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Analysis of cyanolipids and triacylglycerols from sapindaceae seed oils with high-temperature gas chromatography and high-temperature gas chromatography–chemical ionization mass spectrometry

R. Aichholz[a,*], V. Spitzer[b], E. Lorbeer[c]

[a]*Novartis Pharma AG, Research, Core Technology Area/Analytics, CH-4002 Basel, Switzerland*

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

[c]*Institute of Organic Chemistry, University of Vienna, Währinger Strasse 38, A-1090 Vienna, Austria*

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Abstract

The seed oils of three Sapindaceae species have been examined by high-temperature gas chromatography using glass capillary columns coated with the stationary phases SOP-50 (50% diphenyl–50% dimethylpolysiloxane, methoxy-terminated) and OV-225-OH (25% phenylmethyl–25% cyanopropylmethylpolysiloxane, hydroxy-terminated), respectively. The cyanolipid and triacylglycerol fraction could be identified by a combination of high-temperature gas chromatography with a flame ionization and a nitrogen–phosphorus detector and gas chromatography–mass spectrometry in positive and negative chemical ionization mode using NH_3 and CH_4/N_2O as reagent gases. The OV-225-OH phase showed better separation properties than the SOP-50 phase and up to 34 peaks could be separated. The analysis of the fatty acids in the seed oil of *Allophylus edulis* as their methyl esters by high-resolution GC and GC–MS are presented for the first time. © 1997 Elsevier Science B.V.

Keywords: Oils; Sapindaceae spp.; Cyanolipids; Triacylglycerols; Lipids

1. Introduction

Cyanolipids are a group of unusual plant lipids that thus far have been detected only in seed oils from the Sapindaceae. A large number of species from this plant family has been thoroughly investigated for this exotic lipid class and the results have been reviewed elsewhere [1–3]. The cyanolipids occur together with the triacylglycerols in the seed oil and their structures can be classified into four groups [1]. Structures I and II are diesters of 1-cyano-2-hydroxymethylprop-2-ene-1-ol (I) and 1-cyano-2-hydroxymethylprop-1-ene-3-ol (II) and structures III and IV are the monoesters of 1-cyano-2-hydroxymethylprop-1-ene (III) and 1-cyano-2-methylprop-2-ene-1-ol (IV), respectively.

*Corresponding author.

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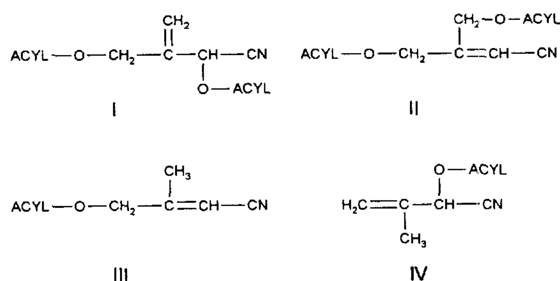

The nitrile moieties are esterified with long-chain fatty acids. In most of the cases, each plant species contains one or two of the four possible structures and the cyanolipid types I and IV are cyanogenic. The toxicological properties of these compounds are still unexplored [4].

The major part of the species of the Sapindaceae produce oily seeds and some are utilized commercially in the countries of origin [5]. Insect repellent properties as well as insecticidal activities of some of these seed oils have also been described [6,7]. As another outstanding peculiarity, most of the Sapindaceae seed oils have an unusually high content of eicosanoic and eicosenoic acids [1–3,5] which are preferentially incorporated in the cyanolipid fractions. In recently published works on four Brazilian Sapindaceae seed oils, it was shown that their monoene fatty acid isomer pattern is unusual in that the rare *cis*-13-eicosenoic acid (paullinic acid) and *cis*-11-octadecenoic acid (*cis*-vaccenic acid) were found at a high level in the fatty acid composition of the total lipids besides other monoenoic acid positional isomers [8,9].

For the detection of cyanolipids in seed oils, various spectroscopic and chromatographic methods have been investigated. It was shown that gas chromatography (GC) with packed columns and flame ionization detection (FID) can be used for the separation of the cyanolipids from the triacylglycerols [1]. Due to the low resolution of this type of column, only components varying in total carbon number (TCN) can be resolved. It is well known, that a separation according to the degree of unsaturation cannot be achieved with this type of column. Although the GC analysis data of a seed oil can give first hints for the presence of unusual lipid constituent, further information about the identity of a cyanolipid must be obtained from other analytical techniques such as ^{1}H NMR or ^{13}C NMR spectroscopy. Only one paper has been published about the analysis of a seed oil of a Sapindaceae species using a WCOT (wall coated open tubular) glass capillary column with OV-1 as stationary phase [10]. Similar to the separation with packed columns, two groups of several peaks have been observed and were attributed to the triacyclglycerol and cyanolipid fractions without further detailed characterization. The authors used the uncorrected area values from the GC analysis to quantify the relation between the triacylglycerols and cyanolipids of the seed oil of *Sapindus trifoliatus*.

In order to extend the knowledge of Sapindaceae seed oils and cyanolipid detection methods, it was considered desirable to investigate some members of this plant family with modern analytical techniques.

From earlier work it is known, that the *Paullinia* species examined in this paper contains the cyanolipid structure I and an unusual C-18 and C-20 monoenoic fatty acid isomer pattern [8,9]. The seed oil from *Allophyllus edulis* contains the same cyanolipid [11] but the high-resolution capillary GC analysis of the fatty acids has not been reported yet. The latter plant occurs in most of the South American countries and its fruits are described as eatable. After fermentation of the fruits a beverage similar to wine can be produced. In Peru the fruit is added to the traditional beverage 'chicha' [12].

In the present work, as part of our investigations on high-temperature gas chromatography (HTGC) of unusual lipids, the cyanolipid and triacylglycerol compositions of the seed oils from *Paullinia elegans*, *Paullinia meliaefolia* and *Allophylus edulis* have been analyzed. The high-temperature analyses were carried out using glass capillary columns coated with the polarizable stationary phases SOP-50 (50% diphenyl–50% dimethylpolysiloxane, methoxy-terminated) [13] and OV-225-OH (25% phenylmethyl–25% cyanopropylmethylpolysiloxane, hydroxy-terminated) [14], respectively. HTGC analysis with polarizable stationary phases enables the separation of acylglycerides by TCN and, within each TCN, separation according to the degree of unsaturation can be achieved [15]. FID and flameless thermoionic nitrogen–phosphorus detection (NPD) were used to illustrate the elution pattern of each lipid sample. For structure elucidaton of the individual lipid species high-temperature gas chroma-

tography–chemical ionization mass spectrometry have been utilized. GC–CI-MS with positive ions (GC–PCI-MS) [16] and negative ions (GC–NCI-MS) [17,18] were already successfully applied in the analysis of triacylglycerols. In a recent paper α-monoacetotriacylglyerols were studied with GC–NCI-MS [19]. This paper describes the first HTGC–CI-MS investigation of intact cyanolipids.

