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SYNTHETIC AND BIOGENIC CALCIUM FATTY ACID SALTS: STOICHIOMETRY AND PHASE TRANSITION BEHAVIOUR

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SUMMARY

Calcium fatty acid salts generally exhibit three endothermic processes. A first irreversible transition of the monohydrates is mainly related to dehydration. Simultaneous DTA/light microscopy investigations show that, after a second irreversible phase transition, continuous melting of the compounds occurs. The appearing mesophases do not represent liquid crystalline phases in the classical sense.
Three different samples of fatty acid compounds precipitating in human gallstones were investigated. The only phase found was Calcium Palmitate Monohydrate.

INTRODUCTION

Calcium fatty acid salts with the general formula $Ca(CH_3(CH_2)_xCOO)_2 \cdot yH_2O$ or analogous unsaturated compounds are of interest because of their technical applications and because of their occasional occurrence in biogenic solids as e.g. human gallstones. The role of these compounds in the in vivo formation of gallstones still remains unclear. In order to elucidate the stoichiometry and the phase transition behaviour of calcium fatty acid salts we have synthesized the pure compounds indicated in fig.1. A comparison of the characteristics of synthetic samples with samples of biological origin should reveal the identity of the compounds precipitated in human gallstones.

RESULTS AND DISCUSSION

All samples were prepared by precipitation of calcium chloride in 50 % aqueous ethanolic solution with the corresponding fatty acid solved in absolute ethanol (adjusted to pH=8 with 2 N NaOH). Drying of the precipitates at 10 torr and 20 °C resulted in stoichiometric monohydrates, whereas the corresponding anhydrates could be prepared by drying at 10^{-3} torr and 60 °C. The stoichiometry was checked by chemical analysis and by dehydration of the compounds on a thermobalance (Perkin-Elmer TGS-2). The start of the dehydration reaction of the monohydrates variies from about 71 °C (Ca-Oleate·$1H_2O$) to about 87 °C (Ca-Laurate·$1H_2O$).
The X-ray powder diffraction diagrams (fig.1) prove that the monohydrates as well as the anhydrates represent more ore less crystalline compounds. The diagrams of the monohydrates of Ca-Laurate, Ca-Myristate, Ca-Stearate and partly also of Ca-Palmitate and Ca-Oleate show reasonable agreement with published data (1). The literature data however are erroneously assigned to belong to the corresponding anhydrates. No X-ray diagrams are published so far which coincide with our diffraction data for calcium fatty acid anhydrates.
Differential Scanning Calorimetry (DSC) data were recorded on a Perkin-Elmer DSC-2 instrument (flowing nitrogen atmosphere, heating rates 10 °C/min., sample weights 1.0 - 2.2 mg). With the exception of Ca-Laurate and Ca-Oleate the compounds investigated generally exhibit three endothermic processes on heating (fig.2 and fig.3). Cooling experiments show that only the third, small DSC peaks at about 190 °C (ΔH values about 1 kcal/mol) are reversibel, whereas the two first peaks between about 90 °C and 140 °C (sum of ΔH values

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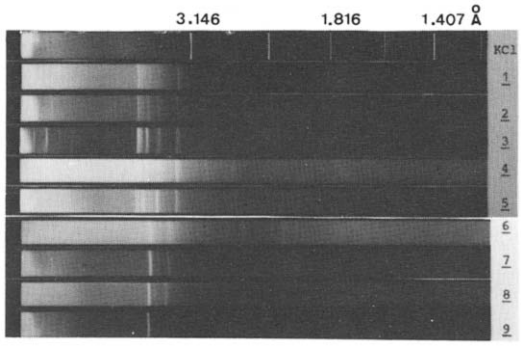

Fig.1. Powder X-ray diffraction films (Guinier-de Wolff camera, Cu-$K_{\alpha 1}$) of:
1 Ca-Laurate·$1H_2O$
2 Ca-Myristate·$1H_2O$
3 Ca-Palmitate·$1H_2O$
4 Ca-Stearate·$1H_2O$
5 Ca-Oleate·$1H_2O$
6 Ca-Laurate
7 Ca-Myristate
8 Ca-Palmitate
9 Ca-Stearate.

about 20 - 40 kcal/mol) are irreversibel. Form and splitting of the first DSC peak are strongly dependent on the sample weight used for the experiment (see fig.4 and fig.5). For instance, the first single peak of Calcium Myristate Monohydrate also clearly splits up into two peaks, if samples of less than 0.5 mg are investigated. Fig.6 gives a plot of transition temperatures and transition enthalpies versus the chain length of the corresponding fatty acid.

