, 1.73, and 2.64 assigned to 2α,2β- and 6-deuterium atoms respectively.

Since $GA_{40}$ (6) possesses a 2α-hydroxy group, its use as a precursor of regiospecifically labelled gibberellins was explored. Treatment of $GA_{40}$ methyl ester (7), prepared by the route of Beeley *et al.*,[5] with phosphorus oxychloride gave 2β-chloro-$GA_9$ methyl ester (8) as the major product. The $^1$H n.m.r. spectrum displayed a triplet of triplets at δ 4.08 ($J_{ax,ax}$ 11 Hz and $J_{ax,eq}$ 6 Hz) due to the axial 2α-H. Reduction of the chloro compound (8) with tri-n-butyltin deuteride gave [2ξ-$^2$H]-$GA_9$ methyl ester (9) containing 0.99 deuterium atoms per molecule. Since the $^2$H n.m.r. spectrum displayed signals at δ 1.55 and 1.73 in the ratio 3 : 1, it was concluded that 75% of the isotope was located at the 2α-position, the remainder at 2β. Since the synthesis of isotopically labelled gibberellins from $GA_{40}$ (6) is both lengthy and unsuitable for 3-hydroxylated derivatives this approach was not pursued.

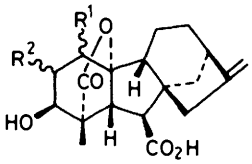
Conjugate reduction of the enone (29) introduces hydrogen at carbons-1, -2, and -3.[6-8] Hanson *et al.*[7,8] reported that reduction of the enone (29) with sodium borohydride in methan[$^2$H]ol, in the presence of copper(I) chloride gave regiospecifically [2β-$^2$H]-3-*epi*-$GA_4$ methyl ester (11). However, in our hands 3-*epi*-$GA_4$ methyl ester ($M^+$, 347), containing 0.87 deuterium atoms per molecule, was obtained the $^2$H n.m.r. spectrum of which indicated that only 60% of the isotope was located at 2β, the remainder at the 2α-position. The $^1$H n.m.r. spectrum in [$^2H_5$]pyridine, displayed a signal at δ 3.9, assigned to 3-H, as a doublet $J_{ax,ax}$ 11 Hz enclosing a smaller doublet, with $J$ 8 Hz, in approximately the ratio 6 : 4, confirming that the 3-*epi*-$GA_4$ methyl ester contained a 6 : 4 mixture of 2β to 2α deuterium atoms.

The above reduction was repeated in t-butyl [$^2$H]alcohol to give 3-*epi*-$GA_4$ methyl ester (18) containing 0.65 atoms of deuterium. Investigation of the $^1$H and $^2$H n.m.r. spectra showed that 70% of the label was located at 2β, the remainder at 2α. Reduction of the enone (29), as described by Beale *et al.*,[6] with sodium borohydride in tetrahydrofuran in the presence of lithium bromide, followed by addition of deuterium oxide gave a mixture of [2α-$^2$H]- and [2β-$^2$H]-3-*epi*-$GA_4$ methyl esters in accord with their results. Finally, reduction of the enone with L-Selectride in tetrahydrofuran gave, on work-up with deuterium oxide, [2-$^2$H]-3-*epi*-$GA_4$ methyl ester containing 0.71 deuterium atoms per molecule of which 45% was located at 2β.

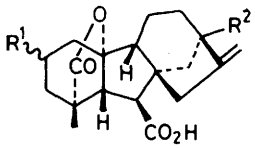
A stereoselective partial synthesis of [2α-$^2$H]gibberellins was achieved from $GA_4$/$GA_7$ *via* 2β-iodo-3-oxo$GA_4$ methyl ester (26). The 2β-iodo ketone (26) was prepared in two ways: (*a*) from the enone (29) treated with L-Selectride in tetrahydrofuran followed by the addition of one equivalent of iodine; and (*b*) from 3-oxo$GA_4$ methyl ester (24) treated with lithium di-isopropylamide followed by iodine. The $^1$H n.m.r. spectrum of the iodo ketone displayed three sets of doublets, centred at δ 2.52, 3.20, and 5.00. The signal at δ 2.52, with $J_{gem}$ 14 Hz and $J_{ax,ax}$ 11 Hz, was assigned to the axial 1β-H while the resonances at δ 3.20 with $J_{gem}$ 14 Hz and $J_{ax,eq}$ 8 Hz, was attributed to the equatorial 1α-H. The doublet of doublets at δ 5.00, with $J_{ax,ax}$ 11 Hz and $J_{ax,eq}$ 8 Hz, was assigned to the axial 2α-H in accord with the proposed structure (26).

