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ANALYSIS OF CHARACTERISTIC HUMAN FEMALE AXILLARY ODORS: QUALITATIVE COMPARISON TO MALES

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Abstract—Odors produced in the human female axillae are of both biological and commercial importance. Several studies have suggested that extracts from female underarm secretions can alter the length and timing of the female menstrual cycle. In addition, more than 1.6 billion dollars are spent annually on products to eliminate or mask the axillary odors. Our recent studies have determined that the characteristic axillary odors in males consist of C_6–C_{11}, saturated, unsaturated and branched acids, with (*E*)-3-methyl-2-hexenoic acid (3M2H) being the major compound in this mixture. The 3M2H appears to be carried to the skin surface bound to two proteins in the axillary secretions. Data reported here show that the same mixture of odorous compounds is found in female axillary secretions, with several minor qualitative differences. Separation of the female apocrine secretions into aqueous and organic soluble fractions demonstrated that 3M2H, and several other members of the acids in the characteristic odor, are released by hydrolysis with base. Electrophoretic separation of the proteins found in the aqueous phase of female apocrine secretions revealed a pattern identical to that seen in males. The qualitative similarity of the acidic constituents making up the characteristic axillary odors of both females and males as well as the proteins present in the aqueous phase suggest a similar origin for axillary odors in both sexes.

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Key Words—Axillary odors, human females, 3-methyl-2-hexenoic acid, androstenol.

INTRODUCTION

Odors produced in the human axillae are of both biological and commercial importance. For example, studies performed in several laboratories have suggested that extracts made from human axillary secretions can alter the length and timing of the human menstrual cycle (Russell et al., 1980; Preti et al., 1986; Cutler et al.; 1986; Stern and McClintock, 1993). In addition, in the United States alone, more than 1.6 billion dollars are spent annually on products to eliminate, mask, or prevent the formation of axillary odors (Ramirez, 1990).

Prior to our studies, investigators examining the nature of axillary odors emphasized the steroid biochemistry occurring in the underarm (Labows, 1988; Gower and Ruparelia, 1993). Radioimmunoassay and gas chromatography–mass spectrometry techniques have been used to identify and trace the origins of volatile, odoriferous steroids in the underarm, such as 5α-androst-16-en-3α-ol (androstenol) and 5α-androst-16-en-3-one (androstenone). The musky, urine-like odors were thought to resemble the odor produced in the axillae (Gower et al., 1994). However, recent studies that employed organoleptic evaluation of gas chromatographic eluants and other analytical techniques, particularly GC-MS, to examine the axillary odor from a combined group of males found that the characteristic odors consist of C_6–C_{11}, saturated, branched, and unsaturated acids, with (*E*)-3-methyl-2-hexenoic acid (*E*-3M2H) being the major component of this mixture (Zeng et al., 1991). The *Z* isomer is also present at about 1/10 the level of the *E* isomer. In terms of relative abundance, these acids, particularly *E*- and *Z*-3M2H are present in far greater quantity than those odoriferous steroids within the acid fraction of the combined male samples; the neutral and basic compounds in these male extracts had little to no odor (Zeng et al., 1991, 1992). Both *E*-3M2H and *Z*-3M2H, as well as a number of other axillary odor components, appear to be carried to the axillary skin surface bound to water-soluble proteins found in apocrine secretions (Zeng et al., 1992; Spielman et al., 1995).

Female axillary secretions and odors have been examined organoleptically and microbiologically (Leyden et al., 1981). These studies suggest that males and females with the same axillary microflora will produce qualitatively similar axillary odors. A greater number of males appear to have the *Corynebacteria* as axillary residents; these bacteria are associated with the strongest axillary odors (Leyden et al., 1981). However, psychophysical studies suggest that the assignment of gender based upon axillary odor is most likely done by intensity and not by overt qualitative differences (Russell, 1976; Doty, 1981).

Studies have examined the volatile steroids present in the female axillary

extracts and made quantitative comparisons in the levels of these to males (Gower et al., 1985; Preti et al., 1987). Our earlier investigation of axillary extracts also suggested that at least one volatile steroid, androstenol, appears to be present in greater concentration during the follicular phase of the menstrual cycle than at any other time (Preti et al., 1987). However, no analyses of secretions from the female axillae have been done to determine whether females contain the same array of acidic compounds in their characteristic odor components. Results of analyses described below demonstrate that both males and females are very similar, although some qualitative distinctions are present.

METHODS AND MATERIALS

Subjects and Collection of Axillary Secretions. Six healthy female donors (ages 24–34; mean = 28.70) were employed to collect the axillary secretions used in this study. The axillae of each subject was sampled to determine its bacterial microflora prior to secretion collection (Leyden et al., 1981). Each subject was found to have lipophilic diphtheroids as normal axillary residents. The latter have been associated with the production of the stronger axillary odors (Leyden et al., 1981). Secretions were collected using 4-in. × 4-in. cotton pads worn in the axillae three times a week as in our previous studies (Preti et al., 1986; Zeng et al., 1991). Briefly, this protocol requires subjects to go without the use of deodorants, antiperspirants, deodorant soaps, or colognes in the axillae. They may wash once a day, only in the morning, with a nonfragranced soap (Ivory) during the collection protocol.

Preparation of Axillary Secretion Extract. A total of 24 pads were extracted to yield the axillary extract. Four pads from each subject were chosen. Extracts were prepared by extracting 12 pads at a time (two per subject) with double-distilled ethanol, as previously described (Cutler et al., 1986; Preti et al., 1987). After removal of the ethanol, pads were subsequently soaked with a mixture of 85:15 chloroform–methanol (15 ml/pad) for 1 hr and then squeezed. (Nanograde solvents were from Mallinkrodt, St. Louis, Missouri; these solvents contain few or no impurities by GC-MS analysis when 100–200 ml is concentrated to ≤ 50 μl.) The two 12-pad extracts yielded 300 ml and 310 ml total volumes, respectively. The two extracts were combined; 1 ml of saturated $NaHCO_3$ was added to this volume, and the extract was concentrated under vacuum to dryness. A 1.5-ml aliquot of doubly distilled water was employed to wash the flask and dissolve all dried solids. This aqueous solution was transferred to a 12-ml conical centrifuge tube in order to isolate the acidic compounds from the bases and neutral components.

Isolation of Acidic Components from Axillary Extract. The aqueous solution was extracted with 1.5 ml of Nanograde chloroform (Mallinkrodt) by vigorous

vortexing for 10 sec. The resulting two layers were separated by centrifugation in a clinical (IEF) centrifuge at top speed for 5 min. The bottom layer, containing the basic and neutral components, was transferred to a 10-ml vial by using a thinly drawn pipet. Two subsequent 1.5-ml aliquots of $CHCl_3$ were used to extract the aqueous layer, and the $CHCl_3$ layers were combined for 4.5 ml total. Fractionation of the basic and neutral compounds was done as described previously (Zeng et al., 1991), and the fractions were stored at −10°C until they were concentrated and analyzed.

