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Analysis of Human Skin Emanations by Gas Chromatography/Mass Spectrometry. 2. Identification of Volatile Compounds That Are Candidate Attractants for the Yellow Fever Mosquito (*Aedes aegypti*)

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Volatile compounds emanated from human skin were studied by gas chromatography/mass spectrometry (GC/MS). The purpose of this study was to identify compounds that may be human-produced kairomones which are used for host location by the mosquito, *Aedes aegypti* (L.). The procedure used to collect volatiles was chosen because of prior knowledge that attractive substances can be transferred from skin to glass by handling. Laboratory bioassays have shown that the residuum on the glass remains attractive to mosquitoes until the compounds of importance evaporate. The sampling and analytical procedures modeled the above-cited process as closely as possible except that the evaporation of compounds from the glass surface was accomplished by thermal desorption from glass beads in a heated GC injection port. This made possible the solventless injection of volatiles onto the column. The compounds were cryofocused on the head of the column with liquid nitrogen prior to GC separation. A single stage of mass spectrometry on a triple quadrupole instrument was used for mass analysis. A combination of electron ionization and pulsed positive ion/negative ion chemical ionization modes on two different GC columns (one polar, one relatively nonpolar) was used to identify most of the 346 compound peaks detected by this technique.

Mosquitoes are nuisance pests to both livestock and humans. In addition to causing annoyance, mosquitoes are vectors of the disease agents that cause malaria, viral encephalitis, dengue fever, filariasis, and dog heartworm.[1,2] The reason for conducting this work is to better understand host attraction by identifying the volatile compounds emanated by humans. The mosquito species that is targeted by this study is the yellow fever mosquito, *Aedes aegypti* (L.), which transmits yellow fever, dengue fever, and dengue hemorrhagic fever.[3]

Common approaches to mosquito control and control of the diseases that they transmit employ the use of chemical insecticides for area-wide mosquito abatement and repellents for personal protection. Because of insecticide resistance in mosquitoes and concern with pollution of the environment with pesticides,[4,5] there has been an increased emphasis on the development of alternative mosquito control technologies. The use of attractant-baited traps/targets is one promising technology currently being considered for this purpose. Mosquitoes use attractants to locate their mates, find hosts for blood feeding, find nectar sources, and locate resting and oviposition sites. Host attractants appear to offer the most immediate promise for utilization in traps/targets. However, effective utilization of attractants for mosquito control requires an understanding of the mechanisms that attract mosquitoes to humans and animals. Ultimately, improved understanding of attraction may also lead to the design of better repellents.

Mosquito Attraction Stimuli. Current knowledge indicates that attraction to (or repulsion from) a potential host involves a behavioral response by the female mosquito to one or more host stimuli, which include visual cues,[6−13] moisture,[6−8,14,15] heat,[6−8,14] carbon dioxide,[6−9,11,13−26] and other host chemical emanations.[6−9,11,14,16,19−26] Although sound (specifically wing-beat frequency) is not a stimulus for host location, it is a short-range attractant for orientation of males to females for mating.[6,27,28]

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Chemical emanations, including and in addition to carbon dioxide, appear to be the most important mechanism used by mosquitoes to locate their hosts.

Unfortunately, few specific host chemical emanations have been identified definitively as mosquito attractants. The compound L-lactic acid was identified as a chemical attractant for *Ae. aegypti* in 1968 from an acetone washing of human skin.[29] It has also been shown that a synergistic attraction response can be produced by combining L-lactic acid with carbon dioxide.[29,30] Since the discovery of *Ae. aegypti* attraction to lactic acid, studies of compounds structurally similar to lactic acid for mosquito attraction have produced mixed results.[20]

The compound 1-octen-3-ol (octenol), present in cow's breath,[31] has been identified as an attractant for some species of mosquitoes. Although the response to octenol by itself is not as great as the response to carbon dioxide by itself, synergism has been demonstrated when both are employed together as attractants for some species of mosquitoes.[21−25] In addition to lactic acid and octenol, other yet-unidentified compounds that emanate from the skin have been shown to play a role in the attraction of *Ae. aegypti* to humans.[32] Therefore, it is believed that the examination of human skin emanations will provide the necessary information to further define the chemical cues by which mosquitoes locate human hosts.

Analysis of Human Odor and Perspiration. Studies of body odor, particularly those odors associated with perspiration, yield knowledge of compounds present on the skin, but not necessarily about the volatiles that are of importance to host-seeking behavior. Perspiration, comprised mainly of water, is a dilute solution of compounds containing salts, involatile compounds, as well as small amounts of volatiles. Analysis by combined liquid chromatography/mass spectrometry (LC/MS) has detected the presence of lactic acid (lactate is a byproduct of exercise), urea, and various amino acids (phenylalanine, leucine, valine, and alanine).[33] However, the vapor pressures of amino acids are presumably too low to permit such compounds to be present at levels detectable by mosquito chemosensilla.

One requirement for mosquito attraction is that the attractant is sufficiently volatile for both short- (typically less than 3 m) and long-range (thought to be up to 60 m) detection by mosquitoes to occur. Human breath does excite and attract *Ae. aegypti*, and the analysis of breath has led to the identification of over 100 compounds.[34] There is some overlap of compounds identified in breath with those from skin; it should be noted, however, that although both skin emanations and breath attract mosquitoes, entirely different processes generate these compounds and the overall compositions are very dissimilar. Previous studies were conducted to identify the odor components of human perspiration, as well as total effluents produced by humans in an enclosed environment;[35−40] over 260 compounds, spanning a wide range of compound classes, have been reported.

Experiments comparing isopropyl alcohol extracts from the fingers of adults and children have been briefly discussed in the literature.[35] This method of collection was reported to provide more material than extraction of fingerprints from glass; however, the time delay regarding collection of fingerprints from glass was not reported. Sampling from glass, as was done in the current study, provides a discriminating benefit with respect to attractant analysis. It is known that at least some of the attractive compounds will be transferred to glass, and these compounds will evaporate, thus enabling orientation of mosquitoes to the glass. The use of glass beads is believed to allow for preferential concentration of oily/waxy residues while minimizing collection of aqueous perspiration.[41]

Other recent work has emphasized the identification of skin emanations that attract mosquitoes, including *Ae. aegypti*.[41,42] One method isolated active components from fractionated ethanol washing of human skin.[42] Lactic acid was confirmed to play a role with at least two additional compounds used in the mosquito−host attraction process. We have previously described a different approach, identifying primarily the volatile to semivolatile components, where these compounds are first transferred to glass then desorbed back off of the glass in the injection port of a gas chromatograph.[41] Recent progress has been made with attraction of *Anopheles gambiae* Giles, an important malaria vector.[43] Observations that this species (and other Anophelines) has preferential body locations from which it takes blood meals has recently led

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to a focus on foot odors.[44] This has resulted in the discovery that Limburger cheese is an attractant, further leading to the examination of bacteria-produced acids as mosquito attractants.[43,45,46] Additionally, incubated sweat was shown to be attractive to *An. gambiae sensu stricto* and that fresh sweat was not attractive.[47]

The purpose of this manuscript is to report the identities of human skin emanations that are desorbed from handled glass and then analyzed using the method described by us in a previous paper.[41] The results reported herein provide the foundation for the next manuscript in this series, in which a comparison is made of emanations from hosts for which *Ae. aegypti* has a markedly different attraction.

