Significance and Taxonomic Value of Iso and Anteiso Monoenoic Fatty Acids and Branched β-Hydroxy Acids in *Desulfovibrio desulfuricans*

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The fatty acids obtained from extractable lipids of the anaerobic sulfate bacterium *Desulfovibrio desulfuricans* were identified. Saturated and monoenoic iso (C_{15}–C_{19}) and anteiso (C_{15}, C_{17}) fatty acids and saturated normal (C_{14}–C_{18}) and monoenoic normal (C_{16}, C_{18}) fatty acids were shown to be present by capillary gas chromatography-mass spectrometry. Iso and anteiso β-hydroxy fatty acids were analyzed as trimethylsilyl ethers in the same way. The position of methyl branches in the monoenoic fatty acids was determined from characteristic fragment ions in the mass spectra of their methyl esters. Disilyloxy methyl esters, prepared by derivatization of the mono unsaturated methyl esters and analyzed by capillary gas chromatography-mass spectrometry, provided the position of double bonds. The monoenoic fatty acids identified in this way were normal ($\Delta7$-$C_{16:1}$, $\Delta9$-$C_{16:1}$, $\Delta9$-$C_{18:1}$, $\Delta11$-$C_{18:1}$), iso ($\Delta7$-$C_{15:1}$, $\Delta9$-$C_{16:1}$, $\Delta9$-$C_{17:1}$, $\Delta11$-$C_{18:1}$, $\Delta11$-$C_{19:1}$), and anteiso ($\Delta7$-$C_{15:1}$, $\Delta9$-$C_{17:1}$). Iso $\Delta9$-$C_{17:1}$ fatty acid is present as the major component. The occurrence of these monoenoic fatty acids in this bacterium is of taxonomical importance.

Desulfovibrio species are omnipresent bacteria in sediments of the marine environment. Our interest in their fatty acids was aroused after analysis of a recent marine sediment in which sulfate-reducing bacteria were important links in the final decomposition of the organic matter. The presence of monoenoic iso C_{15} and iso C_{17} fatty acids (4) and of a set of iso and anteiso β-hydroxy fatty acids (5) in these sediments prompted research into the lipids of *Desulfovibrio desulfuricans*, seen as a possible source of these acids in this sedimentary environment. Moreover, the occurrence of iso and anteiso monoenoic fatty acids among the major fatty acids of *D. desulfuricans* has considerable taxonomic and biochemical significance.

The iso and anteiso saturated fatty acids are widely found in gram-positive bacteria. Gram-negative bacteria very often contain straight-chain fatty acids with one double bond. Such characteristic features in the alkyl chain of fatty acids are used as a guide to classification in bacterial taxonomy (23). Unsaturated iso and anteiso fatty acids are still rare in nature. So far, they have been found in phospholipids of the aerobic gram-positive *Bacillus cereus* (15), as an excretion product of *Myxococcus xanthus* (18), and as an acyl constituent in two antibiotics (25).

Their distribution may prove to be more widespread in the future, when more advanced techniques, such as high-resolution gas chromatography and combined gas chromatography-mass spectrometry, find their way among taxonomically oriented bacteriologists. An exclusive presence as major fatty acids in *Desulfovibrio* species would point, on the other hand, to a very special phylogenetic position with respect to other microorganisms.

Desulfovibrio species are thought to be primitive in character. An early offspring from the phylogenetic tree is indicated by its cytochromes (10) and primitive energy metabolism (14, 29). *Desulfovibrio* species are able to biosynthesize iso and anteiso precursors for processing along the anaerobic chain elongation pathway, as is indicated by the double-bond position in their fatty acids. This points to a dehydrase enzyme system (3) able to handle branched-chain substrates. The presence of two enzyme systems in one bacterium which are found to operate separately in other strains of bacteria could be another one of those "vestiges of ancestral biochemistry" that have been enumerated for the sulfate-reducing bacteria (17).

MATERIALS AND METHODS

Organism and culture conditions. A strain of the sulfate-reducing bacteria *D. desulfuricans* was isolated from the anaerobic black sediment of the

Dutch Wadden Sea (J. H. Vosjan, Ph.D. thesis, University of Groningen, Groningen, The Netherlands, 1975). The criteria of Postgate and Campbell (21) were used for identification, and the purity was checked by using the method of Postgate (20).

The organism was grown in batch culture on the following medium: NaCl, 20 g; K_2HPO_4, 0.1 g; $MgCl_2 \cdot 6H_2O$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; yeast extract, 1 g; tris(hydroxymethyl)aminomethane, 6.06 g; $FeSO_3 \cdot 6H_2O$, 0.003 g; $(NH_4)_2SO_4$, 3.3 g; Li-lactate, 3.3 g; concentrated HCl, 3.8 ml; dissolved in 1 liter of water and distilled over $KMnO_4$. The pH was adjusted to 7.5. Cultures were grown anaerobically at 30°C. The cells of an 18-liter batch culture were harvested by continuous centrifugation at the end of the exponential growth phase. The bacterial pellets (16.1 g) were freeze-dried, and the dried bacteria were stored in vacuum-sealed ampoules.

Extraction and isolation of lipid material. Freeze-dried bacteria (1.882 g) were extracted by a modification of the Bligh and Dyer (2) procedure. The bacterial residue were homogenized subsequently in a Waring blender in 20 ml of water, 40 ml of methanol, and 80 ml of chloroform. After filtration the residue was extracted with 50 ml of methanol. Centrifugation yielded a pellet, which was ultrasonically treated in 30 ml of chloroform. The combined extracts yielded a monophasic solution, which was separated into two layers by addition of 40 ml of water. The chloroform layer, containing total lipids, was washed once with water. A 0.269-g amount of total lipid extract was obtained by solvent evaporation, using a rotatory evaporator.

