On-line Detection of Human Skin Vapors

Pablo Martínez-Lozano[b,*] and Juan Fernández de la Mora[a]

[a] Department of Mechanical Engineering, Yale University, New Haven, Connecticut, USA
[b] SEADM, Parque Tecnológico de Boecillo, Valladolid, Spain

Vapors released by the skin in the hand of one human subject are detected in real time by sampling them directly from the ambient gas surrounding the hand, ionizing them by secondary electrospray ionization (SESI, via contact with the charged cloud from an electrospray source), and analyzing them in a mass spectrometer with an atmospheric pressure source (API-MS). This gas-phase approach is complementary to alternative on-line surface ionization methods such as DESI and DART. A dominating peak of lactic acid and a complete series of saturated and singly unsaturated fatty acids (C_{12} to C_{18}) are observed, in accordance with previous off-line studies by gas chromatography–mass spectrometry. Several other metabolites have been identified, including ketomonocarboxylic and hydroxymonocarboxylic acids. (J Am Soc Mass Spectrom 2009, 20, 1060–1063) © 2009 American Society for Mass Spectrometry

Skin volatiles have been widely studied as potential attractants of mosquitoes [1–5]. Until the recent development of desorption electrospray ionization (DESI) [6] and its variant extractive electrospray ionization (EESI) [7], such studies were dominated by gas chromatography–mass spectrometry (GC-MS) [3, 4, 8–10], which requires sample collection and preconcentration, and is time consuming. Selected ion flow tube–mass spectrometry (SIFT-MS) [11] has provided real-time information on skin emanations for relatively volatile species such as acetone [12]. Proton transfer reaction–mass spectrometry (PTR-MS) claims parts-per-trillion (ppt) sensitivities [13] and has permitted on-line monitoring of UV-induced lipid peroxidation products from human skin and its dependence on the fatty acid composition of the skin [14]. The rich new possibilities for on-line analysis of surfaces brought about by DESI and EESI have been used to continuously monitor the release of caffeine from the skin of a person before and after drinking coffee [15], or of DESI ions from intact bacteria [16]. The present study will continue these promising ES-based approaches relying on an ionization mechanism referred to as secondary ESI (SESI) [17–21], where gas is sampled from the ambient and mixed with the charged cloud of an electrospray of clean solvent. Polar volatile species are then ionized, either directly by the charged electrosprayed drops or by the small ions released after drop evaporation. Therefore, SESI ionizes vapor species and suspended particles [18] directly in the gas phase [22], being complementary with other surface ionization methods such as DESI or direct analysis in real time (DART) [23], which actively desorb charged species from surfaces.

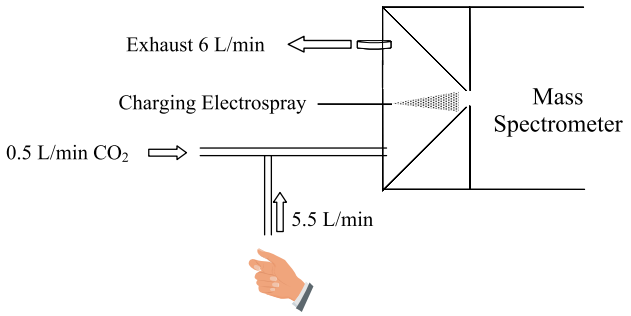

Figure 1. Sketch of the SESI experimental setup to sense skin vapors in real time.

We shall see here that hexadecanoic and octadecanoic acids are released directly from the skin as vapors, without the need for an active desorption.

We have recently used real-time SESI to sense explosive vapors [24] and breath volatiles [22, 25], with lower detection limits approaching 0.2 ppt [24]. In negative ionization of breath [25] and urine [26], we accidentally observed a rich group of skin vapors, primarily organic acids. This interesting finding motivates the present study on negative SESI of skin vapors.

