Partial Synthesis of [17-^{13}C; 15,17-^{3}H]Gibberellin A_{29} from Gibberellin A_3

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The preparation of [17-^{13}C]gibberellin A_{29}, containing 96.8 atoms % ^{13}C, from gibberellin A_3 in fourteen steps in 0.55% overall yield is described. [17-^{13}C; 15,17-^{3}H]Gibberellin A_{29}, containing 90.6 atoms % ^{13}C and with a specific radioactivity of 29.8 mCi mmol^{-1}, is also described.

The ready conversion of 2,O^2-didehydrogibberellin A_{29} by aqueous alkali to a known catabolite of gibberellin A_{29} is noted.

In a study of the metabolism of gibberellins (GAs) in developing seeds of *Pisum sativum* cv. Progress No. 9, Sponsel and MacMillan [1] found that the metabolism of [1β,3α-$^{2}H_2$; 1β,3α-$^{3}H_2$]GA_{29} [see structure (1)], metabolically derived from [1β,3α-$^{2}H_2$; 1β,3α-$^{3}H_2$]GA_{20} [2] [see structure (2)], and the metabolism of [2α-$^{2}H_1$; 2α-$^{3}H_1$]GA_{29} [3] resulted in loss of labelled atoms. For further investigation of the metabolism of GA_{29} (1), this GA was required with labelled atoms in positions other than ring *A*. This paper describes the preparation of 17-labelled GA_{29} from the available fungal GA_3 (4).

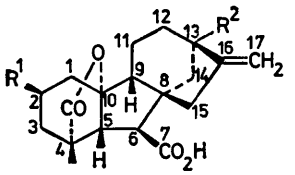
2-Hydroxy-GAs, containing no other hydroxy-group in ring *A*, can be prepared by the addition of acetyl hypobromite to a Δ^1- or Δ^2-ene. This method was first described by Beeley and MacMillan [4] in their synthesis of GA_{40} (3) from the norketone (6). In an earlier synthesis of GA_{29} (1) Beale and MacMillan [3] used a mixture of the Δ^1- and Δ^2-enes (9) and (10), obtained by reduction with tri-n-butylstannane of the 3α-chloro-compound (11). Compound (11) was, in turn, obtained by treatment of the norketone (5) with toluene-*p*-sulphonyl chloride and lithium chloride (*cf.* ref. 5) but this step was low yielding (21%) because of the simultaneous formation of the 13-toluene-*p*-sulphonate (12) which could not easily be converted into GA_{29} (1).[3] Another low yielding (27%) step in the previous synthesis of GA_{29} (1) was the tri-n-butylstannane reduction of the mixture of bromoacetates (13) and (14), obtained from the mixture of olefins (9) and (10), possibly because of lactone opening of the intermediate 1-radical. A new route was therefore sought from GA_3 (4) to the intermediate (7) in which the 13-hydroxy-group was protected for eventual introduction of the 16,17-exocyclic double bond by a Wittig reaction.

Oxidation of the known [2] GA_5 methyl ester 13-acetate (8) offered a direct route to the required intermediate (7). However, with osmium tetraoxide and sodium periodate, a complex mixture was obtained. With 1 mol equiv. of osmium tetraoxide, a mixture (7 : 2 by g.l.c.–mass spectrometry) of the 2β,3β- and the 16ξ,17-diols (15) and (16) was obtained, indicating that the 2-ene was more reactive than the 16-ene (*cf.* ref. 6).

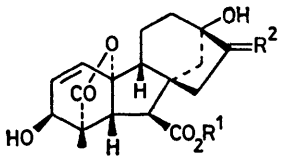
Attention was therefore directed to ring *A* precursors of the 2,3-double bond, and the successful route to GA_{29} (1) is shown in Scheme 1. The mixture (5 : 1 : 1) of alcohols (23)—(25), prepared as previously described [2] by acetylation and reduction of the known [7] enone (22), was oxidised with osmium tetraoxide–periodate to give the norketones (26) and (27) in 64 and 12% yield, respectively. The diketone (28) was also obtained in 0.6% yield; there are precedents [8] for the oxidation of a secondary alcohol by osmium tetraoxide–periodate. The norketone derived from the allylic alcohol (25) was not detected, possibly owing to further oxidation of the 1,2-double bond and loss of the products. The dehydration of the 3-alcohols (26) and (27) to the required intermediate (7) presented difficulties.

Direct dehydration of the 3α-alcohol (26) occurred in refluxing hexamethylphosphoramide [9] in an atmosphere of nitrogen, but only in 23% yield; the corresponding *N*,*N*-dimethylamide was also formed (11%), presumably by a nucleophilic displacement of the methoxy-group in a manner analogous [10] to the formation of dimethylamides from acid chlorides and hexamethylphosphoramide. The toluene-*p*-sulphonates (17) and (18) of the 3α- and 3β-alcohols (26) and (27) were unchanged in refluxing collidine. They were also inert to treatment with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) [11] in refluxing pyridine or tetrahydrofuran, although with DBU in refluxing collidine they did give the required olefin (7) but in low yield. Treatment of the 3α-toluene-*p*-sulphonate (17) with lithium bromide in pyridine at room temperature gave the 3β-bromide (29) and, on prolonged refluxing, gave the required olefin (7), accompanied by some demethylation [12] of the methyl ester. The 3β-bromide (29), on brief refluxing with DBU in pyridine, gave the required olefin (7) in 34% overall yield from the mixture of alcohols (26) and (27). Alternatively and more conveniently, the 3β-bromide (29) was obtained directly by reaction of the 3α-alcohol (26) with phosphoryl bromide in pyridine; some (10%) of the required olefin (7) was also formed. Analogous treatment of the 3β-alcohol (27) gave the required olefin (7). Thus the method adopted (Scheme 1) was treatment of the mixture (5 : 1) of the 3α- and 3β-alcohols (26) and (27) with phosphoryl bromide in pyridine to give, after chromatography, a mixture (2 : 7) of the olefin (7) and the 3β-bromo-compound (29); this mixture was then directly treated with DBU in pyridine to give the olefin (7); a minor amount of the 13-deacylated norketone (30) was also formed and re-acetylated to the 13-acetate (7).

