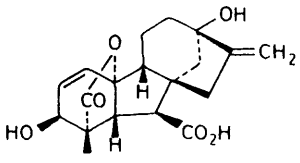
Partial Syntheses of [2α-^{2}H]- and [2α-^{3}H]-Gibberellin A_{29} and [2α-^{2}H,-15,17-$^3H_4$]Gibberellin A_{51} from Gibberellin A_3

By **Michael H. Beale** and **Jake MacMillan,*** School of Chemistry, The University, Bristol BS8 1TS

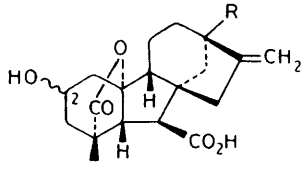
In the partial syntheses of the title compounds gibberellin A_3 (GA_3) is ozonised to the norketone and the methyl ester (12) is chlorinated with lithium chloride and toluene-*p*-sulphonyl chloride to give the 3α-chloro-13-alcohol (15) and its 13-toluene-*p*-sulphonate (14). The 13-alcohol (15) is converted, by tri-n-butylstannane reduction, then reaction with acetyl hypobromite, and reduction with tri-n-butylstannane, into the 2α-acetoxy-16-ketone (23). This intermediate (23) is converted successively into the 16-ene (24), the 2α-alcohol (25), the 2-ketone (26), [2α-^{2}H]- and [2α-^{2}H]-GA_{29} methyl esters (29) and (31), and finally into [2α-^{2}H]- and [2α-^{3}H]-GA_{29} (30) and (32).

An attempt to convert the 3α-chloro-13-toluene-*p*-sulphonate (14) into GA_{29} by analogous reactions was abandoned since tri-n-butylstannane reduction of the 2α-acetoxy-13-toluene-*p*-sulphonates (18) and (19) resulted in extensive loss of the 13-toluene-*p*-sulphonyloxy-group. However the resultant 13-deoxy-compound (22) was converted into [2α-^{2}H,15,17-$^3H_2$]GA_{51} (37) as for the 13-alcohol (15).

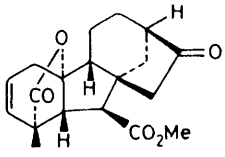
CONTINUING our studies on the preparation of labelled gibberellins for metabolic studies, we report the chemical conversion of gibberellin A_3 (GA_3) (1) into the 2α-$^2H_1$- and 2α-$^3H_1$-derivatives of GA_{29} (2) and into the 2α-$^2H_1$,15,17-$^3H_4$-derivative of GA_{51} (3). Metabolic studies



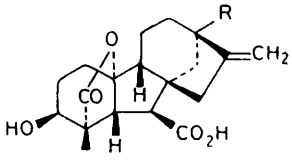
(1) GA_3



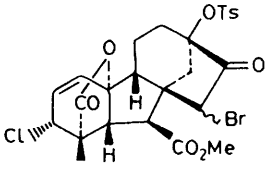
(2) GA_{29}, R = OH, 2β—OH
(3) GA_{51}, R = H, 2β—OH
(4) GA_{40}, R = H, 2α—OH



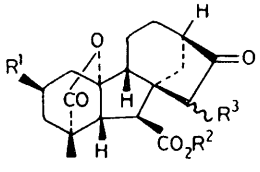
(5)



(6) GA_4, R = H
(7) GA_1, R = OH



(8)



(9) R^1 = H, R^2 = Me, R^3 = Br
(10) R^1 = R^3 = H, R^2 = Me
(11) R^1 = R^2 = R^3 = H

in the seed of *Pisum sativum* cv. Progress No. 9, using the labelled GA_{29}, have already been reported.[1]

Beeley and MacMillan [2] have described the partial synthesis of GA_{40} (4) in which the 2α-hydroxy was introduced by the addition of acetyl hypobromite to the Δ^2-ene (5), derived from GA_4 (6). A similar route was therefore explored for the preparation of GA_{29} (2). However, since GA_1 (7) was not available, the accessible GA_3 (1) was used as starting material.

RESULTS AND DISCUSSION

Gibberellin A_3 (1) was ozonised by the method of Speake [3] (Scheme 1). After chromatography on silica gel, followed by methylation, the norketone (12) was obtained in 70% yield. On one occasion the crude ozonolysis product was methylated, then chromatographed on alumina, to give a mixture of the required norketone (12) and an isomer which was assigned the 6-epimeric structure (13) from the following n.m.r. data.† Comparison of the ^{1}H n.m.r. spectra of the norketone (12) and its epimer (13), showed that the ring A structure was the same in the two compounds; however the 5- and 6-protons formed an AB quartet with J 7.5 Hz in the new compound, compared to J 11 Hz for the corresponding AB quartet in the norketone (12). Also a doublet of doublets at δ 3.11 with J 3.5 and 19 Hz, attributed to one of the 14-protons, was observed in the spectrum of

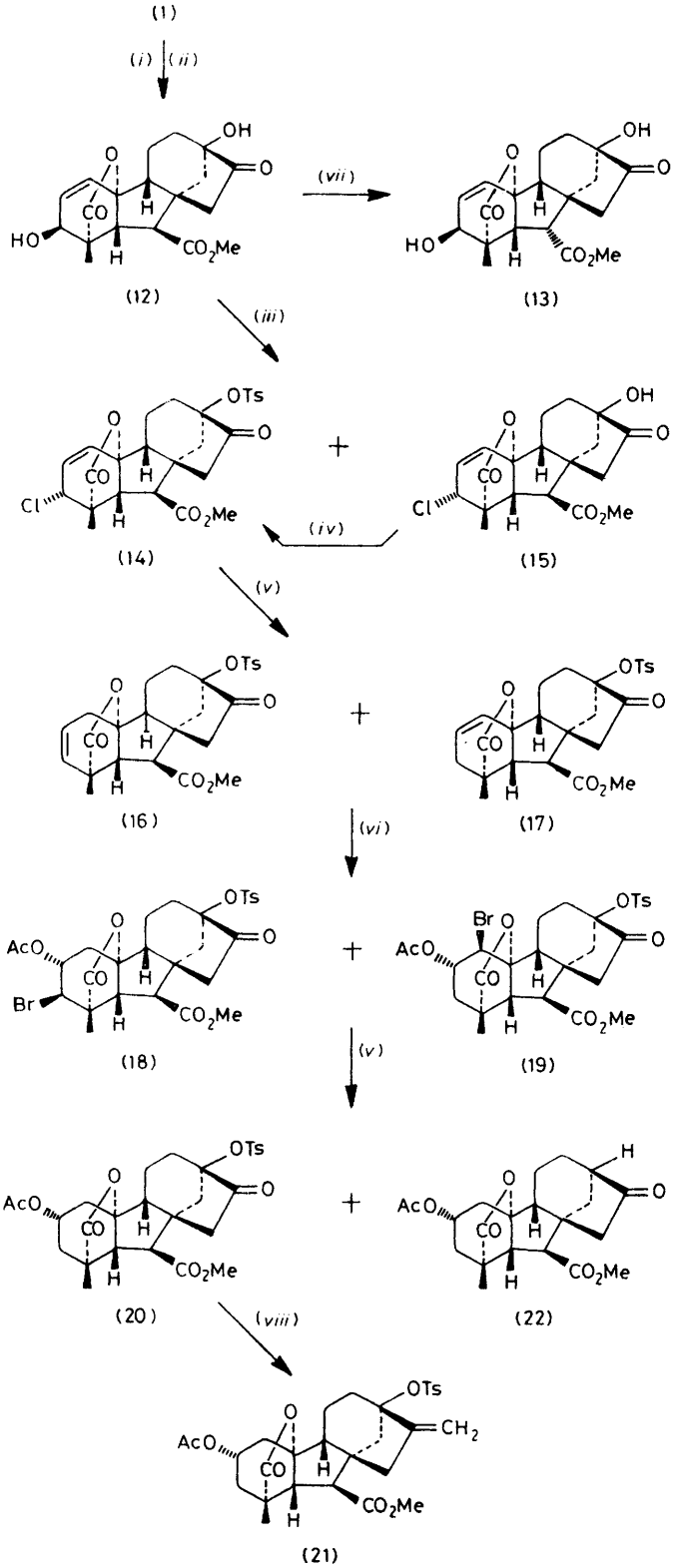
^{13}C N.m.r. of GA_3 norketone (12) and its 6-epimer (13)

