Journal of Chromatography, 441 (1988) 31–44
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1277

CHARACTERIZATION OF A LIPID-RICH FRACTION SYNTHESIZED BY *STREPTOMYCES AVERMITILIS*

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SUMMARY

Isolation of the macrocyclic lactone parasiticide avermectin and other closely related natural products produced by *Streptomyces avermitilis* also yields a lipid-rich fraction. The latter has been characterized by techniques based on gas–liquid chromatography (GLC) and mass spectrometry (MS). Initial examination of the lipid-rich fraction by direct probe electron-impact (EI) MS and packed-column GLC showed that it consists primarily of a mixture of triglycerides possessing C_{14}–C_{17} acyl groups. Further examination of this fraction by capillary column GLC–MS demonstrated that it contains low levels of C_{15}–C_{17} free fatty acids, squalene and diglycerides and, as the major components, at least ten mixed acyl triglycerides (total number of acyl carbon atoms ranging from 43 to 50). Prominent among the triglycerides were a C_{15}–C_{15}–C_{16} species, a C_{15}–C_{16}–C_{16} species and a C_{15}–C_{16}–C_{17} species. Capillary-column GLC and GLC–MS of the fatty acid methyl esters resulting from transesterification demonstrated that the major triglyceride acyl groups are *anteiso*-C_{15} (12-methyltetradecanoyl), *iso*-C_{16} (14-methylpentadecanoyl), *n*-C_{16} (hexadecanoyl) and *anteiso*-C_{17} (14-methylhexadecanoyl). Lower levels of the methyl esters of the following fatty acids were observed: *iso*-C_{14} (12-methyltridecanoic), *n*-C_{14} (tetradecanoic), *iso*-C_{15} (13-methyltetradecanoic), *n*-C_{15} (pentadecanoic), *iso*-C_{17} (15-methylhexadecanoic) and *n*-C_{17} (heptadecanoic). Little evidence was seen for either unsaturated acyl groups or acyl groups of less than 13 or more than 18 carbon atoms. Desorption chemical ionization MS (ammonia reagent gas) analysis confirmed the nature of the lipid-rich fraction, and is an attractive one-step approach for determining the molecular weights and distribution of triglycerides in a mixture.

INTRODUCTION

The avermectins, a family of macrocyclic lactone disaccharides, are produced by *Streptomyces avermitilis*[1–3]. These natural products, especially avermectin B_1, and also its 22,23-dihydro analogue ivermectin (generated from avermectin by chemical reduction[4]), are active against a wide variety of parasites[5–7]. A lipid-rich fraction is

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formed during fermentation in addition to the avermectins. As demonstrated in the Hornings' laboratory[8,9], profiling of complex mixtures (*e.g.*, natural products, such as human urinary steroids[10] and urinary metabolites of aromatic compounds[11]) by capillary column gas–liquid chromatography (GLC) and GLC–mass spectrometry (MS) is a valuable approach to the examination of such samples and characterization of individual components. This paper reports the examination and characterization of the lipid-rich fraction from *S. avermitilis* by a variety of GLC and MS techniques.

EXPERIMENTAL

Materials

HPLC grade methanol, acetone, toluene, hexane and isooctane were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.); dipalmitin, trimyristin, tripalmitin, and tristearin from Sigma (St. Louis, MO, U.S.A.); triolein was obtained from Supelco (Bellefonte, PA, U.S.A.). The methyl esters of 12-methyltridecanoic acid (*iso*-C_{14}), myristic acid (*n*-C_{14}), 12-methyltetradecanoic acid (*anteiso*-C_{15}), pentadecanoic acid (*n*-C_{15}), 14-methylpentadecanoic acid (*iso*-C_{16}), palmitic acid (*n*-C_{16}), and 14-methylhexadecanoic acid (*anteiso*-C_{17}) were obtained as a reference mixture from Supelco. Pentadecanoic, palmitic, heptadecanoic, stearic, and oleic acids were purchased from Sigma, as was squalene. Formation of methyl esters of individual fatty acids was effected via methylation, using diazomethane generated from Diazald (Aldrich, Milwaukee, WI, U.S.A.). Transesterification was carried out overnight at 100°C in methanol (sulfuric acid); the methyl esters were extracted with acetone–isooctane (1:1).

Preparation of the lipid-rich fraction

Whole broth from the *S. avermitilis* fermentation was acidified (pH 2.5) and extracted with toluene at 80°C. The solvent layer, containing avermectins and lipids, was evaporated to give an approximately 1:1 mixture of toluene and oil. Addition of hexane and ethanol to this mixture at 50°C effected precipitation of the avermectins, leaving the oily triglyceride-containing lipid-rich fraction.

