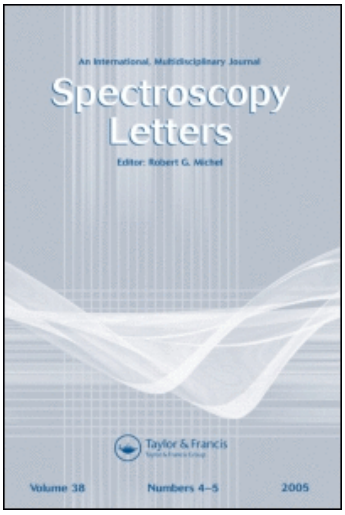

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Intramolecular Hydrogen Bonding in Bilirubin Amides as Detected by Circular Dichroism Spectroscopy

David A. Lightner[a]; Man-Hua Zhang[b]
[a] Department of Chemistry, University of Nevada-Reno, Reno, Nevada [b] Institute of Photographic Chemistry, Academia Sinica Beijing, China

To cite this Article Lightner, David A. and Zhang, Man-Hua(1988) 'Intramolecular Hydrogen Bonding in Bilirubin Amides as Detected by Circular Dichroism Spectroscopy', Spectroscopy Letters, 21: 6, 469 — 480
To link to this Article: DOI: 10.1080/00387018808062727
URL: http://dx.doi.org/10.1080/00387018808062727

SPECTROSCOPY LETTERS, 21(6), 469-480 (1988)

INTRAMOLECULAR HYDROGEN BONDING IN BILIRUBIN AMIDES AS DETECTED BY CIRCULAR DICHROISM SPECTROSCOPY

KEY WORDS:Bilirubin Mono- and Bis-Amides, Intramolecular H-Bonding, Circular Dichroism Spectroscopy

David A. Lightner

Department of Chemistry, University of Nevada-Reno
Reno, Nevada 89557-0020

Man-Hua Zhang

Institute of Photographic Chemistry, Academia Sinica
Beijing, China

ABSTRACT

Analysis of the circular dichroism spectra of bilirubin-IXα amides of *S*-(-)-1-phenylpropylamine reveals a preference in organic solvents for folded, intramolecularly H-bonded conformations involving the amide N-H hydrogen.

INTRODUCTION

(4*Z*,15*Z*)-Bilirubin-IXα (BR-IX), the yellow-orange pigment of jaundice,[1,2] can fold into either of two enantiomeric conformations[1,3] where steric repulsions are minimized[4] and which are stabilized by intramolecular H-bonds. As determined by NMR measurements,[5] they interconvert with an activation energy of ~18 kcal/mole at a rate of 7.2 ±0.4 sec^{-1} (~58°C). BR-IX can thus be viewed as a *racemic* mixture of conformational enantiomers, **P** ⇌ **M** (Fig. 1).[6] In isotropic solvents, its solutions are optically

inactive; however, in the presence of chiral complexation agents, such as serum albumin[7] and optically active amines,[8] the pigment becomes optically active and exhibits circular dichroism (CD) for its intense long wavelength UV-vis absorption near 450 nm. The origin of the induced optical activity can be explained in terms of non-equal populations of **M** and **P** species in the complexes.

The factors that direct the pigment to adopt the folded, enantiomeric conformations of Fig. 1 are only now being clarified. Although intramolecular hydrogen bonding is an important component of conformational stabilization, the tendency to adopt a folded conformation emanates from even more fundamental geometric and steric considerations. Space-filled molecular models and molecular mechanics calculations[4] indicate that *Z*-configuration rubinoid pigments with hydrogens at the nitrogens and at the *meso* carbons tend to adopt folded conformations of the types illustrated as their low energy structures -- even in the absence of the intramolecular hydrogen bonding shown. Even when BR-IX is deprotonated at the propionic acid groups, as in salts with amines[9] or tetraalkylammonium hydroxides,[10] the pigment retains a marked preference for the folded intramolecularly hydrogen-bonded structures of the acid form. And in polar solvents such as dimethylsulfoxide, the chiral conformations of Fig. 1 still predominate, albeit with the propionic acid CO_2H residues tied to their nearest lactam and pyrrole NH groups via bound solvent molecules.[5c] This remarkable preference for a folded conformation is not limited to intramolecularly H-bonded pigments but is found[5c,11] for esters, e.g., BR-IX dimethyl ester, in solvents where they are monomeric (dimethylsulfoxide).