Carbon	(12)	(13)	Carbon	(12)	(13)
1	131.5	130.4	11	17.4	18.3
2	134.5	134.9	12	33.8	35.6
3	69.7	70.6	13	78.7	81.7
4	54.4	53.5	14	42.2	47.7 [b]
5	53.2	51.9 [a]	15	49.0	48.9 [b]
6	52.0	50.6 [a]	16	218.5	216.6
7	172.4	173.3	17		
8	47.8	48.9	18	15.2	15.0
9	51.5	55.9 [a]	19	179.0	179.2
10	90.6	89.4	OMe	52.4	51.1

[a,b] These assignments may be interchanged.

the 6-epimer (13), but was obscured at higher field by the complex signals of the aliphatic protons in the norketone (12). The ^{13}C n.m.r. spectra of the norketone (12) and the 6-epimer (13), shown in the Table, are also consistent with structure (13). The signals were assigned by

† As pointed out by a referee, the data do not exclude a ring C/D-rearranged structure for the isomer of the norketone (12).



SCHEME 1 (*i*) O_3; (*ii*) CH_2N_2; (*iii*) LiCl–p-$MeC_6H_4SO_2Cl$; (*iv*) *p*-$MeC_6H_4SO_2Cl$; (*v*) n-Bu_3SnH; (*vi*) $LiOAc{\cdot}2H_2O$–MeCONHBr–AcOH; (*vii*) alumina; (*viii*) $CH_2{=}PPh_3$

analysis of the partially proton-decoupled spectra and by comparison with published [4,5] data for the gibberellins. Comparison of the spectra revealed that the 14-, 13-, 4-, and 8-C signals of the 6-epimer (13) were at lower field than in the norketone (12). The signals for the 5-, 6- and 9-C atoms at 50.6, 51.9, and 55.9 p.p.m. could not be conclusively assigned, but at least one of these signals was at significantly lower field than in the norketone (12). These n.m.r. data are consistent with structure (13), epimerisation at carbon-6 having occurred during chromatography on alumina.

The norketone (12) was chlorinated with lithium chloride and toluene-*p*-sulphonyl chloride as described by Bearder *et al.*[6] to give a mixture of the 3α-chloro-toluene-*p*-sulphonate (14) and the 3α-chloro-13-alcohol (15). Toluene-*p*-sulphonation of the latter compound (15) gave the 13-toluene-*p*-sulphonate (14) which was then obtained in 52% overall yield from the norketone (12). Attempts to react acetyl hypobromite with the double bond in the 3α-chloro-13-toluene-*p*-sulphonate (14) failed. At room temperature in acetic acid no reaction occurred and, under reflux, a bromo-compound was formed which was assigned the 15-bromo-structure (8) from the presence of a one-proton doublet at δ 4.37 (*J* 3 Hz) in the n.m.r. spectrum. Support for the structure (8) comes from the formation of an analogous compound (9) with a one-proton doublet (δ 4.33, *J* 3 Hz), from GA_9 methyl ester norketone (10) under identical conditions. An α-stereochemistry for the 15-bromo-substituent in these compounds seems probable from the long-range W coupling to one of the 14-protons.

Since the failure of acetyl hypobromite to react with the olefin (14) was probably due to the electron-withdrawing chlorine, the chlorine was removed from (14) by treatment with tri-n-butylstannane in the presence of 2,2′-azobis-(2-methylpropionitrile). As previously reported [6] for the corresponding 3α-chloro-derivative from GA_3 (1), a 3 : 1 mixture of the Δ^2- and Δ^1-enes (16) and (17) was obtained. This mixture, characterised by ^1H n.m.r., reacted smoothly with acetyl hypobromite to give a quantitative yield of the bromo-acetates (18) and (19), characterised principally by ^1H n.m.r. Reduction of the mixed bromo-acetates (18) and (19) with tri-n-butylstannane was expected to give the 2α-acetate (20) exclusively. In the event, although the expected 2α-acetate (20) was the major product, a considerable yield (37%) of the 13-deoxy-analogue (22) was also obtained and identified by comparison with an authentic specimen.[2] This unexpected result, *i.e.* the hydrogenolysis of a bridgehead toluene-*p*-sulphonyloxy-group by tri-n-butylstannane, will be discussed in detail elsewhere.[7]

