The Microbiological Production of Analogues of Mould Metabolites. Part I. Production of Fluorogibberellic acid and Fluorogibberellin A_9 by *Gibberella fujikuro*.

By **John H. Bateson** and **Brian E. Cross,*** Department of Organic Chemistry, The University, Leeds LS2 9JT

1β-Fluoromethyl-10β-formyl-4aα-methyl-8-methylenegibbane-1α-carboxylic acid (3) and 1β-fluoromethyl-4aα-methyl-8-methylenegibbane-1α,10β-dicarboxylic acid (fluorogibberellin A_{12}) (4) have been prepared from 6α,7β,18-trihydroxykaur-16-en-19-oic acid 19,6-lactone (7). The aldehyde (3) was converted by *G. fujikuroi* into 1β-fluoromethyl-2β,4aα,7α-trihydroxy-8-methylenegibb-3-ene-1α,10β-dicarboxylic acid 1,4a-lactone (18) and 1β-fluoromethyl-4aα-hydroxy-8-methylenegibbane-1α,10β-dicarboxylic acid 1,4a-lactone (fluorogibberellin A_9) (15).

INTRODUCTION of fluorine into biologically active molecules often enhances or modifies their activity and has been the subject of much investigation.[1] The replacement of hydrogen in C–H bonds by fluorine provides the opportunity of effecting large changes in electron density, and in properties such as hydrogen-bonding ability, without, in most cases, causing more than minimal changes in molecular size and shape. The preparation of fluoro-derivatives of the gibberellins, which constitute a group of diterpenoid plant hormones with very high and varied biological activities,[2] was therefore undertaken. The total synthesis of fluorogibberellins is not at present practicable, but a combination of chemical and microbiological reactions based on the well defined biosynthetic route[3] to the gibberellins offered two main advantages. First, it could provide a route to otherwise inaccessible compounds which were required for biological testing, and secondly it would enable the simultaneous investigation of the substrate specificity of the enzyme systems responsible for the biosynthesis of the gibberellins.

There are few references to the microbiological production of fluoro-analogues of natural products and none to the use of such methods for the preparation of fluorinated diterpenoids. 4′-Fluoropyrrolnitrin (1) was produced in 25% yield from 6-fluorotryptophan by *Pseudomonas aureofaciens* cultures[4] and *Streptomyces cacaoi* converted 5-fluorouracil into 5-fluoropolyoxin L and 5-fluoropolyoxyin M.[5] On the other hand, 5-fluorotryptophan was not utilised by *Claviceps purpurea*.[6]

The aldehyde (2) is a good precursor of gibberellic acid in fermentations of the fungus *Gibberella fujikuroi*;[7] this paper describes the preparation of the 1β-fluoromethyl analogue (3) of this aldehyde and the products formed when the fluoroaldehyde was fed to *G. fujikuroi*.†

The fluoroaldehyde (3) was prepared from 7,18-dihydroxykaurenolide ‡ (7) which is readily available.[8] The latter was converted into its monotoluene-*p*-sulphonate (8),[8] which on treatment with caesium fluoride in anhydrous *NN*-dimethylacetamide, gave the fluorokaurenolide (9) in 57% yield. The spectroscopic data of the fluoro-compound (see Experimental section) were consistent with structure (9); in particular its ^{1}H n.m.r. spectrum showed the C(18)-protons as two quartets (AB of ABX, J 47 and 9 Hz) and its ^{19}F n.m.r. spectrum exhibited a triplet (J 47 Hz) broadened by long-range coupling (J 2 Hz) with the 6β-proton. The acetate (10) was a minor product from the fluorination reaction.

† For a preliminary account see J. H. Bateson and B. E. Cross, *J.C.S. Chem. Comm.*, 1972, **649**.

‡ *Editorial note.* The convention used in this paper for numbering the C-4 methyl groups of kaurane (C-18 = β; C-19 = α) is opposite to that used in Chemical Abstracts.

[1] *E.g.* 'Carbon–Fluorine Compounds. Chemistry, Biochemistry and Biological Activities,' A CIBA Foundation Symposium, Elsevier, 1972.

[2] *E.g.* L. G. Paleg, *Ann. Rev. Plant Physiol.*, 1965, **16**, 291; P. W. Brian, *Internat. Rev. Cytol.*, 1966, **19**, 229.

[3] B. E. Cross, *Progr. Phytochem.*, 1968, **1**, 195; J. MacMillan, in 'Aspects of Terpenoid Chemistry and Biochemistry,' ed. T. W. Goodwin, Academic Press, London, 1971, p. 153; J. R. Hanson, *Progr. Chem. Org. Nat. Prod.*, 1971, **29**, 406.

[4] M. Gorman, R. L. Hamill, R. P. Elander, and J. Mabe, *Biochem. Biophys. Res. Comm.*, 1968, **31**, 294.

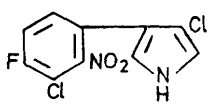
[5] K. Isono, P. F. Crain, T. J. Odiorne, J. A. McCloskey, and R. J. Suhadolnik, *J. Amer. Chem. Soc.*, 1973, **95**, 5788.

[6] W. A. Skinner, J. J. Morris, and J. V. Stevenson, *J. Pharm. Sci.*, 1967, **56**, 396.

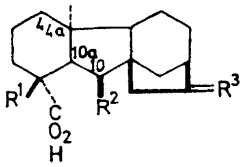
[7] B. E. Cross, K. Norton, and J. C. Stewart, *J. Chem. Soc.* (*C*), 1968, 1054.

[8] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1963, 3783.

