Geometric Isomerisation of Bilirubin-IXα and its Dimethyl Ester

By ANTONY F. MCDONAGH*
(*Department of Medicine and the Liver Center, University of California, San Francisco, California* 94143)
and DAVID A. LIGHTNER* and TIMOTHY A. WOOLDRIDGE
(*Department of Chemistry, University of Nevada, Reno, Nevada* 89557)

Summary Absorbance difference measurements and chromatographic analyses indicate that bilirubin-IXα and its dimethyl ester rapidly form less stable, more polar (5*E*,15*Z*), (5*Z*,15*E*), and (5*E*,15*E*)-configurational isomers on irradiation with visible light.

THE two *meso* double bonds of bilirubin-IXα (BR-IXα) have the *Z* configuration, which allows the molecule to adopt the preferred highly hydrogen-bonded conformation (**1**).[1,2] In principle, three other geometric isomers of BR-IXα should exist: an *E*,*Z* (**2**), a *Z*,*E* (**3**), and an *E*,*E* (**4**). Unlike the natural *Z*,*Z* isomer, these would be unable to form the compact, lipophilic, internally bonded structure (**1**).[1] Dipyrrylmethenes,[3,4] 5,15-biladienes,[5] and 5,10,15-bilatrienes[4] are known to undergo photochemical $Z \rightarrow E$ isomerisation at *meso* bridge positions, often to stable products. However, there is no convincing evidence that BR-IXα undergoes an analogous process, and it is not clear whether the internal hydrogen bonding inhibits the reaction. We now report strongly suggestive evidence that BR-IXα and its dimethyl ester (BRDME-IXα) do form geometric isomers on illumination and that intramolecular hydrogen bonding retards the reaction but does not prevent it.

Brief irradiation of BR-IXα ($1{\cdot}5$—$3{\cdot}0 \times 10^{-5}$ M) in neutral or basic organic solvents† with monochromatic (440 ± 10 nm) or narrow-band blue light generated characteristic difference spectra similar to that previously reported for chloroform solutions.[6] These spectra had a synthesis peak at *ca.* 500 nm, a loss peak at *ca.* 460 nm, and an isosbestic point near 480 nm, and resulted from formation of a new chromophore(s) that overlaps the BR-IXα absorption band (Figure 1). Formation of this chromophore was not prevented by removing oxygen or inhibited by the presence of singlet oxygen quenchers such as 2,3-dimethylbut-2-ene or triethylamine (Figure 1). Therefore, the process is independent of the well known self-sensitised photo-oxygenation of BR-IXα which is a singlet oxygen reaction.[3] On continued irradiation the peak at *ca.* 500 nm (ΔA_{496}) increased rapidly to a maximum and then remained constant (Figure 2) while concomitantly $|\Delta A_{460}|$ increased to a plateau value, demonstrating formation of a photostationary state.‡ In the presence of oxygen the

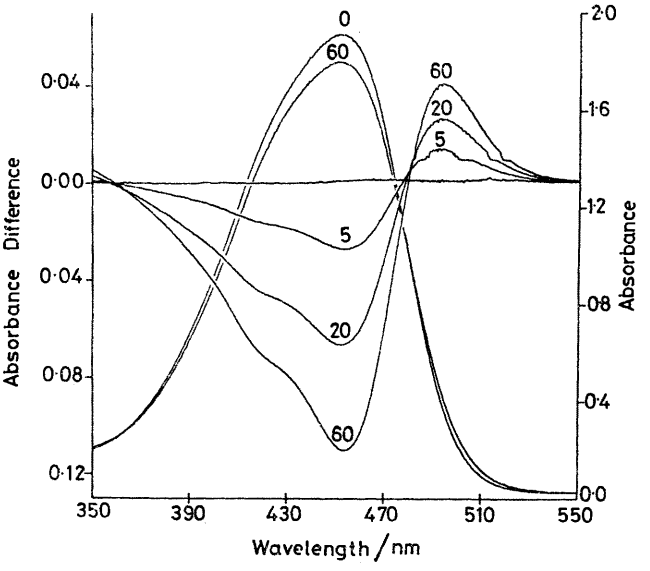


FIGURE 1. Absorbance and absorbance difference spectra obtained on irradiating BR-IXα ($3{\cdot}0 \times 10^{-5}$ M) in $CHCl_3$ containing 1% EtOH, 10% Et_3N, and $4{\cdot}4 \times 10^{-2}$ M 2,3-dimethylbut-2-ene with blue light. Numbers on the curves are the cumulative irradiation times in s.

† Solvents used included 1% EtOH–$CHCl_3$, 1% hexane–$CHCl_3$, benzene, 1% NH_4OH–MeOH and 5—50% Et_3N in 1% EtOH–$CHCl_3$. The monochromatic light source was a filtered 200 W Hg lamp and the narrow-band blue light was a Westinghouse 20 W Special Blue fluorescent tube. Absorbance difference spectra are given as irradiated minus non-irradiated.