Experimental

The experimental system includes a commercial quadrupole time-of-flight mass spectrometer (QTOF; QStar MDS Sciex, Foster City, CA, USA), with its atmospheric pressure entrance slightly modified to create a chamber including an ionization source, simply an enclosed electrospray source facing the MS sampling orifice (Figure 1). Neutral vapors enter the chamber through a ¼-in. inlet tube, are ionized by the ES cloud, and are sucked into the analyzer. A gas flow rate of 6 L/min is driven in and out of the charging chamber by a pump connected to a second outlet ¼-in. tube. The inlet flow is

Address reprint requests to Dr. Juan Fernández de la Mora, Yale University, Department of Mechanical Engineering, P.O. Box 208286, New Haven, CT 06520. E-mail: juan.delamora@yale.edu

* Present address: National Research Council–Institute for Biomedical Technologies, Via Fratelli Cervi, 93. 20090 Segrate (MI) Italy.

1044-0305/09/$32.00
doi:10.1016/j.jasms.2009.01.012

Published online February 27, 2009
Received November 23, 2008
Revised January 12, 2009
Accepted January 19, 2009

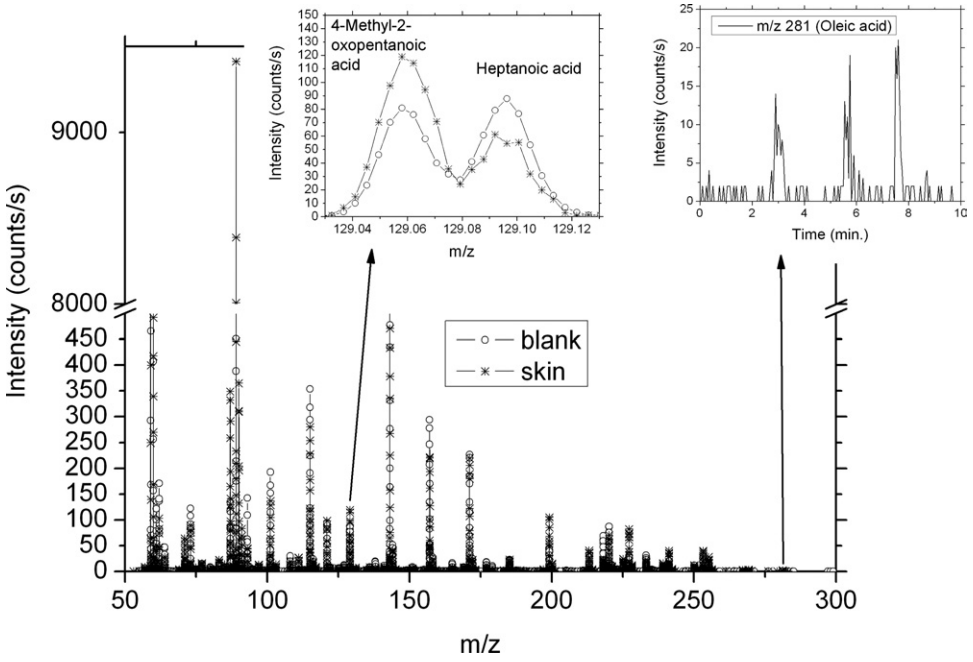

Figure 2. Comparison of mass spectra from ambient air (blank) and skin volatiles, showing a drastic increase in lactic acid concentration (*m/z* 89) from the approaching hand. The left inset magnifies the peaks observed at *m/z* 129 to illustrate the presence of overlapping peaks with opposite response toward the approaching hand. The right inset shows the increase in ion signal for the heaviest ion observed (*m/z* 281; oleic acid), as the subject's hand approaches three times the sampling tube.

a mixture of 0.5 L/min of CO_2 (to minimize sparking in the negative ES) and 5.5 L/min of ambient laboratory air (blank). By simply placing the hand at about 1 cm from the sampling tube and keeping the mouth away from it, the system responds to the skin emanations. Notice that there is no direct contact between the skin and the electrospray drops. The gas and vapors surrounding the hand are drawn into the electrospray chamber as neutrals and are charged there in isolation from the surfaces from which they originate. Figure 1 shows schematically the experimental setup.

We have noted previously that the significant effect of humidity on vapor charging probability requires special precautions to correct for the background when analyzing samples with a high content of water vapor, such as breath [25] and urine [26]. The approach is simpler in the case of skin vapors, since the rise in humidity when placing the palm of the hand in the close vicinity of the sampling tube is relatively small. Indeed, when measured with a hygrometer, it increased from roughly 22 to 26%. Thus, we attribute to skin volatiles (rather than to the spurious effect of humidity) any substantial increment of peak height above the blank. We operated the QTOF in negative mode, and the ES buffer used for the electrospray ionizer of vapors was 0.1% NH_4OH in 1:1 MeOH/H_2O (vol/vol). All the ions sensed (mainly acids) were in the form of a deprotonated vapor (molecular weight minus 1 Da).

