The Microbiological Production of Analogues of Mould Metabolites. Part 2.[1] Production of 9α-Fluorogibberellin A_4, 9α-Fluorogibberellin A_{14}, and other Fluoroterpenoids by *Gibberella fujikuroi*

By **Brian E. Cross** * and **Anton Erasmuson**, Department of Organic Chemistry, The University, Leeds LS2 9JT

ent-15β-Fluorokaur-16-en-19-oic acid (3) has been prepared from xylopic acid and fed to fermentations of *Gibberella fujikuroi* in the presence of AMO-1618. The products have been shown to include 9α-fluorogibberellin A_4 (28), 9α-fluorogibberellin A_{14} (17), 15α-fluorofujenal (23), 15α-fluoro-7β-hydroxykaurenolide (18), a metabolite which may be 15α-fluoro-1β,7β-dihydroxykaurenolide (21), and 1α-carboxy-2β-hydroxy-1β,4aα-dimethyl-8-methylenegibbane-10β,9α-carbolactone (25). In the absence of AMO-1618 *ent*-15β-fluorokaur-16-en-19-ol had no detectable effect upon a fermentation of *G. fujikuroi*.

THREE fluorinated analogues of biosynthetic precursors of the gibberellins have been shown to be transformed by *G. fujikuroi* into novel fluorogibberellins and related metabolites,[1-3] whilst a fourth acts as an enzyme inhibitor [4] in the same biological system. On the other hand some fluorinated *ent*-kaurenes, although biologically active in plant tests, had no detectable effect on the production of metabolites by the fungus.[5] In particular 15α-fluorokaurene (1) was not transformed by *Gibberella fujikuroi* into isolable mould metabolites.[5] However, since kaurenoic acid (2) lies farther along the biosynthetic pathway,[6] and its 15α-fluoro-derivative (3) is readily prepared from xylopic acid (4),[7] the effect of the fluoro-acid on the fermentation has been examined.†

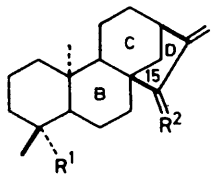
RESULTS AND DISCUSSION

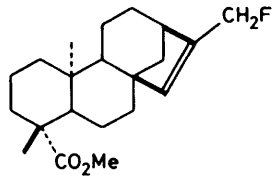
Fluorination of the methyl ester (5) of deacetylxylopic acid (6) [7] with 2-chloro-*NN*-diethyl-1,1,2-trifluoroethylamine (fluoramine) gave a mixture of the fluoro-esters (7) and (10) in the ratio 10 : 1 as shown by n.m.r. spectroscopy (*cf.* refs. 5 and 8) (see Experimental section). Separation of the mixture was difficult and the required ester (7) was more readily prepared [9] by fluorination of the hydroxy-ester (5) with diethylaminosulphur trifluoride. By analogy with the fluorination of other allylic alcohols with fluoramine [5,8] the 15-fluorine atom was assigned the α-configuration, *i.e.* fluorination takes place on the less-hindered α-face of the molecule.

The fluoro-ester (7) was converted into the corresponding acid (3) by reduction with lithium aluminium hydride to the alcohol (8), followed by oxidation with Jones reagent at room temperature (*cf.* ref. 4); on one occasion oxidation at 0 °C gave the aldehyde (9).

Addition of the fluoro-alcohol (8) to a fermentation of *G. fujikuroi* did not appear to affect the production of metabolites or give rise to fluorogibberellic acid, although the fluoro-compound was not recovered.

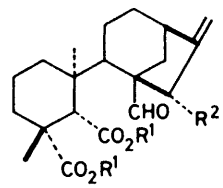
The fluoro-acid (3) was fed to two fermentations of *G. fujikuroi* and in each case AMO-1618 was also added to suppress the production of diterpenoids,[10,11] and hence to avoid the difficulty of separating any fluorinated metabolites from their proton analogues.[1,2] The acidic metabolites from the first of these fermentations tended to give mixed fractions on column chromatography but after methylation, careful p.l.c. gave, as the fastest-running band, the dimethyl fluoro-ester (11). The structure of the latter was deduced from its molecular



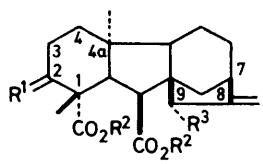


(10)

