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ON THE CONFORMATION OF BILIRUBIN DIMETHYL ESTER FROM CIRCULAR DICHROISM SPECTROSCOPY

KEY WORDS: *Bilirubin, α,α' -Dimethylmesobilirubin-XIII α Dimethyl Ester, Stereochemistry, Circular Dichroism*

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ABSTRACT

Unlike the parent diacid, the dimethyl ester of ($\alpha R, \alpha' R$)-dimethylmesobilirubin-XIII α exhibits only a weak bisignate circular dichroism (CD) spectrum in a wide variety of solvents. This implies a loss of conformation stabilization afforded (in the diacid) by intramolecular hydrogen bonding, and with it the loss of an allosteric effect that forces the parent acid into the P-helicity conformational enantiomer.

INTRODUCTION

Bilirubin, the neurotoxic yellow-orange pigment of jaundice,¹ consists of two dipyrinone chromophores joined at a -CH₂- group (Fig. 1). Rotation of the dipyrinones, like blades of a propeller, about the -CH₂- generates a large array of conformations,^{2,3} only one of which uniquely brings the propionic acid COOH groups and opposing dipyrinone NH and C=O groups into juxtaposition for conformational stabilization through intramolecular hydrogen bonding.

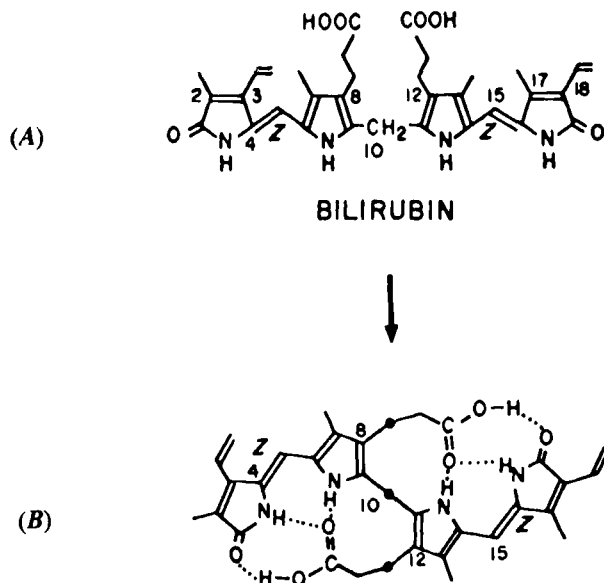


FIGURE 1. (A) Linear representation for bilirubin showing two dipyrromethane chromophores connected by a $-\text{CH}_2-$ group. (B) Planar projection of the intramolecularly hydrogen-bonded conformation adopted following rotation of the two dipyrromethane chromophores about the central $-\text{CH}_2-$ at C_{10} .

Whether such conformational stabilization can be achieved with bilirubin diesters, where the COOH groups are replaced by COOR and the maximum number of hydrogen bonds is thereby reduced by one-third, has been an important question in studies of bilirubin stereochemistry.^{4,5} Recent CD and $^1\text{H-NMR}$ studies of $(\alpha R, \alpha' R)$ -dimethylmesobilirubin-XIII α provided evidence for ridge-tile intramolecularly hydrogen bonded conformations in non-polar, aprotic solvents and for less well-defined structures in polar solvents, such as $(\text{CH}_3)_2\text{SO}$, that engage in hydrogen bonding with solute molecules.⁶ In the following, we show that CD spectroscopy of optically active bilirubin analog, $(+)-(\alpha R, \alpha' R)$ -dimethylmesobilirubin-XIII α and its dimethyl ester provides experimental evi-

dence that bilirubin diesters do not engage effectively in conformation-determining intramolecular hydrogen bonding.

EXPERIMENTAL

Circular dichroism spectra were recorded on a JASCO J-600 spectropolarimeter, and UV-visible spectra were run on a Cary 219 spectrophotometer. All NMR spectra were obtained on a GE QE-300 or QN-300 FT-spectrometer. The deuterated solvents used were 99.9% from Aldrich.

