Cholesta-5,7,9(11)-trien-3β-ol found in plasma of patients with Smith-Lemli-Opitz syndrome indicates formation of sterol hydroperoxide

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Abstract The Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder characterized by accumulation of cholesta-5,7-dien-3β-ol caused by a deficiency of the enzyme desaturating this sterol to cholesterol. In addition to other unusual sterols recently found in plasma of patients with SLOS, namely cholesta-5,8-dien-3β-ol and 19-nor-cholesta-5,7,9(10)-trien-3β-ol we have detected a trienol and we describe here its identification as cholesta-5,7,9(11)-trien-3β-ol by GC–MS and by comparison with a synthetic standard. We tested the possibility that the trienol may be formed by radical oxidation of cholesta-5,7-dien-3β-ol accumulated in plasma of patients with SLOS because it is known to be formed by decomposition of 7-hydroperoxy-cholesta-5,8-dien-3β-ol, which is a product of cholesta-5,7-dien-3β-ol photooxidation. Incubation of cholesta-5,7-dien-3β-ol with rat liver microsomes in the presence of ADP/Fe^{2+} and NADPH gave rise to a number of oxygenated sterols. Among these, analysis by particle-beam LC–MS under CI conditions indicated the presence of 7-hydroperoxy-cholesta-5,8-dien-3β-ol and of cholesta-5,7,9(11)-trien-3β-ol which is known to derive from the oxidation of the 7-hydroperoxide. From these results we conclude that cholesta-5,7-dien-3β-ol accumulated in tissues of patients with SLOS may be oxidized by oxygen radicals giving rise to oxygenated sterols. Some of these compounds may be toxic and may contribute to worsen the pathological picture in patients with SLOS.—**De Fabiani, E., D. Caruso, M. Cavaleri, M. Galli Kienle, and G. Galli.** Cholesta-5,7,9(11)-trien-3β-ol found in plasma of patients with Smith-Lemli-Opitz syndrome indicates formation of sterol hydroperoxide. *J. Lipid Res.* 1966. **37:** 2280–2287.

Supplementary key words 7-dehydrocholesterol • oxysterols • proton nuclear magnetic resonance • mass spectrometry • photooxidation

The Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder (1) characterized by abnormal concentrations of cholesta-5,7-dien-3β-ol ($\Delta^{5,7}$) deriving from a deficiency of the activity of Δ^7-reductase which catalyzes the transformation of the dienol into cholesterol (2, 3). Cholesta-5,8-dien-3β-ol was also found to accumulate in plasma of patients with SLOS (4) and very recently, when this work was already in progress, 19-nor-cholesta-5,7,9(10)-trien-3β-ol ($\Delta^{5,7,9(10)}$), with aromatized ring B, was identified among the unusual sterols of patients with SLOS (5).

Origin of the severe clinical abnormalities associated with SLOS (1, 6–9) has not been clearly established. Toxic effects may be ascribed to cholesterol deficiency in these patients. Nevertheless, the possibility that toxic sterols may originate from accumulated $\Delta^{5,7}$ due to its high reactivity under oxidative conditions should not be disregarded.

We have previously analyzed plasma samples of children (0.3–13 years) with clinical features suggesting SLOS, which was then confirmed by high levels of $\Delta^{5,7}$ determined by GC–MS with selected ion monitoring technique (SIM) of trimethylsilyl ethers (TMS) of sterols (10). We describe now analyses carried out on sterol acetates obtained from some of those plasma samples. $\Delta^{5,8}$ was also identified in all tested children by comparison with an authentic sample (11). Careful interpretation of mass spectra associated with other peaks not commonly present in the GC–MS chromatograms obtained for control subjects suggested the presence of

Abbreviations: GC–MS, gas chromatography–mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; PB, particle beam; CI, chemical ionization; MID, multiple ion detection; SIM, selected ion monitoring; TMS, trimethylsilyl ether; rt, retention time; NMR, proton nuclear magnetic resonance; HPLC, high performance liquid chromatography; $\Delta^{5,7}$, cholesta-5,7-dien-3β-ol; $\Delta^{5,7,9(10)}$, 19-nor-cholesta-5,7,9(10)-trien-3β-ol; $\Delta^{5,7,9(11)}$, cholesta-5,7,9(11)-trien-3β-ol; 7OOH$\Delta^{5,8}$, 7-hydroperoxy-cholesta-5,8-dien-3β-ol; 7αOH, cholest-5-en-3β, 7α-diol; 7βOH, cholest-5-en-3β,7β-diol; 7CO, cholest-5-en-3β-ol-7-one; 5OOH, 5-hydroperoxy-cholest-5-en-3β-ol; 7OOH, 7-hydroperoxy-cholest-5-en-3β-ol; 5,8OOΔ^6, 5α,8α-epidioxycholest-6-en-3β-ol.

