Partial Synthesis of [1β,2β-$^2H_2$]-, [2β-^2H]-, and [2α-^2H]-Gibberellin A_1

Jake MacMillan* and Christine L. Willis
A.F.R.C. Research Group, School of Chemistry, The University, Bristol BS8 1TS

The preparation of [1β,2β-$^2H_2$]GA_1 by [^2H]hydrogenation of GA_3 13-acetate 16,17-epoxide is described. [2β-^2H]Gibberellin A_1 was prepared by [^2H]hydrogenolysis of GA_3 13-acetate methyl ester, iodolactonisation of the 2β-deuteriated product followed by reduction and hydrolysis. Treatment of [2β-^2H]-1β-iodoGA_1 13-acetate with 1,8-diazabicyclo[5.4.0]undec-7-ene gave [2-^2H]GA_3 13-acetate which has been reduced to [2α-^2H]GA_1 either *via* hydrogenation of the corresponding 16,17-epoxide or by hydrogenolysis, iodolactonisation, and reduction. The isotopic labelling, assigned by a combination of ^1H, ^2H, and ^{13}C n.m.r. spectroscopy, was completely stereoselective in all cases.

Functionalisation of ring A of the gibberellins (GAs) plays an important role in the metabolism and biological activity of this family of plant hormones. Of particular importance is modification at C-2. In order to study the stereochemistry of 2β-hydroxylation and 1,2- and 2,3-dehydrogenations we required gibberellins stereospecifically labelled with deuterium and tritium at C-2. We now describe the preparation of [1β,2β-$^2H_2$]-GA_1 (**16**), [2β-^2H]GA_1 (**25**) and [2α-^2H]GA_1 (**28**) required for metabolic studies.

Results and Discussion

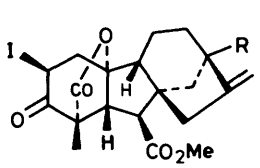
Several routes to [2α-^2H] and [2β-^2H]gibberellins without a 13-hydroxy group have been examined previously by us.[1,2] Only two methods gave greater than 80% stereoselectivity: the reduction of 2β-iodo-3-oxoGA_4 (**1**) with [^2H]tributylstannane to [2α-^2H]-3-oxoGA_4 (**3**)[1] and [1β,2β-$^2H_2$]hydrogenation of GA_7 16,17-epoxide (**29**).[2]

Attempts to apply the former method to the preparation of [2α-^2H]GA_1 (**28**) were unsuccessful. The required 2β-iodo-3-oxoGA_1 (**2**) could not be prepared by treatment of 3-oxoGA_1 (**4**) or 3-oxoGA_1 13-acetate (**5**), with lithium di-isopropylamide followed by iodine (1 equiv.) or by treatment of 3-oxoGA_3 (**8**) or 3-oxoGA_3 13-acetate (**9**) with L-Selectride and iodine. We have previously encountered similar undefined difficulties in reactions involving enolates of 13-hydroxygibberellins.[3]

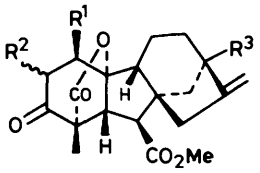
[1β,2β-$^2H_2$]Gibberellin A_1 (**16**) was prepared in an analogous manner[2] to [1β,2β-$^2H_2$]GA_4 (**10**) as shown in Scheme 1. It was necessary to protect the 13-hydroxy group (as the acetate) to avoid the formation of unidentified isomers of GA_1 at the stage of regeneration of the 16,17-double bond. The 13-acetate (**13**) was prepared by hydrolysis of the 3,13-diacetate (**11**), derived from the GA_3 methyl ester (**12**), acetic anhydride and toluene-*p*-sulphonic acid, with potassium carbonate in aqueous methanol. Epoxidation of the 13-acetate (**13**), [^2H]-hydrogenation of the epoxide (**14**), deoxygenation to the alkene (**15**) and hydrolysis gave [1β,2β-$^2H_2$]GA_1 (**16**).†

[1β,2β-$^2H_2$]Gibberellin A_1 13-acetate methyl ester (**15**) contained 1.56 atoms of deuterium. Its ^1H n.m.r. spectrum showed a 1-H singlet at δ 3.83 with $J_{ax,eq}$ 4 Hz, assigned to 3-H. To confirm the 1β,2β-stereochemistry of the deuterium atoms, the 3β-alcohol (**15**) was oxidised with Jones reagent to the ketone (**6**) which was then reduced with sodium borohydride to give [1β,2β-$^2H_2$]-3-*epi*-GA_1 13-acetate (**17**). This 3α-alcohol (**17**) also contained 1.56 atoms of deuterium indicating that no exchange at C-2 had occurred. Its ^2H n.m.r. spectrum displayed signals at δ 1.5 and 2.2, attributed to 1β-^2H and 2β-^2H respectively,[2] whilst a doublet at δ 3.92, $J_{ax,ax}$ 10.8 Hz assigned to 3-H confirmed the presence of the 2β-^2H. Hence *syn* addition of deuterium had occurred from the less hindered β-face at C-1 and C-2. Alkaline hydrolysis of compound (**15**), with prior protection of the 3-hydroxy group as the tetrahydropyranyl ether to avoid epimerisation at C-3,[4] gave the required [1β,2β-$^2H_2$]GA_1 (**16**).

