Partial Syntheses of 2-Hydroxygibberellins; Characterisation of Two New Gibberellins, A_{46} and A_{47}

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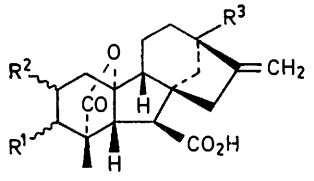
A general method for the conversion of 3-hydroxygibberellins into the methyl esters of 2-hydroxygibberellins is described. Thus the structures of two new gibberellins have been established by partial syntheses of their methyl esters, namely, GA_{46} (12) (*ent*-2α-hydroxygibberell-16-ene-7,19,20-trioic acid) from seed of *Echinocystis macrocarpa* and GA_{47} (10) (*ent*-2β,3α,10-trihydroxy-20-norgibberell-16-ene-7,19-dioic acid 19,10-lactone) from cultures of *Gibberella fujikuroi*, strain GF-1a. The partial syntheses of the methyl esters of gibberellins A_{40} (9), A_{34} (4), and 3-*epi*-A_{34} (11) are described. The anomalous opening of the epoxide (23) is noted.

In higher plants, 2β- (*ent*-2α-) hydroxylation of the gibberellins (GAs) may be a metabolic process of growth-regulating significance.[1] For example, GA_1 (1) is metabolised to GA_8 (2) in six plants;[2] GA_4 (3) is converted into GA_{34} (4) in rice seedlings;[3] and GA_9 (5) and GA_{20} (6) are metabolised to 2-*epi*-GA_{40} (7) and GA_{29} (8), respectively, in developing pea seed.[4] In the fungus, *Gibberella fujikuroi*, 2β- (*ent*-2α-) hydroxylation of GAs has not been observed, although 2α- (*ent*-2β-) hydroxylation occurs to a minor extent. For example GA_9 (5) is converted[5] into GA_{40} (9) by cultures of the mutant

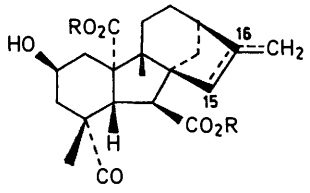
[1] R. J. Patterson and L. Rappaport, *Planta*, 1974, **119**, 183.
[2] L. Rappaport, L. Davies, S. Lavee, R. Nadeau, T. J. Patterson, C. F. Stolp, in 'Plant Growth Substances 1973,' Hirokawa, Tokyo, 1974, p. 314.
[3] R. C. Durley and R. P. Pharis, *Planta*, 1973, **109**, 357.
[4] V. M. Frydman and J. MacMillan, *Planta*, 1975, **125**, 181.
[5] 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.

B1-41a, and 2-*epi*-GA_{34} (10) has been tentatively identified [6] in cultures of the parent strain GF-1a of the mutant B1-41a.

In view of this interest in 2-hydroxy-GAs we have devised a preparative route to them from the more accessible *ent*-3α-hydroxy-GAs. Thereby structures (12)



	R^1	R^2	R^3		R^1	R^2	R^3
(1)	β-OH	H	OH	(7)	H	β-OH	H
(2)	β-OH	β-OH	OH	(8)	H	β-OH	OH
(3)	β-OH	H	H	(9)	H	α-OH	H
(4)	β-OH	β-OH	H	(10)	β-OH	α-OH	H
(5)	H	H	H	(11)	α-OH	β-OH	H
(6)	H	H	OH				



(12) R = H; Δ^{16}
(13) R = H; Δ^{15}
(14) R = Me; Δ^{16}
(15) R = Me; Δ^{15}

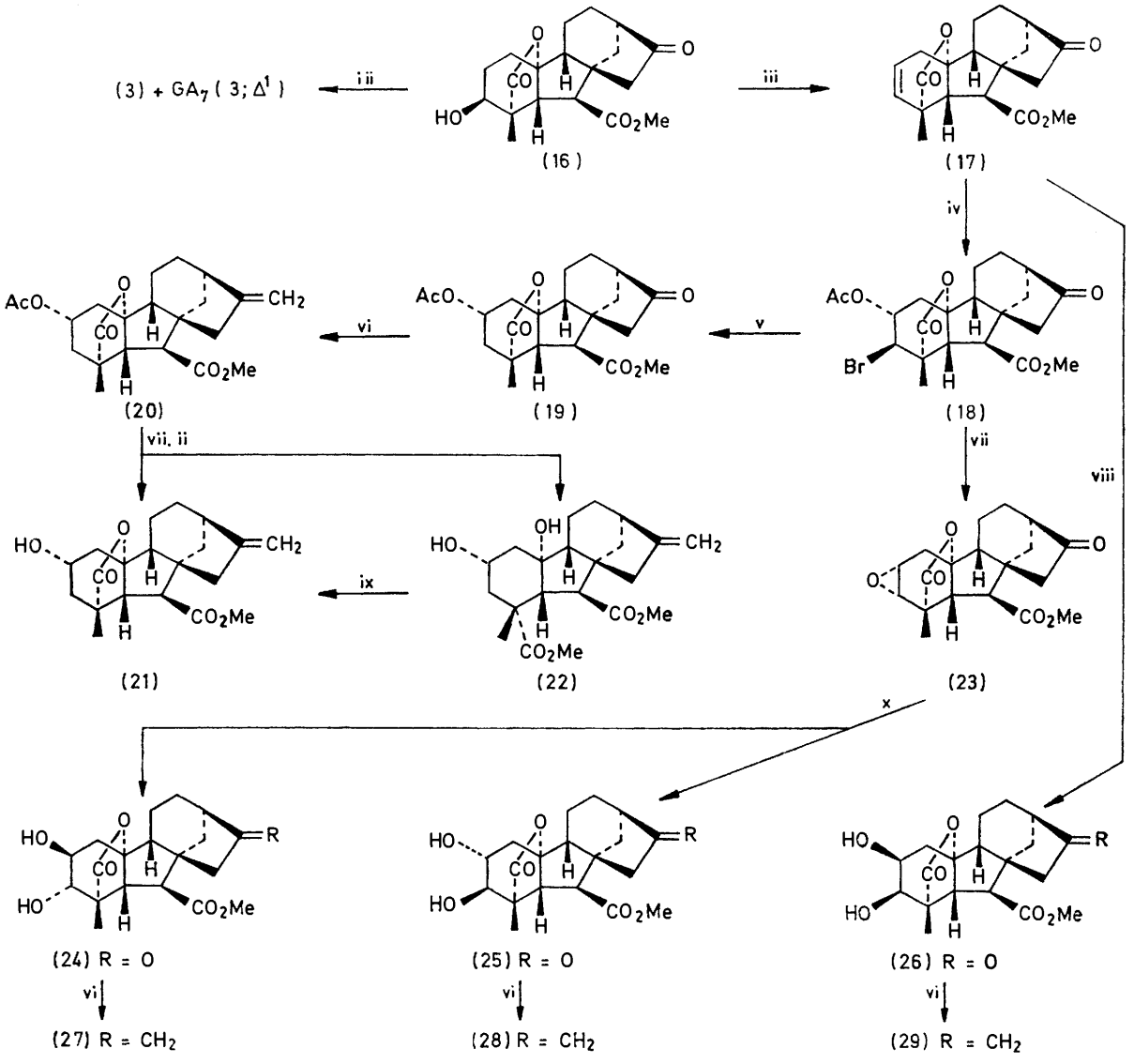
and (13) have been established for two previously unidentified GA-like compounds, detected [7] by g.l.c.–mass spectrometry in the nucellus-endosperm of *Echinocystis macrocarpa*. The former (12) is now allocated [8] the trivial name GA_{46}; the other compound (13) may be an artefact of GA_{46} (12) formed by double-bond isomerisation during extraction. Also the partial synthesis of 2-*epi*-GA_{34} (10) has confirmed the structure tentatively proposed [6] for this fungal metabolite, which is now given [8] the trivial name GA_{47}.