	R^1	R^2
(1)	Me	α-F, β-H
(2)	CO_2H	H_2
(3)	CO_2H	α-F, β-H
(4)	CO_2H	α-H, β-OAc
(5)	CO_2Me	α-H, β-OH
(6)	CO_2H	α-H, β-OH
(7)	CO_2Me	α-F, β-H
(8)	CH_2OH	α-F, β-H
(9)	CHO	α-F, β-H



	R^1	R^2
(11)	Me	F
(12)	Me	H
(13)	H	H



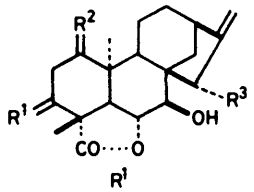
	R^1	R^2	R^3
(14)	α-H, β-OH	Me	F
(15)	α-H, β-OH	Me	H
(16)	O	Me	H
(17)	α-H, β-OH	H	F

formula and the close similarity of its R_F, i.r. ($\nu_{max.}$ 2 708, 1 724br, and 914 cm^{-1}), and n.m.r. spectra with the corresponding data for its proton analogue (12), (see Table 1), except for the protons attached to ring D. The chemical shifts and splitting pattern of the latter were in

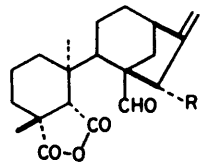
† For a preliminary account see ref. 2.

good agreement with the corresponding protons in the 15α-fluoro-ester (7).[9] It was assumed that the configuration of the fluorine atom in the acid (3) was retained in all the fluorinated metabolites.

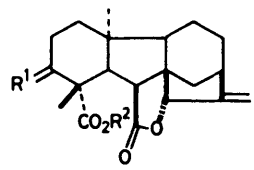
Material from a slower-running band was identified as



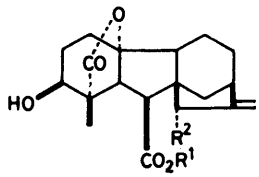
	R^1	R^2	R^3
(18)	H_2	H_2	F
(19)	α-H, β-OH	H_2	H
(20)	H_2	α-H, β-OH	H
(21)	H_2	α-H, β-OH	F
(22)	H_2	H_2	H



	R
(23)	F
(24)	H



	R^1	R^2
(25)	α-H, β-OH	H
(26)	α-H, β-OH	Me
(27)	O	Me



	R^1	R^2
(28)	H	F
(29)	Me	F
(30)	Me	H

the dimethyl ester (14) of 9α-fluorogibberellin A_{14} by its formula (accurate mass measurement) and ^1H n.m.r. spectrum (see Table 2). Furthermore in its ^{19}F n.m.r. spectrum the fluorine atom gave a signal at δ_F 159.49.

A second fermentation was fed with a larger amount of the fluoro-acid (3) in the presence of AMO-1618. Chromatography of the acidic metabolites on Kieselgel gave only poor separations; however, groups of similar fractions were identified by t.l.c. and were combined. P.l.c. then yielded 15α-fluorofujenal (23) with an R_F close to that of fujenal (24). Its structure was readily determined by i.r. spectroscopy ($\nu_{max.}$ 2 705, 1 854, 1 770, 1 732, 1 721, and 895 cm^{-1}) and the n.m.r. spectrum (see Table 1).

A slower-running band afforded an amorphous acid, $C_{20}H_{26}O_5$, shown by its n.m.r. spectrum in [$^2H_5$]pyridine to be a gibberellin [δ 4.24 (d, *J* 12.5 Hz, 10a-H) and 2.83 (d, *J* 12.5 Hz, 10-H)]. It was characterized as its methyl ester whose ^1H n.m.r. spectrum closely resembled that of the dimethyl ester (15) [13] of gibberellin A_{14} (see Table 2), except for the signal at δ 4.61 and the small couplings observed in the 8-methylene group. These data and the i.r. spectrum of the ester ($\nu_{max.}$ 3 520, 1 763, and 1 703 cm^{-1}) suggest structure (26) for the latter. This was strongly supported when irradiation at the frequency of the signal at δ 4.61 caused both methylene doublets to collapse to singlets. Furthermore a similar 7-deoxy-10β,9α-carbolactone, derived chemically from gibberellin A_7, showed a 9β-proton signal at δ 4.85 in the n.m.r. spectrum in [$^2H_5$]pyridine; [14] in this solvent the spectrum of the acid-lactone (25) showed the 9β-proton resonance at δ 4.62. The lactone (25) was assigned the 9α-configuration because steric factors almost certainly exclude a 10β ⟶ 9β-lactone (*cf.* ref. 14).