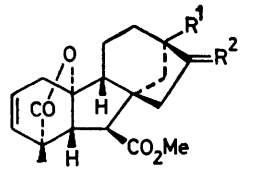
[17-^{13}C]GA_{29} (41) was prepared from the intermediate



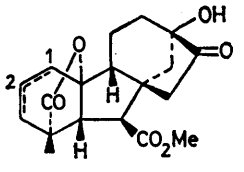
(1) $R^1 = R^2 = OH$
(2) $R^1 = H, R^2 = OH$
(3) $R^1 = OH, R^2 = H$



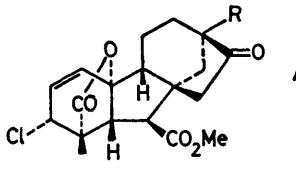
(4) $R^1 = H, R^2 = CH_2$
(5) $R^1 = Me, R^2 = O$



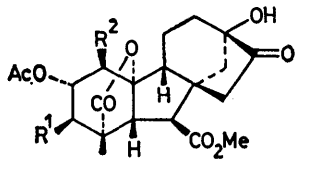
(6) $R^1 = H, R^2 = O$
(7) $R^1 = OAc, R^2 = O$
(8) $R^1 = OAc, R^2 = CH_2$



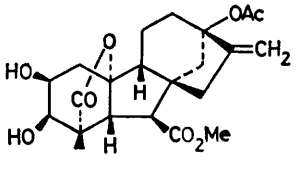
(9) Δ^1-ene
(10) Δ^2-ene



(11) R = OH
(12) R = $OSO_2C_6H_4Me$-*p*



(13) $R^1 = H, R^2 = Br$
(14) $R^1 = Br, R^2 = H$



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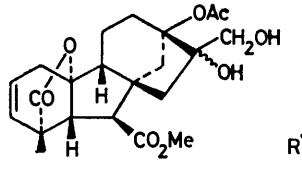

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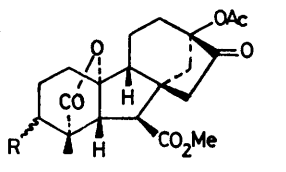
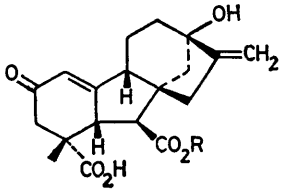
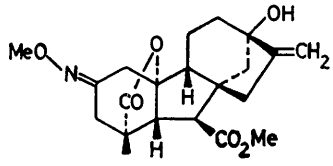


(17) R = α-$OSO_2C_6H_4Me$-*p*
(18) R = β-$OSO_2C_6H_4Me$-*p*



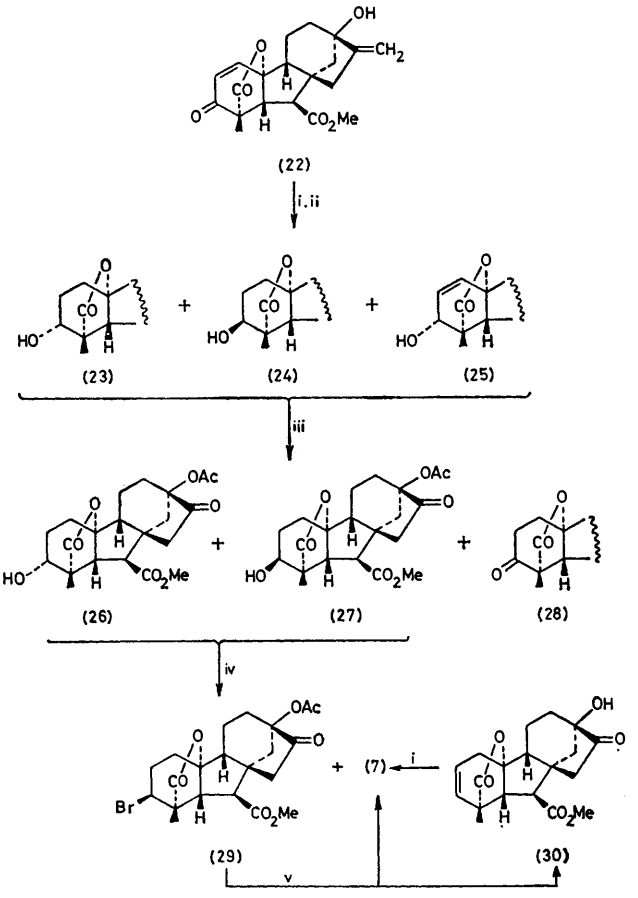
(19) R = Me
(20) R = H



(21)

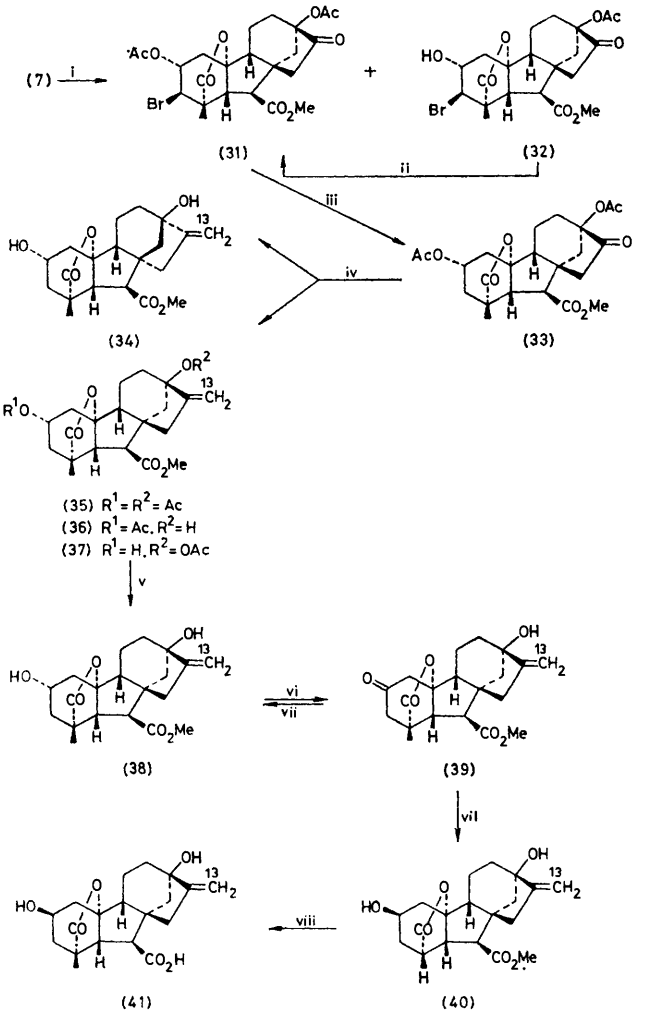
(7) according to Scheme 2. Reaction of the olefin (7) with acetyl hypobromite [13] gave the bromo-acetate (31) together with 11% of the bromohydrin (32) which was re-acetylated to the diacetate (31). The norketone (33), obtained by reductive debromination of the bromo-acetate (31), did not react smoothly with [^{13}C]methylenetriphenylphosphorane, prepared in tetrahydrofuran from [^{13}C]methyltriphenylphosphonium iodide and sodium hydride. A complex mixture of products was also obtained when the same Wittig conditions were applied to the bistrimethylsilyl ether of the diol, prepared after hydrolysis of the diacetate (33) by the method of Brewster *et al.*[14] The only difference between these unsuccessful reactions and numerous successful Wittig reactions, previously performed (*e.g.* ref. 15) in the laboratory, was the use of the phosphonium iodide in place of the phosphonium bromide. Since sodium iodide has appreciable solubility in tetrahydrofuran, it was concluded that the dissolved salt was causing unwanted side reactions. The problem was overcome, in this case, by changing the solvent to benzene, in which sodium iodide is insoluble. This resulted in the formation of the [17-^{13}C]diacetate (35) in 62% yield. The monoacetates (36) and (37) were also formed in 4 and 8% yield; rearrangement of rings *C*/*D* was also evident from the isolation, after hydrolysis, of the diol (34) in trace amounts. The hydrolysis of acetates [4] and rearrangement of rings *C*/*D* [15] during Wittig reactions have been previously noted. The monoacetates (36) and (37) were distinguished by their mass spectra (see later) and by their n.m.r. spectra. The 17-protons in the 13-acetate showed a smaller chemical shift difference (0.16 p.p.m.) than in the 2-acetate (0.27 p.p.m.), which is characteristic [5] of a 13-acetoxy- *versus* a 13-hydroxy-GA. Interestingly the diol (34) also showed a small (0.16 p.p.m.) chemical shift difference for the 17-protons, suggesting that rearrangement of rings *C* and *D* may have the same effect [5] as a 13-acetate or a