Treatment of the iodo ketone (26) with tri-n-butyltin deuteride gave [2α-$^2$H]-3-oxo$GA_4$ methyl ester (25), which on reduction with sodium borohydride afforded as the major product [2α-$^2$H]-3-*epi*-$GA_4$ methyl ester (12), containing 0.82 deuterium atoms per molecule. Signals in the $^2$H n.m.r. spectrum at δ 1.45 and 2.22 in the ratio 10 : 1 indicated that 90% of the isotope was located at 2α. The $^1$H n.m.r. spectrum displayed a doublet at δ 3.9, $J$ 8 Hz due to 3-H, confirming that the reduction of the iodide (26) with tri-n-butyltin deuteride was stereoselective giving mainly [2α-$^2$H]-3-*epi*-$GA_4$ methyl ester (12). A minor product was the correspondingly deuteriated $GA_4$ methyl ester (13). Deoxygenation of [2α-$^2$H]-3-*epi*-$GA_4$ methyl ester by treatment of the 3β-chloro compound (14) with tri-n-butyltin hydride gave [2α-$^2$H]-$GA_9$ methyl ester (10) containing 0.80 atoms of deuterium. The $^2$H n.m.r. spectrum displayed a major signal at δ 1.58 due to 2α-$^2$H confirming the previous results. Hydrolysis of the methyl ester with methanolic sodium hydroxide gave [2α-$^2$H]-$GA_9$ (3).

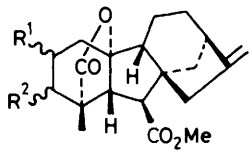
Reduction of the iodo ketone (26) with sodium borodeuteride in methanol or tetrahydrofuran gave 3-*epi*-$GA_4$ methyl ester ($M^+$, 347) containing 0.85 deuterium atoms per molecule. A single signal at δ 3.68 in the $^2$H n.m.r. spectrum combined with the absence of the signal due to 3-H in the



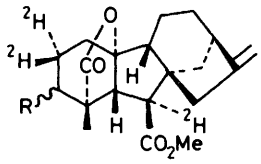
(1) $R^1 = R^2 = \beta\text{-}^2H$
(2) $R^1 = H$, $R^2 = \alpha\text{-}^2H$



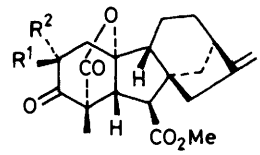
(3) $R^1 = \alpha\text{-}^2H$, $R^2 = H$
(4) $R^1 = R^2 = H$
(5) $R^1 = H$, $R^2 = OH$
(6) $R^1 = OH$, $R^2 = H$



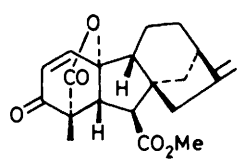
| | $R^1$ | $R^2$ |
|---|---|---|
| (7) | α-OH | H |
| (8) | β-Cl | H |
| (9) | ξ-$^2$H | H |
| (10) | α-$^2$H | H |
| (11) | β-$^2$H | α-OH |
| (12) | α-$^2$H | α-OH |
| (13) | α-$^2$H | β-OH |
| (14) | α-$^2$H | β-Cl |
| (15) | H | α-OH, β-$^2$H |
| (16) | H | α-$^2$H, β-OH |
| (17) | ξ-$^2$H | α-OH, β-$^2$H |
| (18) | ξ-$^2$H | α-OH |
| (19) | ξ-$^2$H | β-OH |
| (20) | H | H |



(21) R = H
(22) R = α-OH
(23) R = β-Cl

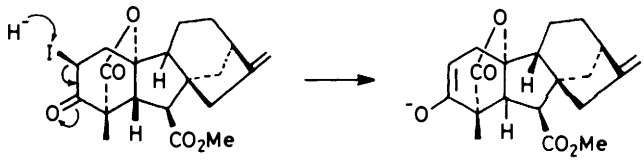


| | $R^1$ | $R^2$ |
|---|---|---|
| (24) | H | H |
| (25) | H | $^2$H |
| (26) | I | H |
| (27) | Br | H |
| (28) | Br | Br |



(29)

$^1$H n.m.r. spectrum demonstrated that the isotope was solely at 3β. A minor product of the reduction was [3-$^2$H]-GA$_4$ methyl ester (16). The $^2$H n.m.r. spectrum displayed a signal at δ 3.85 assigned to 3-$^2$H. As expected, this signal was absent in the $^1$H n.m.r. spectrum. Treatment of the iodo ketone (26) with sodium borohydride in methan[$^2$H]ol yielded 3-*epi*-GA$_4$ methyl ester ($M^+$, 347) containing 0.75 deuterium atoms per molecule. The $^2$H n.m.r. spectrum displayed signals at δ 2.22 and 1.45 in the ratio 6 : 4 assigned to 2β-$^2$H and 2α-$^2$H respectively. The signal at δ 3.9 due to 3-H, in the $^1$H n.m.r. spectrum in [$^2H_5$]pyridine, appeared as a doublet, $J_{ax,ax}$ 11 Hz, enclosing a smaller doublet $J_{ax,eq}$ 7 Hz in accord with the product containing a mixture of 2α- and 2β-deuterium atoms. Reduction of the iodo ketone (26) with sodium borodeuteride in methan[$^2$H]ol gave [2ξ,3-$^2H_2$]-3-*epi*-GA$_4$ methyl ester (17) ($M^+$, 348) with 1.92 deuterium atoms per molecule. The $^2$H n.m.r. spectrum displayed signals at δ 3.68, 2.22, and 1.45 in the ratio 1 : 0.6 : 0.4, due to 3-$^2$H, 2β-$^2$H, and 2α-$^2$H respectively, in accord with the results from the previous reductions. The reduction of the iodo ketone (26) with sodium borohydride in methan[$^2$H]ol therefore gives the same 2α : 2β deuterium ratio as the conjugate reduction of the enone (29). These results suggest that the reaction of the iodo ketone with sodium borohydride proceeds *via* the enolate anion (30) and not by nucleophilic attack of hydride at C-2.



