A new family of very long chain α,ω-dicarboxylic acids is a major structural fatty acyl component of the membrane lipids of *Thermoanaerobacter ethanolicus* 39E

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Abstract A new family of α,ω-dicarboxylic, very long chain fatty acids was isolated and characterized from the lipids of thermophilic anaerobic eubacterium, *Thermoanaerobacter ethanolicus* 39E. After the isolation of the membrane, the fatty acyl components were converted to methyl esters by acid-catalyzed methanolysis. The esterified fatty acyl components were purified by a variety of chromatographic techniques and analyzed by gas chromatography (GC) and GC–mass spectrometry (MS). One of the isolated, esterified α,ω-dicarboxylic, very long chain fatty acids was characterized by mass spectrometry, ^{1}H and ^{13}C NMR spectroscopy and Fourier transform infrared spectroscopy. NMR experiments used included double quantum filtered correlated spectroscopy (DQF-COSY) to establish spin connectivities and polarization transfer (DEPT) to measure the multiplicity of carbon signals split by protons. Based on these results, the structures of the other components could be deduced from their mass spectra. The new family of very long chain fatty acid methyl esters are α,ω-13,16-dimethylheptacosanedioate dimethyl ester (C29), α,ω-13,16-dimethyloctacosanedioate dimethyl ester (C30), α,ω-13,16-dimethylnonacosanedioate dimethyl ester (C31), and α,ω-13,16-dimethyltriacotanedioate dimethyl ester (C32). This family of fatty acids make up about 40% of fatty acyl components of the membrane of *Thermoanaerobacter ethanolicus* 39E. Almost all (> 90%) of the very long chain, α,ω-dicarboxylic fatty acid was α,ω-13,16-dimethyloctacosanedioic acid. A careful analysis of the structures of the α,ω-dicarboxylic acid strongly implies that the synthetic mechanism for formation is by tail-to-tail (ω)coupling of regular *iso*-branched fatty acids across opposite sides of the membrane.—**Jung, S., J. G. Zeikus, and R. I. Hollingsworth.** A new family of very long chain α,ω-dicarboxylic acids is a major structural fatty acyl component of the membrane lipids of *Thermoanaerobacter ethanolicus* 39E. *J. Lipid Res.* 1994. **35:** 1057–1065.

Supplementary key words thermophilic eubacterium • dicarboxylic fatty acids • membrane fluidity • gas chromatography-mass spectrometry • nuclear magnetic resonance • tail-to-tail coupling

The optimum temperature for growth of the thermophilic eubacterium, *Thermoanaerobacter ethanolicus* 39E, is 67–69°C; the maximum temperature at which growth occurs is 76–78°C; the minimum is 42°C (1, 2). The structural basis for the stability of membranes of thermophilic bacteria has been somewhat of a puzzle because of the high proportions of branched fatty acids found in them. A number of physical studies, using either model or natural membranes enriched in typical regular chain branched-chain fatty acids, indicate that these molecules act as membrane "fluidizers" rather that "stabilizers" as would be expected (3). These studies suggest homeophasic rather than homeoviscous adaptation (4, 5) for thermophilic membrane because branched fatty acids cause membranes containing them to be particularly resistant to formation of non-bilayer phases. High temperature induces high degrees of motion of lipids, through which the dynamics or fluidity of the membrane is critically affected. To circumvent this adverse effect, phase transitions of the membrane mediated by branched fatty acids were proposed. It is possible that there are other specific adaptive mechanisms by which membranes of thermophilic organisms maintain optimal dynamic functionality at their high growth temperatures.

It is well acknowledged that, in bacteria, adaptive changes in the membrane structure in response to increased temperature involve the increase in the average chain lengths of fatty acid residues (6, 7), the synthesis of cyclohexane-containing fatty acids (8, 9), and the synthesis of hopanes (10–12). These processes all require the activation of some critical membrane activity which is thermally regulated. In this study, we demonstrate that an

Abbreviations: GC, gas chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; DQF-COSY, double quantum filtered correlated spectroscopy; DEPT, distortionless enhanced polarization transfer; FTIR, Fourier transform infrared spectroscopy.

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adaptive response, which we demonstrated in another organism, *Sarcina ventriculi,* involving the synthesis of very long bifunctional fatty acid species (13), is the established mechanism by which *T. ethanolicus* 39E has adapted for optimum growth at extremely high temperatures.

MATERIALS AND METHODS

Bacterial cultures and membrane isolation

T. ethanolicus 39E was isolated from Ocopus Spring at Yellowstone National Park (14) and is deposited in the American Type Culture Collection (Rockville, MD) as ATCC 33223. The cells were grown on a complex medium that contained yeast extract, trypticase, trace salts, and vitamins (TYE medium) with either 0.5% xylose or glucose as the fermentable carbohydrate under stringent anaerobic condition at 65°C (1). Bacterial membranes were isolated as described before (13).

Total fatty acid analysis

To 1 ml membrane suspension, isolated as described earlier (13), was added 3 ml chloroform followed by 15 ml 5% methanolic HCl solution. The suspension was heated at 72°C for 12 h. Three-ml portions of chloroform were added every 6 h followed by mild sonication for 5 min after each addition. The mixture was then concentrated to dryness on the rotary evaporator and the fatty acid methyl esters were isolated by partitioning the residue between chloroform and water and extraction with chloroform several times. The fatty acid methyl esters isolated above were subjected to gas chromatographic analysis on a 25-m J&W Scientific DB1 capillary column using helium as carrier gas. The temperature program was: 150°C initial temperature, 0.00 min hold time, a first ramp of 3.0 deg/min rate to a temperature of 200°C. A second ramp of 4.0 deg/min was immediately started to a final temperature of 300°C with a hold time of 30 min. GC–MS (gas chromatography–mass spectrometry) analyses were performed on a Jeol JMS-AX505H spectrometer interfaced with a Hewlett-Packard 5890A gas chromatograph.