Although CD has proved to be an excellent probe of pigment stereochemistry, there are almost no examples of the CD of optically active covalent derivatives of BR-IX.[12,13] In the present work we report on the CD of the *diastereomeric* mono- and bis-amides of BR-IX (BR-IX MPA and BR-IX BPA, respectively) prepared from *S*-(-)-1-phenylpropylamine and compare them with CD spectra of the diastereomeric mono and diesters of BR-IX derived from S-(-)-1-phenylethanol (BR-IX MPE and BR-IX DPE, respectively). The former offer an opportunity to explore the importance of the amide N-*H* in intramolecular hydrogen bonding; the latter offer

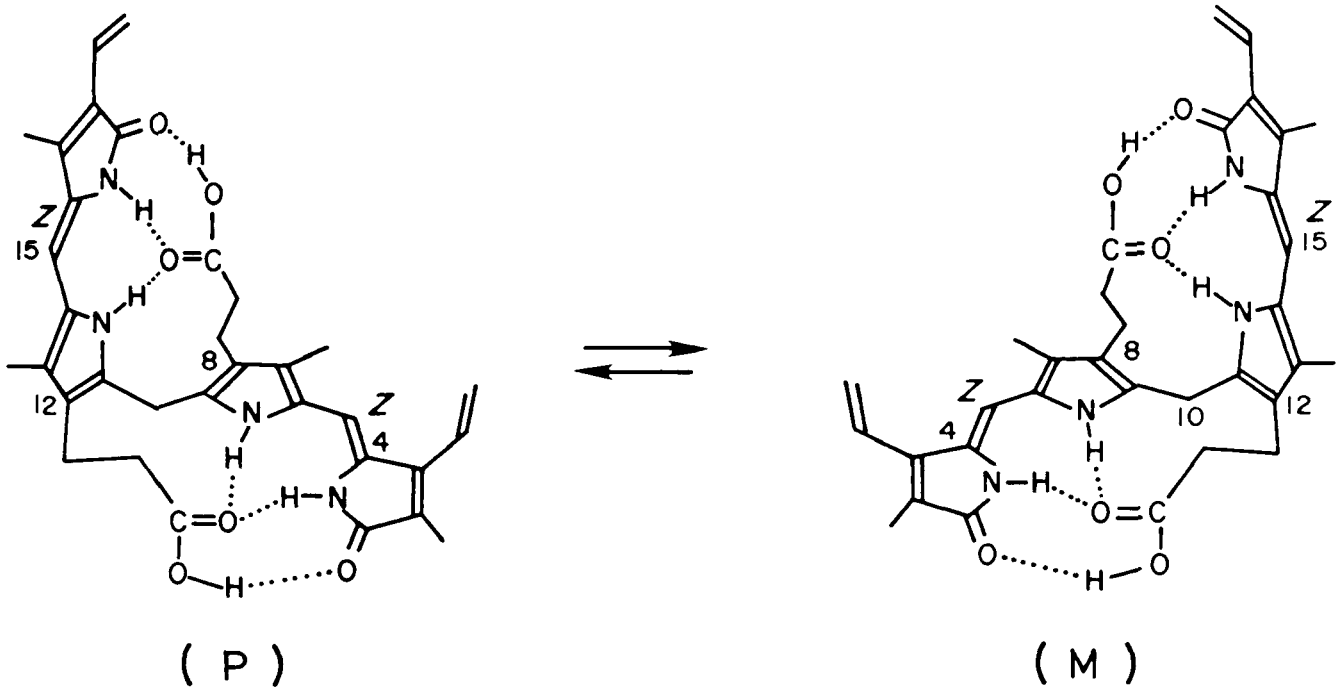

Figure 1. Interconverting, intramolecularly hydrogen-bonded enantiomeric conformations of bichromophoric (4Z,15)-bilirubin-IXa (BR-IX). The orientation of the (long wavelength electric dipole transition moments of) pyrromethenone chromophores of the left conformer corresponds to the **P** (plus helicity) chirality. The right conformer corresponds to **M** (minus helicity).[8a]

important offer comparison standards with reduced numbers of potential intramolecular H-bonds.

EXPERIMENTAL

(4*Z*,15*Z*)-Bilirubin-IXα was obtained from Sigma and purified as described earlier.[14] Its amides with *S*-(-)-1-phenylpropylamine (from Norse Labs, $[\alpha]_D$ = -19° (neat)) were prepared using diphenylphosphoryl azide, as with the amides of dimethylamine;[15] its esters with *S*-(-)-1-phenylethanol (from Aldrich, $[\alpha]_D^{23}$ = -41.3° (neat)) were prepared using carbonyldiimidazole to activate the BR-IX propionic acid groups.[16] Bis- or di-derivatives were separated from mono-derivatives (as mixtures of 8- and 12- monoamides or mono-esters) by column chromatography on Woelm Act. II neutral alumina. The product purity was >99% as determined by HPLC.[17] Circular dichroism spectra were run on a JASCO J-40 intrument; ^{1}H-NMR spectrum were run on an IBM NR-80/AF spectrometer.

