Biosynthesis of fluoroacetate and 4-fluorothreonine by *Streptomyces cattleya*. Glycine and pyruvate as precursors

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Using ^{19}F NMR spectroscopy, ^{13}C-labelled glycines and pyruvates are shown to be incorporated at high levels into fluoroacetate and 4-fluorothreonine by resting cells of *Streptomyces cattleya*; the labelling patterns illustrate a conversion of glycine *via* serine to pyruvate, where the C-2 and C-3 carbon atoms of pyruvate contribute both carbon atoms of fluoroacetate and C-3 and C-4 of 4-fluorothreonine respectively.

Fluorinated natural products are rare in nature.[1,2] The most ubiquitous of this class of natural product is fluoroacetate **1**, which is found in a wide variety of plant species in both arid and tropical regions around the world.[1,3] Only two bacterial strains, both actinomycetes, have been identified with the ability to biosynthesise organofluorine compounds. Fluoroacetate **1** and 4-fluorothreonine **2** are coproduced by *Streptomyces cattleya*[4] when grown in the presence of a source of fluoride and suspensions of resting cells of the organism provide an attractive system in which to explore the biosynthesis of these fluorinated metabolites.[5,6] Nucleocidin **3** was isolated[7] from *Streptomyces calvus* in 1957, although a correct structure for the molecule was not advanced until 1969.[8] Unfortunately more recent attempts to produce the antibiotic in culture have failed.[9] Here we report a study using ^{19}F NMR spectroscopy of the incorporation of [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-glycine and [1-^{13}C]-, [2-^{13}C]-, [3-^{13}C]-pyruvate into both fluoroacetate **1** and 4-fluorothreonine **3** by *S. cattleya*. In each case the labelled precursors were incubated at a final concentration of 10 mm with resting cell suspensions and incorporation of ^{13}C label into the fluorometabolites was examined using GC–MS and ^{19}F NMR spectroscopy after 48 h.

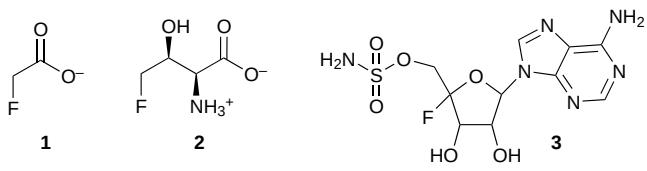
The results of the GC–MS study, which are reported elsewhere,[10] revealed several striking features. A low level of incorporation of ^{13}C label (< 4%) from [1-^{13}C]glycine into fluoroacetate **1** contrasted with a very high level of incorporation (40%) from [2-^{13}C]glycine. Furthermore the labelling patterns in fluoroacetate from [2-^{13}C]- and [1,2-$^{13}C_2$]-glycine were practically identical, indicating that the high proportion (80% of total ^{13}C incorporation) of double incorporation observed in the [1,2-$^{13}C_2$]glycine experiment is not due to the incorporation of glycine as an intact two carbon unit but rather that the C-2 of glycine is recombined during the biosynthesis of fluoroacetate.

It was not possible to assess from the GC–MS data the precise extent of recombination of C-2 of glycine into C-3 and C-4 of 4-fluorothreonine and so the degree of analogy between the labelling pattern in C-3 and C-4 of 4-fluorothreonine and the C-1 and C-2 of fluoroacetate could not be ascertained. The uncertainty was resolved by $^{19}F\{^1H\}$ NMR spectroscopy. A direct comparison of the incorporation of isotope into C-1 and C-2 of fluoroacetate with that into C-3 and C-4 of 4-fluorothreonine was made by $^{19}F\{^1H\}$ NMR spectroscopy after concentration of the supernatant at the end of each experiment. Proton decoupling was advantageous in simplifying the complexity of the ^{19}F NMR signals at −214.6 ppm for fluoroacetate and −229.15 ppm for 4-fluorothreonine, revealing coupling patterns which indicate intact ^{19}F-^{13}C-^{13}C, ^{19}F-^{13}C-^{12}C and ^{19}F-^{12}C-^{13}C combinations ($^1J_{^{19}F\text{-}^{13}C}$ = 165 Hz, $^2J_{^{19}F\text{-}^{13}C}$ = 18 Hz) in the resultant metabolites. The signals for each of the two fluorinated metabolites obtained by $^{19}F\{^1H\}$ NMR spectroscopy always possessed a similar multiplicity indicating that ^{13}C incorporations in terms of magnitude and regiochemistry into C-1 and C-2 of fluoroacetate mirror those into C-3 and C-4 of 4-fluorothreonine. The most obvious interpretation of this observation is that both sets of carbons have an identical biosynthetic origin and that there is a single fluorinating enzyme operating in *S. cattleya*. Our previous studies[5,6] have ruled out a direct interconversion of one of the metabolites to the other, so we are forced to the conclusion that each fluorometabolite arises by metabolism of a (presently unknown) common fluorinated intermediate.

