ORGANO-FLUORINE COMPOUNDS IN PLANTS

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I. ABSTRACT

A brief review is presented of the occurrence of organo-fluorine compounds in plants and of the effects of feeding plants with fluoride. The influence of fluorine location in a molecule on toxicity is discussed and the toxicities of a range of organo-fluorine compounds, both naturally occurring and synthetic, are summarized.

Distribution of fluoroacetate in Acacia georginae *is considered and the various findings are related to the incidence of poisoning of sheep and cattle in the field. Difficulties in detection of organo-fluorine compounds are emphasized and possible areas of further investigation are indicated.*

II. INTRODUCTION

General interest in the carbon-fluorine compounds began to expand in the early 1940's when Marais reported the occurrence of monofluoroacetic acid as the toxic principle in the South African plant *Dichapetalum cymosum* (1). Although the possibilities of such compounds as chemical warfare agents generated some enthusiasm during the war years, it was not until the late 1960's that general interest in the field began to expand. However, during the interval the biological significance of many forms of organically bound fluorine became evident. For example, it was found that the introduction of fluorine into the steroid hormones could greatly enhance their biological action. Furthermore changes could be qualitative as well as quantitative, that is, the type of biological action could be changed as well as the amount.

As interest in organo-fluorine compounds increased, Sir Rudolph Peters and Dr. P. W. Kent thought that a meeting with an interdisciplinary approach might clarify problems and stimulate further work. The result was the "Symposium on Carbon-Fluorine Compounds: Chemistry, Biochemistry, and Biological Activities" held at the Ciba Foundation, London, 13th-15th September 1971. There were twenty-five contributors from almost as many research groups. The papers presented and the discussion sessions were edited and were published in 1972 (2), providing a broad view of the whole field. Continuing interest is illustrated by the list of approximately 650 entries under "fluoro-" in the cumulative index of Biological Abstracts, July-December 1976. It seems relevant, therefore, to review the present knowledge of organo-

fluorine compounds, especially in relation to their occurrence in plants.

III. OCCURRENCE

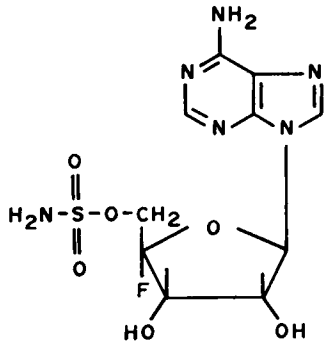
Of the known natural occurrences of organo-fluorine compounds the incidence of fluoroacetate is by far the most extensive. Following the initial identification in *Dichapetalum cymosum* (1) it was also detected in *Acacia georginae* in Australia (3) in the Brazilian plant *Palicourea marcgravii* (4) and in a large number of Australian plants, varieties of *Gastrolobium* and *Oxylobium*, of the family Leguminosae (5,6). All of these plants are toxic to animals, but the most toxic appear to be *Gastrolobium bilobum* and *Oxylobium parviflorum* from West Australia. The dry leaf of these plants has contained up to 1.25% fluoroacetate so that about 30 g fresh leaf would be sufficient to kill a sheep.

The toxicity of another South African plant *Dichapetalum toxicarium* is concentrated mainly in the seed and is predominantly caused by ω-fluorooleic acid. Also present is ω-fluoropalmitic acid and possibly also ω-fluoromyristic and ω-fluorocapric acids (7,8). Further examination of the organo-fluorine compounds extracted from these seeds has resulted in the detection of three other compounds, one of which has been purified (2a). This compound has a molecular weight of 472 and an empirical formula (from high resolution mass spectrometry) of $C_{27}H_{49}O_5F$ although it normally exists as a hydrate in association with $1\frac{1}{2}H_2O$. It probably contains a carbonyl group, four hydroxyls, a $-CH_2-CH_2F$ group, at least sixteen methylene groups and two rings. An interesting feature is the isolation from the same source of a non-fluorinated analogue which shows very similar properties and spectra. The only difference in structure appears to be $-CH_2-CH_3$ in place of $-CH_2-CH_2F$. A further interesting point was that the concentration ratio of fluoro-compound to non-fluoro was approximately 1:10, very similar to the ratio of ω-fluorooleic acid to oleic acid reported in seed from the same plant.

An unusual naturally occurring organo-fluorine compound is the antitrypanosomal antibiotic, nucleocidin, which was first isolated from a Streptomyces and reported in 1957 (9). It was twelve years later before the presence of fluorine in the molecule was demonstrated (10). This compound has been assigned the structure of a nucleoside 1 in which the fluorine is attached to the carbohydrate moiety.