RESULTS AND DISCUSSION

The mono and bis-amides, BR-IX MPA and BR-IX BPA, of bichromophoric bilirubin exhibit intense bisignate CD Cotton effects (CEs) straddling the UV-vis long wavelength transition(s) near 450 nm (Fig. 2). The esters, BR-IX MPE and BR-IX DPE, behave in a qualitatively similar way, except their CE intensities are much reduced, particularly the diester. The CE magnitudes of Fig. 2 may viewed as surprisingly large for inherently symmetric chromophores[18] with remote chiral perturbation, e.g., optically active pyrromethenone amides of xanthobilirubic acid: the amide of *S*-(-)-α-phenethylamine exhibits $\Delta\epsilon_{413}^{max}$ = +1.3 in dimethylsulfoxide, where the pigment is monomeric, and $\Delta\epsilon_{402}^{max}$ = +3.1, $\Delta\epsilon_{446}^{max}$ = -1.3 in chloroform, a solvent in which pyrromethenones form intermolecularly H-bonded dimers.[19] For the amides of BR-IX, the unexpectedly large CEs and their bisignate nature point to an entirely different mechanism of optical activity from that of chiral perturbation of an inherently symmetric chromophore[18] and provide a clue to the pigment's 3-dimensional structure.

Dilute solutions of BR-IX in CH_2Cl_2 consist largely of equimolar concentrations of interconverting conformational enantiomers (**P** and **M**, Fig. 1)[5] and understandably exhibit no optical activity. The amides, too, might be viewed as pairs of interconverting diastereomers (**P′** and **M′**, Fig.