Fatty acids and hydroxy acids in the total lipid extract. A portion of the total lipid extract (34 mg) was saponified in 1 ml of 1 N KOH in 95% ethanol under reflux for 30 min. The non-acid fraction was extracted with diethyl ether. About 13 mg of fatty acids was obtained after acidification and extraction with diethyl ether. These acids were methylated by diazomethane in diethyl ether.

Preparative thin-layer chromatography (TLC) yielded a monocarboxylic and a hydroxy monocarboxylic methyl ester fraction (26). The monocarboxylic methyl esters were analyzed by gas-liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS).

Further characterization was achieved by application of the OsO_4-H_2S method (see below) for the derivatization of the unsaturated methyl esters. Diol methyl esters obtained after TLC separation of the reaction mixture were treated with bis(trimethylsilyl)acetamide (Pierce Chemical Co.) and analyzed by GC-MS as disilyloxy methyl esters. The hydroxy fatty acid methyl esters were treated with bis(trimethylsilyl)acetamide, and the silyloxy derivatives were analyzed by GLC and GC-MS.

OsO_4-H_2S method for preparation of diol esters. About 5 mg of fatty acid methyl esters was taken up in 2 ml of dioxane-pyridine (8:1, vol/vol) in a centrifuge tube. A small crystal of OsO_4 was added, and the solution was occasionally swirled by hand. The yellow solution turned orange-red during 2 h of reaction in the dark at room temperature (about 20°C). Diol fatty acid methyl esters were produced

from osmate esters by bubbling gaseous H_2S through the solution until saturation. Excess H_2S was removed by bubbling nitrogen for 1 h. A black osmium salt was precipitated from suspension by centrifugation. The supernatant was concentrated under a stream of nitrogen to a volume suitable for TLC and/or GC-MS after silylation by bis(trimethylsilyl)acetamide.

TLC separation of reaction products. Separation of reaction products resulting from the OsO_4-H_2S and OsO_4-Na_2SO_3 methods (8, 9) was achieved by silica gel TLC (0.25 mm) using hexane-ethylacetate (7:3, vol/vol) as developer. TLC bands were visualized by iodine vapor and/or 0.05% rhodamine 6G in 95% ethanol. In this TLC system diol fatty acid methyl esters had an R_f of 0.20, whereas saturated methyl esters had an R_f of 0.80.

GLC and GC-MS conditions. GC was carried out on a Perkin-Elmer 990 equipped with 30-m, stainless-steel open tubular columns (ID, 0.25 mm) coated with OV-101. Nitrogen was used as carrier gas.

Monocarboxylic fatty acid methyl esters were analyzed using temperature programming from 140 to 250°C at a rate of 2°C/min, β-silyloxy fatty acid methyl esters were analyzed using temperature programming from 170 to 260°C at a rate of 4°C/min, and disilyloxy fatty acid methyl esters were analyzed using temperature programming from 200 to 300°C at a rate of 2°C/min. Quantitative data were obtained by applying an Infotronics CRS 101 electronic integrator to the gas chromatograph.

GC-MS was carried out using a Varian-MAT 111 instrument equipped with either a 21-m, stainless-steel open tubular column (ID, 0.25 mm) coated with SP 2250 or a 30-m, stainless-steel open tubular column (ID, 0.25 mm) coated with OV-101. The carrier gas used was He. The mass spectrometer was used under standard conditions at 80 eV. Temperature programming was carried out under conditions comparable to those described above for the GLC analysis.

Accurate mass analysis and metastable analysis. For accurate mass analysis, the mixture of disilyloxy methyl esters, obtained by the OsO_4-$NaSO_3$ method, was used. This mixture, consisting almost exclusively of iso and anteiso 9,10-disilyloxy-C_{17} methyl ester and the normal 9,10-disilyloxy-C_{16} methyl ester, was analyzed by direct probe analysis on a MAT 311 A mass spectrometer.

Accurate masses determined were: m/e 111 = C_8H_{15} (experimental, 111.11712; calculated, 111.117370); m/e 201 = $C_{11}H_{25}OSi$ (experimental, 201.17187; calculated, 201.174458); m/e 109 = C_8H_{13} (experimental, 109.10393; calculated, 109.101720); m/e 187 = $C_{10}H_{23}OSi$ (experimental, 187.15146; calculated, 187.158809).

Metastable analysis was applied to this mixture to determine the origin of fragment ions m/e 111, m/e 109, and m/e 97 both with the defocusing technique and with direct analysis of daughter ions (see Fig. 1).

Standard reference compounds. Some saturated normal, iso, and anteiso fatty acid methyl esters were purchased from Applied Science Laboratories.

A mixture of branched fatty acid methyl esters (5), prepared from Mackerel (*Scromber scombrus*) lipids in our laboratory, was also used for comparison.

Mass spectra and equivalent drain length values were obtained from a mixture of normal, iso, and anteiso β-silyloxy fatty acid methyl esters, synthesized from the corresponding fatty acid methyl esters (5). Normal $\Delta9$-$C_{18:1}$, $\Delta7$-$C_{16:1}$, and $\Delta5$-$C_{14:1}$ fatty acid methyl esters were purchased from Applied Science Laboratories. These were used for preparation of corresponding disilyloxy compounds, using the OsO_4-H_2S method. Normal $\Delta9$-$C_{17:1}$, iso $\Delta9$-$C_{17:1}$, 15-deutero-iso $\Delta9$-$C_{17:1}$, and anteiso $\Delta9$-$C_{17:1}$ methyl esters were synthesized, and their mass spectra were used for comparison (J. J. Boon et al., submitted for publication).