(\pm)- α,α' -Dimethylmesobilirubin-XIII α and (+)-($\alpha R,\alpha' R$)-dimethylmesobilirubin-XIII α (100% e.e.) were prepared as previously described.⁶ The dimethyl esters were prepared by activation of the acid functional group with carbonyl diimidazole then treatment with methanol as follows.

To a 25 mL 3-neck round bottom flash equipped with a reflux condenser and N₂ inlet/outlet were added 20 mg (0.0324 mmol) of the (*R,R*) or (*S,S*)- α,α' -dimethylmesobilirubin-XIII α , 44.0 mg (0.27 mmol) of 1,1'-carbonyl diimidazole and 5.0 mL of (CH₃)₂SO (dried and N₂ saturated). The mixture was heated at 55°C and stirred with a magnetic bar under an N₂ atmosphere for 2.5 hours. The 12 mL of methanol (purged with N₂) was added, and the mixture was heated at reflux under N₂ for 2 hours. The mixture was poured into 40 mL of dichloromethane, and the organic phase was washed with 0.2 M sodium bicarbonate (5 x 60 mL), dried over anhydrous sodium sulfate and evaporated to dryness to give a quantitative yield 92% pure by HPLC.⁷ This fairly pure product was chromatographed to afford a very pure product (>98% pure by HPLC, 80% yield) on a Woelm silica gel column eluting with dichloromethane containing 5% methanol. It had mp 230°C (dec.); IR (KBr) ν : 3343, 2924, 1736, 1662 cm⁻¹; ¹H-NMR (CDCl₃) δ : 10.66 (2H, s), 10.21 (2H, s), 5.91 (2H, s), 4.11 (2H, s), 3.67 (6H, s), 3.00 (2H, m), 2.62 (4H, m), 2.32 (4H, q, J=7.2 Hz), 2.09 (6H, s), 1.44 (6H, s), 1.13 (6H, d, J=6.0 Hz), 1.00 (6H, t, J=7.2 Hz) ppm; ¹³C-NMR (CDCl₃) δ : 177.00 (s), 174.27 (s), 147.01 (s), 131.15 (s), 128.87 (s), 123.96 (s), 123.46 (s), 123.05 (s), 117.76 (s), 100.17 (d), 51.52

(q), 41.01 (d), 28.73 (t), 22.81 (t), 17.79 (t), 16.50 (q), 14.71 (q), 9.91 (q), 7.72 (q) ppm.

Anal. Calcd. for $C_{37}H_{48}N_4O_6$ (644.8): C, 68.92; H, 7.50; N, 8.69

Found: C, 68.50; H, 7.51; N, 8.39

RESULTS AND DISCUSSION

Intramolecularly hydrogen-bonded bilirubin (Fig. 1B) folds into either of two ridge-tile shaped conformations, which are non-superimposable mirror images (Fig. 2). Introduction of a methyl group at the α and α' carbons of the propionic acid chains of bilirubin or mesobilirubin-XIII α greatly stabilizes one folded conformer relative to its enantiomer through allosteric action, but only in the *intramolecularly hydrogen bonded structures*. Thus, when mesobilirubin-XIII α is folded and held in the **P**-chirality ridge tile conformation by intramolecular hydrogen bonding, the *pro-S* α and α' hydrogens come in close contact with the pyrrole CH_3 groups at C_7 and C_{13} ; whereas, the *pro-R* hydrogens lie in a sterically less crowded environment (Fig. 2B). In the **M**-helicity conformer the inverse situation obtains, with the *pro-R* α and α' hydrogens being in the sterically more crowded environment. Consequently, replacing the *pro-R* hydrogens by CH_3 groups can be expected to force the conformational equilibrium strongly toward the **P**-helicity conformer. However, in the absence of significant intramolecular hydrogen bonding, the allosteric stabilization of the ridge tile conformations by CH_3 substitution would be lost and minimal conformational enantioselectivity would be expected. The α -methylated mesobilirubins thus serve as excellent chiral probes of pigment conformational stereochemistry.