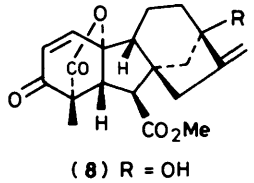
The partial syntheses of [2α-^2H] and [2β-^2H]GA_1 (**28**) and (**25**) were accomplished as shown in Scheme 2. Hydrogenation of GA_7 methyl ester (**20**) in the presence of partially poisoned 10% palladium-on-calcium carbonate is known to give the hydrogenolysis product (**21**).[5] Treatment of GA_3 13-acetate (**13**) in MeOD with deuterium gas in the presence of 10% palladium-on-calcium carbonate partially poisoned with pyridine intro-



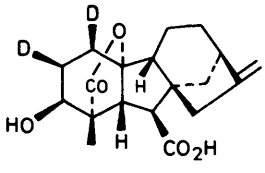
(**1**) R = H
(**2**) R = OH



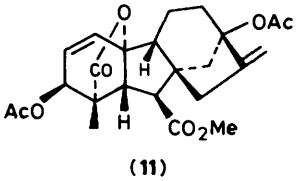
(**3**) R^1 = H; R^2 = α-D; R^3 = H
(**4**) R^1 = R^2 = H; R^3 = OH
(**5**) R^1 = R^2 = H; R^3 = OAc
(**6**) R^1 = D; R^2 = β-D; R^3 = OAc
(**7**) R^1 = H; R^2 = β-D; R^3 = OAc



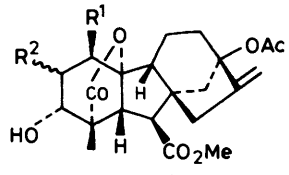
(**8**) R = OH
(**9**) R = OAc



(**10**)

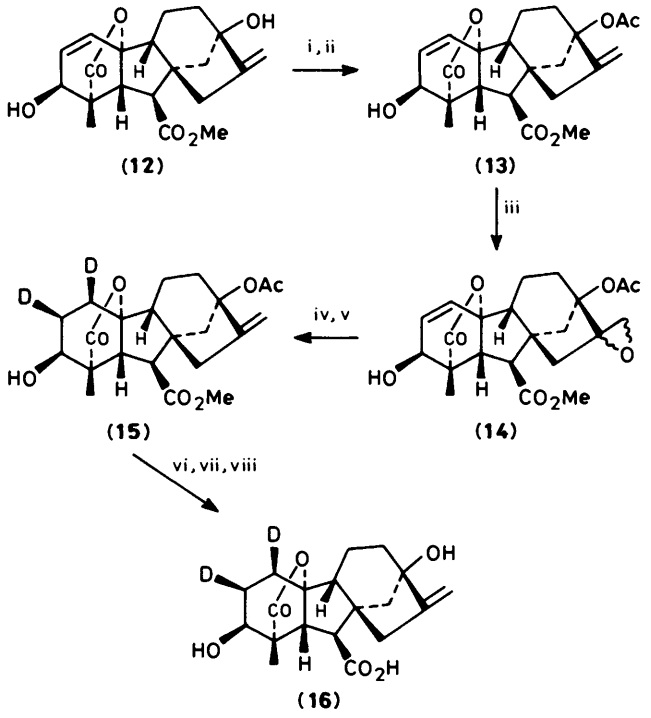


(**11**)

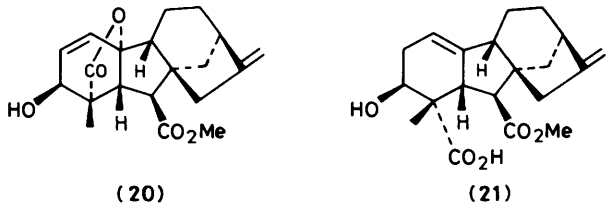


(**17**) R^1 = D; R^2 = β-D
(**18**) R^1 = H; R^2 = β-D
(**19**) R^1 = H; R^2 = α-D

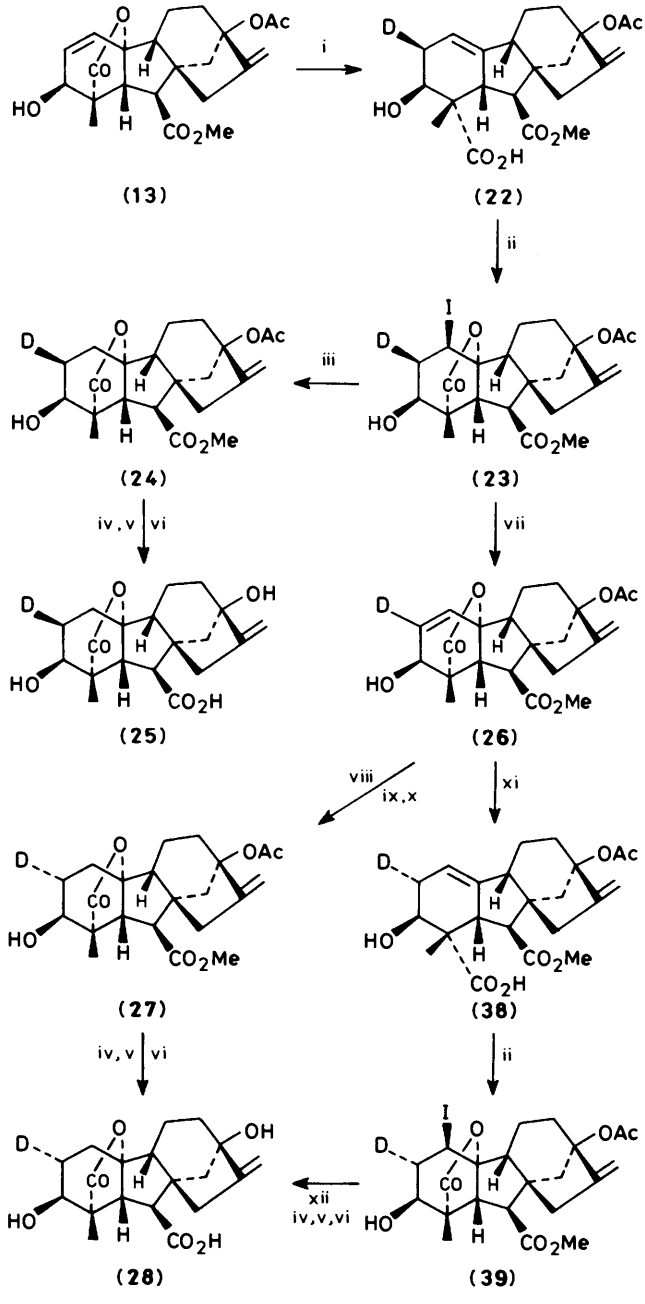
† [^3H]GA_1 and [^3H]GA_4 (20—40 Ci mmol^{-1}) are now available from Amersham International p.l.c.



