Chirality Inversion in a Molecular Exciton

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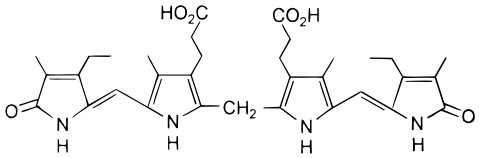
Received September 7, 1999. Revised Manuscript Received November 1, 1999

Abstract: The bichromophoric pigment bilirubin acts as a molecular exciton in its UV−visible and circular dichroism (CD) spectroscopy. The optically active analogue, (*βR*,*β′R*)-dimethylmesobilirubin-XIIIα exhibits intense bisignate CD Cotton effects in the region of its long wavelength UV−vis absorption near 400 nm, with $\Delta\epsilon_{434}^{max}$ +337, $\Delta\epsilon_{389}^{max}$ −186 in the nonpolar solvent $CHCl_3$, and nearly as intense Cotton effects in the polar, hydrogen bonding solvent CH_3OH: $\Delta\epsilon_{431}^{max}$ +285, $\Delta\epsilon_{386}^{max}$ −177. Addition of amines leads to Cotton effect sign inversions: in isopropylamine $\Delta\epsilon_{436}^{max}$ −605, $\Delta\epsilon_{392}^{max}$ +375, due to an inversion of molecular chirality.

Introduction

Bilirubin is a water-insoluble yellow-orange pigment, the end product of heme metabolism.[1,2]



Mesobilirubin-XIIIα

It occurs only in vertebrates (∼300 mg/day in healthy adult humans) and is clinically important for several reasons:[1−3] Its accumulation in blood and extravascular tissue is a useful sign of disease, usually liver disease; it can cause irreversible neurologic damage; it is involved in the formation of gallstones; and it is an endogenous inhibitor of free-radical injury.[4] In the form of its ester conjugates with sugars, it is the principal pigment in bile.

Bilirubin belongs to the class of pigments called "linear tetrapyrroles,"[2,5] but its solution and biological properties do not correlate well with either the linear (Figure 1A) or a porphyrin-like shape in which the polar carboxyl and lactam groups are freely solvated. Bilirubin is conformationally flexible in solution, but one conformation is significantly more stable than all of the others: a folded, ridge-tile structure with intramolecular hydrogen bonds linking the dipyrrinone pyrrole and lactam functions to the propionic carboxyl (or carboxylate) groups (Figure 1).[6−8] Although bilirubin can form helical conformers, they are of relatively high energy, and the linear (Figure 1A) and porphyrin-like conformations are especially high energy.[8,9] The ridge-tile conformation is the only one that has been observed in crystals of bilirubin[6,7] and its carboxylate salts.[10] Early spectroscopic studies, particularly by NMR, strongly suggested that hydrogen-bonded ridge-tile conformers also prevail in solution, even in the dipolar protophilic solvent dimethyl sulfoxide.[11] Such indications were supported recently by $^{13}C\{^1H\}$-heteronuclear Overhauser effect (NOE) measurements[12] and by energy calculations.[8,9] The ridge-tile conformation is dissymmetric, and bilirubin can adopt either of two nonsuperimposable mirror-image conformations (Figure 1B). Both enantiomers occur in the crystal[6,7] and in solution,[13] interconverting rapidly[11] in solution via a succession of nonplanar intermediates in which the hydrogen bonding network is never completely broken.[8] The energetically most favored ridge-tile conformation (with interplanar angle, $\theta \approx 100°$) is *not* rigid; however, it is flexible. Small, low-energy rotations about the C(9)−C(10) and C(10)−C(11) bonds cause θ to open or close somewhat, while maintaining hydrogen bonding.[8] Large rotations, however, break hydrogen bonds and lead to energetically unfavorable conformations. Such large bond rotations are associated with the interconversion of mirror image ridge-tile conformations—a dynamic process that occurs at an experimentally determined rate of ∼5.4 s^{-1} at 37 °C over an experimentally determined barrier of ∼18−20 kcal/mol.[14] The picture of the bilirubin structure is thus one of *flexible* enantiomeric *ridge-tile shapes* that *interconvert rapidly* at room temperature.

Solutions of bilirubin in isotropic media can be thought of as a 50:50 mixture of equilibrating ***M*** and ***P*** conformational enantiomers (Figure 1B). Displacement of the equilibrium toward the ***M*** or ***P*** can be achieved by enantioselective

(1) Chowdury, J. R.; Wolkoff, A. W.; Chowdury, N. R.; Arias, I. M. Hereditary Jaundice and Disorders of Bilirubin Metabolism. In *The Metabolic and Molecular Bases of Inherited Disease*; Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Eds.; McGraw-Hill: New York, 1995; Chapter 67, Vol. II, pp 2161−2208.

(2) McDonagh, A. F. Bile Pigments: Bilatrienes and 5,15-Biladienes. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 6, pp 293−491.

(3) Berk, P. D.; Noyer, C. *Semin. Liver Dis.* **1994**, *14*, 323−394.

(4) Neuzil, J.; Stocker, R. *J. Biol. Chem.* **1994**, *269*, 16712−16719.