The synthetic procedures are illustrated in the Scheme for the preparation of the methyl esters of GA_{40} (9) and GA_{47} (10). The known [9] norketone (16), obtained by oxidation with osmium tetraoxide–periodate of a mixture of GA_4 (3) and GA_7 (3; Δ^1) followed by methylation, was dehydrated to the known [10] olefin (17) with phosphoryl chloride. Reaction of the olefin (17) with *N*-bromoacetamide and lithium acetate [11] gave the bromo-acetate (18). The regio- and stereo-specificity of addition was established by the formation of the epoxide (23) from the bromo-acetate (18) and by comparison of the n.m.r. spectra of the bromo-acetate (18) and the debromo-compound (19), which showed that the 5-proton was deshielded by the 3(*ax*)-bromine atom. The n.m.r. spectrum of the bromo-acetate (18) also showed a very small $J_{2,3}$ value, indicating a half-chair conformation of ring A. The debromo-compound (19), prepared by reduction of the bromo-acetate (18) with tri-n-butyltin hydride, reacted quantitatively with salt-free methylenetriphenylphosphorane, prepared in tetrahydrofuran from methyl triphenylphosphonium bromide and sodium hydride, to give the acetate (20) (80%) and GA_{40} methyl ester (21) (20%). Hydrolysis of the acetate (20) with 1% potassium hydroxide in methanol, followed by methylation, gave GA_{40} methyl ester (21) and the diol (22) in the ratio of 9 : 1. The ratio was 2 : 3 when 5% potassium hydroxide in methanol was used. The diol (22) was lactonised to GA_{40} methyl ester (21) either by heating in xylene or during g.l.c. Since Yamaguchi *et al.*[12] have converted GA_{40} (9) into 2-*epi*-GA_{40} (7) *via* the 2-ketone, the present work also provides a partial synthesis of this higher plant metabolite (7) [4] of GA_9 (5).

Treatment of the *ent*-2β,3β-epoxide (23) with dilute sulphuric acid in tetrahydrofuran gave approximately equal amounts of the *ent*-2α,3β- and -2β,3α-diols, (24) and (25), respectively. This anomalous cleavage of the epoxide (23) to the diequatorial diol (24) will be discussed in a subsequent publication together with other examples. The third isomeric diol (26), the norketone of GA_{34} methyl ester, was prepared from the Δ^2-derivative (17) and osmium tetraoxide. The bistrimethylsilyl (bisTMSi) ethers of each of the three diols (24)—(26) reacted smoothly with methylenetriphenylphosphorane and, after removal of the TMSi groups, the methyl esters (27)—(29) of 3-*epi*-GA_{34} (11), GA_{47} (10), and GA_{34} (4) were obtained. The three diols (27)—(29) had distinctive n.m.r. spectra. The *ent*-2α,3β-diol (27) was distinguished by the typical diaxial $J_{2,3}$ value of 9 Hz, and in the spectra of the diols (28) and (29) the 5-proton was deshielded by the *ent*-3α(*ax*)-hydroxy-group [by comparison with the diol (27)]. The mass spectra of bisTMSi ethers of the diols (27)—(29) had similar fragmentation patterns but showed significant differences in the relative intensities of some fragment ions. For example, the rearrangement ion, $Me_3Si\text{–}\overset{+}{O}\text{=}SiMe_2$, derived from the vicinal TMSiO-groups was least abundant in the case of the bisTMSi ether of the *trans*-diaxial diol (28). The bisTMSi ethers of the three diols (27)—(29) had different g.l.c. retention times and so were readily distinguished by g.l.c.–mass spectrometry. G.l.c. of the underivatised methyl esters (24)—(26) was less satisfactory but their probe mass spectra were different.

