Journal of Chromatography, 163 (1979) 294—299
Biomedical Applications

CHROMBIO. 352

Note

Analysis of human axillary volatiles: compounds of exogenous origin

J. LABOWS* and G. PRETI

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, Pa. 19104 (U.S.A.)

E. HOELZLE

University of Munich, Munich (G.F.R.)

and

J. LEYDEN and A. KLIGMAN

Duhring Laboratories Dept. of Dermatology, University of Pennsylvania, Philadelphia, Pa. 19104 (U.S.A.)

(First received November 7th, 1978; revised manuscript received April 11th, 1979)

Considerable speculation has appeared concerning the possibility of chemical communication among humans. Anecdotal information as well as scientific reports including those dealing with mammalian odor communication have fueled this speculation [1, 2]. The human axilla has been suggested as an area especially suited for the development of odors which may affect human reproductive biology. The following factors present in the axillae aid in odor production: (a) unique secretion from the apocrine gland which can serve as a bacterial substrate, (b) moisture from the eccrine glands, (c) a resident population of bacteria, (d) lipid secretions from the sebaceous gland to serve as an odor fixative, and (e) the presence of hair to assist in odor dispersal [3].

Despite the considerable resources expended on control and masking this odor, little is known concerning the nature of the odoriferous constituents being produced. Our efforts to isolate and identify these odorous compounds were begun by sampling the odor directly by sweeping the axillary headspace and also by collection of odors on cotton pads. These collections have led to the identification of a large number of volatile constituents many of which appear to be synthetic perfume components as well as pollutants in our atmosphere and drinking water. A number of these compounds were both odorous and physiologically active and may be contributing to our normal body odor.

METHODS AND MATERIALS

Subjects

A panel of ten subjects, four male and six female, for the pad study and three subjects (male) for the direct cup sampling (ages 18—23 years) followed a regimen involving use of only a non-bacteriostatic soap for two weeks and no soap 48 h prior to sampling. Previous investigations have shown this protocol to produce strong typical axillary odor in most individuals [4].

In this panel, the protocol gave axillary odors of weak to strong intensity. The odor was rated on a scale of 0—3 by several workers experienced in axillary odors, where the values represent none, discernable, moderate, and strong-unpleasant odors, respectively. Only individuals with strong odors were selected for direct sampling.

Odor collection

Direct sampling of the axillae employed a cup (plastic funnel with PTFE and metal fittings) which was held tightly over the axillae. A small pump was employed to draw room air through an activated charcoal filter and over the axillae. Attached to the exit of the cup was a 6 in. × 1/8 in. stainless-steel tube containing 70 mg of Tenax (Applied Science, State College, Pa., U.S.A.). Collections were 15 min for each axillae on the same tube at a flow-rate of 40—60 ml/min. Prior to collection, a fresh tube of Tenax was employed to sample the same amount of room air.

Collection of the odors used cotton pads which were previously extracted with chloroform—methanol (85:15, v/v), ether, and vacuum dried [5]. For each subject a pad was worn overnight in one axilla, while the other axilla was left uncovered and rated for odor upon the subjects's arrival in the laboratory. After removal, the pad was placed in a glass tube, warmed to 50° and swept with nitrogen (flow-rate 80 ml/min for 16 h) to transfer the volatiles to Tenax. Upon removal of the pads from the collection tube they were odorless, even those which initially had strong odors.

Gas chromatographic and gas chromatographic—mass spectrometric (GC—MS) analyses

The volatiles were thermally desorbed from the Tenax tube by heating in the helium stream at 220° for 10 min. Dry ice was placed around the front 15 cm of the chromatographic column to condense the desorbed volatiles. The chromatograph used was a Perkin-Elmer (PE) 990 equipped with flame ionization detector and a 10 ft × 2 mm I.D. Pyrex 10% Carbowax 20M column for separation. Analysis conditions were as follows: temperature 70° (4 min), 70°—220° (4°/min); helium flow-rate 40 ml/min; injector 250°; detector 250°. The GC—MS system consists of a PE 990 interfaced with a PE/Hitachi RMU-6L mass spectrometer via a Watson—Biemann separator [6]. Structural assignments were based upon mass spectral comparisons with either authentic samples or literature spectra. Relative retention times of unknown and authentic samples were obtained by comparing their elution times with a series of n-C_2—C_{18} fatty acid ethyl esters. This yields a fatty acid ethyl ester (FAEE) index for each compound [7].

