

## THE SYNTHESIS AND DEFLUORINATION OF MONOFLUOROACETATE IN SOME *DICHAPETALUM* SPECIES

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**Key Word Index**—*Dichapetalum*; Dichapetalaceae; fluoride; monofluoroacetate biosynthesis; monofluoroacetate defluorination; fluorocitrate

**Abstract**—Leaves from *Dichapetalum toxicarium* (G. Don) Baill. (Dichapetalaceae) removed from the plant and aqueous extracts prepared from the leaves of *D. heudelotii* (Planch. ex Oliv.) Baill. were able to synthesize monofluoroacetate from NaF. *D. pallidum* (Oliv.) Engl. leaves were not able to synthesize monofluoroacetate from NaF. Aqueous extracts from both *D. toxicarium* and *D. heudelotii* containing monofluoroacetate and possibly other organo-fluorine compounds were defluorinated by a micro-organism from the air. This organism was able to defluorinate fluorocitrate but not difluoroacetate or trifluoroacetate. In *D. toxicarium* monofluoroacetate is present in a form which is easily leached from the leaves, and which is defluorinated if the leaves are kept under herbarium conditions. The monofluoroacetate in this species is not defluorinated if the leaves are dried at 100° and stored in sealed polythene bags.

### INTRODUCTION

In previous papers we have shown that *Dichapetalum toxicarium* [1] and *D. heudelotii* [2] contain monofluoroacetate and that *D. pallidum* [3] does not. As free monofluoroacetic acid was not detected in the plant we assumed that the compound was present as the alkali metal salt [1]. Other workers have reported that *D. cymosum* contained potassium monofluoroacetate [4] and that *Acacia georginae* contained monofluoroacetic acid [5]. Peters and Shorthouse [6] have shown that single cell cultures of *A. georginae* synthesize monofluoroacetate and fluorocitrate from  $10^{-3}$  M NaF solutions. Weinstein *et al.* [7] have shown that axenically grown seedlings of this plant supplied with NaF synthesized monofluoroacetate, and Preuss *et al.* [8] demonstrated that callus tissue of the seedling stems of *A. georginae* also synthesized this compound. However the artificially induced synthesis of monofluoroacetate from  $F^-$  by *Dichapetalum* has not been previously recorded; neither has it been shown conclusively that the leaves can act as the site of synthesis. Hall and

Cain [9] have suggested that monofluoroacetate is synthesized in the rhizosphere, and subsequently absorbed into the plant.

It has been shown that peanuts [10] and lettuce [11] defluorinate monofluoroacetate and that soil bacteria defluorinate monofluoroacetate [12] and fluorocitrate [13] producing inorganic fluorine. This has not been demonstrated for *Dichapetalum* where the fluoro-organic compounds have been reported [14] as remaining stable for several years.

### RESULTS AND DISCUSSION

The monofluoroacetate in *D. toxicarium* was completely removed by soaking the leaves in cold water for 24 hr. This was shown by re-extracting the leaves with boiling water, when analysis of the extract by the A.O.A.C. method [15] previously used [1] showed that no monofluoroacetate was present. No monofluoroacetic acid could be detected in an ether extract of the acidified, cold water extract. However, after neutralizing the aqueous extract and boiling for 30 min, analysis by the modified method [1] of Kawashiro *et al.* [16] showed the monofluoroacetate ion to be present at a concentration of 220 µg/g fresh tissue. Mono-

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Table 1. Concentration of free fluoride ions ( $F^-$ ) in  $\mu\text{g/g}$  fresh tissue retained by the leaves of some *Dichapetalum* species with their petioles standing in  $10^{-3}$  M NaF solution for 10 days

Plant	$F^-$ absorbed from solution	$F^-$ recovered from leaves by soaking	$F^-$ retained by leaves
<i>D. toxicarium</i>	108	65	43*
<i>D. heudelotii</i>	60	11	49
<i>D. pallidum</i>	240	94	146
<i>D. toxicarium</i> in water	41 excreted into water	45	86 lost by leaves

\* Monofluoroacetate was synthesized by the leaves (200  $\mu\text{g/g}$  fresh tissue).

