Journal of Chromatography, 598 (1992) 219–226
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 039

Analysis of sterol esters from alga and yeast by high-performance liquid chromatography and capillary gas chromatography–mass spectrometry with chemical ionization

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(First received December 2nd, 1991; revised manuscript received January 22nd, 1992)

ABSTRACT

Sterol esters from the green alga *Chlorella kessleri* and the yeast *Saccharomyces cerevisiae* were analysed by tandem high-performance liquid chromatography (HPLC–HPLC). Non-polar lipids were separated by normal-phase HPLC into individual classes and the fraction of sterol esters was subsequently separated by reversed-phase HPLC into intact molecular species. Further separation and identification of the fractions after HPLC were effected by capillary gas chromatography–mass spectrometry (cGC–MS) with positive and/or negative chemical ionization. A technique consisting of direct injection of the sample after its passage through the detector into the on-column injector of the gas chromatograph was used to transfer the sample from HPLC–HPLC to cGC–MS. By means of this method it was possible to demonstrate more than 30 sterols in both the alga and the yeast, and more than 20 new sterol esters were detected.

INTRODUCTION

Sterol esters are widely distributed in nature and have been detected in fungi, algae, plants and animals [1], in both vertebrates and invertebrates. With a few exceptions [2] (diseases of the phytosterolaemia type), mammalian and human blood only contains esters of cholesterol with acids and analysis is not complicated.

The separation of cholesterol esters and of other esters on a capillary column has been described several times using either polar or non-polar stationary phases [2–7]. Gas chromatography–mass spectrometry (GC–MS) has also been used several times [6,8–10]. However, it is much more complicated to separate sterol esters, where in a complex mixture critical pairs can be produced owing to the chromatographic behaviour of the sterols and fatty acids (FAs). A frequently used method, hydrolysis of sterol esters with subsequent identification of sterols and fatty acids, does not yield adequate results, with the exception of cholesterol. Therefore, in all sources containing more than one sterol (more FA are always present) it is necessary to separate sterol esters in their intact form.

High-performance liquid chromatography (HPLC) has been used for the separation of cholesterol esters without any problems and with excellent results [11]. On the other hand, the analysis of plant sterol esters [4] is more complicated owing to the formation of critical pairs. Capillary GC (cGC) is more advantageous. It is commonly used, often in combination with mass spectrometry. Unfortunately, electron impact (EI) mass spectra of sterol esters do not yield significant M^+ (molecular ion) or fatty acyl ions [4,8–10]. For these reasons, chemical ionization, *e.g.*, with methane, isobutane and ammonia, has been used. Ammonia was found to be most advantageous [9], especially in the negative ion scanning mode [10]. In this mode ions $[M-H_2O]^+$,

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$[M-RCO_2]^+$ and $[RCO_2]^+$ can be determined from the mass spectra.

On the basis of the above reports, we decided to use tandem HPLC–HPLC and cGC–MS with either positive (PCI) or negative chemical ionization (NCI) with natural mixtures of sterol esters from a yeast and a green alga. To our knowledge, the analysis of sterol esters in yeasts has been described only once [4] and in algae not at all.

EXPERIMENTAL

Preparation of samples and standards

The isolation of total lipids from alga (*Chlorella kessleri*) [12] and yeast (*Saccharomyces cerevisiae*) [13] has already been described.

FAs, ergosterol and thionyl chloride were obtained from Sigma (St. Louis, MO, USA) and other chemicals from Merck (Darmstadt, Germany). The standards ergosteryl palmitate, stearate and oleate were prepared by reaction of the respective acyl chloride (0.15 mmol) (synthesized from thionyl chloride) with the sterol (0.1 mmol) in a solution containing 2 ml of benzene and a few drops of pyridine, as described [9].

The samples after reversed-phase (RP) HPLC were saponified by hydrolysis with 25% KOH in 50% methanol at 90°C for 30 min. The unsaponifiable residue (sterols) was extracted and analysed by GC–MS. After acidification, free fatty acids were extracted and derivatized to methyl esters by reaction with BF_3–methanol [12,13].

Isolation of sterol ester fraction

Total lipids (100 mg) were applied to a Silica-Cart C_{18} plastic cartridge system (*e.g.*, 1-ml tube) (Tessek, Prague, Czechoslovakia) and washed with 10 ml of cyclohexane–diethyl ether (98:2). The mixture of non-polar lipids was evaporated to one tenth of its volume, dissolved in cyclohexane (10% concentration) and used for HPLC–HPLC.