Oxidation of the ester (26) afforded the ketone (27), thus confirming the presence of a secondary hydroxy-group. The n.m.r. spectrum of the ketone (see Table 2)

TABLE 1

N.m.r. data for some protons in fluorinated kaurenoids and their proton analogues in deuteriochloroform at 90 MHz (δ, *J*/Hz)

Compound	5-H	6-H	7-H	13-H	15-H	17-H_2	18-Me *	20-Me *
(12)	2.73 (s)		9.77 (s)	2.79 (m)		5.25 (br) 5.15 (br)	1.28	0.97
(11)	2.53 (s)		9.84 (s)	2.94 (m)	5.16 (d, *J* 55)	5.53 (d, J_{HF} 5) 5.26 (d, J_{HF} 6)	1.32	1.11
(22)	1.76 (d, *J* 6)	4.64 (t, *J* 6)	4.41 (d, *J* 5)			5.02 (br) 4.88 (br)	1.28	0.86
(18)		4.80 (t, *J* 6.5)	*ca.* 4.5 (br, d, *J ca.* 8)	2.78 (br m)	4.92 (d, *J* 59)	5.40 (d, J_{HF} 7) 5.31 (d, J_{HF} 10)	1.33	0.90
(24)	2.63 (s)		9.77 (s)			4.94 (t, *J* 2) 4.84 (br)	1.37	0.87
(23)	2.62 (s)		9.81 (s)	2.89 (m) or 3.03 (m)	5.29 (d, *J* 55)	5.42 (d, J_{HF} 5) 5.35 (d, J_{HF} 6)	1.38	0.91
(20) [16]	2.24 (d, *J* 7)	4.70 (t, *J* 7)	4.40 (d, *J* 7)			4.87 (m) 5.00 (m)	1.28	0.75
(21)	2.07 or 2.14 (d, *J ca.* 6)	4.80 (t, *J* 7)	4.38 (m)	2.78 (m)	4.89 (d, *J* 59)	5.44 (br) 5.36 (br)	1.38	0.98

* Singlet.

P.l.c. of the neutral fraction from the fermentation gave a band containing the sesquiterpene, cyclonerodiol,[12] which was also found in the control fermentation. A later band afforded *ent*-15β-fluoro-7α-hydroxy-19-nor-kaur-16-ene-4β,6β-carbolactone (18), identified by accurate mass measurement, i.r. spectroscopy, and the n.m.r. spectrum (see Table 1).

shows downfield shifts of the 4a-methyl group and the 10a-proton as in the 2-ketone (16) [13] derived from the dimethyl ester of gibberellin A_{14} (see Table 2).

The origin of the 10,9α-lactone (25) is uncertain; one attempt to isolate it from a control fermentation failed,

but further work is needed to exclude the possibility that the compound is a new gibberellin. It is unlikely to be an artefact since the fluoro-acid (3) is stable to acetate in acetic acid for 72 h. However, it may arise by enzymically assisted displacement of the α-fluorine followed by attack of the 10β-carboxyl group at the 9α-position.

Further p.l.c. of later fractions from the Kieselgel column, followed by methylation, gave the methyl ester (29) of 9α-fluorogibberellin A_4 whose structure followed from spectroscopic data (see Table 2 and Experimental section).

The neutral fraction was subjected to p.l.c. and yielded the fluoro-kaurenolide (18), obtained above, and a band of lower R_F from which, after further p.l.c., a compound with an R_F almost identical with that of the 3β,7β-dihydroxykaurenolide (19)[15] was obtained. Its accurate mass spectra gave it the formula $C_{20}H_{27}FO_4$ and its ^1H n.m.r. spectrum (see Table 1) showed that it had the same rings B/C/D structure as the fluorokaurenolide (18). The position of the other hydroxy-group has not been assigned with certainty. In its n.m.r. spectrum the fluoro-diol gave a signal at δ 3.75 ($W_{\frac{1}{2}}$ 8 Hz) which indicated that the second hydroxy-group was secondary, but was inconsistent with a 3β-hydroxy-group as in structure (19) (3α-H at δ *ca.* 4.3)[15,16] or an 11α-hydroxykaurenolide (11β-H at δ 4.27).[16] However, in the n.m.r. spectrum (see Table 1) of the dihydroxykaurenolide (20), also produced by *G. fujikuroi*, the 1α-proton gives rise to a signal at δ 3.58 ($W_{\frac{1}{2}}$ 7 Hz)[16] in good agreement with the new fluorokaurenolide, which has therefore been provisonally assigned structure (21). This could not be confirmed by the chemical shift of the 5-proton,[16] since the signal due to the latter was not readily distinguishable from the methylene envelope, but doublets occurred at δ 2.14, 2.07, and 1.94 each with *J ca.* 7 Hz and one of these is presumably due to the 5-proton. The downfield shift of the 18 and 20 methyl groups in the diol, with respect to these groups in compound (20), casts some doubt on structure (21) and further work is needed to resolve this problem.