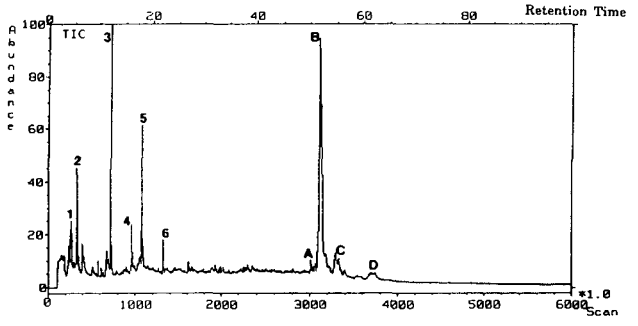

Fig. 1. Total ion chromatogram of GC–MS analysis for the esterified fatty acyl components of the membrane of *T. ethanolicus* 39E. The later eluting cluster of peaks is due to very long chain α,ω-bifunctional fatty acids. 1. $C_{16:1}$ *iso*-branched fatty aldehyde ($OHC(CH_2)_{12}(CH_3)CHCH_3$); 2. $C_{14:0}$ *iso*-branched carboxylic acid methyl ester ($OCH_3CO(CH_2)_{10}(CH_3)CHCH_3$); 3. $C_{15:0}$ *iso*-branched carboxylic acid methyl ester ($OCH_3CO(CH_2)_{11}(CH_3)CHCH_3$); 4. $C_{16:0}$ *iso*-branched carboxylic acid methyl ester ($OCH_3CO(CH_2)_{12}(CH_3)CHCH_3$); 5. $C_{17:0}$ *iso*-branched carboxylic acid methyl ester ($OCH_3CO(CH_2)_{13}(CH_3)CHCH_3$); 6. unknown; A. $C_{29:0}$ α,ω-dicarboxylic dimethyl ester; B. $C_{30:0}$ α,ω-dicarboxylic dimethyl ester; C. $C_{31:0}$ α,ω-dicarboxylic dimethyl ester; D. $C_{32:0}$ α,ω-dicarboxylic dimethyl ester.

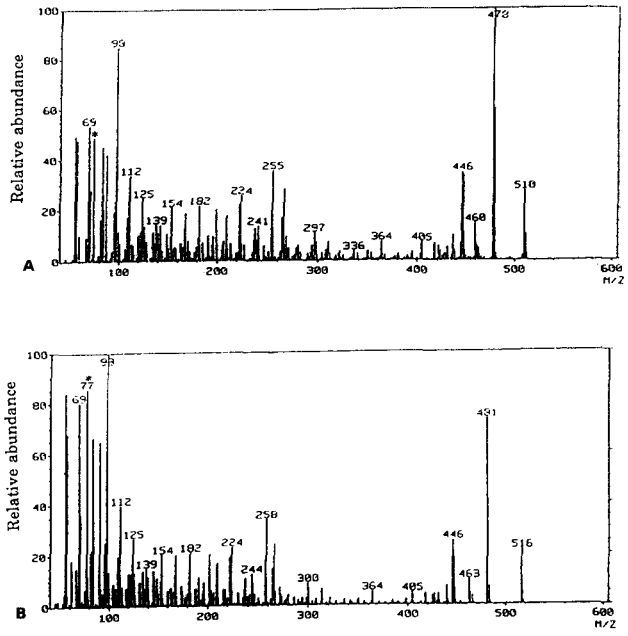

Fig. 2. (A) Electron impact mass spectrum (70 eV) of peak B. Major ions appear at *m/z* 510, 478, and 447. These correspond to the molecular ion (M^+) with the sequential losses of methanol and a methoxy group, respectively. The ion at *m/z* 255 is due to the single bond inductive loss of propene from the major fragment ion at *m/z* 297. (B) The EI mass spectrum of the deuterium-labeled molecules obtained by methanolysis with D-4 methanolic HCl solution. The presence of two carboxylic acid methyl ester groups was confirmed by the mass shifts of 6 units of the molecular ion. Peaks marked with an asterisk are due to the characteristic McLafferty fragment.

Isotope labeling

Isotope labeling was used to aid in deducing exact structures of esterified fatty acyl components by GC–MS. Methyl esters of fatty acid obtained by acid methanolysis were further treated with 5% D-4 methanolic-HCl solution for 6 h at 72°C. Deuterated methyl esters of fatty acids were extracted and analyzed as described before.

Isolation of α,ω-13,16-dimethyloctacosanedioate dimethyl ester

The mixtures of fatty acid methyl esters were applied to preparative silica TLC (thin-layer chromatography) plates which were eluted with chloroform–hexane 1.5:1.0 (v/v). Bands were detected by brief exposure of the plates to iodine. One of the fractions thus identified and found to be containing dicarboxylic acid dimethyl esters (by

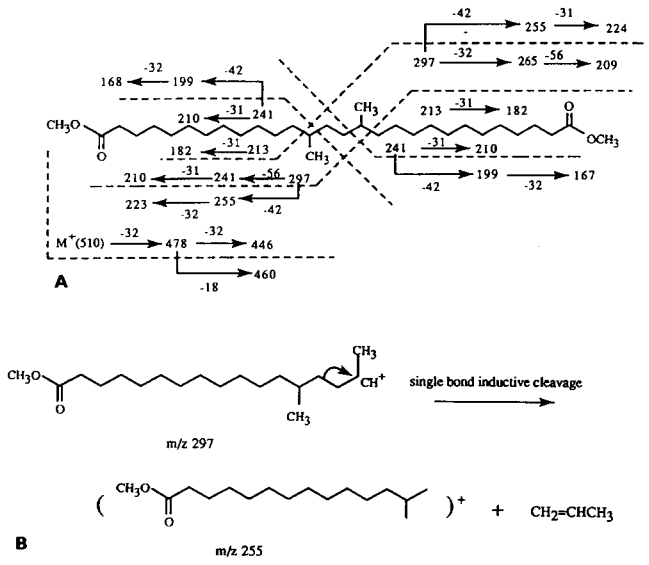

Fig. 3. A: Analysis of the mass spectral fragmentations of peak B. Losses of 18, 31, 32, 42, and 56 correspond to water, methoxy, methanol, propene, and butane, respectively. B: Single bond inductive cleavage fragmentation mechanism explaining the occurrence of the *m/z* 255 ion as a major product.

