ozone in acetic acid at room temperature for three days. The ozonides were decomposed with hydrogen peroxide. The β-methyl malic acid formed was extracted with ether from a pH 3.5–4 aqueous solution of the reaction products (in which the oxalic acid remained), and its di-*p*-bromophenacyl ester, $C_{21}H_{18}O_7Br_2$, was prepared and recrystallized from ethanol. The product (A) had a m.p. of 186–7°.

For comparison with product A, *erythro*-β-methyl malic acid di-*p*-bromophenacyl ester was prepared from *erythro*-1,2-diphenylpropanol [9] in a similar way. The product (B) showed the m.p. 187–8°, which was undepressed on admixture of A.

Threo-β-methyl malic acid di-*p*-bromophenacyl ester was similarly prepared from *threo*-1,2-diphenylpropanol [9]. The product (C) melted at 101–3°.

The IR curves of A and B were superimposable while that of C was distinctly different.

The β-methyl malic acid (II) obtained from dehydrodi-isoeugenol thus being the *erythro*-form, the substituents at the coumarane ring in the latter compound must be arranged as shown in formula (I) (*trans*-configuration).

PMR investigations (S. Forsén) of dehydrodi-isoeugenol, 2,3-dibromocoumarane and dihydrocoumarilic acid showed that the spin coupling between protons attached to carbon atoms 2 and 3 of the oxygen ring is dependent not only on the configuration but also on the nature of the other substituents at those positions.

We intend to publish full details of these investigations in this journal. A grant from *Cellulosaindustriens forskningsstiftelse, 1959 års fond* is gratefully acknowledged.

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Separation and Isolation of Waxes and Sterol Esters of Skin Surface Fat with Thin Layer Chromatography *

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The skin surface fat consists of a mixture of hydrocarbons, sterol esters, waxes, glycerides, free fatty acids and phospholipids [1-3]. New analytic methods, *i.e.* silicic acid chromatography and gas chromatography have elucidated the composition of the skin surface fat [4-7]. However, the separation of the waxes and sterol esters from each other has not satisfactorily succeeded with these methods [6]. The resolution of these fractions with thin layer chromatography is described in this report.

The skin surface fat was eluted with acetone from the thoracic region of the back [6], which had been cleaned by a blank wash five hours earlier. The acetone was removed in a rotating evaporator at 50°C and the residue extracted with hexane. The hexane extract was filtered and evaporated. A sample for thin layer chromatography was taken from the dry residue and the main part was transferred on a silicic acid column to isolate a fraction containing the squalene, the waxes and the sterol esters. The details of the chromatography have been described earlier [6]. The eluate was then evaporated and the residue, 65 mg in total, was dis-

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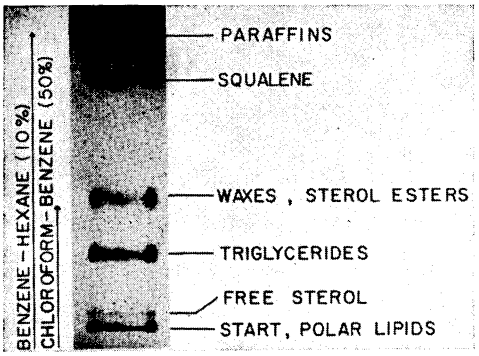

Fig. 1. Separation of human skin surface lipids with thin layer chromatography on Silica Gel G.

solved in hexane and subjected to thin layer chromatography.

The separation pattern of the total skin surface lipids on standard thin layer plates of Silica Gel G (Merck) is shown in Fig. 1. The spots have been made visible by spraying the plates with potassium bichromate-sulphuric acid with subsequent heating for 30 min at 125°C [8]. The fractionation is very similar to that obtained with silicic acid column chromatography [6], but the resolution is better on thin layer plates. However, no sufficient separation of waxes and sterol esters was obtained on Silica Gel G thin layer plates. Replacing the silica gel with Aluminum Oxide G (Merck) and using 1 % benzene-hexane as solvent, all the components of the squalene-sterol ester-wax preparation distinctly separated from each other (Fig. 2 (1)).

For rechromatography of these fractions material was collected from 20 such thin layer plates stained with Rhodamine 6-G (BDH). The areas of the support containing the waxes and the sterol esters, respectively, were scraped off under UV-light and extracted with chloroform. The chloroform was evaporated and the residue eluted with 20 % benzene in hexane in a silicic acid column to eliminate traces of the Rhodamine. A yield of 9 mg of waxes and 1.5 mg of sterol esters was obtained. The purity of the fractions was confirmed by rechromatography on Aluminum Oxide G (Fig. 2—2 and 2—3).

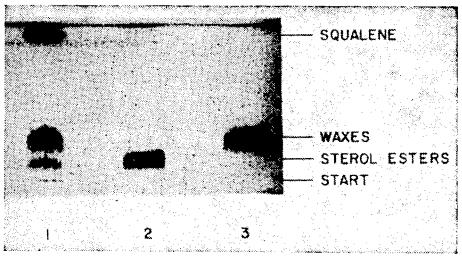

Fig. 2. Thin layer separation of the waxes and the sterol esters of human skin surface lipid on Aluminum Oxide G.
Solvent: 1 % benzene-hexane. (1) Separation of squalene, waxes and sterol esters fractionated with silicic acid solumn chromatography. (2) Test on purity of isolated sterol esters. (3) Test on purity of isolated waxes.

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