EXPERIMENTAL SECTION

Sample Collection, Loading, and Cryofocusing. Experiments used two to five 2.9-mm-diameter glass beads handled by one of four human male subjects, 26, 49, 52, and 61 years in age. The two oldest subjects were tested 16 times each. Each subject handled beads for a total of eight analyses with electron ionization (EI) and eight by chemical ionization (CI). The eight analyses by each ionization mode consisted of four analyses using a HP5 column (Hewlett-Packard, Atlanta, GA) and four using a HP-FFAP column (Hewlett-Packard). The 49-year-old subject was tested on five consecutive days, using both EI and CI modes, and only on an HP-FFAP column, for a total of 10 analyses. The 26-year-old subject provided handled beads for two EI and two CI analyses on each of the two columns employed, for a total of eight analyses. Volunteer subjects were not given any special instructions with regard to diet, handwashing, or application of normal toiletries prior to arriving at the laboratory. However, all subjects used the same soap to wash their arms and forearms in the laboratory when they arrived. The subjects then handled 15−25 beads when their hands were completely dry, 10−20 min after washing. The glass beads were rubbed in the palms and on the backs of the hands by a subject for 10−15 min prior to placing them into an insert. The gloved author held the insert while the ungloved subject placed the beads in the insert.

After loading the beads into a Varian 3400 GC (Varian, Walnut Creek, CA) fritted glass injection insert, the insert was replaced, reversed from normal configuration, in the injection port. The cap, septum, and needle guide were then restored to seal the injection port. The frit kept the beads from dropping down onto the column entrance and provided a means for volatile emanations to be loaded onto the column; the column entrance extended up into the injector insert and was located just below the frit. The injection port was operated in an entirely splitless mode for the duration of the experiment, providing only one exit for volatiles, i.e., through the column. This configuration allows for as many as 12 beads to be placed between the frit (located approximately halfway down the insert) and the GC septum sealing the injection port. Throughout the loading process, the injection port was held at 25 °C to minimize sample loss from evaporation and the head pressure of helium was set to 0 psig to allow for proper alignment of the septum and prevent premature migration of volatiles past the intended point of cryofocusing on the column.

The cryofocusing process involved heating glass beads in the GC injection port to desorb volatiles. Before increasing the helium head pressure, liquid nitrogen (LN_2, bp −196 °C) was placed in a 12-oz Styrofoam cup. The cup was placed in the oven such that the approximately 8 cm of column could be looped in the cup about 15 cm below the point where the column enters the oven from the injection port. The helium pressure was then increased to 20 psig, and the initial desorption phase started. This entailed loading a program to ramp the injection port from 25 to 250 °C over 7.5 min and then holding at 250 °C for 2.5 min. Throughout the cryofocusing phase, the GC oven was set at 25 °C and the transfer line set at 40 °C. Liquid nitrogen was added to the cup as necessary during this 10-min cryofocusing phase. When available, standards were analyzed by direct inlet probe (limited to solids and confirmation by mass spectrum only) or were injected (0.5 μL of a 100−150 ng/μL solution in methylene chloride or hexane) on the HP5 column under the same conditions as the glass bead experiments.

Chromatographic Parameters. After the completion of cryofocusing, a new program was loaded from the computer to the Varian 3400 GC. Once the oven temperature reached equilibrium at the start temperature of the temperature ramp program, the cup containing liquid nitrogen was removed. Analyses involving the comparison of subjects were performed with different ramp programs, depending upon the column employed. The columns were a 25 m × 0.20 mm i.d. HP5 FSOT column ($d_f = 0.33$ μm) or a HP-FFAP 25 m × 0.20 mm i.d. FSOT column ($d_f = 0.33$ μm). For experiments conducted with the HP5 column (35-min run time), the GC ramp consisted of a 1.0-min hold at 40 °C followed by a 10.6-min ramp at 17 °C/min and then a hold at 220 °C for 23.4 min. The transfer line was concurrently ramped from 50 to 220 °C at 20 °C/min over 8.5 min and held at 220 °C for the remainder of the analysis. The ramp program for the FFAP column (45-min run time) consisted of a 1.0-min hold at 40 °C followed by a 17.7-min ramp at 11 °C/min to 235 °C and then a hold for 26.3 min at this temperature. The transfer line was concurrently ramped from 50 to 236 °C at 23 °C/min for 8.1 min and held at 236 °C for the remaining 36.9 min. The column and transfer line were held at their final temperatures for 10.0 min after the recording of data, to bake off any high-boiling components prior to the next analysis.

Mass Spectrometric Parameters. The TSQ70 triple quadrupole mass spectrometer (Finningan-MAT, San Jose, CA) was operated using only a single stage of mass detection. Both EI and pulsed positive ion/negative ion chemical ionization (PPINICI)[48] modes were used for analysis. Acquisition of PPINICI data employed methane reagent gas at 1660−1690 mTorr (indicated) pressure. The ion source and manifold temperatures were 150 and 70 °C, respectively, for CI and 170 and 70 °C, respectively, for EI. The electron energy for CI experiments was 100 eV and that for EI was 70 eV. The third quadrupole was scanned with a scan time of 1 s per scan for PPINCI (m/z 50−650 for positive ions and 10−650 for negative ions) and 0.5 s per scan for EI data (m/z 35−650). The filament emission current was set at 200 μA.