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two additional sterols with retention times longer and shorter than that of cholesterol, respectively. The former compound corresponded to $\Delta^{5,7,9(10)}$, also found by Batta et al. (5) in patients with SLOS while the latter is a trienol, still unreported for patients with SLOS.

Photooxidation of $\Delta^{5,7}$ sterols was described in early investigations on the synthesis of aromatized sterols (12). The reaction was recently reconsidered and formation of cholesta-5,7,9(11)-trien-3β-ols has been reported for both ergosterol and $\Delta^{5,7}$ photooxidation with singlet oxygen (13). On the basis of this knowledge and of the identification of the trienol in plasma as cholesta-5,7,9(11)-trien-3β-ol ($\Delta^{5,7,9(11)}$) by comparison with a synthetic standard, we have considered the possibility that oxidation of $\Delta^{5,7}$ by radicals may occur in vivo when the dienol accumulates. The results reported here suggest that unusual sterols found in plasma of patients with SLOS likely derive from $\Delta^{5,7}$ by radical oxidation with the intermediate formation of a highly reactive product which might have toxicological significance (13).

EXPERIMENTAL

Materials

All chemicals were used of the highest grade commercially available.

Standard sterols

$\Delta^{5,7}$, cholest-5-en-3β,7β-diol (7βOH) and cholest-5-en-3β-ol-7-one (7CO) were purchased from Sigma Chemical Co. (St. Louis, MO). Purity of $\Delta^{5,7}$ was checked by LC–MS under conditions described below that allowed us to avoid thermal degradation and to separate dienols and trienols as well as oxygenated sterols from each other. No impurities were observed at retention times (relative to $\Delta^{5,7}$) of monoenols, dienols, and of 7OOH$\Delta^{5,8}$ and 5,8OOΔ^{6}. Only small impurities at retention times shorter than those of the compounds in which we were interested were present, but could not be identified due to their minute amounts. Based on this finding, 7-dehydrocholesterol was used without any preliminary purification. Cholest-5-en-3β,7α-diol (7αOH) was purchased from Steraloids (Steraloids Inc., Wilton, NH). Oxygenated sterols deriving from cholesterol oxidation were obtained as previously described (14, 15). $\Delta^{5,7,9(11)}$ was synthesized according to Windhaus and Linsert (16) by reaction of $\Delta^{5,7}$ (50 mg/ml in ethanol) with $Hg(CH_3COO)_2$. Purification of the trienol was achieved by column chromatography on silica gel G-silver nitrate (17). Elution of the compound was monitored by GC–MS analysis as TMS. Disappearance of $\Delta^{5,7}$ was accompanied by appearance of a compound giving rise to a peak with 14 min rt. Fractions showing this peak were crystallized from methanol. Proton NMR spectrum was obtained in $CDCl_3$. Chemical shifts expressed in ppm (δ $CHCl_3$ 6.62 ppm) were at: 0.548 (3H, s, C18CH_3), 0.844–0.856 (6H, dd, J = 8.8 Hz, C26 and C27CH_3), 0.906 (3H, d, J = 6.4 Hz, C21CH_3), 1.227 (3H, s, C19CH_3), 3.58 (1H, m, H3), 5.38 (1H, complex signal, H7), 5.49 (1H, complex signal, H11), 5.66 (1H, dd, H6). The purified compound showed a typical UV spectrum with maxima at λ 324.4 nm. (ε = 11.581, c = 74 nmol/ml in ethanol), and at 311.2 and 339.2 nm. Acetylation was carried out with acetic anhydride–pyridine 2:1 (v/v) for 30 min at 60°C. The derivative showed a single peak by GC–MS and its mass spectrum is reported in the results (Fig. 1B). The TMS derivative obtained with trimethylsilyl imidazole–piperidine 1:1 (v/v) had rt 13.4 min and the mass spectrum showed the molecular ion at *m/z* 454 (11%) and the $(M^+ - 90)$ ion at *m/z* 364 (100%).

