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Fluorine-containing natural products

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Abstract

It is now more than 50 years since the first fluorinated natural product was identified. In that time only about a dozen fluorinated natural products have been isolated, the last one over a decade ago. Very little is known about the mechanism of biological fluorination although significant progress has been made in elucidating the pathway by which biosynthesis of fluoroacetate and 4-fluorothreonine occurs in the bacterium *Streptomyces cattleya*. In this article we review the fluorinated natural products and the current status of our understanding of fluorometabolite biosynthesis. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Fluoroacetate; ω-fluorinated lipids; 4-fluorothreonine; Nucleocidin; Fluorocitrate; Sir Rudolph Peters

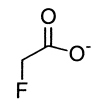
1. Introduction

Plants, fungi and micro-organisms have developed a tremendous capacity for biosynthesis of secondary metabolites and hundreds of thousands of different compounds have been identified, representing an amazingly diverse array of structures [1]. The halogens, chlorine, bromine and even iodine, figure prominently in many of these compounds with ca. 3000 natural products containing the elements reported to date [2].

Surprisingly the most abundant halogen in the earth's crust, fluorine, has been identified as a component of only 13 secondary metabolites of which eight are ω-fluorinated homologues of long chain fatty acids found as co-metabolites in the seeds of the same plant. So formally only six discrete fluorinated natural products have been isolated excluding the fatty acid homologues [3]. The first organofluorine compound identified in 1943 was fluoroacetate, a metabolite of the Southern African plant *Dichapetalum cymosum* [4,5] whilst the most recently discovered, structurally novel, fluorine-containing natural product was 4-fluorothreonine isolated from the bacterium *Streptomyces cattleya* some 14 years ago in 1986 [6]. It is remarkable, particularly in view of the increasingly sophisticated methods of screening and structure elucidation developed in recent years, that in the last decade not a single new class of fluorinated metabolite has been reported.

One particularly intriguing aspect of the few fluorinated metabolites which do exist is the nature of the biological fluorination process. Despite a considerable interest and a variety of speculative suggestions as to the mechanism, no specific details of the biochemistry of fluorination in any organism are known. The best studied system biochemically is fluoroacetate and 4-fluorothreonine biosynthesis in *S. cattleya* and our current understanding of that system [7,8] will be reviewed in Section 8.

2. Fluoroacetate in plants



Fluroacetate is found in a wide variety of plants at low concentrations. However in certain plants it accumulates at very high concentrations (500–8000 ppm on a dry weight basis). It was first identified in 1943 by Marais in South Africa from the shrub, *D. cymosum* [4,5] found to the north of Pretoria. The fresh leaves are particularly toxic and can accumulate fluoroacetate to levels of 250 ppm [9]. In the early spring farmers fence off the shrub to protect their livestock. Where the problem is significant bore holes are

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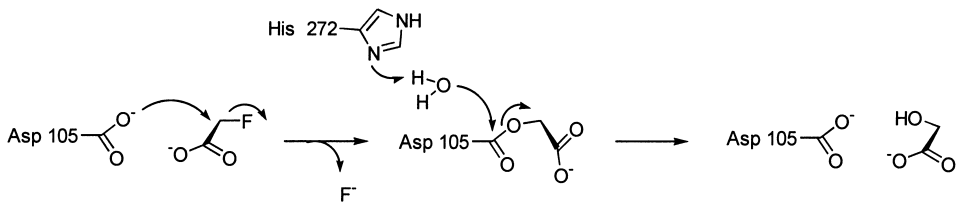
sometime dug and filled with copper sulphate solution to try to kill the plant. However, this is only a temporary measure as the root system of *D. cymosum* is extensively networked many meters below the soil surface. It is not clear whether the extensive root system has evolved to assimilate fluoride from the soil, as many plants in semi-arid regions have deep roots. In Central Africa, for example Tanzania many other fluoroacetate-accumulating plants are found, most belonging to the genus *Dichapetalum. D. braunii* is particularly toxic with seeds which contain 8000 ppm fluoroacetate, the highest level recorded [10].

Fluoroacetate-accumulators are also found in Australia and the toxin has been identified in 35 species from three genera of the Leguminosae, especially *Gastrolobium* and *Oxylobium* [11]. These plants do not have a wide geographic distribution and are generally confined to the south west of Western Australia. *Oxylobium parviflorum* (box poison) and *Gastrolobium bilobum* (heart leaf poison) can contain up to 2600 ppm in their leaves and 6500 ppm in their seeds, rendering them among the most toxic fluoroacetate producers known. Fluoroacetate is found also in a few South American plants, notably *Palicourea marcgravii* which is a significant accumulator with levels of 500 ppm recorded in the seeds and flower stalk [12]. Thus highly toxic fluoroacetate-producing plants are globally distributed with species on several of the major continents, but it is notable that some widely grown crop and forage plants when exposed to fluoride ion can also biosynthesise fluoroacetate albeit at very low non-toxic levels. Soya bean (*Glycine max*) and crested wheat grass (*Agropyron cristatus*) can show concentrations of 4 ppm after growth on 1 mM fluoride solution [13–15]. Therefore it seems likely that many plants have a low level capability to biosynthesis fluoroacetate, although clearly the toxic plants have evolved and enhanced ability presumably for purposes of defence.

