Analysis of Conjugated Octadecatrienoic Acids in *Momordica balsamina* Seed Oil by GLC and ^{13}C NMR Spectroscopy

E.M. Gaydou[a], J. Miralles[b] and V. Rasoazanakolona[a,1]
[a]Laboratoire de Phytochimie, Ecole Superieure de Chimie de Marseille, Avenue Escadrille Normandie-Niemen, 13397 Marseille Cedex 13, France, and [b]Departement de Biologie Vegetale-Faculte des Sciences, Dakar, Senegal.

Separation of conjugated octadecatrienoic acids by open tubular gas liquid chromatography (GLC) was performed using glass capillary columns coated with Carbowax 20 M and with OV-1. The equivalent chain length of geometrical isomers of the conjugated octadecatrienoic acids belonging to the two series $C_{18:3}\Delta^{8,10,12}$ and $C_{18:3}\Delta^{9,11,13}$ were determined. The application of these results to the study of the *Momordica balsamina* seed oil shows that this oil contains two conjugated octadecatrienoic fatty acids in appreciable amounts, punicic acid (50%) and α-eleostearic acid (13%). The isomerization of conjugated acids in *M. balsamina* seed oil was followed for one year. Quantitation of octadecatrienoic acids using GLC gave results similar to those obtained with ^{13}C NMR.

Among the various conjugated fatty acids identified in seed oils, six conjugated octadecatrienoic acids have been reported (1). These acids occur in only a few families (Cucurbitaceae, Bignoniaceae, Compositeae, Rosaceae and Valerianaceae) and have been identified particularly by older methods. More recently, using WCOT gas liquid chromatography (GLC) with a nonpolar liquid phase OV-1 and HPLC with a Zorbax ODS column (2) and ^{13}C nuclear magnetic resonance spectroscopy (3,4), the compositions of fatty acids in oils containing more than one conjugated octadecatrienoic acid were established.

It was observed by Earle et al. (5) and Hopkins and Chisholm (6) that the seed oil of *Momordica balsamina* (Cucurbitaceae) contained a conjugated triene acid, punicic acid (*cis*, *trans*, *cis*-octadeca-9,11,13-trienoic acid). In another species of the same genus (*M. Charantia*) several authors (2,6,7) observed the presence of α-eleostearic (*cis*, *trans*, *cis*-octadeca-9,11,13-trienoic acid). In our investigation of *M. balsamina* seed oil grown in Senegal using a Carbowax 20 M glass capillary column, we have observed the presence of more than two conjugated octadecatrienoic fatty acids. Because the natural co-occurrence of various octadecatrienoic fatty acid isomers has been observed only in *Aleurites fordii* (2) and *Centrathus ruber* (4) we checked to see if the two major trienoic acids are naturally

TABLE 1

Equivalent Chain Length of Geometrical Isomers of Conjugated Octadecatrienoic Methyl Esters. Fatty Acid Composition of *Momordica balsamina* Seed Oil and Isomerization During Storage

Conjugated trienoic acid number	Fatty acid	ECL		Peak area (%)[a]/Months of storage[b]			
		CWX20M	OV1	0[c]	3	6	12
	palmitic			13.6	14.2	13.6	13.2
	stearic			7.5	7.1	8.1	8.0
	oleic			5.1	4.9	5.5	6.0
	linoleic			6.5	6.2	6.9	7.4
1	jacaric[d]	21.50	18.94	—	—	—	—
2	punicic[e]	21.56		50.6	42.6	37.3	27.3
3	α-eleostearic[f]	21.72	18.98	13.1	14.3	15.0	16.7
4	calendic[g]	21.88	19.05	—	—	—	—
5	catalpic[h]	21.91	19.12	2.0	6.0	6.3	9.3
6	β-eleostearic[i]	22.19	19.35	1.1	3.5	5.4	9.6
	others			0.5	1.2	1.8	2.5

[a]Determined using a 25-m glass capillary column coated with Carbowax 20 M at 180 C.
[b]Seed oil stored at ambient temperature.
[c]Freshly extracted with hexane.
[d]*cis,trans,cis*-octadeca-8,10,12-trienoic acid.
[e]*cis,trans,cis*-octadeca-9,11,13-trienoic acid.
[f]*cis,trans,trans,*-octadeca-9,11,13-trienoic acid.
[g]*trans,trans,cis*-octadeca-8,10,12-trienoic acid.
[h]*trans,trans,cis*-octadeca-9,11,13-trienoic acid.
[i]*trans,trans,trans*-octadeca-9,11,13-trienoic acid.

