Tetrahedron Letters Vol. 22, pp 855 - 858
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0040-4039/81/0222-0855$02.00/0

THE CONFIGURATIONS IN SOLUTION OF THE BILIVERDIN IXγ PHORCABILIN AND ISOPHORCABILIN DIMETHYL ESTERS

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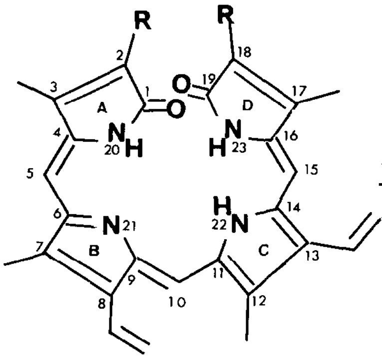
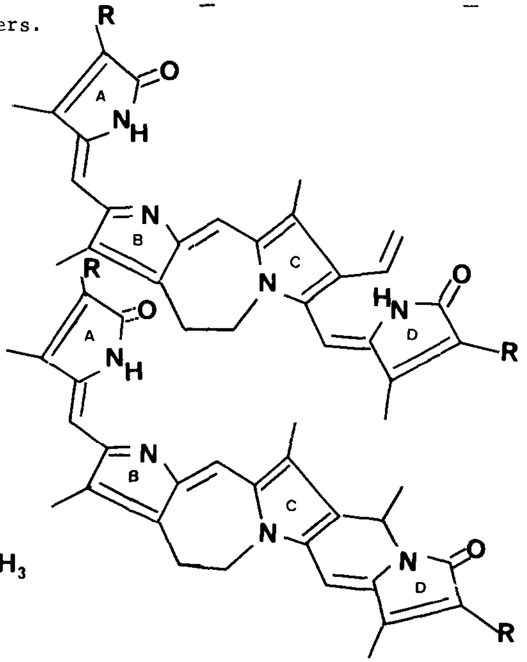
Summary : *The configurations in solution of the title compounds have been attributed by ^1H NMR. Correlations with the absorption spectra show the importance of conformationnal restraints in the spectroscopic properties of the bilatriene system which is usually chosen as chromophore model for the phytochrome phototransformation.*

As model for the chromophore of the phytochrome, extended theoretical calculations have been effected on bilatriene structure to examine the relation between spectroscopic properties and geometry of this tetrapyrrolic system [1-4]. The predominent helical all syn Z configuration of some common biliverdins is now well known [5-6]. Preparation and isolation of biliverdins with EZZ or EZE configuration have been described recently by Falk et al [7]. We report here the results of a structural assignment and a configurationnal study in solution by high frequency ^1H NMR of biliverdin IXγ dimethyl ester 1 and its two isomers 2 and 3 : phorcabilin and isophorcabilin dimethyl esters.



1

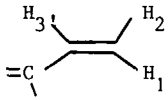
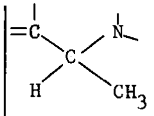


2

3

$R = CH_2CH_2COOCH_3$

TABLE 1 : ^1H NMR chemical shifts (δppm) and coupling constants (JHz) of the biliverdins 1,2,3[(f)]

Subs.	$=\overset{\vert}{C}—CH_3$				$\overset{/}{\underset{\Vert}{C}}-\underset{2}{CH_2}-CH_2COO-CH_3$			$=C—H(b)$									$\overset{/}{\underset{\Vert}{C}}-CH_2\ CH_2-\overset{\vert}{N}-$			
Pos.	3	7	12	17	18			5	10	15	8			13			8	22	13	23
											1'	2'	3'	1'	2'	3'				
1 [(e)]	2.02 s	2.09 s	2.20 s	2.00 s	2.42 s		3.64 s	5.71 s	6.72 s	5.82 s	5.46	5.43 (a)(c)	6.70	5.36	5.37 (a)(c)	6.56	-		-	
2	2.09 s	2.14 s	2.34 s	2.22 s	2.66 m	2.61 m	3.66s 3,68s	5.81 s	7.26 s	5.92 s	-			5.44	5.37 (a)(c)	6.38	3.04 m (c)	4.10 m	-	
3	2.06 s	2.12 s(d)	2.21 s	2.13 s(d)	2.69 m	2.63 m	3.65 s	5.88 s	7.25 s	6.03 s	-			-			2.96 m (c)	4.12 m	5.37 q(a) (c)	4.41 d

(a) coupling constants : 1 : $C_8 - CH = CH_2$: $J_{1'2'} = 1.0$ $J_{1'3'} = 19.0$ $J_{2'3'} = 11.5$, $C_{13} - CH = CH_2$: $J_{1'2'} = 1.0$ $J_{1'3'} = 17.0$ $J_{2'3'} = 12.0$; 2 : $C_{13} - CH = CH_2$: $J_{1'2'} = 1.1$ $J_{1'3'} = 17.5$ $J_{2'3'} = 11.5$; 3 : J = 6.2

(b) proton shift varies with dilution ; (c) double resonance experiment. (d) may be permuted. (e) recently several authors [11,12] have published uncompleted assignements for this compound. (f) in $DCCL_3$ solution, CAMECA 250 MHz.

