Partial Syntheses of Gibberellins A_{45} and A_{63}

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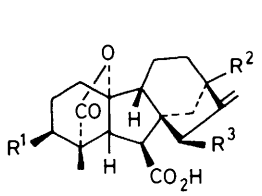
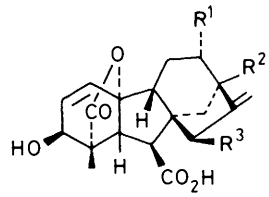
The structures of the higher plant 15β-hydroxy-C_{19}-gibberellins, GA_{45} and GA_{63}, have been established by rational syntheses from gibberellins A_9 and A_4 respectively.

Several 15β-hydroxylated gibberellins (GAs) have been detected in plant extracts. Only two 15β-hydroxy-C_{19} compounds have been previously characterised and therefore allocated GA numbers.[1] These are GA_{45} (**2**), first identified in immature seeds of pear[2,3] (*Pyrus communis*), and GA_{32} (**8**) from immature seeds of *Prunus persica*[4] and *Prunus armeniaca*.[5] Investigation of the biological properties of 15β-hydroxygibberellins has been hindered by the inaccessibility of these gibberellins from their natural sources. Some 15β-hydroxygibberellins have been available through microbiological transformations of 15β-hydroxykaurenoic acid (**9**) by cultures of *Gibberella fujikuroi*.[6,7] A general synthetic method for the introduction of the 15β-hydroxy group into the available fungal gibberellins GA_4 (**3**), GA_7 (**6**), GA_1 (**5**), and GA_3 (**7**) was sought to give a range of 15β-hydroxyGAs for biological evaluation and to confirm their structures.

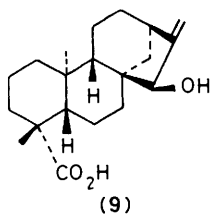
This paper describes the partial syntheses of two gibberellins, GA_{45} (**2**) and 15β-hydroxyGA_4 (**4**). Both have been previously identified in plant extracts (*Pyrus communis*) and the latter is now assigned the gibberellin A number GA_{63}.



	R^1	R^2	R^3
(1)	H	H	H
(2)	H	H	OH
(3)	OH	H	H
(4)	OH	H	OH
(5)	OH	OH	H



	R^1	R^2	R^3
(6)	H	H	H
(7)	H	OH	H
(8)	OH	OH	OH



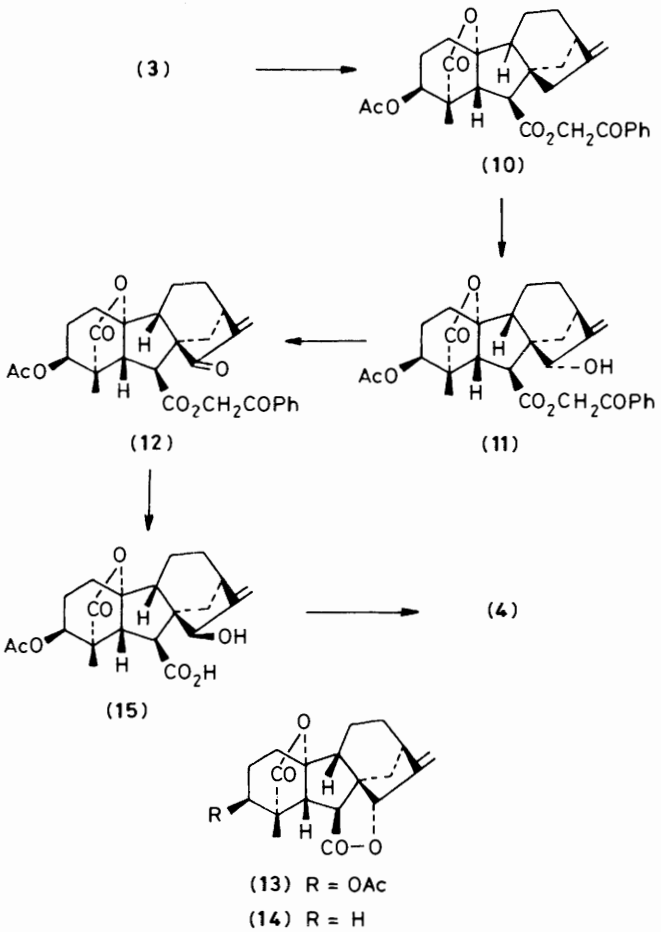
(9)