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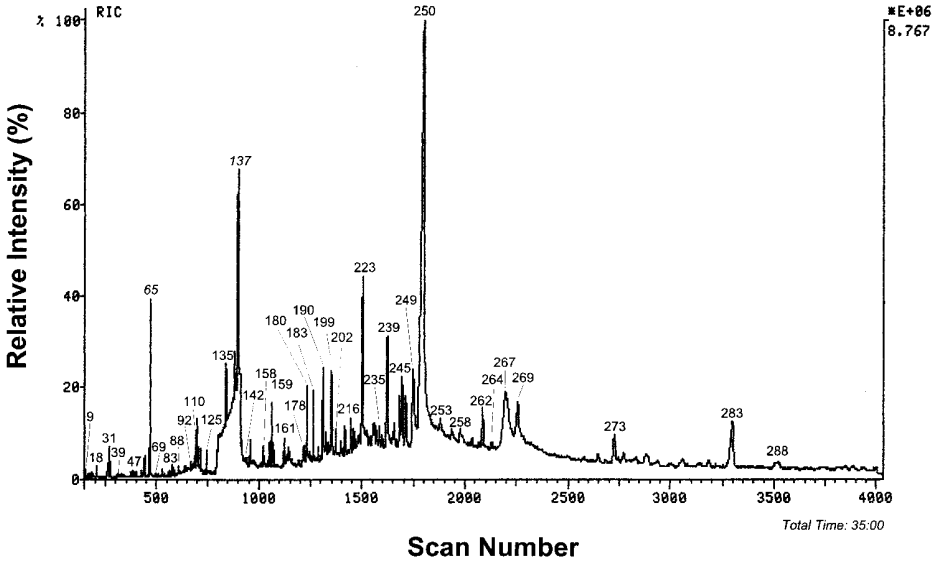

Figure 1. Reconstructed ion chromatogram from the EI analysis of compounds thermally desorbed from five handled glass beads onto a HP5 GC column. Labeled peak numbers correspond to those listed in Table 1.

The conversion dynode was set at −5 kV for positive-ion CI and EI and +5 kV for negative-ion CI. The electron multiplier was set between −1000 and −1200 V.

Prior to analysis on each day, the instrument was tuned with perfluorotributylamine (PFTBA) and GC/MS data for blanks (appropriate number of beads in an insert without rubbing) were recorded. Beads were selected randomly from a beaker containing clean beads. Beads were cleaned with multiple rinses of deionized water, methanol, and methylene chloride and baked out overnight before being reused. Prior to conducting the entire study of the four subjects, an insert was rubbed in a gloved hand to identify any possible compounds introduced from the gloves.

Tentative compound identifications were made by matching sample mass spectra with those of the NBS library for most of the peaks observed in the chromatograms. Manual interpretation was used for some of the identifications, and when available, standards were analyzed, as described earlier in this section.

RESULTS AND DISCUSSION

The Human Profile. A reconstructed ion chromatogram (RIC) of human skin emanations thermally desorbed from glass beads, acquired in EI mode, is presented in Figure 1. The peak numbers represent the elution order of identified peaks. Analysis using the methods employed here resulted in chromatograms that contained as many as 346 discernible peaks. Of these, 303 have been identified with varying degrees of certainty. Many of the more abundant components are labeled by number on the chromatogram. The peak numbers in Figure 1 correspond to those listed in Table 1.

In general, the four subjects studied were similar with respect to composition of emanations, with some exceptions discussed later in this manuscript. Further studies may reveal whether some of these are endogenous or if they are due to deposition on the skin from exogenous sources. Rigorous control of subject diet and exposure to exogenous sources was not performed. Although mosquitoes have little problem deciphering human odor cues amidst an odor profile with volatiles present that are exogenous to humans, the presence of these exogenous substances can complicate the discovery of host attractants. Previous publications have demonstrated the persistence (some cases 2 weeks or more) of exogenous substances, even when great care was taken in the sampling process.[50,51]

Forty-three of the detected component peaks in these analyses could not be classified nor identified from their EI and CI mass spectra; these are not listed in Table 1. Some of the components detected in these analyses raised interesting problems in terms of identification or were not expected to be from a human subject; a few of these are discussed below.

Carboxylic Acids, Alcohols, and Esters of Carboxylic Acids. The majority of the intense peaks observed in the chromatogram are carboxylic acids. Many of the later eluting intense peaks are esters of pentanedioic and hexanedioic acids. Free fatty acids are metabolites formed in the sebaceous glands,[52−55] and a much greater amount result from bacterial activity, through hydrolysis of triglycerides into free fatty acids (FFAs) on the skin.[56] Previous work has shown that 90−99% of FFAs on the living layer of skin are below C_{20} in chain length.[57] Of the total FFAs, hexadecanoic and octadecanoic acids accounted for 75−82 wt %. The monounsaturated FFAs consist mainly of 86% 9-octadecenoic acid and 6−7% 9-hexadecenoic acid. Greater than 95% of the diunsaturated acids is 9,12-octadecadienoic acid. The positional isomers for sites of unsaturation are mainly at the 9- position for

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Table 1. Compounds Present or Suspected of Being Present from Thermal Desorption of Human Skin Emanations from Handled Glass. This List Was Derived from Cryofocused GC/MS Analyses of Four Human Subjects

