Microbiological Production of 9α-Fluorogibberellin A_4, 9α-Fluorogibberellin A_{14}, and other Fluoroterpenoids

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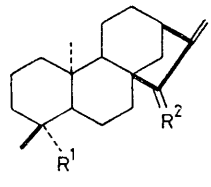
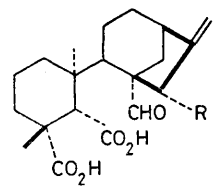
Summary 15α-Fluorokaurenoic acid (**5**) was prepared and fed to fermentations of *Gibberella fujikuroi* in the presence of AMO-1618; the products have been shown to include 9α-fluorogibberellin A_4 (**15**), 9α-fluorogibberellin A_{14} (**12**), 15α-fluorofujenal (**13**), 15α-fluoro-7β-hydroxykaurenolide (**16**), and 1α-carboxy-2β-hydroxy-1β,4aα-dimethylgibb-8-ene-10β,9α-carbolactone (**17**) which may be a new gibberellin.

DURING an investigation of fluorinated kaurenoids[1] as potential inhibitors of the biosynthesis of the gibberellins, 15α-fluorokaurenoic acid (**5**) was prepared from deacetylxylopic acid[2] by the route shown in the Scheme.

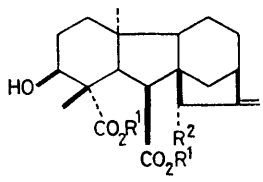


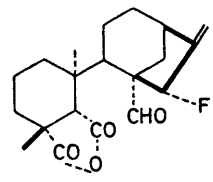


	R^1	R^2
(1);	CO_2H	α-H, β-OH
(2);	CO_2Me	α-H, β-OH
(3);	CO_2Me	α-F, β-H
(4);	CH_2OH	α-F, β-H
(5);	CO_2H	α-F, β-H
(6);	CH_2OH	H_2
(7);	CO_2H	H_2

(8); R = F

(9); R = H





(13)

	R^1	R^2
(10);	Me	F
(11);	Me	H
(12);	H	F

i ii iii iv
(1) → (2) → (3) → (4) → (5)

SCHEME. i, CH_2N_2; ii, Et_2NSF_3–CH_2Cl_2, 0 °C (W. J. Middleton, *J. Org. Chem.*, 1975, **40**, 574); iii, $LiAlH_4$; iv, CrO_3–Me_2CO–H^+.

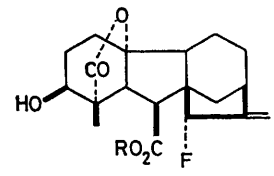
The fluoro-ester (**3**) was also prepared by reaction of the alcohol (**2**) with fluoroamine (*cf.* ref. 3). The 15-fluorine atom was assigned the α-configuration by analogy with the formation of 15α-fluorokaurene from both 15α- and 15β-hydroxykaurenes[1] and on the basis of n.m.r. data.

Addition of the fluoro-alcohol (**4**) to a fermentation[4] of *G. fujikuroi* (ACC 917) did not greatly reduce the yield of gibberellic acid, but since kaurenol (**6**) and kaurenoic acid (**7**) are intermediates in the biosynthesis of the gibberellins,[5] the possibility that (**5**) might act as a precursor of fluorogibberellins[6] was examined.

To avoid the problem of separating fluorogibberellins from their proton analogues[6] (*cf.* ref. 7) the fluoro-acid (**5**) was fed to stirred fermentations[4] of *G. fujikuroi* in the presence of AMO-1618 (10 mg l^{-1}), which blocks the biosynthesis[4,7] of the gibberellins prior to kaurene. In the first fermentation the acid (**5**) (390 mg) was added to a dilute broth (1·5 l of medium[4] diluted to 4 l); in the second fermentation the acid (695 mg) was added to a stronger medium (2·5 l of medium[4] diluted to 4 l). The metabolites were isolated in the usual way[4] and were characterised by comparison of their i.r., n.m.r., and mass spectra (including accurate mass measurements) with those of the corresponding proton analogues.†

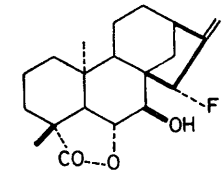
The acids from the first fermentation were methylated and purified by preparative layer chromatography (p.l.c.) on Kieselgel GF 254 in ethyl acetate–chloroform (1:3). The least polar band yielded the dimethyl ester of the 15α-fluoro-analogue (**8**) of the metabolite (**9**).[8] The next band afforded dimethyl 9α-fluorogibberellin A_{14} (**10**) (15 mg).

