A model for micellar aggregates of a bile salt: crystal structure of sodium taurodeoxycholate monohydrate

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Abstract Crystals of sodium taurodeoxycholate monohydrate, $NaC_{26}H_{44}NO_6S \cdot H_2O$, are trigonal, space group $P3_1$, with $a = 18.393(1)$, $c = 7.097(1)$ Å, $V = 2079.3(5)$ Å^3, and $Z = 3$. The structure was solved by direct methods and Fourier techniques and refined by full-matrix least-squares calculations. The final R index is 0.051. The side chair of the anion displays an approximate folded-back conformation. The cyclopentane ring assumes an intermediate conformation between the half-chain and the β-envelope. The sodium ion shows a distorted octahedral coordination with six oxygen atoms, giving rise to ion-ion and ion-dipole interactions. The molecules form helices, characterized by threefold screw axes, with a radius of about 16 Å. The helices are packed in such a way as to be embedded in each other as cog-wheels. The helix found in this crystal structure will be used as a model and checked in the study of the micellar solutions of sodium taurodeoxycholate, following the same strategy satisfactorily employed in the case of sodium deoxycholate.—**Campanelli, A. R., S. Candeloro De Sanctis, E. Giglio, and L. Scaramuzza.** A model for micellar aggregates of a bile salt: crystal structure of sodium taurodeoxycholate monohydrate. *J. Lipid Res.* 1987. **28:** 483–489.

Supplementary key words X-ray diffraction • sodium taurodeoxycholate helices • micellar structure

Many problems are still open in the physical chemistry and structure of micellar aggegates of bile salts owing to contradictory results reported in the literature (1). Two valuable reviews illustrate the physical-chemical properties of this important class of interesting biological compounds (2, 3). Unfortunately, many experimental data cannot be correctly interpreted nor can some forecasts about their behavior be inferred since the structure of their micelles is unknown. For this reason we believe that reasonable structural models, satisfactorily describing the micellar properties of bile salts, are needed. Of course, the study of the solid state easily provides structural models that can be verified in solution, even when the perturbation due to the solvent may change the structure observed in the solid state. In order to minimize this danger, we tried to identify bile salts that increase the micellar size by varying parameters such as ionic strength, pH, and temperature up to the formation of a gel. From this gel a macromolecular fiber can be drawn that transforms into a crystal by aging. When the X-ray diffraction patterns of all these solid and liquid phases show intensity maxima and minima in the same ϑ regions, it is likely that the same structural unit (or a very similar one) occurs both in the solid and liquid phases. Thus, the determination of the crystal structure may give a suitable model to be used in the study of the micellar solutions. This strategy was satisfactorily applied to sodium deoxycholate (NaDC), which satisfies the above-mentioned requirements (4–8). The structural unit is a helix having the inner part filled by cations and water molecules and the outer surface covered by nonpolar groups as the methyls C_{18} and C_{19} (5). Ion-ion ($Na^+ \cdots {}^-COO$) and ion-dipole ($Na^+ \cdots H_2O$) interactions together with a close network of hydrogen bonds strongly stabilize the helix which has been successfully tested in the investigation of NaDC aqueous solutions by means of small-angle X-ray scattering, nuclear magnetic resonance, electron spin resonance, and circular dichroism techniques.

Subsequently, sodium taurodeoxycholate (NaTDC, **Fig. 1**) was identified as another bile salt which fulfills the above mentioned requirements. From its aqueous solutions gels, macromolecular fibers and crystals were obtained. Inspection of preliminary X-ray data regarding the macromolecular fiber and the crystal showed that an assembly of helices is present in these two solid phases.

Abbreviations and nomenclature: taurodeoxycholate, 3α,12α-dihydroxy-5β-cholanoyltaurine; deoxycholate, 3α,12α-dihydroxy-5β-cholan-24-oate. NaTDC, sodium taurodeoxycholate; NaDC, sodium deoxycholate.

