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On the Conformation of Bilirubin Ditaurate

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Summary. The first optically active taurine conjugate of a bilirubin was prepared by reaction of taurine sodium salt with the mixed anhydride formed from reaction of (βS,$\beta' S$)-dimethylmesobilirubin-XIIIα with isobutyl chloroformate. Analysis of the circular dichroism spectra of the conjugate in water and chloroform indicate a conformational preference for the (*M*)-helical ridge-tile conformation, thus providing the first spectroscopic evidence on the conformation of ditaurobilirubins.

Keywords. Taurine; Bilirubin; Dipyrrinone; CD.

Introduction

Taurine ($^{-}O_3SCH_2CH_2NH_3^{+}$), a conditionally essential nutrient important to mammalian development is found in plasma and milk, *inter alia*, and in bile as conjugates of bile acids, *e.g.*, taurocholic acid [1, 2]. It has also been found as a conjugate of the dicarboxylic acid bilirubin (the yellow pigment of jaundice) in the bile of certain fish (yellowtail, red sea bream, and flounder) [3]. It is not found in mammalian bile, however, where the principle bilirubin conjugates excreted into bile are mono and di-conjugates of glucuronic acid [4]. Bilirubin glucuronides are reactive, undergoing acyl migration and facile hydrolysis, and they are not readily available [5]. In contrast, the ditaurate does not undergo either and is far more stable, and it is available commercially. Consequently, it has been used as a surrogate for bilirubin diglucuronide *in vitro* and in animal studies, where it is smoothly excreted by the liver [6, 7]. Although its constitutional structure is known (Fig. 1), little is known of its conformation [8].

In the following, we describe the syntheses of the ditaurate of (βS,$\beta' S$)-dimethylmesobilirubin (**1**) and β-methylxanthobilirubic acid (**2**) (Fig. 2) and a spectroscopic study of **1** designed to provide new information on the three-dimensional structure of ditaurobilirubin.

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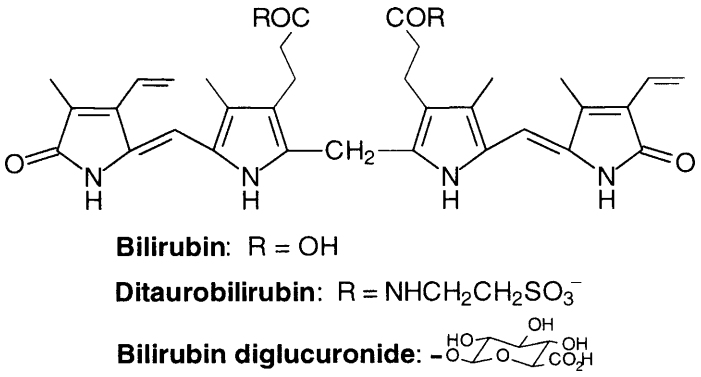

Fig. 1. Linear constitutional structures of bilirubin-IXα and its ditaurate and diglucuronide conjugates

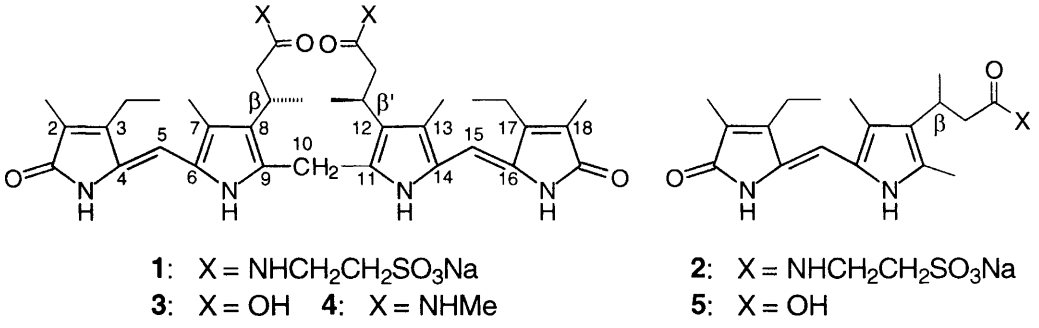

Fig. 2. Ditauro-(βS,$\beta' S$)-dimethylmesobilirubin-XIIIα disodium salt (**1**), tauro-(βS)-methylxanthobilirubinate sodium salt (**2**), their parent carboxylic acids **3** and **5**, and the bis-*N*-methylamide (**4**) of **3**

