

FLUOROACETIC ACID BIOSYNTHESIS: A PROPOSED MECHANISM

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Abstract

A mechanism for the biosynthesis of fluoroacetic acid is presented. This is based on a correlation of phytochemical and taxonomic data together with the established function of pyridoxal phosphate in general amino acid metabolism. Evidence for this hypothesis is based on the reported incorporation of a wide range of synthetic nucleophiles into corresponding β -substituted alanines by plant homogenates. Fluoride ion is considered to be an acceptable nucleophile for a non-specific enzyme synthesizing a range of β -substituted alanines, many of which occur in plants elaborating fluoroacetic acid.

It is considered that, initially, fluoride is covalently bound to a C_3 entity linked to pyridoxal phosphate and which is derived from cysteine, serine, or derivatives. The mechanism for fluoride incorporation is considered to involve direct attack on a carbonium ion but may occur by an exchange process, as nucleophile exchange occurs in β -substituted alanines in closely related plants.

Fluoroacetic acid is considered to be derived from the fluoro- $(C_3$ -pyridoxal phosphate) unit either by (1) transamination and release of fluoropyruvic acid which may produce fluoroacetic acid by an oxidative decarboxylation process, or (2) by decarboxylation, transamination, and hydrolytic release of fluoroacetaldehyde which may be oxidized to fluoroacetic acid.

I. INTRODUCTION

Fluoroacetic acid, a highly toxic natural product, was isolated from *Dichapetalum cymosum* by Marais (1944). Since then it has been found in *Palicourea marcgravi* (Oliviera 1963) and in several Australian members of the Leguminales, namely, *Acacia georginae* (Oelrichs and McEwan 1961), *Gastrolobium grandiflorum* (McEwan 1964), and in numerous *Gastrolobium* and *Oxylobium* species by Cannon (unpublished data) and Aplin (1967).

Although extensive studies have been made on the mode of toxic action of fluoroacetic acid there is a dearth of information, both theoretical and definitive, on the mechanism of biosynthesis of this substance. *Acacia georginae* is a serious cattle poison in the Northern Territory and Queensland. Peters, Murray, and Shorthouse (1965) have stated that, due to fluoroacetate toxicity, "losses of animals on a farm may reach £50,000".

The purpose of this paper is to propose a biosynthetic pathway for the formation of the toxin based on phytochemical and taxonomic relationships, and the established function of pyridoxal phosphate in enhancing the incorporation and exchange of nucleophiles in amino acid metabolism.

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II. DISCUSSION

In recent years many unusual amino acids have been isolated from plant material—particularly from members of the Leguminales. The biosynthesis of many of these has been described in terms of a nucleophilic attack on the β -carbon of serine or cysteine. Table I lists amino acids established to have been derived from cysteine,

