The Fatty Acids of Wax Esters and Sterol Esters from Vernix Caseosa and from Human Skin Surface Lipid

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ABSTRACT

Separation of sterol esters from wax esters in the lipids of vernix caseosa and adult human skin surface was accomplished by column chromatography on MgO. The fatty acids of the sterol esters and wax esters of both samples were separated into saturates and monoenes, and examined in detail by gas liquid chromatography (GLC). The saturated fatty acids of the wax esters of vernix caseosa and of adult human skin surface were remarkably similar. They ranged in chain length from at least C_{11} to C_{30}, six skeletal types being present: straight even, straight odd, iso, anteiso, other monomethyl branched and dimethyl branched. A large number of patterns of monoenes were observed, each pattern consisting of desaturation of a specific chain at Δ6 or Δ9 plus its extension or degradation products. The mole per cent of the total Δ6 and Δ9 patterns of wax ester fatty acid monoenes of vernix caseosa were 87% and 12%, respectively, and 98% and 1%, respectively, for adult human skin surface lipid. The sterol ester fatty acids of vernix caseosa were much different from those of adult human skin surface: vernix caseosa saturates were largely branched and of lengths greater than C_{18}, whereas the saturates of adult human surface lipid resembled the wax ester fatty acids. Of the vernix caseosa monoene patterns, the mole per cent was 30% Δ6 and 70% Δ9, whereas of the adult human skin surface sterol ester fatty acids 89% were Δ6 and 11% Δ9. Chain extension was particularly pronounced in the sterol ester fatty acid monoenes of vernix caseosa amounting to 7-8 C_2 units in some cases. The fatty acids of the sterol esters of both vernix caseosa and adult human skin surface appear to be derived from the sebaceous gland and from the keratinizing epidermis, but those of the wax esters are from the sebaceous glands only.

INTRODUCTION

Sterol esters of skin lipids have evoked considerable interest since Rothman reported that psoriatics have a lower amount than normal (1). Their origin and role are still unresolved problems.

Knowledge of skin sterol esters has been hampered by the difficulty in separating them from the wax esters, for in most chromatographic systems the two ester types migrate as a group. Sterol esters can now be separated from wax esters conveniently by chromatography on magnesium oxide (2).

On the assumption that the fatty acid moieties of each of these ester types would give clues as to the origin and role of these lipids, we used the above technique to separate the sterol esters from the wax esters of the lipids of vernix caseosa and of adult human skin surface. A plausible explanation as to how sterol esters are formed, which would fit all of our results and other relevant data, is that sterols, which are products of epidermis, become esterified primarily (but not entirely) with sebum fatty acids in late phases of keratinization or after keratinization is complete, both in the adult human skin and in vernix caseosa. Some unusual fatty acid unsaturation patterns were also observed, as were some unusual extension patterns of both saturated and unsaturated fatty acids.

EXPERIMENTAL PROCEDURES

Human skin surface lipid was collected daily from a 26-year-old man by the ether scalp soaking technique (3). An aliquot (817.1 mg) from four soaks was chromatographed on a column (205 mm x 44 mm ID) of 134 g silicic acid (Unisil, 100 mesh, Clarkson Chemical Co., Williamsport, Pa.). Fifty milliliter fractions were collected and assayed by thin layer chromatography (TLC). (This was carried out on 250μ layers of silica gel plus magnesium silicate 9:1 developed in hexane-ether 95:5 v/v.) Hexane (550 ml) and 5% benzene in hexane (520 ml) eluted 4.0 mg saturated hydrocarbons and 75.3 mg squalene, respectively. Then 600 ml 20% benzene in hexane eluted 185.1 mg of a mixture of sterol esters plus wax esters, and an additional 550 ml eluted a mixture of 11.80 mg wax esters plus about an equal amount of more polar material. The latter fraction was not included in further work-up.

A 173.7 mg aliquot of sterol esters plus wax esters was chromatographed on a column (92 mm x 44 mm ID) of 85 g MgO (Matheson

TABLE I

Relative Amounts of Wax Esters and Sterol Esters and Their Fatty Acid Moieties in Vernix Caseosa and Adult Human Skin Surface Lipid

Esters	Vernix caseosa lipid				Adult human skin surface lipid			
	Fatty acid moieties, 100%							
	Saturates	Monoenes	Dienes	Polar[a]	Saturates	Monoenes	Dienes	Polar[a]
Wax esters, % of total lipid		15.9%				19.6%		
Straight even	8.5	46.8			16.5	40.7		
Straight odd	1.8	8.1			2.7	4.7		
Iso	6.6	6.6			3.5	15.0		
Anteiso	6.1	5.0			1.8	4.0		
Monomethyl branched[b]	1.9	tr[c]			2.2	tr		
Dimethyl branched[d]	0.1	tr			0.2	tr		
Totals	25.0	66.5	6.0	2.0	26.9	64.4	4.0	4.7
Sterol esters, % of total lipid		25.4%				2.81%		
Straight even	6.8	23.6			22.5	40.5		
Straight odd	0.9	1.3			4.4	4.1		
Iso	38.1	3.8			5.2	7.4		
Anteiso	19.6	0.7			3.3	2.0		
Monomethyl branched[b]	tr	tr			2.3	tr		
Totals	65.4	29.4	3.7	1.5	37.7	54.0	8.3	tr

[a]Polar unidentified material.

