Small-Angle Scattering of X-Rays in Aqueous Solutions of Sodium Salts of Conjugated and Unconjugated Bile Acids

PER EKWALL, KRISTER FONTELL and ARNE NORMAN*

Institute of Physical Chemistry, Åbo Akademi, Åbo, Finland

We have previously shown that aqueous sodium desoxycholate and cholate solutions produce a small angle X-ray pattern [1,2]. Using the same apparatus and technique we have now investigated solutions of sodium salts of a number of conjugated and unconjugated bile acids, which we have found to give rise to similar X-ray patterns. The salts studied are: sodium cholate, desoxycholate, chenodesoxycholate, dehydrocholate, taurocholate, taurodesoxycholate, taurochenodesoxycholate, glycocholate, glycodesoxycholate and glycochenodesoxycholate. The chenodesoxycholic acid and all the conjugated bile acids were synthesized by one of us (A.N.)[3]. The other acids were samples from Hoffmann-La Roche A. G., Basle, which were recrystallized several times before use. Their melting points were: cholic acid, 197° C, desoxycholic acid, 174—175° C, dehydrocholic acid 237° C.

The sodium dehydrocholate solutions (up to 0.4 molal) gave only a dark background; no intensity maximum was evident on the photometer curve. All the other bile salts, however, yielded pronounced intensity maxima, as seen in Figs. 1 and 2, where photometer recordings of the X-ray films obtained with 0.4 molal solutions of the salts are reproduced. It is immediately noted that the three cholates as a group and also the three desoxycholates and the three chenodesoxycholates exhibit closely similar X-ray patterns. Conjugation does not appear to have any greater influence on the location of the intensity maxima. Also the photometric curves for the desoxycholates and chenodesoxycholates are very similar, which indicates that the positions of the hydroxyl groups (3,12 and 3,7) do not exert any significant effect. The number of hydroxyl groups does, however, influence the scattering since marked differences are noted between the photometric curves of the cholate series (3 hydroxyl groups), on one hand, and those of the desoxy- and chenodesoxycholate series (2 hydroxyl groups), on the other.

* Present address: Department of Physiological Chemistry, University of Lund, Lund, Sweden.

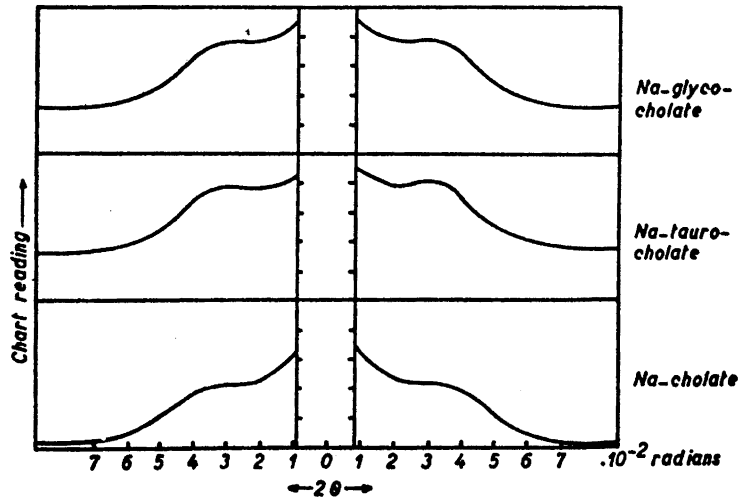

Fig. 1. Photometer recordings of the small angle scattering by 0.4 M aqueous solutions of sodium cholate, taurocholate and glycocholate.

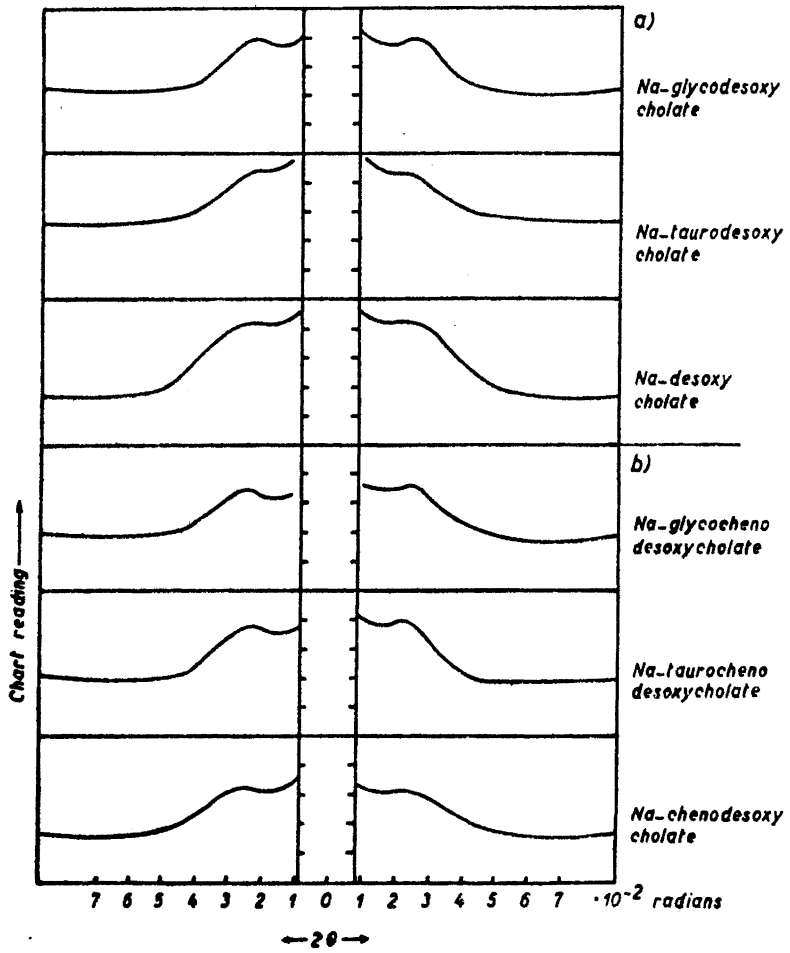

Fig. 2. Photometer recordings of the small angle scattering by 0.4 M aqueous solutions of

a) sodium desoxycholate, taurodesoxycholate and glycodesoxycholate.

b) sodium chenodesoxycholate, taurochenodesoxycholate and glycochenodesoxycholate.