The aqueous layer remaining in the centrifuge tube was acidified by single drop additions of 2 N HCl until a pH of 2 was achieved. The mixture was extracted with $CHCl_3$ (1.5 ml × 2). The $CHCl_3$ layer was separated by centrifugation (as described above) and concentrated to 20 μl prior to analysis by GC-MS.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. A Finnigan 4510 GS-MS data system equipped with a split/splitless injector, a fused silica capillary column, and with capabilities for operation in both electron impact and chemical ionization modes was used for analyses. The columns employed were a 30-m × 0.32-mm-ID fused silica column with a 1.0-μm coating of Stabilwax (cross-bonded polyethylene glycol, Restek Inc., Bellefonte, Pennsylvania), or a 30-m × 0.32-mm-ID fused silica RTX-1 column with a 1.0-μm coating of cross-bonded methylsilicon (Restek Inc.).

The analysis conditions for the Stabilwax column were as follows: 60°C, 4 min, then 3°/min to 220°C; hold at 220°C for 1 hr. Analyses performed with the RTX-1 column used the following conditions: 60°C for 4 min, then 4°/min to 300°C; hold at 300°C for 20 min.

The mass spectrometer was interfaced with a Nova 4X computer that utilized the Super Incos software for data acquisition, analysis, and quantitation. The mass range employed during these analyses was typically m/z 40–400, with one scan per second. A typical run included 3600 scans. The data system also includes the NBS library of 42,000 compounds.

As in our previous studies (Zeng et al., 1991, 1992; Spielman et al., 1995), identifications were based on comparison of unknown spectra with those from synthetic or commercial standards. In addition, the relative chromatographic retention times of unknown and known standards were compared with a mixture of fatty acids ethyl esters (van den Dool and Kratz, 1963).

Collection of Apocrine Secretions. Four of the six female subjects who donated axillary secretions collected on pads agreed to donate apocrine secretions. The axillae was prepared for sampling and apocrine secretions stimulated by an intradermal injection of 1 : 10,000 adrenalin (in physiologic saline) as in our previous reports (Labows et al., 1979b; Zeng et al., 1992). The amount of secretion collected varied greatly with the individual subject (≤ 10 μl).

Separation of Apocrine Secretions in Aqueous and Organic Soluble Fractions. The apocrine secretions collected from each subject were combined by washing the vials with a total of 300 μl of doubly distilled water. Each of the collection vials was then washed with 400 μl of Nanograde chloroform. The chloroform and aqueous layers were combined and vigorously shaken for 10–15 sec. The mixture was centrifuged in a clinical (IEC) centrifuge for 5 min at top speed to form two layers. The bottom chloroform layer was removed in a thinly drawn pipet.

The remaining aqueous layer was extracted two more times with chloroform (2 × 400 μl) and subsequently separated by the same procedure as above. This procedure gave a volume of 1.2 ml of the chloroform solution and 300 μl of aqueous apocrine solution. Aliquots of 10 μl of the aqueous phase and 10 μl of the chloroform layer were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). This is described below.

Hydrolysis of Apocrine Secretion Aqueous Phase. The aqueous apocrine solution was refluxed for 20 min with 1 ml, 5% aqueous sodium hydroxide under a stream of nitrogen. The mixture was allowed to cool to room temperature and acidified with 2 N HCl to pH 2. A slight axillary-like odor developed in the reaction mixture upon acidification. The acidified mixture was extracted with 3 × 150 μl chloroform. The combined chloroform extracts were concentrated to approximately 5 μl for organoleptic evaluation and GC-MS analysis.

Hydrolysis of Apocrine Secretion Organic Phase. The chloroform extract was concentrated to approximately 100 μl and refluxed for 20 min with 5% NaOH in 2 ml of a 1 : 1 solution of methanol–water under a stream of nitrogen; the same conditions were also used for the aqueous-phase hydrolysis. After removal of the solvent, the reaction mixture was acidified with 2 N HCl of a pH of 2; no odor was detected. This acidic solution was extracted with chloroform (3 × 0.5 ml) and concentrated to approximately 5 μl for organoleptic and GC-MS analyses.

Organoleptic Evaluation of Axillary Extracts and Hydrolyzed Apocrine Secretion Samples. Organoleptic evaluation was performed by a panel of three or four judges in a manner similar to that used previously for evaluation of chromatographic eluants (Zeng et al., 1991). Judges were presented in a blind manner with either fractions of axillary extracts or the acidified, hydrolyzed aqueous or organic portions of apocrine secretion and asked which had an odor that most resembled the axillary odor. A small (2- to 5-μl) aliquot of concentrated axillary extract on a filter-paper strip (in a sealed vial) was on hand for reference if requested by a judge.

SDS-PAGE of Apocrine Secretion Aqueous and Organic Soluble Fractions. SDS-PAGE was done according to Laemmli (1970). The stacking gel was 5% acrylamide and the separating gel was 15%. Samples (10 μl) of each fraction

were dried down to approximately 5 μl, then mixed with 5 μl of sample buffer containing 2% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue, and 50 mM Tris HCl at pH 6.8 (Laemmli, 1970). After electrophoresis, the gels were stained with Coomassie blue.

RESULTS

Organoleptic evaluation of the concentrated neutral, base, and acidic components from the pad extract demonstrated that the acidic portion of the extract contained the characteristic axillary odor and most resembled the entire concentrated extract, a result analogous to that found with male samples (Zeng et al., 1991). Consequently, analyses by GC-MS were performed on the components in the acidic fraction. All the pads used in the extracts were collected during the presumed ovulatory portion of the menstrual cycle (±3 days of presumed ovulatory day) as judged by the subjects' basal body temperature charts and cycle length.

Figure 1 shows the reconstructed ion chromatogram (RIC) resulting from the analyses of the acidic components using the Stabilwax column. The part of the chromatogram shown in Figure 1 (bottom) is the area in which the C_6–C_{11} acids elute; these compounds have been shown to constitute the characteristic axillary odor in males. Both the identity of the lettered components and their presence in male extracts are listed in Table 1.

The top part of Figure 1 shows a mass chromatogram of *m/z* 60. This ion is formed by McLafferty rearrangement and is generally the most intense ion in the mass spectrum of aliphatic acids (with an available γ-hydrogen and unsubstituted α carbon) (Budzikiewicz et al., 1967). The large, regularly spaced maxima for peaks showing *m/z* 60 in Figure 1 suggest the presence of a series of *n*-aliphatic acids. Interpretation of the mass spectra of these chromatographic peaks when considered together with their relative retention times (ethyl ester units) (van den Dool and Kratz, 1963) led to the conclusion that these compounds were *n*-organic acids: hexanoic (peak A) through undecanoic acid (peak Gg).

