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Identification of Fatty Acids and Aliphatic Hydrocarbons in *Sarcina lutea* by Gas Chromatography and Combined Gas Chromatography-Mass Spectrometry

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The composition and nature of the fatty acids and hydrocarbons of *Sarcina lutea* were elucidated by gas chromatography and by combined gas chromatography-mass spectrometry. The distribution of fatty acids found in *S. lutea* showed two families of pairs, or dyads, of saturated monocarboxylic acids (C12–C18) with and without methyl branching. These pairs of fatty acids showed a pattern of iso and anteiso structures for C13, C15, and C17, and iso and normal structures for C12, C14, and C16. Only the C18 showed unsaturation. The distribution of hydrocarbons in the range C22–C29 showed two families of tetrads of unsaturated aliphatic hydrocarbons all showing methyl branching. Each tetrad was composed of four isomers identified as two iso olefins and two anteiso olefins. The only difference between the tetrads pertaining to different families was found in the relative gas chromatographic retention times of the last two components of each group.

The hydrocarbons and fatty acids of *Sarcina lutea* have been described by Huston and Albro (2, 10, 11). In their investigations, the extractable lipid material was analyzed by thin-layer and gas chromatography and by infrared spectrophotometry. In the present report, the identification of the fatty acids and hydrocarbons of *S. lutea* is based on data obtained by a new technique of combined gas chromatography-mass spectrometry. Although the identity of some of the fatty acids was confirmed, the nature of some of the fatty acids and of most of the hydrocarbons was found to be significantly different from that previously reported.

MATERIALS AND METHODS

Culture conditions. *S. lutea* (ATCC 533) was cultivated in Erlenmeyer flasks containing Trypticase Soy Broth, *p*H 7.0 (BBL). The cultures were placed in water baths adjusted to 25 C and were continuously aerated for 48 hr. The cells were collected in plastic tubes in an RC-2 Servall centrifuge, washed three times in a 0.15 M NaCl solution, frozen, and then dried over P_2O_5 under vacuum.

Extractions. The method used to extract, fractionate, and analyze the hydrocarbons and fatty acids from bacteria is described in an accompanying report (25). A 1.5-g amount of dried cells was placed in an all-glass Soxhlet-type apparatus and extracted with 50 ml of a benzene-methanol mixture (3:1) for 8 hr. The extract was transferred to a beaker, and the solvent was removed by evaporation at 40 C under a stream of purified nitrogen.

Column fractionation. The extracts were fractionated on silica gel columns into three fractions: the *n*-heptane fraction, containing the aliphatic hydrocarbons (alkanes, olefins, etc.); the benzene fraction, saved for future analysis; and the methanol fraction, containing the fatty acids and glycerides, among other lipids. The *n*-heptane eluate collected from the silica gel column was divided into two subfractions. One subfraction was saved for pigment analysis. The other subfraction was transferred to a glass column (1 by 30 cm). The column was provided with a sintered-glass disc and was filled to a depth of 10 cm with alumina that had been previously activated at 340 C for 24 hr, and washed with 10 ml of *n*-heptane. The aliphatic hydrocarbons, relatively free from pigments, were eluted with *n*-heptane.

Preparation of derivatives. The fatty acids were liberated from the glycerides of the methanol fraction by alkaline hydrolysis (17). Methyl esters of the fatty acids were prepared for gas chromatography and mass spectrometry as previously described (10).

Thin-layer chromatography. Glass plates were spread with Silica Gel G (Stahl) and heat-activated for 2 hr at 120 C. The plates were developed in unlined tanks by the ascending method with petroleum

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ether-diethyl ether-glacial acetic acid (90:10:1) and benzene-*n*-heptane (9:1) as solvents. The components were observed by means of tracer studies (26). The plates were exposed to Ansco-non-screen X-ray film for 1-week periods. They were also sprayed with a saturated solution of potassium dichromate in sulfuric acid and were charred.

The pigment spot (presumably a carotenoid pigment) was eluted with benzene from the plate and was saved for spectrophotometric and mass spectrometric analysis.

Spectrophotometric analysis. The eluted pigment was dissolved in *n*-heptane, and the absorption spectrum determined on a Beckman DB spectrophotometer equipped with a Sargent recorder, model SRL.

Hydrogenation. A portion of the *n*-heptane eluate from a silica gel column was hydrogenated catalytically (Pt) to reduce the pigment and any unsaturated hydrocarbons present. After reduction the hydrogenated compounds were removed from the catalyst by passing the product through a silica gel column as before and eluting with *n*-heptane.

Infrared analysis. Infrared spectra of the *n*-heptane fractions before and after hydrogenation were recorded with an IR 10 infrared spectrophotometer.

Gas chromatography and mass spectrometry. All aliphatic hydrocarbons and fatty acid methyl esters were analyzed on a F & M Model 810 gas chromatograph equipped with a flame ionization detector and an LKB 9000 gas chromatograph-mass spectrometer. All samples were prepared for analysis as previously described (18). The chromatographic analyses were carried out in several stainless-steel capillary columns: (i) a column 31 meters by 0.025 cm (inner diameter) coated with 10% Apiezon L (a high-temperature grease purchased from Applied Science Laboratory, Inc., State College, Pa.), at a nitrogen pressure of 1,430 g/cm^2; (ii) a column 91 meters by 0.076 cm (inner diameter) coated with Polysev (*m*-bis *m*-(phenoxy-phenoxy)-phenoxy benzene; Applied Science Laboratory, Inc.), at a nitrogen pressure of 700 g/cm^2; (iii) a column 310 meters by 0.070 cm (inner diameter) coated with Polysev, at a nitrogen pressure of 933 g/cm^2; (iv) a column 155 meters by 0.076 cm (inner diameter) coated with Igepal CO 990 (nonylphenoxy polyoxyethylene ethyl alcohol; General Aniline and Film Corp., New York, N.Y.), at a nitrogen pressure of 933 g/cm^2.

