Inhibitors of the Biosynthesis of Gibberellins. Part 1. 7-Fluoro-10β-fluoromethyl-1β,8-dimethylgibbane-1α,4aα-carbolactone

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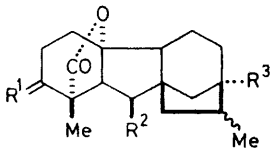
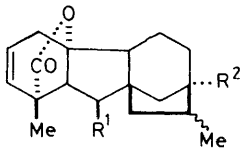
The title compound (1) has been prepared from gibberellic acid; its addition to a fermentation of *G. fujikuroi* led to the accumulation of *ent*-kaurene, and hence suggested that it inhibited the biosynthetic oxidation of kaurene. Compound (1) also retards the conversion of gibberellin A_{12} aldehyde (25) into gibberellins A_{12} (26) and A_{43} (21) by a plant enzyme system.

Two fluorinated precursors in the biosynthesis of the gibberellins by *G. fujikuroi* [1] have been shown to be transformed by the fungus into fluorinated gibberellins [2,3] and other fluorinated diterpenoid metabolites.[3] However, placement of a fluorine atom at a site in the precursor which undergoes microbiological transformation during the biosynthesis [1] could result in a compound which inhibits the biosynthetic reactions. Such an enzyme inhibitor might have valuable biological properties.

RESULTS AND DISCUSSION

The 10β-fluoromethyl derivatives (1) and (27) were selected as potential inhibitors of the later stages in the biosynthesis of the gibberellins, whilst *ent*-7,7-difluorokauranol (31) [4] might be effective at earlier points in the pathway. The 10-fluoromethyl compound (1) was first prepared by converting methyl tetrahydrogibberellate (2) into a mixture of its mono- and bis-toluene-*p*-sulphonates, which was boiled in collidine to give the olefins (10) and (11). Alkaline hydrolysis of both olefins gave the required dihydrogibberellin A_5 (12).[5]





	R¹	R²	R³		R¹	R²
(1)	H_2	CH_2F	F	(10)	CO_2Me	OH
(2)	α-H, β-OH	CO_2Me	OH	(11)	CO_2Me	$OSO_2C_6H_4Me$
(3)	H_2	CO_2H	OH	(12)	CO_2H	OH
(4)	H_2	COF	F	(13)	CO_2H	F
(5)	H_2	CO_2H	F	(14)	CO_2Me	F
(6)	H_2	CH_2OH	OH			
(7)	H_2	CHO	OH			
(8)	H_2	CH_2OH	F			
(9)	α-F, β-H	CO_2Me	F			

Hydrogenation of the latter gave tetrahydrogibberellin A_5 (3) [5] which, on treatment with 2-chloro-*NN*-diethyl-1,1,2-trifluoroethylamine (fluoro-amine), gave the relatively stable fluoro-acid fluoride (4), $\nu_{max.}$ 1 831 and 1 773 cm^{-1}. The latter required boiling with potassium hydroxide in aqueous tetrahydrofuran to give the fluoro-acid (5);[6] consequently the carboxy-group was usually protected during the fluorination of gibberellins (*cf.* ref. 6).

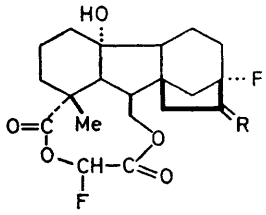
Reduction of the mixture of 8-epimers of the tetrahydro-acid (3) with diborane gave the diols (6); on one occasion the aldehyde (7) was isolated as a minor product. The 8-epimeric diols (6) could, if necessary, be separated by t.l.c. Fluorination of the diols with fluoro-amine gave an oil, $C_{21}H_{28}F_2O_5$, and the 7-fluoro-derivative (8), as major and minor products respectively. The structure of the latter was readily determined spectroscopically (see Experimental section); since the survival of a primary hydroxy-group is unusual during fluorination, compound (8) may arise by breakdown of the major product during work-up.

The oil, $C_{21}H_{28}F_2O_5$, was purified by chromatography which afforded part of the compound as a single C-8 epimer.† Its n.m.r. spectrum included a signal at τ 5.70 (2 H, m, CH_2OCO) and a low-field resonance at τ 3.68 (1 H, d, *J* 50 Hz) which was assigned to the grouping O–CHF–CO. In confirmation the ^{19}F n.m.r. spectrum showed a doublet at δ_F 147.58 (*J* 50 Hz, $\geqslant$C–CHF–C$\leqslant$) and one 7α-fluorine signal at δ_F 160.43, indicating that it was the 8α-methyl epimer (15).[7] Hence the oil was tentatively assigned structure [(15) and (16)], which could arise as shown in the Scheme. Strong supporting evidence for this structure was provided by hydrolysis of the oil with potassium carbonate in aqueous methanol,[8] which gave the fluoro-alcohol (8). The latter was identical with the samples prepared above and below.