The identity of all the peaks labeled in Figure 1 are shown in Table 1. This table also indicates which of these compounds were also found in the characteristic axillary odors from males (Zeng et al., 1991). Figure 2 shows the bottom portion of Figure 1 and the same portion of the chromatogram from the previously published combined male sample (Zeng et al., 1991) to show the relative differences between the male and female samples. Peak L in the bottom portion of Figure 2 is *E*-3M2H, and peak K in the top part of this figure is *E*-3M2H (male sample). Peaks H and G, bottom and top, respectively, are the *Z* isomer. Although *E*-3M2H was the major component in the combined male sample, this

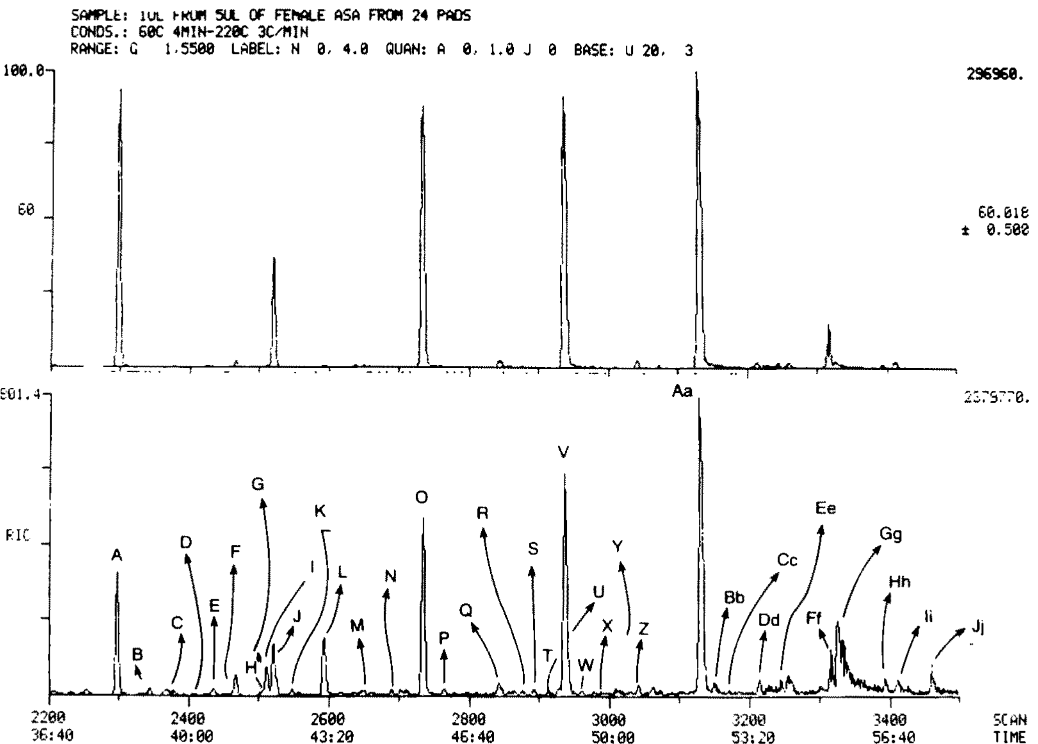
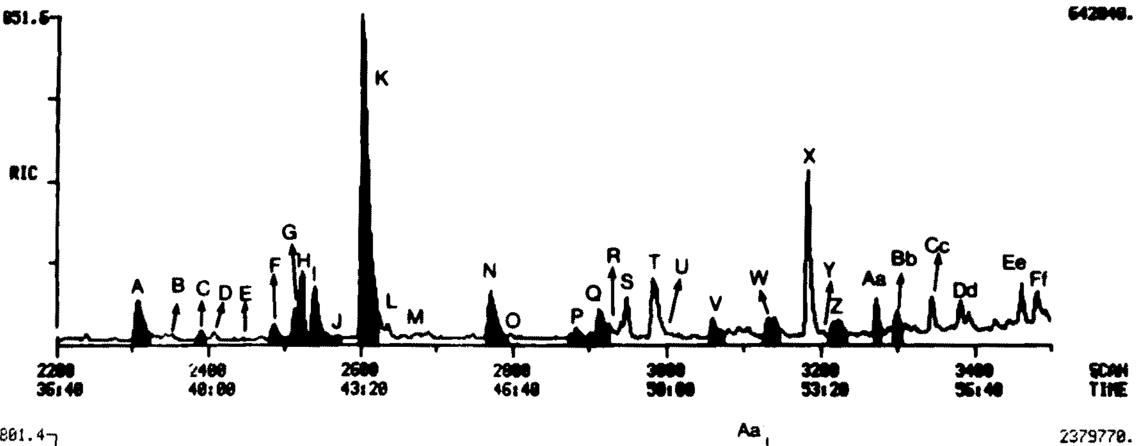


FIG. 1. The top part of the figure shows a plot of m/z 60 (mass chromatogram) in the area where the characteristic axillary odors elute. This ion is prominent in the mass spectra of aliphatic acids with an unsubstituted α-carbon and available γ-hydrogen as discussed in the text. This ion is the base ion for many normal-chain acids. The identity of the labeled peaks in the reconstructed ion chromatogram (bottom portion of the figure) may be found in Table 1, together with their relative retention tiumes.

