The Synthesis of 1-Methyl- and 1α,2α-Methylene-gibberellins

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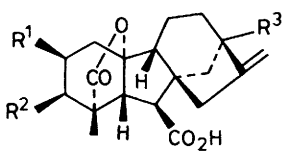
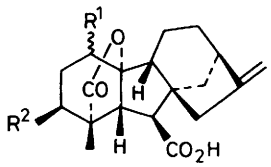
The syntheses of 1α-methyl-, 1β-methyl-, and 1α,2α-methylene-gibberellins required for further investigations into structure–biological activity relationships are described. Thermolysis of the pyrazoline derived from GA_7-3-ketone-7-methyl ester gave, as the major product, the corresponding 1-methyl enone with minor amounts of the 1α,2α-methyleneGA_4-3-ketone-7-methyl ester. The latter compound was reduced and hydrolysed to 1α,2α-methyleneGA_4. Reduction of the 1-methyl enone with sodium borohydride gave 1α-methylGA_4 methyl ester, which was hydrolysed to 1α-methylGA_4, and 1α-methyl-3-*epi*-GA_4 methyl ester which was deoxygenated to give 1α-methylGA_9. The palladium-catalysed reduction of the 1-methyl enone gave 1β-methylGA_4-3-ketone and 1α-methylGA_4-3-ketone which were converted into the corresponding 1β-methylGA_4 and 1α-methylGA_4. The analogous synthesis of the C-1 alkylated 13-hydroxylated gibberellins was improved by the use of a phenacyl ester to protect C-7, thus providing routes to 1β-methylGA_1, 1α-methylGA_1, and 1α,2α-methyleneGA_1.

Incubation of 1α-methylGA_4 with the fungus *Gibberella fujikuroi*, mutant B1-41a, gave 1α-methyl-GA_1 as the sole metabolite. Incubation of 1β-methylGA_4 with the mutant B1-41a gave 1-methylGA_3, 1-methylGA_7, and 1β-methylGA_1 confirming that it is the 1α-hydrogen which is lost in the 1,2-didehydrogenation process in the fungus.

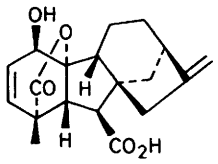
Metabolic studies of the gibberellins (GAs) in higher plants has shown that the biological activity of these plant growth hormones is profoundly altered by hydroxylation. For example GA_{20} (**1**) which occurs in the shoots of maize and pea has low activity in promoting stem extension in the dwarf mutants, *d*-1 of maize[1] and 1*e* of pea.[2] However 3β-hydroxylation of GA_{20} (**1**), a normal process in the tall phenotypes of these plants, gives GA_1 (**2**) which shows high biological activity.[3] Contrarily, 2β-hydroxylation, which also occurs in plants, converts the bio-active GAs, *e.g.* GA_1 (**2**), to bio-inactive GAs, *e.g.* GA_8 (**3**).[4] Furthermore 1β-hydroxylated GAs occur in plants, for example GA_{61} (**6**) and GA_{62} (**8**) in wheat,[5] which show less activity than their non-1-hydroxylated counterparts.[6] Previous investigations[7–9] of the effect on biological activity of introducing 2- and 3-substituents led to the highly active 2,2-dimethylGA_4 (**9**).[10] In extending these studies on structure and biological activity 1α-methyl-, 1β-methyl-, and 1α,2α-cyclopropyl-GAs have now been prepared.



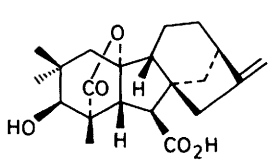
(**1**) $R^1 = R^2 = H$; $R^3 = OH$
(**2**) $R^1 = H$; $R^2 = R^3 = OH$
(**3**) $R^1 = R^2 = R^3 = OH$
(**4**) $R^1 = R^2 = R^3 = H$
(**5**) $R^1 = H$; $R^2 = OH$; $R^3 = H$



(**6**) R^1 = β-OH, $R^2 = H$
(**7**) R^1 = α-OH, $R^2 = OH$



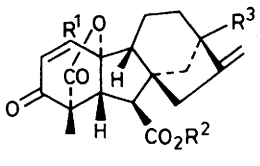
(**8**)



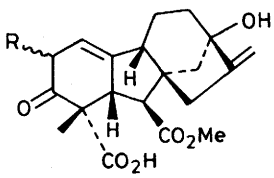
(**9**)

Results and Discussion

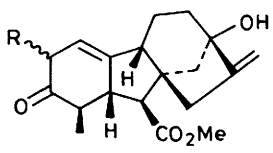
The introduction of a 1-methyl group by conjugate addition of lithium dimethylcuprate or a Grignard reagent to the enones (**10**) and (**11**) was not examined since our previous studies[11] had shown that such reactions with the enone (**11**) gave S_N2'-alkylation at C-2 with displacement of the lactone and products (**15**)—(**18**); no 1-alkylation was observed. Since dialkyl cuprates have been used in the direct displacement of iodides by alkyl groups[12] we did investigate the treatment of 1β-iodoGA_1 methyl ester (**19**)[13] with lithium dimethylcuprate. However



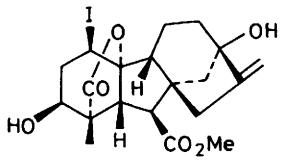
(**10**) $R^1 = H$, $R^2 = Me$, $R^3 = H$
(**11**) $R^1 = H$, $R^2 = Me$, $R^3 = OH$
(**12**) $R^1 = R^2 = Me$, $R^3 = H$
(**13**) $R^1 = Me$, $R^2 = CH_2COPh$, $R^3 = OH$
(**14**) $R = H$, $R^2 = CH_2COPh$, $R^3 = OH$



(**15**) R = β-Me
(**16**) R = α-Me



(**17**) R = β-Me
(**18**) R = α-Me

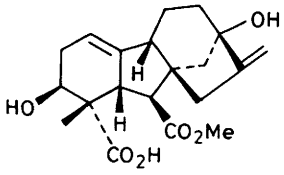


(**19**)

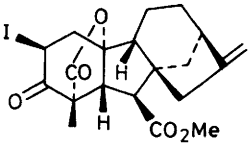
only the elimination product (**20**) was obtained. We had observed previously[14] a similar attack on iodine in the iodo ketone (**21**) by hydride and by bromide ion.

α,β-Unsaturated ketones with diazomethane give pyrazolines which can be thermolysed or photolysed to give 1-methyl enones or cyclopropanes.[15,16] Treatment of the enone (**10**) with

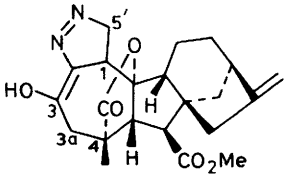
ethereal diazomethane in methanol gave two products. The less polar product, $C_{21}H_{24}N_2O_5$, showed a broad signal at δ6.66 in the ^1H n.m.r. spectrum, attributed to NH and consistent with the 2-pyrazoline (**23**) rather than the expected 1-pyrazoline. The ^{13}C n.m.r. spectrum was also consistent with structure (**23**). The more polar product, $C_{22}H_{26}N_2O_5$, is assigned the structure (**22**) in which expansion of ring A has occurred; the ^{13}C and ^1H n.m.r. spectra indicated that 1Δ ⟶ 2Δ isomerisation of the pyrazoline had not occurred but that conjugation had been achieved by enolisation. Formation of the ring expanded compound (**22**) was avoided by conducting the reaction of the enone (**10**) with diazomethane in acetone, not in methanol.



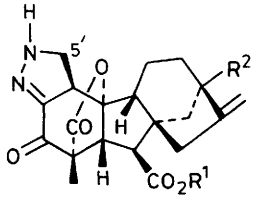
(**20**)



(**21**)



(**22**)



(**23**) R^1 = Me, R^2 = H

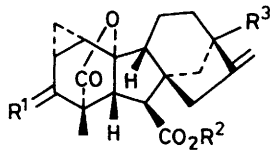
(**24**) R^1 = CH_2COPh, R^2 = OH

Thermolysis of the pyrazoline (**23**) at 170 °C in the presence of potassium dihydrogen orthophosphate[17] gave a product which was homogeneous by capillary g.l.c. and which gave the correct elemental analysis and, in the ^1H n.m.r. spectrum, the expected doublets (*J* 1.5 Hz) at δ 2.08 (1-Me) and δ 5.71 (2-H) for the 1-methyl enone (**12**). However the ^1H n.m.r. spectrum of this product indicated the presence of a minor component shown (see later) to be the 1α,2α-methylene derivative (**25**). The 1-methyl enone (**12**) was obtained pure after repeated recrystallisation.