The lactone (25) is biologically active in tests on Tanginbozu dwarf rice, and in the lettuce hypocotyl and d-5 maize bioassays, but its activity is much less than that of gibberellins A_1, A_3, A_4, and A_9; it is however more active than gibberellin A_{13}.[17]

EXPERIMENTAL

Details of chromatographic materials and conditions used for the determination of physical data, *etc.*, have been reported.[18]

^{19}F N.m.r. spectra were determined for solutions in deuteriochloroform with hexafluorobenzene as internal standard on Bruker HF3 and JEOL FX90Q instruments at 84.66 and 84.305 MHz, respectively; δ_F values were calculated [19] with reference to trichlorofluoromethane using a value of δ_F 162.6 for hexafluorobenzene. Known metabolites were identified spectroscopically.

Methyl Deacetylxylopate (5).—Deacetylxylopic acid [7] was methylated with diazomethane; the *ester* (5) crystallised from aqueous methanol as prisms, m.p. 160—161 °C (Found: C, 75.6; H, 9.7. $C_{21}H_{32}O_3$ requires C, 75.9; H, 9.7%); $\nu_{max.}$ 3 530, 1 700, and 886 cm^{-1}; δ 5.10(br s) and 4.97 (d, *J* 2 Hz, 17-H_2), 3.76 (1 H, s, 15-H), 3.65 (3 H, s, OMe), 2.68 (1 H, m, 13-H), 1.18 (3 H, s, 18-Me), and 0.86 (3 H, s, 20-Me).

Fluorination of Methyl Deacetylxylopate (5) *with* 2-*Chloro*-NN-*diethyl*-1,1,2-*trifluoroethylamine* (*Fluoramine*).—The hydroxy-ester (5) (500 mg) in dichloromethane (10 ml) was stirred at 0 °C, treated with fluoramine (1.5 ml) during 25 min, allowed to reach room temperature, and then stirred for 2 h. Recovery by evaporation *in vacuo*, followed by chromatography of the residue on silica gel (20 g) and elution with ethyl acetate–light petroleum (1 : 25), gave a mixture (478 mg, 95%) shown by its n.m.r. spectrum to consist of methyl *ent*-15β-fluorokaur-16-en-19-oate (7) [9] and methyl *ent*-17-fluorokaur-15-en-19-oate (10) in the ratio 10 : 1. The latter was distinguished by n.m.r. signals at δ 5.48 (d, *J* 5 Hz, 15-H) and 4.86 (d, J_{HF} 47 Hz, 17-CH_2F) (*cf.* ref. 5). The mixture was not separable by crystallisation and was used in the following reaction.

The fluoro-ester (7) was more readily prepared [9] by treating the hydroxy-ester (5) with diethylaminosulphur trifluoride.[20]

ent-15β-*Fluorokaur*-16-*en*-19-*ol* (8).—The fluorester (401 mg) in ether (20 ml) was stirred with lithium aluminium hydride (100 mg) at room temperature for 24 h. Ethyl acetate (1 ml), water (0.2 ml), 2N-sodium hydroxide solution (0.5 ml), and Celite (1 g) were added, and the mixture was

TABLE 2

N.m.r. data (δ) for protons in some fluorogibberellins and their proton analogues in deuteriochloroform solution at 90 MHz