Instrumental analysis

GLC. Packed-column GLC of aliquots of the lipid-rich fraction and reference standards was carried out using a Varian (Walnut Creek, CA, U.S.A.) Model 6000 instrument, equipped with a 40 cm × 4 mm I.D. stainless-steel column, packed with 1% Dexsil 300, 80–100 mesh (Supelco) and a flame-ionization detector (350°C). Samples were flash injected onto silanized glass wool at 350°C. The carrier gas was helium at a flow-rate of 30 ml/min. The column was temperature-programmed from 100 to 200°C at 8°C/min, then from 200 to 340°C at 10°C/min. Capillary column GLC was performed on a Model 6000 instrument equipped with a Model 1075 split/splitless injector (Varian) and a flame-ionization detector (350°C). Aliquots of the lipid-rich fraction and reference standards were chromatographed on a 12 m × 0.32 mm I.D. DB-5, 0.5-μm film thickness column (J & W Scientific, Rancho Cordova, CA, U.S.A.). Split (100:1) injections were carried out at 350°C, using helium as the carrier gas (45 cm/s linear velocity). The initial column temperature was 150°C, and after 2 min the temperature was increased to 250°C at 10°C/min, and then from 250 to 350°C at 5°C/min. Capillary column analysis of methyl esters was carried out with a 50 m

× 0.22-μm I.D. CPS:188, 0.2-μm film-thickness column (Chrompack, Bridgewater, NJ, U.S.A.). Split injections were performed at 275°C with helium carrier gas (40 cm/s linear velocity). The column was temperature-programmed from 100 to 200°C at 5°C/min. The flame detector was operated at 300°C and helium make-up was adjusted to 30 ml/min.

GLC–MS. Capillary column GLC–MS (EI) of aliquots of the lipid-rich fraction and reference standards was performed on a Finnigan-MAT 212 (San Jose, CA, U.S.A.), using a Varian Model 3700 gas chromatograph with an open-split MS interface. The column was 12 m × 0.32 mm I.D. DB-5, 0.5 μm film thickness. Spectra were recorded at 90 eV ionization voltage, and scans (2 s) were recorded from 70 to 900 atomic mass units (a.m.u). Split (10:1) injections were carried out, using helium as the carrier gas (30 cm/s linear velocity). The initial column temperature was 150°C, and after 2 min the temperature was programmed to 350°C at 10°C/min. Capillary column GLC–MS analysis of methyl esters was carried out using a 30 m × 0.32 mm I.D. DB-1, 0.25 μm film thickness column (J & W Scientific). The linear velocity of the helium carrier gas was 30 cm/s; the initial column temperature was 100°C, and after 2 min the temperature was programmed to 200°C at 5°C/min. Scans from 70 to 700 a.m.u. were recorded every 2 s.

Direct probe MS (EI). Analysis of aliquots of the lipid-rich fraction and reference standards was carried out with a LKB Model 9000 (Stockholm) instrument at 70 eV ionization voltage and scanning (10 s) from 50 to 900 a.m.u.

Desorption chemical ionization MS. Analysis of aliquots of the lipid-rich fraction and reference standards was carried out with a Delsi-Nermag Model R10-10C (Houston, TX, U.S.A.) instrument with ammonia reagent gas. Operating conditions included 70 eV ionization voltage, 150°C source temperature, ammonia reagent gas pressure 0.2 Torr, probe gradient 50 to 550 mA at 20 mA/s, and scanning (0.5 s) from 80 to 1000 a.m.u.

RESULTS AND DISCUSSION

Direct-probe EI-MS of the lipid-rich fraction yielded a series of spectra. Those from the early scans were of low total ionization intensity, and the most intense ions of higher mass were found at *m/z* 242, 256, 270 and 410. The last-mentioned ion (accompanied by a signal of *m/z* 395) increased with scan number as the other above-mentioned signals rapidly diminished; however, as the succeeding spectra increased in total ionization, the intensity of the *m/z* 395 and 410 ions also diminished rapidly. The intense spectra (see Fig. 1) were dominated by ions recognizable as arising from triglycerides (see Table I)[12]. For reference purposes, the pertinent fragment ions formed from tripalmitin are included in the table, and it is clear that a mixture of triglycerides, possessing a variety of saturated acyl groups (C_{14}–C_{17}), is present in the fraction. The low-intensity signals, observed in the region of *m/z* 800 in Fig. 1, indicate the presence of triglycerides with molecular weights up to 792 and 806.

Because of the complexity of the lipid-rich fraction, we thought that a profiling approach would facilitate analysis. GLC–MS profiling was employed by Rezanka *et al.*[13] to examine the triglycerides of a green alga, and the triglycerides of peanut oil were characterized recently by Singleton and Pattee[14] using GLC and direct-probe EI-MS. High-temperature packed-column GLC of the lipid-rich fraction showed a