[b]Equivalent chain lengths ranged from 10.5-20.5 (see Table II).

[c]tr = Trace.

[d]Equivalent chain lengths ranged from 11.2 to at least 17.2 (Table II).

TABLE II

Fatty Acids of Wax Esters and Sterol Esters from Vernix Caseosa and from Human Skin Surface Lipid[a]

	Wax esters				Sterol esters			
	Saturates		Hydrogenated monoenes		Saturates		Hydrogenated monoenes	
ECL[c]	VC[b] %	HSL %	VC %	HSL %	VC %	HSL %	VC %	HSL %
11.22	.02	tr						
11.50	.43					tr		
11.65	tr	.17			tr	.21		
12	.77	.96	tr	tr	tr	.64		
12.12	tr	.30				tr		
12.48	2.01	1.24				.54		
12.68	.87	.12			tr	tr		
13	.76	.37	.04	.04	tr	.37		
13.15	tr	tr				tr		
13.20	.44	.24			tr			
13.3						tr		
13.48	.15	tr			tr	.06		
13.66	9.28	5.99	.03	tr	2.25	3.18	tr	tr
14	8.13	13.04	7.62	6.76	1.25	13.69	.60	5.25
14.2	tr	tr	Some?	tr?	Some?	Some?		
14.48	2.85	2.78			tr	2.49		
14.69	16.60	5.32	.63	2.23	6.41	4.75	tr	1.05
15	5.22	6.85	6.86	5.06	1.02	7.12	.70	3.54
15.2	tr	tr	Some?			Some?		
15.49	tr	.07			tr	.20		
15.65	14.63	5.04	8.78	21.56	15.24	4.62	2.02	10.09
16	20.50	40.13	48.39	47.01	5.02	32.25	11.61	45.83
16.2	tr	tr	Some	Some?		Some?		
16.50	2.17	3.99	Some	Some?	tr	3.09	tr	
16.72	5.76	.75	6.92	3.84	3.45	1.31	1.21	2.22
17	.96	2.02	5.13	2.07	.24	2.32	1.93	3.34
17.22	.08	tr	Some?		.05	.07		
17.52	tr	.04		Some		.07		
17.65	.84	.38	.98	1.49	3.65	.60	3.32	2.49
18	3.78	4.83	13.18	8.60	1.29	6.05	32.24	20.76
18.2	tr	tr	Some?			.05		
18.49	tr	tr	.02		.15	.05		
18.6						.07		
18.72	.69	tr	.04	.14	1.32	.21	.40	.22
19	.07	.26	.19	.21	.06	.58	.66	.58
19.66	.76	.24	.09	.09	12.90	1.23	3.38	.52
20	.25	.70	.64	.53	.84	1.68	9.55	1.53
20.4		tr			.60	.01		
20.5	tr	tr				.04	tr?	
20.72	.25	.09	.02	.01	6.80	.55	.39	.06
21	tr	.05	.03	.01	tr	.32	.49	.06
21.47			.02	.03	Some			
21.65	.30	.21	.03	.01	12.43	1.25	1.59	.14
22	.12	.47	.11	.07	.64	1.23	12.47	.06
22.2			.02			Some?		
22.5					.60	.20		
22.74	.15	.15	.01	tr	4.69	.49	.18	.05
23	.02	.16	.02	.01	.20	.26	.35	.06
23.2		tr						
23.3	tr							
23.4	tr		tr			tr		
23.5				tr	Some			
23.64	.35	.52	.01	.04	7.76	1.57	1.88	.27
24	.30	1.11	.07	.05	.95	2.90	9.04	.75
24.2						.07		
24.5	tr	Some?	.01		.60	.05		
24.75	.25	.43	.01	tr?	3.96	1.00	.16	.07
25	.02	.28	.01	tr	tr	.54	.14	.07
25.4					Some?			
25.6	.15	.33	.01	.04	3.38	.88	.64	.26
26	.03	.37	.04	.04	.40	.94	1.43	.19

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TABLE II
(Continued from preceding page)

26.4						.03		
26.7	.02	tr	tr	.02	1.11	.07	tr	tr
27	tr	tr	tr	.02	tr	.04	tr	tr
27.6	.02	tr	.01	.01	.60	.03	tr	tr
28	tr	tr	.02	.01	tr	.03	.50	tr
28.4					tr			
28.7					.14			tr
29			tr			.03	tr	tr
29.5					tr			
29.6								tr
30		tr	.02				.21	
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[a]VC = vernix caseosa, HSL = human surface lipid, tr = trace. Percentage figures are given to two decimal places simply to round out the data and show relative amounts of the minor components. Accuracy is estimated at ± 5%.