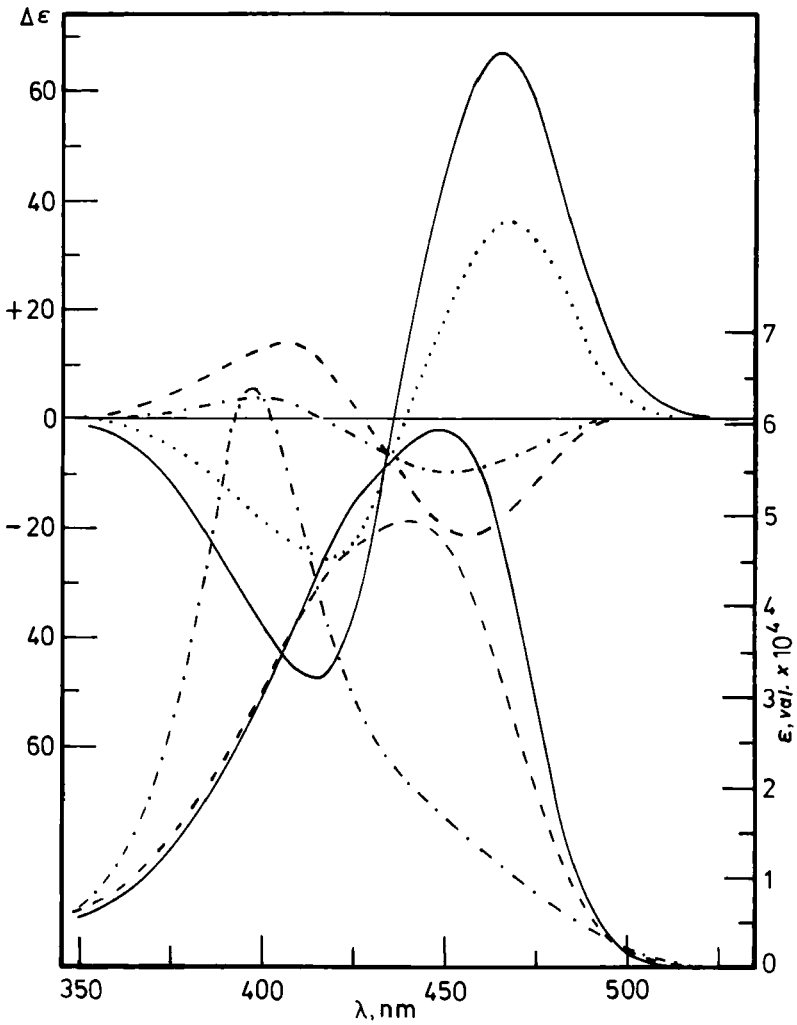

Figure 2. Circular dichroism and UV-visible spectra of 1.5 x 10^{-5} *M* solutions of BR-IX monoamide of *S*-(-)-1-phenylpropylamine (————), the bis-amide (• • • •), BR-IX mono-ester of *S*-(-)-1-phenylethanol (- - - -) and diester (- • - •) in benzene at 21°C. The UV-vis spectra show ϵ values increasing from the bottom of the Figure, the ϵ=0 line. The UV spectra of the mono and bis amides are essentially identical and shown together on the (————) UV curve.

3) that have an enantiomeric disposition of the two pyrromethenone chromophores. Here, added conformational stabilization might come from intramolecular H-bonding involving the propionamide N-H hydrogen. Since the amide N-*H* is less acidic than the propionic acid O*H*, the effectiveness of H-bonding is probably reduced in the amide as compared with the acid, but secondary amides still offer a greater potential for stabilizing the folded conformations of Figs. 1 and 3 than esters or tertiary amides. For diastereomers, ΔG°_{f} (P′) ≠ ΔG°_{f}(M′), and the concentration of P′ will thus not be equal to that of M′, resulting in a net optical activity characteristic of the predominant diastereomer. Large differences in concentration are expected to yield large observed CEs, with an approximate computed theoretical limit of $|\Delta\epsilon| \simeq 260$ mole cm^{-1}.