1,10-double bond on the separation of the 17-proton signals. Otherwise the n.m.r. and mass spectra of the diol (34) were similar to those of 2-*epi*-[17-^{13}C]GA_{29} methyl ester (38), obtained by hydrolysis [14] of the diacetate (35) and the monoacetates (36) and (37).



SCHEME 1 *Reagents*: i, Ac_2O–*p*-$MeC_6H_4SO_3H$; ii, $NaBH_4$–LiBr; iii, OsO_4–$NaIO_4$; iv, $POBr_3$; v, DBU

Oxidation of 2-*epi*-gibberellin A_{29} methyl ester (38) to the 2-ketone (39) by the method of Poos *et al.*[16] was reported by Beale and MacMillan [3] but in low yield. Alternative methods were therefore sought. Pyridinium dichromate [17] in dichloromethane gave incomplete oxidation of [17-^{13}C]-2-*epi*-GA_{29} methyl ester (38). Pyridinium chlorochromate [18] and sodium acetate in dichloromethane gave a mixture (3 : 1 by n.m.r.) of the required ketone (39) and the known [19] enone (19). The required ketone (39) decomposed on g.l.c. columns, was difficult to recover from p.l.c. on silica gel, and readily gave the enone (19) on mild treatment with aqueous alkali. However, it formed an *O*-methyloxime (21), which was characterised by g.l.c.–mass spectrometry of the 13-trimethylsilyl ether. Oxidation of the [17-^{13}C]-GA_{29} methyl ester (38) with pyridinium chlorochromate in dichloromethane, in the absence of sodium acetate, gave the required 2-ketone (39), homogenous by t.l.c., which was directly reduced with sodium borohydride to give equal amounts of the 2α- and 2β-alcohols (38) and (40), separable by p.l.c. The former was recycled to increase the yield of the 2β-alcohol (40), which was demethylated to give [17-^{13}C]GA_{29} (41), containing 96.8 atoms % ^{13}C; high recovery of the GA_{29} required exhaustive extraction of the aqueous acidified reaction mixture presumably because of the high water-solubility of the open-lactone compound, formed as an intermediate in the alkaline hydrolysis.

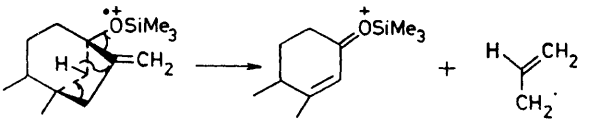


SCHEME 2 *Reagents:* i, LiOAc,$2H_2O$–MeCONHBr; ii, Ac_2O–*p*-$MeC_6H_4SO_3H$; iii, Bu^n_3SnH–$(Me_2C{\cdot}CN)_2N_2$; iv, $^{13}CH_2{=}PPh_3$; v, K_2CO_3–MeOH; vi, $C_5H_5N^+CrO_3Cl^-$; vii, $NaBH_4$; viii, KOH–H_2O–MeOH

This preparation of GA_{29}, which was accomplished from GA_3 (4) in fourteen steps in 0.55% overall yield, was repeated from the norketone (33), using [^{13}C; ^{3}H]-methyltriphenylphosphonium bromide in the Wittig step, to yield [17-^{13}C; 17-^{3}H]GA_{29} containing 90.6 atoms % ^{13}C and with a specific activity of 29.8 mCi mmol^{-1}. The scrambling of the ^{3}H-label between the 15- and 17-positions was presumed by analogy.[20]

In the mass spectra of the trimethylsilyl ethers of the methyl esters (38) and (40) the ions at *m/z* 207, 193, 180,

and 167, characteristic [21] of 13-hydroxy-GAs, occurred at 1 mass unit higher. A weak ion at m/z 465 ($M^+ - 42$), present in the spectra of the methyl esters (38) and (40) and of the unlabelled GA_{29} methyl ester indicates the loss of C-15, -16, and -17 as indicated in Scheme 3.



SCHEME 3

The 17-^{13}C-labelled compounds were characterised by ^{1}H and ^{13}C n.m.r. spectroscopy in which a $J(^1H,^{13}C)$ value of 158 Hz was observed for the 17-H_2 and 17-^{13}C signals.

The metabolism of [17-^{13}C]GA_{29} (41) to the [17-^{13}C]-catabolite (20) in developing seed of *P. sativum* cv. Progress No. 9 has been reported.[22] The intermediacy of the 2-ketone (39) in this metabolic conversion has been discussed and the possibility that the catabolite (20) is an artefact, formed by the observed non-enzymic, base-catalysed opening of the lactone bridge in the 2-ketone (39), has been discounted.[1,23]

EXPERIMENTAL

For general experimental details see refs. 2 and 3. Mass spectral data were obtained by g.l.c.–mass spectrometry unless stated otherwise.

Osmium Tetraoxide Oxidation of ent-13-*Acetoxy*-10-*hydroxy*-20-*norgibberella*-2,16-*diene*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (8).—The diene (8) (5 mg), osmium tetraoxide (2 mg) in tetrahydrofuran (0.1 ml), and water (0.1 ml) were treated at 0 °C with sodium periodate (2 mg). The solution was shaken for 24 h, then evaporated to half-volume and extracted with ethyl acetate at pH 3.0. The product was shown to be a complex mixture by g.l.c.–mass spectrometry of the trimethylsilyl (TMSi) derivative.

The diene (8) (5 mg) and osmium tetraoxide (3.5 mg) in pyridine (0.05 ml) and chloroform (0.05 ml) were left for 4 days. Sodium disulphite (200 mg) in water (2 ml) was added and the mixture was stirred for 30 min. The products were extracted into ethyl acetate at pH 3.0, trimethylsilylated, and analysed by g.l.c.–mass spectrometry. Two products were detected in the ratio 7 : 2 and assigned the structures (15) and (16) on the basis of the following mass spectral data: compound (15) TMSi ether, m/z 564(M^+, 39%), 522(3), 286(10), 217(17), 147(42), 75(30), 73(100), and 43(35); compound (16) TMSi ether, m/z 564(M^+, <1%), 522(2), 448(5), 377(26), 260(11), 147(21), 75(29), 73(100), and 43(41).