compound	identified peak no.[a]	notes[c]	M_r[b]	compound	identified peak no.[a]	notes[c]	M_r[b]
				Carboxylic Acids			
acetic acid	26	S,5,6	60	methyltetradecanoic acid	233	C	242
2-propenoic acid	43	S,6	72	methyltetradecanoic acid	237	C	242
propanoic acid	40	S,5,6	74	pentadecanoic acid	239	S,5,6	242
2-butenoic acid	69	S	86	9-hexadecenoic acid	249	S	254
2-methyl-2-butenoic acid	72	C	100	methylpentadecanoic acid	246	C	256
3-methyl-2-pentenoic acid	81	C	114	hexadecanoic acid	250	S,5,6	256
3-methylpentanoic acid	89	C	116	heptadecenoic acid	255	C	268
hexanoic acid	90	S,6	116	methylhexadecanoic acid	253	C	270
heptanoic acid	109	S,6	130	heptadecanoic acid	258	S,6	270
octanoic acid	139	S,6	144	11-phenoxyundecanoic acid	292	A,E	278
nonanoic acid	158	S,6	158	9,12-octadecadienoic acid	263	S	280
decanoic acid	161	S,6	172	9-octadecenoic acid	267	S,5	282
undecanoic acid	176	A,6	186	methylheptadecanoic acid	264	C	284
dodecanoic acid	190	S,6	200	octadecanoic acid	269	S,5,6	284
methyldodecanoic acid	205	C	214	docosanoic acid	295		340
tridecanoic acid	209	S,6	214	lactic acid	117	S,2,5,6	90
tetradecenoic acid	217	C	226	hexanedioic acid	286	A,E	146
methyltridecanoic acid	220	C	228	heptanedioic acid	291	A,E	160
tetradecanoic acid	223	S,6	228	benzoic acid	142	S,6	122
pentadecenoic acid	238	B	240	4-hydroxybenzoic acid	152	C	138
methyltetradecanoic acid	232	C	242	4-hydroxy-3-methoxybenzoic acid	221	B	168
				Alcohols			
2-butanol	18	C,(1?),2	74	dodecenol	175	A	184
3-methyl-4-penten-2-ol	42	B,C	100	tridecanol	189	A	200
2-hexen-1-ol	59	C	100	1-tetradecanol	210	C,5	214
4-hexen-1-ol	56	C	100	2-hexadecanol	245	C,(5?)	242
1-hexen-3-ol	60	C,(1?)	100	2-heptadecanol	254	B,C	256
2-methyl-3-pentanol	62	B,C	102	cholest-5-en-3-ol	303	S,6	386
1-hepten-3-ol	83		114	phenol	110	S,5,6	94
1-octen-3-ol	111	A,D	128	benzyl alcohol	125	S,5,6	108
2-octen-1-ol	102	C	128	phenylethyl alcohol	141	A,E,5	122
2-methyl-3-octenol	120	B,C	142	2-(2*H*-benzotriazol-2-yl)-4-methylphenol	260	A,D	225
nonenol	134	C	142	ethylene glycol	112	S,2,6	62
3,7-dimethyl-6-octen-1-ol	144	B,C	156	glycerol	131	S,6	92
decenol, substituted	153	D	156	2-(hydroxymethyl)-2-methyl-1,3-propanediol	96	A,E	120
2-decanol	155	S,(5?)	158				
				Aldehydes			
propanal	6	S,2,6	58	nonanal	135	S,5	142
2-methylpropanal	12	6	72	3,7-dimethyl-2,6-octadienal	133	B,C	152
2-methyl-2-butenal	23	B,C	84	decanal	157	B,5	156
2-methylbutanal	31	C,6	86	dodecanal	171	B	184
3-methylpentanal	49	C	100	2-methylhexadecanal	213	A,B	254
heptanal	88	S,2,5	114	benzaldehyde	106	S,5	106
2,2-dimethylhexanal	103	C,(2?)	128	3-hydroxy-4-methylbenzaldehyde	132	B	136
octanal	108	B,5	128	4-phenylmethoxybenzaldehyde	243	A	212
2,4-nonadienal	113	C	138				
				Aliphatics/Aromatics			
pentene	14	B	70	2-methyl-2-undecene	124	D,E	168
4-methyl-2-pentene	17	C,(2?)	84	3-methyl-5-undecene	123	D,E	168
hexane	19	S,1,2,3	86	4-methyl-4-undecene	119	B,C	168
dimethylpentadiene	24	B,C	96	2-methyl-2-dodecene	145	C	182
2-methyl-1-hexene	33	C	98	methyldodecene	147	C	182
heptane	35	B,1,2,3	100	tetradecene	163	C	196
3-ethyl-1,4-hexadiene	45	A,B,(2?)	110	cyclotetradecane	214	E	196
2-methyl-1-heptene	46	C	112	tetradecane	172	B	198
2-octene	55	2	112	pentadecane	181	S	212
3-octene	54		112	hexadecene	196	D,E	224
4-octene	53		112	cyclohexadecane	240	E	224
2,4-dimethylhexane	57	C,(1?),(2?)	114	hexadecane	197	S	226
octane	58	S,1,5	114	heptadecane	215	S,5	240
3,4-nonadiene	63	C	124	9-octadecene	226	C	252
2,6-dimethyl-1-heptene	61	B,C,(1?)	126	methylheptadecane	229	C	254
4-ethyl-3-heptene	80	B,C	126	octadecane	230	S,5	254
4-nonene	85	C,(2?)	126	trimethyl-3-methylenehexadecane	248	A,B	272
nonane	87	S,1,5	128	heneicosane	259		296
N-menth-6-ene	127	A	138	docosane	271	S,5	310
menthane	130	A	140	tricosane	274	5	324
trimethyl-1,5-heptadiene	74	D,E	138	tetracosane	287	A,5	338
2,7-dimethyl-1-octene	84	A,E	140	pentacosane	297		352
5-decene	101	C,(2?)	140	cholesta-3,5-diene	301	A,B	392
decane	114	S,2,5,6	142	triacontene, branched	300	C	410
undecadiene	115	B	152	squalene	299	S,4,6	410

Table 1 (Continued)

