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CONFORMATION OF BILIRUBIN ESTERS FROM CIRCULAR DICHROISM SPECTROSCOPY

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Summary: Optically active esters prepared from the methane-sulfonate of S-(+)-2-butanol and bichromophoric bilirubin-IXα show bisignate circular dichroism curves, with the mono-ester Cotton effects being up to 10-fold more intense than the diester -- data in accord with the presence of folded, partially intramolecularly hydrogen-bonded conformations for mono-esters.

(4Z,15Z)-Bilirubin-IXα (BR-IX), the yellow-orange, hydrophobic and neurotoxic pigment of jaundice, is a dicarboxylic acid produced in abundant quantities by heme catabolism in mammals and transported as a non-covalent association complex with serum albumin to the liver for glucuronidation and subsequent excretion.[1] The structure of the pigment has long been of interest owing to its importance in Nature and its peculiar solubility properties. The latter have been reconciled in terms of its 3-dimensional structure, which shows a strong preference for the folded, intramolecularly hydrogen-bonded conformation (Fig. 1).[2,3] Two enantiomeric conformations are possible, which interconvert with an activation barrier of ~18 kcal/mole.[4,5] Conformational enantiomerism has also been recognized for solutions of BR-IX with chiral solvating agents (CSA) such as amines[6] and albumins,[7] in whose presence the pigment may show extremely large bisignate circular dichroism (CD) Cotton effects (CEs). The factors that govern the non-covalent heteroassociation of the pigment to the CSA remain unclear, but the mechanism of the induced optical activity can be ascribed to unequal concentrations of the (diastereomeric) complexes formed between the CSA and BR-IX enantiomeric conformers A and B (Fig. 1).[7] Thus, solutions of BR-IX, which contain racemic mixtures of pigment enantiomers in isotropic solvents, become optically active through a first order asymmetric transformation.[8]

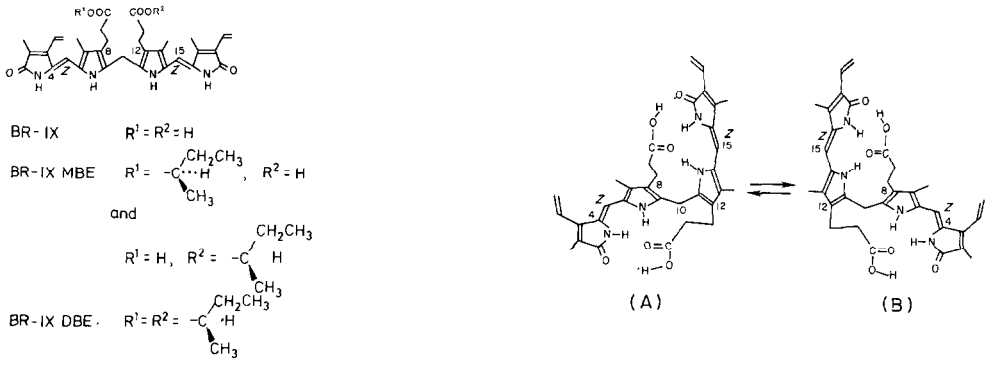


FIGURE 1. (Left) Linear representations of (4Z,15Z)-bilirubin-IXα (BR-IX) and its mono-esters (BR-IX MBE) and di-ester (BR-IX DBE) with (R)-(-)-2-butanol. (Right) Interconverting enantiomeric, intramolecularly hydrogen-bonded conformers of BR-IX.