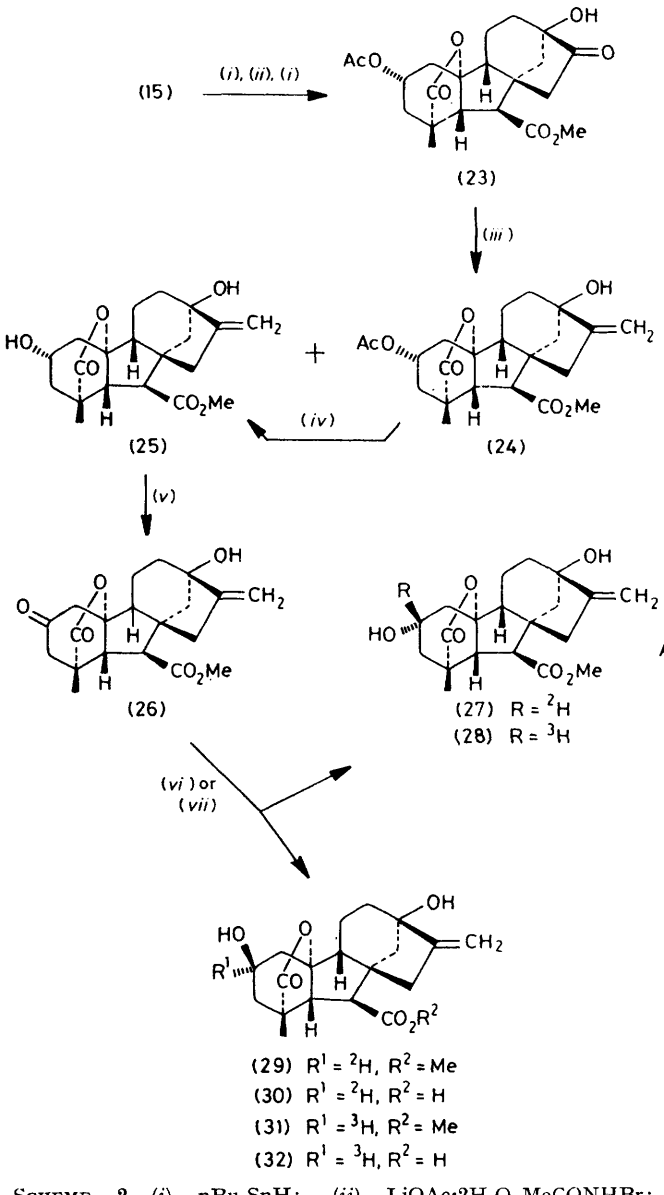
Treatment of the toluene-*p*-sulphonate (20) with a salt-free solution of the ylide, prepared [8] from methyltriphenylphosphonium bromide and sodium hydride in tetrahydrofuran, gave the required olefin (21), but in low yield. In view of this low yield and the loss of the 13-toluene-*p*-sulphonyloxy-group in the tri-n-butylstannane reduction of the bromo-acetates (18) and (19), it was decided to complete the preparation of GA_{29} (2)

using 13-hydroxy-compounds and protection of the 13-hydroxy-group with a trimethylsilyl group at the Wittig stage.

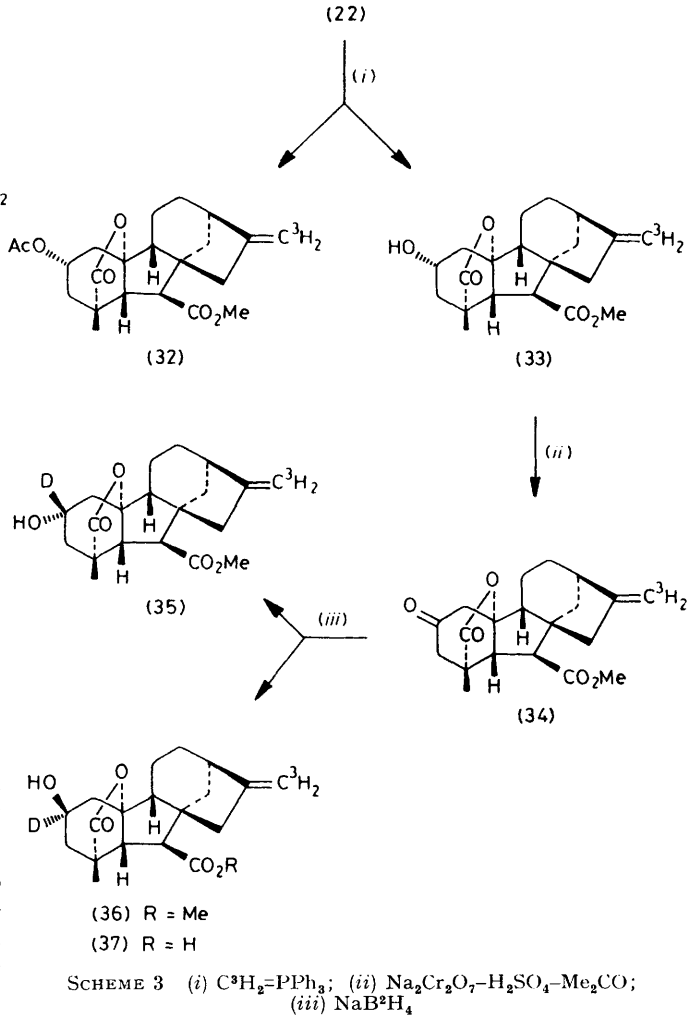
Thus (Scheme 2) the 3α-chloro-13-alcohol (15) was converted into the 2α-acetate (23) by successive reactions with tri-n-butylstannane, acetyl hypobromite, and tri-n-butylstannane. The 13-TMSi-ether, prepared from the 2α-acetate (23) with excess of hexamethyldisilazane and chlorotrimethylsilane in pyridine, was reacted with a salt-free solution of methylenetriphenylphosphorane in tetrahydrofuran. Hydrolysis of the 13-TMSi-ether, and chromatography of the products, gave the 2α-acetoxy-olefin (24) and the corresponding diol (25) in 36 and 10% yield, respectively. Partial hydrolysis of a 2α-acetate during a Wittig reaction has been noted previously by Beeley and MacMillan.[2] The acetate (24) was hydrolysed by 0.01N potassium hydroxide in methanol, to give the diol (25) which was oxidised by chromium trioxide–pyridine in dichloromethane, recovery of the ketone (26) being achieved by dilution of the reaction mixture with ethyl acetate and filtration through a plug of Celite.[9] The ketone (26), pure by t.l.c., was used directly for reduction with sodium borodeuteride in ethanol to obtain *ca.* equal amounts of the epimeric deuterio-2α- and -2β-alcohols (27) and (29). Demethylation of the deuterio-2β-alcohol (29) with 2M-sodium hydroxide in methanol gave [2α-^{2}H]GA$_{29}$ (30) containing 0.97 atoms deuterium per molecule.

Re-oxidation of the deuterio-2α-alcohol (27) with chromium trioxide–pyridine as above and subsequent reduction of the ketone (26) so formed with tritiated sodium borohydride gave the tritio-2α- and -2β-alcohols (28) and (31). Demethylation of (31) gave [2α-^{3}H]-gibberellin A$_{29}$ with a specific activity of 60 mCi mmol^{-1}.

The preparation of labelled GA$_{51}$ (37) from the 2α-acetate (22), fortuitously obtained as described in



SCHEME 2 (*i*) nBu$_3$SnH; (*ii*) LiOAc·2H$_2$O–MeCONHBr; (*iii*) CH_2=PPh_3; (*iv*) KOH–MeOH; (*v*) CrO_3–CH_2Cl_2–C_5H_5N; (*vi*) NaB^{2}H$_4$; (*vii*) NaB^{3}H$_4$



SCHEME 3 (*i*) C^3H_2=PPh_3; (*ii*) $Na_2Cr_2O_7$–H_2SO_4–Me_2CO; (*iii*) NaB^{2}H$_4$

Scheme 1, is outlined in Scheme 3. The route follows that described by Beeley and MacMillan [2] as far as GA_{40} methyl ester (33), which was converted into GA_{51} (37) by the method of Yamaguchi *et al.*[10] The tritium-label was introduced at the Wittig step using [^{3}H]methyltriphenylphosphonium bromide, prepared as described by Bearder *et al.*[8] Since Bearder *et al.*[11] have shown that the analogous Wittig reaction on GA_9 norketone (11) introduced tritium at position 15 (41%) as well as position 17 (59%) it may be assumed that the label is similarly located in GA_{51} (37). The deuterium label was introduced by reduction of the 2-ketone (34) with sodium borodeuteride to give equal amounts of the 2β-^{2}H-2α-alcohol (35) and the 2α-^{2}H-2β-alcohol (36). Hydrolysis of (36) gave [2α-^{2}H,15,17-^{3}H$_4$]GA_{51} (37) with a specific activity of 35 mCi mmol^{-1} and containing 0.95 atoms deuterium per molecule.