To obtain standard 7OOH$\Delta^{5,8}$ and 5,8OOΔ^{6}, photooxidation of $\Delta^{5,7}$ was carried out in absolute ethanol at 2 mg/ml as suggested by Albro, Corbett, and Schroeder (13). Y Eosin sodium salt (Sigma Chemical Co., St. Louis, MO) was added to the solution and the reaction vessel was irradiated using a Thorn Tropical Day light (5500°K) 15W lamp set, providing a total emission of 22000 Lux. Aliquots were collected after 10 and 120 min of reaction and analyzed by PB–LC–MS. At both times two peaks with a 1/3 area ratio (scanning mode) were present in the chromatogram at 6.2 and 8 min, respectively. Mass spectra registered at both rt showed the base fragment at *m/z* 399 corresponding to the $[M + H - 18]^+$ ion for either a hydroperoxydienol or an endoperoxyenol. Both spectra showed also ions at *m/z* 381 and 365. Nevertheless the protonated molecular ion at *m/z* 417 was lacking in the mass spectrum at 6.2 min as observed for the two other tested hydroperoxy sterols, 7OOH and 5OOH. The parent ion was instead present in the spectrum at 8 min. The ethanolic solution was taken to dryness under nitrogen and the residue was redissolved in $CHCl_3$ containing 0.5 mM HCl (13). After 1 h at room temperature, the solution was washed with water and analyzed by PB–LC–MS. As expected from previous data by Albro et al. (13), the peak at 6.2 min had almost disappeared while a new peak was present. Mass spectrum and rt proved the compound originating this peak to be $\Delta^{5,7,9(11)}$. This result further confirmed for the compound with 6.2 min rt the structure of 7OOH $\Delta^{5,8}$ which is known to be degraded to $\Delta^{5,7,9(11)}$ under various conditions (13).

Mass spectrometry

A Hewlett-Packard spectrometer model 5970MSD was used. Analyses were carried out either in scanning mode (50–500 a.m.u.) or in the MID mode. For GC–

MS the mass spectrometer was connected to a HP Mod. 5890 gas chromatography, equipped with an SPB1 capillary column (0.2 mm i.d., 15 m; Supelco Inc.) with electron impact ionization. Analyses of both acetates and TMS of sterols were carried out under programmed temperature (from 100 to 300°C at 30°C/min) after 1 min at 100°C. Helium flow was 1 ml/min and temperatures of injector, transfer line, and ion source were 280, 290, and 250°C, respectively.

For LC/MS a particle-beam interface (HP 5988A) was used. Temperature of the desolvation chamber was 40°C and helium pressure for vaporization was 36–38 psi. Temperature of the ion source was 250°C. The mass spectrometer operated under CI conditions with CH_4 as reacting gas (0.2 torr). Samples dissolved in methanol were injected into a HPLC pump (HP 1050) equipped with a C18-reversed phase cartridge (5 μm particle size, 150 × 21 mm, Merck Chemical Co, Darmstadt, Germany) which was connected to the PB interface. Column was eluted with methanol at 0.4 ml/min flow rate.

Proton NMR

Spectra were obtained in $CDCl_3$ ($CHCl_3$ 7.26 ppm internal reference) using a Bruker AM-500 instrument (Bruker Instruments, Silberpreiten, Germany) operating at 500 MHz.

Patients with SLOS

Plasma samples of three children (GA, CC, and FS) were analyzed. These subjects were part of a previous study (10) and had plasma $\Delta^{5,7}$ exceeding 0.4 mg/dl which was the established limit to include subjects into the group of patients with SLOS. Concentrations of total cholesterol and of $\Delta^{5,7}$ had been determined by SIM injecting 0.2% of the extract obtained from 0.25–0.5 ml of plasma and corresponded to 53 and 48 mg/dl (GA), 26 and 9.8 mg/dl (CC), 61 and 22 mg/dl (FS), respectively. Two age-matched children with total cholesterol 90 and 150 mg/dl and with plasma $\Delta^{5,7} < 0.4$ mg/dl, were used as controls.

Incubation of microsomes

Microsomes were prepared from the liver of male Sprague-Dawley rats as described previously (18). Final suspension was in 20 ml of KCl 0.15 M and contained 3.13 mg protein/ml. Incubation samples contained 2 ml of microsomal suspension and 0.67 ml of a solution containing 48 mM ADP and 5.4 mM $FeSO_4$ as described by Slater and Sawyer (19). Either $\Delta^{5,7}$ or $\Delta^{5,8}$ (32.5 μM final concentration) was added to the incubation medium as suspension (0.25 mg/ml, 1 ml/sample) in 37.2 mM Tris-HCl buffer (pH 8) containing 83.5 mM KCl and 4.2 mg/ml Tween 80 (20). Final volume of the samples was 20 ml. Samples were incubated for 120 min at 37°C in a Dubnoff incubator in open flasks. At the end of the incubation, extraction was carried out with 4 volumes of ethyl acetate–isoamyl alcohol 98.5:1.5 (v/v) twice. The organic phase was filtered over anhydrous sodium sulfate and taken to dryness under nitrogen. Organic residues were dissolved in methanol and aliquots were analyzed by PB–LC–MS in scan and MID conditions. Control samples were: *a*) microsomes incubated under the above described conditions without any added sterol; *b*) zero-time samples in which microsomes, cofactors, and either $\Delta^{5,7}$ or $\Delta^{5,8}$ were added directly to the extraction solvent; and *c*) samples in which either $\Delta^{5,7}$ or $\Delta^{5,8}$ was incubated in the absence of microsomes and cofactors.

