Dimerisation of Bilirubin Anion in Aqueous Solution

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Bilirubin* is insoluble in water but dissolves in sodium hydroxide solution forming a divalent anion (Formula I). The light absorption spectrum of the solution changes with the concentration. The spectrum at very low concentrations is shown in Fig. 1 (full line). With increasing concentration of the anion the spectrum approaches the dotted curve. The spectral shift was studied in a buffer solution containing tris base and tris ascorbic acid salt, with varying pH, temperature, and ionic strength (sodium chloride added). Spectra were recorded in a Bausch & Lomb Spectronic 505 Spectrophotometer, using cells from 0.02 to 10 cm light path, fitted in thermostatic cell holders. The temperature of the solution was measured with a thermocouple located inside the cell.

The spectrum showed no change with variation of pH from 7.5 to 9.0.

At pH 8.25, 25°C and ionic strength 0.1 M (0.01 M tris ascorbate and 0.09 M sodium chloride) the ratio of the extinction coefficients at 520 and 430 nm was found to vary with the concentration of the bilirubin anion as shown in Fig. 2. The observed points are seen to fit the curve which is calculated on the presumption that the spectral shift is due to reversible formation of a dimer ion. The dissociation of this dimer may be written

$$B_2^{4-} \rightleftharpoons 2\,B^{2-}$$

The constant of dissociation at 25°C and ionic strength 0.1 M is found from this curve to 8.5×10^{-5} M.

The constant of dissociation, determined in this way, with varying temperature and ionic strength is pictured in Fig. 3.

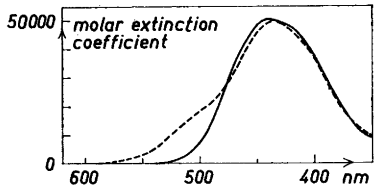


Fig. 1. Light absorption spectra of bilirubin sodium salt in dilute aqueous solution (full line) and of its dimer form (dotted line). The curve for the dimer has been calculated by extrapolation, since the dimer cannot be obtained in pure solution. The ordinates of the dimer spectrum are based on half the molecular weight.

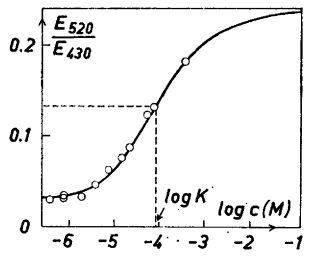


Fig. 2. The ratio of extinction coefficients at 520 and 430 nm as a function of the concentration at 25° and ionic strength 0.1 M. The concentrations are based on the monomer molecular weight. The curve is calculated from the presumption of reversible dimerisation. The dissociation constant may be obtained as the abscissa to the point of half dimerisation.

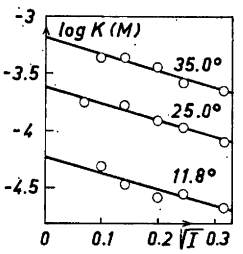
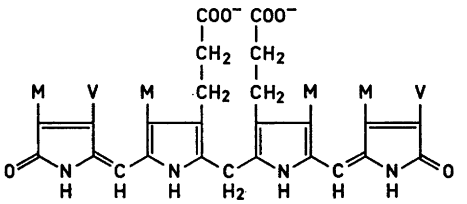


Fig. 3. The dissociation constant as a function of ionic strength and temperature.



Formula I. Bilirubin anion. M = methyl, V = vinyl.

* Bilirubin Sigma (sigma grade) was used. $E^{1\%}_{454} = 1.02 \times 10^3$ in chloroform.

The spectrum does not shift if sodium chloride is replaced by an equimolar concentration of tris ascorbate.

Extrapolation to zero ionic strength at 25°C gives the dissociation constant 2.7×10^{-4} M, or the standard affinity of dissociation $-\Delta G_0 = -4.9$ kcal/mole.

Log K is found to be a linear function of $1/T$. From the slope is calculated $\Delta H = 18.2$ kcal/mole.

The molar standard change of entropy is $\Delta S_0 = (\Delta H - \Delta G_0)/T = 45$ cal/mole degree.

In order to confirm the existence of an equilibrium of dimerisation the phase distribution of bilirubin was studied in the system aqueous buffer-methyl isobutylketone. In this organic solvent bilirubin is soluble as the acid. The light absorption spectrum of this solution shows no change with increasing concentration. The coefficient of distribution varies with pH in quantitative agreement with the following scheme:

B_2^{4-} (in water) $\rightleftharpoons$ 2 B^{2-} (in water)
B^{2-} (in water) + $2H^+ \rightleftharpoons$ BH_2 (in water) $\rightleftharpoons$ BH_2 (in methyl isobutylketone)

The coefficient of distribution at pH = 9.00 was found to vary with the concentration of bilirubin in accordance with this scheme. These findings confirm the dimerisation of the anion in the aqueous phase. The bilirubin acid is present as a monomer in the organic solvent. The numerical value of the dissociation constant, 6×10^{-4} M at 25°C and ionic strength 0.1 M, is different from the value determined above from the spectral shift. This is due to a change of dissociation constant from an aqueous medium to a medium of water saturated with methyl isobutylketone, as observed from a spectral shift which takes place when the ketone is added to the aqueous solution. Determination of the dissociation constant from phase distribution is rather inaccurate, due to instability of bilirubin in this system.

Summary. Reversible dimerisation of the divalent bilirubin anion in aqueous solution has been shown by studies of changes in the light absorption spectrum of bilirubin in buffer solutions and of phase distribution of such solutions by shaking with methyl isobutylketone. The thermodynamic constants have been determined.

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A Novel Synthesis of Isotripiperideine

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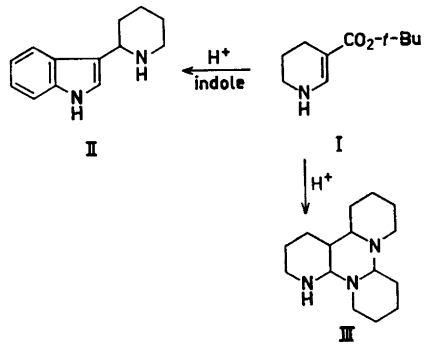
Isotripiperideine, III, was required as starting material in the synthesis of some alkaloids.*

Schöpf and coworkers [1] have studied the synthesis of various piperideine trimers. By treatment of *N*-chloro-piperidine with ethanolic potassium hydroxide they obtained α- or isotripiperideine or sometimes mixtures of the two isomers in moderate yields.

In an earlier paper [2] the preparation of *t*-butyl 1,4,5,6-tetrahydronicotinate, I, was described. When this compound was treated with indole in the presence of dilute acetic acid, hydrolysis, decarboxylation, and condensation occurred with formation of 2′-(3-indolyl)-piperidine, II.

It appeared possible that simple hydrolysis of *t*-butyl ester I would give rise to trimers of piperideine. Actually treatment of I with dilute acetic acid gave a complex mixture of compounds but a large yield (81 %) of isotripiperideine, III, was obtained under somewhat more acidic conditions.



I (5.00 g) was added to a solution of ortho-phosphoric acid (10 g, 89 %, *d.* 1.75) in water (300 ml). The mixture was stirred and heated to reflux in an atmosphere of

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