[6] J. MacMillan and C. M. Wels, *Phytochemistry*, 1974, **13**, 1413.
[7] L. J. Beeley, P. Gaskin, and J. MacMillan, *Phytochemistry*, 1975, **14**, 779.
[8] J. MacMillan and N. Takahashi, *Nature*, 1968, **217**, 170.
[9] J. F. Grove, J. MacMillan, T. P. C. Mulholland, and W. B. Turner, *J. Chem. Soc.*, 1960, 3049.
[10] B. F. Cross, R. H. B. Galt, and J. R. Hanson, *Tetrahedron*, 1962, **18**, 451; J. R. Hanson, *J. Chem. Soc.*, 1965, 3550.
[11] C. H. Robinson, L. Finkenor, D. Gould, and E. P. Oliveto, *J. Amer. Chem. Soc.*, 1959, **81**, 2195.
[12] I. Yamaguchi, M. Miyamoto, H. Yamane, N. Murofushi, N. Takahashi, and K. Fujita, *J.C.S. Perkin I*, 1975, 996.

In the C_{20} GA series the partial synthesis of GA_{46} methyl ester (14) from the fungal GA_{13} (30) was analogous to that shown in the Scheme for GA_{40} methyl ester (21). Reaction of the Δ^2-derivative (31), prepared from GA_{13} (30) as described,[13] with *N*-bromoacetamide and lithium acetate [11] gave the bromohydrin (34) and not the expected 2β-hydroxy-group in the bromohydrin (34). A distinction between the alternative structures (41) and (42) was not attempted for this lactone, which was more rationally prepared from the alcohol (40) with sodium hydride. To prevent formation of the lactone (41) or (42) during the Wittig reaction of the norketone (37), the

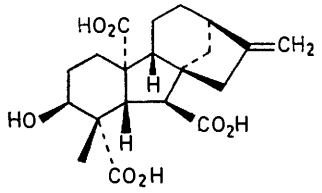


SCHEME Reagents: i, OsO_4–$NaIO_4$; ii, CH_2N_2; iii, $POCl_3$; iv, $MeCO{\cdot}NHBr$–$MeCO_2Li$–$MeCO_2H$: v, Bu^n_3SnH; vi, $Ph_3P{:}CH_2$; vii, KOH–MeOH; viii, OsO_4; ix, heat; x, 2N-H_2SO_4–tetrahydrofuran.

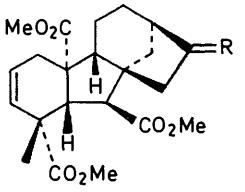
bromo-acetate (35). The bromohydrin (34) had the appropriate n.m.r. spectrum, showing that the 5-proton was deshielded in comparison with that in the debromo-compound (37), obtained by reduction of (34) with tri-n-butyltin hydride. The formation of a lactone [(41) or (42)] by reaction of the norketone (37) with methylene-triphenylphosphorane confirmed the presence of the *ent*-2β-hydroxy-group was protected by formation of the TMSi ether. This protecting group was introduced at the bromohydrin stage, since debromination of the TMSi ether (36) to (38) gave a higher yield than debromination of the unprotected bromohydrin (34). Reduction with sodium borohydride of the 2-ketone (43), obtained by direct oxidation of the TMSi ether (39) with Jones reagent, afforded GA_{46} methyl ester (14) in 25% yield together with the isomeric *ent*-2β-alcohol (40) (58%) and

[13] D. M. Harrison and J. MacMillan, *J. Chem. Soc.* (*C*), 1971, 631.

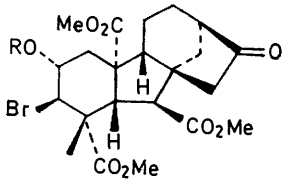
the lactone [(41) or (42)]. Reduction of the *ent*-2-oxo-gibberellanes (43) and (9; 2-oxo) [12] with sodium borohydride gave a greater proportion of axial (*ent*-2β-)



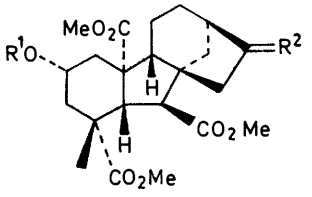
(30)



(31) R = O
(32) R = CH_2
(33) R = $(OCH_2)_2$

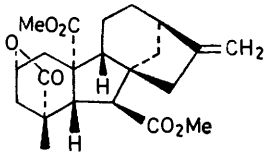


(34) R = H
(35) R = Ac
(36 R = $SiMe_3$



(37) R^1 = H, R^2 = O
(38) R^1 = $SiMe_3$, R^2=O
(39) R^1 = $SiMe_3$, R^2=CH_2
(40) R^1 = H, R^2= CH_2

alcohol than similar reduction of *ent*-3-oxo-gibberellanes.[14] Reduction of the 2-ketone (43) with aluminium isopropoxide gave a slightly better yield of GA_{46} methyl ester (14) (40%) together with unchanged ketone (30%)



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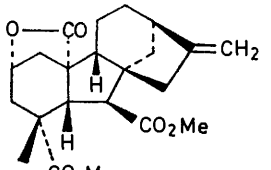
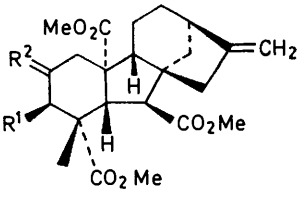
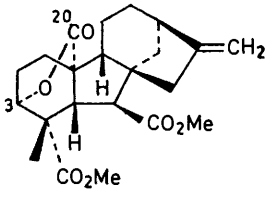


(42)



(43) R^1 = H, R^2 = O
(44) R = OH, R^2 = H, β-OH



(45)

and the lactone (41) or (42) (30%). However, by using aluminium isopropoxide, prepared [15] from aluminium and propan-2-ol in the presence of mercury(II) chloride and carbon tetrachloride, GA_{46} methyl ester (14) was obtained in 80% yield together with 10% of the *ent*-2β-isomer (40) and none of the lactone (41) or (42). The *ent*-2α-orientation of the hydroxy-group in GA_{46} methyl ester was evident from the ready lactonisation of the isomeric *ent*-2β-alcohol (40) and from the $W_{\frac{1}{2}}$ value (12 Hz) of the 2(*ax*)-proton n.m.r. signal. Treatment of GA_{46} methyl ester (14) with iodine in refluxing benzene gave the endocyclic double-bond isomer (15). In an attempt to prepare the isomer (15) treatment of GA_{46} methyl ester (14) with toluene-*p*-sulphonic acid in refluxing benzene gave, unexpectedly, the endocyclic double-bond isomer of the lactone (41) or (42), also obtained by similar treatment of the lactone (41) or (42). The mass spectra of the synthetic GA_{46} methyl ester (14), and its TMSi ether, and of the endocyclic double bond isomer (15), and its TMSi ether, were respectively identical with those of the corresponding derivatives of *y*-hydroxy-GA_{25} and *x*-hydroxy-GA_{25}, previously detected in nucellus-endosperm of *Echinocystis macrocarpa* together with GA_{43} (44).

