Mass Spectrometric Studies of Long Chain Methyl Esters

A Determination of the Molecular Weight and Structure of Mycocerosic acid

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Mass spectra of methyl esters of normal, long-chain fatty acids exhibit a series of peaks corresponding to ionized fragments of the general type $-(CH_2)_n-COOCH_3^+$ with intact methoxycarbonyl group.

The intensity distribution among this group of peaks in the case of methyl *n*-hexacosanoate is shown in Fig. 1 a. The spectrum also shows a large peak at $m/e = 74$, corresponding to an ionized molecule of methyl acetate formed by re-arrangement. This is analogous to the $m/e = 60$ peak found for *n*-carboxylic acids [1]. If the carbon atom in α-position to the methoxycarbonyl group carries one or two alkyl substituents these are included in the re-arranged fragment. A large peak thus appears at $m/e = 88$ for methyl 2-methylhexacosanoate (Fig. 1 b) and at $m/e = 116$ for methyl 2-methyl-2-ethyleicosanoate (Fig. 1 c).

Methyl 3-methyleicosanoate shows a large $m/e = 74$ peak, but the peak at $m/e = 87$ ($n = 2$) is comparatively low because the corresponding fragment can only be formed by the simultaneous breaking of two bonds and rearrangement of one hydrogen atom. On the other hand, $m/e = 101$ is large owing to easy cleavage of the bonds attached to the tertiary carbon atom in position 3.

Methyl 3,5-dimethyltricosanoate (Fig. 1 d) shows for $n = 1$—3 a behaviour very similar to that of the 3-methyl substituted ester just mentioned. The peak for $n = 4$ ($m/e = 129$) is small, as the corresponding fragment, like that of $m/e = 87$, can be formed only by the breaking of two bonds with simultaneous rearrangement.

Two stereo-isomers of methyl 3,4-dimethyldocosanoate have been studied. These give practically identical mass spectra, which show a strong $m/e = 74$ peak and a very large peak at $m/e = 101$. The latter corresponds to the fragment obtained by cleavage between the two tertiary carbon atoms in positions 3 and 4.

Methyl 2,4-dimethylheneicosanoate (Fig. 1 e) shows a weak peak at $m/e = 73$ and a strong one at $m/e = 88$, characteristic of a 2-methyl substituted ester. The peaks at $m/e = 101$ ($n = 3$) and 129 ($n = 5$) are strong, as they arise from cleavage of the bonds on both sides of the tertiary carbon atom in position 4. The peak at $m/e = 115$ is weak, as expected.

The methyl esters all give very strong parent peaks corresponding to the ionized unfragmented molecule. The mass-spectrum thus gives the exact molecular weight.

We have used the mass-spectrometric method to determine the molecular weight and structure of mycocerosic acid isolated from *M. tuberculosis* (human strain H-37) by Anderson and collaborators [2-4]. Ginger and Anderson [4] fractionated the methyl ester by distillation at low pressure. The highest-boiling fraction had m. p. 25°, $[\alpha]_D^{24}$—7.6° (chloroform), d_4^{30} 0.8541, n_D^{30} 1.4509. The analytical data for the acid agreed [4] best with $C_{30}H_{60}O_2$. Analogous or identical acids were later isolated by Chanley and Polgar [5] from a human strain of the bacillus, and by Asselineau [6] from the avirulent strain H 37 Ra. Polgar [7] subjected his compound, called mycoceranic acid, to stepwise degradation, and his results indicated the presence of methyl groups in positions 2, 4, and 6. The acid was considered probably to have 31 carbon atoms.

Cason and Fonken [8] have recently described a similar compound called C_{31}-mycosanoic acid. They report the following analytical data: C 79.89; H 13.50 %, equiv. wt. by titration 475. These data are, however, in better accord with the C_{32} formula (calc. for $C_{32}H_{64}O_2$: C 79.93; H 13.42 %; mol. wt. 480.8) than the C_{31} formula (calc. for $C_{31}H_{62}O_2$: C 79.76; H 13.39 %; mol. wt. 466.8).

The specimen of methyl mycocerosate used in the present work was prepared from a specimen of the acid obtained from Professor R. J. Anderson several years ago. A C-methyl

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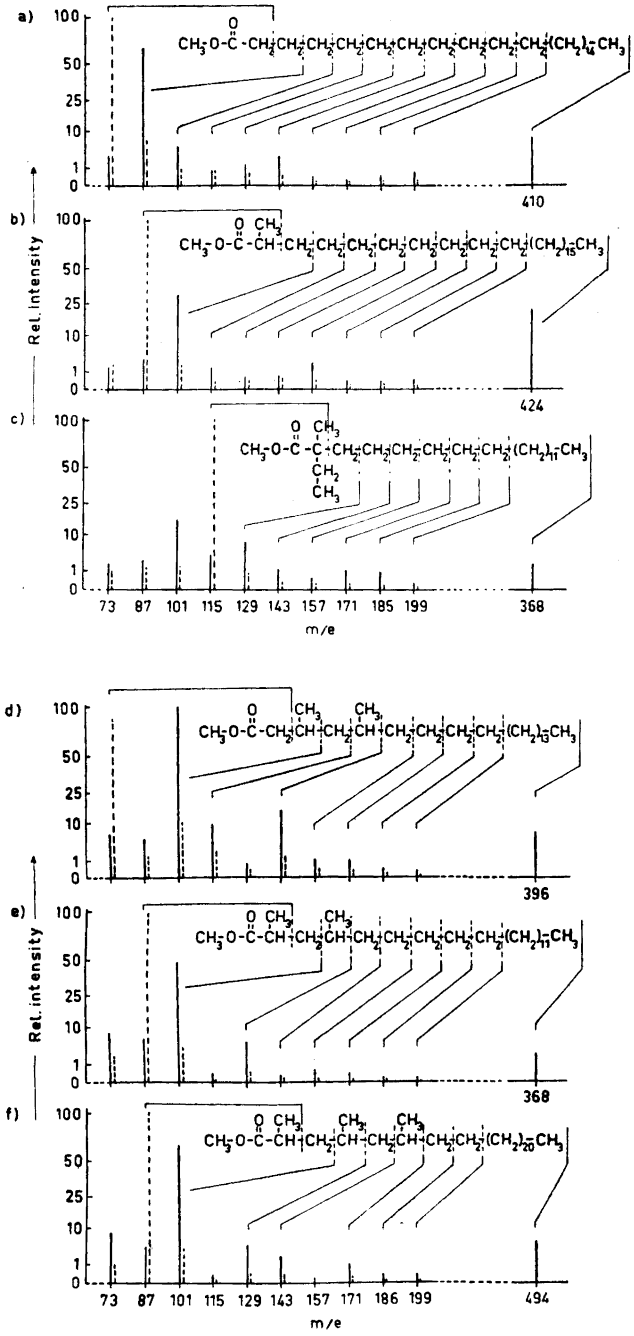

