Pergamon

S0031-9422(96)00140-9

Phytochemistry, Vol. 42, No. 5, pp. 1357–1360, 1996

0031-9422/96 $15.00 + 0.00

FATTY ACID COMPOSITION OF SOME SEED OILS OF THE SAPINDACEAE

VOLKER SPITZER

Faculdade de Farmácia UFRGS, Curso de Pós-Graduação, Avenida Ipiranga, 2752, 90610.000 Porto Alegre/RS, Brazil

(*Received in revised form* 20 *December* 1995)

Key Word Index—*Paullina meliaefolia*; *Urvillea uniloba*; *Cardiospermum grandiflorum*; Sapindaceae; seed oils; eicosenoic fatty acids; paullinic acid; cyanolipids; GC-MS.

Abstract—The fatty acid compositions of the seed oils of *Paullina meliaefolia*, *Urvillea uniloba* and *Cardiospermum grandiflorum* have been analysed as their methyl esters and 4,4-dimethyloxazoline derivatives by GC and GC–mass spectrometry. *cis*-13-Eicosenoic Acid (paullinic acid) and *cis*-11-octadecenoic acid (*cis*-vaccenic acid) were found to be the main components besides other monoenoic acid positional isomers. The stereochemistry of the double bonds was confirmed by IR and ^{13}C NMR spectroscopy. Moreover, the unusual fatty acids C16:2(*c*9, *c*12) and C20:2 (*c*11, *c*14) have been detected as minor compounds. The cyanolipids were identified as 1-cyano-2-hydroxymethylprop-2-ene-1-ol and 1-cyano-2-hydroxymethylprop-1-ene-3-ol derivatives by spectroscopic methods.

INTRODUCTION

The seeds of many species of the Sapindaceae are rich in oil and some are utilized commercially in the countries of origin [1]. Insect repellent properties as well as insecticidal activities of some of their seed oils have also been described [2, 3]. A large number of species from this plant family have been investigated for cyanolipid constituents in the seeds, and the results have been reviewed [4, 5]. Cyanolipids, which occur together with acylglycerides, are a group of unusual plant lipids. Four types of cyanolipid structures, having fatty acids (FAs) esterified with a mono- or di-hydroxynitrile moiety, have been reported [5]. Another interesting feature of the cyanolipid in Sapindaceae seed oils is an unusually high content of eicosanoic and eicosenoic acids [1, 6–14]. It is known that such acids are preferentially incorporated in the nitrile-containing fractions [7, 14]. Seed oils with a substantial amount of very long-chain FAs have attracted attention because of their value for industrial purposes [15]. Furthermore, these compounds can be of chemotaxonomic significance [16].

Most researchers have analysed the FA composition from Sapindaceae seed oils by GC of the methyl esters (MEs) on packed columns and identified the olefinic compounds only by retention time comparison [6–8, 10–12] or by an additional measurement of the mixed melting point of the oxidation product after separation by distillation [1]. It is well known that these methods are not reliable for distinguishing between monoenic FA isomers. In other studies, monoenic FAs have been characterized by GC–mass spectrometric analysis after on-site modification as methoxyl [9] and trimethylsiloxy derivatives [13] or by similiar analysis of their ozonolysis products [17], without a complete chromatographic separation of monoene isomers. Recently, the FA composition of lipid fractions of *Paullinia elegans*, analysed by capillary GC-mass spectrometry of their 4,4-dimethyloxazoline (OXFA) derivatives was reported. This method allows both the unequivocal localization of the position of the double bonds in the FA chain and the GC separation of the corresponding monoene isomers. An unusual pattern of C18:1, C20:1 and C22:1 isomers with paullinic acid (*cis*-13-eicosenoic acid) as the main compound was found [14].