compound	identified peak no.[a]	notes[c]	M_r[b]	compound	identified peak no.[a]	notes[c]	M_r[b]
Aliphatics/Aromatics (Continued)							
benzene	29	S,1,2,3,6	78	ethylbenzene	78	1,2,3	106
toluene	51	S,1,2,3,6	92	propylbenzene	104	(1?)	120
styrene	86	S,2,3	104	4,4′-dimethyl-1,1′-biphenyl	228	A	182
1,4-dimethylbenzene	77	C,1,2,3,5	106				
Amides/Amines/Related							
propanamide	128	S	73	*N*-methyl-*N*-nitroso-1-dodecanamine	275	A	228
N,*N*-diethyl-3-methylbenzamide (DEET)	198		191	*N*,*N*-dimethyl-1-dodecanamine	183		213
N,*N*-bis(2-hydroxyethyl)dodecanamide	185	A,E	287	*N*,*N*-dimethyl-1-tridecanamine	199		227
N,*N*-didodecyl formamide	285	B	381	*N*,*N*-dimethyl-1-tetradecanamine	216		241
1,3-butanediamine	22	B	84	*N*,*N*-dimethyl-1-pentadecanamine	234		255
N,*N*-dimethyl-1,2-ethanediamine	5	D,E	116	*N*,*N*-dimethyl-1-hexadecanamine	244		269
N-ethylcyclopentamine	219	A	113	*N*,*N*-dimethyl-1-heptadecanamine	252		283
N,*N*-dimethyl-3-butoxypropylamine	167	A	159	*N*,*N*-dimethyl-1-octadecanamine	262		297
N,*N*-dimethyl-3-benzyloxypropylamine	170	B	193				
Esters							
2-butenoic acid, butyl ester	38	A,(2?)	128	tetracosanoic acid, methyl ester	298	A,B	382
butanoic acid, methyl ester	39	A,B,2	102	pentanedioic acid, ester	273	D,E	>144
nonanoic acid, methyl ester	184		172	pentanedioic acid, mono(2-ethylhexyl) ester	276	C	244
tridecanoic acid, methyl ester	207	A	228	hexanedioic acid, ester	278	D,E	>160
13-methylpentadecanoic acid, methyl ester	235	C	270	hexanedioic acid, ester branched	283	C,D,E	>160
14-methylpentadecanoic acid, methyl ester	247	C	270	hexanedioic acid, mono(2-ethylhexyl) ester	280	C	258
methylhexadecanoic acid, methyl ester	251	C	284	hexanedioic acid, octyl ester	288	B	258
heptadecanoic acid, methyl ester	257	C	284	heptanedioic acid, dibutyl ester	294	B	272
octadecenoic acid, methyl ester	261	C	296	hydroxybutanoic acid, ethyl ester	52	A	128
hexadecanoic acid, methyl ester	256		270	3-hydroxybenzoic acid, methyl ester	178	C	152
16-methylheptadecanoic acid, methyl ester	266	C	298	4-hydroxybenzoic acid, propyl ester	204	B,C	180
octadecanoic acid, methyl ester	265	B	298	2-hydroxybenzoic acid, phenylmethyl ester	242	B,C	228
hexadecanoic acid, butyl ester	270		312	2,4-dihydroxy-3,6-dimethylbenzoic acid, methyl ester	218	A,E	196
octadecanoic acid, phenyl ester	293		360				
tetradecanoic acid, undecyl ester	282	A,B	382				
Halides/Related							
methyl iodide	9	6	142	1-chlorohexadecane	241	B	260
1-chlorohexane	76	1	120	1,6-dichloro-1,5-cyclooctadiene	160	B,D	176
1-chloroheptane	105		134	benzylchloride	118	S,6	126
1-chlorononane	129		162	3-chlorobenzeneamine	149	C	127
1-chlorododecane	180		204	2-chloro-1-methylethylbenzene	168	D	154
1-chlorotetradecane	202		232	1-chloro-4-(4-methyl-4-pentyl)benzene	169	D	194
1-chloropentadecane	225	B	246	2,3-dichlorobenzeneamine	177	C	161
Heterocyclics							
2-methyl-1*H*-pyrrole	71	(5?)	81	trimethylpyrazine	116	A	122
3-methyl-1*H*-pyrrole	73		81	3-ethyl-2,5-dimethylpyrazine	208	C	136
pyridine	48	S,5,6	79	5-methyl-2-methylthio-4(1*H*)-pyrimidinone	212	A	156
3-pyridinamine	67		94	1-methylpiperazine	121	C	100
4-pyridinamine	66		94	2-ethylpiperazine	224	C	114
4-methyl-2-pyridinamine	95	C	108	2,5-dimethylpiperazine	173	C	114
2-methylpyridine	79	C,2,5	93	oxazole	37		69
4(1*H*)-pyridinone	70		95	2-methylfuran	21		82
6-amino-3-pyridine carboxylic acid	201	B	138	3-methylfuran	20		82
3(1-methyl-2-pyrrolidinyl)pyridine (nicotine)	166	4	162	2,3-dihydro-4-methylfuran	15	B,C,(5?)	84
1,2,3-4-tetrahydroquinoline	154		133	2-furanmethanol	75	(2?)	98
2,3,4-trimethylquinoline	203	A	171	benzofuran	136	2	118
2-ethylpiperidine	122	(2?)	113	dihydro-5-tetradecyl-2(3*H*)furanone	268	B	282
1-piperidineethanol	97	D,E	129	2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	140	A	144
4-piperidinemethanamine	195	A	114				
1-phenyl-3-(1-piperidinyl)-2-buten-1-one	289	A	229	2-methoxy-6-methyl-4*H*-pyran-4-one	206	B	140
1*H*-indole	191	S,6	117	2*H*-1-benzopyran-2-one	179	C	146
indole, substituted	227	D	>117	3-acetyl-6-methyl-2*H*-pyran-2,4(3*H*)dione	164	A	168
4,5-dihydro-2-methyl-1*H*-imidazole	94		84	thiazolidine	211		89
1,5-dimethyl-1*H*-pyrazole	143	C	96	2-methylisothiazole	186	A	99
pyrazine	44		80	5-methyl-2(5*H*)thiophenone	162	A,B	114
2,6-dimethylpyrazine	93	B	108	2-methoxy-5-methylthiophene	236	C	128
Ketones							
butanone	11	S,2,3,5,6	72	2-nonen-4-one	138	B	140
2-pentanone	34	S,2,5	86	2-decanone	146	S	156
3-pentanone	30	S,2,6	86	2-methoxy-2-octen-4-one	150	B,C	162
2-hexanone	36	C	100	6,10-dimethyl-5,9-undecadien-2-one	174	A	194
6-methyl-3,5-heptadien-2-one	82	C	124	3-hydroxyandrostan-11,17-dione	302	A	304
6-methyl-5-hepten-2-one	107	S	126				
Sulfides							
carbon disulfide	10	3,5,6	76	dimethyl disulfide	47	S,5	94

Table 1 (Continued)

compound	identified peak no.[a]	notes[c]	M_r[b]	compound	identified peak no.[a]	notes[c]	M_r[b]
Thio/Thioesters/Sulfonyls							
thiomethane	4	2,5,6	48	*o*-(2-butenylthio)phenol	231	B	180
2-thiopropane	50		76	thiocarbamic acid, butyl ester	32	A,E	127
1-methylthiobutane	194	C,(2?)	104	acetylthiocarbamic acid, methyl ester	193	B	130
1-thiododecane	188	D	202	methanesulfonylchloride	16		114
3-methylthietane	68	A	88	1,1′-sulfonylbis[4-chlorobenzene]	272	B,C	286
3-(methylthio)-1,2-propanediol	165	D	122				
Urea/Related							
urea	25	6	60	thiourea	64	6	76
methylurea	41	6	74	*N*,*N*-dimethylthiourea	91		104
Miscellaneous							
1-isocyanato-3-methylbenzene	192	B	133	1,2,3-trimethoxypropane	156	A	134
1,3-dimethoxybutane	126	A,E	118	1,4-benzenedicarbonitrile	148	A	128
Compounds Present in Background/Blank Analyses							
methyl chloride	1	6	50	3-isopropoxy-1,1,1,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	182		576
tetrachloroethene	7	3,5	164				
1,1-difluoroethane	3	S	66	2,4-bis(1,1-dimethylethyl)phenol	99	C,1,5	206
fluoroethene	2		46	2,5-bis(1,1-dimethylethyl)phenol	98		206
1,1,2-trichloro-1,2,2-trifluoroethene	8		167	2,4-bis(1,1-methylpropyl)phenol	100		206
1-silacyclo-3-pentene	27		84	4,6-di(1,1-dimethylethyl)-2-methylphenol	151	B	220
silacyclopentane	28		86	1,2-benzenedicarboxylic acid, diethyl ester	200	5	222
trimethylsilanol	13		90	1,2-benzenedicarboxylic acid, butylphenylmethyl ester	277		312
hexamethylcyclotrisiloxane	65		222	1,2-benzenedicarboxylic acid, diheptyl ester	279		362
octamethylcyclotetrasiloxane	92		296	1,2-benzenedicarboxylic acid, diisooctyl ester	284		390
decamethylcyclopentasiloxane	137		370	1,2-benzenedicarboxylic acid, bis(2-ethylhexyl ester)	281		390
dodecamethylcyclohexasiloxane	159		444	1,2-benzenedicarboxylic acid, diisononyl ester	290	A	418
tetradecamethylhexasiloxane	187		458	1,2-benzenedicarboxylic acid, undetermined ester	286	D,E	>415
hexadecamethylcycloheptasiloxane	222		532				