In complete agreement with the predictions of the allosteric model and exciton coupling theory, the *R,R*-enantiomer of α,α' -dimethylmesobilirubin-XIII α shows an intense bisignate CD that is characteristic of the **P**-helicity conformational enantiomer (Fig. 3). Although the CD intensity decreases to $\sim 50\%$ of the maximum value with increasing ability of the solvent to participate in hydrogen-bonding, as in CH_3OH , only in $(CH_3)_2SO$ does the intensity

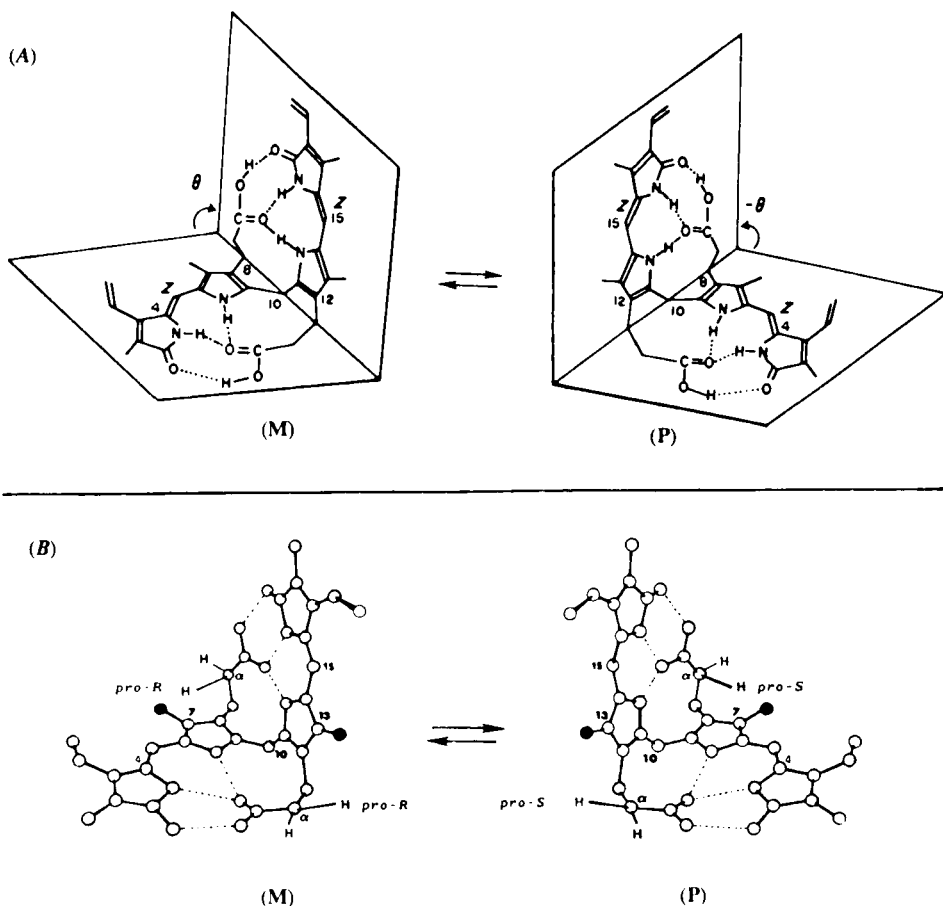


FIGURE 2. (A) Three-dimensional representations for the intramolecularly hydrogen bonded conformations adopting a ridge-tilt shape. There are two enantiomeric ridge-tilt conformers, which interconvert by breaking all six hydrogen bonds, rotating the dipyrinones about the C_{10} - CH_2 - and remaking the hydrogen bonds. The helicity or chirality of the two enantiomers is designated **M** or **P** according to the helical arrangement made by the electric dipole transition moments oriented along the long axis of each of the two dipyrinone chromophores of each molecule. (B) Ball and stick conformational representations for the ridge-tilt shape intramolecularly hydrogen bonded, interconverting enantiomers. The C_7 and C_{13} methyl groups are represented by the darkened circles. The hydrogens on the α and α' - CH_2 - groups are either *pro-R* or *pro-S*, only one of the set is designated. When the **M**-helicity conformer inverts into the **P**-helicity, steric crowding of the *pro-R* hydrogens is relieved and taken up by the *pro-S* hydrogens.

