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Sodium Etiobilirubin-IVγ-C10-sulfonate: A Highly Solvated Bile Pigment Structure Containing Two Different Non-ridge-tile Conformers in the Unit Cell

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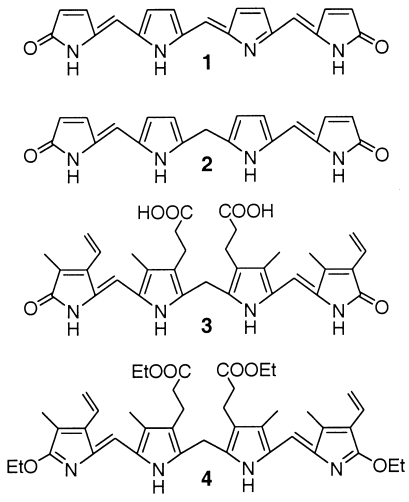
Abstract—The crystal structure of the title compound is the first example of a bilirubin existing in both extended and cyclic conformations and the first bile pigment structure showing two markedly different conformations in the unit cell. In contrast to previous rubin structures the dipyrrinone rings are twisted out of planarity in both conformers. Because of numerous hydrogen-bonding and ionic interactions a highly complex tetrameric structure is observed in which each extended conformer is held pincer-like by another.

Unlike their relatively rigid porphyrin and heme precursors, bile pigments are conformationally flexible molecules.[1] In solution and in the solid state biliverdin and related compounds (with the tetrapyrrole backbone **1**) exist predominantly as helical ('lock-washer') 5,9,14-synperiplanar conformers,[2] although extended 'linear' conformers can occur.[3] In contrast, folded 'ridge-tile' structures, in which the two synperiplanar dipyrrinones lie in separate planes at ~95–120° to each other, have invariably been observed for the corresponding rubins (backbone **2**), such as bilirubin **3**, containing propionic acid substituents at C8 and C12.[4–8] These ridge-tile conformers are stabilized by a network of intramolecular hydrogen bonds linking the propionic carboxy groups to the pyrrole imino and lactam groups. This conformation is dominant in solution[5,6] and has been found even for C10-substituted derivatives both in solution[5,8a] and in the solid state.[8b] The conformational preference of rubins that cannot form intramolecular hydrogen bonds is less clear. Molecular dynamics calculations suggest that the ridge tile conformation is still preferred,[7b] while spectroscopic studies indicate that other conformations are possible.[9] The only relevant crystal structure available is that of 1,19-diethoxybilirubin diethyl ester **4**, which crystallizes in a conformation in which the two planar dipyrrinones are oriented almost perpendicular to each other.[10]



During studies related to bile pigment metabolism in frogs, we synthesized the sulfonated bilirubin analogue etiobilirubin-IVγ-C10-sulfonic acid and isolated its monosodium salt **6**. The crystal structure of this substance presented here is remarkable because of its unusually high degree of solvation and localization of sodium ions within an intermolecular channel, and because the unit cell contains two different conformers, one intercalated inclusion-like into the other and neither having the expected ridge-tile structure.

The sulfonate **6** [ε_{max} (390 nm, $CHCl_3$) 41.4 mmol dm^{-3}] was synthesized by reaction of aqueous sodium bisulfite