It was demonstrated that short-chain aliphatic acids were not transferable from a moist cotton pad (axillary pads absorb ca. 1 g moisture during sample collection). The aliphatic acids gave low to moderate efficiencies (10—50%) from aqueous solutions even when the solutions were acidified and salted. Esters, hydrocarbons, furfuryl alcohol, cresol, and indole were transferred efficiently (30—90%) from both pads and aqueous solution. In other studies, where other specific groups of compounds have been collected on Tenax, high efficiencies have been reported [8].

RESULTS

The volatiles found in the direct sampling of the axillae are listed in Table I. Many of these compounds have been identified in studies of urban air and are thought to arise from "man-made emissions" [9—12]. Room air controls showed the presence of alkylbenzenes and chlorinated ethylenes (Table I). These chemicals have also been identified in an analysis of volatiles from blood plasma [13—15].

TABLE I

CHEMICALS IDENTIFIED ON DIRECT SAMPLING OF AXILLAE

Benzene*, **, ***	Trimethylbenzenes*, **
Toluene*, **, ***	Methylene chloride*
Xylenes*, **, ***	Chloroform**
Ethylbenzene*, **, ***	Limonene*, **
2-Ethylhexanol	6-Methyl-hept-5-en-2-one
Trichloroethylene*, **	Acetone***
Tetrachloroethylene*, ***	

*Found in room air blanks.

**Found in rural and urban air samples (ref. 10).

***Found in normal blood plasma (ref. 11).

Fig. 1 shows a typical GC trace of volatiles collected from the cotton pads. The major identified components are summarized and include a series of isopropyl esters of fatty acids, principally isopropyl myristate and isopropyl palmitate. These esters are known components of deodorant and cosmetic preparations [16]. In addition, commercial samples of palmitic and myristic acids were shown to contain small amounts of other C-12 to C-18 fatty acids as impurities and this may account for their presence in the axillary samples [17]. A number of compounds in Fig. 1 are known fragrance chemicals which are added to soaps and cosmetics. Two compounds, 2-ethylhexanol and diethylphthalate, are commonly found in biological samples [18].

Antioxidants, such as di-*tert.*-butyl-hydroxytoluene (BHT), are found in approximately half of the samples including one subject from direct sampling. In addition, the mass spectral data also suggest the presence of di-*tert.*-butyl-hydroxyanisole.

The aldehydes and isopropyl esters were found in most subjects. Compounds identified from only a few subjects include nonenyl salicylate (T), methyl and