The mass spectra were taken as each component emerged from the column. Ionizing energies used were 70 ev for the fatty acid methyl esters and 20 ev for the aliphatic hydrocarbons. The remaining conditions were the same as previously described (18).

Mass spectrometric analysis of pigments. Samples of the pigment that was eluted from the thin-layer chromatograph plate with benzene were sent to Varian Corp., Analytical Instrument Division, Palo Alto, Calif. The samples were transferred with a small amount of benzene to a capillary tube. The benzene was removed from the sample by evaporation in vacuum. The sample was then introduced into an M-66 mass spectrometer with the use of the solid sample probe. Mass spectra were taken at probe temperatures of 100, 125, 150, 175, 200, and 225 C.

RESULTS

Lipid composition. The cells of *S. lutea* dried over P_2O_5 were found to contain on the average about 1.45% of total extractable lipid material. The amount of aliphatic hydrocarbons in the *n*-heptane eluate from the alumina column (this excludes the pigment) was found to be about 0.25% of the dried cell mass. These values are in close agreement with those reported (1.30% and about 0.27%, respectively) by Huston and Albro (10).

Gas chromatography and mass spectrometry of fatty acid methyl esters. Figures 1 and 2 show two typical gas chromatographic patterns of the fatty acid methyl esters from *S. lutea*. Fifteen of the peaks (numbered from 1 to 15) were identified by both gas chromatography and mass spectrometry. Their identities are listed in Table 1. With the exception of oleic and linoleic acids, all the other fatty acids analyzed were found to be saturated. Typical mass spectra for the compounds corresponding to peaks 7, 8, and 10 are shown in Fig. 3. The patterns shown in Fig. 1 (Igepal CO 990) and Fig. 2 (Apiezon L) correlate well with each other. They also agree with the results of mass spectrometry, which showed that no major unsaturated components are present.

The fatty acids found in *S. lutea* follow a distribution pattern having a predominance of neither even- nor odd-numbered carbon chains. However, careful inspection of the peaks in the chromatograms of Fig. 1 and 2 and their identity as listed in Table 1 shows the existence of pairs of dyads of fatty acids grouped in two distinct families. One of these families is made of fatty acids with an even number of carbon atoms, the pairs being iso and normal C12, C14, and C16 saturated acids; the other family is made of fatty acids with an odd number of carbon atoms, the pairs being iso and anteiso C13, C15, and C17 saturated acids. Some of the C18 and other fatty acids were present in amounts too small to allow proper characterization. As indicated earlier, of all the fatty acids identified, the only unsaturates found were the common oleic and linoleic acids.

As seen from Fig. 1 and 2, the two predominant fatty acids of *S. lutea* are iso and anteiso pentadecanoic acids, which are probably related to the sarcinic acid (branched C15) reported by Akashi and Saito (1). When comparing various fatty acid methyl ester chromatograms and mass spectra, we found that the iso- and anteiso-C15 reported here were identical with the C15 fatty acids of *Bacillus cereus* and *Staphylococcus aureus* (*in preparation*).

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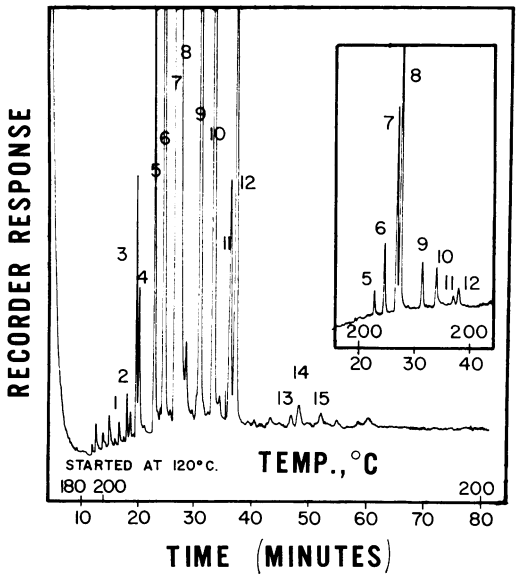

FIG. 1. *Gas chromatographic separation of the fatty acid methyl esters of Sarcina lutea on stainless-steel tubing (155 meters by 0.076 cm) coated with Igepal CO-990. Nitrogen pressure, 933 g/cm²; no split. F & M 810 apparatus equipped with a flame ionization detector. Range, 10²; attenuation, 1. Temperature programmed at approximately 6 degrees per minute from 120 to 200 C, and held isothermally. From 1.5 g of extracted cells, 1/100 of the sample was injected.*

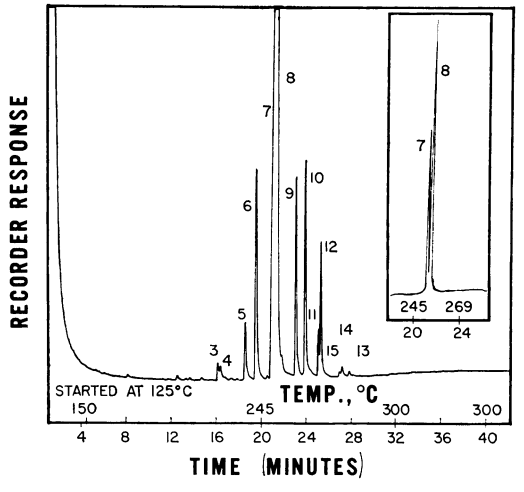

FIG. 2. *Gas chromatographic separation of fatty acid methyl esters of Sarcina lutea on stainless-steel tubing (31 meters by 0.025 cm) coated with 10% Apiezon L. Nitrogen pressure, 1,430 g/cm²; no split. Range, 10²; attentuation, 1. Temperature programmed at approximately 6 degrees per minute from 125 to 300 C, and held isothermally. From 1.5 g of extracted cells; 1/200 of the sample injected.*

