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IDENTIFICATION OF SOME ABNORMAL METABOLITES IN PSORIATIC NAIL USING GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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SUMMARY

A gas chromatographic—mass spectrometric analysis was used to separate and identify abnormal compounds in the nail of psoriatic patients. The nail was extracted with heated ethanol, and the extract was analyzed with and without trimethylsilylation. Tetradecanoic acid octadecyl ester, hexadecanoic acid octadecyl ester and octadecanoic acid octadecyl ester were first identified in the psoriatic nail, but were not detected in normal nail.

INTRODUCTION

Psoriasis is a genetic skin disease characterized by glycogen accumulation, excessive cell proliferation, and incomplete differentiation in lesional epidermis. A number of metabolic abnormalities have been noted in the disease [1—4]. However, attention was largely devoted to the analysis of the psoriatic blood, urine and scale. The authors analyzed the affected nail using gas chromatography—mass spectrometry (GC—MS) and detected some previously unidentified compounds, which could not be detected in the normal nail.

MATERIALS AND METHODS

Chemicals

Trimethylsilylating agents, N,O-bis(trimethylsilyl)acetamide and trimethylchlorosilane were purchased from Pierce (Rockford, IL, U.S.A.). Myristic acid, palmitic acid, oleic acid, stearic acid, cholesterol, oxalyl chloride and octadecyl alcohol were the products of Tokyo Kasei (Tokyo, Japan).

Hexadecanoic acid octadecyl ester was synthesized from octadecyl alcohol and palmitoil chloride. Palmitic acid (9 g) and oxalyl chloride (12 ml) were dissolved in benzene (10 ml) and heated at 60°C for 1 h. After concentration with a rotary evaporator, the distillate under reduced pressure (13 mmHg, 188°C)

was collected, yielding palmitoyl chloride. The latter (4.9 g) and octadecyl alcohol (4.8 g) were dissolved in anhydrous diethyl ether (40 ml) and anhydrous pyridine (3 ml). The mixture was refluxed for 3 h. After washing with hydrogen chloride and concentration with a rotary evaporator, hexadecanoic acid octadecyl ester was purified through a column packed with silica gel 60 F254. The product was confirmed to be pure using ^{1}H NMR spectroscopy.

Sample preparation

Nail samples were obtained from five patients with psoriasis vulgaris and from six healthy adults.

The surface of the nail was discarded and the remainder granulated, 100 mg of the granulated nail was extracted with 10 ml of ethanol at 65°C for 24 h. After concentration with nitrogen gas, the extract was analyzed using GC—MS with and without trimethylsilylation. Trimethylsilylation was performed with 80 μl of N,O-bis(trimethylsilyl)acetamide and 20 μl of trimethylchlorosilane at 30°C for 30 min.

Gas chromatography—mass spectrometry

The instrument used for combined GC—MS consisted of a gas chromatograph, JGC-20K, and a double focusing mass spectrometer, JMS D-300 (JEOL, Tokyo, Japan). The gas chromatograph was equipped with a 3% OV-1 single coiled glass column (2 m × 2 mm I.D.). Carrier gas was helium with a flow-rate of 30 ml/min. The column temperature was programmed from 100°C to 300°C at 4°C/min. Electron impact ionization (EI) mass spectra were recorded under the following conditions: ionizing energy 22 eV, ionization current 300 μA, separator temperature 250°C, ion source temperature 230°C and accelerating voltage 3 kV. Chemical ionization (CI) mass spectra were recorded using methane as a reactant gas, ionizing energy was 250 eV. The other conditions were the same as for EI. High-resolution MS was performed with a data processing system, JMA 2000 (JEOL) with an ionizing energy of 70 eV and a resolution of 5000. Peak matching measurements were performed with perfluorokerosene as a reference compound.