The results presented here correspond to the hand of a single male subject in good health and have been reproduced on different days. Ongoing work (by PML) has confirmed and extended our present conclusions with a wider group of subjects. The subject did not use any perfumes for more than 7 days before the test, but did use underarm deodorant. Some 30 min before the test he washed his hands with water and soap.

Results and Discussion

Figure 2 presents the mass spectrum for the ambient laboratory air (blank) and the overlaid spectrum obtained when placing the hand at about 1 cm from the sampling tube. Note the break in the *y*-axis, forced by the drastic intensity increase from about 1300 to 9400 counts per second of the peak at *m/z* 89, assigned to lactic acid by collision-induced dissociation (CID) based on the web database: www.massbank.jp. Both lactic and pyruvic acids have been observed and identified previously in real time with the pioneering TAGA detector [27] of ambient volatiles developed commercially by Sciex (B. A. Thompson, private communication), perhaps suggesting that their atmospheric pressure chemical ionization (APCI) system might be a match to SESI. Some 20 other peaks rise in Figure 2 clearly above the background. The inset on the right displays the heaviest ion observed, at *m/z* 281, assigned (based on its exact mass and MS/MS spectrum interpretation) to oleic acid. The three peaks shown correspond to the moments when the operator brings his hand to the proximity of the sampling tube. The high resolution of the TOF permits resolving pairs of peaks assigned to fatty acids and keto-acids, observed up to *m/z* 129. The inset on the