TABLE 1. *Fatty acids of Sarcina lutea*

Peak no.	Identification[a]
1	i 12:0
2	12:0
3	i 13:0
4	ai 13:0
5	i 14:0
6	14:0
7	i 15:0
8	ai 15:0
9	i 16:0
10	16:0
11	i 17:0
12	ai 17:0
13	18:0
14	18:1
15	18:2

[a] Symbols: i = iso; ai = anteiso. The first number represents the chain length; the second number represents the number of unsaturations.

of the procedure were followed in the absence of sample, and in which analyses of the media and solvents were carried out, showed either negligible or no measurable quantities of saponifiable material.

Interpretation of the mass spectra. The small peaks preceding the iso-laureate (peak 1) gave either inconclusive or complex spectra; their identification has not been further attempted. The interpretation of the fragmentation patterns of fatty acid methyl esters for purposes of identification has been reported by Beynon (3) and Ryhage and Stenhagen (21). Only the data most pertinent to the fatty acids of *S. lutea* will be discussed here. These data are exemplified by the fragmentation patterns of the compounds in the chromatographic peaks 7, 8, and 10 corresponding to iso-C15, anteiso-C15, and *n*-C16 fatty acids. The mass spectra are shown in Fig. 3.

The major mass peak in spectra of this type (fatty acid methyl esters) is usually formed at m/e 74 (unless the α-carbon is substituted), and corresponds to the fragment ion $(CH_3—O—\underset{\underset{OH}{|}}{C}=CH_2)^+$. This fragment ion is produced by a cyclic rearrangement involving the migration of a γ-hydrogen atom to the carboxyl oxygen of the ester group with a concerted β cleavage (4, 16). The next intense peak is found at m/e 87, $(CH_3—O—\overset{\overset{O}{\|}}{C}—CH_2—CH_2)^+$, and is formed by a simple γ cleavage of the chain (8). The majority of the remaining intense peaks are similar oxygen-containing fragments formed by simple

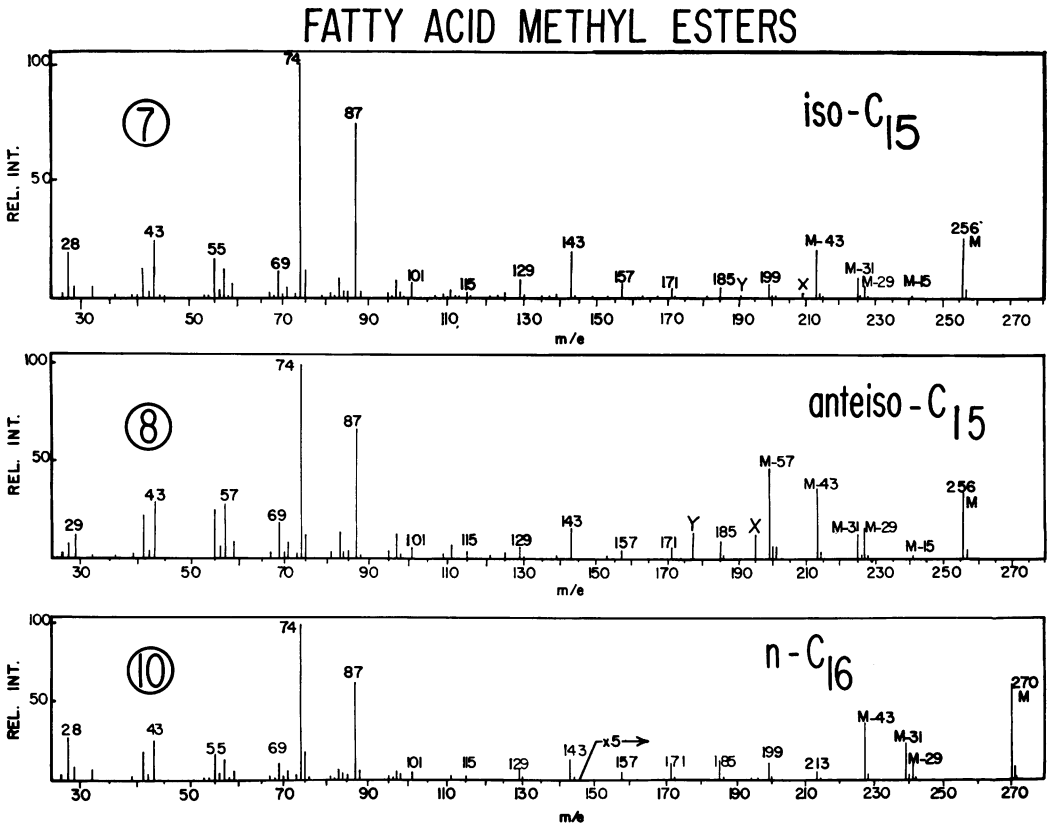

FIG. 3. *Mass spectra of compounds 7, 8, and 10 were obtained with an LKB 9000 gas chromatograph as each of the components emerged from capillary tubing (91 meters by 0.076 cm) coated with Polysev. The components were ionized by electron impact at 70 ev as they entered the ion source. Each peak was scanned within the range of 0.0 to 400 mass units in 10 sec. The spectra were recorded by means of an oscillograph recorder with the chart speed set at 10 cm/sec.*

cleavage of the chain at each of the successive carbon groups; m/e: 101, 115, 129, 143, 157, etc. The ion at m/e 143, $(CH_3—O—\overset{\overset{O}{\|}}{C}—(CH_2)_6)^+$, which gives a relatively high-intensity peak in the mass spectra of long-chain fatty acid esters, originates through a simple 7,8 cleavage. To account for its higher probability, Beynon suggested a rearranged dicyclic structure (3).