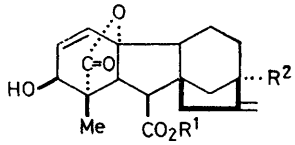
The required difluoride (1), first obtained by fluorination of the diol (6) with diethylaminosulphur trifluoride [9] (DAST), was more conveniently prepared by the following route. The fluoro-acid (5) [6] was readily prepared from methyl tetrahydrogibberellate (2) by treatment with DAST to give the fluoro-olefin (14),[6] which was converted into the fluoro-acid by the literature method.[6] Reduction of the acid (5) with diborane gave the fluoro-alcohol (8), identical with the specimen prepared above, which on treatment with DAST afforded the difluoride (1) [τ 5.53 (2 H, dm, *J* 47 Hz, 10β-CH_2F)].

A minor product from the reaction of methyl tetrahydrogibberellate and DAST exhibited three ^{19}F n.m.r.

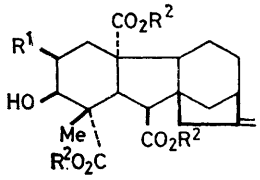
† All other gibberellins were mixtures of 8-epimers.



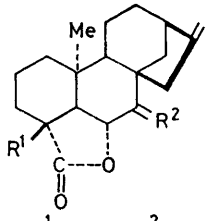
	R
(15)	α-Me,β-H
(16)	α-H, β-Me



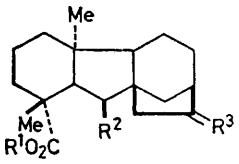
	R^1	R^2
(17)	H	OH
(18)	Me	OH
(19)	Me	H



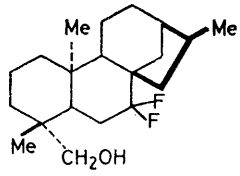
	R^1	R^2
(20)	H	Me
(21)	OH	H



	R^1	R^2
(22)	Me	α-H, β-OH
(23)	CH_2OH	α-H, β-OH
(24)	Me	α-$OSO_2C_6H_4Br$-*p*, β-H



	R^1	R^2	R^3
(25)	H	α-H, β-CHO	CH_2
(26)	H	α-H, β-CO_2H	CH_2
(27)	H	α-H, β-CH_2F	CH_2
(28)	Me	α-H, β-CH_2OH	CH_2
(29)	Me	α-H, β-CH_2OH	H,Me
(30)	Me	CH_2	H,Me



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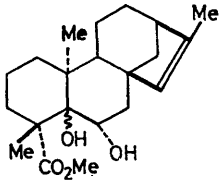

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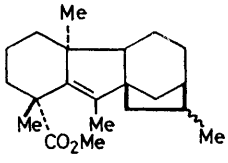
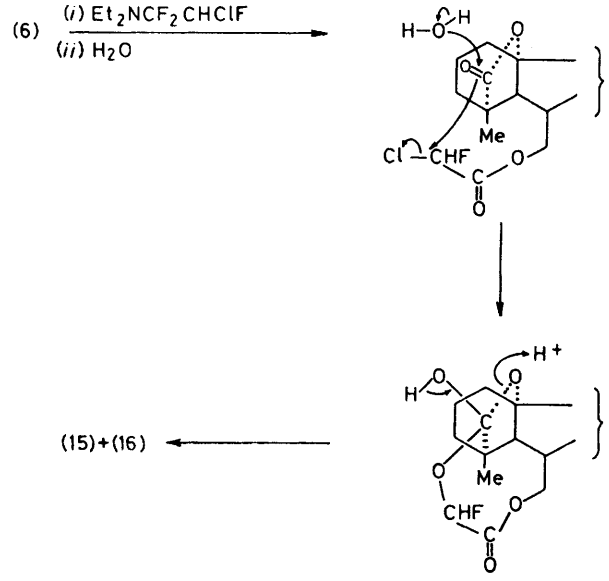


(33)

signals [δ_F 159.36 (7-F in the 8α-Me epimer), 146.95 (7-F in the 8β-Me epimer), and 152.70 (dm, J 47 Hz, 2α-F)]. The 2-fluorine atom was assigned the α-configuration in structure (9) partly because DAST is known [10] to fluorinate alcohols by an S_N2 mechanism, but mainly because 2β-fluorogibberellins give ^{19}F n.m.r. signals at δ_F *ca.* 181 [11] (*cf.* ref. 7).