In an earlier attempt to prepare GA_{46} (12) from GA_{13} (30) the hydroboronation of the ethylene acetal (33) was examined. Without isolation of the intermediates, the total hydroboronation product was oxidised and hydrolysed to a mixture of 16-ketones which was treated with methylenetriphenylphosphorane. The mixture of products was shown by g.l.c.–mass spectrometry to consist of the trimethyl ester of GA_{13} (30), the 20,3-lactone (45), GA_{46} methyl ester (14), and the lactone (41) or (42) in the approximate ratio 14 : 11 : 1.5 : 1. Thus hydroboronation of the olefin (33) gave mainly 3-hydroxylation, with minor amounts of 2-hydroxylation, and, apparently, an appreciable proportion of non-axial attack at both the 2- and 3-positions. Similar results have been reported [16] for some Δ^2-steroids.

EXPERIMENTAL

For general procedures see ref. 7. Except where stated otherwise, i.r. spectra are for Nujol mulls and n.m.r. spectra are for solutions in deuteriochloroform. Low resolution mass spectra were obtained by g.l.c.–mass spectrometry; [7] high resolution data were obtained by using an A.E.I.–G.E.C. MS9 instrument. Mass spectral data for compounds (18), (19), (22)—(29), (33), (34), and (40)—(43) are available in Supplementary Publication No. SUP 21716 (16 pp.).* Light petroleum had b.p. 60—80°.

ent-10-*Hydroxy*-16-*oxo*-17,20-*bisnorgibberell*-2-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (17).—Crude gibberellin A_4 norketone (2.3 g), obtained from a mixture (7 : 3) of GA_4 and GA_7 (2.5 g), was methylated; the product was dissolved in pyridine (60 ml) and treated with phosphoryl chloride (5.5 ml), first at 20 °C for 12 h, then under reflux for 3 h. Work-up in the usual way gave a gum (1.6 g) which

* For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1975, Index issue.

[14] B. E. Cross, J. F. Grove, and A. Morrison, *J. Chem. Soc.* (*C*), 1961, 2498.

[15] A. I. Vogel, 'Elementary Practical Organic Chemistry, Part 1,' Longman, Green, and Co., London and New York, 1957, p. 337.

[16] A. Hassner and C. Pillar, *J. Org. Chem.*, 1962, **27**, 2914.

was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 1). The olefin (17) was recovered from the band at R_F *ca.* 0.5 and crystallised from acetone–light petroleum in prisms (900 mg), m.p. 158—159 (lit.,[10] 160—161°); $\nu_{max.}$ 3 040, 1 778, 1 745, and 1 732 cm^{-1}; τ 8.78 (s, 18-H_3), 7.32 and 7.17 (J_{AB} 10 Hz, 5- and 6-H), 6.28 (s, CO_2Me), and *ca.* 4.25 (m, 2- and 3-H).

ent-2β-*Acetoxy*-3α-*bromo*-10-*hydroxy*-16-*oxo*-17,20-*bisnorgibberellane*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (18).—*N*-Bromoacetamide (400 mg) was added with stirring to the olefin (17) (856 mg) and lithium acetate dihydrate (4 g) in glacial acetic acid (90 ml). Stirring was continued for 3 h, then the mixture was poured into water and extracted with ethyl acetate. The gum (1.1 g) recovered from the ethyl acetate was subjected to p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 1). Elution of the band at R_F *ca.* 0.5 gave a gum (900 mg), which crystallised from acetone–light petroleum to give the *bromo-acetate* (18), m.p. 181—184° (Found: M^+, 468.078. $C_{21}H_{25}{}^{79}BrO_7$ requires M, 468.078); $\nu_{max.}$ (CH_2Cl_2) 1 785 and 1 745 cm^{-1}; τ 8.76 (s, 18-H_3), 7.95 (s, OCOMe), 7.2 (d, J 11 Hz, 6-H), 6.64 (d, J 11 Hz, 5-H), 6.26 (s, CO_2Me), 5.87br (s, 3-H), and 4.65 (m, $W_{\frac{1}{2}}$ 7 Hz, 2-H).

ent-2β-*Acetoxy*-10-*hydroxy*-16-*oxo*-17,20-*bisnorgibberellane*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (19).—Tri-n-butyltin hydride (700 μl) was added to the bromo-acetate (18) (950 mg) in benzene (10 ml). The mixture was stirred at 20 °C for 24 h then poured into water. The product was recovered in ethyl acetate and subjected to p.l.c. on silica gel with ethyl acetate–light petroleum (3 : 2). Extraction of the band at R_F 0.4—0.5 gave the *acetate* (19) (460 mg), m.p. 164—166° (from acetone–light petroleum) (Found: M^+, 390.167. $C_{21}H_{26}O_7$ requires M, 390.168); $\nu_{max.}$ 1 780, 1 750, and 1 730 cm^{-1}; τ 8.88 (s, 18-H_3), 7.97 (s, OCOMe), 7.36 and 7.20 (J_{AB} 10 Hz, 5- and 6-H), 6.26 (s, CO_2Me), and 4.79 (m, $W_{\frac{1}{2}}$ 11 Hz, 2-H).