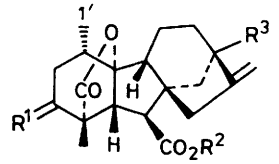
According to literature precedents[18–20] for the enone (**10**), reduction of the 1-methyl enone (**12**) with sodium borohydride and copper(I) chloride occurred from the β-face at C-1 to give a mixture of 1α-methylGA$_4$ methyl ester (**33**), 1α-methyl-3-*epi*-GA$_4$ methyl ester (**34**), and the unsaturated alcohol (**50**). A similar result was obtained by reducing the 1-methyl enone (**12**) with L-Selectride. The unsaturated alcohol (**50**) which is formed in 30% yield can be recycled by oxidation with Jones reagent to the 1-methyl enone (**12**), then reduction to (**33**) and (**34**).

1α-MethylGA$_4$ methyl ester (**33**) was hydrolysed with sodium propanethiolate to give 1α-methylGA$_4$ (**35**); anhydrous conditions were used to prevent epimerisation at C-3.[21] 1α-Methyl-3-*epi*-GA$_4$ methyl ester (**34**) was converted into 1α-methylGA$_9$ (**36**) as described by Beale *et al.*[22] for the preparation of GA$_9$ (**4**) from 3-*epi*-GA$_4$ methyl ester (**57**). Thus treatment of 1α-methyl-3-*epi*-GA$_4$ methyl ester (**34**) with phosphoryl chloride in refluxing pyridine gave the 3β-chloro derivative (**37**). Reduction of (**37**) with tributyltin hydride in the presence of an initiator, followed by hydrolysis, gave 1α-methylGA$_9$ (**36**).

To prepare 1β-methylGA$_4$ (**44**) methods were investigated of directing hydride attack at C-1 in the 1-methyl enone (**12**) from the more hindered α-face. This was partially achieved by treatment of (**12**) with tributylstannane in the presence of

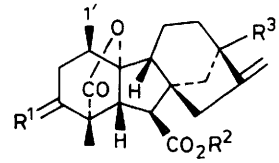


(**25**) R^1 = O, R^2 = Me, R^3 = H
(**26**) R^1 = β-H, α-OH, R^2 = Me, R^3 = H
(**27**) R^1 = β-H, α-OH, R^2 = R^3 = H
(**28**) R^1 = O, R^2 = R^3 = H
(**29**) R^1 = β-OH, α-H, R^2 = R^3 = H
(**30**) R^1 = β-H, α-OH, R^2 = R^3 = H
(**31**) R^1 = β-OH, α-H, R^2 = H, R^3 = OH
(**32**) R^1 = O, R^2 = H, R^3 = OH

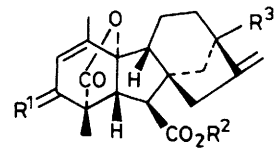


(**33**) R^1 = β-OH, α-H, R^2 = Me, R^3 = H
(**34**) R^1 = β-H, α-OH, R^2 + Me, R^3 = H
(**35**) R^1 = β-OH, α-H, R^2 = R^3 = H
(**36**) R^1 = H_2, R^2 = R^3 = H
(**37**) R^1 = β-Cl, α-H, R^2 = Me; R^3 = H
(**38**) R^1 = H_2, R^2 = Me, R^3 = H
(**39**) R^1 = O, R^2 = Me, R^3 = H
(**40**) R^1 = β-H, α-OH, R^2 = R^3 = H
(**41**) R^1 = O, R^2 = R^3 = H
(**42**) R^1 = β-OH, α-H, R^2 = H, R^3 = OH
(**43**) R^1 = O, R^2 = R^3 = H

tetrakistriphenylphosphinepalladium(0).[23] This reaction resulted solely in reduction of the 1,2-double bond to give a mixture (1:1) of 1α- and the 1β-methylGA$_4$ ketones (**39**) and (**45**). Reduction of this mixture with sodium borohydride, followed by alkaline hydrolysis and purification by reverse-phase h.p.l.c., gave 1β-methyl-3-*epi*-GA$_4$ (**46**), 1α-methyl-3-*epi*-GA$_4$ (**40**), and an inseparable mixture (7:3 by ^1H n.m.r.) of 1β-methylGA$_4$ (**44**) (δ 1.22, d, *J* 7 Hz, 1β-Me) and 1α-methylGA$_4$ (**33**) (δ 0.99, d, *J*, 6 Hz, 1α-Me). Oxidation of the mixture of 1α- and 1β-methylGA$_4$ (**33**) and (**44**) gave the corresponding ketones (**41**) and (**47**) which were separated by reverse-phase h.p.l.c.



(**44**) R^1 = β-OH, α-H, R^2 = R^3 = H
(**45**) R^1 = O, R^2 = Me, R^3 = H
(**46**) R^1 = β-H, α-OH, R^2 = R^3 = H
(**47**) R^1 = O, R^2 = R^3 = H
(**48**) R^1 = β-OH, α-H, R^2 = H; R^3 = OH
(**49**) R^1 = O, R^2 = H, R^3 = OH

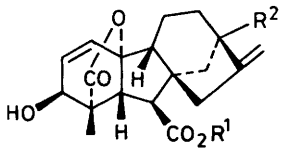


(**50**) R^1 = β-H, α-OH, R^2 = Me, R^3 = H
(**51**) R^1 = β-OH, α-H, R^2 = R^3 = H
(**52**) R^1 = β-OH, α-H, R^2 = H, R^3 = OH

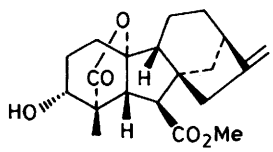
Reduction of 1β-methylGA$_4$ ketone (**47**) with sodium borohydride gave the required 1β-methylGA$_4$ (**44**) as the major product whereas reduction of 1α-methylGA$_4$-3-ketone (**41**) under the same conditions gave mainly 1α-methyl-3-*epi*-GA$_4$ (**40**). The required 1α-methylGA$_4$ (**33**) was prepared by reduction of the corresponding 3-ketone (**41**) with K-Selectride. The differing proportions of 3α:3β-alcohols formed from the reduction of the 1-methylketones (**41**) and (**47**) may be explained by steric interference from the methyl group at C-1. The axial 1β-methyl group hinders the β-face of ring A, thus directing attack from the α-face at C-3 whereas the equatorial 1α-methyl group has little steric effect and hydride attack is directed from the less hindered β-face at C-3.

The assignments of the methyl groups at C-1 in the ^{1}H n.m.r. spectra of (**33**) and (**44**) were confirmed by n.O.e. difference spectroscopy. The doublet at δ 1.22, assigned to the 1β(ax)-methyl group in (**44**), showed an n.O.e. to the doublet (*J* 10 Hz) at δ 3.45, assigned to 5-H; the doublet at δ 0.99, assigned to the 1α(eq)-methyl group in (**33**) showed no n.O.e. to the doublet (δ 3.20, *J* 11 Hz) of the 5-H signal.

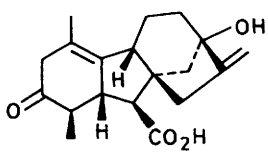
Reduction of the 1-methyl enone (**12**) with tributylstannane and tetrakistriphenylphosphinepalladium(0), also provided a method of isolating the 1α,2α-cyclopropylketone (**25**), present in the crude product from the thermolysis of pyrazoline (**23**). Such reduction of the crude thermolysis product gave a separable mixture of the more polar 1α,2α-cyclopropane (**25**) from the less polar mixture (1:1) of 1α- and 1β-methylGA$_4$ ketones (**39**) and (**45**). Reduction of the cyclopropyl ketone (**25**) with sodium borohydride gave solely the 3α-alcohol (**26**) which was in turn hydrolysed with sodium propanethiolate to the corresponding acid (**27**), then oxidised to the keto acid (**28**). Reduction of the keto acid (**28**) with K-Selectride gave a separable mixture (3:7) of 1α,2α-methyleneGA$_4$ (**29**) and 1α,2α-methylene-3-*epi*-GA$_4$ (**30**). The 1α,2α-stereochemistry of the methylene group was indicated by the formation of an excess of the 3α-alcohol on reduction of the ketone (**28**) with K-Selectride and by ^{1}H n.m.r. of the 3β-alcohol (**29**) which contained a singlet for the 3α-H and of the 3α-alcohol (**30**) which showed a doublet (*J* 7.3 Hz) for the 3β-H. The 1α,2α-stereochemistry of the methylene group was unexpected since it was assumed that diazomethane would add to the least hindered β-face of the enone (**10**), resulting in the 1β-pyrazoline (**23**), the precursor of the 1α,2α-methylene ketone (**25**). However prototropic shift of the 1-proton can occur during thermolysis of the pyrazoline (**23**) resulting in epimerisation at C-1.