3. Tolerance to fluoroacetate

Several studies have revealed that animals that forage in areas where fluoroacetate-producing plants are common have evolved an increased resistance to fluoroacetate compared to animals from areas where plants containing the toxin are not indigenous. This phenomenon is well documented for Australia where it has been observed in a wide range of animals, birds and reptiles [11]. The effect is most dramatic in herbivores and seed eaters which are more directly exposed to the toxin than carnivores. The emu (*Dromaius novaehollandiae*) is the oldest seed eating bird species in Australia and has a very high level of resistance to fluoroacetate with an LD_{50} of 100–200 mg kg^{-1}. In contrast seed–eating birds from regions outside the range of fluoroacetate-producing plant species have an LD_{50} in the range of 0.2–20 mg kg^{-1}. Similarly the brush-tailed possum *Trichosurus vulpecula* of south-western Australia is 150 times less susceptible to fluoroacetate poisoning than the same species in eastern Australia where plant species containing the toxin are not present. The biochemical basis of tolerance is not entirely clear but resistant mammals have high glutathione levels, and a glutathione-requiring defluorinating enzyme which can use fluoroacetate as substrate has been characterised from possum liver [16]. Resistance to fluoroacetate is also found in insects. Those species which browse on vegetation which does not contain fluoroacetate are approximately 40–150 fold more sensitive to the toxin than those species that include vegetation containing fluoroacetate in their diet [11]. Interestingly the caterpillar of the moth, *Sindrus albimaculatus* which feeds on *D. cymosum* can not only detoxify fluoroacetate but can accumulate it, probably in vacuoles, and use it as a defence against predition [17]. In bacteria the mechanism of tolerance has been investigated in some detail. Enzymes capable of defluorinating fluoroacetate have been isolated from several micro-organisms which could grow on fluoroacetate as sole carbon source e.g. *Pseudomonas* spp. [18–20] and *Fusarium solani* [19], but the best characterised system at the mechanistic level is a fluoroacetate dehalogenase from a *Moraxella* sp [21,22]. At the active site of the enzyme the carboxylate group of aspartate-105 acts as a nucleophilic species which attacks fluoroacetate, displacing fluoride and forming an enzyme-bound ester intermediate as shown in Scheme 1. Histidine–272 then acts as a base deprotonating a water molecule and the resulting hydroxyl ion cleaves the ester intermediate releasing glycolate. Consistent with this mechanistic analysis, isotope from $H_2{}^{18}O$ labelled the enzyme, not the released glycolate. The gene encoding for this enzyme has been cloned and expressed in the rumen bacterium *Butyrivibrio fibrisolvens* [23,24]. This organism was subsequently employed to infect the gut of sheep in an attempt to protect the animals against poisoning by fluoroacetate-containing plants in their diet. Early indications are that the genetically modified organism becomes established



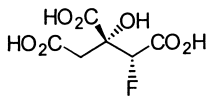
Scheme 1. Proposed mechanism of fluoroacetate dehalogenase from *Moraxella* sp.

sufficiently well to give the sheep a degree of resistance to fluoroacetate.

Plants which accumulate fluoroacetate, often to levels several thousand fold higher than the LD_{50} for most mammals, must possess a resistance mechanism to counter its toxicity. Several theories have been advanced as to the biochemical basis of resistance but no single process has been identified to explain the phenomenon. One suggestion is that their acetyl-CoA synthetase is not located in the mitochondria of plants but may instead be situated in the chloroplast as is the case for spinach (*Spinach oleracea*) [25]. As acetyl-CoA synthesised in the chloroplasts of this species is not transported across the chloroplast membrane it is unlikely that fluoroacetyl-CoA will migrate out of the chloroplast into the mitochondria, the site of citrate synthesis.

On the basis of the powerful inhibition of aconitase by fluorocitrate from *D. cymosum*, it was concluded that the tolerance of this species to fluoroacetate was due to an inability to synthesise fluorocitrate, most probably because its citrate synthetase has a much lower affinity for fluoroacetyl-CoA than acetyl-CoA [26]. However, recent work has demonstrated the presence of a fluoroacetyl-CoA hydrolase enzyme in *D. cymosum* which could not use acetyl-CoA as a substrate [27]. The presence of such an enzyme in fluoroacetate-accumulating plants could explain resistance to autointoxication by the compound.