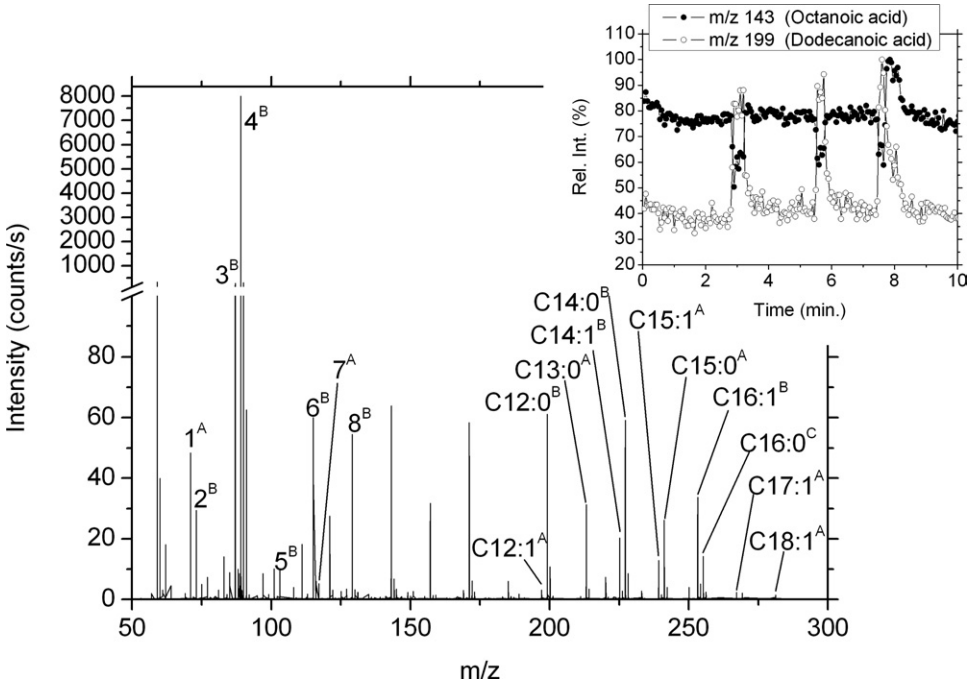

Figure 3. Blank-subtracted mass spectrum resulting from Figure 2. The inset displays the SIM trace for deprotonated octanoic and dodecanoic acids, showing that only the latter can be positively associated to skin emanations. Note on the right-hand side of the graph the complete series of saturated fatty acids from C12:0 to C16:0, as well as unsaturated fatty acids from C12:1 to C18:1. The compounds labeled by peak number are: (1) pyruvaldehyde; (2) glyoxylic acid; (3) pyruvic acid; (4) lactic acid; (5) 4-hydroxybutanoic acid; (6) 3-methyl-2-oxobutanoic acid; (7) 3-hydroxypentanoic acid; (8) 4-methyl-2oxopentanoic acid. A: The structure is known to a reasonable degree of certainty, based on its exact mass and MS/MS spectrum interpretation. In the particular case of fatty acids, the completeness of the series provides further assurance of their identity. B: Identities assigned by CID, using as reference a free web database [www.massbank.jp]. Uncertainty exists in the location of the keto or hydroxyl groups. C: Identification performed by CID against a standard.

left magnifies the region at *m/z* 129, where we clearly distinguish two peaks. Conversely to oleic acid, heptanoic acid decreases below the background level when approaching the hand, whereas the neighboring peak (4-methyl-2-oxopentanoic acid) rises above it.

Figure 3 shows the blank-subtracted spectrum from Figure 2, with assigned peaks indicating the identification procedure. Blank subtraction simplifies the mass spectrum interpretation, but may lead to spurious low-intensity peaks (for example, those at *m/z* 143, 157, and 171, clearly falling below the background in Figure 2). To illustrate this point we represent in the inset the single-ion monitoring (SIM) traces for the peaks at *m/z* 143 (octanoic acid) and 199 (dodecanoic acid), which display opposite responses to the approaching hand. According to our previous observation of short-chain carboxylic acids in breath [25] our explanation for the negative response of octanoic acid and other lighter fatty acids is that they exist in the laboratory background as a result primarily of human breath, rather than skin. This background is not from a spurious individual exhalation near the sampling tube, but is uniformly dispersed across the laboratory. The negative effect of the approaching hand is probably indirect, possibly resulting from the reduction of the ionization probability associated with competition from lactic acid. The subtraction correction method is evidently unreliable in cases when the variation of the peak height is modest (~10%). Conversely, the positive response to dodecanoic acid, more than doubling the background signal, is sure evidence that its source is primarily cutaneous. Starting at dodecanoic acid, we observe a series spaced by 14 Da, clearly associated with CH_2 addition in a hydrocarbon chain. The series continues uninterrupted with well-defined peaks up to deprotonated hexadecanoic (palmitic) acid (C16:0). Another series can be seen located 2 units to the left of a member of the main series, associated with singly unsaturated fatty acids. It extends from C12:1 to C18:1 (missing C13:1), including peaks for palmitoleic (C14:1), myristoleic (C16:1), and oleic acid (C18:1). Note that no special procedures beyond comparing fragmentation patterns with the database were used to verify that the position of the double bonds are those indicated above, corresponding to the natural fatty acids. Some of these precise assignments may therefore need further confirmation, particularly given the richness of skin lipids [28].

The observation of mass spectra from skin volatiles dominated by lactic and fatty acids is consistent with previous studies by GC-MS [29]. In particular, fatty acids beyond C12 are efficiently collected by solvent

extraction, concentration, and injection into the GC [8]. Short-chain fatty acids from C3 to C6 are relatively uncommon in human skin [28], except from the foot [5, 30], and, rarely, from the back and forearm [8]. The discrepancy between the absence in the palm of the hand of fatty acids below C12:0 in our study and the report of Bernier et al. [3] may be attributable to contamination from the high background level of low molecular weight fatty acids originating from exhaled breath. Indeed, we have reported strong signals in breath of the complete series of saturated fatty acids, starting with propanoic (C3:0) and ending in myristic (C14:0) acid [25]. Conversely, when studying fatty acids from breath, one must be careful to avoid interferences from the skin of the operator, from which one can mistakenly conclude that breath contains heavy fatty acids. Thus we conclude that the fatty acid background is primarily from human origin, with the shorter chains originating in breath (and ultimately blood) and the longer ones from skin. We have identified several other compounds, including ketomonocarboxylic and hydroxymonocarboxylic acids (see Figure 3). Interestingly, the complete series of keto-carboxylic acids from C6 to C10 has been observed in breath [25]. Table S1 (available as supplementary material, which can be found in the electronic version of this article) lists identified compounds including the metabolic pathways they are involved in.