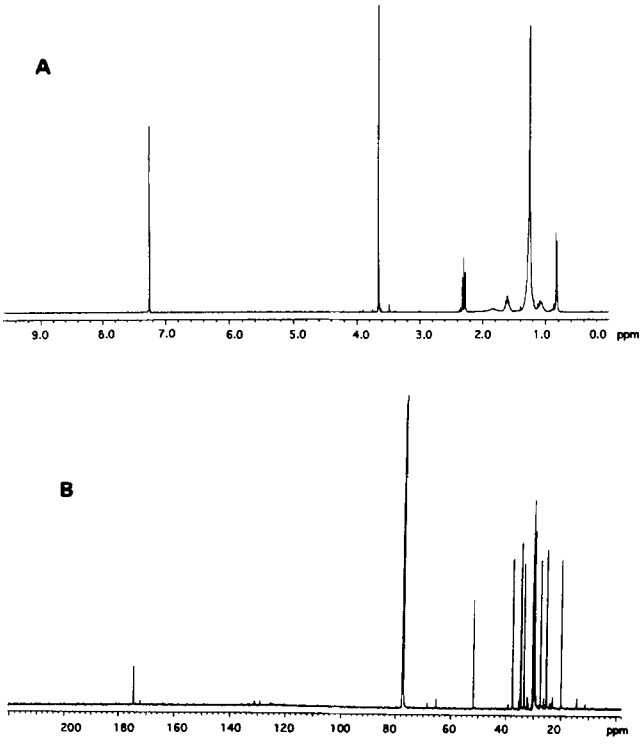

Fig. 4. ^{1}H NMR spectrum of peak B. Signals at 0.83 ppm (d, J = 8.3 Hz) and 1.25 ppm characteristic of the methyl and methylene groups of long chain acyl components. Resonances at 2.30 ppm (t, J = 7.5 Hz) and at 3.65 ppm (s) represent the methylene groups α to the carbonyl and ester methoxy group, respectively. The multiplet at 1.61 ppm was assigned to the protons of the β carbons of the molecule. The signal at δ 7.24 was assigned to chloroform.

GC-MS analysis) was successively subjected to flash column chromatography (15). The column was sequentially eluted with three times the column volume of a mixture of chloroform–hexane 1.5:1 (v/v), 2 volumes of chloroform–hexane 4:1 (v/v), and 1 volume of chloroform at a flow rate of 20 ml/min. Separated components were conveniently detected by spotting 5–10 μl of each fraction on silica TLC plates followed by development with chloroform and charring. Fractions with similar compositions were pooled and concentrated on a rotary evaporator. Each such fraction was dissolved in hexane and then subjected to further analyses.

^{1}H NMR and ^{13}C NMR spectroscopy

Proton NMR spectra were recorded at 500 MHz on solutions in $CDCl_3$. J-correlated data was obtained by 2D DQF-COSY (16) experiments. ^{13}C NMR spectra were recorded at 125 MHz on solutions in $CDCl_3$. DEPT (distortionless enhanced by polarization transfer) experi-

TABLE 1. Analysis of 70 eV electron impact mass spectral fragments of peak B

Structure of Ionic Fragments	Mass	Deuterated Mass
$CH_3OCO(CH_2)_{11}CH(CH_3)CH_2CH_2(CH_3)CH(CH_2)_{11}OCOCH_3$	510	516
$CH_3OCO(CH_2)_{11}CH(CH_3)CH_2CH_2(CH_3)CH(CH_2)_{10}CH = CO$	478	481
$CO = CH(CH_2)_{10}CH(CH_3)CH_2CH_2(CH_3)CH(CH_2)_{10}CH = CO$	446	446
478–18 (H_2O)	460	463
$CH_3OCO(CH_2)_{11}CH(CH_3)CH_2CH_2CH(CH_3)$	297	300
$CO = CH(CH_2)_{10}CH(CH_3)CH_2CH_2CH(CH_3)$	265	268
$CH_3OCO(CH_2)_{11}CH(CH_3)CH_2$	255	258
$CH_3OCO(CH_2)_{11}CH(CH_3)$	241	244
$OC(CH_2)_{11}CH(CH_3)CH_2$	224	224
$CH_3OCO(CH_2)_{11}$	213	216
$OC(CH_2)_{11}$	182	182
$CH_3OC(OH) = CH_2$	74	77
$CH_3OCO(CH_2)_n$; n = 1–13	73 + 14*n	76 + 14*n
$CO = CH(CH_2)_n$; n = 1–13	55 + 14*n	55 + 14*n
CH_3-$(CH_2)_n$; n = 3–12	57 + 14*n	57 + 14*n
$CH_2 = CH$-$(CH_2)_n$; n = 2–11	55 + 14*n	55 + 14*n

ments (17) were performed for establishing ^{13}C chemical shift assignments. Chemical shifts are quoted relative to the chloroform resonance taken as 7.24 ppm for proton and 77 ppm for ^{13}C measurements, respectively.

Fourier transform infrared (FTIR) spectroscopy

Spectra were obtained with a Nicolet model 710 FTIR spectrometer on 10% (w/v) chloroform solution.