HPLC

Two liquid chromatographs were used: a semipreparative Gradient LC System G-I (Shimadzu, Kyoto, Japan) with two LC-6A pumps (0.5 ml/min), an SCL-6A system controller, an SPD ultraviolet detector (206 nm), an SIL-1A sample injector and a C-R3A data processor, with an SGX (spherical silica gel) preprative column (250 mm × 8 mm I.D.; 7 μm particles) (Tessek), and a Sigma 3B analytical (Perkin-Elmer, Norwalk, CT, USA) with an analytical column (250 mm × 4 mm I.D.) packed with SGX C_{18} with 5-μm particles (Tessek).

After injection of 100 μl of solution of total lipids (50 mg/ml) a gradient of propionitrile (PCN)–methyl *tert.*-butyl ether (MTBE) (convex gradient from 100:0 to 90:10 in 20 min) was applied. Through a six-way valve the eluate from the semipreparative column was transferred directly to the second system (Sigma) and elution was then effected with acetonitrile–tetrahydrofuran–methanol using a linear gradient from 40:50:2 to 70:28:2 in 10 min and to 70:20:10 in 8 min.

Individual compounds were identified by determining fatty acid methyl esters and free sterols (by GC–MS) after hydrolysis (see above) and by direct injection of the eluate (collected in a 5-μl microsyringe) in a volume of 2.5 μl.

GC–MS

All GC–MS separations were performed by using a Finnigan MAT (San Jose, CA, USA) Model 1020 B apparatus with EI or PCI and/or NCI.

Sterols. The instrument was equipped with a splitless capillary injector heated to 260°C and a fused-silica capillary column (10 m × 0.25 mm I.D. 0.12-μm film thickness) was coated with chemically bonded, non-polar CP-SIL 5 CB liquid stationary phase (Chrompack Middelburg, Netherlands). The temperature programme was 220°C for 1 min, then increased at 5°C/min to 290°C. The linear velocity of the carrier gas (hydrogen) was 50 cm/s and the ionization energy was 70 eV (EI mode).

Fatty acids (as methyl esters). A Supelcowax 10 fused-silica capillary column (Supelco, Bellefonte, PA, USA) (30 m × 0.25 mm I.D., 0.25-μm film thickness) was used. The temperatures were as follows: splitless injection, 240°C; column, 100°C for 1 min, then increased at 20°C/min to 160°C and at 2°C/min to 220°C. The carrier gas was hydrogen at 36 cm/s and the ionization energy was 70 eV (EI mode).

Sterol esters. The injection temperature (splitless injection) was 100°C and a (Supelcowax 10) fused-silica capillary column (15 m × 0.25 mm I.D., 0.25-μm film thickness) was used. The temperature programme was 100°C for 1 min, then increased at

20°C/min to 230°C and at 2°C/min to 280°C, which was maintained for 10 min. The carrier gas was hydrogen at a linear velocity of 120 cm/s. Ammonia (0.6 Torr) was used as the CI (PCI and/or NCI mode) reagent gas. The spectra were scanned within the range m/z 200–750.

RESULTS AND DISCUSSION

Fatty acids and sterols

The content of fatty acids and sterols in yeasts and algae has been studied many times [12–15], and our results are summarized in Table I. However, intact sterol esters have been investigated only rarely. To our knowledge, only one paper [4] was concerned with the analysis of molecular species of sterol esters in yeasts, *viz.*, in wild and mutant strains. Unfortunately, more than half of the peaks remained unidentified. Esters of fatty acids with ergosterol are major sterol esters.

High-performance liquid chromatography of sterol esters

Only two studies [5,8] were concerned with the chromatographic behaviour of molecular species of both cholesteryl esters and plant sterol esters. Billheimer *et al.* [5] reported the relative retention times (RRTs) of more then 30 sterol esters related to cholesteryl oleate. Because of diversity of both the sterols and acids, the authors derived rules that make it possible to predict, at least partially, chemical structures on the basis of RRTs. In another study [8] plant sterols were chromatographed and the formation of critical pairs was observed.