Conclusions

A study of skin volatiles based on SESI has revealed that a good number of species have sufficient volatility to be detected in real time by commercial API-MS, without the need to use active desorption processes such as those taking place in DESI and DART. Given the speed of the analysis, SESI ionization offers an attractive approach to investigate skin fatty acids.

Acknowledgments

This study has been funded primarily by SEADM. We thank Prof. John B. Fenn for his inspiration on the sensitivity of the electrospray vapor charger and Dr. Bruce A. Thomson (Sciex) and Mr. Gonzalo Fernández de la Mora (SEADM) for numerous ideas and suggestions. We thank J. Rus, J. A. Sillero, M. Hernández, and A. Casado for their support on the experiments. PML expresses his gratitude to Yale University for hosting his postdoctoral visit.

Appendix A
Supplementary Material

Supplementary material associated with this article may be found in the online version at doi:10.1016/j.jasms.2009.01.012.

References

1. Acree, F., Jr.; Turner, R. B.; Gouck, H. K.; Beroza, M.; Smith, N. L-Lactic Acid: A Mosquito Attractant Isolated from Humans. *Science* **1968,** *161,* 1346–1347.
2. Bosch, O. J.; Geier, M.; Boeckh, J. Contribution of Fatty Acids to Olfactory Host Finding of Female Aedes aegypti. *Chem. Senses* **2000,** *25,* 323–330.
3. Bernier, U. R.; Booth, M. M.; Yost, R. A. Analysis of Human Skin Emanations by Gas Chromatography/Mass Spectrometry. 1. Thermal Desorption of Attractants for the Yellow Fever Mosquito (Aedes aegypti) from Handled Glass Beads. *Anal. Chem.* **1999,** *71,* 1–7.
4. Bernier, U. R.; Kline, D. L.; Barnard, D. R.; Schreck, C. E.; Yost, R. A. 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). *Anal. Chem.* **2000,** *72,* 747–756.
5. Knols, B. G. J; Meijerink, J. Odors Influence Mosquito Behavior. *Sci Med.* **1997,** *4,* 56–63.
6. Takáts, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. Mass Spectrometry Sampling under Ambient Conditions with Desorption Electrospray Ionization. *Science* **2004,** *306,* 471–473.
7. Chen, H.; Venter, A.; Cooks, R. G. Extractive Electrospray Ionization for Direct Analysis of Undiluted Urine, Milk and Other Complex Mixtures without Sample Preparation. *Chem. Commun.* **2006,** 2042–2044.
8. Gallagher, M.; Wysocki, C. J.; Leyden, J. J.; Spielman, A. I.; Sun, X.; Preti, G. *Br. J. Dermatol.* **2008,** *159,* 780–791.
9. Curran, A. M.; Rabin, S. I.; Prada, P. A.; Furton, K. G. Comparison of the Volatile Organic Compounds Present in Human Odor Using SPME-GC/MS. *J. Chem. Ecol.* **2005,** *31,* 1607–1619.
10. Penn, D. J.; Oberzaucher, E.; Grammer, K.; Fischer, G.; Soini, H. A.; Wiesler, D.; Novotny, M. V.; Dixon, S. J.; Xu, Y.; Brereton, R. G. Individual and Gender Fingerprints in Human Body Odour. *J. R. Soc. Interface* **2007,** *4,* 331–340.
11. Smith, D.; Španěl, P. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) for On-line Trace Gas Analysis. *Mass Spectrom. Rev.* **2005,** *24,* 661–700.
12. Turner, C.; Parekh, B.; Walton, C.; Španěl, P.; Smith, D.; Evans, M. L. An Exploratory Comparative Study of Volatile Compounds in Exhaled Breath and Emitted by Skin Using Sselected Ion Flow Tube Mass Spectrometry (SIFT-MS). *Rapid Commun. Mass Spectrom.* **2008,** *22,* 526–532.