In the upper end of the spectrum (Fig. 3), there are three distinct peaks whose m/e values correspond to M-43, M-31, and M-29 (M is the mass of the molecular ion). The peak at M-31 is produced by the loss of the methoxy radical with rentention of the positive charge on the large fragment containing the carboxyl group. The M-43 peak, as well as the peak at M-29, originate through an expulsion mechanism of propyl and ethyl groups, respectively, as established by deuterium labeling experiments (22).

With regard to the branched isomers of these compounds, it is important to emphasize that the introduction of an alkyl side chain at a carbon atom other than C-2 does not have any great effect on the overall appearance of the spectrum, except for an increased tendency of the alkyl chain to fragment at the carbon atom bearing the additional substituent. The presence of a methyl side chain is readily determined by means of the peak formed at M-15, which is absent in the normal compounds. Compare fragmentation patterns of compounds 7 and 8 with that of compound 10 (Fig. 3).

In our case, there was no evidence of any appreciable change in the central portion of the fragmentation pattern between the normal and the methyl-branched compounds. This can be accounted for by the methyl substituent being in an iso or anteiso position, so that only the lower and higher end of the spectra will show any

changes; the region between m/e 74 and M-57 remains undisturbed.

The characterization of the iso branching in those molecules is at first unclear because the peak originated by the loss of the isopropyl radical (m/e 43) coincides with the M-43 peak originated by the expulsion mechanism of the propyl group mentioned above (*see* Fig. 3). The best clue to the position of the iso methyl branch is obtained from the presence of a peak at an m/e corresponding to the formation of a ketene ion (m/e 209 peak X in compound 7 of Fig. 3) which is derived from the branched structure through the loss of methanol (22). The loss of water from the ketene ion gives a peak at m/e 209 − 18 = m/e 191 (peak Y in compound 7 of Fig. 3). Although of very low intensity, both peaks X and Y are reliable, since they are completely absent in the spectra of normal fatty acids.

In the case of anteiso long-chain methyl esters, the problem is somewhat simpler; there is a peak at M-57 (compound 8 of Fig. 3), due to loss of the isobutyl radical, which is much higher than the corresponding peak in a normal fatty acid ester. The ketene ion appears here at m/e 195 (peak X of compound 8 of Fig. 3), and by loss of water gives rise to a peak at m/e 177 (peak Y of compound 8 of Fig. 3). A pronounced feature is the higher intensity of the M-29 peak relative to that of the M-31 (loss of the methoxy radical). This is expected since there are now two different processes contributing to the formation of the M-29 ion (the expulsion process mentioned before and a single-bond cleavage next to the methyl substituent).

Gas chromatography and mass spectrometry of aliphatic hydrocarbons. The gas chromatographic pattern for the aliphatic hydrocarbons is shown in Fig. 4 and 5. The identities are given in Table 2. (Typical mass spectra are shown in Fig. 6.) The two patterns agree well with each other, although they are from different columns of unrelated phases. The range of hydrocarbons is from C22 to C29, with the major component being an anteiso-Δ-C25. In Fig. 4, peaks a, b, and c were relatively unstable components that periodically appear in freshly prepared samples. Conclusive mass spectra were not obtained for these components; however, indications were obtained that they are polyunsaturates.

As in the case of the fatty acids, the aliphatic hydrocarbons in *S. lutea* show a distribution having a predominance of neither even- nor odd-numbered carbon chains. However, examination of the two chromatograms (Fig. 4 and 5) and of Table 2 also shows a certain parallelism with the fatty acids. Tetrads (instead of dyads)

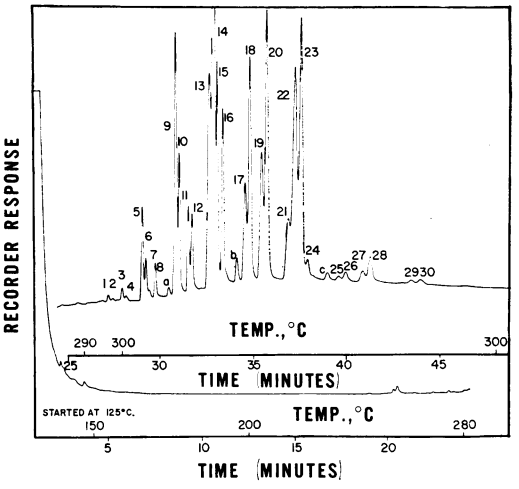

FIG. 4. *Gas chromatographic separation of the hydrocarbons of Sarcina lutea on a stainless-steel column (31 meters by 0.025 cm) coated with 10% Apiezon. Nitrogen pressure, 1,430 g/cm²; no split. F & M 810 apparatus equipped with a flame ionization detector. Range, 10²; attenuation, 1. Temperature programmed at approximately 6 degrees per min from 120 to 300 C, and held isothermally. From 1.5 g of extracted cells; 1.5/100 of the sample was injected.*