Results and Discussion

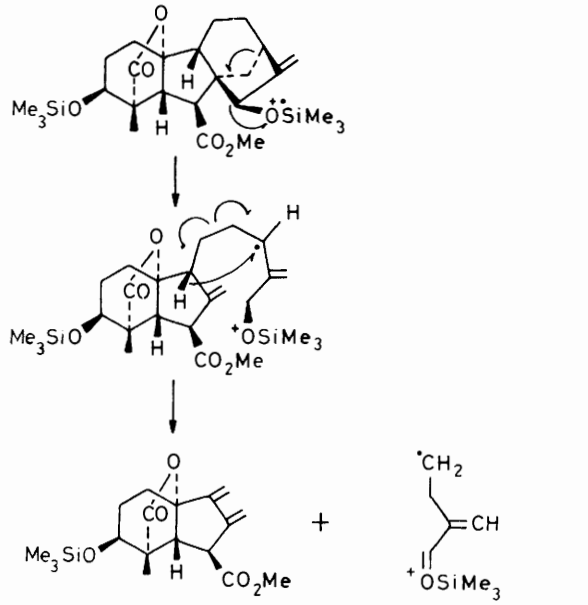
The successful routes to GA_{45} (**2**) and GA_{63} (**4**) are illustrated in Scheme 1 for GA_{63} (**4**). In early work towards the development of a general route to these compounds, no direct means of introducing a 15β-hydroxy group was found. Allylic oxidation at C-15 with selenium dioxide gave the α-alcohol. This reaction has been modified and found to be a convenient means for introducing a 15α-hydroxy group. This reaction requires protection of the 7-CO_2H to prevent the formation of a 7,15α-lactone. Initially the methyl ester was used. The approach to the inversion of stereochemistry at C-15 has centred on oxidation and reduction. Many attempts were made to oxidise the 15α-hydroxy group to an enone. The most successful reagents were found to be activated dimethyl sulphoxide complexes.[8] These reactions are specific to alcohols and avoid oxidation of the adjacent double bond which occurs with many chromium reagents. Initially, the stereospecific reduction of these 15-oxogibberellins concentrated on the use of sodium borohydride in the presence of Ce^{III}, shown by Luche *et al.*[9,10] to give selective 1,2-reductions of conjugated ketones. However, optimisation of conditions still gave complex mixture of 1,2- and 1,4-reduced products as well as reduction of the C-7 methyl ester. Subsequent attempts to hydrolyse the methyl ester, before reduction, using thiolate,[11] butoxide,[12] and hydroxide anions were low yielding and were complicated by facile conjugate-addition of nucleophiles to the D ring enone system. Recourse to the use of phenacyl esters which are easily prepared and readily cleaved by treatment with zinc in acetic acid[13,14] provided a successful route to GA_{45} and 15β-hydroxyGA_4 (GA_{63}), as illustrated in Scheme 1.