Scheme 1 *Reagents:* i, Ac_2O–TsOH; ii, K_2CO_3–MeOH–H_2O (4:1) pH 10; iii, *m*-$ClC_6H_4CO_3H$; iv, D_2–10% Pd-on-$CaCO_3$–THF; v, NaI–NaOAc–Zn–AcOH–H_2O; vi, DHP–TsOH–CH_2Cl_2; vii, 2M-NaOH; viii, TsOH–Me_2CO–MeOH.



duced deuterium specifically from the β-face leading to the [2β-^{2}H]-open lactone (**22**) and an inseparable by-product. Iodolactonisation[6] of the crude deuteriation mixture gave [2β-^{2}H]-1β-iodoGA_1 13-acetate (**23**) containing 0.76 atoms of deuterium. The ^{1}H n.m.r. spectrum of (**23**) displayed signals at δ 4.40 (d, *J* 6 Hz) and 3.98 (d, *J* 4 Hz) attributed to 1-H and 3-H respectively. Reduction of the iodide with tributylstannane gave [2β-^{2}H]GA_1 13-acetate (**24**) whose proton decoupled ^{13}C n.m.r. spectrum showed a reduction in the signal at δ 28.1 confirming that deuterium was located solely at C-2. The ^{2}H n.m.r. of (**24**) in benzene displayed a single peak at δ 1.45 assigned to 2β-^{2}H while the ^{1}H n.m.r. gave a doublet, *J* = 4 Hz at δ 3.80 due to 3-H. To confirm the stereochemistry of isotopic label [2β-^{2}H]GA_1 13-acetate (**24**) was oxidised with Jones reagent to the corresponding ketone (**7**) which on reduction with sodium borohydride gave, as the major product, [2β-^{2}H]-3-*epi*-GA_1 13-acetate (**18**). An incorporation of 0.76 atoms of deuterium per molecule in the acetate (**18**) indicated that no exchange had occurred during this procedure. Examination of the ^{1}H n.m.r. spectrum of (**18**) in [$^2H_5$]pyridine revealed a doublet, $J_{ax,ax}$ 10 Hz at δ 3.95 due to 3-H whilst the ^{2}H n.m.r. displayed one broad signal at δ 2.16 confirming that deuterium was located solely at the 2β-position. Hydrolysis of [2β-^{2}H]-GA_1 13-acetate (**24**), as previously described for [1β,2β-$^2H_2$]-GA_1 13-acetate (**15**), gave [2β-^{2}H]GA_1 (**25**) containing 0.75



Scheme 2 *Reagents:* i, D_2–10% Pd-on-$CaCO_3$–MeOD–[$^2H_5$]pyridine–cyclohexene; ii, I_2–$NaHCO_3$–THF;–CH_2Cl_2; iii, Bu_3SnH–AIBN*; toluene; iv, DHP*–TsOH–CH_2Cl_2; v, 2M-NaOH; vi, TsOH–Me_2CO–MeOH; vii, DBU*–toluene; viii, *m*-$ClC_6H_4CO_3H$; ix, H_2–10% Pd-on-$CaCO_3$–THF; x, NaI–NaOAc–Zn–AcOH–H_2O; xi, H_2–10% Pd-on-$CaCO_3$–MeOH–pyridine; xii, $NaBH_4$–DMSO.

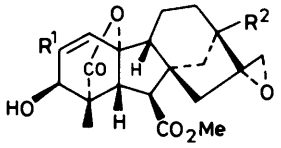
atoms of deuterium in 30% overall yield from GA_3 methyl ester (**12**).

An incorporation of deuterium of 75% into the gibberellin was disappointing since the deuterium gas was 99% pure. Exchange reactions are known to occur on the surface of palladium catalysts.[7] We have previously improved the incorporation of deuterium by pre-washing the catalyst with D_2O;[2]

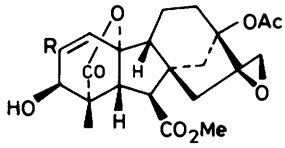
* AIBN = azoisobutyronitrile; DHP = 2,3-dihydropyran; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

however, in this case, since the reaction was conducted in MeOD, no significant enhancement of deuterium incorporation was observed. Similarly, if the catalyst was pre-reduced with deuterium gas, a 78% incorporation of deuterium into [2β-^{2}H]-GA_1 (**25**) was obtained. However, when the catalyst was pre-reduced with deuterium gas in MeOD in the presence of cyclohexene, then poisoned with [$^2H_5$]pyridine prior to the addition of the gibberellin, an incorporation of 92% was achieved.