The use of two different columns during HPLC–HPLC analysis, without intermediate isolation (sample concentration and subsequent injection) has not been described previously for lipids. However, the application of HPLC–cGC was highly successful, with wax and sterol esters in particular [16].

A typical HPLC profile of intact sterol esters iso-

TABLE I

CONTENTS OF FATTY ACIDS AND STEROLS IN ALGA AND YEAST

Fatty acids			Sterols		
Acid[a]	Yeast (mol%)[c]	Alga (mol%)[c]	Sterol[a]	Yeast (mol%)[c]	Alga (mol%)[c]
Lauric	0[c]	2.2±0.2	Zymosterol	13.2±1.3	0
Myristic	2.7±0.2	10.8±1.4	Ergosterol	55.7±3.1	0
Pentadecanoic	0	4.7±0.4	Ergostatetraenol	15.4±0.9	0
Palmitic	25.8±1.5	31.2±2.6	5,7-Ergostadienol	6.5±0.1	0
Palmitoleic	18.7±1.1	7.8±0.9	Cholestadienol	4.3±0.2	0
Hexadecadienoic[b]	0	3.7±0.3	8-Ergostenol	2.3±0.1	0
Hexadecatrienoic[b]	0	3.1±0.2	8(14)-Ergostenol	1.5±0.1	3.1±0.2
Stearic	3.5±0.3	4.6±0.3	Methylergostenol	1.1±0.1	0
Oleic	29.7±2.4	19.8±1.7	Ergostadienol	0	2.8±0.3
Linoleic	19.6±0.8	7.6±0.4	Fungisterol	0	53.7±3.6
Linolenic	0	4.5±0.6	Chondrillasterol	0	9.2±1.2
			Schotenol	0	28.6±2.7
			Stigmastenol	0	2.6±0.2

[a] Trivial names; systematic names are as follows: lauric = dodecanoic; myristic = tetradecanoic; palmitic = hexadecanoic; palmitoleic = 9-hexadecenoic; stearic = octadecanoic; oleic = 9-octadecenoic; linoleic = 9,12-octadecadienoic; linolenic = 9,12,15-octadecatrienoic; zymosterol = 8,24-cholestadien-3β-ol; ergosterol = 5,7,22-ergostatrien-3β-ol; ergostatetraenol = 5,7,22,24(28)-ergostatetraen-3β-ol; 5,7-ergostadienol = 5,7-ergostadien-3β-ol; cholestadienol = 5,7-cholestadien-3β-ol; 8-ergostenol = 8-ergosten-3β-ol; 8(14)-ergostenol = 8(14)-ergosten-3β-ol; methylcholestenol = 4-methyl-8-cholesten-3β-ol; ergostadienol = 7,22-ergostadien-3β-ol; fungisterol = 7-ergosten-3β-ol; chondrillasterol = 24-ethyl-7,22-cholestadien-3β-ol; schotenol = 24-ethyl-7-cholesten-3β-ol; stigmastenol = 24-ethyl-8(14)-cholesten-3β-ol.

[b] Positions of double bonds are 7,10 and 7,10,13, respectively.

[c] Each value represents the means ± S.D. from five analyses.

lated from yeasts is shown in Fig. 1 and Table II. By using the HPLC–HPLC method is was possible to separate individual classes of non-polar lipids in one analysis and by subsequently using the reversed-phase mode it was possible to separate intact sterol esters into individual molecular species. However, in several instances mixtures (critical pairs were present) (see Fig. 1 and Tables II and III).

The occurrence of 37 molecular species of sterol esters from yeast is presented as percentages in Table II. It follows from Fig. 2 that sterol esters with four double bonds and an even number of carbon atoms (44 and 46) are the most frequent. It is also apparent that the strain used tends to preserve a certain membrane fluidity which influences the composition of molecular species characterized roughly by a Gaussian distribution. The combination of polyunsaturated fatty acids with more unsaturated sterols was not found.

In the green alga *Chlorella kessleri* a total of 32 intact sterol esters were identified, of which esters of schotenol with linolenic, linoleic and oleic acids were the most frequent. Fungisterol palmitate was also highly abundant. The representation of molecular species is characterized by a much higher scattering of fungisterol esters as compared with schotenol esters.