---

[1] Present address: Departement de Recherches Technologiques FOFIFA, Ambatobe, B.P. 254, 101 Antananarivo, République Démocratique de Madagascar.

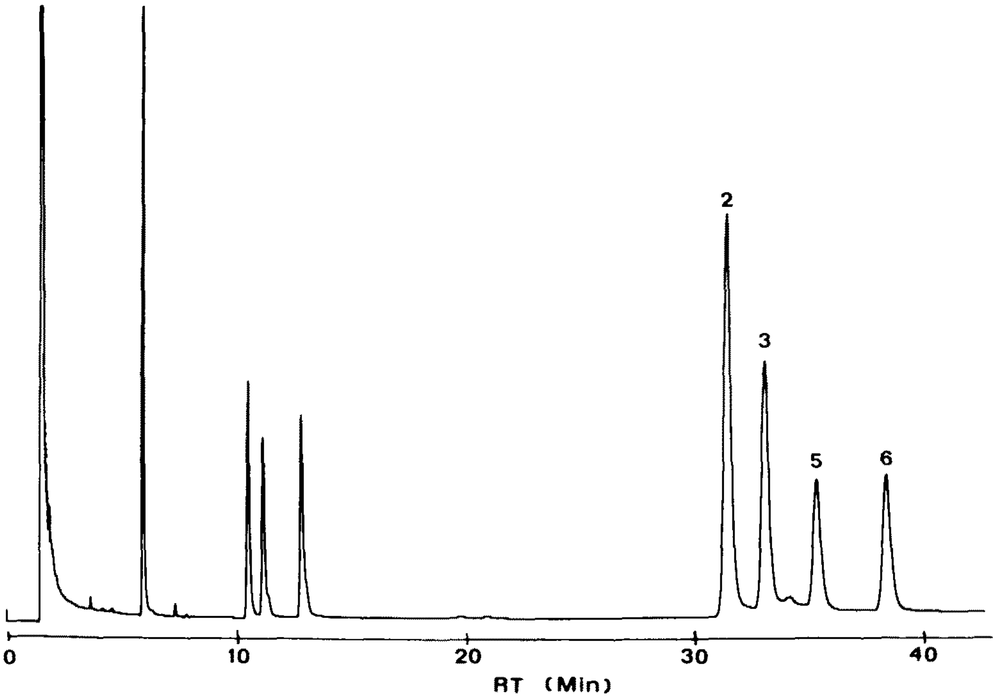

FIG. 1. Capillary GLC chromatogram of fatty acid methyl esters obtained from *Momordica balsamina* seed oil stored at ambient temperature 12 mo. For octadecatrienoic methyl esters identification, see Table 1. Column, 50 m glass capillary coated with Carbowax 20 M; temperature, 190 C; pressure, 0.9 bar; carrier gas, H_2; split 5/100; sample volume 0.4 μl.

