FRACTIONATION OF HUMAN SEBUM FATTY ACIDS

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ABSTRACT. — Lipids extracted from adult male hair collected according to race and scalp condition have been saponified and the mixed fatty acids as such or following fractionation by way of the lead salts were esterified; the resulting methyl esters were submitted to distillation in spinning band columns. Such enriched fractions of human sebum fatty acids were required in special gas chromatographic studies. Proximate analyses are advanced for sebum acids based on the ester distillations.

The lipids elaborated by the sebaceous glands of the skin have been the subject of several investigations and the correlation of composition with metabolism and disease is of importance (Rothman, 1954; Montagna et al., 1963). The properties and constants of hair fat as a sebum source have been reported by this laboratory, among others (Krotoszynski et al., 1956).

The free fatty acids of lipids extracted from hair of adult males were reported to consist of normal saturated and unsaturated acids ranging in chain length from 7-22 carbon atoms on the basis of a distillation approach (Weitkamp et al., 1945); conventional methods, including preliminary studies with lead salt fractionation, were employed by others (Engman and Kooyman, 1934; Ricketts et al., 1951). Through chromatography, James and Wheatley (1956) analyzed the free fatty acids from forearm lipids initially extracted with acetone and noted the presence of odd- and even-numbered straight and branched chain saturated acids in addition to olefinic members. Comparable studies were reported by Boughton et al. (1959a; 1959b). A rather wide variation in free fatty acid content can be observed with sebum, hydrolytic decomposition occurring on storage and being aided in part by microorganism enzymes. Also, the nature of these fatty acids would depend on the component glycerides and sterol or wax esters hydrolyzed. Accordingly, greater attention was directed by this group to the total fatty acids obtained on saponification of sebum (Krotoszynski et al., 1956); gas chromatographic analysis of such mixtures from the hair fat of four normal human subjects was carried out by Haahti (1961).

In the present study, the fatty acids were removed following alkaline hydrolysis of sebum. The mixture as such, or after fractionation by way of the lead salts, was esterified and the esters distilled in spinning band columns. The resulting fractions were required in order to pin-point by subsequent gas chromatographic analysis several components normally present in minute amounts.

EXPERIMENTAL

For the isolation of mixed fatty acids from sebum and the determination of pertinent constants, the pro-

cedures described by Krotoszynski et al. (1956) were followed in all details. Iodine numbers were ascertained by use of bromine and sodium bromide in absolute methanol as the halogenating agent (Kaufmann and Baltes, 1936).

Hair pools from adult white and Negro full-headed and balding males were extracted with petroleum ether (boiling range: 30-60° C.) and the lipids saponified with 20% NaOH in 95% ethanol by heating for 24 hr on the steam bath. Dilution with water and removal of the unsaponifiable components with ethyl ether followed. The aqueous solution on acidification with sulfuric acid yielded the fatty acids. For removal of volatile acids (under 0.10%), the resulting mixture was steam-distilled until distillates were essentially free of acid as judged by titration with alkali. On cooling, the long-chain acids which came to the surface were removed by filtration, dissolved in ether, washed with water until free of mineral acid, and dried over anhydrous sodium sulfate. Processing yielded the mixed acids (MA).

Fractionation of MA by Way of the Lead Salts.—The procedure of Twitchell (1921) was applied for the enrichment of unsaturated fatty acids by lead salt formation. In one experiment, a boiling solution of 35 g lead acetate in 150 ml 95% ethanol containing 2.3 ml acetic acid was added to 50 g MA-WF-Series 2 in 350 ml 95% ethanol. The contents were boiled for 5 minutes, cooled to 25° C., then stored over ice for 20 hr. The solid salt mixture was filtered under vacuum, the filtrate F-S being stored for later use. Washing of the cake with two 10 ml portions of cold 95% ethanol and treatment with 15 ml of 7 N HCl followed. Removal of lead chloride and mineral acid yielded 26.4 g (52.4%) of solid acids (SA). The unsaturated liquid acids (LA) were recovered after vacuum concentration to one-third of the initial volume; yield: 21.6 g (43.3%). Table 1 presents yields and iodine numbers of the respective acid fractions starting with 25-50 g of MA.