The $^{19}F\{^1H\}$ NMR spectrum resulting from incubation of [2-^{13}C]glycine with resting cells is shown in Fig. 1. This spectrum has identical features to that obtained after incubation with [1,2-$^{13}C_2$]glycine which is entirely consistent with the GC–MS data and confirms that only C-2 of glycine is contributing ^{13}C label to the fluorinated metabolites. The similarity in the patterns of the ^{19}F NMR signals of the resultant fluoroacetate and 4-fluorothreonine is striking. In each case the parent (unlabelled) signal (singlet) has a predominant doublet of doublets ($^1J_{^{19}F\text{-}^{13}C}$ = 160 Hz, $^2J_{^{19}F\text{-}^{13}C}$ = 18 Hz, α + β-shift = 0.09 ppm) associated with it due to populations of **1** and **2** (*ca*. 40%) with an intact ^{19}F-^{13}C-^{13}C combination. There is also a doublet ($^1J_{^{19}F\text{-}^{13}C}$ = 165 Hz, α-shift = 0.07 ppm) due to populations (*ca*. 5%) with an intact ^{19}F-^{13}C-^{12}C combination. This is apparent as a small increase in the intensity of the left hand arms of the predominant doublet of doublets. Finally the right hand arm of a doublet (18 Hz, β-shift = 0.02) can be seen at the base of the unlabelled peaks for **1** and **3** due to populations (*ca*. 7%) with an intact ^{19}F-^{12}C-^{13}C combination.

The high level of recombination of C-2 of glycine in both fluorometabolites and very low incorporation of C-1 is most readily explained if glycine is converted to serine or a metabolite of serine such as pyruvate (Scheme 1). In bacteria[11] glycine is cleaved and C-2 is processed *via* N^5, N^{10}-methylene-tetrahydrofolate by serine hydroxymethyltransferase and condensed with another molecule of glycine to generate serine. Further metabolism of serine by serine dehydratase generates pyruvate.

In order to explore this putative relationship further the incorporation of ^{13}C-labelled pyruvates into fluorometabolites by resting cell suspensions of *S. cattleya* was examined by GC–MS and $^{19}F\{^1H\}$ NMR spectroscopy. The results of the GC–MS study are reported elsewhere and indicate that C-2 and C-3 of



1 **2** **3**

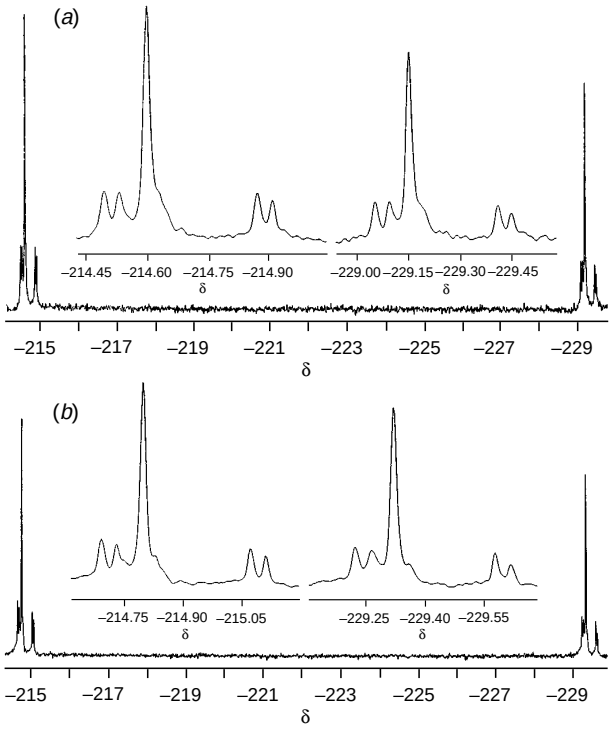
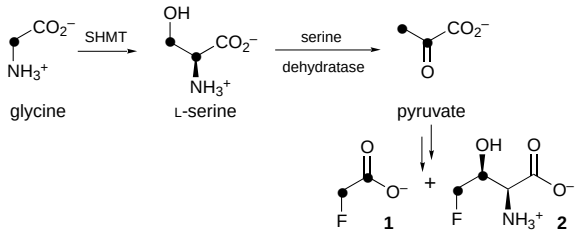


Fig. 1 $^{19}F\{^{1}H\}$ NMR spectrum of fluoroacetate **1** (−214.60 ppm) and 4-fluorothreonine **2** (−229.15 ppm) in the supernatant after incubation of resting cells of *S. cattleya* for 48 h with (*a*) [2-^{13}C]-glycine and (*b*) [1,2-$^{13}C_2$]glycine. The insets show expansions of each signal.



