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Cerumen Composition by Flash Pyrolysis-Gas Chromatography/Mass Spectrometry

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Objective: To assess the chemical composition of cerumen by flash pyrolysis-gas chromatography/mass spectrometry.
Study Design: Collected earwax specimens were fractionated into residue and supernatant by means of deoxycholate. This natural bile acid produces significantly better disintegration of earwax in vitro than do presently available ceruminolytic preparations, and also has demonstrated excellent clinical results in vivo to date.
Patients: The sample for analysis was obtained from a patient with clinical earwax impaction.
Results: The supernatant is composed of simple aromatic hydrocarbons, C_5-C_{17} straight-chain hydrocarbons, a complex mixture of compounds tentatively identified as diterpenoids, and steroids, in particular cholesterol. The residue, on the other hand, produced simple aromatic compounds (including benzenes, phenols, and benzonitriles), C_5-C_{25} straight-chain hydrocarbons, greater relative quantities of nitrogen compounds and phenol, and lesser importance of the (tentatively identified) diterpenoids.
Conclusions: Through the use of the detergent deoxycholate, squalene and a tentatively identified diterpenoid were revealed to be present in a free, unbound state, whereas some steroids and hydrocarbons appeared to be bound to a macromolecular structure by nitrogen linkages or other bonds. Additionally, this study reintroduces detergents as a viable method of earwax removal, specifically the bile acids.
Key Words: Cerumen, Ceruminolytic, Flash pyrolysis-Gas chromatography-Mass spectrometry.

Cerumen is produced by ceruminous glands, sebaceous glands, keratinocytes, and hair from the outer third of the human ear canal (1). Cerumen serves as a protectant from bacteria and fungus as well as a cleaning and lubricating agent for the external auditory canal. However, ceruminous impaction can cause itching, pain, hearing loss, perforated tympanum, tinnitus, dizziness, and increased risk of infection (2-4). Indeed, 150,000 cerumen removals are performed weekly in the United States because of such otologic complications (2).

Upon a literature search, little mention is found of the use of detergents in dissolving earwax. Nevertheless, some detergents disintegrate earwax significantly better than equal aliquots of water or triethanolamine polypeptide oleate-condensate (Cerumenex) (Burkhart CN, Burkhart CG, unpublished data). We have been most impressed with the in vitro results with detergents with a steroid skeleton structure, such as primary and secondary bile acids. More specifically, bile acids in the appropriate mixture offer significantly better disintegration of earwax in vitro than do presently available ceruminolytic preparations. Moreover, these bile acids have demonstrated significant clinical applicability in patients with ceruminous plugs. In this presentation, an analysis of earwax fractionated by centrifugation into residue and supernatant by means of one of these bile acids is assessed.

The composition of earwax has been assessed by several techniques. By gas chromatography and mass spectrometry, long-chain fatty acids, alcohols, cholesterol precursors, squalene, and other long-chain hydrocarbons have been identified (5-7). A technique combining gas chromatography and mass spectrometry revealed saturated and unsaturated long-chain fatty acids, alcohols, squalene, and cholesterol (8). The amino acid composition of earwax with hair and epidermal cells included has also been assessed, but not quantitated (9). The current study of earwax composition used the more refined technique of flash Py-GC/MS to establish the chemical composition of earwax.

Recent studies have described the application of Py- GC/MS to the investigation of the molecular composition of the louse sheath (10), chitin in fresh and fossil invertebrate cuticles (11), and proteinaceous moieties in biologic materials (12). With a minute quantity of studied material, this method permits rapid determination of the chemical constituents of insoluble materials and biopolymers in various biologic tissues.

MATERIALS AND METHODS

A human earwax specimen from a patient with cerumen impaction was centrifuged in a solution of deoxycholate, NaCl, and Na_2HPO_4. The resulting supernatant and residue were freeze-dried and shipped to the SIU Organic Geochemistry Laboratory for molecular characterization. Upon receipt, the two samples were rinsed three times with fresh, high-performance liquid chromatography-grade dichloromethane to remove solvent-extractable organic matter, including substances that might have been added during the initial preparation of the specimen.