RESULTS

Fatty acids of *D. desulfuricans*. Figure 2 presents the gas chromatogram of the fatty acid methyl esters obtained from the total lipid extract of *D. desulfuricans*. The numbers indi-
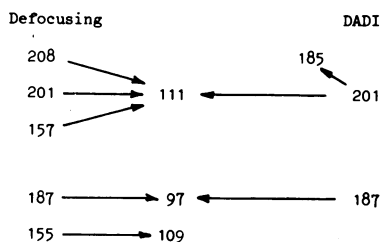

FIG. 1. *Origin of some fragment ions from disilyloxy methyl esters, determined by defocusing and the direct analysis of daughter ions (DADI) technique on a Varian MAT 311 A mass spectrometer.*

cated correspond with those in Table 1, listing the compounds and their relative abundances.

The identification is based on mass spectral data of the methyl esters and the disilyloxy methyl esters, which were prepared to determine the double-bond positions in the monoenoic fatty acid methyl esters. Iso and anteiso methyl branches in the saturated methyl esters can be judged from the mass spectral data by the enhanced intensity of [M+-43] and [M+-57], the intensity reversal of the fragment ions [M+-29] and [M+-31], and the fragment ions [M+-61] and [M+-79] present in the mass spectra of anteiso methyl esters (19). Equivalent chain length values aided in the identification. Straight-chain monoenoic methyl esters are characterized by their parent ion and the high-intensity fragment ions [M+-32], [M+-74], and [M+-116] (22). Branched-chain monoenoic methyl esters present a somewhat different mass spectrum (see Fig. 3A and B). The iso $C_{17:1}$ methyl ester is also characterized by m/e 227 [M+-55], m/e 195 [M+-87], and m/e 177 [M+-105]. The anteiso $C_{17:1}$ methyl ester mass spectrum shows a similar set of fragment ions but is 14 mass units lower. This is pointed out by m/e 213 [M+-69], m/e 181 [M+-101], and m/e 163 [M+-119]. Comparable fragment ions are found in the mass spectra of iso $C_{15:1}$, anteiso $C_{15:1}$, iso $C_{16:1}$, and iso $C_{18:1}$.

The mass spectrum of iso $\Delta11$-$C_{17:1}$ methyl ester isolated from *M. xanthus* (18) presents the same characteristics. The explanation for fragment ion [M+-87], considered by these authors
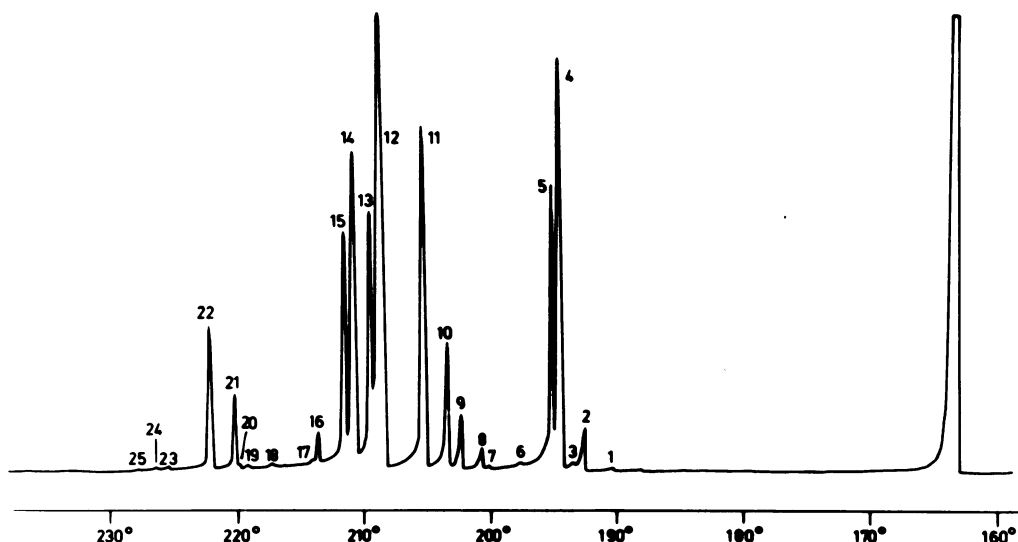

FIG. 2. *Gas chromatogram of the fatty acid methyl esters obtained from the total lipid extract of D. desulfuricans. Numbered peaks are listed in Table 1. A 30-m OV-101-coated capillary column was used for analysis, and column temperature was programmed from 160 to 250°C at a rate of 2°C/min.*