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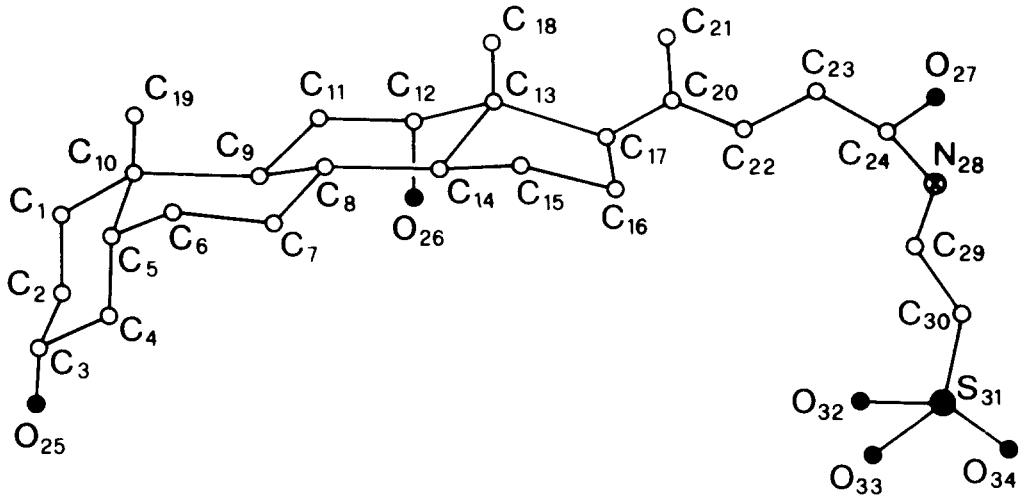

Fig. 1. Atomic numbering of the NaTDC anion. Hydrogen atoms are omitted.

Thus, the solution of the NaTDC crystal structure was judged interesting and was undertaken for the following reasons. *a*) It has been reported that NaTDC, under proper conditions, forms rodlike micelles in aqueous solutions (9, 10). The helix is a characteristic rod-shaped object. *b*) If the helix found in the NaTDC crystal structure will be confirmed in the micellar solutions, a comparison can be made with the helix of NaDC, looking for common motifs in the helical geometry and in the types of interactions. *c*) If the NaTDC micelles are helices, as in the case of NaDC, the idea that the micelles of the bile salts are all helices, even if different among them, takes shape.

EXPERIMENTAL

Sodium taurodeoxycholate monohydrate (Calbiochem) was dissolved in water at room temperature. Acetone was then added until the solution, kept in a closed flask, became cloudy. The colorless crystals obtained after a few days (mp 481–483 K), recrystallized several times from the mixture acetone–water, were prisms elongated along the c axis. The crystal selected for data collection had dimensions $\sim 0.8 \times 0.4 \times 0.1$ mm. Weissenberg and precession photographs showed that the crystals were trigonal, space group $P3_1$ or $P3_2$. The unit cell parameters were refined using angular settings of four independent reflections with their symmetry equivalent ones and their Friedel mates, measured with a Nonius CAD4 diffractometer. The density, measured by flotation in a mixture of benzene and carbon tetrachloride, was 1.29 g · cm^{-3} in agreement with that calculated for 3(NaTDC · H_2O) in the unit cell (1.29 g · cm^{-3}). Three-dimensional X-ray intensity data were collected at room temperature for 4416 reflections having $2\vartheta \leq 140°$, with indices hkl and $\bar{h}kl$. Intensity measurements were made by use of Ni-filtered

TABLE 1. Nonhydrogen atom positional parameters ($\times 10^4$) and U_{eq} ($\times 10^3$), with e.s.d.'s in parentheses