TABLE 1. COMPOUNDS IDENTIFIED IN COMBINED FEMALE (F) AXILLARY EXTRACTS IN COMPARISON TO MALES

Peak	Compound	Molecular weight (kDa)	Retention time (ethyl ester units)	Present in combined female (F) male (M) extract
A	*n*-Hexanoic acid[G]	116	12.20	F/M
B	2-Methylhexanoic acid[G]	130	12.39*	F/M
C	3-Methylhexanoic acid[G]	130	12.55*	F/M
D	(*E*)-3-Methyl-2-pentenoic acid	**114**	**12.60***	**F only**
E	Dimethylsulfone[G] ($C_2H_6SO_2$)	94	12.63	F/M
F	γ-C_8-Lactone[G]	142	12.79	F/M
G	4-Ethylpentanoic acid[G]	130	12.97*	F/M
H	(*Z*)-3-Methyl-2-hexenoic acid[G]	128	13.10*	F/M
I	2-Ethylhexanoic acid	144	13.13	F/M
J	*n*-Heptanoic acid[G]	130	13.22*	F/M
K	2-Methylheptanoic acid[G]	144	13.36*	F/M
L	(*E*)-3-Methyl-2-hexenoic acid[G]	128	13.50*	F/M
M	Phenol	94	13.65	F/M
N	γ-C_9-Lactone[G]	156	13.91	F/M
O	*n*-Octanoic acid[G]	144	14.28*	F/M
P	2-Methyloctanoic acid[G]	158	14.41	F/M
Q	4-Ethylheptanoic acid[G]	158	14.81*	F/M
R	7-Octenoic acid[G]	142	14.95*	F/M
S	2-Piperidone[G]	**99**	**14.55***	**F only**
T	γ-C_{10}-Lactone[G]	170	15.01	F/M
U	*n*-Tetradecanol[G]	214	15.21	F/M
V	*n*-Nonanoic acid[G]	158	15.28	F/M
W	2-Methylnonanoic acid[G]	172	15.38	F/M
X	(*E*)-3-methyl-2-octenoic acid[G]	**156**	**15.45***	**F only**
Y	4-Ethyloctanoic acid[G] ("goat acid")	172	15.64*	F/M
Z	Unsaturated C_9 acid[T]	156	16.04*	F/M
Aa	*n*-Decanoic acid[G]	172	16.28	F/M
Bb	2-Methyldecanoic acid[T,G]	186	16.36	F/M
Cc	Unsaturated C_{10} acid[T]	170	16.46*	F/M
Dd	4-Ethylnonanoic acid[T,G]	186	16.69*	F/M
Ee	9-Decenoic acid[G]	170	16.90*	F/M
Ff	*n*-Hexadecanol	242	17.24	F/M
Gg	*n*-Undecanoic acid[G]	186	17.29	F/M
Hh	4-Ethyldecanoic acid[T,G]	200	17.66	F/M
Ii	10-Undecenoic acid[G]	184	17.76*	F/M
Jj	Benzoic acid[T]	122		

[a]The following symbols are used in this table: T = tentatively assigned by mass spectral data; G = correspondence of mass spectrum and relative chromatographic retention times with commercially available or synthetic sample. *Compounds judged to be important contributors to axillary odor, from data reported in male samples (Zeng et al., 1991).



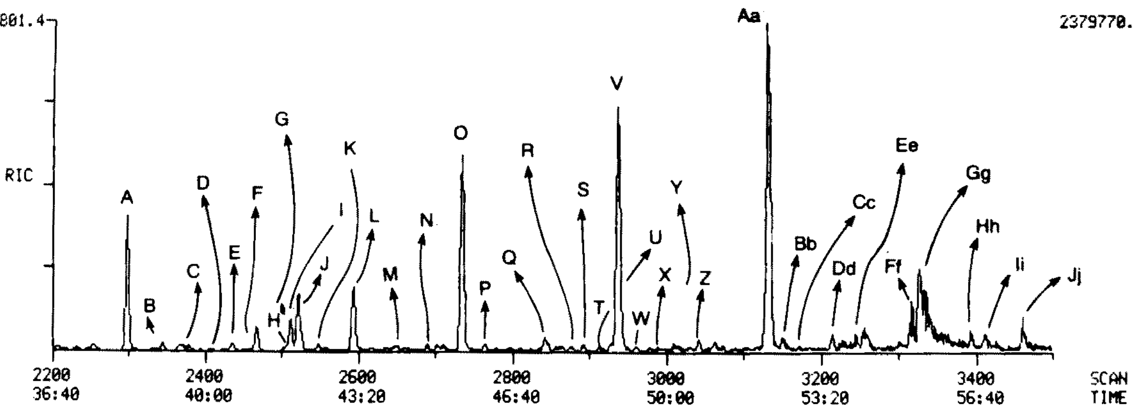


FIG. 2. This figure shows the regions in the reconstructed ion chromatograms where the C_6–C_{11} acids elute for the male (top: Zeng et al., 1991) and female (bottom) axillary extracts. Note the different relative proportions of constituents in the two samples with peak K (*E*-3M2H) being greater than the other components in the male sample (top), while normal acids are greater than peak L (*E*-3M2H) and other components in the female samples (bottom).

is not the case in the combined female sample. In the latter, the *n*-aliphatic acids are present in greater quantity than *E*-3M2H.

Comparison of injections of known amounts of 3M2H suggests ~150 ng/μl of *E*-3M2H is present in the female extract, while the male extract contained ~357 ng/μl of *E*-3M2H. In addition, the *E* to *Z* ratio in the combined female sample is 16:1, indicating a greater relative amount of the *E* vs. *Z* isomer in the female sample than previously reported in males, in which the ratio was 10:1 (Zeng et al., 1991).

The same series of γ-lactones reported in the combined male samples is present in the combined female sample. Although only C_8–C_{10}, γ-lactones are listed in Table 1, several lower members of the homologous series were also found eluting prior to the components in the area of the characteristic odor components.

Several other compounds whose structures were reported in male samples, and which are present here, included 7-octenoic acid (peak R) and two higher homologs (peaks Ee and Ii); goat acid (4-ethyl-octanoic acid, peak Y) and several homologs (peaks G, Q, Dd, and Hh); and 2-methylhexanoic acid (peak B) as well as four higher homologs (peaks K, P, W and Bb). Peak C was found to be 3-methyl-hexanoic acid.

Several subtle, qualitative differences between the male and female samples were seen, as noted in Table 1. Two homologs of the (*E*)-3-methyl-2-hexenoic acid are present in the female sample: 3-methyl-2-pentenoic (3M2P) and 3-methyl-2-octenoic (3M2O) acids. The relative retention times of both the lower and higher homologs matched with those of the synthetic *E* isomers we had in our possession (Zeng et al., 1992). The mass spectrum and relative retention time of peak S corresponded to 2-piperidone, a six-membered lactam.

To determine whether the aqueous soluble molecules from the apocrine secretions of females could be treated with base to yield 3M2H (Zeng et al., 1992), these secretions were separated into water-soluble and organic-soluble compounds as in our previous study (Zeng et al., 1992). Ten microliters of each fraction was saved for electrophoretic separation of putative proteins in each phase using SDS–polyacrylamide gels (Zeng et al., 1992). The rest of the aqueous and organic phases were treated with 5% NaOH to effect hydrolysis, and the acid components liberated by this procedure were isolated as in our previous study on male apocrine secretions (Zeng et al., 1992).

