Anti-Configuration of the $N = N$ Bond in Azobilirubin Pigments (1)

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The conformational and internal energy barriers for rotation of the phenylazo group in azobilirubin pigments were determined by the PCILO method. The calculations showed a restricted rotation of the azo group at 180-300° dihedral angle.

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The chemical breakdown of bilirubin with diazoreagents releases two stable isomers (Scheme 1) frequently used in the study of bile pigments (2). The azo pigments can be separated as the methyl ester derivatives by column chromatography or tlc on silica gel without altering the original *Z* configuration about the methine bridge (3).

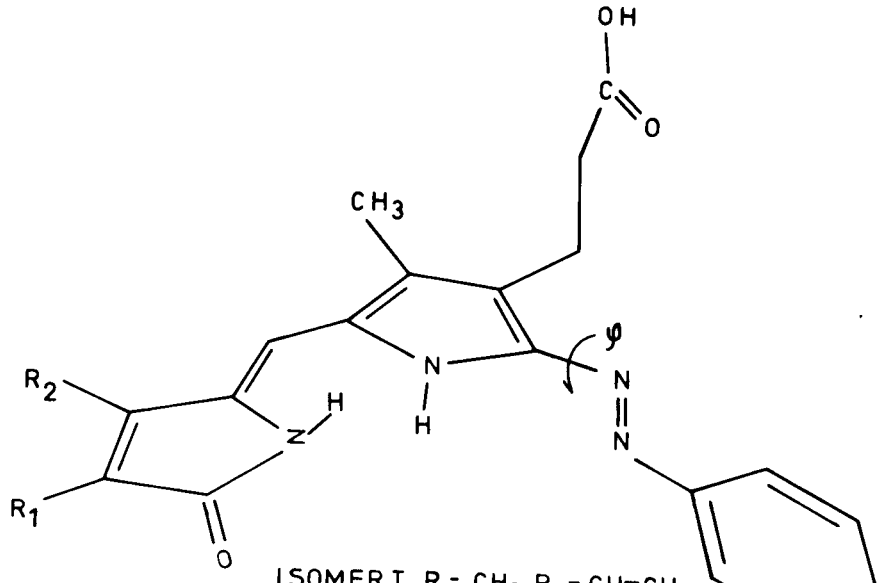
It is well known that diazo coupling reactions generate two geometric isomers about the -N=N- bond. However, because of the instability of the *syn*-isomer, only in a few cases have both the *syn* and *anti* isomers of an azo compound been isolated (4).

The suggestion of a possible *syn-anti* isomerism around the -N=N- bond in azobilirubin derivatives (5) prompted us to analyze these compounds by nmr and to carry out theoretical calculations on models of these isomers.

The nmr spectrum was measured for each azobilirubin isomer which are known to be pure *Z* isomer about the methine bridge (3b,6a,b). The spectra measured using shift reagent $Eu(fod)_3$ also show that the phenylazo grouping has the same conformation about the -N=N- bond. This conformation was again confirmed by ^{13}C nmr where the carbons *o*, *m* and *p* to the phenyl group appear as three single lines in their spectra (6b).

While the sample is configurationally pure, the nmr analysis did not allow us to distinguish whether the sample has the *syn* or *anti* configuration of the azo bond.

In order to define the preferred conformation occurring in these compounds, the Perturbative Configuration using Localized Orbitals (PCILO) method (7) was employed to calculate the energy barrier for internal rotation of the $C\text{-}N{=}N\text{-}C_6H_5$ group. The energy values corresponding to



SCHEME 1

0022-152X/81/040837-03$02.25

the various points resulting from the successive rotation of the azo group in the *anti* form starting from the coplanar position $\phi = 0°$ (8) are plotted against the rotational angle ϕ (Figs. 1 and 2). These values are listed in Table 1. The

