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SULFOXIDES AS CHIRAL COMPLEXATION AGENTS. CONFORMATIONAL ENANTIOMER RESOLUTION AND INDUCED CIRCULAR DICHROISM OF BILIRUBINS.

J. K. GAWROŃSKI, T. POŁOŃSKI and D. A. LIGHTNER*

Department of Chemistry, University of Nevada

Reno, Nevada 89557-0020 USA

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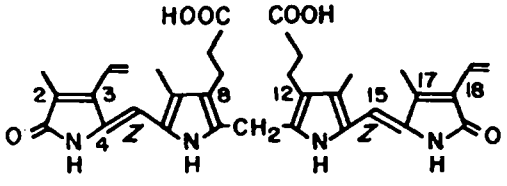
Abstract. Bilirubin-IXα, the end product of porphyrin metabolism in mammals and the neurotoxic yellow-orange pigment of jaundice, exhibits a strong tendency to fold like a book into either of two interconverting enantiomeric conformations, which are further stabilized by intramolecular hydrogen bonding. Bilirubin exhibits optical activity in *R*-(-)-ethylmethylsulfoxide solvent, as seen by moderately strong bisignate circular dichroism Cotton effects ($\Delta\epsilon_{452}^{max}$ = +10.9, $\Delta\epsilon_{404}^{max}$ = -4.5), and in dichloromethane solution in the presence of 2*M* *R*-(+)-methyl-*p*-tolylsulfoxide its circular dichroism spectrum ($\Delta\epsilon_{463}^{max}$ = +11.2, $\Delta\epsilon_{412}^{max}$ = -7.1) is comparably strong. As observed earlier for chiral recognition of bilirubin by optically active amines and serum albumins, the optically active sulfoxide acts as a chiral complexation agent to induce an asymmetric transformation of bilirubin, whose bisignate circular dichroism spectra are characteristic of an exciton splitting arising from interaction of the two component dipyrrinone chromophores.

INTRODUCTION

Normal human metabolism produces and eliminates the cytotoxic yellow-orange pigment of jaundice, (4*Z*,15*Z*)-bilirubin-IXα (bilirubin), at the rate of ~300 mg/individual/day — representing the breakdown of approximately 10^{11} red blood cells per day.[1-3] Important to metabolism is the binding of bilirubin to a variety of proteins involved in its transport from blood to bile, e.g., serum albumin, membrane and cytosolic proteins implicated in hepatic transport or placental uptake, and bilirubin-glucuronyl transferase, which esterifies the bilirubin propionic acid groups to glucuronides.[1-4] Yet despite the many studies involving bilirubin, few have dealt specifically with its structure; so, little is known of its conformation in solution or when bound to proteins.

BILIRUBIN:
(Linear Representation)



