Separation of Human Skin Waxes by Gas Chromatography

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Wax esters occur widely in both plants and animals. They were among the first natural products to be studied in modern times (cetyl alcohol was obtained from sperm oil by Chevreul in 1817¹), but they have received very little attention in recent years. Their study is difficult because the isolation and identification of pure compounds has required prolonged fractionation procedures.

The development of gas chromatographic methods for the separation of steroids² suggested, that related techniques might be developed for work with wax esters. However, waxes usually have a wide molecular weight range, and under isothermal conditions this leads to unduly long retention times for higher members of the series. Further, for many studies compounds containing up to about forty carbon atoms must be separated. Preliminary studies indicated, that suitable methods could be developed by employing a relatively thin film of thermostable liquid phase in the

column, and by using a temperature-programmed procedure. Phases with sufficient thermal stability for this work were found to be silicone polymer SE-30 (General Electric Co.), ethylene glycol isophthalate (EGIP), neopentyl glycol succinate (NGS) and fluoroalkyl silicone polymer QF-1 (Dow-Corning Corp.). Of these, SE-30 was the most useful because of its ability to separate monoene and diene esters from saturated esters. With temperature programming from 100° to 280° and with a 1 % SE-30 column it was found possible to scan a wide molecular weight range, and to separate unsaturated and saturated esters from about C₁₀ to the C₄₀-44 level. Two applications were examined.

Fig. 1 shows a separation of the wax esters of a commercial sample of spermaceti. It is known, that cetyl palmitate is the major component of this material³. This is clearly indicated by the analytical record; the presence of even and odd-numbered homologs is also indicated. The absence of unsaturated esters was confirmed by analytical gas chromatographic examination of the acid and alcohol fractions resulting from hydrolysis of the wax.**

Fig. 2 shows a separation of the human wax esters of the skin surface lipid film. Skin surface lipids were collected by acetone washing, and a wax fraction containing both wax esters and cholesterol esters was separated by chromatography on silicic

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** Early studies on sperm oil indicate that unsaturated compounds are present; these are evidently lost in the commercial isolation process used for spermaceti.

Fig. 1. Separation of components of spermaceti. Conditions: 6 ft. $\times$ 3.4 mm glass coil column; 1 % SE-30 (General Electric Co.) on 100–140 mesh Gas-Chrom P; argon inlet pressure 30 psi; argon ionization detector. Heating rate, 1.73°C/min. The sample was introduced in benzene solution. Carbon numbers refer to total carbon content of wax esters; full scale deflection as shown is 1×10^{-8} amp.

Fig. 2. Separation of components of human skin surface waxes. The conditions were the same as for Fig. 1. Carbon numbers refer to total carbon content of wax esters.

acid⁴. The major wax esters were recognized as a monene and a diene C_{36} ester by comparison with authentic (synthetic) samples of saturated and unsaturated wax esters. Homologs differing by a $-\text{CH}_2\text{CH}_2-$ group are also indicated in the figure. This separation, while giving a precise definition of the molecular weight range of waxes of the human skin surface, indicates only the combined chain length present in the esters; it does not indicate the individual acid or alcohol chain lengths. The ester fraction was saponified, and the distribution of individual acids and alcohols was determined by analytical gas chromatography (the acids were separated as methyl esters, and the alcohols were separated both free and as acetates). The major alcohols were found to be eicosanyl alcohol and a monene C_{20} alcohol. The occurrence of saturated and unsaturated C_{20} alcohols is in agreement with mass spectroscopic work carried out in a study of human hair fat.⁵ The "iso" C_{20} alcohol reported in an earlier study of sebum alcohols⁶ was found only as a relatively minor component. The major acid was found to be palmitoleic acid. These results suggested, that the two chief C_{36} wax components were eicosanyl palmitoleate and eicosenyl palmitoleate (unsaturated bond position unspecified in the alcohol). To confirm this finding, a preparative separation on a milligram scale was carried out for the major C_{36} ester. An analytical examination of the acids and alcohols in the

isolated fraction showed, that this material was in fact almost entirely derived from an eicosenyl alcohol and palmitoleic acid. Small amounts of branched chain acids and alcohols were also present, indicating that some branched chain esters as well as straight chain esters were present originally.

As far as we can determine, unsaturated waxes of the structure found here have not been reported previously, in spite of the almost universal occurrence of long chain wax esters in biological systems.

The gas chromatographic techniques used for this work should prove useful for studying many other natural products.

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Received April 21, 1961.