ent-2β-*Acetoxy*-10-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (*Acetyl GA*$_{40}$ *Methyl Ester*) (20).—Sodium hydride (2 g) was added with stirring under nitrogen to a suspension of methyltriphenylphosphonium bromide (15 g) in tetrahydrofuran (150 ml). After 24 h at 20 °C, the stirring was stopped and the sodium bromide was allowed to settle. A portion (25 ml) of the supernatant was added to the norketone (19) (500 mg) and the solution was stirred for 4 h under nitrogen. Evaporation under vacuum left a residue, a solution of which in ethyl acetate was washed with water, dried, and evaporated to give a crude product (2 g). The latter was fractionated by p.l.c. on silica gel with ethyl acetate–light petroleum (3 : 2). Extraction of the band at R_F *ca.* 0.5 gave *acetyl GA*$_{40}$ *methyl ester* (20) (400 mg), m.p. 120—122° (from acetone–light petroleum) (Found: C, 68.1; H, 7.4. $C_{22}H_{28}O_6$ requires C, 68.0; H, 7.3%); $\nu_{max.}$ 3 090, 1 740, 1 725, and 1 665 cm^{-1}; τ 8.94 (s, 18-H_3), 8.06 (s, OCOMe), 7.4 and 7.24 (J_{AB} 10 Hz, 5- and 6-H), 6.32 (s, CO_2Me), 5.15 and 5.02 (both br, 17-H_2), and 4.78 (m, $W_{\frac{1}{2}}$ 12 Hz, 2-H).

The band at R_F *ca.* 0.3 gave GA_{40} methyl ester (21) (80 mg) (see later for characterisation).

Hydrolysis of ent-2β-*Acetoxy*-10-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (20).—(a) *GA*$_{40}$ *methyl ester* (21). The acetate (20) (40 mg) and potassium hydroxide (17.5 mg) in methanol (2.5 ml) were refluxed for 2.5 h. The mixture was added to water, which was then adjusted to pH 2.5 with concentrated hydrochloric acid. Recovery of the product in ethyl acetate gave a gum (30 mg) which was methylated (CH_2N_2), then subjected to p.l.c. on silica gel with ethyl acetate–light petroleum (3 : 2). Extraction of the band at R_F 0.2—0.35 gave gibberellin A_{40} methyl ester (21) (28 mg), m.p. 136—138° (from acetone–light petroleum) (Found: C, 69.2; H, 7.7. Calc. for $C_{20}H_{26}O_5$: C, 69.5; H, 7.6%), identical (i.r., n.m.r., and mass spectra) with an authentic sample;[12] $\nu_{max.}$ 3 500, 3 080, 1 755, 1 720, and 1 660 cm^{-1}; τ 8.92 (s, 18-H_3), 7.42 and 7.24 (J_{AB} 10 Hz, 5- and 6-H), 6.31 (s, CO_2Me), 5.71 (m, $W_{\frac{1}{2}}$ 11 Hz, 2-H), and 5.15 and 5.03 (both br, 17-H_2).

(b) Hydrolysis of the acetate (20) with potassium hydroxide–methanol (5 : 95 w/v) gave a 2 : 3 mixture of GA_{40} methyl ester (21) and the *diol* (22). The latter compound had a higher R_F value and was obtained as a gum (Found: M^+, 378.206. $C_{21}H_{30}O_6$ requires M, 378.204); $\nu_{max.}$ (CH_2Cl_2) 3 460br, 1 730, 1 700, and 1 660 cm^{-1}; τ 8.81 (s, 18-H_3), 7.85 (d, J 11 Hz, 5-H), 6.49 (d, J 11 Hz, 6-H), 6.27 and 6.24 (both s, 2 × CO_2Me), 5.73 (m, $W_{\frac{1}{2}}$ 8 Hz, 2-H), and 5.16 and 5.08 (both br, 17-H_2). G.l.c.–mass spectrometry (SE-33; 220 °C) gave a single g.l.c. peak with *m/e* values identical with those for GA_{40} methyl ester (21); lactonisation also occurred (g.l.c.–mass spectrometry) when the diol (22) was heated for 5 h in refluxing xylene.

ent-2β,3β-*Epoxy*-10-*hydroxy*-16-*oxo*-17,20-*bisnorgibberellane*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (23).—Potassium hydroxide (225 mg) in methanol (2 ml) was added to a stirred solution of the bromo-acetate (18) (400 mg) in methanol (15 ml). After 2 h stirring, the mixture was added to water. The solid, recovered in ethyl acetate, was essentially homogeneous by g.l.c. Traces of impurity were removed by p.l.c. on silica gel, developed with ethyl acetate–chloroform (1 : 1). Recovery from the band at R_F 0.4—0.5 gave the *epoxide* (23), m.p. 248—252° (from chloroform–light petroleum) (Found: M^+, 346.142. $C_{19}H_{22}O_6$ requires M, 346.142); $\nu_{max.}$ 1 785, 1 745, and 1 730 cm^{-1}; τ 8.64 (s, 18-H_3) 7.36 and 6.99 (J_{AB} 10 Hz, 5- and 6-H), 6.78 (d, J 4 Hz, 3-H), 6.66 (t, J 4 Hz, 2-H), and 6.23 (s, CO_2Me).

Hydrolysis of ent-2β,3β-*Epoxy*-10-*hydroxy*-16-*oxo*-17,20-*bisnorgibberellane*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (23).—The epoxide (23) (60 mg), tetrahydrofuran (6 ml), and 2N-sulphuric acid (2 ml) were heated under reflux for 2 h, then poured into water. The gum obtained by extraction with ethyl acetate (sodium hydrogen carbonate wash) was fractionated by p.l.c. on alumina, developed with ethyl acetate–methanol (4 : 1). The band at R_F 0.45—0.6 yielded the ent-2β,3α-*diol* (25) (23 mg), m.p. 110—115° (solidifying and remelting at 163—165°) (from ethanol) (Found: M^+, 364.153. $C_{19}H_{24}O_7$ requires M, 364.152); $\nu_{max.}$ *ca.* 3 450br and 1 740br cm^{-1}; τ (C_5D_5N) 8.44 (s, 18-H_3), 6.94(d, J 11 Hz, 6-H), 6.33 (s, CO_2Me), 6.20 (d, J 11 Hz, 5-H), 5.7 (br, 3-H), 5.38 (d, J 5 Hz, 2-H).