Thus GA_4 (**3**) was alkylated using phenacyl bromide, potassium hydrogen carbonate, and 18-crown-6 ether in acetonitrile and acetylated with acetic anhydride in pyridine to give the ester (**10**). The 15α-hydroxy group was successfully introduced into the GA_4 phenacyl ester 3-acetate (**10**) using selenium dioxide and t-butyl hydroperoxide.[15] The product, the 15α-alcohol (**11**), readily cyclised on standing in solution to give the 7,15α-lactone (**13**), the formation of which was minimised by immediate submission of the 15α-alcohol (**11**) to Swern oxidation.[8] The desired ketone (**12**) was obtained in reproducible and high yields. Reductive cleavage of the phenacyl group with zinc in acetic acid gave a product (**15**) with a ^1H n.m.r. spectrum which not only confirmed the cleavage of the phenacyl group but also revealed a broad singlet (δ 4.20) consistent with a CHOH group. Combined g.l.c.–mass spectroscopy on the deacetylated (potassium carbonate, methanol) product, as its methyl ester trimethylsilyl ether derivative, indicated a dihydroxy compound with an ion at *m/z* 156 characteristic of the formation of a 15-trimethylsilyloxyGA, as illustrated in Scheme 2. Both the 15α- and 15β-epimers gave almost identical mass spectra as methyl ester trimethylsilyl ethers. However, their g.l.c. retention times were different. They also have different n.m.r.* spectra (see Table). Reduction with zinc in acetic acid provided a new route to the 15β-hydroxylated compound, the proton being introduced from the less hindered α-face. Careful analysis of the product ratios at regular time intervals throughout the course of this reaction showed that the rate of reduction of the 15-ketone was faster than the cleavage of the phenacyl ester. The structure (**16**) for a minor byproduct was supported by the hydrogenation of the α,β-unsaturated ketone (**18**) to give a single g.l.c. component which cochromatographed with the byproduct (**16**) as its methyl ester on g.l.c. (OV210).

Although the mass spectra of the methyl ester trimethylsilyl



Scheme 1. *Reagents*: i, $PhCOCH_2Br$, 18-crown-6 ether, $KHCO_3$, CH_3CN; ii, Ac_2O–pyridine; iii, SeO_2–Bu^tOOH; iv, $(COCl)_2$–DMSO, Pr^i_2EtN; v, Zn–AcOH; vi, K_2CO_3–MeOH



Scheme 2. (After H. Obermann and G. Spitteller, *Chem. Ber.*, 1976, **104**, 3450)

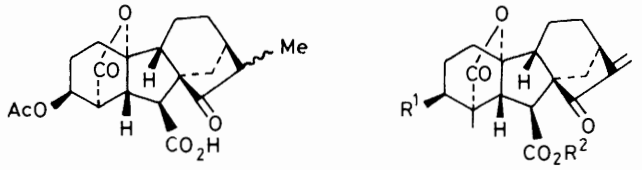
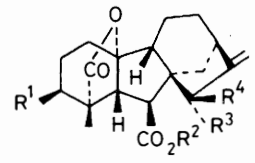


Table N.m.r. data of 15- and 17-H_2 protons for some 15-oxygenated gibberellins in $CDCl_3$

Compounds	15-H	17-H_2
(**19**)	4.08	5.18 and 5.27
(**20**)	3.97	5.10 and 5.13
(**13**)	4.59	5.19 and 5.31
(**14**)	4.54	5.17 and 5.30



	R^1	R^2	R^3	R^4
(**19**)	OAc	Me	OH	H
(**20**)	OAc	Me	H	OH
(**21**)	H	CH_2COPh	H	H
(**22**)	H	CH_2COPh	OH	H

ethers of 15α- and 15β-hydroxyGA_4 are almost identical, their g.l.c. retention times, underivatised mass spectra, n.m.r. spectra and stabilities are quite different. 15β-HydroxyGA_4 (**4**) was assigned gibberellin number GA_{63}, and is identical with a putative gibberellin detected in pear seeds and with a product of the metabolism of *ent*-15α-hydroxykaurenoic acid[16] (**9**) by cultures of *Gibberella fujikuroi*.[6,7]

The synthetic sequence shown in Scheme 1 was also used to prepare GA_{45} (**2**) from GA_9 (**1**), one of the gibberellins detected in seeds of apple and pear.[2,3] The biological activities of the 15β-hydroxygibberellins are currently being investigated.