(**53**) R^1 = CH_2COPh, R^2 = OH
(**54**) R^1 = R^2 = H
(**55**) R^1 = Me, R^2 = OH
(**56**) R^1 = H, R_2 = OH



(**57**)



(**58**)

The corresponding 1α-methyl, 1β-methyl, and 1α,2α-methylene derivatives of GA$_1$ (**42**), (**48**), and (**31**) were prepared from GA$_3$ (**56**) in an analogous series of reactions except that the route was improved by the use of the phenacyl ester to protect the carboxylic acid function. Thus GA$_3$ phenacyl ester (**53**) was oxidised with manganese dioxide to the corresponding enone (**14**) which on treatment with ethereal diazomethane gave the pyrazoline (**24**). Thermolysis of the pyrazoline (**24**) in the presence of potassium dihydrogen orthophosphate gave the 1-methyl enone (**13**) as the major product. The phenacyl ester was not reductively hydrolysed at this stage of the synthesis since treatment of the enone (**12**) with zinc and acetic acid led to allylic displacement of the lactone followed by decarboxylation to (**58**). A similar rearrangement has been observed on the GA$_3$-ketone methyl ester (**11**).[24]

Crude 1-methyl enone phenacyl ester (**13**) was directly reduced with tributyltin hydride in the presence of tetrakistriphenylphosphinepalladium(0) and the resultant mixture treated with zinc and acetic acid. 1α,2α-MethyleneGA$_1$ ketone (**32**) was separated from the reaction mixture by flash chromatography and the 1β-methylGA$_1$ ketone (**49**) and 1α-methylGA$_1$ ketone (**43**) were purified by reverse phase h.p.l.c.

The ketones (**49**), (**43**), and (**32**) were each reduced with K-Selectride to give 1β-methylGA$_1$ (**48**), 1α-methylGA$_1$ (**42**), and 1α,2α-methyleneGA$_1$ (**31**).

The metabolism of 1β-methylGA$_4$ (**44**) and 1α-methylGA$_4$ (**35**) by the fungus *Gibberella fujikuroi* mutant B1-41a was examined. This mutant is effectively blocked for GA biosynthesis[25] but will efficiently metabolise exogenously applied gibberellins as well as unnatural substrates such as 2,2-dimethylGA$_4$ (**9**).[8] 1α-MethylGA$_4$ (**35**), unlike 1β-methylGA$_4$ (**44**), is blocked at the centre for both 1α-hydroxylation[26] and 1α,2α-didehydrogenation[27] which are normal metabolic steps from GA$_4$ (**5**) leading to GA$_{16}$ (**7**) and GA$_7$ (**54**) respectively. It was therefore expected that the only metabolic transformation available to 1α-methylGA$_4$ (**35**) was 13-hydroxylation, whereas 1β-methylGA$_4$ could undergo 1,2-dehydrogenation to the GA$_7$ and GA$_3$ derivatives (**51**) and (**52**). Indeed incubation of 1α-methylGA$_4$ (**35**) with the mutant B1-41a gave 1α-methylGA$_1$ (**42**) as the sole product in *ca.* 20% yield. Incubation of 1β-methylGA$_4$ (**44**) with the mutant B1-41a gave 1-methylGA$_3$ (**52**) as the major metabolite with minor amounts of 1-methylGA$_7$ (**51**) and 1β-methylGA$_1$ (**48**). This result is in agreement with the conclusion[27] that it is the 1α-hydrogen which is lost in the didehydrogenation of GA$_4$ to GA$_7$ in the fungus *Gibberella fujikuroi*.

The biological activities of the 1β-methylgibberellins (**44**) and (**48**), 1α-methylgibberellins (**35**) and (**42**) and 1α,2α-methylenegibberellins (**29**) and (**31**) will be described elsewhere.

Experimental

General experimental details have been described in a previous paper.[8]

ent-3α,10β,13-*Trihydroxy*-1α-*iodo*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**19**).—GA$_3$ methyl ester (**55**) (0.5 g) in methanol (30 ml) and piperidine (1 ml) were stirred with 10% palladium-on-calcium carbonate (30 mg) under an atmosphere of hydrogen for 0.5 h. The mixture was diluted with ethyl acetate and filtered and the solvent was removed under reduced pressure. Purification of the reaction mixture by flash chromatography with ethyl acetate–light petroleum–acetic acid (14:4:1) gave the hydrogenolysis product (**20**) (355 mg), m.p. 232—235 °C (lit.,[28] m.p. 236—238 °C); δ(C_5D_5N) 1.68 (s, 18-H$_3$), 3.70 (s, OMe), 4.53 (br s, 3-H), 5.07 (br s, 17-H), and 5.41 (br s, 17-H and 1-H); *m/z* 362 (*M*$^+$, 10%), 344 (22), 312 (33), 302 (91), 298 (43), 284 (85), 239 (100), 193 (24), 155 (27), 105 (24), and 91 (27).

The hydrogenolysis product (**20**) (350 mg) in tetrahydrofuran (10 ml) and dichloromethane (20 ml) was stirred vigorously with saturated aqueous sodium hydrogen carbonate (30 ml) and iodine (0.5 g) at room temperature for 1 h. The organic phase

was decanted off, diluted with dichloromethane (100 ml), washed with saturated aqueous sodium thiosulphate and then with water, and finally concentrated under reduced pressure. 1β-IodoGA$_1$ methyl ester (**19**) crystallised from acetone–light petroleum (348 mg), m.p. 214—215 °C (lit.,[13] m.p. 209—210 °C); δ(C_5D_5N) 1.52 (2, 18-H_3), 3.10 (d, *J* 10 Hz, 6-H), 3.62 (s, OMe), 4.26 (m, 3-H), 4.53 (d, *J* 10 Hz, 5-H), 4.76 (d, *J* = 4.5 Hz, 1-H), and 5.09 and 5.63 (2 br s, 17-H_2); *m*/*z* 488 (M^+, 49%), 456 (25), 429 (100), 361 (35), 343 (88), 329 (60), 301 (48), 283 (45), and 255 (22).

Treatment of 1β-*Iodo*GA$_1$ *Methyl Ester* (**19**) *with Lithium Dimethylcuprate*.—Copper(I) iodide powder (380 mg) in tetrahydrofuran (15 ml) was stirred with methyl-lithium (1.2M; 4.8 ml) under nitrogen. The solution was cooled to 0 °C and 1β-iodoGA$_1$ methyl ester (**19**) (250 mg) in tetrahydrofuran (5 ml) was added dropwise. Stirring was continued for 3 h and then the reaction mixture was worked up as usual to give a gum which crystallised from ethyl acetate–light petroleum yielding the hydrogenolysis product (**20**), m.p. 234—236 °C (lit.,[28] m.p. 236—238 °C) identical with that obtained in the previous experiment from the hydrogenation of GA$_3$ methyl ester (**55**) in the presence of piperidine.