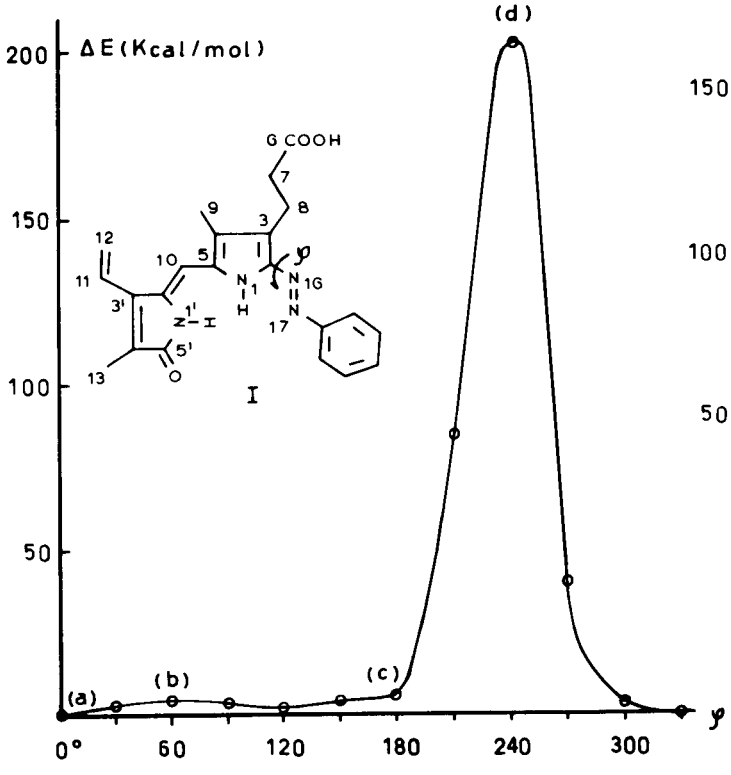


FIGURE 1

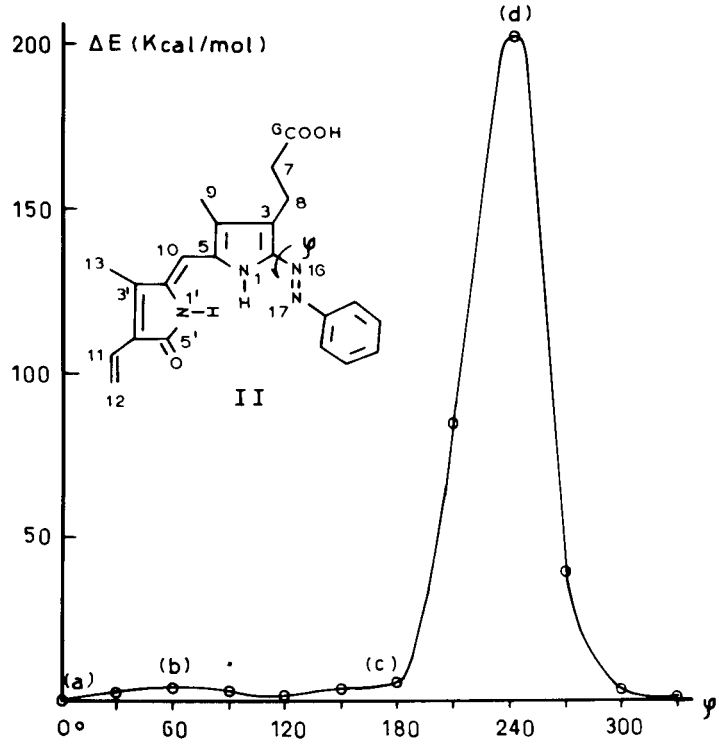


FIGURE 2

energy diagrams show two energy maxima: A low-energy maximum at $\phi = 60°$ and a high-energy maximum at $\phi = 240°$ for each isomer (*b* and *d* in Figures 1 and 2, respectively). The ΔE values for the low energy barrier at $\phi = 60°$ (4.3 kcal/mol for I and 3.9 kcal/mol for II), arise from an electronic interaction between the nitrogen atom in the α positon of the pyrrole ring and the non-bonded electrons of the carboxyl oxygens. As the rotation progresses, the energy of the system increases. The high energy maxima of $\Delta E = 201.6$ kcal/mol and $\Delta E = 201.2$ kcal/mol for the pair of isomers (Figures 1d and 2d), is produced by the small internuclear distance of 1.23 Å determined at $\phi = 240°$ between the nitrogen attached to the phenyl ring N_{17} and the oxygen atoms of the 3-propionic acid side chain. This large energy barrier at 180-300° dihedral angle disrupts the internal rotation process, consequently the free rotation of the phenylazo group around the C_2-N_{16} bond is forbidden by the propionic chain position. However, the group undergoes a stable oscillation motion in the low energy region with the most stable conformation appearing when $\phi = 0°$ where the phenylazo group is *anti* and coplanar with the pyrrole ring.

Table 1

Dihedral Angle	Δ E kcal/mol	
ϕ	I	II
0°	0	0
30°	2.5	2.9
60°	3.9	4.3
90°	3.0	3.4
120°	1.5	1.9
150°	3.6	4.0
180°	5.0	5.4
210°	84.4	84.8
240°	201.2	201.6
270°	39.4	39.8
300°	2.9	3.3
330°	0.1	0.5

The same calculation was repeated with the *syn* isomers. The results indicate that the energy barrier for the internal rotation is considerably higher than with the *anti* isomers. Our results showed that the *anti* form is more stable than the *syn* isomer. In this regard, our calculations parallel the preparative results where only the *anti* isomer is isolated (3b,6a,b). Therefore the specificity of the diazo coupling reaction may be controlled by the same factors

that determine the stability of the isomers.

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REFERENCES AND NOTES

(1) Contribution No. 559 from Instituto de Química UNAM.

(2) D. W. Hutchinson, B. Johnson and A. J. Knell, *Biochem. J.*, **127**, 395 (1970); C. C. Kuenzle, *ibid.*, **119**, 395 (1970).

(3a) F. H. Jansen and M. S. Stoll, *ibid.*, **125**, 585, (1971); (b) M. Salmón, E. Díaz, M. C. Rock and C. Fenselau, *Org. Magn. Reson.*, **8**, 126 (1976).

(4) S. N. Ege and R. R. Sharpe, *J. Chem. Soc.*, (B), 2014 (1971).

(5) F. Compernolle, F. H. Jansen and K. P. M. Heirwegh, *Biochem., J,* **120**, 891 (1970).

(6a) M. Salmón, *J. Heterocyclic Chem.*, **14**, 1101 (1977); (b) M. Salmón, I. Legoff and P. Joseph-Nathan, *ibid.*, **16**, 385 (1979).

(7) J. Langlet and J. P. Malrieu, *J. Am. Chem. Soc.*, **94**, 7254 (1972) and references therein.

(8) The molecular geometry of both isomers used in this study was taken from R. Cetina, M. Rubio, M. Salmón and J. Bernal, *Aust. J. Chem.*, **31**, 1911 (1978); M. Rubio, R. Cetina and M. Salmón, unpublished data.