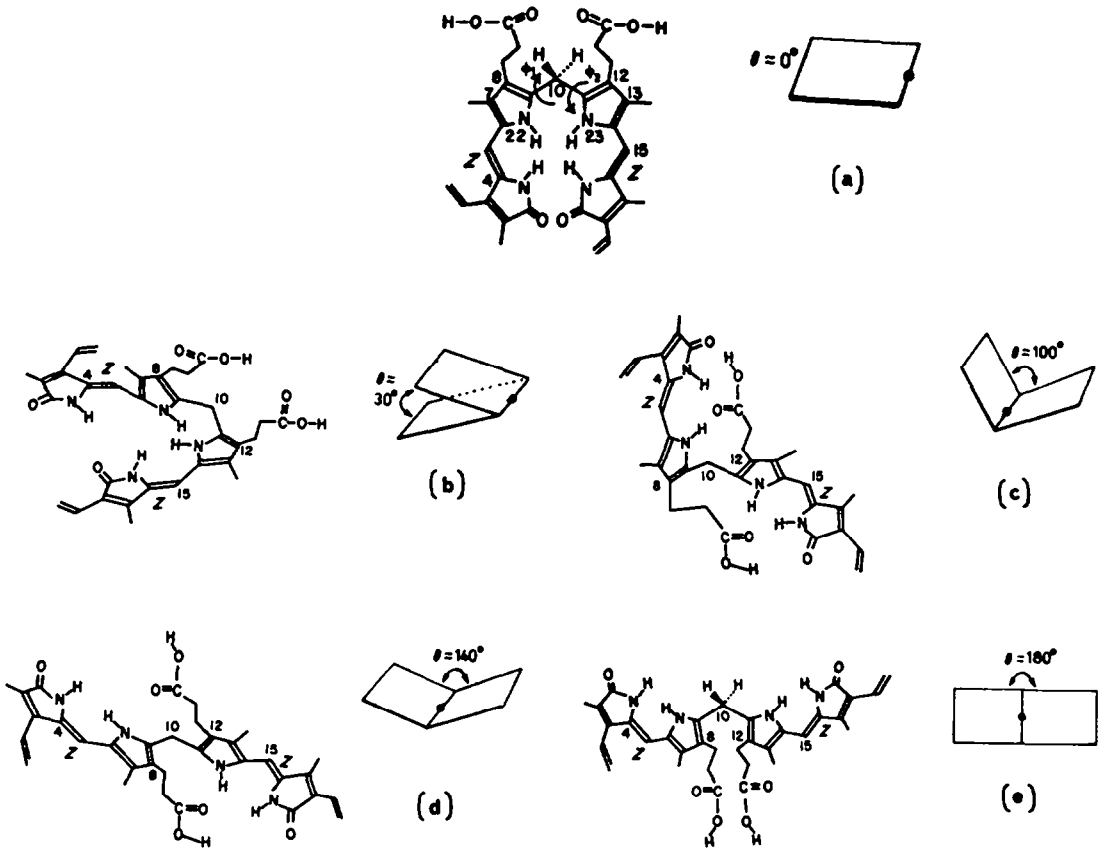


FIGURE 1. Conformational drawings of a few representative three-dimensional structures of (4*Z*,15*Z*)-bilirubin-IXα. The indicated bilirubin conformations are produced by rotations about the C_9-C_{10} and C_{10}-C_{11} carbon-carbon single bonds (rotation angles corresponding to ϕ_1 and ϕ_2, the torsion angles formed by N_{22}-C_9-C_{10}-C_{11} and C_9-C_{10}-C_{11}-N_{23}, respectively). In going from (a) to (e) the change in shape is similar to the opening of a book. The dihedral angle (θ) of intersection of the dipyrrinone planes is represented at the right of each structure, with the line of intersection passing through C_{10}, where C_{10} is represented by •. (a) The porphyrin-like $\theta \simeq 0°$ conformation has both dipyrrinones lying essentially in the same plane and torsion angles $\phi_1 \simeq \phi_2 \simeq 0°$. It suffers considerable steric hindrance associated, *inter alia*, with overlapping lactam centers. (b) The $\theta \simeq 30°$ conformation is skewed or helical with $\phi_1 \simeq \phi_2 \simeq 10$-$20°$. It has a lock-washer-like shape and is shown with the plus (P) chirality. (c) Folded conformations with $\theta \simeq 90$-$110°$ have $\phi_1 \simeq \phi_2 \simeq 60$-$70°$. Intramolecular hydrogen bonding (as shown in Fig. 2) can easily be accommodated in these conformations. The "ridge-tile" folded structure of Fig. 2 was found by X-ray crystallography (ref. 6) to have $\theta \simeq 96°$ or 99° and $\phi_1 \simeq \phi_2 \simeq 61°$. (d) The extended conformation, $\theta \simeq 140°$, arises from an elongation of a folded conformation by increasing the ϕ_1 and ϕ_2 torsion angles to ~140°. (e) The linear conformation, $\theta \simeq 180°$, leads to approximate co-planarity of the dipyrrinone rings by rotations of the ϕ_1 and ϕ_2 torsion angles to ~180°. This conformation suffers from severe steric hindrance between the C_8 and C_{12} propionic acid groups.

The constitutional structure of bilirubin elucidated by Fischer *et al.* some fifty years ago,[5] shows the pigment to consist of two dipyrrinone halves conjoined by a $-CH_2-$ group. The two halves are almost identical and, though covalently linked, can react more or less independently. Aside from a few minor details, such as Fischer's preference for the lactim tautomer (hydroxypyrrole) and an unspecified stereochemistry at C_4 and C_{15}, the *primary* structure remains unchanged today. We now know, through a combination of X-ray crystallographic[6] and solution spectroscopic studies,[7] that the lactam predominates and the 4*Z*,15*Z*-configuration is most stable.[3] This structure is consistent with many of the properties of bilirubin, for example, its yellow color and solubility in dilute aqueous alkali. But it is apparently inconsistent with many other properties of the molecule, such as its high oil-water partition coefficient, its resistance to hepatobiliary excretion and — unusual for a dicarboxylic acid — its insolubility in aqueous $NaHCO_3$ solution.[3] The reason for the apparent inconsistency is that the Fischer structure represents only the constitutional structure of bilirubin, not the conformational structure. Yet, except in a few special circumstances,[6] the conformation of bilirubin remains largely unknown.

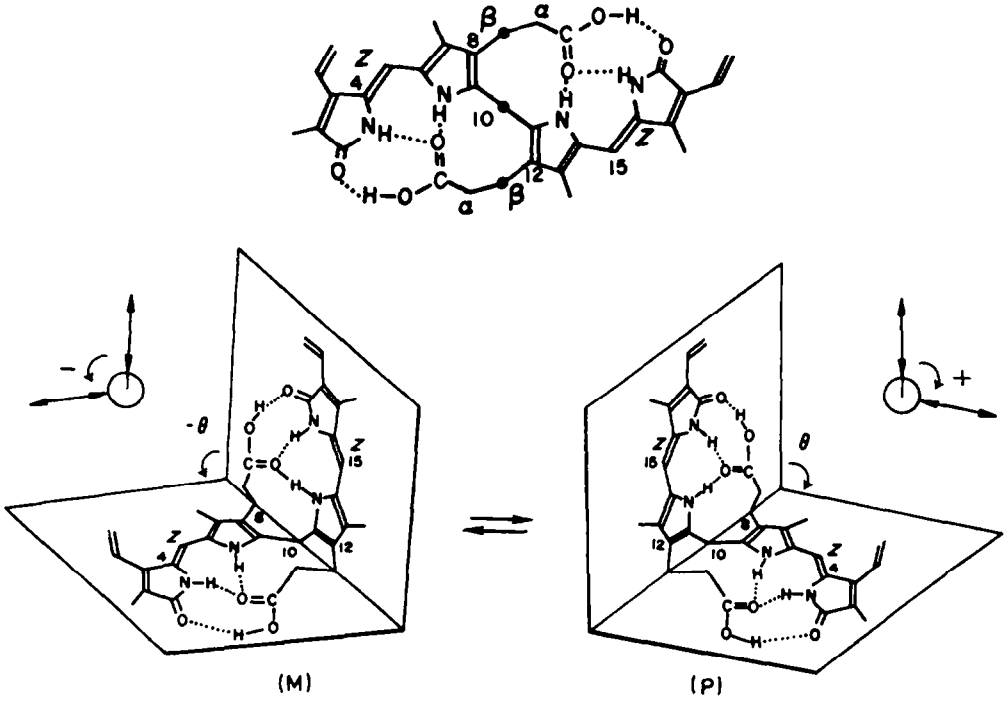


FIGURE 2. (Upper) Planar representation of the intramolecularly hydrogen-bonded structure of bilirubin. Folding along the line passing through the two β-carbons and C_{10} produces mirror image 3-D conformations. (Lower) Interconverting enantiomeric conformations of folded, intramolecularly hydrogen-bonded bilirubin. The interconversion rate (3-95 sec^{-1} at 50-95°C) and activation barrier (~18 kcal/mole) (refs. 10, 11) have been estimated by NMR methods. The chirality of (the electric transition dipole moments of) the bichromophoric enantiomers is shown above.

Because the two dipyrrinones of bilirubin are linked by a tetrahedral $-CH_2-$ group, the pigment must have a bent or folded shape. One such bent shape is shown in the porphyrin-like structure (a) of Fig. 1. However, like the blades of a molecular propeller, e.g., diphenylmethane,[8] the two dipyrrinone chromophores can move rapidly and independently with respect to each other by rotation about the C_9-C_{10} and C_{10}-C_{11} single bonds connecting them to the central $-CH_2-$ (C_{10}). In principle, therefore, many interconvertible folded structures are possible (as illustrated in Fig. 1), with different juxtapositions of the dipyrrinone halves.[9] And, except for the two conformations in which the dipyrrinones are coplanar, each folded structure has a non-superimposable mirror image. Significantly, for a narrow range of folded conformations which allow for close approach of the polar pyrrole NH, amide -NH-C=O and -COOH groups, formation of multiple intramolecular hydrogen bonds becomes possible (Fig. 2).