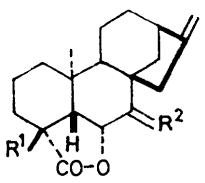
The fluoroaldehyde (3) was prepared from the fluorokaurenolide (9), *via* the sequence (9) ⟶ (11) ⟶ (12) ⟶ (13) ⟶ (3), analogous to the route used to prepare the aldehyde (2) from 7-hydroxykaurenolide



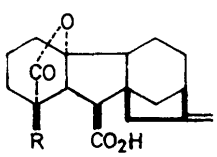
(1)



	R^1	R^2	R^3
(2)	Me	CHO	CH_2
(3)	CH_2F	CHO	CH_2
(4)	CH_2F	CO_2H	CH_2
(5)	Me	CO_2H	CH_2
(6)	Me	CHO	$^{14}CH_2$



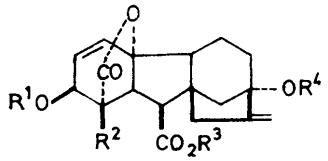
	R^1	R^2
(7)	CH_2OH	α-H, β-OH
(8)	p-$MeC_6H_4\cdot SO_2\cdot OCH_2$	α-H, β-OH
(9)	CH_2F	α-H, β-OH
(10)	CH_2F	α-H, β-OAc
(11)	CH_2F	O
(12)	CH_2F	α-OH, β-H
(13)	CH_2F	α-p-$MeC_6H_4\cdot SO_2\cdot O$, β-H
(14)	Me	α-H, β-OH



	R
(15)	CH_2F
(16)	Me

(14).[7] The ^1H and ^{19}F n.m.r. spectra (see Experimental section) of the fluoroaldehyde were in agreement with structure (3). Oxidation of the fluoroaldehyde with Jones reagent gave the first fluorogibberellin, *viz.* the fluoro-analogue (4) of gibberellin A_{12} (5).[9]

The fluoroaldehyde (3) (1 g) was added to a stirred fermentation (5 l) of *G. fujikuroi* during the gibberellic acid production phase and the acidic and neutral fractions were isolated in the usual way.[10] The neutral fraction yielded fujenal,[3] 7-hydroxykaurenolide (14), and 7,18-dihydroxykaurenolide (7) which, as expected from their biosynthetic pathways,[3] did not contain fluorine. Careful chromatography of the acidic fraction on silica gel afforded a crystalline mixture (30 mg) of the fluorogibberellin A_9 (15) and gibberellin A_9 (16), shown by g.l.c. of the methyl esters to contain 11% of the latter. Preparative layer chromatography (p.l.c.) of this mixture of gibberellins separated the fluorogibberellin (pure by g.l.c. of its methyl ester) as the slower moving band. The structure (15) of the fluorogibberellin A_9 was established by spectroscopic data. First, its ^1H n.m.r. spectrum did not contain a tertiary methyl resonance, but showed two quartets centred at τ 5·46 (AB of ABX, J 47·5 and 9 Hz) assigned to the $-CH_2F$ group and, as with other fluorinated compounds in this series, the 10-proton exhibited long-range coupling (J 2 Hz) with the fluorine atom. Secondly, the mass spectra of the acid and its methyl ester each showed the appropriate molecular ions, and the mass spectrum of the ester also contained fragment ions at *m/e* 316 (base peak, M — MeOH) and 288 (M — HCO_2Me) analogous to, but 18 mass units heavier than, fragment ions given by the methyl ester of gibberellin A_9.[11] The yield of fluorogibberellin A_9 (15) indicates that at least 3% of the fluoroaldehyde (3) was utilised as its precursor. Gibberellin A_9, which is not a precursor of gibberellic acid (17),[12,13] is not normally produced in significant yield under the fermentation conditions employed in this experiment. Hence, it is possible that the presence of high concentrations of the fluoroaldehyde may have overloaded the enzyme system which converts the aldehyde (2) into gibberellic acid.[7]



	R^1	R^2	R^3	R^4
(17)	H	Me	H	H
(18)	H	CH_2F	H	H
(19)	Ac	Me	Me	Ac
(20)	Ac	CH_2F	Me	Ac
(21)	$CCl_3\cdot CH_2\cdot O\cdot CO$	Me	Me	$CCl_3\cdot CH_2\cdot O\cdot CO$
(22)	Me	Me	Me	$CCl_3\cdot CH_2\cdot O\cdot CO$
(23)	$CCl_3\cdot CH_2\cdot O\cdot CO$	CH_2F	Me	$CCl_3\cdot CH_2\cdot O\cdot CO$

Further elution of the silica gel column (see above) of the acidic fermentation products afforded crystalline gibberellic acid. The leading fractions of gibberellic acid were shown by fluorine analysis and by ^1H n.m.r. spectroscopy, to contain *ca.* 28% of the fluorogibberellic acid (18). Calculation revealed that the total yield of the latter was *ca.* 140 mg which corresponds to the

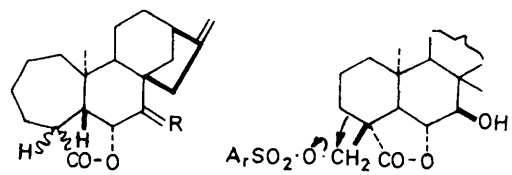
[9] B. E. Cross and K. Norton, *J. Chem. Soc.*, 1965, 1570.
[10] B. E. Cross and P. L. Myers, *Phytochem.*, 1969, **8**, 79.
[11] R. Binks, J. MacMillan, and R. J. Pryce, *Phytochem.*, 1969, **8**, 271; N. Takahashi, N. Murofushi, S. Tamura, N. Waseda, H. Hoshino, T. Tsuchiya, S. Sasaki, T. Aoyama, and E. Watanabe, *Org. Mass Spectrometry*, 1969, **2**, 711.
[12] B. E. Cross, R. H. B. Galt, and K. Norton, *Tetrahedron*, 1968, **24**, 231.
[13] J. MacMillan, personal communication.

