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FLUORIDE METABOLISM IN *DICHAPETALUM TOXICARIUM*

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Abstract—The monofluoroacetate and fluoride ions are present in varying concentrations throughout *Dichapetalum toxicarium* (G. Don) Baill. (Dichapetalaceae). The synthesis of the monofluoroacetate ion takes place in the young leaves of the plant. This ion is stored in the small leaves adjacent to the flowers until withdrawn by the embryo seeds, in which it is converted to long-chain fluoro fatty acids. The fluoride ion is an intermediate in the synthesis of the monofluoroacetate ion and *D. toxicarium* has an unusual ability to withdraw fluoride from a low fluoride environment.

INTRODUCTION

PLANTS in general contain from 0·1 to 10 ppm dry wt of fluorine[1] and only a few plants are known to contain larger amounts of this element. Ornamental camellias contain a fluorine concentration of 790–3060 ppm dry wt[2] and tea 72–300 ppm dry wt,[3] but there is no evidence for the presence of organofluorine compounds in these plants. Those plants known to contain organofluorine compounds are *Acacia georginae*,[4] *Gastrolobium grandiflorum*,[5] *Palicourea marcgravii*,[6] *Dichapetalum cymosum*,[7] all containing monofluoroacetic acid, and *Dichapetalum toxicarium*.[8] The seeds of the latter were shown by Ward, Hall and Peters[9] to contain ω-fluoro-oleic acid, ω-fluoro-palmitic acid and possibly other long-chain fluoro fatty acids in small amounts. However, these workers detected neither an organofluorine compound nor inorganic fluoride in the leaves.

RESULTS AND DISCUSSION

It was shown that there was no free monofluoroacetic acid in the leaves of *D. toxicarium* but that the monofluoroacetate ion (MFA) was present. A single aqueous extraction removed the MFA quantitatively. The absence of long-chain fluoro fatty acids in amounts sufficient to be toxic[10] was shown by feeding rabbits with *D. toxicarium* leaves from which MFA had been removed. The rabbits showed no symptoms of poisoning.

The MFA and inorganic fluoride concentrations in specific organs of *D. toxicarium* are given in Table 1. Although analyses of random *D. toxicarium* samples gave varying absolute

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[4] P. B. OELRICHS and T. MCEWAN, *Nature, Lond.* **190**, 808 (1961).
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[10] R. A. PETERS and R. W. WAKELIN, *Biochem. J.* **71**, 245 (1959).

TABLE 1. THE CONCENTRATIONS OF MFA AND INORGANIC FLUORIDE IN ppm FRESH TISSUE, AND THE PERCENTAGE OF DRY MATTER IN THE SPECIFIC ORGANS OF A FLOWERING *D. toxicarium* PLANT

Organ	MFA conc.	Inorganic fluoride conc.	% dry matter
Young leaves	450	65	28
Young stems	270	30	40
Mature leaves	60	14	40
Mature stems	27	27	49
Old leaves	60	14	39
Leaves adnate to flowers	1100	85	37
Flowers	63	53	26
Tap root	23	30	49
Lateral root	<1	38	55

concentrations, the distribution pattern remained constant. Analysis of young and mature leaves at different times of the year showed that MFA concentration was lowest in the wet season and highest in the hot, dry season (Table 2). The low value for September can partially be explained by the leaching action of heavy rain on the leaves.[11] The gradual increase in concentration of MFA from September to March is probably due to increasing metabolic activity, which is at a maximum just before flowering.[12] The flowering season of *D. toxicarium* extends from February to April but is at a maximum in March. At no time of the year is *D. toxicarium* nontoxic to cattle, although toxicity is lowest during seed production in May and June, when production of young leaves is halted.

TABLE 2. THE VARIATION OF MFA CONCENTRATION IN ppm FRESH TISSUE WITH SEASON, SHOWN BY THE LEAVES OF *D. toxicarium*

Leaves	September	January	March
Young	100	300	450
Mature	11	20	60

It is consistently found that the small leaves, whose petioles are adnate to the peduncles of the inflorescences, and which develope with the cyme, contain the highest concentration of MFA as the flowers open, but that this is drastically reduced (1100–25 ppm fresh tissue) when the flowers are dead. It seems that these leaves act as a storehouse for the embryo seeds which withdraw MFA and convert it to long-chain fluoro fatty acids by the mechanisms suggested by Ward *et al.*[9] A total fluorine value of 170 ppm fresh tissue was found for the mature seeds, which corresponds approximately to the amount of ω-fluoro-oleic acid found in the seeds by Peters *et al.*[13] Our results indicate an ω-fluoro-oleic acid concentration of about 2500 ppm fresh tissue.

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[12] H. FLÜCK, *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 167, Academic Press, London (1963).
[13] R. A. PETERS, R. J. HALL and P. F. V. WARD, *Biochem. J.* **77**, 17 (1960).

Total inorganic fluoride concentrations in the leaves were divided into water soluble and acid soluble-water insoluble fractions. On ashing the sample with calcium oxide after removal of these fractions and MFA, a small residual amount of fluorine was present (Table 3). This was too high to be due to experimental error and indicates the possibility of a small concentration of water and acid insoluble organo-fluorine compounds or a very stable inorganic fluoro complex. These possibilities are being investigated.

TABLE 3. THE CONCENTRATIONS OF FLUORINE IN ppm FRESH TISSUE IN THE YOUNG AND MATURE LEAVES OF *D. toxicarium*

Fluorine type	Young leaves	Mature leaves
Inorganic—water soluble	87	10
Inorganic—water insoluble	20	52
Fluorine from MFA	23	0·5
Residual on ashing	7	4
Total fluorine by ashing	200	65
Difference between total by ashing and total by addition of parts	63	1·5

It was also found (Table 3) that the sum of the partial fluorine concentrations did not agree with the total fluorine determination for the young leaves, the discrepancy being of the order of 60 ppm fresh tissue, but that the agreement was within experimental error for the mature leaves. It therefore seems that a second, water soluble, organo-fluorine compound is present in the young leaves. The identity of this compound is being investigated.

