advantage that under these conditions it is difficult to elicit depressor responses, even when crude extracts are given. A typical comparison of noradrenaline with hypertensin is shown in Fig. 1. While comparisons were made with stock hypertensin, it was of interest to compare it with the most potent pressor substance known.

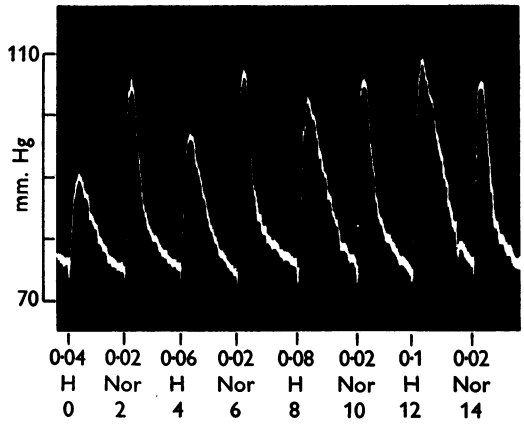

Fig. 1. Comparison of hypertensin with (–)-noradrenaline, showing that 0·02 μg. of noradrenaline is equivalent to between 0·08 and 0·1 ml. of hypertensin. Doses of noradrenaline in μg., of hypertensin in ml. Time in min.

SUMMARY

1. A new method of large scale production of crude hypertensin is described, depending on charcoal adsorption with subsequent elution by glacial acetic acid.

2. A reliable sensitive method of biological assay using the pressor responses in the rat is described.

I would like to express my thanks to Professor G. W. Pickering for continual encouragement, to Mrs P. Brown for technical assistance, and to James Tait, Milfield, Northumberland, for a generous supply of rabbit kidneys.

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The Constitution of the Aliphatic Alcohols in Human Sebum

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Human sebum consists mainly of wax esters, free fatty acids, hydrocarbons and a small amount of glycerides. Whilst the free fatty acids have been investigated in detail (Weitkamp, Smiljanic & Rothman, 1947), little is known about the composition of the other fractions. The unsaponifiable matter has long been known to contain cholesterol (for review of earlier literature, see Čmelik, 1952) and squalene has recently been identified (Sobel, 1949; Mackenna, Wheatley & Wormall, 1952). By chromatographic studies these last authors found 14–20 % of aliphatic alcohols in the unsaponifiable matter, but apart from an impure preparation of eicosanol (?) no individual alcohol was isolated.

The present paper describes an investigation into the aliphatic alcohols of human sebum. Sebum (surface skin 'fat') was obtained by extraction of hair from the heads of African (Bantu) males.

METHODS

Reversed-phase partition chromatography of fatty acids. The method of Howard & Martin (1950) (cf. Silk & Hahn, 1954) was extended to effect the resolution of six normal fatty acids ranging in chain length from six to eleven carbon atoms. It was found unnecessary for the present purpose to protect eluate and developing solvent against atmospheric CO_2; also, nitrogen was not purified before entering the titration vessel. The columns were maintained at tap-water temperature (18–22°). For loading of the columns, the acid mixtures were usually dissolved in the developing solvent. It was necessary to prepare a mull only when the mixed acids differed widely in chain length (such as C_5–C_{12}).

The efficiency of separation is illustrated by the elution curve (Fig. 1) for a chromatogram of a synthetic mixture of pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, hendecanoic and dodecanoic acids (about 7 mg. of each). The C_5 and C_6 acids (unresolved) were eluted with water; C_7 with 10 % (v/v) aqueous acetone, C_8 with 25 %,

C_9 with 40%, C_{10} with 45%, C_{11} with 50% and C_{12} with 55%. The eluate of this particular chromatogram was collected in 1·8 ml. samples and titrated with 0·02 N alkali.

Isolation of total material forming complex with urea. The fraction to be treated with urea was dissolved in 96% (v/v) aqueous ethanol (5–10%, w/v, soln.). An amount of urea was added, equal to about 3 times the anticipated weight of complex-forming material in the fraction. The urea was dissolved by refluxing the solution. After standing overnight at room temperature, the solution was filtered and the precipitate was dried. A second precipitate of urea complex was sometimes obtained by treating the filtrate with more urea, or by further cooling or evaporation to a smaller volume of the filtrate. Complexes were recrystallized from ethanol in which urea (half the weight of the complex) was dissolved. Complexes were decomposed with water, and the alcohol fractions were recovered in ether. Residues which were left in the filtrates were recovered in the same way.

Fractional crystallization of urea complexes. This method was used for resolving mixtures which contained the acetates of a saturated normal, a saturated *iso* and an unsaturated normal alcohol, all having the same number of carbon atoms (cf. Tiedt & Truter, 1952). The prefix '*iso*' denotes a structure containing a terminal *iso*propyl group.