Results and Discussion

Synthesis Aspects

A synthesis of ditaurobilirubin, reported in 1958 involved reaction of bilirubin with ethyl chloroformate to form the mixed anhydride, followed by reaction with taurine in dioxane-triethylamine [9]. Other methods of activation of carboxylic acids for reaction with taurine involve acid chloride, *N*-acylsuccinimide, azide, acyl imidazole, and anhydride intermediates [10]. Given *Jirsa*'s procedure [9] for preparing ditaurobilirubin, we were inclined toward syntheses involving mixed anhydrides [10h–j] and found an attractive variation using isobutyl chloroformate described in the synthesis of a water-soluble taxol [11]. Using this modification and a change of solvent (to *THF*) with the presence of triethylamine as HCl scavenger, we converted **3** cleanly and quantitatively to its mixed anhydride when the solvents and reagents were oxygen-free and anhydrous, and the medium was basic. Addition of sodium taurate in *DMSO* to the mixed anhydride of **3** afforded the desired ditaurorubin **1** in 90% yield, isolated after radial chromatography on silica using a polar mobile phase. In several experiments, **1** crystallized on the chromatography plate (rotor) and could not be moved by the CH_2Cl_2–MeOH eluent – a problem that was

solved by pre-wetting the rotor with a small volume of 10% (v/v) MeOH in CH_2Cl_2 before loading the sample. Using the same procedure, β-methylxanthobilirubic acid (**5**) was converted to its taurate derivative **2** in 86% yield.

NMR and Structure

The constitutional structures and absolute configuration (Fig. 2) of (βS,$\beta' S$)-dimethylmesobilirubin-XIIIα (**3**) [12], its bis-*N*-methylamide (**4**) [13] and β-methylxanthobilirubic acid (**5**) [14] are well established. Consequently, it is not surprising that the ^{13}C-NMR chemical shifts in *DMSO-d_6* of the carbons of the common skeleton are nearly identical (Table 1), especially in comparing **1**, **3** and **4**. The small differences seen, especially in the β-methylpropionic chains, among these three are probably relevant only to changes in the state of ionization and the presence of a sulfonate ion. It is interesting to note that the propionic carbonyl carbon is 1–2 ppm more shielded in **1** and **2** than in **3** and **4** but the α-carbon is more deshielded, and the β-carbons of **1**–**4** have nearly the same chemical shifts. Similar shieldings of carbonyl and α-carbon are found in taurate derivatives of bile acids, *e.g.*, taurodeoxycholate, $\delta_{C=O} = 172.3$ ppm and $\delta_{\alpha CH_2} = 32.7$ ppm relative to the parent bile acid, deoxycholic acid, $\delta_{C=O} = 174.9$ and $\delta_{\alpha CH_2} = 30.8$ ppm.

Table 1. Comparison of ^{13}C NMR assignments (5×10^{-3} *M*) in *DMSO-d_6* at 25°C; δ in ppm of the sodium salt of ditauro-(βS,$\beta' S$)-dimethylmesobilirubin (**1**), sodium tauro-(βS)-methylxanthobilirubinate (**2**), (βS,$\beta' S$)-dimethylmesobilirubin (**3**) and its bis-*N*-methylamide (**4**)

