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ISOTOPE LABELLING IN RING A OF GIBBERELLIN A_{20}†

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Abstract: Full assignments of the ^{1}H-nmr chemical shifts of the ring A protons in gibberellin A_{20} methyl ester 13-acetate have been made on the basis of ^{1}H-, ^{2}H- and ^{13}C-nmr data of various deuteriated derivatives. These assignments have been used to prove that catalytic deuteriogenation of GA_5-16,17-epoxide-13-acetate is a syn-addition from the less hindered β-face accompanied by allylic exchange at C-1 giving isotopic labels at the 1β-, 2β- and 3β- positions. The position and stereochemistry of isotopic labelling was confirmed by comparison with an authentic sample of [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate prepared by methods which introduce deuterium stereoselectively at C-1, C-2 and C-3. The preparation of [2α-^{2}H]GA_{20} and [3α-^{2}H]GA_{20} is described.

INTRODUCTION

Gibberellin A_{20} (1) is a key intermediate in the biosynthesis of gibberellins (GAs) in higher plants. It is the immediate precursor of GA_{29} (2)[1], GA_1 (3)[2,3] and GA_5 (4)[4]. To study the enzymes which catalyse these metabolic conversions GA_{20} (1) is required stereospecifically labelled at carbons -2 and -3 with deuterium and tritium. The partial synthesis of unlabelled GA_{20} (1) from the available fungal GA_3 (5) has been extensively investigated[5-8]. This paper describes the preparation of 1-, 2- and 3-deuteriated derivatives of GA_{20} and the assignments of the chemical shifts of the ring A protons in the nmr spectra of GA_{20} methyl ester 13-acetate (7).

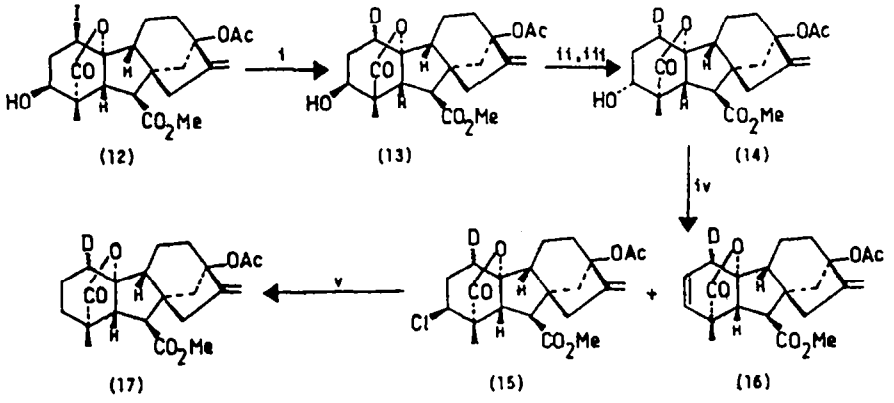
RESULTS AND DISCUSSION

The preparation of GA_{20} derivatives labelled with deuterium at positions -1, -2, and -3, and the discussion of the assignments of the chemical shifts of the protons at these positions, are presented under separate headings. The use of these assignments to determine the stereochemistry of catalytic deuteriogenation of GA_5 (4) is then described.

†This paper is dedicated to Professor R. Raphael, F.R.S., on the occasion of his 65th birthday.

Position -1. [1ξ-^{2}H]Gibberellin A_{20} methyl ester 13-acetate (17), containing 0.86 atoms of deuterium per molecule, was prepared as outlined in Scheme 1. Reduction of 1β-iodoGA_1 methyl ester 13-acetate (12)[9] with tri-n-butyl[^{2}H]stannane gave [1ξ-^{2}H]GA_1 methyl ester 13-acetate (13) which was oxidised with Jones reagent and the resultant 3-ketone (45) was then reduced with sodium borohydride to provide [1ξ-^{2}H]-3-epiGA_1 methyl ester 13-acetate (14). Treatment of the 3α-hydroxy epimer (14) with phosphoryl chloride yielded the 3β-chloride (15) and some (10%) of the olefin (16). Reduction of the 3β-chloride (15) with tri-n-butylstannane gave the required [1ξ-^{2}H]GA_{20} methyl ester 13-acetate (17).

The ^{13}C-nmr spectrum of [1ξ-^{2}H]GA_{20} methyl ester 13-acetate (17) showed a reduction in the intensity of the signal at δ30.40, compared to that of the unlabelled compound (7), confirming the presence of deuterium at carbon-1. Two signals were present in the ^{2}H-nmr spectrum of (17) at δ1.39 and δ2.05 in the ratio 2:1. To assign these signals the ^{2}H-nmr spectra of the four [1ξ-^{2}H] compounds (13), (14) (15) and (17) were compared (Table).



Reagents: (i) nBu_3SnD, AIBN; (ii) BN-CrO_3/H^+; (iii) $NaBH_4$, MeOH, (iv) $POCl_3$; (v) nBu_3SnH, AIBN.

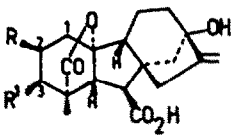
Scheme 1.

TABLE

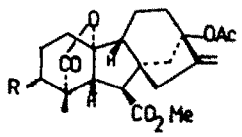
Chemical shifts of the signals in the ^{2}H-nmr spectra ($CHCl_3$)

Gibberellin methyl ester 13-acetate	1α-^{2}H	1β-^{2}H (ratio 1:2)
[1ξ-^{2}H]GA_{20} (17)	2.05	1.39
[1ξ-^{2}H]GA_1 (13)	1.87	1.87
[1ξ-^{2}H]-3-epiGA_1 (14)	2.04	1.51
[1ξ-^{2}H]-3βCl-GA_{20} (15)	1.96	1.81

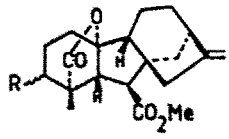
Changes in the functional group at carbon-3 had little effect on the chemical shift of the less intense signal at ~δ2.0 and was therefore assigned to the equatorial 1α-deuteron (proton). However the more intense signal at δ1.39 in [1ξ-^{2}H]GA_{20} methyl ester 13-acetate (17) was shifted downfield in the spectrum of the 3β-chloride (15) to δ1.81 as expected for a 1,3-diaxial relationship and was therefore assigned to the axial 1β-deuterium (proton). These assignments for the chemical shifts of the C-1 protons in (14) are in agreement with those previously found[10] for the non-13-hydroxylated analogue, 3-epiGA_4 methyl ester (27), namely δ1.51 and δ2.05 for the 1β- and 1α-protons respectively. Thus tri-n-butylstannane reduction of a 1-halide occurs predominantly with retention of configuration although with less stereoselectively than the corresponding reduction at carbon-3 (see later). These results show that the 1β- and 1α- protons in GA_{20} methyl ester 13-acetate (7) resonate at δ1.39 and δ2.05 respectively.