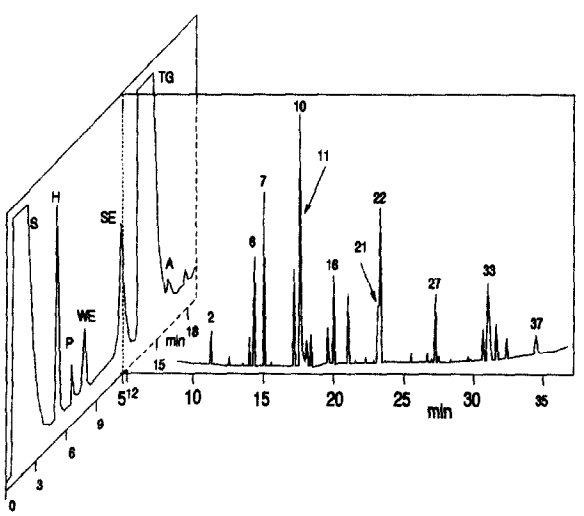

Fig. 1. Semipreparative HPLC of non-polar lipids. Left trace, normal-phase HPLC of non-polar lipids; right, RP-HPLC of sterol ester fraction. For experimental conditions, see text. Peaks: S = solvent; H = hydrocarbons; P = pigments; WE = wax esters; SE = sterol esters; TG = triglycerides; A = alcohols. Peaks 1–37 are molecular species of sterol esters (see Table II).

It follows from Tables II and III that the RRT is mainly affected by the acid moiety of the molecule but that the sterol moiety also plays a certain role (see also ref. 5). In conclusion, the results implicate several rules (see Table IV). It is of interest that individual contributions, *e.g.*, in C_{16} and C_{18} unsaturated acids, are identical. With an increase of the number of double bonds the RRT is always reduced by one quarter (the sterol moiety remains the same). On the other hand, in sterols the introduction of a second double bond (conjugated diene) results in a decrease in RRT by only one fifth (*e.g.*, 5 → 5,7 or 22 → 22,24 (28). We explain this phenomenon by a lower polarity of conjugated dienes with respect to methyl-interrupted double bonds in the fatty acid chain. However, small differences also occur in conjugated dienes, where the rigid homoannular dienes (5,7) have a higher polarity than the diene in the side-chain [22,24(28)]. Compared with the published data [5], we extended our values by further structural elements, *viz.*, the above-mentioned unsaturated acids and sterol dienes. On the basis of information about chromatographic behaviour, it is thus possible to determine at least a tentative chemical structure. However, for conclusive clarification of the structure of molecular species of sterol esters, this tentative structure must be confirmed by means of other methods.

Gas chromatography of sterol esters

Similarly to HPLC, there have been few reports of the separation and identification of intact sterol esters other than cholesterol esters by cGC.

In photosynthetic tissues (freshwater dinoflagellates [7] or celery [17]) sterol esters could be identified on non-polar capillary columns. Also in sediments sterol esters were analysed by cGC–MS with CI (methane) [6], although the shortcomings of a non-polar capillary column, *e.g.*, one chromatographic peak represents a complex mixture of sterol esters consisting of five acyl chains and four sterol moieties, were clearly demonstrated.

The separation of sterol esters on a polar column has been reported in only two papers [2,3]. In one [2], esters of plant sterols and of cholesterol with human serum fatty acids in patients suffering from phytosterolaemia were separated on a capillary col-