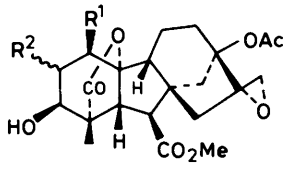
Since catalytic reduction occurs from the less hindered β-face of ring A of the gibberellins this reaction was utilised in the preparation of [2α-^{2}H]GA_1 (**28**) from [2-^{2}H]GA_3 13-acetate 7-methyl ester (**26**). [2β-^{2}H]-1β-IodoGA_1 13-acetate (**23**) containing 0.92 atoms of deuterium per molecule was prepared as outlined in Scheme 2. Treatment of (**23**) with 1,8-diazabicyclo-[5.4.0]undec-7-ene in toluene gave [2-^{2}H]GA_3 13-acetate (**26**) also with 0.92 atoms of deuterium, confirming that all the label was retained in a *trans* diaxial elimination of hydrogen iodide.



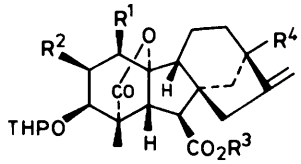
(**29**) $R^1 = R^2 = H$
(**30**) $R^1 = D$; $R^2 = OAc$



(**31**) R = H
(**32**) R = D



(**33**) $R^1 = H$; $R^2 = \alpha$-D
(**34**) $R^1 = R^2 = \beta$-D



(**35**) $R^1 = R^2 = D$; $R^3 = Me$; $R^4 = OAc$
(**36**) $R^1 = H$; $R^2 = D$; $R^3 = Me$; $R^4 = OAc$
(**37**) $R^1 = R^3 = H$; $R^2 = D$; $R^4 = OH$

Protection of the exocyclic double bond as the epoxide (**30**), followed by hydrogenation of the 1,2-double bond, gave [2α-^{2}H]GA_1 16,17-epoxide (**33**). Deoxygenation of (**33**) by the method of Cornforth *et al.*[8] gave [2α-^{2}H]GA_1 13-acetate (**27**) whose ^{1}H n.m.r. spectrum displayed a singlet at δ 3.8 due to 3-H. The stereochemistry of the deuterium labelling was confirmed by the n.m.r. spectra of the corresponding 3-*epi*-alcohol (**19**) obtained by oxidation of the 3β-alcohol (**27**), then reduction, with no loss of isotopic label. The ^{1}H n.m.r. spectrum of [2α-^{2}H]-3-*epi*-GA_1 methyl ester (**19**) in [$^2H_5$]pyridine displayed a doublet, $J_{ax,eq}$ 5 Hz, at δ 3.94 due to 3-H whilst the ^{2}H n.m.r. displayed a single broad signal at δ 1.50 due to 2α-^{2}H. Hydrolysis of [2α-^{2}H]GA_1 13-acetate 7 methyl ester (**27**), as previously described for [1β,2β-$^2H_2$]GA_1 13-acetate 7-methyl ester (**16**), gave [2α-^{2}H]GA_1 (**28**) containing 0.9 atoms of deuterium per molecule.

An alternative route to [2α-^{2}H]GA_1 was by hydrogenolysis of [2-^{2}H]GA_3 13-acetate (**26**) to give the [2α-^{2}H] open-lactone (**38**) which on iodolactonisation gave [2α-^{2}H]-1β-iodoGA_1 13-acetate (**39**). The ^{1}H n.m.r. of the 13-acetate (**39**) revealed a broad singlet at δ 3.98 due to 3-H and a singlet at δ 4.40 assigned to 1-H. Reduction of the iodide (**39**) with sodium borohydride in dimethyl sulphoxide gave [2α-^{2}H]GA_1 13-acetate (**27**) identical to that previously obtained.

The syntheses described in this paper provide 1- and 2-labelled GA_1 with a high incorporation of deuterium with total selectivity. They are superior to the previously described methods such as radical reduction of 2β-iodo-3-oxoGA_4 (**1**) with [^{2}H]tributylstannane which is only *ca.* 90% stereoselective. They have the further advantage that they may be applied to 13-hydroxylated as well as 13-deoxygenated gibberellins.

Experimental

General experimental details have been described in a previous paper.[9]

ent-13-*Acetoxy*-3α,10β-*dihydroxy*-20-*norgibberell*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**13**).—Gibberellin A_3 methyl ester (**12**) (980 mg) was stirred with acetic anhydride (20 ml) in the presence of toluene-*p*-sulphonic acid (3 crystals) for 4 h at room temperature. The mixture was diluted with water, acidified to pH 2 with dilute hydrochloric acid and extracted with ethyl acetate. The extract was washed with water then concentrated under reduced pressure the acetic acid being azeotroped off with toluene. The product was purified by flash chromatography eluting with 30% ethyl acetate in light petroleum to give GA_3 3, 13-diacetate 7-methyl ester (**11**) (950 mg) m.p. 158—160 °C (from ethyl acetate–light petroleum) (lit.,[10] m.p. 167—169 °C); δ 1.14 (s, 18-H_3), 2.03 (s, 13-OAc), 2.12 (s, 3-OAc), 2.78 (d, *J* 11 Hz, 6-H), 3.33 (d, *J* 11 Hz, 5-H), 3.75 (s, OMe), 5.00 and 5.18 (2 × br s, 17-H_2), 5.33 (d, *J* 4 Hz, 3-H), 5.86 (dd, *J* 9, 4 Hz, 2-H), and 6.39 (d, 9 Hz, 1-H); *m/z* 444 (M^+, 2%), 413 (8), 402 (5), 384 (10), 352 (8), 324 (8), 310 (4), 280 (62), 221 (62), and 43 (100).