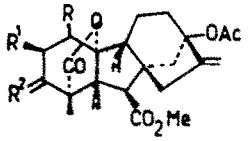
(1) R = R^1 = H
(2) R = OH; R^1 = H
(3) R = H; R^1 = OH
(4) $\Delta^{2,3}$-ene
(5) R^1 = OH; $\Delta^{1,2}$-ene
(6) R = H; R^1 = D



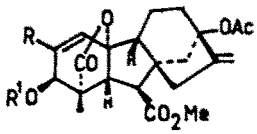
(7) R = H
(8) R = β-D
(9) R = β-Cl
(10) R = β-OH
(11) R = D



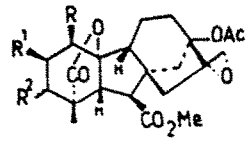
(27) R = α-OH
(28) R = H
(29) R = β-Cl



(45) R = D; R^1 = H; R^2 = O
(46) R = R^1 = β-D; R^2 = O
(47) R = R^1 = H; R^2 = β-Cl, α-D



(48) R = R^1 = H
(49) R = D; R^1 = Ms

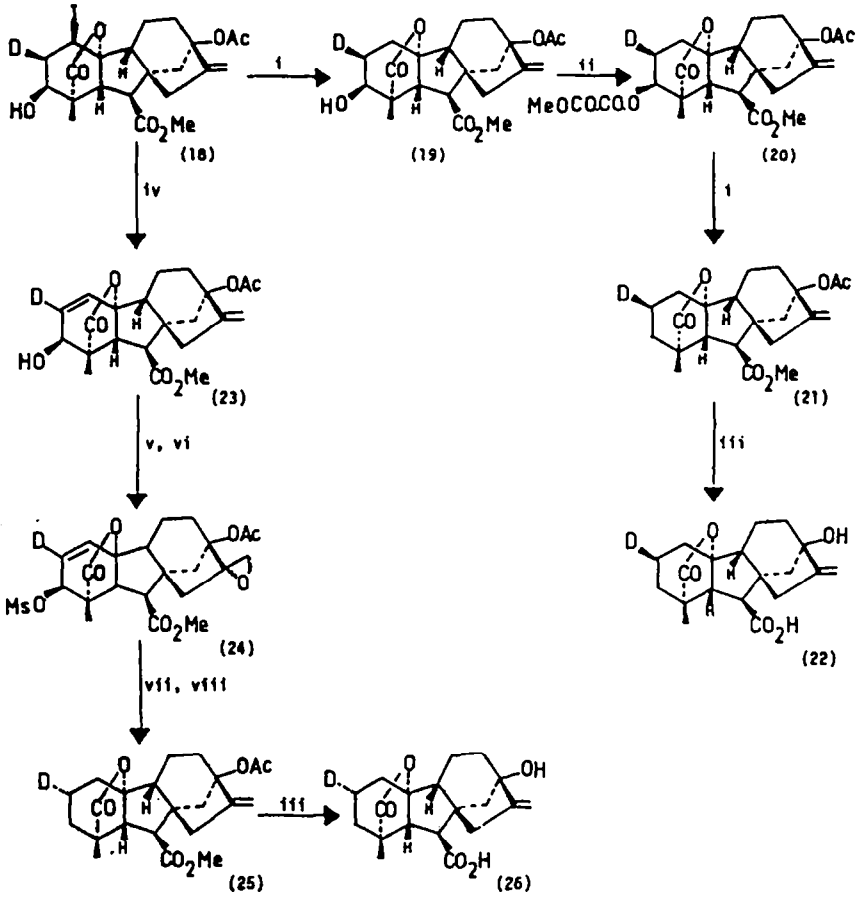


(50) R = R^1 = H; R^2 = α-D
(51) R = R^1 = D; R^2 = β-OH
(52) R = R^1 = R^2 = β-D

Position -2. The 2α- and 2β-proton signals in the nmr spectrum of GA_{20} methyl ester 13-acetate (7) were assigned from the ^{2}H-nmr spectra of the [2β-^{2}H]- and [2α-^{2}H] derivatives (21) and (25).

[2β-^{2}H]Gibberellin A_1 methyl ester 13-acetate (19) has previously been prepared[9] and the stereochemistry of the isotopic label determined by nmr studies. Treatment of (19) with oxalyl chloride and quenching the reaction with methanol gave the ester (20)[11] (Scheme 2). Tri-n-butyl-stannane reduction of (20) gave a 3:5 mixture of [2β-^{2}H]GA_1 methyl ester 13-acetate (19) and the required [2β-^{2}H]GA_{20} methyl ester 13-acetate (21) containing 0.96 atoms of deuterium. Hydrolysis of (21) with aqueous sodium hydroxide gave [2β-^{2}H]GA_{20} (22). [2α-^{2}H]Gibberellin A_{20} (26) was prepared as shown in Scheme 2. [2β-^{2}H]-1β-IodoGA_1 methyl ester 13-acetate (18), containing 0.88 atoms of deuterium per molecule, was prepared from GA_3 (5) as previously described[9] and was de-hydroiodinated with 1,8-diazabicyclo[5.4.0]undec-7-ene, to give [2-^{2}H]GA_3 methyl ester 13-acetate (23) without loss of deuterium. Methanesulphonation of the [2-^{2}H]GA_3 derivative (23) followed by epoxidation gave (24) which was hydrogenated to [2α-^{2}H]GA_{20} methyl ester 13-acetate 16,17-epoxide (50). Deoxygenation of the epoxide (50) followed by hydrolysis of the acetate and ester functions gave [2α-^{2}H]GA_{20} (26, Scheme 2) containing 0.85 atoms of deuterium per molecule.

The ^{13}C-nmr spectra of both [2β-^{2}H]GA_{20} methyl ester 13-acetate (21) and [2α-^{2}H]GA_{20} methyl ester 13-acetate (25) showed a reduction in the intensity of the signal at δ19.11, compared to that of the unlabelled compound (7), confirming the presence of deuterium at carbon-2. The ^{2}H-nmr spectrum of [2β-^{2}H]GA_{20} methyl ester 13-acetate (21) displayed a single broad signal at δ1.73 while that of [2α-^{2}H]GA_{20} methyl ester 13-acetate (25) showed one broad signal at δ1.57. Thus it may be concluded that 2β-H resonates at δ1.73 and 2α-H at δ1.57 in GA_{20} methyl ester 13-acetate (7). These chemical shifts are almost identical to those previously determined[12] for the 2α- and 2β-protons in the 13-deoxy analogue, GA_9 methyl ester (28).



Reagents: (i) nBu_3SnH, AIBN; (ii) $(COCl)_2$ then MeOH; (iii) 2N-NaOH; (iv) DBU; (v) MsCl, pyridine; (vi) mClpBA; (vii) H_2, 10% Pd on $CaCO_3$, MeOH, pyridine; (viii) NaI, NaOAc, Zn, AcOH.

Scheme 2.