RESULTS

Identification of $\Delta^{5,7,9(11)}$ in plasma of patients with SLOS

A typical chromatogram obtained by GC–MS of a patient plasma extract after acetylation is reported in **Fig. 1A.** In addition to cholesterol, $\Delta^{5,7}$ and $\Delta^{5,8}$ (which coelutes with cholesterol), two peaks marked **a** and **b** were present in all tested patients, but undetectable in controls. Mass spectrum of the compound giving rise to peak a showed ions at *m/z* 350 (M^+–60), 237 (M^+–saturated side chain), 195 [M^+–(saturated side chain + 42)] and 183 (loss of ring A) which suggested for the compound the structure of $\Delta^{5,7,9(10)}$ already reported by Batta et al. (5). The mass spectrum of the corresponding TMS showed the molecular ion at *m/z* 440 and a fragmentation pattern as reported (5). The molecular ion (*m/z* 424) and the fragmentation pattern in the spectrum registered at rt of peak **b** suggested that the compound had the backbone of cholesterol with no modifications in rings A and D, but with three double bonds instead of one. Based on these observations we synthesized $\Delta^{5,7,9(11)}$ as described in the Method section. Retention time and mass spectrum of the acetate of the unknown compound were superimposable to those of the acetate of the authentic standard (Fig. 1B and C). Separation of $\Delta^{5,7,9(11)}$ as TMS derivative from cholesterol TMS was not achieved under the tested conditions.

Quantification of the trienol in plasma of patients was not considered. Nevertheless, semiquantitative evaluation based on the peak areas in the total ion chromatogram indicated that concentration of the sterol in plasma of the tested patients (0.5–1 mg/dl) was much more than the plasma concentration of any sterol precursor of cholesterol (desmosterol, lathosterol, Δ^8-cho-