Name	x	y	z	U_{eq} (Å^2)
C_1	−1681 (5)	6111 (4)	6501 (11)	54 (3)
C_2	−1247 (5)	6975 (5)	5607 (12)	59 (3)
C_3	−1711 (5)	6991 (4)	3902 (13)	59 (3)
C_4	−1825 (5)	6307 (4)	2471 (12)	55 (3)
C_5	−2238 (4)	5425 (4)	3364 (11)	47 (3)
C_6	−2314 (4)	4787 (4)	1952 (12)	53 (3)
C_7	−1486 (4)	4834 (4)	1471 (12)	49 (3)
C_8	−1019 (4)	4815 (4)	3236 (10)	41 (3)
C_9	−923 (3)	5482 (3)	4645 (10)	37 (3)
C_{10}	−1789 (4)	5390 (4)	5193 (11)	46 (3)
C_{11}	−404 (4)	5508 (4)	6387 (10)	47 (3)
C_{12}	457 (4)	5616 (4)	5858 (11)	45 (3)
C_{13}	365 (4)	4950 (4)	4431 (10)	39 (3)
C_{14}	−139 (4)	4973 (4)	2756 (10)	39 (3)
C_{15}	−53 (5)	4419 (5)	1292 (12)	56 (3)
C_{16}	846 (4)	4584 (5)	1557 (11)	53 (3)
C_{17}	1182 (4)	5106 (4)	3407 (10)	41 (3)
C_{18}	−54 (4)	4081 (4)	5400 (12)	50 (3)
C_{19}	−2341 (4)	4564 (4)	6238 (12)	53 (3)
C_{20}	1813 (4)	4923 (4)	4403 (11)	44 (3)
C_{21}	2123 (5)	5382 (7)	6317 (12)	76 (3)
C_{22}	2572 (4)	5178 (4)	3099 (11)	50 (3)
C_{23}	3118 (4)	4782 (4)	3625 (12)	50 (3)
C_{24}	2628 (4)	3851 (4)	3269 (11)	47 (3)
O_{25}	−1282 (4)	7798 (3)	3058 (10)	81 (3)
O_{26}	995 (3)	6440 (3)	5111 (8)	51 (2)
O_{27}	2427 (3)	3591 (3)	1627 (9)	63 (3)
N_{28}	2407 (4)	3355 (4)	4755 (11)	62 (3)
C_{29}	1780 (5)	2461 (4)	4726 (13)	64 (3)
C_{30}	897 (5)	2344 (4)	4939 (14)	65 (3)
S_{31}	101 (1)	1305 (1)	5440[a]	51 (1)
O_{32}	419 (3)	1018 (3)	6969 (9)	62 (3)
O_{33}	−45 (3)	820 (3)	3717 (9)	62 (3)
O_{34}	−616 (3)	1378 (4)	5982 (9)	72 (3)
O_w	1194 (8)	2186 (7)	15 (15)	148 (3)
Na	40 (2)	820 (2)	396 (5)	56 (2)

$U_{eq} = 1/3\ (\Sigma_i \Sigma_j U_{ij}\, a^*_i\, a^*_j\, \bar{a}_i \cdot \bar{a}_j)$, where U_{ij} are thermal parameters, expressed as mean-square amplitudes of vibration, and $\bar{a}$ and a^* are unit cell direct axes and reciprocal axes moduli, respectively.

[a] This coordinate was kept fixed during the refinement.