utilisation of >12% of the fluoroaldehyde (3) in the production of fluorogibberellic acid. This figure is close to the incorporation of 15·4% found in feeding experiments with the ^{14}C-labelled aldehyde (6).[7] Gibberellic acid (17) and the fluorogibberellic acid (18) are both very polar and the mixture ran as one spot on t.l.c. in several solvent systems. To reduce its polarity a portion of the mixture was converted into the methyl diacetyl esters (19) and (20). These were separated by p.l.c. [development (×20) in ethanol–benzene (1 : 99)]; the less polar band gave, on recovery, pure methyl diacetylfluorogibberellate (20) whose structure was derived spectroscopically. In particular, its ^{1}H n.m.r. spectrum showed no tertiary methyl resonance, but contained signals typical of a $-CH_2F$ group [τ 5·33 (AB of ABX, J 47 and 9 Hz)] and exhibited long-range coupling between the fluorine atom and both the 3- and 10-protons (J 2 and 3 Hz respectively). Since gibberellic acid is a very sensitive molecule, it was unlikely that the free fluoro-acid could be prepared from its diacetate, and a study of some protecting groups was undertaken. Model experiments showed that methyl gibberellate reacted with 2,2,2-trichloroethyl-chloroformate [14] in pyridine at room temperature to give the biscarbonate (21). The trichloroethoxycarbonyl groups were readily removed in good yield with zinc dust in acetic acid,[14] but attempts to remove these groups with activated zinc in methanol [14] gave *inter alia* the methyl ether (22). Demethylation of methyl gibberellate was achieved by the method of Corey.[15,†] Gibberellic acid containing *ca.* 24% of the fluorogibberellic acid (18) was converted into its methyl ester biscarbonate [(21) + (23)]. However, t.l.c. and p.l.c. investigation of this mixture failed to separate the fluorinated component.

In another attempt to isolate the pure fluorogibberellic acid (18), the mixture with gibberellic acid was subjected to partition chromatography on Sephadex using the method of Vining.[17] A complete separation was not achieved, although the content of fluorogibberellic acid in the leading fractions was enriched to *ca.* 43%. Further efforts to prepare the pure fluorogibberellic acid are in hand.

The work described above has clearly demonstrated that a combination of chemical and microbiological methods can produce significant quantities of otherwise unavailable analogues of mould metabolites. The fluorogibberellin A_9 (15) has been shown to be considerably more active than gibberellin A_9 in the lettuce hypocotyl extension test.[18] Although pure fluorogibberellic acid has not yet been made available for bioassay, very interesting results have been obtained with gibberellic acid containing 30% of the fluoro-acid and full details of the biological activities of these fluorogibberellins have been published.[18]

In one experiment the fluorination of the toluene-*p*-sulphonate (8) was carried out in *NN*-dimethylacetamide, which had been dried over calcium hydride, but had not been redistilled before use. In addition to the fluorokaurenolide (9) the products included a compound, $C_{20}H_{28}O_3$ (20% yield), which on the basis of its i.r. [$\nu_{max.}$ ($CHBr_3$) 3600—3480 (OH), 1765 (γ-lactone), and 1654 and 878 ($=CH_2$) cm^{-1}] and n.m.r. spectra [τ 9·15 (3H, s), 5·5 (2H, m, 6-H and 7-H), and 5·05 and 4·91 ($=CH_2$)] was tentatively assigned the A-homo-structure (24). This structure was supported by oxidation of the compound to a ketone believed to have



R

(24) α-H, β-OH

(25) O

(26)

structure (25) [$\nu_{max.}$ 1770 and 1700 cm^{-1}; τ 5·25 (1H, d, J 7 Hz, 6β-H)]. The A-homo-compound (24) might arise by solvolysis of the toluene-*p*-sulphonate with concomitant migration of the 3,4-bond (26) followed by quenching of the resultant carbonium ion by hydride ion (from residual calcium hydride).

EXPERIMENTAL

Details of chromatographic materials and conditions used for the determination of physical data, *etc.*, are reported in ref. 19. G.l.c. was carried out on a Pye Argon gas chromatograph using a 150 cm column. ^{19}F N.m.r. spectra were recorded with a Bruker HF3 instrument at 84·66 MHz for solutions in deuteriochloroform with hexafluorobenzene as internal standard. ϕ^* Values were calculated [20] with reference to trichlorofluoromethane using a measured value of +162·5 p.p.m. for ϕ^* of hexafluorobenzene.

The 18-*Monotoluene*-p-*sulphonate* (8) *of* 7,18-*Dihydroxykaurenolide*.—Prepared by the literature method,[8] the ester (8) showed τ 9·10 (3H, s, 20-H_3), 7·90 (1H, d, J 7 Hz, 5-H), 7·58 (3H, s, Ar*Me*), 5·89 (2H, s, 18-H_2), 5·61 (1H, d, J 7 Hz, 7α-H), 5·33 (1H, t, J 7 Hz, 6-H), and 4·99br (1H) and 4·86br (1H) ($=CH_2$).

The 18-*Fluorokaurenolide* (9).—The dry monotoluene-*p*-sulphonate (8) (13·2 g), powdered caesium fluoride (previously fused and allowed to cool in a dry-box) (*ca.* 12 g), and *NN*-dimethylacetamide (dried over calcium hydride and redistilled from calcined 4A molecular sieves) (200 ml) were distributed between four dry Carius tubes

† Recent work has shown [16] that the carboxy-group of gibberellic acid is more conveniently protected as its *p*-bromophenacyl ester.

[14] T. B. Windholz and D. B. R. Johnston, *Tetrahedron Letters*, 1967, 2555.

[15] E. J. Corey, T. M. Brennan, and R. L. Carney, *J. Amer. Chem. Soc.*, 1971, **93**, 7316; P. A. Bartlett and W. S. Johnson, *Tetrahedron Letters*, 1970, 4459.

[16] J. H. Bateson and B. E. Cross, *Tetrahedron Letters*, 1973, 1783.

[17] D. W. Pitel, L. C. Vining, and G. P. Arsenault, *Canad. J. Biochem.*, 1971, **49**, 185.

[18] J. L. Stoddart, *Planta*, 1972, **107**, 81.

[19] B. E. Cross and R. E. Markwell, *J. Chem. Soc.* (*C*), 1971, 2980.

[20] R. J. Abraham, D. F. Wileman, and G. R. Bedford, *J.C.S. Perkin II*, 1973, 1027.