EXPERIMENTAL

For general experimental details see refs. 12 and 13.

ent-3α,10β,13-*Trihydroxy*-7-*methoxycarbonyl*-16-*oxo*-17,-20-*bisnorgibberell*-1-*en*-19-*oic Acid* 19,10-*Lactone* (12).[3]—A solution of gibberellin A_3 (2.5 g) in ethyl acetate (200 ml) and acetic acid (90 ml) was cooled to −30 °C and ozonised oxygen (4.8 mg O_3 min^{-1}, determined by KI–$Na_2S_2O_3$ titration) was passed through the solution for 1.25 h. On some occasions, the ozonide precipitated from the reaction mixture before the calculated amount of ozone has been added. When this occurred the precipitate was dissolved by allowing the temperature to rise to −20 °C and by adding more ethyl acetate; the ozonolysis was then completed at −20 °C. Triphenylphosphine (2.25 g) was then added and the solution was allowed to warm to 0 °C. After standing at 0 °C overnight, the solvents were removed under vacuum with the minimum amount of heating, toluene being added to remove the acetic acid azeotropically.

The combined products from two experiments were adsorbed on silica gel by evaporation of a methanolic solution and placed on a column of silica gel (500 g, 65 × 5 cm), made up in benzene. The column was eluted with benzene containing increasing amounts of methanol. After elution of triphenylphosphine oxide (8—9% methanol) and of unchanged gibberellin A_3 (10—13% methanol), fractions eluted with 13—25% methanol were combined and methylated with diazomethane to give the norketone methyl ester (12) (3.56 g), m.p. 230—232 °C (from methanol) (lit.,[3] 230—232 °C); δ([^{2}H$_5$]pyridine) 1.58 (s, 18-Me), 3.16 (d, *J* 11 Hz, 6-H), 3.58 (s, CO_2Me), 3.71 (d, *J* 11 Hz, 5-H), 4.48 (br, $W_{\frac{1}{2}}$ 12 Hz, 3-H; simplified to d, *J* 3.5 Hz on addition of D_2O), 6.12 (dd, *J* 9 and 3.5 Hz, 2-H), and 6.38 (d, *J* 9 Hz, 1-H); *m/e* 362 (*M*$^+$, 35%), 344 (19), 331 (43), 330 (19), 320 (28), 318 (35), 302 (65), 299 (52), 288 (50), 287 (50), 286 (55), 256 (100), 255 (58), 214 (55), 211 (77), 197 (65), 169 (56), 155 (82), and 91 (50).

In one experiment, the crude products from the ozonolysis of gibberellin A_3 (5 g) were methylated with diazomethane and then chromatographed on an alumina column (500 g, 65 × 5 cm, Grade I) made up in benzene. Triphenylphosphine oxide was eluted with 1—1.5% methanol in benzene and gibberellin A_3 with 2—2.5% methanol. The ketone products (2.88 g), eluted with 3—6% methanol, showed two spots of similar intensity by t.l.c. on silica gel, one with a lower R_F value than that of the required norketone methyl ester. Fractional crystallisation from methanol gave a pure compound (150 mg), believed to be 6-*epigibberellin* A_3 *norketone methyl ester* (13), m.p. 226—227 °C (from methanol) (Found: C, 62.9; H, 6.4. $C_{19}H_{22}O_7$ requires C, 63.0; H, 6.1%); $\nu_{max.}$ 3 498, 3 390 (br), 1 761, 1 746, and 1 722 cm^{-1}; δ([^{2}H$_5$]pyridine) 1.64 (s, *J* 18-Me), 3.18 (d, *J* 7.5 Hz, 6-H), 3.21 (dd, *J* 3.5 and 19 Hz, 14ξ-H), 3.64 (s, CO_2Me), 3.80 (d, *J* 7.5 Hz, 5-H), 4.53 (br, $W_{\frac{1}{2}}$ 10 Hz, 3-H; simplified to d, *J* 3.5 Hz, by addition of D_2O); 6.14 (dd, *J* 3.5 and 9 Hz, 2-H), and 6.44, (d, *J* 9 Hz, 1-H); *m/e* (362 (*M*$^+$, 12%), 346 (4), 331 (14), 330 (10), 316 (14), 302 (48), 299 (20), 288 (53), 287 (38), 256 (84), 214 (58), 211 (73), 197 (95), 169 (53), and 155 (100).

Reaction of ent-3α,10β,13-*Trihydroxy*-7-*methoxycarbonyl*-16-*oxo*-17,20-*bisnorgibberell*-1-*en*-19-*oic Acid* 19,10-*Lactone* (12) *with Lithium Chloride and Toluene*-p-*sulphonyl Chloride*.—The norketone (12) (3.88 g) and toluene-*p*-sulphonyl chloride (6.5 g) in pyridine (50 ml) were stirred at room temperature for 2 days. Anhydrous lithium chloride (3.8 g) and toluene-*p*-sulphonyl chloride (600 mg) were added and, after stirring for a further 3 days, the reaction mixture was poured into dilute hydrochloric acid. The product, recovered in ethyl acetate, was chromatographed on silica gel (300 g, 45 × 3.5 cm), and eluted with increasing amounts of ethyl acetate in light petroleum. Elution with 5% ethyl acetate (1 l) and 7.5% ethyl acetate (1 l) gave toluene-*p*-sulphonyl chloride. Fractions eluted with 25—35% ethyl acetate, gave the 3α-*chloro*-13-*toluene*-p-*sulphonate* (14) (1.6 g), m.p. 174—176 °C (from ethyl acetate–light petroleum) (Found: C, 58.1; H, 5.4. $C_{26}H_{27}ClO_8S$ requires C, 58.4; H, 5.1%); $\nu_{max.}$ 1 778, 1 732, 1 600, 1 345, and 1 179 cm^{-1}; δ 1.33 (s, 18-Me), 2.44 (s, Ar–Me), 2.62 and 2.89 (each d, *J* 11.5 Hz, 14-H$_2$), 2.84 and 3.12 (each d, *J* 11 Hz, 5- and 6-H), 3.79 (s, CO_2Me), 4.62 (dd, *J* 1.5 and 2.5 Hz, 3-H), 5.91 (dd, *J* 2.5 and 9 Hz, 2-H), 6.20 (dd, *J* 1.5 and 9 Hz, 1-H), and 7.30 and 7.76 (each d, *J* 8 Hz, Ar-H); *m/e* 534 (M$^+$, 2%), 379 (72), 271 (98), 211 (82), 155 (51), and 91 (100).