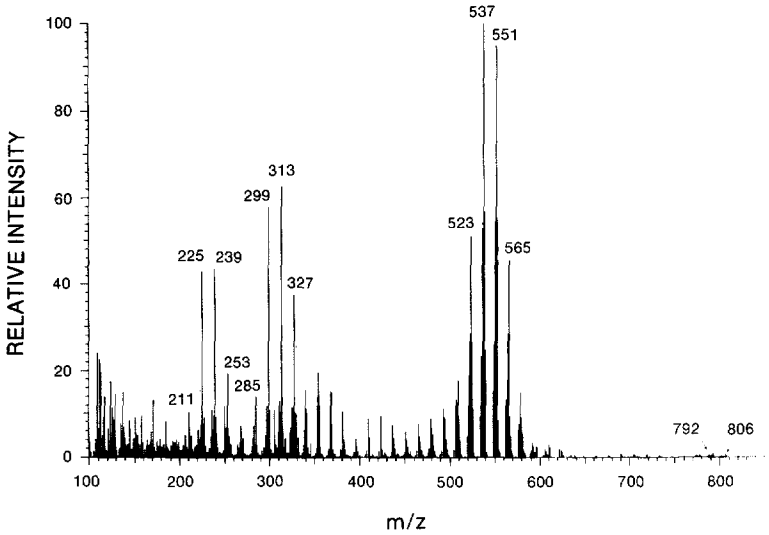

Fig. 1. Typical intense spectrum obtained by analysis of the lipid-rich fraction (*S. avermitilis*) via EI (70 eV) MS. Note the signals of *m/z* 211, 225, 239 and 253 (acylium ions, RCO), 285, 299, 313 and 327 (RCO + 74) and 523, 537, 551 and 565 (M − RCOO), characteristic of triglycerides possessing saturated acyl groups. Instrument operating conditions given in Experimental.

cluster of eight peaks with a retention time spread from somewhat less than that of trimyristin to approximately that of tripalmitin. The fraction thus appeared to contain a mixture of triglycerides ranging from approximately 45 to 51 carbon atoms. The triglycerides account for approximately 90% of the fraction by weight.

In order to obtain MS data on individual components of the lipid-rich fraction, aliquots were examined by capillary column GLC and GLC–MS, employing a short (12-m) DB-5 column; a representative chromatogram is presented in Fig. 2. A cluster of peaks was observed early in the chromatogram. The apparent molecular ions for components 1–4 were *m/z* 242, 256, 256 and 270, respectively, suggesting that these compounds are C_{15}, C_{16} and C_{17} free fatty acids. Each spectrum contained an ion

TABLE I

CHARACTERISTIC ELECTRON IMPACT IONIZATION MASS SPECTROMETRIC FRAGMENTATION IONS OF TRIGLYCERIDES

Triglyceride sample	*m/z*		
	$[RCO]^+$	$[RCO + 74^*]^+$	$[M-RCOO]^+$
Tripalmitin	239**	313***	551
Lipid fraction	211, 225, 239, 253	285, 299, 313, 327	523, 537, 551, 565

* From glycerol moiety.
** $[CH_3(CH_2)_{14}CO]^+$ (*i.e.*, C_{16} acyl).
*** $[CH_3(CH_2)_{14}CO + C_3H_6O_2]^+$.

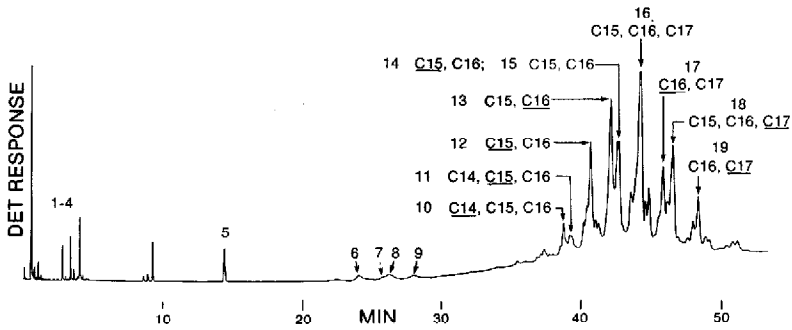

Fig. 2. Chromatogram obtained by temperature-programmed capillary column GLC of the lipid-rich fraction from *S. avermitilis*. Instrument operating conditions given in Experimental. Components 1–4 identified as C_{15}–C_{17} free fatty acids, component 5 as squalene, components 6–9 as diglycerides, and components comprising the complex cluster of peaks from 10–19 as triglycerides (with an indication of the acyl groups present in the latter).

of m/z 73, $[CH_2CH_2COOH]^+$, characteristic of fatty acids; no M − 31 or m/z 74 and 87 ions were observed in the spectra, and thus the compounds are not methyl esters of C_{14}, C_{15} and C_{16} fatty acids. The mass spectrum of component 1 is presented in Fig. 3, and is different from that of authentic *n*-pentadecanoic acid [the spectrum of the former possesses a much more intense ion of m/z 185 (M − 57) than does that of the straight chain acid]. As authentic methyl 12-methyltetradecanoate also yields a mass spectrum possessing an intense M − 57 ion, by analogy component 1 is assigned the structure 12-methyltetradecanoic acid (*anteiso*-C_{15}).

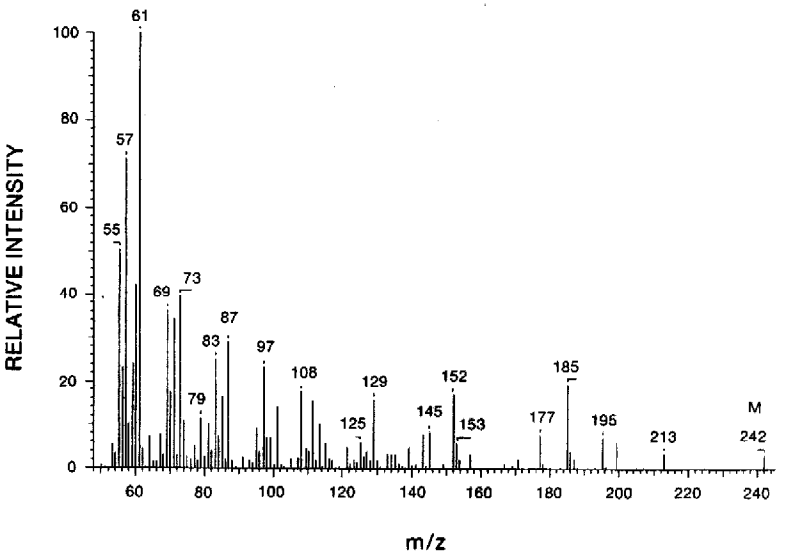

Fig. 3. EI (70 eV) mass spectrum of component 1 (Fig. 2) as obtained by GLC–MS. Instrument operating conditions given in Experimental.