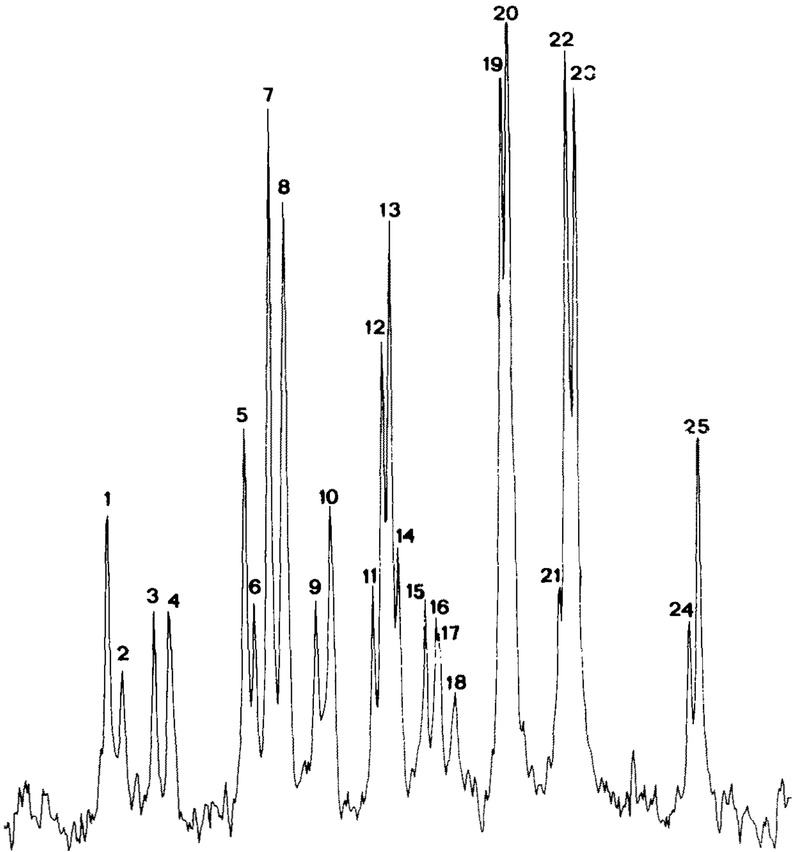

FIG. 2. Partial ^{13}C NMR spectrum (in $CDCl_3$) of the double bonded carbon area obtained from *Momordica balsamina* seed oil stored at ambient temperature 12 mo. For signal number assignment see Table 3.

occurring, or if one of them is formed by isomerization in *M. balsamina* oil.

Another purpose of this work was to determine the equivalent chain length (ECL) of the various geometrical isomers of the conjugated octadecatrienoic acids belonging to the two series 8,10,12 and 9,11,13 using a Carbowax 20 M glass capillary column. For the identification of these acids, we used oils of *Aleurites fordii, Jacaranda acutifolia* and *Punica granatum*, which are known to contain α-eleostearic, jacaric (*cis,trans,cis*-octadeca-8,10,12-trienoic) and punicic acids, respectively. The results were compared with those obtained using other GLC conditions (2). The stability of the *M. balsamina* seed oil stored at ambient temperature was investigated for one year to follow the isomerization process of the various trienoic isomers. The stability of the various geometrical isomers during the transmethylation process and during GLC analyses was checked using ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

EXPERIMENTAL PROCEDURES

Oils extractions. Seeds of *M. balsamina* and *P. granatum* were collected in the Dakar area (Senegal). Seeds were ground to a powder with an electric mill and extracted with hexane using a Soxhlet apparatus. After drying with anhydrous sodium sulfate, hexane was removed by vacuum distillation at low temperature (8). The seeds were found to contain about 20% and 16% of oil, respectively.

Preparation of the methyl esters, isomerizations, oxidations and maleic adducts. Methyl esters were prepared from oils by base-catalyzed transmethylation (9). Isomerization was obtained by addition of a trace amount of iodine in a hexane solution of the methyl esters (10). Oxidative splitting of the acids by permanganate-periodate was accomplished by the Von Rudloff method (11). For the preparation of maleic adducts, the conjugated triene acids were refluxed for 5 hr under nitrogen, with benzene containing maleic anhydride (10).

Gas liquid chromatography (GLC). A Girdel 30 gas chromatograph equipped with a flame ionization detector (FID) and a glass injector was used for the analyses. Three glass capillary columns were used, a 25-m and a 50-m × 0.35-mm coated with Carbowax 20 M (0.15μ phase thickness and a 25-m × 0.30-mm coated with OV-1 (0.15 μ-phase thickness). Temperatures used were 180 or 190 C for the Carbowax 20 M columns and 170 C for the OV-1 column, 200 C for the detector and 220 C for the inlet. The flow rate of hydrogen used as carrier gas was 5 ml/mn with a split ratio of 5/100. The injections averaged about 0.5-1 μl of a 5% solution of methyl esters in hexane. For the tentative identification of oxidative products, azelaic and suberic acid methyl esters (Fluka) were used as standards. For the determination of equivalent chain lengths (ECL), myristic, palmitic, stearic, arachidic and behenic acid methyl esters (Sigma Chemical Co., St. Louis, Missouri) were used as standards.