2. Material and methods

2.1. Seed lipids and fatty acid derivatives

The seed oils from *Paullinia elegans* and *P. meliaefolia* were available from previous work [8,9]. The fruits from *Allophyllus edulis* (Saint-Hilaire) Radlkofer were collected in January 1996 in the region of Cachoeira do Sul, in the Federal State of Rio Grande do Sul, Brazil. Seed oil extraction, transesterification to the fatty acid methyl esters and preparation of the dimethyloxazoline derivatives was carried out as described previously [8].

2.2. GC and GC–MS analysis of the fatty acid methyl esters (FAMEs) of *Allophylus edulis*

The FAME mixture was analyzed by GC and GC–MS using a HP 20M (Hewlett–Packard) fused-silica capillary column (50 m, 0.2 mm I.D.), coated with Carbowax 20M (0.2-μm phase thickness) and a DB 23 (J&W Scientific, Folsom, CA, USA) fused-silica capillary column (30 m×0.25 mm I.D.) coated with 50% cyanopropyl- and 50% methylsilicone (film thickness 0.25 μm) under the same conditions as described previously [8].

The following fatty acid composition (%, w/w) of the total lipids was found: 16:0 (8.1%), 16:1(c9) (5.2%), 18:0 (2.5%), 18:1(c9) (51.8%), 18:1(c11) (3.1%), 18:2(c9,c12) (5.5%), 18:3(n-c9,c12,c15) (1.7%), 20:0 (4.6%), 20:1(c11) (16.2%), 20:1(c13) (1.3%), 20:2 (c11,c14) (0.1%).

2.3. HTGC analysis of the seed oils

The HTGC system consists of a Carlo Erba Mega 5160 equipped with an on-column injector, a constant flow-constant pressure regulator (CP-CF 516) and a FID system, which was fitted with a ceramic jet and heated to 400°C. For the detection of nitrogen containing compounds, the FID system was replaced by a thermoionic NPD 80 FL that was connected to the Control Module NPD 800. The NPD system runs with the following operation parameters: detector temperature: 400°C, source heating current: 2.65 A, polarization voltage: 3.5 V, hydrogen: 3 ml/min, air: 40 ml/min, make-up gas (nitrogen): 27 ml/min.

Separation of the total lipid mixture was carried out on two different duran glass capillary columns of 10 m×0.2 mm I.D. The capillaries were pretreated and coated with a 0.10 μm film of SOP-50 (50% diphenyl–50% dimethylpolysiloxane, methoxy-terminated) and OV-225-OH (25% phenylmethyl–25% cyanopropylmethylpolysiloxane, OH-terminated, available from Ohio Valley Speciality Chemical,Inc., Marietta, OH, USA), respectively. The columns were conditioned at a temperature of 380°C. Detailed descriptions of the capillary column preparation were given in Ref. [13,14].

For analysis the samples were diluted 1:100 (v/v) in isooctane (Merck) for on-column injection and the oven temperature was programmed as follows: 1 min at 70°C, from 70 to 250°C at 12°C/min, from 250 to 380°C at 4°C/min. Hydrogen was employed as carrier gas with a linear velocity of 0.5 m/s. Data acquisition was carried out on a Spectra Physics Labnet System (Spectra Physics Integrator 4270 linked to a PC 3/86 data station).

2.4. HTGC–MS analysis of the seed oils

For HTGC–MS analyses, a Carlo Erba 4160 GC was coupled via a specially designed high-temperature interface (370°C) [20] to a Finnigan 4600 mass spectrometer. Chemical ionization was carried out with the following parameters: ammonia (PCI and NCI) and a CH_4/N_2O (80:20, w/w) mixture (NCI) were used as NCI reagent gases, respectively. The ion source was heated to a temperature of 160°C, filament current: 40 mA, electron multiplier voltage: 1400 V and the acquisition mass range was 250–1000 u in 2 s. Separation during the HTGC–MS analysis was achieved with both types of Duran glass capillary columns using the same temperature program.

3. Results and discussion

3.1. High-temperature gas chromatography

The use of NPD in HTGC analysis is uncommon. Only one citation referring to the analysis of petroporphyrins with alkali flame ionization detection (AFID) was found in the literature [21]. Fig. 1 demonstrates the high-temperature application of FID and NPD in the analysis of the Sapindaceae seed oils on the SOP-50 column. This stationary phase enabled the separation into two peak groups which herein are divided in up to 20 individual peaks. As only the first peak group responded to both detectors in the same manner, it can be concluded that all peaks from this group must represent the nitrogen containing cyanolipid species, while the second peak group can be attributed to the usual triacylglycerols.

The chromatograms obtained from the analyses of the Sapindaceae seed oils examined in this paper

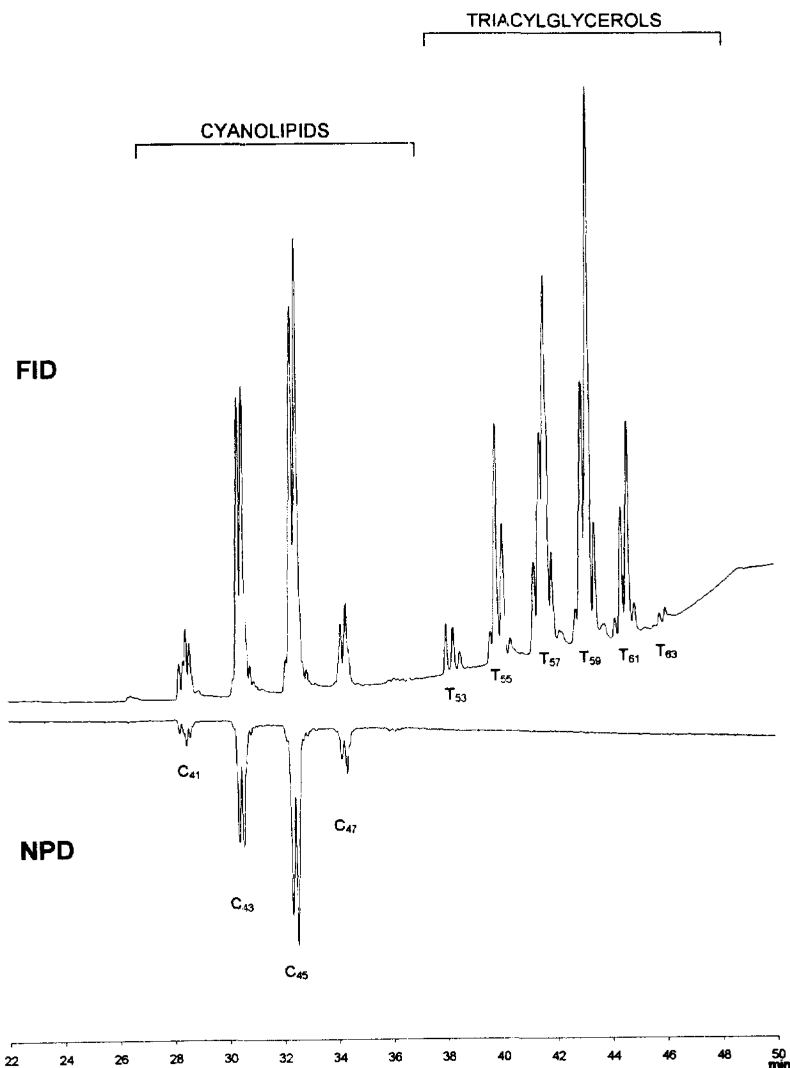

Fig. 1. Partial FID–NPD chromatogram of the seed oil from *Allophylus edulis*. The chromatogram illustrates the determination of the cyanolipid fraction with the nitrogen selective detector. C_{41}–C_{47} and T_{53}–T_{63} referring to the total carbon number of the cyanolipids and triacylglycerols in each peak cluster. Column: 10 m×0.2 mm glass capillary coated with SOP-50.

