terminal residue. In the peptide Try B-2, valine is N-terminal. The amino acid sequence of this fragment is val.gly.leu. (or ileu.)gly.ala.arg. As is seen from Table 2, the residues found in the fragments are in fairly good conformity with the residues found in peptide B. In the preliminary report on the amino acid composition of peptide B (cf. Ref.[2]), threonine was stated to be absent. In subsequent analysis with a somewhat modified procedure [7], threonine was recovered from the hydrolysate of peptide B. This is in agreement with the original finding by Bettelheim [3].

For further elucidation of the structure of peptide B, digestion of this peptide by means of subtilisin has given promising results. After digestion with this enzyme, 6 or 7 small peptides were isolated by chromatography or by electrophoresis.

The complete paper on the amino acid sequence of bovine fibrinopeptides A and B will be published elsewhere.

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1. Scheraga, H. A. and Laskowski, M. *Advances in Protein Chem.* **12** (1957) 1.
2. Blombäck, B. *Acta Physiol. Scand.* **43** (1958) Supplementum 148.
3. Bettelheim, F. R. *Biochim. et Biophys. Acta* **19** (1956) 121.
4. Lorand, L. *Physiol. Revs.* **34** (1954) 742.
5. Blombäck, B. and Vestermark, A. *Arkiv Kemi* **12** (1958) 173.
6. Gladner, J. A., Folk, J. E., Laki, K. and Carroll, W. R. *J. Biol. Chem.* **234** (1959) 62.
7. Sjöquist, J. *Acta Chem. Scand.* (1959) *in press.*
8. Laki, K., Gladner, J. E. and Kominz, D. R. *Seventh Annual Symposium on Blood,* Wayne State University, U.S.A. 1958.
9. Folk, J. E., Gladner, J. A. and Laki, K. *J. Biol. Chem.* **234** (1959) 67.
10. Sjöquist, J. *Arkiv Kemi* (1959) *in press.*
11. Sjöquist, J. *Arkiv Kemi* **11** (1957) 129.
12. Sjöquist, J. *To be published.*
13. Bettelheim, F. R. *J. Am. Chem. Soc.* **76** (1954) 2838.
14. Wallén, P. and Sjöholm, I. *To be published.*

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Synthesis of (—)-Methyl 2D, 4D,6D-Trimethylnonacosanoate and Identification of C_{32}-Mycocerosic Acid as a 2,4,6,8-Tetramethyloctacosanoic Acid

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A mass spectrometric study [1] of the methyl ester of mycocerosic acid isolated by Anderson and co-workers [2-4] from the lipids of tubercle bacilli, indicated a molecular weight of 494, corresponding to the methyl ester of a C_{32}-acid. The mass spectrum furthermore indicated the presence of methyl side chains at positions 2, 4, and 6. These methyl group positions were the same as deduced by Polgar [5] for the closely related or identical compound called mycoceranic acid [6,7]. The levo-rotation of mycoceranic acid and the optical rotations recorded by Polgar for the products obtained in the step-wise degradation suggested an all D optical configuration. For comparison we therefore synthesized (—)-methyl 2D,4D,6D-trimethylnonacosanoate. The synthesis was performed as follows.

n-Eicosylmalonic acid, m. p. 122.8—124.2°, was prepared in a yield of 90 % from ethyl *n*-docosanoate and ethyl oxalate [8]. The reaction of the acid chloride derived from (—)-6-methoxycarbonyl-3L, 5L-dimethylhexanoic acid [9] with the sodium derivative of di-(tetrahydropyranyl) *n*-eicosylmalonate [10] gave (+)-methyl 3D,5D-dimethyl-7-oxooctacosanoate, m. p. 42.8—43.6°, $[\alpha]_D^{25}$ +0.4° (c = 19.3) *,

* All optical rotations were measured in chloroform solution using a 1 dm tube.