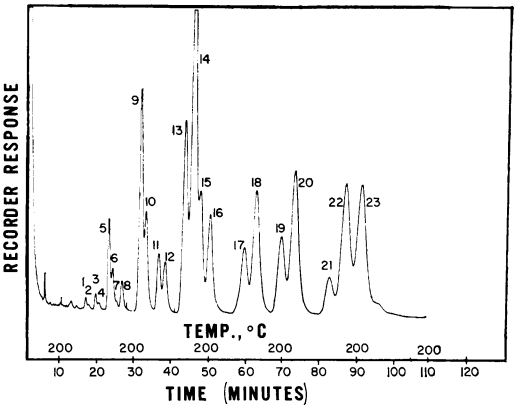

FIG. 5. *Gas chromatographic separation of the hydrocarbons of Sarcina lutea on a stainless-steel column (155 meters by 0.076 cm) coated with Igepal CO 990. Nitrogen pressure, 933 g/cm²; no split. F & M 810 apparatus equipped with a flame ionization detector. Range, 10²; attenuation, 1, Isothermally at 200 C. From 1.5 g of extracted cells; 1/75 of the sample injected.*

of hydrocarbons are grouped in two distinct families. One of these families is made of hydrocarbons with an even number of carbon atoms, the tetrads being four isomers in the following order: iso, anteiso, iso, anteiso of each of the C22, C24, C26, and C28 olefins. The other family

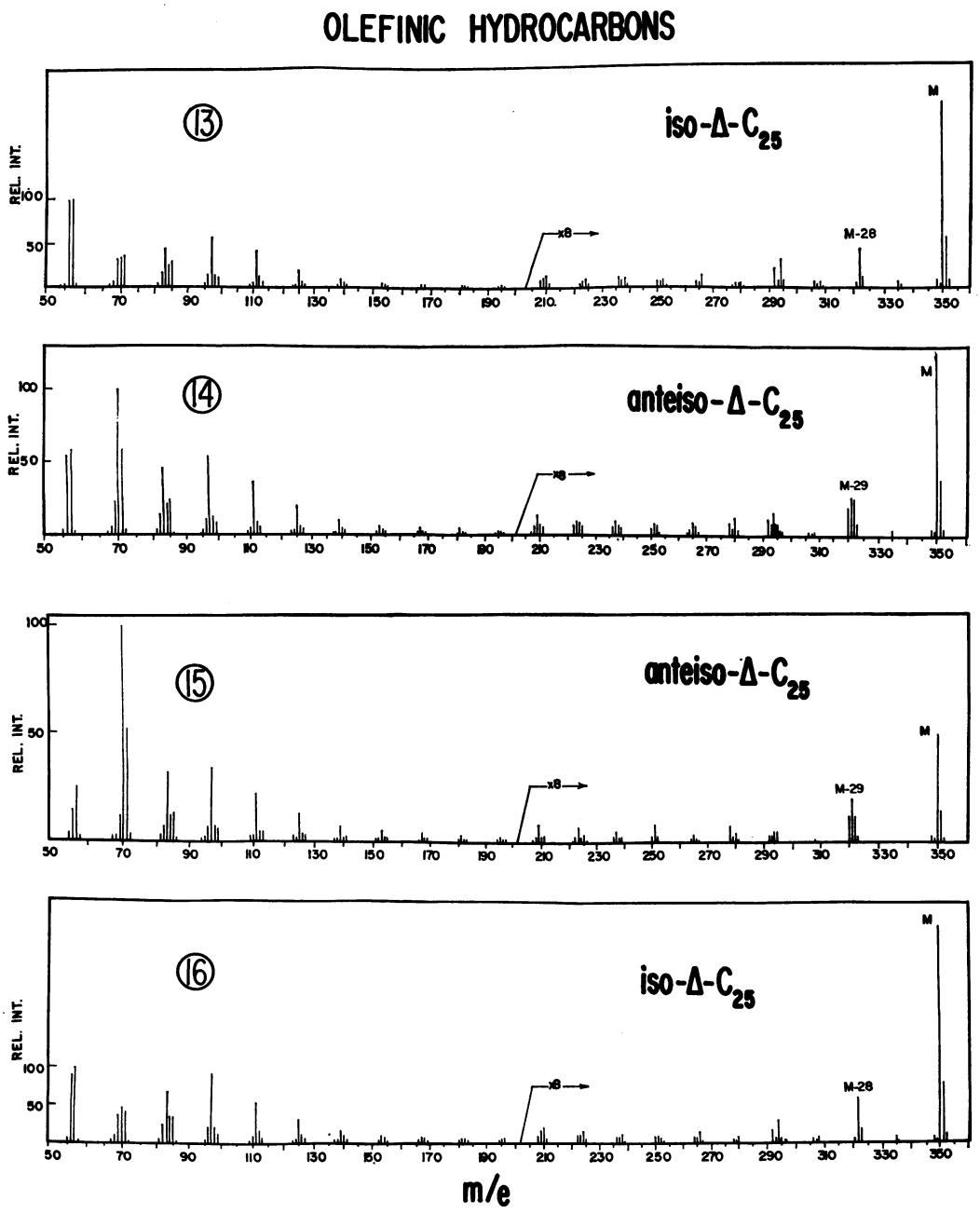

FIG. 6. *Mass spectra of compounds 13, 14, 15, and 16 were obtained with an LKB 9000 gas chromatograph as the components were eluted from capillary tubing (91 meters by 0.076 cm) coated with Polysev. The components were ionized by electron impact at 20 ev as they entered the ion source. Each peak was scanned within the range of 0.0 to 400 mass units in 10 sec. The spectra were recorded by means of an oscillograph recorder with the chart set at 10 cm/sec.*

is made of hydrocarbons with an odd number of carbon atoms, the tetrads being four isomers in the following order: iso, anteiso, anteiso, and iso of each of the C23, C25, and C27 olefins. Only traces of two C29 alkenes (iso and anteiso) were found, although it is possible that with increased sensitivity the four isomers could have been detected.

