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AMPLIFICATION OF OPTICAL ACTIVITY BY REMOTE CHIRAL FUNCTIONALITY. CIRCULAR DICHROISM OF BILIRUBIN *EXO*-VINYL N-ACETYL-L-CYSTEINE ADDUCTS

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Summary: Optically active diastereomers prepared by Markovnikov addition of N-acetyl-L-cysteine to the *exo*-vinyl group of bilirubin-IXα show intense, bisignate circular dichroism spectra in dimethylsulfoxide, as predicted for folded, enantiomeric pigment conformers.

(4Z,15Z)-Bilirubin-IXα (BR-IX), the bichromophoric and cytotoxic yellow-orange pigment of jaundice[2,3] is optically inactive, yet capable of adopting well-characterized enantiomeric conformations (Fig. 1)[3,4] as energy-minimum structures.[5] These folded, intramolecularly hydrogen-bonded conformers[2] which tuck the erstwhile polar lactam and carboxylic acid groups within a lipophilic exterior (and thus explain the unusual solubility properties of the pigment) are in dynamic equilibrium, interconverting at a rate of 3-95 sec^{-1} (50-95°C) with an associated activation barrier of ~18 kcal/mole.[4,5] In the presence of chiral complexation agents that exhibit enantioselective binding, e.g., proteins,[7] amines[8] or cyclodextrins,[9] the proportions of complexed **M** and **P** species are not equal and the solutions therefore become optically active in pigment -- as evidenced by circular dichroism (CD), in some cases extraordinarily intense CD ($\Delta\epsilon$ values ±50-200 $M^{-1} \cdot cm^{-1}$),[8,10] for the BR-IX long wavelength UV-visible transition near 450 nm. Given the potentially enormous Cotton effect (CE) magnitudes associated with either **M** or **P** alone,[8] only small displacements from a 1:1 equilibrium can generate intense CD. For example, as little as a 0.1% enantiomeric excess, corresponding to a 50.05:49.95 equilibrium or $\Delta\Delta G_f^\circ \simeq 1$ calorie/mole, would result in easily detectable CEs with $\Delta\epsilon$ values ±0.2-0.3 $M^{-1} \cdot cm^{-1}$. The amplification afforded by the system thus offers an extraordinarily sensitive way to detect small displacements from equilibrium.

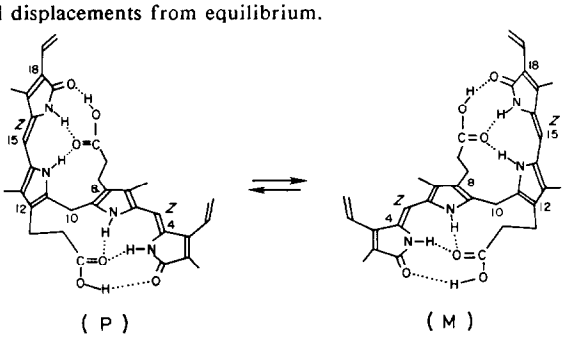
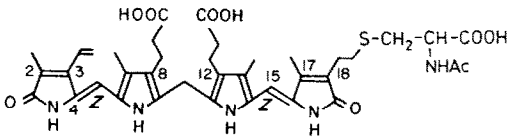


Figure 1. Interconverting, intramolecularly hydrogen-bonded enantiomeric conformers of bichromophoric BR-IX. The (long wavelength electric dipole transition moments, ref. 8, of the component) pyrromethenone chromophores of **P** have a positive (plus) chirality orientation, those of **M** have a negative (minus) chirality.

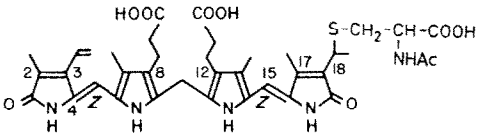
Bilirubin optical activity induced by chiral complexation agents has been studied, especially in connection with protein binding,[7,9] but until recently there were surprisingly few known examples of optically active BR-IX derivatives with the chiral agent covalently bound to the pigment. And those were mainly esters,[11] in which the complete H-bonding pattern of Fig. 1 cannot be fully expressed. The only known examples of optically active derivatives in which the H-bonding pattern is not directly perturbed are the N-acetyl-L-cysteine and glutathione adducts to the *exo*-vinyl group located at C-18;[11] yet, no chiroptical

measurements of these interesting pigments have been reported. In this work we report on the CD of the BR-IX N-acetyl-L-cysteine adducts, which offer an unprecedented opportunity to examine the role of remote stereochemistry on the equilibrium of Fig. 1 and add to our understanding of the 3-dimensional structure of BR-IX in solution.

