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FLUORO FATTY ACIDS IN SEED OIL OF *DICHAPETALUM TOXICARIUM*

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Key Word Index—*Dichapetalum toxicarium*; Dichapetalaceae; seed oil; gas chromatography-mass spectrometry; ω-fluorofatty acids.

Abstract—The fatty acid composition of the seed oil of *Dichapetalum toxicarium* was examined by GC-mass spectrometry. The presence of ω-fluoro-palmitoleic, -stearic, -linoleic, -arachidic and -eicosenoic acids was established, in addition to that of the ω-fluoro derivatives of oleic and palmitic acids previously isolated from this source. Strong evidence was also obtained for the presence of 18-fluoro-9,10-epoxystearic acid. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

A number of tropical and subtropical plant species from Africa, Australia and South America are capable of biosynthesizing the C–F bond, the most common organofluorine compound formed being the highly toxic fluoroacetic acid [1]. In Africa, the biosynthesis of this secondary metabolite appears to be mainly confined to the genus *Dichapetalum*. The compound has been isolated from the leaves or seeds of at least six species, and good toxicological evidence exists for its presence in nine others. Quantities as high as 7000–8000 ppm on a dry weight basis have been recorded in the young leaves and seeds of the East African species *D. braunii* [2]. The shrubby West African species, *D. toxicarium*, is unusual in that, in addition to containing fluoroacetate in the young leaves [3, 4], it accumulates ω-fluorinated lipids in the seeds at concentrations of up to 1800 ppm organic fluorine on a dry weight basis [5, 6]. The principal fluorinated component comprising about 80% of the organic fluorine present and 3% of the seed oil was isolated in 1959 by Peters and co-workers [6, 7] by reverse-phase chromatography on cellulose–liquid paraffin columns and identified as ω-fluoro-oleic acid (18:1). Small quantities of ω-fluoropalmitic acid (16:0) were also isolated and identified in the seed oil using similar techniques [8]. The presence of ω-fluoro derivatives of capric (10:0) and myristic acids (14:0) as minor components was surmised on the basis of the similarity of retention times of certain peaks on a GC trace of the methylated fatty acids of the oil to those calculated for the methyl esters of these compounds, but no unequivocal chemical confirmation of their identity was obtained [8]. Recent work on the more polar components of the fatty acid fraction have resulted in the identification of *threo*-18-fluoro-9,10-dihydroxystearic acid in the oil [9]; it was proposed that this compound arose from ω-fluoro-oleic acid via a 9,10-epoxide. In view of the tentative nature of conclusions drawn in the early work on the seed oil regarding the identity of some of the ω-fluorofatty acids present and also the relative insensitivity of the analytical techniques employed, a quantitative re-examination of the fatty acid composition of the seed oil of *D. toxicarium* has been conducted using GC-mass spectrometry. We report the results in the present paper.

RESULTS AND DISCUSSION

The hexane-extractable oil of seeds of *D. toxicarium* represented 10.7% of the dry weight, and the organic fluorine content of the seeds was 630 ppm. The fatty acid composition of the seed oil is shown in Table 1. ω-Fluoro fatty acid methyl esters (FAMEs) were identified on the basis of their retention times and electron impact (EI) and chemical ionization (CI) mass spectra. CI-mass spectrometry yielded characteristic mass spectra for ω-fluoro-FAME with major ions detected at $[M+H]^+$, $[M+H-HF]^+$, $[M+H-HF-CH_3OH]^+$ and $[M+H-CH_3OH]^+$. Other weaker diagnostic ions, such as $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$, were also present. The base peak

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Table 1. Fatty acid composition of oil from seeds of *Dichapetalum toxicarium*

Fatty acid	% Total fatty acid content*
14:0	0.3
16:0	5.6
16:0 F	0.5
16:1	0.4
16:1 F	Trace
18:0	17.3
18:0 F	2.0
9,10-Epoxy 18:0	Trace
18:1	47.4
18:1 F	9.6
18:2	10.5
18:2 F	0.8
19:1	0.5
20:0	1.9
20:0 F	Trace
20:1	2.2
20:1 F	Trace
22:0	Trace
24:0	0.4
26:0	0.3