Osmium Tetraoxide–Sodium Periodate Oxidation of the Mixture of Acetates (23)—(25).—The mixture (7.2 g) of acetates (23)—(25), obtained [2] in the ratio 5 : 1 : 1 by hydride reduction of the acetate of 3, O^3-didehydrogibberellin A_3 methyl ester (22), was stirred at 0 °C in tetrahydrofuran (65 ml) and water (65 ml). Osmium tetraoxide (15 mg) and sodium periodate (11.5 g) were added with stirring and the mixture was allowed to reach room temperature. After 17 h, the tetrahydrofuran was removed *in vacuo* and the products were extracted into ethyl acetate. The recovered residue (7.09 g) was adsorbed on silica gel (20 g) and placed on a column of silica gel (345 g, 40 × 4 cm), made up in light petroleum. The column was eluted in 400 ml fractions with increasing increments of ethyl acetate in light petroleum. The fraction (68 mg) eluted with 60% ethyl acetate was purified by p.l.c., using ethyl acetate–light petroleum (4 : 1), to give, at R_F *ca.* 0.7, 3, O^3-*didehydro*-GA_1 *norketone methyl ester* 13-*acetate* (28) (45 mg), m.p. 200—203 °C (ethyl acetate–light petroleum) (Found: M^+, 404.145. $C_{21}H_{24}O_8$ requires M, 404.147); $\nu_{max.}$ ($CHCl_3$) 1 787, 1 768, 1 733, and 1 722sh cm^{-1}; δ 1.20(s, 18-H_3), 2.07(s, OCOMe), 2.87(d, J 10 Hz, 6-H), 3.17(d, J 10 Hz, 5-H), and 3.78(s, CO_2Me); m/z (probe) 404(M^+, 21%), 376(6), 362(17), 334(27), 316(26), 302(47), 288(23), 112(48), and 43(100).

The fractions (863 mg) eluted with 65—70% ethyl acetate, gave mainly GA_1 *norketone methyl ester* 13-*acetate* (27), a portion of which was subjected to p.l.c. as above to give, at R_F 0.5—0.6, the pure norketone (27) as a gum (Found: M^+, 406.161. $C_{21}H_{26}O_8$ requires M, 406.163); $\nu_{max.}$ ($CHCl_3$) 3 524, 1 764, and 1 735 cm^{-1}; δ 1.18(s, 18-H_3), 2.08(s, OCOMe), 2.77(d, J 10 Hz, 6-H), 3.27(d, J 10 Hz, 5-H), 3.79(s, CO_2Me), and 3.88(br, s, 3-H); m/z (probe) 406(M^+, 5%), 378(3), 364(9), 363(9), 318(22), 304(15), 272(21), 258(16), and 43(100); m/z (TMSi ether) 478(M^+, 21%), 390(71), 272(92), 258(52), 129(70), 75(71), 73(100), and 43(79).

The fractions (4.66 g) eluted with 75—100% ethyl acetate, gave mainly 3-epi GA_1 *norketone methyl ester* 13-*acetate* (26), part of which was purified by p.l.c. as above to give, at R_F 0.4—0.5, the pure norketone (26), m.p. 198—201 °C (ethyl acetate–light petroleum) (Found: C, 61.3; H, 6.8%; M^+, 406.161. $C_{21}H_{26}O_8$ requires C, 62.1; H, 6.4%; M, 406.163); $\nu_{max.}$ 3 500, 1 767, 1 742, and 1 717 cm^{-1}; δ 1.19(s, 18-H_3), 2.05(s, OCOMe), 2.59(d, J 10 Hz, 6-H), 2.82(d, J 10 Hz, 5-H), *ca.* 3.72(br, s, 3-H), and 3.78(s, CO_2Me); m/z (TMSi ether) 478(M^+, 15%), 450(14), 431(14), 403(26), 390(48), 376(93), 358(41), 349(43), 129(100), 75(54), 73(96), and 43(64).

Preparation of the Toluene-p-*sulphonates* (17) *and* (18).—A mixture (328 mg; 9 : 1) of the 3-alcohols (26) and (27), and toluene-*p*-sulphonyl chloride (610 mg) were dissolved in pyridine (3.5 ml). After 2 days, water was added, the pH was adjusted to 3.0 with 10M-hydrochloric acid, and the products were extracted into ethyl acetate. P.l.c. of the recovered product using ethyl acetate–light petroleum (7 : 3) gave, at R_F 0.4—0.5, the 3α-*toluene*-p-*sulphonate* (17) (277 mg), m.p. 221—222 °C (from chloroform–light petroleum) (Found: C, 60.2; H, 5.8; S, 5.7. $C_{28}H_{32}O_{10}S$ requires C, 60.0; H, 5.7; S, 5.7%); $\nu_{max.}$ 1 790, 1 760, 1 729, 1 180, and 938 cm^{-1}; δ 1.04(18-H_3), 2.05(s, OCOMe), 2.47(s, ArMe), 2.66(d, J 10 Hz, 6-H), 2.80(d, J 10 Hz, 5-H), 3.77(s, CO_2Me), 4.62(dd, J 6 and 10 Hz, 3-H), 7.39 and 7.82(each d, J 8 Hz, 4 × ArH); m/z (probe) 560(M^+, 9%), 532(11), 490(17), 472(52), 458(49), 318(18), 300(26), 286(57), 272(37), 258(29), 91(73), and 43(100).

Elution of the band at R_F 0.5—0.6 gave the 3β-*toluene*-p-*sulphonate* (18) (20 mg), m.p. 235—238 °C (from chloroform); δ 0.88(s, 18-H_3), 2.05(s, OCOMe), 2.48(s, ArMe), 3.15(d, J 10 Hz, 5-H), 3.77(s, CO_2Me), 4.54(m, 3-H), and 7.43 and 7.88(each d, J 8 Hz, 4 × Ar-H); m/z (probe) 560(M^+, 6%), 532(6%), 517(11), 472(40), 458(11), 318(31), 300(25), 286(41), 272(68), 91(68), and 43(100).

Exploratory attempts to prepare ent-13-*Acetoxy*-10-*hydroxy*-16-*oxo*-17,20-*dinorgibberell*-2-*ene*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (7).—(*a*) The 3α-toluene-*p*-sulphonate (17) (13 mg) in collidine (0.4 ml) was refluxed under nitrogen

for 2.5 h. Water was added; extraction into ethyl acetate at pH 3.0 gave the starting material (by n.m.r.).

(*b*) The 3β-toluene-*p*-sulphonate (18) (6 mg) in collidine (0.4 ml) was refluxed under nitrogen for 2.5 h. Work-up as above gave the starting material (by n.m.r.).

(*c*) The 3α-toluene-*p*-sulphonate (17) (20 mg) and 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) (0.006 ml) in tetrahydrofuran (0.5 ml) were refluxed under nitrogen for 2 h. Work-up as in (*a*) gave starting material only.