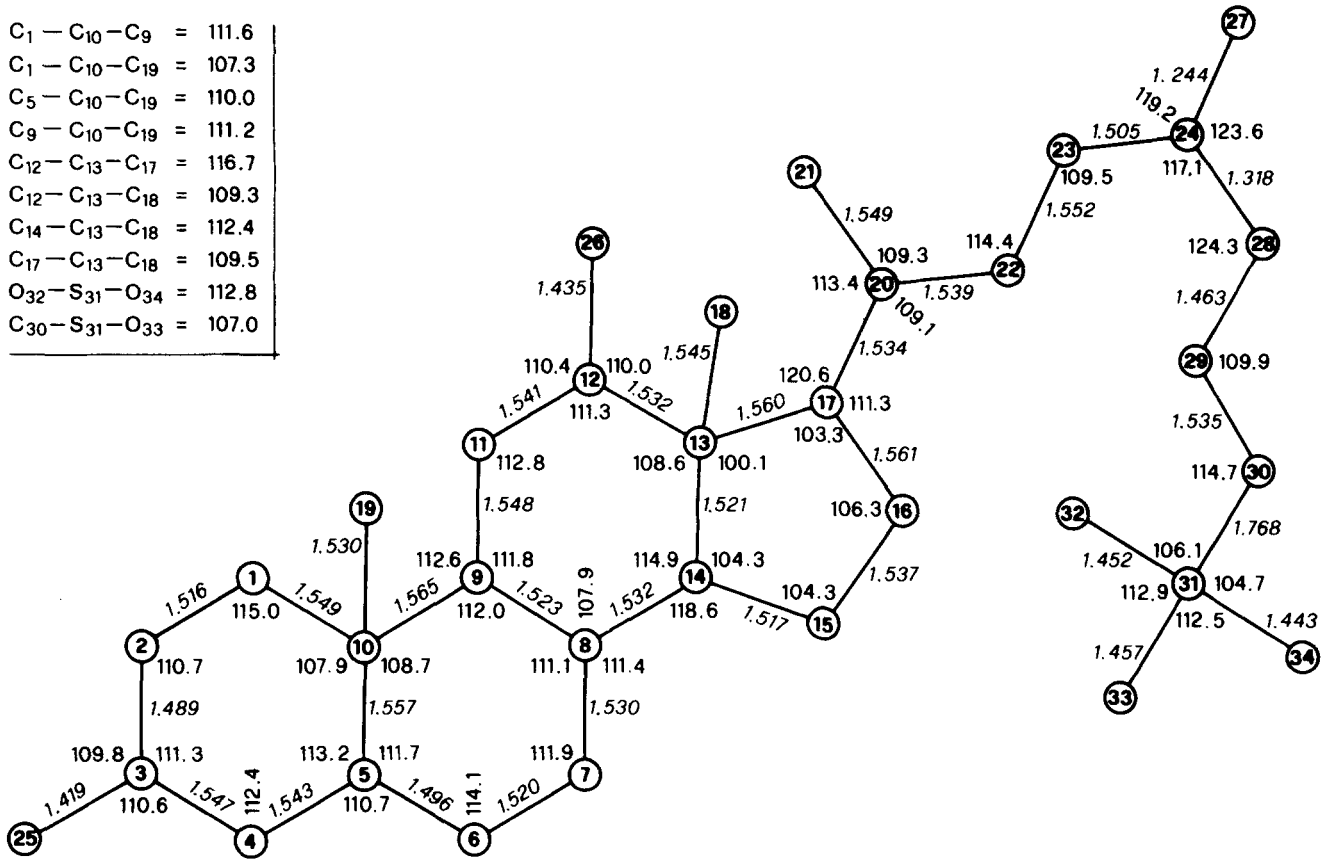

Fig. 2. Bond distances and angles of the NaTDC anion. The average estimated standard deviations are 0.009 Å and 0.5° with maximum values of 0.012 Å and 0.7°, respectively.

CuKα radiation ($\lambda = 1.5418$ Å) and ω-2ϑ technique. Variable scan speed was used: if a first high-speed scan gave $I \leq 1.5\ \sigma(I)$ the reflection was considered "weak" and no other scan was made; otherwise a second scan was performed at appropriate speed. Backgrounds were measured by counting for a quarter the scan time on each side of the reflections.

Four standard reflections were monitored during collection, but negligible decay was observed. The data were corrected for Lorentz and polarization effects, but not for absorption.

The structure was determined using 2633 unique reflections (the merging of equivalent reflections gave an $R_{int} = 0.03$) and by running the MULTAN program (11). A difference-Fourier synthesis was then necessary for a better location of some atoms and revealed the position of

TABLE 2. Torsion angles of NaTDC side chain and ring D together with Δ and φ_m; the estimated standard deviations are in parentheses