TABLE 2. *Hydrocarbons of Sarcina lutea*

Hydrocarbon	Peak no.	Identification[a]
C22	1	iso-Δ-C22
	2	anteiso-Δ-C22
	3	iso-Δ-C22
	4	anteiso-Δ-C22
C23	5	iso-Δ-C23
	6	anteiso-Δ-C23
	7	anteiso-Δ-C23
	8	iso-Δ-C23
C24	9	iso-Δ-C24
	10	anteiso-Δ-C24
	11	iso-Δ-C24
	12	anteiso-Δ-C24
C25	13	iso-Δ-C25
	14	anteiso-Δ-C25
	15	anteiso-Δ-C25
	16	iso-Δ-C25
C26	17	iso-Δ-C26
	18	anteiso-Δ-C26
	19	iso-Δ-C26
	20	anteiso-Δ-C26
C27	21	iso-Δ-C27
	22	anteiso-Δ-C27
	23	anteiso-Δ-C27
	24	iso-Δ-C27
C28	25	iso-Δ-C28
	26	anteiso-Δ-C28
	27	iso-Δ-C28
	28	anteiso-Δ-C28
C29	29	iso-Δ-C29
	30	anteiso-Δ-C29

[a] Symbols: Δ = double bond.

This constitutes a remarkable distribution, with a unique order, of hydrocarbons. The following characteristic features were found: (i) all the components were identified as branched hydrocarbons with methyl substituents at either iso or anteiso positions; (ii) all were identified as monounsaturated olefins (monoenes); (iii) the components appeared in groups of four, with all four components of the same group (tetrad) having the same molecular mass (therefore being isomers); and (iv) the four isomers in the groups with an odd number of carbon atoms were found to emerge from the gas chromatographic columns very close together, whereas the four isomers in the groups with an even number of carbon atoms were better resolved and separated clearly into two pairs.

The identification of these hydrocarbons as branched monounsaturated olefins was made possible or was greatly facilitated by use of an LKB 9000 gas chromatograph mass spectrometer. Of the 18 times that *S. lutea* was grown to study lipid formation, culture conditions (25), etc., the lipid fractions were analyzed eight times by gas chromatography-mass spectrometry with essentially identical results each time.

Interpretation of mass spectra. The outstanding feature revealed by the mass spectral data was the same molecular mass for each of the four gas chromatographic peaks corresponding to a tetrad. The four components of the tetrad were also found to be olefins. This may be seen in Fig. 6, which exemplifies the mass spectra of the four C25 olefins corresponding to the four gas chromatographic peaks 13 (iso), 14 (anteiso), 15 (anteiso), and 16 (iso). Very similar mass spectral data were obtained for the isomers of the other tetrads. For that reason, the mass spectra corresponding to the C25 group, given in Fig. 6, should be considered as representative of the fragmentation patterns found within the other tetrads of four isomers as well.

The first (iso) and second (anteiso) components show in all of the tetrads fragmentation patterns which are virtually identical (except the molecular masses) to the mass spectra of peaks 13 (iso) and 14 (anteiso). (*See* Fig. 6.) The only difference between different groups was found in the relative gas chromatographic retention times of the last two components in each group. Although their mass spectra are also practically undistinguishable from those corresponding to peaks 15 (anteiso) and 16 (iso; Fig. 6), they reverse positions in the olefin chains of even-carbon number.

Since the double bond is rather mobile in olefins and migrates easily via successive shifts of hydride ions and hydrogen radicals, it was not possible to locate its exact position in the chain. The evenness of the central portion of the spectra indicated an iso or anteiso type of branching. To remove the possible interaction of the double bond with the side chain and thus obtain unequivocal data on the exact position of the branch on the chains, the *n*-heptane eluate was hydrogenated.

The mass spectral patterns resulting from the hydrogenated *n*-heptane eluate are shown in Fig. 7 for the C25 tetrad. They demonstrate that: (i) all of the components within each of the isomer groups are methyl-branched paraffins; (ii) the methyl side chain occupies the iso position in two of the four isomers and the anteiso position in the other two; (iii) the mass spectral patterns corresponding to the iso and anteiso paraffins are not only essentially identical within each group (Fig. 7), but also to those of the other groups of the isomers. As in the case of the olefins, the

