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DETECTION AND MEASUREMENT OF FLUOROACETATE IN PLANT EXTRACTS BY ^{19}F NMR

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Abstract—Examination of extracts from seeds and foliage of several species known to contain fluoroacetate, using ^{19}F NMR spectroscopy, has shown the presence of the characteristic FCH_2-signal in most of them and enabled quantitative determination of their fluoroacetate content. No other fluorine-containing plant metabolites were detected; fluoroacetate was not detected in the extracts of several non-toxic species. The limit of detection is estimated to be *ca* 4 μg/g.

INTRODUCTION

Trifluoroacetate was identified as the toxic principle of *Dichapetalum cymosum* in 1944 [1] and it has since been found in three other poisonous *Dichapetalum* species of tropical and southern Africa [2–4]. Toxic plants belonging to the Australian genera *Gastrolobium*, *Oxylobium* and *Acacia* are also known to contain fluoroacetate which was first identified by Cannon [5, 6]. Extensive work by Aplin [7] has shown that only species in the section Racemosae of *Gastrolobium* and *Podolobieae* of *Oxylobium*, which are found only in south-western Western Australia, contain fluoroacetate. The single exception is *G. grandiflorum*, the range for which extends across northern Australia [5]. Also in the Leguminosae is the only other Australian plant to contain fluoroacetate, *Acacia georginae*, occurring in a limited area of central Australia [8]. One other tropical African plant *Spondianthus preussii* [9], and one from central America [10], *Palicouvea maregravii*, also contain fluoroacetate and it has been detected in low concentrations in other plant materials which are not normally regarded as toxic [11–13].

In the initial isolation [14], 3.5 g of the potassium salt of fluoroacetic acid was separated from 10 kg of plant material, but beginning with McEwan in the early 1960's [5, 8] gas chromatography has been the usual assay technique, with recent refinements making the technique extremely sensitive [11–13].

In 1972 Hall suggested that ^{19}F NMR spectroscopy might be useful in studies of this type [15], but none have been reported until now. The method is nowhere near as sensitive as GC, but is easier in practice and offers the opportunity to detect and identify other fluorocompounds which might accompany fluoroacetate.

RESULTS AND DISCUSSION

The method adopted in this work was to chill the plant material with liquid nitrogen and grind it to a powder which was then extracted by stirring at room temperature with aqueous or ethanolic ammonia. The combined filtrates were evaporated and the residue taken up in D_2O for examination by ^{19}F NMR. When foliage was extracted in this way the resulting signals were broad but treatment of the solution with chelating resin resulted in sharp signals, presumably because of the removal of paramagnetic metal ion impurities. This treatment was not necessary with seed extracts. The NMR signal of ammonium fluoroacetate in D_2O was a triplet (J = 47 Hz) occurring at 216 ppm upfield of the internal standard $CFCl_3$. The identity of the compound giving rise to this triplet was established by augmentation of the signal when sodium fluoroacetate was added to the solution, and also by the observation of a doublet (J = 47 Hz) at δ4.90 in the ^{1}H NMR spectrum of a *G. bilobum* extract which had the ^{19}F triplet.

The spectra of extracts were examined carefully but there were no signals which could be assigned to other fluorine-containing compounds. We had in mind the identification of ω-fluorofatty acids in leaves of *Dichapetalum cymosum* and seeds of *D. toxicarium* [16–18]. We examined the ^{19}F NMR spectrum of the model compound 6-fluorohexanoic acid which showed a triplet of triplets (^3J = 48 Hz, ^4J = 24 Hz) at 218 ppm, but hexane and ether extracts of seeds and foliage of the species shown in Table 1 were devoid of such signals. In the early stages of our work we discovered that aqueous ammonia slowly leached trifluoroacetate from magnetic stirrers coated with poly (tetrafluoroethylene) but this did not occur with newly manufactured stirrers and we conclude that the polymer had taken up trifluoroacetic acid during the use of the stirrers over the years in our laboratories. Quantitation of fluoroacetate in plant extracts was achieved by the addition of measured amounts of ammonium *p*-fluorophenylacetate. This substance gave a ^{19}F NMR signal at 116.6 ppm; integration of its area and that of the fluoroacetate signal gave simple quantitation with lower limits of *ca.* 4 μg/g fluoroacetate content when using a 1 g sample of plant material. This compares poorly with 0.1 μg/g for gas chromatography (GC) [19, 20] and

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Table 1. Fluoroacetate detection and estimation

Location	Species	Portion of plant	Extractant	Result	Fluoroacetate content* ppm (μg/g)
S.W. Qld.	*Acacia georginae*	Leaf	aq, NH_3	–	
		Seed	aq. NH_3	+	
	Acacia cambagei	Leaf	hexane, ether, methanol, aq. NH_3	–	
Sth. Africa	*Dichapetalum cymosum*	Leaf	aq. NH_3	+	
Qld.	*Dichapetalum papuanum*	Leaf	aq. NH_3	–	
	Gastrolobium bidens	Leaf	aq. NH_3	–	
W.A.	*Gastrolobium bilobum*	Leaf	aq. NH_3	+	190 ± 20
		Seeds	aq. NH_3	+	440 ± 40
Qld. (Herberton)	*Gastrolobium grandiflorum*	Leaf	EtOH/NH_3	+	
		Stems	EtOH/NH_3	+	
		Seeds	EtOH/NH_3	+	
		Leaf	aq. NH_3	+	
W.A.	*Gastrolobium spinosum*	Leaf	aq. NH_3	–	
W.A.	*Gastrolobium stenophyllum*	Seed	aq. NH_3	+	3470 ± 180
W.A.	*Gastrolobium villosum*	Seed	aq. NH_3	+	310 ± 30
W.A.	*Gastrolobium calycium*	Leaf	aq. NH_3	–	
W.A.	*Gastrolobium oxylobioides*	Leaf	aq. NH_3	–	
W.A.	*Gastrolobium microcarpum*	Leaf	aq. NH_3	+	180 ± 20
W.A.	*Gastrolobium bidens* (*Gastrolobium polystachyum* var.) [27]	Leaf	aq. NH_3	+	115 ± 10
Vic.	*Oxylobium alpestre*	Leaf	aq. NH_3	–	
Vic.	*Oxylobium lanceolatum*	Leaf	aq. N_3	–	
W.A.	*Oxylobium parviflorum*	Leaf	aq. NH_3	+	
		Seed	al. NH_3	+	400 ± 40
Vic.	*Oxylobium procumbens*	Leaf	aw. NH_3	–	
W.A.	*Oxylobium racemosum*	Seed	aq. NH_3	+	2990 ± 160
W.A.	*Oxylobium tetragonophyllum*	Seed	aq. NH_3	+	2370 ± 120