Compound	1β-Me [a]	2-H [b]	4aα-Me [a]	7-H	8-H_2	9-H	10-H [c]	10a-H [c]
(15)	1.20	4.14 (t, *J ca.* 3)	0.70	2.60 (m)	4.87 (br)		2.36	3.32
(14)	1.18	4.13 (m, $W_{\frac{1}{2}}$ 6)	0.73	2.7 (br)	5.43 (d, J_{HF} 7) 5.25 (d, J_{HF} 7)	4.86 dd (*J* 55 and 1)	2.39	3.31
(26)	1.46	4.20 (t, *J* 2.4)	0.70	2.76 (m)	5.26 (d, *J* 1.5) 5.15 (d, *J* 1)	4.61 ($W_{\frac{1}{2}}$ 3)	2.23	3.50
(16)	1.28		1.03		4.87 (br)		2.24	3.48
(27)	1.54		1.02		5.26 (d, *J* 1.5) 5.15 (s)	4.55 ($W_{\frac{1}{2}}$ 4)	1.97	3.72
(29)	1.16	3.84 (m, $W_{\frac{1}{2}}$ 6)		*ca.* 2.7 (m)	5.42 (d, J_{HF} 6.5) 5.32 (d, J_{HF} 6.5)	4.77 (d, *J* 55.5)	2.69	3.26
(30)	1.15	3.85 (m)			4.95 4.85		2.71	3.22

[a] Singlet. [b] Coupling constants and half-height widths are in Hz. [c] Doublet, *J* 10.5—12.5 Hz.

stirred and then filtered. Evaporation of the filtrate *in vacuo* gave the *fluoro-alcohol* (8) which crystallised from light petroleum as needles, m.p. 134—135 °C (288 mg; 78%) (Found: C, 78.5; H, 10.0; F, 6.5. $C_{20}H_{31}FO$ requires C, 78.4; H, 10.2; F, 6.2%); $\nu_{max.}$ 3 390br, 1 051, 1 010, and 995 cm^{-1}; δ_H 5.32 (d, *J* 6 Hz) and 5.22 (d, *J* 7 Hz) (17-H_2), 4.54 (1 H, d, *J* 56 Hz, 15-H), 3.59 (2 H, AB q, *J* 11 Hz, 19-CH_2OH), 2.83 (1 H, m, 13-H), 1.02 (3 H, s, 18-Me), and 0.97 (3 H, s, 20-Me); δ (C_6D_6) 5.36 (d, J_{HF} 6 Hz) and 5.12 (d, *J* 7 Hz, 17-H_2), 4.45 (d, *J* 55 Hz, 15-H), 3.38 (AB q, *J* 10.7 Hz, 19-CH_2OH), 2.67 (m, $W_{\frac{1}{2}}$ 10 Hz, 13-H), 0.92 (s, 18-Me), and 0.81 (s, 20-Me); δ_F 175.63 (d, *J* 55.2 Hz, 15-F).

ent-15β-*Fluorokaur*-16-*en*-19-*al* (9).—The fluoro-alcohol (30 mg) in acetone (5 ml) was treated dropwise at 0 °C with Jones reagent until a permanent brown colouration was obtained; the reaction mixture was then immediately quenched with sodium hydrogensulphite solution. The product was recovered in chloroform and crystallised from methanol as plates of the *fluoroaldehyde* (9) (20 mg; 67%), m.p. 97—102 °C (Found: *m/e*, 304.2203. $C_{20}H_{29}FO$ requires *M*, 304.2202); $\nu_{max.}$ 2 705, 1 720, 1 085, 1 076, 1 065, and 1 019 cm^{-1}; δ 9.77 (1 H, s, CHO), 5.33 (1 H, d, J_{HF} 6 Hz) and 5.23 (1 H, d, J_{HF} 7 Hz) (17-H_2), 4.57 (1 H, d, *J* 55 Hz, 15-H), 2.84 (1 H, m, $W_{\frac{1}{2}}$ 10 Hz, 13-H), 1.02 (3 H, s, 18-Me), and 0.90 (3 H, s, 20-Me); *m/e* 304, 289, 286, 284, and 275.

ent-15β-*Fluorokaur*-16-*en*-19-*oic Acid* (3).—The fluoro-alcohol (8) (100 mg) in acetone (10 ml) was treated with a two-fold excess of Jones reagent, stirred at room temperature for 2.5 h, and then the mixture was quenched with sodium hydrogensulphite solution. Recovery in chloroform gave the *acid* (3) (99 mg; 95%) which crystallised from methanol in prisms, m.p. 191 °C (decomp.) (Found: C, 75.1; H, 9.4; F, 6.3. $C_{20}H_{29}FO_2$ requires C, 75.0; H, 9.1; F, 5.9%); $\nu_{max.}$ 1 694, 1 267, 991, and 912 cm^{-1}; δ 5.32 (d, J_{HF} 6 Hz) and 5.22 (d, J_{HF} 7 Hz) (17-H_2), 4.54 (1 H, d, J_{HF} 56 Hz, 15-H), 2.80 (1 H, m, 13-H), 1.25 (3 H, s, 18-Me), and 0.95 (3 H, s, 20-Me); *m/e* 320 (M^+), 305, 300, 285, and 274.

