ON THE FATTY ACIDS OF PLEUROPNEUMONIALIKE ORGANISMS[1]

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In recent years progressively increasing attention has been given to the pleuropneumonialike organisms and to such similar entities as bacterial protoplasts and the L forms. The chemical composition of PPLO has been of particular interest, not only per se, but for comparison with the composition of other microbial forms. While progress has been made in the analyses of carbohydrate and nitrogenous components, relatively little has been learned of the fatty acid composition of PPLO. Information regarding the lipids of these organisms has been largely limited to per cent total lipid, sterol and phospholipid, and to the proportion of volatile to non-volatile fatty acids (Lynn and Smith, 1960).

The purpose of the present paper is to report analyses of the medium and long chain fatty acids of the 07 strain of PPLO. Samples of the mixed fatty acids of this organism were obtained through the generosity of Dr. Paul F. Smith of the University of South Dakota. The acids were extracted with methanol, saponified, the non-saponifiable matter removed, and the free fatty acids recovered in the usual manner. From the PPLO grown in 55 liters of culture, 190 mg. of mixed fatty acids were finally obtained. With such a limited amount of material, it was essential that the analytical methods to be employed be confined to those that could provide a maximum of information while consuming a minimum of sample. More exhaustive studies must await the availability of larger samples.

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The mixed fatty acid preparation was a yellowish-white waxy solid at room temperature and melted readily when warmed above 40°C. This material was found to have a neutral equivalent of 262 which indicated a preponderance of C_{16} acid. The iodine number, as determined by the Wijs method, was 12, which suggested a monounsaturated acid content of around 10 %. Microhydrogenation experiments gave results which were in agreement with this data.

In anticipation of the probability that the amounts of individual fatty acids recovered by chromatographic means would not be sufficient for further manipulation and study, and in order to obtain some insight into the types of compounds that might be encountered, several infra-red studies were made of the mixed fatty acids. A typical infra-red absorption spectrum is shown in Fig. 1. Such spectra exhibited features characteristic of fatty acids, and

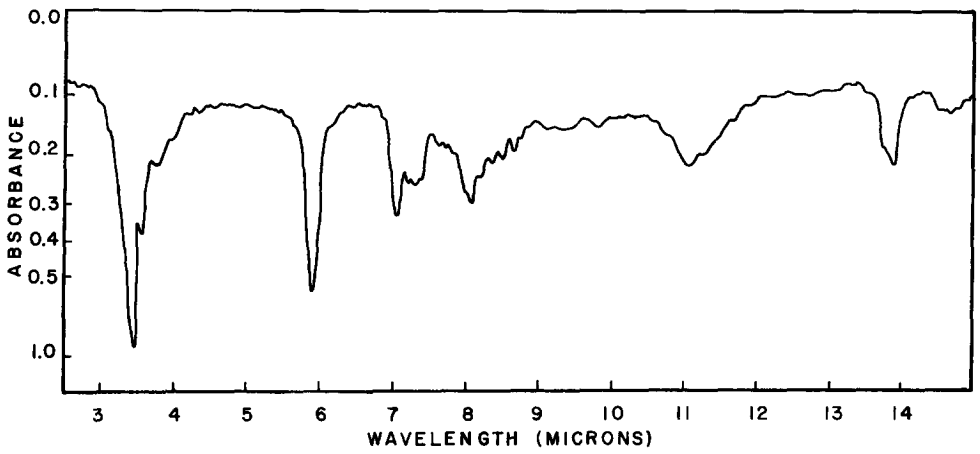

Fig. 1 Infra-red spectrum of mixed PPLO fatty acids

indicated the presence of some compounds containing cis ethylenic bonds. A slight but discernible absorption at 9.8 microns which is characteristic of the cyclopropane ring suggested the possible presence of acids containing this structure. Both cis ethylenic fatty acids and cyclopropane fatty acids have been shown to be major constituents of the lipids of many conventional bacteria (e.g., Hofmann, et al., 1955; O'Leary, 1959; and many others).

The fatty acid composition of this mixture was studied by means of gas-liquid chromatography. The fatty acids were converted to their methyl esters by the use of boron trifluoride in methanol (Metcalfe and Schmitz, 1961). The analyses were performed using a Perkin-Elmer fractometer fitted with a 2 or 4 meter silicone column and thermistor detector under the following conditions: column temperature, 220°C; gas, helium; flow rate, 110 ml./minute. A typical chromatogram is shown in Fig. 2. The per cent of each fraction and its tentative identification based on chromatographic behavior are shown in Table 1.