IV. EFFECTS OF FEEDING PLANTS WITH FLUORIDE

The most likely source of the fluorine bound in organic compounds was fluoride from the soil. Many experiments have supported this idea, at least in the cases of those plants which are known to produce organo-fluorine compounds. For example,



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Weinstein (11) found that *Acacia georginae* seedlings varied in susceptibility to sodium fluoride damage so that he could select those which were most resistant. These fluoride-resistant seedlings produced high concentrations of fluoroacetic acid when incubated with sodium fluoride. Under similar conditions no fluoro-acids were detected in hay, soybean, crested wheat grass, corn, alfalfa or tomato. Peters (12,13) also investigated *Acacia georginae* seedlings and found that more fluorine was absorbed and more "organic" fluorine synthesized when the nutrient was more acid - pH 4.0 compared with pH 6.5.

Homogenates of *Acacia georginae* have also been treated with fluoride by Peters (14). The volatile products of this treatment were difficult to trap and it was suspected that vinyl fluoride might have been liberated. Further investigations showed other volatile fluorine compounds one of which has been tentatively identified as fluoroacetone. Addition of sodium fluoride to the homogenate increased the liberation of volatile fluorine by a factor of ten. Preuss (15) has reported that a tissue culture from a stem section of *Acacia georginae* will also produce fluoro-acetate when fed with sodium fluoride.

Dichapetalum spp. are also capable of converting fluoride into fluoroacetate. For example, isolated leaves of *Dichapetalum toxicarium* produced 200 mg fluoroacetate/kg after ten days with their petioles in 10^{-3} M sodium fluoride (16). It is believed (17) that the fluoroacetate is synthesized in the young leaves of this plant, stored in the small leaves adjacent to the flowers and withdrawn by the embryo seeds to be converted to the long-chain fluoro-acids.

Numerous attempts have been made to detect organo-fluorine

compounds in plants exposed to relatively large amounts of fluoride, either atmospheric or in solution. Results have generally been negative but some indications of the presence of fluoroacetate and fluorocitrate have been found. Lovelace (18) has reported the presence of quite high levels (179 mg fluoroacetate/kg; 896 mg fluorocitrate/kg) in forage crops from a pasture mix which contained *Medicago sativa* and *Agropyron cristatum* collected within two miles of a phosphate plant. However, fluoride levels on the pasture were high and major clinical symptoms were those of fluoride intoxication although blood citrate levels were elevated.

Fluorocitrate, in very small amounts, has also been detected in lettuce plants fed with fluoroacetate (19,2b). Peters (2c) reports trace amounts of fluorocitrate in an extract of commercial tea. Tea, of course, can have very high concentrations of fluoride but previous examinations have failed to detect organo-fluorine compounds. Peters suggests that the presence of trace amounts of fluorocitrate may be rather general in plants and has also identified it in fluorosed bones from cattle (20). It is uncertain whether this fluorocitrate was produced within the animal or stored following ingestion in the forage.

Other fluoro-acids, fluorobutyric and fluorohexanoic acids, have been tentatively identified as metabolic products obtained when brewer's yeast was treated with fluoroacetate (21). No indication of the position of the fluorine atoms in the molecules was given.

V. DISTRIBUTION IN PLANTS

Distribution of organo-fluorine in some fifteen plants has been discussed in detail by Hall (22) who studied plant specimens which were collected in the appropriate parts of the world, air dried, packed in polythene bags and dispatched to England. On arrival the plants were separated into leaf, bark, roots, thick and thin stem, and in some samples seeds. These samples were analyzed for fluoride obtained after a selection of treatments: ashing for total fluoride; acid-labile fluoride; "organic" fluoride; water soluble fluoro-compounds; and fluoro-compounds soluble in alkaline propanol. Hall's major findings were that there were wide variations both within and between plants in both organic and inorganic fluorine and in the ratios between them. The Brazilian plant *Palicourea marcgravii* had far more fluorine in the leaves than any other species and 61% of this was organic. All of the samples of *Acacia georginae* leaf analyzed had rather low organo-fluorine content but this is not unusual since the leaf of this plant is usually of low toxicity. It is used as a drought feed for sheep in the area. The only leaf material of *Acacia georginae* which frequently contains high levels of fluoroacetate is young leaf, particularly leaf of suckers growing from the root stock of grubbed out plants. A surprising finding, however, was the very low organo-fluorine content found in the seed of *Acacia*