Gibberellin A_3 3,13-diacetate 7-methyl ester (**11**) (700 mg) in methanol (40 ml) was stirred with aqueous potassium carbonate (10 ml), pH 10 for 0.5 h at room temperature. Work-up gave GA_3 13-acetate 7-methyl ester (**13**) (610 mg) m.p. 143—145 °C (from ethyl acetate–light petroleum) (lit.,[11] m.p. 148—150 °C); δ 1.23 (s, 18-H_3), 2.03 (s, 13-OAc), 2.79 (d, *J* 11 Hz, 6-H), 3.72 (d, *J* 11 Hz, 5-H), 3.74 (s, OMe), 4.14 (d, *J* 4 Hz, 3-H), 4.99 and 5.17 (2 × br s, 17-H_2), 5.90 (dd, *J* 4, 7 Hz, 2-H), and 6.30 (d, *J* 7 Hz, 1-H); *m/z* 402 (M^+, 23%) 371 (20), 360 (95), 324 (49), 280 (41), 237 (34), and 43 (100).

Epoxidation of Gibberellin A_3 *Methyl Ester* 13-*Acetate* (**13**).—Gibberellin A_3 methyl ester 13-acetate (**13**) (550 mg) in chloroform (20 ml) was treated with *m*-chloroperbenzoic acid (320 mg) overnight at 5 °C. The reaction was worked up to give a gum which, after flash chromatography eluting with 70% ethyl acetate in light petroleum, gave (i) ent-13-*acetoxy*-3α,10β-*dihydroxy*-16α,17-*epoxy*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**31**) (55 mg) as a foam (Found: M^+, 418.1621. $C_{22}H_{26}O_8$ requires *M*, 418.1627); δ 1.22 (s, 18-H_3), 1.99 (s, OAc), 2.77 (d, *J* 11 Hz, 6-H), 2.81 (d, *J* 5 Hz, 17-H), 3.05 (d, *J* 5 Hz, 17-H), 3.23 (d, *J* 11 Hz, 5-H), 3.75 (s, OMe), 4.14 (d, *J* 4 Hz, 3-H), 5.92 (dd, *J* 4, 7 Hz, 2-H), and 6.35 (d, *J* 7 Hz, 1-H); *m/z* 418 (M^+, 2%), 387 (7), 376 (22), 358 (5), 346 (7), 313 (10), 296 (33), 91 (15), and 43 (100), and (ii) ent-13-*acetoxy*-3α,10β-*dihydroxy*-16β,17-*epoxy*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**14**) (450 mg) as a foam (Found: M^+, 418.1623. $C_{22}H_{26}O_8$ requires *M*, 418.1627); δ 1.23 (s, 18-H_3), 2.01 (s, OAc), 2.75 (d, *J* 5 Hz, 17-H), 2.83 (d, *J* 11 Hz, 6-H), 3.11 (d, *J* 5 Hz, 17-H), 3.24 (d, *J* 11 Hz, 5-H), 3.74 (s, OMe), 4.14 (m,

3-H), 5.92 (dd, *J* 4, 9 Hz, 2-H), and 6.33 (d, *J* 9 Hz, 1-H); *m/z* 418 (M^+, 4%), 387 (12), 376 (35), 296 (24), 213 (17), 155 (14), and 43 (100).

ent-13-*Acetoxy*-1α,2α-*dideuterio*-3α,10β-*dihydroxy*-20-*nor*-*gibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**15**).—Gibberellin A_3 13-acetate 16α,17-epoxide (**14**) (150 mg) in tetrahydrofuran (15 ml, freshly distilled) was stirred with 10% palladium-on-calcium carbonate (3 mg, prewashed with D_2O then dried) under an atmosphere of deuterium gas for 1 h at room temperature. The mixture was diluted with ethyl acetate, filtered through Celite and concentrated under reduced pressure and then reduced as follows.

Sodium iodide (300 mg) and sodium acetate (100 mg) were stirred in glacial acetic acid (7 ml) and water (1 ml). Freshly activated zinc dust (300 mg) was added followed by crude [1β,2β-$^2H_2$]GA_1 methyl ester 13-acetate 16α,17-epoxide (**34**) (145 mg) in acetone (2 ml). Stirring was continued for 4 h at room temperature. The mixture was diluted with ethyl acetate, filtered, washed with water and concentrated under reduced pressure. Purification by flash chromatography eluting with 70% ethyl acetate in light petroleum gave [1β,2β-$^2H_2$]GA_1 13-acetate 7-methyl ester (**15**) as a gum (102 mg) containing 1.56 atoms of deuterium per molecule; δ 1.14 (s, 18-H_3), 1.99 (s, 13-OAc), 2.65 (d, *J* 11 Hz, 6-H), 3.21 (d, *J* 11 Hz, 5-H), 3.70 (s, OMe), 3.73 (m, 3-H), and 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,2α-*Dideuterio*-3α,10β,13-*trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**16**).—[1β,2β-$^2H_2$]-Gibberellin A_1 13-acetate 7-methyl ester (**15**) (95 mg) in dichloromethane (10 ml) was stirred with 2,3-dihydropyran (200 μl, freshly distilled from potassium hydroxide pellets) and toluene-*p*-sulphonic acid (2 crystals) for 2 h at room temperature. The reaction mixture was worked-up to give the tetrahydropyranyl ether (**35**) (100 mg) which was refluxed in methanol (3 ml) with 2M-aqueous sodium hydroxide (20 ml) for 6 h. Work-up gave a single product by t.l.c. which in acetone (10 ml) and methanol (1 ml) was stirred with toluene-*p*-sulphonic acid (3 mg) for 3 h at room temperature. The mixture was diluted with ethyl acetate, washed with water and then concentrated under reduced pressure. Purification by flash chromatography, eluting with ethyl acetate–light petroleum–acetic acid (17:2:1) gave [1β,2β-$^2H_2$]GA_1 (**16**) (55 mg) m.p. 258—260 °C (lit.,[12] m.p. 256—260 °C) containing 1.56 atoms of deuterium per molecule; δ{[$^2H_6$]acetone} 1.12 (s, 18-H_3), 2.58 (d, *J* 10 Hz, 6-H), 3.23 (d, *J* 10 Hz, 5-H), 3.73 (d, *J* 4 Hz, 3-H), and 4.88 and 5.20 (2 × br s, 17-H_2); *m/z* [Me ester, $(Me_3Si)_2$ ether] 508 (M^+, 77%), 448 (11), 376 (5), 235 (7), 207 (22), 75 (100), and 73 (65).