TABLE II

CHARACTERISTIC ELECTRON IMPACT IONIZATION MASS SPECTROMETRIC FRAGMENTATION IONS OF DIGLYCERIDES

Diglyceride sample	*m/z*		
	[RCO]$^+$	*[RCO + 74*]*$^+$	*[M−18]*$^+$
Dipalmitin	239**	313***	550
No. 6	225, 239	299, 313	536
No. 7	239	313	550
No. 8	225, 253	299, 327	550
No. 9	239, 253	313, 327	564

* From glycerol moiety.
** $[CH_3(CH_2)_{14}CO]^+$ (*i.e.*, C_{16} acyl).
*** $[CH_3(CH_2)_{14}CO+C_3H_6O_2]^+$.

Another cluster of four components was eluted following the squalene peak; the MS data for these four compounds are presented in Table II, as are data for dipalmitin. The latter possesses a molecular weight of 568, but under our EI-MS conditions, the ion of highest *m/z* value observed is M − 18, *m/z* 550. The most intense signals in the spectrum are those of *m/z* 239 and of this ion plus 74 (*m/z* 313). It is evident from the data in the table that each of the components 6–9 is a diglyceride. Three of these are mixed acyl diglycerides, and only component 7 possesses two identical acyl groups. Whether these are 1,2- or 1,3-diglycerides is not known, as these isomeric species yield equivalent mass spectra[12].

The chromatogram (Fig. 2) is dominated by numerous peaks, arising from components eluted in the triglyceride region. MS data indicate that each triglyceride possesses a variety of acyl groups (there is no guarantee, of course, that these GLC components are homogeneous). A consistent upward shift in the acyl group chain

TABLE III

CHARACTERISTIC ELECTRON IMPACT IONIZATION MASS SPECTROMETRIC IONS ARISING FROM TRIGLYCERIDE COMPONENT 13

Ion	*m/z*	*Intensity (%)*
$[RCO]^+$	211	12
	225	32
	239	41
	253	5
$[RCO+74]^+$	285	8
	299	36
	313	60
	327	19
$[M-RCOO]^+$	523	29
	537	100
	551	88
	565	15
$[M-18]^+$	774	1

lengths of the triglycerides is noted with increasing retention time of the triglycerides (see Fig. 2). Four of the triglycerides exhibited M − 18 ions, making possible the assignment of molecular weights for these components (12–15). Component 12, with an M − 18 ion of *m/z* 760, possesses a molecular weight of 778; components 13–15, on the other hand, exhibit M − 18 ions of *m/z* 774 (and hence, molecular weights of 792). Tripalmitin, with a molecular weight of 806, contains three identical acyl groups with a total of 48 acyl group carbon atoms. By analogy, triglycerides 12 and 13–15 must possess 46 and 47 acyl group carbon atoms, respectively. The mass spectrum of triglyceride 12 possesses an ion of *m/z* 225 (C_{15} acyl) of approximately 2-fold greater intensity than that of *m/z* 239 (C_{16} acyl). The ion of *m/z* 299 dominates the $[RCO + 74]^+$ region of the spectrum [the ion of *m/z* 299 (225 + 74) is approximately twice as intense as the ion of *m/z* 313 (239 + 74)] giving further evidence for the preeminence of the C_{15} acyl group in this triglyceride. Based on these data, component 12 is a C_{15}–C_{15}–C_{16} triglyceride. However, in the spectrum of component 13 (see Table III), the ion of *m/z* 239 is significantly more intense than that of *m/z* 225, and the ion of *m/z* 313 is of greater intensity than that of *m/z* 299. Component 13 is thus probably a C_{15}–C_{16}–C_{16} triglyceride. The spectrum of this component contains $[M - RCOO]^+$ ions of *m/z* 537 and 551, arising from losses involving pentadecanoyl and hexadecanoyl groups. The intense $[M - RCOO]^+$ ions of *m/z* 523 and 537 in the spectrum of component 12 confirm that in this triglyceride the acyl groups are pentadecanoyl and hexadecanoyl.