georginae. While fluctuations occur in the field most analytical examinations have shown concentrations of fluoroacetate around 10-40 mg/kg with occasional samples, especially of young immature seed reaching 400 mg/kg (23). Perhaps of relevance in this context is a more recent finding by Vickery and Vickery (16) related to loss of fluoroacetate from leaf and from extracts of *Dichapetalum toxicarium*. When a sample of leaf was air-dried and kept for two months in humid air the concentration dropped from 600 mg fluoroacetate/kg to 60 mg/kg whereas a replicate sample, oven dried at 100° C and kept for two months in a sealed plastic bag retained the whole 600 mg/kg. Similarly, aliquots of a sterilized aqueous extract were stored either open to the atomosphere or sealed. Fluoroacetate disappeared from the open container but was retained in the sealed aliquot. This loss of fluoroacetate was attributed in both cases to the action of an unidentified micro-organism. Hall's samples could have been similarly attacked.

In many studies problems may arise through the actual compound containing the fluoroacetate moiety within the specific plant. In the seed of *Acacia georginae* the ease of extraction with organic solvents suggests that free monofluoroacetic acid is present (23). Similar solvent extraction data suggest that in the leaf of *Dichapetalum cymosum* the fluoroacetate is present as the potassium salt (1). Recently, Vickery and Vickery (16) have shown that fluoroacetate present in *Dichapetalum toxicarium* leaf is water-extractable but will not partition from the acidified aqueous extract into ether. When the neutralized extract is boiled for 30 minutes and reacidified, fluoroacetic acid could then be extracted with ether. The nature of the initial complex was not determined.

VI. SYNTHETIC FLUORO-COMPOUNDS

Goldman (2d) briefly summarizes some biosynthetic possibilities for the formation of organo-fluorine compounds. Some products of microbial transformations are very difficult to prepare by other means. For example, D(+)-fluorosuccinate is one of the products of metabolism of p-fluorophenylacetate in the protein of *E. coli* when the organism is grown in the presence of the fluoro-compound. Although such transformations are limited by the ability of micro-organisms to grow on substrates containing fluorine, the vast number of micro-organisms available and their adaptability make this approach a promising means of preparation of many kinds of fluorine-containing compounds.

Saunders (2e) has discussed the chemical synthesis of a range of compounds in which the carbon-fluorine bond is present. He states that some 300 compounds were synthesized. Among these were the methyl, ethyl, n-propyl and isopropyl fluoracetates, methyl α-fluoropropionate, methyl α-fluoroisobutyrate, fluoroethanol, 2-fluoroethyl-fluoroacetate, fluoroacetyl chloride, chloroacetyl fluoride, and fluoroacetyl fluoride, as well as a range of ω-fluoro acids and several cyclic compounds. Kent (2f) has reviewed

the preparation of fluorocarbohydrates and has tabulated the properties of numerous fluoro- trioses, tetroses, pentoses, and hexoses and their derivatives. Fluorine can be inserted in place of most hydroxyl groups in the carbohydrate molecule. Fluorine can also be introduced into steroids and Wettstein (2g) has provided a brief review of the methods involved. Once again, one or more fluorine atoms may be introduced into a range of positions within the molecule. Many fluorinated amino acids have also been prepared and 5-fluorouracil and its derivatives have been extensively studied.

VII. TOXICITY

Quantitative toxicity data about the organo-fluorine compounds is not very plentiful. Fluoroacetic acid toxicity has been examined rather thoroughly and the variation in response of different species is illustrated in Table I taken in part from Garner's Veterinary Toxicology (24).

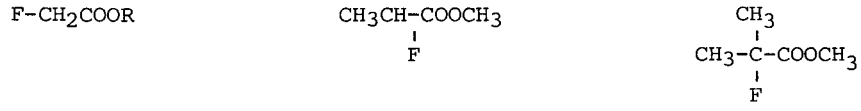
TABLE I

Oral Lethal Doses of Monofluoroacetic Acid

Species	Dose (mg/kg)	Species	Dose (mg/kg)
horse	0.5 - 1.75	cat	0.3 - 0.5
sheep	0.25 - 0.5	fowl	10 - 30
goat	0.3 - 0.7	quail	about 400
pig	0.3 - 0.4	man	4 - 7

Poisoning by fluoro-acetate, in short-term dosing, is essentially cumulative. Six days of dosing at one sixth of a lethal dose per day is usually fatal.

