Notes

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Identification of 9,10-methylenehexadecanoic acid in some aerobic "Actinomycetales" by a combined gas chromatographic–mass spectroscopic technique*

In a survey of the fatty acid components of 43 strains of aerobic *Actinomycetales* the occurrence of small amounts (from traces up to 6 % of the total fatty acids) of an acid different from members of the *normal*, *iso* and *anteiso* series was noticed in 13 organisms[1]. Of these, eleven belonged to the genus *Streptomyces*, one was a *Micromonospora* and one was a *Streptosporangium*. By a combined gas chromatographic and mass spectroscopic microtechnique the unknown compound has now been identified as 9,10-methylenehexadecanoic acid, a cyclopropane fatty acid first observed in *Escherichia coli*[2–5] and later found in several other Gram-negative and in a limited number of Gram-positive bacteria[6].

The unknown methyl ester was isolated from the mixture of fatty acid methyl esters by gas chromatography (Fractovap G, from C. Erba, Milan) using a column of 3 m length and 6 mm internal diameter, packed with 15 % diethylene glycol succinate on silanized Chromosorb W; the column was operated isothermally at 180° with helium as carrier gas. The unknown methyl ester emerged after methyl heptadecanoate, from which it was cleanly separated, and immediately preceded methyl 16-methylheptadecanoate. The purified compound was analyzed by gas chromatography on the same capillary column described in a previous paper[1] and resulted slightly contaminated by methyl 16-methylheptadecanoate. Without further purification it was examined with a combined gas chromatograph–mass spectrometer (LKB 9000, from LKB-Producter AB, Stockholm). A column of 3 m length and 4 mm internal diameter, packed with 2 % Apiezon L on Chromosorb W and operated at 170° with helium as carrier gas, was used; in this system the unknown ester is neatly resolved from methyl 16-methylheptadecanoate. The separator was run at 230° and the ion source at 250°; the electron energy was 70 eV. The mass spectrum taken on the leading edge of the peak corresponding to the unknown ester was consistent with that of either a straight chain monounsaturated or a cyclopropane C_{17} fatty acid. It is indeed known that mass spectra of these two classes of compounds are practically indistinguishable[7,8]. As the chromatographic behaviour of the unknown ester was unchanged after hydrogenation at room temperature with a palladium on carbon catalyst, the presence of a double bond was excluded; strong evidence in favour of a cyclopropane ring was yielded by the infrared spectrum (liquid film) which showed a medium intensity band at 9.8 μ.

In order to locate the position of the cyclopropane ring in the carbon chain the combined chemical and mass spectroscopic technique of POLACHECK *et al.*[9] was adopted. The validity of this technique was confirmed, before application to the

* Dedicated to Professor E. LEDERER on the occasion of his 60th birthday.

unknown ester, with a sample of methyl 9,10-methylenehexadecanoate (obtained through the courtesy of Prof. J. ASSELINEAU), and has also been recently substantiated by a publication of MCCLOSKEY AND LAW[10] which appeared when the results reported in this paper had already been completed. The unknown ester was hydrogenated over Adams catalyst in glacial acetic acid, the solvent removed and the residue analyzed by gas chromatography on the capillary column described in previous work[1]. Two main peaks were observed; the retention time of the first was compatible with that of C_{17} methyl esters branched in a position remote from the terminal methyl group, that of the second corresponded to methyl heptadecanoate. The ratio of the areas of the first to the second peak was 7 to 1. The products present in the two peaks were analyzed by the combined gas chromatograph–mass spectrometer apparatus under the same conditions adopted for the analysis of the compound before hydrogenation. The fragmentation pattern of the material emerging in the first peak was that expected for a mixture of methyl 9- and 10-methylhexadecanoates and was in fact exactly the same observed in the mass spectrometric analysis of the corresponding peak obtained from the authentic sample of methyl 9,10-methylenehexadecanoate run through the same series of operations. The mass spectrum of the material emerging in the second peak confirmed the formation of methyl heptadecanoate. These results unambiguously prove that the unknown acid detected in several aerobic *Actinomycetales* is 9,10-methylenehexadecanoic acid; with the very few milligrams prepared in the course of the present investigation it was impossible to establish whether the compound has the same stereochemistry as the acid isolated from *Escherichia coli*.

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