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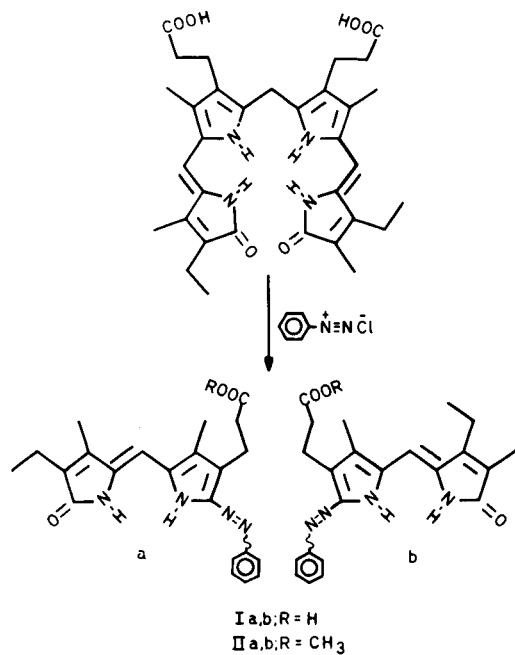
The structure of azomesobilirubin isomers as their methyl esters were determined using nmr and  $\text{Eu}(\text{fod})_3$  as a shift reagent.

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The two azo-pigment derivatives of mesobilirubin formed when it reacts with diazo reagents have been separated by several chromatographic techniques in order to perform some spectroscopic studies (2-4).

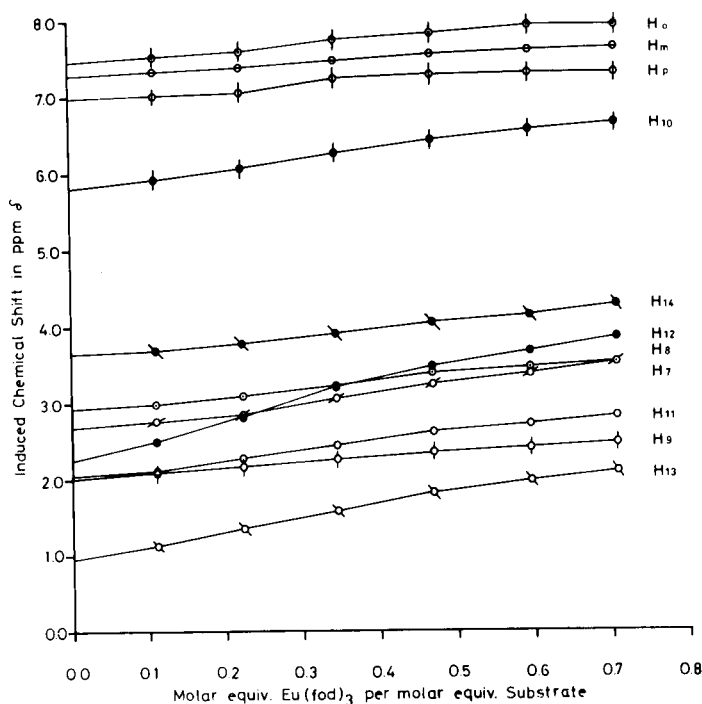
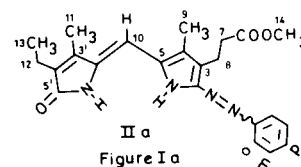
Literature reports showed poor yields in both catalytic reduction of bilirubin to obtain mesobilirubin (5-6) and the diazo reaction to generate the azo-pigments (2-4). The above mentioned difficulties come together with the instability of mesobilirubin to oxygen and light. Unsuitable procedures to have both azo-pigment isomers in large amounts, restrict the feasibility to obtain accurate nmr analysis. This is the purpose of this communication.

The diazotizing reaction cleaves the molecule at the methylene bridge, producing two benzeneazodipyrromethene isomers (2-4) as shown in Scheme 1.



SCHEME 1

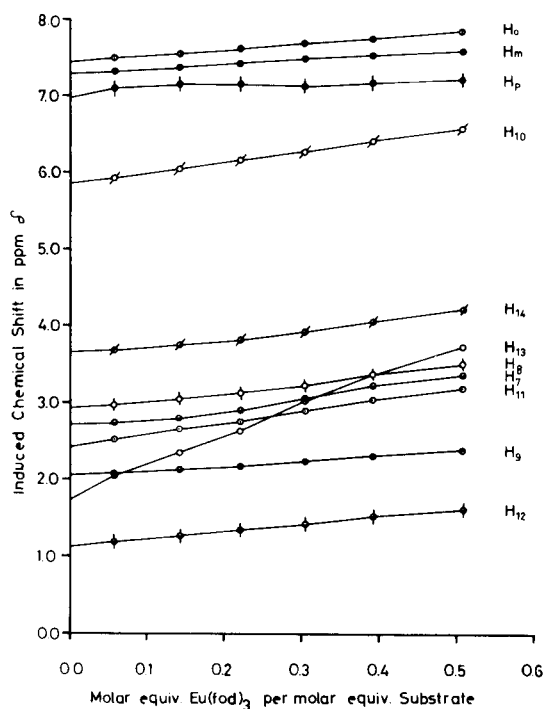
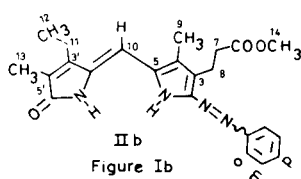
Even though mass spectrometry has provided a considerable amount of structural information for mesoazo-pigments (4), the distinction of methyl-ethyl positional isomers IIa and IIb can not be settled by this technique. The position of the methyl-vinyl substituents in a pair of isomers derived from bilirubin, have been previously assigned by nmr (7); the main interest in this study is to establish the methyl and ethyl positions on the lactam ring in each isomer. Proton assignments and shifts in the