Experimental

General experimental details have been described in a previous paper.[17]

Phenacylation and Acetylation of ent-3α,10-*Dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**3**).—Gibberellin A_4 (**3**) (500 mg) was added to a solution of phenacyl bromide (200 mg), 18-crown-6 ether (50 mg), and potassium hydrogen carbonate (400 mg) in acetonitrile (50 ml) and heated under reflux for 1 h. The reaction mixture was diluted with water (50 ml), and acidified to pH 3, extracted with ethyl acetate (3 × 50 ml), and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave the phenacyl ester. The product was treated with acetic anhydride (1 ml) in pyridine (5 ml) and stirred for 2 h. The reaction mixture was transferred into water (50 ml), acidified to pH 3 with hydrochloric acid and extracted with ethyl acetate (3 × 50 ml). The combined organic layers were evaporated under reduced pressure with the addition of toluene to assist the removal of the residual pyridine and acetic acid by azeotropic distillation. The ester (**10**) (500 mg) was obtained as a gum; δ[$(CD_3)_2CO$], 1.13 (s, 18-H_3), 2.20 (s,

OCOMe), 2.79 (d, *J* 11 Hz, 6-H), 3.23 (d, *J* 11 Hz, 5-H), 4.88 (br s, 3-H), 4.88 and 5.0 (each br s, 17-H_2), 5.57 (m, CO_2CH_2CO), and 7.6 and 8.0 (complex m, aromatic); *m/z* 492 (M^+, 35%), 474 (3), 432 (10), 388 (18), 356 (16), 296 (22), 269 (100), 224 (70), 120 (13), 105 (40), and 91 (12).

Phenacylation of ent-10-*Hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (**1**).—Gibberellin A_9 (**1**) (500 mg) was added to a solution of phenacyl bromide (230 mg), 18-crown-6 ether (50 mg), and potassium hydrogen carbonate (400 mg) in acetonitrile (50 ml) and heated under reflux for 1 h. The reaction mixture was extracted as described above and gave the *ester* (**21**) (450 mg) as a gum (Found: M^+, 434.2116. $C_{27}H_{30}O_5$ requires M^+, 434.2093); δ($CDCl_3$) 1.28 (s, 18-H_3), 2.59 (d, *J* 10.5 Hz, 5-H), 2.91 (d, *J* 10.5 Hz, 6-H), 4.88 and 4.99 (each br s, 17-H_2), 5.37 (m, CO_2CH_2CO), and 7.50 and 7.90 (complex m, aromatic); *m/z* 434 (M^+, 97%), 417 (1), 416 (4), 298 (47), 270 (100), 242 (21), 225 (23), 120 (27), 105 (69), and 91 (44).

General Procedure for the Formation of 15α-*Alcohols.*—Compounds (**10**) and (**21**) (400 mg) in dichloromethane (2 ml) were added to a solution of selenium dioxide (80 mg) and t-butyl hydroperoxide (320 μl) in dichloromethane (10 ml). The mixture was stirred at room temperature for 2 h. Examination by t.l.c. showed complete conversion into a more polar product. The reaction mixture was washed with water, the pH was adjusted to 3.0 with hydrochloric acid, and the solution dried (Na_2SO_4). The solvent was removed to give: from compound (**21**), 15α-*hydroxyGA*$_9$ 7-*phenacyl ester* (**22**); δ($CDCl_3$) 1.22 (s, 18-H_3), 2.58 (d, *J* 9.8 Hz, 5-H), 2.81 (d, *J* 9.8 Hz, 6-H), 4.09 (br s, 15-H), 5.18 and 5.29 (each br s, 17-H_2), 5.27 (d, *J* 16.6 Hz, CO_2CH_2CO), 5.44 (d, *J* 16.6 Hz, CO_2CH_2CO), and 7.60 and 7.90 (complex m, aromatic); and, from compound (**10**), 15α-*hydroxyGA*$_4$ 3-*acetate* 7-*phenacyl ester* (**11**); δ($CDCl_3$) 1.20 (s, 18-H_3), 2.17 (s, OCOMe), 2.78 (d, *J* 10 Hz, 6-H), 3.25 (d, *J* 10 Hz, 5-H), 4.13 (br s, 15-H), 5.01 (br s, 3-H), 5.19 and 5.30 (each br s, 17-H_2), 5.28 (d, *J* 16.6 Hz, CO_2CH_2CO), 5.44 (d, *J* 16.6 Hz, CO_2CH_2CO), and 7.60 and 7.90 (complex m, aromatic). Both compounds readily lactonised on standing to give compounds (**14**) and (**13**) respectively, and therefore could not be analysed fully.