Position-3. A mixture of [3α- and 3β-^{2}H]GA_{20} methyl ester 13-acetate (4) and (8), containing 0.76 atoms of deuterium per molecule, was prepared by the tri-n-butyl[^{2}H]stannane reduction of the known 3β-chloroGA_{20} methyl ester 13-acetate (9)[7]. In the ^{13}C-nmr spectrum the signal at δ34.5 in the unlabelled compound (7) was reduced in intensity in the spectrum of the mixture of the [3ξ-^{2}H] compounds (11), confirming that deuterium was solely on carbon-3. The ^{2}H-nmr spectrum of the [3ξ-^{2}H] mixture (11) displayed signals at δ1.51 and δ1.68 in the ratio 5:1. Beale et al.[7] have shown that tri-n-butylstannane reduction of 3β-chloroGA_9 (29) occurred mainly with retention of configuration. From this analogy the signals at δ1.51 and δ1.68 in the ^{2}H-nmr spectrum of [3ξ-^{2}H]GA_{20} methyl ester 13-acetate (11) were assigned to the 3β- and 3α-deuterons respectively. This was confirmed by the preparation of [3α-^{2}H]GA_{20} (43) and examination of its ^{2}H-nmr spectrum (see later).

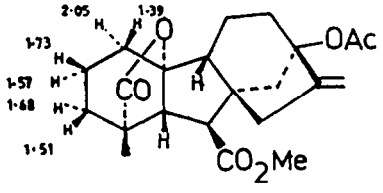
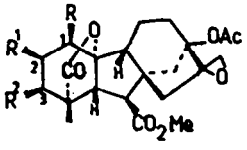
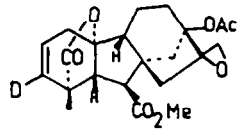


Figure 1.

The full assignments of the ^{1}H-nmr chemical shifts of the ring A protons in GA_{20} methyl ester 13-acetate (7) are shown in Figure 1. These assignments can be used to examine the stereochemistry of isotopic label introduced into GA_{20} methyl ester 13-acetate (7) under various conditions.



(53) R = H; $\Delta^{2,3}$-ene
(54) R^2 = OH; $\Delta^{1,2}$-ene
(55) R = R^1 = D; R^2 = OH



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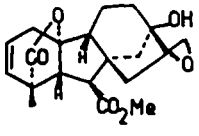

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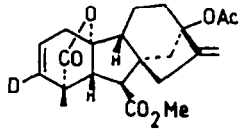
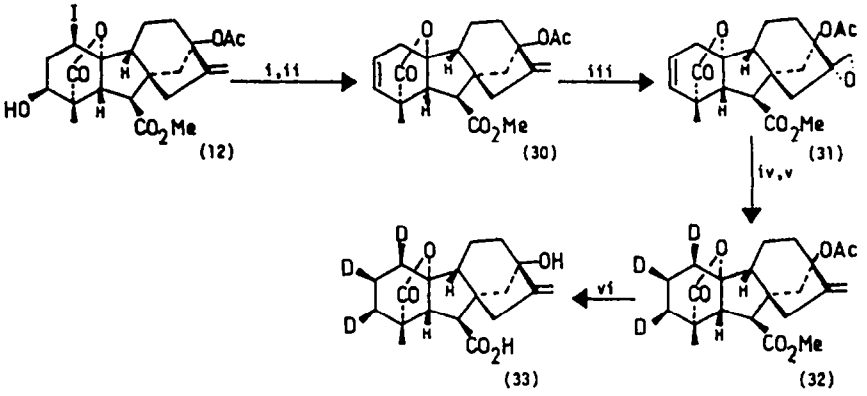


(58)

[1β,2β,3β-$^{2}H_3$]Gibberellin A_{20} (33). The tritiation of GA_5 methyl ester-16,17-epoxide (57) has been used as a method for introducing tritium into ring A of GA_{20} (1)[6]. It has the advantage of giving [^{3}H]GA_{20} with high specific radioactivity, a requirement for the successful examination of enzymatic processes in higher plants. Thus the stereochemistry of isotopic labelling was examined.

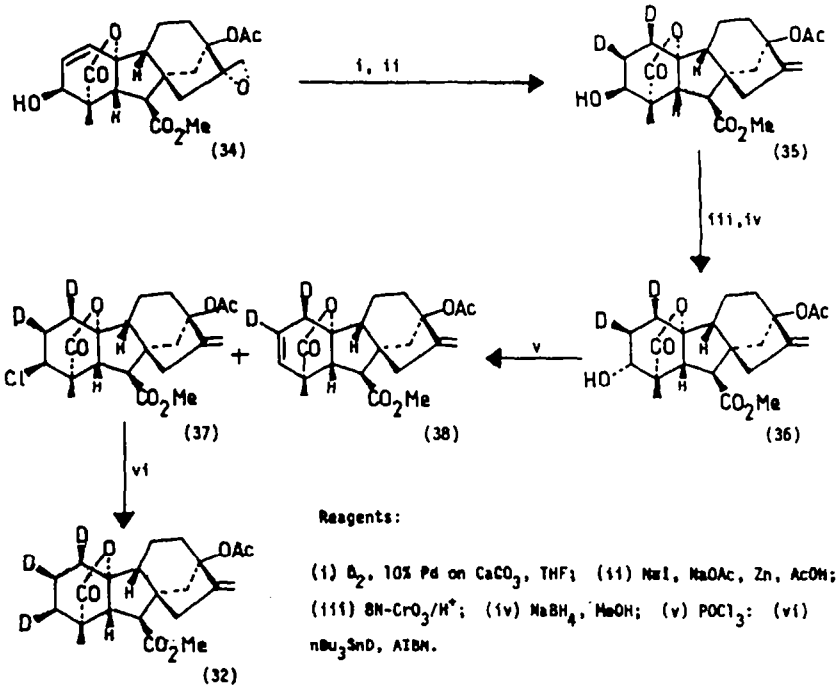
Deuteriogibberellin A_{20} was prepared as outlined in Scheme 3. It was advantageous to protect the 13-hydroxyl group as the acetate; this enhances the yields of the GA_5 derivative (30) on treatment of GA_1 methyl ester 13-acetate (10) with phosphoryl chloride, and prevents the formation of isomers of GA_{20} on regeneration of the exocyclic double bond. The mass spectrum of the deuterioGA_{20} methyl ester 13-acetate revealed an incorporation of 23% 3 deuterium atoms, 41% 2 deuterium atoms and 28% 1 deuterium atom while the ^{13}C-nmr spectrum showed a reduction in the signals at δ19.20, δ30.40 and δ34.50, compared with the ^{13}C-nmr spectrum of the unlabelled compound (7), indicating that deuterium was located at carbons-2, -1 and -3 respectively. Therefore during the reduction of GA_5-16,17-epoxide (31) proton-deuterium exchange at C-1 had occurred; proton-deuterium exchanges have previously been noted on the surface of heterogeneous catalysts[13]. The ^{2}H-nmr of [1,2,3-$^{2}H_3$]GA_{20} methyl ester 13-acetate (32) displayed signals at δ1.41, δ1.50 and δ1.77 in the approximate ratio 1:1:1. These signals were assigned to 1β-^{2}H, 3β-^{2}H and 2β-^{2}H respectively from the established assignments shown in Figure 1. Thus catalytic deuteriogenation of GA_5-16,17-epoxide (31) is a syn-process from the less hindered β-face with exchange at C-1 occurring on the surface of the catalyst.



Reagents: (i) $NaBH_4$, DMSO; (ii) $POCl_3$; (iii) mClpBA; (iv) D_2, 10% Pd on $CaCO_3$, THF; (v) NaI, NaOAc, Zn, AcOH; (vi) 2N-NaOH.