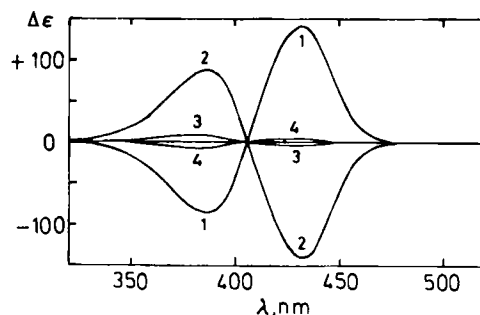


FIGURE 3. Circular dichroism spectra of 2.0×10^{-5} methanol solutions of ($\alpha R, \alpha' R$) and ($\alpha S, \alpha' S$)-dimethylmesobilirubin-XIII α [(1) and (2), respectively] and their dimethyl esters [(3) and (4), respectively] at 20°C.

fall drastically (Table 1). This is consistent with the picture where $(\text{CH}_3)_2\text{SO}$ solvent molecules are bound between the COOH and dipyrnone polar groups thereby decreasing the $\alpha\text{-CH}_3 \mid \text{C}_7\text{-CH}_3$ and $\alpha'\text{-CH}_3 \mid \text{C}_{13}\text{-CH}_3$ steric interactions and hence the conformational enantioselectivity.⁸ Nevertheless, the $\Delta\epsilon$ values in non-polar solvents are close to the theoretically predicted⁹ maximum $\Delta\epsilon$ values, as expected for solvents that do not disrupt the matrix of intramolecular hydrogen bonds.

In marked contrast, ($\alpha R, \alpha' R$)-dimethylmesobilirubin-XIII α dimethyl ester exhibits CD spectra weaker by more than an order of magnitude than those of the parent diacid in most solvents studied. The bisignate shape of the dimethyl ester CD's is still indicative of an exciton coupling origin, but the small Cotton effect magnitudes are in keeping with structures where the allosteric influence of the α, α' methyls is diminished, apparently by a reduced importance of intramolecular hydrogen bonding. The ineffectiveness or lack of such hydrogen bonding in conformational (enantiomer) stabilization is consistent with earlier NMR studies indicating that (1) bilirubin dimethyl ester self-associates through dipyrnone-dipyrnone intermolecular hydrogen bonding in non-polar solvents,¹⁰ such as CHCl_3 , and (2) the propionic ester residues are tethered via

TABLE 1. Circular Dichroism and Ultraviolet-Visible Spectral Data for ($\alpha R, \alpha' R$)-Dimethyl-mesobilirubin-XIII α and Its Dimethyl Ester^a

Solvent	Di-electric Const.	DIMETHYL ESTER				DIACID			
		CD		UV		CD		UV	
		$\Delta\epsilon_{\max}(\lambda_1)$	λ_2 at $\Delta\epsilon=0$	$\Delta\epsilon_{\max}(\lambda_3)$	ϵ_{\max}	$\Delta\epsilon_{\max}(\lambda_1)$	λ_2 at $\Delta\epsilon=0$	$\Delta\epsilon_{\max}(\lambda_3)$	ϵ_{\max}
C ₆ H ₆	2	+ 6.0(416)	393	- 5.0(376)	56,000	+252(436)	408	-135(392)	52,000
CCl ₄	2	+ 4.8(429)	405	- 3.0(390)	53,000	+259(436)	408	-124(392)	54,000
p-dioxane	2	+ 3.2(415)	390	- 2.2(378)	48,000	+230(434)	407	-129(390)	56,400
CHCl ₃	5	+19.7(426)	390	-11.8(375)	50,800	+246(436)	408	-133(398)	56,000
(CH ₃) ₂ CO	21	+ 1.3(440)	411	-1.8(380)	56,000	+226(434)	407	-128(390)	55,600
CH ₃ OH	33	- 3.2(430)	405	+ 7.0(382)	47,000	+141(432)	406	- 85(388)	55,700
CH ₃ CN	37	+ 1.0(430)	397	- 2.0(376)	52,000	+214(429)	403	-121(386)	55,000
HCON(CH ₃) ₂	37	- 6.0(426)	399	+ 8.2(380)	48,000	+ 83(431)	405	- 59(387)	48,000
(CH ₃) ₂ SO	49	-10.5(430)	404	+20.7(384)	49,300	+ 18(428)	406	- 9(393)	48,000
H ₂ O ^b	80	—	—	—	—	+117(425)	399	- 84(379)	45,000
HCONHCH ₃	160	- 7.2(429)	405	+11.9(383)	45,000	+136(428)	402	- 90(385)	45,000

^a Run on 2.0×10^{-5} M solutions at 20°C. ^b From Gordon, A.J.; Ford, R.A. *The Chemist's Companion*, Wiley, NY (1972) pp 4-8.
^c pH 7.4 phosphate buffer.

