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Identification of the lipids and the ant attractant 1,2-dioleoylglycerol in the arils of *Commiphora guillaumini* Perr. (Burseraceae) by supercritical fluid chromatography–atmospheric pressure chemical ionisation mass spectrometry

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Abstract

On-line coupling of supercritical fluid chromatography to atmospheric pressure chemical ionisation mass spectrometry was used to analyse a complex mixture of tri- and di-acylglycerols extracted from the tree *Commiphora guillaumini* Perr. (Burseraceae). The single components, including the ant attractant 1,2-dioleoylglycerol, were identified by mass spectrometry using skimmer-fragmentation in both the positive and the negative mode.

Keywords: *Commiphora guillaumini* Perr.; Supercritical fluid chromatography–mass spectrometry; *Aphaenogaster swammerdami*; Lipids; 1,2-Dioleoylglycerol; Glycerols

1. Introduction

Supercritical fluid chromatography (SFC) is becoming an increasingly attractive chromatographic technique for separations which present difficulties in both HPLC and GC. An example of such a case is the analysis of fats where the separative power of LC is insufficient to separate the large number of closely related acylglycerols, while the high temperatures required for the elution from GC limit the choice of stationary phases to relatively non-polar phases. The emerging techniques for coupling SFC with spectroscopic methods, in particular with NMR [1], are expected to enhance the popularity of SFC as a supplement to LC and GC.

The first on-line-coupling of SFC with a mass spectrometer, described by Smith et al. in 1982 [2], was followed by a number of improved interface designs [3–5] including some that permitted higher flow-rates. Other interfaces, originally developed for HPLC–MS, have been applied for SFC–MS [6,7]. Soon after the first coupling of SFC using packed columns to an APCI-MS by Huang et al. [8] and

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Anacleto et al. [9], reports of capillary SFC–MS appeared as well [10,11].

We have employed SFC–APCI-MS for the determination of the lipid composition in the arils of *Commiphora guillaumini* Perr. (Burseraceae), a tree species native to the dry forest of western Madagascar. *Commiphora guillaumini* is one of the few plants whose seed dispersal is effected only by a limited number of animal species. While a small percentage of the seeds is dispersed by frugivorous tree visitors such as birds and lemurs, the main proportion is distributed by the ant species *Aphaenogaster swammerdami*, which carries fallen seeds into its colonies [12]. Bresinsky observed as early as 1963 [13] that a lipid-containing fraction induced a variety of ants to collect seeds. In 1979, Marshall et al. [14] showed that 1,2-dioleoylglycerol acts as an ant attractant. Other groups [15,16] showed later that in the cases of *Acacia myrtifolia* and *Tetratheca stenocarpa*, 1,2-dioleoylglycerol was the compound which attracted ants. It appeared plausible that this could also be the case for *Commiphora guillaumini*, and thus we investigated extracts of the arils attached to the seeds of *Commiphora guillaumini* for the presence of 1,2-dioleoylglycerol by SFC–MS, at the same time also screening the lipid profile.

2. Experimental

2.1. Chemicals

A triacylglycerol mixture containing the standard substances tripalmitoylglycerol (PPP), tristearoylglycerol (SSS), trioleoylglycerol (OOO), trilinoleoylglycerol (LLL) and trilinolenoylglycerol (LnLnLn), as well as the 1,2- and 1,3-dioleoylglycerol, was purchased from Sigma (Munich, Germany). Cyclohexane and methanol were obtained from Merck (Darmstadt, Germany).

2.2. Samples and sample preparation

Seeds were collected in March 1994 in the forest of Kirindy, 60 km north of Morondava, western Madagascar. The seeds were frozen in liquid nitrogen to facilitate the removal of the aril from the grain. The frozen arils (5 g fresh weight) were then ground with a pestle and mortar and extracted in a Soxhlet apparatus for 5 h with 200 ml of cyclohexane. The extract was filtered, concentrated in a vacuum evaporator to a final volume of 20 ml and stored at −20°C under nitrogen in the dark.