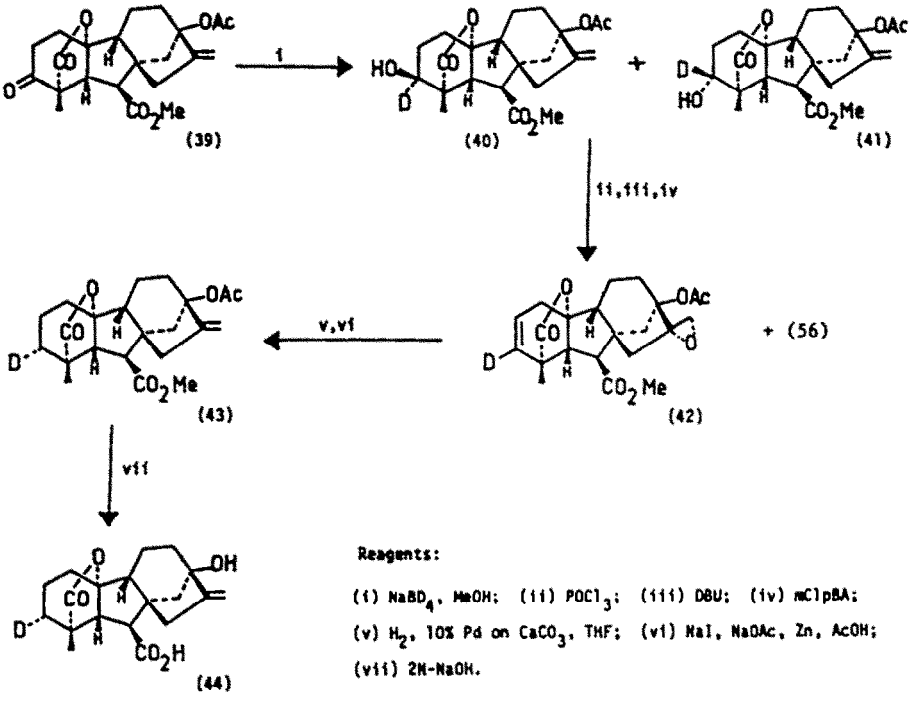
Scheme 3

To confirm the stereochemistry of [1β,2β,3β-$^2H_3$]GA_{20} (33), prepared by the catalytic deuteriogenation of GA_5-16,17-epoxide (31), [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate (32) of known stereochemistry was synthesised (Scheme 4) and the nmr spectra compared. Catalytic deuteriogenation of GA_3 methyl ester 13-acetate 16,17-epoxide (34) has been shown to give [1β,2β-$^2H_2$]GA_1 methyl ester 13-acetate 16,17-epoxide (51)[9]. This reaction was repeated, without pre-washing the 10% palladium on calcium carbonate catalyst with D_2O, to give [1β,2β-$^2H_2$]GA_1 methyl ester 13-acetate (35) containing 1.26 atoms of deuterium. A moderately low incorporation of deuterium was required in order to differentiate between the (broad) ^2H-nmr signals due to 2-^2H and 3-^2H in the final product (2α-^2H resonates at δ1.57 and 3β-^2H at δ1.51). Oxidation of (35) followed by reduction gave [1β,2β-$^2H_2$]-3-epiGA_1 methyl ester 13-acetate (36) with no loss of isotopic label. Treatment of the 3-epimer (36) with phosphoryl chloride afforded the 3β-chloro compound (37), containing 1.32 atoms of deuterium, and the olefin (38), with 0.84 atoms of deuterium per molecule. The loss of deuterium in the formation of (38) from (36) indicated a mixture of cis- and trans-elimination. To check this conclusion it was confirmed that treatment of the chloride (37) with 1,8-diazabicyclo [5.4.0]undec-7-ene gave the olefin (38) containing 1.30 atoms of deuterium per molecule, as expected from a trans elimination of the 3β-chloride and 2α-hydrogen. Reduction of a 3β-chloride with tri-n-butyl[^2H]stannane has been shown to occur with 85% retention of configuration. Thus reduction of [1β,2β-$^2H_2$]3β-chloroGA_{20} methyl ester 13-acetate (37) gave mainly the required [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate (32). The ^2H-nmr spectrum of (32) displayed signals at δ1.40, δ1.50 and δ1.76, assigned to 1β-^2H, 3β-^2H and 2β-^2H respectively, and as expected the signal at δ1.50, due to 3β-^2H, was the most intense. A low intensity signed at δ1.68, assigned to 3α-^2H, was also present. These results confirm that catalytic deuteriogenation of GA_5-16,17-epoxide (31) is a syn-process from the less hindered β-face resulting in isotopic labels at the 1β,2β and 3β positions.



Scheme 4.

[3α-^{2}H]Gibberellin A_{20}. [3α-^{2}H]Gibberellin A_{20} (44) was prepared as shown in Scheme 5. The known GA_1-3-ketone 13-acetate (39) was reduced with sodium borodeuteride to give a mixture of 3-epimeric alcohols (40) and (41) which were treated with phosphoryl chloride and then with 1,8-diazabicyclo[5.4.0]undec-7-ene to give [3-^{2}H]-GA_5 methyl ester 13-acetate (58). Protection of the 16,17-ene in (58) by formation of the mixed epoxides (42) and (56) then catalytic hydrogenation followed by regeneration of the 16,17-double bond gave [3α-^{2}H] GA_{20} methyl ester 13-acetate (43). The [3α-^{2}H] signal in the ^{2}H-nmr spectrum of (43) occurred at δ1.70. Hydrolysis of (43) gave [3α-^{2}H]GA_{20} (44) containing 0.96 atoms of deuterium per molecule.



Scheme 5.

CONCLUSION

The ^{2}H-nmr data of the deuteriated derivatives of GA_{20} methyl ester 13-acetate (7), combined with the ^{13}C- and ^{1}H-nmr of these compounds, permits the assignment of the ring A proton signals as shown in Figure 1. In the course of this work [1β,2β,3β-$^2H_3$]GA_{20} (33, Scheme 3 and 4), [2β-^{2}H]GA_{20} (22, Scheme 2) [2α-^{2}H]GA_{20} (26, Scheme 2) and [3α-^{2}H]GA_{20} (44, Scheme 5) have been prepared with high deuterium incorporation and with proven high stereoselectivity. [3β-^{2}H]-Gibberellin A_{20} (6) has been prepared containing ca. 15% of the [3α-^{2}H]isomer. The use of these labelled compounds in the study of enzymatic conversions of GA_{20} in plants will be presented elsewhere.

EXPERIMENTAL

All solvents were redistilled before use. Light petroleum refers to the fraction boiling at 60-80°C. T.l.c. was carried out on Merck Kieselgel 60HF254 and visualised by spraying with 5% H_2SO_4 in EtOH followed by heating to 120°C for 5 min. For flash chromatography[18] Merck Kieselgel 60 (40-63 μm) was used. N.m.r. chemical shifts were determined in deuteriochloroform unless otherwise stated and are relative to internal tetramethylsilane. G.l.c. and g.c.-mass spec. analyses were on capillary glass WCOT OV-1 columns with derivatisation, where necessary, as methyl esters $SiMe_3$ ethers.