Esterification of Fatty Acids.—

TABLE 1. Separation of Sebum Mixed Fatty Acids by Lead Salts.[1]

Mixed Acids (MA)[2]		Solid Acids (SA)[3]		Liquid Acids (LA)[4]	
Code	Weight, g	%	Iodine Number	%	Iodine Number
WF–Series 2	50.0	52.4	26.2	43.3	62.2
CF–Series 1	50.0	51.6	28.9	46.3	73.8
CR–Series 1	25.0	58.9	25.3	45.0	63.4
CF–Series 2	25.0	50.0	31.3	50.0	77.1

[1] The acid numbers of the LA mixture generally ranged lower than the corresponding SA values. Thus, the numbers for SA and LA from WF Series 2 were 195 and 184, respectively.
[2] In the codes which designate the origin of the sebum or the hair processed, C, W, F and B refer to white, Negro, full-headed and balding, in the order stated.
[3] Obtained from the lead salts insoluble in cold 95% ethanol.
[4] Prepared from acidification of ethanol-soluble lead salts.

MA or acids obtained by way of lead salt fractionation (SA and LA) were converted into esters by treatment with absolute methanol in the presence of hydrogen chloride. Invariably, two layers occurred at the conclusion of the heating period, the lower one containing up to 25% of the total ester content. As the two phases for a given esterified mixture were quite similar in properties, preliminary separation and individual processing was abandoned. Thus, the iodine numbers for the esters in the lower and upper layers from MA-CB-Series 2 were 25.6 and 27.8, respectively. In one experiment, 46.4 g of LA, obtained from CF Series 1 (Table 1), in 175 ml absolute methanol was saturated with a slow stream of hydrogen chloride for 10 min, the mixture refluxed for 19 hr, then freed of methanol under vacuum and 50 ml of ether added. Washing with five 50 ml portions of 5% sodium bicarbonate and four 15 ml volumes of water followed. By removal of ether from the dried solution, 46.3 g of esters resulted.

Spinning Band Column Distillation of Methyl Esters.—A total of 31.6 ml (30.2 g) of esters from MA-WF-Series 1 was distilled in a Podbielniak column (90 cm x 5 mm) with a band rotation of 3250 rpm and at a pressure of 1 mm. The throughput was 90 drops per minute or 18 ml/hr and the product rate or take-off comprised 0.5 ml/hr which was later decreased to 0.35 ml/hr. A total of 69 fractions was collected, with a recovery of 95.0%. The indices of refraction and iodine numbers were ascertained as shown in the curves of Figure 1. The hold-up which amounted to 0.91 g yielded 0.73 g of fatty acids on saponification; the still residue was partly carbonized.

Methyl esters derived from the liquid acids on lead salt fractionation of MA-WF-Series 2 were distilled in a Nestor spinning band column (76 cm x 10 mm) at a pressure of 0.8 mm and a band rate of 1550 rpm. From a charge of 22.8 ml (20.0 g), eighteen 1 ml fractions were collected, accounting for 18.2 g or a 91.0% recovery. The corresponding curves are shown in Figure 2. The densities (d_{30}^{30}) of Fractions 2, 4, 7, 8, 11 and 14 were 0.8632, 0.8628, 0.8604, 0.8604, 0.8599 and 0.8661, respectively.

Paper Chromatography of Volatile Acids.—The volatile acid portions obtained on steam distillation of the aqueous saponification mixtures were neutralized and concentrated to dryness on the steam bath. Several samples were dissolved in water, acidified with a few drops of concentrated hydrochloric acid and applied to paper strips for chromatographic separation by the procedure of Duncan and Porcous (1953). In all instances, the most prominent spot was due to acetic acid, the R_f value averaging 0.12.

