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Received August 29, 2003

The 10-thia analog of mesobilirubin-XIIIα, with the central C(10) CH_2 group replaced by sulfur (**1a**) was synthesized by condensation of neoxanthobilirubic acid (**3**) with sulfur dichloride. Thia-rubin **1a** is a brilliant yellow solid, forming bright yellow solutions with uv-visible absorption maximum from 425-440 nm.

J. Heterocyclic Chem., **40**, 1113 (2003).

Bilirubin, the end product of heme metabolism and the yellow pigment of jaundice [1], is a powerful neurotoxin [2] and anti-oxidant [3]. Structural studies of the pigment have shown it to be composed of two dipyrrinones conjoined to a methylene group [4], and stereochemical studies have shown it to be folded into a ridge-tile shape that is well-stabilized by a matrix of six intramolecular hydrogen bonds linking the two propionic acids to the two opposing dipyrrinones (Figure 1) [5,6]. Thus, the pigment is not conformationally linear but bent about the central sp^3-carbon into a ridge-tile shape. We recently investigated the structure and properties of a bilirubin analog with the C(10) methylene group replaced by a sulfur and found that this thia-rubin (**1b**) had enhanced solubility in nonpolar solvents [7]. Whether this was due to a change in conformation induced by the bonding angles around sulfur (*vs* sp^3-carbon) [8], or whether it was due to the presence of ethyl groups replacing methyls on the lactam end rings was unclear. In order to clarify this important structure-solution property phenomenon, in the following, we describe the synthesis and properties of a true mesobilirubin-XIIIα (**2**) analog (**1a**) with sulfur at position 10 and one methyl group on each end ring.

Construction of the target thiarubin (**1a**) followed the route described for the synthesis of **1b** [7] of Scheme 1. The synthetic strategy [9] involved construction of two identical dipyrrinone halves and condensing them with sulfur dichloride. Thus, two equivalents of 9*H*-dipyrrinone **3** were reacted with sulfur dichloride in dichloromethane under nitrogen at room temperature to yield **1a**. Dipyrrinone **3** (neoxanthobilirubic acid, prepared earlier by reaction of bilirubin in molten resorcinol [10]) was obtained by a smooth, high yield decarboxylation of 9-CO_2H dipyrrinone **4** by heating in molten sodium acetate-potassium acetate [11]. Dipyrrinone **4** was prepared, as described earlier [11] through base-catalyzed condensation of pyrrole α-aldehyde **5** with 4-ethyl-3-methyl-2-pyrrolinone **6**.

The structure of **1a** is consistent with its carbon-13 nmr (Table 1) and mass spectral data, and the method of syn-

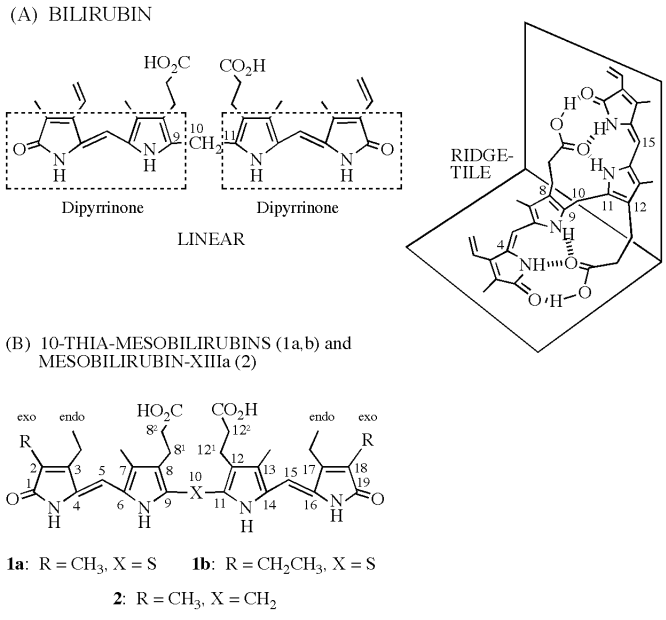
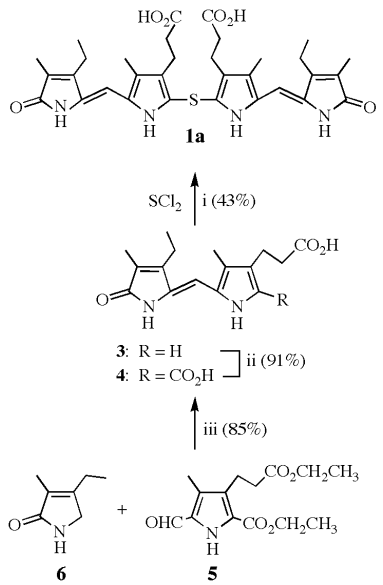