C_{13} - C_{17} - C_{20} - C_{22}	- 177.1 (5)	C_{29} - C_{30} - S_{31} - O_{32}	48.2 (8)
C_{13} - C_{17} - C_{20} - C_{21}	- 55.1 (7)	C_{29} - C_{30} - S_{31} - O_{33}	- 72.5 (8)
C_{17} - C_{20} - C_{22} - C_{23}	- 161.7 (5)	C_{29} - C_{30} - S_{31} - O_{34}	167.8 (7)
C_{20} - C_{22} - C_{23} - C_{24}	68.1 (8)	C_{13} - C_{14}	46.9 (6)
C_{22} - C_{23} - C_{24} - N_{28}	- 112.2 (8)	C_{14} - C_{15}	- 35.8 (7)
C_{22} - C_{23} - C_{24} - O_{27}	65.2 (10)	C_{15} - C_{16}	9.9 (8)
C_{23} - C_{24} - N_{28} - C_{29}	165.6 (8)	C_{16} - C_{17}	18.5 (8)
C_{24} - N_{28} - C_{29} - C_{30}	- 84.3 (11)	C_{13} - C_{17}	- 39.4 (7)
O_{27} - C_{24} - N_{28} - C_{29}	- 11.7 (15)	Δ	9.7
N_{28} - C_{29} - C_{30} - S_{31}	- 166.9 (6)	φ_m	47.1

The values of the torsion angles agree with the convention of Klyne and Prelog (14). The phase angle of pseudorotation Δ and the maximum angle of torsion φ_m are calculated according to Altona, Geise, and Romers (15).