Position	**1**	**2**	**3**	**4**
1,19–CONH	171.98	171.92	172.10	171.96
2,18	122.94	122.50	122.97	123.04
2,18–CH_3	8.10	8.08	8.09	8.12
3,17	147.29	147.20	147.33	147.45
3,17–CH_2CH_3	17.19	17.18	17.18	17.22
3,17–CH_2CH_3	14.83	14.88	14.86	14.84
4,16	128.00	127.19	128.01	127.98
5,15–CH=	97.71	97.65	97.67	97.30
6,14	122.60	121.82	122.48	122.67
7,13	121.43	123.55	121.70	121.02
7,13–CH_3	10.63	10.03	10.65	10.54
8,12	123.34	121.51	123.21	123.14
β,β'–CH	27.46	27.28	26.88	27.20
β,β'–CH_3	20.25	20.12	19.84	21.05
α,α'–CH_2	42.48	42.76	39.41	41.59
α,α'–CO	171.46	170.96	173.68	173.55
$CONHCH_2CH_2SO_3Na$	35.50	35.42	–	–
$CONHCH_2CH_2SO_3Na$	50.47	50.64	–	–
$CONHCH_3$	–	–	–	25.73
9,11	130.16	128.79	130.20	130.57
9–CH_3	–	12.03	–	–
10–CH_2	23.19	–	23.91	21.84

Table 2. Comparison of the proton NMR spectral assignments ($2 \times 10^{-3}\,M$) in *DMSO-d_6* at 25°C; δ in ppm of the sodium salt of ditauro-(βS,$\beta' S$)-dimethylmesobilirubin (**1**), sodium tauro-(βS)-methylxanthobilirubinate (**2**), (βS,$\beta' S$)-dimethylmesobilirubin (**3**) and its bis-*N*-methylamide (**4**)

Site	**1**	**2**	**3**	**4**
α,α'–CONH– or COOH	7.87[a]	7.71[h]	11.98	8.30[p]
21,24–NHCO	9.85	9.79	9.85	10.05
22,23–NH	10.33	10.23	10.10	10.25
–CONHCH_2CH$_2$SO$_3$Na	3.30[b]	3.26[b]	–	–
–CONHCH$_2$CH_2SO$_3$Na	2.52[b]	2.52[i]	–	–
–CONHCH_3	–	–	–	2.50[q]
2,18–CH$_3$	1.75	1.76	1.76	1.73
3,17–CH_2CH$_3$	2.48[c]	2.48[c]	2.44[m]	2.48[m]
3,17–CH$_2$CH_3	1.07[d]	1.07[d]	1.07[n]	1.06[n]
5,15–CH=	5.94	5.91	5.95	5.92
7,13–CH$_3$	2.09	2.06	2.08	2.13
β,β'–CH	3.25[b]	3.12[b]	3.17[b]	3.39[b]
β,β'–CH$_3$	1.04[e]	1.10[j]	0.98[j]	1.16[j]
α,α'–CH$_2$	2.28[f]	2.19[k]	2.40[o]	2.63[r]
	2.39[g]	2.32[l]		
9–CH$_3$	–	2.21	–	–
10–CH$_2$	3.97	–	3.98	3.90

[a] t, $J = 5.3$ Hz; [b] m; [c] q, $J = 7.5$ Hz; [d] t, $J = 7.5$ Hz; [e] d, $J = 7.2$ Hz; [f] *AB*X, $^3J = 7.7$ Hz, $^2J = 13.8$ Hz; [g] A*BX*, $^3J = 8.0$ Hz, $^2J = 13.8$ Hz; [h] t, $J = 5.6$ Hz; [i] t, $J = 7.7$ Hz; [j] d, $J = 7.1$ Hz; [k] *AB*X, $^3J = 6.6$ Hz, $^2J = 13.7$ Hz; [l] A*BX*, $^3J = 8.8$ Hz, $^2J = 13.7$ Hz; [m] q, $J = 7.4$ Hz; [n] t, $J = 7.4$ Hz; [o] *AB*X, $^3J = 8.7$, 8.1 Hz; [p] br.q; [q] d, $J = 4.5$ Hz; [r] *ABX*, $^3J = 10.8$, overlapped