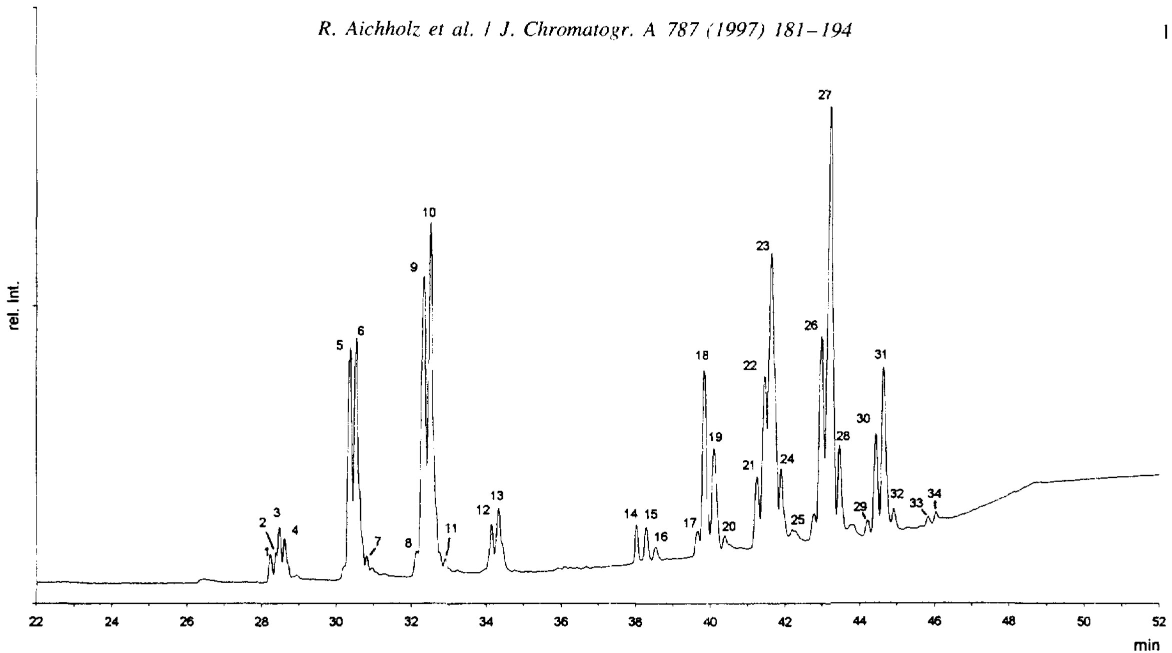

Fig. 2. Partial FID chromatogram of the seed oil from *Allophylus edulis*. Column: 10 m×0.2 mm glass capillary coated with SOP-50.

with the SOP-50 column are presented in Figs. 2–4. The resolution observed on the OV-225-OH phase was slightly different from the SOP-50 phase. Similar observations were made recently with the separation of α-acetotriacylglycerols from Celastraceae seed oils [19]. The elution order on both columns was approximately identical but the comparison of the chromatograms of each seed oil showed a better separation of the individual cyanolipid species for the column coated with OV-225-OH. Especially in

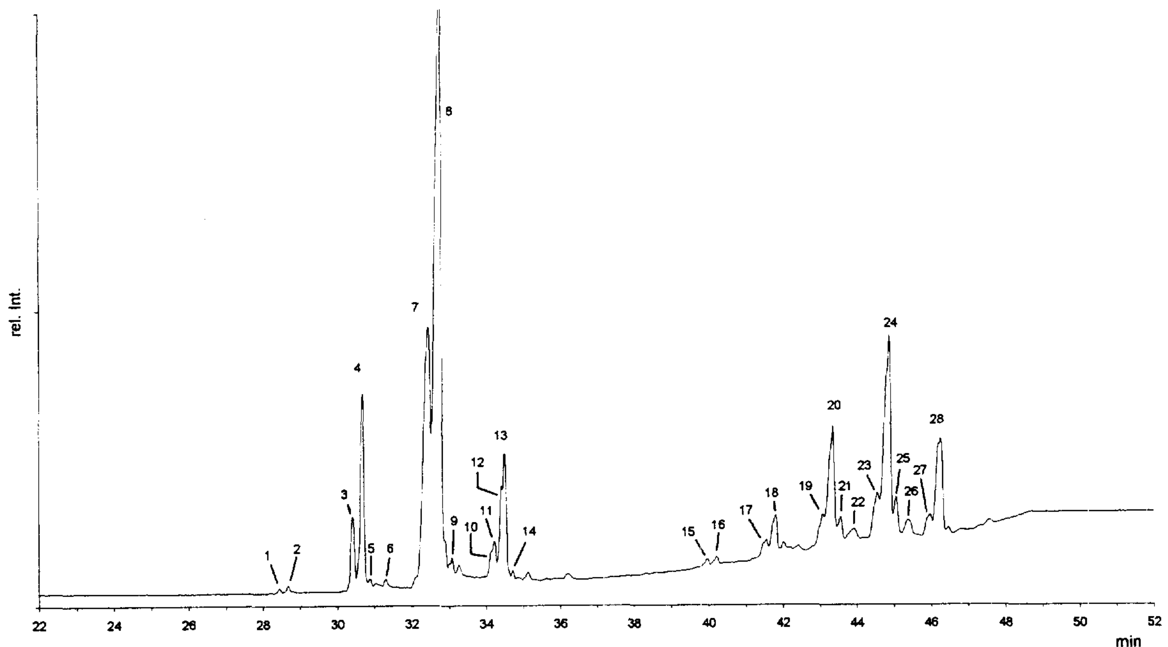

Fig. 3. Partial FID chromatogram of the seed oil from *Paullinia meliaefolia*. Column: 10 m×0.2 mm glass capillary coated with SOP-50.