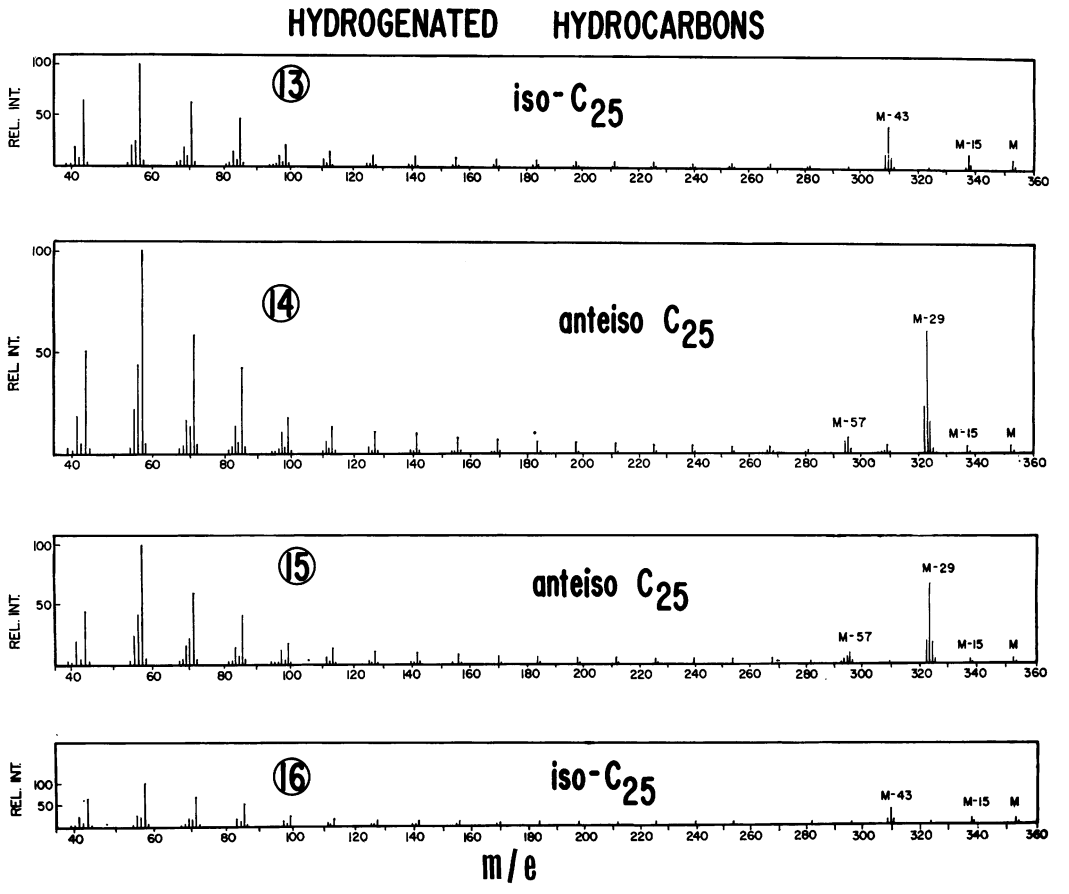

FIG. 7. *Mass spectra of the hydrogenated hydrocarbons. All conditions are the same as in Fig. 6.*

spectra of the C25 paraffins presented in Fig. 7 are representative of the spectra found for all of the other groups of hydrogenated hydrocarbons.

From these data, it can be shown that the first component in the olefin tetrads of *S. lutea* is always an iso olefin, and the second, an anteiso olefin; the third, an iso olefin in the even-numbered carbon chains, corresponds to an anteiso olefin in the odd-numbered chains (*see* Table 2). In accordance with these results, the four peaks within each group of olefin isomers would be expected to collapse into two peaks in the gas chromatogram upon hydrogenation of the sample. The fact that this does not take place and that there is an almost identical reproducibility of the gas chromatographic pattern after hydrogenation of the sample indicates (i) that the double bond does not play a significant role in the resolution of each of the isomers and (ii) that it is necessary to provide for some configurational differences between each of the iso and anteiso isomers, in order to explain the unusual resolution encountered. The possibility of having iso or anteiso configurations on both ends of the molecule cannot be excluded, since this would not change the overall mass spectral pattern. On the other hand, the clear differentiation of iso and anteiso fragmentation patterns indicates that the coexistence of iso and anteiso methyl branching in any one of the molecules is not possible. Therefore, any near-terminal methyl disubstitutions on the olefins must be of the same type, either both iso or both anteiso.

More work will have to be done to locate the position of the double bond and to determine the possible presence of some doubly near-terminal methyl-substituted isomers. The possibility of having a disubstituted molecule is attractive, since it would explain the variation in retention times of the isomers through diastereoisomeric forms.

Thin-layer chromatography. The analytical results obtained by thin-layer chromatography on the migration and distribution of lipids of *S.*

lutea were in accordance with those previously reported (2). An audioradiogram from a thin-layer chromatography plate analysis of the hydrocarbons and fatty acids from cells grown in the presence of 50 μc of palmitate-*1*-^{14}C was obtained (26). The hydrocarbons and fatty acid methyl esters were identified by R_F values and by elution and reanalysis by gas chromatography. The results were in agreement with the gas chromatographic and mass spectrometric results described above.

Infrared spectra of n-heptane eluates. It will be recalled that the *n*-heptane eluate from silica gel chromatography contains not only the hydrocarbons but also the pigments. Infrared spectral analyses of the *n*-heptane eluate and the hydrogenated *n*-heptane eluate are shown in Fig. 8. The absorption for the *n*-heptane eluate between 3,650 to 2,600 cm^{-1} and 1,740 cm^{-1}, owing to stretching vibrations of OH and C=O, respectively, was attributed to the carotenoid pigments that were present. The absorption at other wave numbers shows that olefins are present, as indicated by the double-bond stretching frequency around 1,640 cm^{-1} and the absorptions from 600 to 1,000 cm^{-1}. The bands around 605, 820, and 975 cm^{-1} are indications of *cis* olefins, trisubstituted olefins, and *trans*-disubstituted olefins, most of which belong to the carotenoid pigments. The infrared spectra of the hydrogenated *n*-heptane eluent (Fig. 8) confirms the hydrogenation of all the olefins by the deletion of the absorptions in question.

Pigments. The pigments eluted from a thin-layer plate were identified as carotenoids by their yellowish-orange appearance (13, 19, 20), their