The difluoride (1) was fed to two fermentations of *G. fujikuroi* and the resultant changes in the metabolites produced are shown in the Table. It can be seen that



the difluoride considerably increased the yield of *ent*-kaurene and that the yields of gibberellin A_7 and gibberellin A_{13} also showed some increase.

The accumulation of *ent*-kaurene suggested that the difluoride inhibited the biosynthetic conversion by *G. fujikuroi* of *ent*-kaurene into gibbanes. In Graebe's cell-free enzyme preparation (from *Cucurbita maxima*) the difluoride had little effect on the early stages in the biosynthesis of gibberellins, but inhibited the conversion of gibberellin A_{12} aldehyde (25) into gibberellins A_{12} (26) and A_{43} (21).[12] In contrast the difluoro-alcohol (31) [4] inhibited the conversion of kaurene into kaurenoic acid, but had little effect on the later stages in the biosynthesis of the gibberellins.[12]

The difluoride (1) and the fluoro-alcohol (8) showed no activity in the Tanginbozu dwarf-rice bioassay.[13]

Consideration of the biosynthetic route [1] to the gibberellins suggested that the fluoro-compound (27) might be a better inhibitor than the difluoride (1).

The hydroxy-ester (28),[14] prepared [15–17] from 7-hydroxykaurenolide (22), was treated with fluoroamine and with DAST at 0 °C, but in each case intractable gums were obtained; this may be due, in the latter case, to the reactivity of DAST with terminal methylene groups.[8] Consequently the ester (28) was hydrogenated and then allowed to react with DAST. The resultant ester contained no fluorine and had the formula $C_{21}H_{32}O_2$. Its n.m.r. spectrum showed a methyl singlet at τ 8.24 which was assigned to the allylic methyl group in structure (33). Very weak signals at τ 5.00 and 4.64 suggested the presence of *ca.* 10% of the isomer (30). We have previously encountered difficulties in the fluorination of primary alcohols with DAST.[8]

One large-scale ring-contraction of the *p*-bromobenzenesulphonate (24),[16] followed by reduction of the crude product with sodium borohydride, and methylation with diazomethane, gave the required hydroxy-ester (28). However an ester, $C_{21}H_{32}O_4$, was also isolated; its i.r. spectrum showed $\nu_{max.}$ 3 500, 3 450, 1 700, and 1 662 (C=C) cm^{-1}, and it gave a positive test for a 1,2-diol.[18] Its n.m.r. spectrum included the following signals [8.35br (3 H, s, C=C–Me), 5.33 (1 H, dd, J 4 and 6 Hz, ≥C–C*H*OH–CH_2), and 4.60 (1 H, br s, =C–H)]. Decoupling experiments confirmed that the allylic methyl group was coupled to the proton at τ 4.60. The ^{13}C n.m.r. spectrum of the ester showed two olefinic carbon atoms [δ_C 144.01 (C-16) and 133.35 (C-15)] and two carbon atoms attached to oxygen [δ_C 85.88 (≥C–OH) and 73.14 (>CHOH)]. On the basis of this evidence the ester was tentatively assigned structure (32).

Effect of feeding the difluoride (1) to fermentations of *G. fujikuroi*

Metabolites isolated	Fermentation 1 [a] (mg per 4 l)	Control 1 (mg per 4 l)	Fermentation 2 [b] (mg per 4 l)	Control 2 (mg per 4 l)
(19)	30	0	11	0
(20)	20	0	38	0
ent-Kaurene	33	14	28	4
(22) + (23)	34	60	20	12
Recovered (1)	0		10	

[a] Fed with 40 mg of (1). [b] Fed with 100 mg of (1).