RESULTS AND DISCUSSION

Total fatty acid analyses of *T. ethanolicus* 39E

The total ion chromatogram from the GC-MS analysis of fatty acid methyl esters extracted from cells grown at 67°C is shown in **Fig. 1.** The peaks from 1 to 6 were due to typical membrane fatty acyl components ranging from 14 to 17 carbons. Major regular fatty acids were *iso*-pentadecanoic acid (peak 3, *iso*-$C_{15:0}$) and *iso*-heptadecanoic acid (peak 5, *iso*-$C_{17:0}$). The peaks from A to D, however, corresponded to unusual components of lengths apparently much greater than the usual.

Mass spectrometric analyses of C_{30} - dicarboxylic dimethyl ester

Peak B was the most abundant fatty acyl component (about 36%) of the membrane fatty acids of *T. ethanolicus* 39E. The electron impact mass spectrum of peak B is shown in **Fig. 2.** Fig. 2A showed major ions at *m/z* 510, 478, and 446. These corresponded to the molecular ion of

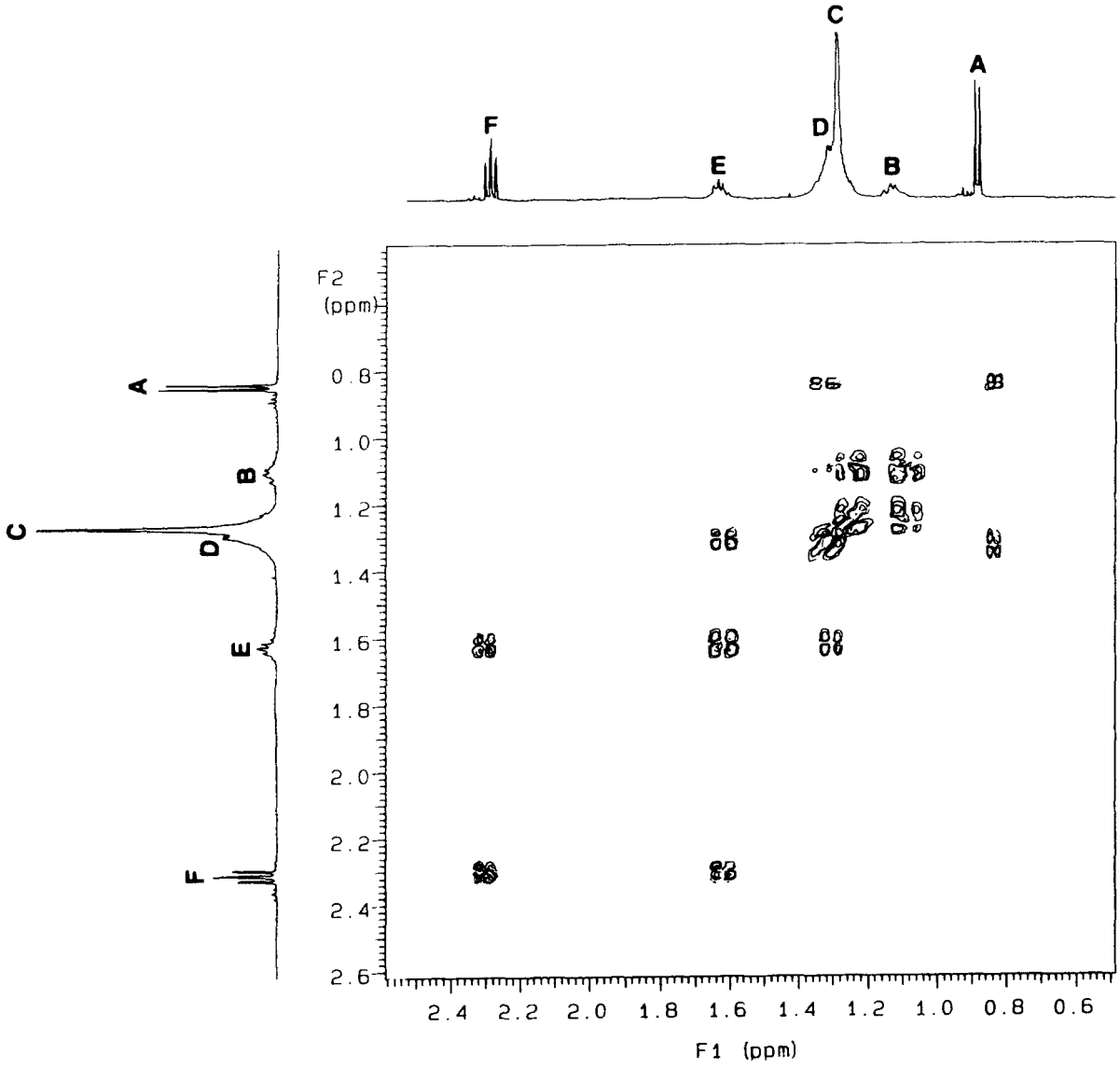

Fig. 5. The ^1H DQF-COSY NMR spectrum (in the region between 0 to 2.6 ppm) of peak B, in $CDCl_3$ at 500 MHz. Correlation analysis showed the relationship among the peaks such as peak A with D, peak D with A and B, peak E with F and C, and peak C with B and E. The doublet at 0.83 ppm (J = 6.3 Hz) was assigned to the branched methyl groups of the fatty acyl chains and the resonance at δ 1.61 to the protons of the β-carbons of the molecule. Resonances at δ 2.30 (t, J = 7.5 Hz) were assigned to the methylene groups α to the carbonyl group ($-CH_2-CO$).