When bilirubin or simple salts of the carboxylate dianion are crystallized, the crystal lattice contains equal numbers of enantiomers shaped like those of Fig. 2.[6] Other structures or conformations are absent. In the crystalline state bilirubin molecules can stretch and vibrate but are otherwise restrained. However, when dissolved in organic solvents, bilirubin is potentially more flexible. It has more conformational freedom and can form hydrogen bonds with solvent molecules, with other bilirubin molecules, or intramolecularly within itself. Despite these many conformational possibilities, spectroscopic studies[10-12] indicate that structures similar to those of Fig. 2 can be maintained in non-polar solvents such as chloroform, dichloromethane and benzene; in polar solvents such as acetonitrile, acetone and ethanol; and even in basified water, where the propionic acid groups are deprotonated. In a strongly hydrogen bonding solvent such as dimethylsulfoxide, it is thought that the intramolecular hydrogen bonds are broken through solvation,[11,13] but the resulting conformation is unknown. Resonance Raman studies point to a conformation different from that adopted in chloroform solvent,[13] possibly an extended conformation,[14] but an evaluation of the segmental motion in the propionic acid chains by NMR T_1 measurements[11] indicates a much more restricted motion than that of a dipyrrinone half molecule, e.g., xanthobilirubic acid (xanthobilirubic acid methyl ester, XBRME, Fig. 3). At present, the solution structure of bilirubin is incompletely resolved and controversial, especially in dimethylsulfoxide, the solvent in which the pigment has its greatest solubility. In the following we show that bilirubin forms chiral association complexes with sulfoxides. The work is important because it demonstrates that the ability to form intramolecular hydrogen bonds remains an essential component of the stereochemistry. And it is the first example where an optically active sulfoxide induces a first-order asymmetric transformation.[15]

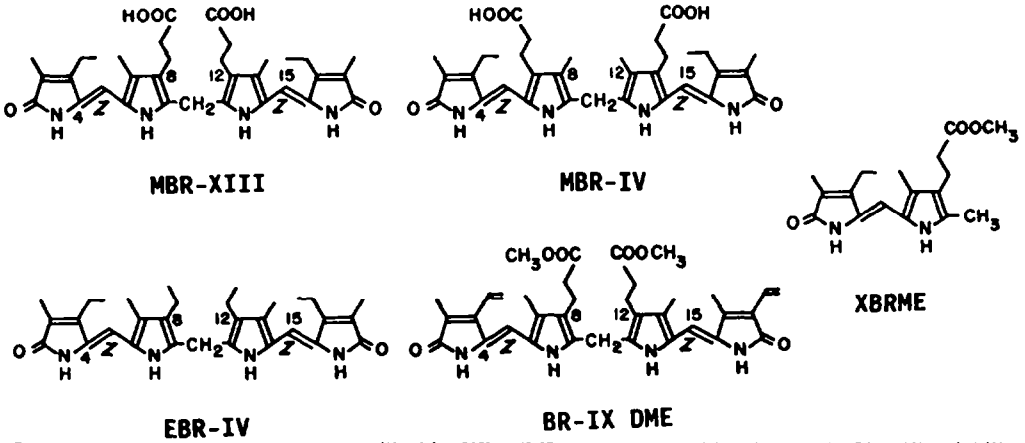


FIGURE 3. Linear representations of mesobilirubin-XIIIα (MBR-XIII) mesobilirubin-IVα (MBR-IV), etiobilirubin-IVγ (EBR-IV) bilirubin-IXα dimethylester (BR-IX DME) and xanthobilirubic acid methyl ester (XBRME).

EXPERIMENTAL

Bilirubin-IXα (Sigma) contained less than 5% of the symmetric IIIα and XIIIα isomers, as determined by high performance liquid chromatography.[16] Bilirubin-IXα dimethyl ester was prepared by reaction with diazomethane, as described previously.[17] Mesobilirubin-XIIIα (MBR-XIII), mesobilirubin-IVα (MBR-IV), etiobilirubin-IVγ (EBR-IV) and xanthobilirubic acid methyl ester (XBRME) were prepared by total synthesis.[18] *R*-(+)-Methyl-*p*-tolylsulfoxide ($[\alpha]_D^{22}$ +143.5° (acetone), ≥98% e.e.) was prepared by reaction of (-)-(1*R*)-menthyl (*S*)-toluene-4-sulfinate (Aldrich, $[\alpha]_D^{20}$ -195° (acetone)) with methyl magnesium bromide, as described earlier.[19] *R*-(-)-Ethylmethylsulfoxide ($[\alpha]_D^{25}$ -15.6 (acetone), 11% e.e.) of known absolute configuration[20] was prepared by asymmetric oxidation of ethylmethylsulfide according to Kagan *et al.*[21] The enantiomeric excess (e.e.) was determined by observing and integrating the methyl signal by ^{1}H-NMR following addition of the 3,5-dinitrobenzamide of 100% e.e. *R*-(+)-α-methylbenzylamine.[22] *R*-(-)-Benzylmethylsulfoxide ($[\alpha]_D^{22}$ -96.6° (ethanol), 91% e.e.) of known absolute configuration[23] was isolated following asymmetric oxidation of benzylmethylsulfide,[21] which gave predominantly the *R*-enantiomer (38% e.e.), then fractional crystallization of the more crystalline racemic sulfoxide to leave 91% e.e. *R*-enantiomer in the mother liquor. *R*-(-) and *S*-(+)-n-butylmethylsulfoxides ($[\alpha]_D^{25}$ -97.3° (acetone) 82 ± 2% e.e. and $[\alpha]_D^{26}$ +68.5 (acetone), 58 ± 2% e.e.) were obtained by resolution[24] of the racemic mixture (prepared by oxidation of n-butylmethylsulfide with one equivalent of 30% hydrogen peroxide in methanol at 0°C[25]) with *S*-1,1′-binaphthalene-2,2′-diol.[26] The enantiomeric excess was determined as above for ethylmethylsulfoxide. The organic solvents used were spectral grade (Fisher or Aldrich). Solutions were prepared by dissolving the pigment in a solution (3*M*, 2*M*, 0.3 *M*, 0.03*M*) of sulfoxide in the appropriate solvent; generally solutions were 2*M* in sulfoxide. All circular dichroism spectra were recorded on a JASCO J-600 spectropolarimeter, and all UV-visible spectra were run on a Cary 219 spectrophotometer.