Surprisingly, there have been few reports of optically active bilirubin derivatives,[9] and no reports of their CD spectra, with the exception of that of an incompletely characterized ester or amide derivative with human serum albumin.[9b,c] Non-optically active esters of BR-IX, however, have been shown to exhibit weak bisignate induced CD spectra in selected optically active solvents, e.g. $\Delta\epsilon^{450}_{max}$ = +0.9, $\Delta\epsilon^{400}_{max}$ = -0.4 for 10μM BR-IX dimethyl ester in R-(-)-butan-2-ol at 298°K.[10] In this report, optically active mono and di-esters (Fig. 1) of BR-IX were synthesized from the methanesulfonate of commercially available (Aldrich) S-(+)-2-butanol using the S_N2 reaction outlined earlier[11] and the bis-tetra-n-butylammonium salt of BR-IX.[12] The mono-ester, which in our hands was an unseparated mixture from alkylation of the C-8 or C-12 propionic acid groups, is optically active by virtue of remote attachment of a chiral group; yet, the pigment exhibits a surprisingly intense CD associated with its long wavelength UV-vis transition near 440 nm (Fig. 2). In contrast, remote chiral perturbation of aromatic chromophores, e.g. (R)-2,2-dimethyl-3-(α-naphthyl)butane: ϵ^{282}_{max} 7,500, $\Delta\epsilon^{280}_{max} \simeq -1$,[13] generally yields only weak CD CEs for their $\pi\rightarrow\pi^*$ excitations.[14] The CD CEs of the BR-IX diester are ~10-fold weaker than those of the mono-ester; thus, derivatization of both pyrromethenone chromophores of the bichromophoric pigment with identical chiral groups does not simply double the CE magnitude. This non-additivity, the unexpectedly large CEs and their bisignate character point to an entirely different mechanism of optical activity from that of chiral perturbation of an inherently symmetric chromophore.[14]

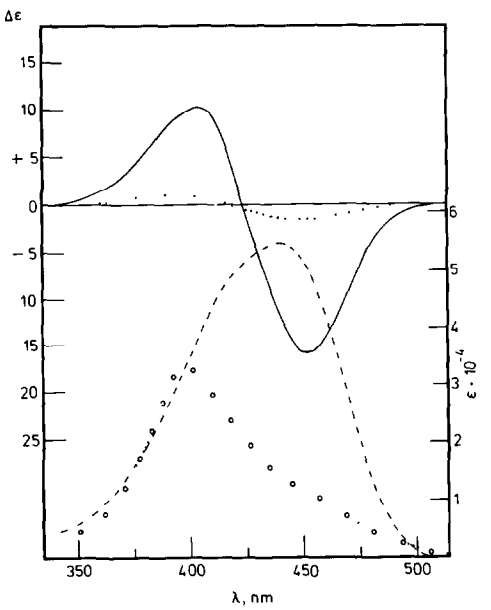
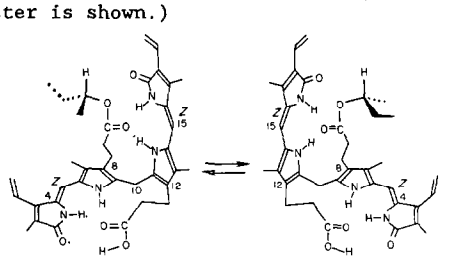


FIGURE 2. (Left) CD spectra of 4 x 10^{-5} M solutions of BR-IX MBE (———) and BR-IX DBE (. . . . .) in CH_2Cl_2 at 20°C. UV spectra of 4 x 10^{-5} M solutions of BR-IX MBE (- - - - -) and BR-IX DBE (o....o) in CH_2Cl_2. Data were run on a JASCO J-600 spectropolarimeter and a Cary 219 UV-vis spectrophotometer.

FIGURE 3. (Below) Interconverting diastereomers of intramolecularly hydrogen-bonded BR-IX MBE. (Only the C-8 ester is shown.)



Dilute solutions of BR-IX in CH_2Cl_2 consist largely of equimolar concentrations of interconverting conformational enantiomers (**A** and **B**, Fig. 1)[3-7] and understandably exhibit no optical activity. NMR evidence suggests that BR-IX monomethyl esters tend to adopt similar conformations,[15] with one half of the pigment intramolecularly H-bonded to the unesterified propionic acid carboxyl group thereby maintaining a folded conformation in which the remaining pyrromethenone moiety is potentially weakly H-bonded to the propionic ester carbomethoxy group. The mono-esters of chiral alcohols, e.g. BR-IX MBE, may thus be seen as a pair of interconverting diastereomers (**A'** and **B'**, Fig. 3) that have an enantiomeric disposition of the

two pyrromethenone chromophores. Since diastereomers typically have $\Delta G_f^\circ(A') \neq \Delta G_f^\circ(B')$, the concentration of **A'** will not be equal to that of **B'**, and solutions of BR-IX MBE will exhibit an optical activity characteristic of the predominant diastereomer.