The band at R_F 0.30—0.45 from p.l.c. gave the ent-2α,3β-*diol* (24) (20 mg), m.p. *ca.* 120° (solidifying and re-melting at 180—184°) (from ethanol) (Found: M^+, 364.153. $C_{19}H_{24}O_7$ requires M, 364.152); $\nu_{max.}$ (CH_2Cl_2) *ca.* 3 600, 1 780, and 1 740 cm^{-1}; τ (C_5D_5N), 8.45 (s, 18-H_3), 6.95 (s, 5- and 6-H; AB-system on addition of D_2O at 7.04 and 6.97, J_{AB} 10 Hz), 6.31 (s, CO_2Me), 5.94 (d, J 9 Hz, 3-H), and *ca.* 5.8 (m, 2-H).

ent-2α,3α,10-*Trihydroxy*-16-*oxo*-17,20-*bisnorgibberellane*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (26).—The olefin (17) (110 mg) and osmium tetraoxide (100 mg) in chloroform (0.5 ml) and pyridine (0.5 ml) were left at 20 °C for 2 days. Sodium disulphite (250 mg) in water (2 ml) was added and the mixture was stirred for 30 min, then added to water. After neutralisation with concentrated hydro-

chloric acid the solution was extracted with ethyl acetate. Evaporation of the extract gave a solid (120 mg), which was recrystallised from methanol to give the ent-2α,3α-*diol* (26), m.p. *ca.* 115—120° (Found: M^+, 364.152. $C_{19}H_{24}O_7$ requires M, 364.152); $\nu_{max.}$ (CH_2Cl_2) 3 590br, 1 775, and 1 740 cm^{-1}; τ (C_5D_5N) 8.48 (s, 18-H_3), 7.03 (d, J 11 Hz, 6-H), 6.33 (s, CO_2Me), 6.19 (d, J 11 Hz, 5-H), and *ca.* 5.75 (m, 2- and 3-H).

ent-2β,3α,10-*Trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (*GA*$_{47}$ *Methyl Ester*) (28).—The *ent*-2β,3α-diol (25) (25 mg) in pyridine (0.5 ml) was treated for 2 h at 20 °C in a sealed tube with hexamethyldisilazane (75 μl) and trimethylsilyl chloride (75 μl). The mixture was evaporated in a stream of dry nitrogen and the residue was extracted twice with ethyl acetate. The extract was passed down a short column of silica gel, then evaporated to yield the bisTMSi ether of the diol (25) as a gum, which was treated with an excess of methylenetriphenylphosphorane in tetrahydrofuran by the procedure described earlier for the norketone (19). The crude product was dissolved in acetic acid–methanol (1 : 4) and, after 24 h, the solution was gently warmed with stirring until hydrolysis of the bisTMSi ether was complete (t.l.c.). Evaporation gave a gum which was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (17 : 2). Extraction of the zone at R_F 0.35—0.45 gave *GA*$_{47}$ *methyl ester* (28) (20 mg), m.p. 242—245° (from methanol) (Found: M^+, 362.174. $C_{20}H_{26}O_6$ requires M, 362.173); $\nu_{max.}$ 3 495, 3 090, 3 400, 1 780, 1 715, and 1 658 cm^{-1}; τ (C_5D_5N) 8.41 (s, 18-H_3), 6.93 (d, J 11 Hz, 6-H), 6.32 (s, CO_2Me), 6.17 (d, J 11 Hz, 5-H), 5.69br (3-H), 5.38 (m, $W_{\frac{1}{2}}$ 10 Hz, 2-H), and 5.1 and 5.0 (each br, 17-H_2).

ent-2α,3β,10-*Trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (3-epi-*GA*$_{34}$ *Methyl Ester*) (27).—Prepared by the methods described in the previous experiment, 3-epi-*GA*$_{34}$ *methyl ester* (27) was obtained from the *ent*-2α,3β-diol (24) as an intractable gum (Found: M^+, 362.173. $C_{20}H_{26}O_6$ requires M, 362.173); $\nu_{max.}$ (CH_2Cl_2) 3 600br, 1 780, 1 740, and 1 660 cm^{-1}; τ (C_5D_5N) 8.43 (s, 18-H_3), 6.94 (s, 5- and 6-H; on addition of D_2O, an AB system at 7.06 and 6.92, J_{AB} 10 Hz), 6.32 (s, CO_2Me), 5.98 (d, J 9 Hz, 3-H), 5.75 (m, $W_{\frac{1}{2}}$ 20 Hz, 2-H), and 5.1 and 5.0 (each br, 17-H_2).

ent-2α,3α,10-*Trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (*GA*$_{34}$ *Methyl Ester*) (29).—Prepared in an analogous manner to the *ent*-2β,3α-isomer (28), *GA*$_{34}$ *methyl ester* (29) was obtained from the *ent*-2α,3α-diol (26) as an intractable gum (Found: M^+, 362.173. $C_{20}H_{26}O_6$ requires M, 362.173); $\nu_{max.}$ (CH_2Cl_2) 3 550br, 1 775, 1 740, and 1 660 cm^{-1}; τ (C_5D_5N) 8.46 (s, 18-H_3), 7.04 (d, J 11 Hz, 6-H), 6.32 (s, CO_2Me), 6.17 (d, J 11 Hz, 5-H), 5.72 (m, 2- and 3-H), and 5.11 and 5.0 (each br, 17-H_2).

