High molecular weight alcohols of human hair lipids*

An analysis of alcoholic components of human scalp and hair lipids by gas chromatography has been advanced by GERSHBEIN AND O'NEILL[1] and good resolution of branched chain alcohols was later afforded by thin-layer chromatography (TLC) followed by gas chromatography[2]; components up to C_{30} were detected. In the present study, techniques were explored for the analysis of alcohols of even higher carbon number in such lipids.

Experimental

Alcohol mixtures. Hair cuttings were carefully collected from full-headed men who abstained from the use of any scalp or hair dressings and extracted with petroleum ether (b.p. 30–60°). The two pools of lipids (F-9 and F-10) were saponified by heating with 20 % sodium hydroxide in 95 % ethanol. Each of the unsaponifiable portions in petroleum ether was chromatographed over alumina (Alcoa F-20) and the column eluted with petroleum ether as such and containing 5 % and 10 % chloroform, then 100 % chloroform and finally absolute methanol, whereby five fractions were isolated. The last one (Fraction V) contained the alcohols and sterol. The procedures for removal and processing of the lipids as presented in greater detail in previous reports[3,4] were followed in all respects.

Fraction V was shaken mechanically with 90 % ethanol at room temperature for periods up to 1 h and the solid product after filtration from the solution rich in the lower alcohols was repeatedly extracted with fresh portions of the ethanol. The insoluble portion (Fraction V-In) made up about 5 % of the fraction or about 0.5 % or less as based on the initial hair lipid mixture.

TLC. Separation of the saturated and olefinic alcohols of Fraction V-In was achieved by TLC. For this purpose, glass plates of 20 × 5 × 0.4 cm were uniformly coated with Silica Gel G at a thickness of 0.25 mm, dried at 25° for 16 h and then heated at 110° for 30 min. The cooled plates were stored over silica gel until use. Samples of the lipid in ether were applied 2.0 cm from the edge of the plate and the latter dried at 25° then placed in a chamber containing 70 % ethanol saturated with silver nitrate and which had been equilibrated for several hours previously. Ascending development was conducted at 25° for 1 h, after which time the straight line solvent front was marked and the plates dried. The respective areas or spots were located by initial charring with sulfuric acid–dichromate mixture. With the latter as guide, chromatoplates were then prepared and the two gel portions removed and the respective pools exhaustively extracted with ethyl ether. Solvent was removed under nitrogen thereby yielding the saturated components which did not migrate from the point of application and the mobile unsaturated alcohol portion. The ratio of unsaturated to saturated components was about 1:3.

Gas chromatography. Fraction V-In and the unsaturated alcohol mixture from TLC separation were acetylated with acetic anhydride and the acetates submitted to temperature programmed chromatography to 400–410° in a Barber-Colman gas chromatograph model 5000 with hydrogen flame detector. The rate of heating was 1.5–2°/min. Resolution of components was performed in U-shaped glass columns

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containing aged or completely stripped SE-30 (2.2 %) on 60–80 mesh Gas Chrom P. The latter was conditioned at 400° for 16 h with helium at 120 ml/min. The samples were injected in ethereal solution and the various peaks identified by comparison with

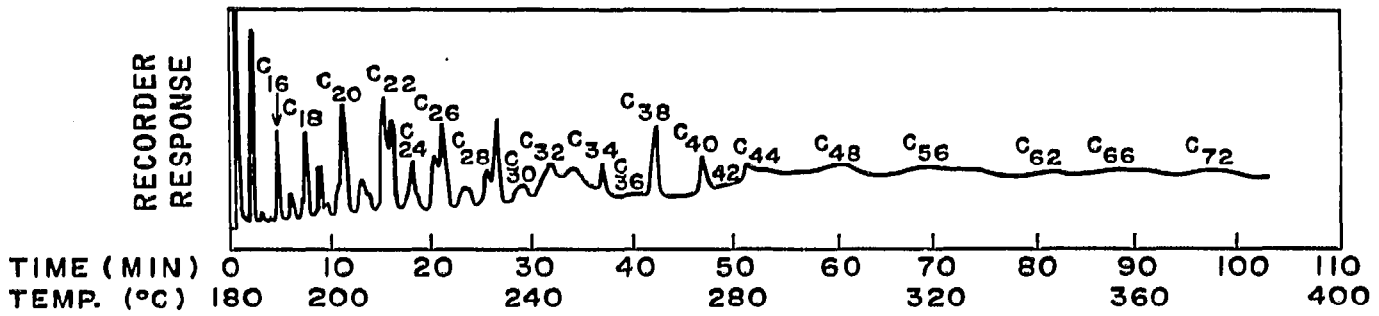

Fig. 1. Gas chromatographic resolution of alcohols of Fraction V-In from pool F-9 in a U-shaped column, 18 in. × 0.6 in. O.D. containing stripped packing, 2.2 % SE-30 on Gas Chrom P; hydrogen flame detector; He pressure: 35 lb.

the elution times. Pertinent chromatograms appear in Figs. 1 and 2. Rubber stoppers or septa became brittle and sintered in the course of the daily runs, a difficulty which was unresolved.