The reconstructed ion chromatograms (RICs) from the analyses of the aqueous and organic phase hydrolysates are shown in Figure 3A and B, respectively. Identity and/or data concerning the labeled peaks are shown in Table 2. Far greater amounts of *E* and *Z*-3M2H are seen in the hydrolysate of the aqueous phase than in the hydrolyzate of the organic phase molecules as indicated in Table 3. In addition, both the ratio of *E* to *Z* isomers in the aqueous hydrolyzate (~11.5:1) and the differences between the amount of *E* isomers in the aqueous

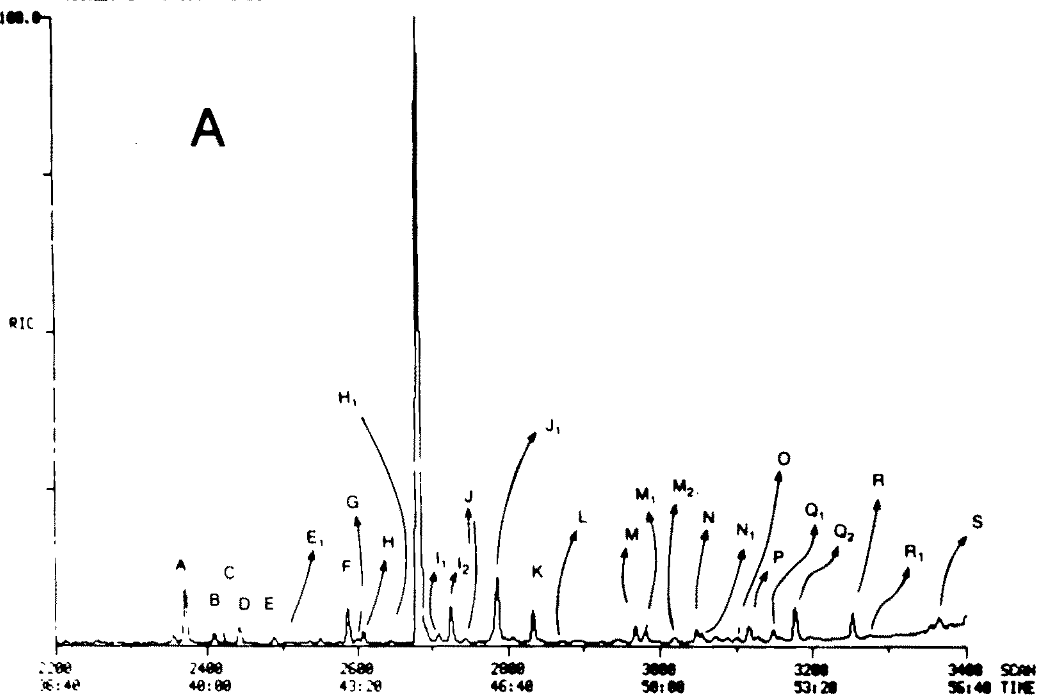


FIG. 3. The reconstructed ion chromatograms of the acidic components generated by the hydrolysis of the aqueous (A) and organic soluble (B) molecules found in female apocrine secretions. The aqueous phase hydrolysate (A), is dominated by the presence of a large amount of *E*-3M2H (peak I). The organic phase hydrolysate (B) contains a number of large lipid components and esters which may be exogenous constitutents.

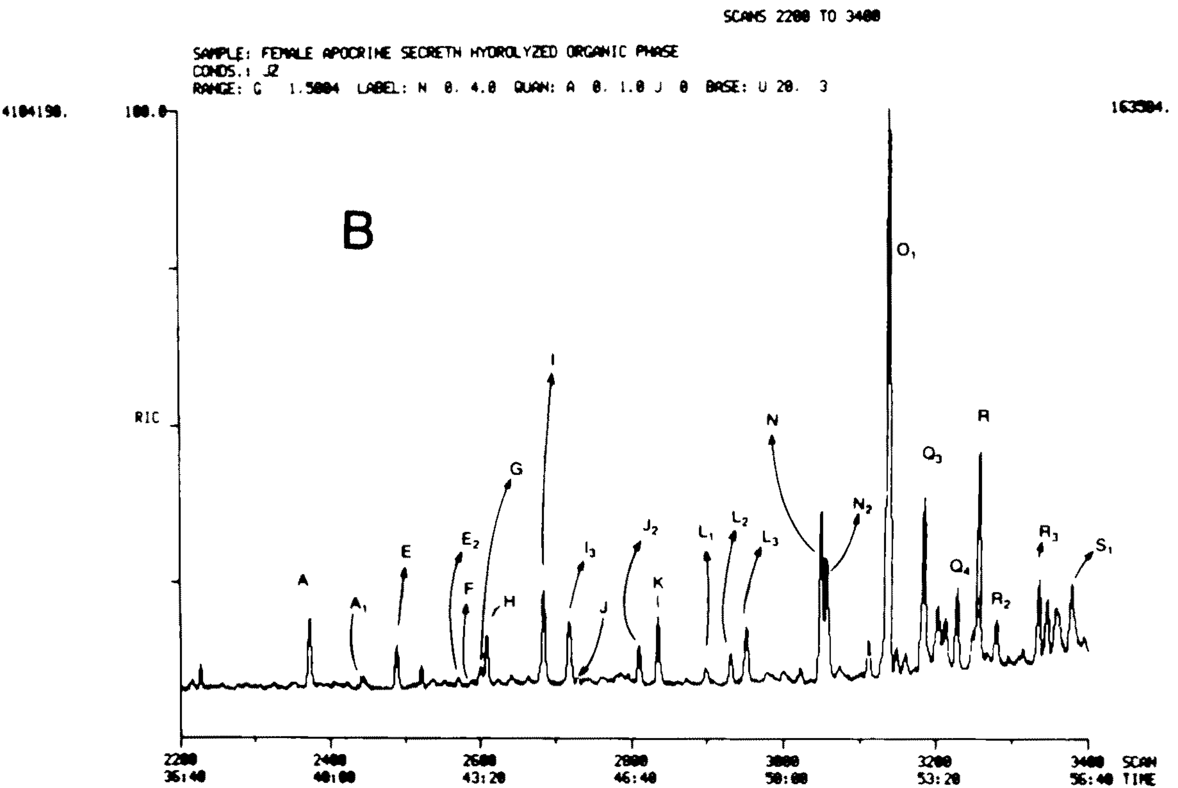


FIG. 3. Continued.