(5) Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*; Springer-Verlag: New York/Wien, 1989.

(6) Bonnett, R.; Davies, J. E.; Hursthouse, M. B.; Sheldrick, G. M. *Proc. R. Soc. London, Ser. B* **1978**, *202*, 249−268.

(7) LeBas, G.; Allegret, A.; Mauguen, Y.; DeRango, C.; Bailly, M. *Acta Crystallogr., Sect. B* **1980**, *B36*, 3007−3011.

(8) Person, R. V.; Peterson, B. R.; Lightner, D. A. *J. Am. Chem. Soc.* **1994**, *116*, 42−59.

(9) Shelver, W. L.; Rosenberg, H.; Shelver, W. H. *Int. J. Quantum Chem.* **1992**, *44*, 141−163.

(10) Mugnoli, A.; Manitto, P.; Monti, D. *Acta Crystallogr., Sect. C* **1983**, *38*, 1287−1291.

(11) Kaplan, D.; Navon, G. *Isr. J. Chem.* **1983**, *23*, 177−186.

(12) (a) Dörner, T.; Knipp, B.; Lightner, D. A. *Tetrahedron* **1997**, *53*, 2697−2716. (b) Nogales, D.; Lightner, D. A. *J. Biol. Chem.* **1995**, *270*, 73−77.

(13) Boiadjiev, S. E.; Person, R. V.; Puzicha, G.; Knobler, C.; Maverick, E.; Trueblood, K. N.; Lightner, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 10123−10133.

(14) Navon, G.; Frank, S.; Kaplan, D. *J. Chem. Soc., Perkin Trans 2* **1984**, 1145−1149.

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Published on Web 12/31/1999

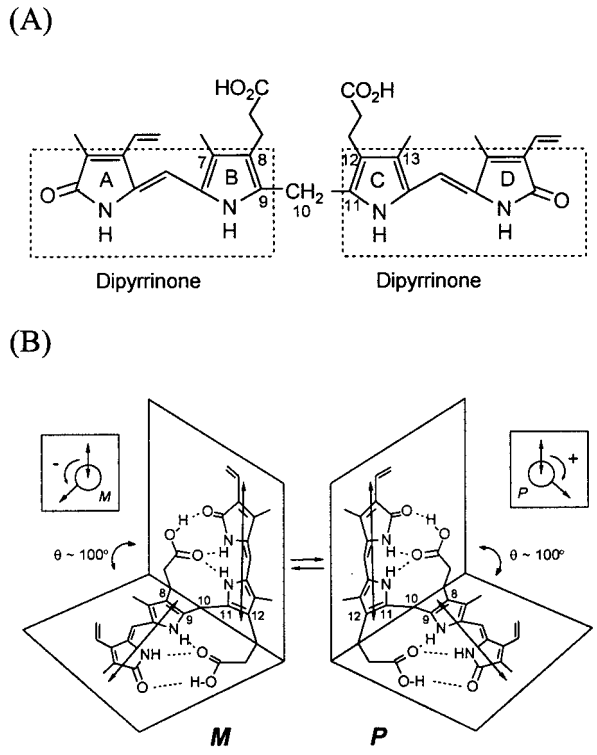
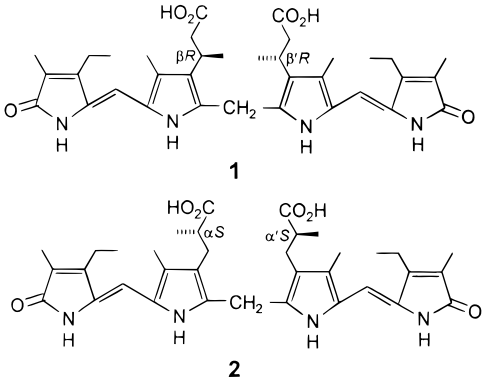


Figure 1. (A) Linear representation of bilirubin showing that the molecule is composed of two dipyrrinone chromophores connected to a common CH_2 unit. (B) The most stable (enantiomeric) conformations of bilirubin, shaped like ridge-tiles and stabilized by a matrix of intramolecular hydrogen bonds that link the dipyrrinones to opposing propionic acids. The double-headed arrows represent the approximate orientation of the electric dipole transition moments associated with the dipyrrinone long-wavelength transitions. Inset: The relative orientations of the dipyrrinone electric dipole transitions of the ***M*** (−) and ***P*** (+) chirality enantiomeric conformations.

complexation with a chiral compound such as quinine,[15] or serum albumin,[16,17] or by the action of intramolecular nonbonded steric repulsions, as has been observed when stereogenic centers are created by methyl substitution at either the α or the *β* carbons of the propionic acid chains.[13,18] It is this latter phenomenon that has attracted our interest because such "intramolecular resolution" can be observed by circular dichroism spectroscopy.[13,18−20]

The optically active compounds of particular interest in the current work are analogues of bilirubin, called mesobilirubins: (*βR,β′R*)-dimethylmesobilirubin-XIIIα (**1**)[13] and (α*S*,α′*S*)-dimethylmesobilirubin-XIIIα (**2**).[18] Mesobilirubins differ from bilirubin in having ethyl rather than vinyl groups (cf, Figure 1A). For the symmetric mesobilirubin-XIIIα, these small differences in structure in the substituents of lactam rings A and D are far removed from the important, conformation-determining intramolecular hydrogen bonding motif of Figure 1B. Thus, mesobilirubin-XIIIα shares many structure-derived similarities with natural bilirubin, including a high lipid/water partition coefficient and similar processing by the liver in animal metabolism.