TABLE I
AMINO ACIDS BIOSYNTHEZIZED FROM CYSTEINE, SERINE, OR *O*-ACETYL SERINE AS PRECURSORS OF NUCLEOPHILE RECIPIENTS

Amino acid	Established nucleophile	Botanical source	References for isolation	References for biosynthesis
Leguminales				
<i>S</i> -Methylcysteine (4)*	Methanethiol	<i>Astragalus</i> ; <i>Phaseolus</i>	Thompson, Morris, and Zacharius (1956); Trelease, Di Somma, and Jacobs (1960)	Wolf, Black, and Downey (1956)
β -Cyanoalanine (3)	Cyanide ion	<i>Vicia</i> ; <i>Lathyrus</i> ; <i>Lupinus</i> ; <i>Lotus</i>	Ressler, Giza, and Nigam (1963); Fowden and Bell (1965); Blumenthal <i>et al.</i> (1968)	Nigam and Ressler (1964); Floss, Hadwiger, and Conn (1965); Blumenthal <i>et al.</i> (1968)
		<i>Glycine</i>	Nartey (1970)	Tschiersch (1964a, 1964b, 1965); Hendrickson and Conn (1969)
Mimosine (7)	3,4-Dihydroxypyridine†	<i>Mimosa</i>	Renz (1936)	Tiwari, Penrose, and Spenser (1967)
Albizzine (5)	‡	<i>Mimosa</i>	Gmelin, Strauss, and Hasenmaier (1958)	
		<i>Acacia</i>	Seneviratne and Fowden (1968a)	
		<i>Albizzia</i>	Reinbothe (1962)	Reinbothe (1962)
		<i>Enterolobium</i>	Gmelin, Strauss, and Hasenmaier (1959)	
Caryophyllales				
β -Orcylalanine (8)	Orsellinic acid	<i>Agrostemma</i>	Hadwiger <i>et al.</i> (1965)	Hadwiger <i>et al.</i> (1965)
Cucurbitales				
β -Cyanoalanine (3)	Cyanide ion	<i>Ecballium</i>	Fowden and Bell (1965)	
β -Pyrazol-1-ylalanine (6)	Pyrazole	Numerous genera	Noe and Fowden (1960); Dunnill and Fowden (1965)	Dunnill and Fowden (1963); Frisch <i>et al.</i> (1967)
Graminales				
β -Cyanoalanine (3)	Cyanide ion	<i>Sorghum</i>	Blumenthal <i>et al.</i> (1968)	
Malpighiales				
β -Cyanoalanine (3)	Cyanide ion	<i>Linum</i>	Tschiersch (1964b)	

* Numbers in parentheses refer to Figure 1 structures.

† Not established as nucleophile, but it is significant that the closely related 5-hydroxyisoleucine (2-carboxy-5-hydroxypiperidine) co-occurs.

‡ Nucleophile not established.

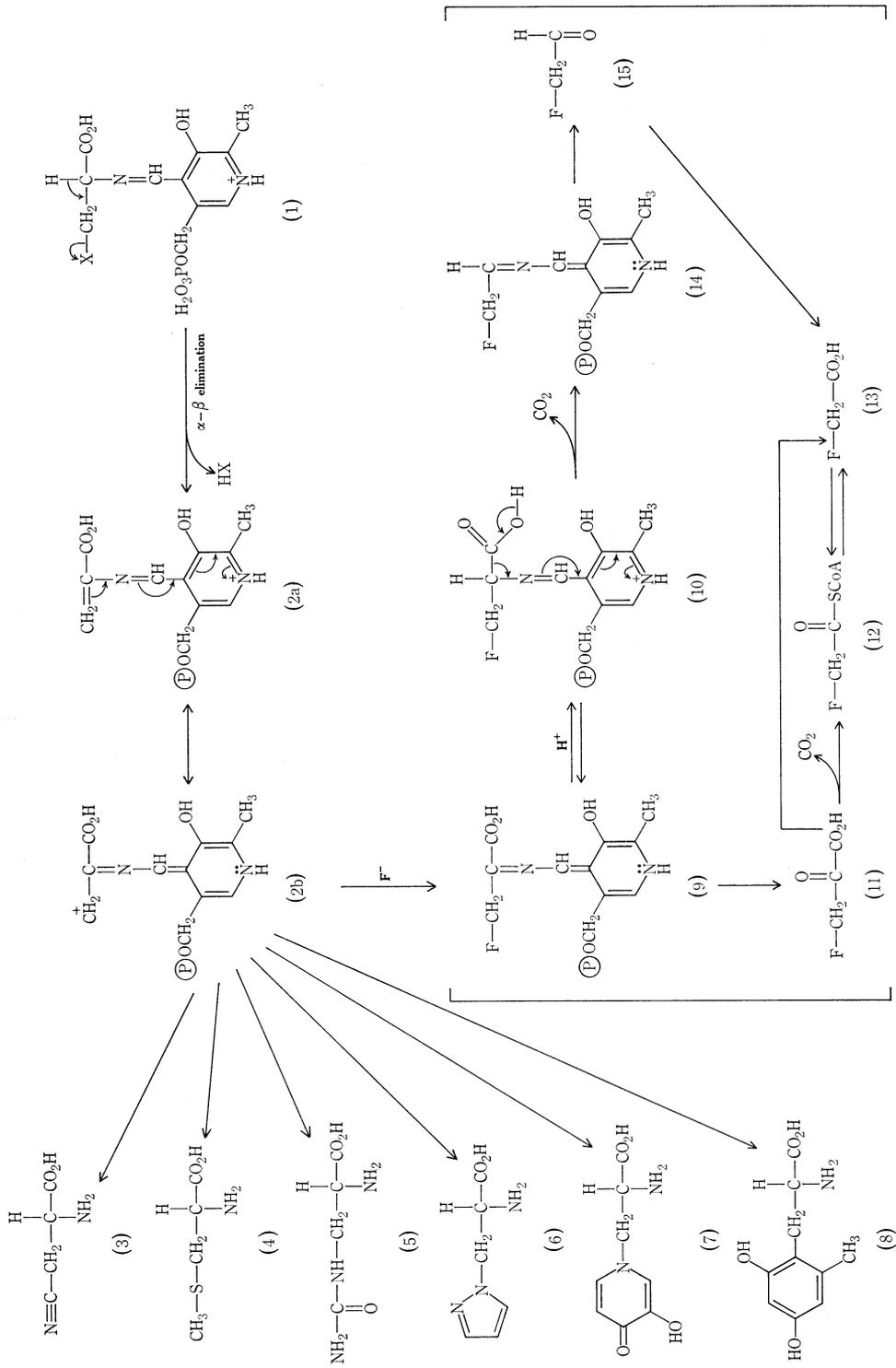
serine, or *O*-acetylserine, together with their plant origins. Examples selected involve nucleophiles in which the negative charge is associated with either nitrogen, carbon, or sulphur. It is proposed that fluoride ion may be incorporated in a similar manner through the action of non-specific enzymes of the β -cyanoalanine synthase type which catalyse the formation of β -cyanoalanine from cyanide ion and cysteine, serine, or *O*-acetylserine (Blumenthal *et al.* 1968; Floss, Hadwiger, and Conn 1965).