TABLE 1. Fatty acids of D. desulfuricans

| Compound	Systematic name	Relative abundance	Peak no. in Fig. 2
Normal $C_{14:0}$	Tetradecanoic	0.008	1
Iso $\Delta7$-$C_{15:1}$	13-Methyltetradec-7-enoic	0.051	2
Anteiso $\Delta7$-$C_{15:1}$	12-Methyltetradec-7-enoic	0.013	3
Iso $C_{15:0}$	13-Methyltetradecanoic	0.583	4
Anteiso $C_{15:0}$	12-Methyltetradecanoic	0.281	5
Normal $C_{15:0}$	Pentadecanoic	0.006	6
Iso $\Delta7$-$C_{16:1}$	14-Methylpentadec-7-enoic	0.002	7
Iso $\Delta9$-$C_{16:1}$	14-Methylpentadec-9-enoic	0.028	8
Iso $C_{16:0}$	14-Methylpentadecanoic	0.058	9
Normal $\Delta7$-$C_{16:1}$	Hexadec-7-enoic	Unresolved trace amount	10
Normal $\Delta9$-$C_{16:1}$	Hexadec-9-enoic	0.150	
Normal $C_{16:0}$	Hexadecanoic	0.503	11
Iso $\Delta9$-$C_{17:1}$	15-Methylhexadec-9-enoic	1.000	12
Anteiso $\Delta9$-$C_{17:1}$	14-Methylhexadec-9-enoic	0.260	13
Iso $C_{17:0}$	15-Methylhexadecanoic	0.447	14
Anteiso $C_{17:0}$	14-Methylhexadecanoic	0.238	15
Normal $C_{17:0}$	Heptadecanoic	0.027	16
Unknown			17
Iso $\Delta11$-$C_{18:1}$	16-Methylheptadec-11-enoic	0.004	18
Iso $C_{18:0}$	16-Methylheptadecanoic	0.002	19
Normal $\Delta9$-$C_{18:1}$	Octadec-9-enoic	0.071	20
Normal $\Delta11$-$C_{18:1}$	Octadec-11-enoic		21
Normal $C_{18:0}$	Octadecanoic	0.142	22
Iso $\Delta11$-$C_{19:1}$	17-Methyloctadec-11-enoic	0.004	23
Anteiso $\Delta11$-$C_{19:1}$	16-Methyloctadec-11-enoic	0.002	24
Iso $C_{19:0}$	17-Methyloctadecanoic	0.001	25
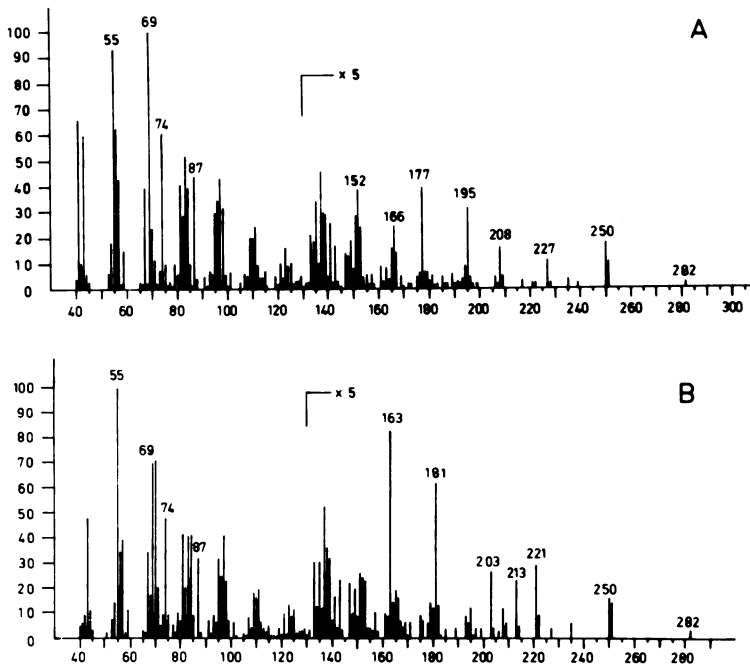
FIG. 3. Mass spectrum of iso $\Delta9$-$C_{17:1}$ fatty acid methyl ester (A) and anteiso $\Delta9$-$C_{17:1}$ fatty acid methyl ester (B). Both mass spectra were obtained by capillary GC-MS of methyl esters obtained from the total lipid extract of D. desulfuricans.

to be loss of $CH_3OCOCH_2CH_2$, is to be criti-
cized. It does not explain the loss of 101 mass
units in the case of the anteiso methyl ester.
Evidence from direct analysis of daughter ions,
applied to synthetic standards and the 15-deu-
tero-iso $\Delta 9$-$C_{17:1}$ methyl ester points to another
mechanism of fragmentation: the tertiary H or
D (in the 15 position) is transferred to the car-
boxyl end of the molecule (J. J. Boon et al.,
submitted for publication). This initial rear-
rangement is followed by the loss of 55 or 69
atomic mass units dependent on, respectively,
the iso or anteiso structure of the unsaturated
fatty acid methyl ester. This is shown in Fig.
3A and B by fragments m/e 227 and m/e 213.

Methanol and water are lost subsequently
from these fragment ions, thus generating m/e
195 and m/e 177 in the case of the iso compound
and m/e 181 and m/e 163 in the case of the
anteiso compound. In this way the iso and an-
teiso structure of branched monoenoic fatty
acid methyl esters can be determined from the
presence of definite fragment ions, whereas in
case of the corresponding saturated esters in-
tensity differences from some fragment ions
must be used for the identification. It should be
noted that [M^+-61] and [M^+-79] are also present
in the mass spectra of anteiso mono-unsatu-
rated methyl esters.

Hydroxy acids of *D. desulfuricans*. Hydroxy
fatty acids were found in the acid fraction ob-
tained after alkaline hydrolysis of the total
lipid extract. Figure 4 presents the gas chro-
matogram of the hydroxy acids analyzed as sil-
yloxy methyl esters. Table 2 lists the com-
pounds identified.