The situation with the mono-esters, where the chiral perturber is covalently attached to the pigment, may be equivalent to that of heteroassociation complexes of BR-IX with chiral agents such as serum albumin[7] and amines,[6] where the CSA is non-covalently attached. The pigment may be viewed as a mo lecular exciton[16] containing two pyrromethenone chromophores held in a chiral orientation. Electrostatic interaction of the pyrromethenone electric transition dipole moments[17] leads to an exciton (Davydov) 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. According to theory, the two exciton transitions always have oppositely-signed CD CEs, as is observed for BR-IX MBE (Fig. 2).

When the residual intramolecular H-bonding is relaxed or broken, the well-defined chiral 3-dimensional structures of Fig. 3 no longer predominate, and the pigment may assume a multiplicity of interconverting (enantiomeric) conformations of essentially the same energy.[18] In these cases, the CD CEs are expected to be weak due to cancellation or to an unfavorable alignment[16] of the pyrromethenone electric transition moments. This situation obtains for BR-IX MBE in selected solvents, e.g. DMSO, and in general for the corresponding diester, BR-IX DBE (Table 1). Our understanding of the CD of the latter is complicated by a monomer dimer equilibrium ($K_A \simeq 1500$ in CH_2Cl_2);[10] however, the BR-IX DBE CD CEs are considerably weaker than those of the mono-esters. And over a 1000-fold concentration range in CH_2Cl_2, the diester CD CEs show a much greater concentration dependence than the CD CEs of the mono-ester -- in keeping with the view that the mono-ester CD is derived from an intrinsic conformation (A',B') and that of the diester from a multiplicity of conformations of monomeric and dimeric species.

Table 1. Circular Dichroism (CD) and Ultraviolet-Visible (UV) Absorption Data for 2-5 x 10^{-5} M Solutions of the Bilirubin-IXα Mono- and Di-esters formed from S-(+)-2-Butanol[a]

Ester	Solvent	CD $\Delta\epsilon_{max}(\lambda_1)$	CD λ_2 at $\Delta\epsilon=0$	CD $\Delta\epsilon_{max}(\lambda_3)$	UV $\epsilon_{max}(\lambda)$
Mono	CH_2Cl_2	+10.2 (405)	424	-15.7 (450)	54,300 (438)
Di		+ 1.3 (400)	421	- 1.7 (450)	33,700 (398)
Mono	Me_2CO[b]	+ 8.2 (405)	423	-12.2 (450)	60,000 (438)
Di		+ 0.6 (400)	420	- 0.86 (450)	34,500 (406)
Mono	THF[b]	+ 4.8 (405)	422	- 7.7 (450)	46,700 (417)
Di		+ 0.8 (400)	418	- 1.0 (445)	38,500 (408)
Mono	DMSO[b]	+ 0.7 (420)		<<0.1	56,800 (454)
Di		+ 1.4 (415)		<<0.1	32,400 (453)

[a] Prepared by S_N2 reaction of BR-IX bis-tetra-n-butylammonium salt with the methanesulfonate of S-(+)-2-butanol (ref. 11). Spectra were run at 20° C within 0.5 hrs of solution preparation using the corresponding esters of racemic 2-butanol as the ($\Delta\epsilon=0$) baseline.
[b] Me_2CO = acetone; THF = tetrahydrofuran; DMSO = dimethylsulfoxide.

The absolute configuration of the predominant mono-ester diastereomer can be predicted (to be **A'**) from exciton coupling theory and a knowledge of the direction of the UV-vis long wavelength electric dipole transition moment.[6] The intensity of the CD CEs can also be calculated approximately (calculated CD curve: $\Delta\epsilon^{455}_{max} \simeq -260$, $\Delta\epsilon^{395}_{max} \simeq +190$)[6] for **A** (≃**A'**) using exciton theory. From these data, one can estimate that **A'** predominates only slightly (~5%) over **B'**.

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