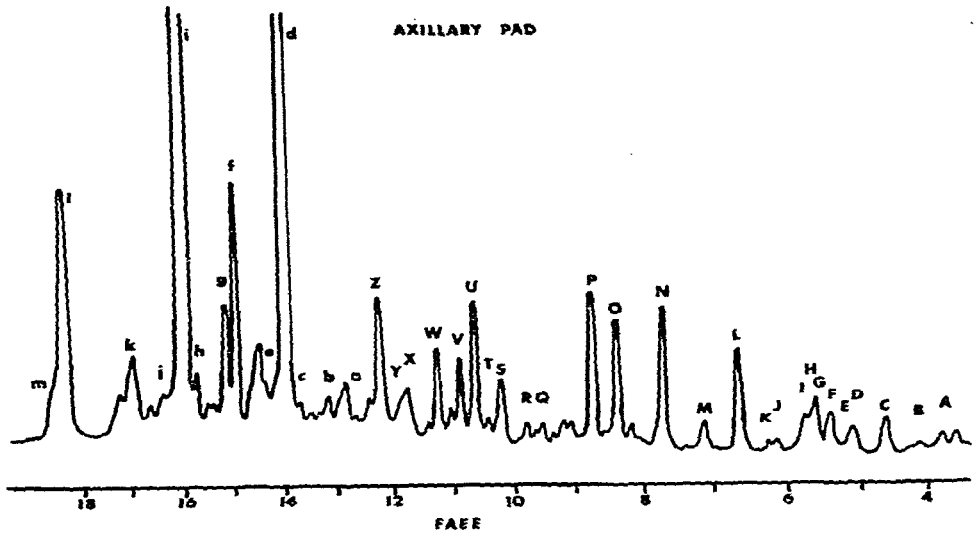

Fig. 1. GC analysis of volatiles from axillary pad concentrated on Tenax and separated on 10 ft. x 2 mm I.D. 10% Carbowax 20M column programmed at 70° (4 min), 70°—220° (4°/min). Ordinate values refer to FAEE Indices. Numbers refer to *m/z* values for assumed molecular ion (*) and significant fragment ions (underlined). T = Tentative. Peaks: A = propylfuran (T), B = tetrachloroethylene, C = hexanal, D = butylfuran, E = xylene, F = C_9-ketone (142*), G = heptanal, H = cyclopentanone (T), I = limonene, K = unknown (122*), L = octanal, mesitylene C_3-alkylbenzene, M = 6-methyl-hept-5-en-2-one, N = nonanal, O = unknown (ketone, T), P = decanal, Q = unknown (204*, 142) R = undecanal, S = furfuryl alcohol, T = unknown (126*, 111), U = naphthalene, unknown (210*, 192), V = heptadecane, W = unknown, X = octadecane, Y = isopropyl laurate, Z = geranyl acetone, unknown (243, 71, 43), a = butylated hydroxytoluene, b = isopropyl tridecanoate, lauryl alcohol (C-12), c = methyl myristate, d = isopropyl myristate, e = *di-tert.*-butyl-hydroxyanisole (T), f = isopropyl pentadecanoate, g = myristyl alcohol (C-14), h = methyl palmitate, i = isopropyl palmitate, j = ethyl palmitate, k = isopropyl heptadecanoate, diethyl phthalate, l = isopropyl stearate, m = nonenyl salicylate (262*, T).

ethyl esters of myristic and palmitic acids, diphenylamine, myristyl propionate (major product in one subject), and C-17, 18, 21, 22, 23, 24 hydrocarbons [19].

In both direct sampling and the pad users, the amounts and type of constituents present did not appear to correlate with the perceived odor intensity of the axillae or the pad. In addition, sampling the odors eluting from various positions of the chromatograph did not reveal any individual constituents which smelled like apocrine odor.

DISCUSSION

Our study to characterize the volatiles associated with axillary odor hav shown that the major volatile constituents identified from the skin surface o the axillae are of exogenous origin. It is believed that many of the observe compounds are emissions from man-made sources in and around Philadelphi These pollutants undoubtedly adhere to and perhaps are concentrated in th unwashed axillary region (particularly hair) of our subjects who spend a majc

ity of their days in an urban atmosphere. Both the atmosphere pollutants and the soap/cosmetic constituents may contribute to the observed skin odors but do not form part of the "natural" odor.

A number of these materials, such as limonene, toluene, benzene and chlorinated hydrocarbons, have been detected in serum and urine samples and may also be excreted to the skin surface through the sweat glands [13—15, 20]. These materials are not observed in our GC investigations of freshly collected sebaceous and apocrine secretions and are most likely of exogenous origin.

Preliminary analyses in our laboratory of sebum from the scalp extracts and "pure" apocrine secretion have shown antioxidants to be present in these samples also. This suggests that compounds such as these arise from food sources and are excreted through the apocrine and sebaceous glands to the skin surface [21]. Man reportedly consumes about 0.1 mg per kg body weight daily of phenolic antioxidants [22]. BHT can be metabolized by the body and has been shown to inhibit the activation of certain carcinogens [22, 23]. However, other materials we have identified, such as the chlorinated ethylenes and benzene (Table I), are suspected carcinogens [24].

A previous investigation of axillary odors employed GC profiles of collected volatiles with subsequent evaluation of individual peaks for malodors. No structure elucidation was performed in this study [25]. Consequently, many of the major constituents could well have been exogenous materials co-eluting with odorous substances.

Other studies have examined the chemical composition of total body volatiles. In one such report, room air was sampled in the presence and absence of individual subjects. The volatile compounds which were identified and thought to arise from sweat included C-1 to C-3 alcohols, acetone, and acetic acid [26]. Another study which examined whole body volatile effluvia identified 135 volatile compounds from a complex GC trace containing about 300–400 constituents [27]. Alkylbenzenes, acetone, heptanal, and 2-ethylhexanol were identified by Ellin et al. [23] and are also reported here. Odorants produced in the axillae are undoubtably major contributors to human whole body odors however, neither our results nor those of Ellin et al. have identified constituents such as androgen steroids and/or C_2—C_5 aliphatic acids which have often been suggested as comprising whole body/axillary odors [2, 28].

Synthetic body odors fashioned by perfumers will often contain short-chain aliphatic acids to give them the "sweaty" note [29]. In our experience, the odor of these compounds alone differs markedly from that of axillary odor; however, they may constitute some part of the "odor bouquet" originating in the axillae. As noted above (Methods and materials) our experiments with short-chain acids showed that they were not transferable from moist cotton pads. Also, other studies suggest free androgen steroids are present in axillary sweat but at such low concentrations that our present collection and analysis procedures would not detect them [28, 29].