RESULTS AND DISCUSSION

Induced Circular Dichroism (ICD). The ICD spectra of bilirubin in *R*-(-)-ethylmethylsulfoxide solvent and in dichloromethane containing 2*M* *R*-(+)-methyl-*p*-tolylsulfoxide are shown in Fig. 4. Although the ICD spectra are now known to be typical of bilirubin in the presence of certain chiral salt forming complexation agents, e.g., optically active amines[12a-c] and proteins, such as human serum albumin,[12d] ICD spectra of bilirubin have never been detected heretofore in the presence of sulfoxides, which are thought to break down the pigment's ridge-tile conformation-stabilizing intramolecular hydrogen bonding matrix (Fig. 2). Typical of bilirubin CDs induced by chiral complexing agents,[12] the ICD is concentration dependent (Table 1). At lower than a 2*M* concentration of *R*-(+)-methyl-*p*-tolylsulfoxide the ICD magnitude decreases, but above 2*M* sulfoxide concentration it does not increase. Interestingly, the ICD magnitudes are quite comparable to those seen with *R*-(-)-2-aminobutane at the same pigment:chiral complexation agent ratio.[12a,b,27] The amine, however, is known to bind strongly to bilirubin through salt formation with the propionic acid groups, but the complex still retains a conformation-stabilizing intramolecular hydrogen-bonding network.[6c] Other optically active sulfoxides are less effective than methyl-p-tolylsulfoxide (Table 2) for inducing circular dichroism in the pigment in dichloromethane solvent at a 7000:1 sulfoxide:bilirubin molar ratio. At larger ratios, the ICD intensities approach those seen for 2*M* methyl-*p*-tolylsulfoxide and neat ethylmethylsulfoxide.

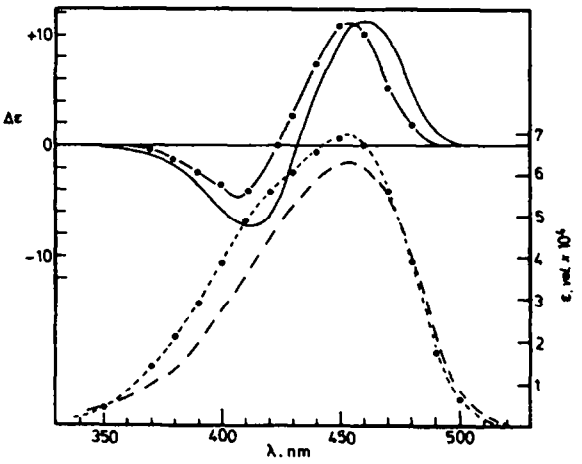


FIGURE 4. Circular dichroism (——) and UV-visible (— — —) spectra of $3x10^{-4}$ *M* bilirubin in dichloromethane containing 2 *M* *R*-(+)-methyl-*p*-tolylsulfoxide. Circular dichroism (o — o — o) and UV-visible (o - - o - - o) spectra of $3x10^{-4}$ *M* bilirubin in *R*-(-)-ethylmethylsulfoxide. The CD data were run at 21°C and are corrected to 100% e.e. of sulfoxide, and the $\Delta\epsilon=0$ line is from the CD spectra in the presence of racemic sulfoxide.

TABLE 1. Circular Dichroism and Ultraviolet-Visible Spectral Data for Solutions of Bilirubin-IXα and with Various Concentrations of *R*-(+)-Methyl-*p*-tolylsulfoxide in Dichloromethane at 20°C.

Sulfoxide Conc.(*M*)	Bilirubin Conc.(*M*)	[Sulfoxide]: [Bilirubin]	CD			UV	
			$\Delta\epsilon^{max}$ (λ_1)	λ_2 at $\Delta\epsilon=0$	$\Delta\epsilon^{max}$ (λ_3)	ϵ^{max}	λ(nm)
3.0	$2.86 x10^{-4}$	~10,000:1	+ 11.2 (465)	433	- 7.0 (412)	66,700	455
2.0	$2.87 x10^{-4}$	~ 7,000:1	+ 11.2 (463)	433	- 7.1 (412)	66,500	452
0.3	$2.91 x10^{-4}$	~ 1,000:1	+ 1.9 (462)	433	- 1.0 (413)	67,000	452
0.03	$3.07 x10^{-4}$	~ 100:1	« 0.1		« 0.1	66,400	453

TABLE 2. Comparison of the Circular Dichroism and Ultraviolet-Visible Spectral Data for Solutions of Bilirubin-IXα and Various *R*-Methylsulfoxides at 20°C.

O ↑ CH_3S-R R =	[Sulfoxide]: [BR-IX]	Solvent	CD			UV	
			$\Delta\epsilon^{max}$ (λ_1)	λ_2 at $\Delta\epsilon=0$	$\Delta\epsilon^{max}$ (λ_3)	ϵ^{max}	λ(nm)
p-Tolyl	~7,000:1	CH_2Cl_2	+ 11.2 (463)	433	- 7.1 (412)	66,500	452
Benzyl	~7,000:1	CH_2Cl_2	+ 1.0 (459)	430	- 0.5 (416)	59,900	454
n-Butyl	~7,000:1	CH_2Cl_2	+ 1.0 (457)	429	- 0.6 (408)	60,700	453
Ethyl	~7,000:1	CH_2Cl_2	« 0.1		« 0.1	62,300	452
	38,500:1	neat	+ 10.9 (452)	424	- 4.5 (404)	69,600	453