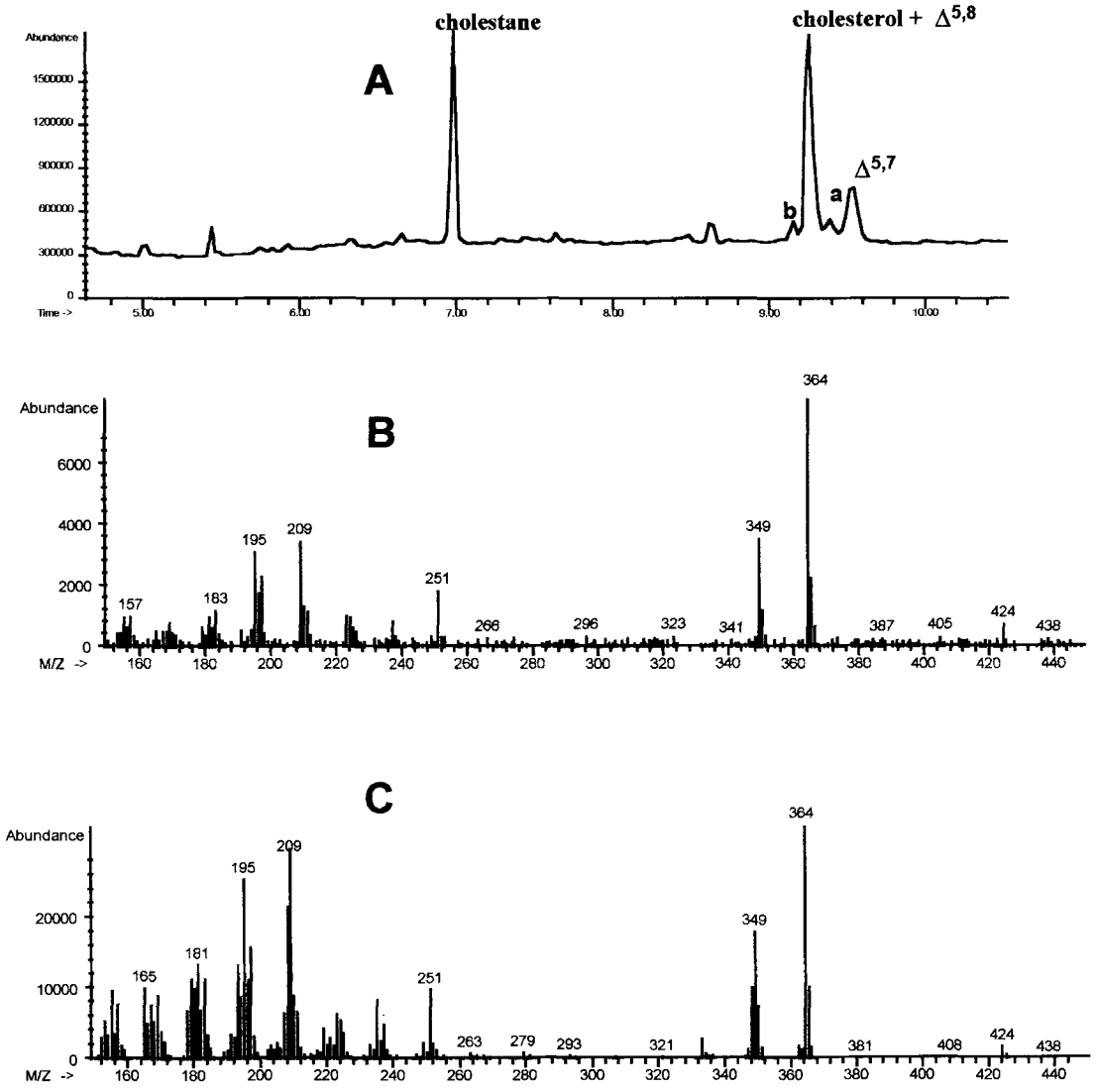

Fig. 1. GC–MS analysis of the acetates of plasma sterols of a patient with Smith-Lemli-Opitz syndrome (patient CC). Analyses were carried out under scan conditions. A: Total ion current. B: EI-mass spectrum of peak b. C: EI-mass spectrum of the acetate of authentic $\Delta^{5,7,9(11)}$.

lesterol) reported to be in the 10–100 μg/dl range (21).

LC–MS analysis of sterols in plasma extracts

Plasma sterols from patients and controls were analyzed by PB–LC–MS under CI conditions. Sensitivity of the detection was much lower than that of GC–MS but LC–MS allowed analysis of hydroperoxysterols without thermal degradation (15). Under the LC–MS conditions used, cholesterol was the most retained among the tested sterols (rt 25 min). CI spectra of all authentic sterols showed the base ion at *m/z* corresponding to the $[M-H_2O + H]^+$ ion: 369 for cholesterol, 367 for dienols as $\Delta^{5,7}$ and $\Delta^{5,8}$ and 365 for $\Delta^{5,7,9(11)}$.

LC–MS analysis of total plasma sterols from patients with SLOS (**Fig. 2**) allowed us to identify cholesterol (rt 25.3 min), $\Delta^{5,7}$ (rt 22.5 min), $\Delta^{5,8}$ (rt 20.3 min), and $\Delta^{5,7,9(11)}$ (rt 16.8 min) by comparison with authentic standards. $\Delta^{5,7,9(11)}$ was well separated from other sterols while an rt very close to that of $\Delta^{5,7}$ was assigned to $\Delta^{5,7,9(10)}$ on the basis of the ion at *m/z* 350 evidenced at this time. In addition to peaks attributable to mono-, di-, and trienols, all patient samples showed minor peaks at retention times characteristic of oxygenated sterols (5–10 min) which were undetectable in control subjects. 5,8OOΔ^6 was identified among these compounds by comparison with the mass spectrum of that of the prepared standard. This finding and previous studies on the formation of $\Delta^{5,7,9(11)}$ trienols from both ergosta-5,7- and cholesta-5,7-dienols (13) prompted us

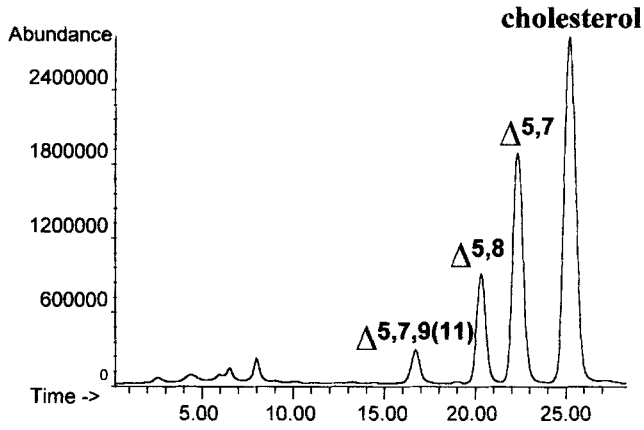

Fig. 2. PB–LC–MS analysis of plasma sterols of a patient with Smith-Lemli-Opitz syndrome. Analysis was carried out under SIM conditions focusing ions at *m/z* 369 (for monoenols), *m/z* 367 (for dienols), *m/z* 365 (for trienols), *m/z* 399 (for hydroperoxydienols), *m/z* 417 (for endoperoxyenols). The chromatogram represents a total ion current under these conditions.

to clarify whether $\Delta^{5,7,9(11)}$ found in patients with SLOS may derive from the oxidation of $\Delta^{5,7}$ accumulated in tissues.