Treatment of the Enone (**10**) *with Ethereal Diazomethane*.—(*a*) *In methanol*. The enone (**10**) (0.5 g) in methanol (30 ml) was treated with an excess of ethereal diazomethane for 0.5 h at room temperature. Removal of the solvent gave a yellow gum which was purified by flash chromatography. Elution with 35% ethyl acetate–light petroleum gave ent-4′,5′-*dihydro*-10β-*hydroxy*-3-*oxo*-1′H-20-*norgibberell*-16-*eno*[1,2-c]*pyrazole*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**23**) (365 mg) which crystallised from ethyl acetate–light petroleum, m.p. 179—181 °C (Found: C, 66.0; H, 6.3. $C_{21}H_{24}N_2O_5$ requires C, 65.6; H, 6.3%) $\nu_{max.}$(Nujol) 3 400, 1 790, 1 740, 1 710, and 1 660 cm^{-1}; δ 1.28 (s, 18-H_3), 2.85 (d, *J* 10 Hz, 6-H), 3.49 (d, *J* 10 Hz, 5-H), 3.60 (m, 5′-H_2), 3.74 (s, OMe), 3.91 (m, 1-H), 4.90 and 5.02 (2 × br s, 17-H_2), and 6.66 (br s, N-H); δ_C 10.40 (C-18), 16.58 (C-11), 31.20 (C-12), 37.05 (C-14), 38.46 (C-13), 44.37 (C-15), 51.84 (C-1), 52.33 (Me), 52.71 (C-5′), 52.87 (C-6 and C-8), 53.14 (C-9), 56.99 (C-5), 63.71 (C-4), 89.93 (C-10), 108.08 (C-17), 144.86 (C-2), 155.59 (C-16), 172.00 (C-7), 173.14 (C-19), 185.22 (C-3); *m*/*z* 384 (M^+, 100%), 356 (9), and 325 (18).

Further elution with 50% ethyl acetate–light petroleum gave the 7-*membered ring* A *pyrazoline* (**22**) which crystallised from ethyl acetate–light petroleum (104 mg), m.p. 192—194 °C (Found: C, 65.9; H, 6.5; N, 7.1. $C_{22}H_{26}N_2O_5$ requires C, 66.4; H, 6.5; N, 7.0%); δ 1.38 (s, 18-H_3), 2.68 (d, *J* 10 Hz, 6-H), 3.16 (d, *J* 10 Hz, 5-H), 3.64 (m, 3a-H_2), 3.76 (s, OMe), 4.70 (m, 5′-H_2), and 4.88 and 5.00 (2 × br s, 17-H_2); δ_C 11.60 (C-18), 16.43 (C-11), 31.21 (C-12), 37.07 (C-14), 38.66 (C-13), 44.40 (C-15), 44.95 (C-9), 49.84 (C-8), 51.61 (C-1), 51.73 (C-4), 52.14 (OMe), 53.87 (C-6), 55.70 (C-5), 58.57 (C-5′), 69.39 (C-3a), 88.69 (C-10), 107.81 (C-17), 136.76 (C-2), 155.82 (C-16), 159.36 (C-3), 172.43 (C-7), and 176.58 (C-19); *m*/*z* 398 (M^+, 18%), 382 (65), 368 (29), 348 (21), 336 (42), 276 (100), 261 (25), 233 (27), 145 (15), and 91 (27).

(*b*) *In acetone*. The enone (**10**) (700 mg) in acetone (50 ml) was treated with an excess of ethereal diazomethane for 0.2 h at room temperature. Removal of the solvent under reduced pressure gave a gum which crystallised from ethyl acetate–light petroleum to give the pyrazoline (**23**) (680 mg) identical with that obtained from the previous reaction.

ent-10β-*Hydroxy*-1-*methyl*-3-*oxo*-20-*norgibberell*-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**12**).—The pyrazoline (**23**) (600 mg) and powdered potassium dihydrogen orthophosphate (120 mg) were heated to 170 °C for 0.5 h. Work-up gave a yellow gum which was purified by flash chromatography. Elution with 20% ethyl acetate–light petroleum gave 1-*methyl*-3-*oxo*GA$_7$ *methyl ester* (**12**) (350 mg) which was crystallised 3 times from ethyl acetate–light petroleum, m.p. 145—146 °C (Found: C, 70.9; H, 6.7. $C_{21}H_{24}O_5$ requires C, 70.8; H, 6.7%); δ 1.27 (s, 18-H_3), 2.08 (d, *J* 1.5 Hz, 1′-H_3), 2.87 (d, *J* 10 Hz, 6-H), 3.33 (d, *J* 10 Hz, 5-H), 3.67 (s, Me), 4.82 and 4.96 (2 br s, 17-H_2), and 5.71 (d, *J* 1.5 Hz, 2-H); *m*/*z* 356 (M^+, 100%), 324 (95), 296 (24), 252 (13), 217 (15), 199 (28), 173 (28), 160 (36), and 91 (16).

Reduction of the 1-*Methyl-enone* (**12**).—(*a*) *With sodium borohydride–copper*(I) *chloride*. The 1-methyl enone (**12**) (300 mg) in methanol (30 ml) was stirred with copper(I) chloride (50 mg). Sodium borohydride (150 mg) was added and stirring continued for 1 h at room temperature. Work-up gave a gum (310 mg) which, by n.m.r. and g.l.c.–mass spectrometry on the trimethylsilyl ethers, was shown to contain *ca.* 30% of the 1-methyl-3-*epi*-GA$_7$ methyl ester (**50**). Hence the reaction mixture in acetone (50 ml) was treated dropwise with Jones reagent for 0.5 h at room temperature. Work-up gave a gum which was again reduced with sodium borohydride–copper(I) chloride. No unsaturated material was apparent by either n.m.r. or g.l.c.–mass spectrometry. Purification of the mixture by flash chromatography gave, with 30% ethyl acetate–light petroleum, ent-3α,10β-*dihydroxy*-1β-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**33**) (25 mg), m.p. 182—185 °C (from ethyl acetate–light petroleum) (Found: M^+, 360.1930. $C_{21}H_{28}O_5$ requires *M*, 360.1936); δ 0.96 (d, *J* 7 Hz, 1′-H_3), 1.12 (s, 18-H_3), 2.70 (d, *J* 11.5 Hz, 6-H), 3.21 (d, *J* 11.5 Hz, 5-H), 3.71 (s, OMe), 3.81 (d, *J* 2 Hz, 3-H), and 4.88 and 5.00 (2 × br s, 17-H_2); *m*/*z* 360 (M^+, 16%), 342 (4), 328 (60), 300 (76), 298 (100), 238 (84), 105 (15), and 91 (27).

Further elution with 35% ethyl acetate–light petroleum gave ent-3β,10β-*dihydroxy*-1β-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**34**) (172 mg) as a gum (Found: M^+, 360.1962. $C_{21}H_{28}O_5$ requires *M*, 360.1936); δ 1.00 (d, *J* 6.5 Hz, 1′-H_3), 1.15 (s, 18-H_3), 2.54 (d, *J* 10.5 Hz, 5-H), 2.78 (d, *J* 10.5 Hz, 6-H), 3.71 (s, OMe), and 4.85 and 4.97 (2 × br s, 17-H_2); *m*/*z* 360 (M^+, 26%), 342 (29), 328 (100), 314 (45), 300 (74), 272 (23) 256 (23), 228 (39), 183 (16), 127 (46), 105 (24), and 91 (42).

(*b*) *With L-Selectride*. The enone (**24**) (250 mg) in tetrahydrofuran (20 ml, freshly distilled) was cooled to −70 °C under nitrogen. L-Selectride (1M; 2 ml) was added dropwise and stirring continued for 0.3 h with warming to −30 °C. Work-up gave a gum which, when analysed by g.l.c.–mass spectrometry on the trimethylsilyl ethers, was shown to be a mixture (1:6:3) of (*a*) 1α-methylGA$_4$ methyl ester (**33**) [*m*/*z* 432 (17), 417 (6), 362 (13), 342 (26), 298 (66), 289 (43), 238 (40), 143 (100), 75 (59), and 73 (71)]; (*b*) 1α-methyl-3-*epi*-GA$_4$ methyl ester (**34**) [*m*/*z* 432 (3), 385 (11), 362 (10), 357 (9), 289 (40), 143 (100), 75 (34), and 73 (26)]; and (*c*) 1α-methyl-3-*epi*-GA$_7$ methyl ester (**50**) [*m*/*z* 430 (20), 398 (6), 386 (30), 325 (93), 311 (40), 236 (100), 75 (59), and 73 (90)].

ent-3α,10β-*Dihydroxy*-1β-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**35**).—Sodium hydride (60% dispersion in oil; 240 mg was washed with light petroleum. Freshly distilled hexamethylphosphoramide (5 ml) was added *via* a syringe under nitrogen. The flask was cooled in ice–water and propanethiol (0.7 ml) was added dropwise with stirring. The reagent was stirred for 1 h and then allowed to settle.