Nuclear magnetic resonance (NMR). The ^{13}C NMR spectrum of *M. balsamina* seed oil (stored one year at ambient temperature) was recorded on a Bruker AC-100 Spectrometer (Ecole Supérieure de Chimie de Marseille). The sample was prepared in a 5-mm o.d. tube by mixing 0.3 ml of oil with 0.5 ml of $CHCl_3$; tetramethylsilane was used as internal standard. The FT^{13}C NMR was measured under the following conditions: frequency, 25.2 MHz; spectral width, 200 ppm; pulse delay, 10 sec; pulse width, 3 μsec; acquisition time, 1.4 sec; number of data points, 6 300. Compositions were calculated by averaging intensities of the same carbon atom as described by Tulloch and Bergter (3).

TABLE 2

^{13}C Chemical Shifts of Double Bond Carbons of Seed Oil from *Momordica balsamina*[a]

Signal number[b]	Chemical shift[c]	Assignment	Relative intensity
1	134.98	α-eleostearic	10.08
2	134.72	catalpic	5.02
3	134.27	β-eleostearic	6.72
4	134.02	β-eleostearic	6.93
5	132.88	α-eleostearic	12.55
6	132.74	catalpic	7.01
7	132.51	punicic	22.40
8	132.28	punicic	19.57
9	131.80	catalpic	7.03
10	131.57	α-eleostearic	10.17
11	130.93	β-eleostearic	7.48
12	130.78	β-eleostearic + catalpic	15.35
13	130.66	β-eleostearic + α-eleostearic	18.87
14	130.56	β-eleostearic	8.49
15	130.12	linoleic	7.10
16	129.95	oleic	6.50
17	129.90	linoleic	5.95
18	129.70	oleic	4.05
19	128.95	punicic	24.49
20	128.85	α-eleostearic + catalpic + punicic	37.37
21	128.10	linoleic	7.05
22	127.96	punicic + linoleic	29.46
23	127.82	punicic	24.07
24	126.09	catalpic	6.49
25	125.95	α-eleostearic	12.57

[a] Determined using a seed oil sample stored 12 mo at ambient temperature.

[b] Signal numbers are taken from Fig. 2.

[c] Chemical shifts ($CDCl_3$) are given in δ values from TMS and were determined at 25.2 MHz.

RESULTS AND DISCUSSION

Equivalent chain length of octadecatrienoic acids. The ECL on Carbowax 20 M glass capillary column of punicic (21.59) and α-eleostearic (21.72) acids were determined using fatty acid methyl esters obtained from *P. granatum* and *A. fordii* seed oils, respectively. The conversion into *trans*-isomers of these two fatty acids using iodine gave an increase of two peak areas which were attributed to catalpic acid (21.91) and the β-eleostearic acid (22.19), as shown in Table 1. The formation of maleic adducts gave a decrease in peak areas having an ECL of 21.72, 21.91 and 22.19, results in agreement with the geometrical attribution *cis,trans, trans; trans,trans,cis,* and *trans,trans,trans,* respectively. The oxidation of all these fatty acids using $NaO_4I/KMnO_4$ shows the presence of azelaic acid by GLC at 95%. Similar results were observed with conjugated octadecatrienoic methyl esters belonging to the series 8,10,12 and obtained from *J. acutifolia* seed oil. The ECL values are summarized in Table 1. The order of elution was in agreement with those given by Takagi and Itabashi (2) using an OV-1 column. Better resolutions of certain conjugated octadecatrienoic methyl esters were obtained using Carbowax 20 M columns.

Fatty acid composition of M. balsamina *seed oil.* The fatty acid composition of freshly extracted *M. balsamina* oil obtained from seeds grown in Senegal is given in Table 1. Results obtained on the various columns are in the same range. Octadecatrienoic fatty acid methyl esters represent 66-67%. Punicic acid is the major one, with 50-51%, and α-eleostearic is found in an appreciable amount (13%). The contents of catalpic and β-eleostearic acids are low in freshly extracted oil (1-2%).

Stability of octadecatrienoic acids during oil storage. Since the all *trans*-fatty acids usually have been considered as artifacts formed by isomerization of the other trienoic acid (12,13), we have studied the relative composition of the four octadecatrienoic acids contained in *M. balsamina* seed oil stored at ambient temperature (20-25 C) for one year. A typical chromatogram is given in Figure 1. Results given in Table 1 show that the relative composition of the *cis,trans,cis*-isomer decreased from 51% to 27% in one year, and the amount of the *trans,trans,cis-* and *trans,trans,trans*-isomers increased from 1-2% to 9-10%. α-Eleostearic acid (*cis,trans,trans*-isomer) increased only slightly from 13% to 17%.