Figure 1. (A) Linear (left) and ridge-tile (right) representations of bilirubin. The latter is the most stable conformation. (B) Bilirubin analogs in linear representations.

Scheme 1 [a]



[a] Reagents and conditions: i, CH_2Cl_2, 25 ∞C, 3 h; ii, KOAc-NaOAc, 165-180 °C, 0.5 h; iii, 4M KOH, CH_3OH, reflux 16 h.

thesis, thus the carbon-13 resonances of **1a** match up well with those of **1b** and with the parent **2**. The presence of the sulfur, curiously, has little influence on the resonances at most of the carbons of the pyrrole rings and is felt most strongly at C(6,14), C(5,15), C(4,16) and C(2,18).

Table 1

^{13}C-nmr chemical shifts (ppm) [a] and assignments of carbons in **1a** and comparison with **1b** [b] and **2** [c].

Carbon		**1a**	**1b**	**2**
1, 19	C=O	172.2	171.8	171.9
2, 18	=C-	124.3	125.4	122.9
2^1, 18^1	CH_3 or CH_2	9.37	16.35	9.15
2^2, 18^2	CH_3	-	13.75	-
3, 17	=C-	147.3	146.8	147.1
3^1, 17^1	CH_2	17.10	16.93	17.15
3^2, 17^2	CH_3	14.71	15.65	14.82
4, 16	=C-	126.5	130.1	127.8
5, 15	CH	96.66	96.72	97.70
6, 14	=C-	125.4	126.3	122.4
7, 13	=C-	121.9	121.8	122.5
7^1, 13^1	CH_3	8.08	9.35	8.07
8, 12	=C-	119.6	119.5	119.3
8^1, 12^1	CH_2	20.03	19.95	19.33
8^2, 12^2	CH_2	34.41	34.23	34.56
8^3, 12^3	CO_2H	173.9	173.8	174.1
9, 11	=C-	130.4	130.4	130.4

[a] In ppm downfield from $(CH_3)_4Si$ for 1 x 10^{-2} M solutions at 22 °C. The numbering system may be found in Figure 1B. [b] Entries for **1b** obtained from reference 7. [c] Entries for **2** obtained from reference 12.

From the ^{1}H-nmr spectra, especially the NH and COOH, chemical shifts in deuteriochloroform, one can usually glean structural information related to intramolecular hydrogen bonding [6,12]. For example, in **2**, where intramolecular hydrogen bonding is firmly established in nonpolar solvents, the pyrrole NH resonates near 9.7 ppm, but in dimethylsulfoxide it shifts downfield to ~10.3 ppm. The lactam NH resonates at higher field in dimethylsulfoxide than in chloroform, as does the carboxylic acid signal. A brief examination of Table 2 indicates that the pyrrole NH resonance of **1a** appears at nearly the same chemical shift as **2**, and close to that of **1b** in chloroform, as do the carboxylic acid OH signals. The lactam NH resonance of **1a** is more deshielded in chloroform than in dimethylsulfoxide, consistent with the behavior of **1b** and **2**. Taken collectively, these data may be taken as evidence that both **1a** and **1b** adopt an intramolecularly hydrogen bonded ridge-tile shape (Figure 1A) in chloroform.