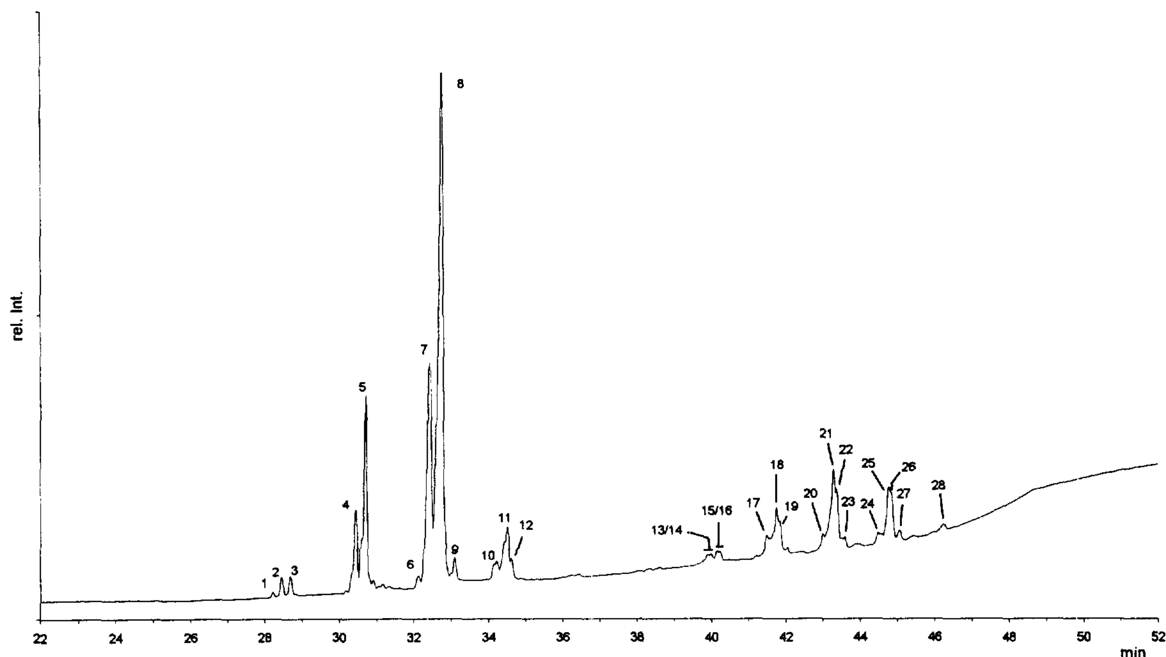

Fig. 4. Partial FID chromatogram of the seed oil from *Paullinia elegans*. Column: 10 m×0.2 mm glass capillary coated with SOP-50.

the seed oil of *Paullinia elegans* some additional minor compounds in the cyanolipid peak group could be detected (Fig. 5). However, the relatively high tendency for column bleeding of the OV-225-OH phase can be a drawback for the quantification of the later eluting triacylglycerols. The nitrogen-containing column bleed at temperatures above 340°C, produced by the cyanopropyl groups, prevented the high-temperature application of NPD with the OV-225-OH columns.

3.2. Mass spectrometry

A detailed identification of each GC peak was made by HTGC–MS analyses in NCI and PCI mode. Due to the fact that the mass spectroscopic behaviour of both lipid classes was found to be different, separate analyses were necessary using CH_4/N_2O and NH_3 chemical ionization, respectively. The interpretation of the NCI mass spectra and the uncorrected FID integral values from the HTGC analysis obtained from each oil sample are summarized in Tables 1–3. As expected, neither the regiospecific distribution of the fatty acids at the nitrile or glycerol backbone nor the position of double bonds in the acyl chains can be proven with this method.

3.3. Mass spectra of the cyanolipids

With CH_4/N_2O as reagent gas mixture, no quasi-molecular ions could be observed for the cyanolipids. However, the fragments due to the individual carboxylate anions appeared with high abundance. By using ammonia as reagent gas, weak quasi-molecular ions ($[M-H]^-$) could be observed. Fig. 6 illustrates as an example, the mass spectrum of a cyanolipid with the CN 45 using ammonia as a reagent gas for chemical ionization. Another ion of low abundance in the high mass range can be attributed to the loss of HCN from the $[M-H]^-$ molecular ion species. The base peak at m/z 309 represents the carboxylate anion from eicosenoic acid and the ion at m/z 386 can be explained by the loss of one molecule of eicosenoic acid from the quasi-molecular ion. Fig. 7 shows the corresponding mass spectrum of a minor peak of the seed oil from *Paullinia elegans* with the TCN 41 (Peak No 2 in