Materials and Methods

The optically active bilirubin analogues (**1** and **2**) used in this work were prepared in 100% ee, as reported previously.[13,18] All circular dichroism (CD) spectra were measured on a JASCO J-600 spectropolarimeter, and all UV−vis spectra were recorded on a Perkin-Elmer Lambda 12 or Cary 219 spectrophotometer, using spectral grade (Fisher Scientific Co., Pittsburg, PA, or Aldrich Chemical Co., Milwaukee, WI) solvents at 23 °C. NMR spectra were obtained on a Varian Unity Plus 500 MHz spectrometer. Unless otherwise noted, $CDCl_3$ solvent was used, and chemical shifts are reported in *δ*, ppm referenced to the residual $CHCl_3$ ^1H signal at 7.26 ppm and the $CDCl_3$ ^{13}C signal at 77.00 ppm. A double-pulsed field gradient spin−echo experiment[21] was used for the $^1H\{^1H\}$-NOE measurements.

Various combinations of **1** or **2** with diethylamine, triethylamine, *n*-propylamine, di-*n*-propylamine, tri-*n*-propylamine, *i*-propylamine, di-*i*-propylamine, tri-*i*-propylamine, and longer chain amines in chloroform, benzene, tetrachloromethane, hexane, methanol, and dimethyl sulfoxide solvents were examined by CD spectroscopy in the course of this study.

Results and Discussion

CD Inversion. The circular dichroism (CD) spectra of **1** in the region of its long wavelength UV−vis absorption near 420 nm typically show a bisignate shape and very large magnitude ($\Delta\epsilon$),[8,13,16,20] as is characteristic of exciton coupling (Figure 2).[22] Such behavior by **1** is found in a wide variety of solvents of wide-ranging polarity and hydrogen bonding ability, from benzene to methanol to *N*-methylformamide.[13] Only in dimethyl sulfoxide are the CD Cotton effects greatly diminished, an observation attributed to intercalation of solvent molecules into the matrix of intramolecular hydrogen bonds (Figure 1B).[11,13,14,23]

Unexpectedly, the CD of **1** in amine solvents, such as isopropylamine (Figure 3) shows an inverted CD couplet, compared with that found in most ordinary organic solvents, except $(CH_3)_2SO$. The inversion also occurs with secondary and tertiary amines; for example, addition of *n*-propyl, di-*n*-propyl, or tri-*n*-propylamine to chloroform solutions of **1** affords a progressive diminution of the Cotton effect seen in $CHCl_3$ and an eventual inversion as the molar ratio of amine/pigment increases (Figure 4). The tertiary amine was less effective than

(15) Lightner, D. A.; Gawroński, J. K.; Wijekoon, W. M. D. *J. Am. Chem. Soc.* **1987**, *109*, 6354−6362.
(16) Blauer, G. *Biochim. Biophys. Acta* **1987**, *109*, 6354−6362.
(17) Lightner, D. A.; Wijekoon, W. M. D.; Zhang, M. H. *J. Biol. Chem.* **1988**, *263*, 16669−16676.
(18) Puzicha, G.; Pu, Y.-M.; Lightner, D. A. *J. Am. Chem. Soc.* **1991**, *113*, 3583−3592.
(19) (a) Boiadjiev, S. E.; Lightner, D. A. *Tetrahedron: Asymmetry* **1996**, *7*, 1309−1322. (b) Boiadjiev, S. E.; Lightner, D. A. *Tetrahedron: Asymmetry* **1997**, *8*, 2115−2129.
(20) For a review of the optical activity of bilirubins and other oligopyrroles, see: Boiadjiev, S. E.; Lightner, D. A. *Tetrahedron: Asymmetry* **1999**, *10*, 607−655.
(21) (a) Stonehouse, J.; Adell, P.; Keeler, J.; Shaka, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 6037−6038. (b) Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T.-L.; Shaka, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 4199−4200.
(22) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy − Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.
(23) (a) Gawroński, J. K.; Połonski, T.; Lightner, D. A. *Tetrahedron* **1990**, *46*, 8053−8066. (b) Trull, F. R.; Shrout, D. P.; Lightner, D. A. *Tetrahedron* **1992**, *48*, 8189−8198.