A portion (1.3 ml) of the supernatant sodium propanethiolate–hexamethylphosphoramide solution was added to 1α-methyl-GA$_4$ methyl ester (**33**) (20 mg) and the solution set aside for 4 h. Work-up gave a gum which on purification by flash chromatography with ethyl acetate–light petroleum–acetic acid (6:12:1) gave 1α-*methylGA*$_4$ (**35**) (12 mg), m.p. 254—256 °C

(from acetone–light petroleum) (Found: C, 69.9; H, 7.8. $C_{20}H_{26}O_5$ requires C, 69.4; H, 7.5%); δ[$(CD_3)_2CO$], 0.94 (d, *J* 6 Hz, 1′-H_3), 1.10 (s, 18-H_3), 2.60 (m, 6-H), 3.24 (d, *J* 11 Hz, 5-H), 3.68 (d, *J* 4 Hz, 3-H), and 4.86 and 4.99 (2 × br s, 17-H_2); δ(C_5D_5N), 0.93 (d, *J* 7 Hz, 1′-H_3), 1.67 (s, 18-H_3), 3.19 (d, *J* 11 Hz, 6-H), 3.91 (d, *J* 11 Hz, 5-H), 4.14 (m, 3-H), and 4.89 and 5.02 (br s, 17-H_2); *m/z* 328 (*M* − 18$^+$, 7%), 300 (9), 284 (91), 274 (11), 239 (15), 105 (18), 91 (21), and 44 (100).

Further elution with ethyl acetate–light petroleum–acetic acid (8:12:1) gave 1α-*methyl*-3-epi-*GA*$_4$ (**40**) (2 mg), m.p. 265—267 °C (from acetone–light petroleum) (Found: C, 69.7; H, 7.3. $C_{20}H_{26}O_5$ requires C, 69.4; H, 7.5%); δ[$(CD_3)_2CO$] 0.97 (d, *J* 6 Hz, 1′-H_3), 1.11 (s, 18-H_3), 2.52 (d, *J* 10.5 Hz, 5-H), 2.69 (d, *J* 10.5 Hz, 6-H), 3.70 (dd, *J* 11 and 6 Hz, 3-H), and 4.85 and 4.99 (2 × br s, 17-H_2); δ (C_5D_5N) 0.93 (d, *J* 7 Hz, 1′-H_3), 1.68 (s, 18-H_3), 2.95 (d, *J* 10 Hz, 5-H), 3.20 (d, *J* 10 Hz, 6-H), 4.05 (m, 3-H), and 4.88 and 4.99 (2 × br s, 17-H_2); *m/z* 346 (*M*$^+$, 34%), 328 (93), 310 (27), 300 (100), 284 (87), 274 (38), 256 (51), and 239 (31).

ent-10β-*Hydroxy*-1β-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**36**).—1α-Methyl-3-*epi*-GA_4 methyl ester (**34**) (150 mg) in pyridine (15 ml) was refluxed with phosphoryl chloride for 0.5 h. Work-up gave a gum, 1α-methyl-3β-chloroGA_9 methyl ester (**37**) (85 mg); δ 0.99 (d, *J* 6.5 Hz, 1′-H_3), 1.18 (s, 18-H_3), 2.73 (d, *J* 10.8 Hz, 6-H), 3.72 (s, OMe), 3.99 (d, *J* 10.8 Hz, 5-H), 4.10 (m, 3-H), and 4.88 and 5.00 (s × br s, 17-H_2).

Crude 1α-methyl-3β-chloroGA_9 methyl ester (**37**) (85 mg) in toluene (50 ml) was refluxed with tributyltin hydride (350 μl) in the presence of α-azoisobutyronitrile (3 mg) for 1 h. The solvent was removed under reduced pressure and purification of the product by flash chromatography eluting with 15% ethyl acetate–light petroleum gave 1α-methylGA_9 methyl ester (**38**) as a foam (52 mg); δ 0.97 (d, *J* 6 Hz, 1′-H_3), 1.06 (s, 18-H_3), 2.56 (d, *J* 11 Hz, 5-H), 2.72 (d, *J* 11 Hz, 6-H), 3.70 (s, OMe), and 4.86 and 4.99 (2 × br s, 17-H_2); *m/z* 344 (*M*$^+$, 18%), 312 (100), 284 (87), 257 (31), 240 (47), 173 (16), and 91 (31).

1α-MethylGA_9 methyl ester (**38**) (50 mg) was refluxed in aqueous sodium hydroxide (2M; 20 ml) and methanol (5 ml) for 6 h. Work-up and purification by flash chromatography eluting with ethyl acetate–light petroleum–acetic acid (1:10:1) gave 1α-methylGA_9 (**36**) (37 mg), m.p. 241—242 °C (from ethyl acetate–light petroleum) (Found: *M*$^+$, 330.1815. $C_{20}H_{26}O_4$ requires *M*, 330.1831); δ 0.98 (d, *J* 6 Hz, 1′-H_3), 1.12 (s, 18-H_3), 2.50 (d, *J* 10.5 Hz, 5-H), 2.75 (d, *J* 10.5 Hz, 6-H), and 4.88 and 5.01 (2 × br s, 17-H_2); *m/z* 330 (*M*$^+$, 15%), 312 (28), 286 (100), 284 (43), 243 (59), 241 (34), 217 (33), 173 (22), 105 (25), and 91 (45).

Reduction of the Enone (**10**) *with Tributyltin Hydride in the Presence of Tetrakistriphenylphosphinepalladium*(0).—1-MethylGA_7-3-ketone 7-methyl ester (**10**) (300 mg) in tetrahydrofuran (15 ml) was stirred with tetrakistriphenylphosphinepalladium(0) (40 mg) under nitrogen. Tributyltin hydride (270 μl) was added dropwise over 3 h with stirring. Work-up gave a black gum which was purified by flash chromatography. Elution with 10% ethyl acetate–light petroleum gave a gum (210 mg) which, by g.l.c.–mass spectrometry, was a 1:1 mixture of the 1β-methylGA_4 ketone methyl ester (**45**) [*m/z* 358 (*M*$^+$, 27%), 326 (100), 314 (50), 298 (42), 239 (32), 217 (62), and 160 (37)] and the 1α-methylGA_4 ketone methyl ester (**39**) [*m/z* 358 (*M*$^+$, 43%), 326 (100), 298 (63), 217 (60), and 160 (52)]. Further elution with 12% ethyl acetate–light petroleum gave ent-10β-*hydroxy*-1β,2β-*methylene*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**25**) (45 mg), m.p. 172—174 °C (from ethyl acetate–light petroleum) (Found: C, 70.8; H, 7.0. $C_{21}H_{24}O_5$ requires C, 70.8; H, 6.7%); δ 1.15 (s, 18-H_3), 2.75 (d, *J* 10 Hz, 6-H), 3.41 (d, *J* 10 Hz, 5-H), 3.72 (s, OMe), and 4.90 and 5.00 (2 × br s, 17-H_2); *m/z* 356 (*M*$^+$, 100%), 324 (49), 296 (34), 284 (68), 269 (37), 241 (40), 115 (25), and 91 (54).