In order to extend our knowledge of the FA composition of the Sapindaceae seed oils, it was considered desirable to investigate more members of this plant family with modern analytical methods. In the present work, as a part of our current research project on seed oils of plants from south Brazil, the complete FA composition of the seed oils of *P. meliaefolia* and *Urvillea uniloba* is described for the first time and that of *Cardiospermum grandiflorum* was re-analysed by high resolution GC–mass spectrometry.

RESULTS AND DISCUSSION

The results of the spectroscopic analysis (IR, ^{1}H and ^{13}C NMR) of the seed oils from *P. meliaefolia*, *U. uniloba* and *C. grandiflorum* and of the GC and GC–mass spectrometric analysis of their total FAs are summarized in Table 1.

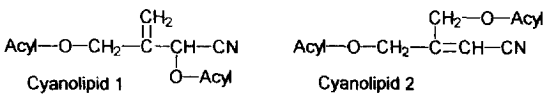
Identification of individual cyanolipids was carried out by comparison of the ^{1}H NMR, ^{13}C NMR and IR spectra obtained from the oils with the spectra of a

Table 1. The fatty acid composition (wt%) and cyanolipid content (mol%) from the seed oils of *Paullinia meliaefolia* (*P.m.*), *Urvillea uniloba* (*U.u.*), *Cardiospermum grandiflorum* (*C.g.*) and *Paullinia elegans* (*P.e.*)

Fatty acid	ECL*	*P.m.*	*U.u.*	*C.g.*	*C.g.* (ref. [1])	*P.e.* (ref. [14])	Diagnostic mass fragments (*m/z*) of the oxazoline derivatives of unsaturated fatty acids (rel. int.)
16:0		1.1	2.5	3.3	4	2.2	
16:1 (*c*9)	16.25	1.5	2.4	1.6	—	3.4	196 (5.2), 208 (10.3), 307 (11.2, $[M]^-$)
16:1 (*c*11)	16.37	0.1	0.2	0.1	—	0.2	224 (4.6), 236 (7.9), 307 (9.4, $[M]^+$)
16:2 (*c*9, *c*12)	16.79	<0.1	<0.1	<0.1	—	—	196 (8.5), 208 (6.5), 236 (9.5), 248 (4.3), 305 (5.8, $[M]^+$)
18:0		0.8	1.5	4.1	1	1.7	
18:1 (*c*9)	18.20	5.3	9.8	13.8	27†	12.2	196 (7.7), 208 (8.8), 335 (9.2, $[M]^+$)
18:1 (*c*11)	18.28	19.7	13.4	21.9	—	19.8	224 (4.2), 236 (5.1), 335 (8.4, $[M]^+$)
18:2 (*c*9, *c*12)	18.61	2.9	5.8	8.7	7†	3.1	196 (9.1), 208 (3.2), 236 (18.2), 248 (1.5), 333 (14.4, $[M]^+$)
18:3 (*c*9, *c*12, *c*15)	19.20	2.7	4.2	4.6	3†	1.8	196 (10.2), 208 (18.8), 236 (8.2), 248 (9.3), 276 (14.2), 288 (7.6), 331 (12.4, $[M]^+$)
20:0		4.0	9.8	2.7	10	4.9	
20:1 (*c*11)	20.16	12.3	15.6	10.8	49	3.7	224 (4.7), 236 (2.6), 363 (9.5, $[M]^+$)
20:1 (*c*13)	20.28	47.1	26.4	25.9	—	44.4	252 (1.5), 264 (1.9), 363 (14.5, $[M]^+$)
20:1 (*c*15)	20.36	0.5	0.2	<0.1	—	0.7	280 (1.3), 292 (2.5), 363 (9.4, $[M]^+$)
20:2 (*c*11, *c*14)	20.59	0.7	1.0	1.2	—	—	224 (0.9), 236 (2.1), 264 (8.5), 276 (2.1), 361 (3.16, $[M]^+$)
22:0		0.1	1.5	0.3	—	0.3	
22:1 (*c*13)	22.17	0.3	1.9	0.2	—	0.4	252 (1.1), 264 (2.2), 391 (13.3, $[M]^+$)
22:1 (*c*15)	22.29	0.2	3.3	0.4	—	0.8	280 (0.7), 292 (1.6), 391 (22.4, $[M]^+$)
Cyanolipid 1		56.3	29.7	58.7		71.4	
Cyanolipid 2		—	3.7	—		—	