(*d*) The 3β-toluene-*p*-sulphonate (18) (8 mg) and DBU (0.006 ml) in pyridine (0.4 ml) were refluxed under nitrogen for 1 h. Work-up as in (*a*) gave the starting material.

(*e*) The 3α-toluene-*p*-sulphonate (17) (90 mg) and DBU (0.045 ml) in collidine (2.5 ml) were refluxed under nitrogen for 2.5 h. Work-up as in (*a*) gave a gum (75 mg) which on p.l.c. using ethyl acetate–light petroleum (7 : 3) gave, at R_F 0.45—0.6, the Δ²-*norketone* (7) (24 mg) (see later for characterisation).

(*f*) The 3α-toluene-*p*-sulphonate (17) (32 mg) and anhydrous lithium bromide (300 mg) in pyridine (10 ml) were refluxed under nitrogen for 13 h. Work-up as in (*a*) and remethylation with ethereal diazomethane gave, on evaporation, a gum which was purified by p.l.c. as in (*e*). Elution of the band at R_F 0.45—0.55 gave the Δ²-norketone (7) (11 mg); the band at R_F 0.55—0.65 gave the 3β-bromo-norketone (29) (5 mg) (see later for characterisation).

(*g*) Treatment of the 3β-toluene-*p*-sulphonate (18) with lithium bromide in pyridine as in (*f*) gave no reaction.

(*h*) Phosphoryl bromide (60 mg) and the 3α-alcohol (26) (50 mg) in pyridine (1 ml) were left for 20 h. The reaction mixture was cooled in ice and water was added slowly. Extraction into ethyl acetate at pH 3.0 gave, on evaporation and p.l.c., the Δ²-norketone (7) (2 mg) and the 3β-bromo-norketone (29) (19 mg).

(*i*) The 3β-bromo-norketone (29) (13 mg) and DBU (0.007 ml) in pyridine (0.4 ml) were refluxed under nitrogen for 1 h. Work-up as in (*a*) gave the pure Δ²-norketone (7) (10 mg).

(*j*) The 3α-alcohol (26) (18 mg) in hexamethylphosphoramide (1 ml) was refluxed under nitrogen for 2 h. Work-up as in (*a*) gave a gum (15 mg) which was subjected to p.l.c. as in (*e*). The band at R_F 0.5—0.6 gave the Δ²-norketone (7) (4 mg). Elution of the band at R_F 0.15—0.2 gave the N,N-*dimethylamide* of the norketone (7) as a gum (2 mg); δ(microcell) 1.24(s, 18-H_3), 2.07(s, OCOMe), 3.04 and 3.15(each s, NMe_2), and 5.78(m, 2- and 3-H); *m/z* 401(M^+, 1%), 373(6), 356(81), 330(17), 213(10), 72(100), 46(18), and 43(29).

ent-13-*Acetoxy*-3α-*bromo*-10-*hydroxy*-16-*oxo*-17,20-*dinorgibberellane*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (29).—A mixture (5 : 1; 4.1 g) of the 3α- and 3β-alcohols (26) and (27) and phosphoryl bromide (5.0 g) in pyridine (70 ml) was left for 20 h. Work-up was carried out as in (*h*) above and the crude product (3.06 g) was adsorbed on silica and placed on a silica column (140 g; 33 × 3 cm) made up in light petroleum. The column was eluted in fractions (each 200 ml) with increasing proportions of ethyl acetate in light petroleum. Elution with 40—75% ethyl acetate gave a mixture (2 : 7 by n.m.r.; 1.84 g) of the Δ²-norketone (7) and the 3β-bromo-norketone (29). P.l.c. of a sample as in (*e*) above gave, at R_F 0.6—0.75, the pure 3β-*bromo-norketone* (29), m.p. 235—245 °C (decomp.) (needles from chloroform–light petroleum) (Found: C, 53.4; H, 5.5; Br, 16.9. $C_{21}H_{25}BrO_7$ requires C, 53.7; H, 5.3; Br, 17.1%); $\nu_{max.}$ 1 776, 1 760, 1 729, 1 232, 1 197, and 920 cm^{-1}; δ 1.23(s, 18-H_3), 2.07(s, OCOMe), 2.78(d, *J* 10 Hz, 6-H), 3.39(d, *J* 10 Hz, 5-H), 3.81 (s, CO_2Me), and 4.29(t, *J* 3 Hz, 3-H); *m/z* (probe) 470 (M^+ + 2, 9%), 468(M^+, 9), 428(21), 426(24), 400(13), 398(13), 382(33), 380(28), 368(25), 366(26), 344(28), 273(97), 213(27), and 43(100).

ent-13-*Acetoxy*-10-*hydroxy*-16-*oxo*-17,20-*dinorgibberell*-2-*ene*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (7).—The mixture (2 : 7; 1.8 g) of the Δ²-norketone (7) and the 3β-bromo-compound (29) from the previous experiment, and DBU (0.45 ml) in pyridine (10 ml) were refluxed under nitrogen for 40 min. Work-up as in exploratory experiment (*a*) afforded the crude product (1.58 g), which was purified by column chromatography on silica (130 g; 30 × 3 cm) as in the previous experiment. Elution with 45—55% ethyl acetate gave the Δ²-*norketone* (7) (1.03 g), crystallised from chloroform–light petroleum as hexagonal plates, m.p. 239—241 °C (transition to rectangular plates, 190—220 °C) (Found: C, 64.1; H, 6.5%; M^+, 388.153. $C_{21}H_{24}O_7$ requires C, 65.0; H, 6.2%; *M*, 388.152); $\nu_{max.}$ 1 780, 1 762, 1 733sh, 1 622, and 1 029 cm^{-1}; δ 1.26(s, 18-H_3), 2.06(s, OCOMe), 2.72(d, *J* 9 Hz, 6-H), 2.87(d, *J* 9 Hz, 5-H), 3.79(s, CO_2Me), and 5.79(m, 2- and 3-H); *m/z* (probe) 388(M^+, <1%), 357(6), 344(33), 302(13), 286(25), 284(54), 240(32), 213(29), 155(21), and 43(100).

Elution with 60—90% ethyl acetate and p.l.c. as in exploratory experiment (*e*) gave the Δ²-norketone (7) (134 mg) at R_F 0.6—0.7. The band at R_F 0.35—0.45 gave the 13-*hydroxy*-Δ²-*norketone* (30) as a gum (69 mg) (Found: M^+, 346.138. $C_{19}H_{22}O_6$ requires *M*, 346.142); $\nu_{max.}$ ($CHCl_3$) 3 492, 1 770, 1 754, 1 712, and 900 cm^{-1}; δ 1.27(s, 18-H_3), 2.76(d, *J* 10 Hz, 6-H), 2.91(d, *J* 10 Hz, 5-H), 3.09(s, disappeared on addition of D_2O, 13-OH), 3.79(s, CO_2Me), and 5.80(m, 2- and 3-H); *m/z* (probe) 346(M^+, 10%), 315(23), 302(85), 286(44), 242(100), 240(28), 213(39), 157(50), 156(52), 155(37), and 143(34); *m/z* (TMSi ether) 418(M^+, <1%), 403(13), 390(44), 362(72), 278(16), 143(100), 75(30), and 73(98).

