[JOINT CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE STANDARD OIL COMPANY (INDIANA) AND THE SECTION OF DERMATOLOGY, DEPARTMENT OF MEDICINE, UNIVERSITY OF CHICAGO]

The Free Fatty Acids of Human Hair Fat

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Introduction

Changes in the chemical composition of the secretions of the sebaceous glands of the scalp, occurring at puberty, have been shown to be responsible for the immunity of adults to *tinea capitis*.[1,2,3] This disease, popularly known as ringworm of the scalp, is a fungus infection caused by *Microsporon audouini*. Growth of the causative fungus is inhibited by the sebum or fat (total ether extract) of the hair of adults at 0.5–0.6% concentration when tested in Saboraud's medium, whereas about five times higher concentration of children's hair fat is required. The fungistatic factor has been shown to be restricted to the free fatty acid fraction of the hair fat and to be largely concentrated in the lowest boiling 5% of that fraction.[1] Recently it has been shown that the active components are normal fatty acids containing 7, 9, 11 and 13 carbon atoms.[3]

In order to collect sufficient material for the present investigation it was necessary to resort to the use of barber shop sweepings. From the dermatological viewpoint this introduced no difficulty since it had been shown that hair fat obtained from barber shop sweepings was identical in fungistatic potency with hair fat from students who had used no hair dressings of any kind in the interval between washing and cutting.[1]

It is evident that barber shop hair will be contaminated with mineral oil and even some fatty oils which are known to be components of hair dressings. However, these fatty oils are originally present as neutral fats and will not appear in the free fatty acid fraction of the hair extract except in so far as hydrolysis may have occurred prior to the extraction. The compositions of commercial fatty oils are such that contamination from that source would be very largely restricted to the C_{16} and C_{18} fractions and for the most part to the unsaturated portions of those fractions. However, examination of the unsaturated acids in the C_{16} and C_{18} fractions revealed that, except for 9, 10-oleic acid, they are isomers of the corresponding unsaturated acids found in the common commercial fatty oils. Since 9,10-oleic acid fits into the general pattern of the hair oil acids there is some reason for supposing that even it is a product of the sebaceous glands rather than of extraneous origin.

The present investigation was directed toward determining the identities of the components of the free fatty acid fraction of human hair fat, particularly those having fungicidal effect. Since the nature of the individual components was totally unknown, emphasis has been placed on isolation and identification rather than on quantitative estimation. Some of the minor components have not been examined in detail.

(1) Rothman, Smiljanic and Shapiro, *Proc. Soc. Expt. Biol. Med.*, **60**, 394 (1945).

(2) Rothman, Smiljanic and Weitkamp, *Science*, **104**, 201 (1946).

(3) Rothman, Smiljanic, Shapiro and Weitkamp, *J. Investigative Dermatol.*, **8**, 81 (1947).

Experimental

Preparation of Material

Barber shop sweepings, consisting principally of the hair of adult males, were collected and after manual separation of extraneous material yielded 45 kg. of hair. Boiling ether was used to extract lipids from the hair in a single batch extraction. The ether solution was washed with cold aqueous 1% potassium hydroxide to remove the free fatty acids as potassium soaps. The free fatty acids, after recovery from the soap solution, were converted to methyl esters by boiling under reflux with methanol saturated with dry hydrogen chloride. Phenols are not esterified under these conditions and were extracted with alkaline 60% ethanol from a petroleum ether solution of the esters. The phenol fraction was not further investigated since it is not fungicidal to *M. audouini*.[1] These phenols are believed not to be produced by the sebaceous glands, but to be of extraneous origin.

The petroleum ether solution of the esters was partially decolorized by percolation through a column packed with Attapulgus clay. The petroleum ether was removed from the esters by careful distillation through an efficient fractionating column in order to minimize loss of the lowest boiling esters. Finally the esters were distilled under reduced pressure (*vide infra*) yielding 240 g. of distillate and 10 g. of a resinous residue. The residue was not further investigated. The distillate, equivalent to 228 g. of free fatty acids, represents an over-all yield of approximately 0.5% based on the weight of original hair.