EXPERIMENTAL

Details of chromatographic materials and conditions used for the determination of physical data, *etc.*, have been reported.[2,19]

Tetrahydrogibberellin A_5.—Treatment of methyl tetrahydrogibberellate (2.0 g) (mixture of 8-epimers) with toluene-*p*-sulphonyl chloride (2.4 g) in pyridine (4 ml) at 20 °C for 6 d gave a mixture of the *mono*- and *bis-toluene*-p-*sulphonates* (2.9 g). A mixture of the 8-epimers of the 2,7-*bis-toluene*-p-*sulphonate* of methyl tetrahydrogibberellate crystallised from ethyl acetate–light petroleum as prisms, m.p. 152—153 °C (Found: C, 60.75; H, 6.05; S, 9.3. $C_{34}H_{40}O_{10}S_2$ requires C, 60.7; H, 5.95; S, 9.5%); $\nu_{max.}$ 1 780, 1 742, 1 599, and 733 cm^{-1}; τ 9.17 (3 H, s, 1β-Me), 9.01 (d, J 7 Hz) and 8.98 (d, J 7 Hz) (8α- and 8β-Me), 7.56 (3 H, s, ArMe), 7.42 (1 H, d, J 10 Hz, 10a-H), 6.95 (1 H, d, J 10 Hz, 10-H), 6.33 (s, OMe), 6.31 (s, OMe), 5.45 (1 H, br, 2α-H), and 2.64 (4 H, m) and 2.30 (4 H, m) (aromatic H).

The mixture of sulphonates was refluxed in collidine for 6 h. Chromatography on silica gel and elution with ethyl acetate–light petroleum (1 : 4) gave the 8-epimeric mixture of the unsaturated 7-toluene-*p*-sulphonates (11) followed by the methyl ester of dihydrogibberellin A_5 (10). Hydrolysis of the latter gave a mixture of the 8-epimers of dihydrogibberellin A_5 (12).[5]

Hydrolysis of the unsaturated 7-toluene-*p*-sulphonates

(800 mg) in tetrahydrofuran (5 ml) and water (16 ml) with potassium hydroxide (1.7 g) under reflux in an atmosphere of nitrogen for 45 h also gave a mixture of the 8-epimers of dihydrogibberellin A_5 (300 mg).

Both samples of dihydrogibberellin A_5 (12) were identical (i.r. spectrum) with an authentic specimen, and on hydrogenation gave a mixture of the 8-epimers of tetrahydrogibberellin A_5 (3) identical with an authentic sample.[5]

Reduction of a Mixture of the 8-*Epimers of Tetrahydrogibberellin* A_5 *with Diborane*.—Tetrahydrogibberellin A_5 (3) (214 mg) in dry tetrahydrofuran (10 ml) was cooled to 0 °C under nitrogen and 1.4M-diborane in tetrahydrofuran (1.3 ml) was added over 25 min. The solution was left to stand at room temperature for 18 h, water (2 ml) was added, and the mixture was evaporated to dryness *in vacuo*. The residue was treated with water (10 ml) and the products were extracted into ethyl acetate. The extracts were washed with sodium hydrogencarbonate solution and water, and evaporated *in vacuo* to give a gum, which on purification by p.l.c., with two developments in ethanol–benzene (1 : 9), afforded a mixture of the 8-epimers of 7-*hydroxy*-10β-*hydroxymethyl*-1β,8-*dimethylgibbane*-1α,4aα-*carbolactone* (6) as a gum (166 mg) (Found: M^+, 320.197 6. $C_{19}H_{28}O_4$ requires M, 320.198 7); $\nu_{max.}$ ($CHCl_3$) 3 609, 3 440, and 1 760 cm^{-1}; τ 9.02 (d, J 7 Hz) and 8.97 (d, J 7 Hz) (8α- and 8β-Me), 8.81 (3 H, s, 1β-Me), and 6.31 (2 H, m, $W_{\frac{1}{2}}$ 8 Hz, CH_2OH). Further p.l.c. in ethanol–benzene–formic acid (10 : 89 : 1) gave the pure 8-epimers as gums.

In one experiment the reduction, followed by isolation in the usual way, gave, in addition to the 8-epimeric diols (6), a mixture of the 8-epimers of 10β-*formyl*-7-*hydroxy*-1β,8-*dimethylgibbane*-1α,4aα-*carbolactone* (7) as a gum (Found: M^+, 318.183 0. $C_{19}H_{26}O_4$ requires M, 318.183 1); $\nu_{max.}$ ($CHCl_3$) 3 410, 2 715, 1 763, and 1 720 cm^{-1}; τ 9.03 (d, J 7 Hz) and 8.93 (d, J 7 Hz) (8α- and 8β-Me), 8.91 (3 H, s, 1β-Me), and 0.21 (1 H, d, J 2 Hz, 10β-CHO).