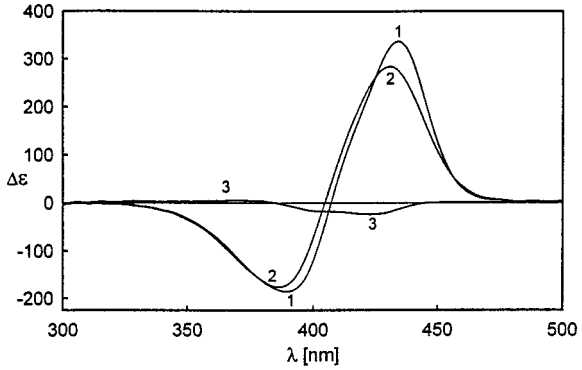


Figure 2. Circular dichroism spectra of ~10^{-5} M ($\beta R,\beta' R$)-dimethylmesobilirubin-XIIIα (**1**) in chloroform (spectrum 1, $\Delta\epsilon_{434}^{max}$ +337, $\Delta\epsilon_{389}^{max}$ −186), methanol (spectrum 2, $\Delta\epsilon_{431}^{max}$ +285, $\Delta\epsilon_{386}^{max}$ −177), and dimethyl sulfoxide (spectrum 3, $\Delta\epsilon_{425}^{max}$ −23, $\Delta\epsilon_{369}^{max}$ +6) solvents at 23 °C.

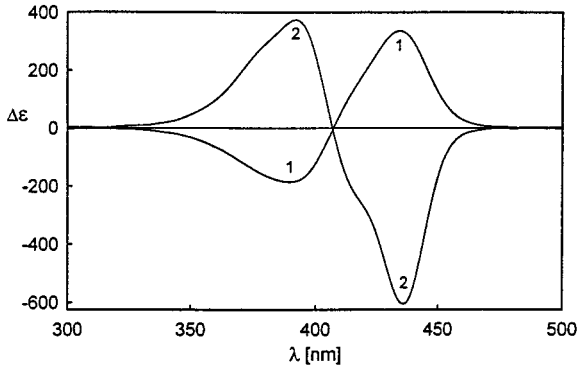


Figure 3. Circular dichroism spectra of ~10^{-5} M ($\beta R,\beta' R$)-dimethylmesobilirubin-XIIIα (**1**) in chloroform (spectrum 1, $\Delta\epsilon_{434}^{max}$ +337, $\Delta\epsilon_{389}^{max}$ −186), and isopropylamine (spectrum 2, $\Delta\epsilon_{436}^{max}$ −605, $\Delta\epsilon_{392}^{max}$ +375) solvents at 23 °C.

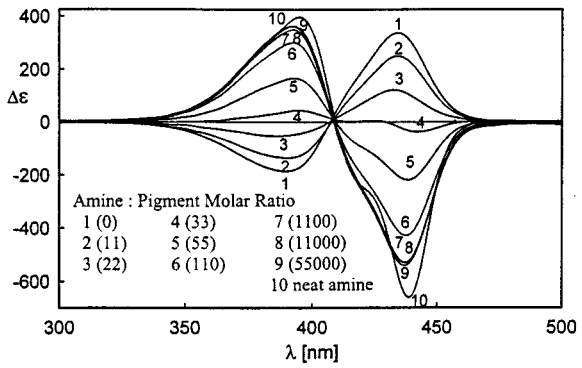


Figure 4. Changes in the CD spectrum of ~10^{-5} M (βR, $\beta' R$)-dimethylmesobilirubin-XIIIα (**1**) upon sequential changing from pure chloroform solvent to pure di-*n*-propylamine solvent. The molar ratios of amine/pigment are given in parentheses for spectra 1−10. Spectrum 1, $\Delta\epsilon_{434}^{max}$ +337, $\Delta\epsilon_{389}^{max}$ −186; spectrum 10, $\Delta\epsilon_{439}^{max}$ −660, $\Delta\epsilon_{395}^{max}$ +400.

the primary in achieving intense Cotton effects, as judged from the molar ratio of amine: pigment, and the secondary amine was most effective (Figure 5). However, the relative effectiveness of the type (primary, secondary, tertiary) of amine was found to vary according to solvent. Consistently, large Cotton effects from **1** could be reproduced in CH_3OH solvent with added amine (Figure 6A), but without sign inversion. Most interestingly, large Cotton effects were also found in $(CH_3)_2$-SO solvent in which the weak Cotton effects typical of this solvent were restored to very intense effects when amine was added (Figure 6B). In various amines studied, the Cotton effects

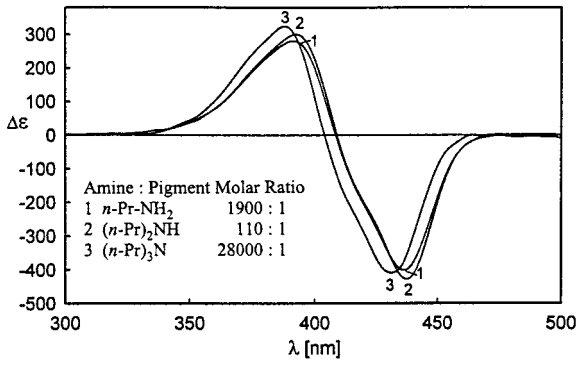


Figure 5. Comparison of the amine/pigment ratios required to produce nearly the same CD curves for ~10^{-5} M ($\beta R,\beta' R$)-dimethylmesobilirubin-XIIIα (**1**) at 23 °C.

