JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1989, p. 2130–2132
0095-1137/89/092130-03$02.00/0
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Vol. 27, No. 9

Identification of Monounsaturated Fatty Acids of *Aerococcus viridans* with Dimethyl Disulfide Derivatives and Combined Gas Chromatography-Mass Spectrometry

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Received 10 April 1989/Accepted 14 June 1989

Location of the double-bond position of monounsaturated fatty acids of *Aerococcus viridans* was accomplished by combined gas chromatography (GC)-mass spectrometry analysis of dimethyl disulfide (DMDS) derivatives. The monoenoic fatty acids from whole bacterial cells were converted to methyl esters and then to DMDS adducts and analyzed by capillary GC-mass spectrometry. The mass spectra of DMDS adducts gave an easily recognizable molecular ion (M^+) and two major diagnostic ions attributable to fragmentation between the two CH_3S groups located at the original site of unsaturation. Two relatively novel acids that distinguish aerococci from bacteria of closely related genera were identified as $C_{16:1}$ ω9c and $C_{16:1}$ ω9t from their mass spectrometry fragmentation patterns and retention characteristics on nonpolar capillary GC columns.

During recent gas chromatographic (GC) studies of the cellular fatty acid of members of the family *Streptococcaceae*, we observed several positional isomers of 16-, 18-, and 20-carbon acids that were well resolved with fused silica capillary GC columns. A relatively novel monounsaturated 16-carbon acid ($C_{16:1}$) was observed in *Aerococcus* and *Streptococcus* spp. but not in other genera of this family. Identification of this acid, by using combined gas chromatography-mass spectrometry (GC-MS) analysis of dimethyl disulfide (DMDS) derivatives to determine the position and geometry of the double bond, is described in this report.

Cells for fatty acid analysis were grown in Todd-Hewitt broth at 30°C for 48 to 72 h, centrifuged, washed, and processed for total cellular fatty acids as described previously (4). The resulting fatty acid methyl ester (FAME) sample was analyzed by GC and GC-MS and then treated with DMDS to convert unsaturated FAME to FAME-DMDS adducts (1, 3). GC separation of FAME and FAME-DMDS adducts was done with a fused silica cross-linked methyl silicone (OV-1) capillary column (50 m by 0.2 mm, inside diameter) installed in a 5880 gas chromatograph equipped with a flame ionization detector (Hewlett-Packard Co., Avondale, Pa.), operated under conditions described previously (3). GC-MC analyses of FAME-DMDS adducts were done with a 4500 mass spectrometer equipped with a Superincos Data System (Finnigan MAT, San Jose, Calif.) and a DB5 capillary column (60 m by 0.25 mm, inside diameter; J & W Scientific, Folsom, Calif.) which was interfaced directly into the ion source. The operating parameters of both the column and MS were identical to those reported previously (3).

The methyl ester derivative of the $C_{16:1}$ acid from *Aerococcus viridans* ATCC 11563 eluted from the OV-1 capillary column at 9.77 min (Fig. 1). Under identical GC conditions, reference standards of $C_{16:1}$ ω7c and $C_{16:1}$ ω7t eluted at 9.83 and 9.87 min, respectively. Although these retention times are within 0.1 min, accurate calculation of equivalent chain-length values (ECL) with the computer permitted precise and reproducible GC resolution of these three $C_{16:1}$ isomers. The ECL of the $C_{16:1}$ FAME of aerococci eluting at 9.77 min (Fig. 1) was 15.773 ± 0.003, versus 15.818 ± 0.003 for $C_{16:1}$ ω7c and 15.865 ± 0.003 for $C_{16:1}$ ω7t. The mass spectra of the $C_{16:1}$ FAME (ECL, 15.773) from *A. viridans* showed a molecular ion (M^+) at *m/e* = 268 and characteristic fragment ions at $M^+ - 32 = 236$ and $M^+ - 74 = 194$, confirming the 16-carbon chain length and one unsaturated bond. Upon hydrogenation and subsequent GC and GC-MS analyses, the $C_{16:1}$ FAME was found to be completely converted to $C_{16:0}$ straight-chain FAME, confirming the absence of any methyl branching in the carbon chain.