1β-*MethylGA*$_4$ *ketone* (**45**) *and* 1α-*MethylGA*$_4$ *ketone* (**39**).—The 1:1 mixture (200 mg) of 1β-methylGA_4 ketone methyl ester (**45**) and 1α-methylGA_4 ketone methyl ester (**39**), obtained from the previous reaction, in methanol (25 ml) was stirred with sodium borohydride (100 mg) for 1 h at room temperature. Work-up gave a gum (200 mg) which was refluxed for 6 h in methanol (5 ml) and 2M-aqueous sodium hydroxide (25 ml). Work-up gave a product (160 mg) which, in acetone (20 ml), was treated dropwise with Jones reagent. Work-up gave a gum which was purified by flash chromatography. Elution with ethyl acetate–light petroleum–acetic acid (4:12:1) gave a gum (105 mg) which, by g.l.c.–mass spectrometry of the methyl esters, was a mixture of 1β-methylGA_4 ketone (**47**) [*m/z* 358 (*M*$^+$, 27%), 326 (100), 314 (52), 298 (44), 239 (32), 217 (64) and 160 (37)] and 1α-methylGA_4 ketone (**41**) [*m/z* 358 (*M*$^+$, 44%), 326 (100), 298 (61), 217 (66), and 160 (58)]. The ketones (**47**) and (**41**) were separated by h.p.l.c. on C_{18} reverse-phase Spherisorb ODS column (25 × 4.5 mm) using methanol–1% aqueous phosphoric acid (6:4) eluting at 2.5 ml min^{-1} and monitoring the u.v. absorption at 210 nm. The eluant was diluted with water, acidified to pH 2 with 2M-hydrochloric acid and extracted with ethyl acetate as usual to give: (*a*) ent-10β-*hydroxy*-1α-*methyl*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**47**) (45 mg), m.p. 227—228 °C (from acetone–light petroleum); *M*$^+$, 344.1618. $C_{20}H_{24}O_5$ requires *M*, 344.1624); δ[$(CD_3)_2CO$] 1.13 (s, 18-H_3), 1.15 (d, partially obscured by 18-H_3, 1′-H_3), 3.20 (d, *J* (10 Hz, 5-H), and 4.88 and 5.00 (2 × br s, 17-H_2); *m/z* 344 (100), 326 (25), 300 (92), 285 (21), 257 (22), 239 (22), 229 (37), 133 (24), 117 (22), 105 (36), and 91 (60); and (*b*) ent-10β-*hydroxy*-1β-*methyl*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**41**) (52 mg), m.p. 232—234 °C (from acetone–light petroleum) (Found: *M*$^+$, 344.1620. $C_{20}H_{24}O_5$ requires *M*, 344.1624); δ[$(CD_3)_2CO$] 1.01 (s, 18-H_3), 1.13 (d, partially obscured by 18-H_3, 1′-H_3), 3.26 (d, partially obscured, 6-H), 3.12 (d, *J* 10 Hz, 5-H), and 4.89 and 5.03 (2 × br s, 17-H_2); *m/z* 344 (*M*$^+$, 100%), 326 (33), 316 (15), 300 (42), 298 (25), 244 (25), 229 (25), 105 (27), and 91 (46).

ent-3α,10β-*Dihydroxy*-1α-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**44**).—1β-MethylGA_4 ketone (**47**) (30 mg) in tetrahydrofuran (15 ml) was stirred with potassium dihydrogen orthophosphate (3 mg) under nitrogen at −70 °C. K-Selectride (1M solution in tetrahydrofuran; 0.5 ml) was added dropwise and stirring was continued for 0.5 h. Work-up gave a gum which was purified by flash chromatography. Elution with ethyl acetate–light petroleum–acetic acid (6:12:1) gave 1β-*methylGA*$_4$ (**44**) (14 mg), m.p. 230—231 °C (from acetone–light petroleum) (Found: *M*$^+$, 346.1772. $C_{20}H_{26}O_4$ requires *M*, 346.1780); δ[$(CD_3)_2CO$], 1.12 (s, 18-H_3), 1.22 (d, *J* 7 Hz, 1′-H_3), 3.45 (d, *J* 10 Hz, 5-H), 3.75 (m, 3-H), and 4.87 and 4.98 (2 × br s, 17-H_2); *m/z* 346 (*M*$^+$, 1%), 328 (16), 300 (15), 284 (100), 256 (8), 239 (12), 184 (8), 183 (8), 119 (7), 105 (9), and 91 (16).

Further elution with ethyl acetate–light petroleum–acetic acid (8:12:1) gave ent-3β,10β-*dihydroxy*-1α-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**46**) (4 mg), m.p. 263—265 °C (from acetone–light petroleum) (Found: C, 68.9, H, 7.2. $C_{20}H_{26}O_5$ requires C, 69.4, H, 7.5%); δ(C_5D_5N) 1.06 (d, *J* 7 Hz, 1′-H_3), 1.72 (s, 18-H_3), 3.27 (br s, 5-H and 6-H), 4.20 (m, 3-H), and 4.92 and 5.01 (2 × br s, 17-H_2); *m/z* 346 (*M*$^+$, 21%), 328 (65), 300 (100), 295 (40), 284 (42), 256 (33), 239 (27), 228 (22), 127 (33), 105 (27), and 91 (48).

Reduction of 1α-*MethylGA*$_4$ *ketone* (**41**).—(*a*) *With sodium borohydride.* 1α-MethylGA$_4$ ketone (**41**) (25 mg) in methanol (10 ml) was stirred with sodium borohydride (15 mg) for 1 h at room temperature. Work-up gave a gum which was fractionated by flash chromatography. Elution with ethyl acetate–light petroleum–acetic acid (6:12:1) gave 1α-methylGA$_4$ (**35**) (3 mg), m.p. 255—257 °C, identical with that previously obtained.

Further elution with ethyl acetate–light petroleum–acetic acid (8:12:1) gave 1α-methyl-3-*epi*-GA$_4$ (**40**) (14 mg), m.p. 267—269 °C, identical with the sample previously obtained.

(*b*) *With K-Selectride.* 1α-MethylGA$_4$ ketone (**41**) (30 mg) in tetrahydrofuran (15 ml) was stirred with potassium dihydrogen orthophosphate (5 mg) under nitrogen at −70 °C. K-Selectride (1M solution in tetrahydrofuran; 0.5 ml) was added dropwise and stirring continued for a further 0.5 h. Work-up gave a gum which was purified by flash chromatography as described above to give 1α-methylGA$_4$ (**35**) (15 mg) and 1α-methyl-3-*epi*-GA$_4$ (**40**) (5 mg) identical with those previously prepared.

ent-3β,10β-*Dihydroxy*-1β,2β-*methylene*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**30**).—1α,2α-MethyleneGA$_4$-3-ketone-7-methyl ester (**25**) (40 mg) in methanol (10 ml) was stirred with sodium borohydride (20 mg) for 1 h at room temperature. Work-up gave a gum which was a single product by g.l.c.–mass spectrometry, 1α,2α-methylene-3-*epi*-GA$_4$ methyl ester (**26**) (38 mg); δ 0.6 and 0.92 (m, 2 × cyclopropyl H), 1.11 (s, 18-H$_3$), 2.64 (d, *J* 10.5 Hz, 5-H), 2.74 (d, *J* 10.5 Hz, 6-H), 3.71 (s, OMe), 4.18 (d, *J* 7 Hz, 3-H), and 4.87 and 4.99 (2 × br s, 17-H$_2$); *m*/*z* 358 (*M*$^+$, 23%), 326 (74), 298 (100), 284 (41), 243 (49), 197 (39), and 91 (49).

Sodium hydride (60% dispersion in oil; 240 mg) was washed with light petroleum. Freshly distilled hexamethylphosphoramide (5 ml) was added *via* a syringe under nitrogen. The flask was cooled in ice and propanethiol (0.7 ml) was added dropwise with stirring. The reagent was stirred for 1 h and then allowed to settle.

A portion (2.6 ml) of the supernatant sodium propanethiolate–hexamethylphosphoramide solution was added to the crude 1α,2α-methylene-3-*epi*-GA$_4$ methyl ester (**26**) (37 mg) and the solution was set aside for 4 h. Work-up gave a gum which on purification by flash chromatography with ethyl acetate–light petroleum–acetic acid (8:12:1) gave 1α,2α-*methylene*-3-epi-*GA*$_4$ (**30**) (29 mg) as a foam (Found: *M*$^+$, 344.1624. C$_{20}$H$_{24}$O$_5$ requires *M*, 344.1659); δ[(CD$_3$)$_2$CO] 0.6 and 0.95 (m, 2 × cyclopropyl H), 1.09 (s, 18-H$_3$), 2.58 (partially obscured doublet, 5-H), 2.73 (d, *J* 11 Hz, 6-H), 4.15 (*d*, *J* 7 Hz, 3-H), and 4.87 and 4.99 (2 × br s, 17-H$_2$); *m*/*z* 344 (*M*$^+$, 10%), 326 (13), 308 (5), 298 (52), 287 (28), 270 (25), 229 (27), 170 (19), 91 (33), and 28 (100).

ent-3α,10β-*Dihydroxy*-1β,2β-*methylene*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**29**)—1α,2α-Methylene-3-*epi*-GA$_4$ (**30**) (27 mg) in acetone (10 ml) was treated dropwise with Jones reagent for 1 h at room temperature. Work-up gave 1α,2α-*methyleneGA*$_4$-3-*ketone* (**28**) (26 mg) as a gum (Found: *M*$^+$, 342.1492. C$_{20}$H$_{22}$O$_5$ requires *M*, 342.1467); δ[(CD$_3$)$_2$CO] 1.11 (s, 18-H$_3$), 1.28 (m, 2 × cyclopropyl H), 2.72 (d, *J* 10 Hz, 6-H), 3.43 (d, *J* 10 Hz, 5-H), and 4.85 and 4.95 (2 × br s, 17-H$_2$); g.l.c.–mass spectrometry (methyl ester) 356 (*M*$^+$, 100%), 324 (50), 296 (32), 284 (60), 269 (42), 241 (40), 115 (25), and 91 (58).