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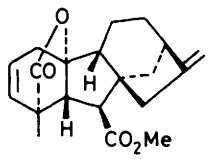

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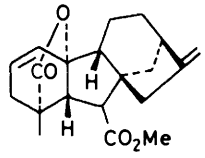

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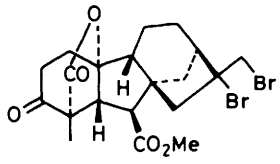
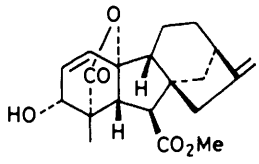


(33)



(34)

The ready formation of the enolate anion (30) from the iodo ketone (26) was utilised in the preparation of 2β-bromo-3-oxoGA$_4$ methyl ester (27). Treatment of the iodo ketone (26) with lithium bromide in tetrahydrofuran gave, in quantitative yield, the 2β-bromo ketone (27). The $^1$H n.m.r. spectrum displayed three sets of doublets centred at δ 2.34, with $J_{gem}$ 14 Hz and $J_{ax,ax}$ 11 Hz, assigned to the axial 1β-H; at δ 3.20 with $J_{gem}$ 14 Hz and $J_{ax,eq}$ 8 Hz due to the equatorial 1α-H; and at δ 4.77, $J_{ax,ax}$ 11 Hz and $J_{ax,eq}$ 8 Hz, assigned to 2α-H in accord with the substitution occurring with retention of configuration. Other routes to the monobrominated product were unsatisfactory. For example treatment of the ketone (24) with lithium di-isopropylamide followed by *N*-bromosuccinimide gave, as the major product, 2,2-dibromo-3-oxoGA$_4$ methyl ester (28), while treatment of (24) with bromine gave the dibrominated product (33) in which addition across the exocyclic double bond had occurred.

Reduction of the 2β-bromo ketone (27) with tri-n-butyltin deuteride gave the [2-$^2$H]-ketone (25) which on reduction with sodium borohydride gave, as the major product [2α-$^2$H]-3-*epi*-GA$_4$ methyl ester ($M^+$, 347) (12), identical with that from the 2β-iodo ketone (26) but with higher deuterium incorporation (0.94 deuterium atoms per molecule). A minor product from the reduction of either the iodo ketone (26) or bromo ketone (27) was [2α-$^2$H]-GA$_4$ methyl ester (13). However, an improved yield of the 3β-alcohol was obtained by treatment of the bromo ketone (27) with tri-n-butyltin deuteride followed by reduction with aluminium isopropoxide yielding [2α-$^2$H]-GA$_4$ methyl ester (13) ($M^+$, 347) containing 0.94 atoms of deuterium per molecule. A major signal in the $^2$H n.m.r. spectrum in benzene at δ 1.67 [1] and collapse of the signal at δ 3.8 to a doublet $J$ 3 Hz in the $^1$H n.m.r. spectrum again indicated that the reduction was stereoselective giving mainly [2α-$^2$H]-GA$_4$ methyl ester (13). Hydrolysis of the methyl ester with sodium propanethiolate, to prevent epimerisation at C-3,[9] gave [2α-$^2$H]-GA$_4$ with 0.9 deuterium atoms per molecule of which 90% was located at the 2α position.

The observed reduction of the 2β-bromo and 2β-iodo ketones (27) and (26) and of 2β-chloroGA$_9$ methyl ester (8) with tri-n-butyl[$^2$H]stannane to give predominantly the [2α-$^2$H]epimers (25), (25), and (10) respectively is perhaps surprising since the α-face is usually regarded as more hindered than the β-face. The absence of a 3α-hydroxy group in the substrates (26), (27), and (8) may be an important determinant.

## Experimental

General experimental details have been described in a previous paper.[1] Where indicated a Brüker 400 MHz n.m.r. spectrometer was used.

ent-2,2,6-*Trideuterio*-10β-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (21).—[2,2,6-$^2$H$_3$]-3-*epi*-GA$_4$ methyl ester (22) (80 mg), phosphoryl chloride (0.6 ml), and pyridine (20 ml) were refluxed for 3 h. The mixture was poured into water, acidified with hydrochloric acid, and extracted with ethyl acetate. P.l.c. of the oily product with ethyl acetate–light petroleum (1 : 1) gave, at $R_F$ 0.8, [2,2,6-$^2$H$_3$]-3β-chloroGA$_9$ methyl ester (23) (40 mg) as a gum; δ 1.19 (s, 18-H$_3$), 2.68 (d, *J* 10.5 Hz, 6-H), 3.24 (d, *J* 10.5 Hz, 5-H), 3.67 (s, OMe), 4.12 (s, 3-H), and 4.86 and 4.96 (2 br s, 17-H$_2$). A solution of the 3β chloro compound (23) (35 mg), tri-n-butylstannane (12 μl), and a crystal of 2,2′-dimethylazopropionitrile in benzene (10 ml) was refluxed for 5 h under nitrogen. After removal of the solvent under reduced pressure the product was purified by p.l.c. with ethyl acetate–light petroleum (1 : 1) to give, at $R_F$ 0.8, [2,2,6-$^2$H$_3$]-GA$_9$ methyl ester (21) (20 mg), m.p. 134—136 °C (lit.,[10] m.p. 136 °C) containing 2.58 deuterium atoms per molecule; δ 1.18 (s, 18-H$_3$), 2.54 (s, 5-H), 3.70 (s, OMe), 4.85 and 4.95 (2 br s, 17-H$_2$); $\delta_{2H}$ (CHCl$_3$) 1.55 (2α-$^2$H), 1.73 (2β-$^2$H), and 3.64 (6-$^2$H); *m*/*z* 333 (*M*$^+$, 7%), 301 (100), 273 (68), 246 (32), 229 (45), and 220 (22).