The material (about 10 g.) was dissolved (2%, w/v, soln.) in benzene containing 35% (v/v) of ethanol. Urea (in most cases 2 g.) was added to the solution and dissolved under reflux. After standing overnight at room temperature, the solution was filtered from the precipitated urea complex. The filtrate was again treated with urea (2 g.) to yield a second precipitate of urea complex. This procedure was continued until the precipitate consisted of urea only. The complexes were separately decomposed with water and the acetates recovered.

Chromatography on acid-washed alumina. Alumina (Peter Spence, Widnes, grade H) was washed with hot 2% (w/v) aqueous HNO_3 and then with warm water (distilled) and reactivated at 200°.

The unresolved mixture (about 0·5 g.) of an ω-hydroxy ester and an unhydroxylated ester dissolved in hexane (5 ml.) was passed through a column (12 × 1 cm.) of acid-washed alumina (10 g.) and eluted successively with hexane (35 ml.), hexane containing 10% (v/v) of dry ether (40 ml.) and dry ether (80 ml.). The eluate was collected in 10 ml. fractions which were evaporated and weighed. The unhydroxylated ester was contained in the hexane eluate and the ω-hydroxy ester in the ether eluate.

Isolation of cholesterol through the oxalic acid complex. The method was essentially that of Pickard & Seymour (1945); the detailed procedure was as follows. The cholesterol-containing material was dissolved in anhydrous benzene (20%, w/v, soln.). Anhydrous oxalic acid (10% of the cholesterol-containing material) was added and the solution refluxed for 1 hr. The precipitated complex was filtered off the next day and washed with cold benzene. It was decomposed with ethanolic KOH solution and cholesterol was recovered in ether. The residual material was recovered in the same way.

Infrared absorption spectra. The spectra were recorded with a Perkin Elmer Infrared Spectrometer Model 12C; rock salt prism; path length, 0·5 mm. Alcohols (1·5%, w/v, in CCl_4) were examined in the 7·25 μ. region for the presence of *iso* and normal structures (Freeman, 1952). Unsaturated alcohols (1·5%, w/v, in CS_2) were examined in the 10·35 μ. region for *trans* configurations and in the 10·1 μ. region for terminal double bonds (Freeman, 1953).

EXPERIMENTAL AND RESULTS

Melting points are given corrected. They were measured in capillary tubes immersed in a paraffin bath which was electrically heated at a rate of 1°/6 min.

Extraction of sebum

Hair (28 kg. in batches of about 1·5 kg.) from Africans (Bantu) was obtained from the prison authorities. It was dried at 65° and 20 mm. Hg for 16 hr. and subsequently extracted continuously for 8 hr. with hot commercial 'isoheptane' in a stainless-steel percolator. The extracts were left to stand until the suspended solids settled. The

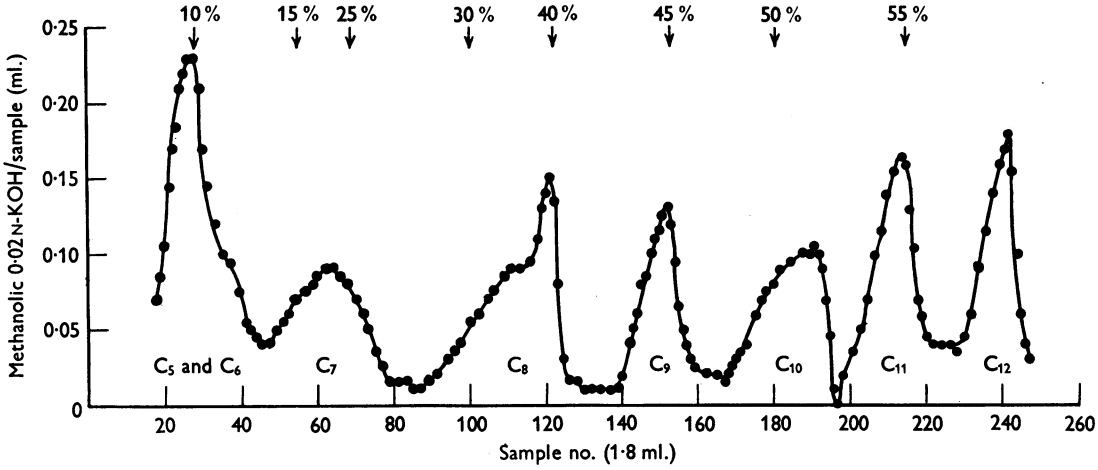


Fig. 1. Resolution of a mixture of pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, hendecanoic and dodecanoic acids (all *n* acids). Column 75 × 0·8 cm. Hold-up, 23 ml. Changes of solvent (%, v/v, aqueous acetone) are indicated by the arrows.

solutions were siphoned off, the solids washed with 'isoheptane' and the combined solutions evaporated to give crude sebum (1450 g.).