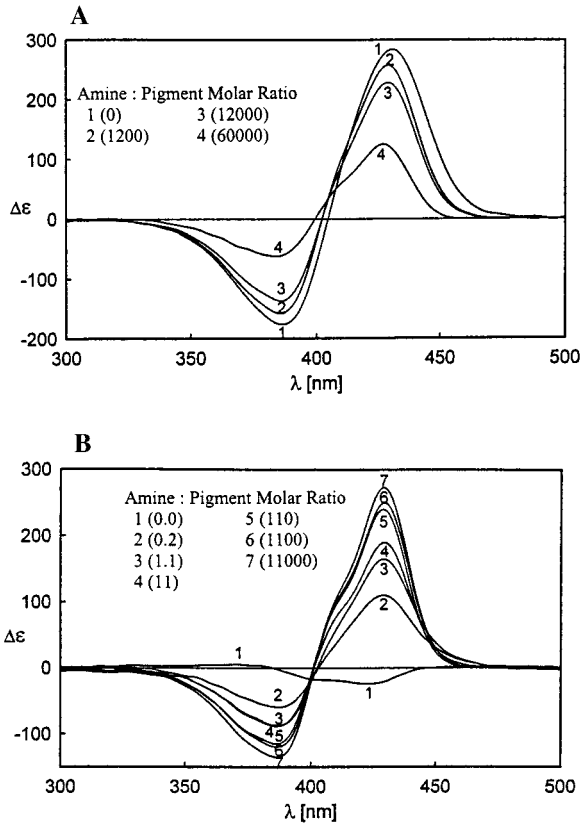


Figure 6. Circular dichroism spectra of ~10^{-5} M ($\beta R,\beta' R$)-dimethylmesobilirubin-XIIIα (**1**) in (A) methanol or (B) dimethyl sulfoxide and the influence of added di-isopropylamine on the spectra at 23 °C. The amine/pigment molar ratios are given in parentheses for each of numbered curves. (A) Spectrum 1: $\Delta\epsilon_{431}^{max}$ +285, $\Delta\epsilon_{386}^{max}$ −177; spectrum 4: $\Delta\epsilon_{426}^{max}$ +127, $\Delta\epsilon_{384}^{max}$ −63. (B) Spectrum 1: $\Delta\epsilon_{425}^{max}$ −23, $\Delta\epsilon_{369}^{max}$ +6; spectrum 7: $\Delta\epsilon_{429}^{max}$ +273, $\Delta\epsilon_{386}^{max}$ −135.

of **1** had the same signed sequence and comparable magnitudes, and the inverted Cotton effect signs seen for **1** in amine solvents were also found for **2**.

The data of Figure 4 suggest an acid−base reaction between the propionic acids of **1** and the added amine. In this case, however, the amine must remain strongly coordinated to the acid because the carboxylate dianion of **1**, formed by acid−base reaction with tetra-*n*-butylammonium hydroxide gave no sign inversion or weakening of intensity (Figure 7A) in common organic solvents, but in $(CH_3)_2SO$ the Cotton effects were restored to considerable intensity. The latter suggests that the unusually weak Cotton effects seen for **1** (but not its dianion) arise from interaction of solvent with the carboxylic acid proton,