Discussion

For fractionation of MA esters toward distillation and use in special gas chromatographic separations, the efficacy of the lead salt method was explored. However, this procedure is replete with inherent difficulties as can be noted from the constants for the "saturated" groups of acids (Table 1). With the latter, salts of the olefinic acids are precipitated from cold ethanol along with

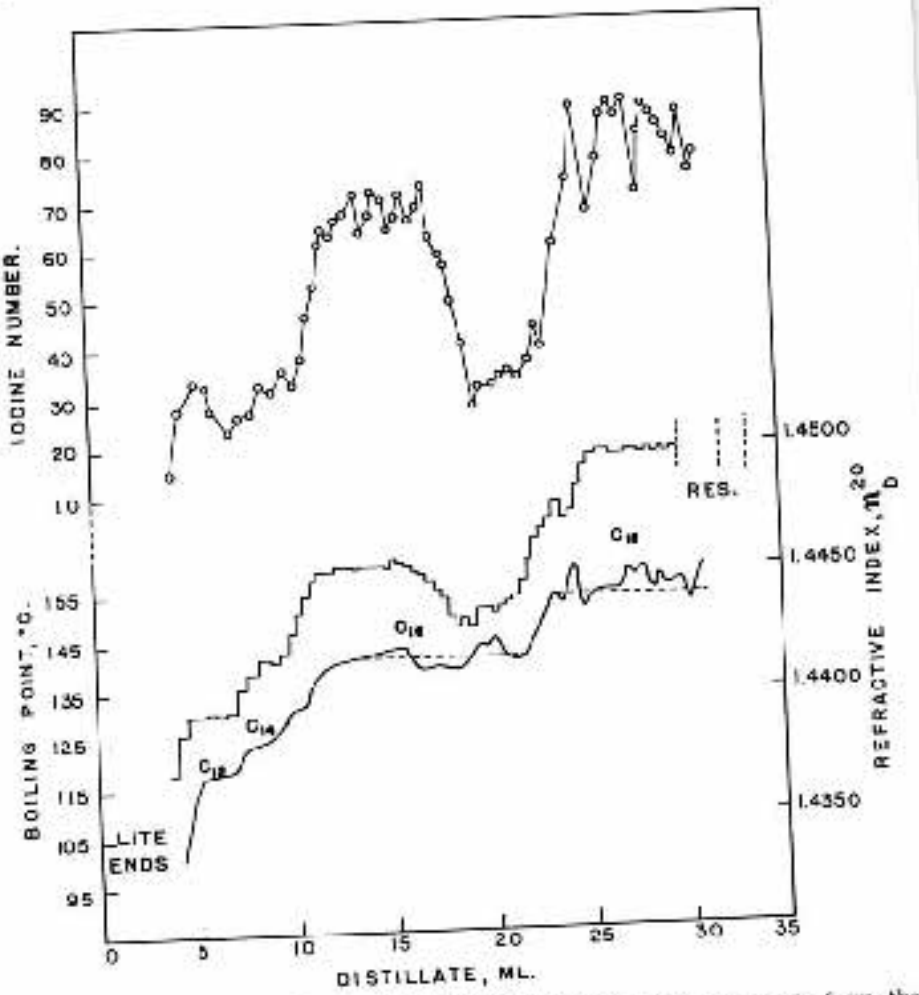

FIGURE 1.—Indices of refraction and iodine numbers for ester cuts from the mixed acids (MA) derived from WF-Series 1 with boiling points at a pressure of 1 mm. The dotted line on the distillation curve represents more idealized boiling ranges.

the saturated long-chain members. Also, homologs of shorter chain length, C_{12}—C_{16}, are incompletely precipitated. The neutralization equivalents of the acids from the ethanol-soluble salts invariably ranged lower than those of the "saturated" or solid acids. In this regard, no marked improvement was evident when lithium salts were substituted and in common with methods involving salt formation, complexes with urea or thiourea produced no clean-cut fractionation of MA, although many products were isolated. Since the iodine number of

the most saturated mixtures obtained by these procedures were similar, indicating no greater advantage for any one method, the acids were separated by way of the lead salts prior to esterification of LA or the "unsaturated" acids.

An effective distillation of MA esters was performed with a pool originating from white full-headed male sebum (WF-Series 1) in a spinning band column at a pressure of 1 mm. The outstanding plateaus noted in the boiling curve for the resulting 69 cuts (Fig. 1) were also reflected in the indices of refraction and iodine numbers. The predominent carbon chain represented by a given plateau was ascertained by comparison with literature values and nomographs. Fractions richer in saturated esters showed lower values with concomitant variations in refractive indices. From the data, one may assume that esters with more than one double bond are present in the C_{18} mixtures. However, assuming that the unsaturated acids in each group all contained only one double bond, a proximate composition was derived for the methyle esters as shown in Table 2; odd-carbon homologs as such were deleted in the groups although small amounts of such acids do occur based on gas chromatography and as might be implied from the distillation curve. Whereas the presence of homologs containing more than eighteen carbon atoms could not be adequately justified in this case, they were undoubtedly present in the light of constants obtained with esters from similar still residues.