Scheme 1 Proposed pathway accounting for the labelling of the fluorometabolites by [2-^{13}C]glycine and ^{13}C-labelled pyruvates

pyruvate are probably incorporated as a unit into fluoroacetate.[10] The supernatants from these experiments were analysed by $^{19}F\{^{1}H\}$ NMR spectroscopy to assess the extent of the similarity in the pattern of incorporation into the two metabolites. As with the experiments conducted with labelled glycines, the signals obtained from each of the fluorometabolites by $^{19}F\{^{1}H\}$ NMR spectroscopy always possessed a similar multiplicity indicating that ^{13}C incorporation into C-1 and C-2 of fluoroacetate paralleled exactly that into C-3 and C-4 of 4-fluorothreonine.

Fig. 2 shows the $^{19}F\{^{1}H\}$ NMR spectrum obtained following incubation of [3-^{13}C]pyruvate with resting cell suspensions. It is clear that each of the signals assigned to fluorometabolites **1** (−214.8 ppm) and **2** (−229.3 ppm) has satellites ($^{1}J_{^{13}C\text{-}^{19}F}$ = 165 Hz) associated with it. These correspond to a population (*ca*. 30%) of molecules in each case which possess an intact ^{19}F-^{13}C-^{12}C combination. No other labelled populations are apparent. Therefore we can conclude that C-3 of pyruvate has regiospecifically labelled the fluoromethyl groups of **1** and **2**. Similarly the resultant spectrum (not shown) from the [2-^{13}C]pyruvate feeding experiment had satellites associated with each fluorometabolite resonance, indicative of a two bond coupling between F and ^{13}C ($^{1}J_{^{13}C\text{-}^{19}F}$ = 18 Hz). Thus C-2 of pyruvate has regiospecifically labelled C-1 of fluoroacetate and C-3 of 4-fluorothreonine (*ca*. 40%). Thus C-2 and C-3 of pyruvate contribute C-1 and C-2 of fluoroacetate and C-3 and C-4 of fluorothreonine as summarized in Scheme 1.

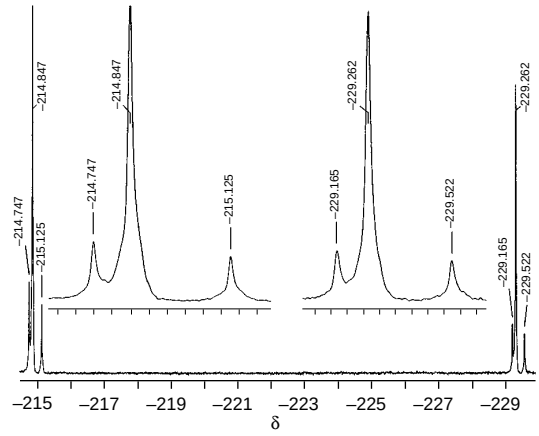


Fig. 2 $^{19}F\{^{1}H\}$ NMR spectrum of fluoroacetate **1** (−214.8 ppm) and 4-fluorothreonine **2** (−229.3 ppm) in the supernatant after incubation of resting cells of *S. cattleya* for 48 h with [3-^{13}C]pyruvate. The insets show expansions of each signal

Although these results show clearly that pyruvate is a highly effective precursor of the fluorometabolites in resting cells of *S. cattleya*, it cannot be concluded that pyruvate, or a closely related metabolite such as β-hydroxypyruvate,[12] is the substrate for fluorination. Studies using stable and radiolabelled precursors[6,12] have shown that glycerol is a good precursor in fluoroacetate biosynthesis. The fluorination substrate must therefore be situated between pyruvate and glycerol on the glycolytic pathway. The very high incorporations observed into the fluorometabolites from labelled pyruvate may owe more to the tight channelling of intermediates in this pathway than the proximity of pyruvate to the carbon substrate for fluorination. The stereochemical processing of glycerol prior its incorporation into the fluorometabolites **1** and **2** is consistent with this hypothesis.[13]

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Footnote

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