The mass spectra agree with those of stan-
dards (11). Iso, anteiso, and normal β-silyloxy

methyl esters cannot be distinguished by their
mass spectra (5). Comparison with ECL values,
obtained from a mixture of iso and anteiso β-
silyloxy methyl esters, aided in the identifica-
tion. The α,β-unsaturated methyl ester identi-
fied is considered to be the result of elimination
of water from the β-hydroxy methyl esters after
TLC separation. The other methyl esters found
in trace quantity in this fraction are considered
to be impurities resulting from tailing on the
TLC plate. The relative amount of the β-hy-
droxy fatty acids as compared to the fatty acids
is small.

Usefulness of the disilyloxy derivatives. Un-
equivocal determination of the double-bond po-
sition in mono-unsaturated methyl esters is
possible by modification of the double bond with
OsO_4 and ultimate analysis of the reaction
products as disilyloxy methyl esters by GC-MS.

The orginal double-bond position is marked
by high-intensity mass spectral fragment ion
peaks generated as a result of the cleavage of
the carbon bond between the two silyloxy

TABLE 2. *Hydroxy fatty acids of D. desulfuricans*

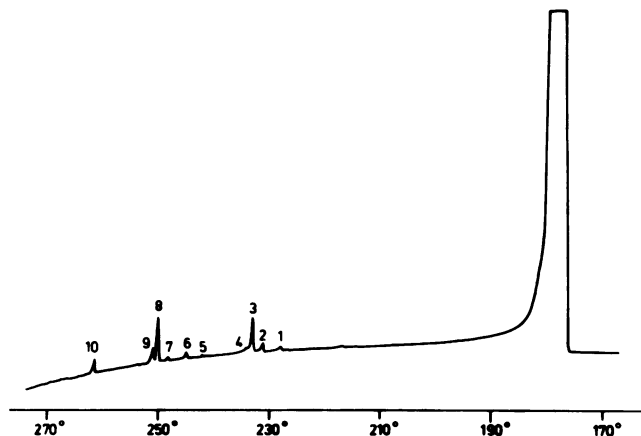| Compound	Peak no. in Fig. 4
β-OH-iso $C_{15:0}$	3
β-OH-anteiso $C_{15:0}$	4
β-OH-iso $C_{16:0}$	5
β-OH-normal $C_{16:0}$	6
β-OH-iso $C_{17:0}$	8
β-OH-anteiso $C_{17:0}$	9
β-OH-normal $C_{18:0}$	10
α,β-Unsaturated methyl ester	1
Normal $C_{16:0}$ methyl ester	2
Normal $C_{18:0}$ methyl ester	7

FIG. 4. *Gas chromatogram of the β-hydroxy acids of D. desulfuricans analyzed as trimethylsilyloxy methyl esters on a 30-m OV-101-coated capillary column. Column temperature was programmed from 170 to 270°C at a rate of 4°C/min. Numbered peaks are listed in Table 2.*

groups (8, 11). So far, this method has been applied to single pure compounds of various kinds (11, 16, 31). We applied this modification reaction to a mixture of saturated and mono-unsaturated methyl esters. An important modification of the earlier procedure used is the reduction of osmates. The Na_2SO_3-methanol-water method was replaced by a reduction with H_2S (1) to avoid the loss of volatile compounds, to increase ease of handling, and to make possible a scaling down to small samples. The reaction mixture obtained can be separated by TLC into saturated methyl esters and diol methyl esters or, alternatively, can be analyzed as such. The latter approach saves time and volatile compounds but is dependent on the composition of the mixture analyzed (unless the mass spectral data acquisition is computerized). Both approaches have been used successfully in this work on *Desulfovibrio* acids.

In the course of the mass spectral examination of the disilyloxy compounds, no direct obvious differences were observed between the mass spectra of normal and branched-chain compounds. However, in Fig. 5, comparing the normal and iso 9,10-disilyloxy C_{16} methyl ester, an enhancement of fragment m/e 97 is observed in the mass spectrum of the iso compound. This phenomenon is also found in the other branched disilyloxy compounds (Table 3).

It could be shown by accurate mass measurements and the application of defocusing and the direct analysis of daughter ions technique that m/e 187 and m/e 201 produce, m/e 97 and m/e 111 (see Fig. 1), respectively. Fragment ions m/e 187 and m/e 201 are ions consisting of the alkyl part of the disilyloxy compound containing one trimethylsilyloxy group. Preterminal branching in this alkyl part is apparently enough to enhance the loss of trimethylsilanol (90 mass units) from these fragment ions.

DISCUSSION

D. desulfuricans can be characterized by its straight-chain and branched-chain fatty acids. Preliminary results by TLC and field desorption MS indicate that these fatty acids are present in phospholipids, plasmalogens, and as acylglucosamine derivatives. The phospholipid composition is in qualitative agreement with data on other *Desulfovibrio* species (19). The branched-chain fatty acids of *D. desulfuricans* are the most characteristic part of its fatty acids. About 34% of all fatty acids present show the unusual combination of methyl branching and unsaturation in the alkyl chain.