a C_{30}-α,ω-dicarboxylic dimethyl ester (M^+) with the sequential losses of methanol (CH_3OH) and methoxy (CH_3O) groups, respectively. The predominant ion at *m/z* 297 suggested dimethyl branching and represents the structure obtained by fragmentation at a secondary carbon (**Fig. 3A**). The ion at *m/z* 265 was assigned to the loss of methanol (CH_3OH) from the ion at *m/z* 297. The proposed positions for the placement of the methyl groups were supported by the presence of the strong peak at *m/z* 255 corresponding to the loss of propene from the ion at *m/z* 297 by way of an inductive effect (Fig. 3B) (18). The ion at *m/z* 199 was similarly assigned to the loss of propene ($CH_2 = CHCH_3$) from the other secondary cationic fragment at *m/z* 241. The characteristic McLafferty fragment ion at *m/z* 74 ($CH_3OC(OH) = CH_2$) was also detected. Fig. 2B shows the electron impact mass spectrum of the deuterium-labeled molecule obtained by methanolysis with the D-4 methanol-HCl solution (to produce the trideuterated methyl ester). The most striking change was the shift of molecular ion by 6 mass units confirming the presence of two methoxy groups. Two trideuteromethanol (CD_3OH) losses from the molecular ion (516 *m/z*) to give ions at *m/z* 481 and 446 were also observed. The ions at *m/z* 258 ($CD_3OCO(CH_2)_{11}$ $CH(CH_3)CH_2$) and 300 ($CD_3OCO(CH_2)_{11}$ $CHCH(CH_3)CH_2CH_2CH(CH_3)$) corresponded to the deuterated *m/z* 255 ($CH_3OCO(CH_2)_{11}$ $CH(CH_3)CH_2$) and 297 ($CH_3OCO(CH_2)_{11}$ $CH(CH_3)CH_2$ $CH_2CH(CH_3)$) ions, respectively. Fig. 3A and **Table 1** explain the origins of most other fragmentation products.

NMR-FTIR analyses of C_{30} - dicarboxylic acid dimethyl ester

The ^1H NMR spectrum (**Fig. 4**) of peak B contained signals at 0.83 and 1.25 ppm characteristic of the methyl and methylene groups, respectively, of the long chain fatty acyl components. Ester methoxy groups (CH_3O-) were assigned to a singlet at δ 3.65. The spin correlations of the peaks in the region between 0 and 2.6 ppm were investigated by 2D-NMR DQF-COSY spectroscopy (**Fig. 5**). Correlation analysis showed the relationship between peaks A and D, peak D with both A and B, peak E with F and C, and peak C with B and E (Fig. 5). The doublet at 0.83 ppm (J = 6.3 Hz) (peak A) was assigned to the branched methyl groups of the fatty acyl chain. Resonances at δ 1.61 (peak E) were assigned to the protons β to the carbonyl group of the molecule. Resonance at δ 2.30 (t, J = 7.5 Hz) (peak F) were assigned to the methylene function α to the carbonyl group ($-CH_2-CO$). **Fig. 6** shows the ^{13}C-DEPT spectrum of peak B. All 32 carbons (16 pairs) were assigned by this analysis. The presence of a signal for one methine carbon (CH) confirmed the presence of the two branching methyl groups in the structure. The signals for the methoxy groups (δ 50.9) and branching methyl groups (δ 19.2) were readily discernible.

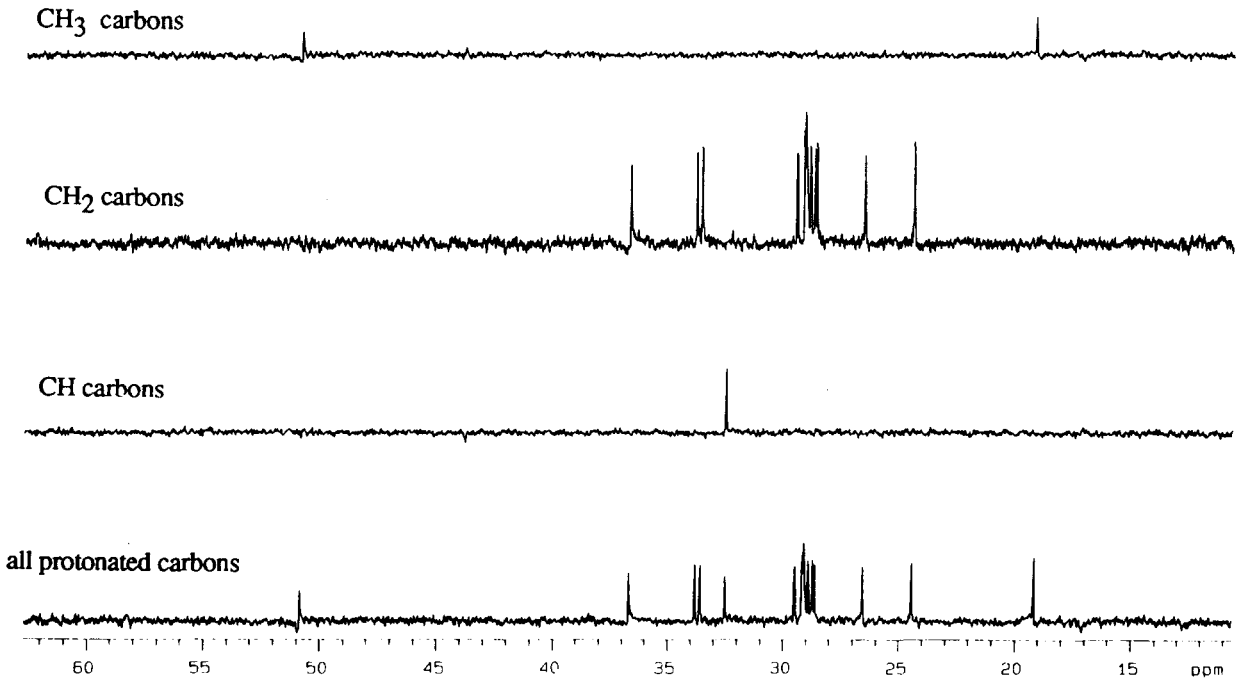

Fig. 6. ^{13}C-DEPT spectrum of peak B in $CDCl_3$. The lower trace represents all protonated carbons. The presence of one methine carbon (CH) and two methyl carbons (CH_3) confirmed the two branched methyl groups and two methoxy groups in the structure (the molecule is symmetrical and each signal represents two identical carbons).