Fluorination of the Hydroxy-acid (3) *with Fluoro-amine*.—The acid (125 mg) in dichloromethane (5 ml) was treated with fluoro-amine (0.25 ml) at 0 °C over 25 min, then allowed to reach room temperature and stirred for a further 65 min. Evaporation *in vacuo*, followed by purification by p.l.c. [development in ethanol–benzene–formic acid (10 : 89 : 1)] gave the acid fluoride (4) as a gum (90 mg); $\nu_{max.}$ ($CHCl_3$) 1 831 (COF) and 1 773 cm^{-1}; *m/e* 194 ($M^+ - 44$).

Hydrolysis of the Acid Fluoride (4).—The acyl fluoride (90 mg) in tetrahydrofuran (10 ml) and water (10 ml) was refluxed with potassium hydroxide (100 mg) for 4 h. Evaporation *in vacuo*, acidification with dilute hydrochloric acid, and recovery in ethyl acetate gave the fluoro-acid (5) as prisms, m.p. 202—205 °C, identical (i.r. spectrum) with an authentic sample.[6]

Fluorination of the 8-*Epimeric Mixture of Diols* (6) *with Fluoro-amine*.—The diols (185 mg) in dry dichloromethane (20 ml) were cooled to 0 °C and fluoro-amine (0.9 ml, 1.08 g) was added over 20 min. The mixture was stirred at 0 °C for 1 h, at room temperature for a further 2 h, and then the solvent was removed *in vacuo* to afford an oil which was purified by chromatography on silica gel (40 g). Elution with ethyl acetate–light petroleum (3 : 17) gave a mixture of the 8-epimers of 10β-(2′-*fluoro*-2′-*hydroxyacetoxymethyl*)-4aα-*hydroxy*-7α-*fluoro*-1β,8-*dimethylgibbane*-1α,2′-*carbolactone* [(15) and (16)] as a gum (75 mg) (Found: C, 62.5; H, 7.4; F, 9.5. $C_{21}H_{28}F_2O_5$ requires C, 63.3; H, 7.0; F, 9.5%); $\nu_{max.}$ ($CHCl_3$) 1 770(br) and 967 cm^{-1}; τ 9.02 (d, J 7 Hz) and 8.98 (d, J 7 Hz) (8α- and 8β-Me), 8.78 (3 H, s, 1β-Me), 5.70 (2 H, m, 10β-CH_2O), and 3.68 (1 H, d, J 50 Hz, O–CHF–CO).

Further elution with ethyl acetate–light petroleum (2 : 3) gave a mixture of the 8-epimers of 7-*fluoro*-10β-*hydroxymethyl*-1β,8-*dimethylgibbane*-1α,4aα-*carbolactone* (8) which crystallised from ethyl acetate–light petroleum as prisms, m.p. 176—181 °C (Found: C, 70.7; H. 8.7; F, 5.9. $C_{19}H_{27}FO_3$ requires C, 70.8; H, 8.4; F, 5.9%); $\nu_{max.}$ 3 540, 1 761, 934, and 880 cm^{-1}; τ 9.02 (d, J 7 Hz) and 8.96 (d, J 7 Hz) (8α- and 8β-Me), 8.82 (3 H, s, 1β-Me), and 6.30 (2 H, m, 10β-CH_2OH); *m/e* 278.

Hydrolysis of the Mixture of 8-*Epimers of the Macrocycle* [(15) *and* (16)].—The macrocycle (50 mg) in hot methanol (5 ml) was treated with a 10% aqueous solution of potassium carbonate (1 ml) for 20 min. Glacial acetic acid (2 ml) was added and the mixture was stirred at room temperature for 45 min. Recovery in ethyl acetate gave a gum (46 mg) which crystallised on standing. It was identical (i.r. spectrum) with the fluoro-alcohol (8).

Fluorination of the Mixture of 8-*Epimeric Diols* (6) *with Diethylaminosulphur Trifluoride* (*DAST*).—The diols (45 mg) in dry dichloromethane were treated with DAST (150 mg, 0.075 ml) at 0 °C for 30 min. Water was added and the products were recovered in dichloromethane. Removal of the solvent *in vacuo* followed by purification by p.l.c. [development in chloroform–light petroleum (1 : 1)] afforded a mixture of the 8-epimers of 7-*fluoro*-10β-*fluoromethyl*-1β,8-*dimethylgibbane*-1α,4aα-*carbolactone* (1) as a gum (35 mg) which solidified on standing, m.p. 87—98 °C [Found: *m/e* 280.200 1. $C_{19}H_{26}F_2O_2$ requires ($M - CO_2$), 280.200 2] and was identical (i.r. and n.m.r. spectra) with the authentic sample prepared below.