Trimethyl ent-3α-*Bromo*-2β-*hydroxy*-16-*oxo*-17-*norgibberellane*-7,19,20-*trioate* (34).—The Δ^2-olefin (31) (590 mg), prepared by the method of Harrison and MacMillan,[13] *N*-bromoacetamide (190 mg), lithium acetate dihydrate (2 g), and glacial acetic acid (20 ml) were stirred at 20 °C for 3 h. After the addition of ethyl acetate (100 ml), the mixture was washed with water, dried, and evaporated under vacuum to give a gum (770 mg) which was fractionated by p.l.c. on silica gel with ethyl acetate–light petroleum (3 : 2). Elution of the band at R_F 0.4—0.5 gave the *bromohydrin* (34) as an intractable gum (400 mg), pure by g.l.c. of the trimethylsilyl ether (Found: M^+, 500.104. $C_{22}H_{29}{}^{79}BrO_8$ requires M, 500.104); $\nu_{max.}$ (CH_2Cl_2) 3 430br, 1 740, and 1 700 cm^{-1}; τ 8.54 (s, 18-H_3), 7.42 (d, J 12 Hz, 5-H), 6.35 (d, J 12 Hz, 6-H), 6.35, 6.30, and 6.06 (each s, 3 × CO_2Me), 5.71 (q, with D_2O, J 5 Hz, 2-H), and 5.18 (d, J 5 Hz, 3-H).

Trimethyl ent-2β-*Hydroxy*-16-*oxo*-17-*norgibberellane*-7,19,20-*trioate* (37) *and its Trimethylsilyl Ether* (38).—Trimethylsilylation of the bromohydrin (34) (680 mg), by the procedure described earlier, gave the TMSi ether of the bromohydrin (34) as a solid (680 mg), which was stirred in benzene (11 ml) at 18 °C for 24 h with tri-n-butyltin hydride (450 μl). Evaporation under vacuum yielded a gum which was fractionated by p.l.c. on silica gel with ethyl acetate–light petroleum (2 : 3). Extraction of the band at R_F 0.4—0.5 gave the TMSi ether (38) as a homogeneous (g.l.c.) but intractable gum (550 mg) which was used directly for the following Wittig reaction. It was characterised by g.l.c.–mass spectrometry: *m/e* 494 (M^+, <1%), 479(100), 462(24), 434(2), 419(8), 372(4), and 312(11), and by hydrolysis with acetic acid–methanol (1 : 4) at 18 °C for 12 h to give the *alcohol* (37), m.p. 118—119° (from acetone–light petroleum) (Found: C, 62.4; H, 6.9. $C_{22}H_{30}O_8$ requires C, 62.5; H, 7.2%); $\nu_{max.}$ 3 490, 1 760, 1 725, and 1 713 cm^{-1}; τ 8.8 (s, 18-H_3), 7.85 (d, J 12 Hz, 5-H), 6.33 (d, J 12 Hz, 6-H) 6.33, 6.24, and 6.13 (each s, 3 × CO_2Me), and 5.86 (m, $W_{\frac{1}{2}}$ 12 Hz, 2-H).

Trimethyl ent-2β-*Hydroxygibberell*-16-*ene*-7,19,20-*trioate* (40).—A portion (20 ml) of a solution of methylenetriphenylphosphorane in tetrahydrofuran prepared as described earlier was added to the TMSi ether (38) (508 mg) and the mixture was stirred at 20 °C for 4 h under nitrogen, then evaporated under vacuum. The residue was dissolved in ethyl acetate and the solution was washed with water, dried, and evaporated. The residual gum (2.0 g) was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 3). Recovery from the band at R_F 0.5—0.6 gave the TMSi ether (39) (370 mg), *m/e* 492 (M^+, <1%), 477(64), 460(100), 310(38), 283(24), 282(23), 223(50), 75(18), and 73(46).

The TMSi ether was hydrolysed with acetic acid–methanol (1 : 4) at room temperature for 12 h to give the *alcohol* (40), m.p. 105–106° (from light petroleum) (Found: C, 66.1; H, 7.85. $C_{23}H_{32}O_7$ requires C, 65.7; H, 7.7%); $\nu_{max.}$ 3 450, 1 725, 1 705, and 1 660 cm^{-1}; τ 8.82 (s, 18-H_3), 7.88 (d, J 12 Hz, 5-H), 6.46 (d, J 12 Hz, 6-H), 6.36, 6.25, and 6.14 (each s, 3 × CO_2Me), 5.87 (m, $W_{\frac{1}{2}}$ 12 Hz, 2-H), and 5.09br (17-H_2).

The Lactone [(41) *or* (42)].—The alcohol (40) (30 mg) in tetrahydrofuran (3 ml) was heated gently for 1 min with sodium hydride. After a further 12 h at 18 °C, the mixture was added to water. Extraction with ethyl acetate gave the lactone (41) or (42) (25 mg), m.p. 155—157° (from acetone–light petroleum) (Found: C, 68.05; H, 7.5. Calc. for $C_{22}H_{28}O_6$: C, 68.0; H, 7.3%); $\nu_{max.}$ 3 075, 1 770, 1 732, and 1 660 cm^{-1}; τ 8.69 (s, 18-H_3), 7.54 (d, J 12 Hz, 5-H), 6.43 (d, J 12 Hz, 6-H), 6.34 and 6.28 (both s, 2 × CO_2Me), 5.32 (t, J 5 Hz, 2-H), and 5.16 and 5.05 (both br, 17-H_2).

Trimethyl ent-2-*Oxogibberell*-16-*ene*-7,19,20-*trioate* (43).—The TMSi ether (39) (370 mg) in acetone (15 ml) was oxidised by Jones reagent at 0 °C for 45 min. The usual work-up gave the *ketone* (43) as a gum (340 mg) (Found: M^+, 418.198. $C_{23}H_{30}O_7$ requires M, 418.199); $\nu_{max.}$ (CH_2Cl_2) 1 730 and 1 660 cm^{-1}; τ 8.69 (s, 18-H_3), 6.36, 6.32, and 6.22 (each s, 3 × CO_2Me), 6.09 (d, J 12 Hz, 6-H), and 5.17 and 5.06 (both br, 17-H_2).