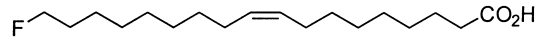
4. Fluorocitrate



Fluoroacetate is toxic because it is converted in vivo to (2*R*, 3*R*)-fluorocitrate which arises by condensation of fluoroacetyl-CoA with oxaloactate by citrate synthetase, the enzyme that normally feeds acetyl-CoA into the citric acid cycle. Therefore many plants which can accumulate low levels of fluoroacetate also contain fluorocitrate [13–15]. Even commercial tea can contain up to 30 ppm on a dry weight basis [28]. Rudolph Peters in 1953, was the first to propose [29] a mechanism for the toxicity of fluoroacetate involving its conversion to fluorocitrate, a process that he termed 'lethal synthesis'. It emerges that the enzymatic process is highly stereospecific and generates only the (2*R*, 3*R*)-stereoisomer of fluorocitrate. The stereoselectivity of this enzymatic reaction has been partly attributed [30] to the relative stabilities of the possible enol intermediates which are generated during the condensation of fluoroacetyl-CoA with oxaloacetate. Fluorocitrate is a potent inhibitor of aconitase, the enzyme which succeeds citrate synthase in the citric acid cycle and converts citrate to isocitrate. It is a little perverse perhaps that of the four possible stereoisomers of fluorocitrate, only the (2*R*, 3*R*) isomer, the compound generated during the enzymatic reaction, is toxic. This was revealed by some elegant work of Kun and Dummell in 1969 [31], who prepared and purified each of the four stereoisomers and demonstrated that only the (2*R*, 3*R*)-isomer was an inhibitor of aconitase. A recent study [32] has revealed that (2*R*, 3*R*)-fluorocitrate is converted to fluoro-*cis*-aconitate by aconitase, which, in enzyme-bound form, is attacked by hydroxyl ion to generate 4-hydroxy-*trans*-aconitate as illustrated in Scheme 2.

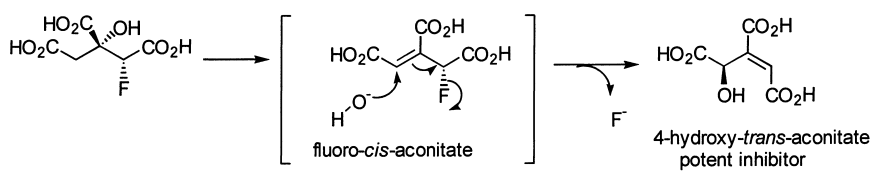
This product has a very high binding affinity for aconitase and is a competitive inhibitor. The crystal structure of the enzyme with bound inhibitor was solved [32] adding significant credence to this mechanism. Additionally fluorocitrate appears to covalently bind to proteins involved in citrate transport across the mitochondrial membrane, and this provides another and possibly more important rationale for its acute toxicity [33].

5. ω-Fluorinated fatty acids

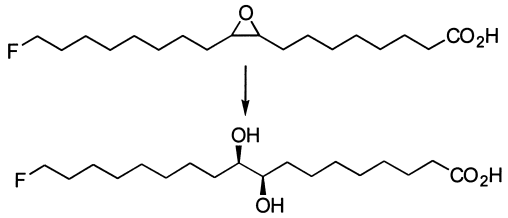


ω-fluorooleic acid

The seeds of a shrub, *Dichapetalum toxicarium*, indigenous to Sierra Leone and other parts of West Africa were once widely used by witchdoctors to conduct trials by ordeal. The plant is frequently responsible for livestock losses and the local name, ratsbane, indicates its widespread use as a rodenticide. When ingested the seeds cause loss of co-ordination, paralysis of the lower limbs and ultimately death. Recognising the similarly of the symptoms to those induced by fluoroacetate, Peters and co-workers [34,35] investigated the nature of the toxic principle present. Seeds of *D. toxicarium* were found to contain up to 1800 $\mu g\ g^{-1}$ organic fluorine, of which a negligible amount was in the form of fluoroacetate. The main fluorinated component, which comprised 80% of the total organic fluorine present



Scheme 2. The mechanism of fluorocitrate inhibition by citrate synthatase.



Scheme 3.