Figure 1 shows the pathway for a number of novel amino acids in which the structure (1) shows the initial pyridoxal phosphate-activation of serine ($X = OH$), cysteine ($X = SH$), *O*-acetylserine ($X = OCOCH_3$), and may also involve phosphoserine ($X = OPO_3H_2$). Figure 1 includes (in square brackets) the proposed process for the fluoride ion. One mode involves the formation of pyridoxamine phosphate-bound fluoropyruvate (9) which equilibrates with pyridoxal phosphate-bound fluoroalanine (10). One possible mechanism may involve fluoropyruvate (11) derived hydrolytically from (9) and conversion to fluoroacetyl coenzyme A (12) or directly to fluoroacetic acid (13) by an oxidative decarboxylation. Fluoroacetic acid derived by the latter process may be converted to fluoroacetyl coenzyme A. Subsequent metabolism of fluoroacetyl coenzyme A may involve (i) incorporation as the terminal group of long-chain ω -fluoro fatty acids which have been isolated from *Dichapetalum* (Peters and Hall 1959; Peters *et al.* 1960; Peters, Hall, and Ward 1964), or (ii) condensation with oxaloacetate to produce fluorocitrate as in *Agropyron* (Yu and Miller 1970).

An alternative mode involves the decarboxylation of pyridoxal phosphate-bound fluoroalanine (10) producing pyridoxamine phosphate-bound fluoroacetaldehyde (14). Hydrolysis of the latter to fluoroacetaldehyde (15) and subsequent oxidation to fluoroacetic acid may occur. The relative importance of these alternatives, based on biochemical analogies, is discussed later.