Work-up comprised addition to water, acidification with HCl to pH 3 and recovery in ethyl acetate.

Reduction of 1β-IodoGA$_1$ methyl ester 13-acetate (12). 1β-Iodogibberellin A_1 methyl ester 13-acetate (12) (0.48 g) in toluene (50 ml) was refluxed with [^{2}H]-tri-n-butylstannane (350 μl) in the presence of 2,2'-azobis(2-methylpropionitrile) for 1h. The solvent was removed in vacuo and purification of the product by flash chromatography, eluting with 70% ethyl acetate in light petroleum gave [1ξ-^{2}H]GA$_1$ methyl ester 13-acetate (13) (310 mg) m.p. 137-140°C (from ethyl acetate-light petroleum) (the unlabelled compound was previously isolated[14] as a gum) containing 0.90 atoms of deuterium per molecule. ^{2}H-nmr: δ ($CHCl_3$) 1.87 (br.s) ^{1}H-nmr: δ 1.14 (s, 18-H_3), 2.03 (s, 13-OAc), 2.70 (d, J=11Hz, 6-H), 3.22 (d, J=11Hz, 5-H), 3.72 (s, -OMe), 3.83 (m, 3-H), 4.99 and 5.14 (2 br.s, 17-H_2); m/z 405 (M^+, 10%), 363 (43), 283 (23), 55 (15) and 43 (100).

ent-1ξ-Deuterio-13-acetoxy-3β,10β-dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (14). [1ξ-^{2}H]Gibberellin A_1 methyl ester 13-acetate (13) (0.28 g) in acetone (30 ml) was treated dropwise with Jones reagent for 1h at room temperature. Work-up gave a gum which was immediately reduced with sodium borohydride (500 mg) in methanol (30 ml) with stirring for 0.75h. Work-up, followed by flash chromatography and elution with 35% ethyl acetate in light petroleum gave sequentially unchanged [1ξ-^{2}H]GA$_1$ methyl ester 13-acetate (13) (50 mg), then [1ξ-^{2}H]-3-epiGA$_1$ methyl ester 13-acetate (14) (200 mg) 141-143°C (Lit.[7] m.p. of unlabelled compound 143-145°C) containing 0.89 atoms of deuterium per molecule; ^{2}H-nmr: δ ($CHCl_3$) 1.51 (br.s, 1β-^{2}H) and 2.14 (br.s, 1α-^{2}H) in the ratio 7:3; ^{1}H-nmr: δ 1.18 (s, 18-H_3), 2.02 (s, 13-OAc), 2.56 (d, J=10 Hz, 5-H), 2.76 (d, J=10Hz, 6-H), 3.64 (m, 3-H), 3.73 (s, -OMe), 4.98 and 5.13 (2 br.s, 17-H_2); m/z 405 (M^+, 11%), 363 (41), 345 (14), 285 (14), 91 (15) and 43 (100).

ent-1ξ-Deuterio-13-acetoxy-10β-hydroxy-20-norgibberell-16-ene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (17). [1ξ-^{2}H]-Epigibberellin A_1 methyl ester 13-acetate (14) (300 mg) in pyridine (20 ml) was refluxed with phosphoryl chloride (0.5 ml) for 1h. Work-up gave a gum which on purification by flash chromatography eluting with 25% ethyl acetate in light petroleum gave sequentially: (a) [1ξ-^{2}H]GA$_5$ methyl ester 13-acetate (16) (50 mg) m.p. 131-134°C (from ethyl acetate-light petroleum (Found: M^+, 387. 1815 $C_{22}H_{25}O_6D$ requires M, 387. 1792); ^{1}H-nmr: δ 1.23 (s, 18-H_3), 2.02 (s, 13-OAc), 2.67 (d, J=10Hz, 6-H), 2.81 (d, J=10Hz, 5-H), 3.73 (s, -OMe), 5.00 and 5.13 (2 br.s, 17-H_2), 5.67 (d,d, J=2Hz and 9Hz, 3-H) and 5.80 (d,d, J=3Hz and 9Hz,2-H); m/z 387 (M^+,8%), 347 (26), 284 (26), 283 (100), 224 (25), 233 (24) and 43 (92); and (b) [1ξ-^{2}H]3β-chloroGA$_{20}$ methyl ester 13-acetate (15) (250 mg) m.p. 140-143°C (lit.[7] m.p. of unlabelled compound 125-127°C); ^{2}H-nmr: δ ($CHCl_3$) 1.81 (br.s, 1β-^{2}H) and 1.96 (br.s, 1α-^{2}H) in the ratio 7:3; ^{1}H-nmr: δ 1.19 (s, 18-H_3), 2.03 (s, 13-OAc), 2.71 (d, J=10.5 Hz, 6-H), 3.30 (d, J=10.5 Hz, 5-H), 3.73 (s, -OMe), 4.12 (m, 3-H). 5.00 and 5.16 (2 br.s, 17-H_2) m/z 425 (M^+, 3%), 423 (15), 381 (42), 363 (16), 283 (20), 91 (15) and 43 (100).

[1ξ-^{2}H]3β-Chlorogibberellin A_{20} methyl ester 13-acetate (15) (200 mg) in toluene (20 ml) was refluxed with tri-n-butylstannane (300 μl) in the presence of 2,2'-azobis(2-methylpropionitrile) for 0.6h. Evaporation of the solvent in vacuo and purification of the product by flash chromatography eluting with 20% ethyl acetate in light petroleum gave [1ξ-^{2}H]GA$_{20}$ methyl ester 13-acetate (17) (140 mg) m.p. 113-115°C (lit.[7] m.p. of unlabelled compound 111-113°C) containing 0.86 atoms of deuterium per molecule; ^{2}H-nmr: δ ($CHCl_3$) 1.39 (br.s, 1β-^{2}H) and 2.05 (br.s, 1α-^{2}H) in the ratio 2:1; ^{1}H-nmr; δ 1.08 (s, 18-H_3), 2.02 (s, -OAc), 2.57 (d, J=10 Hz, 5-H), 2.71 (d, J=10Hz, 6-H), 3.72 (s, -OMe), 4.98 and 5.13 (2 br.s, 17-H_2); ^{13}C-nmr: δ 17.03 (C-18), 17.22 (C-11), 19.11 (C-2), 30.50 (C-1), 34.39 (C-3), 38.26 (C-12), 42.99 (C-14), 45.36 (C-15), 48-89 (C-4), 49.52 (C-8), 51.43 (C-6), 51.88 (-OCH_3), 53.05 (C-9), 58.56 (C-5), 78.17 (C-13), 92.62 (C-10), 107.08 (C-17), 156.86 (C-16), 173.05 (C-7), 179.00 (C-19); m/z 389 (M^+, 21%), 347 (100), 329 (40), 301 (33), 287 (38), 285 (40) and 43 (91).

ent-2α-Deuterio-10β,13-dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone (22). [2β-^{2}H]-1β-Iodogibberellin A_1 methyl ester 13-acetate (18) (0.5 g) in toluene (50 ml) was refluxed with tri-n-butylstannane in the presence of 2,2'-azobis(2-methylpropionitrile) for 1h. The solvent was removed in vacuo and purification of the product by flash chromatography eluting with 50% ethyl acetate in light petroleum gave [2β-^{2}H]GA$_1$ methyl ester 13-acetate (19) (350 mg) containing 0.88 atoms of deuterium per molecule.