RESULTS

Total ion monitoring chromatograms of the trimethylsilylated extract from psoriatic and normal nail are shown in Figs. 1 and 2, respectively. A number of high peaks were recognized in the psoriatic chromatogram. Peaks 23, 28, 30, 31 and 37 were identified as myristic acid, palmitic acid, oleic acid, stearic acid and cholesterol, respectively. The mass spectra of the peaks were compared with the mass spectra obtained in our laboratory from the derivatives of the authentic compounds.

The EI mass spectrum of peak 29 is shown in Fig. 3. The molecular weight was found to be 342 by recording the CI mass spectrum. The peak was assumed to be octadecanol. The EI mass spectrum of peak 39 is shown in Fig. 4. The ion at *m/e* 480 was confirmed to be the molecular ion by recording the CI mass spectrum. High-resolution MS data indicated that the elemental formulae of the

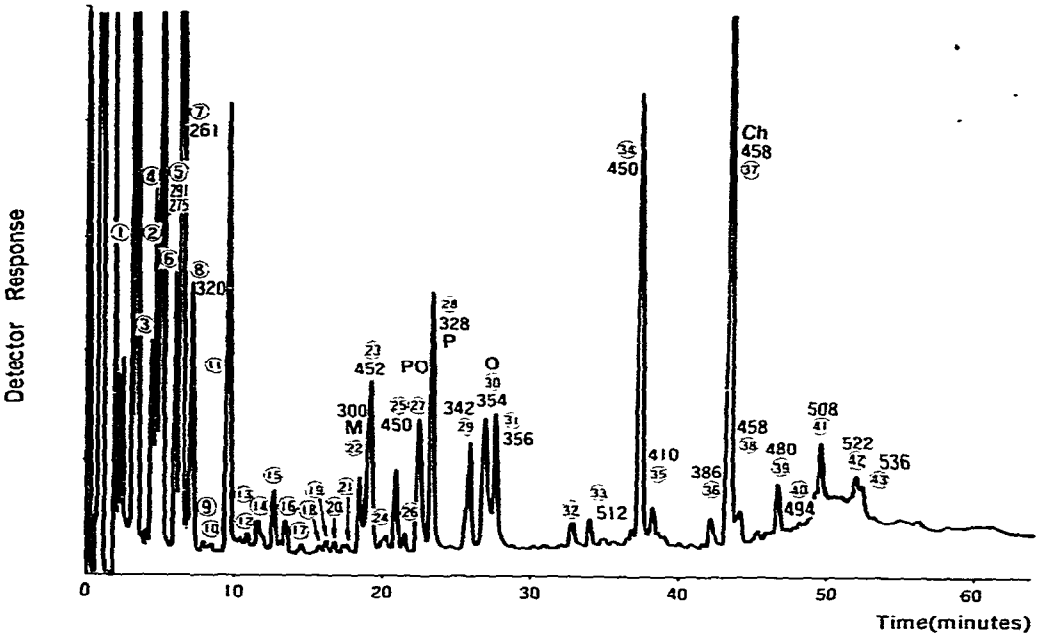

Fig. 1. Total ion chromatogram of the nail extract of psoriasis patients treated (before treatment). Many peaks were seen and they were numbered for easy comparison and evaluation. The figure on each peak indicates the molecular weight of the substance. Peaks: 23, myristic acid; 28, palmitic acid; 30, oleic acid; 31, stearic acid; 37, cholesterol.