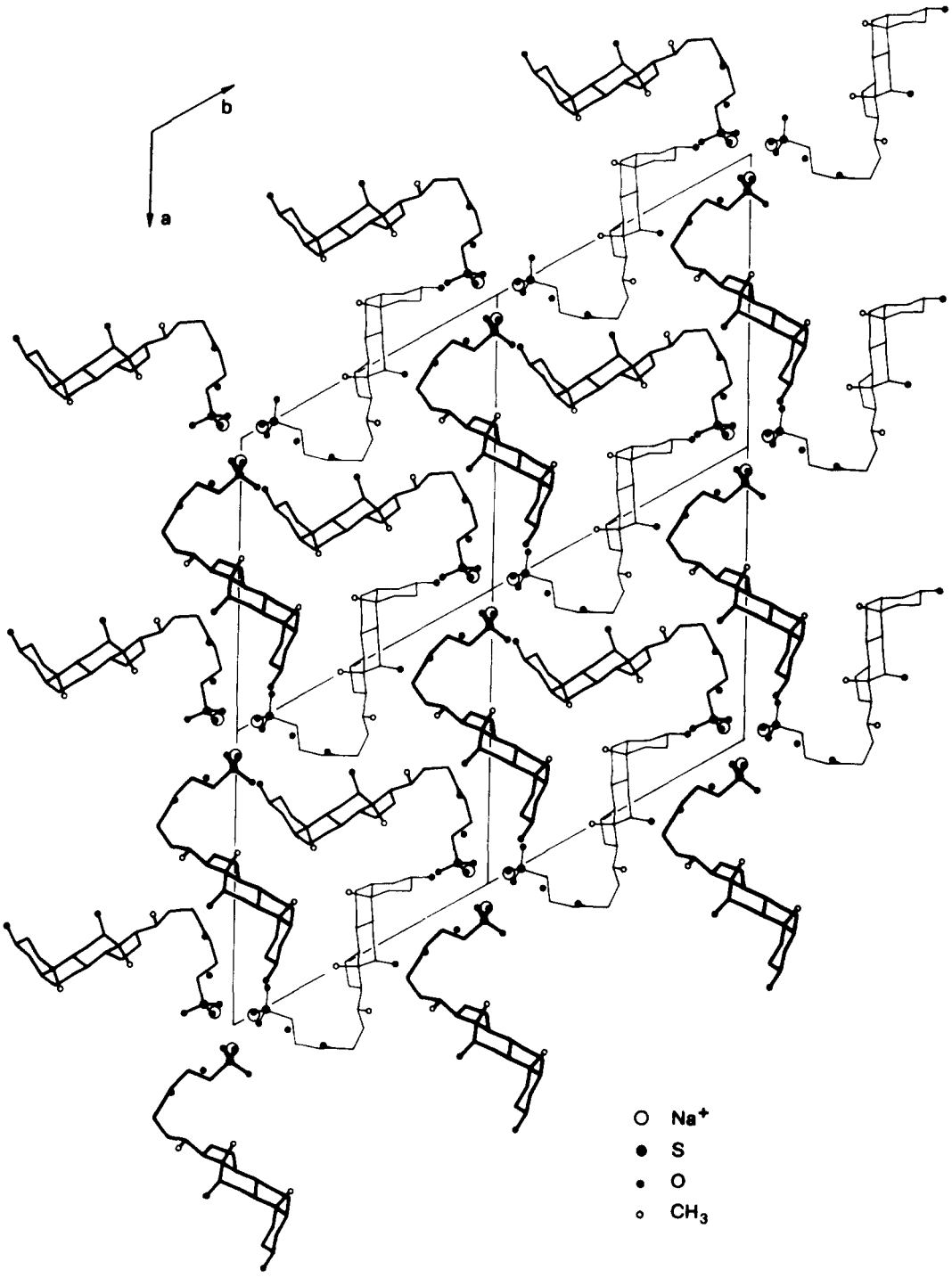

Fig. 3. Crystal packing of NaTDC helices viewed along c . A thicker line represents a molecule nearer to the observer.

a water molecule. A subsequent thermogravimetric analysis, carried out with a TGS-2 Perkin-Elmer thermobalance, confirmed this finding.

The program SHELX (12) was used for the refinement of the structure. Scattering factors and anomalous dispersion coefficients were taken from International Tables for X-Ray Crystallography (13).

The hydrogen atoms of the NaTDC molecule were generated at the expected positions. The two hydroxylic hydrogens were found in a difference-Fourier map, while the water hydrogens were not introduced in any calculation. The positional parameters of the hydrogen atoms were not refined and their thermal parameters were taken to be equal to the isotropic ones of the parent atoms. The

full-matrix refinement was carried out with 1950 reflections having $I > 1.5\sigma(I)$ and with the weighting scheme $w = k/[\sigma^2(F) + G \cdot F^2]$, where the value of G was refined at each least-squares cycle. The final values of k and G were 0.49 and 0.0076, respectively. The maximum and minimum height in the final difference Fourier synthesis was 0.2 and -0.2 e • Å^{-3}. The final reliability factors were $R = 0.051$ and $R_W = 0.059$; S (defined as $[\Sigma w (|F_O| - |F_C|)^2/(m - n)]^{1/2}$, where m is the number of reflections and n is the number of parameters refined) equals 0.52.

RESULTS

The final atomic coordinates and the equivalent isotropic thermal parameters together with their estimated standard deviations are reported in **Table 1.** Bond distances and angles are given in **Fig. 2.** The torsion angles of the side chain and ring D are listed in **Table 2.** The crystal packing is shown in **Fig. 3.** The sodium ion is coordinated to six oxygen atoms, belonging to four SO_3^- and one hydroxyl group and to one water molecule **(Fig. 4).** These six ligands form a distorted octahedron. The donor-acceptor distance in the hydrogen bonds is reported in **Table 3.**

DISCUSSION

The oxygen of the water molecule has thermal parameters and estimated standard deviations of the coordinates so high (see Table 1) that a slight positional disorder may be considered possible. However, the formation of two hydrogen bonds (see Table 3) and the ion-dipole interaction with Na^+(see Fig. 4) cast no doubt about the location and the presence of the water molecule, which was also detected by thermal analysis.

The conformation of the D-ring is intermediate between the half-chair and the β-envelope symmetry, as observed in some inclusion compounds of deoxycholic acid and in rubidium deoxycholate where the torsion angle C_{17}-C_{20}-C_{22}-C_{23} is approximately *trans* (4, 16, 17). This is the case of NaTDC ($-161.7°$, see Table 2) so that two important interactions between side chain and ring D, controlling their conformation, involve the two hydrogen atoms of $C_{16}$and one of C_{22}. The best interaction energy corresponds to $\Delta \cong 15°$(16), which agrees with the value of 9.7° found in NaTDC. The side chain conformation is such as to fold back the polar head and is characterized by the torsion angles C_{20}-C_{22}-C_{23}-C_{24}, C_{22}-C_{23}-C_{24}-N_{28}, and C_{24}-N_{28}-C_{29}-C_{30} which greatly differ from the extended conformation (see Table 2).