Fermentations of G. fujikuroi ACC 917 *to which Fluorokaurenoids were added.*—Fermentation conditions have been described,[5,10] except that 1.5 l of medium was diluted to 4 l and extra glucose (50 g) was added.

The fluoro-compounds in ethanol solution (*ca.* 50 ml), previously sterilized by means of a Seitz filter, were added when the concentration of ammonium ions in the medium was *ca.* 0. The fermentations were harvested after a further 72—96 h and the metabolites were isolated as previously described.[10]

Fermentation 1. The fluoro-alcohol (8) (142 mg) was added. The yields of crude acidic and neutral fractions were very similar to those of the control. Crystalline gibberellic acid (120 mg) was isolated from the ' fed ' acidic fraction, but contained no fluorine (microanalysis). The non-crystalline acids also contained no fluorine (microanalysis) and gave methyl gibberellate on methylation and chromatography. None of the fluoro-alcohol was recovered from the neutral fraction.

Fermentation 2. AMO-1618 (40 mg) in water (50 ml) was added to both fermenters A and B 21 h after inoculation. The fluoro-acid (3) (390 mg) was added to fermenter A 27 h later. The fermenters were harvested after a further 94 h. (The pH of the culture filtrates from fermenters A and B were 3.25 and 3.41, respectively, at harvest).

Fermenter A afforded 299 mg of crude acids and 72 mg of neutrals, whereas fermenter B gave only 73 mg of acidic metabolites and 68 mg of neutral material.

The acidic fraction from fermenter A was chromatographed on silica gel (12 × 2 cm) and eluted with ethyl acetate–light petroleum (1 : 1) and with ethyl acetate. T.l.c. showed that all the fractions were mixtures and were only poorly separated by p.l.c. Consequently the acidic fractions were combined (210 mg) and were methylated with diazomethane and then purified by p.l.c. using ethyl acetate–chloroform (1 : 3) as eluant.

Material recovered from a band of R_F 0.8 afforded the *dimethyl fluoro-ester* (11) (7.6 mg), which crystallised from methanol, m.p. 148—153 °C (Found: *m/e*, 394.2155. $C_{22}H_{31}FO_5$ requires *M*, 394.2155); $\nu_{max.}$ ($CHCl_3$) 2 708, 1 724br, 1 126, and 914 cm^{-1}; δ_F 167.5 (dm, *J* 54 Hz, 15-F); *m/e* 394, 376, 363, 362, 356, 344, 342, and 227.

Material from the band at R_F 0.4 gave *dimethyl* 9α-*fluoro*-2β-*hydroxy*-1β,4aα-*dimethyl*-8-*methylenegibbane*-1α,10β-*dicarboxylate* (14) as a solid (15 mg) (Found: *m/e* 394.2159. $C_{22}H_{31}FO_5$ requires *M*, 394.2155); $\nu_{max.}$ ($CHCl_3$) 3 510, 3 420, 1 723, 1 602, 1 130, 974, and 915 cm^{-1}; δ_F 159.49 (dm, *J* 56 Hz, 9-F); *m/e* 394, 376, 362, 356, 344, 342, and 324.

Five small fractions were obtained from other bands but could not be identified.

The neutral fraction from fermenter A was purified by p.l.c. Development with ethyl acetate–chloroform (1 : 3) afforded bands at R_F 0.2, 0.3, and 0.7. Material from the band at R_F 0.3 was cyclonerodiol [12] (7.8 mg), which was also found (6.8 mg) in the neutral fractions from fermenter B.

Elution of the band at R_F 0.7 gave material (4 mg) which on t.l.c. (development with chloroform) ran slightly faster than 7-hydroxykaurenolide. It crystallised from ethyl acetate–light petroleum as prisms, m.p. 185 °C (decomp.), of ent-15β-*fluoro*-7α-*hydroxy*-19-*norkaur*-16-*ene*-4β,6β-*carbolactone* (18) (Found: *m/e* 334.1940. $C_{20}H_{27}FO_3$ requires *M*, 334,319,44); $\nu_{max.}$ 3 540, 1 771, 1 091, 934, and 907 cm^{-1}; *m/e* 334, 319, 316, 314, and 306.