The dried, solvent-rinsed samples were subjected to analytical Py-GC/MS, using a CDS 120 Pyroprobe, coupled to an HP 5890 gas chromatograph with an HP 5970 mass selective detector and a 50 m HP-1 column (0.2 mm internal diameter, film thickness 0.33 μm). Small, measured amounts (0.35 mg of the supernatant and 0.42 mg of the residue) underwent pyrolysis in a flow of helium for 20 seconds within a platinum coil at 610°C, as measured by a thermocouple in the sample holder. The gas chromatography oven was operated under the following program: isothermal for 5 minutes at 40°C; temperature programmed at 5°C/minute to 300°C and then isothermal for 20 minutes. The mass spectrometer was run in full scan mode, across a mass range of 50 to 450 Da. Identification of the 152 principal chromatographic peaks was made by evaluation of the mass spectral and retention time data, with comparisons with the results of previous analyses in this laboratory and with a computerized library of about 250,000 mass spectra. The relative quantities of the principal compounds were estimated by measuring their peak areas on the total ion chromatograms. The results (Table 1) are expressed relative to the summed area of all detected peaks (about 400 in each case) on the respective total ion chromatograms, in parts per thousand (%). These values are not absolute quantities. They are presented as a convenience, for better appreciation of the contribution of each compound to the complex mixtures produced upon pyrolysis of the samples.

RESULTS

Dissolution of the wax occurred quickly, as visualized by a yellowish color of the deoxycholate solution, swelling, and floating of keratin. After 2 to 5 hours, approximately half of the earwax was dissolved. Microscopic examination of the suspension revealed only floating dead cells and no other particles in the yellow solution. The Py-GC/MS data are summarized in graphic form on the total ion chromatograms of the supernatant and residue samples (Fig. 1). The essential mass spectral data for the 152 principal compounds, the

compound identifications (where possible), and the relative quantities are presented in Table 1. Peak numbers are denoted by square brackets in the text.

Aliphatic compounds

Hydrocarbons are very important compounds in the pyrolysates of both samples. Straight-chain (normal) hydrocarbons (the *n*-alk-1-ene and *n*-alkane series, the peak numbers of which are underlined in the figures) are prominent in the 20- to 40-minute retention time range (Fig. lA). These are normal hydrocarbons with chains from 10 to 17 carbon atoms long, with the C_{11}, C_{12} , C_{14} , C_{15}, and C_{17} alkenes [30, 41, 59, 62, 70] particularly strong. The normal hydrocarbons are even more important in the residue pyrolyzate. In addition to the hydrocarbons noted in the supernatant pyrolyzate, the residue produced longer chain compounds, up to C25, with the C22-C24 n-alkanes and n-alkenes [112, 114, 121, 123, 132] especially noteworthy (Fig. 1b).

All *n*-alk-1-enes produce a strong mass fragment of 55 Da and *n*-alkanes produce one at 57 Da. Chromatograms displaying only the sum of these two masses provide a clear, convenient way of displaying the normal hydrocarbon distributions (Fig. 2), without the interference of the other types of compounds present. The significant differences between the samples are readily apparent on these mass 55+57 traces. The longer chain hydrocarbons (C_{18}-C_{25}) are prominent in the residue pyrolyzate (Fig. 2b), but barely detectable at all in the supernatant pyrolyzate (Fig. 2a). The high relative abundance of *n*-alkanes and *n*-alkenes indicates the earwax has a strongly paraffinic character, especially the residual fraction. The term "wax" is therefore very appropriate.

Interestingly, the aliphatic character of the residue is further underscored by the presence of several long-chain nitrogenous compounds. The C_{16}- and C_{18}-alkylnitriles [80, 99] are like normal alkanes, except that the carbon at one end of the chain is bound to a nitrogen (-CN). Similarly, the two alkylamides [108, 127], tentatively identified also as the C_{16} and C_{18}, are long hydrocarbon chains terminated at one end with a (-$CONH_2$) group (Fig. 2b).

Aromatic compounds.

Simple monoaromatic compounds [9, 18, 22, 23] dominate the lower molecular weight fraction of the supernatant pyrolyzate, i.e., the left side of Fig. 1a. Several two-ring aromatic hydrocarbons are also important, including indenes [38, 58] and possible biphenyl derivatives [73, 77]. Toluene and dimethylbenzene [9, 18] are also prominent on the residue's pyrogram (Fig. 1b). In contrast to the supernatant, however, the residue produced relatively much more phenols [especially 21, 29], pyrrole [7], benzenenitriles [31, 42] and indoles [48, 57], indicating significant structural differences between the two samples.

Nitrogen compounds.

Dipeptides are conspicuously absent from both pyrolyzates. Such compounds, which consist of two linked amino acids, are common fragments generated by protein pyrolysis. This indicates that skin proteins, such as keratin and collagen, are not major constituents of the cerumen specimen. Nitrogenous structures are nevertheless important in the residue (but not in the supernatant) and may indicate the presence of amino acids in some form, in the wax itself or perhaps in foreign matter or parasitic microbes. (Small hairs were noted during the rinsing of the residue, but every effort was taken to avoid them when packing the material into the sample holder prior to analysis.) The above-noted pyrroles may be produced from the pyrolysis of the amino acid proline, the benzonitriles (and perhaps, in part, the benzenes and styrene) from phenylalanine, and the indole from tryptophan. In fact the phenols, although they do not contain nitrogen themselves, may have been produced by the pyrolysis

of the amino acid tyrosine. The presence of the alkylnitriles and alkylamides is also intriguing. It may imply that at least some of the long hydrocarbon chains are bound into the macromolecular structure by nitrogen linkages.