ent-2ξ-*Deuterio*-10β-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (9).—GA$_{40}$ methyl ester (7) (100 mg), phosphoryl chloride (0.4 ml), and pyridine (10 ml) were refluxed for 1 h. The reaction mixture was then poured into water which was acidified with hydrochloric acid and extracted with ethyl acetate. P.l.c. of the resultant gum with ethyl acetate–light petroleum (1 : 1) gave, at $R_F$ 0.7, 2β-chloroGA$_9$ methyl ester (8) (54 mg), m.p. 136—137 °C (Found: C, 66.5; H, 7.0. $C_{20}H_{25}ClO_4$ requires C, 65.8; H, 6.9); δ 1.13 (s, 18-H$_3$), 2.64 (d, *J* 10.5 Hz, 6-H), 2.75 (d, *J* 10.5 Hz, 5-H), 3.70 (s, OMe), 4.08 (tt, *J* 11 Hz and *J* 6 Hz, 2-H), and 4.83 and 4.96 (2 br s, 17-H$_2$); *m*/*z* 364 (*M*$^+$, 11%), 332 (97), 304 (100), 277 (24), 260 (56), 181 (24), 169 (32), 155 (35), 105 (32), and 91 (63).

Band $R_F$ 0.8 gave a mixture of 2β-chloroGA$_9$ methyl ester (8) and the unsaturated compounds (31) and (32) identified by $^1$H n.m.r. spectroscopy.

A solution of the 2β-chloro-compound (8) (50 mg), tri-n-butyltin deuteride (0.2 ml) and a crystal of 2,2′-dimethyl-2,2′-azopropionitrile in benzene (15 ml) was refluxed for 1.5 h. After removal of the solvent under reduced pressure the product was purified by p.l.c. with ethyl acetate–light petroleum (1 : 1). The band at $R_F$ 0.7 gave [2ξ-$^2$H]-GA$_9$ methyl ester (9) (37 mg), m.p. 136—138 °C (lit.,[10] m.p. 136 °C) (0.99 deuterium atoms per molecule); $\delta_{2H}$ (400 MHz; CHCl$_3$) 1.76 [2β-$^2$H] and 1.58 [2α-$^2$H]; $\delta_{1H}$ 1.18 (s, 18-H$_3$), 2.54 (d, *J* 10.5 Hz, 5-H), 2.68 (d, *J* 10.5 Hz, 6-H), 3.70 (s, OMe), and 4.82 and 4.92 (2 br s, 17-H$_2$); *m*/*z* 331 (*M*$^+$, 7%), 299 (100), 271 (70), 244 (34), 227 (51), and 218 (23).

*Reduction of* ent-10β-*Hydroxy*-3-*oxo*-20-*norgibberell*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (29).—(a) *With sodium borohydride, copper*(I) *chloride in methan*[$^2$H]*ol.* To a stirred solution of the enone (29) (100 mg) and copper(I) chloride (150 mg) in methan[$^2$H]ol (15 ml) was added sodium borohydride. After 1.5 h the mixture was poured into water, acidified with hydrochloric acid, and extracted with ethyl acetate. P.l.c. of the recovered product with ethyl acetate–light petroleum (6 : 4) gave, from the band at $R_F$ 0.5, [2ξ-$^2$H]-3-*epi*-GA$_4$ methyl ester (18) (55 mg) with 0.87 deuterium atoms per molecule, m.p. 169—170 °C (lit.,[11] m.p. 166—167 °C); $\delta_{2H}$ (CHCl$_3$) 2.25 [2β-$^2$H] and 1.45 [2α-$^2$H]; $\delta_{1H}$ (CDCl$_3$) 1.18 (s, 18-H$_3$), 2.52 (d, *J* 10.5 Hz, 5-H), 2.73 (d, *J* 10.5 Hz, 6-H), 3.68 (m, 3-H), 3.70 (s, OMe), and 4.85 and 4.97 (2 br s, 17-H$_2$); $\delta_H$ (C$_5$D$_5$N) 1.53 (s, 18-H$_3$), 2.82 (d, *J* 10.5 Hz, 5-H), 3.03 (d, *J* 10.5 Hz, 6-H), 3.71 (s, OMe), 3.94 (d, *J* 11 Hz, enclosing further d, *J* 7 Hz, 3-H), 4.91 and 5.00 (2 br s, 17-H$_2$); *m*/*z* 347 (*M*$^+$, 38%), 329 (79), 315 (100), 301 (55), 287 (62), 269 (27), and 242 (30). Extraction of the band $R_F$ 0.6 gave [2ξ-$^2$H]GA$_4$ methyl ester (19) (14 mg), m.p. 171—173 °C (lit.,[12] m.p. 176 °C) containing 0.84 deuterium atoms per molecule; $\delta_{2H}$ (C$_6$H$_6$), 1.43 [2β-$^2$H] and 1.67 [2α-$^2$H]; $\delta_{1H}$ (CDCl$_3$) 1.22 (s, 18-H$_3$), 2.79 (d, *J* 10.5 Hz, 6-H), 3.08 (d, *J* 10.5 Hz, 5-H), 3.70 (s, OMe), 3.81 (m, 3-H), and 4.85 and 4.95 (2 br s, 17-H$_2$); *m*/*z* 347 (*M*$^+$, 8%), 329 (17), 315 (100), 285 (55), 269 (44), 242 (23), and 225 (75).