Fermentation 3. The more concentrated medium described in ref. 5 was used. AMO-1618 (40 mg) in water (50 ml) was added to both fermenters 27 h after inoculation, and the fluoro-acid (3) (694 mg) was fed to fermenter A 47 h after inoculation. The fermenters were harvested after a further 100 h. The culture filtrates were acidified and worked-up in the usual way [5,10] to give the following results: fermenter A, acidic metabolites 676 mg, neutral metabolites 216 mg; fermenter B, acidic metabolites 174 mg, neutral metabolites 99 mg. Chromatography of the crude acids from fermenter A on a Kieselgel column and elution with ethyl acetate–light petroleum–formic acid, with gradually increasing concentration of ethyl acetate, and collection of 10-ml fractions, gave a poor separation. Groups of fractions were re-combined on the basis of t.l.c. evidence that they contained common major product(s).

P.l.c. of fractions 112—122 from the column [development with ethyl acetate–chloroform–formic acid (30 : 69 : 1)] gave two main bands. Material recovered from a band of R_F 0.8—0.9 had the same R_F as fujenal and crystallised from methanol in prisms (25 mg) of 15α-*fluorofujenal* (23), m.p. 194—197 °C (Found: *m/e*, 348.1740. $C_{20}H_{25}FO_4$ requires *M*, 348.1737); $\nu_{max.}$ 2 705, 1 854, 1 770, 1 732, 1 721, 1 010, 975, and 895 cm^{-1}; *m/e* 330, 328, 320, and 300.

A band at R_F 0.3 yielded 1α-*carboxy*-2β-*hydroxy*-1β,4aα-*dimethyl*-8-*methylenegibbane*-10β,9α-*carbolactone* (25) as an amorphous solid (23 mg) (Found: *m/e* 346.1785. $C_{20}H_{26}O_5$

requires *M*, 346.1780), δ([$^2H_5$]pyridine) 5.24 (2 H, m, $W_{\frac{1}{2}}$ 7 Hz, 17-H_2), 4.78 (1 H, br, $W_{\frac{1}{2}}$ 6 Hz, 2α-H), 4.62 (1 H, br, $W_{\frac{1}{2}}$ 4.5 Hz, 9β-H), 4.24 (1 H, d, *J* 12.5 Hz, 10a-H), 2.83 (1 H, d, *J* 12.5 Hz,10-H), 2.12 (3 H, s, 1β-Me), and 1.10 (3 H, s, 4aα-Me); *m/e* 346, 328, 313, 310, and 300.

Its *methyl ester* (26), prepared with diazomethane in methanol–ether, crystallised from methanol as needles, m.p. 280—281 °C (Found: M^+, 360.1932. $C_{21}H_{28}O_5$ requires *M*, 360.1937); $\nu_{max.}$ 3 520, 1 763 (γ-lactone), 1 703, and 902 cm^{-1}; *m/e* 360, 345, 342, 329, 328, and 300.

P.l.c. of column fractions 136—141 [development in ethyl acetate–chloroform–formic acid (30 : 69 : 1)] gave a band at R_F 0.25. Elution of the latter followed by methylation, afforded 9α-*fluoro*-2β-*hydroxy*-10β-*methoxycarbonyl*-1β-*methyl*-8-*methylenegibbane*-1α,4aα-*carbolactone* (29) which crystallised from methanol as plates, m.p. 179—180 °C (Found: M^+, 364.1682. $C_{20}H_{25}FO_5$ requires *M*, 364.1686); $\nu_{max.}$ 3 535, 1 746, 1 737, 1 020, and 923 cm^{-1}; δ_F 160.17 (dm, *J* 56 Hz, 9-F); *m/e* 364, 346, 344, 333, 326, 316, and 312.

P.l.c. of column fractions 30—111, 123—135, and 142—150 [development in ethyl acetate–chloroform (28 : 72)] gave a further quantity of the crude lactone (25) (44 mg) which was purified by methylation and p.l.c. (development in chloroform) to afford the pure compound (19 mg).

P.l.c. of column fractions 158—172 yielded gibberellic acid (40 mg); gibberellic acid production had probably begun before the AMO-1618 was added to this fermentation.