Swern Oxidation of Allylic Alcohols.—Oxalyl chloride (78 μl) was added to dry dichloromethane (20 ml) in a dry flask under nitrogen and cooled to −72 °C. After 10 min, dimethyl sulphoxide was added and allowed to react for 3 min before the addition of the 15α-alcohol (200 mg) in dichloromethane (2 ml). The reaction mixture was stirred at −72 °C for 45 min and at −60 °C for a further 15 min. Di-isopropylethylamine (1.0 ml) was added dropwise and the reaction mixture was then allowed to warm to ambient temperature. The solvent was removed under reduced pressure and the products separated by flash column chromatography [18] eluting with 20—50% ethyl acetate in light petroleum.

(*a*) Oxidation of 15α-*hydroxyGA*$_9$ 7-*phenacyl ester* (**22**). The first compound eluted was ent-10-*hydroxy*-20-*nor*-15-*oxogibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*phenacyl ester* (**17**) (100 mg) obtained as a gum (Found: M^+, 448.1875. $C_{27}H_{28}O_6$ requires M^+, 448.1886); δ[$(CD_3)_2CO$] 1.22 (s, 18-H_3), 2.72 (d, *J* 10.3 Hz, 5-H), 2.87 (d, *J* 10.3 Hz, 6-H), 5.05 (d, *J* 16.6 Hz, CO_2CH_2CO), 5.39 and 5.93 (each br s, 17-H_2), 5.51 (d, *J* 16.6 Hz, CO_2CH_2CO), and 7.50 and 7.90 (complex m, aromatic); *m/z* 448 (M^+, 2%), 420 (1), 349 (2), 329 (60), 313 (43), 239 (27), 105 (100), 91 (26), and 77 (30).

The second compound eluted, *GA*$_9$ 7,15α-*lactone* (**14**) (50 mg), was crystallised from acetone–light petroleum as needles m.p. 218—220 °C (Found: M^+, 314.1516. $C_{19}H_{22}O_4$ requires M^+, 314.1518); $\nu_{max.}$($CHCl_3$) 3 050, 2 950, 1 780, and 880 cm^{-1}; δ($CDCl_3$) 1.36 (s, 18-H_3), 2.30 (d, *J* 8.5 Hz, 5-H), 2.66(d, *J* 8.5 Hz, 6-H), 4.54 (br s, 15-H) and 5.17 and 5.30 (each br s, 17-H_2); *m/z* 314 (M^+, 65%), 270 (17), 242 (11), 226 (100), 214 (15), 211 (52), 183 (28), 169 (23), 129 (23), and 91 (40).