*Equivalent chain length measured at 190° on a HP20M capillary column (see Experimental).
†Double bond position was not specified.

sample of cyanolipid 1, isolated from *P. elegans* and with literature data [14, 18]. In all oil samples examined herein, cyanolipid type 1 could be detected and in the oil of *U. uniloba* a small amount of cyanolipid type 2 was also found. These observations are in good agreement with published data [7,19].



The FAs of the seed oils were identified as their MEs by GC and GC–mass spectrometry using standards. To ensure accuracy in the location of the double bonds in unsaturated FAs, OXFA derivatives were additionally examined by GC–mass spectrometry. The double bond positions in these compounds can be easily deduced from the mass spectrum by characteristic 12 amu breaks at the olefinic bond in the homologous 14 amu sequence of the saturated part of the chain. Additional information is provided by two more intense peaks produced by allylic cleavage on both sides on the double bond [20]. As *cis*- and *trans*-FAs cannot always be fully separated by capillary GC [21], IR and ^{13}C NMR spectra of the FAME mixture were also carefully examined in order to detect the presence of *trans*-FAs. The typical *trans*-acid IR band between 900 and 1000 cm^{-1} [22] and a ^{13}C NMR signal at *ca* δ 32.5, characteristic for allylic carbon atoms of *trans*-unsaturated acids [23], were absent in all spectra. Instead of the latter, an abundant signal at δ 27.3 was observed in the ^{13}C NMR spectrum, which can be assigned to the allylic carbon atoms of *cis*-olefins [23]. Thus, only *cis*-acids were detectable in all seeds oils.

The observed FA patterns (Table 1) can be considered as unusual. The major FAs are mono-unsaturated (*n*-7)-type isomers with *cis*-11-octadecenoic acid (*cis*-vaccenic acid) and *cis*-13-eicosenoic acid (paullinic acid) as principal compounds. All monoenic FAs appeared with at least two positional isomers. In seed oils the principal monoene FAs usually have 18 or more carbon atoms and are of the (*n*-9)-type (oleic acid type). *cis*-Vaccenic acid is indeed widely spread in plants, but in seed lipids it occurs in lower concentrations (i.e. 0.5–2%) [24]. With the exception of our previous work on *P. elegans* [14], a high content of *cis*-vaccenic acid has never been reported for Sapindaceae seed oils. Higher contents (up to 15%) of this compound in seed fats are only known from some members of the families Asclepediaceae and Connaraceae [25] and also from various pulp lipids [24]. In contrast to *cis*-vaccenic acid, paullinic acid is very rare in the plant kingdom and was only found as a trace compound in some seed oils of the family Protaceae [26], in rapeseed oil [27] and in the seed oil of *Sapindus trifoliatus* (Sapindaceae) [13]. It was recently discovered in higher concentrations (44%, Table 1) in the seed lipid fractions of *P. elegans* in our laboratory [14]. A high yield of C20:1 monoene FAs had already been reported in seed oils of the Sapindaceae. In most of the cases, however, these have been identified without further characterization of the double bond positions [6–8, 10–12] or were analysed as *cis*-11-eicosenoic acid [1, 9, 13, 17] without a complete chromatographic isomer separation. So, for the seed oils from *P. meliaefolia* (72%) and *U. uniloba* (50%), only the total contents of C20 FAs were described in the literature without distinguishing between saturated or unsaturated compounds [7]. In Table 1 the literature

data [1] from the FA composition of the seed oil of *C. grandiflorum*, which were obtained by using packed CG columns and distillation to separate the FAs, is compared with our analysis data. It can be observed that both data are in good agreement, but that the literature data [1] lacked the complete identification of the monoene isomers and some minor FAs.