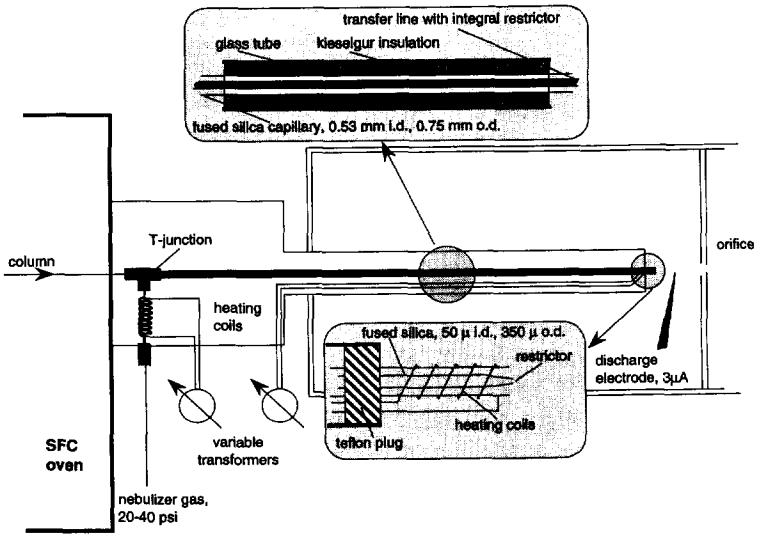

Fig. 1. Schematic diagram of the SFC–MS interface.

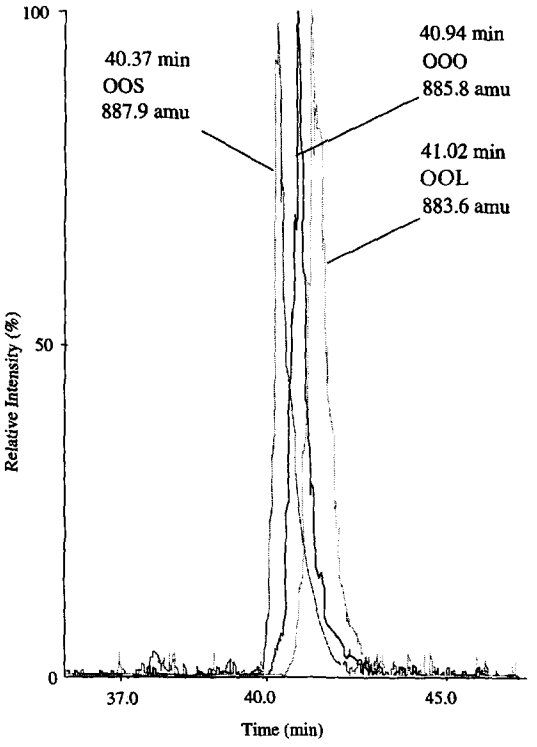

Fig. 2. SFC chromatogram of the hexane extract: reconstructed ion-current traces corresponding to the triacylglycerols: dioleoylstearoylglycerol (OOS) m/z=887.9, trioleoylglycerol (OOO) m/z=885.9 and dioleoyllinolylglycerol (OOL) m/z=883.6. Chromatographic conditions: Column, 25 cm × 250 μm I.D. fused-silica, packed with benzoylamidopropyl–Nucleosil (d_p 5μ); mobile phase, CO_2; T=70°C; P=20 MPa, 1 min isobar, 0.5 MPa/min to 40 MPa.

2.3. SFC and MS interface

The SFC was self-assembled and consisted of an ISCO μLC-500 Micro-Flow-Pump with pressure/density programme facilities (ISCO Software Chemsearch-SFC), and a Dani 3900 GC oven. The carrier fluid was pure CO_2 (Messer Griesheim, FRG, 99.99 vol% purity). A Valco CI 4W injection valve equipped with a capillary retention gap to allow solvent-venting was used to inject ca. 60 nl of sample dissolved in methylene chloride onto a fused-silica capillary column 25 cm × 250 μm I.D. packed with benzoylamidopropyl–Nucleosil (i.e. Nucleosil–NH_2, particle diameter 5μ, capped by reaction with benzoyl chloride). The separation was carried out at 70°C with pressure programming from 20.0 MPa (1 min) at 0.5 MPa/min to 40.0 MPa.