[2β-^{2}H]GA$_1$ methyl ester 13-acetate (19) (176 mg) was refluxed with oxalyl chloride (50 μl) in tetrahydrofuran (2 ml) for 1.5h. Methanol was added then the mixture diluted with water, acidified with dilute hydrochloric acid and extracted with ethyl acetate as usual to give the crude ester (20) (118 mg). The ester (20) (110 mg) in toluene (10 ml) was refluxed with tri-n-butylstannane (300 μl) in the presence of 2,2'-azobis (2-methylpropionitrile) for 2h. The solvent was removed in vacuo and purification of the product by flash chromatography eluting with 50% ethyl acetate in light petroleum gave [2β-^{2}H]GA$_{20}$ methyl ester 13-acetate (21) (53 mg) identified by its ^{1}H-nmr, ^{2}H-nmr and mass spectra.

[2β-^{2}H]GA_{20} methyl ester 13-acetate (21) (50 mg) in methanol (1 ml) and aqueous sodium hydroxide (5 ml, 2N) was refluxed for 7h. Work-up gave a gum which was purified by flash chromatography eluting with 50% ethyl acetate in light petroleum containing 1% acetic acid to give [2β-^{2}H]GA_{20} (22) (5.6 mg) containing 0.93 atoms of deuterium per molecule; ^{1}H-nmr: δ [$(CD_3)_2CO$] 1.05 (s, 18-H_3), 2.58 (s, 5- and 6-H), 4.95 and 5.36 (2 br.s, 17-H_2). m/z (Me ester, $SiMe_3$ ether) 419 (M^+, 100%), 404 (13), 390 (4), 375 (51), 360 (15), 302 (13), 207 (29) and 73 (16).

ent-2-Deuterio-13-acetoxy-16β,17-epoxy-3α,10β-dihydroxy-20-norgibberell-1-ene-7,19-dioic Acid 3-Methanesulphonyl Ester 7-Methyl Ester 19,10-Lactone (24). [2β-^{2}H]18-Iodogibberellin A_1 methyl ester 13-acetate (18) (365 mg, containing 0.88 atoms of deuterium per molecule) in toluene (10 ml) was refluxed with 1,8-diazabicyclo[5.4.0]undec-7-ene (370 μl) for 1h. The toluene was removed *in vacuo* and the product purified by flash chromatography eluting with ethyl acetate:light petroleum (1:1) to give [2-^{2}H]GA_3 methyl ester 13-acetate (23) (258 mg) containing 0.87 atoms of deuterium; m.p. 144-146°C (Lit.[16] m.p. of unlabelled compound 148-150°C); ^{1}H-nmr: δ 1.25 (s, 18-H_3), 2.02 (s, -OAc), 2.81 (d, J=10.5 Hz, 6-H), 3.22 (d, J=10.5 Hz, 5-H), 3.72 (s, -OMe), 4.15 (s, 3-H), 5.00 and 5.17 (2 br.s, 17-H_2) and 6.31 (s, 1-H); m/z 403 (M^+, 5%), 372 (6), 361 (13), 343 (14), 311 (9), 298 (14), 281 (18), 238 (19), 91 (17) and 43 (100).

[2-^{2}H]Gibberellin A_3 methyl ester 13-acetate (23) (250 mg) in pyridine (10 ml) was stirred with methanesulphonyl chloride (200 μl) for 2h at room temperature. The usual work-up followed by purification by flash chromatography eluting with 35% ethyl acetate in light petroleum gave [2-^{2}H]GA_3 methyl ester 3-mesylate 13-acetate (49) (175 mg) containing 0.87 atoms of deuterium; ^{1}H-nmr: δ 1.28 (s, 18-H_3), 2.03 (s, -OAc), 2.79 (d, J=11Hz, 6-H), 3.10 (s, -OMs), 3.31 (d, J=11Hz, 5-H), 3.75 (s, -OMe), 5.02 (br.s, 17-H), 5.06 (s, 3-H), 5.19 (br.s, 17-H) and 6.47 (s, 1-H); m/z 439 ($M-42^+$, 2%), 358 (11), 341 (27), 299 (17), 281 (87), 222 (70), 156 (22), 79 (23) and 43 (100).

[2-^{2}H]Gibberellin A_3 methyl ester 13-acetate 3-mesylate (49) (150 mg) in chloroform (15 ml) was treated with m-chloroperbenzoic acid (80 mg) overnight at room temperature. The mixture was diluted with chloroform, washed with saturated aqueous sodium hydrogen carbonate and then with water and concentrated *in vacuo*. Purification by flash chromatography eluting with 65% ethyl acetate in light petroleum gave [2-^{2}H]gibberellin A_3 methyl ester 3-mesylate 13-acetate 16α,17-epoxide (24) as a gum (108 mg) containing 0.86 atoms of deuterium per molecule. (Found: M^+, 497.1461 $C_{23}H_{27}O_{10}S\ ^2H$ requires M, 497.1465); ^{1}H-nmr: δ 1.25 (s, 18-H_3), 2.00 (s, -OAc), 2.68 (d, J=11Hz, 6-H), 2.70 (d, J=4Hz, 17-H), 2.96 (d, J=4Hz, 17-H), 2.98 (s, -OMs), 3.22 (d, J=11Hz, 5H), 3.72 (s, -OMe), 4.91 (s, 3-H) and 6.35 (s, 1-H); m/z 497 (M^+, 2%), 466 (4), 455 (13), 398 (8), 297 (100), 198 (27), 156 (41), 79 (18) and 43 (63).

ent-2β-Deuterio-10β,13-dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone (26). [2-^{2}H]-Gibberellin A_3 methyl ester 3-mesylate-13-acetate-16α,17-epoxide (24) (100 mg) in ethyl acetate (15 ml) and pyridine (0.5 ml) was stirred under an atmosphere of hydrogen in the presence of 10% palladium on calcium carbonate (40 mg) for 4h at room temperature. The mixture was diluted with ethyl acetate and filtered. The filtrate was concentrated *in vacuo* to give a gum (105 mg).

Sodium iodide (400 mg) and sodium acetate (150 mg) in acetic acid (15 ml) were stirred with freshly activated zinc (200 mg). The above crude product (105 mg) in acetone (5 ml) and water (0.5 ml) was added dropwise and stirring continued for a further 4h at room temperature. Work-up followed by purification by flash chromatography eluting with 30% ethyl acetate in light petroleum gave [2α-^{2}H]GA_{20} methyl ester 13-acetate (25) (30 mg) containing 0.86 atoms of deuterium m.p. 114-116°C (Lit.[7] m.p. of unlabelled compound 111-113°C); ^{2}H-nmr: δ ($CHCl_3$) 1.57 (2α-^{2}H); ^{1}H-nmr: δ 1.08 (s, 18-H_3), 2.02 (s, -OAc), 2.57 (d, J=10Hz, 5-H), 2.71 (d, J=10Hz, 6-H), 3.73 (s, -OMe), 4.98 and 5.12 (2 br.s, 17-H_2); m/z 398 (M^+, 24%), 347 (100), 329 (42), 301 (32), 287 (45), 285 (40) and 43 (89).