(b) *With sodium borohydride, copper*(I) *chloride in t-butyl* [$^2$H]*alcohol.* To a stirred solution of the enone (29) (100 mg) and copper(I) chloride (150 mg) in t-butyl [$^2$H]alcohol was added sodium borohydride. After 1.5 h the mixture was poured into water, acidified with hydrochloric acid, and extracted with ethyl acetate. P.l.c. of the recovered product with ethyl acetate–light petroleum (6 : 4) gave, from the band at $R_F$ 0.5, [2ξ-$^2$H]-3-*epi*-GA$_4$ methyl ester (18) (45 mg) with 0.67 deuterium atoms per molecule; $\delta_{2H}$ (CHCl$_3$) 2.25 [2β-$^2$H] and 1.45 [2α-$^2$H]. Extraction of band $R_F$ 0.6 gave [2ξ-$^2$H]GA$_4$ methyl ester (19) (9 mg) with 0.65 deuterium atoms per molecule; $\delta_{2H}$ (C$_6$H$_6$), 1.45 [2β-$^2$H] and 1.67 [2α-$^2$H]. Extraction of band $R_F$ 0.8 gave unchanged starting material (20 mg).

(c) *With sodium borohydride and lithium bromide in tetrahydrofuran with deuterium work-up.* To a suspension of sodium borohydride (20 mg) and anhydrous lithium bromide (55 mg) in dry tetrahydrofuran (10 ml), cooled to 0 °C, was added the enone (29) (100 mg). After 1 h at 0 °C, the mixture was poured into deuterium oxide, acidified with deuterium chloride, and extracted with ethyl acetate. P.l.c. of the product as previously described gave [2ξ-$^2$H]-3-*epi*-GA$_4$ methyl ester (18) (60 mg) containing 0.75 deuterium atoms per molecule; $\delta_{2H}$ (CHCl$_3$) 2.22 [2β-$^2$H] and 1.45 [2α-$^2$H]. Extraction of the band at 0.6 gave a mixture of GA$_4$ methyl ester (19) and 3-*epi*-GA$_7$ methyl ester (34) which were not further purified.

(d) *With* L-*Selectride in tetrahydrofuran with deuterium work-up.* To a solution of the enone (29) (100 mg) in dry tetrahydrofuran (20 ml), cooled to −70 °C, was added dropwise L-Selectride (0.8 ml; 1M-solution in tetrahydrofuran). After 0.5 h the reaction was worked up as described in (c) to give [2ξ-$^2$H]-3-*epi*-GA$_4$ methyl ester (18) (87 mg) containing 0.71 deuterium atoms per molecule; $\delta_{2H}$ (CHCl$_3$) 2.22 [2β-$^2$H] and 1.45 [2α-$^2$H].

ent-2α-*Iodo*-10β-*hydroxy*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (26).—(a) 3-OxoGA$_4$ methyl ester (24) (200 mg) in tetrahydrofuran (15 ml) was stirred with lithium di-isopropylamide [13] (1.1 ml; 0.5M-solution) under nitrogen. The mixture was cooled to −70 °C. Iodine (120 mg) in tetrahydrofuran (2 ml) was titrated into the mixture which was then poured into water, acidified with hydrochloric acid, and extracted with ethyl

acetate. The extract was washed with aqueous sodium thiosulphate and the solvent removed under reduced pressure. Purification of the product by column chromatography, gave on elution with 12% ethyl acetate–light petroleum, 2β-*iodo*-3-*oxo*$GA_4$ *methyl ester* (26) (140 mg), m.p. 148—150 °C (Found: C, 52.0; H, 4.8. $C_{20}H_{23}IO_5$ requires C, 51.1; H, 4.9); $\delta_H$ 1.23 (s, 18-$H_3$), 2.52 (dd, *J* 14 Hz, *J* 11 Hz, 1β-H), 2.81 (d, *J* 10.5 Hz, 6-H), 3.20 (dd, *J* 14 Hz, *J* 8 Hz, 1α-H), 3.22 (d, *J* 10.5 Hz, 5-H), 3.72 (s, OMe), 4.85 and 4.95 (2 br s, 17-$H_2$), and 5.00 (dd, *J* 11 Hz, *J* 8 Hz, 2α-H); *m/z* 470 ($M^+$ 43%), 438 (42), 344 (37), 312 (62), 284 (30), 131 (100), 130 (98), 127 (35), 121 (88), and 91 (36).