[a] Peak numbers are assigned only to peaks identified wholly or partially and represent the elution order on an HP5 column. [b] Listed with each compound is the corresponding relative molecular mass (M_r). [c] Explanation of notes: (S) Confirmed by comparison of retention time and/or mass spectrum to that of the standard. (A) Identity of the compound is questionable, although the most reasonable identity is listed. The EI library search purity value is low (less than 750) either from the absence of characteristic masses in the sample mass spectrum or the presence of additional masses in the sample spectrum compared with the library spectrum. (B) The M_r of this compound is known from CI analysis. The actual structure given is questionable due to differences in fragmentation pattern of the library mass spectrum compared with the sample mass spectrum. (C) The structure is known to a reasonable degree of certainty. Uncertainty exists in the location of the substituted functional group and/or in the double-bond location. (D) The class or base structure of this compound is characterized by a specific EI fragmentation pattern. The information from CI may not be available to assist in determining the identity of this compound. (E) The M_r from CI analysis for this compound is questionable. (1) Reported previously in breath.[34] (2) Reported previously as a volatile human effluent.[39] (3) Reported previously as a volatile in human blood.[49] (4) Reported previously as a component of fingerprint residue.[35] (5) Reported previously as a volatile human odor.[40] (6) Reported previously as a volatile human odor or present in effluent.[36−38] (?) Compound reported previously is a different isomer of the listed compound.

hexadecenoic and octadecenoic acids, and 9,12- for octadecadienoic acid. These three compounds comprise greater than 90% of the unsaturated isomers, and all of the above-mentioned compounds are readily seen here. Although the method used in this paper is not amenable to the detection of hydroxy acids larger than L-lactic acid, there are α-hydroxy acids on the skin, ranging from C_{14}−C_{26}, with C_{16} and C_{18} as the primary constituents. Internally branched FFAs were not detected, except possibly in the case of methyltetradecanoic acid; this is probably a result of the low sample volume collected combined with the low amount of these compounds present. Although the sensitivity was not adequate to provide information on trace levels of fatty acids, it is important to keep in mind that the method described herein involves little sample preparation and no concentration or cleanup steps. Lipids greater than 18 carbons in backbone chain length are less intense under the chromatographic conditions of this manuscript. These were condsidered less important because they are thought to be too involatile to factor into mosquito attraction.

Comprehensive discussions of all six series variations of FFAs can be found elsewhere.[55,58,59] The primary fatty acids are straight-chain, even-numbered carbon backbones, split approximately evenly between fully saturated and monounsaturated compounds. This series accounts for approximately 70% of the FFAs. Straight-chain, odd-numbered carbon-backboned saturated and monounsaturated FFAs comprise another 10%. About 10% contain an iso- methyl branch at the terminus opposite the acid moiety, while 3% have an anteiso- methyl branch three carbons in from the terminus opposite the acid functional group. Internally branched monomethyl fatty acids constitute 3% of FFAs, and the remaining 4% consists of diunsaturated straight-chain and dimethyl-branched FFAs.[58] Inspection of Table 1 indicates that several methyl-branched fatty acids were observed, and these are probably the iso- configurations, although additional effort was not made to identify the actual position of branching. There are three methyl-branched tetradecanoic acid isomers observed; therefore, at least one of these has an internal methyl branch.

When the chromatography and recording of mass spectral information was continued past the final retention time of Figure 1, a series of fatty acid lipid esters was observed but is not presented here; these were baked off of the column between

(58) Nicolaides, N. *Science (Washington, D.C.)* **1974**, *186*, 19−26.

(59) Nicolaides, N.; Apon, J. M. B. *Biomed. Mass Spectrom.* **1977**, *4*, 336−46.

experiments. The pattern of carboxylic acid peaks in this chromatogram and many of the chromatograms acquired using these parameters appears to be similar to that observed from bacteria.[43] Moreover, it is expected that some discrimination exists in this method against highly volatile, low relative molecular mass acids (as well as the more volatile compounds of other compound classes). Lactic acid, for example, was difficult to detect in EI mode while other hydroxy acids, such as tartaric acid, and oxo acids, such as pyruvic acid, were not detected by this method. Although not observed here, tartaric and pyruvic acids are known to be present in skin emanations.[38]

Numerous short-chain alcohols were observed, and most of these were unsaturated. Alcohols on the skin result from the reduction of FFAs present on the skin.[58] Here is a contrast between the large number of unsaturated alcohols observed in this study and the absence of unsaturated alcohols in previous studies.[39,40] Unfortunately, the location of unsaturation and location of the alcohol moiety in most of these compounds remains uncertain. A known attractant for some species of mosquitoes, 1-octen-3-ol,[21] is identified tentatively here and has been noted as a skin emanation in a recent publication.[60] There were two octenol isomers observed in this study, and one could possibly be 1-octen-3-ol as noted in Table 1.

The relatively intense, saw-tooth-shaped peak located at a scan number between 800 and 900 in Figure 1 (retention time between 8 and 10 min) presented an interesting problem with respect to identification, ironically because of the large amount of this compound present in the analysis. Because of the inability to locate a plausible mass-to-charge value for the molecular ion in the EI mass spectrum for this compound, a clue from the peak shape and relative molecular mass information from PCI and NCI were used to assist in the identification. The shape of this peak is similar to lactic acid (addressed below); thus, it was expected that this compound may also be a hydroxy acid or, at least, highly polar.