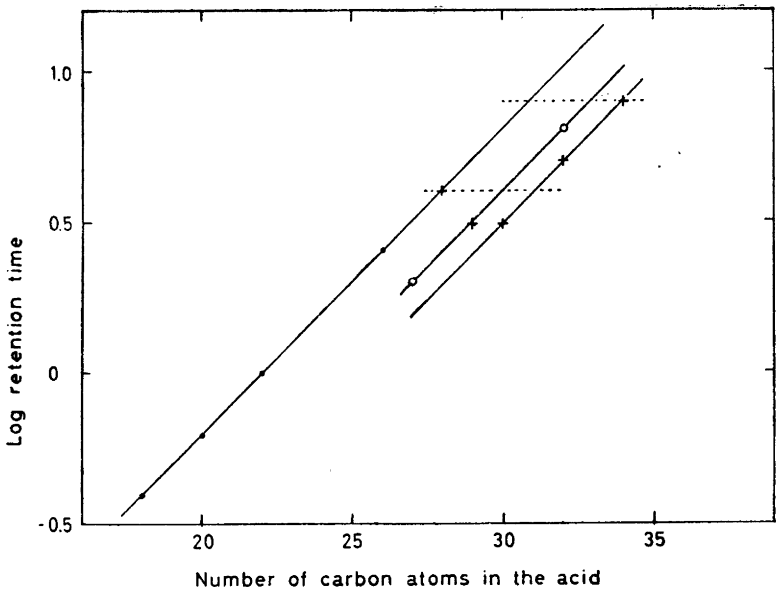
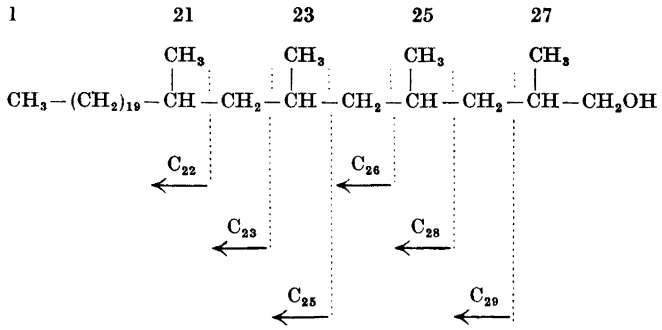


Fig. 1. The relations between the logarithm of the retention time and the number of carbon atoms in the acid in the gas chromatography of the methyl esters on silicone at 260°. ●, Methyl esters of normal chain acids; O, methyl esters of 2,4,6-trimethyl-substituted acids; +, fractions from methyl mycocerosate. Dotted horizontal lines are drawn through points corresponding to fractions of unknown molecular weight.

in a yield of 52 %. The reduction of the oxo-group was carried out by the desulphurization of the ethylene mercaptal [11]. (+)-Methyl 3D,5D-dimethyloctacosanoate, m. p. 41.2—42.0°, $[\alpha]_D^{25}$ +1.5° (c = 6.0), was obtained in a yield of 95 %. Saponification gave the free acid, m. p. 59.9—60.2°.

Hunsdiecker degradation [12] of the silver salt of this acid gave (—)-1-bromo-2D,4D-dimethyl-heptacosane, m. p. 40—41°, $[\alpha]_D^{21}$ —0.5° (c = 5.8), in a yield of 81 %. The bromide was converted into the iodide by means of sodium iodide in acetone. The reaction between the iodide and the sodium derivative of diethyl methylmalonate, followed by saponification and decarboxylation, gave a mixture of 2D, 4D,6D- and 2L,4D,6D-trimethylnonacosanoic acids in a yield of 72 %. The methyl esters were prepared by means of diazomethane, and the two stereo-isomers separated by means of chromatography on magnesium trisilicate. The separation was followed by measuring the optical rotation. Repeated chromatography finally yielded a levo-rotatory fraction, the rotation of which was unchanged on further chromatography. (—)-Methyl 2D,4D,6D-trimethylnonacosanoate thus obtained had m. p. 37.9—38.2°, $[\alpha]_D^{20}$ —7.2° (c = 5.5).