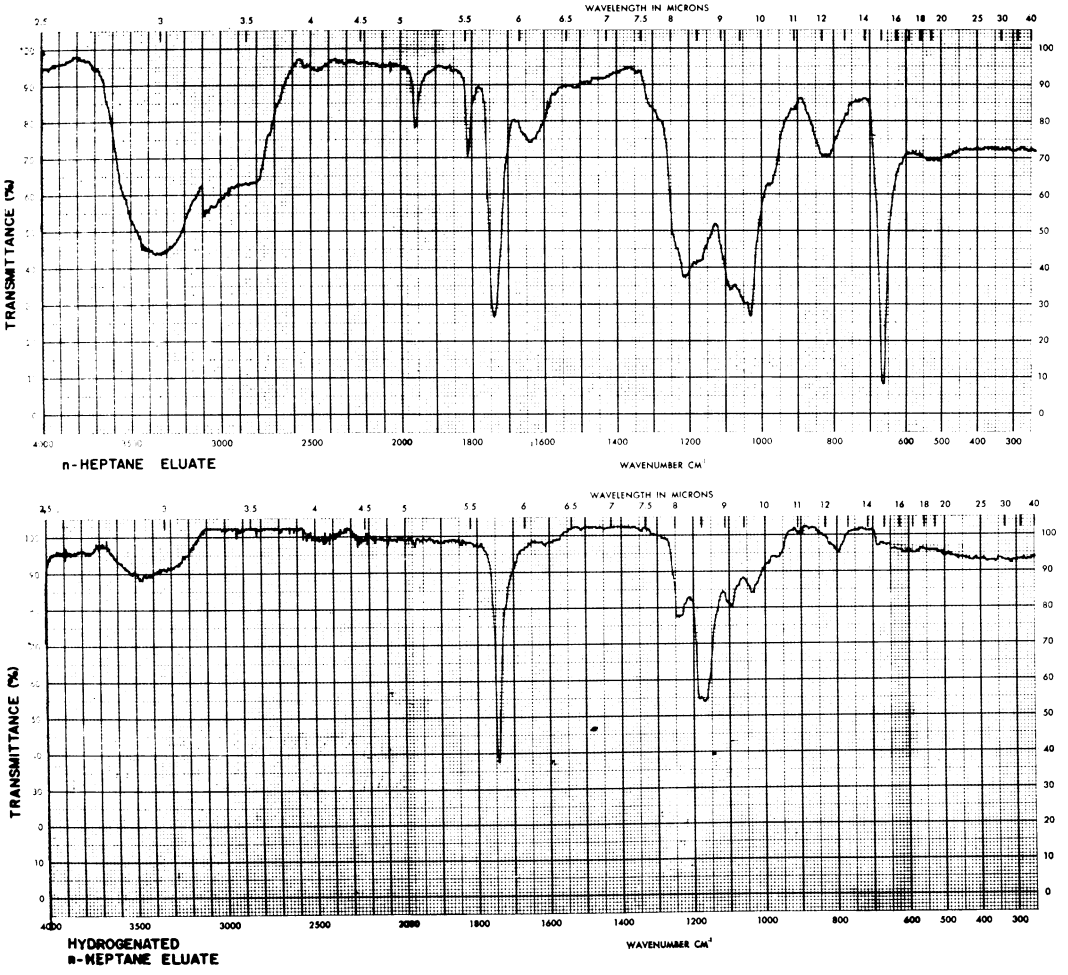

FIG. 8. *Infrared transmission spectra of the n-heptane eluate and the hydrogenated n-heptane eluate recorded on an IR 10 infrared spectrophotometer.*

R_F values on thin-layer plates, and by their positive color test with 30% $SbCl_3$ in chloroform (7). Identification was also based on recent information published on *S. lutea* (2) and on the correlation of spectrophotometric studies (Fig. 9) with those already reported (5, 12, 19, 23, 24). The carotenoid absorbing at 480, 451, and 423 mμ was identified by Takeda and Ohta (23, 24) as a new xanthophyll, which they called sarcenaxanthine. On the other hand, Dyer (9) reported that *trans*-β-carotene has a maximal absorption at 452 and 478 mμ. Although the mass spectral analysis is far from complete, we have obtained results which are in accordance with the high molecular mass number 704 recently reported (15). Our fragmentation pattern suggests the presence of at least one oxygen atom, which is in line with the infrared analysis previously mentioned. Such a mass unit number is significantly different from some compounds previously proposed in a review by Liaaen Jensen (13), and throws some doubt on the previous identification of some of the carotenoids in *S. lutea* (19, 23, 24). As indicated by recent investigations (6, 14, 15), more work will have to be done for the complete identification of some of the "carotenoid" pigments.

DISCUSSION

The distribution patterns of the fatty acids and hydrocarbons of *S. lutea* are unique. The former constitute two families of pairs or dyads of saturated fatty acids (C12–C18) with and without methyl branching, and the latter constitute two families of tetrads of unsaturated aliphatic hydrocarbons (C22–C29) all showing methyl branching.

Comparison of the fatty acids with the hydrocarbons yields the following observations. For each dyad or pair of isomers of fatty acids, there is a tetrad or quadruplet of isomers of hydrocarbons. Whereas the range of carbon numbers for the fatty acids is approximately from 12 to 18, the range for the hydrocarbons is approximately from 22 to 28, the difference being uniformly 10, as if indicating that the hydrocarbons are derived from the acids by the addition of 10 carbon atoms. Whereas the major component of the fatty acids is an anteiso-C15, the major component of the hydrocarbons is an anteiso-C25. Whereas essentially all the fatty acids are saturated (except oleic and linoleic), all the identified hydrocarbons are unsaturated, showing in all cases one double bond.

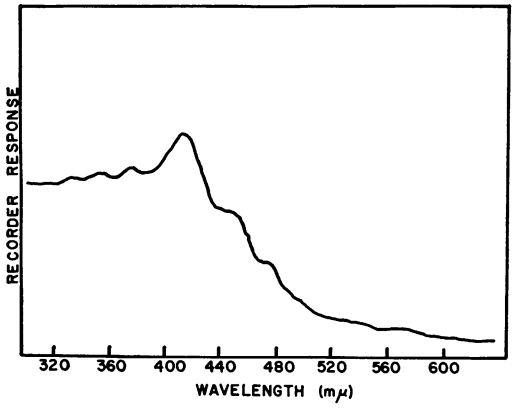

FIG. 9. *Spectrophotometric analysis of the pigment eluted from a thin-layer chromatographic plate. The pigment was suspended in n-heptane.*

These relationships appear to indicate that the hydrocarbons are generated by a unique pathway which involves the fatty acids or some common precursors to both types of compounds. Work is in progress to determine by means of derivatives and combined gas chromatography-mass spectrometry the position of the double bond and the exact configuration of the hydrocarbons. An accompanying paper describes the use of radioactive precursors to ascertain the metabolic pathways for biosynthesis of fatty acids and hydrocarbons in *S. lutea* (26).

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