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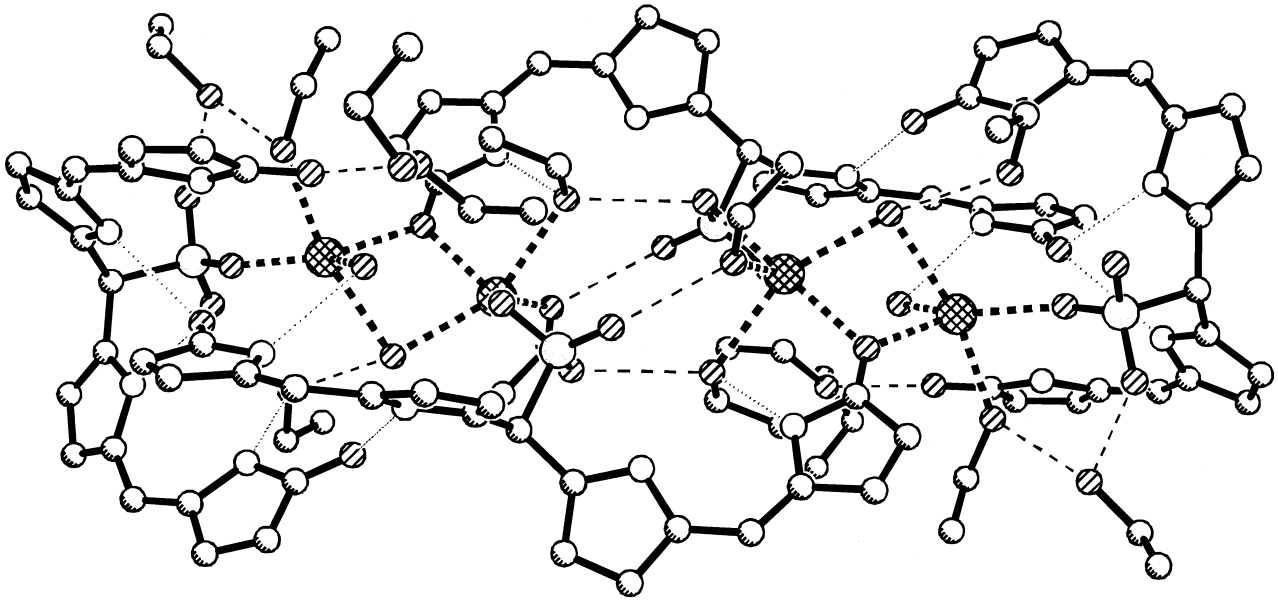
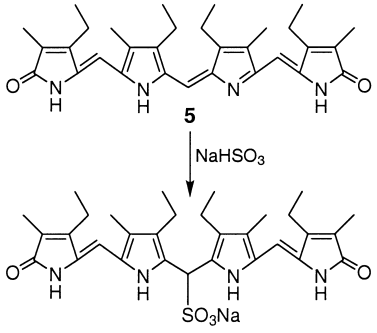


Figure 1. The molecular structure of the tetrameric aggregates formed by **6** in the crystal. Hydrogen atoms, methyl and ethyl substituents, and disordered positions have been omitted for clarity. Dashed solid lines indicate bonding interactions involving the sodium cations, dashed lines hydrogen bonding interactions involving oxygen atoms and dotted lines those involving nitrogen atoms.

with etiobiliverdin-IVγ **5** in $CHCl_3$/MeOH and crystallized from EtOH/*n*-hexane.[11,12] The product had MS and ^1H NMR spectra consistent with the assigned structure, but failed to give satisfactory elemental analyses because of its high degree of solvation and its tendency to decompose during vacuum drying. A crystal structure analysis of **6** was performed as a low temperature study at 130 K.[13]



The salt crystallizes as a tetramer (Fig. 1) in which pigment dimers of extended and claw-like conformers are linked together by multiple ionic and hydrogen bonding interactions involving the sodium and sulfonate ions, solvent molecules, and the pyrrinone NH/O groups. Hydrophobic groups cover the exterior surface of the tetramer, and a polar channel, running between the dimers is stuffed with solvent of crystallization. Four water molecules and 14 ethanol molecules of solvation were found to be directly associated with each tetramer. One hexane molecule and additional water residues were located in the void between the tetramers.

The difficult packing, the complex hydrogen bonding system, and disorder in some chromophore side chains and in the solvate molecules made the refinement difficult and gave a structure with relatively high R-values. This has been observed for most related structures and is often a limiting factor in obtaining reliable structural data for linear tetrapyrroles.[1,15] Despite the unsatisfactory refinement the X-ray analysis confirms the assigned structure of **6** unambiguously as the monosodium salt of a C10 sulfonate.

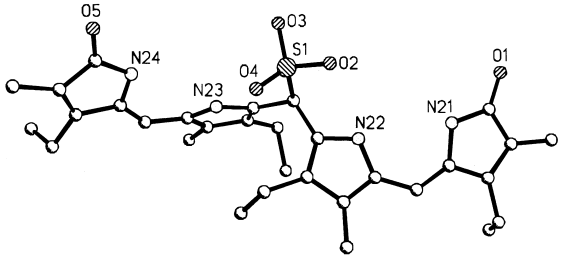


Figure 2. View of the extended, linear conformer of **6** in the crystal.