The 13-hydroxy-Δ²-norketone (30) (67 mg) and toluene-*p*-sulphonic acid (*ca.* 1 mg) in acetic anhydride (0.5 ml) were left for 20 h. Addition of water, extraction into ethyl acetate, and evaporation of the extract gave the Δ²-norketone (7) (73 mg).

ent-2β,13-*Diacetoxy*-3α-*bromo*-10-*hydroxy*-16-*oxo*-17,20-*dinorgibberellane*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (31).—*N*-Bromoacetamide (570 mg) was added to the Δ²-norketone (7) (1.23 g) and lithium acetate dihydrate (5.7 g) in acetic acid (45 ml) with stirring. After 3.5 h, water was added, the product was extracted into ethyl acetate, and the extract was evaporated *in vacuo*. The crude product (1.75 g) was purified by silica column chromatography (120 g; 29 × 3 cm) as in the previous experiments. Elution with 45—55% ethyl acetate afforded the pure 2α,13-*diacetoxy*-3β-*bromo-norketone* (31) (1.06 g), crystallised from chloroform-light petroleum as rectangular plates, m.p. 218—220 °C (changing to needles >200 °C) (Found: M^+, 526.086. $C_{23}H_{27}{}^{79}BrO_9$ requires *M*, 526.083); $\nu_{max.}$ 1 777, 1 764sh, 1 746, 1 242, 1 040, and 924 cm^{-1}; δ 1.24(s, 18-H_3), 208(s, 2 × OCOMe), 2.82(d, *J* 11 Hz, 6-H), 3.41(d, *J* 11 Hz, 5-H), 3.81(s, CO_2Me), 4.15 (s, 3-H), and 5.38(br, t, *J* 3 Hz, 2-H); *m/z* (probe) 528(M^+ + 2, 11%), 526(M^+, 11), 486(15), 484(16), 458(15), 456(15), 398(11), 396(11), 366(41), 364(41), 317(66), 285(100), 213(64), 211(57), and 43(⩾100).

Elution with 60—75% ethyl acetate, evaporation of the eluate, and p.l.c., using ethyl acetate–light petroleum (4 : 1) gave, at R_F 0.7—0.8, the 2α,13-diacetoxy-3β-bromo-nor-

ketone (31) (180 mg). Elution of the band at R_F 0.4—0.5 gave the 13-*acetoxy*-3β-*bromo*-2α-*hydroxy*-*norketone* (32) as a gum (165 mg) (Found: M^+, 484.072. $C_{21}H_{25}{}^{79}BrO_8$ requires M, 484.073); $\nu_{max.}$ ($CHCl_3$) 3 500, 1 780, 1 764, 1 736, and 923 cm^{-1}; δ 1.26(s, 18-H_3), 2.08(s, OCOMe), 2.82(d, J 10 Hz, 6-H), 3.40(d, J 10 Hz, 5-H), 3.57(d, J 4 Hz, disappeared on addition of D_2O, 2-OH), 3.82(s,CO_2Me), 4.22(s, 3-H), and 4.50(m, 2-H); *m/z* (probe) 486(M^+ + 2, 5%), 484(M^+, 5), 458(12), 456(13), 398(16), 396(15), 360(14), 317(44), 285(55), 213(30), 211(23), and 43(100); *m/z* (TMSi ether) 558(M^+ + 2, 1%), 556(M^+, 1), 488(13), 486(13), 389(35), 285(82), 213(53), 75(71), 73(72), and 43(100).

The 13-acetoxy-3β-bromo-2α-hydroxy-norketone (32) (162 mg) and toluene-*p*-sulphonic acid (*ca.* 2 mg) in acetic anhydride (2 ml) were left for 4 h. Addition of water, extraction into ethyl acetate, and evaporation gave the 2α,13-diacetoxy-3β-bromo-norketone (31) (165 mg).

ent-2β,13-*Diacetoxy*-10-*hydroxy*-16-*oxo*-17,20-*dinorgibberellane*-7,20-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (33).—The bromoacetate (31) (1.31 g), tri-n-butylstannane (1.2 ml), and 2,2′-azobis(2-methylpropiononitrile) (5 mg) in benzene (30 ml) were refluxed under nitrogen for 40 min. The solvent was evaporated off and the crude product fractionated (each 200 ml) by silica column chromatography (130 g; 28 × 3 cm) as described earlier. Elution with 55—90% ethyl acetate afforded the 2α,13-diacetoxy-norketone (33) (1.05 g), crystallised from chloroform-light petroleum as needles, m.p. 218—220 °C (Found: C, 61.4; H, 6.5%; M^+, 448.174. $C_{23}H_{28}O_9$ requires C, 61.6; H, 6.3%; M, 448.173); $\nu_{max.}$ 1 782, 1 761, 1 741, and 928 cm^{-1}; δ 1.13(s, 18-H_3), 2.06(s, OCOMe), 2.08(s, OCOMe), 2.70(d, J 10 Hz, 6-H), 2.84(d, J 10 Hz, 5-H), 3.82(s, CO_2Me), and 5.23(m, 2-H); *m/z* (probe) 448(M^+, 8%), 420(8), 378(16), 318(22), 286(100), 272(9), 258(11), 240(11), 213(17), and 43(84).

ent-2β,10,13-*Trihydroxy*-16-*oxo*-17,20-*dinorgibberellane*-7,20-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester*.—The 2α,13-diacetoxy-norketone (33) (20 mg) and anhydrous potassium carbonate (20 mg) were stirred in methanol (10 ml) for 15 h. 'Amberlite' IR-120(H) ion-exchange resin (200 mg) was added and stirring continued for 30 min. The supernatant solution was removed and evaporated to yield the 2α,13-*dihydroxy*-*norketone* as a gum (15 mg) (Found: M^+, 364.149. $C_{19}H_{24}O_7$ requires M, 364.152); $\nu_{max.}$ ($CHCl_3$) 3 550br, 3 380br, 1 778, 1 753, and 1 734 cm^{-1}; δ 1.13(s, 18-H_3), 2.57(s, disappeared on addition of D_2O, 2 × OH), 2.69 (d, J 10 Hz, 6-H), 2.84(d, J 10 Hz, 5-H), 2.80(s, CO_2Me), and 4.39(m, 2-H); *m/z* (probe) 364(M^+, 4%), 346(9), 336(5), 333(11), 304(19), 286(100), 213(37), and 157(27); *m/z* (TMS ether) 508 (M^+, 1%), 493(6), 480(25), 452(79) ,405(16), 390(11), 143(48), 130(22), 75(52), and 73(100).

