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5,9-NONADECADIENOIC ACIDS IN *MALVAVISCUS ARBOREUS* AND *ALLAMANDA CATHARTICA*

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Key Word Index—*Allamanda cathartica*; Apocynaceae; *Malvaviscus arboreus*; Malvaceae; 5,9-nonadecadienoic fatty acids.

Abstract—The phospholipid fatty acid composition of *Malvaviscus arboreus* DC. (Malvaceae) and *Allamanda cathartica* L. (Apocynaceae) was studied. The fatty acids, 17-methyl-5,9-octadecadienoic acid, 16-methyl-5,9-octadecadienoic acid, and 5,9-nonadecadienoic acid were identified in the phospholipid (mainly phosphatidylcholine) extract of *M. arboreus* by GC-MS. 17-Methyl-5,9-octadecadienoic acid was also identified in *A. cathartica*. This is the first report of *iso-anteiso* branched Δ5,9 fatty acids from a plant source.

INTRODUCTION

The study of the fatty acid composition of higher plants has resulted in the identification of several unusual structures [1, 2]. For example, the first reported conjugated straight-chain fatty acid, namely 9,11-octadecadienoic acid, was isolated from an *Ixora* species [3]. Sterculic acid, namely ω-(2-*n*-octylcycloprop-1-enyl)octanoic acid, was isolated from *Sterculia foetida*, and this unveiled for the first time a cyclopropene ring in a fatty acid [3, 4]. Another cyclopropene fatty acid, malvalic acid, was later isolated from *Malva veticillata* in 1957 by Vickery and co-workers [5]. There are also examples in the literature of unusual ethylene-interrupted fatty acids, such as 5,9-hexadecadienoic acid and 5,9-octadecadienoic acid, which were identified in slime mold and conifer leaves [6].

The phospholipid fatty acid composition of higher plants has also been studied. For example, phosphatidylcholine, a predominant phospholipid in higher plants, contains most of the common occurring fatty acids, in particular palmitic acid, linoleic acid, and linolenic acid. Considerable amounts of other very long-chain saturated fatty acids (C_{20}-C_{26}) were reported by Takahashi and co-workers in phosphatidylserines from eighteen species of higher plants such as *Bougainvillaea* sp. [7].

In the present paper, we describe the identification of three $\Delta^{5,9}$ nonadecadienoic acids, namely 17-methyl-5,9-octadecadienoic acid, 16-methyl-5,9-octadecadienoic acid, and 5,9-nonadecadienoic acid, from the flowers of *Allamanda cathartica* L. (Apocynaceae), and *Malvaviscus arboreus* DC. (Malvaceae). This is the first report of *iso-anteiso* branched $\Delta^{5,9}$ fatty acids from a plant source. We also present the phospholipid fatty acid composition of these flowers for literature comparison.

The bright yellow flower *Allamanda cathartica* L. has also been called Golden Trumpet because it resembles a trumpet in shape [8]. In 1954, Arthur and Hui isolated vitamin C and ursolic acid from its leaves [9]. This ornamental plant has also been widely studied for its iridoid content [10–12]. Alamandin, an anti-leukemic iridoid lactone, was characterized in *A. cathartica* by Kupchan *et al.* in 1974 [12]. The red flower *Malvaviscus arboreus* DC. [8] is a native of Mexico and Brazil, and it was introduced in Puerto Rico as an ornamental plant. Some plants of the family Malvaceae were studied by Akramov (e.g., *Althaea officinalis*) and the seeds have a low phospholipid content [13]. *M. arboreus* was studied by Gottsberg *et al.* and asparagine was found to be its most abundant amino acid [14].

RESULTS AND DISCUSSION

The phospholipid composition of *A. cathartica* L. and *M. arboreus* DC. was examined by TLC. The main phospholipids were identified as phosphatidylcholine (PC) with some phosphatidylinositol (PI).