The ^{1}H-NMR (Table 2) of **1**–**4** in *DMSO-d_6* also exhibit considerable similarity, as might be anticipated. The lactam and pyrrole NH chemical shifts are in the normal region for rubins and dipyrrinones in this solvent, and the amide NHs of the taurine (**1** and **2**) and bis-*N*-methylamide (**4**) lie in the expected range. Consistent with the NMR data and interpretations of *Navon* [15] for bilirubin in *DMSO-d_6*, segmental motion within the β-methylpropionamide chains of **1**, **2**, and **3** is indicated by the similar J_{AX} and J_{BX} coupling constants of diastereotopic α-hydrogens of the –CH$_X$(CH$_3$)–CH$_A$H$_B$–C=O segment. In addition, N*O*E measurements indicate that the β (and β')-methyl is near the C(7)/C(13) ring methyl and the C(10)H$_2$ in **1**. Ideally, one would wish to examine the ^{1}H-NMR of **1** in CDCl$_3$ solvent, in which the rubin conformation is typically restricted to either of the two enantiomeric ridge-tile conformations [12, 13], but the insolubility of **1** precluded such measurements.

Analysis of Conformation by Circular Dichroism Spectroscopy

The optical activity of **1**–**4** enables one to measure their circular dichroism (CD) spectra and extract information on conformation [12, 13, 16, 17]. The most stable conformation of bilirubin and mesobilirubin-XIIIα is shaped like a ridge-tile or half-opened book and is stabilized by a network of intramolecular hydrogen bonds formed when the carboxylic acid groups embrace the opposing dipyrrinones

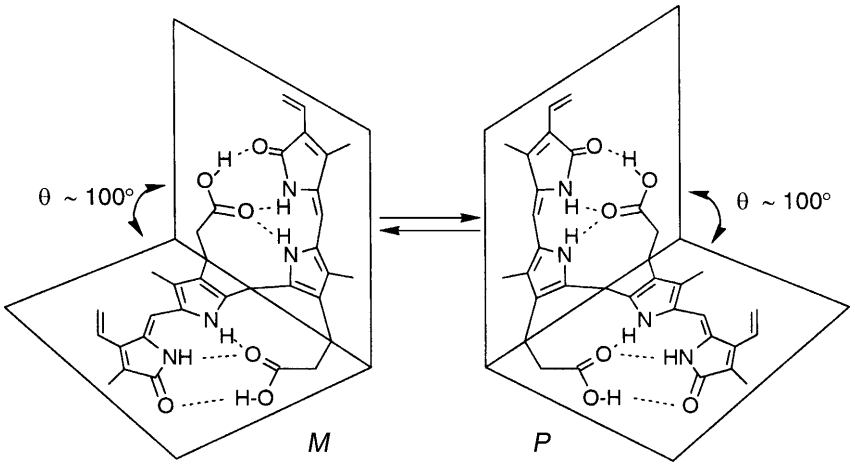

Fig. 3. Bilirubin 3-D conformational structures shaped like ridge-tiles of left (*M*) and right (*P*) handed chirality, are isoenergetic, non-superimposable mirror images (enantiomers); dashed lines are hydrogen bonds

[12, 16, 17]. There are two such ridge-tiles, equi-energetic and interconverting in solution over barriers of $\sim 80\,\mathrm{kJ/mol}$ [16] (Fig. 3). Earlier, we showed that β and β' methyl groups (**3**) can act through nonbonded steric interactions to displace the equilibrium toward either the (*M*) or the (*P*)-helical conformer, which results in the observation of bisignate CD curves for the long wavelength transition [12]. Since bilirubins, with their two dipyrrinone chromophores may be viewed as molecular excitons, exciton coupling theory [18] may be used to predict the (*M*) or (*P*)-helicity of the intramolecularly hydrogen-bonded ridge-tile (Fig. 3) from the signed order of the bisignate CD couplet [19]. In **3**, the (*M*)-helical ridge-tile conformation is confirmed by the intense negative chirality bisignate CD [12]. With *ent*-**3** a mirror image positive chirality bisignate CD confirms the (*P*)-helical ridge tile [12]. Thus CD spectroscopy of $\beta S,\beta' S$-dimethylrubins can be used in conformational analysis and in confirming intramolecular hydrogen bonding [16].