Additional evidence for hydrogen bonding comes from nuclear Overhauser effect (nOe) ^{1}H-nmr experiments which show a weak nOe from the carboxylic acid hydrogen to lactam NH in deuteriochloroform solvent, similar to that observed in other related rubins [6,7,11,12]. Also in keeping with the *syn-Z*-dipyrrinone structure of **1a**, strong nOes were detected between the lactam and pyrrole NHs and between the C(5/15) hydrogen(s) and the C(7/13) methyls and C(3,17) ethyl CH_2s.

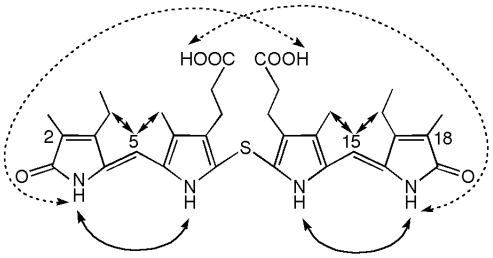


Figure 2. NOEs found in deuteriochloroform for thia-rubin **1a** are shown by curved, double-headed arrows. Weak nOes are shown by curved, dashed arrows. Except for the acid (COOH) to NH nOes, all other nOes are visible in hexadeuteriodimethylsulfoxide solvent as well.

The uv-visible spectral data of **1a** (Table 3) are very similar to the spectral characteristics of the parent, **2**, indicating that these molecular excitons [6] adopt very similar conformations in solution and that the change from C(10) methylene to sulfur induces only minor conformational changes.

Further insight into the conformation of **1a** relative to that of **2** comes from molecular dynamics calculations [13] that predict stable, global energy minimum conformations shaped like ridge-tiles and with each dipyrrinone hydrogen-bonded to an opposing propionic acid (Figure 2). The main difference between the structures of **1a** and its parent **2** lies with the bond angles around the central atom: sulfur

Table 2

Comparison of ^{1}H-nmr chemical shifts NH and COOH hydrogens from **1a**, **1b** and **2** in deuteriochloroform and hexadeuteriodimethylsulfoxide.

Compd	δ (ppm) $(CD_3)_2SO$ N(21), (N24) (lactam)	N(22), N(23) (pyrrole)	COOH	δ (ppm) $CDCl_3$ N(21), (N24) (lactam)	N(22), N(23) (pyrrole)	COOH
1a	10.01	10.83	11.96	10.88	9.77	13.66
1b	9.99	10.73	11.96	10.94	9.89	13.61
2	9.74	10.28	11.87	10.57	9.74	13.62

Table 3

Comparison of the solvent dependence on the uv-visible spectral data of **1a** and **2**

	ε_{max} (λ_{max}, nm) [a]			
	Chloroform	Acetone	Methanol	DMSO
1a	55,700 (437)	42,000 (425)	42,500 (425)	55,300 (424)
2	50,300 (431)	49,700 (427)	50,600 (425)	52,500 (428)

[a] At 22 °C, concentration ~ 1.6 x 10^{-5} M; λ in nm, ε in liters.mol^{-1}.cm^{-1}.

vs sp^3-carbon. The consequences of the smaller bond angle (91.5°) of the former *vs* ~102° of the latter, compensated for somewhat by the longer S-C bond length (~1.8 Å) *vs* C-C (~1.5 Å), causes the dipyrrinones to fold more toward one another such that the interplanar (dihedral) angles between the two dipyrrinones is reduced from ~96° in **2** to ~77° in **1a**.

