Mass-Spectrometric Evidence for Methyl and Ethyl Esters of Long-Chain Fatty Acids in Ox Pancreas

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As has been reported previously, methyl esters of long-chain fatty acids occur naturally in various tissues of mammals (Dhopeswarkar & Mead, 1962; Leikola, Nieminen & Salomaa, 1965; Fischer, Sauk & Kabara, 1966). In these studies methyl esters were identified by both gas-liquid and thin-layer chromatography and by chemical analysis. In this paper a mass-spectrometric study of the esters is reported, which has shown the presence of ethyl esters in addition.

The extraction and all other procedures were performed without using methanol or ethanol so that artifacts might be avoided. Such artifacts can be produced quite easily, as we have found, in agreement with others (Fischer *et al.* 1966; Czeglédi-Jankó, 1965; Lindlar & Wagener, 1964; Skořepa, Hrabák, Mareš & Novák, 1967).

Fresh ox pancreas was obtained at the slaughterhouse and 30 g. of parenchymatous tissue was immediately homogenized with about 300 ml. of chloroform. The tissue was extracted for 60 min. with intermittent stirring, and the solvent was then removed by filtration and evaporated on a water bath under a stream of nitrogen. The extract was chromatographed on a column of alumina, and on a thin layer of silica gel by the technique described by Leikola *et al.* (1965). Individual fractions were detected with 2,7-dichlorofluorescein on the border of the plate. The second fraction before the second front was shown by gas-liquid chromatography to contain long-chain fatty acids as methyl esters. This material was scraped from the plate and extracted with chloroform, and undissolved material was removed by centrifugation. The extract was then rechromatographed under the same conditions. After detection and extraction as before the chloroform was evaporated under the flow of nitrogen and the pure extract was dissolved in light petroleum and sealed in an ampoule under nitrogen.

The mass-spectrometric study was performed with mass spectrometer LKB 9000 (LKB Produkter AB, Stockholm, Sweden) coupled through a two-step molecule jet separator with a gas chromatograph. The column was 2.7 m. $\times$ 1.5 mm. internal diam. packed with 5% SE-30 on 100-120 mesh Gas-Chrom Q (Applied Science Laboratories Inc., State College, Pa., U.S.A.). The temperature was 200° in the flash-heater, column and separator. The carrier gas was helium at 20 ml./min. To obtain mass spectra the acceleration voltage was kept constant at 3500 v and the energy of electrons was 70 eV.

Eight peaks (*A-E*) were resolved, which gave molecular masses 270, 284, 264, 298, 310, 312 and 314 respectively; the mass of the last peak was not recorded. Peaks *A* and *C* were methyl esters of hexadecanoic acid and octadecanoic acid respectively, and peaks *B*, *D* and *E* corresponded to ethyl esters of hexadecanoic acid, octadecanoic acid and octadecenoic acid respectively (Fig. 1).

This finding supports the previous evidence of naturally occurring methyl esters in mammalian tissues demonstrated by other methods (Dhopeswarkar & Mead, 1962; Leikola *et al.* 1965; Fischer *et al.* 1966), and, in addition, establishes the presence of ethyl esters. The origin and physiological role of these compounds are not clear.

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Fig. 1. Mass spectra of the peaks resolved by gas-liquid chromatography from a neutral-lipid fraction of ox pancreas. *A* and *C*, Methyl esters of hexadecanoic acid and octadecanoic acid respectively; *B*, *D* and *E*, ethyl esters of hexadecanoic acid, octadecanoic acid and octadecenoic acid respectively.