Diterpenoids (tentatively identified).

A major feature of the supernatant's pyrogram is a pronounced hump in the 48-61 minute range (Fig. 1a). This is due to a cluster of perhaps a hundred or more compounds which were too similar for the chromatograph to separate completely. These compounds are also present in the pyrolyzate of the residue, but are relatively much less abundant (Fig. 1b). Examination of the mass spectra of the strongest peaks (compounds in the [87-144] range, Table 1) further confirms their chemical similarity. For example, they each produced mass fragments of 91 and 105 Da, as seen in the mass spectra of two typical examples (Fig. 3). The mass 91 chromatogram (Fig. 4b) closely resembles the total ion chromatogram (Fig. 4a) over the 48-61 min. range, indicating that a large majority of the compounds in that portion of the supernatant pyrolyzate belong to same chemical "family". The mass spectra of practically all of these compounds have molecular weights of either 254, 256, 282, 284 or 310 Da (Table 1, Figs 3a-b, 4c-g). A compound of mass 254 could be derived from one of mass 256 by simply converting a (C-C) single bond to a double (i.e., by losing 2 hydrogen atoms). Masses 254, 282 and 310 differ by 28 Da, as do masses 256 and 284. This could be accounted for by adding or subtracting ethyl groups ($-CH_2-CH_2-$). The precise identity of these compounds is unknown, but it is apparent that are all closely related structurally, being minor variations on the same theme.

The mass spectrum of the monocyclic diterpenoid retinol (Vitamin A, Fig. 3c) bears a strong resemblance to those of the compounds in question (for example, compounds [95] and [117], Fig. 3a-b). A molecule of β-carotene might be described as two linked retinol molecules, without the (-OH) groups. The low mass end of its spectrum also resembles those of the compounds in question (Fig. 3d). This line of evidence suggests that these compounds are diterpenoids, perhaps derived from the pyrolysis of one or more carotenoid precursors. The simple benzenes [9, 18, 22, 23] so abundant in the supernatant pyrolyzate could also derive, at least in part, from pyrolytic cleavage and aromatization of the rings at the end of the carotene molecule. Carotenoid compounds are common plant pigments, imparting characteristic colors to carrots and to autumn foliage, for example. Such a compound may be responsible for the yellowish color of the supernatant. Interestingly, the residue was not yellow and its pyrolyzate had relatively much less of the diterpenoids (Fig. 1).

Squalene.

The supernatant was also pyrolyzed in its as-received, unrinsed state. The resulting pyrogram was very similar that produced after solvent rinsing, except that the strongest peak (by far) was due to squalene. Squalene [146] is a linear triterpenoid hydrocarbon and resembles β-carotene (Fig. 3d), but with a slightly longer chain and without the rings. Like the carotenoids, squalene is common in nature and is a known component of cerumen. Traces of squalene are still present in the pyrolyzate of the rinsed supernatant and residue (Fig. 1), suggesting that the solvent extraction was not 100% effective in removing this very abundant compound.

Steroids.

Cholesterol [152] and several other less abundant, unidentified steroids [147, 148, 151] are a significant feature of the supernatant pyrolyzate (Fig. 1a). Deoxycholate was added to the original sample prior to centrifugation, thus the possibility of steroid contamination must be considered. However, there was essentially no qualitative or

quantitative difference in the steroids detected in the pyrolyzates of the "as-received" and solvent-rinsed supernatant. This suggests that the steroids detected are in some way bound to the wax structure, rather than being present as free compounds (like squalene). This would be all the more likely if the centrifuged fractions had been rinsed prior to freeze-drying, permitting the removal of the cholate ions. These steroids are relatively less abundant in the residue pyrolyzate, although two other possible steroids [149, 150] are somewhat more prominent (Fig. 1b).