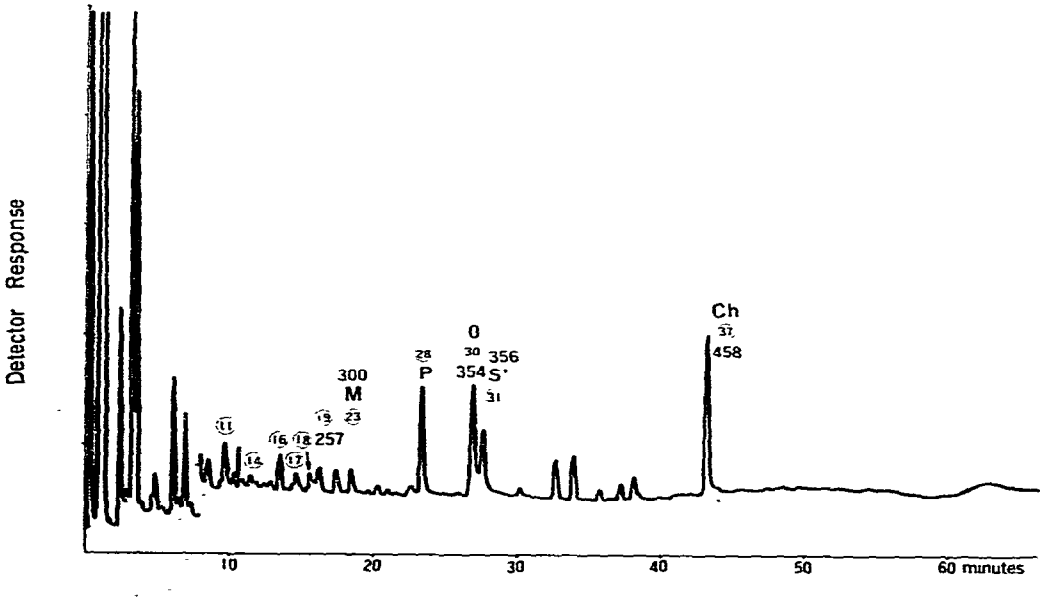

Fig. 2. Total ion chromatogram of a normal nail. Compared to psoriasis the number of peaks is smaller. The number of peaks which also appeared in Fig. 1, and those determined from retention time of the mass chromatogram were identical. Peaks: M, myristic acid; P, palmitic acid; O, oleic acid; S, stearic acid; Ch, cholesterol.

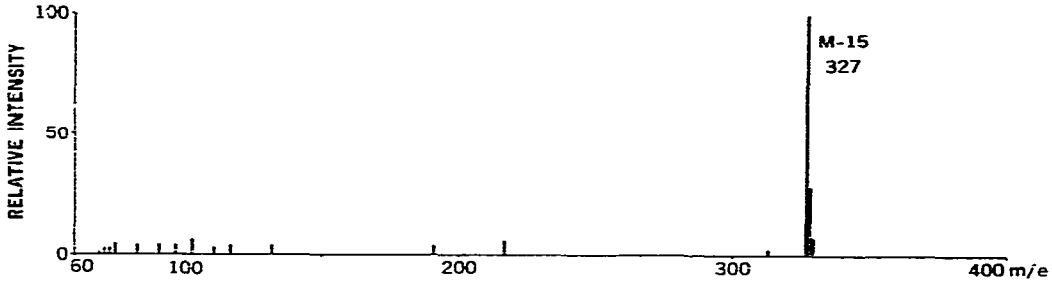

Fig. 3. EI mass spectrum of peak 29. From the determination M = 342, and from retention time of the chromatogram, the peak was identified as octadecanol.