A normal broadband ^{1}H-decoupled ^{13}C spectrum (not shown) contained a resonance at δ 174.2 for the carbonyl carbon. The FTIR spectrum (not shown) contained very strong signals for methylene asymmetrical stretching at 2928 cm^{-1} and symmetrical stretching at 2856 cm^{-1}. The asymmetric stretching of methyl groups occurred at 3017 cm^{-1}. The symmetric bending vibration of methyl groups appeared at 1378 cm^{-1} and asymmetric bending at 1458 cm^{-1}. An absorption band at 1378 cm^{-1} arose from the asymmetrical bending of the methyl C–H bonds. The scissoring band of the methylene groups occurred at 1463 cm^{-1}. The methylene twisting and wagging vibrations occurred at 1119–1378 cm^{-1}. The characteristic C = O absorption band of the aliphatic ester group appeared at 1733 cm^{-1}. There was no evidence for alkyl chain unsaturation in the molecular structure.

Mass spectrometric analyses of C_{29}, C_{31}, and C_{32} dicarboxylic dimethyl esters

Fig. 7A shows the electron impact (70 eV) mass spectrum of peak A. Major ions appear at *m/z* 496, 464, and 432. These corresponded to the molecular ion of a C_{29}-α,ω-dicarboxylic dimethyl ester (M^+) with the sequential losses of two molecules of methanol (CH_3OH) (**Fig. 8**). The major ion at *m/z* 281 represents the structure ob-

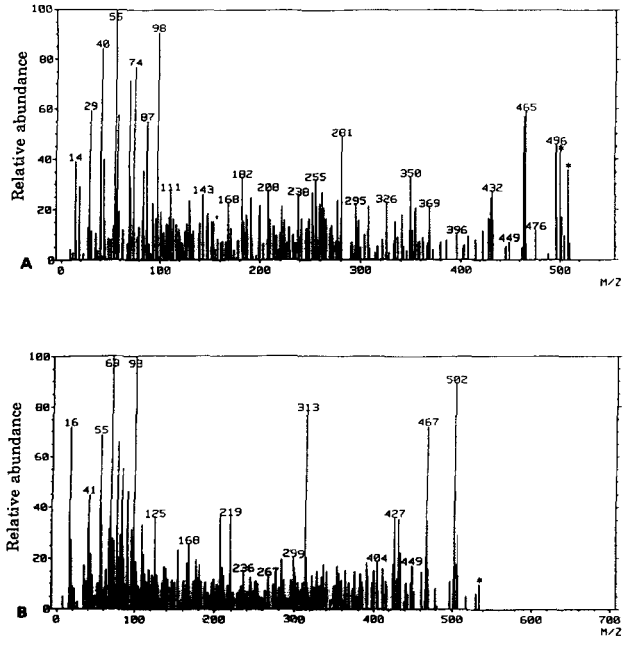

Fig. 7. (A) Electron impact mass spectrum (70 eV) of peak A. Major ions at *m/z* 496, 464, and 432 correspond to the molecular ion (M^+) with the sequential losses of two methanol molecules. The characteristic McLafferty fragment of aliphatic esters appears at 74 (*m/z*). (B) The EI spectrum of the deuterium-labeled molecule obtained by deuteration with D-4 methanolic-HCl solution. The presence of two carboxylic methyl ester functions is confirmed by isotope labeling. Peaks labelled with an asterisk are in the background. The prevalence of background peaks in these spectra is due to the very low amount of peak A (Fig. 1).

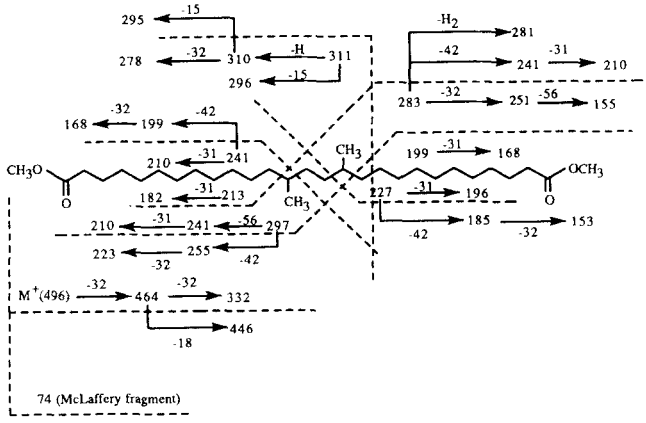

Fig. 8. Mass fragmentation pattern of peak A. Losses of 15, 18, 31, 32, 42, and 56 correspond to a methyl group, water, a methoxy group, methanol, ketene, and butane, respectively.

tained by the loss of one hydrogen molecule from the secondary cation at *m/z* 283. The ion at *m/z* 255 was assigned to the loss of propene (CH_2 = $CHCH_3$) from the ion at *m/z* 297 by way of a single bond inductive cleavage mechanism. The ion at *m/z* 241 was similarly assigned to the loss of propene from the secondary cationic fragment