(i.d. 2 cm); all operations were performed in a dry-box. The tubes were sealed and heated in a furnace at 140—150° for a 16 h. Evaporation (40° at 10 mmHg) followed by recovery in ethyl acetate gave a gum, which was chromatographed on silica gel (700 g; 36 × 6 cm). Elution with ethyl acetate–light petroleum (5 : 35), followed by crystallisation from ethyl acetate–light petroleum, gave 7β-*acetoxy*-18-*fluoro*-6α-*hydroxykaur*-16-*en*-19-*oic acid* 19,6α-*lactone* (10) as needles or square plates (250 mg), m.p. 183—184° (Found: C, 70·2; H, 7·85; F, 5·3. $C_{22}H_{29}FO_4$ requires C, 70·2; H, 7·8; F, 5·05%), $\nu_{max.}$ ($CHBr_3$) 1769, 1742, 1656, and 883 cm^{-1}, τ (90 MHz) 8·94 (3H, s, 20-H_3), 7·93 (3H, s, OAc), 7·81 (1H, d, *J* 7 Hz, 5-H), 5·58 (2H, 2 × 4 lines, *J* 47 and 9 Hz, 18-CH_2F), 5·26 (td, *J* 7 and $^5J_{HF}$ 1 Hz, 6β-H), 5·09br (1H) and 4·95br (1H) (=CH_2), and 4·20 (1H, d, *J* 7 Hz, 7α-H).

Continued elution with the same solvent system gave 18-*fluoro*-6α,7β-*dihydroxykaur*-16-*en*-19-*oic acid* 19,6α-*lactone* (9) which crystallised from ethyl acetate–light petroleum as prisms (4·54 g), m.p. 180·5—182·5° (Found: C, 71·7; H, 8·1; F, 5·7. $C_{20}H_{27}FO_3$ requires C, 71·8; 8·1; F, 5·7%), $\nu_{max.}$ 3510, 1773, 1649, and 904 cm^{-1}; τ (90 MHz) 9·08 (3H, s, 20-H_3), 7·88 (1H, d, *J* 6·5 Hz, 5-H), 5·63 (1H, d, *J* 6·5 Hz, 7α-H), 5·59 (2H, 2 × 4 lines, *J* 47 and 9 Hz, 18-CH_2F), 5·34 (1H, td, *J* 6·5 and $^5J_{HF}$ 1 Hz, 6β-H), and 5·14br (1H) and 4·99br (1H) (=CH_2), ϕ^* 222·4 (td, *J* 47 and *ca.* 2 Hz, 18-F), *m/e* 334 (M^+, 4%), 301 (33), 283 (10), 273 (17), 223 (19), 155 (21), 127 (33), 107 (40), 105 (56), 91 (97), and 33 (6).

Elution with ethyl acetate–light petroleum (3 : 17 and 1 : 4) gave unchanged toluene-*p*-sulphonate (1·53 g).

A similar fluorination experiment with the monotoluene-*p*-sulphonate (11·5 g), but employing *NN*-dimethylacetamide which had been dried only by standing over calcium hydride gave, on eluting the column with ethyl acetate–light petroleum (5 : 35), 7β-acetoxy-18-fluorokaurenolide (10) (250 mg) followed by 18-fluoro-7β-hydroxykaurenolide (9) (3·33 g) as before. However, continued elution with the same solvent system gave prisms (1·48 g), m.p. 188—190°, believed to be the 7β-*hydroxy*-A-*homo*-18-*norkaurenolide* (24) (Found: C, 76·05; H, 8·75; F, 0·0. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·85%), $\lambda_{max.}$ end-absorption only, τ 9·15 (3H, s, 20-H_3), 5·5 (2H, m, $W_{\frac{1}{2}}$ 6 Hz, 6-H and 7-H), and 5·05br (1H) and 4·91br (1H) (=CH_2), τ (pyridine) 9·17 (3H, s, 20-H_3), 7·4 (1H, m, $W_{\frac{1}{2}}$ 8 Hz, 13-H), 6·92 (1H, dt, 15α-H), 5·35 (2H, m, $W_{\frac{1}{2}}$ 10 Hz, 6-H and 7-H), and 4·96 (2H, m, =CH_2).

Its acetate, prepared with acetic anhydride in pyridine, was an intractable foam, $\nu_{max.}$ 1776, 1745, 1651, and 879 cm^{-1}, τ 9·07 (3H, s, 20-H_3), 7·91 (3H, s, OAc), 5·47 (1H, t, *J* 6·5 Hz, 6β-H), 5·10br (1H) and 4·97br (1H) (=CH_2), and 4·18 (1H, d, *J* 6·5 Hz, 7α-H).

Oxidation of the A-*Homo*-18-*norkaurenolide* (24).—The norkaurenolide (100 mg) in dichloromethane (10 ml) was oxidised with an excess of the chromium trioxide–pyridine (1 : 2) reagent.[21] Recovery in ethyl acetate followed by crystallisation from ethyl acetate–light petroleum gave the 7-*oxo*-A-*homo*-18-*norkaurenolide* (25) as prisms (94 mg), m.p. 225—227° (decomp.) (Found: C, 76·2; H, 8·0. $C_{20}H_{26}O_3$ requires C, 76·4; H, 8·3%), $\nu_{max.}$ 1770, 1700, 1657, 909, and 893 cm^{-1}, τ 9·31 (3H, s, 20-H_3), 5·25 (1H, d, *J* 7 Hz, 6β-H), and 5·11br (1H) and 4·95br (1H) (=CH_2), *m/e* 314 (M^+, 14%), 299 (100), 257 (27), 211 (22), 171 (23), 123 (80), and 105 (49).