The minor fatty acids C16:2 (*c*9, *c*12) and C20:2 (*c*11, *c*14), observed in all samples analysed herein (Table 1), are also unusual compounds. They have been reported for some Asclepediaceae and Ephedraceae seed oils [28], but never in Sapindaceae oils.

Since the FA patterns of the Sapindaceae seed oils (Table 1) showed a remarkable uniformity in terms of their high content of the unusual C18:1 and C20:1 monoenic isomers, it is proposed that this can be of chemotaxonomical significance. Unusual FAs which accumulate in the seeds of higher plants can be of chemotaxonomical interest and their presence or absence may indicate closer or more distant relationships between the considered species [29].

Considering the results presented here, the data of the previous publications on Sapindaceae seed oils is probably incomplete. Therefore, it would be interesting to re-investigate all Sapindaceae seed lipids with adequate analytical methods.

EXPERIMENTAL

Oil extraction. Fruits from *P. meliaefolia* A. L. Jussieu, *C. grandiflorum* Swartz and *U. uniloba* Radlkofer were collected and identified by the botanist Marcos Sobral (UFRGS) in May and June 1995 in the National Park of Turvo and in the region of Santana de Boa Vista in the Federal State of Rio Grande do Sul, south Brazil. Air-dried, crushed seeds were extracted in a Soxhlet apparatus for 4 hr with petrol. After extraction, solvent was removed by vacuum distillation at 30°, flushed with N_2 and stored at −18° until used. Oil yields were 38.4% (*P. meliaefolia*), 35.2% (*C. grandiflorum*) and 34.6% (*U. uniloba*).

Derivatization. Transesterification to FAMEs was carried out with NaOMe in dry MeOH (0.5 N) as described in ref. [30]. OXFA derivatives of total FAs were prepd after hydrolysis of the oil with 1 N KOH in 95% EtOH as described in ref. [20].

GC and GC-MS. A gas chromatograph, equipped with a FID (240°) and a split/splitless injector (240°, split 1:100) with glass insert was used for FAME analysis. H_2 was the carrier gas at an inlet pressure of 150 kPa. Sepn of the compounds was achieved on a HP 20M (Hewlett Packard) fused silica WCOT capillary column (50 m × 0.2 mm i.d.; 0.2 μm film thickness) at 190°. For estimation of ECL values, R_ts were measured from the time of elution of solvent, considered as unretained solute. GC-MS analyses were done with an ionization energy of 70 eV, a source temp. of 225° and an interface temp. of 220°. The sepn of the FAME and DMOX derivatives was carried out on a DBWAX (J&W Scientific) fused silica WCOT capillary column (60 m × 0.25 mm i.d.; 0.25 μm film thickness) at 205°. He was used as carrier gas (1 bar). To check on purity of each GC peak, MS were taken at various parts of each peak.

Spectroscopy. IR spectra of oils and FAMEs were recorded as liquid films. ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra were run in $CDCl_3$ soln with TMS as int. standard. The relative composition of cyanolipids in seed oils was determined by ^{1}H NMR integral analysis [18].

Acknowledgements—The author thanks Simone Quintana de Oliveira for technical assistance, Dr V. U. Costa and Mônica Zucolotto (IQ/UFRGS, Brazil) for performing NMR experiments, and Marcos Sobral for collection and identification of plant material. The author is also grateful to the German Academic Exchange Service (DAAD), Bonn, Germany, for a scholarship as visiting professor, and the Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany (GTZ) for financial support (PN 87.2061.7-02.300).