The sensitivity of the amide CDs to solvent dielectric and H-bonding may be seen in the data of Table 1, which reveal reduced CEs for the higher dielectric solvents, or even CE sign reversals (CH_3OH) and the largest CEs for solvents (benzene) that interfere least with H-bonding. Unexpectedly, the bis-amides exhibit slightly larger CEs in benzene and dichloromethane than the mono-amides, which retain intramolecular H-bonding via one propionic acid group. Here it might be seen that the second chiral amine reinforces the diastereomeric selectivity exerted by the first. However, in more polar, H-bonding solvents the relative CD intensities are reversed, suggesting that amide intramolecular H-bonds are more sensitive to intermolecular polarity and H-bonding. Overall, the data are consistent with a picture in which solvation (intramolecular H-bonding involving bound solvent molecules[5c]) reduces the tendency toward (pigment) enantioselectivity exerted by the attached chiral amine.

The amide CD data may be contrasted with those of the closely analogous esters derived from *S*-(-)-1-phenylethanol (Table 2), where the mono-esters, like the mono-amides retain partial intramolecular H-bonding through the underivatized propionic acid carboxyl group.[20] Here the CE magnitudes are smaller than the mono-amides, reflecting possibly a lesser diastereomeric selectivity associated with the one center of intramolecular H-bonding of the mono-ester *vs* the two centers of the mono-amide. When intramolecular H-bonding is reduced further, as in the diester, the CEs weaken even more. And unlike the bis-amides, the diester CEs are gener-

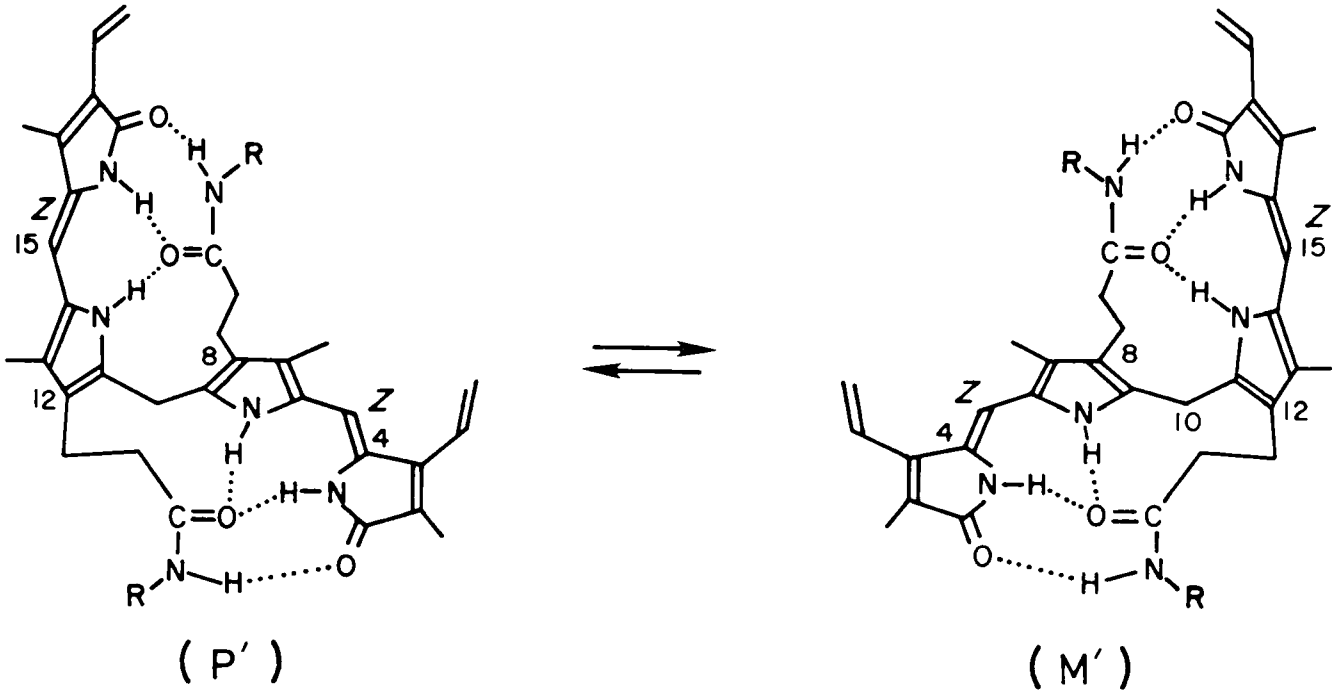

Figure 3. Folded, intramolecularly hydrogen-bonded conformations of the bis-amide of BR-IX with S-(-)-1-phenylpropylamine [R = $CH(CH_2CH_3)C_6H_5$]. The interconverting diastereomers are enantiomeric in the pigment skeleton, as in Fig. 1.