Table 2. Changes in free fluoride ion concentration ( $F^-$ ) with time for aqueous extracts of *Dichapetalum heudelotii* leaves containing added  $F^-$  in the form of NaF

Added $F^-$ ( $\mu\text{g/ml}$ )	1	2	3	4	Days 5	6	7	8	9	Total loss of $F^-$ ( $\mu\text{g/ml}$ )
80*	80	77	73	68	66	65	64	64	64	16
68	68	66	63	61	58	56	53	51	49	19
50	50	50	49	47	47	46	45	44	43	7

\* Monofluoroacetate was synthesized in this extract (9  $\mu\text{g/ml}$ ).

Table 3. Loss of monofluoroacetate (MFA) in  $\mu\text{g/g}$  dry tissue from the leaves of *Dichapetalum toxicarium*

Initial MFA concn	Subsequent treatment of leaves	Final MFA concn
1800	(a) Dried between sheets of newspaper and kept 6 months in herbarium at Njala, Sierra Leone	200
1800	(b) As above, but kept 2 yr	100
160	(a) Dried between sheets of newspaper and kept 2 yr in herbarium at Njala	<1
160	(b) As above, fumigated and kept 2 yr in herbarium at Kew, U.K.	<1
600	(a) Air-dried and kept 2 months in humid air	60
600	(b) Oven-dried at $100^\circ$ and kept 2 months in sealed polythene bag	600

fluoroacetate is therefore not present in the plant as a simple salt.

Fresh, young leaves of *D. toxicarium* which had been soaked in cold water to remove monofluoroacetate were left with their petioles in  $10^{-3}$  M NaF for 10 days. Analysis of the leaves at the end of this time showed that they had synthesized monofluoroacetate (200  $\mu\text{g/g}$  fresh tissue). *D. heudelotii* and *D. pallidum* leaves did not synthesize monofluoroacetate under these conditions. *D. toxicarium* leaves with their petioles in distilled water did not synthesize monofluoroacetate but excreted free fluoride ions into the water (41  $\mu\text{g/g}$  fresh tissue). The amounts of free fluoride ion lost from solution, together with those recovered by soaking the leaves in cold water for 24 hr, are given in Table 1. The concentration of free fluoride ions was determined with a fluoride ion specific electrode.

Although the leaves of *D. heudelotii* did not appear to synthesize monofluoroacetate, aqueous extracts of the leaves containing added  $F^-$  in the form of NaF gradually decreased in free fluoride ion concentration over 10 days (Table 2), and in one case monofluoroacetate was synthesized (9  $\mu\text{g/ml}$ ).

Young leaves from bushes of *D. toxicarium* containing a high concentration of monofluoroacetate when pressed and kept between sheets of herbarium paper gradually lost this ion, and after 2 yr it could no longer be detected in some specimens (Table 3). Extracts prepared from air dried specimens of young *D. toxicarium* leaves which had been kept for 2 yr contained concentrations of monofluoroacetate ranging from <1  $\mu\text{g/g}$  to 100  $\mu\text{g/g}$  dried tissue, whereas leaves from the same bushes, when fresh, had contained from 160–1800  $\mu\text{g/g}$  dried tissue. The loss took place much

Table 4. Concentration of  $F^-$  before and after defluorination of the monofluoroacetate and other organo-fluorine compounds (O-F) present in aqueous extracts prepared from the leaves of *Dichapetalum*

Plant	$F^-$ initial	$F^-$ final	$F^-$ calc.*	$F^-$ (O-F)
<i>D. toxicarium</i> (fresh, young)	0.7	24.3	7.4	16.2
<i>D. toxicarium</i> (dried 2 yr, young)	19.0	25.0	7.4	16.9
<i>D. toxicarium</i> (dried 2 yr, mature)	6.8	7.0	0.2	6.1
<i>D. heudelotii</i> (fresh, young)	0.4	3.0	0.2	2.4
<i>D. heudelotii</i> (dried 2 yr)	0.6	1.6	0.1	1.1

\*  $F^-$  concentration calculated as due to the defluorination of the monofluoroacetate present in the fresh leaves.  
All concentrations in  $\mu\text{g/ml}$ .