Formation of oxygenated sterols by incubation of rat liver microsomes with $\Delta^{5,7}$

Results of LC–MS analysis of extracts from incubation of activated microsomes are shown in **Fig. 3.** It clearly appears that several compounds giving rise to peaks at rt values of oxygenated sterols were present in the samples (Fig. 3A and 3C). Most of them were likely derived from the oxidation of endogenous microsomal cholesterol because they were present in extracts from control incubations carried out without any added sterol but absent in zero-time incubation controls. The presence of 7OOH, 7αOH, 7βOH, although not well separated from each other (peaks a in Fig. 3), and of 7CO (peak b in Fig. 3) was confirmed by comparison of their mass spectra with those of authentic standards. A similar pattern was observed when microsomes were incubated with $\Delta^{5,8}$, while additional peaks were detected when the incubation was carried out in the presence of $\Delta^{5,7}$. We focused our attention on the two peaks marked X and Y in Fig. 3 that clearly derived from $\Delta^{5,7}$ because they were absent both in extracts from microsomes incubated without any added sterol (Fig. 3C) and in standard $\Delta^{5,7}$ incubated in the absence of microsomes and cofactors. Rt of compounds X and Y corresponded to those of 7OOH$\Delta^{5,8}$ and of 5,8OOΔ^{6} prepared by photooxidation of $\Delta^{5,7}$. The coinjection of extracts from microsomes incubated with $\Delta^{5,7}$ and $\Delta^{5,7}$ photooxidation products (Fig. 3B and 3E) confirmed this finding. Moreover, mass spectra of the two synthetic compounds and those registered at the retention times of peaks X and Y (**Fig. 4**) were superimposable. Trace amounts of $\Delta^{5,7,9(11)}$ were also detected in extracts from microsomes incubated in the presence of $\Delta^{5,7}$.

DISCUSSION

This report clearly establishes the presence of $\Delta^{5,7,9(11)}$ in plasma of patients with SLOS based on retention time and mass spectrum in the GC–MS analysis of the acetate that corresponded to those found for the synthetic compound prepared by treatment of $\Delta^{5,7}$ with $Hg(CH_3COO)_2$ (16). No detectable amounts of the trienol were found in the two control children. $\Delta^{5,8}$ and $\Delta^{5,7,9(10)}$ were also found in plasma of the tested patients according to the observations recently reported by Honda et al. (4) and Batta et al. (5). The possibility that $\Delta^{5,7,9(11)}$ may be formed from $\Delta^{5,7}$ during sample handling was excluded because $\Delta^{5,7,9(11)}$ was absent in extracts of plasma of healthy subjects spiked with either $\Delta^{5,7}$ or $\Delta^{5,8}$. For the former sterol the results were in agreement with those reported by Batta et al. (5) to exclude the formation of $\Delta^{5,7,9(10)}$ during sample extraction and analysis.

Detailed work on oxidation of ergosterol by photochemically produced singlet oxygen was reported by Albro et al. (13) who found that 7-hydroperoxy-ergosta-5,8-dien-3β-ol is formed by this reaction in addition to the already reported 5,8-epidioxy-ergosta-6-en-3β-ol. The former compound was described to be unstable in acidic solution and when kept in solvent for a long time giving rise under both conditions to ergosta-5,7,9(11)-trien-3β-ol. Similar results were also obtained by these authors starting from $\Delta^{5,7}$ (13). This prompted us to check whether this oxidation may occur in a biological medium. Incubation of $\Delta^{5,7}$ with activated rat liver microsomes was carried out and ethyl acetate extracts obtained after incubation were analyzed by PB–LC–MS with chemical ionization. By this procedure extracts obtained from samples incubated with $\Delta^{5,7}$ were shown to contain a number of oxygenated sterols in addition to those deriving from microsomal cholesterol. Among these, 7OOH$\Delta^{5,8}$ and 5,8OOΔ^{6} were clearly identified from their mass spectra which were compared to those of oxygenated sterols obtained by photochemical oxidation of $\Delta^{5,7}$. In contrast, incubation of $\Delta^{5,8}$ with activated rat liver microsomes did not cause the formation of oxygenated sterols in addition to those deriving from cholesterol. These findings and the absence in blank samples incubated with $\Delta^{5,7}$ but without microsomes suggest an enzymatic nature for the conversion of $\Delta^{5,7}$ in the presence of microsomes.