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Allamanda cathartica L

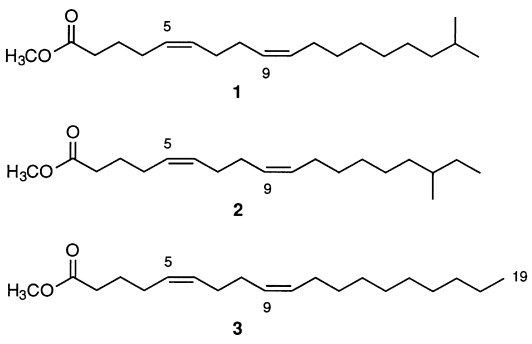
The complete phospholipid fatty acid composition of *A. cathartica* is shown in Table 1. These were characterized by GC-MS as methyl esters, with the double bond positions established by dimethyl disulfide (DMDS) and pyrrolidide derivatization followed by mass spectrometry [15]. The previously unreported 17-methyl-5,9-octadecadienoic acid was also identified and constituted 0.1% of the total fatty acids from *A. cathartica*. Comparison of the mass spectrum of the corresponding methyl ester **1** with that of other similarly reported *iso*-$\Delta^{5,9}$ methyl esters, with longer chain-lengths, permitted its facile characterization [16]. Methyl 17-methyl-5,9-octadecadienoate (**1**) showed a M^+ at *m/z* 308 and a base peak at *m/z* 81, characteristic of the $\Delta^{5,9}$ di-unsaturation [16]. The equivalent chain length value of **1** was calculated to be 18.33, and 0.33 agrees well with the fractional values of other *iso*-$\Delta^{5,9}$ methyl esters with other chain-lengths [16]. There are no reports of *iso*-$\Delta^{5,9}$ fatty acids from the phospholipids of higher plants.

Malvaviscus arboreus DC

Table 1 also shows the complete phospholipid fatty acid composition of *M. arboreus*. Three novel fatty acids were present in *M. arboreus* in very low abundance, and these were identified as 17-methyl-5,9-octadecadienoic acid, 16-methyl-5,9-octadecadienoic acid, and 5,9-nonadecadienoic acid. All of these acids were identified as methyl esters by their mass spectra since they shared a common molecular ion (M^+) at *m/z* 308 and identical base peaks at *m/z* 81, characteristic of $\Delta^{5,9}$ fatty acid methyl esters [17]. The equivalent chain-length (ECL) value for methyl 17-methyl-5,9-octadecadienoate (**1**) was calculated to be 18.31, for methyl 16-methyl-5,9-octadecadienoate (**2**) the ECL value was 18.48, and for the straight-chain methyl 5,9-nonadecadienoate (**3**) the ECL value was 18.57. The fractional values are in agreement with those previously reported for similar $\Delta^{5,9}$ methyl esters with other chain-lengths [16]. Identification of the normal-chain $\Delta^{5,9}$ isomer was also achieved by means of a plot of retention time vs. number of carbon atoms for the $\Delta^{5,9}$ methyl esters present in *M. arboreus*. This ranged in length between C_{19} and C_{24}, since methyl 5,9-eicosadienoate and methyl 5,9-tetracosadienoate were also identified. Such a plot afforded a straight line. Hydrogenation of the whole fatty acid methyl ester mixture was key for locating methyl branching, since the ECL values of the corresponding hydrogenated methyl esters were calculated to be 18.62 for methyl 17-methyloctadecanoate and 18.72 for methyl 16-methyloctadecanoate, typical values of *iso-anteiso* pairs [16]. The original double bond stereochemistry was confirmed to be *cis* by IR spectroscopy since there was no absorption in the 960–980 cm^{-1} region, indicative of a *cis* configuration for all the fatty acid double bonds in *M. arboreus* [17]. To the best of our knowledge, the *iso-anteiso* $\Delta^{5,9}$–19:2 family has not been identified before in nature. Moreover, this is the first report of *iso-anteiso* $\Delta^{5,9}$ fatty acids from the phospholipids of higher plants.

A common feature in the phospholipids from the two Dicotiledoneae flowers analyzed was the considerable amounts of palmitic acid and linoleic acid. However, very long-chain saturated fatty acids, mainly between C_{24}-C_{28}, were also identified. Saturated 2-hydroxy fatty acids were common in these two flowers, but the most unusual was the finding of these α-hydroxy acids as phospholipid components.

The identification of several $\Delta^{5,9}$ fatty acids in these flowers is of interest. Some of these are known compounds that have not been identified before in the phospholipids of higher plants, e.g. 5,9-eicosadienoic and 5,9-tetracosadienoic acids from *M. arboreus*. Three other $\Delta^{5,9}$ fatty acids are novel, being characterized as 17-methyl-5,9-octadecadienoic acid, 16-methyl-5,9-octadecadienoic acid, and 5,9-nonadecadienoic acid, respectively. These three $\Delta^{5,9}$ acids present uncommon features in the fatty acids from plants, namely odd-chains and *iso-anteiso* methyl branching, and raise questions with respect to their biosynthetic origin. Also, a possible bacterial origin for these acids can not be excluded.