Reaction of the Fluoro-alcohol (8) *with DAST*.—The alcohol (18 mg) in dichloromethane at 0 °C was treated with an excess of DAST for 30 min. Addition of water and recovery of the product in dichloromethane gave the difluoride (1) (15 mg), identical (i.r. spectrum) with the specimen prepared above.

Reaction of a Mixture of the 8-*Epimers of Methyl Tetrahydrogibberellate* (2) *with DAST*.—DAST (2.62 ml, 5.22 g) was added to methyl tetrahydrogibberellate (5.1 g), suspended in dry dichloromethane (10 ml), and cooled to 0 °C with stirring for 45 min, by which time the suspension had dissolved. Water (20 ml) was added and the reaction mixture was stirred vigorously for 30 min at room temperature. The products were recovered in dichloromethane and on evaporation in *vacuo* gave a foam which was chromatographed on silica gel (250 g). Elution with ethyl acetate–light petroleum (1 : 9) gave a mixture of the 8-epimers of the 7-fluoro-olefin (14), which crystallised from ethyl acetate–light petroleum as needles (1.25 g) (26%), m.p. 134—140 °C, identical (i.r. and n.m.r. spectra and g.l.c.) with an authentic specimen.[6] Further elution with ethyl acetate–light petroleum (1 : 9) gave a number of mixed fractions (by g.l.c.) which were re-chromatographed on silica gel. Elution with ethyl acetate–light petroleum (1 : 9) gave a fraction (104 mg), which consisted of one component (by g.l.c.), and which was more polar than the fluoro-ester (14) obtained above. It crystallised from ethyl acetate–light petroleum as prisms, m.p. 154—158 °C, of a mixture of the 8-epimers of 2α,7-*difluoro*-10β-*methoxycarbonyl*-1β,8-*dimethylgibbane*-1α,4aα-*carbolactone* (9) [Found: *m/e* 337.160 9. $C_{20}H_{26}F_2O_6$ requires (M – OMe), 337.161 5]; $\nu_{max.}$ 1 777 and 1 739 cm^{-1}; τ 9.03 (d, J 7 Hz) and 8.99 (d, J 7 Hz) (8α- and 8β-Me), 8.80 (3 H, s, 1β-Me), 6.24 (3 H, s, OMe), 5.70

(1 H, dm, $W_{\frac{1}{2}}$ 9 Hz, 2β-H); δ_F 159.36 (m, 7-F), 146.95 (m, 7-F),[7] and 152.70 (dm, J_{HF} 47 Hz, 2α-F).

Hydrogenation and Hydrolysis of a Mixture of the 8-Epimers of the Fluoro-olefin (14).—The fluoro-olefin (1.5 g) and 10% palladium–carbon (700 mg) in ethyl acetate (70 ml) were shaken in hydrogen. Recovery gave a gum which was hydrolysed with potassium hydroxide (1.4 g) in tetrahydrofuran (10 ml) and water (10 ml) under reflux. Work-up in the usual way and crystallisation from ethyl acetate–light petroleum afforded a mixture of the 8-epimers of the fluorogibberellin (5) as prisms, m.p. 204—209 °C (lit.,[6] 205—207 °C); τ 8.95 (m, 8α- and 8β-Me), 8.77 (3 H, s, 1β-Me), 7.08 (1 H, d, *J* 8 Hz, 10a-H), and 6.73 (1 H, d, *J* 8 Hz, 10-H), identical (i.r. spectrum) with an authentic sample.[6]

Diborane Reduction of a Mixture of the 8-Epimers of the Fluoro-acid (5).—The fluoro-acid (300 mg) in dry tetrahydrofuran (5 ml) was cooled to 0 °C under nitrogen, and 1.3M-diborane in tetrahydrofuran (1.2 ml) was added over 30 min. The reaction mixture was stirred at 0 °C for 1.5 h and allowed to come to room temperature during 18 h. Water (10 ml) was added and the solvent was removed *in vacuo*. Recovery in ethyl acetate gave a solid (280 mg) which crystallised from ethyl acetate–light petroleum as prisms, of a mixture of the 8-epimers of 7-fluoro-10β-hydroxymethyl-1β,8-dimethylgibbane-1α,4aα-carbolactone (8), m.p. 158—173 °C, identical (i.r. and n.m.r. spectra) with the sample prepared above.