Extract from plasma of a patient with SLOS was ana-

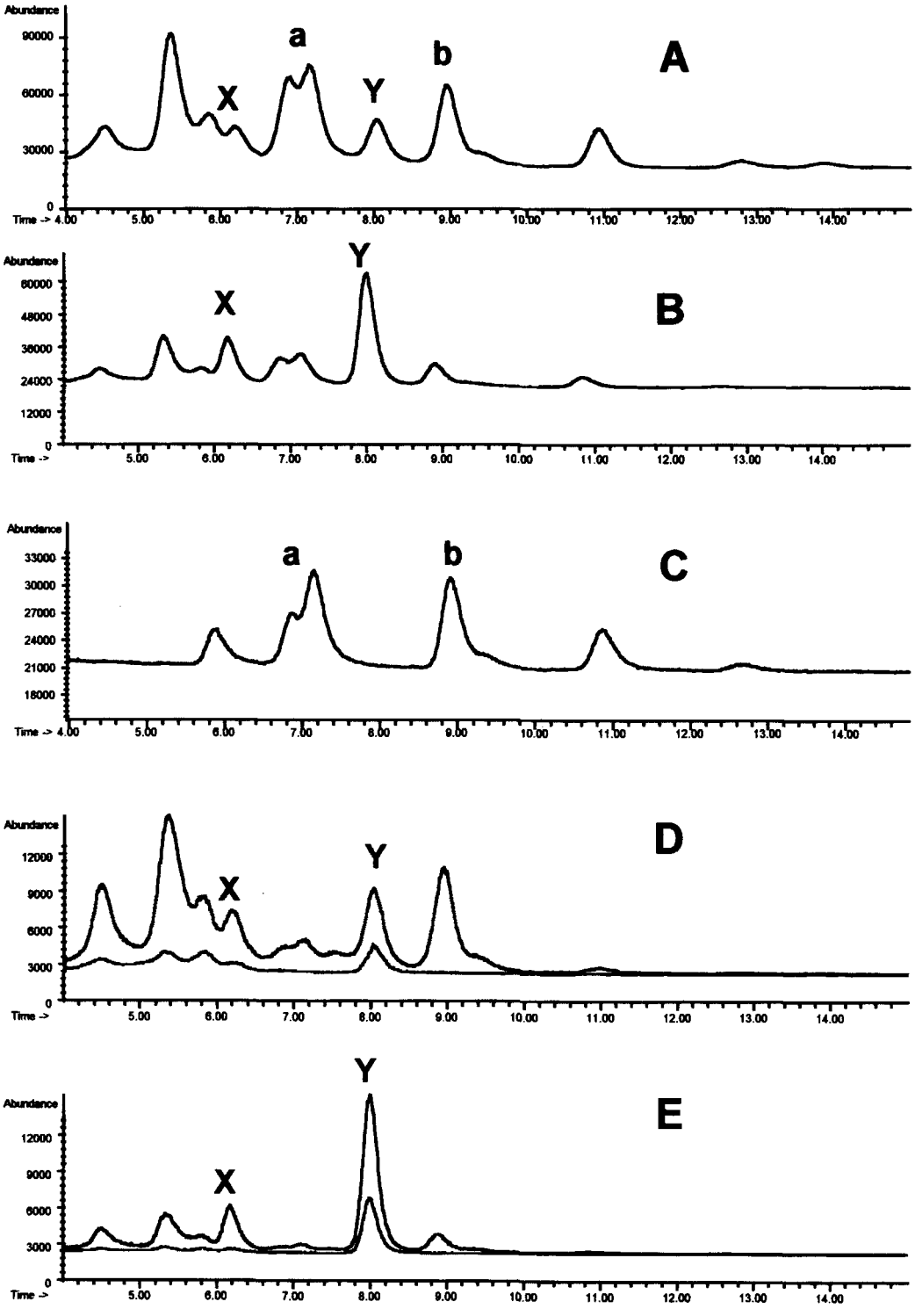

Fig. 3. PB–LC–MS analysis of extracts from rat microsomes incubated under different conditions. Analyses were carried out under SIM conditions as reported in Fig. 2. A: Total ion current from rat microsomes incubated in the presence of $\Delta^{5,7}$. B: Total ion current from rat microsomes incubated in the presence of $\Delta^{5,7}$ coinjected with $\Delta^{5,7}$ photooxidation products. C: Total ion current from rat microsomes incubated without any added sterol. D: Ion chromatograms at *m/z* 399 (solid line) and 417 (dotted line) of the analysis reported in panel A. E: Ion chromatograms at *m/z* 399 (solid line) and 417 (dotted line) of the analysis reported in panel B.