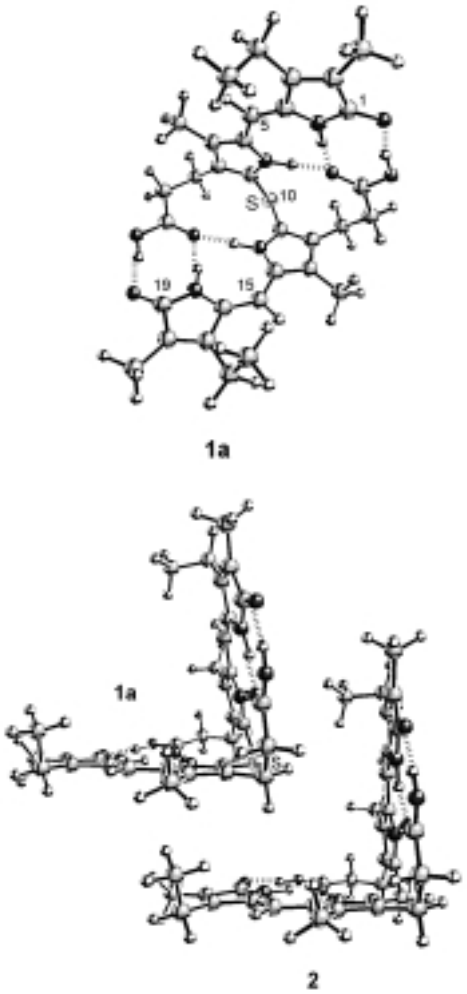


Figure 3. Ball and Stick representation for the energy minimized hydrogen-bonded structure of **1a** (top); edge-view comparisons of the ridge-tiles of **1a** and **2** (below).

The tlc and hplc data reveal interesting facets of structure. On silica gel tlc using dichloromethane-methanol (99:1 by vol) as eluent, the R_f values are **1a**: 0.5, **1b**: 0.8 and **2**: 0.5. By reverse phase hplc, the retention times are **1a**: 17.6 minutes, **1b**: 28 minutes and **2**: 18 minutes. Clearly **1b** is much less polar (more lipophilic) than **1a** or **2**, which exhibit essentially identical polarity or lipophilicity.

Knowing that the preferred general ridge-tile shape of **1a**, **1b** and **2** differ little from that of bilirubin (Figure 1A), we now know from tlc and hplc data that the strongly increased lipophilicity of **1b** is due to the presence of the extra ethyl groups on the end rings. Whether it is due specifically to the presence of *exo* ethyls (irrespective of whether *endo* ethyls are present) is as yet unclear.

EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity Plus or GE QE-300 spectrometer operating at 300 MHz (proton) and 125 MHz and 75 MHz (C-13), respectively in deuteriochloroform and hexadeuteriodimethylsulfoxide solvents. Chemical shifts are reported in ppm referenced to the residual chloroform proton signal at 7.26 ppm and C-13 signal at 77.23 ppm unless otherwise noted. Infrared spectra were recorded on a Perkin-Elmer model 1610-FT infrared spectrophotometer. GC-MS analyses were carried out on a Hewlett-Packard Model 5890A ion selective detector equipped with a DB-1 (100% dimethylpolysiloxane) column. High resolution mass spectral determinations were conducted at the Nebraska Center for Mass Spectrometry. All ultraviolet-visible spectra were recorded on a Perkin-Elmer λ-12 spectrophotometer: a stock solution of (~8.0 x 10^{-4} *M*) was prepared by dissolving an appropriate amount of the pigment in 2 mL of dimethylsulfoxide. A 100 µL volume of this stock solution was diluted to 5 mL with different solvents (Table 3). The final concentration of the solution was approximately 1.6 x 10^{-5} *M*. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Analytical thin layer chromatography (tlc) was carried out on J. T. Baker silica gel IB-F plates (125 µm layer). For hplc analysis, the uv-vis detection was set at 435 nm, and the column was a Beckman-Altex ultrasphere-IP 5 µm C-18 ODS column (25 x 0.46 cm). The elution solvent was 0.1 *M* di-*n*-octylamine acetate in 5% aqueous methanol, and the column temperature ~ 34 °C. All solvents were reagent grade obtained from Fisher or Aldrich. Sulfur dichloride was obtained from Aldrich, and deuterated chloroform and dimethylsulfoxide were from Cambridge Isotope Laboratories. The spectral data were obtained in spectral grade solvents, and hplc grade solvents were dried and purified according to standard procedures [14]. Compounds 4-ethyl-3-methyl-1*H*-pyrrolin-2-one **6** and ethyl 5-carboethoxy-2-formyl-3-methyl-1*H*-pyrrole-4-propanoate **5** were synthesized according to literature procedures [11,15]. The Ball and Stick drawings were created from the atomic coordinates of the molecular dynamics structures using the Müller and Falk "Ball and Stick" program [13] for Macintosh. The molecular dynamics calculations were carried out on Silicon Graphics Octane workstation, with Gasteiger Hückel calculation of electronic charge, using Tripos force-field in SYBYL v. 6.9 [13].