[2α-^{2}H]Gibberellin A_{20} methyl ester 13-acetate (25) (30 mg) in methanol (0.5 ml) and 2N-sodium hydroxide (15 ml) was refluxed for 6h. The usual work-up gave a gum which crystallised from acetone:light petroleum to give [2α-^{2}H]GA_{20} (26) (12 mg) containing 0.87 atoms of deuterium m.p. 234-236°C (Lit.[17] m.p. of unlabelled sample 232-233°C); ^{1}H-nmr: δ [$(CD_3)_2CO$] 1.05 (s, 18-H_3), 2.58 (s, 5- and 6-H), 4.95 and 5.35 (2 br.s, 17-H_2). m/z (7-methyl ester 3,13-diTMSi ether) 419 (M^+, 100%), 404 (10), 375 (68), 360 (17), 302 (12), 207 (29), and 73 (19).

ent-3ξ-Deuterio-13-acetoxy-10β-hydroxy-20-norgibberell-16-ene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (11). 3β-Chlorogibberellin A_{20} methyl ester 13-acetate (9) (200 mg) in toluene (20 ml) was refluxed with [^{2}H]tri-n-butylstannane (200 μl) in the presence of 2,2'-azobis(2-methylpropionitrile) for 1h. Evaporation of the solvent *in vacuo* and purification of the product by flash chromatography eluting with 30% ethyl acetate in light petroleum gave [3ξ-^{2}H]GA_{20} methyl ester 13-acetate (11) (135 mg) m.p.113-114°C (Lit.[7] m.p. of unlabelled compound 111-113°C) containing 0.76 atoms of deuterium per molecule; ^{2}H-nmr: δ ($CHCl_3$) 1.51 (br.s, 3β-^{2}H) and 1.68 (br.s, 3α-^{2}H) in the ratio 5:1.

ent-1α,2α,3α-Trideuterio-10β,13-dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 19,10-Lactone (33). 1β-Iodogibberellin A_1 methyl ester 13-acetate (12) (500 mg) in dimethylsulphoxide (20 ml) was stirred with sodium borohydride (150 ml) for 2h at room temperature. Work-up gave crude GA_1 methyl ester 13-acetate (10) (345 mg) which was refluxed in pyridine with phosphoryl chloride (350 μl) for 1h. Work-up followed by purification by flash chromatography eluting with 25% ethyl acetate in light petroleum gave GA_5 methyl ester 13-acetate (30) (150 mg). GA_5 methyl ester 13-acetate (30) (400 mg) in chloroform (30 ml) was treated with m-chloroperbenzoic acid (200 mg) for 12h at 5°C. The reaction mixture was diluted with chloroform, washed sequentially with aqueous sodium sulphite, aqueous sodium hydrogen carbonate and water, then the solvent was removed *in vacuo*. Purification by flash chromatography eluting with 40% ethyl acetate in light petroleum gave GA_5 methyl ester 13-acetate-16α,17-epoxide (31) (270 mg) as a gum; ^{1}H-nmr: δ 1.22 (s, 18-H_3), 2.01 (s, 13-OAc), 2.70 (d, J=10Hz, 6-H), 2.75 (d, J=5Hz, 17-H), 2.82 (d, J=10Hz, 5-H), 3.11 (d, J=5Hz, 17-H), 3.73 (s,-OMe), 5.80 (m, 2-H and 3-H).

Gibberellin A_5 methyl ester 13-acetate-16α,17-epoxide (31) (70 mg) in tetrahydrofuran (15 ml) was stirred with 10% palladium on calcium carbonate (3 mg) under an atmosphere of deuterium for 1h at room temperature. The mixture was diluted with ethyl acetate and filtered. The filtrate was concentrated in vacuo and deoxygenated as follows. Sodium iodide (400 mg) and sodium acetate (150 mg) in glacial acetic acid (15 ml) and water (0.5 ml) were stirred with freshly activated zinc (200 mg). Crude [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate-16α,17-epoxide (52) (from above) in acetone (5 ml) was added and stirring continued for 4h at room temperature. The reaction mixture was diluted with ethyl acetate and filtered; the filtrate was washed with water then concentrated in vacuo. Purification by flash chromatography eluting with 30% ethyl acetate in light petroleum gave [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate (32) (48 mg) containing 23% 3 deuterium atoms, 41% 2 deuterium atoms and 28% 1 deuterium atom, m.p. 110-113°C. (Lit.[7] m.p. of unlabelled compound 111-113°C); ^2H-nmr: δ ($CHCl_3$) 1.41 (br.s, 1β-^2H), 1.50 (br.s, 3β-^2H) and 1.77 (br.s, 2β-^2H); ^1H-nmr: δ 1.08 (s, 18-H_3), 2.02 (s, -OAc), 2.56 (d, J=10Hz, 5-H), 2.71 (d, J=10Hz), 6-H), 3.72 (s, -OMe), 4.98 and 5.12 (2 br.s, 17-H_2); m/z 391 (M^+, 3%), 359 (5), 349 (22), 303 (15), 287 (20) and 43 (100).

[1β,2β,3β-$^2H_3$]Gibberellin A_{20} methyl ester 13-acetate (32) (35 mg) was hydrolysed as previously described for [2α-^2H]GA_{20} methyl ester 13-acetate (25) to give [1β,2β,3β-$^2H_3$]GA_{20} (33) (19 mg) containing 24% 3 deuterium atoms 41% 2 deuterium atoms and 28% 1 deuterium atom; m/z (Me ester, $SiMe_3$ ether) 421 (M^+, 15%), 406 (5), 375 (40), 304 (6), 235 (10), 207 (32), 75 (46) and 73 (100).

ent-1α,2α-Dideuterio-13-acetoxy-3α,10β-dihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (35). Gibberellin A_3 methyl ester 13-acetate (48) (1 g) in chloroform (10 ml) was stirred with m-chloroperbenzoic acid (0.6 g) for 12h at 0°C. The usual work-up followed by purification by flash chromatography eluting with 70% ethyl acetate in light petroleum gave a mixture of the known[9] α-epoxide (34) and β-epoxide (54) (843 mg). The mixture of epoxides (34) and (54) (840 mg) in tetrahydrofuran (15 ml) was stirred under an atmosphere of deuterium in the presence of 10% palladium on calcium carbonate (100 mg) for 1h at room temperature. The catalyst was removed by filtration and the solvent evaporated under reduced pressure to give a mixture of the epoxides (51) and (55) which was deoxygenated under Cornforth[15] conditions as earlier described for [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate 16α,17-epoxide (52). Purification of the product by flash chromatography eluting with 50% ethyl acetate in light petroleum gave [1β,2β-$^2H_2$]GA_1 methyl ester 13-acetate (35) as a gum (462 mg) containing 1.26 atoms of deuterium per molecule; ^1H-nmr: δ 1.14 (s, 18-H_3), 1.99 (s, 13-OAc), 2.65 (d, J=11Hz, 6-H), 3.21 (d, J=11Hz, 5-H), 3.70 (s, -OMe), 3.73 (m, 3-H), 4.90 and 5.05 (2 br.s, 17-H_2); m/z 406 (M^+, 19%), 378 (44), 364 (87), 346 (28), 304 (17), 284 (31) and 43 (100).