The SFC column was interfaced to the mass spectrometer via a home-made coupling (see Fig. 1) based upon the design of the standard heated nebulizer interface for LC–APCI-MS as described by Thomas et al. [11]. From the T-junction, the 50-μm I.D. fused-silica transfer line from the SFC column (including integral restrictor) was led through a 530-μm I.D. fused-silica capillary through which heated air was passed. This served both to keep the transfer line at an elevated temperature and to provide the nebulizer gas required for the APCI source. The concentric fused-silica capillaries were contained in a 6-mm O.D. glass tube filled with Kieselgur as heat insulator. The restrictor itself was additionally heated over a length of ≈1 cm by a coil positioned around the 530-μm sheath capillary. The distance between the discharge needle and the restrictor was approximately 2.5 cm and between discharge needle and orifice, ca. 2 cm. Auxilliary gas was not necessary.

2.4. Mass spectrometry

Mass spectra were recorded on a Sciex API III triple quadrupole mass spectrometer (Sciex, Toronto, Canada), with a modified APCI ion source as described above and a m/z range of 2400 a.m.u. Calibration was carried out with a NaI solution. Spectra were recorded in positive and negative mode (dwell time, 1 ms; step size, 0.1–0.5 a.m.u.; data acquired to disk). For data aquisition, data processing and the control of the mass spectrometer, a MacIntosh IIx was employed. The current on the discharge needle was held at 3 μA.

3. Results and discussion

3.1. Supercritical fluid chromatography

The benzoylamide stationary phase proved more effective than the more common cyanopropyl phase for the separation of the triacylglycerols according to their degree of unsaturation. Baseline separation of OOO, LLL, LnLnLn and SSS is achieved with a 25-cm packed capillary. The resolution of this col-