TABLE 2. COMPOUNDS IDENTIFIED IN HYDROLYZED FEMALE APOCRINE SECRETION: AQUEOUS PHASE MOLECULES OR ORGANIC PHASE MOLECULES[a]

Peak	Compound	Molecular Weight (kDa)	Found in aqueous phase (a) or organic phase (o) hydrolysate
A	*n*-Hexanoic acid[G]	116	a/o
A_1	Benzyl alcohol	108	o
B	Unknown[b]		a
C	2-Methylhexanoic acid[G]	130	a
D	Unknown[c] plus (*E*)-3-methyl-2-		a
	pentenoic acid[G]	114	a
E	δ-Lactone[T,d]		a/o
E_1	Dimethylsulfone[G]	94	a
E_2	δ-Lactone[T,d]		o
F	(*Z*)-3-Methyl-2-hexenoic acid[G]	128	a/o
G	2-Ethyl-hexanoic acid[G]	144	a/o
H	*n*-Heptanoic acid[G]	130	a/o
H_1	2-Hexenoic acid[T,e]	114	a
I	(*E*)-3-Methyl-2-hexenoic acid[G]	128	a/o
I_1	C_7-unsaturated branched acid[T,f]	128	a
I_2	C_7-unsaturated branched acid[T,g]	128	a
I_3	δ-Lactone[T,d]		o
J	Phenol[G]	94	a
J_1	C_7-unsaturated branched acid[T,h]	128	a
J_2	Aliphatic alcohol[T,i]		o
K	*n*-Octanoic acid[G]	144	a/o
L	2-Methyl-octanoic acid[G]	158	a
L_1	Cyclohexane carboxylic acid[T,j]	128	o
L_2	Pentadecanoic acid methylester[T]	256	o
L_3	δ-Lactone[T,d]		o
M	7-Octenoic acid[G]	142	a
M_1	C_8-unsaturated acid[T,k]	142	a
M_2	C_8-unsaturated acid[T,l]	142	a
N	*n*-Nonanoic acid[G]	158	a/o
N_1	2-Methylnonanoic acid[G]	172	a
N_2	Unknown		o
O	(*E*)-3-Methyl-2-octenoic acid[G]	156	a
O_1	Palmitic acid methyl ester[G]	270	o
P	δ-Lactone[T,d]		o
Q_1	C_9-unsaturated acid[T,m]	156	a
Q_2	C_9-unsaturated acid[T,n]	156	a
Q_3	9-Hexadecenoic acid methylester	268	o
Q_4	Aliphatic alcohol[T,o]		o
R	*n*-Decanoic acid[G]	172	a/o
R_1	2-Methyldecanoic acid[G]	186	a
	Unsaturated C_{10} acid[T,p]	170	a

TABLE 2. Continued.

Peak	Compound	Molecular Weight (kDa)	Found in aqueous phase (a) or organic phase (o) hydrolysate
R_2	C_{17}-methyl branched methylester[T]	284	o
R_3	Heptadecanoic acid methylester[G]	284	o
S	Unknown long-chain alcohol[T]		o
S_1	Unsaturated C_{17} acid methylester[T]	282	o

[a]The following symbols are used in this table: T = tentatively assigned from mass spectral data; G = correspondence of mass spectrum and relative chromatographic retention times with commercially available or synthetic standards.

[b]Unknown seen only in aqueous phase hydrolysate: *m/z* (relative intensity) 45(20), 49(100), 51(31), 63(37), 77(2), 82(11), 84(4), 97(4), 112(42), 114(16): Chlorine containing?

[c]Unknown seen only in aqueous phase hydrolysate: *m/z* (rel. intensity) 41(45), 43(28), 55(100), 56(10), 56(10), 57(7), 59(10), 61(12), 69(38), 71(43), 81(12), 87(12), 97(41), 103(5), 105(4), 114(16), 115(12), 148(5): Sulfur containing?

[d]Proposed δ-lactones, seen only in organic phase, suggested by large ion an *m/z* 99 but few other ions suggestive of structure.

[e]Proposed 2-hexenoic acid, seen only in aqueous phase hydrolysate: *m/z* (rel. intensity) 41(48), 42(52), 43(22), 45(34), 55(35), 57(10), 60(11), 68(22), 71(8), 73(100), 74(12), 99(22), 114M$^+$(10).

[f]C_7 unsaturated branched acid, only in aqueous phase: *m/z* (rel. intensity) 41(90), 43(20), 45(10), 53(11), 55(54), 60(5), 67(35), 68(20), 69(100), 71(52), 82(22), 83(28), 85(6), 87(5), 95(6), 100(3), 113(10), 128(57). Isomeric with 3M2H?

[g]C_7 unsaturated branched acid, only in aqueous phase: *m/z* (rel. intensity) 41(82), 42(18), 43(30), 55(45), 57(12), 60(13), 67(30), 68(25), 69(100), 71(41), 82(42), 83(35), 95(8), 100(5), 113(12), 128(15). Isomeric with 3M2H?

[h]C_7 unsaturated branched acid, only in aqueous phase: *m/z* (rel. intensity) 41(30), 43(50), 55(11), 57(21), 71(100), 72(9), 73(4), 85(7), 100(1), 128(1).

[i]Aliphatic alcohol, only in organic phase hydrolysate: relative retention time suggests a C_{13} alcohol.

[j]Proposed cyclohexane carboxylic acid, seen only in the organic phase hydrolysate: *m/z* (rel. intensity) 41(55), 42(9), 43(11), 55(100), 56(29), 68(12), 69(29), 73(83), 83(50), 87(3), 99(9), 100(2), 110(4), 128(17).

[k]C_8 unsaturated acid, seen only in the aqueous phase: *m/z* (rel. intensity) 41(51), 43(37), 53(11), 55(100), 56(11), 57(11), 67(25), 68(10), 69(55), 71(19), 81(15), 82(62), 83(33), 96(18), 111(3), 124(9), 142(20). Isomeric with 7-octenoic acid?

[l]C_8 unsaturated acid, seen in the aqueous phase: *m/z* (rel. intensity) 41(71), 42(15), 43(54), 45(20), 54(16), 55(69), 56(33), 57(28), 67(28), 68(8), 69(18), 71(18), 73(9), 82(49), 83(21), 84(2), 85(100), 87(15), 96(16), 100(13), 111(2), 113(5), 124(19), 128(6), 142(1).

[m]C_9 unsaturated acid, seen in the aqueous phase: *m/z* (rel. intensity) 41(92), 42(25), 43(65), 45(31), 55(97), 57(29), 67(25), 68(35), 69(100), 71(32), 81(48), 83(38), 85(21), 96(65), 97(15), 114(42), 120(12), 138(1), 156(23).

[n]C_9 unsaturated acid, seen only in the aqueous phase. Mass spectrum reported previously (Zeng et al., 1992).

[o]Long-chain alcohol only in the organic phase, relative retention time suggests a C_{15} alcohol.

[p]C_{10} unsaturated acid, seen only in the aqueous phase: *m/z* (rel. intensity) 41(20), 43(100), 45(12), 55(20), 56(28), 57(6), 68(7), 69(13), 81(6), 96(5), 110(8), 114(10), 128(4), 152(3), 170(5).