‡ Prolonged irradiation caused a decrease in the synthesis peak at *ca.* 500 nm and an increase in the loss peak at *ca.* 460 nm, due to destruction of pigment.

initial rate of the reaction is faster than the competing photo-oxygenation and the photostationary state is reached before losses due to photo-oxygenation become noticeable, as reflected in Figure 2.

When irradiated solutions were kept in the dark at room temperature, $|\Delta A|$ at *ca.* 500 and *ca.* 460 nm slowly decreased without loss of the isosbestic point indicating quantitative reversal of the reaction. The reversal was slow in neutral or basic solutions (*e.g. ca.* 6%/h in 1% NH_4OH–MeOH) and accelerated on heating. The reversal was also accelerated by adding I_2 ($8{\cdot}7 \times 10^{-7}$ M) or a trace of CF_3CO_2H. The latter caused instantaneous reversal and completely removed the difference spectrum. Partial reversal was also achieved photochemically. When BR-IXα in 1% EtOH–$CHCl_3$ was irradiated to photoequilibrium with 440 nm light and then was irradiated with 510 nm light, $|\Delta A|$ at *ca.* 500 and *ca.* 460 nm decreased to new photostationary levels during the second irradiation, reflecting reformation of BR and formation of a new photoequilibrium mixture. Irradiation of BRDME-IXα in 1% EtOH–$CHCl_3$ at 400 nm, close to its λ_{max}, gave difference spectra equivalent to those from BR-IXα, but shifted to shorter wavelengths (synthesis peak 420, loss peak 395 nm). The spectra decayed slowly in the dark and disappeared completely on adding CF_3CO_2H.

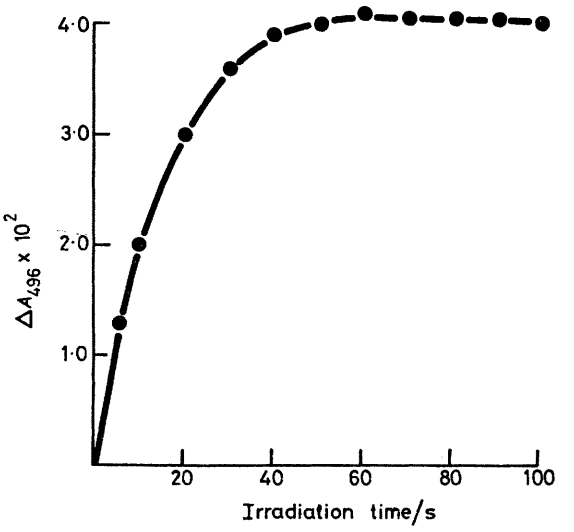
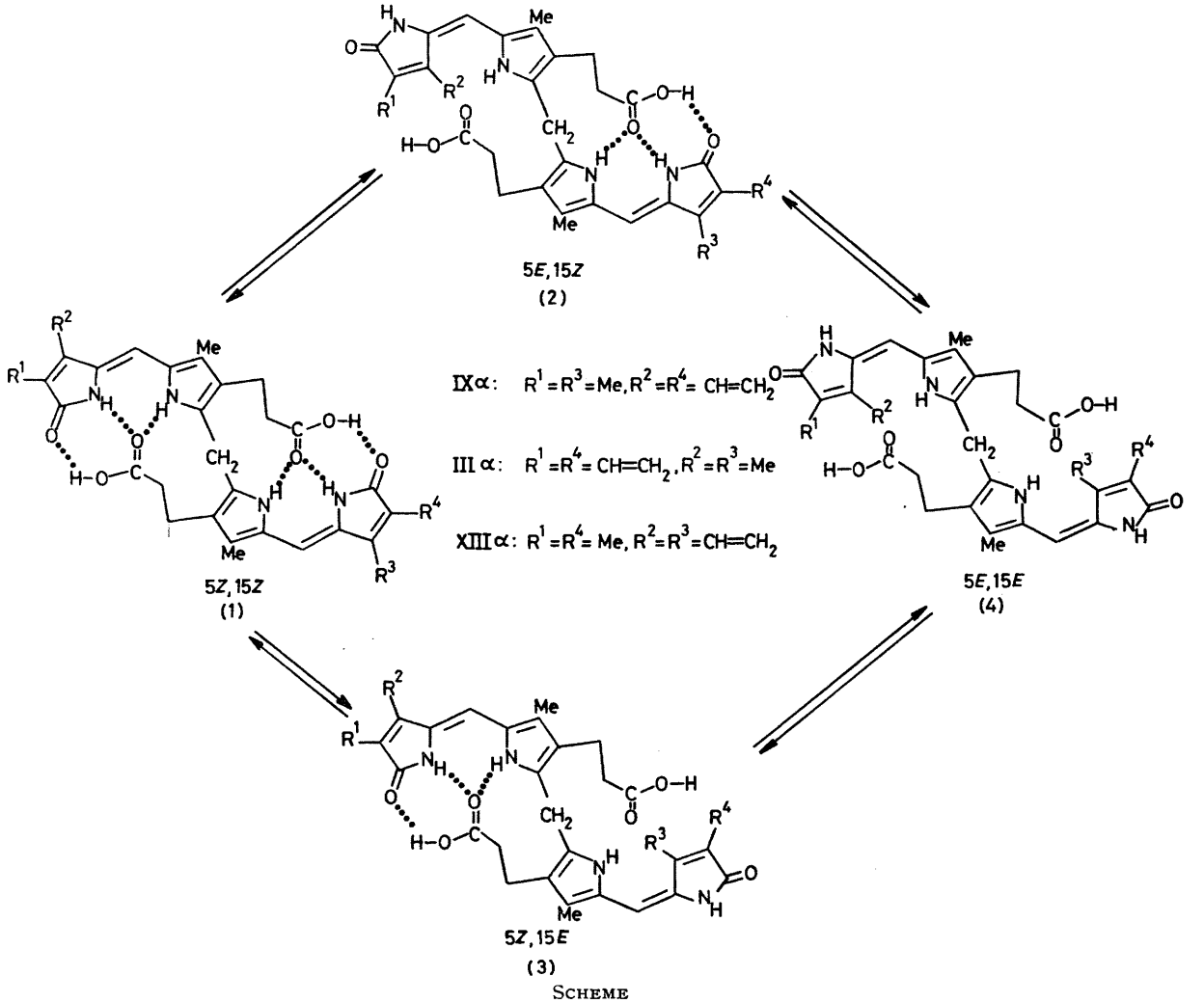