The first transition of the monohydrates at about 90 - 120 °C is mainly due to the dehydration process as demonstrated by TG/DTA experiments (Stanton Redcroft Simultaneous Thermal Analysis System STA-780, flowing nitrogen atmosphere). As an example, the TG/DTA data for Ca-Myristate·xH_2O are given in fig.7. Simultaneous DTA/light microscopy investigations were carried out in flowing nitrogen on a Mettler TA 2000 unit combined with an Olympus Vanox microscope. Some minor changes in the macroscopic morphology of the calcium fatty acid monohydrate powders were observed in connection with the first DTA peak (mainly assigned to dehydration). No changes however were seen during the second transition of the monohydrates or during both two first transitions of the anhydrates. Continuous sintering and melting of all compounds started after the two first transitions, obviously not evident from the DTA traces with the exception of the small reversible "melting" peaks at about 190 °C. At about 150 °C all samples were transparent, but no textures or interference colors typical for liquid crystalline phases could be observed. Therefore we conclude that the appearing mesophases do not represent liquid crystalline phases in the classical sense.

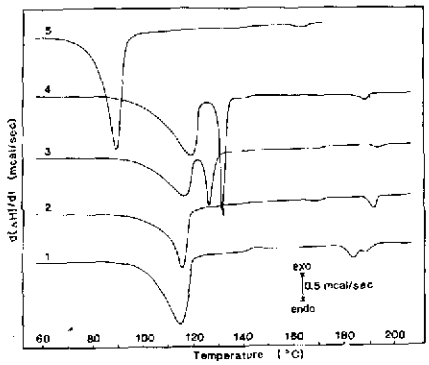

Fig.2. DSC curves of monohydrates: 1:Ca-Laurate, 2:Ca-Myristate, 3:Ca-Palmitate, 4:Ca-Stearate, 5:Ca-Oleate.

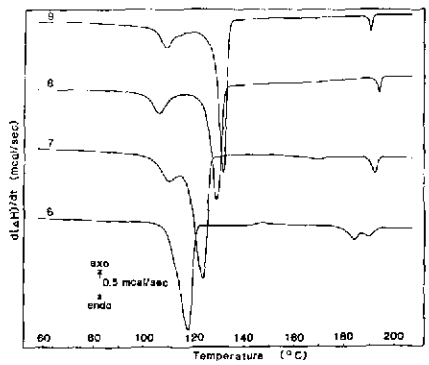

Fig.3. DSC curves of anhydrates: 6:Ca-Laurate, 7:Ca-Myristate, 8:Ca-Palmitate, 9:Ca-Stearate.

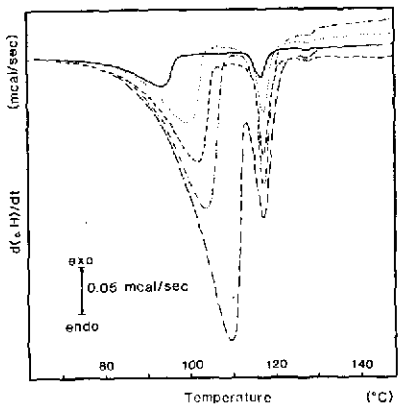

Fig.4. Ca-Myristate·1H_2O: Influence of sample weight on the DSC curves: 0.053 mg, 0.115 mg, 0.195 mg, 0.310 mg, 0.495 mg.

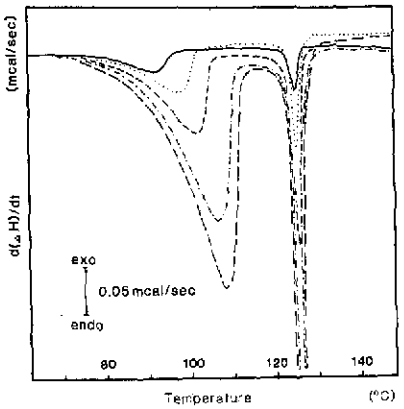

Fig.5. Ca-Palmitate·1H_2O: Influence of sample weight on the DSC curves: 0.050 mg, 0.110 mg, 0.200 mg, 0.365 mg, 0.485 mg.

Occasionally biogenic fatty acid compounds are found in human gallstones, often in the form of white, flowerlike spheroids (see fig.8) with diameters of about 0.2 mm. On the basis of (poorly defined) X-ray powder data it has been suggested, that these compounds represent Ca-Palmitate or α-palmitic acid (2). We have investigated samples isolated from three different gallstones. Energy Dispersive Analysis of X-rays (fig.8) proves the occurrence of calcium within the spheroids, in contrast to the surrounding calcium-free cholesterol matrix. Comparison of DSC data with data from synthetic samples (fig.9) confines the choice to two possible compounds, Ca-Palmitate·1H_2O or Ca-Myristate·1H_2O. Massspectrometric analysis (fig.10) finally indicate palmitic acid. The fatty acid compounds isolated from human gallstones therefore represent Ca-Palmitate Monohydrate.