Fractions eluted with 50—100% ethyl acetate, gave the 13-*hydroxy-compound* (15) (1.1 g), m.p. 198—200 °C (from ethyl acetate) (Found: C, 59.3; H, 5.5. $C_{19}H_{21}ClO_6$ requires C, 59.9; H, 5.6%); $\nu_{max.}$ 3 510, 1 780, 1 753, and 1 736 cm^{-1}; δ 1.35 (s, 18-Me), 2.83 and 3.09 (each d, *J* 11 Hz, 5- and 6-H), 3.75 (s, CO_2Me), 4.63 (dd, *J* 1.5 and 2.5 Hz, 3-H), 5.92 (dd, *J* 2.5 and 9 Hz), and 6.24 (dd, *J* 1.5 and 9 Hz, 1-H); *m/e* 380 (*M*$^+$, 14%), 256 (72), 241 (59), 240 (57), 197 (52), and 155 (100).

The 13-hydroxy-compound (15) (1.1 g) and toluene-*p*-sulphonyl chloride (2.5 g) in pyridine (12 ml) were stirred at room temperature. Further additions of the sulphonyl chloride were made after 2 days (1.0 g) and 4 days (1.5 g) and the reaction was worked up after 6 days. Chromatography of the product on silica gel (200 g, 32 × 3.5 cm), as above, gave, from fractions eluted with 25—40% ethyl acetate in light petroleum, the 13-toluene-*p*-sulphonate (14) (1.4 g), identified by its n.m.r. spectrum.

Reaction of ent-3α-*Chloro*-10β-*hydroxy*-7-*methoxycarbonyl*-16-*oxo*-13-*toluene*-p-*sulphonyloxy*-17,20-*bisnorgibberell*-1-*en*-19-*oic Acid* 19,10-*Lactone* (14) *with Acetyl Hypobromite*.—The 13-toluene-*p*-sulphonate (14) (250 mg), lithium acetate dihydrate (900 mg), and *N*-bromoacetamide (90 mg) in acetic acid (12 ml) were refluxed for 10 min. The reaction mixture was poured into water and the product, recovered in ethyl acetate, was subjected to p.l.c. on silica gel

developed with ethyl acetate–light petroleum–acetic acid (70 : 30 : 1). The single band at R_F 0.65 was extracted with methanol to yield the 15ξ-*bromo-compound* (8) (183 mg), m.p. 229—230 °C (from acetone–light petroleum) (Found: C, 51.1; H, 4.5. $C_{26}H_{26}BrClO_8S$ requires C, 50.9; H, 4.3%); $\nu_{max.}$ 1 790, 1 770, 1 740, 1 600, 1 338, and 1 177 cm^{-1}; δ 1.48 (d, 18-Me), 2.46 (s, Ar–Me), 2.86 and 3.21 (each d, *J* 11 Hz, 5- and 6-H), 3.76 (s, CO_2Me), 4.37 (d, *J* 3 Hz, 15ξ-H), 4.59 (dd, *J* 1.5 and 2.5 Hz, 3-H), 5.93 (dd, *J* 2.5 and 9 Hz, 2-H), 6.31 (dd, *J* 1.5 and 9 Hz, 1-H), 7.33 and 7.81 (each d, *J* 8 Hz, Ar-H).

A similar reaction at room temperature for 3 h gave only starting material.

Reaction of ent-10β-*Hydroxy*-7-*methoxycarbonyl*-16-*oxo*-*bisnorgibberellan*-19-*oic Acid* 19,10-*Lactone* (10) *with Acetyl Hypobromite*.—The norketone (10) (56 mg), lithium acetate dihydrate (200 mg), and *N*-bromoacetamide (20 mg) in acetic acid (6 ml) were refluxed for 25 min and worked up as in the previous experiment. P.l.c. of the product on silica gel with ethyl acetate–light petroleum (1 : 1) gave, from the band at R_F 0.50, a crude product (30 mg), believed to be the 15ξ-bromo-norketone (9); δ 1.24 (s, 18-Me), 2.30—2.86 (8-line complex, assigned to 5-, 6-, and 14-protons), 3.78 (s, CO_2Me), and 4.33 (d, *J* 3 Hz, 15ξ-H); *m/e* 412/410 ($M^+ + 2$ and M^+, 1%), 381/379 (1.5), 309/7 (5), and 286 (100).

Reduction of ent-3β-*Chloro*-10β-*hydroxy*-7-*methoxycarbonyl*-16-*oxo*-13-*toluene*-p-*sulphonyloxy*-17,20-*bisnorgibberell*-1-*en*-19-*oic Acid* 19,10-*Lactone* (14) *with Tri-n-butylstannane*.—The chloro-compound (14) (3 g) in benzene (150 ml) was refluxed for 1 h with tri-n-butylstannane (6 ml) and 2,2′-azobis-(2-methylpropionitrile) (20 mg). The benzene was removed under vacuum, and the residual oil chromatographed on silica gel (200 g, 32 × 3.5 cm), made up in light petroleum and eluted with increasing amounts of ethyl acetate in light petroleum. After elution of tin-containing compounds, elution with 25—50% ethyl acetate gave a mixture (1.85 g) of ent-10β-*hydroxy*-7-*methoxycarbonyl*-16-*oxo*-13-*toluene*-p-*sulphonyloxy*-17,20-*bisnorgibberell*-2-*en*-19-*oic acid* 19,10-*lactone* (16) and the Δ-1-*isomer* (17) in the ratio of 3 : 1 by n.m.r. Crystallisation from aqueous methanol gave a 3 : 1 mixture of isomers, m.p. 150—154 °C (Found: C, 62.3; H, 5.9. $C_{26}H_{28}O_8S$ requires C, 62.4; H, 5.6%) $\nu_{max.}$ 1 772, 1 739, 1 600, 1 348, and 1 172 cm^{-1}; δ(for 2-ene) 1.24 (s, 18-Me), 2.42 (s, Ar–Me), 2.92—2.56 (complex m, 5-H, 6-H, and 14-H_2), 3.72 (s, CO_2CH_3), 5.75 (m, H-2 and H-3), 7.23 and 7.70 (each d, *J* 8 Hz, Ar-H); δ(for 1-ene) 1.20 (s, 18-Me) and 6.10 (d, *J* 10 Hz, H-1); *m/e* 500 (M^+, 2%), 469 (7), 456 (29), 396 (18), 345 (96), 317 (37), 285 (57), 211 (44), 197 (51), 155 (35), and 91 (100).

