Fluoroacetaldehyde: a precursor of both fluoroacetate and 4-fluorothreonine in *Streptomyces cattleya*

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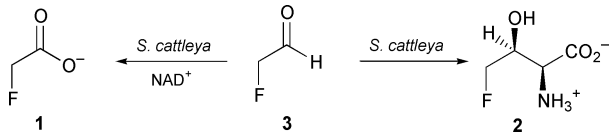
Fluoroacetaldehyde is converted to fluoroacetate and 4-fluorothreonine in *Streptomyces cattleya* indicating that it is the biosynthetic precursor of both of these secondary metabolites.

Organic compounds containing a C–F bond are extremely rare in living organisms; only about a dozen have been identified, the majority in plants.[1] Fluoroacetate (FAc) **1** is the most widespread fluorinated natural product and its biosynthesis in plants has been the subject of investigation and some speculation for the past 50 years. Despite this interest little progress has been achieved in elucidating the mechanism of fluorination or the metabolic route involved.[2] However the discovery that FAc **1** and L-4-fluorothreonine (4-FT) **2** are secondary metabolites of *Streptomyces cattleya* has provided a convenient microbial system in which to study the biosynthesis of the C–F bond.[3]

Our investigations into the origin of these metabolites in *S. cattleya*[4–7] have consistently shown that, in terms of magnitude and regiochemistry, isotope incorporations into C-1 and C-2 of FAc **1** always mirror those into C-3 and C-4 of 4-FT **2** from a given isotopically labelled precursor. This finding suggests a single fluorination enzyme in *S. cattleya*. As the possibility of direct conversion of one fluorometabolite to the other has been eliminated,[4,5] each fluorometabolite must therefore arise by further metabolism of a common fluorinated intermediate. We now report results which indicate that this metabolic precursor is fluoroacetaldehyde **3**.

An aqueous sample of fluoroacetaldehyde **3** was prepared by PDC oxidation of fluoroethanol in dichloromethane, followed by distillation of the volatiles into water. The preparation contained both fluoroacetaldehyde **3** and fluoroethanol (ratio ~1:3). The fluoroacetaldehyde was characterized as the DNP derivative.[8] Incubation of resting cells suspensions[7] of *S. cattleya* with the fluoroacetaldehyde preparation (2 mM with respect to fluoroacetaldehyde in 50 mM MES buffer, pH 6.5, containing 176 mg wet wt cells ml^{-1}) resulted in the efficient and almost stoichiometric conversion of fluoroacetaldehyde **3** to FAc **1** over a 3 h period (Scheme 1). Analysis of the culture supernatant by ^{19}F-NMR indicated that only the fluoroacetaldehyde **3** was converted to FAc **1**, the fluoroethanol remaining unmetabolised. Previous studies have demonstrated[5] that fluoroethanol is metabolically inert in *S. cattleya* and this was confirmed by control experiments with resting cells which did not reveal any oxidation of fluoroethanol to fluoroacetate. To further investigate the conversion of fluoroacetaldehyde **3** to FAc **1** cell-free extracts of *S. cattleya* were prepared with a French press. The extract oxidised fluoroacetaldehyde to fluoroacetate only in the presence of NAD^+ ($NADP^+$ was ineffective), demonstrating that an aldehyde dehydrogenase mediated the conversion. The purified enzyme[9] also oxidised acetaldehyde, chloroacetaldehyde, glycolaldehyde and propionaldehyde (34, 79, 116 and 30% of the enzyme activity with fluoroacetaldehyde respectively) indicating that *S. cattleya* possesses a relatively non-specific NAD^+-dependent aldehyde dehydrogenase which efficiently converts fluoroacetaldehyde **3** to FAc **1**.