Spectral Shifts of the Isomer IIa Protons  
Induced by Added  $\text{Eu}(\text{fod})_3$

Proton	$\delta_0$	$\delta$ SR (a)					
		A	B	C	D	E	F
H <sub>13</sub>	0.95	1.12	1.34	1.57	1.81	1.96	2.08
H <sub>9</sub>	2.01	2.08	2.16	2.25	2.33	2.39	2.45
H <sub>11</sub>	2.04	2.11	2.27	2.43	2.61	2.70	2.80
H <sub>12</sub>	2.25	2.50	2.82	3.20	3.46	3.65	3.83
H <sub>7</sub>	2.68	2.76	2.85	3.05	3.23	3.36	3.50
H <sub>8</sub>	2.93	2.99	3.09	3.22	3.38	3.45	3.50
H <sub>14</sub>	3.65	3.69	3.78	3.90	4.03	4.12	4.25
H <sub>10</sub>	5.81	5.92	6.06	6.25	6.41	6.53	6.61
H <sub>p</sub>	6.99	7.02	7.05	7.23	7.26	7.27	7.26
H <sub>m</sub>	7.28	7.33	7.38	7.46	7.53	7.57	7.59
H <sub>o</sub>	7.46	7.52	7.59	7.73	7.80	7.89	7.88

(a)  $\delta_0$  in absence of added  $\text{Eu}(\text{fod})_3$ . A with 0.111 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; B, with 0.223 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; C, with 0.345 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; D, with 0.471 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; E, with 0.597 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; F, with 0.708 molar equivalents of  $\text{Eu}(\text{fod})_3$ . Induced Chemical Shifts in ppm from internal  $\text{Me}_4\text{Si}$ .



Spectral Shifts of the Isomer IIb Protons  
Induced by Added  $\text{Eu}(\text{fod})_3$

Proton	$\delta_0$	$\delta$ SR (a)					
		A	B	C	D	E	F
H <sub>12</sub>	1.12	1.19	1.27	1.35	1.42	1.53	1.62
H <sub>13</sub>	1.74	2.04	2.35	2.63	3.02	3.36	3.72
H <sub>9</sub>	2.06	2.08	2.13	2.17	2.24	2.32	2.39
H <sub>11</sub>	2.42	2.52	2.66	2.76	2.90	3.04	3.18
H <sub>7</sub>	2.72	2.74	2.80	2.90	3.06	3.22	3.36
H <sub>8</sub>	2.94	2.97	3.05	3.13	3.22	3.34	3.50
H <sub>14</sub>	3.66	3.68	3.75	3.81	3.92	4.05	4.21
H <sub>10</sub>	5.86	5.93	6.05	6.16	6.27	6.41	6.56
H <sub>p</sub>	6.98	7.10	7.15	7.15	7.12	7.16	7.19
H <sub>m</sub>	7.29	7.32	7.37	7.43	7.48	7.52	7.57
H <sub>0</sub>	7.45	7.50	7.55	7.61	7.68	7.74	7.83

(a)  $\delta_0$  in the absence of added  $\text{Eu}(\text{fod})_3$ ; A, with 0.057 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; B, with 0.142 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; C, with 0.221 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; D, with 0.304 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; E, with 0.392 molar equivalents of  $\text{Eu}(\text{fod})_3$ ; F, with 0.507 molar equivalents of  $\text{Eu}(\text{fod})_3$ .

two azo-pigments IIa and IIb, are based on initial and lanthanide induced chemical shifts as is detailed in Figure 1a and 1b. The enolizable carbonyl on the lactam ring, has been shown to be favored to complex the shift reagent  $\text{Eu}(\text{fod})_3$  (7). Consequently, pronounced shifts in the signals of protons from  $\alpha$  substituents to the lactam carbonyl group are observed.

In Figure 1a it can be seen that methyl and methylene from the ethyl group have the largest change in chemical shifts, suggesting that an ethyl substituent is attached near to the complexed substrate carbonyl function. The opposite occurs in isomer IIb Figure 1b, where the nearest methyl group  $\alpha$  to the lactam carbonyl group has a significant change. It can be concluded that the nmr shift reagent  $\text{Eu}(\text{fod})_3$  is a suitable tool to solve in a reliable manner, structural problems in these types of compounds.

#### EXPERIMENTAL

Isomers IIa and IIb were obtained following the method previously described (3,4). Nmr spectra were taken in deuteriochloroform to which TMS was added as an internal reference as well as to provide the internal lock signal in a Varian HA-100 Spectrometer. Solid  $\text{Eu}(\text{fod})_3$  (8) was added in known amounts to isomer IIa (35 mg. in 0.4 ml. of deuteriochloroform) and isomer IIb (35.5 mg. in 0.4 ml. of deuteriochloroform) until a first order spectrum was obtained. The peaks are given in  $\delta$  values.

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#### REFERENCES AND NOTES

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