R-(+)-methyl-*p*-tolylsulfoxide is most effective as a chiral complexation agent for bilirubin in non-polar solvents, a behavior similar to that seen with optically active amines and bilirubin.[12a-c,27] However, even in hydrogen-bonding solvents such as methanol and dimethylsulfoxide, interestingly, bilirubin exhibits an ICD, albeit of the inverted sign sequence in the latter solvent (Table 3). The persistent ICD with *R*-(+)-methyl-*p*-tolylsulfoxide contrasts markedly with the behavior *S*-(+)-2-aminobutane in the same solvents,[27] where the ICD is vanishingly small in dimethylsulfoxide and only very weak in methanol. Thus, methyl-*p*-tolylsulfoxide apparently exerts a surprisingly persistent tendency to complex with bilirubin, even in solvents where the (salt) complex with 2-aminobutane no longer exhibits diastereoselection. A similar behavior may be noted for mesobilirubin-XIIIα (MBR-XIII, Table 2), which,

like bilirubin, has propionic acid groups at C-8 and C-12. In marked contrast, when the propionic acids are replaced by ethyl (EBR-IV, Table 3), the ICD Cotton effect signs become inverted, but with little reduction in magnitude. Thus, the presence of propionic acid groups at C_8 and C_{12} can be seen to exert a unique effect on the ICD. This effect is very probably associated with the pigment's ability to adopt a folded and intramolecularly hydrogen-bonded conformation because EBR-IV (with no CO_2H groups) cannot form the hydrogen-bonding matrix of Fig. 2 — irrespective of solvent. This interpretation is supported by the observation that MBR-IV, which has propionic acid groups located at C_7 and C_{13} (positions from which intramolecular hydrogen bonding (Fig. 2) is impossible), gives ICD data rather similar to those of EBR-IV, but not to those of bilirubin or MBR-XIII. Apparently it is both the presence of propionic acid groups and their location which is of fundamental importance in determining the ICD of bilirubin and MBR-XIII. This conclusion is further supported by the observation that (methyl) esterification of bilirubin reverses the ICD signs: the ICDs of bilirubin dimethyl ester have the same signs and nearly the same magnitudes as those of MBR-IV and EBR-IV, but the signs are opposite to those of bilirubin and MBR-XIII (Table 3). The importance of the unique sulfoxide complexation, which we assume involves S→O···H-N hydrogen bonding[7,17] can be contrasted with the type of salt formation complexation important to the chiral amine-induced ICDs with bilirubin (Table 4). The sulfoxide chiral complexation produces ICDs for both bilirubin and its dimethyl ester, but the amines are not generally effective with the ester.

TABLE 3. Circular Dichroism and Ultraviolet-Visible Spectral Data for Solutions of Bilirubin Pigments and *R*-(+)-Methyl *p*-tolylsulfoxide at 20°C. (Pigment:sulfoxide ratio is 1:7,000).

Pigment	Pigment Conc.(*M*)	Solvent	CD $\Delta\epsilon^{max}$ (λ_1)	CD λ_2 at $\Delta\epsilon$=0	CD $\Delta\epsilon^{max}$ (λ_3)	UV ϵ^{max}	UV λ(nm)
Bilirubin	2.87×10^{-4}	CH_2Cl_2	+ 11.2 (463)	433	- 7.1 (413)	66,500	452
	9.70×10^{-5}	CH_3OH	+ 6.8 (463)	433	- 3.5 (416)	58,200	452
	1.40×10^{-4}	CH_3CN	+ 4.8 (464)	430	- 1.8 (416)	60,000	455
	2.87×10^{-4}	HCONHMe	+ 4.0 (459)	430	- 1.6 (414)	62,700	453
	2.91×10^{-4}	$(CH_3)_2SO$	- 0.7 (456)	444	+ 1.7 (398)	68,700	457
MBR-XIII	3.07×10^{-4}	CH_2Cl_2	+ 9.7 (441)	412	- 5.5 (397)	63,100	428
	1.53×10^{-4}	CH_3OH	+ 8.0 (442)	413	- 4.4 (392)	58,100	428
	1.72×10^{-4}	CH_3CN	+ 5.1 (441)	411	- 1.5 (399)	66,900	428
EBR-IV	3.41×10^{-4}	CH_2Cl_2	- 11.5 (427)	398	+ 8.5 (380)	45,200	384
	4.12×10^{-4}	CH_3OH	- 7.2 (432)	402	+ 5.4 (386)	40,000[b]	379
	3.45×10^{-4}	CH_3CN	- 9.4 (423)	395	+ 6.9 (378)	38,000[a]	384
MBR-IV	2.73×10^{-4}	CH_2Cl_2	- 7.0 (430)	402	+ 5.4 (373)	29,300	395
	3.24×10^{-4}	CH_3OH	- 6.1 (430)	404	+ 5.3 (387)	30,200[c]	395
	2.90×10^{-4}	CH_3CN	- 6.9 (424)	398	+ 5.5 (387)	29,700	383
BR-IX DME	2.82×10^{-4}	CH_2Cl_2	- 3.6 (453)	425	+ 5.9 (398)	35,500	423
	2.86×10^{-4}	CH_3OH	- 2.5 (457)	432	+ 5.2 (402)	36,300[d]	418
	3.09×10^{-4}	CH_3CN	- 3.5 (450)	424	+ 5.7 (395)	33,000	410
XBRME	5.83×10^{-4}	CH_2Cl_2	« 0.1		+ 0.62 (394)	27,660	402
	5.54×10^{-4}	CH_3OH	« 0.1		+ 0.35 (392)	37,000	403
	5.00×10^{-4}	CH_3CN	« 0.1		+ 0.40 (400)	31,000	405

[a] 30,000 (416 sh); [b] 40,005 (427); [c] 29,000 (420); [d] 35,000 (440 sh)

Exciton Coupling Origin of Bisignate ICDs and Bilirubin Conformation. The bisignate shape of the ICD spectra, with moderately strong $\Delta\epsilon$ values, is characteristic of the bichromophoric pigments studied here but not of the monochromophoric analog, xanthobilirubic acid methyl ester (XBRME), whose $|\Delta\epsilon|$ values are monosignate and an

order of magnitude weaker. CD spectra with two oppositely-signed CEs straddling high intensity (large ϵ) UV-visible bands are typical of excited state (dipole) interaction, called exciton coupling, between two proximal chromophores with little orbital overlap.[28] In the present case, the component dipyrrinone chromophores of the bichromophoric rubinoid pigments have strongly allowed long wavelength electronic transitions (ϵ_{410}^{max} ~30,000) but only a small interchromophoric orbital overlap in the folded conformation (~100° dihedral angle) of Fig. 1c. The orbital overlap increases and the exciton chirality decreases as the interplanar (dihedral) angle (θ) between the pyrromethenone chromophores tends toward 0° (planar helical) or toward 180° (linear extended). In the former case, the band should tend to sharpen and blue shift as the transition probability to the lower energy exciton state decreases; in the latter case, the long wavelength UV-visible band of the pigment should tend to sharpen and red-shift as the transition probability to the higher energy exciton state decreases.[28,29] Concomitant changes would attend the CD spectra, with the shape changing from bisignate with roughly equal intensities to monosignate, with most of the intensity in one of CD couplets.[30] Thus the bisignate CD curves of Fig. 4, with comparably intense $\Delta\epsilon$ values for each member of the exciton couplet, offer a compelling argument for the folded conformation.