Comparison of two different methods of octadecatrienoic acid determination. Quantitation of octadecatrienoic acids may be a problem using GLC because of the high reactivity of some conjugated trienes. Isomerization during the transesterification of the triacylglycerols or in the glass capillary columns and decomposition at the high temperature of the column may occur. Tulloch (4) presented the use of ^{13}C NMR spectrometry as a rapid, nondestructive and quantitative method of analysis of seed oils containing conjugated unsaturated acids. By this method, acids could be detected at a level of 1% and estimated at a level of 2%. Satisfactory spectra were obtained for *M. balsamina* seed oil, and the double bonded carbon area spectrum is given in Figure 2. Chemical shifts and relative intensities are listed in Table 2. Values observed for chemical shifts were fairly close to those reported (4). The comparison between GLC and ^{13}C NMR analysis is presented in Table 3. The fatty acid compositions obtained using these two different methods are in remarkably good agreement, showing therefore that GLC can be used for quantitation of conjugated octadecatrienoic acids contained in oils.

TABLE 3

Comparison of Two Different Methods for the Fatty Acid Quantitation in *Momordica balsamina* Seed Oil[a]

Fatty acid	Method GLC[b]	 ^{13}C NMR[c]
palmitic + stearic	21.1	21[d]
oleic	6.2	7
linoleic	7.6	9
punicic	27.2	29
α-eleostearic	16.8	15
catalpic	9.2	9
β-eleostearic	9.5	10
others	2.4	—
sum of octadecatrienoic acids	64.6	63[e]

[a] Determined using a seed oil sample stored 12 mo at ambient temperature.

[b] Determined using a 50-m WCOT glass capillary column coated with Carbowax 20 M at 190 C.

[c] Determined using relative intensities given in Table 3.

[d] Values obtained by difference.

[e] Value obtained using the relative intensity of octadecatrienoic acids at 13.96 ppm and the other fatty acids (palmitic, stearic, oleic, linoleic) at 14.13 ppm for the terminal CH_3 of the various fatty acids.

ACKNOWLEDGMENTS

This work was supported in part by a grant for V.R. from the C.I.E.S. (France). The authors thank A. Archavlis for ^{13}C NMR, A.R.P. Ramanoelina for the gift of *A. fordii* and *J. acutifolia* seed oils and G. Mallet for technical assistance.

REFERENCES

1. Hopkins, C.Y., in *Topics in Lipid Chemistry*, edited by F.D. Gunstone, Vol. 3, Elek Science, London, 1972, pp. 37-87.
2. Takagi, T., and Y. Itabashi, *Lipids 16*:546 (1981).
3. Tulloch, A.P., and L. Bergter, *Ibid. 14*:996 (1979).
4. Tulloch, A.P., *Ibid. 17*:544 (1982).
5. Earle, F.R., E.H. Melvin, L.H. Mason, C.H. Van Etten, I.A. Wolff and Quentin Jones, *J. Am. Oil Chem. Soc. 36*:304 (1959).
6. Hopkins, C.Y., and M.J. Chisholm, *Can. J. Chem. 40*:2078 (1962).
7. Verma, J.P., and J.S. Aggarwal, *J. Indian Chem. Soc. 33*:357 (1956).
8. Wolff, J.P., *Manuel d'analyse des corps gras*, Azoulay, Paris, 1968, pp. 358-359.
9. Luddy, F.F., R.A. Barford and R.W. Riemen-Schneider, *J. Am. Oil Chem. Soc. 37*:447 (1960).
10. Chisholm, M.J., and C.Y. Hopkins, *J. Org. Chem. 27*:3137 (1962).
11. Kates, M., in *Techniques of Lipidology*, North Holland Publishing Company, Amsterdam, 1975, p. 175.
12. Conacher, H.B.S., F.D. Gunstone, G.M. Hornby and F.B. Padley, *Lipids 5*:434 (1970).
13. Du Plessis, L.M., and S. Vladar, *S. Afr. J. Sci. 70*:183 (1974).

[Received June 6, 1986]