The determination of the double-bond position in these fatty acids was considered necessary to understand aspects of their biosynthesis and to increase their taxonomical value. There-
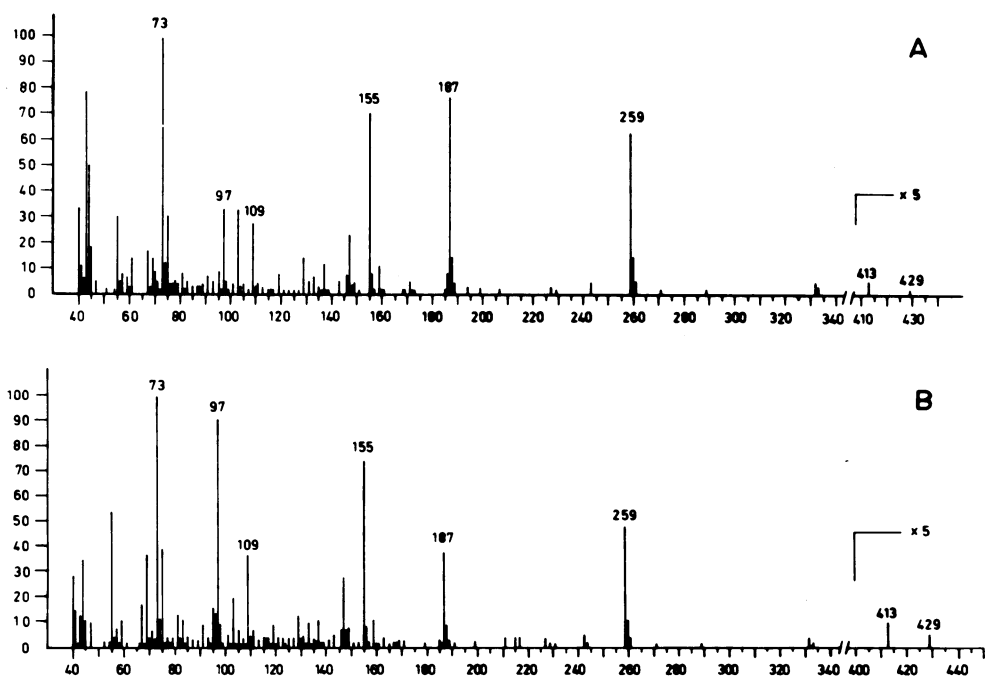

FIG. 5. *Mass spectrum of normal 9,10-disilyloxy C_{16} methyl ester (A) and iso 9,10-disilyloxy C_{16} methyl ester (B).*

TABLE 3. *Major fragment ions and relative intensity of some normal and branched-chain disilyloxy fatty acid methyl esters*

Original compound	R_y		R_y-90		R_y-90/R_y	R_x	
	Fragment ion	Intensity (%)	Fragment ion	Intensity (%)		Fragment ion	Intensity (%)
Branched[a] $\Delta 7$-$C_{15:1}$	201	36	111	69	1.91	231	57
Branched $\Delta 9$-$C_{16:1}$	187	39	97	91	2.34	259	48
Normal $\Delta 9$-$C_{16:1}$	187	79	97	33	0.42	259	63
Branched $\Delta 9$-$C_{17:1}$	201	39	111	60	1.54	259	69
Branched $\Delta 11$-$C_{18:1}$	187	11	97	53	4.82	287	6
Normal $\Delta 9$-$C_{18:1}$	215	13	125	4	0.31	259	10
Normal $\Delta 11$-$C_{18:1}$	187	47	97	35	1.34	287	14
Branched $\Delta 11$-$C_{19:1}$	201	9.7	111	40	4.13	287	11

[a] Branched = iso or anteiso.

fore, we modified an existing technique used for determination of double bonds in single pure compounds in such a way that it could be used for mixtures of fatty acids. The anaerobic metabolism of *Desulfovibrio* species indicates that the so-called "anaerobic pathway of monoenoic fatty acid synthesis" is the biosynthetic mechanism to consider. Bloch and co-workers thoroughly investigated this pathway for a strain of *Escherichia coli* (K-12) (3) and for *Clostridium kluyveri* (25). Other microorganisms investigated by using this pathway have been summarized in a review (12).

The usual fatty acid synthesis sequence (30) is split at the β-hydroxy C_{10} level by a D-β-hydroxydecanoyl thioester dehydrase (7) in *cis*-β,γ-decenoyl-ACP and *trans*-α,β-decenoyl-ACP.

The *cis*-β,γ-decanoyl derivative is elongated and leads to $\Delta 5$-$C_{12:1}$, $\Delta 7$-$C_{14:1}$, $\Delta 9$-$C_{16:1}$, and $\Delta 11$-$C_{18:1}$, the so-called *cis*-vaccenic acid family. The double bonds in the fatty acids of *Desulfovibrio* make certain that this biosynthetic principle is operative not only on straight-chain, but also on branched-chain fatty acids in these bacteria.

The presence of the branched-chain vaccenic acid family in *Desulfovibrio* must have a direct relation to the properties of its D-β-hydroxydecanoyl thioester dehydrase enzyme. Assuming that a dehydrase similar to the one in *E. coli* and *C. kluyveri* is present in *D. desulfuricans*, then an 8-methyl-β-hydroxydecanoyl-ACP is the precursor for anteiso $\Delta 7$-$C_{15:1}$ and anteiso $\Delta 9$-$C_{17:1}$. The 9-methyl-β-hydroxydecanoyl-ACP is the precursor for iso $\Delta 7$-$C_{15:1}$, iso $\Delta 9$-$C_{17:1}$, and $\Delta 11$-$C_{19:1}$. The precursors for iso $\Delta 7$-

$C_{16:1}$ and that for iso $\Delta 9$-$C_{16:1}$ and iso $\Delta 11$-$C_{18:1}$ are the 10-methyl-β-hydroxyundecanoyl-ACP and the 8-methyl-β-hydroxynonanoyl-ACP, respectively. The *E. coli* dehydrase (24) was shown to be highly specific for the straight-chain β-hydroxydecanoyl-ACP derivative; C_9 and C_{11} were much less active, whereas C_8 and C_{12} were almost inert substrates.

