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ATTEMPTED MICROBIOLOGICAL PRODUCTION OF ω-FLUOROAVENACIOLIDE BY *ASPERGILLUS AVENACEUS*

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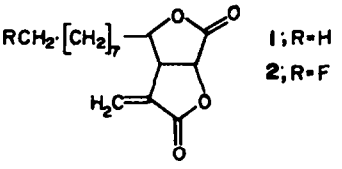
Abstract—12-Fluoro-3-oxododecanoic acid has been synthesised; neither this acid nor 10-fluorodecanoic acid were converted into fluoroavenaciolide by cultures of *Aspergillus avenaceus*.

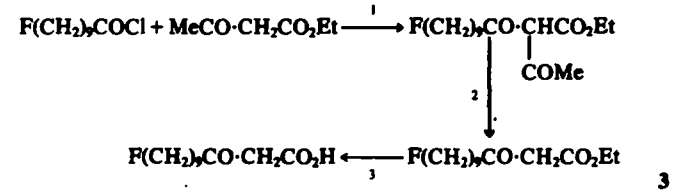
FOLLOWING our successful microbiological production of two fluorogibberellins,[1] we have attempted to utilise similar principles in the preparation of fluorinated analogues of polyketide derived mould products (see Ref. 2). *Aspergillus avenaceus* G. Smith produces the antifungal metabolite[3] avenaciolide (1) which is reported to be biosynthesised from 3-oxododecanoic and succinic acids,[4] and appeared therefore to be an appropriate organism for our experiments.

It seemed reasonable to assume that the end of the long alkyl chain of 3-oxododecanoic acid plays a relatively minor role in the biosynthesis of avenaciolide. On this basis, 12-fluoro-3-oxododecanoic acid was chosen as a suitable analogue of this precursor for feeding to cultures of *A. avenaceus*. The 12-fluoro-keto-acid was prepared from 10-fluorodecanoic acid[5] by the sequence shown in Fig. 1 (see Refs 6–8) and was found to be stable in the culture medium. An attempt to hydrolyse the ester 3 in acetic acid solution[7] gave 11-fluoro-2-oxoundecane.



1; R=H
2; R=F

Still cultures of *A. avenaceus* were fed with 12-fluoro-3-oxododecanoic acid (Table 1) in ethanol and dimethylsulphoxide solutions. The yields of avenaciolide in both the fed and solvent-blank cultures were greatly reduced and abnormal white growths appeared on the top of the mycelial mats. Subsequently investigations revealed that in contrast to many other fungi,[9] *A. avenaceus* would only grow normally if the concentration of ethanol was below 1.5%, but the fungus was unaffected by relatively large concentrations of sodium fluoroacetate (Table 1). The small amounts of avenaciolide from these feeds



1, Na in C_6H_6. 2, NaOEt-EtOH. 3, 1% NaOH solution.

Fig. 1.

Table 1. Feeding experiments with *A. avenaceus*

Substrate		Solvent		No. of flasks	Yield of crude avenaciolide	Yield of pure avenaciolide	Microanalyses
Compound	mg/flask		ml/flask	fed	mg/flask	mg/flask	Found:
12-Fluoro-3-oxododecanoic acid	25	EtOH	2·5	8	—	trace	—
12-Fluoro-3-oxododecanoic acid	30	Me_2SO	4	5	~12	3	—
Ethyl 12-fluoro-3-oxododecanoate	50	EtOH	0·5	3	60	—	C, 67·85; H, 8·3; F, 0% *
10-Fluorodecanoic acid	50	EtOH	4	1	16	9	C, 67·5; H, 8·55; F, 0%
10-Fluorodecanoic acid	50	EtOH	0·5	3	38	—	C, 67·85; H, 8·5; F, 0%
Sodium fluoroacetate	3–80	H_2O	0·25–6	6	20	13	C, 67·85; H, 8·4; F, 0%

* Calc. for $C_{15}H_{22}O_4$: C, 67·6; H, 8·3%

were examined by NMR and mass spectroscopy, and where possible by microanalysis, but no evidence for the presence of the ω-fluoro-analogue (2) was obtained. Ethyl 12-fluoro-3-oxododecanoate (3) was also fed (Table 1) since it was possible that the ester might enter the cells and then undergo hydrolysis to give the keto-acid *in situ*; a good yield of avenaciolide was obtained, but it contained no detectable amount of the fluoro-analogue.

If 3-oxododecanoic acid is a precursor of avenaciolide then it is reasonable to assume that decanoic acid might act as a "starter" unit (see Ref. 10). Consequently 10-fluorodecanoic acid was fed to the fungus (Table 1) and the avenaciolide was isolated, but again there was no evidence that ω-fluoroavenaciolide had been produced. It was concluded that *A. avenaceus* is probably incapable of utilising the fluoro-acids for the production of significant amounts of fluoroavenaciolide (2).

EXPERIMENTAL

Details of chromatographic materials and conditions used for the determination of physical data, etc. have been reported.[1]

10-*Fluorodecanoic acid.* Prepared by the literature method,[5] the acid, m.p. 49–49.5°, showed τ 7.60 (2H, t, *J* 6.5 Hz, 2-H_2) and 5.51 (2H, dt, *J* 47 and 6 Hz, 10-H_2); ϕ^* 218.7 (7 lines, 10-F).

Ethyl 2-acetyl-12-fluoro-3-oxododecanoate and ethyl 12-fluoro-3-oxododecanoate. 10-Fluorodecanoyl chloride, prepared by treating the acid (5.0 g) with thionyl chloride in benzene under reflux followed by evaporation *in vacuo* was a gum, ν_{max}(film) 1800 cm^{-1}.