For component 19, acyl ions of *m/z* 239 and 253 are observed, the latter of greater intensity; the presence of these C_{16} and C_{17} fragments is mirrored by the observed $[RCO + 74]^+$ ions of *m/z* 313 and 327 (of greater intensity). Component 19

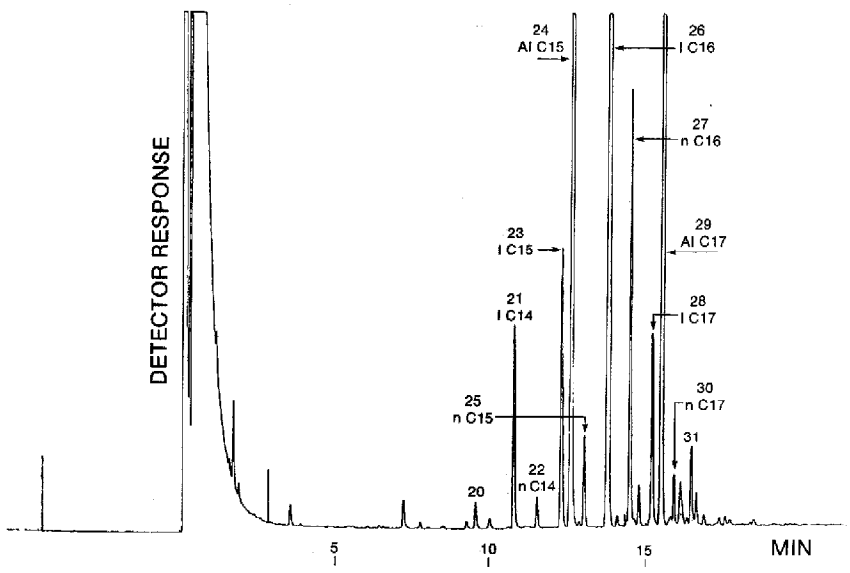

Fig. 4. Chromatogram obtained by temperature-programmed capillary column GLC of the fatty acid methyl esters, arising from the lipid-rich fraction following transesterification. The components are identified as indicated. Instrument operating conditions given in Experimental.

is evidently a C_{16}–C_{17}–C_{17} triglyceride, with a molecular weight of 834. Although no M − 18 ion is observed for this triglyceride, the signals at *m/z* 565 and 579 can be assumed to arise from $[M - RCOO]^+$ ions. Simple mathematical manipulation of the mass values, *i.e.*, M = 565 + 255, 565 + 269, 579 + 255, or 579 + 269, leads to molecular weights of 820, 834 (twice) and 848. The middle value agrees with that predicted on the basis of the *m/z* values of the acylium ions, *i.e.*, two C_{17} and one C_{16} acyl groups (sum of 50 acyl carbon atoms). This approach is similar to that used recently by Evershed and Goad[15] in their work on the GLC–MS of intact steryl esters found in natural sources. No molecular ions are observed for these compounds, but the structures of the esters can be deduced from the *m/z* values for fragment ions characteristic of the sterol and fatty acid. Of course, a steryl ester can possess only one fatty acid moiety, whereas the situation is three times more complex for triglycerides.

To generate additional information concerning the nature of the triglyceride acyl groups, an aliquot of the lipid-rich fraction was subjected to transesterification to form a mixture of fatty acid methyl esters. Temperature-programmed GLC analysis of the resulting methyl esters on a 50-m CPS:188 column gave the chromatogram shown in Fig. 4. On the basis of retention time comparisons with reference standard fatty acid methyl esters the components designated 21, 22, 24–27 and 29 were tentatively identified as the methyl esters of the following fatty acids: *iso*-C_{14} (12-methyltridecanoic acid; 21), *n*-tetradecanoic (myristic) acid (22), *anteiso*-C_{15} (12-methyltetradecanoic acid; 24), *n*-pentadecanoic acid (25), *iso*-C_{16} (14-methylpentadecanoic acid; 26), *n*-hexadecanoic (palmitic) acid (27) and *anteiso*-C_{17} (14-methylhexadecanoic acid; 29). The components designated 23, 28 and 30 exhibited retention time behavior (log retention time *vs.* carbon number plots) consistent with their being the methyl esters of *iso*-C_{15}, *iso*-C_{17} and *n*-heptadecanoic acids, respectively. No evidence was found for the presence in this sample of more than trace amounts of unsaturated fatty acid methyl esters.

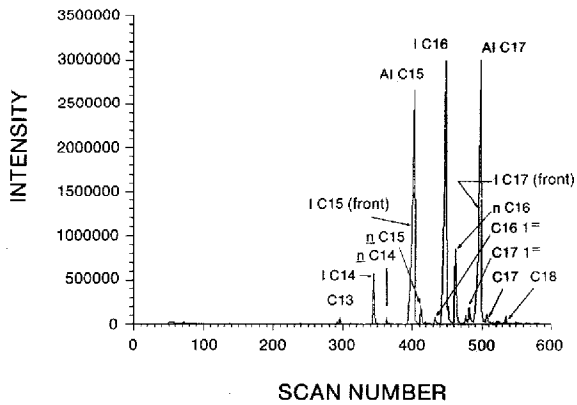

Fig. 5. Chromatogram obtained by temperature-programmed capillary column GLC–MS of the fatty acid methyl esters, arising from the lipid-rich fraction following transesterification (same sample as examined in Fig. 4). The components are identified as indicated. Instrument operating conditions are given in Experimental.