In the bis-*N*-methylamide **4**, negative chirality intense bisignate CD is also observed, confirming the (*M*)-helical ridge-tile conformation as well as intramolecular hydrogen bonding between dipyrrinones and the propionamide groups [13]. Stabilized ridge-tile conformations thus results not only from propionic carboxylic acid to dipyrrinone hydrogen bonding but from hydrogen bonding between propionamides and dipyrrinones as well. Since taurorubin **1** is a propionamide in the same sense as **4**, we determined its CD spectra for use in conformational analysis (Fig. 4).

Table 3 summarizes the CD spectra of **1** in solvents of a wide range of polarity and compares the data to those of **3** [12] and **4** [13]. In *pH* 7.4 0.1 *M* phosphate buffer, a large negative chirality bisignate CD is found for **1**, as predicted from the stereochemistry of the β,β' stereocenters of the propionamide groups and considerations of nonbonded steric interactions in the (*M*)-helical intramolecularly hydrogen-bonded ridge-tile ditaurate conformation (Fig. 4). Comparison of the CD with that of **3** in the same buffer (**4** is insoluble), shows that the CD intensity is lower in **1**, suggesting that intramolecular hydrogen bonding is weakened and

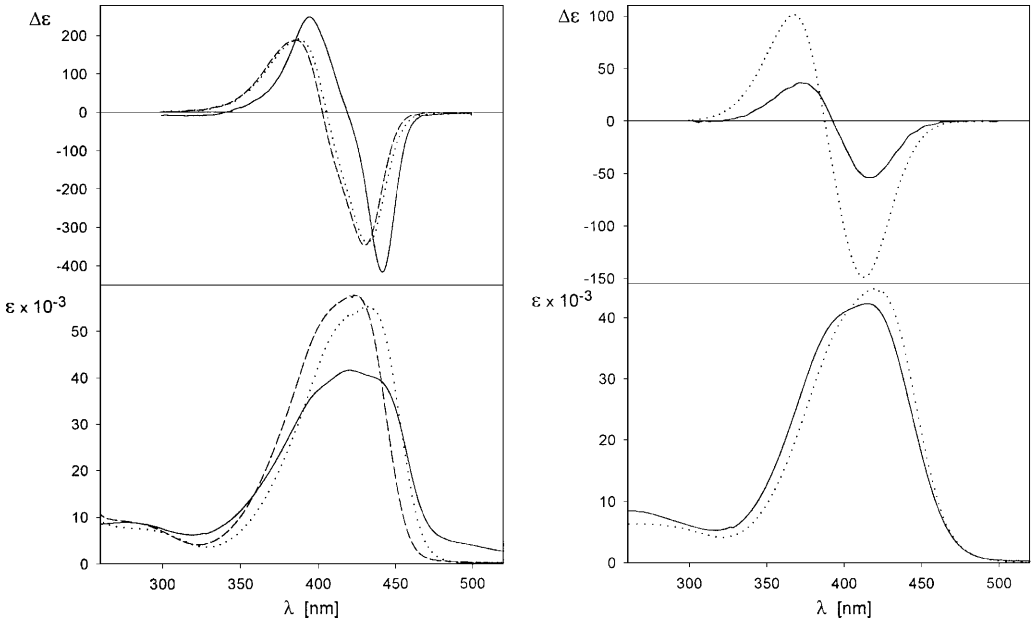

Fig. 4. Circular dichroism (upper) and UV-visible spectra (lower) of **1** (——), **4** (- - - - -) and their parent diacid **3** (· · · ·) in $CHCl_3$ (left) and in *pH* 7.4 phosphate buffer (right)

Table 3. Circular dichroism and ultraviolet-visible spectral data from $2 \times 10^{-5} M$ solutions of ditauro-(βS,$\beta' S$)-dimethylmesobilirubin (**1**), (βS,$\beta' S$)-dimethylmesobilirubin (**3**) and its bis-*N*-methylamide (**4**) at 22°C[a]