In reactions that are highly regiospecific for addition to the *exo*-vinyl group of BR-IX, Manitto and Monti[12] showed how Markovnikov adducts can be prepared from alcohols or thiols photochemically or by acid-catalyzed addition. In our hands, acid-catalyzed or photochemical addition of N-acetyl-L-cysteine to BR-IX proceeded as described[12] to yield purified adduct that ran on reverse-phase HPLC[13] as a single entity. However, on careful examination using silica TLC (using Analtech 250μ analytical plates with $CHCl_3$:CH_3OH:CH_3CO_2H, 90:10:1, v/v/v as irrigant), the adduct showed the presence of three components (R_f 0.58, 0.41, 0.35), which were isolated and examined spectroscopically. The fastest-moving component constituted ~10% of the mixture; whereas, the two slower moving components were present in equal amounts. ^{1}H-NMR measurements[14] confirmed the presence of the *endo*-vinyl group on all three adducts and the *Z*-configuration[13] at C_4-C_5 and C_{15}-C_{16}; consequently, the adducts may be seen as originating from regioselective addition to the *exo*-vinyl group of BR-IX. The structures were assigned by NMR to an anti-Markovnikov adduct as the minor product (R_f 0.58) and to two diastereomeric Markovnikov adducts as the major isomers.[14] The latter are enantiomeric at the newly formed chiral center at C-18[1].

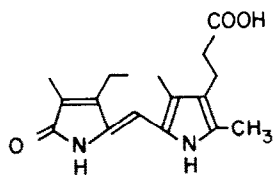


ANTI-MARKOVNIKOV ADDUCT



MARKOVNIKOV ADDUCT

The CD spectra of the three adducts are shown in Fig. 2. Unlike those of mono and diesters of BR-IX with optically active alcohols, which are weak ($|\Delta\epsilon| \simeq 1$) and apparently monosignate in DMSO solvent,[11] those of the adducts are intense and bisignate. The data for these new derivatives, which possess two planar pyrromethenone chromophores, contrast markedly with those from other (planar) mono-chromophoric aromatic substances perturbed by attached, remote chiral groups, e.g., for the ^1L_a transition of (*R*)-2,2-dimethyl-3α-naphthylbutane: $\Delta\epsilon_{280}^{max} \simeq -1$;[15] and for the isolated pyrromethenone chromophore of xanthobilirubic acid ester from *R*-(−)-phenethyl alcohol: $\Delta\epsilon_{410}^{max} \simeq -3$, $\epsilon_{410}^{max} \simeq 30{,}000$ (DMSO).[16] In the latter, the monomeric pyrromethenone is probably twisted by about 40° about the C_5-C_6 bond;[17] yet, the chiral center apparently is ineffective in selecting one skew conformation over the other, and the system behaves like a chirally perturbed inherently symmetric chromophore rather than a dissymmetric chromophore.[18] The unexpectedly large CD Cotton effects (CEs) of the N-acetyl-L-cysteine adducts and their bisignate character point to an entirely different mechanism of optical activity from that of chiral perturbation of an inherently symmetric (pyrromethenone) chromophore.[18] In this mechanism the bichromophoric pigment may be viewed as a molecular exciton. Electrostatic interaction of the pyrromethenone electric dipole transition moments may lead to exciton splitting of the molecular excited state resulting in two long wavelength UV-vis transitions, one higher in energy and one lower in energy, with the separation dependent on the relative orientation and strength of the transition moments.[19] If the two chromophores adopt a non-coplanar orientation and if the orbital overlap between them is small, the two exciton transitions will have oppositely signed CD CEs, typically flanking the UV-vis band(s), exactly as observed in Fig. 2. And the signed order of the CEs can provide information on



XANTHOBILIRUBIC ACID

the absolute stereochemistry of orientation of the chromophores,[8,19] hence the structure of the pigment. Thus, when the long wavelength member of the CD couplet has a (+) CE, P-chirality is indicated, and when it has a (-) CE, M-chirality obtains.