Table 5. The increase in  $F^-$  concentration in  $\mu\text{g/ml}$  with time in aqueous extracts prepared from *Dichapetalum toxicarium* leaves

Extract	Days					
	1	4	8	12	16	20
Heated at 100°, open to air	0.5	0.7	4.8	7.6	12.0	12.0
Cold, open to air	0.5	1.1	6.4	7.8	8.0	8.0
Heated at 100°, sealed	0.5	—	—	—	—	0.6

more rapidly if the leaves were surrounded by moist air at 25–30°, but could be halted by drying the leaves at 100° and sealing in polythene bags.

Aqueous extracts at pH 7, prepared from the leaves of *D. toxicarium* and *D. heudelotii* containing monofluoroacetate, gradually lost this ion if left in the air for 15 days, and the free fluoride ion concentration increased (Table 4). There was no differ-

ence in the final concentration of free fluoride ions or the time required to attain this value, between extracts in which the leaves remained, and those in which they were removed after 24 hr. With both extracts there was a lapse of 48 hr before the free fluoride ion concentration began to increase. A greater concentration of free fluoride ions was generated than that calculated from the defluorination of the monofluoroacetate present in the solutions (Table 4). It seems therefore that other organo-fluorine compounds are being defluorinated. This is in agreement with our previous observation [1] that water soluble organo-fluorine compounds other than monofluoroacetate are present in the young leaves of *D. toxicarium*.

The defluorinating agent was a micro-organism in the air. This was shown by dividing a boiled extract of *D. toxicarium* leaves containing monofluoroacetate into two portions, A and B. A was sealed and B left open to the air for 20 days, when

Table 6. The percentage of added sodium monofluoroacetate (25  $\mu\text{g/ml}$ ) defluorinated in 12 days in aqueous extracts prepared from the leaves of *Dichapetalum*, determined from the increase in  $F^-$  in  $\mu\text{g/ml}$ 

Plant	$F^-$ initial	$F^-$ final*	$F^-$ difference	Defluorination (%)
<i>D. toxicarium</i> (fresh, young)	0.4	2.5	2.1	42
<i>D. toxicarium</i> (dried 2 yr, young)	25.0	25.0	0	0
<i>D. toxicarium</i> (dried 2 yr, mature)	7.0	8.0	1.0	20
<i>D. heudelotii</i> (fresh)	0.4	0.8–1.9	0.4–1.5	8–30
<i>D. heudelotii</i> (dried 2 yr)	1.4	2.7	1.3	26

\* Difference between the free fluoride ion concentration finally determined in the extract containing added monofluoroacetate and that determined in the control extract.

Table 7. The percentage of added sodium fluorocitrate (70  $\mu\text{g/ml}$ ) defluorinated in 12 days in aqueous extracts prepared from the leaves of *Dichapetalum*, determined from the increase in free fluoride ion concentration ( $\text{F}^-$ ) in  $\mu\text{g/ml}$ 

Plant	$\text{F}^-$ initial	$\text{F}^-$ final*	$\text{F}^-$ difference	Defluorination (%)
<i>D. toxicarium</i> (air dried, young)	0.8	2.0	1.2	24
<i>D. heudelotii</i> (air dried, young)	0.6	2.0	1.4	28

\* Difference between the free fluoride ion concentration finally determined in the extract containing added fluorocitrate and that determined in the control extract.

determination of the free fluoride ion concentrations in the two extracts showed that B had been defluorinated and that A had not (Table 5). This micro-organism was found to be present in the air of Sierra Leone and Kenya.

Extracts from fresh, young *D. toxicarium* leaves; air-dried, mature *D. toxicarium* leaves which had been kept for 2 yr; and air-dried *D. heudelotii* leaves which had also been kept for 2 yr, all containing added sodium monofluoroacetate, were partially defluorinated. However a similar extract from air dried, young *D. toxicarium* leaves which had been kept for 2 yr was not defluorinated. This was probably because a high concentration of free fluoride ions was already present. No defluorination took place when NaF (1000  $\mu\text{g/ml}$ ) was added to an extract prepared from the fresh, young leaves of *D. toxicarium* containing monofluoroacetate. Microbial activity was also halted by the addition of NaCN (1000  $\mu\text{g/ml}$ ). However the addition of a small concentration of free fluoride ions (0.7  $\mu\text{g/ml}$ ) to the extract stimulated microbial activity and there was no time lag of 48 hr before the free fluoride ion concentration began to increase. The overall increase in free fluoride ion concentration in the extract was similar to that previously observed.