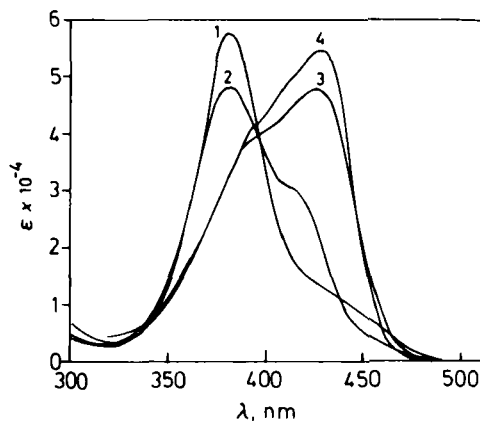


FIGURE 4. UV-visible spectra of $2 \times 10^{-5} M$ solutions of ($\alpha R, \alpha' R$)-dimethylmesobilirubin-XIII α dimethyl ester in benzene (1), chloroform (2), methanol (3) and dimethylsulfoxide (4) solvents at 20°C.

bound solvent molecules to the opposing dipyrinone NH groups in hydrogen bonding solvents such as $(\text{CH}_3)_2\text{SO}$.^{5,11} In (1) the pigment probably adopts a helical shape, where the propionic ester chains are not immobilized by intramolecular hydrogen bonding, and thus the allosteric model (Fig. 2) fails. In (2), with intramolecular hydrogen bonding being swollen by $(\text{CH}_3)_2\text{SO}$ solvent participation,⁸ the allosteric effect on enantiomeric conformational selection is diminished. Further evidence for the suggest conformations in CHCl_3 and $(\text{CH}_3)_2\text{SO}$ comes from the UV-visible spectra (Fig. 4). In $(\text{CH}_3)_2\text{SO}$ the dimethyl ester spectrum is essentially the same as that of the parent diacid, and the conformation adopted in these solvents is thought to be folded¹¹ (into the shape shown in Fig. 2, more or less). In contrast, the hypsochromic UV-visible spectral shift seen in CHCl_3 can be ascribed to a helical conformation adopted by the *intermolecularly* hydrogen bonded dimer.⁵ The spectral shifts are consistent with those expected from the exciton model, where the two dipyrinone electric dipole transition moments go from an essentially perpendicular align-

ment in the folded conformer present in $(\text{CH}_3)_2\text{SO}$ to an almost parallel alignment¹² associated with a helical conformation in CHCl_3 .

The origin of the (weakened) bisignate CD spectra of the dimethyl ester in a variety of solvents comes from electrostatic coupling of the induced electric dipole transition moments of the two component dipyrnone chromophores.⁹ However, neither the hydrogen bonding model of Fig. 2, nor the allosteric conformational selectivity due to methyl substitution, that when taken together produce the very intense CD spectra of the parent acid, appear to be present in the dimethyl ester. Without these conformation-determining factors, one cannot predict the CD Cotton effect signs, but from the Cotton effect signs one can predict that the helicity of the two transition moments is **P** for the ester in non-polar or non-hydrogen bonding solvents (as it is for the parent diacid in all the solvents studies),⁶ but the helicity changes from **P** to **M** in hydrogen bonding solvents. The exact conformational changes of the diester that accommodate such changes are at present unclear.