In *D. desulfuricans* the dehydrase preferentially attacks the normal β-OH-C_{10} compound, judged from the preponderance of normal $\Delta 9$-$C_{16:1}$ and normal $\Delta 11$-$C_{18:1}$. The enzyme is presumably not hindered by the presence of the methyl group in the 9-methyl-C_{10} derivative, which leads to a large relative abundance of the odd iso compounds.

The 8-methyl-C_{10} compound (alternatively "interpreted" by the enzyme as an 8-ethyl-C_9 compound) and the 8-methyl-C_9 and the 10-methyl-C_{11} derivatives are less suitable substrates. Only very small amounts of the normal $\Delta 9$-$C_{18:1}$ and normal $\Delta 7$-$C_{16:1}$ are present, which means that the β-OH-C_{12} compound is a very poor substrate. These conclusions, based on indirect evidence, need further confirmation with the purified dehydrase. The presence of several dehydrases for the generation of straight- and branched-chain fatty acids cannot be ruled out.

Little comparative work has been done concerning the fatty acids of *Desulfovibrio* species, although there is a trend in bacteriology to use fatty acid spectra for taxonomical purposes (23). Unfortunately, extracts from bacteria sometimes suffer from the absence of detailed structural identification. The excellent GLC traces of the fatty acids of *Desulfovibrio vulgaris* Hil-

denborough, *D. desulfuricans* Essex 6, and *D. desulfuricans* Berre Sol in the work of Han (J. C.-Y. Han, Ph.D. thesis, University of California, Berkeley, 1970), however, allow comparison with our results.

The presence of the anaerobic pathway of monoenoic fatty acid synthesis was shown in these bacteria by GC identification of double bonds in the straight-chain C_{16} and C_{18} acids, using the corresponding standards. Although iso and anteiso $C_{15:0}$ and $C_{17:0}$ fatty acids were identified, the identification of several major peaks, which correspond in our view with the monoenoic iso and anteiso fatty acids, was not reported. The GLC trace of the Hildenborough strain shows close resemblance to our species of *D. desulfuricans*. A comparative study of the fatty acids in several species of *Desulfovibrio* and, preferably, also in species of *Desulfotomaculum* (21) would be of taxonomical and biochemical interest. This approach would enable the evaluation of the ability of these organisms to use branched-chain β-hydroxy C_9, C_{10}, and C_{11} compounds as substrates for their β-hydroxydecanoyl thioester dehydrase. The properties of the dehydrase enzyme system itself can also be used as a means to understand the relationship between the bacterial strains that use the anaerobic pathway of monoenoic fatty acid synthesis.

The distribution of this pathway is not restricted to anaerobic microorganisms but follows evolutionary lines (24). This pathway has been shown to occur in aerobic, facultative anaerobic, anaerobic, and photosynthetic microorganisms, e.g., *Pseudomonas fluorescens* (24), *E. coli* K-12 (3), lactobacilli (27), pediococci (27), *C. kluyveri* (24), *Rhodospirillum rubrum* (32), and *Rhodopseudomonas sphaeroides* (32). Branched-chain fatty acids have not been reported among the fatty acids of these microorganisms. If their dehydrase enzyme systems were able to use branched-chain substrates, one could think of a relationship with *Desulfovibrio* species, which are thought to be close to "the" ancestral mother microbe (17).

The composition of fatty acids present in phospholipids, which play an important role in the cell membrane of microorganisms, determines to a large extent the fluidity of the membrane. This fluidity is necessary for the proper function of membrane-related metabolic processes (28). Several bacteria are able to change the degree of unsaturation of their lipids upon lowering the growth temperature by enhanced desaturase activity (13). Another way to lower the melting temperature of lipids is to increase the amount of iso and anteiso fatty acids in these lipids. The amount of branched saturated

and unsaturated fatty acids in phospholipids of *D. desulfuricans* is surprisingly high in view of the growth temperature in the culture (30°C). Several explanations for this phenomenon, such as inability to regulate fatty acid composition in the cell membrane lipids, a special structure of the cell wall, the influence of culture factors, etc., are under investigation.

The branched-chain β-hydroxy fatty acids present a problem in respect to their origin and role in the organism. The analytical procedure points to a free or esterified (via the carboxyl group) occurrence in the total lipid extract. No free hydroxy acids have been observed in the neutral lipids after column chromatography.

β-Hydroxy fatty acids are present in the cell wall of gram-negative bacteria, usually bound to the lipid A component (6). This lipid is usually not extractable by mild organic solvents. Further work is necessary to pinpoint these hydroxy acids in the bacterium.