FIGURE 2. Increase in absorbance at 496 nm (irradiated minus non-irradiated) during irradiation of BR-IXα as described in Figure 1.



SCHEME

We conclude that BR-IXα and BRDME-IXα undergo a rapid reversible photochemical reaction which does not directly involve solvent or oxygen. Because the absorbances of starting material and products overlap, the reaction yields an equilibrium mixture whose composition depends on the irradiation wavelength and the solvent. For BR-IXα, added base (NH_4OH, Et_3N) accelerated the reaction and increased the photoequilibrium concentration of products. Furthermore, photoproduct formation from BRDME-IXα was faster than from unmethylated BR-IXα in the same solvent. Therefore, the forward reaction is facilitated by factors (ionisation, methylation) that reduce the hydrogen bonding present in the un-ionised free acid.

The spectroscopic observations were supported and extended by chromatographic studies. Three yellow photoproducts, two major and one minor, were detected by h.p.l.c. on silica when BRDME-IXα was irradiated (410 nm) in 5% EtOH–toluene. The two major products were formed in roughly equal amounts and had similar retention times, and all three products were more polar than BRDME-IXα. In contrast, the symmetrically substituted isomers BRDME-IIIα and BRDME-XIIIα (Scheme) gave only two photoproducts, one major and one minor. Photoequilibration and thermal reversal of the reaction was clearly demonstrated by h.p.l.c. for all three BRDME isomers. The photoproducts from BR-IXα decomposed to BR-IXα on silica, but were detectable by polyamide t.l.c. and reverse-phase h.p.l.c. However, complete resolution into the expected three components was not achieved. Analysis of irradiated solutions of BR-IXα at photoequilibrium showed, in addition to BR-IXα, one major yellow band and traces of another, both more polar than BR-IXα. Unlike BR-IXα these products were readily soluble in MeOH. The major photoproduct reverted to BR-IXα rapidly on irradiation and more slowly when kept in solution at room temperature in the dark.

The most plausible, if not the only explanation for these observations is geometric isomerisation of BR-IXα and BRDME-IXα at the *meso* double bonds (Scheme).§ This is consistent with the reversibility of the reaction and the formation of the three products from BRDME-IXα compared to only two from each of the corresponding IIIα and XIIIα isomers. Presumably the main photoproduct detected chromatographically from BR-IXα is an unresolved mixture of *E,Z* and *Z,E* isomers. Under the conditions reported, the photoequilibrium lies well to the left so that $[Z,Z] >> [E,Z] = ca.\ [Z,E] > [E,E]$.

This reaction is important for three reasons. (a) It occurs readily in BR-IXα solutions exposed to light but is undetectable by silica chromatography and difficult to detect by simple absorbance measurements. (b) It accompanies other photochemical reactions of BR-IXα and may complicate the kinetics and mechanism(s) of these. (c) It indicates that, despite extensive hydrogen bonding, BR-IXα can be converted into more polar configurational isomers thereby providing an explanation for the sudden biliary excretion of bile pigment observed when jaundiced rats[7] and babies[8] are irradiated with visible light.

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§ The Scheme presented summarises the overall photochemical transformations but omits intermediate structures that may be particularly important for the free acid. For example, thermal reversion of (5*E*,15*Z*)-BR-IXα would be expected to lead first to a (5*Z*,15*Z*)-conformer (not shown) which is less extensively hydrogen bonded than the more stable (5*Z*,15*Z*)-conformer that is finally obtained.

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