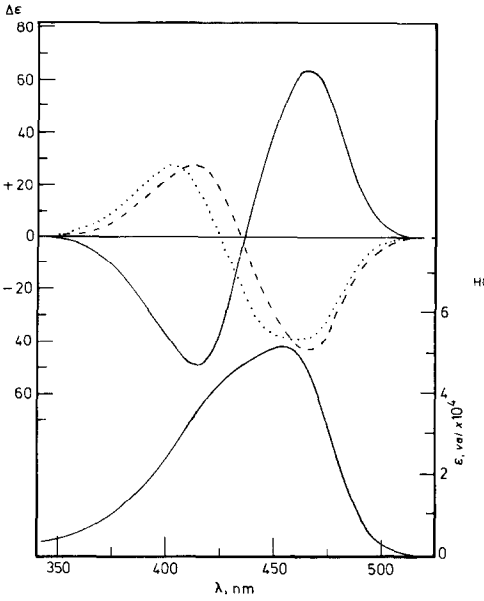


Figure 2. (Left) Bisignate circular dichroism spectra of the diasteriomeric Markovnikov adducts of N-acetyl-L-cysteine to the *exo*-vinyl group of BR-IX (————, R_f 0.41), (- - - -, R_f 0.35), and the anti-Markovnikov adduct (• • • •, R_f 0.58) in dimethylsulfoxide (DMSO) at 22°C. The corresponding UV-vis spectra have essentially the same shape and intensity, but λ_{max} of the anti-Markovnikov isomer is displaced to 448 nm. The curve for the Markovnikov isomers is shown rising from the ϵ=0 base line. Pigment concentrations were 1.1-1.3 x 10^{-5} M. Similarly intense CD spectra are obtained in $CHCl_3$ and CH_3OH solvents.

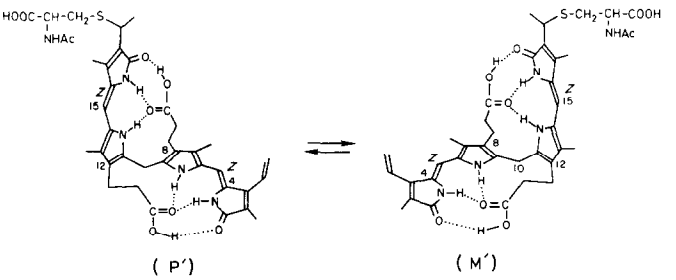


Figure 3. (Above) Interconverting, intramolecularly hydrogen-bonded diastereomeric conformers formed by Markovnikov addition of N-acetyl-L-cysteine to the *exo*-vinyl group of BR-IX at C-18.

Since addition of N-acetyl-L-cysteine to the *exo*-vinyl group is unlikely to disrupt the crucially-important hydrogen-bonding matrix, each adduct must form a pair of interconverting diastereomers (**P'** and **M'**, Fig. 3) that have an enantiomeric disposition of the two pyrromethenone chromophores. Because diastereomers typically have $\Delta G_f°(\mathbf{P'}) \neq \Delta G_f°(\mathbf{M'})$, the concentrations of **P'** and **M'** will not be equal and the solutions will exhibit optical activity and CD characteristics of the predominant diastereomer. For the Markovnikov adduct with R_f 0.35 and the anti-Markovnikov adduct, the predominant conformer is **M'**; for the Markovnikov adduct with R_f 0.41, the predominant conformer is **P'**; and the spectra indicate enantiomeric excesses of 15-25%.[8]

The CD data are important because they provide independent evidence for the persistence of chiral conformations of BR-IX in DMSO, a solvent that hydrogen-bonds to N-*H* groups.[20] Although the data are mute on the question of the persistence of intramolecular hydrogen-bonding in the conformers, earlier studies of BR-IX conformation in DMSO by NMR[4] concluded that the pigment adopts folded conformations, akin to those of Figs. 1 and 3 but with the propionic acid CO_2H groups linked to their nearest lactam and pyrrole groups via bound solvent molecules. Just how the presence of a remote chiral group so effectively determines the predominant pigment conformer (**P'** or **M'**) is unclear. It may be noted that the *R,S* and *S,S* Markovnikov diastereomers exhibit oppositely signed CD curves; so, apparently the configuration at the newly formed chiral center at C-18[1] plays an important conformer-selecting role, possibly by directing the polar amino acid groups into participation in intramolecular hydrogen-bonding, possibly by orienting dipole-dipole interactions of the type proposed for amides of 2,3-dihydro-bilatrienes-*abc*.[21] Futher investigation of the stereochemistry of these and other *exo*-vinyl adducts, e.g., glutathione, by CD and NMR spectroscopy and molecular mechanics methods, is underway.

Interestingly, the N-acetylcysteine adducts of this paper were proposed by Manitto *et al.*[22] as possible intermediates in the mechanism of phototherapy for neonatal jaundice. Although there is no evidence for their formation *in vivo*, we have found in preliminary studies that they are readily excreted in bile unchanged when infused into normal rats and into rats lacking bilirubin glucuronyl transferase (homozygous Gunn rats). Thus, the remote N-acetylcysteine functionality makes the molecule polar enough to be excreted without conjugation. In terms of excretion, therefore, these adducts mimic bilirubin glucuronides[2,3] and may be useful models for them.