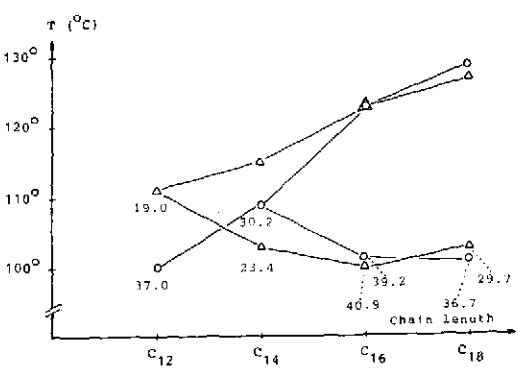

Fig.6. Plot of T_{eo} (extrapolated onset temperature) of DSC peaks versus chain length of the corresponding fatty acid. Numbers in the diagram indicate the total enthalpy ΔH (kcal/mol) of the transitions.
o Monohydrates
Δ Anhydrates

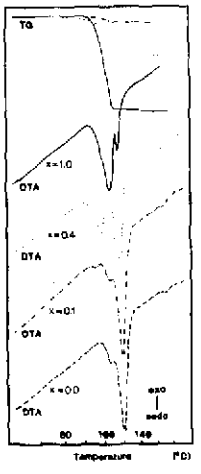

Fig.7. TG/DTA measurements of Ca-Myristate·xH_2O with x=1.0, 0.4, 0.1 and 0.0. Sample weigths: 6.6 - 8.9 mg.

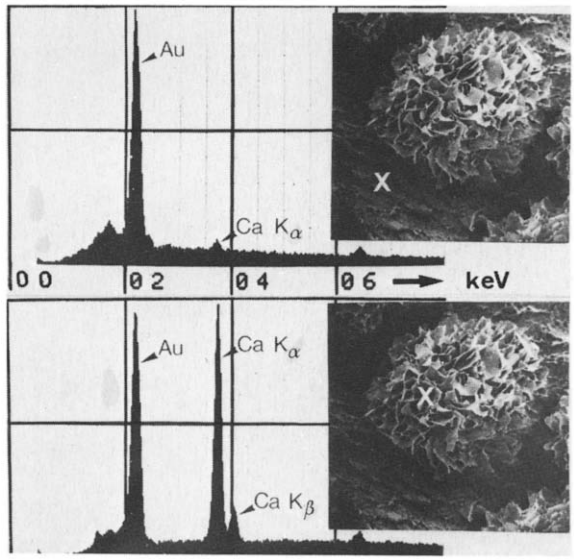

Fig.8. EM photographs of a calcium fatty acid spheroid from a human gallstone and corresponding Energy Dispersive Analysis of X-rays. Spectra were obtained from the regions indicated with "X".

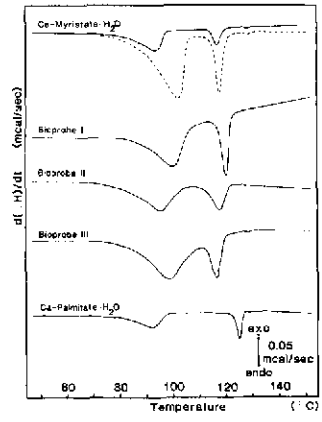

Fig.9. DSC curves of biogenic fatty acid compounds compared to sythetic samples. Sample weights: 0.05 - 0.20 mg.

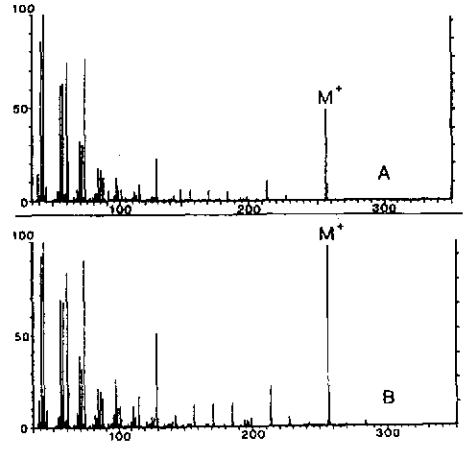

Fig.10. Mass spectra of synthetic (A) and of biogenic (B, Bioprobes I and II, see fig.9) fatty acids prepared by treatment of the calcium salts with conc. HCl.

REFERENCES

(1) JCPDS, Powder Diffraction File. Cards No. 9-851, 9-852, 5-012, 5-010 and 5-287, Swarthmore, PA.
(2) D.J.Sutor, Gut 11,618-619 (1970).