Isolation and Identification of Component Acids

The esters were fractionally distilled at 2 mm. pressure through a modified Stedman column[4,5] having an efficiency

TABLE I

APPROXIMATE COMPOSITION OF THE METHYL ESTERS

Carbon atoms	% Present	% Unsaturated
7	0.07	0
8	.15	0
9	.20	0
10	.33	0
11	.15	Trace
12	3.5	4 (−2H)
13	1.4	3 (−2H)
14	9.5	15 (−2H)
15	6.0	25 (−2H)
16	36.0	50 (−2H)
17	6.0	67 (−2H)
18	23.0	80 (−2.4H)
20	8.5	85 (−2.5H)
22	2.0	..
Bottoms	4.0	..

(4) Weitkamp and Brunstrum, *Oil & Soap*, **18**, 47 (1941).

(5) Weitkamp and Oblad (to Standard Oil Co. (Ind.)), U. S. Patent 2,325,818.

of approximately 100 theoretical plates. From the distillation curve, shown in Fig. 1, it is apparent that the component acids are not restricted to the usual series containing even numbers of carbon atoms. Chain lengths in the even series range from 8 to 22 carbon atoms and in the odd series, from 7 to 17 carbon atoms. The odd series of acids comprises about one-seventh of the total, a small but highly significant proportion in view of the fact that odd acids are 2.5 to 150 times as effective against *M. audouini* as the adjacent even acids.[3] The approximate composition of the methyl esters is shown in Table I.

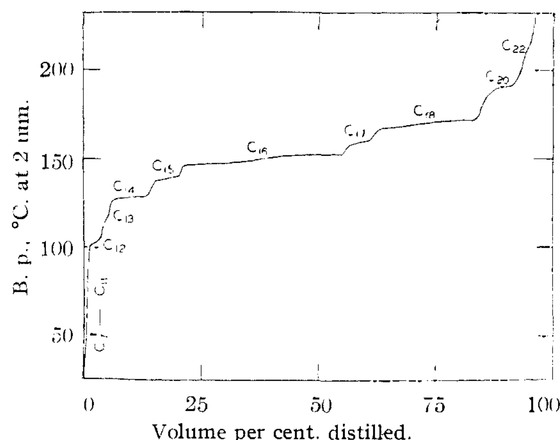

Fig. 1.—Distillation of the methyl esters of the free fatty acid fraction of human hair oil.

In order to obtain maximum separation of adjacent boiling esters, the technique of amplified distillation was applied.[6,7] Fractions from the original distillation were refractionated in the presence of a mineral oil having the appropriate boiling range. The course of the distillation was followed by determining saponification values on incremental (5 ml.) fractions of the distillate.

In Fig. 2 is shown the amplified distillation of the C_7 to C_{11} fraction. This fraction amounted to only 2.2 g. of esters or about 0.9% of the total ester mixture. The straight line distillation curve results from the use of a specially prepared mineral oil. Saponification values were determined on 5-ml. fractions and were plotted as ordinates (Fig. 2) to obtain the stepwise saponification value curve. Each hump in the saponification value curve represents a different ester, or group of esters in case the boiling points of two or more esters happen to coincide. The area under the curve is a measure of the number of moles of ester present in the mixture. The boiling point corresponding to a peak in the saponification value curve provides a clue to the identity of a given ester when the homologous series to which the ester belongs is known.

Following saponification, the several fractions corresponding to a given ester were recombined, except that fractions which were obviously mixtures of adjacent boiling components and fractions having negligible saponification values were discarded. The combined soap solutions, after adjustment of the alcohol concentration to 60%, were freed of mineral oil by washing with hexane. The free fatty acids were recovered from the soaps in the usual manner.

The various acid fractions thus obtained, each consisted primarily of a normal monobasic saturated acid and usually one or more normal monobasic unsaturated acids. This is in contrast with neutral wool fat or lanolin which contains saturated acids most of which have branched chains.[8]

Saturated Acids.—The individual saturated acids, C_{12} to C_{20}, were separated from their unsaturated analogs and

(6) Weitkamp, *J. Am. Oil Chem. Soc.*, **24**, 236 (1947).