(b) The enone (29) (200 mg) in tetrahydrofuran (20 ml) was cooled to −70 °C and L-Selectride (0.7 ml; 1M-solution) was added dropwise. After 0.5 h, iodine (125 mg) in tetrahydrofuran (2 ml) was added and the reaction mixture immediately worked up and purified as described above to give 2β-iodo-3-oxo$GA_4$ methyl ester (26) (170 mg), m.p. 151—153 °C (Found: C, 51.5; H, 4.8. $C_{20}H_{23}IO_5$ requires C, 51.1; H, 4.9).

ent-2β-*Deuterio*-10β-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (12).—A solution of the 2β-iodo ketone (26) (50 mg) and a crystal of 2,2′-dimethyl-2,2′-azopropionitrile in benzene (15 ml) were heated. Tri-n-butyltin deuteride (50 μl) was added and the mixture was refluxed for 3 h. The solution was poured into water and worked up as previously described to give mainly [2α-]$H_2$-3-oxo$GA_4$ methyl ester (25).

The product from the above reaction, without further purification, was stirred with sodium borohydride in methanol. After 1.5 h the mixture was worked up as before to give a gum which after purification by p.l.c. with ethyl acetate–light petroleum (6 : 4) afforded, from the band at $R_F$ 0.5, [2α-$^2$H]-3-*epi*-$GA_4$ methyl ester (12) (28 mg), m.p. 168—169 °C (lit.,[11] m.p. 166—167 °C) containing 0.82 deuterium atoms per molecule; $\delta_{2H}$ 1.45 [2α-]$H_2$ and 2.22 [2β-$^2$H]; $\delta_{1H}$ ($C_5D_5N$) 1.52 (s, 18-$H_3$), 2.82 (d, *J* 10.5 Hz, 5-H), 3.02 (d, *J* 10.5 Hz, 6-H), 3.72 (s, OMe), 3.92 (d, *J* 7 Hz, 3-H), and 4.91 and 4.99 (2 br s, 17-$H_2$).

Elution of the band at $R_F$ 0.65 gave [2α-$^2$H]-$GA_4$ methyl ester (13) (7 mg) containing 0.77 deuterium atoms per molecule; $\delta_{2H}$ 1.67 [2α-$^2$H].

[2α-$^2$H]-3-*epi*-$GA_4$ methyl ester (12) (25 mg), phosphoryl chloride (150 μl), and pyridine (10 ml) were refluxed for 3 h. Work-up and purification by p.l.c. with ethyl acetate–light petroleum (1 : 1) gave, at $R_F$ 0.8, [2α-$^2$H]-3β-chloro$GA_9$ methyl ester (14) (18 mg) as a gum; δ 1.19 (s, 18-$H_3$), 2.72 (d, *J* 10.5 Hz, 6-H), 3.28 (d, *J* 10.5 Hz, 5-H), 3.72 (s, OMe), 4.12 (d, *J* 3 Hz, 3-H), and 4.88 and 4.98 (2 br s, 17-$H_2$).

A solution of [2α-$^2$H]-3β-chloro$GA_9$ methyl ester (14) (15 mg), tri-n-butyltin deuteride (70 μl) and a crystal of 2,2′-dimethyl-2,2′-azopropionitrile in benzene (10 ml) was refluxed for 3 h. Work-up and purification by p.l.c. with ethyl acetate–light petroleum (1 : 1) gave, at $R_F$ 0.75, [2α-$^2$H]-$GA_9$ methyl ester (10) (10 mg), m.p. 134—136 °C (lit.,[10] m.p. 136 °C) containing 0.80 deuterium atoms per molecule; $\delta_{1H}$ (400 MHz) 1.58 [2α-$^2$H]; *m/z* 331 ($M^+$ 7%), 299 (100), 271 (70), 244 (34), 227 (51), 218 (23), 160 (19), and 191 (14).

[2α-$^2$H]-$GA_9$ methyl ester (10) (8 mg) in methanol (2 ml) and aqueous 2M-sodium hydroxide (5 ml) were refluxed for 8 h. The methanol was removed under reduced pressure and the aqueous residue diluted with water, adjusted to pH 2, and extracted with ethyl acetate. Removal of the solvent under reduced pressure gave a gum which was heated on a steam-bath for 0.5 h to re-form the lactone. The gum was diluted with ethyl acetate and backwashed with aqueous sodium hydrogencarbonate. Re-acidification of the aqueous solution and extraction with ethyl acetate gave [2α-$^2$H]-$GA_9$ (3) which crystallised from ethyl acetate–light petroleum as needles (4 mg), m.p. 205—207 °C (lit.,[10] m.p. 208—211 °C), containing 0.80 deuterium atoms per molecule; *m/z* 299 ($M^+$ − 18, 43%), 273 (100), 230 (75), 204 (50), 160 (30), 105 (23), and 91 (25).