Oxidation of 18-*Fluoro*-7β-*hydroxykaurenolide* (9).—The fluorokaurenolide (4·45 g) was oxidised with an excess of the chromium trioxide–pyridine (1 : 2) reagent in dichloromethane. Recovery in ethyl acetate followed by crystallisation from ethyl acetate–light petroleum gave 18-*fluoro*-6α-*hydroxy*-7-*oxokaur*-16-*en*-19-*oic acid* 19,6α-*lactone* (11) as needles (4·15 g), m.p. 241—242° (Found: C, 72·0; H, 7·5; F, 5·95. $C_{20}H_{25}FO_3$ requires C, 72·3; H, 7·6; F, 5·7%), $\nu_{max.}$ 1777, 1708, 1662, and 892 cm^{-1}, τ (90 MHz) 9·24 (3H, s, 20-H_3), 7·32 (1H, d, *J* 7 Hz, 5-H), 5·50 (2H, 2 × 4 lines, *J* 47·5 and 9 Hz, 18-CH_2F), 5·07 (1H, d, *J* 7 Hz, 6β-H), and 5·10br (1H) and 4·94br (1H) (=CH_2), *m/e* 332 (M^+, 73%), 183 (8), 155 (37), 135 (19), 127 (23), 107 (27), 105 (45), and 91 (100).

Reduction of the Fluoroketone (11) *with Sodium Borohydride.*—The fluoroketone (4·1 g) in methanol (500 ml) was treated with sodium borohydride (5·5 g), added in portions at 0°, over 30 min. The mixture was stirred at room temperature overnight. Glacial acetic acid (30 ml) was added, and the methanol was removed *in vacuo*. Recovery in ethyl acetate gave 18-*fluoro*-6α,7α-*dihydroxykaur*-16-*en*-19-*oic acid* 19,6α-*lactone* (12) which crystallised from ethyl acetate–light petroleum as prisms (3·97 g), m.p. 176—178° (Found: C, 71·95; H, 8·25; F, 5·75%; *m/e* 334. $C_{20}H_{27}FO_3$ requires C, 71·8; H, 8·1; F, 5·7%; *M*, 334), $\nu_{max.}$ 3540, 1763, 1552, and 890 cm^{-1}, τ (90 MHz) 8·85 (3H, s, 20-H_3), 7·46br (1H, m, $W_{\frac{1}{2}}$ 8 Hz, 13-H), 5·92 (1H, d, *J* 7·5 Hz, 7β-H), 5·54 (2H, 2 × 4 lines, *J* 47 and 9 Hz, 18-CH_2F), 5·10 (1H, t, *J* 7·5 Hz, 6β-H), and 5·12br (1H) and 5·01br (1H) (=CH_2), ϕ^* 220·8 (td, *J* 47 and 4 Hz, 18-F); *m/e* 334 (M^+, 68%), 182 (32), 180 (33), and 127 (100).

Preparation of the Toluene-p-*sulphonate* (13) *of* 18-*Fluoro*-7α-*hydroxykaurenolide.*—The fluorokaurenolide (12) (3·9 g) was treated with toluene-*p*-sulphonyl chloride (8·2 g) in dry pyridine at room temperature for 10 days. Recovery in ethyl acetate gave a gum which was chromatographed on silica gel (15 × 6 cm). Elution with ethyl acetate–light petroleum (1 : 9 and 3 : 17) gave gummy crystals which were rechromatographed on a similar column. Elution with the same solvent followed by crystallisation from ethyl acetate–light petroleum gave 18-*fluoro*-6α-*hydroxy*-7α-p-*tolylsulphonyloxykaur*-16-*en*-19-*oic acid* 19,6α-*lactone* (13) (4·83 g) as needles, m.p. 140—142° (Found: C, 66·4; H, 6·95; F, 3·8; S, 6·65. $C_{27}H_{33}FO_5S$ requires C, 66·4; H, 6·75; F, 3·9; S, 6·55%), $\nu_{max.}$ 1780sh, 1769, 1666, 1600, 1365, and 1176 cm^{-1}, τ (90 MHz) 9·07 (3H, s, 20-H_3), 7·99 (d, *J* 6 Hz, 5-H), 7·62 (3H, s, Ar*Me*), 5·62 (2H, 2 × 4 lines, *J* 48 and 9 Hz, 18-CH_2F), 5·17 (t, *J ca.* 6·5 Hz, 6β-H), 5·13br (1H) and 5·03br (1H) (=CH_2), and 5·0 (d, *J* 7 Hz, 7β-H).

Ring Contraction of the 18-*Fluoro*-7α-*toluene*-p-*sulphonate* (13).—The toluene-*p*-sulphonate (4·0 g) and potassium hydroxide pellets (50 g) in t-butyl alcohol (500 ml) were were refluxed for 2·5 h in an atmosphere of nitrogen. The solvent was removed *in vacuo*, water was added, and the solution was extracted with ethyl acetate to give a 'neutral' fraction as a gum (1·85 g). The aqueous alkaline solution was acidified at 0° to pH 2 with concentrated hydrochloric acid, and extracted with ethyl acetate to give an acidic fraction as a gummy solid (240 mg).

The 'neutral' fraction was chromatographed on silica gel (30 × 3·5 cm). Elution with ethyl acetate–light petroleum (5 : 35 and 3 : 17) gave an intractable gum

[21] R. Ratcliffe and R. Rodehourst, *J. Org. Chem.*, 1970, **35**, 4000.

(75 mg). Elution with ethyl acetate–light petroleum (3 : 17 and 1 : 4) gave 1β-*fluoromethyl*-10β-*formyl*-4aα-*methyl*-8-*methylenegibbane*-1α-*carboxylic acid* (3) as a gum (1·43 g), which foamed *in vacuo* (Found: *m/e* 334·1949. $C_{20}H_{27}FO_3$ requires *M*, 334·1944), $\nu_{max.}$ ($CHCl_3$ film) 3420—2640, 2730, 1712br, 1662, and 880 cm^{-1}, τ (90 MHz) 9·17 (3H, s, 4aα-Me), 7·96 (d, *J* 12·5 Hz, 10a-H), 6·71 (1H, dd, 10-H), 5·72 (2H, 2 × 4 lines, *J* 47·5 and 9 Hz, 1β-CH_2F), 5·18br (1H) and 5·08br (1H) (=CH_2), and 0·23 (1H, dd, *J* 4·5 and $^6J_{HF}$ 1·5 Hz, CHO), ϕ^* 224·7 (t, *J* 47·5 Hz, CH_2F).