[b]Traces of material to ECL's of 10.50 were seen for this sample.

[c]ECL = equivalent chain length (7). The ECL's listed are an average of all eight samples. Where only one decimal is listed the error is estimated at ± .05 for all samples: where two decimals are given maximum error for all samples is ± .04 and usually ± .02 or less.

Coleman and Bell MX-66 catalytic grade 200 mesh adsorptive powder) and eluted with 20 ml hexane and 190 ml 1% acetone in hexane before anything emerged. An additional 80 ml, 140 ml and 550 ml of 1% acetone in hexane eluted 107.25 mg wax esters, 1.14 mg of a mixture of wax esters plus sterol esters and 27.05 mg of a mixture of sterol ester plus cholesterol and wax alcohol respectively. Finally an additional 450 ml 1% acetone in hexane plus 350 ml 10% acetone in hexane eluted 20.23 mg wax alcohol plus sterol. The 27.05 mg fraction was then rechromatographed on 12 g of silicic acid on a column (120 x 16 mm ID) and sterol esters (21.79 mg) easily separated from the free alcohols plus sterols (5.96 mg). This gave a final recovery of 107.25 mg wax esters, 1.14 mg of an equal mixture of sterol esters and wax esters, 21.79 mg sterol esters and 26.19 mg of a mixture of free alcohol and free sterol in proportion of ca. 5:1 (assayed by TLC), making a total of 156.37 mg recovered. The unrecovered 17.3 mg from the original 173.7 mg was presumably held on the column as fatty acid salts of magnesium. Subsequently it was shown that when fines were removed from the magnesium oxide (which was not done in this chromatogram) less than 5% hydrolysis occurred (2). However the proportion of alcohol to sterol recovered indicated that no preferential hydrolysis of either ester class occurred.

An aliquot (26.9 mg) of the wax ester fraction and the entire sterol ester fraction were then individually saponified (10% KOH in 90% ethanol refluxed 3 hr under nitrogen); the saponification mixture was diluted with water, then acidified with 6N H_2SO_4 and extracted with hexane. The unsaponifiables were then separated from the fatty acids quantitatively by column chromatography on 4.6 g Florisil (Floridin Co., Tallahasee, Fla.) after a batch of the adsorbent had been washed extensively with distilled water, activated at 120 C for 16 hr, then 7% water added (4). Column dimensions were 105 x 50 mm ID. For the sterol ester separation of saponified products, after hexane had eluted a tiny trace of material, 60 ml of $CHCl_3$ eluted 12.32 mg of sterols and 35 ml chloroform-methanol-formic acid (88%) in the proportion 90:5:5 v/v/v eluted 8.31 mg of fatty acids. The wax esters yielded 14.83 mg of wax alcohols and 13.57 mg of fatty acids in a similar chromatogram. The fatty acids from each group of esters were then esterified with methanolic BF_3 and separated into saturates, monoenes, dienes and "polar" material on $AgNO_3/SiO_2$ columns by techniques already reported (5). The saturates were analyzed by GLC and the monoenes were collected by preparative GLC and analyzed by hydrogenation, GLC and ozonolysis by procedures also previously reported (5). Nothing further was done with the polar material or dienes (except as indicated below).

Vernix caseosa (11.82 g) was obtained from a Caucasian male to yield 1.370 g lipid. This was analyzed by a very similar procedure, and details have been reported (6).

RESULTS AND DISCUSSION

The wax ester fatty acids of both vernix caseosa and adult human skin surface show a remarkable similarity. Not only do these esters make up nearly the same percentage of their corresponding lipid samples (Table I), but their fatty acid moieties show a similar composition

TABLE III

Positions Isomers of Fatty Acid Monoenes in Wax Esters of Vernix Caseosa and Adult Human Skin Surface Lipid