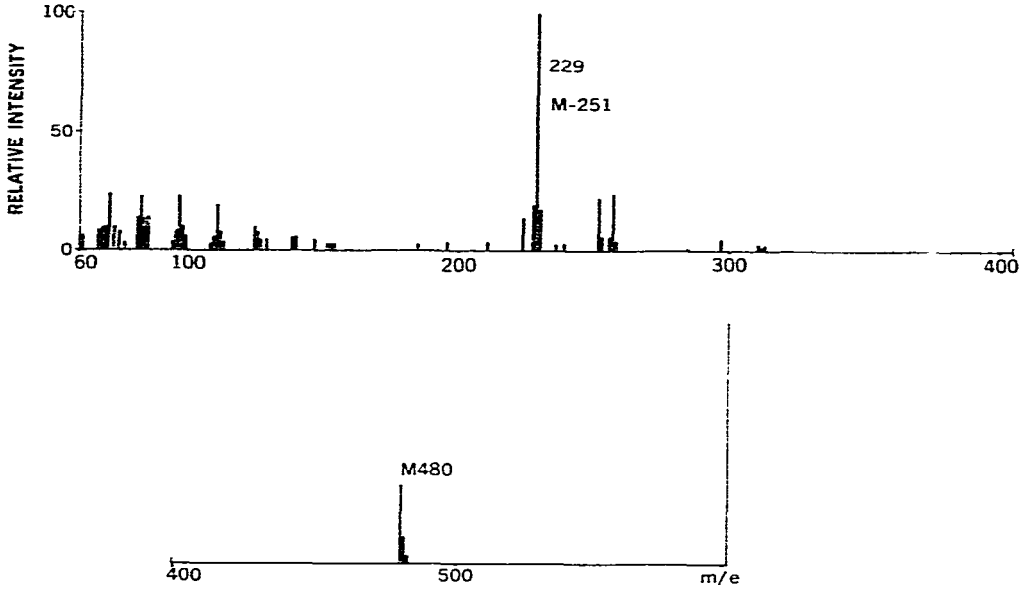

Fig. 4. Mass spectrum of peak 39. M was assumed to be 480. Peaks at *m/e* 229 (M — 251) and 252 are evident.

molecular ion and the ion at *m/e* 229 were $C_{32}H_{64}O_2$ and $C_{14}H_{29}O_2$, respectively. The ion at *m/e* 229 was assumed to be $CH_3(CH_2)_{12}C(OH)O^+H$ formed through double rearrangement of protons. The compound was then identified as tetradecanoic acid octadecyl ester. The EI mass spectrum of peak 41 is shown in Fig. 5. The CI mass spectrum indicated that the molecular ion of the compound was 508. The elemental formula of the compound was $C_{34}H_{68}O_2$, which was derived from high-resolution data. Peak 41 was identified as hexanoic acid octadecyl ester by comparing with the EI mass spectrum of the authentic compound. The EI mass spectrum of peak 43 is presented in Fig. 6. The molecular ion was confirmed to be 536 by recording the CI mass spectrum. The elemental formula of the compound was $C_{36}H_{72}O_2$, identifying peak 43 as octadecanoic acid octadecyl ester. The heights of peak 29 and peaks 39—43 have diminished with improvement of the psoriatic lesions.