*Trace indicates presence at less than 0.3%.

for saturated ω-fluoro-FAME was attributable to the ion $[M+H-HF]^+$, whereas for unsaturated ω-fluoro-FAME the most dominant ion was at $[M+H]^+$. EI mass spectra of saturated ω-fluoro-FAME gave a weak $[M]^+$, with major fragmentation ions at *m/z* 74 and 87 due to a McLafferty rearrangement. EI mass spectra of unsaturated ω-fluoro-FAME gave no $[M]^+$, but weak ions at $[M-OCH_3]^+$ and $[M-HF]^+$ were present.

Spectral data for the various ω-fluoro-FAME identified by GC-mass spectrometry at an ionizing energy of 70 eV are as follows: methyl 16-fluoropalmitate (16:0 F). CIMS, *m/z* (rel. int.): 329 $[M+C_3H_5]^+$ (3), 317 $[M+C_2H_5]^+$ (7), 289 $[M+H]^+$ (41), 287 $[M-H]^+$ (19), 269 $[M+H-HF]^+$ (100), 257 $[M+H-CH_3OH]^+$ (10), 237 $[M+H-HF-CH_3OH]^+$ (13). EIMS, *m/z* (rel. int.): 288 $[M]^+$ (2), 87 $[C_4H_7O_2]^+$ (55), 74 $[C_3H_6O_2]^+$ (100). Methyl 16-fluoropalmitoleate (16:1 F). CIMS, *m/z* (rel. int.): 327 $[M+C_3H_5]^+$ (7), 315 $[M+C_2H_5]^+$ (10), 287 $[M+H]^+$ (100), 267 $[M+H-HF]^+$ (32), 235 $[M+H-HF-CH_3OH]^+$ (66). EIMS, *m/z* (rel. int.): 266 $[M-HF]^+$ (5), 255 $[M-OCH_3]^+$ (5). Methyl 18-fluorostearate (18:0 F). CIMS, *m/z* (rel. int.): 357 $[M+C_3H_5]^+$ (4), 345 $[M+C_2H_5]^+$ (8), 317 $[M+H]^+$ (51), 315 $[M-H]^+$ (36), 297 $[M+H-HF]^+$ (100). EIMS, *m/z* (rel. int.): 316 $[M]^+$ (4), 87 $[C_4H_7O_2]^+$ (61), 74 $[C_3H_6O_2]^+$ (100). Methyl 18-fluoro-oleate (18:1 F). CIMS, *m/z* (rel. int.): 355 $[M+C_3H_5]^+$ (3), 343 $[M+C_2H_5]^+$ (12), 315 $[M+H]^+$ (100), 295 $[M+H-HF]^+$ (44), 283 $[M+H-OCH_3]^+$ (21), 263 $[M+H-HF-CH_3OH]$ (48). EIMS, *m/z* (rel. int.): 294 $[M-HF]^+$ (7), 283 $[M-OCH_3]^+$ (9). Methyl 18-fluorolinoleate (18:2 F). CIMS, *m/z* (rel. int.): 353 $[M+C_3H_5]^+$ (6), 341 $[M+C_2H_5]^+$ (9), 313 $[M+H]^+$ (50), 293 $[M+H-HF]^+$ (100), 281 $[M+H-OCH_3]^+$ (65), 261 $[M+H-HF-CH_3OH]^+$, (22). EIMS, *m/z* (rel. int.): 292 $[M-HF]^+$ (7), 281 $[M-OCH_3]^+$ (7). Methyl 20-fluoroarachidate (20:0 F). CIMS, *m/z* (rel. int.): 385 $[M+C_3H_5]^+$ (3), 373 $[M+C_2H_5]^+$ (7), 345 $[M+H]^+$ (64), 343 $[M-H]^+$ (31), 325 $[M+H-HF]^+$ (100), 313 $[M+H-CH_3OH]^+$ (14), 293 $[M+H-HF-CH_3OH]^+$ (19). EIMS, *m/z* (rel. int.): 344 $[M]^+$ (4), 87 $[C_4H_7O_2]^+$ (61), 74 $[C_3H_6O_2]^+$ (100). Methyl 20-fluoroeicosenoate (20:1 F). CIMS, *m/z* (rel. int.): 343 $[M+H]^+$ (100), 323 $[M+H-HF]^+$ (53), 311 $[M+H-CH_3OH]^+$ (30), 291 $[M+H-HF-CH_3OH]^+$ (58). EIMS, *m/z* (rel. int.): 322 $[M-HF]^+$ (5), 311 $[M-OCH_3]^+$ (6).