EXPERIMENTAL

Instrumentation

Gas chromatography (GC) spectra were recorded on a Hewlett Packard (Palo Alto, CA, USA) 5890A gas chromatograph equipped with a flame-ionization detector and a DB-1 (J & W Scientific, Folsom, CA, USA) nonpolar fused silica capillary column (30 m × 0.25 mm i.d.) containing dimethylpolysiloxane (carrier gas He). GC-mass spectrometry (GC-MS) data was collected in a 5972A MS ChemStation (Hewlett–Packard) equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS) crosslinked with 5%-phenyl methylpolysiloxane. The temperature program was as follows: 130° for 2 min and then increased at 3°/min to 270° and maintained for 40 min.

Table 1. The Phospholipid Fatty Acid Composition of *A. cathartica* and *M. arboreus* following Chemical Derivatisation

Fatty Acids	Abundance (% by wt.)	
	A. cathartica	*M. arboreus*
Tetradecanoic (14:0)	1.7	1.2
Pentadecanoic (15:0)	—	0.6
9-Hexadecenoic (16:1)	1.4	—
Hexadecanoic (16:0)	30.4	34.3
2-Methylhexadecanoic (17:0)	—	0.3
15-Methylhexadecanoic (*i*-17:0)	—	0.2
14-Methylhexadecanoic (*ai*-17:0)	—	0.2
Heptadecanoic (17:0)	0.3	1.3
9,12,15-Octadecatrienoic (18:3 *n*-3)	7.3	8.6
9,12-Octadecadienoic (18:2 *n*-6)	35.4	31.8
9-Octadecenoic (18:1)	0.5	—
11-Octadecenoic (18:1)	0.4	—
Octadecanoic (18:0)	7.8	9.4
17-Methyl-5,9-octadecadienoic (*i*-19:2)*	0.1	0.2
2-Methyloctadecanoic (19:0)	—	0.2
16-Methyl-5,9-octadecadienoic(*ai*-19:2)*	—	0.2
5,9-Nonadecadienoic (19:2)*	—	0.2
9-Nonadecenoic (19:1)	0.2	—
Nonadecanoic (19:0)	0.3	0.3
5,9-Eicosadienoic (20:2)	—	0.2
11,14-Eicosadienoic (20:2)	0.2	—
7-Eicosenoic (20:1)	0.2	—
11-Eicosenoic (20:1)	1.0	0.2
13-Eicosenoic (20:1)	0.1	—
Eicosanoic (20:0)	2.9	1.4
Heneicosanoic (21:0)	0.7	—
13-Docosenoic (22:1)	0.1	—
15-Docosenoic (22:1)	0.1	—
Docosanoic (22:0)	1.9	—
5,9-Tetracosadienoic (24:2)	—	0.3
Tetracosanoic (24:0)	0.9	1.4
Pentacosanoic (25:0)	0.2	—
Hexacosanoic (26:0)	0.2	0.3
Heptacosanoic (27:0)	—	0.1
Octacosanoic (28:0)	—	0.2
2-Hydroxyhexadecanoic (16 h:0)	0.3	—
2-Hydroxyoctadecanoic (18 h:0)	0.6	1.8
2-Hydroxyeicosanoic (20 h:0)	0.9	—
2-Hydroxydocosenoic (22 h:1)	0.3	—
2-Hydroxydocosanoic (22 h:0)	1.9	1.3
2-Hydroxytricosanoic (23 h:0)	0.2	0.2
2-Hydroxytetracosenoic (24 h:1)	0.3	0.8
2-Hydroxytetracosanoic (24 h:0)	1.0	1.6
2-Hydroxypentacosanoic (25 h:0)	—	0.1
2-Hydroxyhexacosenoic (26 h:1)	—	0.1
2-Hydroxyhexacosanoic (26 h:0)	—	0.1

* Not previously identified in nature.

Plant material

Allamanda cathartica was collected at the University of Puerto Rico, Río Piedras Campus, in February, 1994, and *Malvaviscus arboreus* was collected in Río Piedras, Puerto Rico, in April, 1994. Vouchers of these flowers are mantained at the Herbarium of the University of Puerto Rico, Río Piedras (#96-01 and #96-05).

Extraction and isolation of phospholipids

The flowers of the plants (ca. 30–45 g) were washed with water, carefully cleaned of all debris and cut into small pieces. Immediate extraction after collection with 300 mL of chloroform-methanol (1:1, v/v) yielded the total lipids (200–300 mg). The neutral lipids, glycolipids and phospholipids (40–60 mg) were separated by column chromatography on silica gel (60–200 mesh) using the procedure of Privett *et al*. [18]. The phospholipid classes were fractionated by preparative thin-layer chromatography (TLC) using Si gel 60 and $CHCl_3$-MeOH-NH_4OH (65:35:5 by vol) as solvent affording mainly phosphatidylcholine. The fatty acyl components of these phospholipids were obtained as their methyl esters by reaction with methanolic hydrogen chloride [19].