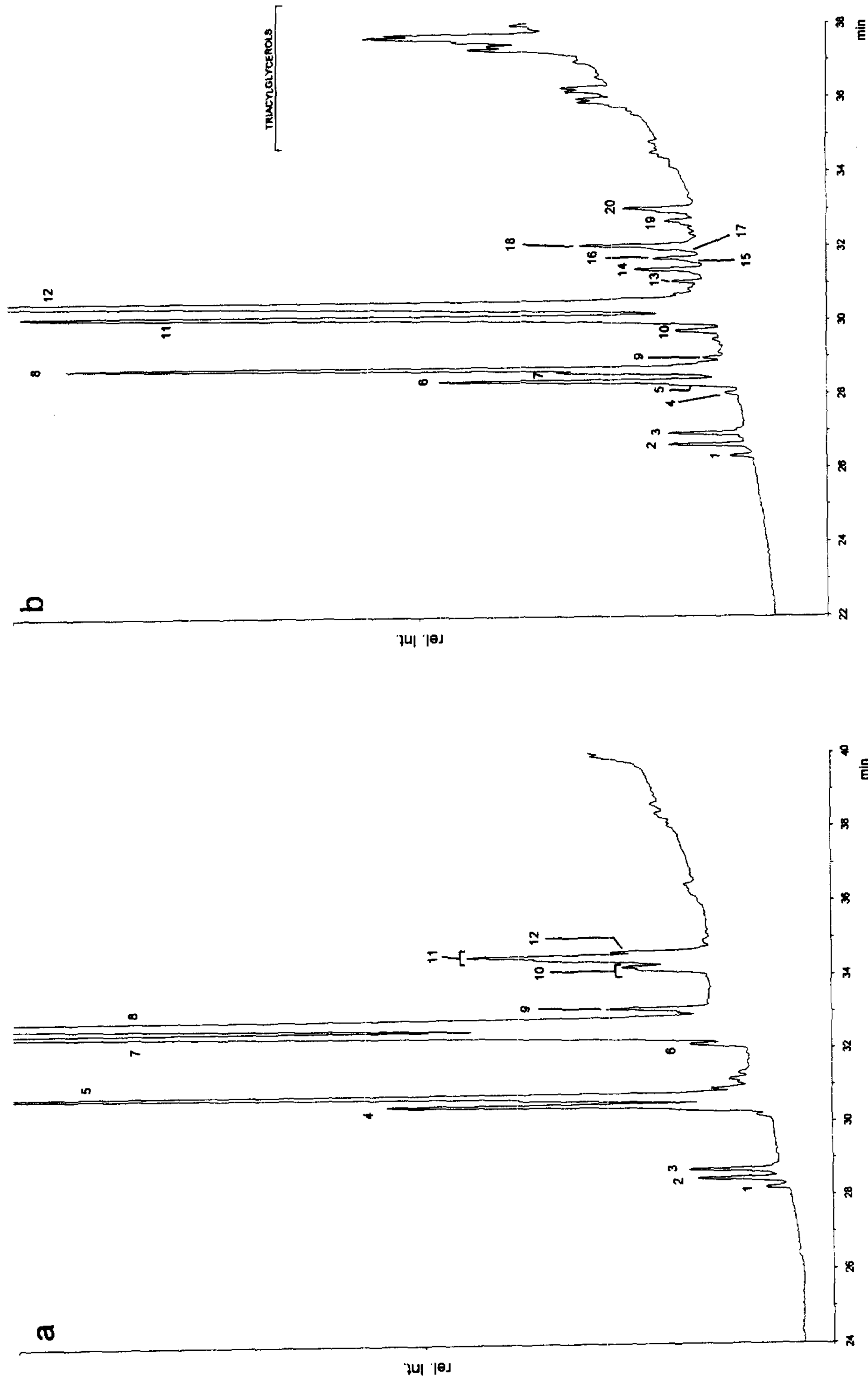

Fig. 5. Partial FID chromatograms of the cyanolipid fraction of the seed oil from *Paullinia elegans*. (a) Column: 10 m×0.2 mm glass capillary coated with SOP-50 and (b) Column: 10 m×0.2 mm glass capillary coated with OV-225-OH.

Table 1
Identification of the cyanolipid and triacylglycerol composition of the seed oil of *Allophylus edulis* analyzed on the SOP-50 column. The peak numbers correspond to Fig. 2

Peak number	Molecular mass	TCN:n[a]	Cyanolipid[b]	FID[c] %
1	645	41:0	16:0/20:0	0.48
2	643	41:1	16:0/20:1[d]	0.43
	643	41:1	18:0/18:1	
3	643	41:1	16:1/20:0	1.04
	641	41:2	18:1/118:1	
4	641	41:2	16:1/20:1	0.91
5	671	43:1	18:1/20:0	5.78
6	669	43:2	18:1/20:1[d]	7.02
	669	43:2	16:1/22:1	
7	667	43:3	18:2/20:1	0.15
8	701	45:0	20:0/20:0	0.54
9	699	45:1	20:0/20:1[d]	8.08
	699	45:1	18:1/22:0	
10	697	45:2	20:1/20:1[d]	10.62
	697	45:2	18:1/22:1	
11	699	45:1	20:0/20:1[d]	0.13
	697	45:2	18:1/22:1	
	697	45:2	18:2/22:0	
12	729	47:0	20:0/22:0	1.27
	727	47:1	20:0/22:1	
13	725	47:2	20:1/22:1	2.53
			Cyanolipids total (FID %)	38.98
			Triacylglycerol[b]	
14	832	53:1	16:0/n.i./n.i.[e]	1.02
15	830	53.2	16:1/n.i./n.i.	0.94
16	n.i.	–	–	0.41
17	860	55:1	n.i./n.i./n.i.	0.54
18	858	55:2	16:0/18:1/18:1	4.87
19	856	55:3	16:1/18:1/18:1	2.92
20	854	55:4	n.i./n.i./n.i.	0.63
21	888	57:1	16:0/18:1/20:0	1.75
22	886	57:2	16:0/18:1/20:1	4.91
23	884	57:3	18:1/18:1/18:1[f]	10.63
	884	57:3	16:1/18:1/20:1	
24	882	57:4	18:1/18:1/18:2	1.52
25	n.i.	–	–	0.86
26	914	59:2	18:1/18:1/20:0	6.11
27	912	59:3	18:1/18:1/20:1	12.85
28	910	59:4	18:1/18:2/20.1	1.80
29	n.i.	–	–	0.31
30	942	61:2	18:1/20:0/20:1	2.20
31	940	61:3	18.1/20:1/20:1	7.28
32	938	61:4	n.i./n.i./n.i.	0.56
33	970	63:2	n.i./n.i./n.i.	0.16
34	968	63:3	n.i./n.i./n.i.	0.22
			Triacylglycerol total (FID %)	61.08
			Total (FID %)	99.98

[a] TCN is the total carbon number and n the combined number of double bonds in the acyl chains of the fatty acids of a cyanolipid or triacylglycerol.

[b] The position of the fatty acid residues has not been determined.

[c] Uncorrected proportions of the cyanolipids and triacylglycerols obtained with the flame ionization detector.

[d] The cyanolipids exhibiting the most abundant carboxylate anions.

[e] n.i. = not identified.

[f] The triacylglycerol exhibiting the most abundant $[M-H]^-$ ion.

Table 2
Identification of the cyanolipid and triacylglycerol composition of the seed oil of *Paullinia meliaefolia* analyzed on the SOP-50 column. The peak numbers correspond to Fig. 3

Peak number	Molecular mass	TCN:*n*[a]	Cyanolipid[b]	FID[c] %
1	643	41:1	16:1/20:0[d]	0.07
	643	41:1	16:0/20:1	
2	641	41:2	16:1/20:1	0.10
3	671	43:1	18:1/20:0	1.96
4	669	43:2	18:1/20:1	5.12
5	667	43:3	18:2/20:1	0.14
6	665	43:4	18:3/20:1	0.15
7	699	45:1	20:0/20:1	14.42
8	697	45:2	20:1/20:1	31.47
9	693?	45:4?	20:1/20:3?	0.88
10	727	47:1	20:0/22:1	–[e]
11	727	47:1	20:1/22:0	2.05
12	725	47:2	20:1/22:1	–[e]
13	725	47:2	20:1/22:1	5.02
14	721	47:4?	20:1/22:3?	0.20
			Cyanolipids total (FID %)	61.58
			Triacylglycerol[b]	
15	858	55:2	16:0/18:1/18:1	0.37
16	858	55:2	16:0/18:1/18:1	0.57
17	886	57:2	n.i./n.i./n.i.[f]	0.90
18	884	57:3	18:1/18:1/18:1[e]	8.21
19	914	59:2	18:0/18:1/20:1	0.51
20	912	59:3	18:1/18:1/20:1	8.07
21	910	59:4	18:1/18:2/20:1	1.01
22	908	59:5	n.i./n.i./n.i.	1.44
23	942	61:2	18:1/20:0/20:1	2.75
24	940	61:3	18:1/20:1/20:1	10.80
25	938	61:4	18:2/20:1/20:1	1.17
26	936	61:5	20:1/n.i./n.i.	1.45
27	970	63:2	20:0/20:1/20:1	1.25
28	968	63:3	20:1/20:1/20:1	4.75
			Triacylglycerol total (FID %)	36.92
			Total (FID %)	98.52

[a] TCN is the total carbon number and *n* the combined number of double bonds in the acyl chains of the fatty acids of a cyanolipid or triacylglycerol.
[b] The position of the fatty acid residues has not been determined.
[c] Uncorrected proportions of the cyanolipids and triaclyglycerols obtained with the flame ionization detector.
[d] The cyanolipid with the most abundant carboxylate anions.
[e] Shoulder of the following Peak, areas were summarized.
[f] n.i. = not identified.