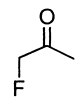
and 3% of the seed oil, was identified as ω-fluorooleic acid ($C_{18:1F}$) but small amounts of ω-fluoropalmitic acid ($C_{16:0F}$) were also isolated. More recent studies [36] using GC/MS have demonstrated the presence in the seed oil of an additional five ω-substituted fluoro acids, $C_{16:1F}$, $C_{18:0F}$, $C_{18:2F}$, $C_{20:0F}$ and $C_{20:1F}$. The positions of the double bonds in the unsaturated ω-fluoro compound have been located by GC/MS of the picolinyl esters and it has been established that the $C_{20:1F}$ acid is present as two isomers with unsaturation at the 9 and 11 positions respectively [37]. *Threo*-18-fluoro-9,10-dihydroxystearic acid has also been isolated from *D. toxicarium* seed oil accounting for about 1% of the organic fluorine present [38]. This compound is presumably a metabolite of ω-fluorooleic acid derived via the 9, 10-epoxide of the acid as shown in Scheme 3.

The pattern of ω-fluorofatty acids in the seed oil in terms of chain length and unsaturation reflected that of their non-fluorinated analogues although the latter were 5- to 10-fold more abundant. This similarity suggests that the ω–fluoro compounds arise from a common precursor unit which is presumably fluoroacetyl-CoA. It would appear that broad substrate specificity of the fatty acid synthase in this plant allows it to utilise fluoroacetyl-CoA in place of acetyl-CoA for the initial condensation with malonyl acyl carrier protein (malonyl-ACP) in the first stage of fatty acid synthesis. The restriction of fluorine to the terminal position implies that either fluoromalonyl-CoA is not readily formed by the acetyl-CoA carboxylase of the plant or that the substrate specificity of the malonyl-ACP transacylase does not allow the incorporation of fluoromalonyl-ACP instead of malonyl-ACP during chain elongation.

Given the substrate flexibility of the enzyme involved in the initial condensation of fatty acid biosynthesis in this plant, it is difficult to explain why more fluoroacetate-producing plants do not also elaborate long chain ω-fluoro fatty acids. Interestingly Peters and Hall [39] reported the presence of a long chain fatty acid with the gas chromatographic characteristics of ω-fluorooleic acid in an organo-fluorine-containing oil exracted from *D. cymosum*. However, the compound did not appear to exhibit in vivo the toxicity of ω-fluorooleic acid and these workers tentatively concluded that it was a C_{17} or C_{18} fluorofatty acid differing from those previously known. These observations suggest that a reinvestigation using modern techniques of the leaves of *D. cymosum* and indeed other fluoroacetate-containing plants for the presence of small amounts of long chain ω-fluorofatty acids might be rewarding.

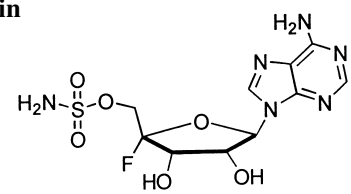
The toxicity of even numbered ω-fluorofatty acids can be attributed to β-oxidation to fluoroacetate [40]. Catabolism of odd numbered fatty acids by this pathway will not lead to formation of fluoroacetate and accordingly such acids have relatively low toxicity [40]. On a fluorine basis the even numbered fatty acids are more toxic than fluoroacetate. This may be due to more efficient uptake by the cell of these highly lipophilic compounds which can even be toxic by direct absorption through the skin. Alternatively the intra-mitochondrial conversion of ω-fluorofatty acids to fluoro-citrate may be more effective than that of fluoroacetate.

6. Fluoroacetone



Peters and Shorthouse conducted a series of experiments in the late 1960's [41,42] exploring fluoride metabolism in the fluoroacetate accumulator, *Acacia georginae*, and other plants. Homogenates of *A. georginae* fortified with 1 mM fluoride, ATP and pyruvate and incubated at 30°C showed losses of fluoride of up to 34% of the total fluoride originally present over a period of 2 h. These losses were attributed to the biosynthesis of volatile fluoro-organics [41]. On passing the volatiles through acidic, 2,4-dinitrophenylhydrazine solution, approximately 13% of the volatilised fluorine was trapped as a fluorinated 2,4-dinitrophenylhydrazone derivative which showed an identical retention time on paper chromatography to the hydrazone derivative of fluoroacetone [42]. However, the comment is made in the paper the "The presence of fluoroacetaldehyde cannot be ruled out, although it seems improbable in view of its instability". Although not known to Peters at the time, fluoroacetaldehyde is in fact quite stable in aqueous solution existing as a hydrate. In view of the fact that fluoroacetaldehyde has subsequently been strongly implicated in the biosynthesis of the fluorometabolites in *S. cattleya* (see Section 8) it seems conceivable that the fluorinated hydrazone derivative isolated in this experiment was actually that of fluoroacetaldehyde. Hence the identification of fluoroacetone as a natural product must be regarded as insecure.