Fatty acid derivatives Hydrogenations were carried out as previously described [16]. The double bond positions of the mono- and dienoic fatty acids were determined by preparing the corresponding dimethyl disulfide derivatives as detailed in Dunkelblum *et al*. [15]. The double bond positions of the polyunsaturated fatty acids were determined by preparing the corresponding *N*-acylpyrrolidide derivatives as previously described [16]. Mass spectral data for the novel methyl esters follows.

Methyl 17-*methyl*-5,9-*octadecadienoate* (**1**) GC-MS (70 eV) *m*/*z* (rel. int) 308 $[M]^+$ (12), 294(11), 277(6), 263(4), 251(3), 237(8), 209(3), 205(4), 186(3), 179(7), 167(3), 165(32), 163(10), 150(13), 137(16), 135(18), 123(24), 120(22), 111(16), 109(43), 107(23), 97(30), 95(96), 87(43), 81(100), 74(41), 69(47), 67(80), 55(78).

Methyl 16-*methyl*-5,9-*octadecadienoate* (**2**) GC-MS (70 eV) *m*/*z* (rel. int) 308 $[M]^+$ (10), 292(3), 277(4), 265(2), 237(4), 213(3), 199(4), 179(4), 166(3), 164(11), 153(7), 151(14), 149(10), 143(13), 137(13), 135(12), 129(7), 123(19), 121(14), 111(12), 109(31), 107(13), 95(69), 87(40), 81(100), 74(47), 69(37), 67(51), 55(60).

Methyl 5,9-*nonadecadienoate* (**3**) GC-MS (70 eV) *m*/*z* (rel. int) 308 $[M]^+$(5), 294(8), 263(8), 251(3), 237(9), 207(8), 199(4), 185(3), 180(4), 165(7), 163(12), 151(11), 149(21), 139(20), 137(18), 135(17), 125(11), 123(23), 121(19), 111(17), 109(42), 95(70), 87(47), 81(100), 74(56), 69(47), 67(71), 55(82).

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REFERENCES

1. Hui, W. H. and Szeto, S. K., *Phytochemistry*, 1967, **6**, 443.
2. Piatelli, M. and Imperato, F., *Phytochemistry*, 1970, **9**, 2557.
3. Sukumaran Kartha, A. R. and Menon, K. N., *Proceedings of the Indian Academy of Science*, 1943, **17**, 11.
4. Nunn, J. R., *Journal of the Chemical Society*, 1952, 313.
5. Macfarlane, J. J., Shenstone, F. S. and Vickery, J. R., *Nature*, 1957, **179**, 830.
6. Harwood, J. L., in *Plant Acyl Lipids*: *Structure*, *Distribution and Analysis*, *The Biochemistry of Plants*: *A Comprehensive Treatise*, ed. P. K. Stumpf and E. E. Conn. Academic Press, New York, 1980, pp. 1–55.
7. Takahashi, N. and Sato, N., Murata, N., *Biochimica et Biophysica Acta*, 1984, **795**, 147.
8. Bailey, L. H., in *Hortus Third*: *A Concise Dictionary of Plants Cultivated in the United States and Canada*. MacMillan Publishing Company, New York, 1976.
9. Arthur, H. R. and Hui, W. H., *Journal of the Chemical Society*, 1954, 2782.
10. Kupchan, S. M., Dessertine, A. L., Blaylock, B. T. and Bryan, R. F., *Journal of Organic Chemistry*, 1974, **39**, 2477.
11. Cobb, A. L. and Coppen, J., *Phytochemistry*, 1983, **22**, 125.
12. Anderson, J. E., Chang, C. J. and McLaughlin, J. L., *Journal of Natural Products*, 1988, **51**, 307.
13. Akramov, S. T., Mukhamedova, K. S. and Akbarov, R. R., *Khimiya Prirodnykh Soedinenii*, 1979, **2**, 223.
14. Gottsberg, G., Arnold, T. and Linskens, H. F., *Bot. Acta*, 1989, **102**, 141.
15. Dunkelblum, E., Tan, S. H. and Silk, R. J., *Journal of Chemical Ecology*, 1985, **11**, 265.
16. Carballeira, N. M. and Negrón, V., *Journal of Natural Products*, 1991, **54**, 305.
17. Doumenq, P., Guiliano, M., Bertrand, J. C. and Mille, G., *Applied Spectroscopy*, 1990, **44**, 1355.
18. Privett, O. S., Dougherty, K. A., Erdahl, W. L. and Stolyhwo, A., *Journal of the American Oil Chemical Society*, 1973, **50**, 516.
19. Carreau, J. P. and Dubacq, J. P., *Journal of Chromatography*, 1978, **151**, 384.