(7) Axe and Bratton, THIS JOURNAL, **59**, 1424 (1937).

(8) Weitkamp, *ibid.*, **67**, 447 (1945

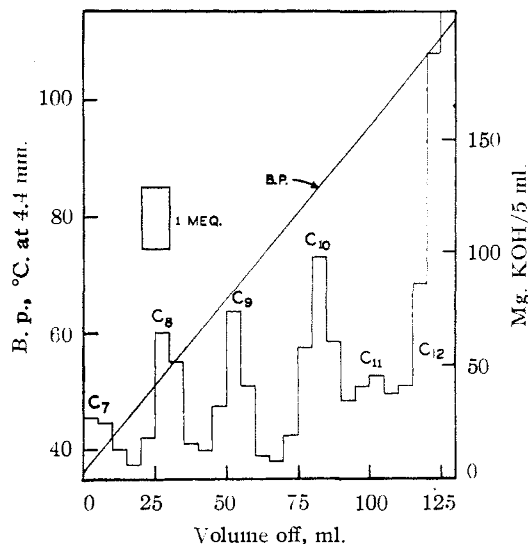

Fig. 2.—Amplified distillation of the methyl esters of the C_7-C_{11} fraction of the free fatty acids of human hair oil.

purified by crystallization from solvents (ether, acetone and hexane) and identified by means of their melting points (Table II) and the boiling points of their methyl esters. In each instance admixture of the acid with an authentic specimen failed to depress the melting point. Pelargonic acid (C_9) was converted to the amide which was similarly purified and identified (Table II, note *b*).

TABLE II

SATURATED ACIDS

Name	Carbon atoms	M. p. (°C., cor.) Found[a]	Lit.	Neut. equiv. Found	Calcd.
n-Heptanoic, enanthic	7		−9[9]		
n-Octanoic, caprylic	8		16[9]		
n-Nonanoic, pelargonic[b]	9		12.5[9]		
n-Decanoic, capric	10		31.19[10]		
n-Hendecanoic	11		29.5[9]		
n-Dodecanoic, lauric	12	43.6	43.71[11]	201.2	200.3
n-Tridecanoic	13	41.5	41.55[10]	214.7	214.3
n-Tetradecanoic, myristic	14	53.4	54.4[11]	228.7	228.4
n-Pentadecanoic	15	52.4	52.26[10]	242.3	242.4
n-Hexadecanoic, palmitic	16	62.3	62.9[11]	256.8	256.4
n-Heptadecanoic, margaric	17	60.3	60.85[11]	269.2	270.4
n-Octadecanoic, stearic	18	69.0	69.6[11]	283.2	284.5
n-Eicosanoic, arachidic	20	74.7	75.35[11]	311.6	312.5
n-Docosanoic, behenic	22		79.95[11]		

[a] Temperatures of complete melting were determined by the method of Francis and Piper.[11] [b] Pelargonamide, m. p. found, 97.3–97.7°; authentic specimen, 98.2°; mixed 97.3°. Nitrogen content found, 8.90%; calcd., 8.91%.

Unsaturated Acids.—The unsaturated acids were freed of saturated acids as completely as possible by crystallization of the latter from ether at temperatures of −50° or lower.[12] The following discussion concerns the unsaturated portion of the respective fractions.

Only one of the mono-ethenoid acids was isolated in a

(9) Heilbron, "Dictionary of Organic Compounds," Oxford University Press, New York, N. Y., 1943.

(10) Meyer and Reid, THIS JOURNAL, **55**, 1577 (1933).

(11) Francis and Piper, *ibid.*, **61**, 577 (1939).