The effect of glass column length on the separation was also explored. A column of 6 in. gave poor resolution and greater combination of peaks, whereas long columns of about 72 in. were responsible for loss of higher molecular weight alcohols, in agreement with the findings reported by KUKSIS[5]. Resolution of components was generally effective with column lengths of 36–48 in. except for the higher components and for which, by far, the best results were achieved with 24 in. glass columns. It is imperative that vibration of the column to settle the packing be avoided as this procedure fractures particles exposing adsorptive sites; rather gentle tapping is recommended.

For Fraction V-In from one pool (F-9), the mass spectrum obtained on heating to 300° showed small but recognizable peaks up to about mass 750 (C_{52}). A minor

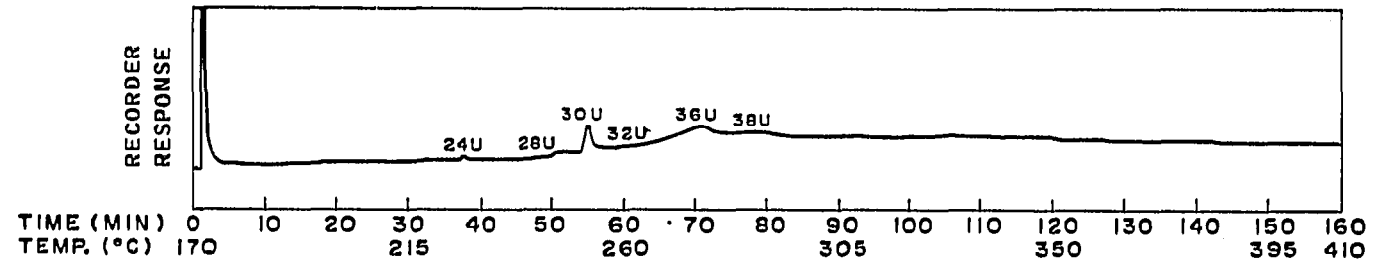

Fig. 2. Gas chromatographic analysis of unsaturated alcoholic components from TLC resolution of Fraction V-In of pool F-10. Temperature programming was carried out as per Fig. 1 except for the column size (30 in. × 0.6 in. O.D.).

portion of material was left in the sample probe. Even longer chain alcohols would have very small peaks and since the amount of components in the range of C_{72} is quite minute, the data do not preclude their presence.

Discussion

By temperature programmed chromatography, both saturated and unsaturated components occurred in the same peaks based on carbon number. It was not possible

to detect the unsaturated moieties by hydrogenation and rerunning the chromatography. Accordingly, the TLC method was instituted and the migrating unsaturated components eluted from the chromatoplates and the resulting acetates analyzed. Previous workers have employed plates impregnated with silver nitrate for such separations but a drawback is the rapid darkening of such plates. This difficulty was circumvented by employing the silver salt as complexing agent in the developing medium.

As will be noted from the chromatogram (Fig. 1), straight chain odd and even alcohols of C_{18} to C_{72} were present in Fraction V-In and possibly, branched components. Attempts at rechromatography in more "efficient" columns resulted in further shouldering and therefore, an exhaustive identification of the higher alcohols cannot be advanced presently. The olefinic members ranged up to about C_{38} or possibly even higher. Sterol was present in small amounts as observed in the gas chromatograms and by colorimetric analysis.

A combination of solvent extraction with TLC and gas chromatography techniques, shown to be of value in the elucidation of sebum higher alcoholic composition, might also be applied profitably to other natural products.

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1 L. L. GERSHBEIN AND H. J. O'NEILL, *J. Invest. Dermatol.*, 47 (1966) 16.
2 E. J. SINGH, L. L. GERSHBEIN AND H. J. O'NEILL, *J. Invest. Dermatol.*, 48 (1967) 96.
3 L. L. GERSHBEIN AND B. K. KROTOSZYNSKI, *J. Gas Chromatog.*, 3 (1965) 378.
4 L. L. GERSHBEIN, B. K. KROTOSZYNSKI AND E. J. SINGH, *J. Chromatog.*, 27 (1967) 431.
5 A. KUKSIS, *J. Am. Oil Chemists' Soc.*, 42 (1965) 269.

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