The Lipids of Hydrogen-Oxidizing Bacteria: Occurrence of *cis*-9,10-Methylene Hexadecanoic Acid in *Hydrogenomonas* H 16

Hydrogen-oxidizing bacteria are able to oxidize molecular hydrogen with oxygen as the hydrogen acceptor and to utilize the energy thus obtained for the assimilation of carbon dioxide. *Hydrogenomonas* is the most representative genus of these bacteria. This genus has been intensively studied with respect to its morphological, physiological and biochemical properties[1]. Its lipids, however, have not been investigated – with the exception of poly-β-hydroxybutyrate (PHB).

If carbon dioxide is assimilated under storage conditions' (e.g. if the cells are kept in a nitrogen-free mineral solution), *Hydrogenomonas* cells accumulate large amounts of PHB as intracellular granules[2]. PHB can be completely extracted with chloroform. Part of other cellular lipids is also extracted by this procedure, while the remaining 'free' lipids can be subsequently extracted with more polar solvents (e.g. ethanol/diethyl ether). The latter lipids are apparently more strongly bound to cellular constituents than the former. In the present investigation, only the 'loosely bound' lipids other than PHB have been studied.

Hydrogenomonas strain H 16 was grown chemolithotrophically in a homogeneous suspension at 28 °C and supplied with a gas mixture of hydrogen, oxygen, and carbon dioxide as described by SCHLEGEL et al.[3]. The bacteria were harvested after growth under storage conditions, washed with water, lyophilized, weighed, and extracted with chloroform, yielding a mixture of the accumulated PHB and other 'loosely bound' liqids (non PHB). PHB was separated from non-PHB by precipitation with diethyl ether. Details on culture conditions, on extraction and separation procedures, as well as the yields of lipids in relation to the bacterial growth phases, will be described in a forthcoming paper[4]. On column chromatography[5] the non-PHB were fractionated into neutral lipids (eluted with chloroform) and polar lipids (eluted with methanol).

Thin-layer chromatography of the polar lipids with 2 different solvent systems (diisobutyl ketone/formic acid/water 40:15:2, v/v/v[6] and chloroform/methanol/glacial acetic acid/water 50:25:8:4, v/v/v/v/[7]) revealed only one component. Its colour reaction was found to be positive with phosphorus spray reagent[8] and with ninhydrin spray[9]. Co-chromatography with various pilot samples indicated phosphatidyl ethanolamine. The widespread occurrence of this phospholipid in bacteria has been reported repeatedly[10]. The fatty acid composition of the phosphatidyl ethanolamine was studied by gas-liquid chromatography (GLC). As shown in Figure 1, the major fatty acid is a C_{17} cyclopropane acid. The presence of a propane ring was supported by the following facts: a) No change of retention time in GLC after mild hydrogenation, indicating absence of unsaturation. b) After refluxing with a solution of hydrochloric acid in methanol a marked decrease of the GLC peak was observed. This phenomenon is said to be characteristic for cyclopropane fatty acids[11]. c) The ratio of retention time of unknown/retention time of $C_{16:0}$ methyl ester is app. 1.6 and therefore identical with the ratio of retention time of lactobacillic acid methyl ester/retention time of $C_{18:0}$ methyl ester as observed in previous studies on *Brucella* fatty acids[12]. This observation indicates a cyclopropane acid with a chain length of 17 carbon atoms.

The mixture of fatty acid methyl esters was hydrogenated in order to decrease the number of components. The cyclopropane acid methyl ester was subsequently isolated by preparative GLC (using an Autoprep apparatus equipped with a 240 cm column of 10% ethylene glycol adipate polyester on Chromosorb 40–60 mesh, at 175 °C, helium flow rate 200 ml/min). The following facts are consistent with the presence of a *cis*-cyclopropane ring in the isolated fatty acid methyl ester: a) Mass spectrometry shows a parent peak at *m/e* 282 indicating the lack of 2 hydrogen atoms with respect to a normal alkanoic acid methyl ester. This lack must be due to an alicyclic struc-

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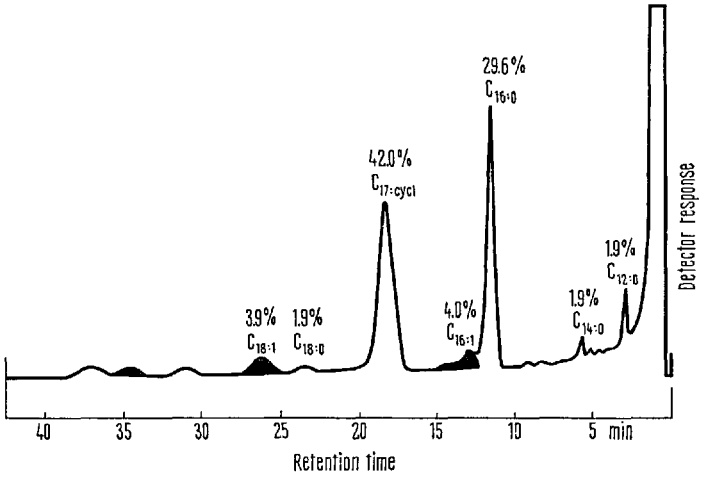

Fig. 1. Gas-liquid chromatogram of fatty acid methyl esters of 'loosely bound' phosphatidyl ethanolamine of *Hydrogenomonas* H 16. Pye Argon chromatograph, 120 cm glass column with 10% ethelene glycol adipate polyester on Chromosorb WS, 80–100, 177° mesh column temperature, gas flow 60 ml/min. Black areas: unsaturates (disappearing after catalytic hydrogenation).

ture since unsaturation has been excluded. b) IR-spectroscopy (as film on a Perkin Elmer model 125) revealed a characteristic band at 9.8 μm.