*Reduction of* ent-2α-*Iodo*-10β-*hydroxy*-20-*nor*-3-*oxogibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (26).—(a) *With sodium borohydride in methanol.* To a stirred solution of the iodo ketone (26) (80 mg) in methanol (15 ml) was added sodium borodeuteride (20 mg). After 1.5 h the mixture was poured into water, acidified with hydrochloric acid, and extracted with ethyl acetate. P.l.c. of the recovered product with ethyl acetate–light petroleum (6 : 4) gave, from the band at $R_F$ 0.45, [3-$^2$H]-3-*epi*-$GA_4$ methyl ester (15) (50 mg) containing 0.85 deuterium atoms per molecule; $\delta_{2H}$ ($CHCl_3$) 3.7 [3-$^2$H]; $\delta_{1H}$ ($C_5D_5N$) 1.53 (s, 18-$H_3$), 2.82 (d, *J* 10.5 Hz, 5-H), 3.03 (d, *J* 10.5 Hz, 6-H), 3.72 (s, OMe), and 4.91 and 5.00 (2 br s, 17-$H_2$); *m/z* 347 ($M^+$ 22%), 329 (93), 315 (100), 301 (54), 287 (70), 269 (33), 242 (3), and 285 (22). Elution of the band at $R_F$ 0.6 gave [3-$^2$H]$GA_4$ methyl ester (16) (13 mg) containing 0.77 deuterium atoms per molecule; $\delta_{2H}$ ($C_6H_6$) 3.85 [3-$^2$H]; $\delta_{1H}$ 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), and 4.85 and 4.95 (2 br s, 17-$H_2$).

(b) *With sodium borohydride in methan*[$^2$H]*ol.* To a stirred solution of the iodo ketone (26) (80 mg) in methan[$^2$H]ol (10 ml) was added sodium borohydride (30 mg). After 1.5 h the reaction was worked up as described in (a) to give a 6 : 4 mixture of [2β-$^2$H]- and [2α-$^2$H]-3-*epi*-$GA_4$ methyl esters (11) and (12) (55 mg), containing 0.75 deuterium atoms per molecule; $\delta_{2H}$ 2.2 [2β-$^2$H] and 1.45 [2α-$^2$H]; $\delta_{1H}$ ($C_5D_5N$) 1.53 (s, 18-$H_3$), 2.82 (d, *J* 10.5 Hz, 5-H), 3.03 (d, *J* 10.5 Hz, 6-H), 3.71 (s, OMe), 3.94 (d, *J* 11 Hz, enclosing further d, *J* 7 Hz, 3-H), and 4.91 and 5.00 (2 br s, 17-$H_2$). Elution of the p.l.c. band at $R_F$ 0.6 gave [2ξ-$^2$H]-$GA_4$ methyl ester (19) (14 mg) containing 0.71 deuterium atoms per molecule; $\delta_{2H}$ ($C_6H_6$), 1.43 [2β-$^2$H] and 1.67 [2α-$^2$H].

(c) *With sodium borodeuteride in methan*[$^2$H]*ol.* To a stirred solution of the iodo ketone (26) (80 mg) in methan[$^2$H]ol (15 ml) was added sodium borodeuteride (20 mg). After 1.5 h the reaction was worked up as in (a) to give [2ξ,3-$^2H_2$]-3-*epi*-$GA_4$ methyl ester (17) (42 mg) containing 1.92 deuterium atoms per molecule; $\delta_{2H}$ ($CHCl_3$), 1.45 [2α-$^2$H], 2.22 [2β-$^2$H], and 3.68 [3-$^2$H]; *m/z* 348 ($M^+$, 55%), 330 (99), 316 (100), 302 (42), 288 (56), and 270 (23).

ent-16,17-*Dibromo*-10β-*hydroxy*-3-*oxo*-20-*norgibberellane*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (33).—3-Oxo$GA_4$ methyl ester (24) (150 mg) in tetrahydrofuran (20 ml) was stirred with lithium di-isopropylamide (0.9 ml; 0.5M solution) under nitrogen. The mixture was cooled to −70 °C and bromine (500 μl) was titrated into the solution. The solution was then poured into water, acidified with hydrochloric acid, and extracted with ethyl acetate. Removal of the solvent under reduced pressure followed by p.l.c. of the resulting mixture with ethyl acetate–light petroleum (1 : 1) gave, from the band at $R_F$ 0.7, 16,17-*dibromo*-3-*oxo*$GA_4$ *methyl ester* (33) (30 mg), m.p. 257—259 °C (Found: C, 47.9; H, 4.9. $C_{20}H_{24}Br_2O_5$ requires C, 47.6; H, 4.8); δ 1.17 (s, 18-$H_3$), 2.81 (d, *J* 10.5 Hz, 6-H), 3.11 (d, *J* 10.5 Hz, 5-H), 3.70 (s, OMe), and 3.88 (s, 17-$H_2$); *m/z* 504/502/500 ($M^+$, 25%), 472/470/468 (54), 424/422 (68), 392/390 (100), 343 (35), 283 (49), 199 (36), and 91 (36).