The melting point of the synthetic ester was about 17° higher than that of methyl mycocerosate (m.p. 20.8—21.2° [1]), and the mass spectra, although very similar, were not identical. It therefore became necessary to reinvestigate the natural product. Gas chromatography on silicone at a temperature of 260° showed the presence of two major and four minor components. The former (designated A and B in the following) had shorter retention times (10.2 and 16.9 min, respectively) than the methyl ester of the synthetic 2,4,6-trimethyl-substituted C_{32}-acid (21.3 min). The mass spectrometric examination of the isolated fractions A and B showed that A was a mixture of two compounds with molecular weights of 452 and 466, respectively. Fraction B (65 % of the total) appeared to consist of a single compound of molecular weight 494. In the diagram of Fig. 1



the point for the compound of molecular weight 452 falls on the same line as those for the methyl esters of the C_{27} and C_{32}-2,4,6-trimethyl-substituted acids. The positions of the compounds of molecular weights 466 and 494 in the diagram indicate that they possess still more branched structures. The mass spectrum of fraction B (M = 494) showed high peaks at m/e = 88 and 101, and very small peaks at m/e = 74, 115, and 157, which indicates methyl side chains at positions 2, 4, and 6. The mass spectrum of the ester suggested the presence of a methyl group also at position 8, but the relevant peaks were small. A comparison between the mass spectra given by the alcohol derived from synthetic methyl 2,4,6-trimethylnonacosanoate and that derived from fraction B provided conclusive evidence, however, that the main component (B) of methyl mycocerosate is the methyl ester of a 2,4,6,8-tetramethyloctacosanoic acid. The mass spectrum of 2,4,6-trimethylnonacosanol-1 shows a series of marked peaks due to hydrocarbon fragments formed by breaking the bonds to the tertiary carbon atoms. The C_{25}, C_{26}, C_{28}, and C_{29} groups of peaks are thus marked, whereas the C_{24}, C_{27}, and C_{30} peaks, which can only arise from fragments formed by simultaneous cleavage of two bonds, are of low height. In the alcohol derived from fraction B, the C_{22}, C_{23}, C_{25}, C_{26}, C_{28}, and C_{29} groups are marked, and the C_{21}, C_{24}, C_{27}, and C_{30} groups of peaks are low. The observed peak height distribution indicates the presence of methyl side chains at positions 21,23,25, and 27 from the hydrocarbon end of the molecule.

The alcohols were prepared on the milligram scale by reduction of the methyl esters by means of lithium aluminium hydride [13]. The alcohol (crude product) from fraction B had m.p. $\sim$ 35°, and alcohol derived from a partially separated mixture of the two stereo-isomeric methyl 2,4,6-trimethylnonacosanoates melted at 53°.

Components corresponding to fractions A and B are present also in the methyl esters derived from the *Test* and the *Canetti* strains of the tubercle bacilli. Our results indicate the occurrence of normal chain acids with 22, 24, 26, and 28 carbon atoms, 2,4,6-trimethyl-substituted acids with 25, 27, and 29 carbon atoms, and 2,4,6,8-tetramethyl-substituted acids with 30, 32, and 34 carbon atoms in the strains studied.

Full details of this work will be published later.

1. Asselineau, J., Ryhage, R. and Stenhagen, E. *Acta Chem. Scand.* **11** (1957) 196.
2. Anderson, R. J. *J. Biol. Chem.* **97** (1932) 639.
3. Wieghard, C. W. and Anderson, R. J. *J. Biol. Chem.* **126** (1938) 505.
4. Ginger, L. G. and Anderson, R. J. *J. Biol. Chem.* **157** (1945) 203.
5. Polgar, N. *J. Chem. Soc.* **1954** 1011.
6. Chanley, J. D. and Polgar, N. *J. Chem. Soc.* **1954** 1003.
7. Asselineau, J. *Compt. rend.* **237** (1953) 1804.
8. Org. Synth. **34** 13.
9. Ahlquist, L., Asselineau, J., Asselineau, C., Serck-Hanssen, K., Ställberg-Stenhagen, S. and Stenhagen, E. *Arkiv Kemi. In press.*
10. Bowman, R. E. and Fordham, W. D. *J. Chem. Soc.* **1952** 3945.
11. Wolfrom, M. L. and Karabinos, J. V. *J. Am. Chem. Soc.* **66** (1944) 909.
12. Hunsdiecker, H. and Hunsdiecker, C. *Ber.* **75** (1942) 291.
13. Nystrom, R. F. and Brown, W. G. *J. Am. Chem. Soc.* **69** (1947) 2548.

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