Further WF esterified fractions but of greater olefinic acid content were derived from the liquid acids (LA) of the lead salt treatment. A proximate analysis based on distillation behavior (Fig. 2) is given in

TABLE 2.—Proximate Analyses of Methyl Esters from WF Sebum Pools.[1]

Chain Length[2]	Percent of Total	Unsaturation[3] in Fraction, %	Unsaturation[3] in Total, %
MA-WF-Series 1			
12	1.7	14.1	0.24
14	21.2	27.8	5.9
16	42.8	48.2	23.0
18	25.3	86.8	21.9
Bottoms	9.0		
LA-WF-Series 2			
14	2.9	12.1	3.5
16	13.5	42.8	5.7
18	42.2	74.3	31.3
Bottoms	39.7	101.5	25.0
	7.2		

[1] The values are based on the data of Figures 1 and 2.
[2] Small amounts of odd carbon acids are also present in the main groups.
[3] Calculated on the basis that only mono-olefinic acids are present.

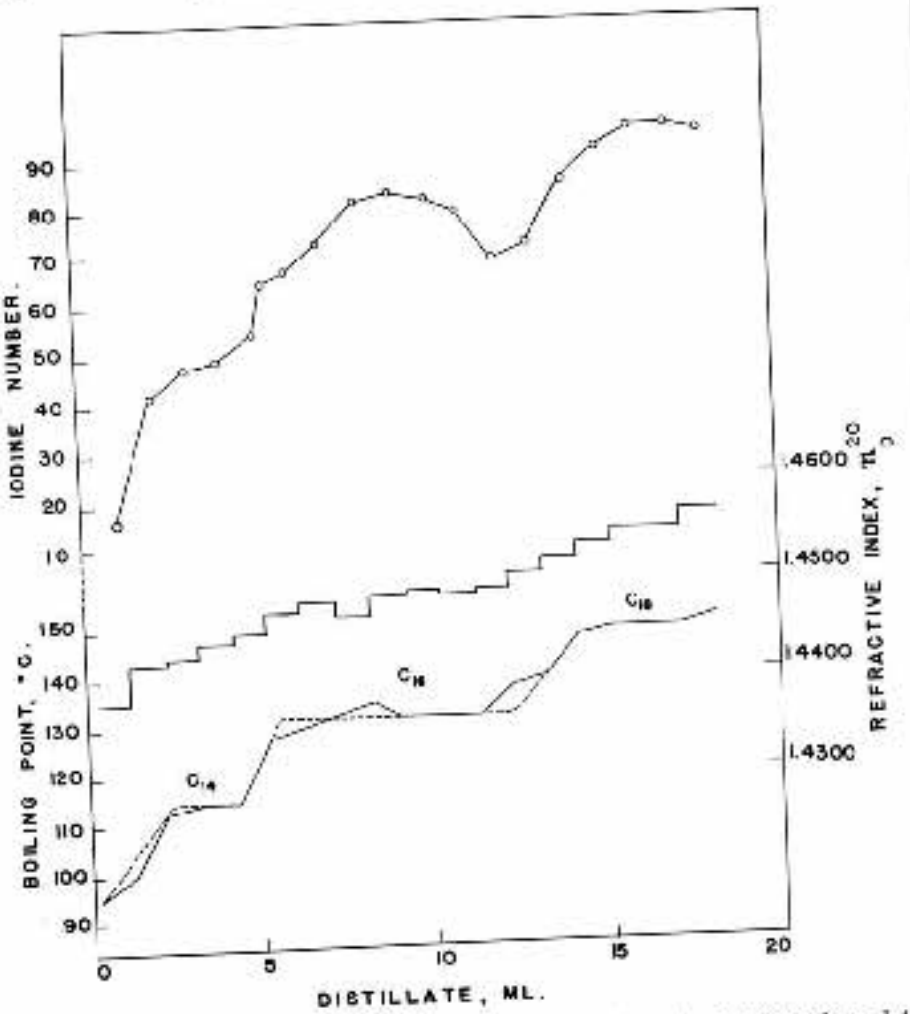

FIGURE 2.—Refractive indices and iodine numbers for ester fractions from LA-WP-Series 2 with boiling points at 0.80 mm pressure. The liquid or "unsaturated" acids are derived from lead salt fractionation of MA.