Preparation of the Fluorogibberellin A_{12} (4).—The fluoroaldehyde (3) (70 mg) in pure acetone (10 ml) was treated with an excess of Jones reagent (0·1 ml) at 0° for 1 h. Excess of the reagent was destroyed with methanol, the solvents were evaporated *in vacuo*, and water (10 ml) was added to the residue. Recovery in ethyl acetate followed by chromatography on silica gel (8 × 0·5 cm) and elution with ethyl acetate–light petroleum (3 : 17 and 1 : 4) afforded a gum which crystallised from ethyl acetate–dichloromethane–light petroleum to give 1β-*fluoromethyl*-4aα-*methyl*-8-*methylenegibbane*-1α,10β-*dicarboxylic acid* (4) (26 mg), m.p. 164—167° (Found: C, 68·0; H, 7·85; F, 5·2. $C_{20}H_{27}FO_4$ requires C, 68·5; H, 7·8; F, 5·4%), $\nu_{max.}$ 3200—2600, 1720, 1707, and 900 cm^{-1}, *m/e* 350 (M^+, 4%), 332 (20), 330 (18), 271 (27), 269 (38), 183 (23), 127 (41), 115 (67), and 91 (100).

Production of Fluorogibberellins by Gibberella fujikuroi.—*G. fujikuroi* ACC. 917 was grown in stirred fermentation (5 l) as previously described,[10] using B.D.H. silicone antifoaming agent. When the concentration of ammonium ion in the medium was approximately zero, a solution of the fluoroaldehyde (3) (1·06 g) in ethanol (55 ml), previously sterilised by means of a Seitz filter, was added. The fermentation was harvested after a further 96 h and the neutral and acidic fractions were isolated as previously described.[10]

The neutral fraction (0·94 g) was chromatographed on silica gel (15 × 1·5 cm). Gradient elution with ethyl acetate–light petroleum gave fujenal (45 mg) followed by 7β-hydroxykaurenolide (84 mg) and 7β,18-dihydroxykaurenolide (92 mg). The metabolites were identified by their i.r. spectra. For each metabolite microanalysis showed F, 0·0%. The fluoroaldehyde (3) and cyclonerodiol could not be detected by t.l.c. analysis.

The acidic fraction (4·87 g) was chromatographed on silica gel (30 × 3·5 cm) (250 ml fractions). Elution with ethyl acetate–light petroleum (3 : 17) (fractions 8—10) gave fujenal. Elution with ethyl acetate–light petroleum (1 : 3 and 7 : 13) (fractions 17—24) gave a gum (Found: F, 4·9%), which on t.l.c. [acetic acid–di-isopropyl ether (1 : 19)] showed *inter alia* a green spot of similar polarity to gibberellin A_9.

Further elution with ethyl acetate–light petroleum (7 : 13 to 11 : 9) gave gums, with only fraction 28 (160 mg) showing a high fluorine content (Found: F, 1·25%). Continued elution with ethyl acetate–light petroleum (3 : 2) and then with ethyl acetate gave crude gibberellic acid (*ca.* 900 mg). The fractions were crystallised from ethyl acetate–light petroleum and on analysis for fluorine gave the results in the Table.

Fraction no.	Weight (mg)	F (%)	Fluorogibberellic acid (%)	Fluorogibberellic acid (mg)
35	47	1·40	27	12
36	200	1·50	29	58
37	182	0·90	17	31
38—41	197	0·80	15	30
42—44	68	0·70	13	9

(Fluorogibberellic acid, $C_{19}H_{21}FO_6$ requires F, 5·2%).

Fraction 36, m.p. 216—218°, had an i.r. spectrum almost identical with that of gibberellic acid, τ [$(CD_3)_2CO$] 8·77 (s, 1β-Me), 7·32 (1H, d, *J* 11 Hz, 10a-H), 6·74 (1H, d, *J* 11 Hz, 10-H), 5·95 (1H, d, *J* 4 Hz, 2α-H), 5·10br (1H) and 4·75br (1H (=CH_2), 4·09 (1H, dd, 3-H), and 3·58 (1H, d, *J* 9 Hz, 4-H). The integral intensity of the methyl singlet at τ 8·77 corresponded to 2·15 ± 0·15H, *i.e.* to the presence of 29 ± 4% of fluorogibberellic acid, whilst weak signals at τ 6·5 and 5·7 (AB of ABX, $J_{AX} = J_{BX}$ *ca.* 46 Hz) and ϕ^* (pyridine) 231·3 (t, *J* 46 Hz), corresponded to $-CH_2F$ (Found: *m/e* 346 and 364. $C_{19}H_{22}O_6$ requires *M*, 346. $C_{19}H_{21}FO_6$ requires *M*, 364).

A sample (2 mg) from fractions 35—36 was methylated with ethereal diazomethane in the usual way. T.l.c. comparison with methyl gibberellate using two solvent systems [ethanol–benzene (3 : 97) and acetic acid–di-isopropyl ether (1 : 99) (development × 3 in both cases)] showed no separation.

To reduce the polarity, the ester was acetylated with acetic anhydride in pyridine at room temperature for 14 days. Recovery gave a gum which was compared with authentic methyl diacetylgibberellate by t.l.c. [development × 10 with ethanol–benzene (0·75 : 99·25)]. This produced a clear separation of the fluorinated triester (20) (R_F 0·56) from its unfluorinated analogue (R_F 0·49).

