IDENTIFICATION OF PRISTANE IN HUMAN SEBUM AND RELATED LIPID SOURCES

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The presence of paraffinic hydrocarbons in human hair and skin surface lipids has been reported by several workers (Gershbein et al, 1967; Gershbein and Krotoszynski, 1965; Haahti, 1961; Henseke and Schieffer, 1961; Nicolaides and Rothman, 1953; MacKenna et al, 1952) but their occurrence as an authentic class had been previously questioned (Boughton et al, 1955; Nicolaides and Kellum, 1965). In the present study, analyses were directed toward a specific hydrocarbon, pristane (2,6,10,14-tetramethylpentadecane) which might represent an effective index for authenticating the presence of various hydrocarbon species. Pristane has been identified in wool wax (Mold et al, 1964), in marine lipids as basking shark liver oil (Blumer et al, 1963), in certain petroleum and coal tar mixtures (Bendoraitis et al, 1962; Kochlofl et al, 1963), and recently, in some human tissue including skin (Avigan et al, 1967). In addition to hair and skin surface lipids, allied mixtures as vernix caseosa and ovarian dermoid cyst lipids were also investigated as these products are not as readily contaminated by environmental factors. The presence of pristane in each of the four sources was confirmed by gas chromatography-mass spectrometry, thereby lending support to the contention that paraffinic hydrocarbons are integral components of sebaceous lipids.

METHODS

Skin surface lipids were obtained on alternate days from balding white men, 35-50 yr in age, by passage of ethyl ether-moistened pledgets over the scalp and wiping the surface dry (PBA). Lipids (PCF-7-L) were also extracted with ethyl ether from a large pool of hair cut with degreased scissors from Negro men (Gershbein and Krotoszynski, 1965; Gershbein and O'Neill, 1966). Chloroform-methanol (2:1) was employed for the extraction of the ovarian dermoid cysts immediately after surgical excision and for the vernix caseosa lipids scraped from the newborn. It must be emphasized that with no exceptions, the glassware and equipment as well as the cotton pledgets were exhaustively degreased with chloroform-methanol and ethyl ether and all solvents were of AR grade and distilled before use. All volunteers abstained from the application of any pomades or hair dressings.

The lipid samples were saponified by heating with 20 wt % NaOH in 95% ethanol under nitrogen and the unsaponifiable portion (UNS) removed with ether. The hydrocarbon fraction was concentrated by chromatography of the UNS in petroleum ether (b 30-60°) over activated alumina (Alcoa F-20) and elution of the column with the same solvent to yield fraction I (Gershbein et al, 1967; Gershbein and Krotoszynski, 1965). GC analyses of fraction I samples dissolved in n-hexane (200 $\mu g/\mu l$) were carried out in a Perkin-Elmer 881 gas chromatograph equipped with a hydrogen flame detector and containing dual 3/16" x 12' columns of OV-I on 80/100 mesh Chromosorb G-HP (Supelco). The column was temperature-programmed from 150-300° at a linear rate of 2°/min. The equivalent chain length (ECL) of a purified pristane sample (J. C. Martens & Company, Bergen, Norway) was found to be 17.5. On chromatography of the fractions from the various lipid pools, the component exhibiting an ECL of 17.5 was split before the hydrogen flame ionization detector with 20% of the sample being directed to a Hitachi Perkin-Elmer model RMU-6D mass spectrometer. The mass spectrum of the ECL-17.5 peak was obtained by scanning up to m/e 350 at a scan rate of 15 sec and at a standard ionization potential of 70 eV. The mass spectra obtained for each of the ECL-17.5 component in each mixture was then compared with that obtained for a purified pristane sample and with published spectra (Mold et al, 1964; Bendoraitis et al, 1962).

RESULTS

As identified in Table 1, the mass spectra for component ECL-17.5 of fractions I from the four lipid sources simulated both the pure pristane and reference spectra, thereby substantiating the presence of pristane in each pool. The differences in relative intensities noted in the table are not unexpected due to the minor inconsistencies resulting from statistical variations in ion detection experienced with rapid scan and the usual instrument differences. No attempt was made to determine the exact amount of the component in the pools since it occurred at low levels ($\sim$0.01%), coupled with the fact that the gas chromatographic separation was optimized solely for the detection of pristane. The GC and mass spectral data did reveal, however, the presence of a complex hydrocarbon mixture containing normal odd and even-numbered hydrocarbons and branched homologs in each of the lipid products screened.

