Free-energy Barrier of Conformational Inversion in Bilirubin

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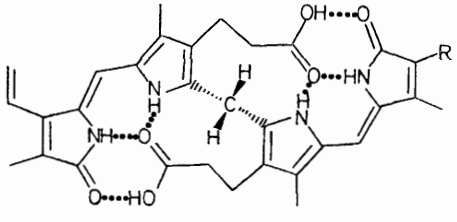
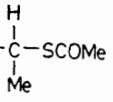
Summary Bilirubin exists in chloroform solution as two enantiomeric conformations (**1** and its mirror image) separated by a free-energy barrier of 17·9 ± 0·5 kcal mol^{-1}.

IT has long been recognised that bilirubin is stabilised by intramolecular hydrogen bonds.[1] To account for certain spectral data and chemical anomalies Knell *et al.*[2] have recently proposed the dissymmetric conformation (**1**) as a secondary structure of the pigment in non-polar solvents and in the solid state. The n.m.r. study reported here strongly supports such an assumption, showing that two interconvertible enantiomeric forms of bilirubin (probably **1** and its mirror image) must occur in chloroform solution; it also allows the free-energy barrier of conformational inversion to be calculated.

The ^{1}H n.m.r. spectrum (100 MHz; hexamethyldisiloxane reference) of (**2**)[3] in anhydrous $CDCl_3$ at room temperature showed the methyl signals of the Me–COS– and Me–CH(Ar)–S– groups as a doublet (centred at δ 2·18, Δν 3·1 Hz) and a doublet of doublets (centred at δ 1·50, Δν 3·4 Hz, J 7·0 Hz), respectively. As the temperature was raised to 53 ± 3 °C, the doublet at 2·18 merged to a broad singlet and



(1) R = -CH=CH$_2$

(2) R = 

the system of peaks at δ 1·50 became a doublet (still with J 7·0 Hz),† thus indicating the occurrence of two exchanging populations of conformers; further, the broadened lineshapes observed over this temperature range showed an approximately equal distribution of the molecules between the two populations.

These data are best explained by assuming a restricted rotation of the dipyrromethene units of (**2**) with respect to each other; such a restriction causes four stereoisomeric forms of (**2**) (two pairs of enantiomers) to exist as a consequence of the presence of two chiral elements in the molecule: *i.e.* the 'helical' conformation (*R*,*S*)[4] of the tetrapyrrolic skeleton and the asymmetric carbon atom (*R'*,*S'*) in the side chain. The rate constant at the colescence temperature for the interconversion of two diastereomeric conformers of (**2**) was then calculated[5] from the above spectral data [equations (1) and (2)].

$$(RR')[\text{or } (SS')] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (SR')[\text{or } (RS')] \quad (1)$$

$$k_1 = ca.\ k_{-1} = k = 7{\cdot}2 \pm 0{\cdot}4\ \text{s}^{-1}\ (T_c = 53 \pm 3\ ^\circ\text{C}) \quad (2)$$

The free-energy of activation obtained from k using the Eyring equation ($\Delta G^{\ddagger} = 17{\cdot}9 \pm 0{\cdot}5$ kcal mol^{-1}, the transmission coefficient being taken as unity)[5] represents the barrier of conformational inversion in (**2**) and, by implication, in bilirubin itself (**1**). In fact, bilirubin in chloroform shows the same u.v. spectrum and i.r. absorption bands [in the range 3600—2900 (NH, OH) and 1700—1600 cm^{-1} (amide and carboxy CO groups)] as compound (**2**).[3]

It must be pointed out that the above value of the free-energy of activation is consistent with the cleavage of four strong and two weaker hydrogen bonds per molecule.[2,6]

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† There should be two different coalescence temperatures, one for each methyl group; in practice they were not distinguishable.

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