Fluorination of a Mixture of the 8-Epimers of the Fluoro-alcohol (8) *with DAST*.—The fluoro-alcohol (220 mg) in dry dichloromethane (5 ml) was treated with DAST (0.2 ml, 400 mg) at 0 °C for 30 min. Water (10 ml) was added and the products were recovered in dichloromethane. Removal of the solvent *in vacuo* gave a gum which was purified by p.l.c. [development in ethanol–benzene (1 : 49)]. Recovery of the material in the major band in the usual way afforded a mixture of the 8-epimers of 7-*fluoro*-10β-*fluoromethyl*-1β,8-*dimethylgibbane*-1α,4aα-*carbolactone* (1) which crystallised from methanol as plates, m.p. 97—99 °C (Found: C, 70.1; H, 8.15; F, 11.6. $C_{19}H_{26}F_2O_2$ requires C, 70.4; H, 8.0; F, 11.7%); $\nu_{max.}$ 1 770 and 930 cm^{-1}; τ 9.01 (d, *J* 6 Hz) and 8.96 (d, *J* 6 Hz) (8α- and 8β-Me), 8.81 (3 H, s, 1β-Me), and 5.53 (2 H, dm, J_{HF} 47 Hz, 10β-CH_2F).

Preparation of the Hydroxy-ester (28).—The ester was prepared from 7-hydroxykaurenolide [20] by the literature method.[14–17]

On one occasion ring-contraction of the 7α-*p*-bromobenzenesulphonate (24) [16] (2.0 g) in t-butyl alcohol (100 ml) and water (2.5 ml), with potassium hydroxide (8.75 g) under reflux for 2 h, and work-up in the usual manner, gave a gum which was reduced with sodium borohydride in methanol. The products were methylated with diazomethane and chromatographed on Kieselgel (100 g). Elution with ethyl acetate–light petroleum (1 : 19) gave the hydroxy-ester (28) which was identified by its n.m.r. spectrum. Elution with ethyl acetate–light petroleum (8 : 92) afforded *methyl* 5ξ,6α-*dihydroxy*-ent-*kaur*-15-*en*-19-*oate* (32) (400 mg), which crystallised from ethyl acetate–light petroleum in prisms, m.p. 165—166 °C (Found: C, 72.6; H, 9.25. $C_{21}H_{32}O_4$ requires C, 72.4; H, 9.3%); $\nu_{max.}$ 3 500, 3 450, 1 700, and 1 662 cm^{-1}; τ 8.95 (3 H, s, 20-Me), 8.68 (3 H, s, 18-Me), 8.35br (3 H, s, 17-Me), 6.24 (3 H, s, OMe), 5.33 (1 H, dd, *J* 6 and 4 Hz, 6-H), and 4.60 (1 H, br s, 15-H) [irradiation at the frequency of the signals at τ 7.45 (7-H_2) caused the double doublet at τ 5.33 to collapse to a broad singlet; irradiation at the frequency of the signal at τ 8.35 caused the singlet at τ 4.60 to become sharp]; δ_C 180.75 (C-19), 144.01 (C-16), 133.35 (C-15), 85.88 (C-5), and 73.14 (C-6).

Reaction of the Hydroxy-ester (28) *with Fluoro-amine*.—The alcohol (100 mg) in dichloromethane (10 ml) was stirred with fluoro-amine (0.2 ml) at 0 °C, and then at 20 °C for 1.5 h. Work-up in the usual manner gave an intractable gum.

Hydrogenation of the Hydroxy-ester (28).—The ester in ethyl acetate was shaken in hydrogen in the presence of 10% palladium–charcoal until uptake of hydrogen ceased. Recovery gave a mixture of the 8-epimers of methyl 10β-hydroxymethyl-1β,4aα,8-trimethylgibbane-1α-carboxylate (29) which was used without purification.