Separation of sebum into free acids, acids released from combination by saponification and unsaponifiable matter

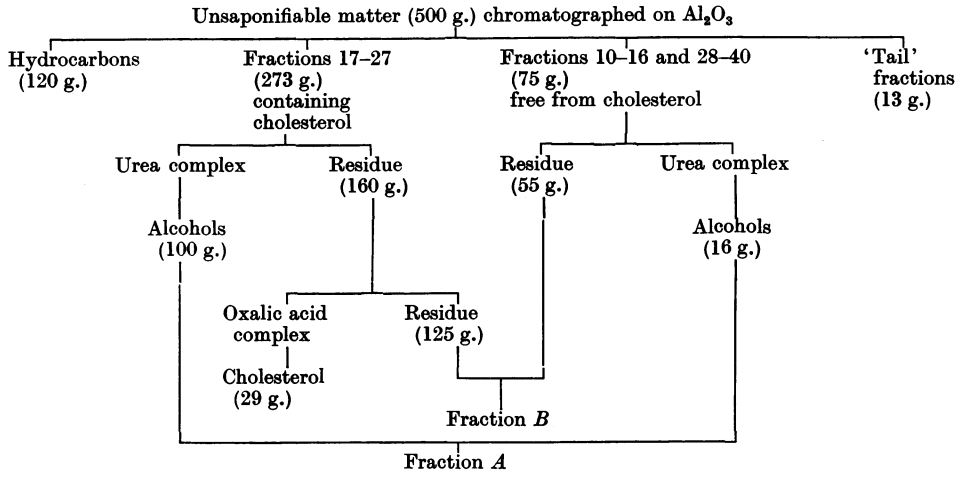
A solution of crude sebum (1335 g.) in hexane (8 l.) was extracted in a separating funnel with 60% aqueous ethanol (4 l.) containing KOH (200 g.). The ethanolic extract, after being continuously extracted with hexane, was acidified (H_2SO_4) and the free acids (458 g.) were obtained by extraction with hexane. Further extraction of the ethanolic solution with ether gave a dark resinous material (24 g.). The combined hexane solutions which contained the neutral material were evaporated to a smaller volume (2 l.). Ethanol (2 l.) containing KOH (170 g.) was added and the mixture stirred for 24 hr. at 40°. Saponification was complete under these conditions. The acids released from combination (285 g.) were separated from the unsaponifiable matter (533 g.) by extraction of the latter with hexane from a solution of the saponified mixture in 60% (v/v) aqueous ethanol. A final continuous extraction (48 hr.) with hexane gave another portion of unsaponifiable matter (14 g.) which was not investigated further.

Separation of hydrocarbons, aliphatic alcohols and cholesterol from the unsaponifiable matter

The non-aromatic hydrocarbons were separated from the unsaponifiable matter by chromatography. Unsaponifiable matter (500 g.) in hexane (10 l.) was passed through a column (35 × 15 cm.) of alumina (Peter Spence, Type H; 5 kg.) and eluted with hexane (12·5 l.), ether (37·5 l.), ether containing 2% (v/v) of 96% (v/v) aqueous ethanol (15 l.), ether containing 10% of 96% ethanol (15 l.), 96% ethanol (10 l.), and finally 96% ethanol containing 10% (v/v) of aqueous 9·5N-HCl (10 l.). The eluate was collected in 2·5 l. fractions (44 in all), samples from which were evaporated to dryness and examined. The hydrocarbons (120 g.) were contained in the fractions (2–9) which were eluted with hexane and subsequently combined. The 'tail' fractions (41–44; 13 g.) which were eluted with ethanolic HCl were set aside. The remaining fractions were combined into a cholesterol-containing group (17–27; 273 g.) and a cholesterol-free group (10–16 and 28–40; 75 g.).

The cholesterol-containing (273 g.) and the cholesterol-free (75 g.) grouped fractions were separately treated with urea; aliphatic alcohols (100 and 16 g. respectively; subsequently combined and referred to as fraction *A*), and residual fractions (160 and 55 g. respectively) were obtained.

The residual fraction (160 g.) which contained cholesterol was treated with oxalic acid; cholesterol (29 g.) and a residual fraction (125 g.) were obtained. The latter was combined with the cholesterol-free residual fraction above (55 g.). This combined fraction is referred to as fraction *B*. These fractionations are shown in the diagram below:



Acetylation of fractions A and B

Fraction *A* (aliphatic alcohols; 116 g.) was heated with acetic anhydride (350 g.) on a water bath for 4 hr. The excess of solvent was distilled off under vacuum to give the acetates (133 g.). A portion of fraction *B* was similarly converted into acetates (30 g.).

Distillation of the acetylated fractions A and B

Two separate batches (30 and 103 g.) of the acetylated fraction *A* were distilled at 1 mm. Hg through a Piros & Glover micro spinning-band column (cf. Horn & Hougen, 1953). The distillation curve for the larger batch is shown in Fig. 2 (curve *A*).

The acetylated portion of fraction *B* (30 g.) was distilled through the same column (Fig. 2, curve *B*). Only about half of this material distilled over below 240° mM.