Visual inspection of PCI data revealed that a M_r of 92 was plausible due to the abundance of the apparent $[M + H]^+$ ion at m/z 93. However, the NCI mass spectrum did not contain an abundant $[M - H]^-$ ion at m/z 91. The NCI mass spectrum did contain an ion at m/z 183 which was postulated to be the $[M_2 - H]^-$ ion of this compound. When the library searching was restricted to compounds of 92 Da, a tentative identification of 1,2,3-propanetriol (glycerol) was made. The mass spectra did not match well, but final confirmation was achieved by comparing the sample EI mass spectrum to that of a standard of glycerol introduced into the ion source via a direct-inlet probe. When the ion source was saturated under these conditions, it was demonstrated that formation of a proton-bound dimer by self-CI had occurred, both here and previously, upon elution of glycerol during sample analyses. The presence of self-CI-produced fragments of this peak in the EI mass spectrum of the analysis in Figure 1 did not permit successful library matching. The glycerol peak from all other subjects was at least two orders in magnitude smaller than the amount detected from this subject. It was later determined that the high glycerol level was attributable to the Sta-Sof-Fro hair and scalp spray used by this subject; glycerol is one of the primary ingredients in the product. This example is a clear case of a substance of exogenous origin being introduced, or at least greatly increased in amount present, on the skin. A strict routine and abstinence from hygiene products would most likely have reduced the exogenous substances; however, some substances persist in excess of 2 weeks on the skin.[50] This persistence and the difficulty with keeping volunteers on a strict identical routine make it difficult to perform an analysis without some exogenous compounds being present. Although this complicates the analysis by the addition of compounds that most likely have no effect on the host-seeking process of mosquitoes, the compounds of interest are still present and, except for the siloxanes discussed later and the above case of glycerol, in significantly greater amounts than exogenous substances.

A poorly chromatographed peak in these experiments was located approximately at scan 680 (retention time of 6 min). The peak is typically one of the largest observed when examined in PCI and NCI modes, but fails to produce a significant peak in EI mode. Examination in NCI mode indicates that this peak is lactic acid due to its relative molecular mass and characteristic fragment ions.[41] Lactones have been detected on the skin,[40] although none are reported here. Some of these may constitute a portion of the 43 compounds that are still unidentified.

Aldehydes, Aliphatics, Aromatics, and Ketones. Chromatographic peaks for the aldehydes became more intense as the series progressed from propanal to nonanal, then became less intense. Some substituted aldehydes were observed. Although nonanal coeluted with glycerol on an HP5 column under the conditions described previously, it was readily identifiable due to its intensity. Curiously, butanal, hexanal, and undecanal were not observed in the described series which runs from propanal to dodecanal. These three aldehydes have not been observed in previous work on human effluents,[39] but were observed in microscale purge and trap GC/MS analysis of human emanations in our laboratory.[41]

There are numerous alkanes and alkenes present below decane and possibly others at levels too low for detection. Additionally, it is believed that some of these compounds were lost to rapid evaporation because of the high volatility of low relative molecular mass compounds. When samples were analyzed with a GC program ramped to a higher temperature and with a shorter column than reported here, both squalene and cholesterol were observed as intense peaks and they elute after octacosane and triacontane, respectively, on a DB-1 column. The analyses reported in this manuscript were typically ended just after pentacosane eluted.

Benzene, toluene, styrene, and some alkyl-substituted benzenes were found on handled beads. These have been reported elsewhere as being present in human breath and on human skin;[34,39] however, these are most likely exogenous. Additionally, dimethyl biphenyl is the best library match to peak 228; however, given the low purity value of the EI library search, this identity is questionable.

Few ketones were observed, with most of the ketone peaks attributed to those between butanone and decanone. A trace amount of hydroxyandrostandione was identified in one of the analyses, but the identity of this compound is also suspect. Acetone was not observed but is believed to be present during the handling of glass and then lost due to its high volatility.

(60) Cork, A.; Park, K. C. *Med. Vet. Entomol.* **1996**, *10*, 269−76.

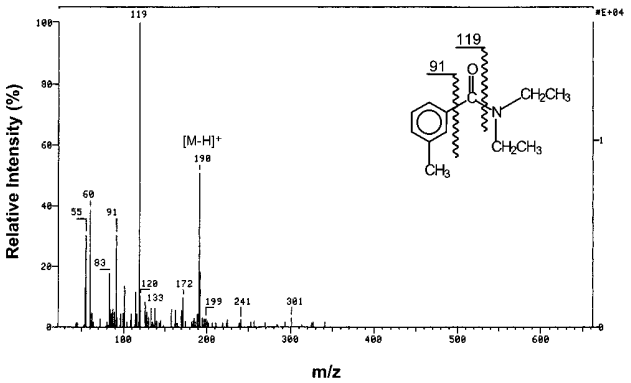

Figure 2. Electron ionization mass spectrum of the GC peak corresponding to *N,N*-diethyl-3-methylbenzamide (DEET). The mass spectrum is from an analysis of five handled glass beads.

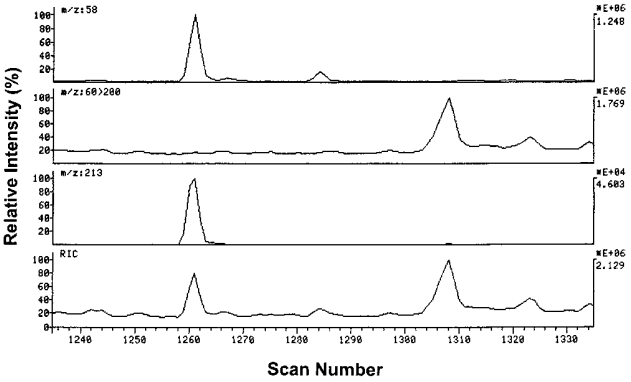

Figure 3. Mass chromatograms of (a) *m*/*z* 58, (b) *m*/*z* 60−200, (c) *m*/*z* 213, and (d) the reconstructed ion chromatogram for the GC peak (scan 1261) corresponding to *N,N*-dimethyl-1-dodecanamine. The chromatograms are from an EI analysis of three handled glass beads.

Amides, Amines, and Heterocycles. Very few amides were found in the samples. The identification of *N,N*-diethyl-3-methylbenzamide (DEET) on the skin of some of the subjects was unexpected and certainly of exogenous origin. Three of the four subjects were involved in routine tests involving topical application of DEET to the skin. Therefore, it is plausible that residual DEET on the skin remained after repeated washing and was transferred to the glass beads. An accurate account of the persistence of this chemical is not possible, but it is believed that tests were conducted with DEET during the week prior to the attractant emanation study. This equates to DEET being detectable by the method employed herein for 3−8 days after application on the skin. The EI mass spectrum for this identified compound from an analysis of one of the human subjects is presented in Figure 2.