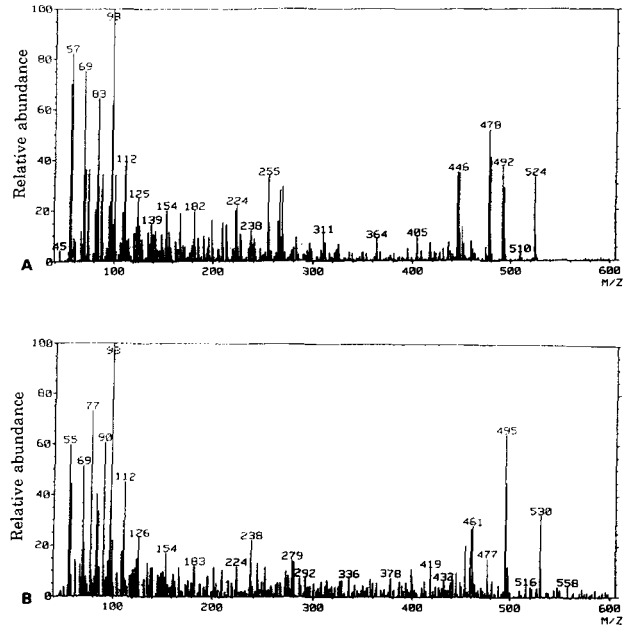

Fig. 9. (A) Electron impact mass spectrum (70 eV) of peak C. The major ions at *m/z* 524, 492, and 461 corresponded to the molecular ion (M^+) with the sequential losses of methanol and a methoxy group, respectively. The major ion at *m/z* 478 was due to the loss of a methoxy from the fragment at *m/z* 509 which was produced after the loss of a methyl group from the molecular ion (M^+). Another major ion at *m/z* 446 was due to the loss of methanol from the fragment at *m/z* 478. The characteristic McLafferty fragment of aliphatic ester appears at 74 (*m/z*). (B) The EI spectrum of the labeled molecule obtained by deuteration with D-4 methanolic-HCl solution. The presence of two carboxylic acid methyl ester groups is confirmed by isotope labeling.

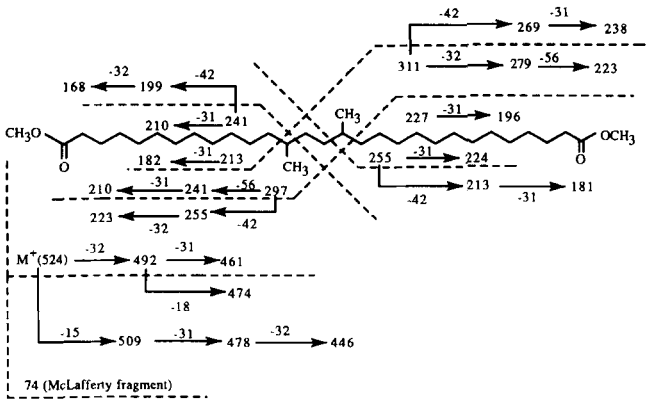

Fig. 10. Mass spectral fragmentation pattern of peak C. Losses of 15, 18, 31, 32, 42, and 56 correspond to a methyl group, water, a methoxy group, methanol, ketene, and butane, respectively.

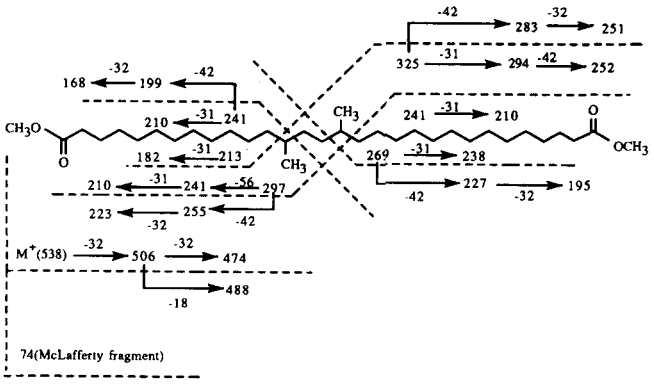

Fig. 12. Mass fragmentation pattern of peak D. Losses of 15, 18, 31, 32, 42, and 56 correspond to a methyl group, water, a methoxy group, methanol, ketene, and butane, respectively.

at *m/z* 283. The characteristic McLafferty fragment ion at *m/z* 74 was again detected. Fig. 7B shows the electron impact mass spectrum of the deuterium-labeled molecule obtained by methanolysis with D-4 methanol-HCl solution to produce the trideuterated methyl ester. The most striking change was the shift of the molecular ion by 6 mass units. This confirmed the presence of two methoxy groups in the molecule. The loss of two molecules of trideutero-methanol (CD_3OH) from the molecular ion (502 *m/z*) to give ions at *m/z* 467 and 432 was also observed. Fig. 8 shows most of the other fragments and their origin. **Fig. 9** shows the electron impact mass spectrum of peak C. Major ions appeared at *m/z* 524, 492, and 461. These corresponded to the molecular ion of a C_{31}-α,ω-dicarboxylic acid dimethyl ester (M^+) with the sequential losses of methanol (CH_3OH) and a methoxy (CH_3O)

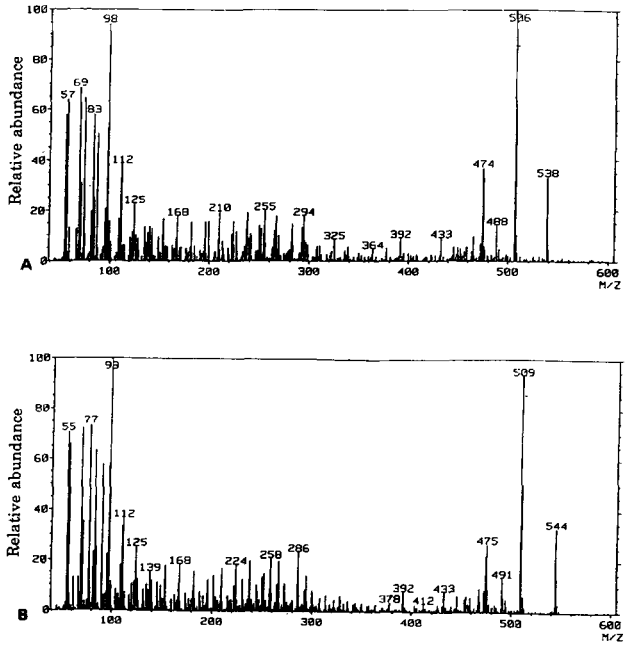

Fig. 11. (A) Electron impact mass spectrum of peak D. The major ions at *m/z* 538, 506, and 475 correspond to the molecular ion (M^+) with the sequential losses of methanol and a methoxy group respectively. The ions at *m/z* 255 and 283 are due to the single bond inductive loss of propene from the major fragment ions at *m/z* 297 and 325, respectively. (B) The EI spectrum of the labeled molecule obtained by deuteration with D-4 methanolic-HCl solution. The presence of two carboxylic acid methyl ester groups is confirmed by isotope labeling.