Fig. 1. Schematic diagram showing relative intensities of peaks corresponding to fragments containing an intact methoxycarbonyl group in mass-spectra of long-chain methyl esters. The heights of the vertical lines are proportional to the square root of the observed intensity, with the intensity of the strongest peak arbitrarily set at 100. Unbroken vertical lines represent normal fragments, and dotted lines re-arranged fragments in which the m/e is equal to m/e of the normal fragments plus one unit.
a) Methyl *n*-hexacosanoate b) Methyl 2-methylhexacosanoate c) Methyl 2-methyl-2-ethyleicosanoate d) Methyl 3,5-dimethyltricosanoate e) Methyl 2,4-dimethylheneicosanoate f) Methyl mycocerosate

group determination by a modified Kuhn-Roth method on the acid as received gave 3.33 equiv. of acetic acid, indicating the presence of four methyl groups [9]. The acid (887 mg) was converted into methyl ester by means of diazomethane, and the ester purified by chromatography on neutralized and slightly deactivated aluminium oxide. The ester fractions with the same refractive index (n_D^{25} 1.4520) were combined (589 mg), and distilled at 0.2 mm pressure: b. p. (air-bath temp.) 220—225°. The distilled methyl ester had m. p. 20.8—21.4°, $[\alpha]_D^{21.5}$ —7.8° (chloroform; *c*, 7.7); $n_D^{21.5}$ 1.4535; $d_4^{21.5}$ 0.8550.

The infra-red spectrum in the sodium chloride region was identical with that of methyl mycoceranate recently isolated by one of us (J.A.) from the *Test* strain of *M. tuberculosis* and with that of a specimen of methyl mycoceranate obtained from Dr. N. Polgar, Dyson Perrins Laboratory, Oxford, in 1953.

The purified methyl mycocerosate gave an excellent mass spectrum with a parent peak at $m/e = 494$ corresponding to the empirical formula $C_{33}H_{66}O_2$. There was no indication of the presence of homologous impurity. The empirical formula of the acid is thus $C_{32}H_{64}O_2$. In order to determine the nature and positions of the side chains the relevant parts of the mass spectrum were examined (Fig. 1 f). The low peak at $m/e = 74$ and strong peak at $m/e = 88$ show the presence of a methyl group in position 2. The low peak at $m/e = 115$ and the strong peaks at $m/e = 101$ and 129 indicate a further methyl side-chain in position 4 (*cf.* Fig. 1 e). Finally, the peak at $m/e = 157$ is very weak compared with those at $m/e = 143$ and 171, indicating the presence of a third methyl group in position 6. The methyl group positions derived for mycocerosic acid are thus the same as found for mycoceranic acid by Polgar [6].

A direct comparison with methyl mycoceranate from the *Test* strain was unfortunately not possible as the original sample had been converted into alcohol ("mycoceranol", m. p. 35.3—36.0°, $[\alpha]_D^{17}$ + 3.5° (chloroform)) before we had found that the methyl esters were especially suited to structure analysis. A mass-spectrometric study of the alcohol indicates the empirical formula $C_{32}H_{66}O$, and a comparison with the spectra of synthetic alcohols, including 2,4-dimethyleicosanol-1, shows that mycoceranol has methyl side-chains in positions 2, 4, and 6.

The results reported above make us believe that, apart from possible stereochemical differences (which cannot be studied by mass-spectrometric methods), mycocerosic, mycoceranic, and C_{31}-mycosanoic acids have the same structure, *viz.* that of 2,4,6-trimethylnonacosanoic acid.

We are indebted to Professor R. J. Anderson, Yale University, for the specimen of mycocerosic acid, and to Mrs. Stina Ställberg-Stenhagen for synthetic branched-chain acids. We also wish to thank Professor Einar Hammarsten for making possible the construction of a mass-spectrometer suitable for the study of structure problems in organic chemistry. Details of the instrument and details of the methyl ester and alcohol spectra will be given in forthcoming papers. The expenses of this work have been defrayed in part by grants from the *Swedish Medical Research Council.*

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Gradient Elution Analysis of Hop Bitter Substances

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Studies in this laboratory [1] have shown recently that the humulones may be separated by reversed-phase partition chromatography with carbon tetrachloride as the stationary phase and 61 % (w/w) aqueous methanol as eluant. In the same manner the lupulones were resolved using 69 % (w/w) methanol as the moving phase.

Subsequent work has shown that both humulones and lupulones can be separated