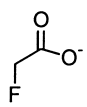
7. Nucleocidin

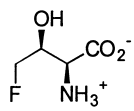


Nucleocidin is an intriguing organo-fluorine natural product with a broad spectrum of antibiotic activity, although it

has proved too toxic for clinical use. The compound was first isolated in 1957 from an organism *Streptomyces calvus* obtained from an Indian soil sample [43]. However, it was not until 1969 that it was appreciated that the compound contained a fluorine atom and the correct structure, 4′-fluoro-5′-*O*-sulphamoyladenosine, was deduced [44]. The location of the fluorine atom at the 4-position of the ribose moiety appears to preclude biosynthesis from a fluoroacyl fragment suggesting that the nature of the fluorination process in *S. calvus* may be quite different from that of all other fluorinated natural products discovered to date. It is therefore particularly frustrating that recent attempts in several laboratories ([45] and Harper, unpublished results) have failed to reisolate this metabolite from cultures of *S. calvus* grown from freeze dried organisms obtained from several culture collections. This has thwarted attempts to elucidate the mechanism by which fluorination occurs in the bacterium. The gene encoding nucleocidn biosynthesis, or at least an essential part of it, appears to have been lost. Such genetic impairment of an organism can sometimes occur if a metabolic trait is plasmid-encoded due to loss of the plasmid during the freeze drying process used in preservation of micro-organisms in culture collections. Hence unless the native organism is reisolated from soil this fascinating metabolite can probably be classed as extinct!

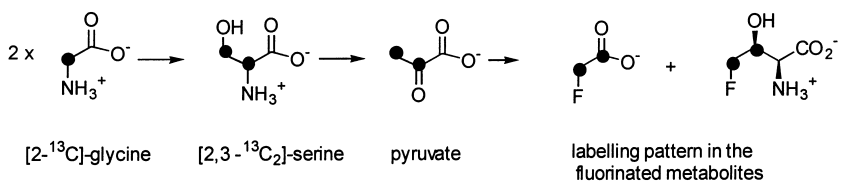
8. 4-Fluorothreonine and fluoroacetate biosynthesis in *Streptomyces cattleya*



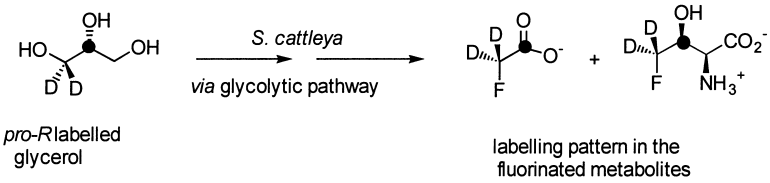


In 1986 during the course of studies optimising the production of the β-lactam antibiotic thienamycin by *S. cattleya* on a complex medium, Sanada et al. [6] discovered that, in the presence of fluoride, both 4-fluorothreonine and fluoroacetate accumulated in the medium. In microbial assay the amino acid acted as an antimetabolite of L-threonine suggesting an analogous stereochemistry. This has now been confirmed by asymmetric synthesis [46] which has demonstrated that natural 4-fluorothreonine has the (2*S*, 3*S*) configuration. Reid et al. [7] examined biosynthesis of fluorometabolites by *S. cattleya* using ^{19}F NMR and demonstrated production of both 4-fluorothreonine and fluoroacetate in millimolar concentrations when the organism was grown on a chemically defined medium with glycerol and glutamate as the C sources. They showed that cells harvested in the early stationary phase (after 6 or 8 days growth) when suspended in buffer containing 2 mM NaF would biosynthesise fluorometabolites for at least 96 h thus providing for the first time a convenient system in which to study fluorometabolite biosynthesis. This development opened the way to experiments on the incorporation of isotopically labelled precursors into the fluorometabolites to delineate the metabolic pathway prior to the fluorination reaction. Both ^{13}C- and ^{2}H-labelled precursors have been employed and incorporation of label into the fluorometabolites determined by ^{19}F {H} NMR and GC/MS using the selected ion monitoring mode. Particularly high incorporation from glycine was observed with C-2 of glycine contributing both carbon atoms of fluoroacetate and C-3 and C-4 of 4-fluorothreonine [8,47]. The incorporation pattern is consistent with cleavage of glycine to generate N^5, N^{10}-methylenetetrahydrofolate which is then condensed with another molecule of glycine to form serine in a reaction catalysed by serinehydroxymethyl transferase. Serine is subsequently converted to pyruvate by serine dehydratase. The ready incorporation of label from [3-^{13}C]-serine and [3-^{13}C]-pyruvate into the fluoromethyl carbons of both fluorometabolites confirmed this interpretation and experiments with other possible precursors indicated further metabolism of pyruvate through oxaloacetate to the glycolytic pathway intermediate, phosphenol pyruvate, prior to incorporation into fluorometabolites [see Scheme 4]. An important finding of this investigation was the striking similarity between the labelling pattern in C-1 and C-2 of fluoroacetate and C-3 and C-4 of 4-fluorothreonine recorded in experiments with every labelled precursor betokening the common origin of both sets of carbons and the existence of a single fluorinating enzyme in *S. cattleya*.