Table 1. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 1.0-1.7 x 10^{-5} M Solutions of Bilirubin Mono- and Bis-Amides of S-(-)-1-Phenylpropyl Amine at 21°C.

Amide	Solvent	CD $\Delta\epsilon_{max}(\lambda_1)$	λ_2 at $\Delta\epsilon$=0	$\Delta\epsilon_{max}(\lambda_3)$	UV ϵ_{max} (λ nm)
Mono	Benzene	-35.5 (420)	442	+46.5 (460)	64,600 (452)
Bis		-53.2 (405)	426	+75.3 (458)	57,600 (457)
Mono	Dichloro-	-21.3 (415)	437	+31.6 (465)	54,400 (450)
Bis	methane	-39.0 (415)	437	+45.4 (465)	55,400 (448)
Mono	Acetone	-14.0 (415)	434	+21.0 (465)	53,800 (452)
Bis		-17.2 (409)	429	+30.3 (458)	59,400 (447)
Mono	Aceto-	-16.3 (410)	431	+19.0 (460)	45,600 (443)
Bis	nitrile	- 8.8 (410)	431	+12.3 (460)	36,500 (440)
[a]Mono	Methanol	+16.4 (410)	427	-25.2 (460)	55,800 (450)
Bis		+12.2 (407)	429	-11.2 (458)	58,300 (450)
Mono	Methyl-	+ 2.4 (400)	---	« 0.1	51,900 (452)
Bis	sulfoxide	+ 1.7 (440)	---	« 0.1	66,800 (450)

[a] $\Delta\epsilon_{max}$ = -47.4 (505 nm), $\Delta\epsilon$ = 0 at 476 nm.

ally considerably weaker than the corresponding mono-derivatives. Although diesters may assume the folded conformations of Fig. 1,[4] they do not achieve this with the added benefit of full H-bonding stabilization; consequently, our understanding of the CD of diesters is correspondingly complicated. Moreover, since the diesters CEs show a much greater concentration dependence than do mono-esters,[12] mono-amides or bis-amides over the range 10^{-5} -10^{-7} *M*, we assume that their CD may not be derived intrinsically from conformations such as those in Figs. 1 and 3.

The situation with mono-esters, mono-amides and bis-amides, with the chiral perturber covalently attached to the pigment may be seen as equivalent to that of the heteroassociation complexes of BR-IX with chiral agents such as serum albumin[7] and amines[8] which act as chiral complexing agents and induce also CD. In either case, the bichromophoric pigment

Table 2. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Spectral Data for 1.3-2.2 x 10^{-5} M Solutions of Bilirubin Mono- and Di-Esters of S-(-)-1-Phenylethanol at 21°C.

Amide	Solvent	CD $\Delta\epsilon_{max}(\lambda_1)$	CD λ_2 at $\Delta\epsilon=0$	CD $\Delta\epsilon_{max}(\lambda_3)$	UV ϵ_{max} (λ nm)
Mono	Benzene	-13.6 (400)	419	+25.8 (450)	50,900 (438)
Di		- 2.4 (400)	414	+ 6.7 (455)	57,700 (400)
Mono	Dichloro-	-13.6 (405)	428	+16.2 (455)	51,100 (438)
Di	methane	- 3.4 (400)	416	+ 9.6 (452)	63,600 (398)
Mono	Acetone	- 6.7 (410)	424	+15.7 (455)	47,100 (438)
Di		« 0.1	---	+ 7.8 (445)	47,400 (400)
Mono	Aceto-	- 2.8 (410)	430	+ 5.0 (460)	45,100 (416)
Di	nitrile	« 0.1	---	+3.4 (410-455)	46,500 (394)
[a]Mono	Methanol	+ 5.5 (410)	440	- 2.1 (460)	75,100 (448)
Di		+ 5.2 (410-455)	---	« 0.1	55,700 (450)
Mono	Methyl-	+ 3.9 (410-455)	---	« 0.1	52,600 (454)
Di	sulfoxide	+ 8.2 (410-455)	---	« 0.1	62,300 (454)