Fig. 4). The dominant fragments at *m/z* 255 and 309 can easily be attributed to the carboxylate anions of palmitic and eicosenoic acid, respectively. The presence of the ions with a low abundance at *m/z* 253, *m/z* 311, *m/z* 281 and 283 led to the conclusion that these peaks must be due to a mixture of one predominant and two minor cyanolipids. All isomers have the TCN 41, the same degree of unsaturation and thus a common molecular mass.

In comparison with the chemical ionization with CH_4/N_2O, the chemical ionization with NH_3 is advantageous as the lifetime of the filament is improved and no interfering background ions in the mid-mass range are observable.

Table 3
Identification of the cyanolipid and triacylglycerol composition of the seed oil of *Paullinia elegans* analyzed on the SOP-50 column. The peak numbers correspond to Fig. 4

Peak number	Molecular mass	TCN:n[a]	Cyanolipid[b]	FID[c] %
1	645	41:0	16:0/20:0	0.14
2	643	41:1	16:1/20:0	0.56
	643	41:1	16:0/20:1	
3	643	41:2	16:1/20:1[d]	0.54
	641	41:2	18:1/18:1	
4	671	43:1	18:1/20:0	3.98
5	669	43:2	18:1/20:1[d]	9.28
6	701	45:0	20:0/20:0	0.74
7	699	45:1	20:0/20:1	13.08
8	697	45:2	20:1/20:1[d]	31.13
	697	45:2	18:1/22:1	
9	697	45:2	20:1/20:1	0.63
10	729	47:0	20:0/22:0	1.28
	727	47:1		
11	727	47:1	20:1/22:1	3.79
12	n.i.	–	–	0.60
			Cyanolipids total (FID %)	65.75
			Triacylglycerol[b]	
13	858	55:2	16:0/18:1/18:1	0.38
14	858	55:2	n.i./18:1/18:1	0.38
15	856	55:3	16:1/18:1/18:1	0.41
16	856	55:3	16:0/18:1/n.i.[e]	0.43
17	886	57:2	n.i./n.i./n.i.[e]	0.65
18	884	57:3	18:1/18:1/18:1	–[f]
19	884	57:3	16:1/18:1/20:1	8.21
20	914	59:2	18:0/18:1/20:1	0.24
	914	59:2	18:1/18:1/20:0	
21	912	59:3	18:1/18:1/20:1	–[f]
22	912	59:3	18:1/18:1/20:1	10.30
23	910	59:4	18:1/18:2/20.1	0.47
24	942	61:2	18:1/20:0/20:1	0.83
25	940	61:3	18:1/20:1/20:1	–[f]
26	940	61:3	18:1/20:1/20:1	6.90
27	938	61:4	18:2/20:1/20:1	0.73
28	968	63:3	20:1/20:1/20:1	0.69
			Triacylglycerol total (FID %)	24.62
			Total (FID %)	96.37

[a] TCN is the total carbon number and n the combined number of double bonds in the acyl chains of the fatty acids of a cyanolipid or triacylglycerol.
[b] The position of the fatty acid residues has not been determined.
[c] Uncorrected proportions of the cyanolipids and triaclyglycerols obtained with the flame ionization detector.
[d] The cyanolipid with the most abundant carboxylate anions.
[e] n.i. = not identified.
[f] Shoulder of the following peak, areas were summarized.

For further verification of the molecular mass of the cyanolipids, the analyses were repeated with PCI using NH_3 as a reagent gas. This ionization method provided dominant quasi-molecular ions $[(M+NH_4)^+]$. However, the sensitivity was inferior in comparison with the NCI mode and the determination of the molecular mass of the cyanolipids in a concentration range less than 1% was difficult.

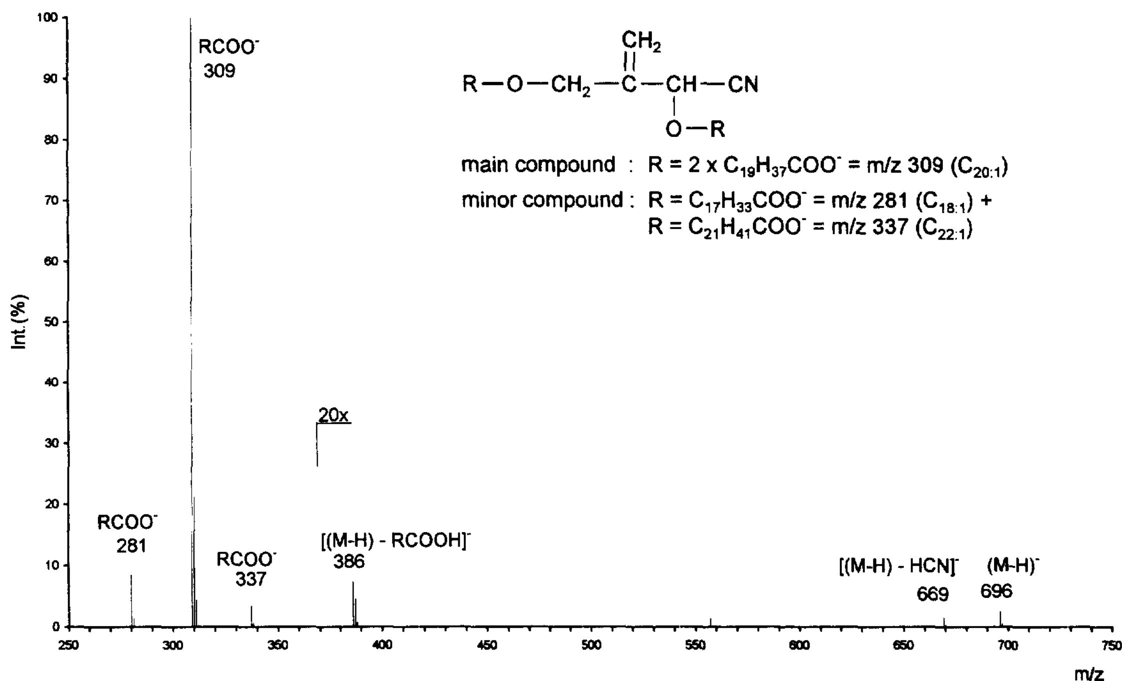

Fig. 6. Mass spectrum obtained by the HTGC–NCI-MS of the cyanolipid mixture corresponding to peak No. 8 in Fig. 3 using ammonia as reagent gas. The peak consists of one predominant cyanolipid and one minor cyanolipid with the same molecular mass and degree of unsaturation, respectively.