Toxicity of other fluoro-acids has been determined by Saunders (25,2e) and related to the toxicity of fluoroacetic acid. Compounds in the remaining discussion have been classified simply as "toxic," meaning of the same order of toxicity as fluoroacetate, or as "non-toxic," meaning the compound is of a low order of toxicity and does not cause fluoroacetate type intoxication. Methyl, ethyl, propyl, and isopropyl esters of fluoroacetic acid (2) are toxic. On the other hand methyl α-fluoropropionate (3) and methyl α-fluoroisobutyrate (4) are non-toxic.



2 toxic

3 non-toxic

4 non-toxic

There is a striking alternation in the toxicities of the ω-fluorocarboxylic esters:

FCH_2COOR	toxic
$F(CH_2)_2COOR$	non-toxic
$F(CH_2)_3COOR$	toxic
$F(CH_2)_4COOR$	non-toxic
$F(CH_2)_5COOR$	toxic
$F(CH_2)_7COOR$	toxic
$F(CH_2)_{10}COOR$	non-toxic

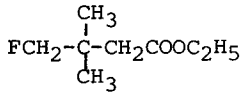
These toxicities are consistent with the β-oxidation of fatty acids in the living body:

$$RCH_2CH_2COOH \longrightarrow RCOOH + CH_3COOH$$

In the series $F(CH_2)_nCOOH$ when n is odd the end product is FCH_2COOH but when n is even the compound is oxidized to the non-toxic FCH_2CH_2COOH. The toxicity is dependent on the production of FCH_2CO^- grouping. This finding is supported by data on the acyl halides and ethyl fluoroformate.

FCH_2COCl fluoroacetyl chloride	toxic
$ClCH_2COF$ chloroacetyl fluoride	non-toxic
FCH_2COF fluoroacetyl fluoride	toxic
$FCOOC_2H_5$ ethyl fluoroformate	non-toxic

Compounds in which the β-position is blocked so that β-oxidation cannot occur are also non-toxic. Structures 5-8 are illustrations.



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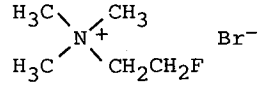

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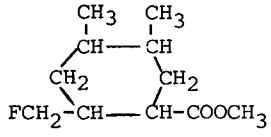

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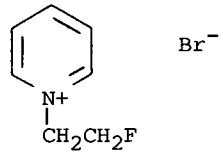

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On the other hand 2-fluoroethanol is readily oxidized to $F\text{-}CH_2COOH$ and is toxic. The ester 2-fluoroethyl fluoroacetate has greatly enhanced toxic properties - about twice that of methyl fluoroacetate, weight for weight. Fluoroacetamide and several substituted amides are also toxic:

FCH_2CONH_2 fluoroacetamide	toxic
$FCH_2CONHCH_3$ N-methyl fluoroacetamide	toxic
$FCH_2CONHCH_2CH_2Cl$ N-chloroethyl fluoroacetamide	toxic
$FCH_2CON(CH_2CH_2Cl)_2$ N,N—di—(chloroethyl) fluoroacetamide	toxic

The LD_{50} values for intraperitoneal injection of a range of α-fluoromalonates and α-fluoroalkanoic acids have been reported by Pattison and co-workers (27). Fluoro-steroids have a wide range of biological effects. Wettstein (2h) has provided a review of their hormonal activities.

VIII. FIELD TOXICITY OF PLANTS

In field situations, the actual concentration of organo-fluorine compounds in a plant varies and consequently so does the toxicity. The actual hazard to livestock is related to both the toxicity and the likelihood that the animals will consume sufficient to harm them. Assessment of possible consumption is a management problem but toxicity can be related to analytical determinations. However, if the analysis is specific for fluoroacetate then other toxic fluorine compounds may be missed or the extraction process may not be adequate to remove all the biologically available fluoroacetate. On the other hand determination of total organic fluorine could include compounds which are relatively harmless.

Acacia georginae is rather unusual for a toxic plant in that it is often freely eaten by animals without harmful effect. Local graziers believe that some areas in which *Acacia georginae* is predominant are "poison" while others are "safe." Analytical investigation has so far found no significant differences between selections of plants from the two classifications. However, such distinctions may have a factual basis, since marked variations in fluoroacetate content have been observed between plants even though there seems to be a similar range of values present in groups of plants from the different areas.

In my opinion, *Acacia georginae* produces symptoms of toxicity in animals only when at least one of the following applies:

(a) Animals have no other feed.
(b) Animals are severely stressed by heat, exercise, or fright.
(c) Pods are available in sufficient quantity so that animals consume large amounts.
(d) Suckers of high fluoroacetate content are available.
(e) Fluoroacetate levels in leaf or pod are unusually high.