ent-1α,2-Dideuterio-13-acetoxy-10β-hydroxy-20-norgibberell-2,16-diene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (38). [1β,2β-$^2H_2$]Gibberellin A_1 methyl ester 13-acetate (35) (728 mg) in acetone was stirred with Jones reagent for 1h at room temperature. Work-up gave the crude ketone (46) which was immediately reduced with sodium borohydride (200 mg) in methanol (25 ml) at room temperature to give a mixture of the 3α- and 3β-alcohols (35) and (36). Purification by flash chromatography, eluting with 65% ethyl acetate in light petroleum, gave the required [1β-2β-$^2H_2$]-epiGA_1 methyl ester 13-acetate (36) (460 mg) containing 1.29 atoms of deuterium; ^2H-nmr: δ ($CHCl_3$) 1.5 (br.s, 1β-^2H) and 2.2 (br.s, 2β-^2H); ^1H-nmr: δ (d_5-pyridine) 1.55 (s, 18-H_3), 2.03 (s, -OAc), 2.86 (d, J=10Hz, 5-H), 3.10 (d, J=10Hz, 6-H), 3.70 (s, -OMe), 3.95 (d, J=10Hz, 3-H), 5.06 and 5.30 (2 br.s, 17-H_2); m/z 406 (M^+, 9%), 364 (33), 346 (11), 304 (15), 91 (18) and 43 (100).

[1β,2β-$^2H_2$]EpiGA_1methyl ester 13-acetate (36) (430 mg) in pyridine (20 ml) was refluxed with phosphoryl chloride (0.5 ml) for 0.5h. Work-up followed by flash chromatography eluting with 18% ethyl acetate in light petroleum gave sequentially: (i) [1β,2β-$^2H_2$]3β-chloroGA_{20} methyl ester 13-acetate (37) (210 mg) containing 1.32 atoms of deuterium; ^1H-nmr: δ 1.18 (s, 18-H_3), 2.03 (s, -OAc), 2.70 (d, J=11Hz, 6-H), 3.29 (d, J=11Hz, 5-H), 3.73 (s, -OMe), 4.12 (d, J=4Hz, 3-H), 4.99 and 5.16 (2 br.s, 17-H_2); m/z 424 (M^+, 8%), 382 (48), 364 (17), 305 (8), 284 (21) and 43 (100): (ii) [1β,2-$^2H_2$]GA_5 methyl ester 13-acetate (38) (35 mg) containing 0.84 atoms of deuterium; ^1H-nmr: δ 1.22 (s, 18-H_3), 2.02 (s, -OAc), 2.67 (d, J=10Hz, 6-H), 2.80 (d, J=10Hz, 5-H), 3.73 (s, -OMe), 4.98 and 5.13 (2 br.s, 17-H_2), 5.75 (m, 2-H and 3-H); m/z 388 (M^+, 3%), 358 (2), 346 (9), 314 (3), 284 (24), 143 (10), 91 (15) and 43 (100).

[1β,2β-$^2H_2$]-3β-ChloroGA_{20} methyl ester 13-acetate (37) (50 mg) in pyridine (3 ml) was refluxed with 1,8-diazabicyclo(5.4.0)undec-7-ene for 2h. The usual work-up gave on purification by flash chromatography eluting with 18% ethyl acetate in light petroleum [1β,2-$^2H_2$]GA_5 methyl ester 13-acetate (38) (18 mg) containing 1.30 atoms of deuterium.

ent-1α,2α,3α-Trideuterio-13-acetoxy-10β-hydroxy-20-norgibberell-16-ene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (32). [1β,2β-$^2H_2$]3β-Chlorogibberellin A_{20} methyl ester 13-acetate (37) (200 mg) in toluene (30 ml) was refluxed with [^2H]-tri-n-butylstannane (300 μl) in the presence of 2,2'-azobis(2-methylpropionitrile) for 1h. Purification of the product by flash chromatography eluting with 18% ethyl acetate in light petroleum gave [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate (32) (120 mg) containing 2.25 atoms of deuterium; ^2H-nmr: δ ($CHCl_3$) 1.41 (br.s, 1β-^2H), 1.50 (br.s, 3β-^2H) and 1.78 (br.s, 2β-^2H); m/z 391 (M^+, 7%), 359 (5), 349 (28), 303 (15), 287 (21) and 43 (100).

ent-3β-Deuterio-10β,13-dihydroxy-20-norgibberell-2,16-diene-7,19-dioic Acid 19,10-Lactone (44). Gibberellin A_1 methyl ester 3-ketone 13-acetate (39) (635 mg) in methanol (25 ml) was stirred with sodium borodeuteride (84 mg) for 1h at room temperature. Work-up gave a mixture of the 3α-alcohol (41) and 3β-alcohol (40) which was refluxed with phosphoryl chloride (1 ml) in pyridine (30 ml) for 1h. Work-up gave a mixture of the 3β-chloro compound (47) and the $\Delta^{2,3}$-ene (58). The mixture of (47) and (58) was refluxed with 1,8-diazabicyclo(5,4,0)-undec-7-ene (0.5 ml) in pyridine (10 ml) for 2.5h to give, after purification by flash chromatography eluting with 18% ethyl acetate in light petroleum, [3-^2H]GA_5 methyl ester 13-acetate (58) (158 mg) containing 0.96 atoms of deuterium.

[3-^{2}H]Gibberellin A_5 methyl ester 13-acetate (58) (158 mg) in chloroform (20 ml) was treated with m-chloroperbenzoic acid (95 mg) for 3.5h at room temperature. Work-up followed by purification by flash chromatography eluting with 70% ethyl acetate in light petroleum gave a mixture of the α-epoxide (42) and β-epoxide (56) (85 mg).

The mixture of epoxides (42) and (56) (85 mg) in tetrahydrofuran (3 ml) was stirred with 10% palladium on calcium carbonate (catalytic) under an atmosphere of hydrogen for 0.5h at room temperature. The mixture was diluted with ethyl acetate and filtered. Concentration of the filtrate *in vacuo* gave crude [3α-^{2}H]GA_{20} methyl ester 13-acetate 16,17-epoxide (50) (70 mg). Without further purification the exocyclic double bond was regenerated by the method of Cornforth[15] as previously described for [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13-acetate 16α,17-epoxide (52) to give [3α-^{2}H]GA_{20} methyl ester 13-acetate (43) (52 mg) containing 0.96 atoms of deuterium. The product was identified by its ^{1}H-nmr and mass spectra.

Hydrolysis of [3α-^{2}H]GA_{20} methyl ester 13-acetate (43) (50 mg) as previously described for [1β,2β,3β-$^2H_3$]GA_{20} methyl ester 13 acetate (32) gave [3α-^{2}H]GA_{20} (44) (8.5 mg) containing 0.96 atoms of deuterium m.p. 228-229°C (Lit.[17] m.p. of unlabelled compound 232-233°C) m/z (Me ester, $SiMe_3$ ether) 419 (M^+, 100%), 404 (10), 375 (44), 360 (13), 302 (10), 238 (7), 207 (25), 75 (16) and 73 (7)

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