ent-13-*Acetoxy*-1α,2α-*dideuterio*-3β,10β-*dihydroxy*-20-*nor*-*gibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**17**).—[1β,2β-$^2H_2$]Gibberellin A_1 13-acetate 7-methyl ester (**15**) (40 mg) in acetone (10 ml) was treated dropwise with Jones reagent (2 ml) for 0.5 h at room temperature. Work-up gave a gum which was immediately stirred with sodium borohydride (15 mg) in methanol (10 ml) for 1 h at room temperature. The reaction mixture was worked up to give two products which were separated by flash chromatography. Elution with 35% ethyl acetate in light petroleum gave [1β,2β-$^2H_2$]GA_1 13-acetate 7-methyl ester (**16**) (7 mg), identical to that previously obtained. Further elution with 40% ethyl acetate in light petroleum gave [1β,2β-$^2H_2$]-3-epiGA_1 13-acetate 7-methyl ester (**17**) (22 mg) m.p. 147—149 °C (lit.,[13] m.p. 143—145 °C) containing 1.54 atoms of deuterium; δ_D 1.5 and 2.2; δ_H[$^2H_5$]-pyridine}, 1.55 (s, 18-H_3), 2.03 (s, OAc), 2.86 (d, *J* 10 Hz, 5-H), 3.10 (d, *J* 10 Hz, 6-H), 3.70 (s, OMe), 3.95 (d, *J* 10 Hz, 3-H), and 5.06 and 5.30 (2 × br s, 17-H_2); *m/z* 406 (M^+, 9%), 364 (33), 346 (11), 304 (15), 286 (13), 257 (10), 91 (18), and 43 (100).

ent-13-*Acetoxy*-2α-*deuterio*-3α,10β-*dihydroxy*-1β-*iodo*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**23**).—Gibberellin A_3 13-acetate 7-methyl ester (**13**) (700 mg) in MeOD (10 ml) and pyridine (1 ml) was stirred for 1 h at room temperature with 10% palladium-on-calcium carbonate (4 mg) under an atmosphere of deuterium. The mixture was diluted with ethyl acetate and filtered. The filtrate was washed with aqueous sodium hydrogen carbonate and the aqueous layer then acidified to pH 2 with dilute hydrochloric acid and extracted with ethyl acetate to give the crude hydrogenolysis product (**22**) as a gum (572 mg). Without further purification, this gum in dichloromethane (10 ml), aqueous sodium hydrogen carbonate (15 ml) and tetrahydrofuran (10 ml) was vigorously stirred with iodine (300 mg) for 0.5 h at room temperature. The mixture was diluted with water and extracted with ethyl acetate. The extract was washed with aqueous sodium thiosulphate and then with water and concentrated under reduced pressure to give [2β-^2H]-1β-iodoGA_1 13-acetate 7-methyl ester (**23**) (530 mg) m.p. 163—165 °C (from ethyl acetate–light petroleum) containing 0.76 atoms deuterium per molecule (Found: M^+, 531.0914. $C_{22}H_{26}O_7I^2H$ requires *M*, 531.0866); δ 1.19 (s, 18-H_3), 2.03 (s, OAc), 2.70 (d, *J* 10.5 Hz, 6-H), 3.75 (s, OMe), 3.85 (d, *J* 10.5 Hz, 5-H), 3.98 (d, *J* 4 Hz, 3-H), 4.41 (d, *J* 6 Hz, 1-H), and 5.04 and 5.17 (2 × br s, 17-H_2); *m/z* 531 (M^+, 9%), 489 (26), 430 (6), 298 (7), 222 (12), 128 (13), 105 (8), 91 (13), and 43 (100).

ent-2α-*Deuterio*-3α,10β,13-*trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**25**).—[2β-^2H]-1β-IodoGA_1 13-acetate 7-methyl ester (**23**) (200 mg) in toluene (30 ml) was refluxed with tributylstannane (250 μl) in the presence of the initiator 2,2′-azo-2-methylpropionitrile (5 mg). The solvent was removed under reduced pressure and the product was purified by flash chromatography eluting with ethyl acetate–light petroleum (1:1) to give [2β-^2H]GA_1 13-acetate 7-methyl ester (**24**) as a gum (140 mg) containing 0.76 atoms deuterium per molecule; δ_D (benzene) 1.45; δ_H 1.14 (s, 18-H_3), 2.02 (s, OAc), 2.71 (d, *J* 10.5 Hz, 6-H), 3.23 (d, *J* 10.5 Hz, 5-H), 3.72 (s, OMe), 3.83 (d, *J* 4 Hz, 3-H), and 4.99 and 5.14 (2 × br s, 17-H_2); *m/z* 405 (M^+, 9%), 363 (45), 345 (14), 303 (15), 283 (25), 179 (29), 135 (66), and 43 (100).