Ethyl acetoacetate (3.9 g) in benzene (30 ml) was treated with Na (605 mg) and then the above acid chloride in benzene was added dropwise. The mixture was refluxed for 15 min, poured onto ice, and acidified with H_2SO_4 (5%), EtOH (25 ml) was added, the layers were separated, and the aqueous phase was extracted with benzene. The combined extracts were washed with H_2O-EtOH (9:1); recovery afforded an orange oil (8.1 g) containing ethyl 2-acetyl-12- fluoro-3-oxododecanoate.

The oil and Na (650 mg) in EtOH (20 ml) were left at room temp. for 12 hr and were poured onto ice and acidified with 2N H_2SO_4. Recovery in ether gave a red gum (7.7 g) which was chromatographed on silica gel (24 × 4 cm). Elution with EtOAc—light petroleum (1:49) gave unchanged *ethyl 2-acetyl-12-fluoro-3-oxododecanoate* as an oil (Found: *m/e* 302.1896. $C_{16}H_{27}FO_4$ requires: *M*, 302.1893), ν_{max} 1740 br; τ 7.63 (3H, s, COMe), 7.65 (2H, t, *J* 6.5 Hz, 4-H_2), 5.82 (2H, q, *J* 6.5 Hz, OCH_2Me) and 5.55 (2H, dt, *J* 47 and 6.5 Hz, 12-H_2).

Elution with EtOAc—light petroleum (1:19) afforded *ethyl-12-fluoro-3-oxododecanoate* (3; 1.71 g) which crystallised at low temp. as rosettes, m.p. 27–29° (Found: *m/e* 260.1789. $C_{14}H_{25}FO_3$ requires: *M*, 260.1788), ν_{max} 3420, 1742, 1720, 1650 and 800 cm^{-1}; τ 7.45 (2H, t, *J* 6 Hz, 4-H_2), 6.57 (2H, 's, 2-H_2), 5.77 (2H, q, *J* 7 Hz, OCH_2Me) and 5.52 (2H, dt, *J* 47 and 6 Hz, 12-H_2).

Further elution gave 10-fluorodecanoic acid.

12-*Fluoro-3-oxododecanoic acid.* The crude ester (200 mg) from the preceding experiment was stirred with NaOH soln (1%; 30 ml) at room temp. for 20.5 hr. Careful acidification with dil. HCl precipitated 12-*fluoro-3-oxododecanoic acid* which was collected by filtration, m.p. (rapid heating) 73–75° (decomp.) (Found: C, 61.3; H, 9.05; F, 8.95%; *m/e* 232. $C_{12}H_{21}FO_3$ requires: C, 62.0; H, 9.1; F, 8.2%; *M*, 232), ν_{max} 3200 br, 1725 and 1700 cm^{-1}; τ 7.31 (t, *J* 7 Hz, 4-H_2), 6.46 (s, 2-H_2) and 5.55 (2H, dt *J* 48 and 6 Hz, 12-H_2). (All samples of the keto-acid contained small amounts of 11-fluoroundecan-2-one).

The keto-acid was recovered after an ethanolic soln (23 mg in 2.5 ml) was added to sterile culture medium (200 ml) and left to stand for 50 hr.

Its *methyl ester*, prepared with diazomethane, was a solid (Found: *m/e* 246.1631. $C_{13}H_{25}FO_3$ requires: *M*, 246.1624).

11-*Fluoroundecan-2-one.* Ethyl 12-fluoro-3-oxododecanoate (1.55 g) in glacial HOAc (30 ml) was stirred at room temp. and conc. HCl was added until the soln became turbid. After 60 hr the soln was evaporated *in vacuo* and the product was recovered in ether. Chromatography on silica gel (20 × 2 cm) and elution with EtOAc—light petroleum (1:39) yielded 11-*fluoroundecan-2-one* (955 mg) as an oil (Found: *m/e* 188.1578. $C_{11}H_{21}FO$ requires: *M*, 188.1576), ν_{max}(film) 1720 cm^{-1}; τ 7.9 (3H, s, 1-H_3), 7.61 (2H, t, *J* 7 Hz, 3-H_2) and 5.56 (2H, dt, *J* 48 and 6 Hz, 11-H_2).

Feeding experiments. Aspergillus avenaceus G. Smith (C.M.I. 16140) was grown as a surface culture[3] on Czapek-Dox medium (200 ml) in 1 l penicillin vessels at 26°. The fluoro-acids were dissolved in the appropriate solvent, sterilised by means of a Seitz filter, and added to 6–7 day old cultures. The flasks were harvested after a further 7–8 days and the avenaciolide was isolated by the literature method.[3] The yield of crystalline avenaciolide from control flasks increased, during the course of the investigation, from *ca.* 20 to *ca.* 80 mg/flask. Blank cultures were treated with sterile solvent only.

Avenaciolide from fed cultures (see Table 1) was crystallised once and then examined by TLC, IR, NMR, and where possible by microanalysis for fluorine. All the samples were subjected to mass spectroscopy with the multiplier set at greatly increased gain in a search for ions at *m/e* 284 and 285† ($C_{15}H_{21}FO_4$ requires *M*, 284). In every case the avenaciolide from fed cultures was indistinguishable from a pure reference sample.

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†Avenaciolide gives a small molecular ion, but the M + 1 ion is much more intense.