1α,2α-MethyleneGA$_4$ ketone (**28**) (25 mg) in tetrahydrofuran (10 ml) was stirred with potassium dihydrogen orthophosphate (2 mg) under nitrogen with cooling to −70 °C. K-Selectride (1M solution in tetrahydrofuran; 0.5 ml) was added dropwise and stirring continued for a further 0.5 h. Work-up gave 2 products by t.l.c. which were separated by flash chromatography. Elution with ethyl acetate–light petroleum–acetic acid (6:12:1) gave 1α,2α-*methyleneGA*$_4$ (**29**) (5 mg) as a gum (Found: *M*$^+$, 344.1643. C$_{20}$H$_{24}$O$_5$ requires *M*, 344.1624); δ[(CD$_3$)$_2$CO] 0.50, 0.67, and 0.88 (m, 2 × cyclopropyl H), 1.09 (s, 18-H$_3$), 2.55 (d, *J* 11 Hz, 6-H), 3.07 (d, *J* 11 Hz, 5-H), 3.77 (s, 3-H), and 4.87 and 4.95 (2 × br s, 17-H$_2$); *m*/*z* 344 (*M*$^+$, 100%), 326 (35), 300 (55), 257 (32), 229 (61), 173 (29), and 91 (98). Further elution with ethyl acetate–light petroleum–acetic acid (8:12:1) gave 1α,2α-methylene-3-*epi*-GA$_4$ (**30**) (12 mg) as a foam, identical by n.m.r. and m.s. to the sample previously obtained.

ent-4′,5′-*Dihydro*-10β,13-*dihydroxy*-3-*oxo*-1′H-20-*norgibberell*-16-*eno*[1,2-c]*pyrazole*-7,19-*dioic Acid* 7-*Phenacyl Ester* 19,10-*Lactone*.—Gibberellin A$_3$ (**56**) (1 g) and potassium hydrogen carbonate (600 mg) in acetonitrile (25 ml) were refluxed with 1-bromoacetophenone (700 mg) and 18-crown-6-ether (70 mg). Work-up gave a gum (980 mg) which was stirred in acetone (50 ml) with activated manganese dioxide (5 g) for 4 h at room temperature. The reaction mixture was diluted with acetone (200 ml) and filtered through Celite. Removal of the solvent under reduced pressure gave a gum which was fractionated by flash chromatography. Elution with 40% ethyl acetate–light petroleum gave *GA*$_3$-3-*ketone*-7-*phenacyl ester* (**14**) (930 mg), m.p. 94—96 °C (from ethyl acetate–light petroleum) (Found: *M*$^+$, 462.1670. C$_{27}$H$_{20}$O$_7$ requires *M*, 462.1678); δ 1.36 (s, 18-H$_3$), 3.08 (d, *J* 10 Hz, 6-H), 3.54 (d, *J* 10 Hz, 5-H), 5.02 and 5.34 (2 × br s, 17-H$_2$), 5.41 (m, C*H*$_2$COPh), 6.05 (d, *J* 8 Hz, 2-H), 7.35 (d, *J* 8 Hz, 1-H), and 7.58 and 7.90 (m, CH$_2$CO*Ph*); *m*/*z* 462 (*M*$^+$, 9%), 343 (6), 326 (9), 299 (100), 253 (29), 105 (64), and 91 (26).

GA$_3$-3-ketone-7-phenacyl ester (**14**) (900 mg) in acetone (25 ml) was treated with ethereal diazomethane for 0.5 h at room temperature. The solvent was removed under reduced pressure to give a gum which was purified by flash chromatography. Elution with 75% ethyl acetate–light petroleum gave the GA$_3$-[1,2-c]*pyrazole ketone*-7-*phenacyl ester* (795 mg), m.p. 145—147 °C (from ethyl acetate–light petroleum) (Found: *M*$^+$ − 28, 476.1852. C$_{28}$H$_{28}$N$_2$O$_7$ requires *M* − 28, 476.1835; δ 1.34 (s, 18-H$_3$), 3.00 (d, *J* 10 Hz, 6-H), 3.57 (d, *J* 10 Hz, 5-H), 3.60 and 3.90 (m, 5′-H$_2$ and 1-H), 5.00 and 5.29 (2 × br s, 17-H$_2$), 5.42 (m, C*H*$_2$COPh), 7.03 (br s, NH), and 7.60 (m, CH$_2$CO*Ph*); *m*/*z* 476 (*M*$^+$ − 28, 6%), 313 (59), 268 (22), 105 (37), 91 (14), 77 (18), and 43 (100).

ent-10β,13-*Dihydroxy*-1-*methyl*-3-*oxo*-20-*norgibberella*-1,16-*diene* 7-*Phenacyl Ester* 19,10-*Lactone* (**13**).—The pyrazoline (**24**) (790 mg) and potassium dihydrogen orthophosphate (300 mg) were heated to 170 °C under nitrogen for 0.5 h. Flash chromatography of the product eluting with 65% ethyl acetate–light petroleum gave 1-*methylGA*$_3$-3-*ketone*-7-*phenacyl ester* (**13**) (380 mg), m.p. 115—117 °C) (3 times from ethyl acetate–light petroleum) (Found: *M*$^+$, 476.1851. C$_{28}$H$_{28}$O$_7$ requires *M*, 476.1835; δ 1.35 (s, 18-H$_3$), 2.13 (d, *J* 1.5 Hz, 1′-H$_3$), 3.10 (d, *J* 11 Hz, 6-H), 3.55 (d, *J* 11 Hz, 5-H), 5.03 and 5.37 (2 × br s, 17-H$_2$), 5.40 (m, CH$_2$COPh), 5.82 (d, *J* 1.5 Hz, 2-H), and 7.45 (m, CH$_2$COPh); *m*/*z* 476 (*M*$^+$, 8%), 457 (4), 313 (100), 268 (40), 120 (13), 105 (51), and 91 (21).

Reduction of the Crude Thermolysis Product with Tributyltin Hydride in the Presence of Tetrakistriphenylphosphinepalladium(0).—Crude 1-methyl 3-oxoGA$_3$ 7-phenacyl ester (**13**) (300 mg) in tetrahydrofuran (15 ml) was stirred with tetrakistriphenylphosphinepalladium(0) (40 mg) under nitrogen. Tri-n-butyltin hydride (300 μl) was added dropwise over 3 h with stirring. Work-up gave a black gum which was purified by flash chromatography. Elution with 65% ethyl acetate–light petroleum gave a mixture (270 mg) which, in glacial acetic acid (30 ml), was stirred with freshly activated zinc dust for 4 h at room temperature. The mixture was diluted with ethyl acetate and filtered. The filtrate was concentrated under

reduced pressure and purified by flash chromatography. Elution with ethyl acetate–light petroleum–acetic acid (10:10:1) gave a mixture of 1β-methylGA$_1$ ketone (**49**) and 1α-methylGA$_1$ ketone (**43**) (80 mg). Further elution with ethyl acetate–light petroleum–acetic acid (11:9:1) gave ent-10β,13-*dihydroxy*-1β,2β-*methylene*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**32**) (24 mg) as a gum (Found: M^+, 358.1420. $C_{20}H_{22}O_6$ requires M, 358.1416); δ[$(CD_3)_2CO$] 1.11 (s, 18-H_3), 2.72 (d, J, 10 Hz, 6-H), 3.45 (d, J 10 Hz, 5-H), 4.92 and 5.24 (2 × br s, 17-H_2); m/z 358 (M^+, 91%), 340 (35), 330 (21), 313 (34), 270 (41), 255 (26), 231 (34), 214 (39), 136 (93), 121 (54), and 55 (100).