A homologous series of tertiary amines was observed in some of the samples from subjects, spanning from *N,N*-dimethyl-1-dodecanamine to *N,N*-dimethyl-1-octadecanamine. Some of these amines eluted at retention times near those of similarly structured carboxylic acids, and their peaks were often hidden under the more abundant acids. Examination of EI reconstructed ion chromatograms showed only two significant ions were present (Figure 3). These ions were the base peak at *m*/*z* 58 (due to α cleavage of the chain) and the corresponding odd-mass molecular ion in the mass spectrum of Figure 4. Library matching initially did not provide the correct identity for most of these homologous

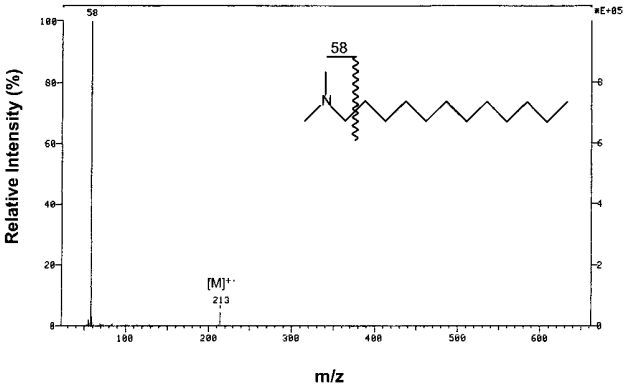

Figure 4. Electron ionization mass spectrum of the GC peak corresponding to *N,N*-dimethyl-1-dodecanamine. The mass spectrum is from an analysis of three handled glass beads.

amines. Upon inspection of library mass spectra for suspected amines, it was determined that in addition to the 12-carbon or greater chain, substitutions of anything larger than methyl groups on the nitrogen would result in a base peak of higher mass-to-charge value in the mass spectrum. Once the proper restrictions were imposed on the library search, identifications of these amines were achieved readily for this series. Evidence for the endogenous or exogenous origin of these long-chain amines does not exist.

Many heterocyclic structures were observed. Substituted pyrroles, pyridines, piperidines, pyrazines, piperazines, and furans were detected in this study and are believed to be of human origin.[39] Pyridines and methylpyridines have been found to be elevated in the breath of subjects with periodontal disease.[61] An interesting finding was the presence of nicotine, albeit a low level, on beads handled by some subjects. It is possible that these may be related to tobacco use, rather than being deposited on skin from exogenous sources; however, there are insufficient data to support or refute this in this case. Nicotine has been observed on the skin in previous studies.[35]

Halides, Sulfur-Containing, and Urea-Related Compounds. In addition to the observation of methyl iodide, which could not be accounted for as background, other haloalkanes were observed. An incomplete series of chloroalkanes from chlorohexane to chlorohexadecane was observed. Some of these chloroalkanes were reported previously in human breath.[34]

It is expected that breath contains relatively higher amounts of malodorous sulfides than are present on the skin.[61] Several sulfides are produced by bacteria and known to be endogenous.[34,40,61] Carbon disulfide and dimethyl disulfide were readily observed in this study, while dimethyl trisulfide is not listed here because it was only observed using purge and trap analysis. A few mercaptans, such as thiomethane and 2-thiopropane, are also present, as is urea.

Miscellaneous and Background Compounds. Some residual halides and chlorofluorocarbons were observed, but the background/blank samples mainly consisted of siloxanes believed to come from the column stationary phase and phthalate contaminants. Siloxanes were identified readily in analyses of volatile

(61) Kostelc, J. G.; Preti, G.; Zelson, P. R.; Stoller, N. H.; Tonzetich, J. *J. Periodontal Res.* **1980**, *15*, 185−92.

organic compounds in the cabin air of the Skylab spacecraft.[62] These siloxanes are not expected to be endogenous. These two series of compounds dominated chromatograms from the analysis of blank beads. Hexamethylcyclotrisiloxane and octamethylcyclotetrasiloxane were often readily observed in sample analyses (see the italicized peak numbers *65* and *137* of Figure 1).

One component, 1,1-difluoroethane (Dust-Off), was introduced into the system because it was employed to cool the glass injection port liner between runs prior to introducing a sample. When the glass injection port liner was reinserted and the analysis conducted, latent difluoroethane eluted off of the column relatively early and was usually one of the most intense peaks. The retention time of this component was used, for qualitative purposes only, as a means to correlate some of the repetitions. This compound is not listed in the table since it was not introduced into the system via transfer from the skin to glass beads.

CONCLUSIONS

The compounds present on skin were similar among the human subjects, but the relative amounts of many compounds differed between subjects. This was expected since skin lipids were already known to be very similar for all humans. Observations based on these experiments indicate that some compounds, one of which is L-lactic acid, in the emanations of a human, can vary in abundance by an order of magnitude on consecutive days. The abundance of other compounds, such as dodecanoic and tetradecanoic acids, tend to change little when compared on consecutive days.

A wide range of compound classes was identified, and some unexpected compounds which were observed and identified were later explained as being exogenous in origin. Exogenous compounds are expected to be the most difficult problem that will need to be overcome before meaningful results can follow from comparison of different subjects or comparison of the same subject over consecutive days.

At least 346 distinct peaks were detected by cryofocused GC/MS analyses. Of the compound peaks detected, 43 were unidentified while 303 were identifed by standard or identified tentatively by library and spectral interpretation. There were 26 compounds confirmed to be of background origin, leaving 277 compounds as candidate attractants for *Ae. aegypti*. The number of compounds observed makes for a difficult and tedious bioassay process. Even with such a large number, there is no guarantee that all of the important compounds have been detected, and it is strongly believed that they have not been.

The next phase of this work will focus upon an approach to more selectively determine candidate volatiles that may be factors in mosquito attraction. Experiments will be performed to compare skin emanations between two subjects that differ markedly in attraction, to compare day to day variations in emanations, to correlate these to attraction measured by bioassay and, on the basis of knowledge that handled glass remains attractive less than 6 h, to identify compounds that are decreased significantly after 6 h of storage on glass beads.

(62) Liebich, H. M.; Bertsch, W.; Zlatkis, A.; Schneider, H. J. *Aviat. Space Environ. Med.* **1975**, *46*, 1002−7.

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