In work in Japan, Tamura et al. [48] demonstrated that label from [2-^{13}C]-glycerol was incorporated into C-1 of fluoroacetate by *S. cattleya*. The entry of glycerol into the glycolytic pathway is mediated by glycerol kinase, en enzyme which phosphorylates the *pro*-(*R*) hydroxymethyl group of glycerol (but not the *pro*-(*S*) hydroxymethyl group) to generate *sn*-glycerol-3-phosphate. Nieschalk et al. [49] prepared the enantiotopically labelled (*R*) [1-$^{2}H_2$]- and (*S*) [1-$^{2}H_2$]-glycerols, and compared their incorporation into the fluorometabolites of *S. cattleya*. Both deuterium atoms from the *pro-R* arm of glycerol but neither from the *pro-S* arm



Scheme 4. Incorporation of glycine and serine into fluoroacetate and 4-fluorothreonine.



Scheme 5. Incorporation of glycerol into fluoroacetate and 4-fluorothreonine.

were incorporated into the fluoromethyl groups of the fluorometabolites. This stereochemical outcome is consistent with activation of glycerol by glycerol kinase and entry into the glycolytic pathway. It is significant that the phosphorylated *pro*-(*R*) hydroxymethyl group is ultimately transformed to the fluoromethyl group. The retention of both deuterium atoms indicates that there is no oxidation at this centre and that whatever the mechanism of the fluorination reaction there is formal replacement of the phosphate group by a fluorine atom (Scheme 5).

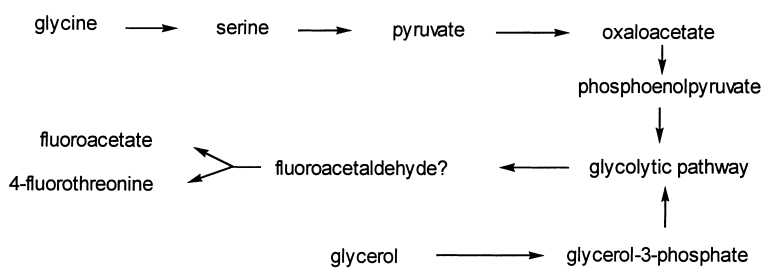
In recent unpublished work we have examined the effect of various unlabelled intermediates in the glycolytic pathway between glycerol and pyruvate on the incorporation of label from [2-^{13}C]-glycerol and [2-^{13}C]-pyruvate into the fluorometabolites. The greatest dilution was observed in the presence of dihydroxyacetone and D-glyceraldehyde and their corresponding phosphate derivatives suggesting that one of these triose phosphates or a closely related C_2 metabolite is the most likely candidate as the substrate for the fluorinating enzyme. It seems likely that a common C_2 fluorine containing intermediate contributes both carbon atoms of fluoroacetate and C-3 and C-4 of 4-fluorothreonine as neither fluorometabolite is derived by metabolism of the other [7]. There is mounting evidence that fluoroacetaldehyde may fulfill this role. Not only do cell extracts of *S. cattleya* have a very efficient NADH-dependent fluoroacetaldehyde dehydrogenase which oxidises fluoroacetaldehyde to fluoroacetate, but cell suspensions incubated with fluoroacetaldehyde and fluoride show increased formation of 4-fluorothreonine (Moss, Murphy, Hamilton, O'Hagan and Harper, unpublished) (Scheme 6).

Further investigations are currently underway in our laboratories to elucidate the biosynthetic role of fluoroacetaldehyde in fluoroacetate and 4-fluorothreonine biosynthesis in *S. cattleya*.

9. Outlook

The discovery of another fluorinated metabolite is clearly overdue. In view of the apparent rarity of these compounds — particularly from bacterial sources which provide by far the most convenient systems in which to investigate biological fluorination — it is particularly frustrating that one of the bacterial fluorinating systems discovered, the nucleocidin-biosynthesis system of *S. calvus* appears to have been lost from the arena of scientific investigation. Doubtless the native organism still exists in the soil environment somewhere in the world but whether it is ever isolated again and available for study in the laboratory remains to be seen. Also, as the identification in 1967 by Peters and Shorthouse of fluoroacetone in *A. georginae* homogenates must be regarded as insecure in the light of our present knowledge concerning the stability of fluoroacetaldehyde, it appears that this rare group of exotic natural products is becoming rarer as the years go by.