TABLE 3. AMOUNTS OF 3-METHYL-2-HEXENOIC ACID LIBERATED FROM FEMALE APOCRINE SECRETION BY NaOH HYDROLYSIS

	Amount (ng/μl)	
	E isomer	*Z* isomer
Aqueous phase	244.7	21.2
Organic phase	1.4	0.01

phase (>200×) vs. the organic phase were almost identical to the ratios found in comparable male samples (Zeng et al., 1992).

As may be further observed in Figure 3A and Table 2, many of the same components seen in the combined female pad extract are apparently liberated by the hydrolysis procedure, albeit at different relative proportions than those in the male samples (Zeng et al., 1992). The organic soluble molecules contain many compounds that are lipophilic in nature, such as long-chain methyl esters and alcohols (peaks O_1, Q_3, Q_4, R_2, R_3, S_1) and may have been present in the apocrine secretions and extracted into the chloroform. A number of these compounds have been previously identified as compounds of exogenous origin in axillary area (Labows et al., 1979c).

Although 3M2H and normal C_6–C_{11} acids are present, their levels are far lower in the organic hydrolysate than in the aqueous phase hydrolysate (see Table 3). Further, the hydrolysate from the aqueous soluble molecules contain traces of *E*-3-methyl-2-pentenoic and *E*-3-methyl-2-octenoic acids (3M2O) as well as 7-octenoic acid. A number of the compounds found only in the aqueous phase appear to be acids whose molecular weights and mass spectral fragmentation patterns suggest that they are isomeric with 7-octenoic acid (M), 3M2H (I), and 3M2O (O): isomers of M are M_1 an M_2; isomers of 3M2H are I_1, I_2, and J_1; and isomers of O are Q_1 and Q_2. Further, the mass spectrum of Q_2 appears to be similar to that of an unknown C_9-unsaturated acid discussed in our previous studies on males (Zeng et al., 1991, 1992).

Other interesting compounds are the 2-hexenoic acid (tentatively identified from its mass spectrum) and peaks B and D found in aqueous phase hydrolyzate. The fragmentation of the latter two (see Table 2) suggest that they may be chlorine- (peak B) or sulfur-containing (D) compounds.

The results of the electrophoretic separation shown in Figure 4 were analogous to that found in males. The figure showing the gel on which the male apocrine secretion proteins were separated (lanes 2 and 3) [shown previously in Zeng et al. (1992)] was placed side-by-side with the female samples for com-

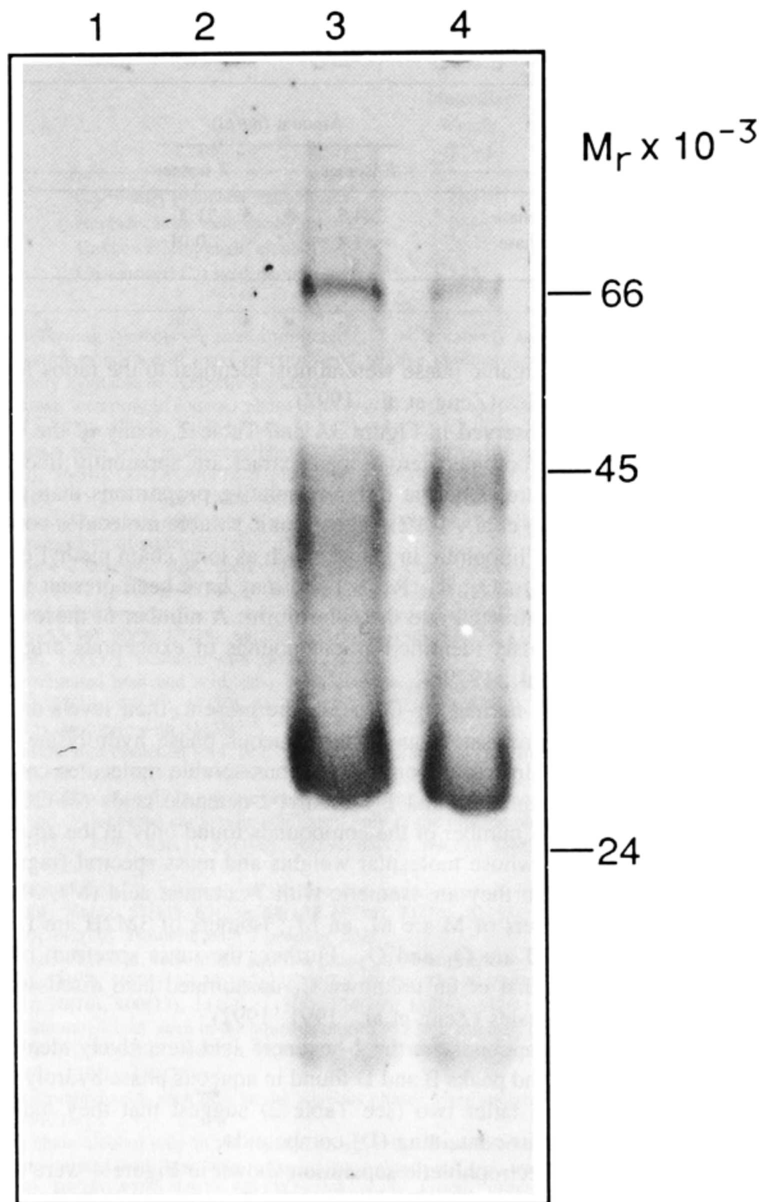


FIG. 4. The SDS-PAGE profile of the unhydrolyzed organic (lanes 1 and 2) and aqueous phases (lanes 3 and 4) from female (lanes 1 and 4, respectively) and male (lanes 2 and 3, respectively) apocrine secretions. The aqueous phase of both sexes display very similar profiles. Molecular weight markers were used to generate the scale to the right.

parison (lanes 1 and 4). The aqueous phase from female apocrine samples appears rich in proteins (lane 4), while the organic phase demonstrated no staining indicative of protein constituents (lane 1). The major protein bands in the aqueous phase are found at 26, 45, and 66 kDa. In male apocrine secretions, the proteins found at apparent molecular masses of 26 kDa and 45 kDa have been shown to carry 3M2H to the skin surface (Spielman et al., 1995).

The acid fraction of the combined pad extract as well as the apocrine secretion aqueous and organic phase hydolysates were examined for the presence of volatile steroids and/or steroid sulfates by GC-MS using a previously developed analysis protocole on a bonded methyl silicon column (Zeng et al., 1992; Preti et al., 1987). The combined pad extract and the aqueous phase hydrolysate contained androstenol (5α-androst-16-en-3α-ol) but no detectable amounts of androstenone (5α-androst-16-en-3-one). Neither of these volatile steroids was seen in the organic phase hydrolysate. The approximate levels of androstenol (i.e., when compared to injection of standards) were 3.5 ng/μl for the acid fraction from the combined pad extract and 0.81 ng/μl for the hydrolyzate from the aqueous soluble molecules from female apocrine secretions. Each of the samples analyzed contained detectable amounts (no quantitation attempted) of 17-oxo-5-androsten-3α-yl sulfate (dehydroepiandrosterone sulfate) and 17-oxo-5α-androstan-3α-yl sulfate (androsterone sulfate); these two sulfates were detected as their pyrolysis products (Labows et al., 1979b; Preti et al., 1987). In addition, each of the three samples contained the free steroids androsterone and dehydroepiandrosterone.