The acids from the second fermentation were chromatographed on a Kieselgel column, but the separation was poor. P.l.c. of material from groups of adjacent fractions in ethyl acetate–chloroform (3:7) gave (i) 15α-fluorofujenal (**13**), (ii) an acid, $C_{20}H_{26}O_5$ (23 mg), and (iii) 9α-fluorogibberellin A_4 (**15**) (20 mg) which was characterised as its methyl ester. The neutral fractions yielded 15α-fluoro-7β-hydroxykaurenolide (**16**).

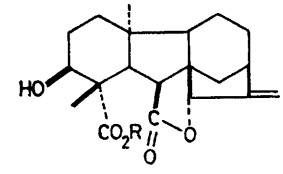


(14); R = Me

(15); R = H



(16)



(17); R = H

(18); R = Me

The amorphous acid, $C_{20}H_{26}O_5$, was characterised as its methyl ester which showed ν_{max} 3 520, 1 763, and 1 703 cm^{-1}, δ0·70 (s, 4aα-H_3), 1·46 (s, 1β-H_3), 2·23 (d, *J* 12 Hz, 10-H), 3·50 (d, *J* 12 Hz, 10a-H), 4·20 (t, *J* 2·4 Hz, 2-H), 4·61 (m, 9-H), and 5·15 (d, *J* 1 Hz) and 5·26 (d, *J* 1·5 Hz) (8-H_2). The n.m.r. data are in good agreement with those of the ester of gibberellin A_{14} (**11**),[9] except for the signal at δ4·61 and the small couplings observed in the 8-CH_2 group, and together with the i.r. spectrum suggest structure (**18**) for the ester. This was strongly supported when irradiation of the resonance at δ4·61 caused both of the methylene doublets to collapse to singlets.

The origin of the acid (**17**) is uncertain; it is unlikely to be an artefact since the fluoro-acid (**5**) is stable to acetate ions in acetic acid for 72 h, but it may be a new gibberellin,

† The ^1H n.m.r. spectra of compounds (**3**), (**4**), (**5**), (**10**), and (**14**) all show a doublet due to the >C*H*F grouping at δ4·45—4·86 (J_{HF} *ca.* 55 Hz).

since in the second fermentation the AMO-1618 was added after gibberellin production had begun.

The work described above shows that a combination of chemical and microbiological methods (*cf.* ref. 6) may be applicable to the production of a number of otherwise inaccessible analogues of mould metabolites.

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[1] R. E. Banks, J. H. Bateson, B. E. Cross, and A. Erasmuson, unpublished work.
[2] D. E. U. Ekong and A. U. Ogan, *J. Chem. Soc.* (*C*), **1968**, 311.
[3] J. H. Bateson and B. E. Cross, *J.C.S. Perkin I*, **1974**, 2409.
[4] B. E. Cross and P. Myers, *Phytochemistry*, **1969**, **8**, 79.
[5] J. MacMillan and R. J. Pryce in 'Phytochemistry', ed. L. P. Miller, vol. **3**, ch. **11**, van Nostrand, New York, 1973.
[6] J. H. Bateson and B. E. Cross, *J. C. S. Perkin I*, **1974**, 1131.
[7] M. W. Lunnon, J. MacMillan, and B. O. Phinney, *J.C.S. Perkin I*, **1977**, 2308.
[8] B. E. Cross, R. H. B. Galt, J. R. Hanson, P. J. Curtis, J. F. Grove, and A. Morrison, *J. Chem. Soc.*, **1963**, 2937.
[9] B. E. Cross, *J. Chem. Soc.* (*C*), **1966**, 501.