Of special interest are the CD data obtained from $(\text{CH}_3)_2\text{SO}$ solvent. NMR studies in $(\text{CD})_3\text{SO}$ of both bilirubin and its dimethyl ester (1) indicate a very limited motional freedom of the propionic side-chain in both (compared to the independent fast motion in the chain of a dipyrnone methyl ester),¹¹ (2) render improbable direct hydrogen bonding between the propionic residues and the dipyrnone NH groups. Both the CO_2H and CO_2CH_3 residues are thought to be tied to the nearest dipyrnone NH residues by bound $(\text{CH}_3)_2\text{SO}$ solvent molecules. Presumably this solvent greatly reduces the forced enantioselectivity of the α -methyl groups in the parent acid and leads to an unusually diminished CD intensity. One might therefore expect the same CD for the diester and the diacid. And indeed its CD intensity is quite comparable in magnitude to that of the diacid, stronger even than those of the diester in other hydrogen bonding solvents. Surprisingly however, the Cotton effect signs are opposite to those of the parent diacid. So even here, for steric reasons as yet unclear, the diester and diacid conformations differ.

CONCLUSIONS

Comparison of the CD spectra of ($\alpha R, \alpha' R$)-mesobilirubin-XIII α dimethyl ester and its parent diacid indicate that the diester adopts different conformations from those favored by the diacid in both non-polar and in polar, hydrogen bonding solvents. Although the helicity of the exciton-interacting electric dipole transition moments can be known from the CD spectra, the exact diester conformations responsible are unknown.

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REFERENCES

1. For a recent reviews see Gollan, J.L. (guest ed.) *Seminars in Liver Disease*, **1988**; 8(2).
2. Falk, H., Müller, N. Kraftfeldrechnungen an Gallenfarbstoffen: Die Energiehyperfläche Rubinoider Pigmente, *Monatsh. Chem.* **1982**, 112, 1325-1332; Force Field Calculations on Linear Polypyrrole Systems, *Tetrahedron* **1983**, 39: 1875-1885.
3. Lightner, D.A., Person, R.V., Peterson, B.R., Puzicha, G., Pu, Y-M., Bojadziev, S. Conformational Analysis and Circular Dichroism of Bilirubin, the Yellow Pigment of Jaundice. In *Biomolecular Spectroscopy II* (Nafie, L., Birge, R., eds.), Seattle, WA: SPIE, **1991**; in press.
4. (a) Lightner, D.A., Trull, F.R. Intermolecular Hydrogen Bonding and Dimeric Structures in Bilirubin Esters from ^1H -NMR Spectroscopy. *Spectrosc. Lett.* **1983**; 16: 785-803.
(b) Lightner, D.A., Zhang, M-H., Trull, F.R. Novel Synthesis of Bilirubin Mono-Esters. *Tetrahedron Lett.* **1987** 28, 4033-4036.
5. Kaplan, D., Navon, G. NMR Spectroscopy of Bilirubin and its Derivatives. *Israel J. Chem.* **1983**, 23: 177-186.

6. Pu, Y-M., Puzicha, G., Lightner, D.A. Allosteric Regulation of Conformational Enantiomerism. Bilirubin. *J. Am. Chem. Soc.* **1991**, *113*, in press.
7. McDonagh, A.F., Palma, L.A., Trull, F.R., Lightner, D.A. Phototherapy for Neonatal Jaundice. Configurational Isomers of Bilirubin. *J. Am. Chem. Soc.* **1982**, *104*: 6865-6867.
8. Gawroński, J.K., Pofonski, T., Lightner, D.A. Sulfoxides as Chiral Complexation Agents. *Tetrahedron*, **1990**, *46*: 8053-8066.
9. Lightner, D.A., Gawronski, J.K., Wijekoon, W.M.D. Complementarity and Chiral Recognition: Enantioselective Complexation of Bilirubin. *J. Am. Chem. Soc.* **1987**, *109*: 6354-6362.
10. Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*, Springer-Verlag-Wien, New York, 1989.
11. Kaplan, D., Navon, G. Studies of the Conformation of Bilirubin and its Dimethyl Ester in Dimethylsulphoxide Solutions by Nuclear Magnetic Resonance. *Biochem. J.* **1982**, *201*, 605-613.
12. Kasha, M. Rawls, H.R., El-Bayouni, M.A. The Exciton Model in Molecular Spectroscopy. *Pure Appl. Chem.* **1965**, *11*, 371-392.

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