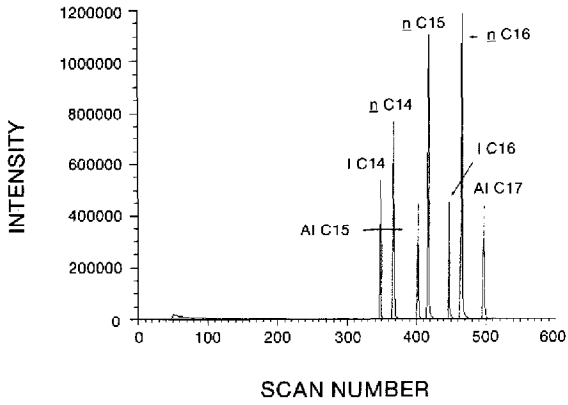

Fig. 6. Chromatogram obtained by temperature-programmed capillary column GLC–MS of reference mixture of fatty acid methyl esters. Components are identified as indicated. Instrument operating conditions same as for Fig. 5.

As dependence solely on retention times for identification purposes can readily lead to misassignment of structure, the mixture of fatty acid methyl esters was examined by capillary GLC–MS using a 30-m DB1 column (Fig. 5). The proposed structures for components 21, 22, 24–27 and 29 were confirmed, as their mass spectra and retention times matched those of the reference standards (compare Figs. 5 and 6). No analogous peak was observed (see Fig. 5) for component 23 noted in Fig. 4. Further, the mass spectrum for the compound eluted just prior (scan number 482) to the methyl ester of *anteiso*-C_{17} (Fig. 5) was not that of a saturated C_{17} fatty acid

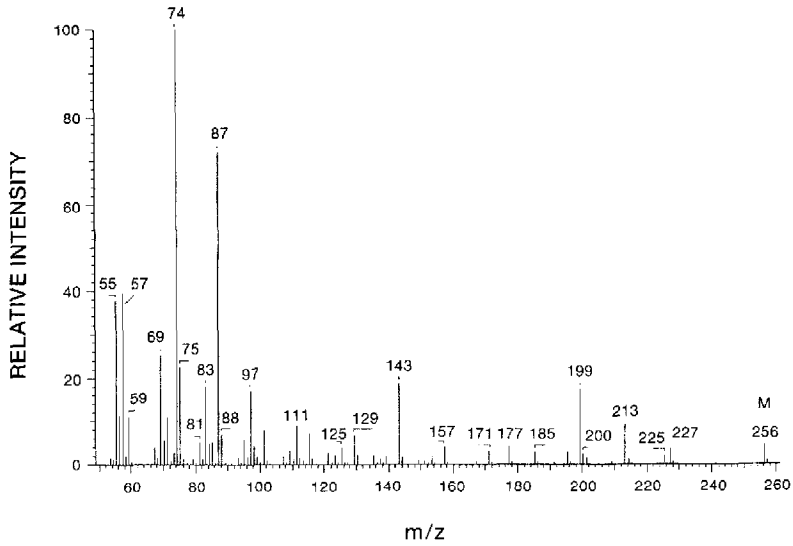

Fig. 7. EI (70 eV) mass spectrum taken at the mid point (scan 403) of the GLC component peak centered near scan number 400 and identified in Fig. 5 as the methyl ester of 12-methyltetradecanoic acid (AI C15). Instrument operating conditions given in Experimental.

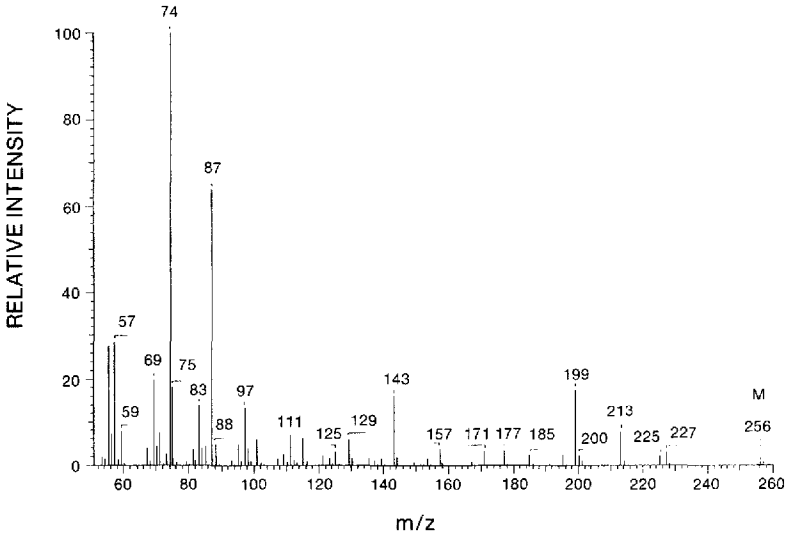

Fig. 8. EI (70 eV) mass spectrum of authentic methyl 12-methyltetradecanoate (AI C15), as obtained by GLC–MS. Instrument operating conditions same as for Fig. 7.

methyl ester, but rather that of a monounsaturated C_{17} fatty acid methyl ester. The mass spectrum of the component eluted at scan number 432 (just prior to methyl 14-methylpentadecanoate) indicates that this compound is the methyl ester of a C_{16} monounsaturated fatty acid. Thus, GLC–MS with the 30-m column possessed decreased resolving power (compared to GLC with the 50-m column) such that com-