Pigment	Solvent	CD			UV	
		$\Delta\varepsilon_1^{max}(\lambda_1)$	λ at $\Delta\varepsilon = 0$	$\Delta\varepsilon_2^{max}(\lambda_2)$	ε^{max}	λ^{max}
1	H_2O	− 54.7 (420)	394	+ 36.8 (375)	42 300	413
3[b]	*pH* 7.4	− 150.4 (423)	398	+ 95.2 (379)	44 500	416
1	CH_3OH	− 56.9 (421)	397	+ 32.1 (381)	50 700	424
3		− 285.4 (431)	405	+ 177.1 (386)	56 600	425
4		− 290.2 (426)	399	+ 153.9 (382)	64 800	424
1	$HCON(CH_3)_2$	− 27.5 (415)	394	+ 10.7 (377)	52 300	419
3		− 246.1 (429)	404	+ 164.8 (386)	53 100	421
4		− 316.9 (427)	399	+ 165.1 (382)	58 400	421
1	$(CH_3)_2SO$	+ 3.6 (426)	–	+ 3.4 (381)	48 600	425
3		+ 23.0 (425)	385	− 5.8 (369)	55 900	425
4		− 178.9 (421)	394	+ 83.4 (379)	60 300	421
1	CH_2Cl_2	− 318.4 (440)	417	+ 208.6 (393)	38 400	424
3		− 319.2 (433)	407	+ 179.9 (389)	54 800	430
4		− 339.6 (427)	400	+ 182.3 (383)	58 100	424
1	$CHCl_3$	− 420.1 (442)	418	+ 245.7 (395)	38 700	421
3		− 337.3 (434)	407	+ 186.2 (389)	55 500	431
4		− 347.7 (431)	403	+ 187.2 (386)	57 600	428

[a] All solutions of **1** contained 2% *v*/*v* of CH_3OH; solutions of **3** and **4** contained 2% *v*/*v* of $CHCl_3$; [b] 0.1 *M* phosphate buffer *pH* 7.40

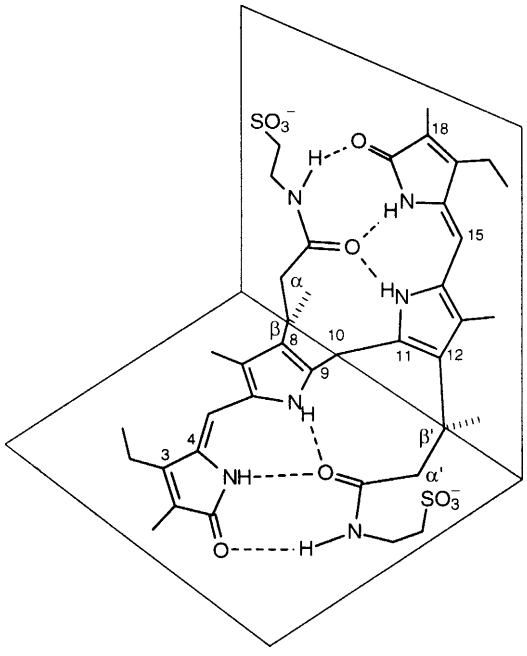

Fig. 5. Most stable 3-D conformational structure of **1**. Dashed lines are hydrogen bonds

with it, a less well-defined preference for the (*M*)-helical ridge-tile conformation. Similarly, in methanol and in dimethylformamide **1** again exhibited a strong negative chirality bisignate CD, but the CD spectra of **3** and **4** were more intense. In contrast, in nonpolar methylene chloride and chloroform solvents, the CD intensities of **1**–**3** were comparably very high. The data from *DMSO* have typically represented a special case of solvent insertion into the hydrogen bonding network [12, 17, 20]. Taken collectively and comparatively, the CD data point to a *strong preference* for the intramolecularly hydrogen-bonded ridge-tile conformation of ditaurorubin **1** (Fig. 5) in nonpolar solvents and the high probability of the same conformation in water and other polar solvents.