13. Lindinger, W.; Hansel, A.; Jordan, A. On-line Monitoring of Volatile Organic Compounds at pptv Levels by Means of Proton-Transfer-Reaction Mass-Spectrometry (PTR-MS): Medical Applications, Food Control and Environmental Research. *Int. J. Mass Spectrom. Ion Process.* **1998,** *173,* 191–241.
14. Steeghs, M. M. L.; Moeskops, B. W. M.; van Swam, K.; Cristescu, S. M.; Scheepers, P. T. J.; Harren, F. J. M. On-line Monitoring of UV-induced Lipid Peroxidation Products from Human Skin In Vivo Using Pproton-Transfer Reaction mass Spectrometry. *Int. J. Mass. Spectrom.* **2006,** *253,* 58–64.
15. Chen, H.; Wortmann, A.; Zhang, W.; Zenobi, R. Rapid In Vivo Fingerprinting of Nonvolatile Compounds in Breath. *Angew. Chem. Int. Ed.* **2007,** *46,* 580–583.
16. Song, Y.; Talaty, N.; Tao, W. A.; Pan, Z.; Cooks, R. G. Rapid Ambient Mass Spectrometric Profiling of Intact, Untreated Bacteria Using Desorption Electrospray Ionization. *Chem. Commun.* **2007,** 61–63.
17. Whitehouse, C. M., Levin, F., Meng, C. K., Fenn, J. B. *Proceedings of the 34th ASMS Conference on Mass Spectrometry and Allied Topics*; Denver, CO, 1986; p 507.
18. Fuerstenau, S., Kiselev, P., Fenn, J. B. Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics; Dallas, TX, June 1999 (ThOE 3:00).
19. Wu, C.; Siems, W. F.; Hill, H., Jr. Secondary Electrospray Ionization Ion Mobility Spectrometry/Mass Spectrometry of Illicit Drugs. *Anal. Chem.* **2000,** *72,* 396–403.
20. Tam, M.; Hill, H., Jr. Secondary Electrospray Ionization-Ion Mobility Spectrometry for Explosive Vapor Detection. *Anal. Chem.* **2004,** *76,* 2741–2747.
21. Lee, C. Y.; Shiea, J. Gas Chromatography Connected to Multiple Channel Electrospray Ionization Mass Spectrometry for the Detection of Volatile Organic Compounds. *Anal. Chem.* **1998,** *70,* 2757–2761.
22. Martínez-Lozano, P.; Fernández de la Mora, J. Electrospray Ionization of Volatiles in Breath. *Int. J. Mass Spectrom.* **2007,** *265,* 68–72.
23. Cody, R. B.; Laramee, J. A.; Durst, H. D. Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. *Anal. Chem.* **2005,** *77,* 2297–2302.
24. Martínez-Lozano, P.; Rus, J.; Fernández de la Mora, G.; Hernández, M.; Fernández de la Mora, J. Secondary Electrospray Ionization (SESI) of Ambient Vapors for Explosive Detection at Concentrations below Parts per Trillion. *J. Am. Soc. Mass Spectrom.* **2009,** *20,* 287–294.
25. Martínez-Lozano, P.; Fernández de la Mora, J. Direct Aanalysis of Fatty Acid Vapors in Breath by Electrospray Ionization and Atmospheric Pressure Ionization-Mass Spectrometry. *Anal. Chem.* **2008,** *80,* 8210–8215.
26. Martínez-Lozano, P. *Proceedings of the 56th ASMS Conference on Mass Spectrometry and Allied Topics*; Denver, CO, 2008 (WP 517).
27. Lane, D. A.; Thomson, B. A. Monitoring a Chlorine Spill from a Train Derailment. *J. Air Pollution Control Assoc.* **1981,** *31,* 122–127.
28. Nicolaides, N. Skin Lipids: Their Biochemical Uniqueness. *Science* **1974,** *186,* 19–26.
29. Puhvel, S. M.; Reisner, R. M.; Sakamoto, M. Analysis of Lipid Composition of Isolated Human Sebaceous Gland Homogenates after Incubation with Cutaneous Bacteria: Thin-Layer Chromatography. *J. Invest. Dermatol.* **1975,** *64,* 406–411.
30. Kanda, F.; Yagi, E.; Fukuda, M.; Nakajima, K.; Ohta, T.; Nakata, O. Elucidation of Chemical Compounds Responsible for Foot Malodour. *Br. J. Dermatol.* **1990,** *122,* 771–776.