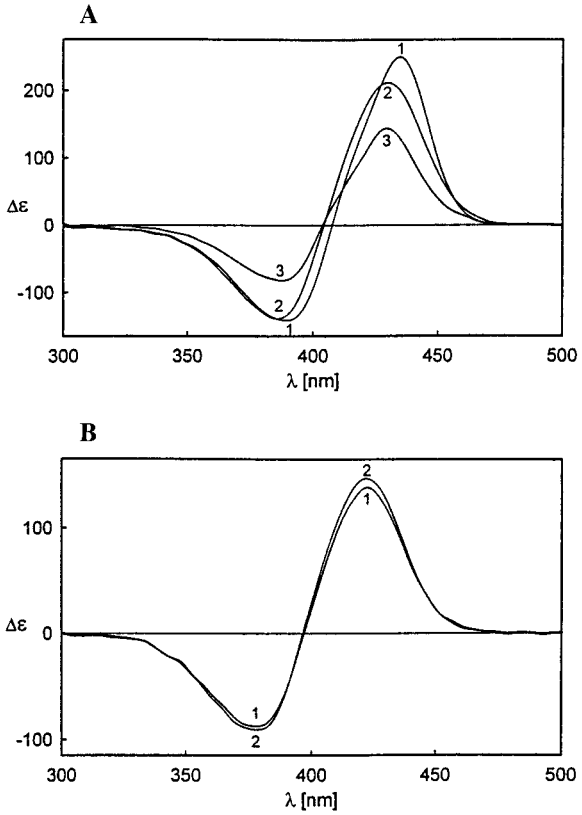


Figure 7. Circular dichroism spectra of the bis-tetra-*n*-butylammonium salt of (*βR*,*β′R*)-dimethylmesobilirubin-XIIIα (**1**) (~10^{-5} M) in (A) chloroform (spectrum 1, $\Delta\epsilon_{435}^{max}$ +251, $\Delta\epsilon_{390}^{max}$ −142), methanol (spectrum 2, $\Delta\epsilon_{429}^{max}$ +213, $\Delta\epsilon_{387}^{max}$ −140), and dimethyl sulfoxide (spectrum 3, $\Delta\epsilon_{430}^{max}$ +145, $\Delta\epsilon_{388}^{max}$ −83); and (B) phosphate buffered water at pH 7.40 (spectrum 1, $\Delta\epsilon_{422}^{max}$ +139, $\Delta\epsilon_{378}^{max}$ −87) and pH 8.60 (spectrum 2, $\Delta\epsilon_{422}^{max}$ +147, $\Delta\epsilon_{378}^{max}$ −90) at 23 °C.

and the intense Cotton effects, seen for the dianion in organic solvents as well as in water (Figure 7B), indicate that the structural and stereochemical elements of **1** that form the basis for its CD spectra continue to be responsible even when its carboxylic acid groups are deprotonated.

Pigment Stereochemistry. The dominant, conformation-determining structural element of **1** and **2** is the intramolecular hydrogen bonding so characteristic of natural bilirubin (Figure 1B) and its mesobilirubin-XIIIα analogue. In such energetically favored intramolecularly hydrogen-bonded ridge-tile conformations, the propionic acid chain CH_2 hydrogens are diastereotopic. In ball-and-stick representations of **1** (Figure 8) and **2**, it is easy to recognize that the *pro-S* hydrogens of the ***M***-helical conformation lie in a much less sterically crowded environment than *pro-R*.[13,18] However, in the ***P***-helical conformation, the *pro-S* hydrogens lie in a more sterically crowded environment than *pro-R*. Thus, a methyl substituent at either the α or *β* propionic acid carbon will preferentially reside in the less sterically crowded environment, and the remaining α or *β* hydrogen will adopt the more crowded. This means that with an α*R* or *βR* configuration the pigment will be forced to adopt the ***P***-helical conformation, whereas an α*S* or *βS* configuration will tilt the balance toward the ***M***.[8,13,18,20]

Exciton Chirality. Correlations between pigment stereochemistry (***M*** and ***P***) and CD spectroscopy have been drawn on the basis of exciton coupling theory.[8,13,15,18,20,22] Bilirubin is a molecular exciton with two independent dipyrrinone chromophores conjoined to a CH_2 group (Figure 1A). As in diphenylmethane and other molecular propellers, the dipyrrinones of bilirubin may rotate about the central CH_2 to generate a wide variety of conformations.[8] Molecular mechanics calculations showed that the ridge-tile conformation lies at a global energy minimum and that other conformations are much higher in energy.[8] In the crystal and in solution, bilirubin was shown experimentally to prefer this hydrogen-bonded shape (Figures 1B and 8) to all others.[6,7,11,12] From analyses and calculations[8,15] in the exciton coupling frame for two dipyrrinone chromophores connected to and rotating about a CH_2, the ***M***-helical conformer (Figure 1B) is predicted to exhibit a *negative* exciton chirality (long wavelength negative, short wavelength positive for the CD couplet corresponding to the pigment's long wavelength absorption band(s) near 420 nm), and the ***P***-helical conformation is predicted to exhibit a *positive* exciton CD couplet (long wavelength positive, short wavelength negative). Independently, the absolute configuration of **1** was determined to be (*βR*,*β′R*).[13] That of **2** was determined to be (α*S*,α′*S*) by NMR methods.[18] The absolute configuration correlates nicely with their CD data in $CHCl_3$ and the predictions of the exciton chirality rule:[8,13,18,22] a positive exciton chirality from **1**[13] (Figures 4−8), a negative from **2**.[18] What accounts for the CD sign inversions found in amine solvents?

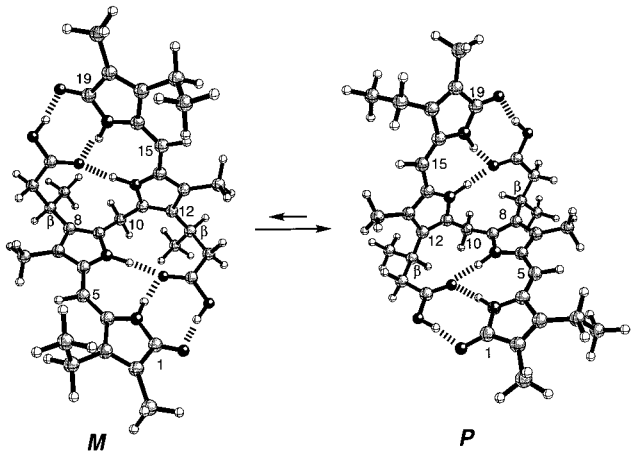


Figure 8. Ball-and-stick conformational drawings of (*βR*,*β′R*)-dimethylmesobilirubin-XIIIα (**1**) in the ***M*** and ***P*** helical intramolecularly hydrogen-bonded ridge-tile conformations.