TABLE II

COMPOSITION OF STEROL ESTERS FROM YEAST

Peak No.[a]	Sterol ester	RRT[b,c]	Concentration (mol%)[d]
1	Ergostatetraenyl palmitoleate	0.762	0.1 ± 0.1
2	Ergosteryl linoleate	0.789	2.3 ± 0.2
3	Ergostatetraenyl oleate	0.833	0.7 ± 0.1
4	Cholestadienyl oleate	0.865	0.2 ± 0.1
5	Zymosteryl linoleate	0.880	1.5 ± 0.2
6	Ergostatetraenyl myristate	0.891	7.4 ± 0.3
7	Ergosteryl palmitoleate	0.914	12.5 ± 1.2
8	Ergostadienyl linoleate	0.934	0.3 ± 0.1
9	Ergostatetraenyl palmitate	0.988	6.9 ± 0.3
10	Ergosteryl oleate	1.000	18.3 ± 1.4
11	Cholestadienyl palmitoleate	1.002	1.3 ± 0.1
12	Zymosteryl palmitoleate	1.018	2.4 ± 0.1
13	Ergosteryl myristate	1.069	2.7 ± 0.2
14	Ergostadienyl palmitoleate	1.081	2.1 ± 0.1
15	Cholestadienyl oleate	1.095	2.5 ± 0.1
16	Ergostatetraenyl stearate	1.096	3.4 ± 0.3
17	Zymosteryl oleate	1.115	4.6 ± 0.3
18	Ergost-8 (14)-enyl linoleate	1.130	0.2 ± 0.1
19	Ergost-8-enyl linoleate	1.157	0.4 ± 0.1
20	Methylergostenyl linoleate	1.175	0.2 ± 0.1
21	Ergostadienyl oleate	1.183	3.4 ± 0.3
22	Ergosteryl palmitate	1.186	8.0 ± 1.2
23	Zymosteryl myristate	1.193	0.1 ± 0.1
24	Ergostadienyl myristate	1.266	0.6 ± 0.1
25	Cholestadienyl palmitate	1.300	0.7 ± 0.1
26	Ergoste-8 (14)-enyl palmitate	1.310	0.2 ± 0.1
27	Ergosteryl stearate	1.315	5.1 ± 0.4
28	Zymosteryl palmitate	1.323	0.4 ± 0.2
29	Methylergostenyl palmitoleate	1.360	0.2 ± 0.2
30	Ergost-8-enyl palmitoleate	1.400	0.3 ± 0.1
31	Ergostadienyl palmitate	1.404	0.1 ± 0.1
32	Ergoste-8(14)-enyl oleate	1.432	1.4 ± 0.1
33	Ergostadienyl stearate	1.441	5.1 ± 0.7
34	Zymosteryl stearate	1.465	0.3 ± 0.1
35	Ergost-8-enyl oleate	1.465	1.8 ± 0.2
36	Methylergostenyl oleate	1.488	1.3 ± 0.3
37	Ergostadienyl stearate	1.556	1.0 ± 0.1

[a] For peak numbers, see Fig. 1.
[b] Relative retention times from RP-HPLC; ergosterol oleate has a retention time of 17.25 min.
[c] S. D. was in the range 0.0001–0.0021.
[d] Each value represents the means ± S. D. from six analyses.

umn. In the other only cholesterol esters were separated.

Gas chromatography–mass spectrometry of sterol esters

The only method suitable for the identification of sterol esters after cGC is MS. During EI ionization the M^+ (molecular ion) is not recognizable and, therefore, either PCI or NCI is used. Wakeham and Frew [6] used PCI (methane) and individual sterol esters were identified on the basis of split ions ($[RCOOH_2]^+$, $[M-RCOO]^+$ and $[M-RCOO-H_2O]^+$). Unfortunately, M^+ could not be identified even with PCI (methane).

TABLE III

COMPOSITION OF STEROL ESTERS FROM ALGA

Sterol ester	RRT[a,b]	Concentration (mol%)[c]
Fungisteryl hexadecatrienoate	0.554	6.6 ± 0.2
Schotenyl hexadecatrienoate	0.598	2.9 ± 0.1
Fungisteryl linolenate	0.617	0.4 ± 0.1
Ergostadienyl palmitoleate	0.762	0.1 ± 0.1
Schotenyl linolenate	0.665	19.1 ± 2.3
Chondrillasteryl linoleate	0.723	1.3 ± 0.2
Ergostadienyl linoleate	0.723	0.4 ± 0.1
Fungisteryl hexadecadienoate	0.738	4.0 ± 0.2
Schotenyl hexadecadienoate	0.797	1.1 ± 0.1
Fungisteryl linoleate	0.822	1.3 ± 0.1
Schotenyl linoleate	0.887	13.9 ± 2.6
Ergostadienyl oleate	0.928	0.5 ± 0.2
Chondrillasteryl oleate	0.928	1.6 ± 0.1
Fungisteryl palmitoleate	0.947	2.8 ± 0.2
Schotenyl palmitoleate	1.022	1.0 ± 0.1
Ergostenyl oleate	1.044	0.4 ± 0.1
Fungisteryl pentadecanoate	1.052	4.5 ± 0.3
Fungisteryl oleate	1.054	0.5 ± 0.1
Schotenyl laurate	1.083	0.6 ± 0.1
Fungisteryl myristate	1.110	6.8 ± 0.4
Stigmastenyl oleate	1.128	0.5 ± 0.1
Schotenyl pentadecanoate	1.134	0.4 ± 0.1
Schotenyl oleate	1.139	15.0 ± 2.7
Chondrillasteryl palmitate	1.168	2.7 ± 0.3
Stigmastenyl myristate	1.187	0.3 ± 0.2
Schotenyl myristate	1.198	1.1 ± 0.1
Ergostenyl palmitate	1.217	0.5 ± 0.1
Fungisteryl palmitate	1.237	15.5 ± 3.4
Stigmastenyl palmitate	1.325	0.8 ± 0.1
Schotenyl palmitate	1.328	3.3 ± 0.3
Fungisteryl stearate	1.375	1.5 ± 0.2
Schotenyl stearate	1.479	3.3 ± 0.5