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References and Notes

1. (a) University of Nevada; (b) University of California.
2. D.A. Lightner and A.F. McDonagh, *Accounts Chem. Res.* **17**, 417-424 (1984).
3. A.F. McDonagh and D.A. Lightner, *Pediatrics* **75**, 443-455 (1985).
4. (a) D. Kaplan and G. Navon, *Isr. J. Chem.* **23**, 177-186 (1983).
   (b) G. Navon, S. Frank and D. Kaplan, *J.C.S. Perkin Trans II*, 1145-1149 (1985).
5. H. Falk and N. Müller, *Tetrahedron* **39**, 1875-1895 (1983).
6. P. Manitto and D. Monti, *J.C.S. Chem. Commun.*, 122-123 (1976).
7. For leading references see G. Blauer, *Isr. J. Chem.* **23**, 201-209 (1983).
8. D.A. Lightner, J.K. Gawronski and W.M.D. Wijekoon, *J. Am. Chem. Soc.* **109**, 6354-6362 (1987).
9. D.A. Lightner, J.K. Gawronski and K. Gawronska, *J. Am. Chem. Soc.* **107**, 2456-2461 (1985).
10. D. Harmatz and G. Blauer, *Arch. Biochem. Biophys.*, **170**, 375-386 (1975).
11. D.A. Lightner, M-H. Zhang and F.R. Trull, *Tetrahedron Lett.* **28**, 4033-4036 (1987).
12. (a) P. Manitto and D. Monti, *Experientia* **28**, 379-380 (1972).
    (b) P. Manitto and D. Monti, *Experientia* **29**, 137-139 (1973).
13. A.F. McDonagh, L.A. Palma, F.R. Trull and D.A. Lightner, *J. Am. Chem. Soc.* **104**, 6860-6867.
14. Partial 300 MHz ^{1}H-NMR spectra of the N-acetyl-L-cysteine adducts in d_6-DMSO, all three have *endo*-vinyl, -CH_X = CH_aH_b 6.80 (H_X, dd, J = 17, 11 Hz), 5.59 (H_a, d, J_{aX} = 11 Hz), 5.55 (H_b, d, J_{bX} = 17 Hz); R_f 0.58: 6.05 (1H, s, C(5)*H*), 6.18 (1H, s, C(15)*H*), 1.80 (3H, s, NHCOCH_3), 1. (2x2H, 2xt, J = 7 Hz, -CH_2-CH_2-S-); R_f 0.41: 6.02 (1H, s, C(5)*H*), 6.20 (1H, s, C(15)*H*), 1.80 (3H, s, NHCOCH_3), 1.40 (3H, d, J = 7 Hz, CH_3-CH-S-); R_f 0.35: 6.08 (aH, s, C(5)*H*), 6.20 (1H, s, C(15)*H*), 1.80 (3H, s, NHCOCH_3), 1.41 (3H, d, J = 7 Hz, CH_3-CH-S-) ppm.
15. P. Salvadori, O. Piccolo, C. Bertucci, R. Menicagli and L. Lardicci, *J. Am. Chem. Soc.*, **102**, 6860-6861 (1980).
16. D.A. Lightner, M. Reisinger and W.M.D. Wijekoon, *J. Org. Chem.* **52**, 5391-5395 (1987).
17. (a) For leading references see: H. Falk, K. Grubmayr, G. Höllbacher, O. Hofer, A. Leodolter, F. Neufingerl and J.M. Ribó, *Monatsh. Chem.* **108**, 1113-1130 (1977).
    (b) H. Falk, K. Grubmayr, U. Herzig and O. Hofer, *Tetrahedron Lett.*, 559-562 (1975).
18. For leading references see: C.W. Deutsche, D.A. Lightner, R.W. Woody and A. Moscowitz, *Annu. Rev. Phys. Chem.* **20**, 407-448 (1969).
19. N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy -- Exciton Coupling in Organic Stereochemistry*, University Science Books, Mill Valley, CA (1983).
20. (a) K.C. Tewari, F.K. Schweighardt, J. Lee and N.C. Li, *J. Magn. Reson.* **5**, 238-247 (1971).
    (b) J.N. Spencer, J.E. Gleim, M.L. Hackman, C.H. Blevins and R.C. Garrett, Jr., *J. Phys. Chem.* **82**, 563-571 (1978).
21. K. Grubmayr and M. Widhalm, *Monatsh. Chem.* **118**, 837-843 (1987).
22. (a) P. Manitto, D. Monti and E. Garbagnati, *Il Farmaco* **XXVII**, 999-1002 (1972).
    (b) E. Garbagnati and P. Manitto, *J. Pediatrics* **83**, 109-115 (1973).

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