The ketones (**49**) and (**43**) were separated by h.p.l.c. on a C_{18} reverse-phase Spherisorb ODS column (25 × 4.5 mm) using methanol–1% aqueous phosphoric acid (1:1) eluting at 2.5 ml min^{-1} and monitoring the u.v. absorption at 210 nm. The eluant was diluted with water, acidified to pH 2 with 2M-hydrochloric acid and extracted with ethyl acetate to give: (i) ent-10β,13-*dihydroxy*-1α-*methyl*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**49**) (32 mg) as a gum (Found: M^+, 360.1582. $C_{20}H_{24}O_6$ requires M, 360.1573); δ[$(CD_3)_2CO$] 1.15 (s, 18-H_3), 1.45 (d, J 7 Hz, 1′-H_3), 2.74 (d, J 8 Hz, 6-H), 3.22 (d, J 8 Hz, 5-H), and 4.91 and 5.19 (2 × br s, 17-H_2); m/z 360 (M^+, 47%), 342 (100), 315 (27), 289 (22), 245 (24), 216 (28), 163 (32), 149 (35), 135 (57), and 121 (22); and (ii) ent-10β,13-*dihydroxy*-1β-*methyl*-3-*oxo*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**43**) (28 mg), m.p. 221—223 °C (from acetone–light petroleum) (Found: M^+, 360.1584. $C_{20}H_{24}O_6$ requires M, 360.1573); δ[$(CD)_3)_2CO$] 1.08 (s, 18-H_3), 1.12 (d, J 6.5 Hz, 1′-H_3), 2.81 (d, J 11 Hz, 6-H), 3.15 (d, J 11 Hz, 5-H), and 4.90 and 5.25 (2 × br s, 17-H_2); m/z 360 (M^+ 59%), 342 (73), 290 (82), 289 (76), 231 (37), 216 (40), 163 (100), and 135 (41).

ent-3α,10β,13-*Trihydroxy*-1α-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**48**).—1β-MethylGA$_4$ ketone (**49**) (25 mg) in methanol (10 ml) was stirred with sodium borohydride (15 mg) for 1 h at room temperature. Work-up gave a gum which on purification by flash chromatography and eluting with ethyl acetate–light petroleum–acetic acid (12:8:1) gave 1β-*methylGA*$_1$ (**48**) (8 mg), m.p. 171—172 °C (from acetone–light petroleum) (Found: M^+, 362.1727. $C_{20}H_{26}O_6$ requires M, 362.1729); δ$(CD_3)_2CO$ 1.13 (s, 18-H_3), 1.21 (d, J 7 Hz, 1′-H_3), 2.60 (d, J 10 Hz, 6-H), 3.48 (d, J 10 Hz, 5-H), 3.78 (m, 3-H), and 4.92 and 5.21 (2 × br s, 17-H_2); m/z 362 (M^+, 19%), 344 (100), 317 (18), 316 (16), 298 (21), 135 (26), 105 (13), and 91 (23).

ent-3α,10β,13-*Trihydroxy*-1β,2β-*methylene*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**31**).—1α,2α-MethyleneGA$_1$-3-ketone (**32**) (15 mg) in tetrahydrofuran (5 ml) was stirred with potassium dihydrogen orthophosphate (3 mg) under nitrogen at −70 °C. K-Selectride (150 μl, 1M solution in tetrahydrofuran) was added dropwise and stirring was continued for a further 0.5 h. Work-up gave a gum which was purified by h.p.l.c. on C_{18} reverse-phase Spherisorb ODS column (25 × 4.5 mm) as previously described eluting with methanol–1% phosphoric acid (4:6) to give 1α,2α-*methyleneGA*$_1$ (**31**) (3 mg) as a gum (Found: M^+, 360.1614. $C_{20}H_{24}O_6$ requires M, 360.1573); δ[$(CD_3)_2CO$] 0.52 and 0.71 (m, 2 × cyclopropyl H), 1.09 (s, 18-H_3), 2.35 (d, J 10 Hz, 6-H), 3.09 (d, J 10 Hz, 5-H), 3.77 (s, 3-H), and 4.90 and 5.20 (2 × br s, 17-H_2); m/z [Me ester, $(Me_3Si)_2$ ether], 518 (M^+, 85%), 503 (7), 309 (9), 238 (6), 208 (16), 193 (10), 129 (20), 75 (100), and 73 (74).

ent-3α,10β,13-*Trihydroxy*-1β-*methyl*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**42**).—1α-MethylGA$_1$ ketone (**43**) (25 mg) in tetrahydrofuran (5 ml) was stirred with potassium dihydrogen orthophosphate (4 mg) under nitrogen at −70 °C. K-Selectride (200 μl, 1M solution in tetrahydrofuran) was added dropwise and stirring was continued for a further 0.5 h. Work-up gave a gum which was purified by flash chromatography. Elution with ethyl acetate–light petroleum–acetic acid (12:8:1) gave 1α-*methylGA*$_1$ (7 mg), m.p. 181—183 °C (from acetone–light petroleum) (Found: M^+, 362.1760. $C_{20}H_{26}O_6$ requires M, 362.1729); δ[$(CD_3)_2CO$] 0.94 (d, J 6 Hz, 1α′-H_3), 1.09 (s, 18-H_3), 2.60 (d, J 11 Hz, 6-H), 3.26 (d, J 11 Hz, 5-H), 3.76 (m, 3-H), and 4.87 and 5.22 (2 × br s, 17-H_2); m/z (Me ester, Me_3Si ether) 520, 505, 448, 376, 375, 235, 207, 193, 143, and 73.

Microbiological Conversion of 1α-*MethylGA*$_4$ (**35**) *into* 1α-*MethylGA*$_1$ (**42**).—A conical flask (1 l) containing 40% I.C.I. solution (500 ml) was inoculated with *Gibberella fujikuroi* mutant B1-41a culture (5 ml) and maintained under the usual conditions[29] for 6 days. The mycelium was obtained by filtration under sterile conditions and resuspended in 0% I.C.I. solution[25] (500 ml) containing 1α-methylGA$_4$ (**35**) (15 mg) which had been added in acetone (0.5 ml). The culture was maintained for 8 days and then filtered and washed with water. The filtrate was acidified to pH 2 with 2M-hydrochloric acid and extracted with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and then with water. The aqueous phase was acidified to pH 2 and then extracted with ethyl acetate. The extract was washed with water and concentrated under reduced pressure. The acidic extract was purified by flash chromatography with ethyl acetate–light petroleum–acetic acid (10:10:1) to give crude 1α-methylGA$_1$ (**4**) which was further purified by h.p.l.c. on a C_{18} reverse-phase Spherisorb ODS column (25 × 4.5 mm) using methanol–1% aqueous phosphoric acid (1:1) eluting at 1 ml min^{-1} and monitoring the u.v. absorption at 210 nm. The eluant containing 1α-methylGA$_1$ (**4**) was diluted with water, acidified to pH 2 with 2M-hydrochloric acid, and extracted with ethyl acetate as usual to give 1α-methylGA$_1$ (**42**) (3 mg), m.p. 181—183 °C identical by n.mr. and m.s. to the sample previously obtained.

Incubation of 1β-*MethylGA*$_4$ (**44**) *with* Gibberella fujikuroi *Mutant B*1-41*a*.—A conical flask (250 ml) containing 40% I.C.I. solution (50 ml) was inoculated with *Gibberella fujikuroi* mutant B1-41a culture (1 ml) and maintained under the usual conditions[29] for 6 days. The mycelium was obtained by filtration under sterile conditions and resuspended in 0% I.C.I. solution[25] (50 ml) containing 1β-methylGA$_4$ (**44**) (1 mg) which had been added in methanol (0.5 ml). The culture was maintained for 8 days and then filtered and washed with water. The filtrate was worked up as described in the previous experiment. The crude product was methylated and trimethylsilylated then analysed by g.l.c.–mass spectrometry. The major product was 1-methylGA$_3$ (**32**) [m/z 518 (M^+, 74%), 503 (6), 459 (2), 401 (4), 379 (6), 207 (23), 180 (10), 167 (9), 75 (100), and 73 (71)], and minor amounts of 1-methylGA$_7$ (**51**) [m/z 430 (M^+, 3%), 398 (19), 360 (12), 332 (30), 216 (20), 159 (19), 117 (21), 97 (20), 75 (64), and 44 (100)], and 1β-methylGA$_1$ (**48**) [m/z 520 (M^+, 67%), 448 (49), 376 (36), 375 (23), 207 (35), 157 (7), 143 (25), 75 (100), and 73 (58)].

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