(12) Foreman and Brown, *Oil & Soap*, **21**, 183 (1944).

state approaching purity. This was the C_{16} member, m. p. 9–10°; iodine value found, 97.4; calcd., 99.8. Lower members were not isolated for the reason that below C_{16} separation of the saturated acids becomes progressively more incomplete due to increased solubility in ether, particularly of the odd members of the series. Above C_{16} complex mixtures of unsaturated acids were encountered. The proportion of unsaturated acid in each of the fractions is shown in Table I. The C_7 to C_{10} fractions were completely saturated. The C_{11} fraction showed only a trace of unsaturation, and as shown in Table I, the degree of unsaturation increased with increasing molecular weight.

In order to characterize the unsaturated acids, oxidation was carried out in two steps. The crude unsaturated acid was first oxidized with 30% hydrogen peroxide in anhydrous formic acid.[13] The resulting *cis*-dihydroxy acid was washed with hexane to remove residual saturated acids and recrystallized to constant melting point from ether. The dihydroxy acids are polymorphic and exhibit two well-defined melting points (Table III). The higher melting form was regularly obtained by crystallization from a solvent or by seeding the melt at any temperature between the two melting points. The low melting form was obtained by cooling the melt in a 1-mm. glass capillary melting point tube.

TABLE III

UNSATURATED ACIDS

Carbon atoms	Location of unsaturation	Melting points (°C., cor.) of *cis*-dihydroxy derivative: High melting form	Low melting form	Monobasic acid from oxidation
11	?			
12	?			
13	?			
14	5,6			C_9
15	6,7	109.5–110	82.8–83.1[a]	C_9
16	6,7	110.5–111	91–91.5	C_{10}[c]
17	6,7	111.7–112.5	88.9–89.5[b]	C_{11}
17	8,9			C_9
18	6,7			C_{12}[d]
18	8,9			C_{10}
18	9,10			C_9
18	diene			C_9[e]
20	?			

[a] Neutralization equivalent found, 273.6; calcd., 274.4. [b] Neutralization equivalent found, 302.9; calcd., 302.4. [c] Melting point found, 30°. Not depressed by admixture with capric acid. [d] Melting point found, 43°. Not depressed by admixture with lauric acid. [e] Ordinary 9,10-12,13-linoleic acid would have yielded caproic acid.

The dihydroxy acids were oxidized in boiling acetone with the quantity of potassium permanganate calculated to cleave the double bond and oxidize the cleavage products to monobasic and dibasic acids.[14] The acidic products of oxidation were taken up in hexane and chilled at 0° to precipitate the dibasic acids. In order to establish the location of the double bond, it is sufficient to identify only one of the products of oxidation. In this case, the monobasic acid was selected. It should be noted in passing that some secondary oxidation to shorter chained monobasic acids was observed.

In each instance, except C_{18}, the monobasic acids from oxidation were distilled with a mineral oil diluent (kerosene) in an efficient fractionating column operated at 18 mm. pressure. The azeotropic elimination curve, *i. e.*, the increasing ratio of acid to oil as the distillation progresses, serves as an identifying characteristic of the acid, provided the homologous series to which the acid belongs is known. This method of identification is particularly applicable to small quantities (50–500 mg.) of relatively simple mixtures. Minor components will be detected provided they are *lower* boiling than the main component.[6] In the case of the C_{18} oxidation products it was necessary to use the more difficult amplified distillation of methyl esters because of the complexity of the mixture and because the minor components were the *higher* boiling ones.

In order to establish the homologous series to which the monobasic oxidation products belong, two of the acids were isolated, recrystallized to purity and identified as normal fatty acids. The chain lengths of the various monobasic fatty acids identifiable as primary oxidation products are listed in the last column of Table III.

The dibasic oxidation products were not examined in detail. However, melting points on crude specimens discount the possibility of branched chains. Hence, it can be concluded that the unsaturated acids of hair oil have normal structures.

The location of the double bonds in the various unsaturated acids presents an intriguing pattern. The shortest chained monobasic acid obtained from any of the oxidations is C_9. Should this be a completely general phenomenon, the lowest acid to exhibit unsaturation would be the C_{11} acid, as was found to be the case. The preferred location of unsaturation in the C_{15} and higher acids seems to be between the 6th and 7th carbon atoms, counting the carboxyl group as No. 1.