Reaction of the Mixture of 8-Epimers of the Hydroxy-ester (29) *with DAST*.—The alcohol (149 mg) in dry dichloromethane (4 ml) was treated with DAST (200 mg) at 0 °C for 20 min. Water (5 ml) was added, and the product was recovered in dichloromethane. Evaporation of the solvent gave a mixture of the 8-epimers of *methyl* 1β,4aα,8,10-*tetramethylgibb*-10-*ene*-1α-*carboxylate* (33) as a gum (80 mg) (Found: M^+, 316.239 6. $C_{21}H_{32}O_2$ requires *M*, 316.240 2); $\nu_{max.}$ 1 730 cm^{-1}; τ 9.20 (3 H, s, 4α-Me), 9.00 (d, *J* 7 Hz) and 8.95 (d, *J* 7 Hz) (8α- and 8β-Me), 8.50 (3 H, s, 1β-Me), 8.24 (3 H, s, 10-Me), and 6.53 (3 H, s, OMe); weak singlets at τ 5.00br and 4.64br indicated that the product contained *ca.* 10% of the isomer (30).

Fermentations of Gibberella fujikuroi. *Fermentation Conditions*.—*G. fujikuroi* ACC 917 was grown in stirred fermentation as previously described.[8] The difluoride (1) in ethanol (10 ml), previously sterilized by means of a Seitz filter, was added when the concentration of ammonium ions in the medium was *ca.* zero. The fermentations were harvested after a further 96 h and the neutral and acidic fractions were isolated as previously described.[21] Metabolites were identified by spectroscopy.

Control fermentations. Ethanol (10 ml) was added to the control fermentations.

Fermentation 1. The difluoride (1) (40 mg) was added. The neutral fraction (339 mg) was chromatographed on silica gel (16 g). Elution of the column with light petroleum gave *ent*-kaurene (33 mg) which crystallised from methanol as prisms, m.p. 47—49 °C. Further elution with ethyl acetate–light petroleum (1 : 4 and 3 : 7) gave 7β,18-dihydroxykaurenolide (23) which crystallised from ethyl acetate–light petroleum as needles (34 mg), m.p. 208—211 °C.

Crystallisation of the acidic fraction (696 mg) gave gibberellic acid (292 mg). Methylation of the mother liquors and chromatography on silica gel (30 g) using ethyl acetate–light petroleum (1 : 19) as eluent gave a gum which was purified by p.l.c. [5 developments in ethanol–benzene (1 : 49)]. Recovery of the band of the higher R_F afforded the trimethyl ester of gibberellin A_{13} (20 mg) (20) which crystallised from acetone–light petroleum as prisms, m.p. 146—150 °C. Recovery of the band of lower R_F gave the methyl ester of gibberellin A_7 (19) (30 mg) which crystallised from acetone–light petroleum as prisms, m.p. 151—155 °C. Methyl gibberellate (72 mg) was obtained by elution of the column with ethyl acetate.

Control fermentation 1. The yield of crystalline gibberellic acid was 149 mg. Methylation of the mother-liquors and purification by p.l.c. gave methyl gibberellate (47 mg). Purification of the neutral metabolites (559 mg) by chromatography on silica gel (18 g) afforded *ent*-kaurene

as a gum (14 mg) (identified by g.l.c.), followed by 7β-hydroxykaurenolide (22), which crystallised from ethyl acetate–light petroleum as prisms (40 mg), m.p. 187—189 °C, and 7β,18-dihydroxykaurenolide (20 mg).

Fermentation 2. The difluoride (1) (100 mg) was added. The neutral metabolites (236 mg) were chromatographed on silica gel (20 g). Elution with light petroleum gave *ent*-kaurene (28 mg), which was identified by g.l.c. Ethyl acetate–light petroleum (1 : 4) eluted a gum which was purified by p.l.c. to yield 7β-hydroxykaurenolide (22) (20 mg) and the difluoride (1) (10 mg).

The acidic fraction (688 mg) gave crystalline gibberellic acid (110 mg). Methylation of the mother liquors in the usual way followed by chromatography on Kieselgel (50 g) and elution with ethyl acetate–light petroleum (1 : 4) gave the trimethyl ester of gibberellin A_{13} (38 mg) and the methyl ester of gibberellin A_7 (11 mg). Elution with ethyl acetate gave methyl gibberellate (30 mg).

Control fermentation 2. Chromatography of the neutral metabolites (125 mg) on silica gel (20 g) and elution with light petroleum afforded *ent*-kaurene (4 mg). Elution with ethyl acetate followed by purification by p.l.c. [ethanol–benzene (1 : 9)] gave 7β-hydroxykaurenolide (12 mg).

The acidic fraction (671 mg) gave gibberellic acid (156 mg). Methylation of the mother-liquors in the usual way and p.l.c. [ethanol–benzene (1 : 9)] gave methyl gibberellate (88 mg).

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