TABLE 2 : Scheme of effective nuclear Overhauser effects experiments (a)

Irradiation	Effect on	1	2	3
$C_3 - CH_3$ ....>	HC_5 =	10	18	20
$C_7 - CH_3$ ....>	HC_5 =	13	16	19
$C_{12} - CH_3$ ....>	HC_{10} =	8	10	(b)
$C_{17} - CH_3$ ....>	HC_{15} =	12	12	8
$C_{13} - CH$ ....>	$H-C_{15}$ =	7	no effect	-
$C_8 - C = CH_2$.......>	$H-C_{10}$ =	10(c)	-	-

(a) Solutions (1 = 0.02M, 2 = 0.01M, 3 = 0.01M) in $DCCl_3$ which had been passed through neutral alumina (activity III) were degazed in several freeze pump thaw cycles with ultrasound stirring.(b) pertubed by $HCCl_3$ (c) negative effect.

These neobiliverdins, prepared from 1 by intramolecular reactions, have rigid configurations between the rings B-C (anti E) for 2 and between the rings B-C (anti E) and C-D (anti Z) for 3. These two compounds have been previously isolated by Bois-Choussy and Barbier [8,9] but the NMR assignements were not completly resolved and so configurational studies were not possible.

The biliverdin 1 had been isolated from haemin oxidation followed by esterification [10]. The phorcabilin 2 was prepared from 1 and the isophorcabilin from 2 [8,9].

Mass spectra by field desorption exhibit the same molecular peak (M^+) at m/e = 610 for these three pigments. TLC (Silica - acetone, $HCCl_3$ 15/85) gives the following R_f : 1 = 0,57, 2 = 0,17, 3 = 0,08.

Absorption spectra characteristics in methanol at room temperature are summarized as follows : 1 λ^1_{max} 372 nm (ε = 45000 mol^{-1} l cm^{-1}) λ^2_{max} 650 nm (13500), 2 λ^1_{max} 300 nm (13000) λ^2_{max} = 550 nm (45000), 3 λ^1_{max} = 300 nm (9500) λ^2_{max} = 605 nm (60000). Spectra in aprotic solvents such as chloroform or benzene are very similar without significative changes. The biliverdin 1, as all the biliverdins, shows two absorption bands in 370 and 650 nm regions with an absorptivity ratio of 3.3 while 2 and 3 have a weak band at lower wavelength and a more intense band at higher wavelength with an absorptivity ratio of 0,28 for 2 and 0,15 for 3. These results agree with the calculation of the optical properties of biliverdin in various configurations which assume a large decrease of the oscillator strength for the absorption band between 300 and 400 nm if biliverdin had a central methine in anti E configuration.

The substitution patterns and the predominant configurations all syn Z for 1, syn Z anti E anti Z for 2 and syn Z anti E anti Z for 3 are assigned on the basis of an ^1H NMR study (Tables 1-2) including spin decoupling and nuclear Overhauser effect experiments correlated with the assignements of the biliverdin IXα dimethyl ester reported by Schaffner [5] and Falk [6] groups. The three N-H protons of 1 present a very broad signal centered towards 9,5 ppm. No signal for N-H protons of 2 and 3 are detectabled probably on account of their pyrrolic type which is usually subjected to a large broadening effect.

In the phorcabilin 2 the restraints at the central methine bridge (anti E) included in a seven membered ring entails a large hypsochromic shift (100 nm). Such a situation supports the view that this neobiliverdin may best be regarded as two condensed pyrromethen-5 (1H) ones rather than substituted pyrromethenes, like bilirubin. The angle between the planes of the rings B and C may be larger and more constrained than in 1 and so a less planar conjuguated system is indicated. In the isophorcabilin 3, the additional restraints at the external methine bridge (anti Z) included in a six membered ring, on the contrary, increases the planearity between the rings C and D, consequently one observes a bathochromic shift (55 mn) with respect to phorcabilin 2.

The results show the importance that conformational restraints can have in the spectroscopic properties of bilatrienes. The relevance of these findings to the understanding of the phytochrome phototransformation is evident if one considers that the protein may modify the chromophore conformations [13] by intramolecular interactions [14].

Acknowledgements : We thank M. REUTENAUER for running the Cameca 250 MHz NMR spectra. (Centre Grenoblois de Résonance Magnétique - D.R.F. - C.E.A. - BP 85 X - 38041 GRENOBLE Cedex).

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(Received in France 25 November 1980)