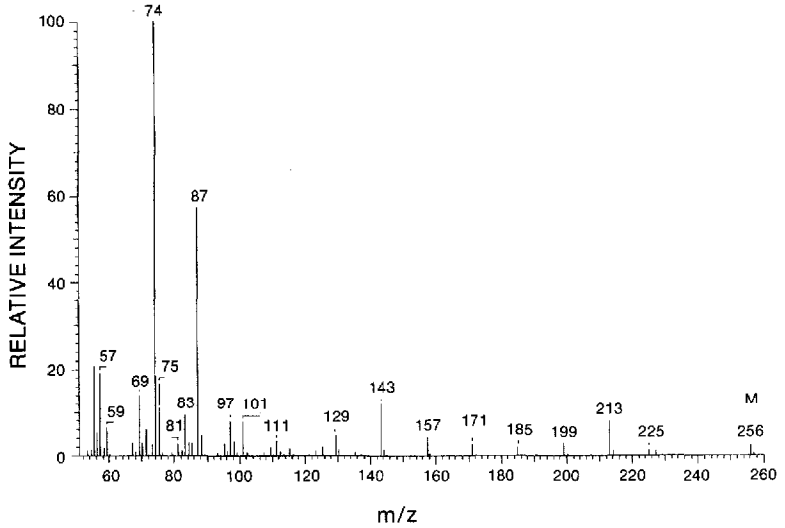

Fig. 9. EI (70 eV) mass spectrum, taken on the leading edge (scan 395) of the GLC component peak centered near scan number 400; identified in Fig. 5 as the methyl ester of 13-methyltetradecanoate acid (I C15). Note the enhanced intensity for the ion of m/z 213 (M−43) relative to that of the ion of m/z 199 (M−57) as compared to the spectrum in Fig. 7. Instrument operating conditions same as in Fig. 7.

ponents 23 and 28 (Fig. 4) were no longer eluted as distinct zones from components 24 and 29, respectively. Close examination of mass spectra gathered across the intense peaks designated *anteiso*-C_{15} and *anteiso*-C_{17} (Fig. 5) demonstrated that these are not homogenous. The major component of the peak centered near scan number 400 is indeed the methyl ester of *anteiso*-C_{15} (compare Figs. 7 and 8), but the leading edge of the *anteiso*-C_{15} peak contains *iso*-C_{15} fatty acid methyl ester (see Fig. 9; note the $M-43$ (C_3H_7) ion of m/z 213, characteristic of an iso acid methyl ester). A mass chromatographic plot of ion intensities [m/z 199 ($M-57$) and m/z 213 ($M-43$)] *vs.* scan number (380–420) is presented in Fig. 10. The I_{213}/I_{199} ratio is greater than unity for scans from *ca.* 393 to 398 ($M-C_3H_7$ from the *iso*-C_{15} methyl ester is more intense than $M-C_4H_9$), whereas at later scans (*ca.* 399–405) the ratio is less than unity (the later-eluted methyl ester of *anteiso*-C_{15} yields a more intense $M-C_4H_9$ ion, characteristic of an *anteiso* methyl ester). In an analogous fashion, it was demonstrated that the methyl ester of *iso*-C_{17} is present in the leading edge of the *anteiso*-C_{17} peak. These two *iso*-fatty acid methyl esters were not separated from their *anteiso* analogues. The peak designated *iso*-C_{16}, on the other hand, was found to be homogenous. The component designated as C_{18} (Fig. 5) and 31 (Fig. 4), based on its mass spectrum, is a saturated C_{18} acid methyl ester. Its retention time and mass spectrum are distinct from those of methyl stearate, and as it yields an intense $M-43$ ion with no $M-57$ ion it is probably the *iso*-C_{18} acid methyl ester. The component designated C_{13} (Fig. 5) and 20 (Fig. 4), on the basis of its mass spectrum (M^+, 228), is a saturated C_{13} fatty acid methyl ester. The component designated C_{17} (Fig. 5) and 30 (Fig. 4) possesses a retention time and mass spectrum comparable to that of methyl *n*-heptadecanoate.

The evidence generated by direct-probe EI-MS, packed-column GLC and capillary column GLC–MS analysis of the lipid-rich fraction, and capillary column GLC and GLC–MS of the fatty acid methyl esters resulting from transesterification, demonstrate that this fraction is predominantly a mixture of mixed acyl [mainly saturated C_{15}–C_{17} (*n*-, *iso*- and *anteiso*-)] triglycerides. Desorption chemical ionization (DCI)

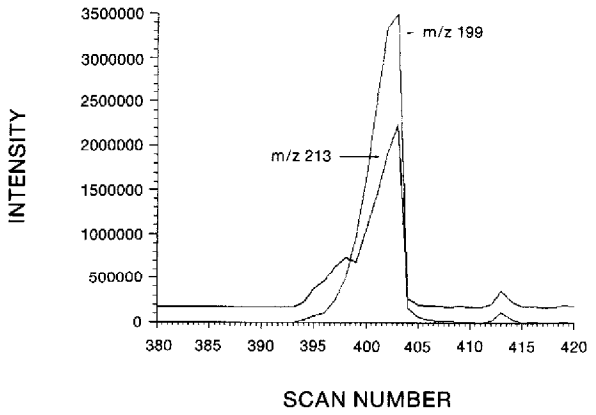

Fig. 10. Mass chromatogram for ions of m/z 199 and 213, produced from data (scans 380–420) generated by GLC–MS of the fatty acid methyl esters arising from the lipid-rich fraction following transesterification (Fig. 5). A Finnigan-MAT SS200 data system was used to generate the mass chromatogram.