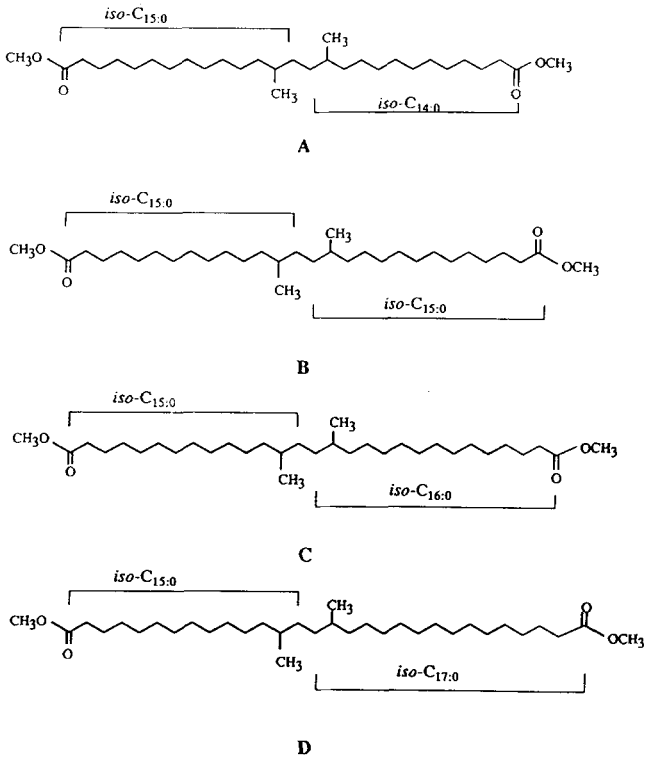

Fig. 13. The determined structures of the members of the family of very long chain α,ω-dicarboxylic acid dimethyl esters. A: α,ω-13,16-dimethylheptacosanedioate dimethyl ester (C29); B: α,ω-13,16-dimethyloctacosanedioate dimethyl ester (C30); C: α,ω-13,16-dimethylnonacosanedioate dimethyl ester (C31); D: α,ω-13,16-dimethyltriacotanedioate dimethyl ester (C32).

group, respectively (**Fig. 10**). The ions at *m/z* 478 and 447 corresponded to the sequential loss of methanol and methoxy groups, respectively, from the parent ion after loss of a methyl group (*m/z* 509). The ion at *m/z* 311 represents the ion obtained by fragmentation of the alkyl chain. The ion at *m/z* 269 was assigned to the loss of propene from the ion at *m/z* 311 by way of single bond inductive cleavage mechanism. The strong peak at *m/z* 255 corresponded to the loss of propene from the ion at *m/z* 297. The characteristic McLafferty fragment ion at *m/z* 74 was also detected. Fig. 9 shows the electron impact mass spectrum of the deuterium-labeled molecule obtained by methanolysis with D-4 methanol-HCl solution (to produce the trideutero methyl ester). The most striking change was again the shift of the molecular ion by 6 mass units, confirming the presence of two trideutero-methyl groups. Loss of two molecules of CD_3OH from the molecular ion (530 *m/z*) to give ions at *m/z* 495 and 447 was also observed. Fig. 10 shows the origin of the other fragmentation products.

The electron impact mass spectra of peak D and its trideutero derivative are shown in **Fig. 11.** A complete rationalization of the fragmentation is given in **Fig. 12.**

Fig. 13 shows the structures of the members of the new family of α,ω-dicarboxylic acids in *T. ethanolicus* 39E. They are α,ω-13,16-dimethylheptacosanedioate dimethyl ester (C29), α,ω-13,16-dimethyloctacosanedioate dimethyl ester (C30), α,ω-13,16-dimethylnonacosanedioate dimethyl ester (C31) and α,ω-13,16-dimethyltriacotanedioate dimethyl ester (C32).

A recent study of the membrane of a strict anaerobic, facultative acidophilic eubacterium, *Sarcina ventriculi,* demonstrated that α,ω-dicarboxylic fatty acid components are synthesized as a general response to the perturbation of the membrane structure by the addition of exogenous organic solvents or increasing temperature (13). In this study we showed that the membrane of *Thermoanaerobacter ethanolicus* 39E contains α,ω-dicarboxylic fatty acyl components similar to those formed in *S. ventriculi.* The critical difference is the position of internal methyl branches. In the case of *S. ventriculi* the positions of the methyl branches indicate that the very long chain fatty acids might be formed by ω-1 tail-to-tail coupling of the fatty acid chains from opposite leaflets of the membrane. The difference in the position of internal methyl branches observed in this study can be easily rationalized if one considers the fact that there are predominantly *iso*-branched regular fatty acids in this organism and ω-coupling occurs since the ω-1 position is hindered. Fig. 13 shows the correlation in structure between the α,ω-dicarboxylic acids and the regular fatty acids. By far the most abundant regular chain fatty acid in *T. ethanolicus* 39E is *iso*-pentadecanoic acid. This fatty acid would then be a component in all of the ω-coupling steps to synthesize the α,ω-dicarboxylic acids. The positions of the methyl group in all four very long chain acids (Fig. 13) can be rationalized based on the structures of the regular fatty acids if ω-coupling is invoked. The phenomenon of lipid chain coupling suggested here has very high significance because it represents a new, potentially general, mechanism for adaptation to extreme stress in bacteria.

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