Monoene carbon skeleton[a]	Mole % of total monoenes	Mole % at Δ positions indicated[b]											
		Δ4	Δ5	Δ6	Δ7	Δ8	Δ9	Δ10	Δ11	Δ12	Δ13	Δ14	Δ15
Vernix caseosa													
n-14	8.59	1	tr	96	1	1	1	tr					
n-16	48.88		tr	88	tr	5	6	tr	1	tr	tr		
n-18	12.04	nd	nd	14	tr	26	35	1	21	nd	3		
n-20	0.54		tr	4	3	2	2	33	9	3	44		
n-22	0.08	NA											
n-24 to 30	0.10	NA											
n-13	.06	NA											
n-15	7.31	tr	tr	99	tr	tr	1	nd					
n-17	4.91		tr	76	4	7	7	tr	6	tr			
n-19	.17	NA											
n-21 to 27	.04	NA											
i-14	.04	NA											
i-16	8.84	tr	1	98	1	tr	tr	tr	tr				
i-18	.90		2	64	5	23	6	tr					
i-20 to 28	.13	NA											
ai-15[c]	.67		3	97	tr	tr	tr	tr					
ai-17[c]	6.62		tr	100	tr	tr							
ai-19 to 27	.08	NA											
Adult human skin surface lipid													
n-14	7.57	nd	nd	100	nd	nd	nd	nd	nd	nd			
n-16	47.17	nd	tr	95	tr	4	1	tr	tr	tr	tr	tr	tr
n-18	7.81	nd	tr	38	2	52	5	3	tr	tr	tr	tr	
n-20	.44		nd	nd	11	9	3	61	6	10	tr	tr	
n-22 to 28	.12	NA											
n-13	.05	NA											
n-15	5.36		nd	100	nd								
n-17	1.97		tr	75	7	17	1	tr	nd				
n-19	.18		nd	55	13	38	8	34	2				
n-21 to 27	.03	NA											
i-16	21.63			100									
i-18	1.35		2	39	2	57							
i-20	.08		6	7	1	24	4	59	tr	tr			
i-22 to 28	.08	NA											
ai-15	2.36		1	97	1	1							
ai-17	3.66	nd	nd	100	nd								
ai-19[c]	.12			47	9	44							
ai-21 to 27	.02	NA											

[a]n- = normal, i- = iso, ai- = anteiso.

[b]tr = Trace, nd = not detected, NA = not analyzed. All structures were determined by analysis of the aldehydes and aldehyde esters formed on reductive ozonolysis (5).

[c]Structure determined by analysis of aldesters only.

both with regard to the amount of saturates and unsaturates (Table I) and to the types of chains (Table II). The chains range from an equivalent chain length (ECL) (7) of 10.50-30.00, the chain types being straight even, straight odd, iso, anteiso, other monomethyl branched and dimethyl branched. These chain types have all been identified by mass spectrometry (6). The similarity in composition of such a diverse group of acids for these two entirely different samples argues against any significant contamination of either sample. However some differences in distribution (Table I) as well as in double bond patterns (to be discussed) does exist.

The sterol esters of vernix caseosa, on the other hand, make up not only a significantly greater percentage of total lipid than do the sterol esters of adult human skin surface lipid, i.e., 25.4% vs. 2.8%, but the fatty acid moieties of the two samples are considerably different. In this regard the sterol ester fatty acids of adult human skin surface resemble more closely those of the wax esters than they do those of

the sterol esters of vernix caseosa. In vernix caseosa sterol esters the per cent saturated fatty acids is exceedingly high, i.e., 65% (Table I). Most of these saturated acids are branched, iso making up 38% and anteiso nearly 20%. This high amount of saturates is in marked contrast to the other samples which have much more straight even monoenes (∿40%). The sterol ester fatty acids of vernix caseosa differ in another respect from those of the other samples in that they have a higher proportion of the longer chain lengths. For example, 60% of the saturates and 43% of the monoenes are above C_{18} for vernix caseosa, whereas only 16% of the saturates and 5% of the monoenes are above C_{18} for adult human skin surface. The fatty acids of the wax esters on the other hand, have only ca. 5% saturates and 1% monoenes above C_{18} for both samples.

Both vernix caseosa lipid and adult human skin surface lipid have methyl branched acids other than iso and anteiso for both wax esters and sterol esters. The location of these methyl branches is predominantly at position 4, but it also occurs on even C-atoms greater than 4 (6).

Our data show a rough correspondence in chain length distribution with those of Kärkkäinen et al. (8); however identifications of the exact structures are at considerable variance. What they have frequently stated as "iso" we have tabulated as ECL's of 0.45-0.52 which were found to be mixtures of monomethyl branched acids. The basis for their identification as "iso" or "anteiso" must have been solely from GLC retention data, and the hazards of that have been discussed (9).

The molar per cents of the double bond position isomers for each chain length monoene of the wax ester fatty acids of vernix caseosa and adult human skin surface are given in Table III; those for the sterol esters of both samples are given in Table IV. A probable manner in which these position isomers could be biosynthesized can be given if we make four reasonable assumptions: (a) There are two desaturase systems, one placing a double bond between the sixth and seventh C-atoms from the carboxyl group (designated as Δ6), and the other between the ninth and tenth C-atoms (designated as Δ9). (b) Both desaturase systems can desaturate all the major skeletal chain types of the fatty acids found, i.e., straight even, straight odd, iso and anteiso. We have also found that the other mono and dimethyl branched chain types show unsaturation (unpublished), but their structures have not yet been elucidated, and they are not included in this discussion of position isomer data. (c) Chain lengths primarily of C_{14} to C_{20} (usually those occurring in greatest amounts) are the substrates upon which the desaturase systems work. (d) After desaturation has occurred the chains can be further extended by C_2 units or degraded by C_2 units, the chain extension process predominating. Each initial substrate plus its extension or degradation products constitute what we are here calling a "pattern." The total moles of a pattern can be computed from data given in Tables III and IV. Thus the mole per cent of the 14Δ6 pattern would include, for example, not only the moles of the initial substrate, $C_{14:\Delta 6}$, but also all its extension and degradation products, i.e., $C_{16:\Delta 8}$, $C_{18:\Delta 10}$, $C_{20:\Delta 12}$, $C_{12:\Delta 4}$, etc., if these were present. For the various patterns occurring in the wax ester fatty acids of vernix caseosa and of adult human skin surface lipid, Table V gives the mole per cent of the total monoenes in the pattern as well as the number of molecular species in each pattern. Table VI gives the same data for the sterol ester fatty acids of both samples.