Reaction of the Mixture of the Olefins (16) *and* (17) *with Acetyl Hypobromite*.—The mixture of olefins (1.85 g) was dissolved in acetic acid (50 ml) together with lithium acetate dihydrate (10 g) and *N*-bromoacetamide (1.0 g). The solution was stirred at room temperature for 3.5 h, poured into water, and then extracted thoroughly with ethyl acetate. The oil, recovered from the ethyl acetate, was chromatographed on silica gel (200 g, 32 × 3.5 cm) which was eluted with increasing amounts of ethyl acetate in light petroleum. Fractions eluted with 20—50% ethyl acetate gave a mixture (2.14 g) of ent-2β-*acetoxy*-3α-*bromo*-10β-*hydroxy*-7-*methoxycarbonyl*-16-*oxo*-13-*toluene*-p-*sulphonyoxy*-17,20-*bisnorgibberellan*-19-*oic acid* 19,10-*lactone* (18) and its ent-2β-*acetoxy*-1α-*bromo-isomer* (19) in the ratio 3 : 1 by n.m.r. Crystallisation from aqueous methanol gave a 1 : 1 mixture of the two isomers, m.p. 138—141 °C (Found: C, 52.1; H, 4.9. $C_{28}H_{31}BrO_{10}S$ requires C, 52.6; H, 4.9%); $\nu_{max.}$ 1 788, 1 773, 1 740, 1 600, 1 348, and 1 176 cm^{-1}; δ (for the 3β-bromo-isomer) 1.26 (s, 18-Me), 2.05 (s, OCOMe), 2.46 (s, ArMe), 2.96—2.50 (complex m, 6-H and 14-H_2), 3.41 (d, *J* 11 Hz, 5-H), 3.81 (s, CO_2Me), 4.13 (s, 3-H), 5.35 (br, 2-H), and 7.81 and 7.35 (each d, *J* 8 Hz, Ar-H); δ (for the 1β-bromo-isomer) 1.16 (s, 18-Me), 3.37 (d, *J* 11 Hz, 5-H), and 4.25 (s, 1-H); *m/e* (M^+ absent) 483/485 (12%), 455/457 (5), 395/397 (6), 211 (27), 197 (25), 155 (37), 91 (100), and 43 (64).

Reduction of the Mixture of Bromo-acetates (18) *and* (19) *with Tri-n-butylstannane*.—The foregoing 3 : 1 mixture (2.1 g) in benzene (150 ml) was refluxed for 1 h with tri-n-butylstannane (4 ml) and 2,2′-azobis-(2-methylpropionitrile) (15 mg). The residue, obtained by evaporation of the solvent, was chromatographed on silica gel (200 g, 32 × 3.5 cm), made up in light petroleum and eluted with increasing concentrations of ethyl acetate. After elution of tin-containing compounds in early fractions, the crude product (1.01 g) was eluted with 55—90% ethyl acetate and was fractionated by p.l.c. on silica gel GF_{254}, developed with acetone–light petroleum (2 : 3). Elution of the band at R_F 0.65 gave methyl *ent*-2β-acetoxy-10β-hydroxy-7-methoxycarbonyl-16-oxo-17,20-bisnorgibberellan-19-oic acid 19,10-lactone (22) (201 mg), identical to an authentic sample.[2]

Elution of the band at R_F 0.55 gave ent-2β-*acetoxy*-10β-*hydroxy*-7-*methoxycarbonyl*-16-*oxo*-13-*toluene*-p-*sulphonyloxy*-17,20-*bisnorgibberellan*-19-*oic acid* 19,10-*lactone* (20) (341 mg) m.p. 228—230 °C (from acetone–light petroleum) (Found: C, 59.9; H, 6.1. $C_{28}H_{32}O_{10}S$ requires C, 60.0; H, 5.8%); $\nu_{max.}$ 1 777, 1 728, 1 600, 1 345, and 1 178 cm^{-1}; δ 1.11 (s, 18-Me), 2.00 (s, OCOMe), 2.42 (s, Ar-Me), 2.52 and 2.70 (each d, *J* 11.5 Hz, 14-H_2), 2.63 and 2.82 (each d, *J* 11 Hz, 5- and 6-H), 3.75 (s, CO_2Me), 5.15 (br, $W_{\frac{1}{2}}$ 12 Hz, 2-H), and 7.27 and 7.73 (each d, *J* 8 Hz, Ar-H); *m/e*, 560 (M^+, 1%), 405 (68), 286 (60), 285 (75), 91 (100), and 43 (66).

Methyl ent-2β-*Acetoxy*-10β-*hydroxy*-7-*methoxycarbonyl*-13-*toluene*-p-*sulphonyloxy*-20-*norgibberell*-16-*en*-19-*oic Acid* 19,10-*Lactone* (21).—To the norketone (20) (45 mg) was added a solution (1.5 ml) of methylenetriphenylphosphorane, prepared as described by Bearder *et al.*[8] from sodium hydride (332 mg of a 60% suspension in oil), tetrahydrofuran (15 ml), and methyltriphenylphosphonium bromide (1.5 g). The solution was stirred at room temperature for 4 h and then refluxed for 1 h, all in an atmosphere of nitrogen gas. The solvent was removed *in vacuo* and the residue was partitioned between water and ethyl acetate. P.l.c. of the product from the ethyl acetate on silica gel, developed with ethyl acetate–light petroleum (3 : 2), gave, from the band at R_F 0.40, the required *olefin* (21) (10 mg), m.p. 187—188 °C (from acetone–light petroleum) (Found: C, 62.5; H, 6.2. $C_{29}H_{34}O_9S$ requires C, 62.4; H, 6.1%); δ 1.08 (s, 18-Me), 2.00 (s, OCOMe), 2.42 (s, Ar-Me), 2.56 and 2.73 (each d, *J* 11 Hz, 5- and 6-H), 3.68 (s, CO_2Me), 5.15 (br 2-H), 5.01 and 5.26 (each br, 17-H_2), and 7.22 and 7.67 (each d, *J* 8 Hz, Ar-H); *m/e* 558 (M^+, 0.6%), 403 (28), 386 (27), 361 (37), 311 (21), 269 (30), 91 (100), and 43 (45).

ent-[2β-$^2H_1$]-2α,10β-*Dihydroxy*-20-*norgibberellane*-7,19-*dioic Acid* 19,10-*Lactone* ([2α-$^2H_1$]*Gibberellin* A_{29}) (30).—(a) ent-2β-*Acetoxy*-10β,13-*dihydroxy*-7-*methoxycarbonyl*-16-*oxo*-17,20-*bisnorgibberellan*-19-*oic acid* 19,10-*lactone* (23). The 3α-chloro-compound (15) (1.06 g), tri-n-butylstannane (2

ml), and 2,2′-azobis-(2-methylpropionitrile) (10 mg), in benzene (70 ml), were refluxed for 2 h. The gum, obtained by evaporation, was chromatographed on silica gel (200 g, 30 × 3.5 cm), eluted with increasing amounts of ethyl acetate in light petroleum. Tin-containing compounds were eluted with 0—10% ethyl acetate. Further elution (10—80% ethyl acetate) gave a 3 : 1 mixture (983 mg), characterised, by ^{1}H n.m.r., as the 13-hydroxy-analogues of the 3 : 1 mixture of the toluene-*p*-sulphonates (16) and (17) described earlier.

This mixture (983 mg), lithium acetate dihydrate (5 g), and *N*-bromoacetamide (500 mg) in acetic acid (25 ml) were stirred at room temperature for 3 h. Addition of water, and recovery of the product in ethyl acetate, gave a gum (1.36 g) which was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (7 : 3) to give a 3 : 1 mixture, characterised by ^{1}H n.m.r. as the 13-hydroxy-analogues of the corresponding mixture of 13-toluene-*p*-sulphonates (18) and (19).