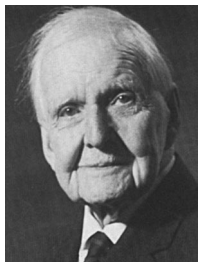
A clear challenge for the new millennium is to isolate and purify a fluorination enzyme for the first time. At this stage *S. cattleya* appears the most likely organism with which to achieve this breakthrough. With the ever more important role that selectively fluorinated bioactive compounds are playing in the pharmaceutical and agrochemical industries the biotechnological prospects for such an enzyme are exciting. The genes encoding defluorination enzymes have already been introduced into host organisms so it is reasonable to expect that the identification of the gene cluster responsible for fluorination and fluorometabolite biosynthesis in *S. cattleya* should allow host organisms to be engineered with the ability not just to produce fluoroacetate and 4-fluorothreonine but to generate completely novel fluorinated products with biological activity e.g. antibiotics. The prospects of applying the results of Nature's small but



Scheme 6. Overview of the metabolic relationships between primary metabolites and fluorinated secondary metabolites of *S. cattleya*.

successful experiment on fluorination to the service of mankind look bright.

Appendix



Sir Rudolph A Peters FRS (1889–1982) towers as the most significant figure in the investigation of natural organo-fluorine compounds and their biological effects in the 20th century. In the early 1940's as Professor of Biochemistry at Oxford, he became interested in the biological effects of fluoroacetate when the compound was identified as the toxic principle of the plant *D. cymosum*. He demonstrated that fluoroacetate was converted to fluorocitrate in vivo, and that the toxic effects of fluoroacetate were associated with the accumulation of citrate in cells. He coined the term 'lethal synthesis' for this process and showed that the citric acid cycle enzyme, aconitase was inhibited by fluorocitrate. The precise details of the mechanism by which this inhibition occurs are only now emerging. Peters also identified ω-fluorooleic acid as the major toxic lipid present in seeds of *D. toxicarium*, a West African plant. Towards the end of his long scientific career in the late 1960's, operating from the Department of Biochemistry at Cambridge he, with his co-worker Mary Shorthouse, involved himself in studies of the metabolism of inorganic fluoride to organo-fluorine compounds in plants. They demonstrated that fluoroacetate and fluorocitrate are biosynthesised in a variety of plants at low levels and proposed that some plants e.g. *A. georginae* can convert fluoride to volatile fluoro-organic compounds. This latter idea has not been further explored. Progress has been made in elucidating the biosynthetic route by which fluorometabolites are formed in bacteria, but our understanding of fluoroacetate and fluorometabolite biosynthesis in plants has developed little since the pioneering contributions of Peters and remains a potentially very fruitful area of investigation.