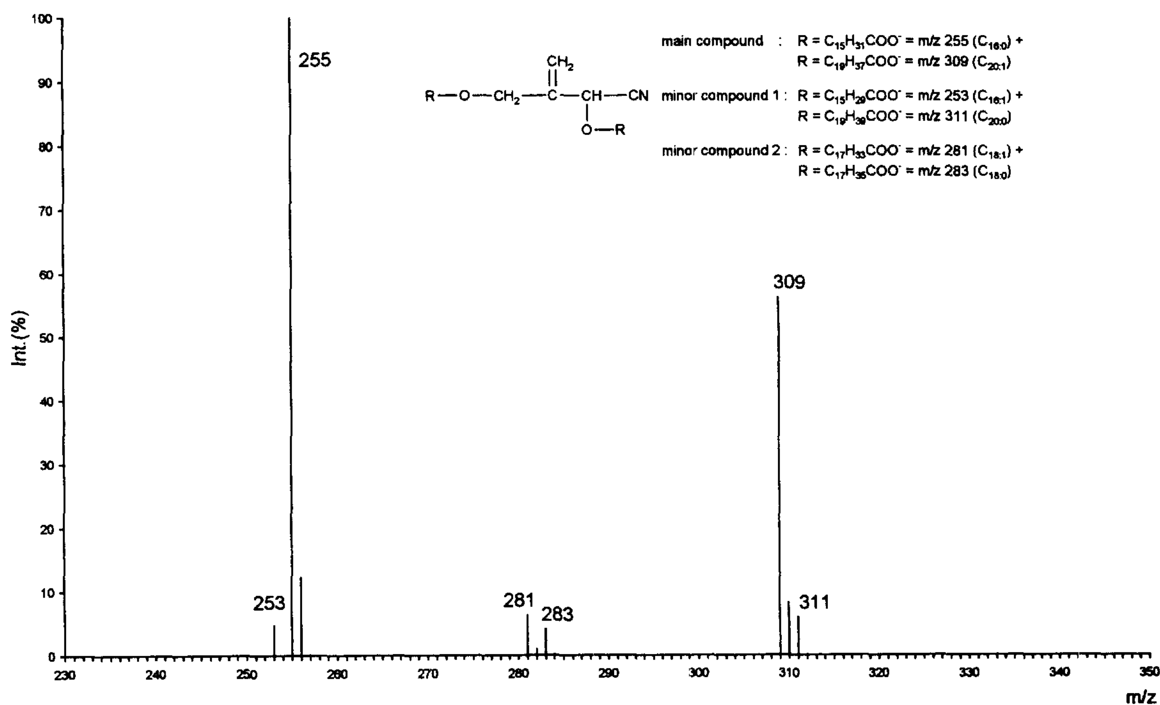

Fig. 7. Carboxylate anions of the peak No. 2 in Fig. 3, obtained by the HTGC–NCI-MS using ammonia as reagent gas, shows the composition of one predominant cyanolipid and two minor cyanolipids with the same TCN and unsaturation.

The minor peaks (peak Nos. 13, 14, 19 and 20, see Fig. 5b) in the cyanolipid group from the seed oil of *Paullinia elegans*, which where separated on the OV-225-OH column, showed the same quasi molecular ions [$(M+NH_4)^+$] and carboxylate anions [$(RCOO)^-$] as the main peaks (Peak No. 6, 8, 11 and 12, see Fig. 5b). The FID integral values and interpretation of the CI mass spectra are given in Table 4. In the oil samples of *Paullinia meliaefolia* and *Allophylus edulis*, these additional isomer peaks could not be detected. It is highly probable that these peaks can be attributed to the cyanolipid structure type II, as the other known cyanolipids III and IV are monoesters with a lower molecular mass. Due to the low concentration of these minor peaks, the corresponding cyanolipid could not be detected in previous work using NMR for identification of the cyanolipid structure [8].

3.4. Mass spectra of the triacylglycerols

In contrast to the observations in literature [17,18], all the obtained NCI mass spectra (NH_3 and CH_4/N_2O) of the triacylglycerols showed an abundant $[M-H]^-$ molecular ion species, enabling the easy determination of the corresponding molecular mass. This observation is in agreement with investigations of triacylglycerols using NCI-MS with direct probe insertion [22–24]. However, the sensitivity was significantly inferior in comparison with the cyanolipids. Fig. 8 shows the respective total ion current of the sample *Paullinia meliaefolia*. Further-

Table 4
Identification of the cyanolipid composition of the seed oil of *Paullinia elegans* analyzed on the OV-225-OH column. The peak numbers correspond to Fig. 5

Peak number	Molecular mass	TCN:*n*[a]	Cyanolipid[b]	FID[c] %
1	645	41:0	16:0/20:0	0.34
2	643	41:1	16:0/20:1[d]	0.61
	643	41:1	16:1/20:0[d]	
	643	41:1	18:0/18:1	
3	641	41:2	16:1/20:1	0.58
4	673	43:0	18:0/20:0	0.23
5	671	43:1	18:1/20:0	1.08
6	671	43:1	18:1/20:0	5.23
7	669	43:2	18:1/20:1	3.50
8	669	43:2	18:1/20:1	12.46
9	667	43:3	18:2/20:1	0.26
10	701	45:0	20:0/20:0	0.87
11	699	45:1	20:0/20:1	19.67
	699	45:1	20:0/20:1	
12	697	45:2	20:1/20:1	26.13
13	671	43:1	18:1/20:0	0.35
14	669	43:2	18:1/20:1	1.26
15	729	45:0	20:0/22:0	0.97
16	727	45:1	20:1/22:0	
17	725	45:2	20:1/22:1	1.65
18	725	45:2	20:1/22:1	
19	699	43:2	20:0/20:1[d]	0.52
	699	43:2	18:1/22:0	
20	697	43:2	20:1/20:1[d]	1.11
	697	43.2	18:1/22:1	
			Cyanolipids total (FID %)	99.41

[a] TCN is the total carbon number and *n* the combined number of double bonds in the acyl chains of the fatty acids of a cyanolipid or triacylglycerol.
[b] The position of the fatty acid residues has not been determined.
[c] Proportions of the cyanolipids and triaclyglycerols obtained with the flame ionization detector.
[d] The cyanolipid with the most abundant carboxylate anions.