*Calculated as μg sodium fluoroacetate ('1080') per g dry plant material.

high performance liquid chromatography [21] and 0.005 μg/g for GC/MS [11, 12], but the NMR method is easier since derivatisation of the fluoroacetate is unnecessary and NMR facilities are commonly available in chemistry departments.

Variability in the fluoroacetate content of toxic plant materials is widely acknowledged, so it is interesting to compare the results in Table 1 with those in the literature. Leaves of *G. bilobum* have been shown to contain up to 2650 μg/g of fluoroacetate [22]. Our sample had less than one tenth of this, but somewhat more in the seeds for which no comparisons are available. Foliage of *O. racemosum* and *O. tetragonophyllum* [23] have been found to contain 1500 and 750 μg/g of the toxin, respectively, but there is considerably more of the toxin in the seeds of these species. Fluoroacetate was not detected in foliage of *G. villosum*, although the species is toxic [24] nor in *G. calycinum* (reported [25] up to 400 μg/g) nor *G. oxylobioides* (reported [26] 1050 μg/g). In *G. microcarpum* and *G. bidens* (a variety of *G. polystachyum* [27] the levels of fluoroacetate in our samples (180 and 115 μg/g) were below those reported by Aplin [26] (600 μg/g) for the former species. The latter is known to be toxic [24] but its fluoroacetate content has not been established.

The Australian *Dichapetalum* and the Victorian species *O. alpestre*, *O. lanceolatum* and *O. procumbens* are not known to be toxic so it was not surprising that fluoroacetate was not detected in them. *Acacia cambagei* is a non-toxic species which closely resembles *A. georginae*, but we were unable to detect fluoroacetate in the leaves of either. Only some seeds of the latter species were found to contain it, these being large enough for individual assay.

Samples of soil from the foot of an *O. parviflorum*, the leaves of which contained appreciable quantities of fluoroacetate, were also examined by the NMR method, but no fluoro-compounds were detected.

EXPERIMENTAL

Plant material. Aerial parts of the following plants were collected in the vicinity of Perth, W. A. and identified by T. E. H. Aplin at the W. A. Herbarium: *G. bilobum*, *G. spinosum*, *G. calycinum*, *O. parviflorum*, *G. oxylobioides*, *G. microcarpum*, *G. bidens* (*G. polystachyum*). *A. georginae* and *A. cambagei* with which it forms a continuous range were collected in the Georgina River basin in S.W. Queensland and identified by L. Pedley (Queensland Herbarium specimens *Pedley* 5152, 5183, 5203, 5209 and 5214). *A. georginae* seeds from the Northern Territory 240 km N.E. of Alice Springs were also obtained from Nindethana Seed Service, Woogenilup, W.A. 6234. Seeds of *O. parviflorum*, *G. stenophyllum*, *G. villosum*, *O. racemosum* and *O. tetragonophyllum*, collected near Perth, were also supplied by Nindethana. From Queensland, in the vicinity of Cairns, *G. grandiflorum* was obtained by J. Clarkson near Herberton and *D.*

papuanum by B. P. M. Hyland at Atherton. In Victoria, three species were obtained through C. C. J. Culvenor, CSIRO Animal Health Laboratory: *O. alpestre* from the Victorian alps, *O. procumbens* near Daylesford, and *O. lanceolatum* at Maranoa Gardens in a Melbourne suburb. All were authenticated by Herbarium officers. The South African species *D. cymosum* was collected in the vicinity of Pretoria and an extract provided by Prof. N. Grobbelaar.

Extraction and NMR measurements. A sample of plant material (1 g) was crushed in a mortar after treatment with liquid N_2, transferred to an Erlenmeyer flask and stirred with a magnetic stirrer bar for 24 hr with an NH_3 soln (1 ml 0.880 NH_3, 9 ml H_2O or EtOH). The liquid was decanted and the plant material extd twice more with NH_3 soln. The combined exts were stirred with Chelex resin (1 g) for 24 hr (only in case of leaf extracts), filtered and evapd on a rotary evaporator. The residue was taken up in H_2O–D_2O (1:1, 5 ml) and this soln was used for NMR analyysis. For quantitative work, an aliquot (0.5 ml) of a soln of ammonium *p*-fluorobenzoate (10 mg in 5 ml) was added. ^{19}F NMR spectra were recorded on a spectrometer operating at 84.66 MHz (500–15 000 scans, digital resolution 0.15 Hz) or one operating at 282 MHz (1000–4000 scans, digital resolution 2.2 Hz) with a small amount of $CFCl_3$ added to the soln as a zero std.

6-Fluorohexanol acid was synthesized from hexane-1,6-diol by first making 6-fluorohexanol as described in ref. [28] and then oxidation [29]. The properties of the product agreed with those in the lit. [30].

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