References

[1] Comprehensive Natural Products Chemistry, vol. 1–6, Pergamon Press, Oxford, 1999.
[2] G.W. Gribble, Acc. Chem. Res. 31 (1998) 141.
[3] D.B. Harper, D. O'Hagan, Nat. Prod. Rep. 11 (1994) 4.
[4] J.S.C. Marais, Onderstepoort J. Vet. Sci. Anim. Ind. 18 (1943) 203.
[5] J.S.C. Marais, Onderstepoort J. Vet. Sci. Anim. Ind. 20 (1944) 67.
[6] M. Sanada, T. Miyano, S. Iwadare, J.M. Williamson, B.H. Arison, J.L. Smith, A.W. Douglas, J.M. Leisch, E. Inamine, J. Antibiotics 39 (1986) 259.
[7] K.A. Reid, J.T.G. Hamilton, R.D. Bowden, D. O'Hagan, L. Dasaradhi, M.R. Amin, D.B. Harper, Microbiology 141 (1995) 1385.
[8] J.T.G. Hamilton, C.D. Murphy, M.R. Amin, D. O'Hagan, D.B. Harper, J. Chem. Soc. Perkin Trans. 1 (1998) 759.
[9] J. Tannock, Rhod. J. Agric. Res. 13 (1975) 67.
[10] D. O'Hagan, R. Perry, J.M. Lock, J.J.M. Meyer, L. Dasaradhi, J.T.G. Hamilton, D.B. Harper, Phytochemistry 33 (1993) 1043.
[11] L.E. Twigg, D.R. King, OIKOS 61 (1991) 412.
[12] R.J. Hall, New Phytol. 71 (1972) 855.
[13] C.J. Lovelace, G.W. Miller, G.W. Welkie, Atmos. Environ. 2 (1968) 187.
[14] M.H. Yu, G.W. Miller, Environ. Sci. Technol. 4 (1970) 492.
[15] R.A. Peters, M. Shorthouse, Phytochemistry 11 (1972) 1339.
[16] R.J. Mead, A.J. Oliver, D.R. King, Aust. J. Biol. Sci. 32 (1979) 15.
[17] J.J.M. Meyer, D. O'Hagan, Chem. Br. 28 (1992) 785.
[18] P. Goldman, J. Biol. Chem. 240 (1965) 3434.
[19] J.R.L. Walker, B.C. Lien, Soil. Biol. Biochem. 13 (1981) 231.
[20] H. Kawasaki, K. Miyoshi, K. Tonomura, Agric. Biol. Chem. 45 (1981) 543.
[21] H. Kawasaki, N. Tore, K. Tonomura, Agric. Biol. Chem. 45 (1981) 29.
[22] J.-Q. Liu, J.T. Kurihara, S. Ichiyama, M. Miyagi, S. Tsunasawa, H. Kawasaki, K. Soda, N. Esaki, J. Biol. Chem. 273 (1998) 30,897.
[23] K. Gregg, C.L. Cooper, D.J. Schafer, H. Sharpe, C.E. Beard, G. Allen, J. Xu, Bio/Technology. 12 (1994) 1361.
[24] K. Gregg, Trends Biotechnol. 13 (1995) 418.
[25] D.N. Kuhn, M. Knauf, P.K. Stumpf, Arch. Biochem. Biophys. 209 (1981) 441.
[26] J.N. Eloff, B. von Sydow, Phytochemistry 10 (1971) 1409.
[27] J.J. Meyer, N. Grobbelaar, R. Vleggaar, A.I. Louw, J. Plant. Physol. 139 (1992) 369.
[28] R.A. Peters, M. Shorthouse, Phytochemistry 11 (1972) 1337.
[29] R.A. Peters, R.W. Wakelin, P. Buffa, L.C. Thomas, Proc. Roy. Soc. B 40 (1953) 497.
[30] D. O'Hagan, H.S. Rzepa, J. Chem. Soc., Chem. Commun. (1994) 2029.
[31] E. Kun, R.J. Dummel, Methods Enzymol. 13 (1969) 632.
[32] H. Lauble, M.C. Kennedy, M.H. Emptage, H. Beinert, C.D. Stout, Proc. Natl. Acad. Sci. USA 93 (1996) 13,699.
[33] E. Kun, E. Kirsten, M.L. Sharma, Proc. Nat. Acad. Sci. 74 (1977) 4942.
[34] R.A. Peters, P.J. Hall, P.F.V. Ward, N. Sheppard, Biochem. J. 77 (1960) 17.
[35] P.F.V. Ward, R.J. Hall, R.J. Peters, Nature 201 (1964) 611.
[36] J.T.G. Hamilton, D.B. Harper, Phytochemistry 44 (1997) 1179.
[37] W.W. Christie, J.T.G. Hamilton, D.B. Harper, Chem. Phys. Lipids 97 (1998) 41.
[38] D.B. Harper, J.T.G. Hamilton, D. O'Hagan, Tetrahedron Lett. 31 (1990) 1776.
[39] R.A. Peters, R.J. Hall, Nature 187 (1960) 573.
[40] F.L.M. Pattison, Industrial Toxic Agents Toxic Aliphatic Fluorine Compounds, Elsevier Monographs, Elsevier, Amsterdam, 1959.
[41] R.A. Peters, M. Shorthouse, Nature 216 (1967) 80.
[42] R.A. Peters, M. Shorthouse, Nature 231 (1971) 123.
[43] S.O. Thomas, V.L. Singleton, J.A. Lowery, R.W. Sharpe, L.M. Preuss, J.N. Porter, J.H. Mowat, N. Bohonos, Antibiotics Ann. 1956-1957 (1956) 716.
[44] G.O. Morton, J.E. Lancaster, G.E. Van Lear, W. Fulmor, W.E. Meyer, J. Am. Chem. Soc. 91 (1969) 1535.
[45] A.R. Maguire, W-d. Meng, S.M. Roberts, A.J. Willetts, J. Chem. Soc., Perkin Trans. (1993) 1795.
[46] M.R. Amin, D.B. Harper, J.M. Moloney, C.D. Murphy, J.A.K. Howard, D. O'Hagan, Chem. Commun. (1997) 1471.
[47] J.T.G. Hamilton, M.R. Amin, D.B. Haper, D. O'Hagan, Chem. Commun. (1997) 797.
[48] T. Tamura, M. Wada, N. Esaki, K. Sato, J. Bacteriol. 177 (1995) 2265.
[49] J. Neischalk, J.T.G. Hamilton, C.D. Murphy, D.B. Harper, D. O'Hagan, Chem. Commun. (1997) 799.