It is not implied that the biosynthetic scheme proposed here is the only one which can explain the data. Other desaturase systems (for example, desaturating between the seventh and eighth C-atoms) or decarboxylating enzymes may be present, which could also explain some of the products. The proposed scheme, however, does have the merit of simplicity and comprehensiveness, and postulates biochemical processes known to exist. We are not aware of any evidence which would necessitate mechanisms additional to those postulated here.

The large number of position isomers present (Tables III and IV) is truly a striking feature. For the wax esters of adult human skin surface lipid, nearly all the monoenes (98%) are of the Δ6 type (Tables II and V). Vernix caseosa wax ester fatty acids also show a preponderance of the Δ6 patterns (87%), but Δ9 patterns are appreciable (12%) (Table V). Of the latter the 16Δ9 and the 18Δ9 make up the largest proportion (5.78 and 4.26 mole %, respectively). The Δ9 patterns are far more prevalent among the sterol ester fatty acids of vernix caseosa, making up ∿70%; but for adult human skin surface, only 11% of the sterol ester fatty acids are Δ9 (Table VI).

It is highly probable that vernix caseosa sterol ester fatty acids originate from both keratinizing epidermis and sebum. Much evidence supports this conclusion: sterol esters are major components of epidermis (10-12); the major fatty acid monoene of epidermis is oleic acid, $C_{18:\Delta 9}$ (13); acids of the Δ6 type (from sebum only) and the Δ9 type (from both sebum and epidermis) occur in total vernix

TABLE IV

Position Isomers of Fatty Acid Monoenes in Sterol Esters of Vernix Caseosa and Adult Human Skin Surface Lipids

Monoene carbon skeleton[a]	Mole % of total monoenes	Mole % at Δ positions indicated[b]																		
		Δ4	Δ5	Δ6	Δ7	Δ8	Δ9	Δ10	Δ11	Δ12	Δ13	Δ14	Δ15	Δ16	Δ17	Δ18	Δ19	Δ20	Δ21	Δ22	Δ23
Vernix caseosa																					
n-14	0.78			100																	
n-16	13.50			62	2	2	31	nd	3												
n-18	37.15		nd	2	nd	10	64	1	22	tr	1										
n-20	9.20			nd	nd	1	1	5	12	1	67	nd	13	nd	nd						
n-22	11.04			nd	nd	nd	nd	nd	nd	2	13	1	65	tr	19						
n-24	7.42								nd	tr	tr	7	17	tr	73	nd	3				
n-26[d]	1.09												nd	9	11	nd	74	nd	6		
n-28[d]	.34															11	5	nd	75	7	2
n-30[d]	.15																		pres	pres	maj
n-13	tr		NA																		
n-15	.87		3	84	8	1	4	nd													
n-17	2.12			25	3	14	45	3	10												
n-19	.66		tr	2	2	22	35	13	19	1	6	tr									
n-21	.44					nd	2	4	3	3	41	8	42								
n-23[d]	.31									nd	14	10	25	4	48						
n-25[d]	.12											nd	16	10	27	9	38				
n-27	tr																				
i-14	tr		NA																		
i-16	2.34		tr	100	tr																
i-18	3.48		nd	25	4	64	7														
i-20	3.24		tr	3	1	29	32	34	1												
i-22	1.40				nd	1	1	19	18	55	6										
i-24[d]	1.53								nd	8	11	79	5								
i-26[d]	.50											nd	14	80	6						
i-28	tr		NA																		
ai-15	tr		NA																		
ai-17	1.25		nd	100	tr	tr	tr														
ai-19	.41		nd	6	4	61	20	3	4												
ai-21	.37				nd	5	10	85	tr												
ai-23[d]	.16							nd	5	90	5										
ai-25[d]	.13											maj									
Adult human skin surface lipid																					
n-14	6.07	3	tr	97	tr																
n-16	47.11	nd	1	95	1	2	1	nd													
n-18	19.34		3	14	2	46	33	2	tr	tr	nd										
n-20	1.30		tr	4	5	7	2	45	21	7	9	nd	nd								
n-22	.47			2	4	3	7	4	tr	30	41	6	3	tr	tr						
n-24	.55				4	tr	3	3	3	2	tr	42	40	3	tr	nd					

