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Letter to the Editor

Identification of fatty acids longer than C_{32} in a sulphate-reducing bacterium by reversed-phase high-performance liquid chromatography and gas chromatography–mass spectrometry

Sir,

The development of improved analytical techniques has made it possible to obtain more detailed information about sources of very long-chain fatty acids (VLCFAs). The presence of VLCFAs in the microbial, plant and animal kingdom has recently been reviewed[1]. In prokaryotes, with the exception of mycobacteria[2–5], VLCFAs hardly occur. *Lactobacillus*[6], in which VLCFAs up to C_{30}, either saturated or monoenoic, could be identified, is one of the few exceptions. In a recent paper[7] we described the possible selective enrichment of a fatty acid (FA) mixture by VLCFAs using a combination of reversed-phase high-performance liquid chromatography (RP-HPLC) and gas chromatography–mass spectrometry (GC–MS). In previous work on VLCFAs in sulphate-reducing bacteria[8], FAs up to C_{34} were demonstrated by means of a special chromatographic technique. In this work we tried to enrich FAs from the bacterium *Desulfotomaculum* sp. by a combination of RP-HPLC and GC–MS techniques.

EXPERIMENTAL

Fatty acid methyl esters (FAMEs) were obtained from the sulphate-reducing bacterium *Desulfotomaculum* sp. strain 43 as described[8]. Portions of 50 mg of FAMEs were injected four times repeatedly and 172 μg of VLCFAs (C_{33} and higher) were obtained.

RP-HPLC

A semi-preparative separation of FAMEs was carried out by using a G-1 gradient LC system (Shimadzu, Kyoto, Japan) with two LC-6A pumps (4 ml/min), an SIL-1A sample injector and a C-R3A data processor. An SPLC-18 semi-preparative column (25 cm × 10 mm I.D.) (Supelco, Gland, Switzerland) was employed. The column was first eluted with methanol for 60 min, then with diethyl ether for 15 min and finally with methanol for 15 min. The fraction collected up to 27 min was discarded and that obtained within the interval 27–90 min was used for further analysis.

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GC–MS

The FAMEs (fraction 27–90 min) were analysed on a Finnigan MAT (San Jose, CA, U.S.A.) 1020 B instrument with an SPB-1 column (15 m × 0.25 mm I.D.) with a 0.25-μm film thickness (Supelco). The injection temperature was 100°C. The column temperature was programmed from 100°C (maintained for 1 min) at 20°C/min to 230°C and at 3°C/min to 320°C. The carrier gas was helium at a flow-rate of 70 cm/s.

RESULTS AND DISCUSSION

Fig. 1 shows that by semi-preparative RP-HPLC it was possible to separate all FAMEs longer than 33 carbon atoms. Although high FAME homologues were involved, by means of GC on a non-polar capillary column it was possible to separate straight-chain FAMEs. We were not able to obtain evidence that the abrupt decrease in the concentration of higher homologues resulted from incomplete enrichment of because they were not adequately recovered from the column. Nevertheless, we think that their concentration in the original sample was very low and, therefore, they could not be enriched to a greater extent.

From the mass spectra it was possible to identify peaks up to 40:0, *i.e.*, up to FAMEs of tetracontanoic acid. The complete mass spectrum of peaks 33–36 was obtained, indicating that in the spectra 33:0–36:0 FAME molecular ions (M^+) and corresponding splits in the region M^+ are always present. This situation is illustrated in Fig. 2. It can be seen that M^+ reaches almost 90% of the base peak (*i.e.*, the ion of *m/z* 74), isotope peak $(M + 1)^+$, *i.e.*, the ion of *m/z* 551 amounts to two fifths of its abundance. The ions of *m/z* 521 (M − 29), 519 (M − 31) and 507 (M − 43) confirmed the structure of methyl ester of hexatriacontanoic acid. As described earlier[7], ions of general formula $(CH_2)_nCOOCH_3$, where $n = 2, 6, 10, \ldots$, are also present and *m/z* assumes values of 87, 143, 199, ... In longer homologues (C_{37} and higher) a sharp decrease in their content in the mixture occurs and, unfortunately, the corresponding ions are not present in the M^+ region. We think that this phenomenon can be explained by a large decrease in the ion current of these homologues, resulting in a loss of the

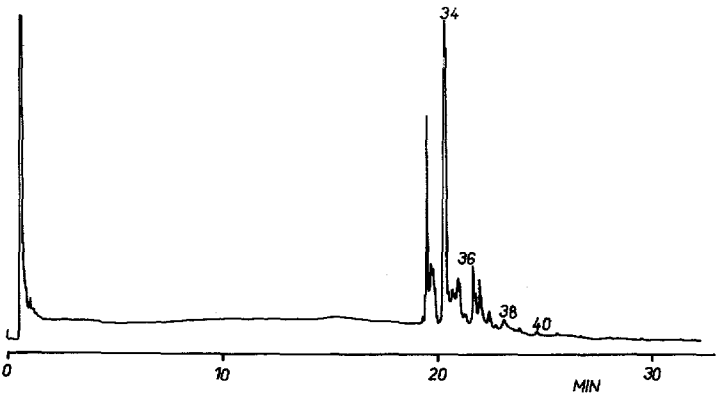

Fig. 1. GC–MS of total ion current of VLCFAs longer than 32. The peaks above 18.5 min are methyl esters; only even-carbon fatty acids are indicated, the numbers on the peaks representing the number of carbon atoms.

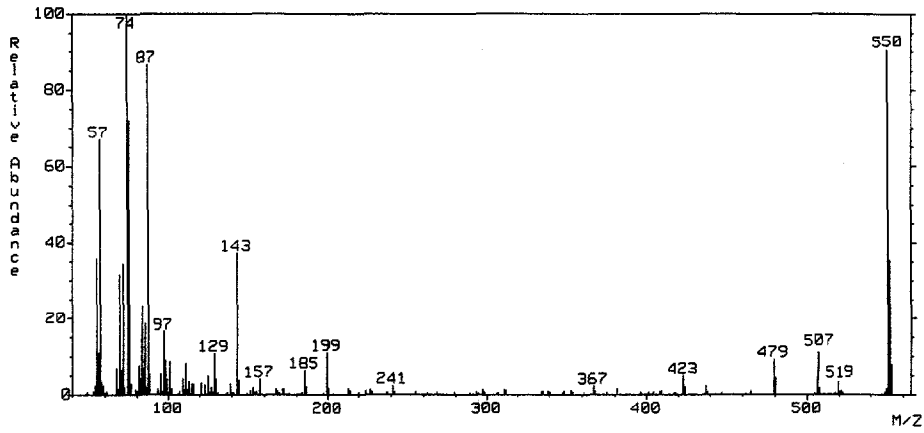

Fig. 2. Mass spectrum of 36:0 FAME.

diagnostic ions in the M^+ region. The structure of these higher homologues can be deduced either from values of the equivalent chain length (ECL) or from the mass spectra.

The mass spectra contained ions of up to *ca.* *m/z* 200, and an ion of *m/z* 74, *i.e.*, typical of saturated methyl esters (formed by McLafferty rearrangement), was also present. Compared with prokaryotes, only mycobacteria can compete in the length of VLCFAs with this sulphate-reducing bacterium. It is concluded that the so far highest FAMEs, *i.e.*, VLCFAs of up to 40:0, could be demonstrated.

The combination of RP-HPLC and high-temperature capillary GC–MS thus extends the analytical possibilities of both methods and contributes to the acquisition of new information on biological materials.

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