Table 2. The C_{14}-moieties occurred at a somewhat lower level and the percentage unsaturation of each of the main groups increased markedly in contrast to the MA esters (Table 2). Homologs with less than 14 atoms were slightly, if at all, enriched with essentially no gross alteration in unsaturation.

The minute amounts of acids removed by steam distillation of the acidified saponifiable mixtures were shown by paper chromatography to consist principally of acetic acid. This is in line with the findings of Brouwer and Nijkamp (1952) on the volatile acids of hair grease obtained from a number of animals and

also man. One or two acids appeared to be characteristic of a species, although most of the homologs of the series could be detected. In human hair fat, acetic and caprylic acids were predominant, the remaining members occurring in traces. As the C_5-acid lies at the extreme limit of steam-distillability, its recovery was probably too small for adequate identification in the present instance.

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Literature Cited

Boughton, B., and V. R. Wheatley. 1959a. The fatty acid composition of surface skin fats ("sebum") of normal human subjects. J. Invest. Dermatol. 33:49-57.

Boughton, B., R. M. B. MacKenna, V. R. Wheatley, and A. Wormall. 1959b. The fatty acid composition of the surface skin fats ("sebum") in acne vulgaris and seborrheic dermatitis. J. Invest. Dermatol. 33:57-65.

Brouwer, E., and H. S. Niekamp. 1952. Volatile acids in the secretion products (hair grease) of the skin. Biochem. J. 52:54-58.

Duncan, R. E. B., and J. W. Portenous. 1953. The identification and determination of the lower straight-chain fatty acids by paper partition chromatography. Analyst 78:641-646.

Eneman, M. F., and D. J. Kooyman. 1934. LXVII. Lipids of the skin surface. Arch. Dermatol. 29:12-19.

Haahti, E. 1961. Major lipid constituents of human skin surface with special reference to gas-chromatographic methods. Scand. J. Clin. Lab. Invest. 13: suppl. 59, 108 pp.

James, A. T., and V. R. Wheatley. 1956. Studies of sebum 6. The determination of the component fatty acids of human forearm sebum by gas-liquid chromatography. Biochem. J., 63:269-273.

Kaufmann, H. P., and J. Baltes. 1936. Dienesynthesen auf dem Fettgebiet. I. Die Dienzahl der Fette. Fette u. Seifen 43:93-97.

Kritchevsky, B. K., L. I. Goldsmith, and S. B. Nebelman. 1956. Properties of hair fat from adult males according to race and hair condition. J. Invest. Dermatol. 26:311-316.

Montagna, W., R. A. Ellis, and A. F. Silver, editors. 1963. Advances in Biology of Skin. Vol. IV. The Sebaceous Glands. Macmillan Company, New York. 260 pp.

Nicolaides, N., and S. Rothman. 1953. Studies on the chemical composition of human hair fat. II. The overall composition with regard to age, sex and race. J. Invest. Dermatol. 31:9-14.

Ricketts, C., J. R. Squire, and E. Tuckley. 1951. Human skin lipids with particular reference to the self-sterilizing power of the skin. Clin. Sci. 10:89-110.

Rothman, S. 1954. Physiology and Biochemistry of the Skin. University of Chicago Press, Chicago. 742 pp.

Twitchell, E. 1921. The precipitation of solid fatty acids with lead acetate in alcoholic solution. J. Ind. Eng. Chem. Ind. 13:806-807.

Wertkamp, A. W., A. M. Smiljanic, and S. Rothman. 1947. The free fatty acids of human hair fat. J. Am. Chem. Soc. 69:1936-1939.

Zehender, F. 1946. Über den Gehalt an Triglyceriden menschlichen Hautfalg. Helv. chim. Acta. 29:973-979.

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