The C_{11}, C_{12} and C_{13} unsaturated acids were not examined because of the small amounts present. Only a single isomer was identifiable in the C_{14}, C_{15} and C_{16} fractions. The C_{17} fraction contains about 80% of the 6,7 isomer and 20% of the 8,9 isomer. As a consequence the melting points listed in Table III for *cis*-6,7-dihydroxyheptadecanoic acid are tentative, since it is not known with certainty that all of the 8,9 isomer was eliminated by recrystallization.

The C_{18} fraction is relatively complex. The crude material had an iodine value of 104.5 and was found by ultraviolet absorption analysis[15] to have the following composition:

Oleic acid	80.8%
Linoleic acid	14.8
Linolenic acid	1.8
Stearic acid	2.6
Tetraenes	0.0

The linoleic and linolenic acids were not separately investigated. Attempts to prepare pure oleic acid by low temperature crystallization or to prepare pure *cis*-dihydroxystearic acid by peroxyformic acid oxidation were unsuccessful. Permanganate oxidation of the crude C_{18} fraction yielded C_9, C_{10} and C_{12} monobasic acids in the approximate molar ratio 4:3:2. Hence, the most abundant oleic acid is the ordinary 9,10 variety. Next in order of abundance is the 8,9-isomer which is a homolog of the 8,9-heptadecenoic acid found in minor proportions in the C_{17} fraction. The least abundant of the oleic acids is the 6,7 isomer.

Since 9,10-oleic acid is such a common component of animal and vegetable fats, particularly of olive oil, it is possible that it may be partly or wholly an extraneous component of the free fatty acid fraction of hair fat.

The pattern of unsaturation is illustrated schematically in Fig. 3. The double bonds in bold face type are those whose positions were actually determined. Two isomeric heptadecenoic acids and three octadecenoic acids are indicated. The structures shown for the C_{11}–C_{13} acids are predicted structures.

It is probable that oxidation of the linoleic acid was the source of some of the C_9 monobasic acid obtained from the oxidation of the C_{18} fraction. If so, the linoleic acid would have its double bonds in the 6,7 and 9,10 positions

(13) Swern, Billen, Findley and Scanlan, THIS JOURNAL, **67**, 1786 (1945).

(14) Armstrong and Hilditch, *J. Soc. Chem. Ind.*, **44**, 437 (1925).

(15) Ultraviolet absorption analysis by B. W. Beadle, American Meat Institute, Chicago, Illinois.

rather than in the usual 9,10 and 12,13 positions. Furthermore, ordinary 9,10–12,13-linoleic acid would have yielded the C_6 monobasic acid when oxidized, but no such acid was recovered. Therefore, it seems likely that the linoleic acid of hair fat bears the same relation to 6,7-oleic acid as ordinary linoleic acid bears to 9,10-oleic acid. In any case the double bonds are separated by a CH_2 group since the acid is alkali isomerizable to the conjugated form.

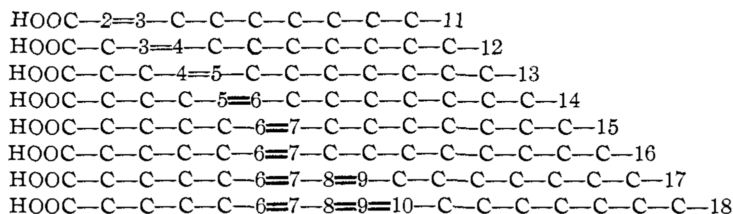

Fig. 3.—Position of double bonds.

Acids Other than the Normal Series.—In the C_7 and C_{11} fractions there were traces of acids which were insoluble in cold hexane. The amounts were insufficient for purification. Microanalyses on the crude hexane soluble and insoluble fractions were obtained.[16] The hexane soluble portion of the C_7 fraction was a monobasic acid as judged from the equivalence of its molecular weight in camphor to its neutralization equivalent. Its carbon and hydrogen contents were those of a C_7 saturated monobasic acid, presumably straight-chained.