GC-MS of the DMDS adduct of the $C_{16:1}$ FAME showed major ions at *m/e* = 189 and *m/e* = 173, attributable to fragmentation between the two CH_3S groups located at the original site of unsaturation (Fig. 2). The *m/e* = 173 ion is the aliphatic fragment (ω), and the *m/e* = 189 ion is the fragment containing the carboxyl end (Δ) of the molecule. The molecular ion at *m/e* = 362 is easily recognizable, and the other major ion at *m/e* = 157 is due to the loss of methanol from the Δ fragment (*m/e* 189 − 32 = *m/e* 157). This fragmentation pattern clearly shows the location of the double bond at the ω9 (or Δ7) position. GC-MS of the DMDS adduct of the slower-eluting $C_{16:1}$ FAME in Fig. 1 (ECL, 15.835 ± 0.003) gave a fragmentation pattern identical to that shown in Fig. 2. Therefore, assignment of *cis* and *trans* geometry was possible by GC, since the *cis* isomer eluted before the *trans* isomer on nonpolar capillary columns, both as the FAME and the FAME-DMDS adduct (1, 3). Thus, the combined data from GC and GC-MS clearly establish that the $C_{16:1}$ acids of *A. viridans* are $C_{16:1}$ ω9c and $C_{16:1}$ ω9t.

Tentative identification of the other acids of *A. viridans* listed in Fig. 1 was accomplished by exact GC retention time matches (ECL) with authentic standards; their identities were subsequently confirmed by GC-MS analysis of FAME and FAME-DMDS adducts. Determination of the position of the cyclopropane ring in the 19-carbon cyclopropane acid ($C_{19:0}$ cyc) was accomplished with the picolinyl ester derivative, prepared as described by Harvey (2) and analyzed by GC-MS. The resulting mass spectrum was identical to a

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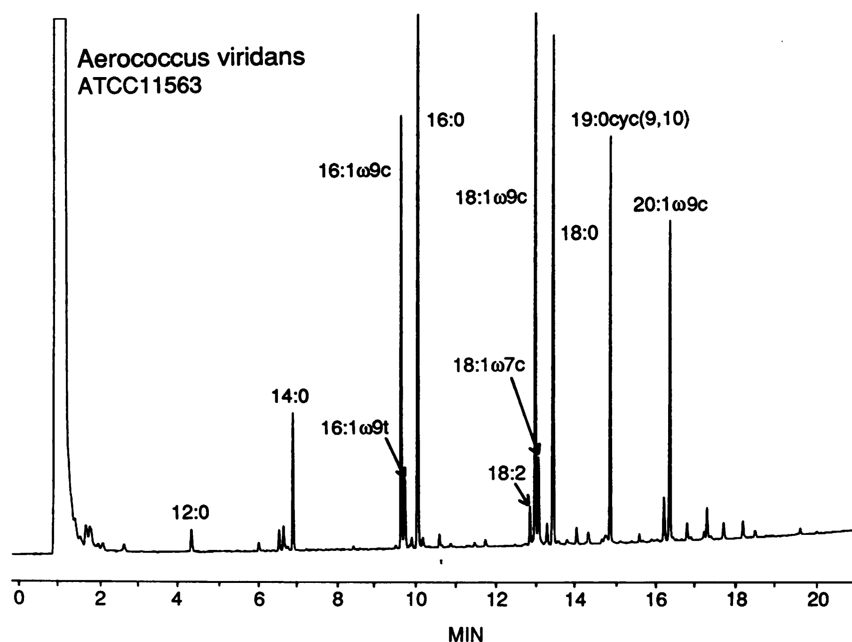

FIG. 1. Gas chromatogram of methylated fatty acids of *A. viridans* ATCC 11563. Fatty acids are designated as follows: number before colon, number of carbon atoms; number after colon, number of double bonds; ω, double-bond position from hydrocarbon end of carbon chain; c, *cis* isomer; t, *trans* isomer; cyc, cyclopropane ring.