TABLE 4. Comparison of Circular Dichroism and Ultraviolet-Visible Spectral Data for Solutions of Bilirubin-IXα Dimethyl Ester (a) and Bilirubin-IXα (b) and Chiral Complexing Agents at 20°C.

Complexing Agent	[Complexing Agent]: [Pigment]	Solvent	Pigment	CD			UV	
				$\Delta\epsilon^{max}$ (λ_1)	λ_2 at $\Delta\epsilon$=0	$\Delta\epsilon^{max}$ (λ_3)	ϵ^{max}	λ(nm)
R-(+)-Methyl *p*-Tolylsulfoxide	7,000:1	CH_2Cl_2	(a)	- 3.6 (453)	425	+ 5.9 (398)	35,500	423
			(b)	+11.2 (463)	433	- 7.1 (412)	66,500	452
R-(-)-2-Amino butane	10,000:1	CH_2Cl_2	(a)	« 0.1	—	« 0.1	59,000	410
			(b)	+ 7.8 (474)	440	- 6.5 (420)	57,500	455
R-(-)-β-Phenyl-isopropylamine	10,000:1	CH_2Cl_2	(a)	« 0.1	—	« 0.1	54,000	411
			(b)	+14.0 (4.74)	438	-12.0 (418)	57,400	462

In the intramolecular exciton represented by the folded conformation, the dipyrrinones interact through their locally excited states by resonance splitting [electrostatic interaction of the local transition moment dipoles (Fig. 2)]. The dipyrrinone-dipyrrinone intramolecular exciton splitting interaction produces two long wavelength transitions in the ordinary (UV-visible) spectrum and two corresponding bands in the CD spectrum.[12c] One band is higher in energy and one is lower in energy, with the separation dependent on the strength and relative orientation of the dipyrrinone electric dipole transition moments.[31] As seen in their UV-visible spectra, the two electronic transitions overlap to give the characteristically broadened absorption bands of bilirubin pigments. As seen in their CD spectra, however, the two exciton transitions are always oppositely signed, as predicted by theory,[28] and thus give rise to bisignate CEs. In contrast to UV-visible absorption bands, which may show only slight broadening when the exciton splitting energy is small, when two oppositely-signed curves overlap in the CD, there is considerable cancellation in the region between the band centers with the net result that the *observed* bisignate CE maxima are displaced from the actual locations of the (uncombined) CD transitions[12c] and typically are seen to flank the corresponding UV-visible band(s). This is amply illustrated by the induced CD and UV-visible spectra of Fig. 4 where the CEs are characteristic of exciton systems.

Bisignate ICDs might also arise in bichromophoric bilirubins if each dipyrrinone acted independently to produce CEs of opposite signs. The optical activity could thus be attributed to asymmetric perturbation or induced

dissymmetry of the chromophore — through the action of a chiral ligation ligating agent. However, in agreement with Blauer[32] we tend to believe that this mechanism is unimportant for explaining the observed large CEs of bilirubin-protein complexes because: (i) the monochromophore molecular analog xanthobilirubic acid methyl ester shows only a weak monosignate ICD, and (ii) the ICD couplets for the bichromophoric molecules are always of opposite sign, as required by the exciton model (Table 3). If the chromophores were acting independently, one should expect to find monosignate ICDs but never strong bisignate ICDs.

Bilirubin Structure and the Origin of Induced Optical Activity. Bilirubin and its analogs are capable of adopting a large number of chiral conformations, which have been mapped and evaluated by molecular orbital calculations.[7,33] These calculations and space-filled molecular models indicate that Z-configuration rubinoid pigments tend to adopt folded conformations (Fig. 1c) as their low energy structures, even without invoking intramolecular hydrogen bonding. Thus, molecular mechanics calculations[7,33] reveal minimum energy conformations with essentially planar dipyrrinone units oriented by torsion angles N_{22}-C_9-C_{10}-C_{11} and C_9-C_{10}-C_{11}-N_{23} (ϕ_1 and ϕ_2, respectively) with values ranging from 60° to 120°. Intramolecular steric interactions tend to destabilize somewhat a wide spectrum of conformations, including the helical, skewed, extended and linear (Fig. 1). There are only a few shallow local minima on the energy hypersurface, and two deeper, global minima that correspond to the folded conformation of Fig. 1c or its enantiomer.[7,33] Intramolecular hydrogen bonding involving either the C_8/C_{12} propionic acid, or its methyl ester, and an opposing dipyrrinone further lowers the energy of the folded conformations. For bilirubin and MBR-XIII, the folded, intramolecularly hydrogen-bonded ("ridge-tile") conformers (Fig. 2) are calculated to be ~16 kcal/mole more stable than the corresponding folded (Fig. 1c conformers without hydrogen bonding.[33] Surprisingly perhaps, the folded, intramolecularly hydrogen-bonded conformers of the dimethyl ester of BR-IX, where only four hydrogen bonds are possible (between the carbomethoxy carbonyl oxygen and the lactam and pyrrole N-H groups), are calculated to be ~6 kcal/mole more stable than the corresponding folded conformer without bonds. Apparently even residual hydrogen bonding is a conformation-stabilizing force.