MS was employed by Rezanka *et al.*[13] to complement their GLC–EI-MS examination of triglycerides from the green alga *Chlorella kessleri*. Using methane as reagent gas, these authors obtained relative intensities for pseudomolecular (MH) ions of up to *ca.* 30–40%. We have found that with ammonia as reagent gas the pseudomolecular ($M+NH_4$) ions of triglycerides are the base peaks with very little production of fragmentation ions. DCI (NH_3) spectra show intense M + 18 ions of *m/z* 740, 824, 902 and 908 and little else for trimyristin, tripalmitin, triolein and tristearin, respectively. Analysis of the lipid-rich fraction by this technique gave the results illustrated in Fig. 11. A distribution of M + 18 ions for a series of triglycerides of molecular weights from 736 to 848 (*m/z* 754–866, M + 18 ions) centered at *m/z* 810 (molecular weight 792) and 824 (molecular weight 806) was observed (along with evidence for low levels of squalene and diglycerides). This result indicates that the peak triglyceride composition corresponds to 47 (C_{15}, C_{16}, C_{16} or equivalent) and 48 (three C_{16} or equivalent, *e.g.*, C_{15}, C_{16}, C_{17}) acyl carbon atoms, or 50 and 51 total carbon atoms. The overall DCI spectral profile reflects the presence of members of a series of mixed triglycerides at each appropriate molecular weight from 736 to 848 (43 to 51 acyl carbon atoms). These results are in line with those generated by the other approaches used in this study. DCI-MS (NH_3) is an attractive one-step means of determining the molecular weight and distribution of triglycerides present in a crude mixture.

The existence of M + 18 − 2 ions can be noted in Fig. 11. As these ions are not artifacts of the analysis, they indicate the presence of triglycerides containing one monounsaturated acyl group as minor components in the mixture. Of interest is the observation that these ions are far more pronounced with the triglycerides of highest molecular weight (*e.g.*, the ions of *m/z* 864 and 866 are roughly equal in intensity) than with the lower-molecular-weight triglycerides. For a 1:1 mixture of a triglyceride containing three saturated acyl groups and the analogous triglyceride in which one

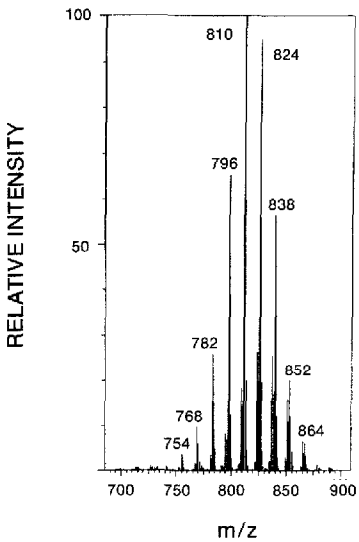

Fig. 11. Typical intense spectrum obtained by analysis of the lipid-rich fraction via DCI-MS with NH_3 reagent gas. Instrument operating conditions given in Experimental.

of the acyl groups is monounsaturated there would be only one monounsaturated acyl group (or monounsaturated fatty acid methyl ester) for five saturated acyl groups.

The general existence of triglycerides and other neutral lipids in several *Streptomyces* species has been reported[16–20]. According to these authors, the triglyceride content of various species comprises a variable portion of the extractable lipids, ranging from just a few percent of the total to a majority of the neutral lipids. Fatty acid substitution within triglycerides is variable. The substitutions include C_{12}–C_{18} straight-chain acids as well as several *iso* and *anteiso* acids of similar molecular weight. It is reported[16,19] generally that iso-branching is more common in acids which bear an even number of carbon atoms, while anteiso-branching is more often observed in acids with an odd number of carbon atoms. This is in good agreement with our results from *S. avermitilis*. Ballio *et al.*[16] reported that the preponderant fatty acids from the triglycerides of *Streptomyces* spp. are 12-methyltridecanoic acid, 12- and 13-methyltetradecanoic acids and 14-methylpentadecanoic acid, whereas for *S. avermitilis* the major triglyceride fatty acids are 12-methyltetradecanoic, 14-methylpentadecanoic, *n*-hexanoic and 14-methylhexadecanoic.

In addition, we found the iso-branched 13-methyltetradecanoic acid and the analogous *iso*-C_{17} acid, but not as predominant triglyceride components. Detection in *S. avermitilis* of only small amounts of unsaturated fatty acids (C_{16} and C_{17}), acid chain lengths from C_{13} to C_{18}, and low levels of diglycerides is in line with earlier reported observations for Streptomycetes. Our finding of squalene in a Streptomycete confirms the report by Grafe *et al.*[21]. Newer analytical techniques such as those employed in our current study and other recent work[13–15,19,21] facilitate the investigation of the nature of complex natural product mixtures such as triglycerides.

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