REFERENCES

1. Hopkins, C. Y. and Swingle, R. (1967) *Lipids* **2**, 258.
2. Khan, N. W. Y., Ahmad, F., Ahmad, I. and Osman, S. M. (1983) *J. Am. Oil Chem. Soc.* **60**, 949.
3. Nishizawa, M., Adachi, K. and Hayashi, Y. (1983) *Tetrahedron Letters* **24**, 4447.
4. Hegnauer, R. (1973) *Chemotaxonomie der Pflanzen*, Vol. VI, p. 271. Birkhäuser Verlag, Basel.
5. Hegnauer, R. (1990) *Chemotaxonomie der Pflanzen*, Vol. IX, p. 486. Birkhäuser Verlag, Basel.
6. Mikolajczak, K. L., Smith, C. R. and Tjarks, L. W. (1970) *Lipids* **5**, 672.
7. Mikolajczak, K. L., Smith, C. R. and Tjarks, L. W. (1970) *Lipids* **5**, 812.
8. Seigler, D. S., Mikolajczak, K. L., Smith, C. R., Jr. and Wolf, I. A. (1970) *Chem. Phys. Lipids* **4**, 147.
9. Gowrikumar, G., Mani, V. V. S. and Lakshminarayana, G. (1976) *Phytochemistry* **15**, 1566.
10. Nahrstedt, A. and Hübel, W. (1978) *Phytochemistry* **17**, 314.
11. Abdel Wahab, S. M. and Selim, M. A. (1985) *Fitoterapia* **56**, 167.
12. Mustafa, J., Gupta, A., Agarwal, R. and Osman, S. M. (1986) *J. Am. Oil Chem. Soc.* **63**, 671.
13. Ucciani, E., Mallet, F. and Zahra, J. P. (1994) *Fat Sci. Technol.* **96**, 69.
14. Spitzer, V. (1995) *J. High Resolut. Chromatogr.* **18**, 413.
15. Baumann, H., Bühler, M., Fochem, H., Hirsinger, F., Zoebelein, H. and Falbe, J. (1988) *Angew. Chem.* **100**, 41.
16. Spitzer, V., Marx, F., Maia, J. G. S. and Pfeilsticker, K. (1990) *Fat Sci. Technol.* **92**, 165.
17. Nishizawa, M., Adachi, K., Sastrapradja, S. and Hayashi, Y. (1983) *Phytochemistry* **22**, 2853.
18. Seigler, D. S. (1974) *Phytochemistry* **13**, 841.
19. Seigler, D. S. and Kawahara, W. (1976) *Biochem.*

Syst. Ecol. **4**, 263.
20. Zhang, J. Y., Yu, Q. T., Liu, B. N. and Huang, Z. H. (1988) *Biomed. Environ. Mass Spectrom.* **15**, 33.
21. Firestone, D. and Sheppard, A. (1992) *Advances in Lipid Methodology—One*, p. 273. The Oily Press, Dundee, U.K.
22. Christie, W. W. (1989) in *Gas Chromatography and Lipids*, p. 147. The Oily Press, Ayr, U.K.
23. Gunstone, F. D. (1994) *Prog. Lipid Res.* **33**, 19.
24. Shibahara, A., Yamamoto, K., Nakayama, T. and Kajimoto, G. (1987) *J. Am. Oil Chem. Soc.* **64**, 397.
25. Badami, R. C. and Patil, K. B. (1981) *Prog. Lipid Res.* **19**, 119.
26. Vickery, J. R. (1971) *Phytochemistry* **10**, 123.
27. Haeffner, E. W. (1970) *Lipids* **5**, 430.
28. Smith, C. R. (1970) in *Occurrence of Unusual Fatty Acids in Plants, Progress in the Chemistry of Fats and Other Lipids* (Holman, R. T., ed.), Vol. XI, pp. 137–177. Pergamon Press, Oxford, U.K.
29. Aitzetmüller, K. (1993) *J. High Resolut. Chromatogr.* **16**, 488.
30. W. W. Christie (1989) *Gas Chromatography and Lipids*, p. 69. The Oily Press, Ayr, U.K.