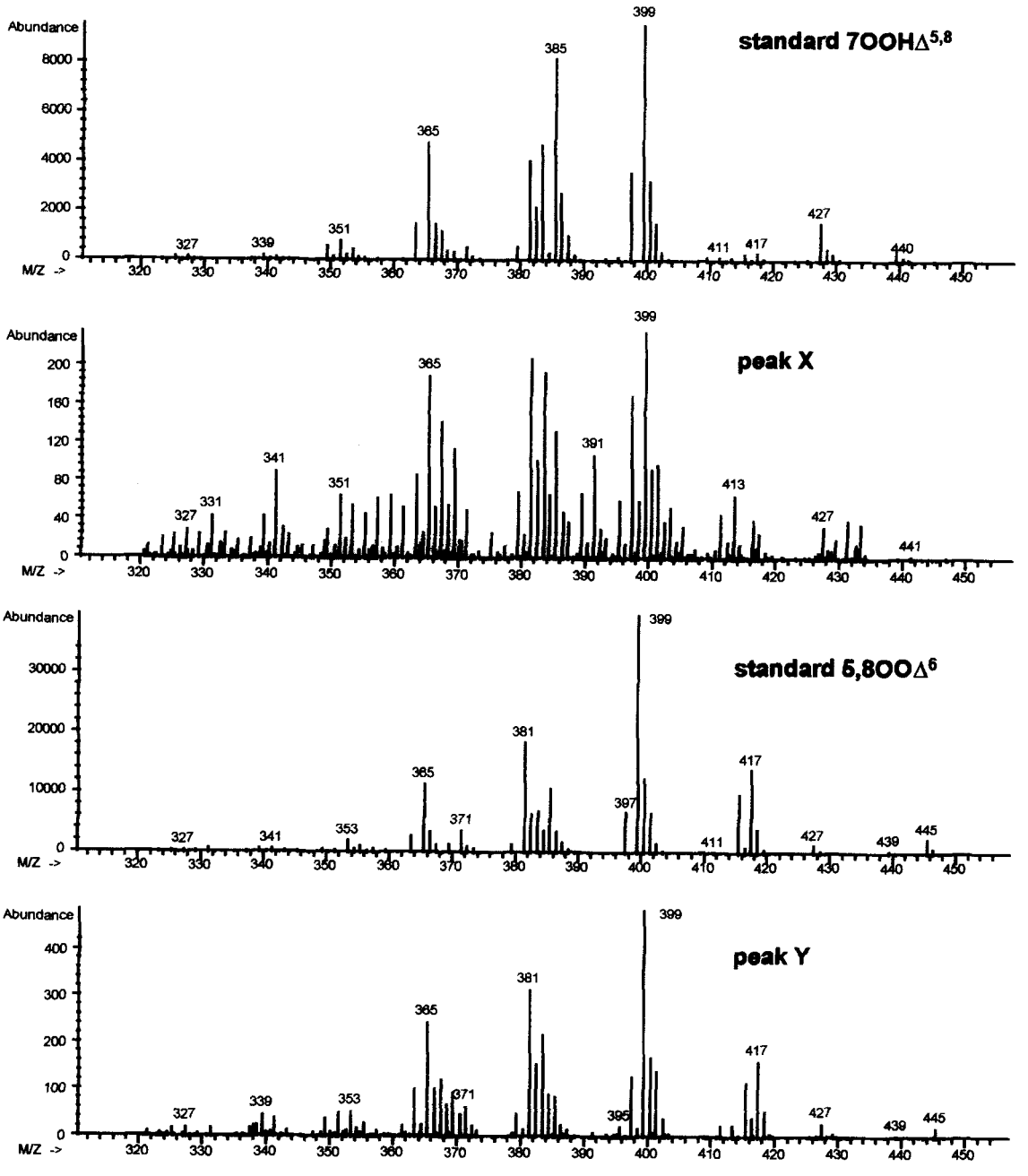

Fig. 4. Mass spectra obtained from PB–LC–MS analysis of $\Delta^{5,7}$ photooxidation products and of peaks labeled X and Y in Fig. 3. Analyses were carried out under scan conditions.

lyzed by LC–PB–MS. A peak at retention time of 5,8OOΔ^6 was present while 7OOHΔ^{5,8} was not detectable. The presence of $\Delta^{5,7,9(11)}$ suggests the possibility that degradation of the hydroperoxy compound may have occurred. On the other hand, the aromatic $\Delta^{5,7,9(10)}$ may also derive from $\Delta^{5,7}$ because aromatization of ring B was reported to occur by degradation of a dimer formed in the photooxidation of the dienol (12). Although the plasma sample had been stored at −80°C, oxidation of $\Delta^{5,7}$ during storage cannot be completely excluded because no antioxidants were added to plasma at the time of blood withdrawal. Based on these considerations it is difficult to establish at present whether the observed oxidation of $\Delta^{5,7}$ may take place in vivo as a consequence of abnormal concentration of oxygen radicals in patients with SLOS. Certainly, de-

composition of 7OOH$\Delta^{5,8}$ to $\Delta^{5,7,9(11)}$ produces hydrogen peroxide which may have toxicological significance (13). Therefore, in patients with SLOS, there may be the need to enhance pharmacologically antioxidant defenses.

In conclusion, we have shown here that accumulation of $\Delta^{5,7}$ in tissues occurring in SLOS may result in its oxidation by oxygen radicals with formation of 5,8OOΔ^{6} and 7OOH$\Delta^{5,8}$. Due to its low stability (13) 7OOH$\Delta^{5,8}$ may give rise to $\Delta^{5,7,9(11)}$ that has been here identified among the unusual sterols in plasma of patients with SLOS.

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