The origin of the induced optical activity and ICD may thus be understood simply in terms of non-equimolar concentrations of the diastereomeric complexes (M•A and P•A) formed between the chiral complexing agent (A), in this case a sulfoxide, and the pigment enantiomeric structures (M and P) — as in the unique, hydrogen-bonded conformations of Fig. 2 or any other chiral conformations, such as those P-helicity conformers of Fig. 1 and their enantiomers. The position of the conformational equilibrium (M $\rightleftarrows$ P) for the uncomplexed mirror images M and P is expected to be unperturbed from $K_{eq} = 1$ to first approximation, neglecting anisotropic solvation effects. However, the conformational equilibrium (M•A $\rightleftarrows$ P•A) between the heteroassociation complexes of M and P with a chiral sulfoxide A is expected to have $K_{eq} \neq 1$ because the complexes are diastereomeric, with different ΔG_f°. Thus, the net concentration of M species [(M) + (M•A)] will not be equal to that of the P species [(P) + (P•A)], and the solutions will exhibit optical activity for the pigment. Optically active complexing agents that bind tightly to and are highly selective in forming heteroassociation complexes with one bilirubin enantiomer are expected to generate the most intense optical activity. This has already been noted for optically active amines,[12] where ICD $|\Delta\epsilon|$ values as large as 210 can be found, and for serum albumins, with which bilirubin exhibits intense ICD Cotton effects (CEs) typically in the range $|\Delta\epsilon| \simeq 20\text{-}50$ $l \cdot mole^{-1}\,cm^{-1}$,[30] although values as high as $|\Delta\epsilon| \simeq 250$ have been published.[34] The large $\Delta\epsilon$ are invariably found for bilirubin and its analogs with propionic acid groups at C_8 and C_{12}, e.g., MBR-XIII of Fig. 3, but not with analogs with relocated propionic acid groups (MBR-IV), or no propionic *acid* groups (EBR and dimethyl esters). They typically show no ICD or only weak ICDs with $|\Delta\epsilon|$ less than one order of magnitude. However, even with bilirubin, chiral complexation agents that either do not have a large affinity constant or exhibit little selectivity

for one enantiomer also generate the weaker ICDs.

Hydrogen Bonding in Bilirubin-Sulfoxide Complexes. The presence of intramolecular hydrogen bonding in bilirubin has been confirmed experimentally in the crystal[6] as well as in non-polar solvents such as chloroform,[11] which is known to act as a hydrogen bond donor to amide carbonyls.[35] In such solvents the pigment preferentially adopts the well-defined folded and intramolecularly hydrogen-bonded chiral conformations represented in Fig. 2. In contrast, the conformation is less clear in polar solvents such as dimethylsulfoxide, which is known to participate as a hydrogen bond acceptor, e.g., to pyrrole and lactam N-H groups (Fig. 5).[7,36] Although the ICD data of Fig. 4 and Tables 1-3 provide evidence for the presence of chiral conformations for the various bilirubin pigments, they do not, as such, define their structure(s). Resonance Raman[13] and NMR[11,36] studies have shown that dimethylsulfoxide interferes considerably with the characteristic intramolecular hydrogen bonding in bilirubin. Whether all of the intramolecular hydrogen bonds of the pigment are broken by the sulfoxide or whether the sulfoxide simply inserts into the matrix of hydrogen bonds is controversial. Using NMR, Navon and Kaplan[11] showed that in dimethylsulfoxide solution the segmental motion of the propionic side chains of bilirubin and even its dimethyl ester is very limited, contrasting markedly with the independent fast motion of the propionic side chain of vinylneoxanthobilirubic acid methyl ester, a dipyrrinone analog of XBRME (Fig. 3) and a model for one-half of bilirubin but with its propionic group not involved in hydrogen bonding, except to solvent. The picture provided thereby for bilirubin and even its dimethyl ester is one where chiral pigment conformations, e.g., Fig. 1c still predominate, albeit with the propionic acid or ester residues linked to the nearest lactam amide and pyrrole NH groups via bound solvent molecules. This picture is also supported by resonance Raman studies in dimethylsulfoxide.[13] Together with molecular mechanics calculations,[7,33] the data are consistent with the presence of folded pigment conformations (Fig. 1c) persisting in the presence of sulfoxides. NMR chemical shifts of the lactam and pyrrole N-*H*[11,17] suggest at least partial hydrogen bonding to the sulfoxide; so, a complex between, e.g., EBR-IV and ethylmethylsulfoxide might be represented as shown in Fig. 5. In this complex, there is no opportunity for intramolecular hydrogen bonding, and pigment-to-pigment intermolecular hydrogen bonding[7,36,37] is known to be unimportant in the presence of sulfoxides. Similar complexes might be represented for any of the tetrapyrrole pigments of Table 3, with steric interactions determining the extent of enantioselection between M and P chiral conformations. Although those cannot be specified more exactly at present, it might be assumed that they account for the diastereoselection contributing to the origin of the pigment ICD.

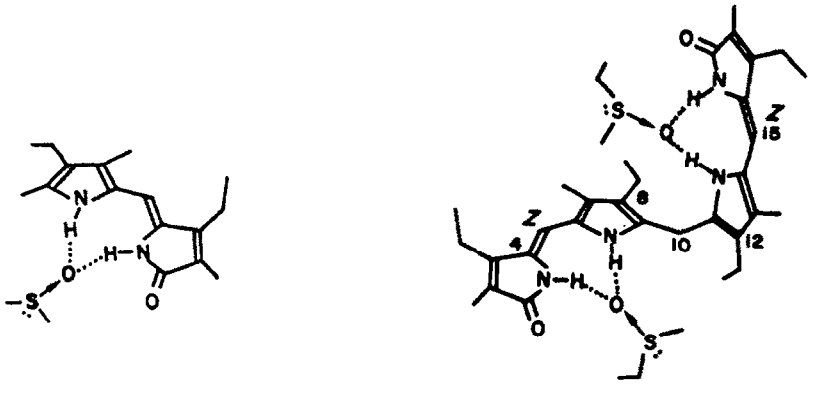


FIGURE 5. (Left) Dipyrrinone hydrogen-bonded to dimethylsulfoxide. (Right) Ethylmethylsulfoxide-bound etiobilirubin-IVγ in an M-helicity folded conformation.