1 **3** **2**

Scheme 1

Sanada *et al.*[3] proposed that 4-FT **2** might arise by an aldol condensation between fluoroacetaldehyde and glycine, such that the latter contributes C-3 and C-4 of the amino acid. However, in experiments using [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-glycine we have demonstrated[7] that glycine is not the condensing nucleophile as it is not incorporated directly into C-1 and C-2 of 4-FT **2**, so at present the origin of C-1 and C-2 of 4-FT **2** is unknown. Nevertheless fluoroacetaldehyde is an attractive precursor for C-3 and C-4 of 4-FT **2**. To establish a role for this compound in 4-FT **2** biosynthesis, its effect on 4-FT **2** production by resting cell suspensions of *S. cattleya* was measured by monitoring concentrations in culture supernatants using an HPLC assay.[10] The data from this experiment are presented in Fig. 1. When resting cell suspensions were incubated over a period of 6 h with the fluoroacetaldehyde–fluoroethanol preparation (176 mg wet wt cells ml^{-1} in 50 mM MES buffer pH 6.5, 2 mM fluoroacetaldehyde, 6 mM fluoroethanol), 4-FT **2** concentrations increased linearly with time ultimately attaining 49 μM. In suspensions with no supplementation or those incubated with 6 mM fluoroethanol alone, concentrations of 4-FT **2** did not exceed 7 μM after 6 h. Suspensions supplemented with 2 mM NaF (the optimal F^- concentration for fluorometabolite biosynthesis[4]) yielded 26 μM 4-FT **2** over the same time period. When 2 mM FAc **1** was

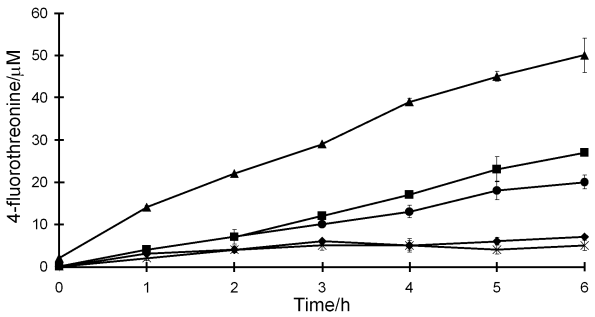


Fig. 1 L-4-Fluorothreonine **2** concentration in supernatant of resting cell suspensions of *S. cattleya* after addition of (a) fluoroacetaldehyde (2 mM)–fluoroethanol (6 mM) (▲); (b) NaF (2 mM) (■); (c) fluoroacetate (2 mM) (●); (d) fluoroethanol (6 mM) (**x**); (e) no supplementation (⧫). Data determined by HPLC analysis.[10] Error bars indicate the SD of triplicate samples.

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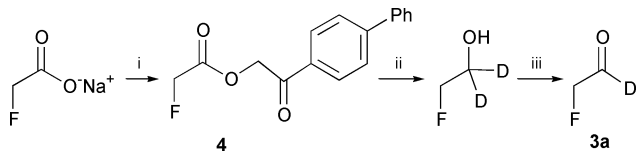
incubated with cell suspensions a slight enhancement of 4-FT **2** biosynthesis was observed (19 μM after 6 h). As previous investigations involving incubation of [$^2H_2$]-fluoroacetate with cell suspensions of *S. cattleya* had demonstrated no direct conversion of FAc **1** to 4-FT **2**, this increase can probably be attributed to defluorination of FAc **1** and subsequent *de novo* synthesis of 4-FT **2** from F^-. Such cell suspensions are known to have a limited defluorinating activity with FAc **1**.[5] Thus fluoroacetaldehyde clearly stimulates 4-FT **2** biosynthesis in *S. cattleya* almost doubling the rate recorded in suspensions incubated under optimal F^- concentrations, an enhancement which is not explicable in terms of metabolism *via* fluoroethanol or fluoroacetate.