Fluorine-rich fractions from the column (115 mg) were converted into the crude methyl diacetyl esters (gum; 134 mg). The gum was applied to three p.l.c. plates (40 × 20 cm) which were developed with ethanol–benzene (1 : 99) (development × 20 of 25 cm). Recovery of material from the less polar band in acetone, followed by crystallisation from ethyl acetate–light pereoleum, gave 2β,7-*diacetoxy*-1β-*fluoromethyl*-4aα-*hydroxy*-10β-*methoxycarbonyl*-8-*methylenegibbane*-1α-*carboxylic acid* 1,4aα-*lactone* (20) (14 mg), m.p. 155—158° (Found: C, 61·95; H, 6·05; F, 3·95%; *m/e* 462·1694. $C_{24}H_{27}FO_8$ requires C, 62·3; H, 5·9; F, 4·1%; *M*, 462·1690), $\nu_{max.}$ ($CHBr_3$) 1783, 1732, 1660, and 899 cm^{-1}, τ (90 MHz), 8·00 (3H, s, OAc), 7·92 (3H, s, OAc), 6·93 (1H, dd, *J* 11 and $^5J_{HF}$ 3 Hz, 10-H), 6·50 (1H, d, *J* 11 Hz, 10a-H), 6·29 (3H, s, CO_2Me), 5·33 (2H, 2 × 4 lines, *J* 47 and 9 Hz, $-CH_2F$), 5·00br (1H) and 4·82br (1H) (=CH_2), 4·56br (1H, d, *J* 4 Hz, 2α-H), 4·12 (1H, d of dd, *J* 9, 4, and $^5J_{HF}$ 2 Hz, 3-H), and 3·60 (1H, dd, 4-H).

In a repetition of the fermentation, the fluoroaldehyde (1·2 g) was added to a stirred fermentation (6 l). Chromatography of the crude acidic fraction (5·79 g), followed by crystallisation, gave the following fractions of gibberellic acid (i) (234 mg) (Found: F, 1·25%), (ii) (186 mg) (Found: F, 0·85%). Later fractions contained no fluorine.

Isolation of the Fluorogibberellin A_9 (15).—Fractions 17—24 from the silica gel column of the preceding experiment were rechromatographed on silica gel (20 × 1·5 cm). Elution with ethyl acetate–light petroleum (1 : 4) followed by recrystallisation from chloroform–light petroleum gave needles (30 mg) of nearly pure fluorogibberellin A_9 (15), $\nu_{max.}$ 3170, 1744, 1735sh, 1658, 1160, 1184, and 884 cm^{-1}, τ 7·37 (d, *J* 11 Hz, 10a-H), 7·02 (1H, dd, *J* 11 and $^5J_{HF}$ 2 Hz, 10-H), 5·46 (2H, 2 × 4 lines, *J* 47·5 and 9 Hz, 1β-CH_2F), and 5·13br (1H) and 5·02br (1H) (=CH_2), *m/e* 334 ($C_{19}H_{23}FO_4$ requires *M*, 334).

A sample (1 mg) was converted into its methyl ester with diazomethane. Comparison with authentic gibberellin A_9 methyl ester by g.l.c. (at 190° using 5% SE30 on 80—90 mesh Anakrom; argon flow: 50 ml min^{-1}) showed the gibberellin fraction to be a mixture of the methyl ester of fluorogibberellin A_9 (retention time 15·75 min) (89%) and the methyl ester of gibberellin A_9 (retention time 13·75 min) (11%). T.l.c. comparison of the two acids with acetic acid–di-isopropyl ether (1 : 99) as eluant (development × 3) showed that the fluorogibberellin A_9 was more polar (R_F 0·61) than gibberellin A_9 (R_F 0·71).

P.l.c. of part of the above mixture (10 mg) in the same solvent system (development × 3) followed by recovery of material from the major (more polar) band in acetone gave an oil which crystallised from carbon tetrachloride as needles (8·5 mg) of 1β-*fluoromethyl*-4aα-*hydroxy*-8-*methyl*-*enegibbane*-1α,10β-*dicarboxylic acid* 1α,4aα-*lactone* (15), m.p. 204—205° (Found: C, 67·95; H, 6·8%; *m/e* 334·1572. $C_{19}H_{23}FO_4$ requires C, 68·2; H, 6·9%; *M*, 334·1580), g.l.c. (as above) of its methyl ester showed a purity >99·5%. The methyl ester showed *m/e* 348 (M^+, 9%), 316 (100), 288 (52), and 105 (11).

Attempted Separation of the Fluorogibberellic Acid–Gibberellic Acid Mixture.—(a) *By partition chromatography.* Fractions 35 and 36 from the experiment described above were chromatographed on Sephadex G-25 (85 g; 95 × 2·5 cm) in benzene–ethyl acetate–acetic acid–water (55 : 25 : 30 : 50) as described by Pitel *et al.*[17] The first fractions containing gibberellic acid were enriched in the fluoro-analogue (Found: F. 2·25%; equivalent to a content of 43 ± 4% of fluorogibberellic acid).

(b) *By p.l.c. of the biscarbonate esters* (21) *and* (23). Gibberellic acid containing 24% of the fluoro-acid was converted into the biscarbonate esters as described below. P.l.c. in ethanol–benzene (1 : 499) failed to separate the fluoro-compound.

Reaction of Methyl Gibberellate with 2,2,2-*Trichloroethyl Chloroformate.*—Methyl gibberellate–chloroform solvate (320 mg) in dry pyridine (8 ml) was stirred with the chloroformate (1 ml) at room temperature for 7 days. The dark red solution was poured into an excess of iced dilute hydrochloric acid, and the solution was extracted with chloroform. The extracts were washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and with water. Recovery gave a gum, which was chromatographed on silica gel (10 × 1 cm). Elution with ethyl acetate–light petroleum (1 : 19) gave a gum which crystallised from benzene as prisms (504 mg) of 4aα-*hydroxy*-10β-*methoxycarbonyl*-1β-*methyl*-8-*methylene*-2β,7α-*bis*-(2,2,2-*trichloroethylcarbonyldioxy*)*gibb*-3-*ene*-1α-*carboxylic acid* 1α,4aα-*lactone* (21), m.p. 162—164·5° (Found: C, 43·8; H, 3·6; Cl, 29·7. $C_{26}H_{26}Cl_6O_{10}$ requires C, 43·9; H, 3·7; Cl, 30·0%), $\nu_{max.}$ 1786, 1760, 1734, 1667, 1242, and 903 cm^{-1}, τ 8·75 (3H, s, 1β-Me), 7·24 (1H, d, *J* 10·5 Hz, 10-H), 6·63 (1H, d, *J* 10·5 Hz, 10a-H), 6·25 (3H, s, OMe), 5·29 (2H, s, $-OCH_2CCl_3$), 5·21 (2H, s, $-OCH_2CCl_3$), 4·95br (1H) and 4·75br (1H) ($=CH_2$), 4·78 (1H, d, *J* 4 Hz, 2α-H), 4·08 (1H, dd, 3-H), and 3·56 (1H, d, *J* 9·5 Hz, 4-H).