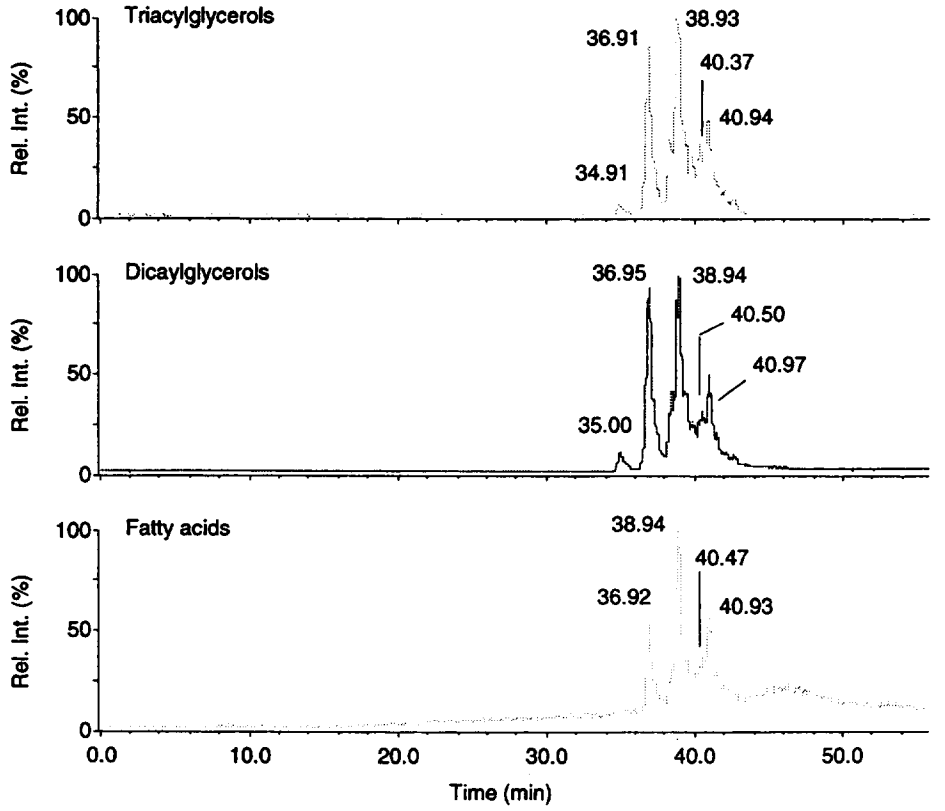

Fig. 3. Comparison of chromatograms of the hexane extract recorded with different windows. Upper: triacylglycerols, orifice voltage (U_o)=+50 V, positive mode; middle: diacylglycerol fragments, U_o=+120 V, positive mode; lower: fatty acid fragments, U_o=−120 V, negative mode. Chromatographic conditions as in Fig. 2.

umn proved insufficient for the separation of the many components of the fat extract from *Commiphora guillaumini* with conventional flame ionisation detection. However, reconstructed ion chromatograms revealed that partial separation of the very similar components present was indeed achieved (see Fig. 2).

The diacylglycerols were eluted later than all triacylglycerols and with good separation of the positional isomers 1,2- and 1,3-dioleoylglycerol. Comparison of retention times with standard substances indicated the presence of 1,2-dioleoylglycerol.

3.2. Mass spectrometry

In general, protonated molecular ions are expected in APCI and these were indeed found. In addition, NH_4^+ adduct ions were present (see Fig. 4) and both species were used to identify the molecular masses of the acylglycerols. A total of seventeen components could be distinguished. The usual way of identifying these seventeen components would be fragmentation by tandem MS methods. Thus it would have been necessary to perform seventeen daughter scan and/or one parent or neutral-loss scan for each expected fatty acid, quite a long procedure considering that each SFC run takes approximately 50 min.

As an alternative to the more time-consuming tandem MS method for the identification of these compounds, fragmentation with high orifice voltage was employed [17–19]. Previous experiments with standards confirmed that in the case of tri- as well as of diacylglycerols, loss of the carboxylate moieties occurs at orifice voltages above 80 V. Operation

Table 1
Retention time (min), $[M+H]^+$ found, mass of the diacylglycerol and fatty acid fragments (a.m.u.), approximate amount of each component calculated from the peak area of the reconstructed ion current chromatogram and the nominal structure

t_R	$[M+H]^+$ a.m.u.	Diglyceride fragments	Fatty acid fragments	Assigned structure[a]	Amount (%)
35.07	807.7	551.4	255.3	PPP	1.9
36.79	835.6	551.4; 579.4	255.3; 283.4	PPS	0.2
36.88	833.7	551.4; 577.2	255.3; 281.2	PPO	12.9
36.88	831.7	551.4; 575.4	255.3; 279.4	PPL	11.1
38.31	861.7	577.3; 579.4; 605.3	255.3; 281.2; 283.2	POS	6.0
38.79	859.8	577.3; 603.4	255.2; 281.3	POO	17.4
38.77	857.5	575.4; 601.2	255.4; 279.4; 281.3	PLO	11.8
39.41	887.9	601.3; 607.4	279.4; 283.3	LSS	4.7
39.80	855.6	575.4; 599.4	255.3; 279.4; 281.3	POLn	8.1
39.85	889.8	605.4; 607.2	281.2; 283.3	OSS	2.7
40.39	887.9	605.2; 603.2	281.2; 282.3	OOS	3.9
40.9	885.8	603.2	281.3	OOO	8.3
41.02	883.6	601.4; 603.2	279.4; 281.3	OOL	5.2
42.08	881.8	599.5; 601.4	279.4; 281.3	OLL	2.6
42.69	879.8	599.3	279.4	LLL	1.8
46.47	577.5		255.3; 281.2	OP	0.4
49.41	603.5		281.2	OO	0.9

[a] All positional permutations possible.

