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Identification of Δ6-monounsaturated fatty acids in human hair and nail samples by gas-chromatography–mass-spectrometry using ionic-liquid coated capillary column

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ABSTRACT

Lipids found in human sebum contain specific fatty acids such as sapienic (*cis*-6 16:1), *cis*-8 18:1 and sebaleic (*cis*-5, *cis*-8 18:2) acids. These fatty acids belong to the n-10 series and the initial step involved in their synthesis is the desaturation of palmitic acid by the Δ6-desaturase to form sapienic acid. The occurrence in human hair and nail of sapienic (*cis*-6 16:1), *cis*-8 18:1 and sebaleic (*cis*-5, *cis*-8 18:2) acids has not been reported to our knowledge nor has the formation of Δ6-monounsaturated fatty acids from other saturated fatty acids such as stearic acid. The pre-requisite for such identification is the ability to separate *cis*-6 from *cis*-8 monounsaturated fatty acid derivative (*i.e. cis*-6 18:1 from *cis*-8 18:1 methyl esters) by gas-chromatography (GC) and such separation is not achievable using cyanoalkyl based highly polar capillary columns. In the present study, we used the 100 m SLB-IL 111 ionic liquid based capillary column recently commercialized by Supelco (Bellefonte, PA). The identification was performed by gas-chromatography–mass-spectrometry (GC–MS) with electronic impact (EI) ionization using 4,4-dimethyloxazoline (DMOX) derivatives. Baseline separation between critical *cis*-6 18:1 and *cis*-8 18:1 isomers was obtained allowing unambiguous identification based on MS fragmentation and pure standards. In sebum, hair and nail samples, sapienic, *cis*-8 18:1 and sebaleic acids were found and more importantly, petroselinic acid was identified in these human tissues for the first time. In addition, we identified in hair and nail lipids *cis*-6 14:1, *cis*-6 15:1, *iso*-*cis*-6 16:1, *aiso*-*cis*-6 17:1 and *cis*-6 17:1 as their DMOX derivatives based on molecular ion as well as diagnostic ion fragments at *m*/*z* 167, 180 and 194. Possible biosynthesis scenario is postulated to explain the occurrence of these Δ6-monounsaturated fatty acids in human sebum, hair and nail lipids.

1. Introduction

In human, fatty acids from the n-10 series have been reported to occur in sebum [1,2] as well as lipid lung surfactants [3]. The main n-10 fatty acids found in these samples are sapienic (*cis*-6 16:1), *cis*-8 18:1 and sebaleic (*cis*-5, *cis*-8 18:2) acids. The initial step of the formation of this series involved the desaturation of palmitic acid by the Δ6-desaturase to form sapienic acid [1]. This biosynthetic pathway seems to be specific to these tissues when exposed to the environment but their biological significance has not been extensively investigated to date [2].

The occurrence of sapienic (*cis*-6 16:1), *cis*-8 18:1 and sebaleic (*cis*-5, *cis*-8 18:2) acids has not been reported to our knowledge in human hair and nail lipids. In addition, it can be hypothesized that the formation of Δ6-monounsaturated fatty acids may occur from other saturated fatty acids such as stearic acid. The pre-requisite for such identification is the ability to separate *cis*-6 from *cis*-8 monounsaturated fatty acid derivative such as petroselinic (*cis*-6 18:1) from *cis*-8 18:1 acid methyl esters by gas-chromatography (GC). Such separation cannot be achieved using cyanoalkyl based highly polar capillary columns.

Delmonte et al. [4] reported significant improvement of critical separation of geometric and positional isomers using the recently released 100 m SLB-1L 111 ionic-liquid coated capillary column (Supelco, Bellefonte, PA). The authors evaluated the performance of this column to separate 14:1, 16:1 18:1, 20:1 and 18:3 isomers and compared the results obtained with SP-2560 (Supelco, Bellefonte, PA) and CP-Sil 88 (Varian, Middelburg, The Netherlands). From our perspective, the more significant progress achieved by Delmonte et al. [4] is the baseline separation of petroselinic (*cis*-6 18:1) from *cis*-7/*cis*-8 18:1 acid methyl esters. This improvement