P.l.c. of the neutral metabolites from fermenter A [development with ethyl acetate–chloroform (1 : 9)] gave a number of bands. The fluorokaurenolide (18) was recovered from a band at R_F 0.37 and was identical (n.m.r. spectrum) with the specimen from Fermentation 2. A band at R_F 0.4 yielded cyclonerodiol. Bands of lower R_F were combined and subjected to further p.l.c. in ethyl acetate–chloroform (3 : 7). Elution of a band at R_F 0.3 gave a solid (*ca.* 4 mg) with almost the same R_F as 3β,7β-dihydroxykaurenolide (19). It may be ent-15β-*fluoro*-1β,7α-*dihydroxy*-19-*norkaur*-16-*ene*-4β,6β-*carbolactone* (21) (Found: M^+, 350.1886. $C_{20}H_{27}FO_4$ requires *M*, 350.1893), *m/e* 350, 332, 330 (base peak), 315, 314, and 312.

Oxidation of the Hydroxy-lactone (26).—The lactone (*ca.* 20 mg) in acetone (3 ml) was oxidised with an excess of Jones reagent (0.5 ml) at room temperature for 1.5 h. The product was recovered in the usual way and purified by p.l.c. (development with chloroform). Material from a band at R_F 0.3—0.35 crystallised from methanol as needles of 1α-*methoxycarbonyl*-1β,4aα-*dimethyl*-8-*methylene*-2-*oxogibbane*-10β,9α-*carbolactone* (27), m.p. 185—188 °C (Found: *m/e*, 358.1772. $C_{21}H_{26}O_5$ requires *M*, 358.1780); $\nu_{max.}$ 1 758, 1 729, 1 711, and 926 cm^{-1}; *m/e* 358, 340, 330, 326, and 312.

Stability of the Fluoro-acid (3).—The fluoro-acid was recovered after (*a*) standing in a solution of sodium acetate in acetic acid for 72 h, and (*b*) treatment with boiling methanol.

Attempt to Isolate the 10,9α-*Lactone* (25) *from a Control Fermentation* (*by J. Tideswell*).—The gibberellic acid was crystallised out (*cf.* ref. 5) from the acidic metabolites from one control fermentation (grown in the absence of AMO-1618),[5,10] and the residues were methylated and purified by p.l.c. A band with the same R_F as the lactone (26) was found, but on isolation it gave an intractable gum and none of the required compound could be detected.

We thank the S.R.C. for a research grant and Dr. P. R. Brook for the JEOL spectra.

[0/1961 *Received*, 22*nd December*, 1980]

REFERENCES

[1] Part 1, J. H. Bateson and B. E. Cross, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1131.
[2] B. E. Cross and A. Erasmuson, *J. Chem. Soc., Chem. Commun.*, 1978, 1013.
[3] B. E. Cross and P. Filippone, *J. Chem. Soc., Chem. Commun.*, 1980, 1097.
[4] K. Boulton and B. E. Cross, *J. Chem. Soc., Perkin Trans. 1*, 1981, 427.
[5] R. E. Banks, J. H. Bateson, B. E. Cross, and A. Erasmuson, *J. Chem. Research*, 1980, (*S*) 46; (*M*) 0817.
[6] P. Hedden, J. MacMillan, and B. O. Phinney, *Annu. Rev. Plant Physiol.*, 1978, **29**, 149.
[7] D. E. U. Ekong and A. U. Ogan, *J. Chem. Soc., C*, 1963, **311**.
[8] J. H. Bateson and B. E. Cross, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2409.
[9] B. E. Cross, A. Erasmuson, and P. Filippone, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1293.
[10] B. E. Cross and P. L. Myers, *Phytochemistry*, 1969, **8**, 79.
[11] M. W. Lunnon, J. MacMillan, and B. O. Phinney, *J. Chem. Soc., Perkin Trans. 1*, 1977, 2308.
[12] B. E. Cross, R. E. Markwell, and J. C. Stewart, *Tetrahedron*, 1971, **27**, 1663.
[13] B. E. Cross, *J. Chem. Soc. C*, 1966, 501.
[14] N. S. Kobrina, E. P. Serebryakov, V. F. Kucherov, G. Adam, and B. Voigt, *Tetrahedron*, 1973, **29**, 3425; E. P. Serebryakov and V. F. Kucherov, *Tetrahedron*, 1976, **32**, 2599.
[15] J. H. Bateson and B. E. Cross, *J. Chem. Soc., Perkin Trans. 1*, 1972, 1117.
[16] P. Heddon, J. MacMillan, and M. J. Grinsted, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2773.
[17] J. L. Stoddart, personal communication.
[18] B. E. Cross, M. R. Firth, and R. E. Markwell, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2930.
[19] R. J. Abraham, D. F. Wileman, and G. R. Bedford, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1027.
[20] W. J. Middleton, *J. Org. Chem.*, 1975, **40**, 574.