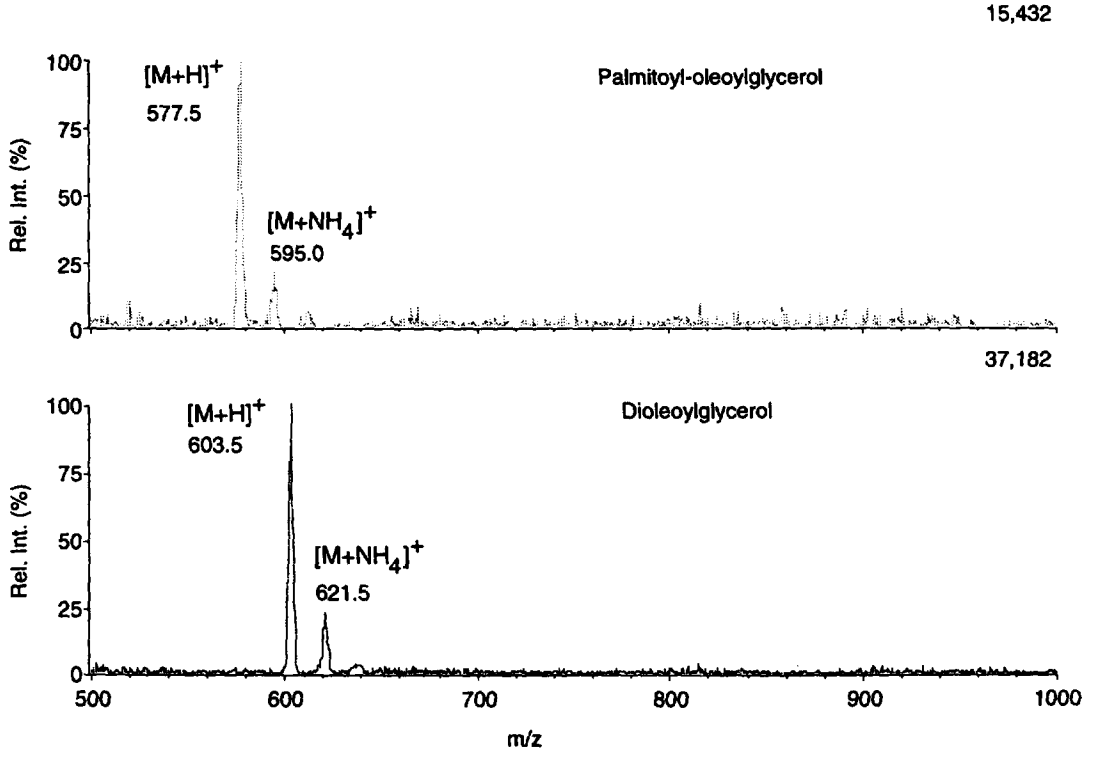

Fig. 4. APCI-mass spectra of the two diacylglycerols found in *Commiphora guillaumini*. (U_o = +50V).