ent-2,2-*Dibromo*-10β-*hydroxy*-3-*oxo*-7-*norgibberell*-16-*ene* 7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (28).—The

above experiment was repeated, adding *N*-bromosuccinimide in place of bromine, to give in 20% yield 2,2-*dibromo*-3-*oxo*$GA_4$ *methyl ester* (28) as a gum (Found: C, 47.7; H, 4.8. $C_{20}H_{22}Br_2O_5$ requires C, 47.8; H, 4.4); δ 1.32 (s, 18-$H_3$), 2.81 (d, *J* 10.5 Hz, 6-H), 3.32 (d, *J* 10.5 Hz, 5-H), 3.48 (d, *J* 14 Hz, 1β-H), 3.78 (s, OMe), 3.84 (d, *J* 14 Hz, 1α-H), 4.85 and 4.95 (2 br s, 17-$H_2$); *m/z* 504/602/500 ($M^+$, 100%), 472/470/468 (58), 424/422 (75), 392/390 (37), 363 (27), 342 (30), 317 (22), 297 (52), 284 (29), and 239 (56).

ent-2α-*Bromo*-10β-*hydroxy*-3-*oxo*-7-*norgibberell*-16-*ene* 7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (27).—2β-Iodo ketone (26) (100 mg) in tetrahydrofuran (10 ml) was stirred with lithium bromide (50 mg) for 4 h at room temperature. The mixture was poured into water and extracted with ethyl acetate. The extract was washed with aqueous sodium thiosulphate and concentrated under reduced pressure to give 2β-*bromo*-3-*oxo*$GA_4$ *methyl ester* (27) which crystallised from ethyl acetate–light petroleum (89 mg) m.p. 158—160 °C (Found: C, 57.3; H, 5.5. $C_{20}H_{23}BrO_5$ requires C, 56.7; H, 5.4); δ 1.27 (s, 18-$H_3$), 2.34 (dd, *J* 14 Hz, *J* 11 Hz, 1β-H), 2.80 (d, *J* 10.5 Hz, 6-H), 3.20 (dd, *J* 14 Hz, *J* 8 Hz, 1α-H), 3.24 (d, *J* 10.5 Hz, 5-H), 3.73 (s, $^-$OMe), 4.77 (dd, *J* 11 Hz, *J* 8 Hz, 2α-H), and 4.88 and 5.00 (2 br s, 17-$H_2$); *m/z* 424/422 ($M^+$, 33%), 392/390 (100), 364/362 (40), 344 (14), 312 (28), 239 (92), 189 (19), and 91 (18).

ent-2β-*Deuterio*-3α,10β-*dihydroxy*-20-*norgibberell*-16-*ene* 7,19-*dioic Acid* 19,10-*Lactone* (2).—A solution of the 2β-bromo ketone (27) (200 mg), tri-n-butyltin deuteride (500 μl) and a crystal of 2,2′-dimethyl-2,2′-azopropionitrile in benzene (25 ml) was refluxed for 1.5 h. The solvent was removed under reduced pressure to give [2-$^2$H]-3-oxo$GA_4$ methyl ester (25) which was reduced without further purification.

Aluminium foil (3 g) and mercuric chloride (30 mg) were suspended in propan-2-ol (50 ml). After heating to reflux, carbon tetrachloride (0.5 ml) was added and the solution refluxed for 3 h. The above [2α-$^2$H]ketone (25) was added and refluxing was continued. After 3 h, addition to dilute hydrochloric acid and extraction with ethyl acetate gave an oil which was purified by p.l.c. Recovery from the band $R_F$ 0.5 gave the required [2α-$^2$H]-$GA_4$ methyl ester (13) (55 mg) containing 0.94 deuterium atoms per molecule; $\delta_{2H}$ ($C_6H_6$) 1.65 [2α-$^2$H], 1.47 [2β-$^2$H]; $\delta_{1H}$ 1.22 (s, 18-$H_3$), 2.79 (d, *J* 11 Hz, 6-H), 3.08 (d, *J* 11 Hz, 5-H), 3.70 (s, OMe), 3.82 (d, *J* 3 Hz, 3-H), and 4.85 and 4.95 (2 br s, 17-$H_2$); *m/z* 347 ($M^+$, 9%), 329 (18), 315 (100), 285 (57), 269 (44), 242 (23), and 225 (76). To a solution of sodium hydride (200 mg) in hexamethylphosphoramide (5 ml) was added propanethiol (0.7 ml) at 0 °C. After 1 h the above mixture (2.2 ml) was added to [2α-$^2$H]-$GA_4$ methyl ester (13) (40 mg). After 4 h the mixture was poured into dilute hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated sodium hydrogen carbonate; acidification of the aqueous phase and extraction with ethyl acetate gave, after purification by p.l.c. with chloroform–ethyl acetate–acetic acid (5 : 4 : 1) at $R_F$ 0.6, [2α-$^2$H]-$GA_4$ (2) (18 mg) containing 0.90 deuterium atoms per molecule; δ [$(CD_3)_2C{=}O$] 1.12 (s, 18-$H_3$), 2.84 (d, *J* 11 Hz, 6-H), 3.23 (d, *J* 11 Hz, 5-H), 3.98 (d, *J* 3 Hz, 3-H), and 4.85 and 4.95 (2 br s, 17-$H_2$); *m/z* 315 ($M - 18^+$, 16%), 287 (18), 271 (100), and 226 (23).

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