After C_{22} the side chain shows a great flexibility, except at the peptide group, due to the lack of bulky substituents at the carbon atoms. Thus, the side chain conformation

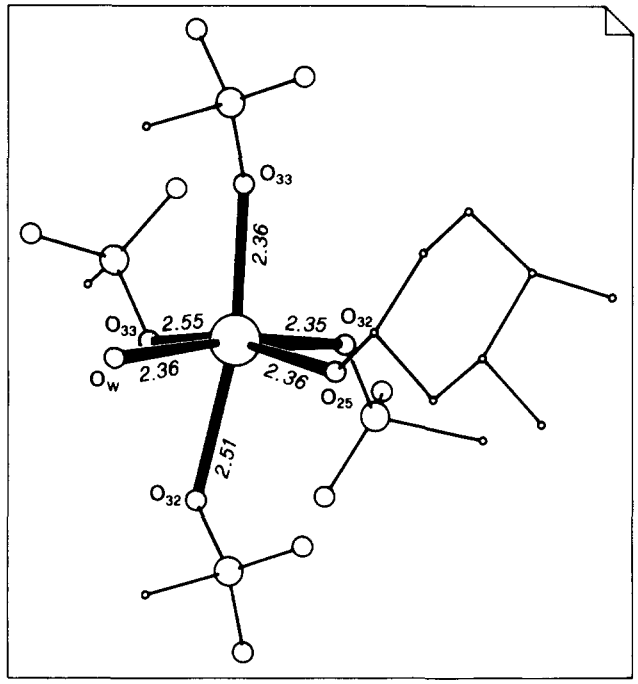

Fig. 4. Coordination of the sodium ion viewed along b. The Na^+... O distances (Å) are reported.

is mainly governed by its ability to form hydrogen bonds together with ion-ion and ion-dipole interactions in the crystal.

The peptide group seems to deviate from the planarity supposing that the nitrogen bonds lie in a plane, since the torsion angle O_{27}-C_{24}-N_{28}-C_{29} is $-11.7°$ instead of 0°. The SO_3^- and C_{30} methylenic groups are approximately staggered, the torsion angle C_{29}-C_{30}-S_{31}-O_{34} being 167.8° instead of 180°.

The crystal packing viewed along c is shown in Fig. 3. The crystal structure consists of helices (hereafter indicated as H_3), generated by rotation about the threefold axes of symmetry (3_1) and composed by three NaTDC molecules within c, embedded one inside the other as cog-wheels. The H_3 helix is clearly visible, for example, at the bottom left corner of Fig. 3. The sodium ions and water molecules fill the interior of H_3 giving rise both to hydrogen bonds and to ion-ion and ion-dipole interactions (see Fig. 4 and Table 3) which stabilize the helix. Each SO_3^- group is surrounded by four Na^+ with S ···Na^+ distances ranging between 3.48 and 3.68 Å. The van der Waals interactions in H_3 are very weak since the corresponding intermolecular distances are large and much greater, generally, than the sum of the van der Waals radii of the atoms involved. The shortest contacts occur between the molecules at (x, y, z) and (x, y, z + 1). A large empty space between two neighboring molecules along the helical path is observed. It is plain that the molecule is somewhat S-shaped in order to reduce the

TABLE 3. O · · · O distances (Å) in the hydrogen bonds; the estimated standard deviations are in parentheses

$O_w \cdots O_{27}$	2.696 (11)	$O_{26} \cdots O^{b}_{27}$	2.984 (8)
$O_w \cdots O^{a}_{32}$	2.874 (12)	$O_{34} \cdots O^{c}_{25}$	2.702 (10)

Symmetry code: a) x, y, z − 1; b) y − x, 1 − x, z + ⅔; c) y − x − 1, −x, z + ⅔.

empty space and to realize a better packing. The SO_3^- and the O_{25}–H groups are the nearest and the farthest group of the anion from the helical axis, respectively, whereas the empty spaces are filled with other anions arranged in reverse way. Thus, an alternative point of view suggests that the crystal is formed by helices, composed by six NaTDC molecules within c and hereafter indicated as H_6, connected by H_3 helices (see **Fig. 5).** Six hydrogen bonds and three $Na^+ \cdots O_{25}$ ion-dipole and van der Waals interactions stabilize the formation of H_6 from $H_3 + 3$ NaTDC.