The fact that the bile salt solutions yield small-angle X-ray scattering patterns shows that an association of the ions to colloidal aggregates, micelles, occurs in their solutions. This result is in agreement with earlier conclusions drawn on the basis of studies in which other methods have been employed [4,5]. Also the finding that conjugation does not greatly influence the micelle formation is in accordance with our previous experience [4,5]. The fact that sodium dehydrocholate solutions do not give rise to an intensity maximum in the small angle X-ray pattern agrees with the results of other physicochemical measurements which indicate that association in these solutions sets in only after a very high concentration, about 0.12 M, has been reached, *i. e.* the tendency of these anions to form micelles is weak [4].

1. Ekwall, P. and Fontell, K. *Acta Chem. Scand.* **10** (1956) 327.
2. Ekwall, P. and Fontell, K. *IX Nord. Kemistmötet, Aarhus, 1956.*
3. Norman, A. *Arkiv Kemi* **8** (1955) 331.
4. *Cf.* Refs. 1—8 in Ref. [1]
5. Ekwall, P. and coworkers. *Unpublished experiments.*

Received December 19, 1956.

Fractionation of Methyl Cellulose Hydrolysate

INGEMAR CROON and BENGT LINDBERG

Institutionen för Träkemi, Kungl. Tekniska Högskolan, Stockholm, Sweden

The distribution of substituents in cellulose derivatives has previously been studied by indirect analytical methods (*cf.* Ref.[1]), the results of which may be rather uncertain and open to some doubt. Spurlin [1] suggested that cellulose ethers should be studied by a different method, involving hydrolysis to glucose and glucose ethers and the fractionation of these by some chromatographic technique. The actual isolation and identification of these products would offer a more reliable basis for calculations. Several authors [2-5] have reported partial fractionations of the products obtained by hydrolysis of methyl cellulose, *e. g.* the fractionation of the three monomethylated glucoses or the fractionation of glucose, mono-, di- and trimethyl ethers. In the present paper the complete fractionation of the eight products in such a hydrolysate is reported. The fractionation was performed on a carbon column using the gradient elution technique. Lindberg and Wickberg[6] separated some methylated sugars by this method and we have recently reported a similar fractionation of the hydrolysate from a hydroxyethyl cellulose[7]. Two components, 6- and 3-*O*-methyl-D-glucose, did not separate by this method but were easily separated by paper chromatography. All components were obtained in a state of purity and identified by their behaviour in paper chromatography and paper electrophoresis, by comparison with authentic samples and by determination of m.p. and optical rotation. Some of them crystallised (see Table 1) and the values for their melting points and specific rotations were in good agreement with those previously recorded.

Experimental. The methyl cellulose investigated was prepared from cotton linters and had a D.S. of 0.90 (OCH_3 15.85, 15.91, 15.97). A sample (1.5 g) was treated with cold 72 % sulphuric acid (16.7 ml). After 30 min at 0° the sample had dissolved completely. It was then diluted with water to 150 ml and heated under nitrogen, first overnight at 60° and finally for 3 h at 100°. The sulphuric acid was neutralised with barium carbonate, the salts removed by filtration and the solution concentrated to dryness (1.460 g). No cellobiose could be detected in the hydrolysate. An aqueous solution of the hydrolysate (50 ml) was added to the top of a carbon column (43 × 3.5 cm) which was then eluted with the following solvents.

Water		1 000 ml
Aqueous ethanol	0 — 8 %	5 000 »
» »	8 — 25 »	2 500 »
» »	25 — 50 »	3 000 »

Fractions (26 ml) were collected and investigated by paper chromatography, paper electrophoresis and determination of the optical rotation (2 dm tube). The result of the fractionation is given in Fig. 1. The 6- and 3-*O*-methyl ethers did not separate well, the former is eluted very slightly faster, and they both contribute to the third peak in Fig. 1, which has a somewhat irregular shape. A small part of the 2-*O*-methyl-D-glucose (second peak) was mixed with the 6-*O*-methyl-D-glucose. Fractions containing s_0 (glucose), s_2(2-*O*-methyl-D-glucose), s_2+s_6, s_6+s_3, s_{26}, s_{36}, s_{23} and s_{236} respectively, were combined, taken to dryness and weighed. In the mixed fraction s_2+s_6, the rather small amount of s_2 was estimated from the shape of the curves. In the mixed fraction s_6+s_3, the proportion between the components was determined by paper chromatography, using methyl ethyl ketone-water (15:1) as solvent. An analytical determination, following the procedure of Hirst, Hough and Jones [8] and a preparative separation on thick filter paper, gave figures in reasonably good agreement, 2.32, 2.43 and 2.26:1, respectively. The yields of each component together with characterising data are given in Table 1.

In a preliminary experiment a larger amount (3.0 g) of methyl cellulose was hydrolysed in the same way and the components fractionated on the same column. A steeper gradient was