DISCUSSION

This study reintroduces detergents as a viable method of earwax removal. We have been most impressed by the in vitro results with detergents with a steroid skeleton structure, such as primary and secondary bile acids. These acids, which include cholate and deoxycholate, are nontoxic surfactants found naturally in the gastrointestinal tract. To better disperse earwax, these bile acids are placed in alkaline solution provided by sodium phosphate in the presence of 0.1 M sodium chloride. Cholate and deoxycholate attach themselves along hydrophobic areas, exposing its hydrophilic tail, which pulls hydrophobic particles into solution. These bile acids dissolve lipids by forming micelles. Additionally, the acids surround hydrophobic parts of membrane-bound protein and move them into solution. Sodium chloride was added to insure a large aggregation number and small critical micellization concentration for the bile acids. The concentration of cations significantly affects anionic detergents like cholate and deoxycholate. These bile acids in vitro quickly penetrate earwax, as visualized by the yellowish color of the solution and the lack of cohesion of the remaining particulate matter. Given the visible discernible disintegration of earwax within 5 minutes with cholate or deoxycholate, approval for clinical human use was sought and granted. Our initial clinical studies in patients with ceruminous plugs have produced excellent dissolution of earwax in vivo, with no ill effects to the ear membrane after a 5-minute application of the bile acids. In this presentation, an analysis of earwax by pyrolysis-gas chromatography/mass spectrometry fractionated by centrifugation into residue and supernatant achieved by bile acids has revealed interesting results.

This analysis of earwax by Py-GC/MS revealed the supernatant to be composed of simple aromatic hydrocarbons, C_5-C_{17} straight-chain hydrocarbons, squalene, a complex mixture of compounds tentatively identified as diterpenoids, and steroids, in particular cholesterol. The residue, on the other hand, produced simple aromatic compounds (including benzenes, phenols, and 'benzonitriles), C_5-C_{25} straight-chain hydrocarbons, greater relative quantities of nitrogen compounds and phenol, and lesser importance of the (tentatively identified) diterpenoids.

The absence of dipeptides indicates that skin proteins, such as keratin from epidermal cells, were avoided in our analysis. As noted above, we attempted to remove all hair follicles from the cerumen during preparation of the studied sample, and exclude any follicular component of earwax from our analysis. Nitrogenous structures are nevertheless important in the residue, and they may indicate the presence of amino acids, in some form, in the wax itself or perhaps in foreign matter or parasitic microbes. The pyrroles may be produced from the pyrolysis of the amino acid proline, the benzonitriles (and perhaps, in part, the benzenes and styrene) from phenylalanine, the indole from tryptophan, and the phenols from tyrosine (although phenols do not contain nitrogen themselves).

The possible presence of carotenoids in earwax is interesting but not unexpected. Retinoids are secreted by other glands, such as the lacrimal glands of rabbits (13). Additionally, oral administration of high doses of the aromatic retinoid etretinate to dogs results in epidermal hyperplasia and increased ceruminous gland activity, which are both

related to ceruminous plug formation (14). Thus, in addition to their possible presence in earwax, carotenoids may be involved in the pathogenesis of ceruminous impaction.

Almost all but the very high molecular weight hydrocarbons and nitrogen-containing compounds were at least partially suspended in the deoxycholate solution. Because only a finite number of micelles can form from the limited amount of deoxycholate in solution, further rinses may allow more of the residue to be suspended. Nevertheless, alternative detergents may be needed to suspend the longer-chain fatty acids, and bonds may need to be hydrolyzed to suspend the nitrogen containing hydrocarbons and bound steroids.

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TABLE 1. Pyrolysis-gas chromatography/mass spectrometry of earwax supernatant and residue, after dichloromethane rinse.

Peak	Retention	Compound (if known)	Characteristic mass spectral peaks						supernat.	resid.
	Time (min)		M+	base	m/z	m/z	m/z	m/z	rel. ‰	rel. ‰
1	4.33	pentene	70	55					1.3	
2	6.98	methylcyclopentadiene	80	79					5.8	
3	7.37	hexadiene	82	67					4.2	
4	7.43	benzene	78	78					1.5	
5	8.78	n-hept-1-ene	98	56	70				3.3	
6	10.03	pyridine	79	79					1.7	
7	10.51	pyrrole	67	67						1.1
8	10.62	methylcyclohexane	96	81					4.0	
9	11.45	toluene	92	91					16.3	24.0
10	11.57	cyclopentadiene, dimethyl	94	79					5.0	
11	11.93	cyclohexene, methyl	96	81	67				1.4	
12	12.92	n-oct-1-ene	112	55	70				4.5	
13	13.97	methylpyrrole	81	80						2.9
14	15.51	ethylbenzene	106	91					1.9	2.3
15	15.89	1,3- & 1,4-dimethylbenzenes	106	91					3.2	
16	16.39	cyclohexene, dimethyl	110	67	81	95			3.4	
17	16.62	styrene	104	104	78				2.6	2.8
18	16.84	1,2-dimethylbenzene	106	91					10.2	5.6
19	17.20	n-non-1-ene	126	56	55	69			2.2	2.6
20	17.67	n-nonane	128	57	85				1.2	
21	19.94	phenol	94	94					2.9	11.8
22	20.38	1-ethyl-2-methylbenzene	120	105					4.4	1.6
23	20.91	styrene, methyl	118	117					4.2	3.2
24	21.00	1,2,4-trimethylbenzene	120	105					1.7	
25	21.18	dec-1-ene	140	55	70				3.3	3.7
26	21.62	decane		57	71				1.8	
27	22.70	indene	116	115					2.9	
28	22.71	2-methylphenol	108	108	107					4.1
29	23.42	4- & 3-methylphenols	108	107					4.3	10.1
30	24.84	undec-1-ene	154	55	70				3.0	4.6
31	25.01	benzeneacetonitrile	117	117	90	116				4.2
32	25.23	undecane	156	57	71				3.2	2.3
33	26.02	2,4-dimethylphenol	122	107					2.4	2.5
34	26.10	C5-benzene	148	91					1.5	
35	26.50	indene, methyl	130	115					2.2	
36	26.59	4-ethylphenol	122	107					3.2	2.4
37	26.68	indene, methyl	130	115					4.5	
38	26.99	indene, methyl	130	115					3.7	
39	27.62	naphthalene	128	128					1.3	
40	27.67	decan-2(?)-one	156	58	71				1.3	
41	28.21	dodec-1-ene		55	69				4.7	7.4
42	28.37	benzenepropanenitrile	131	91						2.5
43	28.57	dodecane	170	57	71				2.8	1.6
44	28.97	C3-phenol	136	121					2.0	1.2
45	29.92	C3-styrene isomer	146	131	91				3.5	
46	30.21	C2-indene	144	129					3.3	
47	30.36	C2-indene	144	129					2.8	
48	30.46	indole	117	90					3.1	9.9
49	31.01	C3-phenol	136	121					1.6	
50	31.20	2-methylnaphthalene	142	142					1.3	
51	31.20	C2-indene	144	129					1.2	