[a] $\Delta\epsilon_{max}$ = -13.4 (490 nm), $\Delta\epsilon$ = 0 at 468 nm.

may be viewed as a molecular exciton[8] containing two pyrromethenone chromophores held in a chiral orientation, as in Figs. 1 and 3. Electrostatic interaction of the pyrromethenone electric dipole transition moments[20,21] leads to an exciton splitting of the molecular excited state resulting in two long wavelength electronic transitions, one higher in energy and one lower in energy, with the separation dependent on the relative orientation and strength of the transition moments. According to theory,[22] to two exciton transitions always have oppositely-signed CD CEs, typically flanking the UV-vis transition(s),[8,22] as is observed (Fig. 2). The absolute configuration of the predominant diastereomer can be predicted from exciton coupling theory and a knowledge of the pyrromethenone long wavelength UV-visible transition moment.[8a] Thus, BR-IX MPA,

BR-IX BPA and BR-IX MPE are predicted to have an excess of the pigment with **P** handedness in all the solvents of Table 1, except in CH_3OH, where the **M** chirality conformer predominates. From the intensity of the CE and using the calculated CEs for **P** or **M**,[8a] we estimate a diastereomeric excess of 12% for BR-IX MPA in CH_2Cl_2 and 17% for BR-IX BPA in CH_2Cl_2. In contrast, BR-IX MPE shows a 6% diasteriomeric excess in CH_2Cl_2.

CONCLUSIONS

BR-IX tends to assume either of two folded, intramolecularly H-bonded conformations that interconvert by breaking and remaking all 6 H-bonds (Fig. 1) and may thus be seen as a racemic mixture. When the conformation-determining propionic acid carboxyl groups are derivatized as secondary amides, intramolecularly H-bonded conformations may still be retained (Fig. 3), but when the amide possesses a chiral center, the erstwhile interconverting enantiomers become diastereomers, and the mixture is no longer racemic. More importantly, the equilibrium is no longer necessarily 1:1, $[P'] \neq [M']$, and the optical activity associated with the mixture can be expected to show unusual bisignate CD CEs associated with exciton coupling of the two pyrromethenone chromophores (Fig. 2 and Tables 1 and 2). Since the intrinsic CEs are typically very large for the enantiomeric conformers, moderate displacements from a 1:1 equilibrium will result in substantial CEs, and only small displacements will still result in significant CEs. Similarly, mono-amides and mono-esters, which still retain at least 50% of the intramolecular H-bonds through the underivatized propionic acid carboxyl group, can exhibit conformational diastereomerism when the amine or alcohol portion contains a chiral center. Here, too, conformations such as those of Fig. 3 appear to be essential in understanding the origin of the bisignate CDs.

ACKNOWLEDGMENT

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

REFERENCES

1. Lightner, D.A.; McDonagh, A.F. *Accounts Chem. Res.* **1984**; 17: 417-424.

2. McDonagh, A.F.; Lightner, D.A. *Pediatrics* **1985**; 75: 443-455.

3. (a) Bonnett, R.; Davies, J.E.; Hursthouse, M.B.; Sheldrick, G.M. *Proc. R. Soc. London Ser.* **1978**; B 202, 249-268.
   (b) LeBas, G.; Allegret, A.; Mauguen, Y.; DeRango, C.; Bailly, M. *Acta Crystallogr.* **1983**; C39: 3007-3011.