n-26	.14						tr	1	6	nd	2	2	4	49	37	tr	nd
n-28	tr	NA															
n-15	3.83		tr	100	tr	nd	nd	nd									
n-17	3.26		tr	60	6	25	9	tr	tr								
n-19	.52			4	3	33	27	22	11	nd							
n-21	.06						tr	25	13	28	34	tr					
n-23	.06									17	14	41	28				
n-25	.06											22	15	31	32		
i-16	10.37	tr	1	98	1	tr	nd										
i-18	2.30		tr	30	2	68	tr										
i-20	.44		nd	tr	2	43	2	48	tr	tr							
i-22[d]	.11		*nd*	tr	tr	*tr*	*tr*	22	tr	78	tr						
i-24	.19						tr	2	tr	11	tr	87					
i-26	.17						nd	5	14	1	1	13	2	66	tr		
ai-15	1.13	*nd*	2	97	1	nd											
ai-17	2.18		tr	98	tr	2	tr										
ai-19	.19				pres	maj	pres										
ai-21	.06	NA															
ai-23	.03	NA															
ai-25[c]	.06							nd	3	2	4	79	3	6	3		

[a]See Table III.

[b]See Table III.

[c]See Table III.

[d]Structure determined by analysis of aldehydes only.

TABLE V

Double Bond Patterns of Wax Ester Fatty Acids in Vernix Caseosa Lipid and in Adult Human Skin Surface Lipid

	Vernix caseosa lipid patterns,[a] mole % of monoenes (no. molec. species[c])				Adult human skin surface lipid patterns[a] mole % of monoenes (no. molec. species[c])			
Pattern initiator[b]	Straight even	Straight odd	Iso	Anteiso	Straight even	Straight odd	Iso	Anteiso
12Δ6	.09(2)							
13Δ6		tr(2)						.02(1)
14Δ6	10.81(4)				9.73(4)			
15Δ6		7.58(2)		.65(2)		5.75(3)		2.30(1)
16Δ6	46.42(4)		8.87(2)		49.14(3)		22.45(3)	
17Δ6		3.74(2)		6.62(1)		1.55(2)		3.71(2)
18Δ6	1.70(2)		.57(2)		3.01(2)		.54(2)	
19Δ6						.01(1)		.06(1)
20Δ6	.02(1)						.01(1)	
Total	59.04	11.32	9.44	7.27	61.98	7.31	23.00	6.09
	Total Δ6 = 87.07 mole %[d]				Total Δ6 = 98.28 mole %[d]			
14Δ9	.94(3)							
15Δ9		.36(2)						
16Δ9	5.78(4)				.47(3)			
17Δ9		.34(2)				.03(2)		.02(1)
18Δ9	4.26(4)		.14(2)		.42(3)			
19Δ9		.20(2)		.02(2)		.15(2)		.02(1)
20Δ9	.01(3)		.14(2)		.17(3)		.03(2)	
21Δ9						.02(2)		.01(1)
22Δ9	.02(1)		.02(1)		.05(2)		.03(2)	
Total	11.01	.90	.30	.02	1.11	.20	.06	.05
	Total Δ9 = 12.23 mole %[d]				Total Δ9 = 1.42 mole %[d]			

[a]A "pattern" is here defined as an initial fatty chain substrate plus its extension or degradation products by C_2 units (see text).

[b]Chain length and double bond position of the substrate initiating the pattern (see text).

[c]The figures in ( ) are the number of molecular species in the pattern (see text).

[d]The difference between the sum of Δ6 + Δ9 patterns and 100.00% is the moles not analyzed.

caseosa lipid (14); and fetal sebum contains the 16Δ9 pattern (15). In the comedo, too, much evidence supports the conclusion that the sterol esters are derived from sebum as well as from epidermal fatty acids (16). If all the oleic acid present in the sterol ester fatty acids of vernix caseosa is derived from keratinizing epidermis, there still remains a very large portion (~46 mole %) of other Δ9 acids, and many of these, especially those of the 16Δ9 and 14Δ9 patterns as well as the iso and anteiso monoenoic acids, are most likely of sebum origin. It is noteworthy that rat surface lipid, also primarily of sebaceous gland origin, shows mainly the 16Δ9 pattern (5).

It is apparent from Tables III-VI that the straight even fatty acids of vernix caseosa sterol esters have been extended the maximal number of moles compared to the fatty acids of any of the other samples. Of these straight even acids, six times as many are extended in the Δ9 series as in the Δ6 series (Table VII). In both series the C_{16} substrate is maximally extended. These extensions are undoubtedly occurring in the sebaceous gland. A large amount of substrate $C_{18:\Delta 9}$ (23.80 mole %) is present, compared to the moles extended (3.94 mole %); but this is not so for the 16Δ9 series, i.e., 4.19 moles of substrate compared to 28.13 moles extension products. This implies that some oleic acid is in a pool not extended as are the other Δ9 chains. This pool is very likely the sterol esters of the keratinizing epidermis as discussed above. Chain extension to as many as 7 or 8 C_2 units is by no means common among lipids of internal tissues.