In addition to pristane, the sebum samples also displayed other peaks in the GC elution sequence which resembled a homologous series of pristane-like components. Mass spectrometry of the two succeeding major peaks eluting after pristane,

Table 1

Mass Spectrographic Data for Pristane in Various Lipid Samples

m/e	Pure Pristane	Mold, et al[a] (Literature Data)	Bendoraitis, et al[b] (Literature Data)	Sebum PCF-7-L	Sebum PBA	Vernix Caseosa	Ovarian Dermoid Cyst
	Samples Analyzed (Relative Intensities of Peaks)						
41	58	43	-	46	56	46	53
43	87	100	-	Off scale	82	82	67
55	45	32	34	45	46	29	37
57	100	98	100	100[c]	100	100	100
69	25	18	16	40	30	25	57
71	95	61	70	Off scale	55	79	54
83	7	5.4	5	27	21	8	9
85	57	24	26	46	31	31	21
97	9	4.9	5	26	17	7	9
99	20	8.1	8	16	7	10	6
112	26	25	12	15	5	14	7
113	33	27	16	18	6	18	8
127	11	4.3	5	9	4	6	3
141	4	2.5	2	5	2	2	2
155	4	4.8	2	4	2	3	2
169	1	0.6	0.5	2	1	0.5	1
182	6	-	4	4	1	4	2
183	13	23	10	8	3	12	4
197	0.7	1.2	0.5	1	1	0.5	d
211	0.1	0.4	0.2	1	0.6	0.2	d
225	0.5	1.1	0.3	0.7	0.5	0.5	d
239	0.4	1.2	0.3	0.7	0.4	0.4	d
253	0.7	3.0	0.7	0.7	0.1	0.5	d
268 (M^+)	0.6	22	0.2	0.4	0.1	0.8	d

[a] Mold, et al, Biochemistry 3, 1293(1964).
[b] Bendoraitis, et al, Anal. Chem. 34, 49(1962).
[c] Approximated from m/e = 55 and 58 because peak was off-scale (4460 units peak height).
[d] Not detectable above background.

indicated a series of saturated hydrocarbons containing one, two and three additional methylene units in excess of that of pristane. Authentic standards for the confirmation of such structures were lacking. A series of olefinic components exhibiting the C_{19} pristane skeleton, but containing one, two, three and four double bonds were also suggested by mass spectrometry by scanning the front edge of the pristane peak in the GC elution sequence. The occurrence of such entities was indicated by a series of four parent ion fragments, each reduced by two mass units. The levels of the four olefinic components in the ovarian dermoid cyst and vernix caseosa lipids were greatly reduced or absent and accordingly, could not be confirmed by mass spectrometry. The possibility of these olefinic parent ion fragments representing naphthenic structures is minimized by the fact that the elution sequence of the corresponding C_{19} ring structures exhibit ECL values of approximately 20.5 on SE-30.

DISCUSSION

Hitherto the unequivocal demonstration of paraffins as definite entities in sebum has been handicapped by possible contamination from exogenous sources. However, dietary hydrocarbons can be elaborated by the sebaceous gland as shown by Nicolaides (1966). In his study, the feeding of ^{14}C-1-octadecane to the rat led to the presence of unaltered octadecane as well as labelled fatty acids of C_{14} to C_{20} in the skin lipids. In earlier studies, Blumer and Thomas (1965) reported the occurrence of pristane and small amounts of the unsaturated isoprenoid C_{19}-isomer and C_{20}-phytadienes in marine organisms, notably, zooplankton on which the basking shark feeds. The primary source of pristane was reported to be the phytyl group of chlorophyll which originated from the marine photosynthetic organisms (Avigan and Blumer, 1968).

It is generally presumed that pristane and its precursors, phytanic and phytenic acids also arise from dietary sources in mammalian tissue. Baxter et al (1967), employing ^{14}C-labelled phytol, showed that it could be absorbed through the intestine by way of the lymphatic system. Subsequently, Baxter and Steinberg (1967) reported the absorption of ^{14}C-pheophytin 'a' isolated from tobacco leaf in rats following force feeding. Their data indicated that 1-2% of the chlorophyll phytol was absorbed, a finding which also applied to non-labelled spinach extracts. Whether or not dietary sources are directly responsible for the presence of pristane and its related structural entities found in the sebaceous lipids is not known. However, if the origin is dietary, then chain lengthening and dehydrogenation mechanisms must be involved to account for the various saturated homologs and unsaturated isomers noted in this study. At present, it does not seem reasonable to postulate that the pristane skeleton can be dehydrogenated to the corresponding branched chain olefinic components (isoprenoids) suggested by our mass spectrometric findings. Alternatively, since the sebaceous gland is an active site for squalene biosynthesis and isoprenoid condensation reactions, this gland might also be considered as a possible alternate synthetic route for these unique isoprenoid hydrocarbons.

In view of the fact that the isoprenoid biosynthetic pathway is intimately involved in certain cardiovascular ailments and with the nervous disorder, Refsum's disease, presumably by the inability to degrade phytanic acid (Avigan et al, 1966), the presence of these isoprenoid metabolites in sebum may well have direct clinical significance. A more direct and comprehensive study of the origin and metabolism of such components would also be important in the screening of certain dermatological disorders.

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