The H_3 helical model could be suitable for representing the NaTDC micelle in aqueous solution. In fact, the NaTDC micelles were described as objects growing in a rod-like fashion by increasing the NaCl concentration and by decreasing the temperature (9). The quasielastic light scattering measurements were in agreement with prolate ellipsoids with a semiminor axis of about 15 Å when the hydrodynamic radius of the micelles had values within the range 15–60 Å. The radius of the H_3 and H_6 helices is about 16 and 17 Å, respectively, in satisfactory agreement with the 15 Å value of the semiminor axis. A possible $H_3 \rightleftarrows H_6$ equilibrium can be found in aqueous solution, especially if the addition of NaCl causes the subtraction of water molecules, very likely located inside the empty spaces, to the H_3 helix which becomes ready to receive other NaTDC molecules. Both H_3 and H_6 helices have a lateral surface covered by polar and nonpolar groups, at variance with the hydrophobic one of the NaDC helix. Therefore, polar interactions among micelles or among micelles and solubilizates must also occur and polar solubilization sites must be detected (5, 6). Moreover, experimental data suggest that the bile salt molecules form micelles by progressive and continuous association in water and in low ionic strengths and by cooperative association in high ionic strengths (3). The helix has the advantage of satisfying both types of growth, since it can grow both by a step-wise addition of molecules and by welding of helices.

Work is in progress to check the validity of this helical model in the study of the NaTDC aqueous solutions.

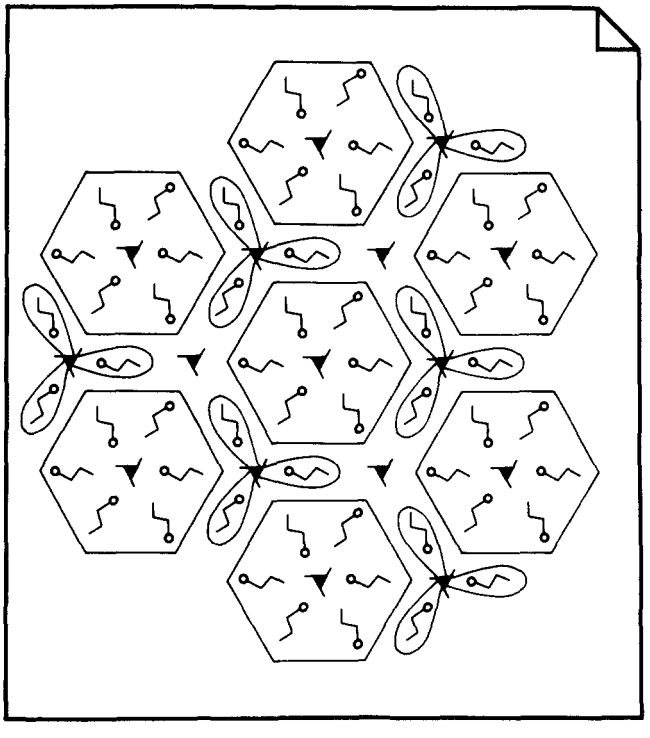

Fig. 5. Schematic drawing of the NaTDC crystal packing considered as an assembly of H_6 and H_3 helices. Only the threefold screw axes at the corners of the unit cells are shown for sake of simplicity. The taurodeoxycholate anion is schematically represented as a tadpole (the small open circle is the SO_3^- group). The cations and the water molecules are omitted. The hexagons and the trilobate figures circumscribe H_6 and H_3 helices, respectively.

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