TABLE 1. (contd.)

Peak	Retention	Compound (if known)	Characteristic mass spectral peaks>>>>>						supernat.	resid.
	Time (min)		M+	base	m/z	m/z	m/z	m/z	rel. ‰	rel. ‰
52	31.34	tridec-1-ene		55	69				2.4	3.9
53	31.67	tridecane		57	71				2.3	3.9
54	31.67	1-methylnaphthalene	142	142					2.1	
55	32.82		158	98	111	125				5.5
56	33.07	C14-alkadiene?		68	81	55			1.9	
57	33.33	methylindole	131	130						3.6
58	33.87	C3-indene	158	143	128	115			3.8	
59	34.27	tetradec-1-ene		55	69				5.3	6.4
60	34.56	tetradecane		57	71				2.4	3.2
61	35.03	1,7 or 1,6 dimethylnaphthalene	156	156	141				3.4	
62	37.02	pentadec-1-ene		55	69				5.6	5.7
63	37.29	pentadecane		57	71				2.4	2.6
64	39.48	C3-naphthalene	170	155					1.3	
65	39.60	hexadec-1-ene		55	69				3.6	4.4
66	39.84	hexadecane		57	71					2.4
67	41.34	unspecified alkene		55	69				2.3	
68	41.73	sim. perhydronaphthofuran	216	201						5.6
69	41.84		214	199	160				3.6	2.7
70	42.05	heptadecene		55	69				7.2	6.4
71	42.26	heptadecane		57	71					6.9
72	42.28	phenol, octyl?	206	107					4.6	
73	42.52	biphenyl deriv.?	212	197	158				3.6	
74	43.20	biphenyl, dimethyl	182	167					3.4	
75	44.35	octadecene		55	69				1.5	3.1
76	44.49	octadecane		57	71				2.1	2.2
77	44.65	phenanthrene, dihydro,methyl ?	194	179	178				3.4	
		OR benzene,methylphenylethenyl?								
78	45.78	bicyclic?? C15H20O2??	232	232	91	199	214		2.7	
79	45.90	sim. to [77]	194	179					1.8	
80	46.19	hexadecanenitrile		55	70	97	110			4.5
81	46.20	methoxyfluorene? (+??)	196	196	181	152			3.7	
82	46.31	Dipyrrolo pyrazine dione,	194	70						7.9
		octahydro-? (C10H14N2O2)?								
83	46.53	nonadecene		55	69					3.0
84	46.73	nonadecane		57	71					3.4
85	47.58	cycloalkane?		55	69	83	197		4.0	
86	47.62	alkylpyridine?		107	120	134				3.0
		OR phenylalkylpyridine?								
87	48.33	diterpenoid?	240	240	225	130	91		3.9	
88	48.62	eicosene		55	69					5.7
89	48.64	diterpenoid?	256	256	91	79	241		5.0	
90	48.80	eicosane		57	71					3.0
91	48.98	diterpenoid?	256	91	79	147	240	105	4.2	
92	49.07	diterpenoid?	256	256	91	105	241	105	6.1	
93	49.18	diterpenoid?	256	256	91	79	241	105	8.1	3.3
94	49.33	diterpenoid?	254	254	91	131	146	239	5.8	
95	49.44	diterpenoid?	256	256	91	241	79	105	9.7	
96	49.77	diterpenoid?	254	254	91	144	131	105	12.6	4.9
97	49.98	diterpenoid?	256	256	91	201	79	105	13.9	
98	50.09	diterpenoid?	254	254	91	129	199	105	7.8	
99	50.43	octadecanenitrile		57	97	110				5.2
100	50.48	diterpenoid?	256	91	79	105	131	201	10.0	
101	50.61	heneicosene		55	69					4.8

TABLE 1. (contd.)