[a] See Table II.
[b] S.D. was in the range 0.0001–0.0021.
[c] Each value represents the mean ± S.D. from five analyses.

In another study [9] different ionization gases and different ionization temperatures were compared and ammonia was found to be the gas of choice. When using PCI with ammonia, pseudo-molecular $[M + NH_4]^+$ ions could be detected. Therefore, it is advantageous to use NCI [10] with ammonia, during which three major ions, *viz.*, $[M - RCO_2H - H]^-$, $[RCOO]^-$ and $[RCO_2 - H_2O]^-$, are produced.

For GC–MS analysis the peaks were always collected in a microsyringe at the elution maximum from the reversed-phase column. Table V presents retention times and important ions of the identified molecular species of sterol esters. It was found that on the polar capillary column with Supelcowax 10 it is possible to separate even critical pairs that are not separated by RP-HPLC. This is due primarily to different interactions [*e.g.*, hydrogen bonds between the stationary phase (polyethylene glycol) and polar groups of the sterol ester]. From the chromatographic data for all the sterols (not shown), it was possible, similarly to RP-HPLC, to derive rules of chromatographic behaviour even for cGC on a polar column (Table IV). Mass spectra of sterol esters were measured in both the positive and negative modes. PCI was selected according to ref. 9. As shown in Table V, pseudo-molecular ions were always identified, roughly with an intensity of 40–70% of the base peak $[M + NH_4 - RCO_2H]$. In the NCI mode three major ions were detected (Table V and Fig. 3) [8,9].

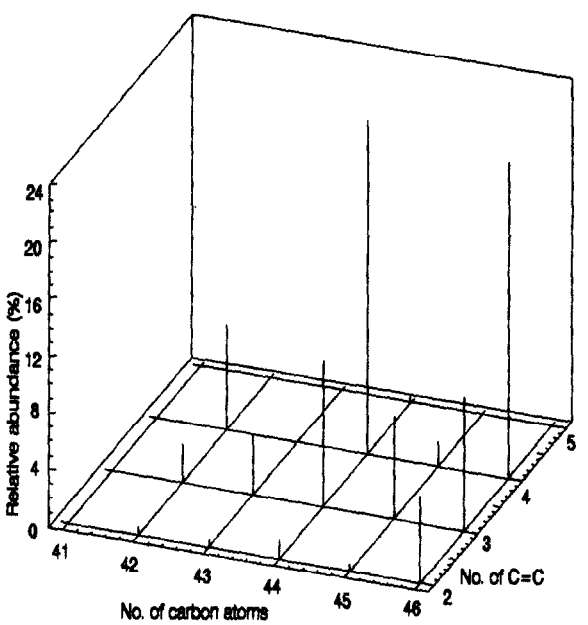

Fig. 2. Relationship between number of double bonds, total carbon atoms and relative abundance of intact steryl esters from yeast. The data were calculated from Table II.