This mixture (870 mg) in benzene (50 ml) was reduced by tri-n-butylstannane (1.7 ml) and 2,2′-azobis-(2-methylpropionitrile (10 mg) as described earlier. P.l.c. of the crude product twice on silica gel with ethyl acetate–light petroleum–acetic acid (80 : 20 : 1) gave the pure (t.l.c., g.l.c., and n.m.r.) 2-*acetoxy-compound* (23) as a gum (200 mg) (Found: M^+, 406.162. $C_{21}H_{26}O_8$ requires M, 406.163); $\nu_{max.}$ (CH_2Cl_2) 3 540, 1 770, 1 752, and 1 735 cm^{-1}; δ 1.12 (s, 18-Me), 2.03 (s, OCOMe), 2.65 and 2.83 (each d, J 11 Hz, 5- and 6-H), 3.77 (s, CO_2Me), and 5.23 (br, $W_{\frac{1}{2}}$ 12 Hz, 2-H); *m/e* 406 (M^+, 6%), 286 (64), and 43 (100).

(b) ent-2β-*Acetoxy*-10β,13-*dihydroxy*-7-*methoxycarbonyl*-20-*norgibberell*-16-*en*-19-*oic acid* 19,10-*lactone* (24). The preceding norketone (180 mg), hexamethyldisalazane (675 μl), and trimethylsilyl chloride (675 μl) in pyridine (4.5 ml) were allowed to stand at room temperature for 2 h. The solution was evaporated at room temperature in a stream of nitrogen gas and the residue, in ethyl acetate, was filtered through a short column of Celite. The filtrate was evaporated in a stream of nitrogen gas and the residue was treated with salt-free methylenetriphenylphosphorane in tetrahydrofuran (9 ml), prepared from sodium hydride (740 mg of suspension in oil), methyltriphenylphosphonium bromide (3.2 g), and tetrahydrofuran (24 ml). After stirring at room temperature for 1 h, acetone (1 ml) was added and the solution was evaporated *in vacuo*. The product, recovered in ethyl acetate, was stirred at room temperature with acetic acid (2 ml) and methanol (8 ml) until hydrolysis of the TMSi-ester was complete (2 h, t.l.c. monitoring) The product, recovered from the solution, was subjected to p.l.c. on silica gel with ethyl acetate–light petroleum (4 : 1). Elution of the band at R_F 0.65 with methanol gave 2-*acetyl*-2-*epigibberellin* A_{29} *methyl ester* (24) as a gum (65 mg) (Found: M^+, 404.181. $C_{22}H_{28}O_7$ requires M, 404.184); $\nu_{max.}$ (CH_2Cl_2) 3 570, 1 775 and 1 732 cm^{-1}; δ 1.09 (s, 18-H_3), 2.02 (s, OCOMe), 2.58 and 2.74 (each d, J 11 Hz, 5- and 6-H), 3.71 (s, CO_2Me), 5.16 (br, $W_{\frac{1}{2}}$ 16 Hz, 2-H), and 4.91 and 5.20 (each br, 17-H_2); *m/e* 404 (M^+, 20%), 362 (12), 345 (29), 344 (33), 312 (100), 303 (27), 289 (29), 105 (37), and 91 (47).

Material from the band at R_F 0.25 was re-chromatographed twice with the same solvent system to yield 2-epigibberellin A_{29} methyl ester (25) (16 mg) (see next section for characterisation).

(c) ent-2β,10β,13-*Trihydroxy*-7-*methoxycarbonyl*-20-*norgibberell*-16-*en*-19-*oic acid* 19,10-*lactone* (25). The preceding acetate (24) (60 mg) and potassium hydroxide (27 mg) in methanol (3.75 ml) were heated under reflux for 2.5 h. The usual work-up gave 2-*epigibberellin* A_{29} (25) as a gum (49 mg), pure by g.l.c. (Found: M^+, 362.172. $C_{20}H_{26}O_6$ requires M, 362.173); $\nu_{max.}$ (CH_2Cl_2) 3 560, 1 775, and 1 731 cm^{-1}; δ 1.12 (s, 18-Me), 2.62 and 2.75 (each d, J 11 Hz, 5- and 6-H), 3.73 (s, CO_2Me), 4.27 (br, $W_{\frac{1}{2}}$ 16 Hz, 2-H), and 4.92 and 5.23 (each br, 17-H_2); *m/e* 362 (M^+, 10%), 312 (58), 277 (100), 201 (66), 149 (79), 91 (53), and 77 (95).

(d) Chromium trioxide (120 mg) in methylene dichloride (3 ml) and pyridine (180 μl) were stirred at 0 °C for 10 min then 2-epigibberellin A_{29} methyl ester (25) (60 mg) in methylene dichloride (1.5 ml) was added. After 10 min ethyl acetate was added and the mixture was filtered through a short column of Celite into water. Separation of the organic layer and evaporation *in vacuo* gave a gum which was dissolved in ethanol (9 ml) and stirred at room temperature for 2 h with sodium borodeuteride (75 mg). The reaction mixture was poured into water, which was acidified to pH 3.0. Recovery in ethyl acetate and purification by p.l.c. on silica gel with ethyl acetate–light petroleum (4 : 1) gave, at R_F 0.30, [2α-^{2}H]*gibberellin* A_{29} *methyl ester* (29) (11 mg. 0.97 atoms deuterium per molecule), pure by g.l.c. and identified by g.l.c.–mass spectrometry of the bis-(TMSi)-ether; *m/e* (underivatised) 363 (M^+, 14%), 345 5), 331 (50), 303 (35), 231 (36), 217 (59), and 149 (100).

The band at R_F 0.20 yielded [2β-^{2}H]-2-*epigibberellin* A_{29} *methyl ester* (27) (14 mg, 0.86 atoms deuterium per molecule), admixed with 6% (g.l.c.) of the isomer (29).

(e) [2α-^{2}H]*Gibberellin* A_{29} (30). The foregoing methyl ester (29) (11 mg), methanol (3.25 ml), and 2M-sodium hydroxide (3.25 ml) were refluxed for 24 h. The methanol was removed *in vacuo* and the aqueous residue diluted with water and acidified to pH 3.0. Extraction into ethyl acetate and recovery gave a gum which, after heating at 80 °C under nitrogen for 20 min, was partitioned between ethyl acetate and aqueous saturated sodium hydrogencarbonate. Evaporation of the organic layer gave starting material (2 mg).

Acidification of the alkaline solution to pH 3.0 with concentrated hydrochloric acid and extraction with ethyl acetate gave [2α-^{2}H]*gibberellin* A_{29} (30) (4 mg) containing 0.97 ^{2}H atoms per molecule, pure by g.l.c. of its tris-TMSi-derivative, and identified by g.l.c. and g.c.–m.s. comparison of its MeTMSi derivative with a standard spectrum.