The tetramer is held together by ionic interactions between the Na^+ and SO_3^- groups (Na1–O7 2.318 Å, Na2–O2 2.621 Å). The coordination sphere of the sodium ions is saturated by ethanol (N1–O2S 2.376 Å, Na2–O3S 2.288 Å, Na2–O4S 2.502 Å) and water molecules (Na1–O1H 2.331 Å, Na1–O2H 2.472 Å, Na2–O2H 2.48 Å) and by bonding with pyrrinone carbonyl oxygen atoms (Na1–O5 2.428 Å, Na2–O3 2.473 Å, Na2–O5 2.384 Å). Additional stabilization is achieved by using ethanol and water molecules as connectors for hydrogen bonding systems. Examples are the connection of Na1 via a two ethanol chain to the SO_3^- group of the claw conformer (O2S–O5S 2.709 Å, O5S–O8 2.637 Å), of Na2 via a one ethanol bridge to the SO_3^- group of the extended conformer (O4S–O3 2.872 Å), and of Na1 via a water (O2H) to Na2. The ethanol situated between Na2 and O3 is also coordinated to N24 (N24–O4S 2.884 Å), while the water molecule O1H connects Na1 to N21 (2.873 Å). Additionally O2H is bonded to a further ethanol (O2H–O6S 2.750 Å), that in turn is bonded to N28 (2.970 Å).

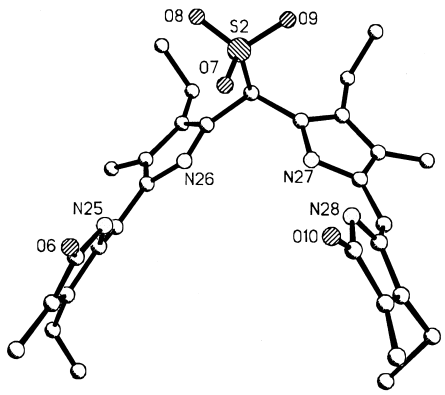


Figure 3. View of the claw conformer of **6** in the crystal.

The two monomers in each dimer of the unit cell have markedly different conformations that have not been observed previously in crystal structures of bilirubins (1,19-dioxo-biladienes-*ac*). One has an extended, 'linear', conformation (Fig. 2); the other, a symmetrical folded, claw-like conformation (Fig. 3). The two monomers form an inclusion complex in which one lactam ring of an extended conformer is intercalated within the wide cleft located between the two dipyrrinone arms of a claw conformer (Fig. 4). This interaction is facilitated both by the specific conformation of the claw conformer and by hydrogen bonding interactions. The carbonyl oxygen atom O1 of the extended conformer is connected in bidendate fashion to N26 (2.872 Å) and N27 (2.909 Å) of the claw conformer. Additionally, a short intermolecular contact is observed between N22 and O10 (2.917 Å).

In both conformers the dipyrrinone moieties deviate much more from planarity than observed in other bilirubin structures. The interplanar dihedral angles between the two pyrrole rings in each dipyrrinone are 26.3° (N21/N22) and 27.7° (N23/N24) in the extended conformer and 31.7° (N25/N26) and 34.2° (N27/N28) in the claw conformer. This is also shown by the twist angles about the bridge C=C bond (ψ_1, ψ_2) within each dipyrrinone (e.g., $\psi_1 = \angle$C4,C5,C5,N22). These are 17.9 and 21.8° in the linear conformer and 26.7 and −30.5° in the claw conformer. In bilirubin the respective values are 17.5 and −2.7°.[4a] Presumably, distortion energy in **6** is more than offset by stabilizing non-bonded interactions within the tetramer.

As shown, the relative orientations of the two dipyrrinone arms in each conformer are quite different. The angle between the two 11 macrocycle atom planes (for each dipyrrinone) is 61.9° in the linear and 88.9° in the folded conformer, respectively. Even more revealing are the dihedral angles involving the *meso* carbon C10 connecting the two dipyrrinone units. According to Sheldrick the angles ϕ_1 (∠N22,C9,C10,C11) and ϕ_2 (∠C9,C10,C11,N23) are expected to be approx. 0° for a porphyrin, $\phi_1 \approx \pm 10°$ and $\phi_2 \approx \pm 10°$ for a helical conformation, $\phi_1 \approx 60°$ and $\phi_2 \approx 60°$ for the ridge-tile conformation, $\phi_1 \approx 180°$ and $\phi_2 \approx 180°$ for a linear conformation and $\phi_1 \approx \pm 90°$ and $\phi_2 \approx 0°$ for a perpendicular conformation.[10] In the present case, the extended conformer (Fig. 2) exhibits $\phi_1 = 137°$ and $\phi_2 = 100.2°$ while the folded conformer (Fig. 3) has an ϕ_1 angle of 67.3° and a ϕ_2 angle of −61.1°. Thus, the two conformations found here deviate substantially from the four classical predicted conformational extremes.