The co-occurrence, in *Acacia* species (Seneviratne and Fowden 1968a), of seven different β -substituted alanines suggests that a non-specific synthase in these plants may function to catalyse the incorporation of a variety of nucleophiles. In this respect it is noteworthy that *Acacia georginae*, from which fluoroacetic acid was isolated (Oelrichs and McEwan 1961), also elaborates β -acetylaminoalanine, albizziine (5), and djenkolic acid [3,3'-(methylenedithio)dialanine]. The latter substance and *S*-carboxyethylcysteine sulphoxide have been shown to derive from serine in *Acacia podalyriaefolia* (Seneviratne and Fowden 1968b). Also Wada and Toyota (1965) have shown that silkworm digestive fluid contains β -aminoalanine and not insignificant concentrations of serine and free ammonia. Furthermore, the soya bean (*Glycine*), another member of the Leguminales, has been shown to synthesize both fluoroacetate (Cheng *et al.* 1968), and asparagine via β -cyanoalanine (3) (Nartey 1970), and *Agropyron* and *Sorghum*, both of the order Graminales, produce fluoroacetate (Yu and Miller 1970), and β -cyanoalanine (Blumenthal *et al.* 1968) respectively.

This non-specificity referred to above is further exemplified below. Hendrickson and Conn (1969) have shown that a β -cyanoalanine synthase from *Lupinus angustifolia* could also catalyse the incorporation of methanethiol into *S*-methylcysteine (4), the conversion of *O*-acetylserine into cysteine, and the exchange of cysteine sulphhydryl groups. Dunnill and Fowden (1963) have established that the biosynthesis of β -pyrazol-1-ylalanine (6) in members of the Cucurbitaceae involves the nucleophile pyrazole reacting with serine. Frisch *et al.* (1967) have shown that a range of plant species and microorganisms (*Phaseolus aureus* [Leguminales], *Chlorella vulgaris*, *Saccharomyces cerevisiae*, and *Escherichia coli*), have the capacity to convert administered pyrazole to β -pyrazol-1-ylalanine (6). Furthermore, Frisch *et al.* (1967) have demonstrated this lack of specificity in *Cucumis sativus* by the incorporation of a range of synthetic nucleophiles (4-nitropyrazole, 1,2,4-triazole, indole, purine, and



others) into the corresponding β -substituted alanines with serine functioning as the recipient. This is an example of an enzyme, normally involved in some basic metabolic process, functioning in a non-specific manner in the presence of an acceptable substrate.

The proposed pathway involves the formation of a pyridoxal phosphate-bound recipient (2). The pyridoxal phosphate-enzymes serine dehydratase and cysteine desulphhydrase catalyse the α - β eliminations shown in Figure 1 (Metzler and Snell 1952). These eliminations would lead to a product which would be expected to exhibit mesomerism, (2a) \leftrightarrow (2b), and the partial carbonium ion character of (2b) would determine the site of nucleophilic attack. In this respect it is relevant that Tschiersch (1965) has shown that the incorporation of cyanide into β -cyanoalanine (3) by *Vicia sativa* homogenates is enhanced by pyridoxal phosphate.

The amino acids listed in Table 1 are derived predominantly from the order Leguminales and it is significant that fluoroacetate has been found in the genera *Acacia*, *Gastrolobium*, *Oxylobium*, and *Glycine*, all of which are in this order. An exception, however, *Dichapetalum cymosum* (order Rosales), from which fluoroacetate was first isolated, has been shown to metabolize serine in an unusual way in that it produces *N*-methylserine (Eloff and Grobbelaar 1969).

Relevant to the soya bean-production of β -cyanoalanine (3), Cheng *et al.* 1968, have reported that the fluoride content of the amino acid fraction of soya bean plants producing fluoroacetate (after fumigation with hydrogen fluoride) was higher than that of the controls. By analogy with natural β -substituted alanines (3)–(8) β -fluoroalanine may exist and could be derived hydrolytically from (10).