(*b*) *Oxidation of* 15α-*hydroxyGA*$_4$ 3-*acetate* 7-*phenacyl ester* (**11**). The first compound eluted was ent-3α-*acetoxy*-10-*hydroxy*-20-*nor*-15-*oxogibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*phenacyl ester* (**12**) (103 mg), obtained as a gum (Found: M^+, 506.1960. $C_{29}H_{30}O_8$ requires M^+, 506.1940); δ($CDCl_3$) 1.21 (s, 18-H_3), 2.14 (s, OCOMe), 2.86 (d, *J* 11 Hz, 6-H), 3.36 (d, *J* 11 Hz, 5-H), 5.01 (br s, 3-H), 5.06 (d, *J* 16.6 Hz, CO_2CH_2CO), 5.01 and 5.96 (each s, 17-H_2), 5.53 (d, *J* 16.6 Hz, CO_2CH_2CO), and 7.50 and 7.90 (complex m, aromatic); *m/z* 506 (M^+, 2%), 492 (1.5), 402 (3), 387 (24), 372 (100), 268 (31), 224 (28), 105 (62), 91 (22), and 77 (25).

The second compound eluted was GA_4 7,15α-*lactone* 3-*acetate* (**13**) (50 mg) obtained as a gum (Found: M^+, 372.1569. $C_{21}H_{24}O_6$ requires M^+, 372.1573); δ($CDCl_3$) 1.34 (s, 18-H_3), 2.12 (s, OCOMe), 2.62 (d, *J* 8.8 Hz, 6-H), 2.90 (d, *J* 8.8 Hz, 5-H), 4.59 (br s, 15-H), 5.03 (br s, 3-H), and 5.19 and 5.31 (each br s, 17-H_2); *m/z* 372 (M^+, 100%), 284 (22), 268 (39), 240 (23), 204, (36), 104 (24), and 43 (82).

Reduction of 15-*Ketones with Zinc and Acetic Acid.*—Zinc dust (activated in 2M-hydrochloric acid, washed with methanol and light petroleum) was added to the starting material in acetic acid (5 ml) and stirred at room temperature for 1 h. The excess of zinc was filtered off and the acetic acid removed with toluene and methanol under vacuum. The product was purified by flash column chromatography,[18] eluting with 20—50% ethyl acetate in light petroleum with 1% acetic acid.

(*a*) *Reduction of* 15-*oxo-GA*$_9$ 7-*phenacyl ester* (**17**). Reduction of compound (**17**) (46 mg) yielded ent-10,15α-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**2**) (31 mg) as a gum (Found: M^+, 332.1626. $C_{19}H_{24}O_5$ requires M^+, 332.1623); δ[$(CD_3)_2CO$] 1.05 (s, 18-H_3), 2.58 (m, 5- and 6-H_2), 4.16 (br s, 15-H) and 5.04 (m, 17-H_2); *m/z* 332 (M^+, 12%), 314 (100), 286 (23), 242 (22), 230 (25), 183 (24), and 117 (22).

(*b*) *Reduction of* 15-*oxo-GA*$_4$ 3-*acetate* 7-*phenacyl ester* (**12**). Reduction of compound (**12**) (80 mg) yielded 15β-*hydroxy GA*$_4$ 3-*acetate* (**15**) (50 mg) as a gum. The product was dissolved in methanol (5 ml). Potassium carbonate (10 mg) was added and stirred at room temperature for 12 h. The product was washed with acidified water (20 ml) and extracted with ethyl acetate (3 × 50 ml). The extract was backwashed, dried (Na_2SO_4) and the solvent removed under reduced pressure to yield ent-3α,10,15α-*trihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**4**) (40 mg) as a gum (Found: M^+, 348.1560. $C_{19}H_{24}O_6$ requires M^+, 348.1573); $\nu_{max.}$($CHCl_3$), 3 450, 1 765, and 1 710 cm^{-1}; δ[$(CD_3)_2CO$] 1.12 (s, 18-H_3), 2.62 (d, *J* 11.2 Hz, 6-H), 3.17 (d, *J* 11.2 Hz, 5-H), 3.70 (br s, 3-H), 4.16 (br s, 15-H), and 5.03 and 5.06 (each br s, 17-H_2); *m/z* 348 (M^+, 7%), 330 (97), 314 (19), 302 (25), 284 (25), 257 (26), 240 (30), 228 (30), 157 (33), 129 (40), 91 (71), and 41 (100).

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