Peak	Retention	Compound (if known)	Characteristic mass spectral peaks>>>>>						supernat.	resid.
	Time (min)		M+	base	m/z	m/z	m/z	m/z	rel. ‰	rel. ‰
102	50.63	diterpenoid?	256	91	105	79	131	147	5.9	
103	50.78	heneicosane		57	71					3.6
104	50.83	diterpenoid?	254	254	91	105	79	131	5.7	
105	51.08	diterpenoid?	252	144	91	254	105	128	7.4	
106	51.37	diterpenoid?	284	255	91	105	161		15.3	3.5
107	51.43	diterpenoid?	284	284	255	91	79	105	8.9	
108	51.64	hexadecanamide		59	72					6.8
109	51.96	diterpenoid?	284	284	255	91	79	105	10.0	
110	52.08	diterpenoid?	284	218	91	79	105	255	9.0	
111	52.36	diterpenoid?	284	91	105	255			7.1	
112	52.50	docosene		55	69					5.1
113	52.65	diterpenoid?	284	284	255	91	79	105	13.5	
114	52.65	docosane		57	71					7.0
115	52.76	diterpenoid?	282	282	91	105	253	267	6.5	
116	52.95	diterpenoid?	296	91	282	105	79	253	5.5	
117	53.17	diterpenoid?	284	284	255	91	79	105	20.4	
118	53.40	diterpenoid?	284	91	79	105	255		11.1	
119	53.54	diterpenoid?		91	105				14.9	
120	53.99	alkene or alkanol		55	69	83	97			5.5
121	54.31	tricosene		55	69					12.2
122	54.45	diterpenoid?	282	282	91	105	174	267	7.7	
123	54.46	tricosane		57	71					4.8
124	54.76	diterpenoid?	300	231	121	91	282		14.2	6.6
125	54.94	diterpenoid?	296	282	91	105	267		14.5	
126	55.33	diterpenoid?	282	282	91	105	231		7.0	
127	55.38	octadecanamide		59	72					10.7
128	55.42	diterpenoid?	282	282	91	231	121	267	9.8	
129	55.60		326	326	325				9.7	
130	55.90	diterpenoid?	282	282	91	105	255	267	9.5	
131	56.03	diterpenoid?	296	91	105	255			4.7	
132	56.05	tetracosene		55	69					7.2
133	56.08	diterpenoid?	296	91	231	105			6.1	
134	56.17	tetracosane		57	71					4.1
135	56.45	diterpenoid?	300	271	91	230			8.8	
136	56.68	Methyl phenyl acetamido-tetrahydroisoquinoline??	280	222	178	194	280		3.8	
137	57.73	pentacosene		55	69					2.9
138	57.85	pentacosane		57	71					2.0
139	58.09	diterpenoid?	310	295	91	105			4.3	2.5
140	58.64	diterpenoid?	310	295	91	105			3.7	
141	59.09	diterpenoid?	310	295	91	105			1.7	
142	59.62	steroid? (sim. cholene)	328	328	231	255	161		3.1	
143	60.04	steroid?	328	328	199	255			2.7	
144	60.29	diterpenoid?	312	312	297	255	91			2.8
145	61.57	sim. to squalene		69	81					3.2
146	64.24	squalene (C30H50)		69	81				1.7	3.7
147	67.26	steroid (sim cholestadiene)	368	368	147	260			5.7	6.7
148	71.26	steroid	356	255	341	302			3.4	3.1
149	71.46	steroid?		337	322	255				8.7
150	72.13	steroid?		283	255					8.0
151	72.78	steroid	356	301	314	255	159		2.5	
152	73.98	cholesterol	386	386	301	275	253		20.9	

FIG. 1. Total ion current chromatograms of the supernatant and residue pyrolyzates. See Table 1 for peak identification.