Trelease, Di Somma, and Jacobs (1960) have identified *S*-methylcysteine (4) in *Astragalus* (Leguminales) and Thompson, Morris, and Zacharius (1956) have found this amino acid in *Phaseolus* (Leguminales). It is known to originate from serine and methanethiol by the action of a pyridoxal-requiring enzyme in yeast (Wolf, Black, and Downey 1956).

Deamination of pyridoxal-bound fluoroalanine (10) to fluoropyruvate (11) is paralleled by the known conversion of *S*-methylcysteine (4) to methylmercaptopyruvate in yeasts (Maw and Coyne 1968). Eisman, Lee, and Winer (1965) have shown that fluoropyruvate is readily converted to 3-fluorolactate by beef heart lactic dehydrogenase, indicating that the substitution of hydrogen by fluorine in these substrates is acceptable to this particular enzyme. A similar acceptability may apply to plant tissue conversion of fluoropyruvate to fluoroacetate by oxidative decarboxylation.

Figure 1 shows an alternative process for deriving fluoroacetate from pyridoxal-bound fluoroalanine (10), namely by initial decarboxylation to (14) followed by hydrolysis. In this case fluoroacetaldehyde (15) would be expected and this may be oxidized by an appropriate aldehyde dehydrogenase. Evidence for this pathway is based on the following: (i) the conversion of *S*-methylcysteine to *S*-methylcysteamine by yeasts (Maw and Coyne 1968); (ii) the formation of ethylamine and ethanolamine by pyridoxal phosphate-dependent decarboxylation of alanine and serine respectively by *Ecballium* (Crocomo and Fowden 1970) which also produces β -cyanoalanine (Fowden and Bell 1965) and β -pyrazol-1-ylalanine (Noe and Fowden 1960).

Relevant to both mechanisms in Figure 1, Barnsley (1964) has shown that *S*-methylcysteine (4) is metabolised in the rat to both methylmercaptopyruvate and

methylmercaptoacetate. This metabolism is analogous to the proposed conversion of (10) to (11) and (13) respectively.

An α - β elimination has been suggested as the mechanism for producing the pyridoxal bound-recipient. A further example of such a process is provided by Tudball, Thomas, and Bailey-Wood (1971), who describe the enzymic conversion of serine-*O*-sulphate to pyruvate. By an α - β elimination an extended conjugation is derived permitting the pyridinium ion (2a) to function as an electron sink which would facilitate the acceptance of the nucleophile. Such a function of pyridoxal phosphate has been suggested for racemization and other processes in amino acid metabolism (Metzler, Ikawa, and Snell 1954). The recipient may, however, possess a β -substituent (X = hydroxy, sulphhydryl, phosphoryl, or acetoxy) all of which are excellent leaving groups.

Further evidence for a role of nucleophiles in the biosynthesis of these amino acids is provided by the enzymic synthesis of *S*-methylcysteine (4) from mimosine (7) and methanethiol when these are incubated with preparations from *Leucaena leucocephala* (Leguminales) seedlings (Murakoshi *et al.* 1970). This exchange of β -substituent suggests a reversible enzyme-catalysed nucleophilic addition process, as 3,4-dihydropyridine was eliminated. In the absence of methanethiol, mimosine was converted to pyruvate and ammonia. This is compatible with the report of Tiwari, Penrose, and Spenser (1967) who found that mimosine is derived from serine and a lysine-derived nucleophile and it is significant that the latter authors found mimosine and 5-hydroxypipicolinic acid to co-occur (see second footnote, Table 1). Murakoshi *et al.* (1970) have shown that a number of other sulphur-containing nucleophiles also exchange with the mimosine β -substituent. In this respect it is noteworthy that Seneviratne and Fowden (1968a) have found some of these amino acids in *Acacia* species.

The wide range of nucleophile acceptability, the occurrence of enzyme-catalysed nucleophile-exchange reactions in species of the Leguminales, and the phytochemical and taxonomic relationships of fluoroacetic acid-producing plants with the abovementioned species suggests that fluoride may mimic these nucleophiles and be incorporated *de novo*, or by exchange processes, and then further metabolize as described in Figure 1.

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