Isolation of six saturated normal alcohols

By crystallizations (from methanol, ethanol or acetone, as suitable) of the appropriate distillation fractions (flats C_{14}, C_{16}, C_{18}, and fraction *b* of flat C_{20}; Fig. 2), pure acetates of tetradecan-1-ol, hexadecan-1-ol, octadecan-1-ol, and eicosan-1-ol were obtained. Impure 1-tetracosanyl acetate was obtained by crystallization of fraction *f* of flat C_{24}. Fractional crystallization of urea complexes was applied to fraction *d* of flat C_{22}. The first two complexes obtained were recovered to

yield an impure preparation of 1-docosanyl acetate. The acetates were hydrolysed with ethanolic KOH and the recovered alcohols crystallized to purity (methanol, ethanol, hexane, ether). Analytical data for the alcohols and their acetates are given in Table 1.

Isolation of three saturated iso *alcohols*

Fractions *a*, *c* and *e* (Fig. 2), combined with the corresponding distillation fractions from the smaller batch (30 g.) of acetylated fraction *A*, were separately fractionated by crystallization of urea complexes. Fraction *a* (14 g.) was treated successively with 4 × 10 g. and 4 × 2 g. of urea. The acetate of 18-methylnonadecan-1-ol was recovered from the last (eighth) urea complex; the residue left after urea fractionation contained an unsaturated alcohol, as shown by its high iodine value, but this residue was not used as a source of eicos-10-en-1-ol since a better source (see below) was available. Fractions *c* (10 g.) and *e* (8 g.) were treated with 2 g. lots of urea and divided into 13 (numbered *c* 1–*c* 13) and 11 (numbered *e* 1–*e* 11) urea complexes respectively. Complexes *c* 8 and *e* 8 were decomposed to give the acetates of 20-methylheneicosan-1-ol and 22-methyltricosan-1-ol respectively. The acetates were hydrolysed and the recovered *iso* alcohols crystallized to purity. Analytical data are given in Table 1. The terminal *iso*propyl structure of the three *iso* alcohols was confirmed by their infrared absorption spectra which showed the characteristic splitting of the 7·25 μ. band (Freeman, 1952). The isolated 18-methylnonadecan-1-ol and a synthetic specimen (Horn & Hougen, 1953) had identical infrared spectra.

Isolation and identification of three unsaturated normal alcohols

Eicos-10-*en*-1-*ol*. The single flat which was obtained by distillation of fraction *B* (Fig. 2) contained the acetate of an unsaturated C_{20} alcohol.

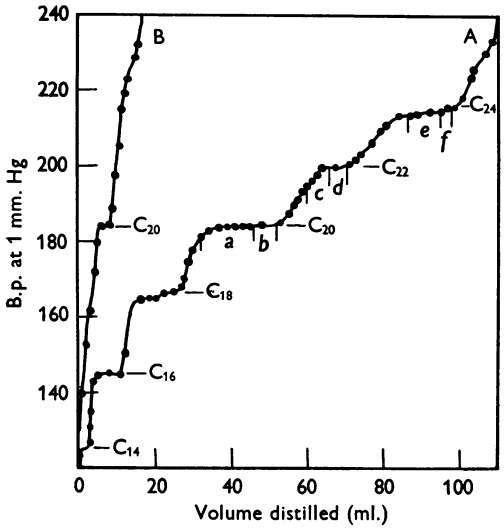


Fig. 2. Distillation curves (for conditions see text): *A*, acetates of the urea complex-forming material of sebum unsaponifiable matter (hydrocarbons excluded); *B*, acetates of the residual unsaponifiable matter.

A sample of the crude acetate was hydrogenated (Pd on $BaSO_4$ in glacial acetic acid) and took up 1·03 mol. prop. of H_2 (based on eicosenyl acetate). The hydrogenated sample melted at 39–41°. The acetate was saponified and the recovered alcohol crystallized from hexane and then methanol to m.p. 64·2–64·6°, unchanged on admixture with authentic eicosan-1-ol. (Found: C, 80·65; H, 14·4. Calc. for $C_{20}H_{42}O$: C, 80·5; H, 14·2%.)

The total fraction of eicosenyl acetate (about 2 g. from 30 g. of acetylated fraction *B*) was saponified, and the recovered alcohol (1·95 g.) crystallized from methanol at −60° to m.p. 23·5–24·4° (analysis, see Table 1). Examination of the infrared spectra revealed that the double bond had the *cis* configuration and that there was no terminal double bond or terminal *iso*propyl group.

The alcohol (1·3 g.) was oxidized with performic acid in acetic anhydride according to the method of Fietelson (1950) for the oxidation of oleyl alcohol. The oxidation product was saponified to give crude trihydroxyeicosane (1·29 g.). Crystallization from hexane–methanol and from ether–methanol gave *trihydroxyeicosane* having m.p. 86·0–86·3°. (Found: C, 72·9; H, 12·9. $C_{20}H_{42}O_3$ requires C, 72·7; H, 12·8%.)