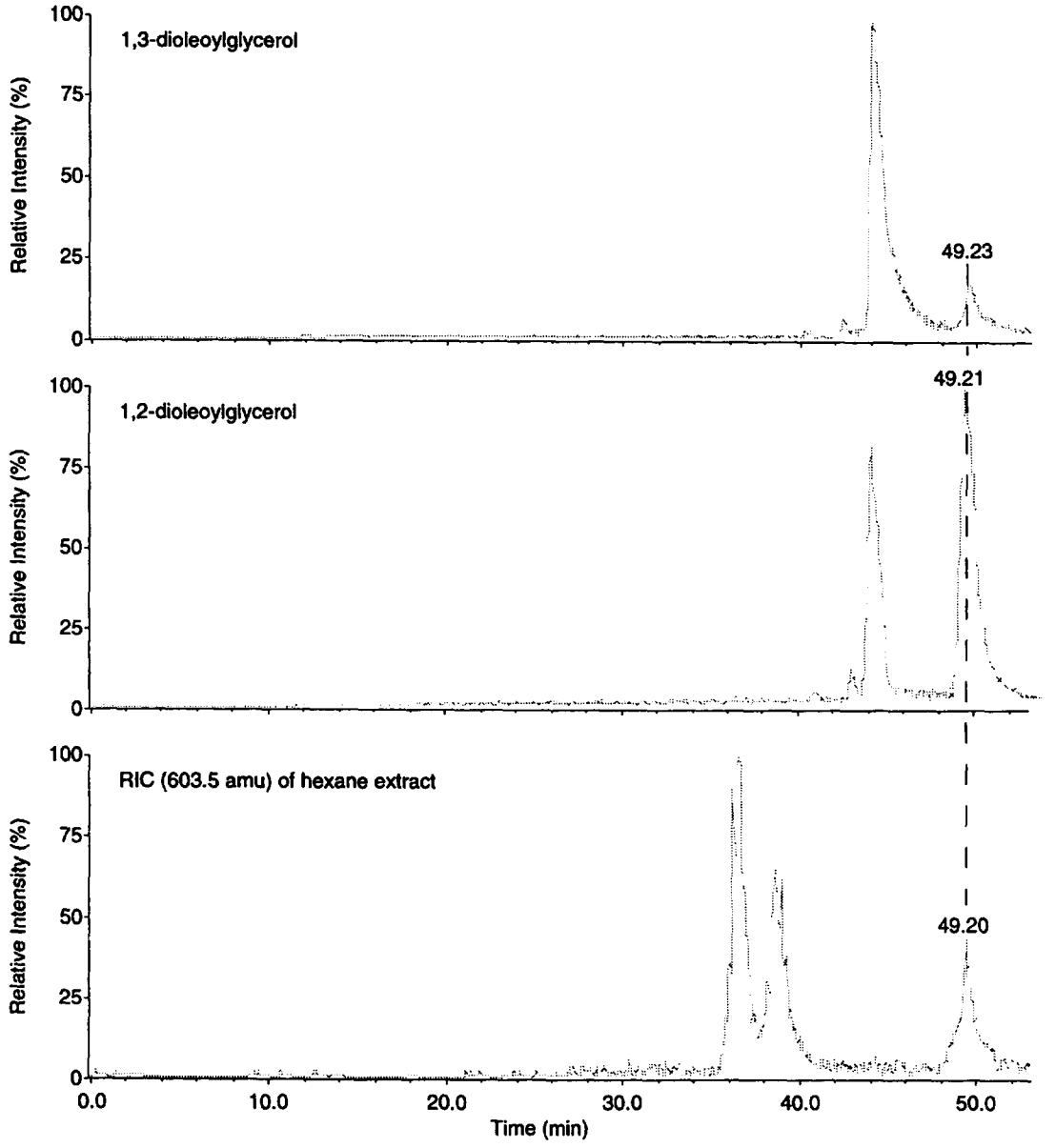

Fig. 5. Total ion current (TIC) chromatograms of 1,3-dioleoylglycerol standard (upper), 1,2-dioleoylglycerol standard (middle) and the reconstructed ion current (RIC) chromatogram (m/z 603.5) of the hexane extract (lower). Chromatographic conditions as in Fig. 2.

under these conditions in the positive mode thus provides not only the masses of the triacylglycerols with lowered signal intensities, but also the masses of the so formed di- and monoacylglycerols. Complementary information can be gained by skimmer fragmentation in the negative mode where the deprotonated fatty acids are obtained. The signal-to-noise ratio in the lower mass range is much better in the negative mode than in the positive mode.

Since the mass difference between a saturated and an unsaturated fatty acid is only 2 a.m.u., accurate mass measurements were required. After a full mass-range scan in the positive mode, the range of 820 to 920 a.m.u. was scanned with a step size of 0.1 a.m.u.

to determine the exact molecular masses of all triacylglycerols. In the following run, the orifice voltage was increased to 120 V to provoke fragmentation. The resulting diacylglycerols were also detected within a 100 a.m.u. window. For confirmation of the diacylglycerol assignment, another run in the negative mode with a mass window corresponding to the free fatty acids was performed. The reproducibility of the retention times was sufficient to allow a direct comparison between the three chromatograms (as seen from Fig. 3).