Sheep, on a good diet, seem to be able to handle an intake of about 2 mg fluoroacetate per day and perhaps more although animals on poorer rations seem to be more susceptible. Jarret and Packham in 1956 reported that lucerne or gluten seemed to protect animals against continued dosing with sublethal amounts of fluoroacetate (2 mg daily). Recent work at the Animal Research Institute (28) indicates that any protection by gluten or urea against continued ingestion of fluoroacetate is minimal and would be essentially useless as a safeguard against field intoxication.

Dichapetalum cymosum is very poisonous in spring (mid-August to the end of November) and autumn (March to May) because at these times the plant produces highly toxic new leaves (29). At no time is the plant non-toxic. Similarly, *Dichapetalum toxicarium* is always toxic but the toxicity is lowest during the initial stages of seed production in May and June, when production of young leaves is halted. Low fluoroacetate content of leaf in September has been attributed to the leaching action of heavy rain (17).

IX. DETECTION OF FLUORINE

Fluorine is ubiquitous in the environment. In fact, fluorine-free situations exist only in systems from which the fluorine has been rigorously excluded. Consequently if fluorine is not detected in a given system, the methods of detection should be checked for sensitivity and the procedures used examined for processes during which fluorine could be lost. Fluorine can form bonds with carbon which vary from extremely strong to very weak and the compounds formed cover the whole range of organic chemistry. Thus the detection technique must be designed specifically for the compounds involved or be sufficiently general to handle all possible compounds. Fortunately, high resolution mass spectrometry and some of the newer techniques of nuclear magnetic resonance spectroscopy are capable of providing the necessary generality. Sensitivity in the NMR techniques has been vastly increased by the use of Fourier transform techniques which have improved the signal to noise ratio by 10^3. Location of the fluorine has also been facilitated by the development of heteronuclear spin decoupling so that the proton spectrum can be recorded while the resonance frequency of the fluorine is being irradiated. Any protons coupled to the fluorine are then detected. The major remaining limitations are in the processing of materials before insertion into the instruments. Fluorine compounds may be readily lost, either by

volatility, by difficulties in extraction, by absorption on to other components of the system or even, in low concentrations, by adsorption on to the walls of the containers. If biological activity is not lost along with the fluorine there is no problem, but if organo-fluorine compounds are present it is likely that their loss is also a loss of activity.

X. CONCLUSION

The range of information presented in this paper has illustrated that the presence of organo-fluorine compounds is not restricted to the higher plants. The data show that many systems are capable of synthesizing the carbon-fluorine bond or converting the compounds formed into others. It seems unlikely that this capacity has not been utilized extensively in natural processes especially since incorporation of fluorine can greatly enhance biological activity. One area in which this possibility could be investigated is the field of mold intoxications - a rapidly expanding area of knowledge. Toxic isolates of many molds have been reported and their biological actions described. It is apparent that many of the toxins involved are present in very small amounts and are extremely active. Naturally, isolation and purification of the active materials have proven difficult. It is possible that some of the difficulties could be caused by the presence of fluoro- and non-fluoro- analogues of ranges of closely related biologically active compounds. Small changes in location of a fluorine atom might cause little change in chemical or physical property but could alter biological activity both qualitatively and quantitatively.

It seems possible also that the range of biological activity available through adjustments of fluorine atom location could be a factor in the resistance of some plants to attack by micro-organisms. Even if nature has neglected this area of activity research workers now are actively synthesizing and testing fluorine compounds to try to develop more effective means of combating the destructive effects of micro-organisms. Only one fluorine-containing antibiotic, nucleocidin, has been mentioned. Others must follow.

The similarity in size between the fluorine and hydrogen atoms has been used in explanation of the way in which compounds such as fluoroacetate pass through metabolic pathways until their modifications cause a lethal blockage [the "lethal synthesis" described by Peters (30)]. Many attempts are being made to use this approach in the treatment of cancer. One idea is to try to set up a "lethal synthesis" specific for the cancer cell. In other words, compounds are being made which, it is hoped, will be rejected by normal cells but accepted into the metabolism of cancer cells and then kill by blocking essential pathways.

Workers involved with the chemistry of biologically active materials should therefore be aware of the possible presence of

fluorine and be aware of the difficulties in detection of fluorine compounds. In short, remember to look for organo-fluorine in biologically active systems.

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