Although the model represented above can, in principle, be expected to obtain for bilirubin and all of the tetrapyrrole pigments of Fig. 3, the data of Table 3 show that the ICD CE signs are the same only for EBR-IV, MBR-IV and BR-IX DME. Both EBR-IV and MBR-IV share a common characteristic: neither can participate in intramolecular hydrogen bonding, and both give very similar ICD spectra. The CE intensities are substantially less with BR-IX DME, however, which is thought to participate weakly in intramolecular hydrogen bonding.[11,33] Interestingly, in the corresponding solvents the ICD CEs are inverted for bilirubin and MBR-XIII, which are the only pigments of the set that can participate strongly in intramolecular hydrogen bonding. These data may thus be taken as *prima facie* evidence for the (at least residual) importance of intramolecular hydrogen bonding in bilirubin and MBR-XIII in the complex with sulfoxide. If complexes with sulfoxide-to-pigment hydrogen bonding of the type important for producing the chiral sulfoxide-induced ICDs of EBR-IV, MBR-IV and BR-IX DME (Fig. 5) are present with bilirubin and MBR-XIII, their ICD contributions are outweighed by those originating from sulfoxide complexes which incorporate some degree of intramolecular hydrogen bonding. The structure of the intramolecularly hydrogen-bonded complex cannot be determined from the ICD data alone. However, because Navon and Kaplan showed by NMR that very limited segmental motion occurs in the propionic acid chain for bilirubin and its dimethylester in dimethylsulfoxide,[11] one is tempted to link (1) the carboxyl hydrogen through intramolecular hydrogen bonding to the bound sulfoxide molecule, or (2) the carbonyl group to the sulfoxide group by dipole-dipole association, as speculated on in Fig. 6.[37,38] For bilirubin as well as MBR-XIII the ICD contributions from new conformations such as those of Fig. 6 would dominate those such as in Fig. 5 and control the CE signs. And in the case of BR-IX DME, the contributions from a conformer such as that of Fig. 6 (right) would reduce, but not overcome, the dominant contributions from structures such as those of Fig. 5. The absolute configuration of such a complex would follow from an interpretation of the signed order of the ICD Cotton effects, but it should be noted that ICD $|\Delta\epsilon|$ values of ~10 reflect a diastereomeric excess of only ~4%[12c] for the complex.

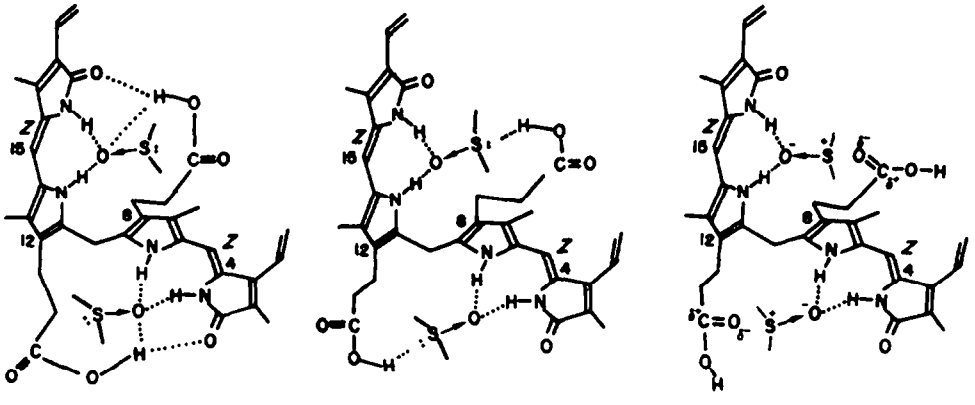


FIGURE 6. Speculative models for (left and middle) intramolecularly hydrogen-bonded conformation of a P-helicity bilirubin-sulfoxide complexes and (right) intramolecularly C=O···S→O dipole-dipole linked hydrogen-bonded conformation.

Absolute Configuration from ICDs. Exciton coupling theory provides a way to assign the absolute configuration, e.g., folded conformations akin to either M or P (Fig. 2), of the pigment in the predominant heteroassociation complex. The handedness or screw sense that the electronic transition moments of the coupled dipyrrinone chromophores make with each other correlates with signed order of the bisignate ICD CEs.[12c] A right-handed screw sense

(positive chirality) of the transition moment leads to a (+) longer wavelength CE followed by a (-) shorter wavelength CE. For a left-handed screw sense (negative chirality) the CE signs are inverted: (-) at the longer wavelength and (+) at the shorter wavelength component of the bisignate CE.[28] Since the direction of the electric dipole transition moment in the dipyrrinone chromophore has been calculated in theoretical studies[31] to lie along the longitudinal axis of the planar conjugated π-system, the exciton model can predict the CE signs of the structurally well-defined diastereomers, such as M•A and P•A, as well as the enantiomers M and P. In those folded conformations, the relative orientations of the two dipyrrinone electric dipole moments constitute a left-handed chirality for M•A and M and a right-handed chirality for P•A and P (with or without hydrogen bonding). Thus, theory predicts a predominance of the right-handed diastereomeric complex, e.g., P•A (e.g., Fig. 6) for solutions of bilirubin and MBR-XIII in the presence of the *R*-configuration sulfoxides of this work since the bisignate ICDs show a long wavelength (+) followed by a short wavelength (-) CE (Fig. 4). Similarly, the M•A complex (Fig. 5) predominates in solutions of EBR-IV, MBR-IV and BR-IX DME with (*R*)-sulfoxide.

CONCLUDING COMMENTS

For the first time, chiral recognition by sulfoxides has been shown to effect a first order asymmetric transformation of a pair of interconverting conformational enantiomers. Enantioselective binding by chiral sulfoxides by means of intermolecular hydrogen bonding generates diastereomeric complexes (Figs. 5 and 6) in which one diastereomer (M•A or P•A) is favored over the other. As a consequence, bilirubin solutions become optically active in pigment and exhibit bisignate ICDs the intensity of which depends on the binding constant and enantioselectivity. The solutions are, of course, optically active in sulfoxide, but the sulfoxide UV/CD transitions near 240 nm are far removed from those exhibited by bilirubins near 450 nm. Although the origin of the enantioselective interaction between pigment and sulfoxide appears to be general for bilirubin-like tetrapyrroles, an additional hydrogen bonding factor weighs in and controls the Cotton effect signs of the ICD when the pigment has propionic *acid* groups at C_8 and C_{12}, as in the natural product bilirubin and its symmetric analog, mesobilirubin-XIIIα: intramolecular hydrogen bonding involving the carboxylic acid hydrogen. It is well-known that bilirubin and related rubins with propionic acid ($-CH_2CH_2CO_2H$) [or even propionate ($-CH_2CH_2CO_2^-$)] groups located at C_8 and C_{12} prefer to adopt either of two intramolecularly hydrogen-bonded enantiomeric conformations (Fig. 2), and as such may be viewed as racemic mixtures of interconverting mirror image structures. When a sulfoxide inserts itself into the hydrogen-bonding matrix, the N-H•••O=C bonds are thought to be replaced by S→O•••H-N, and the C=O•••H-O bonds may be replaced by S→O•••H-O hydrogen bonds, or ^+C-O^- ^+S-O^- dipole-dipole attraction (Fig. 6).

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