Chirality Inversion. The signed order of the CD exciton couplet is governed by the relative helicity of the relevant two electric dipole transition moments, one from each chromophore.[8,22] For bilirubins and mesobilirubins, the two chromophores are dipyrrinones, and each has a strongly allowed long-wavelength $\pi \rightarrow \pi^*$ transition oriented along the long axis of the chromophore ($\epsilon_{410}^{max} \approx 37\,000$). In the exciton model, these electronic transitions, when not aligned strictly in line or in parallel,[8] couple electrostatically to yield two long-wavelength transitions in the UV−vis spectrum and two corresponding bands in the CD spectrum. One band is typically higher in energy and one lower, with the splitting being dependent on the strength and relative spatial orientation of the transition moments. When observed by UV−vis spectroscopy, the two transitions overlap to give the typically broadened and sometimes split long-wavelength absorption band found in bilirubins. In the CD spectra, however, the two exciton transitions are oppositely signed, and thus the CD curve typically exhibits bisignate Cotton effects − as predicted by theory.[8,15,22] It is thus the relative orientation of the two electric dipole transition moments that is important, of crucial importance in assigning absolute stereochemistry and conformation by CD spectroscopy.

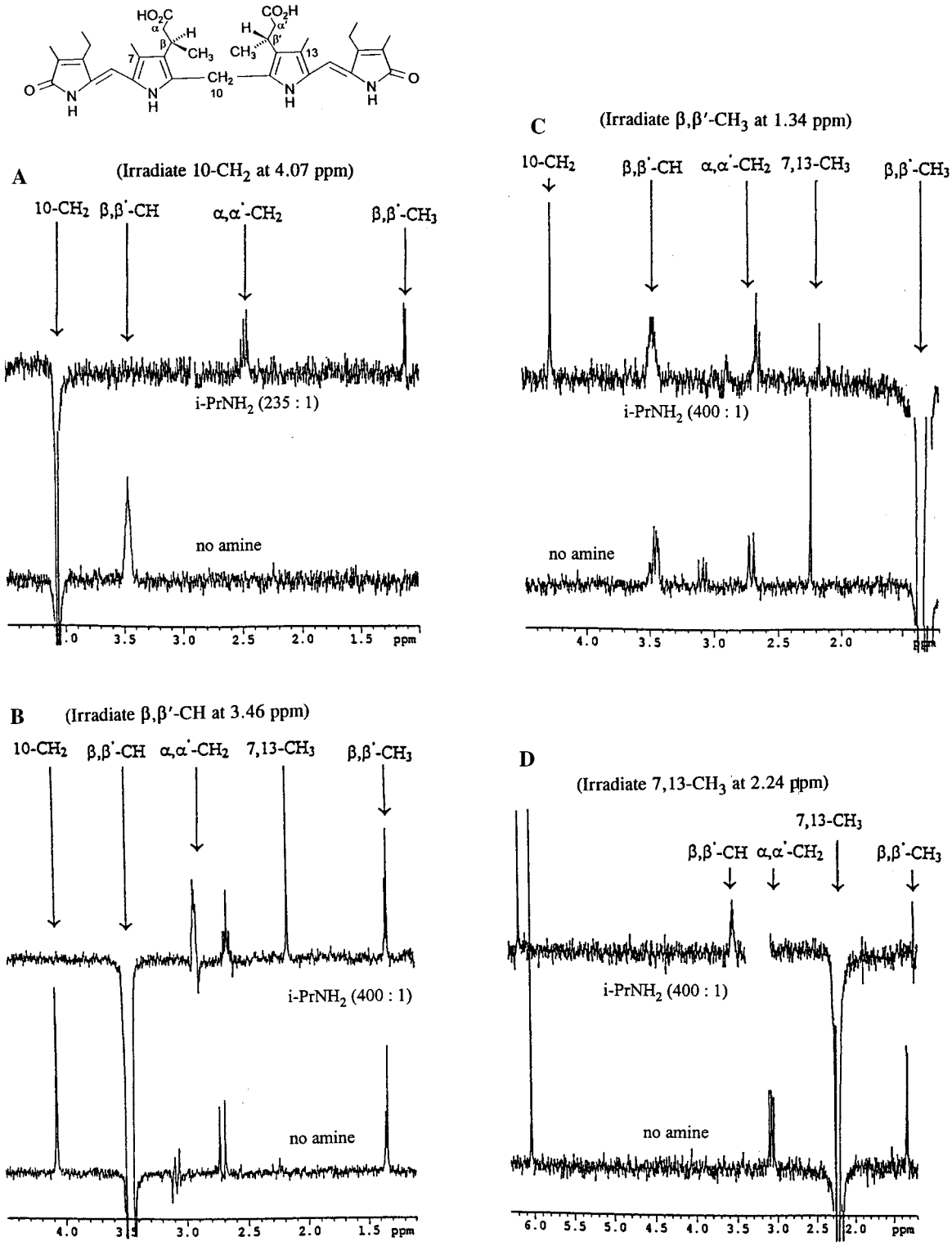


Figure 9. Comparison of $^1H\{^1H\}$-nuclear Overhauser effects seen in (*βR,β′R*)-dimethylmesobilirubin-XIIIα (**1**) in *i*-propylamine and in $CDCl_3$ at 25 °C by irradiating (A) the C(10)-methylene at 4.07 ppm, (B) the *β,β′*-methines at 3.46 ppm, (C) the *β,β′*-methyls at 1.34 ppm, and (D) the C(7)/C(13) ring methyls at 2.24 ppm.