The results of the investigation confirm ω-fluoro-oleic and ω-fluoropalmitic acids as the major fluoro fatty acid components of the seed oil comprising 75% and 15%, respectively, of the total fluorolipid content of the seed. The proportion of ω-fluoro-oleic acid found was very similar to that of 80% reported by Peters and co-workers [6–8]. Although no evidence was obtained for the presence of either ω-fluorocapric or ω-fluoromyristic acid, which were tentatively identified in the oil by Ward *et al.* [8] on the basis of GC retention times, 10% of the total fluoro fatty acid content of the oil was found to be located in ω-fluorostearic and ω-fluorolinoleic acids, compounds not previously reported as natural products. Other hitherto unrecorded ω-fluoro fatty acids identified, albeit in trace quantities, were ω-fluoro derivatives of palmitoleic, arachidic and eicosenoic acids. No fluoro fatty acid was detected in the absence of its non-fluorinated analogue, the latter being about 5- to 10-fold more abundant than the former. Of particular interest amongst the non-fluorinated fatty acids was the presence of trace amounts of 9,10-epoxystearic acid, which has been detected as a minor component in several other seed oils [10–12]. This compound and its ω-fluoro analogue have been proposed as possible precursors of 9,10-dihydroxystearic and its ω-fluoro derivative, respectively, both of which were isolated from *D. toxicarium* seed oil by Harper *et al.* [9]. Neither of the dihydroxy compounds were detected in this batch of seeds using the saponification–CH_2N_2 derivatization or the more sensitive BF_3–MeOH transesterification–silylation techniques. However, use of the latter technique followed by GC-mass spectrometry in the single-ion monitoring mode led to the identification of a peak at a slightly longer retention time than that observed for the derivative of the 9,10-epoxy compound giving fragmentation ions at *m/z* 233 and 259 in the ratio that would be expected of the silyl derivatives of the methyl esters of 18-fluoro-9-methoxy-10-hydroxystearic and 18-fluoro-9-hydroxy-10-methoxystearic acids. This observation provides strong evidence for the presence of 18-fluoro-9,10-epoxystearic acid in the seed oil. The failure to detect dihydroxy compounds in the oil from this batch of seed was unexpected, but the formation of the compounds may be associated with a particular stage of development of the seed. Miwa *et al.* [13] have clearly demonstrated that 12,13-epoxyoleic acid and 12,13-dihydroxyoleic acid undergo interconversion during maturation of seeds of *Veronica anthelmintica*, resulting in major changes in the relative concentrations of the two compounds.

EXPERIMENTAL

Plant material. Fruits of *D. toxicarium* [G. Don] Baill. were collected in January 1990 in Sierra Leone from bushes growing on Kortright Hill overlooking the village of Kissy. Voucher specimens are lodged at the Herbarium in the Botany Department of Fourah Bay College, University of Sierra Leone (acquisition no. RSM/CM/1). Prior to chemical analyses, the outer hardened seed coats of the fruit were broken open and the seeds removed. Seeds were ground in a blender and stored at −20°.