Reduction of Trimethyl ent-2-*Oxogibberell*-16-*ene*-7,19,20-*trioate* (43); *Preparation of GA*$_{46}$ *Methyl Ester* (14).—(a) *By aluminium isopropoxide prepared* in situ.[15] Aluminium (400 mg) and mercury(II) chloride (7 mg) in dry propan-2-ol (13 ml) were heated to boiling. Carbon tetrachloride (7 μl)

was added and the mixture was boiled for 4 h. The ketone (43) (130 mg) was added in propan-2-ol (1 ml) and the mixture was refluxed for a further 2 h. The solvent was then removed *under vacuum*, water was added, and the resulting slurry was extracted with ethyl acetate. Material recovered from the ethyl acetate extract was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 1). The band at R_F *ca.* 0.4 was removed and extracted to give *GA_{46} trimethyl ester* (14) (95 mg), m.p. 138—140° (from acetone–light petroleum) (Found: C, 65.6; H, 8.0. $C_{23}H_{32}O_7$ requires C, 65.7; H, 7.7%); $\nu_{max.}$ 3 550, 1 730, 1 710, and 1 660 cm^{-1}; τ 8.79 (s, 18-H_3), 7.77 (d, J 13 Hz, 5-H), 6.37, 6.32, and 6.26 (each s, 3 × CO_2Me), 6.12 (d, J 13 Hz, 6-H), 5.74 (m, $W_{\frac{1}{2}}$ 23 Hz, 2-H), and 5.16 and 5.08 (both br, 17-H_2). The mass spectra of the methyl ester and the TMSi ether were identical with those reported [7] for *y*-hydroxy GA_{25} trimethyl ester, detected in seed of *Echinocystis macrocarpa*.

(b) *By aluminium isopropoxide*. The ketone (43) (2.5 mg), aluminium isopropoxide (400 mg), and propan-2-ol (5 ml) were refluxed for 5.5 h. The mixture was periodically distilled to half volume, then more propan-2-ol (10 ml total) was added. The mixture was evaporated to 3 ml and water was added. Extraction with ethyl acetate gave unchanged ketone (43) (30%), the *ent*-2α-alcohol (GA_{46} methyl ester) (14) (40%), and the lactone (41) or (42) (30%), identified by g.l.c.–mass spectrometry of the trimethylsilylated total product.

(c) *Sodium borohydride*. The ketone (1 mg) in methanol (0.25 ml) was reduced with sodium borohydride (*ca.* 2 mg) in the usual manner. The product, analysed by g.l.c.–mass spectrometry after trimethylsilylation, comprised GA_{46} methyl ester (14) (25%), the *ent*-2β-alcohol (40) (58%), and the lactone (41) or (42) (17%).

Trimethyl ent-2α-*Hydroxygibberell*-15-*ene*-7,19,20-*trioate* (15).—Gibberellin A_{46} trimethyl ester (14) (2 mg) and iodine (1 mg) were refluxed for 12 h in benzene (2 ml). After the addition of ethyl acetate, the solution was washed with aqueous sodium thiosulphate, then water, and dried. Evaporation gave the 15-*ene* (15), characterised by g.l.c.–mass spectrometry of the methyl ester and the TMSi ether. The mass spectra of these derivatives were identical with those reported [7] for the same derivatives of *x*-hydroxy GA_{25}, detected in extracts of seed of *Echinocystis macrocarpa*.

Trimethyl ent-2β-*Hydroxygibberell*-15-*ene*-7,19,20-*trioate*.—The lactone [(41) or (42)] (2 mg) and toluene-*p*-sulphonic acid (1 mg) in benzene (2 ml) were refluxed for 6 h. After addition of ethyl acetate the product was recovered in the usual way and characterised by g.l.c.–mass spectrometry. The same product was obtained by similar treatment of GA_{46} trimethyl ester (14).

Trimethyl ent-10-*Hydroxy*-16-*oxo*-17-*norgibberell*-2-*ene*-7,19,20-*trioate Ethylene Acetal* (33).—The olefin (33) [13] (100 mg), toluene-*p*-sulphonic acid (3 mg) and ethanediol (1 ml) in benzene (15 ml) were heated under reflux for 6 h with use of a Dean–Stark water separator. The usual work-up gave the *acetal* (33) (109 mg), m.p. 171—172° (from acetone–light petroleum) (Found: C, 64.3; H, 7.35. $C_{24}H_{32}O_8$ requires C, 64.3; H, 7.2%); $\nu_{max.}$ 3 030 w and 1 722 cm^{-1}; τ 8.72 (s, 18-H_3), 7.61 (d, J 13 Hz, 5-H), 6.44, 6.35, and 6.23 (each s, 3 × CO_2Me), 6.14 [m, 6-H and $(OCH_2)_2$], 4.44 (q, J 2 and 10 Hz, 3-H), and 4.19 (oct, J 2, 6, and 10 Hz, 2-H).

Hydroboronation of the Acetal (33) *and Analysis of the Products*.—(a) *Hydroboronation*. 0.5M-Diborane in tetrahydrofuran (50 μl) was added to a solution of the acetal (33) (200 mg) in tetrahydrofuran (100 μl) and the mixture was kept for 30 min at 18 °C in a sealed tube. Aqueous 5% sodium hydroxide (100 μl) and hydrogen peroxide (30% w/v; 50 μl) were added and the biphasic mixture was left for 30 min at 18 °C. After addition of water the gummy product was recovered in ethyl acetate and used directly.

(b) *Deacetalisation*. The foregoing gum in acetone (200 μl) was treated with one crystal of toluene-*p*-sulphonic acid. After 12 h at 18 °C dilute aqueous sodium hydrogen carbonate was added and the gummy product was recovered in ethyl acetate.

(c) *Wittig reaction*. The gum from (b) was treated with a slight excess of a solution of methylenetriphenylphosphorane in dry tetrahydrofuran prepared as described earlier. The product was fractionated by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 1). The zone at R_F 0.15—0.8 was extracted to give a gum which was analysed by g.l.c.–mass spectrometry. The following products were identified (% of total g.l.c. peak areas in parentheses): the olefin (32) (7%), GA_{18} (30) methyl ester (47%), the lactone (45) (37%), GA_{46} methyl ester (14) (5%), and the lactone (41) or (42) (3%).

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