(f) [2-^{3}H]*Gibberellin* A_{29} (32). The mixture (14 mg) of [2β-^{2}H]-2-epigibberellin A_{29} methyl ester (27) and [2α-^{2}H]-gibberellin A_{29} methyl ester obtained from the borodeuteride reduction above was oxidised with chromium trioxide–pyridine in methylene chloride in the normal way to give the 2-ketone (26). The ketone was immediately reduced with tritiated sodium borohydride (100 mCi) in ethanol as above. The two ^{3}H alcohols (28) and (31) thus obtained were separated by p.l.c. as before and the [2α-^{3}H]*gibberellin* A_{29} *methyl ester* (31) hydrolysed with 2N NaOH in methanol to give [2α-^{3}H]gibberellin A_{29} (32) (100 μg, 60 mCi mmol^{-1}) identical by g.l.c. (trisTMSi and MeTMSi derivatives) to the deuterio-derivative above.

ent-[2α-^{2}H; 15,17-^{3}H]-2α,10β-*Dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* ([2-^{2}H; 15,17-$^3H_2$]-*Gibberellin* A_{51}) (37).—(a) A solution of [$^3H_2$]methylenetriphenylphosphorane in tetrahydrofuran (12 ml) was prepared from sodium hydride (370 mg of a 60% suspension in oil) and [^{3}H]methyltriphenylphosphonium bromide (1.6 g)

which, in turn, was prepared by the method of Bearder *et al.*[7] from methyltriphenylphosphonium bromide (1.78 g), acetonitrile (12 ml), triethylamine (2.5 ml), and [^{3}H]water (0.25 ml, 1.25 Ci).

(b) ent-[15,17-^{3}H]-2β,10β-*Dihydroxy*-7-*methoxycarbonyl*-20-*norgibberell*-16-*en*-19-*oic acid* 19,10-*lactone* (33) *and the corresponding* 2-*acetate* (32). The foregoing solution (9 ml) of [$^3H_2$]methylenetriphenylphosphorane was added to the norketone (22) (260 mg) and the solution was stirred at room temperature for 3.5 h. Acetone (1 ml) was added and the solvents were removed *in vacuo*. The residue was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (7 : 3). Extraction of the band at R_F 0.70 yielded 2-*acetyl*-[15,17-$^3H_4$]*gibberellin* A_{40} *methyl ester* (32) (40 mg) with a specific activity of 35 mCi mmol^{-1} and an identical mass spectrum to that of an authentic sample.[2] The band at R_F 0.40 gave [15,17-$^3H_4$]gibberellin A_{40} methyl ester (33) (73 mg) with specific activity 35 mCi mmol^{-1}. The TMSi ether had a mass spectrum identical with that of an authentic specimen.[2]

(c) ent-[2β-^{2}H;15,17-^{3}H]-2α,10β-*Dihydroxy*-7-*methoxycarbonyl*-20-*norgibberell*-16-*en*-19-*oic acid* 19,10-*lactone* (36) *and the corresponding epimer* (35). [15,17-$^3H_4$]Gibberellin A_{40} methyl ester (33) (73 mg) in acetone (1 ml) was oxidised with Jones reagent at 0 °C for 5 min. Addition of methanol and the usual work-up gave a gum, t.l.c. showing complete conversion to one product, less polar than the starting material.

The gum in ethanol (10 ml) was treated with sodium borodeuteride at room temperature for 2 h. The reaction mixture was poured into water which was acidified to pH 3.0 with 3M-hydrochloric acid. The gum, recovered in ethyl acetate, was subjected to p.l.c. on silica gel with ethyl acetate–light petroleum (3 : 2) to give two bands. The band at R_F 0.25 gave [2β-^{2}H; 15,17-$^3H_4$]*gibberellin* A_{40} *methyl ester* (35) (31 mg) with 0.83 atoms deuterium per molecule, and the band at R_F 0.3 yielded [2α-^{2}H, 15,17-$^3H_4$]-*gibberellin* A_{51} *methyl ester* (36) (24 mg) with 0.95 atoms deuterium per molecule. Both compounds had specific activity 35 mCi mmol^{-1}, and were identified by g.l.c.–mass spectrometric comparison of the TMSi-ethers with authentic samples of the unlabelled compounds.

(d) ent-[2β-^{2}H, 15,17-^{3}H]*Gibberellin* A_{51} (37). The preceding methyl ester (36) (24 mg), methanol (7.5 ml) and 2M-sodium hydroxide (7.5 ml) were refluxed for 19 h. The usual work-up, and recovery of the product in ethyl acetate, gave a gum which was heated at 80 °C for 30 min under a stream of nitrogen. The product in ethyl acetate was extracted with saturated aqueous sodium hydrogencarbonate. Evaporation of the ethyl acetate gave starting material (6 mg). Acidification of the aqueous extract to pH 3.0 with 3M-hydrochloric acid and extraction with ethyl acetate gave pure [2α-^{2}H; 15,17-$^3H_4$]*gibberellin* A_{51} (37) (16 mg) with 0.95 atoms deuterium per molecule and with specific activity 35 mCi mmol^{-1}.

We thank the S.R.C. for a Research Studentship (M. H. B.) and I.C.I. Ltd. for a generous gift of gibberellin A_3.

[0/506 *Received*, 2*nd April*, 1980]

REFERENCES

[1] V. M. Sponsel and J. MacMillan, *Planta*, 1978, **144**, 69.
[2] L. J. Beeley and J. MacMillan, *J.C.S. Perkin I*, 1976, 1022.
[3] R. N. Speake, P. M. Bourn, J. F. Grove, T. P. C. Mulholland, B. K. Tidd, and W. Klyne, *J. Chem. Soc.*, 1963, 154.
[4] R. Evans, J. R. Hanson, and M. Siverns, *J.C.S. Perkin I*, 1975, 1514.
[5] I. Yamaguchi, N. Takahashi, and K. Fujita, *J.C.S. Perkin I*, 1975, 992.
[6] J. R. Bearder, P. S. Kirkwood, and J. MacMillan, *J.C.S. Perkin I*, in the press.
[7] M. H. Beale and J. MacMillan, *J. Chem. Res.*, 1980, (*S*) 289; (*M*) 3536.
[8] J. R. Bearder, V. M. Frydman, P. Gaskin, J. MacMillan, C. M. Wels, and B. O. Phinney, *J.C.S. Perkin I*, 1976, 173.
[9] R. K. Hill, J. A. Joule, and L. J. Loeffler, *J. Amer. Chem. Soc.*, 1962, **84**, 4951.
[10] I. Yamaguchi, M. Miyamoto, H. Yamane, N. Murofushi, N. Takahashi, and K. Fujita, *J.C.S. Perkin I*, 1975, 996.
[11] J. R. Bearder, V. M. Frydman, P. Gaskin, I. K. Hatton, W. E. Harvey, J. MacMillan, and B. O. Phinney, *J.C.S. Perkin I*, 1976, 178.
[12] M. H. Beale and J. MacMillan, *J.C.S. Perkin I*, 1980, 877.
[13] M. H. Beale, P. Gaskin, P. S. Kirkwood, and J. MacMillan, *J.C.S. Perkin I*, 1980, 885.