The biosynthetic relationship between fluoroacetaldehyde **3** and 4-FT **2** was further investigated using [1-$^2H_1$]-fluoroacetaldehyde **3a**, a synthesis of which was developed, by an adaptation of a previously reported synthetic route for isotopically labelled ethanol[11] (Scheme 2). The [1-$^2H_1$]-fluoroacetaldehyde preparation contained 5% [1,2-$^2H_2$]-fluoroacetaldehyde as an impurity. [1-$^2H_1$]-Fluoroacetaldehyde **3a** was administered as above to cell suspensions of *S. cattleya* and the incorporation of deuterium into C-3 of 4-FT **2** was monitored by GC-MS[12] over time.



Scheme 2 i. *p*-Phenylphenacyl bromide, 18-crown-6, acetonitrile–toluene (1:1); ii. LiAl^2H_4, diethyl ether, 2-phenoxyethanol quench, distillation; iii. PDC, diethyl ether–dichloromethane (1:9).

The GC-MS data presented in Table 1 show substantial incorporation of a single ^2H atom into (C-2+C-3+C-4) of 4-FT **2** after 7 h. This label can only reside on the C-3–C-4 fragment of 4-FT **1** as there is no significant labelling at C-2. During the same time period there was also a small but significant incorporation (1.7%) of two ^2H atoms into these positions of the amino acid. It is revealing that the ratio of *M*+1:*M*+2 for (C-3+C-4) directly reflects the ratio (~20:1) of $^2H_1$:$^2H_2$ label in the fluoroacetaldehyde administered to the cells. Similar results were obtained for the samples incubated for 20 h. These findings provide compelling evidence that fluoroacetaldehyde **3** is incorporated as an intact unit into C-3 and C-4 of 4-FT **2**. Interestingly the incubation of resting cell suspensions with 0.25 mM chloroacetaldehyde resulted in the detection in the supernatant of small quantities of an amino acid with a mass spectrum similar to that predicted for 4-chlorothreonine.[13] This observation provides further support for the participation of fluoroacetaldehyde in an aldol condensation leading to 4-FT **2** biosynthesis.

Table 1 ^2H Incorporation into 4-FT **2** after labelled fluoroacetaldehyde ([1-^2H]-, 95%; [1,2-$^2H_2$]-, 5%) was incubated with resting cell suspensions of *S. cattleya*. The data are corrected for natural abundances. Means and standard deviations were obtained from 10 replicate analyses

	^2H-Incorporation (%)			
	(C-1+C-2)		(C-2+C-3+C-4)	
Incubation time (h)	M+1	M+2	M+1	M+2
7	< 0.5	< 0.5	33.5 ± 0.09	1.7 ± 0.05
20	< 0.5	< 0.5	22.4 ± 0.10	1.2 ± 0.04

In conclusion we have demonstrated that cell free extracts of *S. cattleya* contain a fluoroacetaldehyde dehydrogenase which efficiently converts fluoroacetaldehyde to FAc **1**. Fluoroacetaldehyde also markedly enhances the biosynthesis of 4-FT **2** by resting cells of *S. cattleya*. Furthermore incubation of resting cells with ^2H-labelled fluoroacetaldehyde indicates incorporation of the compound as an intact unit into C-3 and C-4 of 4-FT **2**. These observations are consistent with a role for fluoroacetaldehyde as the common intermediate in the biosynthesis of both fluorometabolites and it may emerge that fluoroacetaldehyde is the immediate post fluorination product.