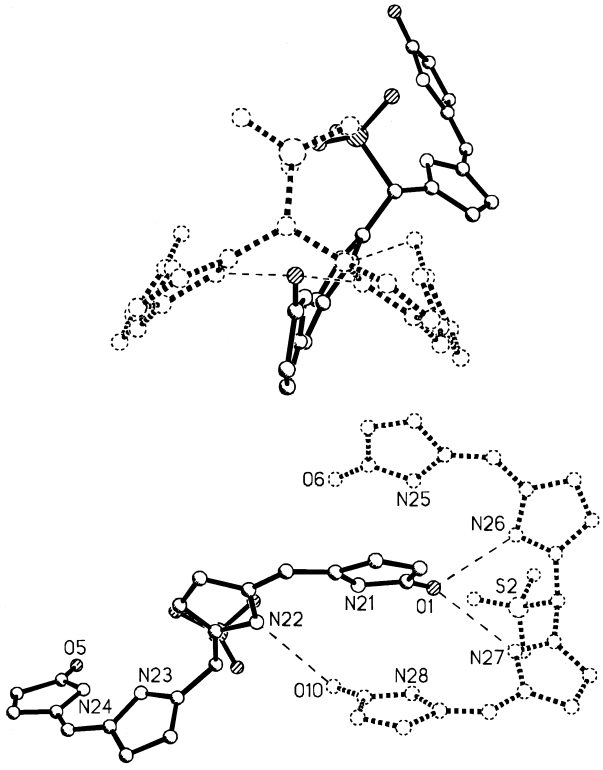


Figure 4. Different views of the intercalation complex formed between the claw (dashed lines) and extended conformer (solid) of **6**. Hydrogen bonding interactions are shown by dashes.

The present structure is the first example of a rubin existing in both extended and folded conformations and the first bile pigment structure showing two markedly different conformers in the unit cell. It contrasts markedly with previous rubin structures in that in both conformers the dipyrrinone rings are twisted out of planarity. Remarkably, each extended conformer is held pincer-like by the other folded conformer (Fig. 4).

Together with Sheldrick's structure[10] of diethoxy-bilirubin dimethyl ester the present example shows that non-ridge-tile structures may prevail for bilirubins when intramolecular hydrogen bonding to propionic or amide functions at C8 and C12 is precluded. For such compounds, the classical ridge-tile structure is not necessarily the preferred conformation, at least in the solid state. The presence of two conformers in crystals of **6** suggests that the compound may also be conformationally heterogeneous in solution, as suggested for bilirubin dimethyl ester.[9]

Acknowledgements

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12. Synthesis and spectra of **6** will be reported elsewhere.
13. Single crystals grown from EtOH/*n*-hexane were immersed in hydrocarbon oil (Paraton N®); a single crystal was selected, mounted on a glass fiber and placed in the low-temperature N_2 stream.[14a] Intensity data were collected on a Syntex $P2_1$ instrument with graphite filtered Cu-K_α radiation ($\lambda = 1.54178$ Å) at 130 K with 2θ–θ-scans. Intensities were corrected for Lorentz and polarization effects. An empirical absorption correction was applied using the program XABS2[14b] and disregarding extinction effects. The structure was solved with Direct Methods using the SHELXS-97 program.[14c] Data were refined against $|F^2|$ with the program SHELXL-97 using all data.[14c] The following experimental data were obtained: $[C_{31}H_{39}N_4O_5SNa]_4 \cdot 14C_2H_5OH \cdot 4.6 H_2O \cdot 1C_6H_{14}$. FW = 3166.92, yellow parallel-piped, size 0.8×0.6×0.5 mm, monoclinic, $P2_1/c$, $a = 12.162(3)$, $b = 20.033(5)$, $c = 35.378(9)$ Å, $\beta = 93.28(3)°$, $V = 8693(4)$ Å^3, $Z = 2$ (2 indep. mol.), $d_{calcd} = 1.210$ Mg m^{-3}, μ(Cu K_α) = 1.216 mm^{-1}, $T_{min} = 0.44$, $T_{max} = 0.58$, $\theta_{max} = 56.35°$, 11405 independent reflections, $R_{int} = 0.0354$, 8873 reflections with $I > 2.0\sigma(I)$, 968 parameters, R1 (all data) = 0.1314, wR2 (all data) = 0.3264, R1 (obsd. data) = 0.1053, $\rho_{max} = 1.067$ e Å^{-3}. The structure showed severe disorder in one sulfonate group, some ethyl side chains and most ethanol molecules of solvation. Crystallographic details have been deposited at the Cambridge Crystallographic Data Centre, Cambridge, CB2 1EZ, UK; deposition number 154809.
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