This product, combined with a less pure second crop from the mother liquors (1·0 g.), was oxidized in benzene solution with lead tetra-acetate and a stream of air according to Mendel & Coops (1939; cf. Horn & Hougen, 1953). The recovered acid reaction product (0·28 g.) was esterified with methanol–H_2SO_4 and the mixed esters (0·27 g.) were separated by chromatography on acid-washed alumina into unhydroxylated ester (0·089 g.) and ω-hydroxy ester (0·124 g.).

The unhydroxylated ester was hydrolysed to give the acid (0·080 g.). A portion of this acid (approx. 9 mg.) was examined by reversed-phase partition chromatography, and a single peak was obtained with no traces of neighbouring homologues. A second chromatogram was run with the unhydroxylated acid (4 mg.) in admixture with authentic decanoic acid (4 mg.). The total material was eluted in a single peak and the unhydroxylated acid was therefore decanoic acid.

The ω-hydroxy ester (0·124 g.) was saponified to give the acid (0·116 g.). The acid (in 40%, v/v, aqueous acetone) was passed through the chromatographic column in order to free it from any admixed unhydroxylated acid. The recovered acid was crystallized from hexane–benzene to m.p. 69–69·5° (10-hydroxydecanoic acid has m.p. 75–76° according to Chuit & Hausser, 1929). The acid was dissolved in aqueous alkali and oxidized with 5% (w/v) aqueous $KMnO_4$ at 50°. The oxidation product was worked up in ether, crystallized from water and extracted with boiling hexane to give a product having m.p. 127–129° (raised to m.p. 130–132° on admixture with sebacic acid of m.p. 133°). (Found: C, 59·8; H, 8·4. Calc. for $C_{10}H_{18}O_4$: C, 59·4; H, 9·0%.) Paper chromatograms of C_8, C_9 and C_{10} dibasic acids, of the acid oxidation product and of this last in admixture with authentic sebacic acid, were run according to the method of Long, Quayle & Stedman (1951), except that the papers were sprayed with bromophenol blue and citric acid (Kennedy & Barker, 1951). The C_8 dibasic acid gave R_F, 0·46; C_9 acid, 0·51; C_{10} acid, 0·615; oxidation product, 0·575; a mixture of the oxidation product and C_{10} acid gave a single spot (R_F, 0·585). Although the sebacic acid obtained by the oxidation of the 10-hydroxydecanoic acid was not entirely pure, there can be no doubt about its identity.

Table 1. *Properties of the isolated alcohols and their acetates*

Calc. values for acetyl, C and H percentages are in brackets.

	Acetates				Alcohols					
	M.p. (°)	Acetyl (%)	C (%)	H (%)	M.p. (°)	C (%)	H (%)	X-ray long crystal spacings (Å)		Minimum percentage present (g./100 g. non-saponifiables)
Tetradecan-1-ol	13·4–14·0	17·0	74·6	12·6	37·7–38·0	78·2	14·0	—	39·6	0·2
	14·0[a]	(16·8)	(74·9)	(12·6)	38·26[g]	(78·4)	(14·1)	—	39·7[f]	—
Hexadecan-1-ol	24·3–24·5	15·3	76·1	12·7	49·3–49·4[b]	79·1	14·1	36·8	44·0	1·4
	24·2[a]	(15·1)	(76·0)	(12·8)	49·62[g]	(79·3)	(14·1)	37·4[f]	44·9[f]	—
Octadecan-1-ol	32·7–32·9	13·9	77·05	13·0	58·0[b]	79·9	14·15	41·2	—	2·5
	32·8[a]	(13·75)	(76·9)	(12·9)	57·98[g]	(79·9)	(14·2)	41·3[f]	50·2[f]	—
Eicosan-1-ol	39·7–39·8	13·0	77·5	13·0	65·05	80·4	14·2	45·4	—	3·0
	40 ± 0·5[a]	(12·6)	(77·6)	(13·0)	65·5[g]	(80·5)	(14·2)	—	—	—
Docosan-1-ol	47·6–47·8[d]	—	—	—	70·3–70·4	81·0	14·1	49·8	—	1·3
	49–51[c]	—	—	—	70·6[g]	(81·0)	(14·2)	49·95[f]	—	—
Tetracosan-1-ol	*ca.* 53·5[d]	—	—	—	75·5–75·7	81·3	14·2	53·2	—	2·0
	57[a]	—	—	—	74·8[g]	(81·3)	(14·2)	54·0[f]	—	—
18-Methylnonadecan-1-ol	30·9–32·9[d]	—	—	—	49·2–49·4[b]	80·3	14·2	—	—	0·5
					50·3[e]	(80·5)	(14·2)	—	—	—
20-Methylheneicosan-1-ol	—	—	—	—	55·4–55·9	80·9	14·3	—	—	0·4
					57·1[e]	(80·9)	(14·2)	—	—	—
22-Methyltricosan-1-ol	—	—	—	—	62·4–62·7	81·15	14·3	—	—	0·5
					62·4[e]	(81·3)	(14·2)	—	—	—
Eicos-10-en-1-ol	—	—	—	—	23·5–24·4	81·0	13·7	—	—	0·6
						(81·0)	(13·6)	—	—	—
Docos-12-en-1-ol	—	—	—	—	—	81·4	13·8	—	—	0·4
						(81·4)	(13·7)	—	—	—
Tetracos-14-en-1-ol	—	—	—	—	43·3–43·5	81·6	13·8	—	—	0·5
						(81·7)	(13·7)	—	—	—

[a] Phillips & Mumford (1934).
[b] The m.p. was unchanged by admixture with an authentic sample.
[c] Tiedt & Truter (1952).
[d] The acetate was not obtained pure.
[e] Murray & Schoenfeld (1952).
[f] Malkin (1930).
[g] Ralston (1948).