As fluorocitrate was also defluorinated (Table 7) but not difluoroacetate or trifluoroacetate when added to extracts from the leaves of *D. toxicarium* and *D. heudelotii*, it seems that the micro-organism can only utilize compounds in which carbon is monosubstituted by fluorine.

#### EXPERIMENTAL

**Extraction and detection of monofluoroacetate.** After soaking the leaves in  $\text{H}_2\text{O}$  for 24 hr the aq. extract was filtered, acidified with 1 M  $\text{H}_2\text{SO}_4$ , extracted with  $\text{Et}_2\text{O}$  and the  $\text{Et}_2\text{O}$  extract analysed for monofluoroacetic acid by the A.O.A.C. method [15] previously used [1]. As no monofluoroacetic acid was detected,

the aq. extract remaining after the  $\text{Et}_2\text{O}$  extraction was neutralized with 1 M NaOH and heated at  $100^\circ$  for 30 min. After cooling, the extract was acidified and extracted with  $\text{Et}_2\text{O}$ . Analysis of the  $\text{Et}_2\text{O}$  extract showed monofluoroacetic acid to be present. A further leaf extract was divided into two portions, one of which was boiled for 30 min. Both extracts were acidified,  $\text{Et}_2\text{O}$  extracted and the  $\text{Et}_2\text{O}$  extracts analysed for monofluoroacetic acid.

**Synthesis of monofluoroacetate.** The young leaves were gathered in the early morning and transported to the laboratory in polythene bags. They were immediately submerged in cold, distilled  $\text{H}_2\text{O}$  and left for 24 hr. The leaves were removed, washed several times with  $\text{H}_2\text{O}$  and dried with blotting paper. They were divided into three portions by weight: (1) was analysed for monofluoroacetate as described above; (2) was left with the petioles standing in  $10^{-4}$  M NaF solution (100 ml); and (3) was left with the petioles in distilled  $\text{H}_2\text{O}$  (100 ml). The beakers containing the leaves were placed near a window and regularly rotated. After 10 days the free fluoride ion concn in the solns (which were at pH 7 and had been kept at 100 ml by adding distilled  $\text{H}_2\text{O}$  when necessary) was determined with a fluoride ion specific electrode. The leaves were soaked for 24 hr in a known volume of distilled  $\text{H}_2\text{O}$  and the free fluoride ion concn in the aq. extract determined. The monofluoroacetate concn in the aq. extract was determined by the modified method [1] of Kawashiro *et al.* [16].

**Defluorination of organo-fluorine compounds.** Extracts were prepared by soaking the leaves in cold distilled  $\text{H}_2\text{O}$  for 24 hr in a closed, dark container. Ratios of ca 50 g fr leaves to 250 ml  $\text{H}_2\text{O}$  and 5 g dried leaves to 100 ml  $\text{H}_2\text{O}$  were used. Except in one instance the leaves were removed and the solns filtered. The extracts were left in transparent containers open to the air and were only stirred during free fluoride ion determinations. In the case of added substances the extracts were divided into two and one portion used as a control. The sodium salts of monofluoroacetate (2 mg), difluoroacetate (2 mg), trifluoroacetate (2 mg) and fluorocitrate (7 mg) were added to each 100 ml of the extract.

**Defluorinating agent.** An aq. extract containing monofluoroacetate was prepared by boiling the leaves of *D. toxicarium* for 2 hr. The extract was filtered and divided into two portions, A and B, which were boiled for a further 15 min. Portion A was sealed with a heat sterilized stopper and portion B left open to the air. The free fluoride ion concn in B was determined when the soln had cooled to room temp., and was then determined every 4 days. On the 20th day the free fluoride ion concn in A was determined.

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