The hexane insoluble C_7 acid was likewise a monobasic acid. High carbon and low hydrogen contents clearly indicated an aromatic nucleus and the boiling point of the methyl ester limited the choice to benzoic acid, a common preservative in cosmetic preparations. The proportion present in the total free fatty acid fraction is on the order of 0.02–0.03%.

The C_{11} fraction, in addition to a presumably normal saturated fatty acid and traces of an unsaturated acid, contained a hexane insoluble substance having a molecular weight much higher than its neutralization equivalent and carbon and hydrogen contents suggestive of a saturated dibasic acid having eight carbon atoms, probably suberic acid. Its content in the total free fatty acid fraction does not exceed 0.05%.

(16) Microanalyses by T. S. Ma, Department of Chemistry, University of Chicago.

Summary

The free fatty acid fraction of human hair fat has been examined and found to contain normal saturated and unsaturated fatty acids, ranging in chain length from 7 to 22 carbon atoms. The normal saturated and unsaturated fatty acids having odd carbon contents appear to have been obtained from a natural source for the first time. The 6,7 position appears to be the characteristic location of the double bond in the unsaturated acids, although some 8,9 and other isomers are present. The unique character of most of the acids precludes the possibility of extensive extraneous origin.

WHITING, INDIANA RECEIVED JANUARY 20, 1947

[CONTRIBUTION FROM THE LABORATORIES OF G. D. SEARLE AND COMPANY]

1-Dialkylaminoalkylaminoisoquinolines[1]

BY RICHARD A. ROBINSON

As our part of the current intensive search for new antimalarial drugs[2] we undertook the synthesis of basically alkylated aminoisoquinolines, a field in which other groups had not shown an interest.[2a] Although this field, in our hands, proved to be unproductive as a source of active antimalarial drugs, several interesting chemical syntheses were achieved, and they will be discussed here and in succeeding papers.

We have synthesized basically alkylated 1-aminoisoquinolines (II) in which variations were made in two directions: (1) variations in the side chain of otherwise unsubstituted dialkylaminoalkylaminoisoquinolines, and (2) the further substitution of 1-(γ-diethylaminopropylamino)-isoquinoline with chloro or methoxyl groups.

The basic side chains were introduced through the reaction of 1-chloroisoquinolines (I) with the desired dialkylaminoalkylamine. The 1-chloroisoquinolines were synthesized by a variety of methods which, in their final step, depended on the conversion of an isoquinoline N-oxide (III), or an isocarbostyril derivative (IV) to the corresponding 1-chloroisoquinoline by the action of phosphorus oxychloride. 1-Chloroisoquinoline was prepared from isoquinoline. Isoquinoline was first converted to isocarbostyril according to the procedure of Chichibabin and Kursanova[3] and the latter substance reacted with phosphorus oxychloride to give 1-chloroisoquinoline.[4]

7-Methoxy-1-chloroisoquinoline and 1,7-dichloroisoquinoline were derived from the known 7-methoxyisoquinoline which was prepared as described by Fritsch.[5] 7-Methoxyisoquinoline was converted to its N-oxide derivative which reacted with phosphorus oxychloride to yield 7-methoxy-1-chloroisoquinoline. This method was suggested

(1) Presented before the organic division of the American Chemical Society, Sept., 1946.

(2) This work was undertaken in coöperation with the Survey of Antimalarial Drugs of the National Research Council. The results of antimalarial screening tests on the compounds here reported will be found in "Antimalarial Drugs 1941–45," Edwards Brothers, Ann Arbor, Michigan, 1946.

(2a) Drake and Peck, THIS JOURNAL, **68**, 1309 (1946), have recently reported several chloro derivatives of 1-(δ-diethylaminoisobutylamino)-isoquinoline. None of their compounds was duplicated in this work.

(3) Chichibabin and Kursanova, *J. Russ. Phys.-Chem. Soc.*, **62**, 1211 (1930).

(4) Gabriel and Colman, *Ber.*, **33**, 985 (1900).

(5) Fritsch, *Ann.*, **286**, 1 (1895).