[2β-^2H]Gibberellin A_1 13-acetate methyl ester (**24**) (100 mg) in dichloromethane (10 ml) was stirred with 2,3-dihydropyran (250 μl) and toluene-*p*-sulphonic acid (1 mg) for 2 h at room temperature. Work-up gave a gum (160 mg) containing the crude tetrahydropyranyl ether (**36**) which was refluxed in methanol (2 ml) and 2M-sodium hydroxide (20 ml) for 6 h. Work-up gave the acid (**37**). The crude hydrolysis product (**37**) in acetone (10 ml) and methanol (1 ml) was stirred with toluene-*p*-sulphonic acid (3 mg) for 3 h at room temperature. Work-up gave a gum which by flash chromatography, eluting with ethyl acetate–light petroleum–acetic acid (7:2:1), gave [2β-^2H]GA_1 (**25**) (67 mg), m.p. 254—257 °C (lit.,[12] m.p. 256—260 °C) (from acetone–light petroleum) containing 0.75 atoms of deuterium per molecule.

ent-13-*Acetoxy*-2α-*deuterio*-3β-10β-*dihydroxy*-20-*norgib*-*berell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**18**).—[2β-^2H]Gibberellin A_1 13-acetate 7-methyl ester (**24**) (40 mg) in acetone (10 ml) was treated dropwise with Jones reagent (2 ml) for 0.5 h at room temperature. Work-up gave a gum which was immediately stirred with sodium borohydride (15 mg) in methanol (10 ml) for 1 h at room temperature. The reaction mixture was worked-up to give two products which

were separated by flash chromatography. Elution with 35% ethyl acetate in light petroleum gave [2β-^{2}H]GA_1 13-acetate 7-methyl ester (**24**) (5 mg), identical to that previously obtained. Further elution with 40% ethyl acetate in light petroleum gave [2β-^{2}H]-3-epiGA_1 13-acetate 7-methyl ester (**18**) (28 mg), m.p. 142—146 °C (lit.,[13] m.p. 143—145 °C) (from ethyl acetate–light petroleum), containing 0.76 atoms of deuterium per molecule; δ_D 2.16 [2β-^{2}H]; δ_H\{[$^2H_5$]pyridine\} 1.55 (s, 18-H_3), 2.02 (s, OAc), 2.87 (d, *J* 10 Hz, 5-H), 3.10 (d, *J* 10 Hz, 6-H), 3.69 (s, OMe), 3.95 (d, *J* 10 Hz, 3-H), and 5.06 and 5.30 (2 × br s, 17-H_2); *m/z* 405 (M^+, 10%), 363 (45), 345 (15), 285 (16), 209 (13), 167 (22), 91 (21), and 43 (100).

ent-13-*Acetoxy*-2-*deuterio*-3α,10β-*dihydroxy*-20-*norgibberell*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**26**).—Cyclohexene (0.5 ml) in MeOD (10 ml) was stirred with 10% palladium-on-calcium carbonate (20 mg) under an atmosphere of deuterium for 0.5 h at room temperature. [$^2H_5$]-Pyridine (0.5 ml) was added followed by GA_3 13-acetate 7-methyl ester (**13**) (500 mg) in MeOD (5 ml). Stirring under an atmosphere of deuterium was continued for a further 1 h and then the mixture was diluted with ethyl acetate, filtered and the filtrate concentrated under reduced pressure. The above crude product in dichloromethane (10 ml), aqueous sodium hydrogen carbonate (15 ml) and tetrahydrofuran was stirred vigorously with iodine (250 mg) for 0.5 h at room temperature. The mixture was diluted with water and extracted with ethyl acetate. The extract was washed with aqueous sodium thiosulphate and then with water and concentrated under reduced pressure to give [2β-^{2}H]-1β-iodoGA_1 13-acetate 7-methyl ester (**23**) (360 mg), m.p. 163—165 °C (from ethyl acetate–light petroleum) containing 0.92 atoms of deuterium per molecule, identical to that previously obtained.

[2β-^{2}H]-1β-IodoGA_1 13-acetate 7-methyl ester (**23**) (350 mg) in toluene (5 ml) was refluxed with 1,8-diazabicyclo[5.4.0]-undec-7-ene (350 μl) for 0.5 h. The toluene was removed under reduced pressure and the product purified by flash chromatography eluting with ethyl acetate–light petoleum (1:1) to give [2-^{2}H]GA_3 13-acetate 7-methyl ester (**26**) (262 mg) containing 0.92 atoms of deuterium per molecule; m.p. 147—149 °C (lit.,[11] m.p. 148—150 °C); δ 1.25 (s, 18-H_3), 2.03 (s, OAc), 2.80 (d, *J* 10.5 Hz, 6-H), 3.22 (d, *J* 10.5 Hz, 5-H), 3.74 (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 (5), 361 (13), 343 (13), 311 (9), 298 (14), 281 (18), 238 (16), 91 (15), and 43 (100).

ent-2β-*Deuterio*-3α,10β,13-*trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**28**).—[2-^{2}H]Gibberellin A_3 13-acetate 7-methyl ester (**26**) (250 mg) was protected as the 16,17-epoxide, hydrogenated, deprotected, and hydrolysed as previously described for the preparation of [1β,2β-$^2H_2$]GA_1 (**16**) to give [2α-^{2}H]GA_1 (**28**) (35 mg), m.p. 257—259 °C (lit.,[12] m.p. 256—260 °C) containing 0.90 atoms of deuterium per molecule.