4. (a) Falk, H.; Müller, N. *Monatsh. Chem* **1982**; 112: 1325-1332.
   (b) Falk, H.; Müller, N. *Tetrahedron* **1983**; 39: 1875-1885.
   (c) Person, R.; Polonski, T.; Lightner, D. Unpublished results.

5. (a) Kaplan, D.; Navon, G. *Isr. J. Chem.* **1983**; 23: 177-186.
   (b) Navon, G.; Frank, S.; Kaplan, D. *J.C.S. Perkin Trans II* **1984**: 1145-1149.
   (c) Kaplan, D.; Navon, G. *Biochem. J.* **1982**; 201: 605-613.
   (d) Manitto, P.; Monti, D. *J.C.S. Chem. Commun.* **1976**: 122-123.

6. Lightner, D.A. In: Heirwegh, K.P.M., and Brown, S.B., eds. *Bilirubin.* CRC Press, Boca Ratan, FL: **1982**, Vol I: 1-58.

7. (a) For leading references see Blauer, G. *Isr. J. Chem.* **1983**; 23: 201-209.
   (b) Lightner, D.A.; Reisinger, M.; Landen, G.L. *J. Biol. Chem.* **1986**; 261: 6034-6038.

8. (a) Lightner, D.A.; Gawronski, J.K.; Wijekoon, W.M.D. *J. Am. Chem. Soc.* **1987**; 109: 6354-6362.
   (b) Lightner, D.A.; An, J.Y. *Tetrahedron* **1987**; 43: 4287-4296.

9. Mugnoli, A.; Manitto, P.; Monti, D. *Acta Crystallogr. Sect. C* **1983**; C38: 1287-1291.

10. Lightner, D.A.; Ma, J-S.; Wu, X-X. *Spectroscopy Lett.* **1986**; 19: 311-320.

11. Carey, M.C.; Spivak, W. In: Ostrow, J.D., ed. *Bile Pigments and Jaundice.* Marcel Dekker, Inc, New York **1986**: 81-132.

12. Lightner, D.A.; Zhang, M.H.; Trull, F.R. *Tetrahedron Lett.* **1987**; 28: 4033-4037.

13. Kuenzle, C.C.; Gitzelman-Cumarasamy, N.; Wilson, K.J. *J. Biol. Chem.* **1976**; 251: 801-807.

14. McDonagh, A.F. In Dolphin, D., ed. *The Porphyrins.* Academic Press, New York **1979**, Vol. VI: 293-491.

15. Lightner, D.A.; Adams, T.C.; Ma, J.S. *Tetrahedron* **1984**; 40: 4253-4260.

16. Compernolle, F. *FEBS Lett.* **1980**; 114: 17-20.

17. McDonagh, A.F.; Palma, L.A.; Trull, F.R.; Lightner, D.A. *J. Am. Chem. Soc.* **1982**; 104: 6860-6867.

18. Deutsche, C.W.; Lightner, D.A.; Woody, R.W.; Moscowitz, A. *Annu. Rev. Phys. Chem.* **1969**; 20: 407-448.

19. Lightner, D.A.; Reisinger, M.; Wijekoon, W.M.D. *J. Org. Chem.* **1987**; 52: 5391-5395.

20. Blauer, G; Wagnière, G. *J. Am. Chem. Soc.* **1975**; 97: 1949-1954.

21. Falk, H.; Grubmayr, K.; Höllbacher, G.; Hofer, O.; Leodolter, A.; Neufingerl, F.; Ribó, J.M. *Monatsh. Chem.* **1977**; 108: 1113-1130.

22. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy - Exciton Coupling in Organic Stereochemistry.* University Science Books, Mill Valley, CA **1983**.

Date Received: 04/12/88
Date Accepted: 05/13/88