From the results in Tables 1–3 it seems certain that MFA is synthesized in the young leaves. It is unlikely that the mature leaves are the main sites of synthesis and MFA transported to, and stored in, the young leaves, as happens with the products of photosynthesis,[14,15] since the water soluble, inorganic fluoride concentration in the mature leaves is much lower than in the young leaves. The synthesis of MFA is probably intimately connected with photosynthesis, although it is unlikely that fluorosugars are formed to be subsequently broken down to give MFA. If this were so, a high concentration of MFA would be expected in the roots, which require an adequate supply of sugars to provide energy for solute absorption.[16]

The toxicity of MFA to animals is due to conversion to fluorocitrate, which inhibits aconitase.[17] Treble *et al.*[18] found that plant aconitase is much less inhibited by fluorocitrate than that of animals. However, *D. toxicarium* must have evolved a mechanism whereby MFA does not interfere with the Kreb's cycle at all. It has been found that no such cycle takes place in the chloroplasts.[19] Thus it is reasonable to propose that the synthesis of MFA takes place in the chloroplasts and involves water-soluble inorganic fluoride. The reaction may involve photochemical fluorination of acetate (Marais[20] found acetate in the leaves of

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[16] F. M. EATON and H. G. JOHAM, *Plant Physiol.* **19,** 507 (1944).
[17] R. A. PETERS, *Proc. Roy. Soc.* **139B,** 143 (1952).
[18] D. H. TREBLE, D. T. A. LAMPORT and R. A. PETERS, *J. Biol. Chem.* **85,** 113 (1962).
[19] G. G. LATIES, *Arch. Biochem.* **27,** 404 (1950).
[20] J. S. C. MARAIS, *Onderstepoort J. Vet. Sci. Animal Ind.* **18,** 202 (1943).

D. cymosum), or substitution of fluorine for hydrogen at some early stage during photosynthesis, with subsequent conversion of the product to MFA.

TABLE 4. PLANTS WHICH CONTAIN NO MFA AND A FLUORIDE CONCENTRATION OF <2 ppm DRY WT GROWING IN THE VICINITY OF *D. toxicarium*

Plant	Family
Mareya micrantha (Benth.) Müll. Arg.	Euphorbiaceae
Morinda geminata DC.	Rubiaceae
Dichrostachys glomerata (Forsk.) Chiov.	Mimosaceae
Harungana madagascariensis Lam. ex Poir.	Hypericaceae
Myrianthus serratus (Trécul) Benth. and Hook.f.	Moraceae
Urena lobata L.	Malvaceae
Triclisia patens Oliv.	Menispermaceae
Rauvolfia vomitoria Afzel.	Apocynaceae
Macaranga barteri Müll.Arg.	Euphorbiaceae
Bertiera spicata (Gaertn.f.) Wernham	Rubiaceae
Vismia guineensis (L.) Choisy	Hypericaceae
Ochthocosmus africanus Hook.f.	Ixonanthaceae

The concentration of fluorine in the environment of *D. toxicarium* is low: that in the soil varies from 1 to 6 ppm and the Taia river water nearby contains 0·05 ppm. Analysis of a random sample of plants (Table 4) growing in the vicinity of *D. toxicarium* showed they contained no MFA and in each case the fluoride concentration was less than 2 ppm dry wt. As it has been shown that the synthesis of MFA can be induced in plants grown in a high fluoride environment,[21] it seems that the key to the synthesis of MFA by *D. toxicarium* lies in the ability of this plant to absorb and store fluoride from the soil in amounts sufficient to initiate the synthesis.

EXPERIMENTAL

Materials. All plants were collected within an area of 20 km^2 at Njala, Sierra Leone. Diurnal variations were minimized by collection in the early morning, and for the analyses given in Table 1 variations due to extrinsic factors were minimised by taking all organs from a single, 120 cm, flowering bush at 9 a.m. in March.

Methods. All plant samples were placed in polythene bags immediately on harvesting, weighed and dried overnight at 105°. Extractions for MFA analyses were with water at 90° for 2 hr. Qualitative analysis for MFA was by the method given in *Methods of Analysis*.[22] Neither the difluoroacetate ion nor the trifluoroacetate ion gave a positive result. The chloroacetate ion, the only ion known to interfere,[22] was shown to be absent by extracting the acidified, aqueous extract of the plant sample with ether, and then extracting the ethereal solution with excess 1 M NaOH and refluxing for 2 hr. No chloride ion could be detected. Quantitative analysis was based on the method of Kawashiro *et al.*[23] The coloured solutions were compared visually with reproducible sets of standard solutions.

Preparation of the samples for fluoride analysis were as follows: Total fluorine was determined by ashing with CaO at 600° for 3 hr. Water soluble fluoride was extracted with water at 90° for 2 hr. It was found that by keeping the temperature below 100° the amount of coloured impurities extracted was minimized. Acid soluble-water insoluble fluoride was extracted with 5% v/v perchloric acid at 90° for 2 hr. In all cases the fluoride was estimated by the method of Willard and Winter.[24] This was proved to be quantitative by ashing a known amount of pure MFA with CaO at 600° and analysing for the resulting fluoride.

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[22] *Methods of Analysis* (10th Edition), p. 399, Association of Official Agricultural Chemists, Washington, D.C. (1965).
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Key Word Index—Dichapetalum toxicarium; Dichapetalaceae; fluoride metabolism; monofluoracetate; fluoro fatty acids.