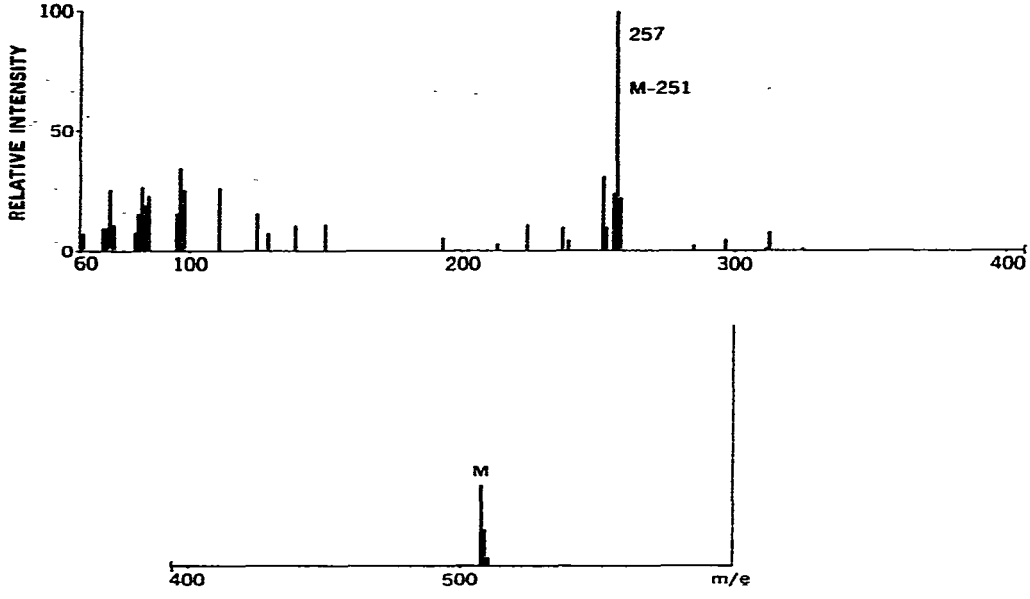

Fig. 5. Mass spectrum of peak 41. *m/e* 508 (M) and 257 (M — 251) were observed and as with peak 39, *m/e* 252 was evident.

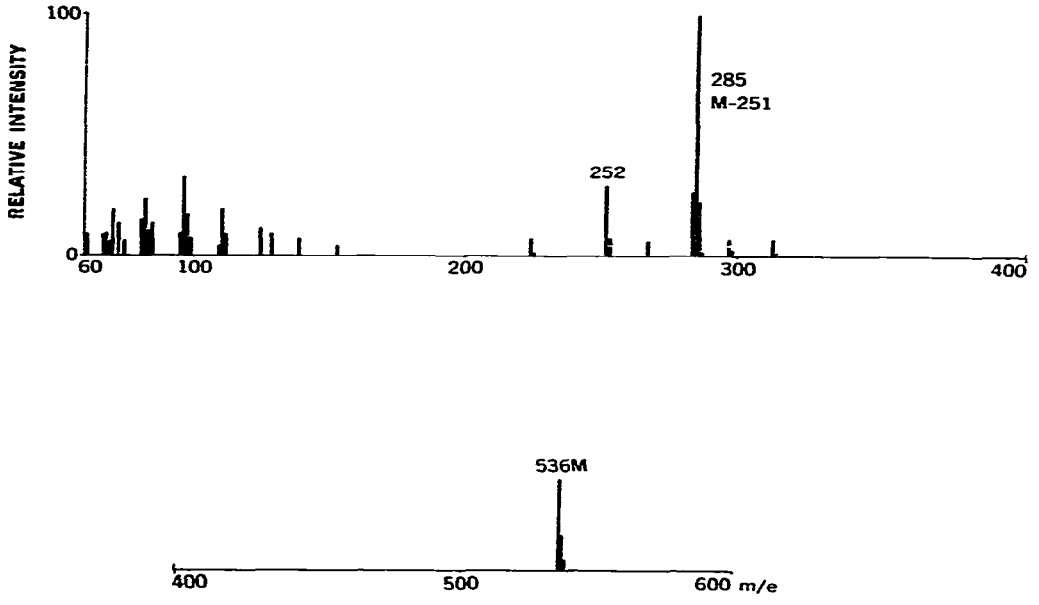

Fig. 6. Mass spectrum of peak 43. *m/e* 536 (M) and 285 (M — 251) were observed. As with peaks 39 and 41, *m/e* 252 was evident.

DISCUSSION

Since the first successful treatment of psoriatic patients with hemodialysis [5], others have also confirmed the effectiveness of the method in a number of retrospective studies on regular hemodialysis patients complicated with the disease [6]. Peritoneal dialysis was also reported to be effective to the psoriatic lesion [7]. However, a recent study reported total ineffectiveness of hemodialysis [8]. In our hospital five out of thirteen patients who were suffering from the disease for a long time and had tried all conventional methods of treatment, remitted completely. These results lead us to a hypothesis that some noxious metabolite(s) accumulate(s) in the blood of psoriasis patients and that the substance is removed by hemodialysis.

The authors detected three abnormal metabolites in the psoriatic nail: tetradecanoic acid octadecyl ester, hexadecanoic acid octadecyl ester and octadecanoic acid octadecyl ester. These esters could not be detected in normal nail nor in psoriatic ultrafiltrate of blood. The origin and its physiological significance are yet unknown.

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