Docos-12-*en*-1-*ol*. As already described, fraction *c* of distillation flat C_{22} (Fig. 2) was divided into thirteen urea complexes. The acetate which was recovered from complex *c*12 was hydrolysed to give an unsaturated C_{22} alcohol (see Table 1). The double bond in this alcohol had the *cis* configuration, as shown by its infrared absorption spectrum, and there was no terminal double bond or terminal *iso*-propyl group.

A sample of the alcohol was hydrogenated and took up 0·88 mol. prop. of hydrogen (based on docosenol). The recovered hydrogenated sample was crystallized from ether to m.p. 70·1–70·2°, unchanged on admixture with authentic *n*-docosanol. (Found: C, 81·0; H, 14·3. Calc. for $C_{22}H_{46}O$: C, 80·9; H, 14·2%.)

A preparation of docosenol (containing also some 20-methylheneicosanol) was obtained from the pooled urea complexes *c*11–*c*13 and the most pentane-soluble part of the recovered alcohols from complexes *c*9 and *c*10. This material (1·55 g.) was oxidized with performic acid and the product saponified to give *trihydroxydocosane* (1·27 g.). Crystallization from ethyl acetate, followed by many extractions with boiling hexane to remove monohydric alcohol, and one crystallization from ether, gave fine prisms, m.p. 87·4–88·1°. (Found: C, 73·9; H, 12·7. $C_{22}H_{46}O_3$ requires C, 73·7; H, 12·9%.)

This product was pooled with a second crop obtained from the mother liquors (total 0·66 g.) and oxidized with lead tetra-acetate and air. The recovered acid reaction product (0·52 g.) was converted into the methyl esters (0·43 g.), and the latter were chromatographed on acid-washed alumina to give unhydroxylated ester (0·15 g.) and ω-hydroxyester (0·2 g.).

The acid (0·093 g.) which was recovered from the unhydroxylated ester was examined by reversed-phase partition chromatography and found to be decanoic acid with no traces of neighbouring homologues. A mixed chromatogram with authentic decanoic acid confirmed this. The acid which was recovered from the first chromatogram was converted into its *p*-bromophenacyl ester, which was crystallized from aqueous ethanol to m.p. 65·1–65·4° (*p*-bromophenacyl decanoate has m.p. 66° according to Hopkin & Williams (1950)). Admixture with authentic *p*-bromophenacyl decanoate gave no depression of the melting point, whereas authentic *p*-bromophenacyl nonanoate depressed the melting point considerably.

The ω-hydroxy ester (0·2 g.) was crystallized from pentane to m.p. 33·1–33·8° (methyl 12-hydroxydodecanoate has m.p. 34–34·5° according to Chuit & Hausser (1929)). Chromatography of the ester on acid-washed alumina followed by crystallization from pentane failed to raise the melting point of the ester. The ester was saponified and the recovered ω-hydroxy acid crystallized (benzene, hexane–ethyl acetate) to m.p. 78·1–81·7° (12-hydroxydodecanoic acid, m.p. 84–85° (Chuit & Hausser, 1929)).

Tetracos-14-*en*-1-*ol*. As described before, fraction *e* of distillation flat C_{24} (Fig. 2) was divided into eleven urea complexes. The acetate contained in complex *e*10 was saponified to give an unsaturated C_{24} alcohol. The alcohol was crystallized (methanol, acetone, benzene) to m.p. 43·3–43·5° (see Table 1). The infrared absorption spectrum proved the double bond of the alcohol to have the *cis* configuration, and that no terminal double bond or *iso*-propyl group was present.

A sample of a less pure preparation of the alcohol (m.p. 41·6–42·0°) was hydrogenated and took up 1·06 mol. prop. of hydrogen (based on tetracosenol). The hydrogenated sample was recovered and crystallized from ether to m.p. 75·1–75·3°, unchanged on admixture with authentic tetracosanol. (Found: C, 81·2; H, 14·4. Calc. for $C_{24}H_{50}O$: C, 81·3; H, 14·1%.)

A preparation (1·6 g.) of tetracosenol (containing some 22-methyltricosanol) was obtained from the pooled urea complexes *e*8–*e*11. The material was oxidized with performic acid and the product saponified to give *trihydroxytetracosane* (1·5 g.), crystallized from hexane, ether at −25° and methanol to m.p. 89–90·4°. (Found: C, 74·5; H, 13·1. $C_{24}H_{50}O_3$ requires C, 74·55; H, 13·0%.)