Reaction of Gibberellic Acid with 2,2,2-*Trichloroethyl Chloroformate.*—Gibberellic acid (300 mg) was treated with the chloroformate (1 ml) in dry pyridine (8 ml) at room temperature for 7 days. Recovery, as before, gave a gum which was chromatographed on silica gel (10 × 1 cm). Elution with ethyl acetate–light petroleum (1 : 9) gave a gum which crystallised from ethyl acetate–light petroleum as prisms of the 2,7-*biscarbonate* of gibberellic acid, m.p. 134—136° (Found: C, 42·8; H, 3·4; Cl, 30·1. $C_{25}H_{24}Cl_6O_{10}$ requires C, 43·1; H, 3·5; Cl, 30·5%), $\nu_{max.}$ 3240br, 1775sh, 1760br, 1732, 1666, 1240, and 900 cm^{-1}, τ 8·73 (3H, s, 1β-Me), 6·66 (1H, d, *J* 10·5 Hz, 10a-H), 5·27 (2H, s, $-OCH_2CCl_3$), 5·21 (2H, s, $-OCH_2CCl_3$), 4·95br (1H) and 4·76br (1H) ($=CH_2$), 4·77 (1H, d, *J* 4 Hz, 2α-H), 4·07 (1H, dd, 3-H), and 3·56 (1H, d, *J* 9·5 Hz, 4-H).

Attempted Removal of the Trichloroethoxycarbonyl Groups from the Ester (21).—The ester (180 mg) in absolute methanol (10 ml) was refluxed with activated zinc dust (180 mg) for 100 min.[14] The solution was filtered, the solid was washed with methanol, and the combined filtrates were evaporated to give a solid which was purified by p.l.c. Development with ethanol–benzene (3 : 97) gave *inter alia* a major band from which the material was recovered in acetone. It crystallised from dichloromethane to give prisms (71 mg), m.p. 248—249°, of 4aα-*hydroxy*-2β-*methoxy*-10β-*methoxycarbonyl*-1β-*methyl*-8-*methylene*-7-(2,2,2-*trichloroethylcarbonyldioxy*)*gibb*-3-*ene*-1α-*carboxylic acid* 1, 4a-*lactone* (22) (Found: C, 50·1; H, 4·7; Cl, 23·6. $C_{24}H_{27}Cl_3O_8,0{\cdot}5CH_2Cl_2$ requires C, 49·7; H, 4·8; Cl, 23·9%), $\nu_{max.}$ 1777, 1753, 1731, 1663, and 904 cm^{-1}, τ 8·80 (3H, s, 1β-Me), 7·24 (1H, d, *J* 10·5 Hz, 10-H), 6·68 (1H, d, *J* 10·5 Hz, 10a-H), 6·25 (3H, s, OMe), 6·19 (3H, s, OMe), 5·28 (2H, s, 7-OCH_2CCl_3), 4·95br (1H) and 4·75br (1H) ($=CH_2$), 4·86 (d, *J* 4 Hz, 2α-H), 4·08 (1H, dd, 3-H), and 3·58 (1H, d, *J* 9·5 Hz, 4-H).

Recovery of material from a band close to the origin gave impure methyl gibberellate (32 mg), identified by its n.m.r. spectrum.

Removal of the Trichloroethoxycarbonyl Groups from the Ester (21) *with Zinc in Acetic Acid.*—The ester (125 mg) was stirred with zinc dust (500 mg) in glacial acetic acid (4 ml) at room temperature for 6 h. The solution was filtered, the residue was washed with dichloromethane, and the filtrate was washed with sodium hydrogen carbonate solution and water. Recovery, followed by crystallisation from chloroform–light petroleum, gave methyl gibberellate (67 mg), identified by its i.r. spectrum.

Demethylation of Methyl Gibberellate (cf. *Ref.* 15).—A flask was charged with finely ground lithium hydride (300 mg) and flushed with dry, oxygen-free argon. Redistilled hexamethylphosphoramide (10 ml) was distilled into the flask from calcined 4A molecular sieves, followed by an excess of propane-1-thiol (*ca.* 1 ml). The mixture was stirred at room temperature in an atmosphere of argon, until gas evolution had ceased (*ca.* 1 h). An excess of the reagent (*ca.* 3 ml) was decanted through a plug of glass wool onto methyl gibberellate–ethyl acetate solvate (200 mg) and the suspension was stirred for 12 h, by which time the reaction mixture had become homogeneous. The solution was poured into an excess of dilute hydrochloric acid and was extracted with ethyl acetate. The extract was washed with sodium hydrogen carbonate solution and the aqueous layer was neutralised with dilute hydrochloric acid at 0°. Recovery in ethyl acetate gave a gum (168 mg) which crystallised from acetone–light petroleum as prisms (142 mg) (92%) of gibberellic acid, which was identified by its i.r. spectrum.

We thank Mr. I. S. Nixon [Imperial Chemical Industries (Pharmaceuticals Division)] for a gift of gibberellic acid and the S.R.C. for a Research Studentship (to J. H. B.).

[3/2576 *Received*, 20*th December*, 1973]