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Notes and references

1 D. O'Hagan and D. B. Harper, *J. Fluorine Chem.*, 1999, **100**, 127.
2 D. B. Harper and D. O'Hagan, *Nat. Prod. Rep.*, 1994, **11**, 4.
3 M. Sanada, T. Miyano, S. Iwadare, J. M. Williamson, B. H. Arison, J. L. Smith, A. W. Douglas, J. M. Liesch and E. Inamine, *J. Antibiotic.*, 1986, **39**, 259.
4 K. A. Reid, R. D. Bowden and D. B. Harper, in *Naturally Produced Organohalogens*, eds. A. Grimvall and E. W. B. de Leer, Kluwer Academic Publishers, Dordrecht, 1995, pp. 269-279.
5 K. A. Reid, J. T. G. Hamilton, R. D. Bowden, D. O'Hagan, L. Dasaradhi, M. R. Amin and D. B. Harper, *Microbiology*, 1995, **141**, 1385.
6 J. Nieschalk, J. T. G. Hamilton, C. D. Murphy, D. B. Harper and D. O'Hagan, *J. Chem. Soc., Chem. Commun.*, 1997, 799.
7 J. T. G. Hamilton, C. D. Murphy, M. R. Amin, D. O'Hagan and D. B. Harper, *J. Chem. Soc., Perkin Trans. 1*, 1998, 759.
8 A dinitrophenylhydrazine (DNP) derivative of fluoroacetaldehyde **3** was prepared by adding the aqueous solution of fluoroacetaldehyde to the DNP solution and the resultant precipitate filtered and recrystallised from ethanol. Melting point: 143–144 °C; ^1H NMR δ_H (400 MHz, $CDCl_3$): 11.17 (1H, s, N*H*), 9.14 (1H, s, (C-3)-*H*), 8.36 (1H, m, (C-5)-*H*), 7.98 (1H, m, (C-6)-*H*), 7.64 (1H, dd, $^3J_{HH}$ = 5.2 Hz, $^3J_{HF}$ = 10.4 Hz, (C-7)-*H*), 5.17 (2H, dd, $^2J_{HF}$ = 46.4 Hz, $^3J_{HH}$ = 5.2 Hz, (C-8)-*H*); δ_C (100 MHz, $CDCl_3$): 80.9 (C-8, d, $^1J_{CF}$ = 166.4 Hz), 114.6 (C-6), 123.2 (C-3), 130.1 (C-5), 138.9 (C-7), 143.5 (C–NO_2), 143.7 (C–NO_2), 144.7 (C-1); δ_F (376 MHz, $CDCl_3$): −222.61 (dt, $^2J_{FH}$ = 46.4 Hz, $^3J_{FH}$ = 6.4 Hz); HRMS (EI) $C_8H_7FN_4O_4$: Calculated (M^+) 242.04513, found 242.0451.
9 Details of the purification of this enzyme will be reported elsewhere.
10 4-FT **2** in culture supernatants was determined using a fluorimetric HPLC procedure similar to that described by L. A. Sternson, J. F. Stobaugh and A. J. Repta *Anal. Biochem.*, 1985, **144**, 233. Precolumn derivatisation with *o*-phthalaldehyde–mercaptopropionic acid reagent was followed by elution from a reversed phase HPLC column. The detection limit of the method was 2 μM in the culture supernatant.
11 D. E. Cane, T.-C. Laing and H. Hasler, *J. Am. Chem. Soc.*, 1982, **104**, 7274.
12 For studies of incorporation of ^2H into 4-FT the per-trimethylsilylated derivative was prepared and analysed by GC-MS.[7] The MS was employed in the selected ion monitoring mode measuring ion currents at *m/z* 218 (M), 219 (M+1) and 220 (M+2) for incorporation onto (C-1+C-2) and at *m/z* 236 (M), 237 (M+1) and 238 (M+2) for incorporation onto (C-2+C-3+C-4).
13 GC-EIMS data for per-trimethylsilyl derivative of 4-chlorothreonine: *m/z* = 218 (100), 252 (45), 254 (15), 73 (38), 116 (14), 147 (10), 354 (3), 356 (1). 4-Chlorothreonine has been reported as a metabolite of *Streptomyces* sp. OH–5093 by H. Yoshida, N. Arai, M. Sugoh, J. Iwabuchi, K. Shioiomi, M. Shinose, Y. Tanaka and S. Omura, *J. Antibiot.*, 1994, **47**, 1165.

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