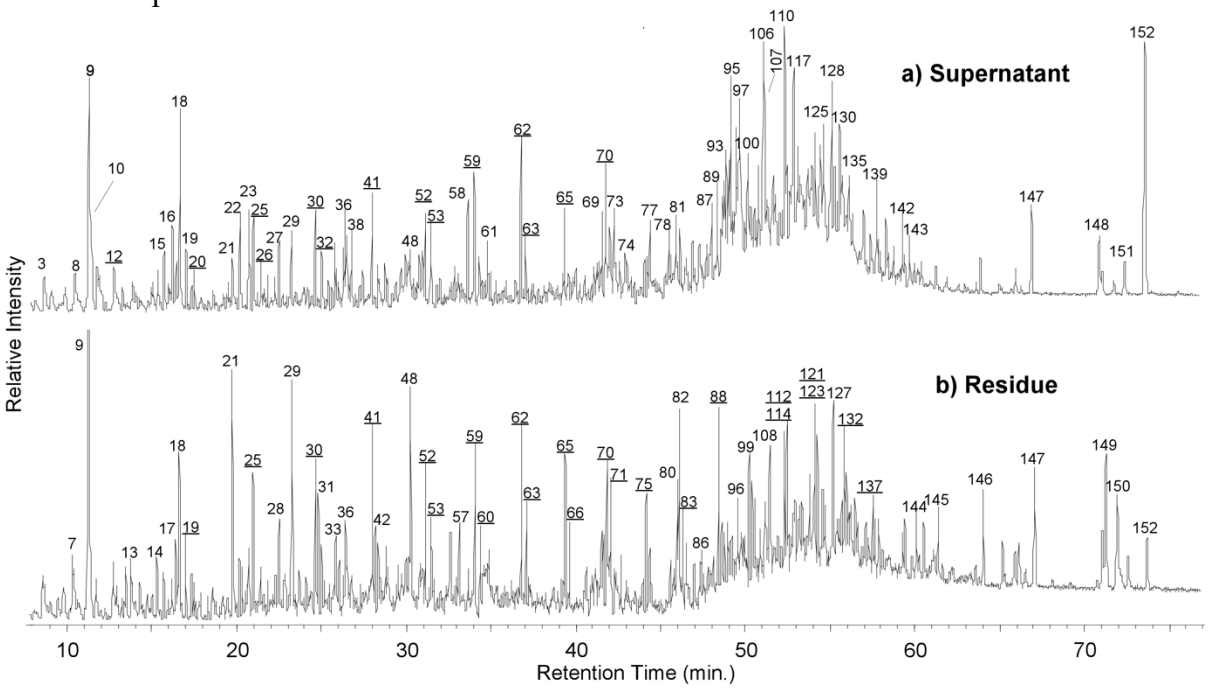

FIG. 2. Mass chromatograms (m/z 55 + 57) for the supernatant and residue pyrolyzates. See Table 1 for peak numbers.