Wilkinson has published data on the double bond position of the fatty acids of sterol esters of adult human skin surface lipid (17). For the major chain lengths that he found, our data are in general agreement. We have, however, found a good many more components, both of a given chain length and for branched and other skeletal types occurring in small amounts. Downing and Green have published double bond position isomer data for all the fatty acids of vernix caseosa obtained by saponification of the entire lipid sample (14). They have remarked on the uniqueness of the 16Δ9 pattern, and in general

TABLE VI

Double Bond Patterns of Sterol Ester Fatty Acids in Vernix Caseosa Lipid and in Adult Human Skin Surface Lipid

	Vernix caseosa lipid patterns[a] mole % of monoenes (no. molec. species[c])				Adult human skin surface lipid patterns[a] mole % of monoenes (no. molec. species[c])			
Pattern initiator[b]	Straight even	Straight odd	Iso	Anteiso	Straight even	Straight odd	Iso	Anteiso
12Δ6	.02(3)							
13Δ6		.13(6)						
14Δ6	1.62(6)				7.36(7)			
15Δ6		1.17(6)		.01(2)		4.83(6)		1.15(3)
16Δ6	13.42(8)		8.01(6)		54.85(7)		12.30(6)	
17Δ6		.70(3)		2.09(5)		2.15(5)		2.38(3)
18Δ6	.82(3)		2.20(4)		2.83(5)		.94(6)	
19Δ6		.01(1)		.04(2)		.02(1)		tr (1)
20Δ6			.11(2)		.08(3)		.01(4)	
22Δ6					.01(3)		.01(1)	
Total	15.88	2.01	10.32	2.14	65.13	7.00	13.26	3.53
	Total Δ6 = 30.35 mole %				Total Δ6 = 88.92 mole %[d]			
14Δ9	4.37(7)							
15Δ9		.66(6)						
16Δ9	32.32(8)				.60(6)			
17Δ9		1.44(6)		.02(2)		.40(6)		.01(3)
18Δ9	28.01(8)		.46(6)		7.58(7)		.10(5)	
19Δ9		.39(6)		.09(4)		.37(6)		.02(4)
20Δ9	.09(2)		1.67(5)		.90(6)		.18(6)	
21Δ9		.02(2)		.05(3)		.02(3)		tr (3)
22Δ9			.04(2)		.70(5)		.02(5)	
23Δ9								tr (1)
24Δ9					.05(4)		.02(3)	
26Δ9					.02(2)			
	64.79	2.51	2.17	.18	9.85	.79	.32	.03
	Total Δ9 = 69.65 mole %				Total Δ9 = 10.99 mole %[d]			

[a]See Table V.
[b]See Table V.
[c]See Table V.
[d]See Table V.

their results are consistent with ours. Ansari et al. (15,5) found independently that the 16Δ9 pattern occurred in the monoenoic fatty acids of the alkane diol diesters of vernix caseosa. Since these diesters are sebum components they concluded that the 16Δ9 desaturase system was a sebaceous gland activity. Since Downing and Greene's sample included epidermal as well as sebaceous gland lipids, they were unable to determine the origin of the 16Δ9 pattern. Double bond positions of the wax ester fatty acids of either vernix caseosa or of adult human surface have hitherto not been reported, nor have they been reported for the sterol esters of vernix caseosa.

It is obvious from the foregoing that the sterol esters of vernix caseosa, which constitute ca. 25% of the lipid, are a very complex fraction. This complexity characterizes not only the fatty acid moieties but also the sterols themselves. For instance, Miettienen and Lukkäinen showed that vernix caseosa lipid contains at least eight additional sterols besides cholesterol (18). Of this group the esters of lanostenol (which constitutes less than 2% of the total) would be expected to migrate with the wax esters in our separation scheme, because models of this sterol show that it cannot present much flat surface to the absorbent, MgO, the basis of the separation (2). Although the Liebermann-Burchard test of the wax esters of vernix caseosa did give the yellow color characteristic of lanosterol, GLC of the total wax ester fraction showed extremely little material in the region of lanosterol palmitate or stearate (unpublished) so that contamination of the wax esters by lanosterol esters must be very slight.

The same authors (18) also examined the sterols of amniotic fluid, meconium, placenta and maternal serum, and showed that the sterols of vernix caseosa, amniotic fluid and meconium bore a much closer similarity to each other than they did to those of the placenta or

TABLE VII

Sterol Ester Fatty Acid Chain Extension Pattern of the Straight Even Acids of Vernix Caseosa