Each retention time listed in Table 1 represents a peak or in some cases a shoulder. The experimentally determined molecular mass of the intact triacylglycerol together with the masses of the diacylglycerols and of the fatty acids obtained as a result of skimmer fragmentation are shown. Since it is not possible to determine the positional isomers of the triacylglycerols by MS, the assigned structure represents one of the possible positional permutations.

3.3. Identification of 1,2-dioleoylglycerol

The high sensitivity of the system allowed the detection of even minor components such as the two diacylglycerols which also gave easily interpretable mass spectra as is shown in Fig. 4.

With mass spectrometry it is possible to identify the diacylglycerol and the constituent fatty acids but not the positional isomer. The presence of 1,2-dioleoylglycerol rather than 1,3-dioleoylglycerol was confirmed by comparison of retention times with standard compounds, monitoring the ions of 603.5 a.m.u. Ion chromatograms of these standard compounds and the hexane extract are shown in Fig. 5. The 1,2-dioleoylglycerol standard is seen to be contaminated with the 1,3 isomer. The agreement between the retention times of the dioleoylglycerol found in the chromatograms and the 1,2-dioleoylglycerol standard confirms its presence in the arils of seeds from *Commiphora guillaumini*.

4. Conclusions

The combination of SFC with APCI-MS proves a powerful method for the separation and identification of lipids. Even complex mixtures of chemically very similar compounds in widely differing amounts can be separated and determined in a relatively short time. Skimmer fragmentation of lipids provides mass spectra of equal or better quality than are obtained from tandem MS methods. Measurement in the negative mode provides fatty acid patterns for each lipid and completes the information.

The presence of the known ant attractant 1,2-dioleoylglycerol in arils of the seeds of *Commiphora guillaumini* was confirmed.

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References

[1] K. Albert, U. Braumann, L.-H. Tseng, G. Nicholson and E. Bayer, Anal. Chem., 66 (1994) 3042.
[2] R.D. Smith, W.D. Felix, J.C. Fjeldstedt and M.L. Lee, Anal. Chem., 54 (1982) 1883.
[3] J. Cousin and P.J. Arpino, J. Chromatogr., 398 (1987) 125.
[4] H.T. Kalinoski, H.R. Udseth and R.D. Smith, J. Chromatogr., 394 (1987) 3.
[5] R.D. Smith and H.R. Udseth, Anal. Chem., 59 (1987) 13.
[6] K. Matsumoto, S. Tsuge and Y. Hirata, Anal. Chem., 58 (1986) 3.
[7] J.B. Crowther and J.D. Henion, Anal. Chem., 57 (1985) 2711.
[8] E.C. Huang, J.D. Henion and T.R. Covey, J. Chromatogr., 511 (1990) 257.
[9] J.F. Anacleto, L. Ramaley, R.K. Boyd, S. Pleasance, M.A. Quilliam, P.G. Sim and F.M. Benoit, Rapid Commun. Mass Spectrom., 5 (1991) 149.
[10] L.N. Tyrefors, R.X. Moulder and K.E. Markides., Anal. Chem., 65 (1993) 2835.
[11] D. Thomas, P.G. Sim and F.M. Benoit, Rapid Commun. Mass Spectrom., 8 (1994) 105.
[12] K. Böhning-Gaese, B. Gaese and S.B. Rabemanantsoa, Ecotropica, submitted.
[13] A. Bresinsky, Bibliotheca Bot., 126 (1963) 1.
[14] D.L. Marshall, A.J. Beattie and W.E. Bollenbacher, J. Chem. Ecol., 5 (1979) 335.
[15] B.A. Skidmore and E.R. Heithaus, J. Chem. Ecol., 14 (1988) 2185.

[16] C.R. Brew, D.J. O'Dowd and I.D. Rae, Oecologia, 80 (1989) 490.
[17] M. Hans, D. Waidelich, T. Schöffmann and E. Bayer, Org. Mass Spectrom., 27 (1992) 995.
[18] K.L. Duffin, J.D. Henion and J.J. Shieh, Anal. Chem., 63 (1991) 1781.
[19] M.G. Ikonomou, A.T. Blades and P. Kebarle, Anal. Chem., 63 (1991) 1989.