A less pure preparation of trihydroxytetracosane (0·95 g.) was oxidized with lead tetra-acetate and air, and the acid oxidation products (0·56 g.) were converted into the methyl esters (0·5 g.). The esters were chromatographed on acid-washed alumina to give unhydroxylated ester (0·15 g.) and ω-hydroxy ester (0·2 g.).

The unhydroxylated ester was hydrolysed to the acid (65 mg.) and the latter was examined by chromatography on the reversed-phase column. A small fraction of the total material (10–15%) was eluted with 45% acetone and the rest of the material with 50% acetone. The acid contained in the second peak was recovered and converted into its *p*-bromophenacyl ester, m.p. 65·3–65·5° (*p*-bromophenacyl decanoate, m.p. 66°) unchanged on admixture with an authentic specimen of *p*-bromophenacyl decanoate. (Found: Br, 21·5. Calc. for $C_{18}H_{25}O_3Br$: Br, 21·7%.) Confirmation that the unhydroxylated acid fraction was substantially decanoic acid was obtained by a chromatogram of this material admixed with authentic decanoic acid. The smaller fraction of the unhydroxylated acid material which was eluted in front of decanoic acid was recovered and melted at 40°.

The ω-hydroxy ester (0·2 g.) was crystallized from hexane to m.p. 41·7–43·0°. The ester was saponified and the recovered acid crystallized from hexane to m.p. 75–85·5° (14-hydroxytetradecanoic acid, m.p. 91–91·5°; Chuit & Hausser, 1929). (Found: C, 68·85; H, 11·6. Calc. for $C_{14}H_{28}O_3$: C, 68·8; H, 11·55%.) The acid in 45% aqueous acetone was passed through the reversed-phase column, but there was no improvement in the melting point of the acid recovered from the eluate.

DISCUSSION

The sebum was obtained by extraction of hair from a large number of people, and it might therefore have been contaminated with extraneous matter such as hair dressings, though these are not normally accessible to the subjects from whom the hair was obtained. Hair dressings consist mostly of neutral fats (glycerides) and mineral oils (cf. Weitkamp *et al.* 1947), and such materials do not yield a high percentage of long-chain alcohols on saponification. Contamination of the material reported upon here is therefore not likely to be appreciable in amount.

Twelve aliphatic alcohols of human sebum were identified. It is not possible to state with certainty the relative proportion of each present, since intermediate fractions and residues obtained during the lengthy fractionation contain unknown amounts of the various compounds; however, an indication of

the minimum percentages present is given in the last column of Table 1. The alcohols belong to three homologous series, namely: saturated normal alcohols (tetradecan-1-ol, hexadecan-1-ol, octadecan-1-ol, eicosan-1-ol, docosan-1-ol, tetracosan-1-ol), saturated *iso* alcohols (18-methylnonadecan-1-ol, 20-methylheneicosan-1-ol, 22-methyltricosan-1-ol) and unsaturated normal alcohols (eicos-10-en-1-ol, docos-12-en-1-ol, tetracos-14-en-1-ol).

Isolation of the mixed alcohols was effected by precipitation of their urea complexes from unsaponifiable matter that had previously been freed from non-aromatic hydrocarbons. Because the formation of urea complexes was not quantitative, a smaller fraction of alcohols was left in the residue. The mixed alcohols (as acetates) were separated by distillation into six fractions of increasing molecular weight (crude C_{14}, C_{16}, C_{18}, C_{20}, C_{22} and C_{24} alcohols). Each of the three lower-boiling fractions consisted of one saturated normal alcohol only. The three higher-boiling fractions were mixtures of normal, *iso* and unsaturated alcohols, which could not be resolved by distillation. These mixtures were separated by fractional crystallization of their urea complexes. One of the unsaturated alcohols (C_{20}) was present in appreciable amount in the residual unsaponifiable matter which did not form a urea complex, and was isolated from this fraction by distillation of the acetates; iodine values of the distillation fractions indicated that there were unsaturated alcohols present of chain lengths lower than C_{20} and higher than C_{24}, but the quantities were too small for their isolation. Some of these distillation fractions, however, deposited small amounts of crystals which in two of the fractions were most probably anthracene and chrysene (Hougen, 1954).