TABLE IV

CONTRIBUTION OF UNSATURATION IN THE FATTY ACID AND STEROL AND OF ADDITIONAL METHYLENE GROUP IN THE FATTY ACID OR STEROL ON THE RETENTION TIMES OF STERYL ESTERS BY MEANS RP-HPLC AND CGC

No. of C=C	Fatty acid[a]	
	RP-HPLC	cGC
0	1.00	1.00
1	0.77±0.02	1.07±0.02
2	0.60±0.01	1.18±0.03
3	0.43±0.01	1.31±0.05

No. of C=C	Position of C=C	Sterol	
		RP-HPLC	cGC
0	–	1.00	1.00
1	5	0.84±0.02	1.08±0.01
1	8	0.84±0.01	1.06±0.02
1	8(14)	0.81±0.01	1.07±0.01
1	22	0.83±0.02	1.11±0.01
2	5,7	0.67±0.03	1.34±0.00
2	22,24(28)	0.70±0.01	1.27±0.00

Difference in CH_2	Fatty acid		Sterol	
	RP-HPLC	cGC	RP-HPLC	cGC
0	1.00	1.00	1.00	1.00
1	1.15±0.03	1.09	1.08±0.03	1.06

[a] *cis*, Methylene interrupted.

TABLE V

GC–MS OF STEROL ESTERS AFTER RP-HPLC (FROM 16 TO 19 min) (FIG. 1) ON POLAR CAPILLARY COLUMN

Sterol ester	*m/z*				
	RRT[a]	$[M+NH_4]^{+b}$	$[M-RCO_2H_2]^{-c}$	$[RCO_2]^{-c}$	$[RCO_2-18]^{-c}$
Ergosteryl myristate	0.874	624	377	227	209
Zymosteryl palmitoleate	0.895	638	365	253	235
Cholestadienyl palmitoleate	0.902	638	365	253	235
Ergostadienyl palmitoleate	0.930	652	379	253	235
Ergostatetraenyl palmitate	0.943	650	375	255	237
Ergosteryl oleate	1.000	678	377	281	263

[a] Relative to ergosteryl oleate (retention time 12.48 min).
[b] PCI-MS.
[c] NCI-MS.

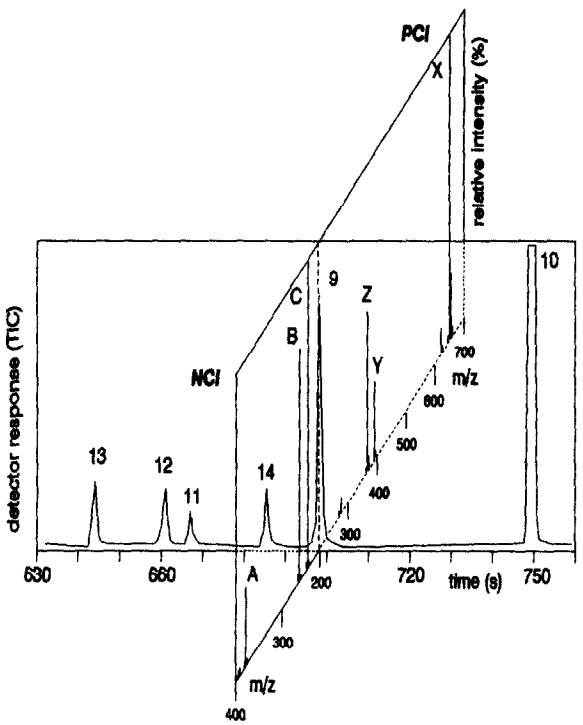

Fig. 3. cGC–MS with PCI and NCI. Trace from left to right, total ion current of partial chromatogram. Front mass spectrum, NCI; rear mass spectrum, PCI. Numbers represent molecular species in Table II. Letters are peaks of the following structures (relative abundance to base peak, in %): A = $[M-RCOOH]^-$ (25); B = $[RCOO]^-$ (80); C = $[RCOO-H_2O]^-$ (100); X = $[M+NH_4]^+$ (100); Y = $[M+NH_4-RCOOH]^+$ (25); Z = $[M+H-RCOOH]^+$ (56).

CONCLUSIONS

By using HPLC–HPLC and cGC–MS with PCI and/or NCI it was possible to identify 32 and 37 sterol esters in green alga and yeast, respectively.

The rules for RP-HPLC and cGC of sterol esters were derived by a combination of HPLC–HPLC and cGC–MS (with PCI and/or NCI). For the first time sterol esters in green algae were demonstrated and the spectrum of known sterol esters in the yeasts was greatly extended. By means of HPLC–HPLC it is possible to identify at least tentatively molecular species of sterol esters; occassional critical pairs can then be further separated and their structure can be fully verified by cGC–MS (with PCI and/or NCI). In a few hours it is thus possible to identify completely even complex mixtures such as sterol esters from microorganisms.

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