(4*Z*,15*Z*)-8,12-Bis-(2-carboxyethyl-3,17-diethyl-2,7,13,18-tetramethyl-10-thia-(21*H*-22*H*-23*H*-24*H*)-1,19-dioxobilin (10-thiamesobilirubin-XIIIα) (**1**).

To a 100 mL, 3-neck round-bottom flask, equipped with a nitrogen inlet, a magnetic stirrer, and a rubber septum, were added neoxanthobilirubic acid **3** (100 mg, 0.34 mmol) and CH_2Cl_2 (30 mL). To the resulting suspension was added a drop of dimethylsulfoxide (to enhance solubility), and the system flushed with nitrogen several times. Next, sulfur dichloride (11 µL, 17.6 mg, 0.17 mmol) was injected using a microsyringe and the mixture left to stir at ambient temperature for 3 h. The reaction was quenched with water, and the resulting yellow precipitate collected by filtration and washed several times with ethanol. Final purification was achieved by dissolving the solid in hot dimethyl sulfoxide followed by dropwise addition of water, precipitating a bright yellow solid, which upon drying *in vacuo* gave 45 mg (43%) of the desired rubin **1a**. It had m.p. 250-258 dec; IR (KBr) ν (cm^{-1}) 3401, 2966, 2919, 2872, 2366, 2319, 1701, 1684, 1625, 1390, 1219, 1008; ^{1}H-nmr (dimethylsulfoxide-d_6): δ 1.08 (6H, t, *J* = 7.3 Hz), 1.78 (6H, s), 1.99 (6H, s), 2.04 (4H, t, *J* = 7.3 Hz), 2.53 (4H, q, *J* = 7.3 Hz), 2.69 (4H, t, *J* = 7.3 Hz), 5.91 (2H, s), 10.01 (2H, br, s), 10.83 (2H, br, s), 11.96 (2H, br, s) ppm; ^{13}C-nmr data are in Table 1. *Anal*. HRMS (FAB, 3-NBA): Calcd for $C_{32}H_{38}N_4O_6S$ (606.2512); Found 606.2494, Δ = 1.8 mDa, error 3.1 ppm.

2,7-Dimethyl-3-ethyl(10*H*)-dipyrrin-1-one-8-propionic acid (Neoxantho-bilirubic acid) (**2**).

This is a known dipyrrinone [10], and was prepared more recently [11] by an aldol condensation reaction of 4-ethyl-3-methyl-1*H*-pyrrolin-2-one **6** and ethyl 5-carboethoxy-2-formyl-3-methyl-1*H*-pyrrole-4-propanoate **5** in the presence of 4 *M* KOH in refluxing methanol. The initial dipyrrinone dicarboxylic acid (**4**), obtained in 85% yield, was smoothly decarboxylated in molten KOAc-NaOAc [11] to afford a gratifying 91% yield of **3**, which had m.p. 244-245 °C (Lit. [10] m.p. 244-246 °C). ^{1}H-nmr (dimethylsulfoxide-d_6): δ 1.04 (3H, t, *J* = 7.3 Hz), 1.74 (3H, s), 1.99 (3H, s), 2.52 (4H, q, *J* = 7.3 Hz), 5.92 (1H, s), 6.70 (1H, s), 9.67 (1H, br, s), 10.50 (1H, br, s), 12.02 (1H, br, s) ppm; ^{13}C-nmr (dimethylsulfoxide-d_6): δ 8.06, 9.04, 14.75, 17.20, 20.43, 34.57, 97.80, 119.44, 121.20, 122.43, 123.55, 123.82, 128.83, 147.50, 172.15, 174.16 ppm.

Acknowledgments.

We thank the U.S. National Institutes of Health (HD 17779) for generous support of this work.

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