ent-13-*Acetoxy*-2β-*deuterio*-3β,10β-*dihydroxy*-20-*norgib*-*berell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**19**).—[2α-^{2}H]Gibberellin A_1 13-acetate 7-methyl ester (**27**) (40 mg) was oxidised with Jones reagent and reduced with sodium borohydride as previously described for [2β-^{2}H]GA_1 13-acetate 7-methyl ester (**24**) to give [2α-^{2}H]-3-*epi*-GA_1 13-acetate 7-methyl ester (**19**) (23 mg), m.p. 148—150 °C (lit.,[13] m.p. 143—145 °C) (from ethyl acetate–light petroleum), containing 0.89 atoms of deuterium per molecule; δ_D 1.50 (br s, 2α-^{2}H); δ_H\{[$^2H_5$]pyridine\} 1.54 (s, 18-H_3), 2.02 (s, OAc), 2.88 (d, *J* 10 Hz, 5-H), 3.10 (d, *J* 10 Hz, 6-H), 3.70 (s, OMe), 3.96 (d, *J* 5 Hz, 3-H), and 5.06 and 5.30 (2 × br s, 17-H_2); *m/z* 405 (M^+, 9%), 363 (45), 345 (19), 285 (16), 209 (13), 167 (23), 91 (25), and 43 (100).

ent-13-*Acetoxy*-2β-*deuterio*-3α,10β-*dihydroxy*-1β-*iodo*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**39**).—[2-^{2}H]Gibberellin A_3 13-acetate (**26**) (250 mg) in methanol (10 ml) and pyridine (1 ml) was stirred for 1 h at room temperature with 10% palladium-on-calcium carbonate (8 mg) under an atmosphere of hydrogen. The mixture was diluted with ethyl acetate and filtered. The filtrate was washed with aqueous sodium hydrogen carbonate and the aqueous layer acidified to pH 2 with dilute hydrochloric acid and extracted with ethyl acetate to give the crude hydrogenolysis product (**38**) as a gum (165 mg). Without further purification this gum in dichloromethane (10 ml), aqueous sodium hydrogen carbonate (15 ml) and tetrahydrofuran (10 ml) was vigorously stirred with iodine (90 mg) for 0.5 h at room temperature. The mixture was diluted with water and extracted with ethyl acetate. The extract was washed with aqueous sodium thiosulphate and then with water and concentrated under reduced pressure to give [2α-^{2}H]-1β-iodoGA_1 13-acetate 7-methyl ester (**39**) (145 mg), m.p. 163—165 °C (from ethyl acetate–light petroleum) containing 0.9 atoms deuterium per molecule; δ 1.18 (s, 18-H_3), 2.03 (s, OAc), 2.71 (d, *J* 10.5 Hz, 6-H), 3.73 (s, OMe), 3.86 (d, *J* 10.5 Hz, 5-H), 3.98 (br s, 3-H), 4.40 (s, 1-H), and 5.04 and 5.16 (2 × br s, 17-H_2); *m/z* 531 (M^+, 10%), 489 (32), 430 (12), 298 (10), 222 (15), 128 (20), 105 (12), 91 (18), and 43 (100).

Reduction of [2α-^{2}H]-1β-*IodoGA*$_1$ 13-*Acetate* (**39**).—[2α-^{2}H]-1β-IodoGA_1 13-acetate 7 methyl ester (**39**) (130 mg) in dimethyl sulphoxide (10 ml) was stirred with sodium borohydride (20 mg) for 2 h at room temperature. Work-up followed by purification by flash chromatography eluting with 40% ethyl acetate in light petroleum gave [2α-^{2}H]GA_1 13-acetate 7-methyl ester (**27**) (58 mg) identical by n.m.r. and mass spectrometry to the sample previously obtained.

Acknowledgements

We are grateful to Dr. E. Curzon of Warwick University for 400 MHz ^{1}H, ^{2}H, and ^{13}C n.m.r. spectra.

References

1 J. MacMillan and C. L. Willis, *J. Chem. Soc., Perkin Trans. 1*, 1984, 357.
2 J. MacMillan and C. L. Willis, *J. Chem. Soc., Perkin Trans. 1*, 1984, 351.
3 D. A. Taylor, Ph.D. Thesis, Bristol 1983.
4 J. MacMillan and R. J. Pryce, *J. Chem. Soc., C*, 1967, 740.
5 N. Murofushi, I. Yamaguchi, H. Ishigooka, and N. Takahashi, *Agric. Biol. Chem.*, 1976, **40**, 2471.
6 E. J. Corey, T. M. Brennan, and R. L. Carney, *J. Am. Chem. Soc.*, 1971, **93**, 7316.
7 J. Turkevich, D. O. Schissler, and P. Isra, *J. Phys. Chem.*, 1951, **55**, 1078.
8 J. W. Cornforth, R. H. Cornforth, and K. K. Mathew, *J. Chem. Soc.*, 1959, 112.
9 M. H. Beale, J. MacMillan, C. R. Spray, D. A. Taylor, and B. O. Phinney, *J. Chem. Soc., Perkin Trans. 1*, 1984, 541.
10 B. E. Cross, *J. Chem. Soc.*, 1960, 3022.
11 L. Lombardo, L. N. Mander, and J. V. Turner, *Aust. J. Chem.*, 1981, **34**, 745.
12 J. MacMillan, J. C. Seaton, and P. J. Suter, *Tetrahedron*, 1960, **11**, 60.
13 M. H. Beale and J. MacMillian, *J. Chem. Soc., Perkin Trans. 1*, 1980, 877.

Received 17*th June* 1985; *Paper* 5/1009