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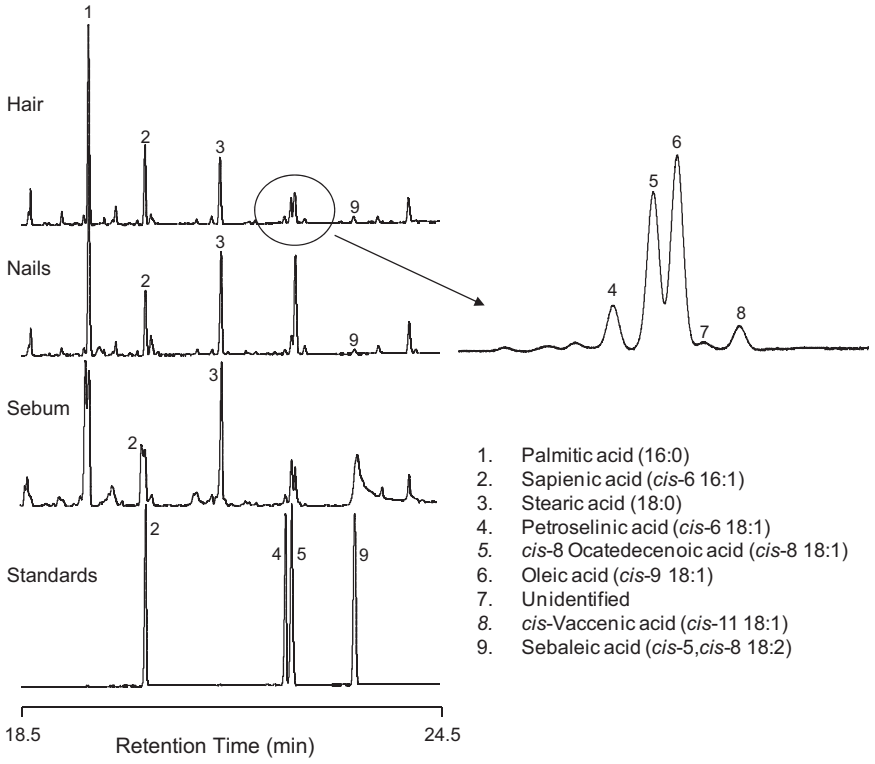

Fig. 1. Fatty acid methyl ester (FAME) profile of hair, nails and sebum samples showing the occurrence of a complex pattern (left panel) of *cis*-octadecenoic acid isomers comprising petroselinic acid (*cis*-6 18:1), *cis*-8 18:1, oleic acid (*cis*-9 18:1) and *cis*-vaccenic (*cis*-11 18:1) acid. Analysis performed on ionic liquid coated capillary column SLB-IL 111 (Supelco). Standard FAME of *cis*-6 16:1, *cis*-6 18:1, *cis*-8 18:1 and *cis*-5,*cis*-8 18:2 were obtained from Lipidox (Sweden).

allows conducting a thorough examination of the occurrence of specific Δ6-monounsaturated fatty acids such as petroselinic acid in human sebum, hair and nail lipids.

2. Materials and methods

2.1. Chemicals and reagents

2-Amino-2-methyl-1-propanol was obtained from Sigma–Aldrich (Saint Louis, USA) and methanol/HCl (3 N) from Supelco (Bellefonte, PA). Pure methyl *cis*-6 16:1, *cis*-6 18:1, *cis*-8-18:1 and *cis*-5, *cis*-8 18:2 acid esters were obtained from Lipidox (Sweden).

2.2. Sampling procedure

Hairs, nails and a superficial skin sample were collected from human volunteers ($N = 6$) following the procedure described hereafter. Hair sample collection: approximately 20 hairs including the hair follicles were collected from females scalp using tweezers. The hairs were further cut with a clean razor blade in order to obtain approximately 1 cm length from the hair follicle. The 1 cm hairs including hair follicles were further pooled in a 15 mL falcon tube before derivatization. Nail sample collection: 1–2 mL of finger nails were cut with a scissor from the ten fingers of the subject and pooled in a 15 mL falcon tube before derivatization. Superficial skin sample: superficial skin samples were collected using tape stripping methodology. Tape stripping is a commonly used method to investigate the stratum corneum (SC) physiology. The SC is the superficial layer of the epidermis which consists of corneocytes embedded in lipid bilayers. Present within these lipids are the ones produced in the sebum. Briefly, regular adhesive tape (Scotch MagicTM Tape), 2 cm wide and 5 cm long was used. The tape was applied to the skin on the human volunteer's right cheek, rubbed lightly to assure adhesion and then pulled off with one fluent and decisive movement. This was repeated 10 times on the same spot. And the 10 strips were pooled in a 50 mL falcon tube to be processed for extraction.

2.3. Preparation of fatty acid methyl esters (FAME)

Hair and nail samples (>20 mg) were placed in a mortar and crushed into very small pieces under very cold conditions (liquid nitrogen-dry ice). Pulverized samples were then transferred into 10 mL screw cap test tubes with methanol (2 mL), methanol/HCl (2 mL, 3 N) and hexane (1 mL). After vigorous shaking, the methylation was performed at 100 °C for 60 min and shaken vigorously every 20 min. After cooling-down to room temperature, water (2 mL) was added and tubes were centrifuged at 1200 × *g* for 5 min. If necessary, sample was further concentrated before GC analysis. Sebum samples were extracted from tape by immersion and homogeneization in hexane (1 mL) and methanol (2 mL) for 1 min. After removing the tape from the tube, methanol/HCl (2 mL, 3 N) was added and methylation was conducted as described for hair and nail samples.

2.4. Preparation of 4,4-dimethyloxazoline (DMOX) derivative for GC–MS analysis

DMOX were prepared as previously described with slight modification [5]. Briefly, FAME were dried over nitrogen and mixed with 2-amino-2-methyl-1-propanol (0.5 mL) and heated at 190 °C overnight. After cooling down to room temperature, hexane (2 mL) and water (2 mL) were added and the tube was vigorously shaken and centrifuged at 1000 rpm for 2 min. The organic phase was recovered and hexane evaporated using nitrogen. Sample was diluted in fresh hexane and analyzed by GC–MS.