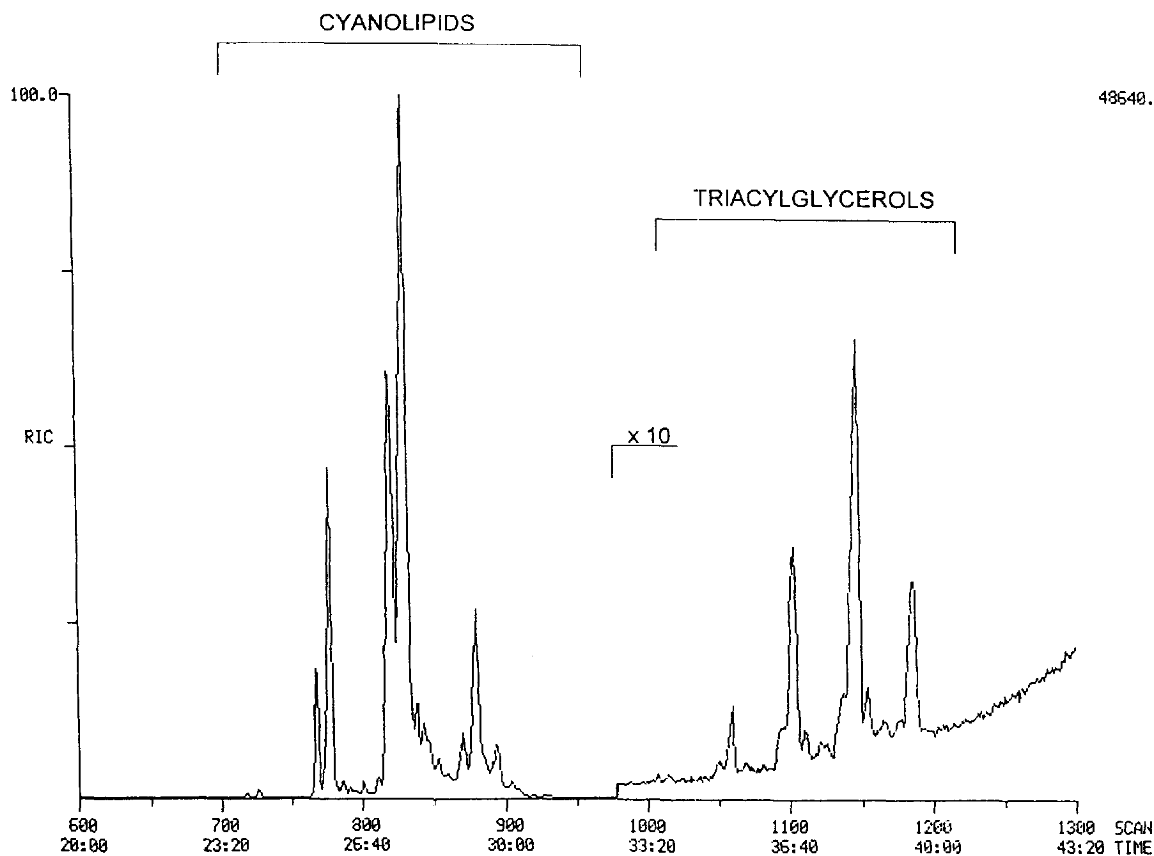

Fig. 8. Total ion current chromatogram obtained by the HTGC–NCI-MS analysis of *Paullinia meliaefolia* using ammonia as reagent gas.

more, the intensity of the carboxylate anions was low and consequently, the identification of the fatty acids in minor compounds was impossible. In Fig. 9 the NCI mass spectra of peak 23 in the *Allophylus edulis* seed oil (see Fig. 2) are presented. It can be observed, that this GC peak contains one predominant triacylglycerol (18:1/18:1/18:1) and one minor triacylglycerol (16:1/18:1/20:1) differing in the chain length of the esterified fatty acid acyl residues. Some of the triacylglycerols peaks are broadened due to a incomplete separation of isomers (e.g. peak Nos. 18/19 and 21/22, Fig. 4). The HTGC–NCI-MS analysis shows only one quasi molecular ion and the identical m/z values for the carboxylate anions. This observation led to the conclusion that the peaks must be a mixture of triacylglycerols with a different regiospecific distribution of the fatty acids (*sn*-2 vs. *sn*-1/3) or different position of the unsaturation in the fatty acid chain.

4. Conclusion

In this paper we described the HTGC and HTGC–MS analyses of three Sapindaceae seed oils. Two different types of capillary columns coated with SOP-50 and OV-225-OH, respectively, were employed. Both types of stationary phases enabled the separation of the lipid species according to their number of carbon atoms and within a carbon number according to their degree of unsaturation. The OV-225-OH column showed an improved separation of the cyanolipids. The use of FID and NPD in capillary GC at high-temperatures enabled the differentiation of cyanolipids and triacylglycerols. HTGC–MS using the positive and negative mode provided a useful approach to study complex lipid mixtures. Individual species of cyanolipids and triacylglycerols belonging to their quasi molecular ions and/or their carboxylate anions could be characterized. Further-

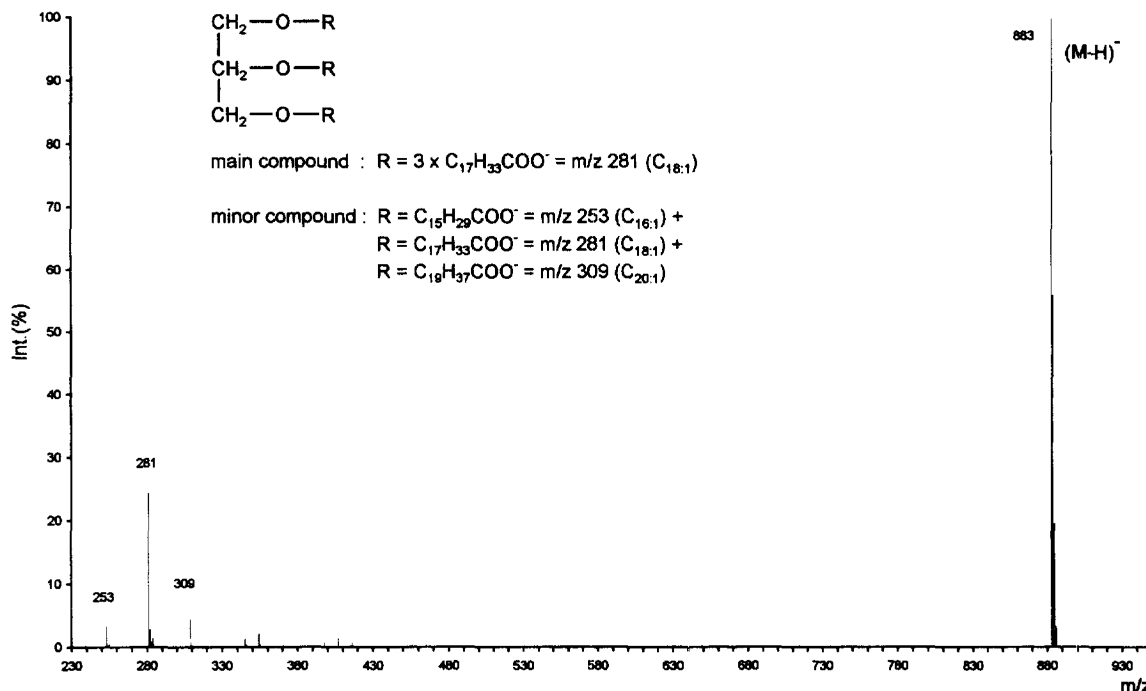

Fig. 9. Mass spectrum obtained through the HTGC–NCI-MS of the cyanolipid mixture corresponding to peak No. 23 in Fig. 2 using ammonia as reagent gas.

more, isomeric lipid compounds which cannot be separated by HTGC can be sucessfully distinguished by HTGC–MS.

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