DISCUSSION

The results of our study demonstrate that men and women with similar axillary microflora are qualitatively similar in the array of volatiles produced. The characteristic axillary odor in the combined female axillary pads resides with the acidic compounds; the neutral/basic compounds possess little or no odor. The major components in both sexes were the same, the normal C_6–C_{11} acids and *E*-3M2H; however, as noted above, the relative proportions are reversed in the females' sample vs. the males'; in the latter, the *E*-3M2H was present in larger amounts than any of the normal C_6–C_{11} acids. The female sample also had a different ratio of E:Z isomers for 3M2H in the pad extracts; males have a 10:1 ratio, while a 16:1 ratio was seen in the female pad extract.

The combined pad extracts from both males and females did not employ internal standardization; therefore, the amounts of 3M2H and androstenol noted in the results are estimated, post-hoc, from comparison with injections of known amounts of standard compounds in the GC-MS system. The recovery of 3M2H and androstenol during extraction in combined pad samples is estimated to be 59% and 67%, respectively (Zeng et al., 1991; Preti et al., 1987).

Some subtle differences between males and females were noted in the constituents found in the combined pad extract acid fraction. For example, the female sample contained traces of *E*-3M2H and Z-3M2O and 2-piperidone. The latter compound is a six-membered ring lactam and is most likely not present as such in the pads, but may arise from a thermally induced cyclization of the compound δ-amino pentanoic acid (in the injector of the GC). We cannot explain why this compound was not seen in males.

In our previous study with the acid fraction from the combined male pad extract, we only determined that androstenol and androstenone were present via mass spectrometric evidence and retention times (Zeng et al., 1991). A re-examination of these data and comparison to standard injections of these steroids analyzed near the time frame this sample was run suggests that both compounds were present at an estimated level of ~ 0.5 ng/μl of extract. Consequently, the acid fraction from the combined female pad extract appears to have more androstenol than the acid fractions of the combined male pad extract; however, the female sample had no detectable amount of androstenone. It is likely that most of these steroidal molecules are extracted into the neutral components.

The female pads used in this study came from the presumed ovulatory portion of the menstrual cycle of each subject. Consequently, the relative proprotions of the various components discussed here may differ in other parts of the menstrual cycle. Further, it is not known whether one donor contributed more than others to the levels of 3M2H, C_6–C_{11} acids, androstenol, and other compounds. This remains to be determined by the analyses of daily axillary samples from individual donors.

In contast to the female pad extract, the dominant component in the acids liberated from the aqueous soluble molecules was 3M2H, not normal C_6–C_{11} acids. This may reflect the individual contributions of one or more donors and/or possibly the time in the menstrual cycle. Because of the individual variability in the amount of apocrine secretion that could be collected, it was not possible to pool apocrine secretions from distinct times in the menstrual cycle; the results above represent the hydrolysis of a pooled sample from donors at different times in the menstrual cycle.

Another factor that may influence the relative proportions of compounds is the formation from axillary bacteria vs. liberation of these compounds by NaOH. The bacteria may add their own array of metabolites to the odor as it "matures" under the arm or release components from the apocrine secretions in a far different manner than does a base hydrolysis (Zeng et al., 1992).

The γ-lactones listed in Table 1 are minute components, as seen in Figure 1. They are most likely due to the metabolic action of lipophilic yeasts such as *Pityrosporum* species. We postulate this origin for γ-lactones, as we did for similar compounds in male axillary samples. These organisms are found in areas of human skin that contain large numbers of sebaceous glands, such as the

axillae and scalp (Leyden et al., 1991; Labows et al., 1979a). *Pityrosporum ovale* species isolated from the scalp have been shown to produce γ-lactones in the presence of human sebum; these lactones have a pleasant odor (Labows et al., 1979a).

Unlike the base-hydrolyzed male aporcine phase (Zeng et al., 1992), the comparable female samples studied here yielded androstenol after hydolysis. Whether this means that androstenol is secreted bound to a different, base-labile precursor or that there is more of the same precursor present in females than males and therefore has a greater probability of interacting with base and liberating the free steroid is not known.

Small amounts of C_6–C_{11} normal-chain acids and 3M2H were present in the organic phase hydolysate. This suggests that their precursors may have been present in minute quantity in the organic phase (slight solubility) because the separation of apocrine secretions into two phases may not be totally complete. Another possibility is that the volatile compounds were present at low levels (below olfactory threshold) in the apocrine secretion (Labows et al., 1979b; Gower and Ruparelia, 1993) prior to hydrolysis. As may be seen in Table 2, there are a number of lipophilic compounds (long-chain alcohols and δ-lactones) that were most likely present in the secretion at the times of collection (perhaps in the hair follicle near the skin surface) and partitioned in the organic phase and/or were formed during the hydrolysis reaction. Some compounds present in the organic phase, as noted above, such as methyl esters, may be present as artifacts from past use of cosmetic products (Labows et al., 1979c); however, we cannot explain why these esters remain after a base hydrolysis, except that the hydrolytic conditions may not have been harsh enough for total ester conversion to their respective acids.

The qualitative similarity in the bouquet of volatile organic acids that make up the characteristic female and male axillary odors suggest a similar origin and mechanism for odor production in males and females. In addition, experiments reported here, which utilized female apocrine secretions, demonstrate that: (1) the characteristic axillary odors can be released from the aqueous soluble molecules while only traces of 3M2H are present in the organic soluble molecules, (2) approximately 200$\times$ more 3M2H is liberated by hydrolysis of the aqueous phase molecules than the organic phase molecules, and (3) the electrophoretic profile of proteins in the aqueous soluble molecules from female and male apocrine secretion is qualitatively similar. Consequently, the water-soluble components of apocrine secretions carry 3M2H and other components of the characteristic axillary odor to the surface where they are liberated by the resident, axillary microflora, particularly the lipophilic diphtheroids. Since similar arrays of proteins are present in the female and male apocrine secretion aqueous phase, we may assume that individual proteins at 26 kDa and 45 kDa carry 3M2H out to skin surface, as has been found for males (Spielman et al., 1995). Further

support for this comes from preliminary immunohistochemical data, which reveal these two proteins in the apocrine glands of females and males (Spielman et al., 1994).

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