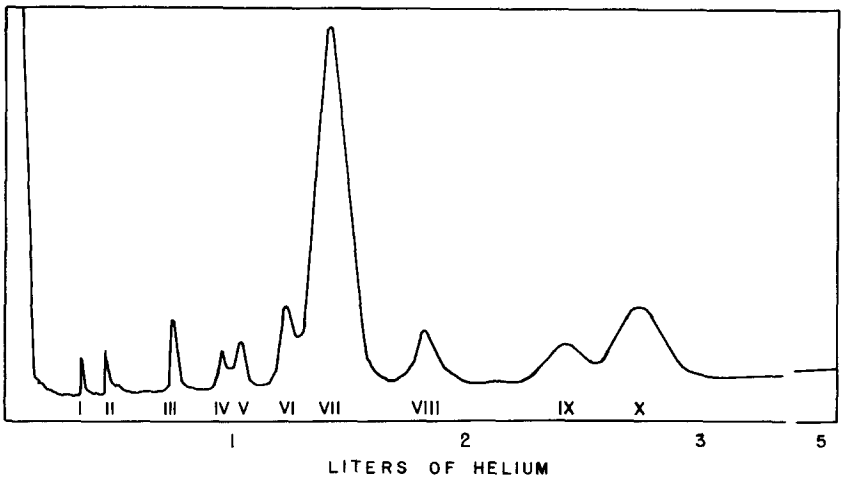

Fig. 2 Typical gas-liquid chromatogram of PPLO fatty acid methyl esters.

TABLE 1

FRACTION	PER CENT OF TOTAL FATTY ACIDS	TENTATIVE IDENTIFICATION
I	1.1	C_{10} saturated
II	1.3	C_{12} "
III	3.0	C_{14} "
IV	1.8	Unknown
V	2.4	C_{15}
VI	6.0	C_{16} unsaturated
VII	56.5	C_{16} saturated
VIII	5.3	C_{17}
IX	7.1	C_{18} unsaturated
X	15.5	C_{18} saturated

It was possible to identify fractions I, II, III, VI, VII, IX and X by comparisons with available authentic standards. The chromatographic behavior of fraction VIII was identical to that exhibited by the cyclopropane fatty acid found in Escherichia coli (O'Leary, 1959; Dauchy and Asselineau, 1960). This compound has now been shown to be cis-9,10-methylene hexadecanoic acid (Kaneshiro and Marr, 1961). The acid comprising fraction VIII is presumed to be similar if not identical to this compound. Fraction V exhibits the mobility expected of a C_{15} acid, and may well be a C_{15} cyclopropane acid similar to the one found in Clostridium butyricum by Goldfine and Bloch (1961).

The fatty acid whose identity is most interesting and least certain is the one comprising fraction IV. Its chromatographic behavior indicates that it is some type of C_{15} compound, but one not identical with that in fraction V. Available information on saturated cyclopropane C_{15} and branched-methyl C_{15} acids indicates that fraction IV is neither of these. On the basis of data now available, it appears that fraction IV is an unsaturated C_{15} acid. Four types of compounds could answer this description: a cyclopropene acid; or a cyclopropane, branched-methyl or straight chain acid with a double bond somewhere along the chain. If this fraction does prove to be a C_{15} unsaturated acid, and particularly if it is a cyclopropene acid, it may well prove to be a major clue to the as yet obscure biosynthetic pathway to the cyclopropane acids. Such unsaturated acids, while never before observed in microbial lipids, have been suggested as possible precursors of the cyclopropane acids found in many bacterial species. Reexaminations of many of the author's analyses of bacterial fatty acids have uncovered evidence of similar fractions, notably in E. coli and other Gram-negative bacteria.

Several overall differences may be noted between the fatty acid contents of PPLO and those of many bacterial species. The proportion of unsaturated to saturated acids is much lower in PPLO than in "whole" microorganisms, i.e., those having a cell wall. The unsaturated acid content in this strain of PPLO is approximately 13 %, while in many bacteria it ranges from 30 to 60 % and even higher. There is a total absence of any C_{19} cyclopropane acid

while such occur frequently in bacteria. Lastly, among the saturated acids, the percentages of C_{10}, C_{12}, C_{14} and C_{18} compounds are quite similar to those encountered in bacteria, but the percentage of C_{16} acid is greatly elevated, amounting to approximately twice the usual bacterial concentration.

These differences suggest that the distribution of fatty acids in microbial cells may not be homogeneous, but that instead various cellular structures may be rich in some acids and poor in others. Accordingly, it would appear that a rewarding line of research would be the study of the fatty acid contents of the individual cellular components such as the cell wall, membrane, protoplast and so on as contrasted with the total fatty acid contents of the whole cell.

Studies along these lines are now in progress in the author's laboratory, and will be reported in more detail as soon as feasible.

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