The saturated normal alcohols were identified by their melting points, elementary compositions, and X-ray long crystal spacings, and in some cases by acetyl values and the melting points and elementary compositions of the acetates. The *iso* alcohols were identified by their melting points, elementary compositions and infrared absorption spectra. There was sufficient material for measurement of the X-ray long crystal spacing of only one of the *iso* alcohols (C_{24}). This alcohol gave no clear X-ray pattern, and an impurity such as tetracosan-1-ol might therefore have been present in the sample. The unsaturated alcohols had one double bond and an unbranched chain as shown by their hydrogenation to the corresponding saturated normal alcohols. The double-bond positions were determined by oxidative cleavage of the alcohols into two acid fragments (ω-hydroxy acids and unhydroxylated acids) followed by the identification of these acids. The possibility was considered that the unsaturated alcohols were mixtures of double-bond position isomers (cf. double-bond position isomers in the free fatty acids of sebum; Weitkamp *et al.* 1947), in which case the acid fission products would be homologous mixtures. The technique of reversed-phase partition chromatography of fatty acids (C_{12}–C_{18} acids (Howard & Martin, 1950); C_{20}–C_{24} acids (Silk & Hahn, 1954)) was extended to include six shorter-chain normal acids (C_6–C_{11}); the unhydroxylated acid degradation products were analysed by this method. The C_{20}, C_{22} and C_{24} unsaturated alcohols each yielded decanoic acid with no traces of its homologues. In one case (degradation product from C_{24} alcohol) a small fraction of unidentified material appeared in the eluate in front of the decanoic acid. This unidentified material was neither a homologue of decanoic acid nor was it 14-hydroxytetradecanoic acid, as was shown by its position in the elution curve and by the melting point of the recovered material. Although this material was not identified, its presence clearly does not influence the argument about the structure of the tetracosenol component. The identification of decanoic acid as a degradation product of the C_{20}, C_{22} and C_{24} alcohols proved their structures to be eicos-10-en-1-ol, docos-12-en-1-ol and tetracos-14-en-1-ol respectively. The corresponding derived ω-hydroxy acids (10-hydroxydecanoic, 12-hydroxydodecanoic and 14-hydroxytetradecanoic acids) proved difficult to purify by crystallization. It was considered sufficient to identify one of them (10-hydroxydecanoic acid) by oxidation to the dibasic acid (sebacic acid).

The saturated normal alcohols of sebum are common constituents of natural waxes. The *iso* alcohols are apparently not of wide occurrence, but have been found in wool wax (Murray & Schoenfeld, 1952). The unsaturated alcohols have not been described before, but somewhat similar alcohols have been found in the seed oil of *Simmondsia chinensis* (eicos-11-enol and docos-13-enol; Green, Hilditch & Stainsby, 1936) and in sperm blubber oil (eicos-9-enol; Toyama, 1938).

In many natural waxes the acids and alcohols are structurally related, and this may suggest a common path in the biosynthesis of these two classes of compounds. Sheep sebaceous secretion (wool wax) is a striking illustration of this phenomenon as the acids and aliphatic alcohols belong to the same four homologous series of normal, *iso*, *ante-iso* and α-hydroxy compounds (Knol, 1954). A similar relationship does not exist between the alcohols and the free fatty acids of human sebum. Acids of odd- as well as even-numbered chain lengths are present, whilst *iso* acids have not been found (Weitkamp *et al.* 1947). The double bonds are differently situated in the acids and the alcohols. The 'combined' acids of sebum have not yet been reported upon in the literature.

SUMMARY

1. Twelve aliphatic alcohols of human sebum have been isolated and identified. They belong to three homologous series as follows: saturated normal alcohols (tetradecan-1-ol, hexadecan-1-ol, octadecan-1-ol, eicosan-1-ol, docosan-1-ol, tetracosan-1-ol), saturated *iso* alchols (18-methylnonadecan-1-ol, 20-methylheneicosan-1-ol, 22-methyltricosan-1-ol) and unsaturated normal alcohols (eicos-10-en-1-ol, docos-12-en-1-ol, tetracos-14-en-1-ol).

2. The reversed-phase partition chromatographic technique for resolution of fatty acid mixtures (Howard & Martin, 1950) has been extended to include six shorter-chain acids having odd and even numbers of carbon atoms.

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The Estimation of Unsaturated Fatty Acids by Reversed-Phase Partition Chromatography

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During an investigation of the metabolism of fat-containing seeds, a method was required for the analysis of milligram quantities of fatty acids. From a number of column-chromatographic methods (Boldingh, 1950; Cropper & Heywood, 1953; Nijkamp, 1953, 1954; Simmons & Quackenbush, 1953), the Howard & Martin (1950) reversed-phase technique was selected for further investigation. This method is effective for the separation of even-numbered straight-chain saturated fatty acids (from C_{12} to C_{18}). It has recently been extended to similar acids from C_{10} to C_{24} (Popják & Tietz, 1954; Silk & Hahn, 1954). Savary & Desnuelle (1953) have investigated the behaviour of oleic and linoleic acids on similar columns and show that these acids are separable. Linoleic acid is eluted rather less rapidly than myristic acid, and oleic acid at the same rate as palmitic acid. In order to separate these saturated and unsaturated acids, Savary and Desnuelle employed ice-cold dilute alkaline permanganate oxidation to convert the unsaturated acids into the corresponding hydroxy acids, which are eluted more rapidly than palmitic acid. The di- and tetra-hydroxy stearic acids are then separated by reversed-phase chromatography on columns of purified castor oil.

We have investigated the behaviour, on Howard & Martin columns, of a large number of unsaturated