Reaction of ent-2β,13-*Diacetoxy*-10-*hydroxy*-16-*oxo*-17,20-*dinorgibberellane*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (33) *with a* [^{13}C]*Methylenephosphorane*.—Sodium hydride (870 mg; 60% suspension in oil) was washed several times with light petroleum then added to [^{13}C]methyltriphenylphosphonium iodide (3.8 g; m.p. 186—187.5 °C), prepared from [^{13}C]methyl iodide (96.8 atoms % ^{13}C), in tetrahydrofuran (30 ml). After being stirred for 14 h in nitrogen (green colour), the supernatant was removed and evaporated under vacuum, and dry benzene (20 ml) was added. The supernatant (10 ml) was added to the 2α,13-diacetate (33) (455 mg) under nitrogen. After 16 h, the reaction was quenched with acetone and the crude product, obtained by evaporation, was fractionated by silica gel column chromatography (125 g; 27 × 3 cm) in the usual way. Elution with 55—65% ethyl acetate in light petroleum gave the [17-^{13}C]2α,13-*diacetate* (35) (270 mg), m.p. 167—168 °C (from chloroform–light petroleum) (Found: C, 64.6; H, 6.6. $^{12}C_{23}{}^{13}C_1H_{30}O_8$ requires C, 64.7; H, 6.7%); $\nu_{max.}$ 1 780, 1 738, and 878 cm^{-1}; δ_H 1.11(s, 18-H_3), 2.04(s, 2 × OCOMe), 2.64(d, J 10 Hz, 6-H), 2.79(d, J 10 Hz, 5-H), 3.77 (s, CO_2Me), 5.00 and 5.16 (each br, d, J 158 Hz, 17-H_2), and 5.22(m, 2-H); δ_C (noise-decoupled, proton-decoupled) 107.815 (17-C), (noise-decoupled only, t, J 158 Hz); *m/z* 447(M^+, 7%), 416(6), 405(83), 387(20), 345(42), 327(18), 313(57), 285(37), 283(47), and 43(100).

Elution with 70—100% ethyl acetate gave a mixture which on p.l.c., using ethyl acetate–light petroleum (7 : 3), afforded, at R_F 0.5—0.6, the [17-^{13}C]-2α,13-diacetate (35) (10 mg) and, at R_F 0.4—0.5, the [17-^{13}C]-2α-*acetoxy*-13-*alcohol* (36) as a gum (17 mg); δ 1.12(s, 18-H_3), 2.04(s, OCOMe), 2.61(d, J 10 Hz, 6-H), 2.75(d, J 10 Hz, 5-H), 3.76(s, CO_2Me), 4.99(br, d, J 158 Hz, 17-H), 5.26(m, 2-H), and 5.29(br, d, J 158 Hz, 17-H); *m/z* (TMSi ether) 477(M^+, 100%), 462(12), 418(18), 376(21), 371(21), 209(13), 208(35), 181(8), 168(15), 75(38), 73(69), and 43(13).

Elution of the p.l.c. band at R_F 0.3—0.4 gave the [17-^{13}C]-13-*acetoxy*-2α-*alcohol* (37) as a gum (34 mg); δ 1.10(s, 18-H_3), 2.03(s, OCOMe), 2.62(d, J 10 Hz, 6-H), 2.76(d, J 10 Hz, 5-H), 3.75(s, CO_2Me), 4.31(m, 2-H), and 5.00 and 5.14(each br, d, J 158 Hz, 17-H_2); *m/z* (TMSi ether) 477(M^+, 24%), 446(8), 435(53), 420(51), 402(64), 373(27), 358(30), 283(59), 224(63), 143(35), 75(100), 73(41), and 43(35).

Elution of the p.l.c. band at R_F 0.2—0.3 gave starting material (33) (54 mg) and, at R_F 0.1—0.2, a mixture (37 mg). Hydrolysis of the mixture by stirring with anhydrous potassium carbonate (50 mg) in methanol (3 ml) for 14 h, and work-up with 'Amberlite' IR-120(H) (500 mg) as before gave the pure C/D-*rearranged* [17-^{13}C]-*diol* (34) as a gum (26 mg) (Found: M^+, 363.176. $^{12}C_{19}{}^{13}CH_{26}O_6$ requires M, 363.176); $\nu_{max.}$ ($CHCl_3$) 3 600, 1 775, 1 731, and 1 643w cm^{-1}; δ 1.15(s, 18-H_3), 3.72(s, CO_2Me), 4.33(m, 2-H), 4.81 and 4.97(each br, d, J 158 Hz, 17-H_2); *m/z* (probe) 363(M^+, 25%), 345(35), 331(40), 317(21), 313(91), 303(28), 299(28), 285(100), 240(53), 136(37), and 91(41); *m/z* (TMSi ether) 507(M^+, 100%), 492(7), 460(10), 376(25), 304(27), 239(10), 208(34), 168(17), 75(29), and 73(60).

ent-[17-^{13}C]-2β,10,13-*Trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (38).—The [17-^{13}C]-2α,13-diacetate (35) (240 mg) and a mixture (51 mg) of the [17-^{13}C]monoacetates (36) and (37) were dissolved in methanol (18 ml) and stirred with anhydrous potassium carbonate (400 mg) for 20 h. Addition of Amberlite IR-120(H) resin (4 g) and the usual work-up gave [17-^{13}C]-2-epi-GA_{29} *methyl ester* (38) (225 mg; 97.1 atoms % ^{13}C), crystallised from acetone–light petroleum as needles, m.p. 181—183 °C (Found: C, 66.3; H, 7.3%; M^+, 363.174. $^{12}C_{19}{}^{13}CH_{26}O_6$ requires C, 66.3; H, 7.2%; M, 363.176); $\nu_{max.}$ 3 513, 1 757, 1 715, and 1 641w cm^{-1}; δ 1.11(s, 18-H_3), 2.61(d, J 10 Hz, 6-H), 2.76(d, J 10 Hz, 5-H), 3.74(s, CO_2Me), 4.33(m, 2-H), and 4.98 and 5.28(each br, d, J 158 Hz, 17-H_2); *m/z* (probe) 363(M^+, 24%), 345(20), 331(24), 317(15), 313(100), 304(33), 285(37), and 240(18); *m/z* (TMSi ether) 507(M^+, 100%), 492(7), 478(4), 465(3), 460(11), 376(37), 304(40), 209(23), 208(53), 75(36), and 73(92).

ent-[17-^{13}C]-10,13-*Dihydroxy*-2-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* (39).—(*a*) [17-^{13}C]-2-*epi* GA_{29} methyl ester (38) (18 mg) and pyridinium dichromate [17] (16 mg) in dichloromethane (0.3 ml) were stirred for 3 h. Diethyl ether (*ca.* 5 ml) was added with

stirring and the insoluble material was removed by centrifugation. The supernatant solution was analysed by mass spectrometry, which revealed that only partial oxidation had occurred.