The localization of the propane ring was determined by GLC (Aerograph model 204 A equiped with a 240 cm column of 10% SE-30 on Chromosorb W, programming of temperature 100–260°, rise of 2°/min, N_2 flow rate 200 ml/min) after oxidative degradation by refluxing with potassium permanganate in acetone[13]. A series of homologous fatty acids up to $C_{7:0}$ and from $C_{10:cycl}$ to $C_{17:cycl}$ was obtained (see Figure 2). From the absence of C_8 and C_9 fatty acids, the C_{17} cyclopropane fatty acid was decided to be 9,10-methylene hexadecanoic acid. The change of character of the acids with increase of carbon number from straight chain to cyclopropane was clearly demonstrated by co-chromatography of a series of even numbered straight chain alkanoic acid methyl esters up to $C_{16:0}$.

The neutral lipid fraction of non-PHB consisted of a variety of components. Only small amounts of long-chain fatty acids were obtained by saponification. GLC of these fatty acids (as methyl esters) revealed C_{17} cyclopropane acid as the major component (98.4%, w/w) besides palmitic acid (1.6%, w/w).

C_{17} cyclopropane acid has been detected in a number of bacteria, predominantly gram-negative (listed in[12]), the localization of the propane ring, however, has not been determined in all these cases. In cases known so far, the propane ring is situated between C-9 and C-10.

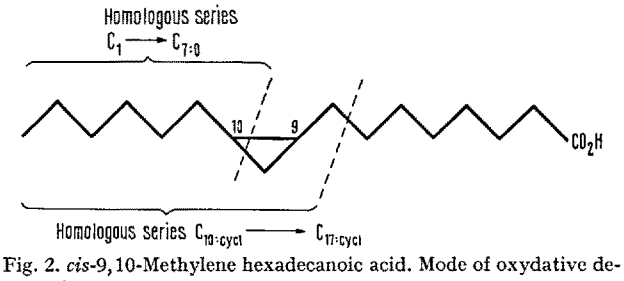

Fig. 2. *cis*-9,10-Methylene hexadecanoic acid. Mode of oxydative degradation.

It is well established[14] that *cis*-cyclopropane acids in bacteria are synthesized by transfer to the corresponding *cis*-monoenoic acid of a methylene group from S-adenosyl methionine. It is worthy of note that both *cis*-9,10-methylene hexadecanoic acid and lactobacillic acid (i.e. *cis*-11,12 methylene octadecanoic acid, present in a variety of bacterial species) belong to the same ω-family (with propane ring at the same position from the terminal methyl group). This fact is consistent with the finding that bacteria can synthesize long-chain monoenoic fatty acids by elongation of already unsaturated precursors[15].

Zusammenfassung. Knallgasbakterien (*Hydrogenomonas* H 16) wurden unter Speicherbedingungen in Submerskultur herangezogen. Die dabei gespeicherte Poly-β-hydroxybuttersäure wurde zusammen mit anderen «lokker gebundenen» Lipiden mit Chloroform extrahiert. Die daraus abgetrennten polaren Lipide bestanden ausschliesslich aus Phosphatidyläthanolamin. Die Analyse der Fettsäurezusammensetzung dieses Phosphatids ergab einen Gehalt von 42% (bezogen auf das Gewicht der Gesamtfettsäuren) einer C_{17}-Cyclopropansäure. Durch spektroskopische Untersuchungen und oxydativen Abbau wurde letztere als *cis*-9,10-Methylenhexadecansäure identifiziert.

O. W. Thiele[16]

Physiologisch-Chemisches Institut der Universität, Humboldtallee 7, D-34 Göttingen (Germany), 3 June 1971.

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Optical Rotatory Dispersion of Proline-Rich Peptides from the Venom of *Bothrops jararaca*

The observation that the venom of the Brazilian snake Bothrops jararaca potentiates bradykinin[1] and also inhibits the enzyme[2] that converts angiotensin I into angiotensin II led to the isolation[3,4] of a series of peptides responsible for these activities. Subsequently, the sequences of peptides I–V (Table I) were elucidated and proved by synthesis[4]. Compound VI is a synthetic peptide prepared to determine what is the active 'core' of IV, while VII is an equiactive analog of the pentapeptide isolated by Greene and Ferreira[3] to which they assigned the structure Pyr-Lys-Trp-Ala-Pro.

A conspicuous feature of the venom peptides I-V is the frequency with which proline occurs in them. Compound VI, a pentapeptide, contains two proline residues, while there is only one in peptide VII. Yet, a second structural feature is equally remarkable: in each peptide isolated from the venom so far, pyroglutamic acid is the N-terminal residue. Pyroglutamic acid resembles proline; its pyrrolidone ring is probably even more rigid than the pyrrolidine in proline. The combination of several constrained areas in a sequence could result in a more or less well-defined geometry of the chain and therefore it was intriguing to explore, with the aid of ord and cd spectra, the existence of a preferred conformation in these peptides.

Not quite unexpectedly, the ord spectra of peptides I-VII resemble those of proline oligomers[6] and of polyproline[7], though no mutarotation could be observed in acetic acid. All spectra (Figure) exhibit, as a principal feature, a trough around 215 nm, although the mean residue rotation at this wavelength is different from pep-

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