The amine-promoted sign inversion of the CD couplet seen in Figures 3−6 can be explained by two different mechanisms. The simplest attributes it to an inversion of molecular chirality, from ***M*** to ***P*** (Figure 1B), which necessarily causes a sign change in the dihedral angle subtended by the electric transition dipole moments from each dipyrrinone. Less obvious, but as noted previously,[8] in bilirubins, book-like opening or closing of the ridge-tile shape causes the relevant dipyrrinone electric dipole transition moments to change orientation so as to open or close the dihedral angle between them (Figure 1B inset). Opening the ridge-tile has an interesting consequence: The dihedral angle opens and passes through 180° before the molecular chirality inverts from ***M*** to ***P***. That is, one can have an inversion of exciton chirality without having an inversion of molecular chirality.[8,24]

Conformation from NOEs. To determine the conformation (***M*** or ***P***) of **1** in amine solvents, we turned to an examination

of selected nuclear Overhauser effects (NOEs). From an examination of molecular models of **1**, it becomes clear that in the energetically favored ***P***-helical conformation the βR methyl groups lie in a relatively nonhindered environment and near the methyls at C(7) and C(13), whereas the βR methine CH lies close to the C(10) methylene (Figure 9). On the other hand, in the less favored ***M***-helical conformation, the βR methyls lie in a sterically crowded environment buttressed against the C(10) methylene, whereas the βR methine CH lies in an unhindered environment and near the C(7) and C(13) methyls. From NOE measurements of **1** in $CDCl_3$ solvent it is thus not surprising to find an enhancement of the β,β'-CH signals upon irradiation of the C(10) CH_2 (Figure 9A) (but no enhancement of the β,β'-CH_3s) and an enhancement of the C(10) CH_2 upon irradiation of the β,β'-CHs (Figure 9B) (but no enhancement at the C(7) and C(13) CH_3s). Nor is it surprising to find an NOE between the β,β'-CH_3s and the C(7) and C(13) CH_3s (Figures 9C and D) but no NOE between the β,β'-CH_3s and the C(10) CH_2 (Figure 9C) or between the C(7) and C(13) CH_3s and the β,β'-CHs (Figure 9D).

With added amine sufficient to cause a strong exciton chirality inversion of **1** (Figures 4−6), the NOE results are characteristic of an ***M***-helicity conformation, signifying that the amine has caused an inversion of molecular chirality to match the sign inversion of the CD couplet. Now NOEs are found (i) between the C(10) CH_2 and the β,β'-CH_3s (Figures 9A and C), but not between the C(10) CH_2 and the β,β'-CHs (Figures 9A and B), and (ii) between the β,β'-CHs and the C(7) and C(13) CH_3s (Figures 9B and D).

Concluding Comments

We have found an unusual example of exciton chirality inversion with change of solvent. Strikingly, the characteristic positive exciton chirality found in typical organic solvents containing ($\beta R,\beta' R$)-dimethylmesobilirubin-XIIIα (**1**) (and the negative exciton chirality found in ($\alpha S,\alpha' S$)-dimethylmesobilirubin-XIIIα (**2**)) is inverted upon adding amines. In such amines studied, mainly alkylamines (primary, secondary, and tertiary), the Cotton effects magnitudes meet or exceed the largest seen in nonpolar solvents such as CCl_4, benzene, etc., but the signs are opposite. While the results of NOE analyses clearly indicate an inversion from the energetically favored ***P***-helical conformation to ***M*** attends the change of solvent to amine, it is not entirely clear how the amine acts to invert the conformation. It is clear, however, that the mechanism does not involve simply the expected acid−base reaction. Rather, the complex formed between amine and **1** (or **2**) is probably some sort of tight ion pair (carboxylate anion−ammonium cation) where the (protonated) amine is dragged into the matrix of intramolecular hydrogen bonds that preserves the ridge-tile conformation of the pigment. In the pigment−amine complex, apparently new nonbonded steric interactions are introduced, and these operate in opposition to the bias imposed by the α and β methyls on the propionic acids of **1** and **2**. These findings may have relevance to understanding the chirality of bilirubin in its complex with serum albumins (that are involved in transport of the pigment in the circulation in vivo).

Acknowledgment. We thank the National Institutes of Health (HD 17779) for generous support and the National Science Foundation (CHE-9214294) for assistance in purchasing the 500 MHz NMR spectrometer used in this work. Special thanks go to Mr. Lew Cary for his assistance in the NMR experiments. Dr. Stefan E. Boiadjiev is on leave from the Institute of Organic Chemistry of the Bulgarian Academy of Sciences, Sofia, Bulgaria.

JA993242B

(24) Pu, Y.-M.; McDonagh, A. F.; Lightner, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 377−380.