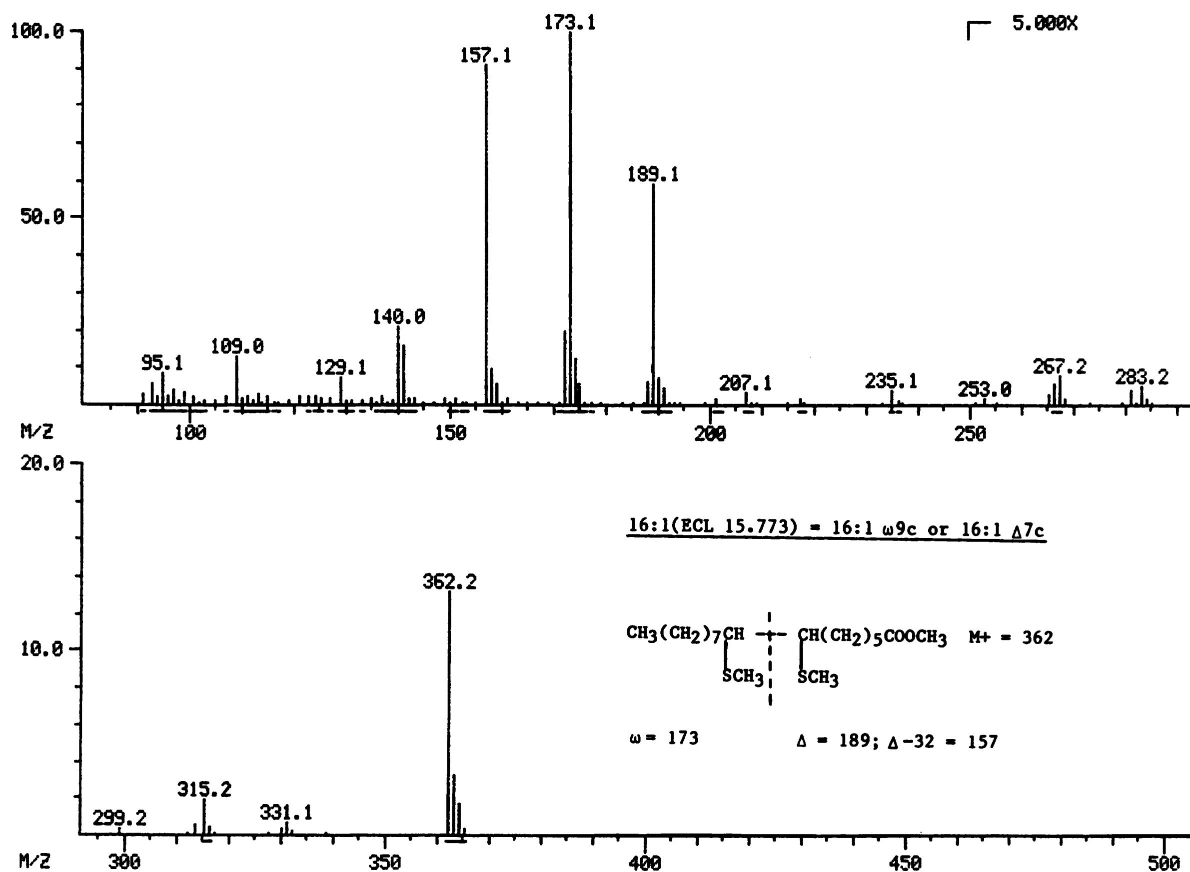

FIG. 2. Mass spectra of the DMDS adduct of the $C_{16:1}$ ω9c FAME from *A. viridans*. The ω fragment is the aliphatic end of the molecule, and the Δ fragment is the carboxylic end of the molecule. M^+, Molecular ion.

reference standard of 9,10-methyleneoctadecanoic (dihydrosterulic) acid, which showed an abundant odd electron ion at $m/e = 247$ resulting from cleavage through the ring at the 9,10 position. In contrast, the picolinyl ester derivative of *cis*-11,12-methyleneoctadecanoic (lactobacillic) acid, which most often occurs in bacteria, showed an abundant odd electron ion at $m/e = 275$, consistent with ring cleavage at the 11,12 position. These two cyclopropane acids as well as $C_{16:1}$ ω9c, $C_{16:1}$ ω9t, and $C_{20:1}$ ω9c are well resolved as FAME on nonpolar capillary columns. Each of 36 *Aerococcus* strains and 175 *Streptococcus* strains contained $C_{16:1}$ ω9c, but only *Aerococcus* strains contained $C_{16:1}$ ω9t. The usefulness of cellular fatty acids for identifying aerococci and for distinguishing various related genera will be detailed in a future report.

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