Experimental

Circular dichroism spectra were recorded on a Jasco J-600 spectropolarimeter, and UV-visible spectra on a Perkin Elmer Lambda 12 spectrophotometer. All solutions for CD and UV-vis measurements of **1** contained 2% (by volume) of CH_3OH and those of **3** and **4** contained 2% of $CHCl_3$. NMR spectra were recorded on Varian Unity Plus spectrometer operating at a proton frequency of 500 MHz. Chemical shifts are reported in δ (ppm) and referenced to the CHD_2SOCD_3 signal at 2.49 ppm (^1H) and $(CD_3)_2SO$ at 39.50 ppm (^{13}C). A *J*-modulated spin-echo experiment was used to obtain carbon multiplicities. Radial chromatography was carried out on Merck silica gel PF-254 with $CaSO_4$ preparative thin layer grade, using a Chromatotron (Harrison Research Inc., Palo Alto, CA). High-resolution FAB mass spectra were obtained at the Nebraska Center for Mass Spectrometry, University of Nebraska, Lincoln, for samples which were $> 95\%$ pure by NMR.

Commercial reagents and HPLC grade solvents (Aldrich or Fisher) were dried and purified following standard procedures [21]. Isobutyl chloroformate was distilled prior to use, and the reactions were carried out under Ar and light protection. Sodium taurate was synthesized as previously described [10i].

Disodium bis-tauro-($\beta S,\beta' S$)-dimethylmesobilirubinate-XIIIα (**1**, $C_{39}H_{52}N_6O_{10}S_2Na_2$)

To a suspension of 62 mg (0.1 mmol) of diacid **3** [12] in 4 cm^3 of anhydrous THF and 84 mm^3 (0.6 mmol) of Et_3N was added isobutyl chloroformate (77 mm^3, 0.59 mmol), and the mixture was stirred for 1.5 h. Then it was transferred to a solution of 59 mg (0.4 mmol) of sodium taurate (prepared according to *Nuzzo et al.* [10i]) in 2 cm^3 of anhydrous *DMSO*. The mixture was stirred for 1.5 h under aspirator vacuum with occasional heating to 35–40°C to remove *THF*. Then Et_3N (84 mm^3, 0.6 mmol) was added, and stirring was continued at ambient temperature for 24 h. A solution of 50 mg (0.6 mmol) of $NaHCO_3$ in 0.3 cm^3 of H_2O was added, and the mixture was kept under vacuum for 1 h. The residue was diluted with 1 cm^3 of CH_3OH and 5 cm^3 of CH_2Cl_2 and purified by radial chromatogrpahy (gradient 7–20% v/v CH_3OH in CH_2Cl_2). The most polar bright yellow fractions were combined, and the solid obtained after evaporation was triturated with 1 cm^3 of CH_3OH and 5 cm^3 of anhydrous Et_2O. The precipitate was collected by filtration and dried under vacuum at 50°C to afford **1**. Yield 79 mg (90%); mp $>$ 260°C (dec); ^{13}C and ^1H NMR in Tables 1 and 2; HRMS (FAB, 3-NBA): calcd. for *bis*-sodium salt: $C_{39}H_{52}N_6O_{10}S_2Na_3$ $(M+Na)^+$ 897.2879; found: 897.2912, $\Delta = 3.2$ mDa, error -3.6 ppm.

Sodium tauro-β-methylxanthobilirubinate (**2**, $C_{20}H_{28}N_3O_5SNa$)

Following the same procedure as above for **1**, and using the same equivalent ratios, 0.2 mmol of β-methylxanthobilirubic acid [14] afforded **2**. Yield 77 mg (86%); mp $>$ 260° (dec); HRMS (FAB, 3-NBA): calcd. for $C_{20}H_{28}N_3O_5SNa_2$ $(M+Na)^+$ 468.1545; found 468.1539, $\Delta = 0.6$ mDa, error 1.2 ppm. ^{13}C and ^1H NMR in Tables 1 and 2.