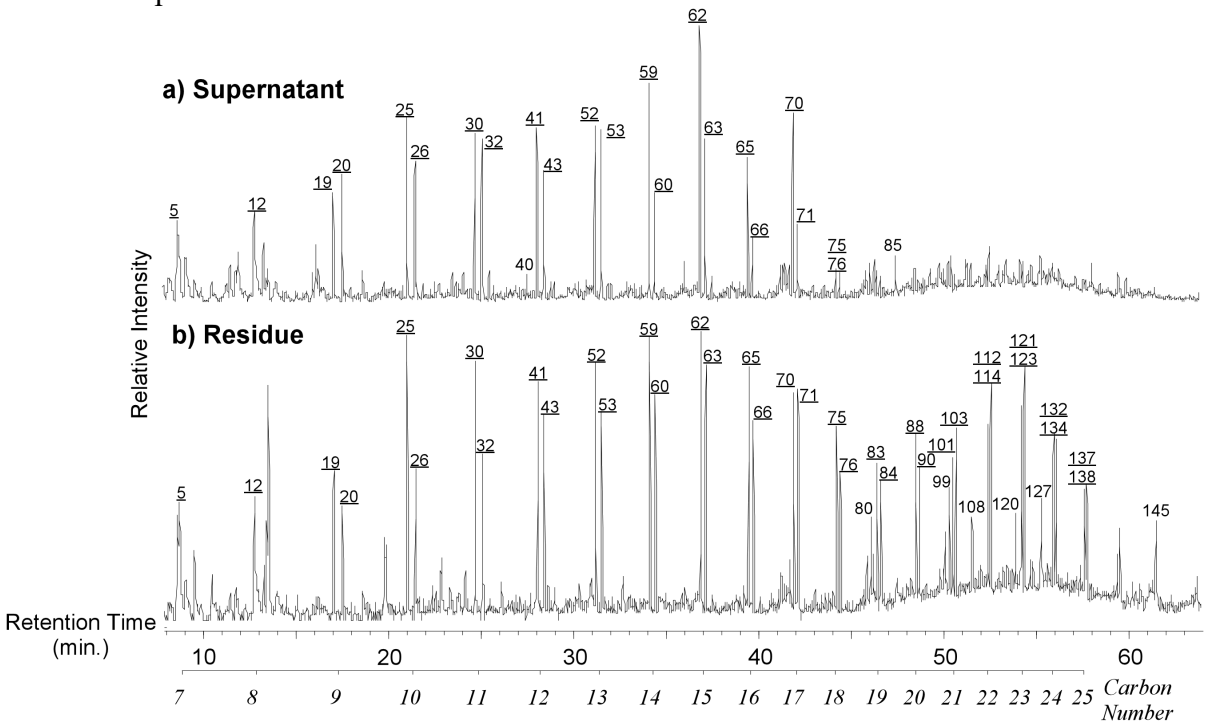

Figure 3. Selected mass spectra.

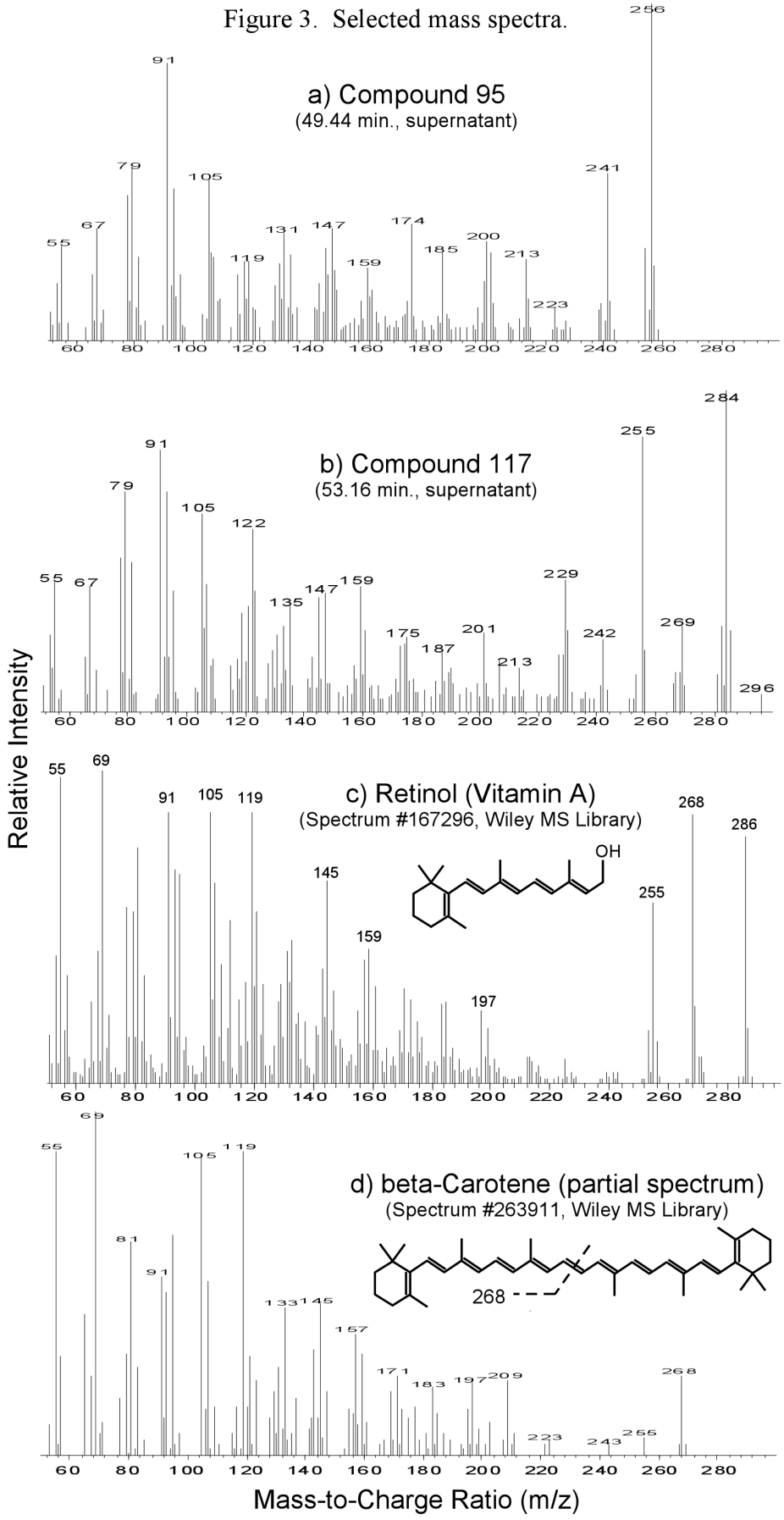

FIG. 4. Total ion current and individual mass chromatograms showing suspected diterpenoid compounds in the supernatant pyrolyzate.