The presence of large amounts of exogenous compounds in the axillae have led us to examine alternate schemes for identification of the volatiles responsible for axillary odor. The most promising approach appears to be the generation of the characteristic odor by in vitro manipulation of pure apocrine secretion by heating and bacterial action. Odorants produced by these techniques are currently being investigated.

ACKNOWLEDGEMENTS

This research was supported in part by grant number 1 RO1 AM22023-01 from the National Institute of Arthritis, Metabolism and Digestive Disease.

REFERENCES

1 D. Muller-Schwarze and M.M. Mozell, Chemical Signals in Vertebrates, Plenum Press, New York, 1977.
2 A. Comfort, The Likelihood of Human Pheromones in M.C. Brich (Editor), Pheromones, North-Holland, Amsterdam, 1974, pp. 386—396.
3 W. Montagna and P. Parakkal, Structure and Function of Skin, Academic Press, New York, London, 1974.
4 J. Hurley and W. Shelley, The Human Apocrine Gland in Health and Disease, Thomas, Springfield, Ill., 1960.
5 G. Preti and G.R. Huggins, J. Chem. Ecol., 1 (1975) 361.
6 J.T. Watson and K. Biemann, Anal. Chem., 37 (1965) 844.
7 H. van den Dool and P.D. Kratz, J. Chromatogr., 11 (1963) 463.
8 G. Holzer, J. Oro and W. Bertsch, J. Chromatogr., 126 (1976) 771.
9 P. Ciccioli, G. Bertoni, E. Brancaleoni, R. Fratarcangeli and F. Bruner, J. Chromatogr., 126 (1976) 757.
10 J.P. Mieure and M.W. Dietrich, J. Chromatogr. Sci., 11 (1973) 559.
11 B. Versino, M. deGrott and F. Geiss, Chromatographia, 7 (1974) 302.
12 G. Holzer, H. Shanfield, A. Zlatkis, W. Bertsch, P. Juarez, H. Mayfield and H.M. Liebich, J. Chromatogr., 142 (1977) 755.
13 D. Dowty, D. Charlisle, J. Laseter and F. Gonzalez, Biomed. Mass Spectrom., 2 (1976) 142.
14 H.N. Liebich and J. Wöll, J. Chromatogr., 142 (1977) 505.
15 A. Zlatkis, W. Bertsch, D.A. Bafus and H.M. Liebich, J. Chromatogr., 91 (1974) 379.
16 Harry's Cosmeticology, 1, 6th ed., Chemical Rubber Co., Cleveland, Ohio, 1973.
17 J.P. Guilott, M.C. Martini and J.Y. Gianffret, J. Soc. Cosmet. Chem., 28 (1977) 377.
18 E. Jellum, P. Størseth, J. Alexander, P. Helland, O. Stokke and E. Teig, J. Chromatogr., 126 (1976) 487.
19 U.P. Schlynegger, Biochim. Biophys. Acta, 260 (1972) 339.
20 K.E. Matsumoto, D.H. Partridge, A.B. Robinson, L. Pauling, R.A. Flath, T.R. Mon and R. Teranishi, J. Chromatogr., 85 (1973) 31.
21 J. Labows, unpublished results.
22 J.L. Branen, J. Amer. Oil Chem. Soc., 52 (1975) 59.
23 L.W. Waftenberg, Amer. J. Dig. Dis., 19 (1974) 947.
24 Registry of Toxic Effects of Chemical Substances, DHEW publication No. 78-104-A,B, 1977.
25 A. Dravnieks, J. Soc. Cosmet. Chem., 26 (1975) 551.
26 V.P. Savina, N.L. Sokolov and E.A. Ivanov, Kosm. Biol. Aviakosm Med., 9 (1976) 76.
27 R.L. Ellin, R.L. Farrand, F.W. Oberst, C.L. Crouse, N.B. Billups, W.S. Koon, N.P. Musselman and F.R. Sidell, J. Chromatogr., 100 (1974) 137.
28 B.W.L. Brooksbank, R. Brown and J.A. Gustafsson, Experientia, 30 (1974) 864.
29 J. Amoore, Chemical Senses and Flavor, 2 (1977) 267.
30 C. Warren, personal communication.
31 M. Julesz, Acta Med. Acad. Sci. Hungar., 25 (1968) 273.
32 R. Claus and W. Alsing, J. Endocrinol., 28 (1976) 483.
33 D.B. Gower, J. Steroid Biochem., 3 (1972) 45.