(*b*) Pyridinium chlorochromate [18] (25 mg) and sodium acetate (12 mg) in dichloromethane (0.5 ml) were stirred for 10 min, and a solution of [17-^{13}C]-2-*epi*-GA_{29} methyl ester (38) (23 mg) in dichloromethane (0.5 ml) was added. Stirring was continued for 1 h and the mixture was worked up as in (*a*). Spectroscopic analyses revealed a mixture (*ca.* 3 : 1) of [17-^{13}C]-2,O^2-*didehydro*-GA_{29} *methyl ester* (39) and the enone (19) [19] (Found: M^+, 361.160. $^{13}C^{12}C_{19}H_{24}O_6$ requires *M*, 361.160); $\nu_{max.}$ ($CHCl_3$) 3 620, 3 434br, 1 781, 1 728, 1 667, 1 616, 1 040, and 872 cm^{-1}; δ [for (39)] 1.20(s, 18-H_3); δ [for (19)] 1.27(s, 18-H_3) and 5.37(t, *J* 5 Hz, 1-H); δ (common signals) 3.81(s, CO_2Me), and 5.01 and 5.28(each br, d, *J* 158 Hz, 17-H_2).

(*c*) Experiment (*b*) was repeated without the addition of sodium acetate and using pyridinium chlorochromate (270 mg) and [17-^{13}C]-2-*epi*-GA_{29} methyl ester (38) (171 mg) in dichloromethane (15 ml) to give pure [17-^{13}C]-2,O^2-*didehydro*-GA_{29} *methyl ester* (39), homogeneous by t.l.c.; *m/z* (probe) 361(M^+, 81%), 343(5), 329(100), 304(47), 302(37), 277(24), 256(30), 164(34), and 137(39). The ketone (39) (0.5 mg) was treated with methoxyamine (1 mg) in pyridine (0.25 ml) at room temperature for 40 h; the usual work-up gave the methyloxime (21); *m/z* (TMSi ether) 462(M^+, 100%), 447(9), 431(18), 403(9), 376(44), 236(18), 208(29), and 73(48).

ent-[17-^{13}C]-2α,10,13-*Trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* 7-*Methyl Ester* ([17-^{13}C]GA_{29} *Methyl Ester*) (40).—The oxidation product from the preceding experiment (*c*), without further purification, was evaporated and immediately dissolved in ethanol (12 ml). Sodium borohydride (250 mg) was added and the mixture was stirred for 2 h. The solution was evaporated, water was added, and the products were extracted into ethyl acetate at pH 3.0. The products obtained by evaporation of the organic solvent *in vacuo* were fractionated by p.l.c., using ethyl acetate–light petroleum (4 : 1), to give at R_F 0.35—0.45, [17-^{13}C]GA_{29} *methyl ester* (40) (97.9 atom % ^{13}C) as a gum (42 mg); δ 1.10(s, 18-H_3), 2.63(d, *J* 10 Hz, 6-H), 2.74(d, *J* 10 Hz, 5-H), 3.76(s, CO_2Me), 3.98(m, 2-H), and 4.98 and 5.27(each br, d, $J_{H^{13}C}$ 158 Hz, 17-H_2); *m/z* (TMSi ether) 507(M^+, 100%), 492(11), 465(3), 448(5), 390(8), 376(13), 304(19), 209(17), 208(34), 194(7), 181(6), 168(12), 75(37), and 73(49).

Elution of the band at R_F 0.25—0.35 gave the 2α-alcohol (38) (34 mg). Re-oxidation, reduction, and p.l.c. gave more of the required 2β-alcohol (40) (10 mg) and the 2α-alcohol (38) (10 mg).

ent-[17-^{13}C]-2α,10,13-*Trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([17-^{13}C]GA_{29}) (41).—[17-^{13}C]GA_{29} methyl ester (40) (52 mg) in methanol (5 ml) and aqueous potassium hydroxide (10%; 5 ml) was refluxed for 16 h. The methanol was evaporated off and the residual solution was extracted with ethyl acetate. The aqueous layer was adjusted to pH 3.0 and was extracted with ethyl acetate; the solvent was evaporated off and the residue was heated at 80 °C for 0.5 h to close the lactone. Repetition of the above partition procedure and evaporation of the ethyl acetate solution of acidic products gave [17-^{13}C]GA_{29} (41) (96.8 atoms % ^{13}C; 92% pure by g.l.c.) as a gum (16 mg); *m/z* (TMSi ether) 565(M^+, 74%), 550(22), 448(34), 309(16), 208(21), 75(100), and 73(90).

ent-[17-^{13}C; 15,17-^{3}H]-2α,10,13-*Trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([17-^{13}C, 15,17-^{3}H]GA_{29}).—(*a*) A suspension of sodium hydride (683 mg of 50% dispersion in oil; washed with light petroleum) and [^{13}C, ^{3}H]methyltriphenylphosphonium bromide (2.46 g) [prepared [15] from $Ph_3P^{13}CH_3Br,3H_2O$ (1.75 Ci; 0.35 ml), triethylamine (3.5 ml), and acetonitrile (16 ml)] in tetrahydrofuran (20 ml) was stirred overnight. The above phosphorane solution (2.5 ml) was added to the norketone diacetate (33) (47 mg). After stirring for 1 h, acetone was added and the solvents were evaporated off. The residue in ethyl acetate–light petroleum (7 : 3) was eluted through a short column of silica gel to give a mixture of the tritiated di- and mono-acetates (35)—(37) (40 mg).

(*b*) The above mixture of acetates in methanol (3 ml) was stirred for 20 h with potassium carbonate (62 mg). Amberlite IR-120(H) resin (620 mg) was added and after 15 min the solution filtered and evaporated to give [17-^{13}C, 15,17-^{3}H]-2-*epi*-GA_{29} methyl ester, pure by g.l.c. of its TMSi derivative.

(*c*) The diol from (*b*), in dichloromethane (3 ml), was added to pyridinium chlorochromate (71 mg) in dichloromethane (1 ml). After 1 h ether was added and the solution filtered and evaporated to give the tritiated ketone (39), which was reduced immediately with sodium borohydride (50 mg) in ethanol (10 ml) for 2 h. The mixture was evaporated and the residue was partitioned between ethyl acetate and 2M-hydrochloric acid. Recovery from the ethyl acetate followed by p.l.c. using ethyl acetate–light petroleum (4 : 1) gave at R_F 0.5 [17-^{13}C,15,17-^{3}H]GA_{29} methyl ester (40) (6.5 mg), and at R_F 0.4 the tritiated 2α-alcohol (38) (8 mg).

(*d*) [15,17-^{3}H, 17-^{13}C]GA_{29} methyl ester (6.5 mg) in methanol (1 ml) and 2N-sodium hydroxide (3 ml) was refluxed for 6 h. The methanol was evaporated off and the aqueous residue was acidified with 2N-hydrochloric acid. Ethyl acetate was added and the mixture was rapidly stirred for 6 h. The layers were separated and the aqueous portion was further extracted with ethyl acetate in the same way for 1 and 3 days. The combined ethyl acetate extracts were then extracted with 2N-sodium hydroxide. Acidification of the aqueous layer, followed by recovery in ethyl acetate, gave [17-^{13}C, 15,17-^{3}H)GA_{29} (41) (5 mg) (90.6 atoms % ^{13}C; 29.8 mCi mmol^{-1}), pure by radio-g.l.c.

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