notes on methodology

Crystallization of sodium taurocholate

J. Lon Pope*

Department of Biochemistry, University of Florida, College of Medicine, Gainesville, Florida 32601

SUMMARY Success in crystallizing sodium taurocholate from ethanol by the addition of ether is critically dependent on the water content of the system. Two crystalline forms of sodium taurocholate were obtained with melting points of 180°C and 225–235°C respectively.

KEY WORDS sodium taurocholate · crystallization conditions · polymorphism

Since conjugated bile acids cannot be readily isolated and crystallized from bile, they are usually prepared from the free acids by the method of Norman (1), that is, the tributylammonium salt of a free bile acid is treated with ethyl chloroformate to give an anhydride, which then reacts with glycine or taurine to yield the conjugated acid. The latter, either as the free acid or as the sodium salt, is then crystallized from aqueous ethanol or from other solvents.

Difficulties were encountered in this laboratory and elsewhere, however, in preparing by this method crystalline NaTC with a melting point in the reported range of 225–235°C. Firstly, the newly synthesized NaTC usually separated as an amorphous material from the mother liquor on the addition of ethyl acetate at the first precipitation step, or of ether at a later precipitation step. Secondly, in one preparatory batch, the crystalline product which was finally obtained after repeated solution and reprecipitation of the amorphous product had a melting point of about 180°C.

By a slight modification of the method, good crystalline preparations of NaTC with the reported melting point were readily obtained. Dioxane, tri-*n*-butylamine, and ethyl chloroformate were freshly redistilled as specified by Norman (1). Reagent grade ethyl acetate, anhydrous ether, and anhydrous ethanol were used without further purification. Taurine was recrystallized by the procedure of Cortese (2), and cholic acid (Nutritional Biochemicals Corporation, Cleveland, Ohio) was recrystallized two or three times by the procedure of Hofmann (3). When the NaTC failed to crystallize from the cooled ethanol–ethyl acetate solution, it was precipitated as a gum by the addition of about 3 volumes more of cold ethyl acetate. After the supernatant solution had been poured off, the residue was allowed to dry and was then dissolved in 90% ethanol.[1] Ether was added until incipient cloudiness was perceptible and the mixture was cooled from room temperature to 2°C in a refrigerator. If the NaTC again failed to crystallize, it was precipitated once more as a gum by the addition of about 3 volumes of cold ether. This cycle of dissolving the gum in 90% ethanol and reprecipitating it by the addition of ether was repeated until crystals were finally obtained from the aqueous ethanol solution at room temperature in the presence of just enough ether to cause incipient cloudiness. The mixture was then cooled to 2°C to increase the yield of crystals. This preparation melted at 225–235°C, and could be recrystallized readily in 70–80% yield from the same solvent.

Our difficulty in obtaining a crystalline product was apparently caused by an inadequate amount of water in the crystallizing solvent. Many years ago Cortese and Bashour (4) stated that the aqueous ethanol should have a water concentration of 9–12% before the addition of ether. Later Cortese (5) emphasized that crystallization will not occur unless enough water is present. Anderson (6) used 88% ethanol for crystallizing NaTC. The "90% ethanol" specified in Norman's method was originally prepared by mixing 90 volumes of absolute ethanol and 10 volumes of water. Because of the decrease in volume that occurs, the resulting solution is about 91.5% ethanol.[1]

Brief tests were run in which precipitation conditions encountered in the NaTC preparation procedure were simulated using crystalline synthetic NaTC (mp 225–235°C), ether, and aqueous ethanol having ethanol concentrations of 89, 90, 91, 91.5, and 92%. Crystallization occurred readily from 89 and 90% ethanol, with difficulty from 91 and 91.5% ethanol, and not at all from 92% ethanol.

If the conjugation reaction does not proceed to completion, sodium cholate remains in the reaction mixture; this was found not to interfere with the crystallization of NaTC.

Under various conditions, three different crystalline forms of NaTC with different melting points have been

Abbreviations: NaTC, sodium taurocholate; TLC, thin-layer chromatography.

* Present address: Department of Biochemistry, Faculty of Medical Sciences, University of Medical Sciences, Bangkok, Thailand.

[1] The percentage of ethanol is expressed as, and is numerically equal to, the volume in milliliters of 100% ethanol contained in 100 ml of aqueous ethanol at room temperature. Accordingly, *n*% ethanol was prepared by diluting *n* ml of 100% ethanol with sufficient water to yield 100 ml of aqueous ethanol at room temperature. Since the volume of these fluids decreases on mixing, volume per cent values may be misleading.

isolated. In our studies, melting points of 180°C and 225–235°C were obtained for NaTC preparations which behaved identically on Silica Gel G TLC [these gave single spots having R_f values of 0.05–0.10 using Solvent II in the analytical system of Hofmann (7)]. Earlier, Tanaka (8) presumably isolated the same forms, mp 180°C and mp 230–231°C, whereas Cortese and Bashour (4) isolated a form that melted at 130–145°C, as well as the one melting at 225–235°C. Norman (1) reports only the form with a 225–235°C melting point, and notes the difficulty in obtaining the melting points of the sodium salts of the bile acids. Hofmann (9) also calls attention to the limited value of melting point determinations of bile acids, in view of the frequent occurrence of polymorphic forms, of the formation of mixed crystals or inclusion compounds, and of the tendency of bile acids to cocrystallize with solvent.

This work was supported by a grant-in-aid (GB-2164) from the National Science Foundation and a grant-in-aid (A-1278-09) from the National Institutes of Health.

Manuscript received 4 August 1966; accepted 2 November 1966.

References

1. Norman, A. 1955. *Arkiv Kemi*. **8:** 331.
2. Cortese, F. 1936. *J. Am. Chem. Soc*. **58:** 191.
3. Hofmann, A. F. 1963. *Acta Chem. Scand*. **17:** 173.
4. Cortese, F., and J. T. Bashour. 1937. *J. Biol. Chem*. **119:** 177.
5. Cortese, F. 1937. *J. Am. Chem. Soc*. **59:** 2532.
6. Anderson, I. G. 1962. *Nature*. **193:** 60.
7. Hofmann, A. F. 1962. *J. Lipid Res*. **3:** 127.
8. Tanaka, T. 1933. *Z. Physiol. Chem*. **220:** 39.
9. Hofmann, A. F. 1964. The Function of Bile Salts in Fat Absorption. Thesis. University of Lund, Sweden.