Carbon chain length	Substrate C_{14}:Δ6	Δ6 Extension patterns						Δ9 Extension patterns							
		Extension		Substrate C_{16}:Δ6	Extension		Substrate C_{18}:Δ6	Extension		Substrate C_{14}:Δ9	Extension		Substrate C_{16}:Δ9	Extension		Substrate C_{18}:Δ9	Extension	
		C_2 units	mole %		C_2 units	mole %		C_2 units	mole %		C_2 units	mole %		C_2 units	mole %		C_2 units	mole %
14	.78									not detected	1	.40	4.19				-1	.27
16		1	.27	8.37							2	.37		1	8.17	23.80		
18		2	.37		1	3.71	.73				3	1.21		2	6.16		1	1.10
20		3	.09		2	.46		1	.09		4	2.10		3	7.17		2	1.44
22		4	.11		3	.22					5	.22		4	5.42		3	1.26
24					4	.52		3	tr		6	.06		5	.81		4	.12
26					5	.10					7	.01		6	.25		5	.02
28					6	.04					8	tr		7	.15		6	tr
30					7	tr												
Total			.84			5.05			.09			4.37			28.13			3.94
		Total mole % Δ6 extended = 6.00[a]									Total mole % Δ9 extended = 36.44							

[a]Includes .02 mole % of C_{12}Δ6 pattern not tabulated.

maternal serum. Thus the similarity of the sterol patterns found in vernix caseosa, amniotic fluid and meconium could result from the fact that during intrauterine life the fetus drinks amniotic fluid into which skin cells and surface lipids have been released, but that these substances are not absorbed. However the authors also point out that the intestinal mucosa, being a tissue of ectodermal origin, may synthesize sterols that resemble those of the skin.

Sterol esters of high molecular weight fatty acids are also not well absorbed. This might be a reason as to why they are made, or, if others are made too, why only esters of high molecular weight acids remain in vernix caseosa. The high carbon content of the sterol esters undoubtedly helps to provide a waxy film of low water solubility (along with other sebum components). This prevents excessive wetting of the fetal skin. Esterification of the sterols of vernix caseosa might also provide a means for removing most of the free cholesterol resulting from epidermal lipid. This would help regulate the amount of free cholesterol that gets into the amniotic fluid.

In the case of adult human skin surface lipid, the sterol esters seem to be simply the product of a residual esterase activity set free by the lysosomal enzymes of dying, keratinizing epidermal cells. This implies that the sterol esters of both the adult human skin surface lipid or of vernix caseosa are secondary products. Free sterols are built up by the living epidermis and are then esterified primarily with sebum acids but also with some acids released from the epidermis in late stages of keratinization or after keratinization is complete.

Yardley (19) has suggested that sterol esters of essential fatty acids are necessary compounds for keratinization. We examined the dienoic fatty acids of adult human skin surface sterol esters briefly and found that, of all the acids esterified to sterols, the C_{18} dienes make up at most 1%. If linoleic acid constitutes the same proportion of the dienes of sterol esters as it does of all the dienes of adult human surface lipid (20), then it must make up only ca. 0.25%. Arachidonic acid, if present, would have appeared in the polar fraction of the acids of Table I. Since only a trace of total polar material was recovered in the sterol esters of adult human skin surface lipid, if any, only minute amounts of arachidonic acid must be present. Thus, if sterol esters of essential fatty acids are to be important for keratinization, they would have to be used in extremely small amounts. We are not aware of any evidence that this is so.

REFERENCES

1. Rothman, S., Arch. Derm. 62:814 (1950).
2. Nicolaides, N., J. Chromatogr. Sci. 8:717 (1970).
3. Nicolaides, N., and R.C. Foster, JAOCS 33:404 (1956).
4. Carroll, K.K., J. Lipid Res. 2:135 (1961).
5. Nicolaides, N., and M.N.A. Ansari, Lipids 3:403 (1968).
6. Nicolaides, N., Ibid. 6:901 (1971).
7. Miwa, T.K., K.L. Mikolajczak, F.R. Earle and I.A. Wolff, Anal. Chem. 32:1739 (1960).
8. Kärkkäinen, J., T. Nikkari, S. Ruponen and E. Haahti, J. Invest. Derm. 44:333 (1965).
9. Nicolaides, N., and T. Ray, JAOCS 42:702 (1965).
10. Kooyman, D.J., Arch. Derm. Syph. 25:444 (1932).
11. Nicolaides, N., JAOCS 42:691 (1965).
12. Nieminen, E., E. Leikola, M. Koljonen, U. Kiistala and K.K. Mustakallio Acta Dermatovener. 47:327 (1967).
13. Ansari, M.N.A., N. Nicolaides and H.C. Fu, Lipids 5:838 (1970).
14. Downing, D.T., and R.S. Greene, J. Invest. Derm. 50:380 (1968).
15. Ansari, M.N.A., H.C. Fu and N. Nicolaides, Lipids 5:279 (1970).
16. Nicolaides, N., M.N.A. Ansari, H.C. Fu and D.G. Lindsay, J. Invest. Derm. 54:487 (1970).
17. Wilkinson, D.I., Ibid. 53:34 (1969).
18. Miettienen, T.A., and T. Lukkäinen, Acta Chem. Scand. 22:2603 (1968).
19. Yardley, H.J., Brit. J. Derm. 81(Suppl. 2):29 (1969).
20. Nicolaides, N., and M.N.A. Ansari, Lipids 4:79 (1969).

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