LITERATURE CITED

1. **Barton, D. H. R., and D. Elad.** 1956. Colombo Root Bitter principles. Part I. The functional groups of columbin. J. Chem. Soc., p. 2085–2090.
2. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917.
3. **Bloch, K.** 1969. Enzymatic synthesis of monounsaturated fatty acids. Acc. Chem. Res. 2:193–202.
4. **Boon, J. J., J. W. de Leeuw, and P. A. Schenck.** 1975. Organic geochemistry of Walvis Bay diatomaceous ooze. I. Occurrence and significance of the fatty acids. Geochim. Cosmochim. Acta 39:1559–1565.
5. **Boon, J. J., F. de Lange, P. J. W. Schuyl, J. W. de Leeuw, and P. A. Schenck.** 1977. Organic geochemistry of Walvis Bay diatomaceous ooze. II. Occurrence and significance of the hydroxy fatty acids. *In* E. Campos (ed.), Advances in organic geochemistry 1975, Proceedings of the 7th International Meeting on Organic Geochemistry in Madrid.
6. **Braun, V., and K. Hantke.** 1974. Biochemistry of bacterial cell envelopes. Annu. Rev. Biochem. 43:89–121.
7. **Brock, D. J. H., L. R. Kass, and K. Bloch.** 1967. β-Hydroxydecanoyl thioester dehydrase. II. Mode of action. J. Biol. Chem. 242:4432–4440.
8. **Capella, P., and C. M. Zorzut.** 1968. Determination of double bond position in monounsaturated fatty acid esters by mass spectrometry of their trimethylsilyloxy derivatives. Anal. Chem. 40:1458–1464.
9. **Criegee, R.** 1936. Osmiumsäure-ester als Zwischenprodukte bei Oxydationen. Justus Liebigs Ann. Chem. 522:75–96.
10. **Dickerson, R. E., and R. Timkovich.** 1975. Cytochrome C, p. 397–547. *In* P. D. Boyer (ed.), The enzymes, vol. 11A. Academic Press Inc., New York.
11. **Eglinton, G., D. H. Hunneman, and A. McCormick.** 1968. Gaschromatographic-mass spectrometric studies of long chain hydroxy acids. I. The mass spectra of the methylesters trimethyl-silylethers of aliphatic

hydroxy acids. A facile method of double bond location. Org. Mass Spectrom. 1:593–611.

12. **Erwin, J. A.** 1973. Comparative biochemistry of fatty acids in eukaryotic microorganisms, p. 41–145. *In* J. A. Erwin (ed.), Lipids and biomembranes of eukaryotic microorganisms. Academic Press Inc., New York.

13. **Fulco, A. J.** 1969. The biosynthesis of unsaturated fatty acids by *Bacilli*. I. Temperature induction of the desaturation. J. Biol. Chem. 244:889–895.

14. **Gottschalk, G.** 1968. The stereospecificity of the citrate synthase in sulfate-reducing and photosynthetic bacteria. Eur. J. Biochem. 5:346–351.

15. **Kaneda, T.** 1972. Positional preference of fatty acids in phospholipids of *Bacillus cereus* and its relation to growth temperature. Biochim. Biophys. Acta 280:297–305.

16. **Karlander, S. G., K. A. Karlsson, H. Leffler, A. Lilja, B. E. Samuelson, and G. O. Steen.** 1972. The structure of sphingomyelin of the honey bee (*Apis mellifera*). Biophys. Biochim. Acta 270:117–131.

17. **Le Gall, J., and J. R. Postgate.** 1973. The physiology of sulphate-reducing bacteria, p. 81–134. *In* A. H. Rose and D. W. Tempest (ed.), Advances in microbial physiology, vol. 10. Academic Press Inc., New York.

18. **Norén, B., and G. Odham.** 1973. Antagonistic effects of *Myxococcus xanthus* on fungi. II. Isolation and characterisation of inhibitory lipid factors. Lipids 8:573–583.

19. **Makula, R. A., and W. R. Finnerty.** 1975. Phospholipid composition of *Desulfovibrio* species. J. Bacteriol. 120:1279–1283.

20. **Postgate, J. R.** 1965. Enrichment and isolation of sulphate reducing bacteria, p. 190–205. *In* H. G. Schlegel (ed.), Anreicherungkultur und Mutantenauslese. Gustav Fischer Verlag, Stuttgart.

21. **Postgate, J. R., and L. L. Campbell.** 1966. Classification of *Desulfovibrio* species, the nonsporulating sul-

fate-reducing bacteria. Bacteriol. Rev. 30:732–738.

22. **Ryhage, R., and E. Stenhage.** 1960. Mass spectrometry in lipid research. J. Lipid Res. 1:361–390.

23. **Shaw, N.** 1974. Lipid composition as a guide to classification of bacteria, p. 63–109. *In* D. Perlman (ed.), Advances in applied microbiology, vol. 17. Academic Press Inc., New York.

24. **Scheuerbrandt, G., and K. Bloch.** 1962. Unsaturated fatty acids in microorganisms. J. Biol. Chem. 273:2064–2068.

25. **Shoji, J., and H. Otsuka.** 1969. Studies on Tsushimycin. II. The structures of constituent fatty acids. J. Antibiot. 12:473–479.

26. **Skipski, V. P., A. F. Smolowne, R. C. Sullivan, and M. Barclay.** 1965. Separation of lipid classes by thin-layer chromatography. Biochim. Biophys. Acta 106:386–396.

27. **Uchida, K., and K. Mogi.** 1972. Cellular fatty acid spectra of *Pediococcus* species in relation to their taxonomy. J. Gen. Appl. Microbiol. 18:109–129.

28. **Vessey, D. A., and D. Zakim.** 1974. Membrane fluidity and the regulation of membrane bound enzymes, p. 138–175. *In* E. Quagliarello, F. Palmieri, and T. P. Singer (ed.), Horizons in biochemistry and biophysics, vol. 1. Addison-Wesley Publishing Co., Reading, Mass.

29. **Vosjan, J. H.** 1970. ATP generation by electron transport in *Desulfovibrio desulfuricans*. Anthonie van Leeuwenhoek 36:585–587.

30. **Wakil, S. J. (ed.).** 1970. Lipid metabolism. Academic Press Inc., New York.

31. **Wolff, R. E., G. Wolff, and J. A. McCloskey.** 1966. Characterisation of unsaturated hydrocarbons by mass spectrometry. Tetrahedron 22:3093–3101.

32. **Wood, B. J. B., B. W. Nichols, and A. T. James.** 1965. The lipids and fatty acid metabolism of photosynthetic bacteria. Biochim. Biophys. Acta 106:261–273.