Extraction and isolation of fatty acids. Ground seed material (0.5 g) was placed in a glass-stoppered tube (50 ml) and EtOH (8.5 ml) and 60% KOH (1 ml) added and the mixture heated at 70° for 2 hr. After cooling, the saponified extract (1 ml) was acidified with 10% H_3PO_4 (1 ml) and extracted × 2 with hexane (5 ml). The hexane frs were combined, evapd to dryness under a stream of N_2 and methylated using CH_2N_2–Et_2O prior to GC-MS analysis.

For extraction and identification of epoxy and dihydroxy fatty acids an alternative transesterification [14] and derivatization [15] technique was also employed. This procedure gave derivatives not only more amenable to GC but also yielding more characteristic spectra than those given by the saponification–CH_2N_2 procedure. Ground seed material (0.5 g) was suspended in 14% BF_3 in MeOH–benzene (9:1, 10 ml) and heated for 18 hr at 60° in a glass-stoppered tube (50 ml). After cooling, H_2O was added and the mixt. extracted with hexane (2 × 10 ml). The extracts were combined, evapd to dryness under a stream of N_2 and reconstituted in hexane (5 ml). A portion of the extract (200 μl) was taken to dryness, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA, 200 μl) added and the soln allowed to stand at room temp. for 5 min prior to analysis by GC-MS.

GC-MS analysis. FAMEs were identified and quantified by GC-MS using a mass selective detector. For analysis of FAMEs prepd by the CH_2N_2 method, the GC was fitted with a Chrompack CP-Sil-19 fused silica WCOT capillary column (25 m × 0.25 mm ID) with 95% methyl, 7% phenyl, 7% cyanopropyl, 1% vinylpolysiloxane as bonded phase. The carrier gas was He (flow rate 0.75 ml min^{-1}). MS were acquired in both the EI and CI modes using an ionization energy of 70 eV. Methane was used as reagent gas for CI. For quantitative analysis, the sample (1 μl) was injected in the split mode employing a split ratio of 20:1, whereas for identification purposes in both CI and EI modes splitless injection was used. After injection of the sample (injector temp., 250°), the oven was held at 30° for 1 min, then programmed at 10° min^{-1} up to 300° and held at this temp. for 10 min. Ion currents between m/z 30 and 500 were monitored and individual FAMEs were identified from their EI and CI MS and GC R_t values. To quantify each FAME the total ion current in the EI mode was expressed as a percentage of the sum of the ion currents associated with all FAMEs in the extract with an abundance greater than 0.3% of the total. Standard mixts of non-fluorinated FAMEs were utilized for calibration. As authentic standards of the ω-fluoro fatty acids were not available, the total ion current associated with the mass spectrum of a ω-fluoro fatty acid was assumed to be identical to that of its non-fluorinated analogue.

For analysis of MSTFA derivatives of FAMEs prepd by the transesterification procedure, the GC was fitted with an HP Ultra 2 WCOT capillary column (25 m × 0.25 mm i.d.) with 95% methyl, 5% phenyl polysiloxane as the bonded phase. Temp. programming was identical to that described above, except that the initial oven temp. was held at 100° rather than 30°.

Determination of organic and inorganic fluorine. Organic fluorine was determined as fluoride after ashing by the method in ref. [16]. The sample (0.5 g) was placed in a Pt crucible (15 ml) with 1 M LiOH (0.5 ml) and 1 M Mg succinate (0.6 ml). The contents of the crucible were evapd to dryness at 100° and pre-ashed by heating on a hot plate. The ashing process was completed over a spirit burner and inorganic fluoride in the residue determined by the procedure in ref. [17]. To the sample was added deionized H_2O (1 ml), 1 M H_2SO_4 (1 ml) 0.5 M trisodium citrate–0.5 M KNO_3 buffer (8 ml) and fluoride concn measured using a fluoride-ion-selective electrode coupled with an Orion double junction reference electrode. Standard fluoride solns used in calibration were prepd using 1 M H_2SO_4 (1 ml), 10 and 100 ppm aq. NaF (1 ml) and 0.5 M trisodium citrate–0.5 M KNO_3 buffer (8 ml).

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