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References

[1] Huxtable RJ (2000) Adv Exptl Med Biol **483**: 1
[2] Smayda R (1999) J Appl Nutrition **51**: 27
[3] Sakai T, Tabata N, Watanabe K (1990) Agric Biol Chem **54**: 2047
[4] Chowdury JR, Wolkoff AW, Chowdury NR, Arias IM (2001) In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The Metabolic and Molecular Bases of Inherited Disease vol II. McGraw-Hill, New York, p 3063
[5] Lightner DA, McDonagh AF, Wijekoon WMD, Reisinger M (1988) Tetrahedron Lett **29**: 3507
[6] Kajihara T, Tazuma S, Yamashita G, Kajiyama G (1999) J Gastroenterol Hepatol **14**: 578
[7] Jansen PLM, Van Klinken JW, Van Gelder M, Ottenhoff R, Elferink RPJO (1993) Am J Physiol **265**: G445
[8] Hillig KJD, Morris MD (1984) J Raman Spectrosc **15**: 288
[9] Jirsa M, Večerek B (1958) Hoppe-Seyler's Z Physiol Chem **311**: 87
[10] a) Turnbull LB, Johnson III CP, Chen YH, Sancilio LF, Bruce RB (1974) J Med Chem **17**: 45; b) Mimura T, Nakamura Y, Nishino J, Sawayama T, Komiya T, Deguchi T, Kita A, Nakamura H, Matsumoto J (1992) J Med Chem **35**: 602; c) Haberfield P, Cincotta JJ (1990) J Org Chem **55**: 1334; d) Wakamiya T, Yamanoi K, Kanou K, Shiba T (1987) Tetrahedron Lett **28**: 5887; e) Huynh-ba T, Philippossian G (1987) J Agric Food Chem **35**: 165; f) Ernst B, Wagner B (1989) Helv Chim Acta **72**: 165; g) Bock MG, DiPardo RM, Evans BE, Freidinger RM, Rittle

KE, Payne LS, Boger J, Whitter WL, LaMont BI, Ulm EH, Blaine EH, Schorn TW, Weber DF (1988) J Med Chem **31**: 1918; *ibid* (1987) **30**: 1853; h) Gulyas J, Sebestyen F, Hercsel-Szepespataky J, Furka A (1987) Org Prep Proc Int **19**: 64; i) Nuzzo RG, Haynie SL, Wilson ME, Whitesides GM (1981) J Org Chem **46**: 2861; j) Küchler B, Voß G, Gerlach H (1991) Liebigs Ann Chem 545

[11] Zhao Z, Kingston DGI, Crosswell AR (1991) J Nat Prod **54**: 1607

[12] Boiadjiev SE, Person RV, Puzicha G, Knobler C, Maverick E, Trueblood KN, Lightner DA (1992) J Am Chem Soc **114**: 10123

[13] Boiadjiev SE, Person RV, Lightner DA (1993) Tetrahedron Asymm **4**: 491

[14] Boiadjiev SE, Anstine DT, Lightner DA (1995) J Am Chem Soc **117**: 8727

[15] a) Kaplan D, Navon G (1982) Biochem J **201**: 604; b) Kaplan D, Navon G (1983) Israel J Chem **23**: 177

[16] Person RV, Peterson BR, Lightner DA (1994) J Am Chem Soc **116**: 42

[17] a) Boiadjiev SE, Lightner DA (2000) J Am Chem Soc **122**: 11328; b) Boiadjiev SE, Lightner DA (1999) Tetrahedron Asymm **10**: 2535

[18] Harada N, Nakanishi K (1983) Circular Dichroic Spectroscopy – Exciton Coupling in Organic Stereochemistry. University Science Books, Mill Valley CA

[19] Lightner DA, Gawroński JK, Wijekoon WMD (1987) J Am Chem Soc **109**: 6354

[20] Kaplan D, Navon D (1983) Israel J Chem **23**: 177

[21] Armarego WLF, Perrin DD (1996) Purification of Laboratory Chemicals, 4th ed, Butterworth-Heinemann, Oxford