# Oxidative Cleavage of the Haem System: The Four Isomeric Biliverdins of the IX Series

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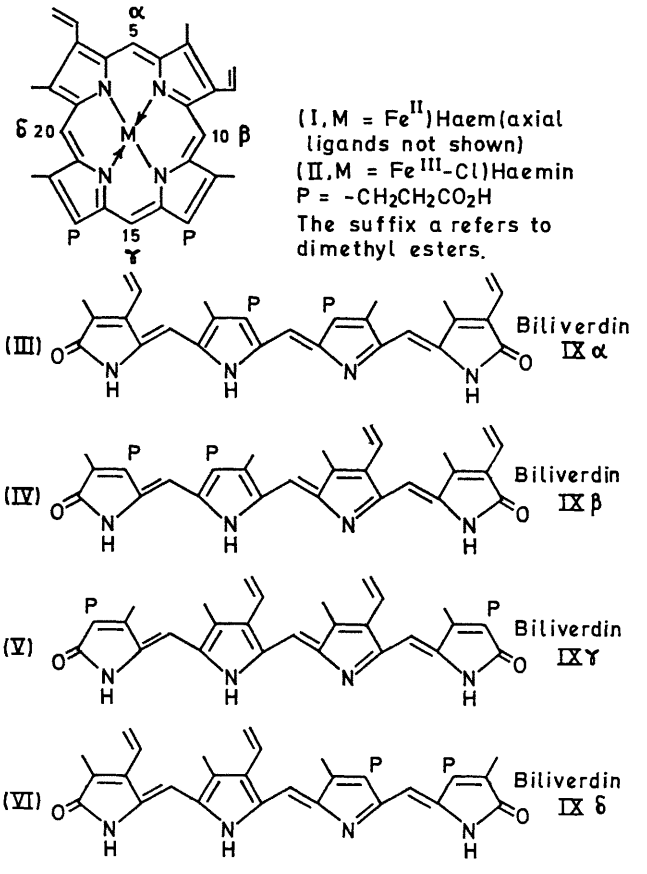
*Summary* Coupled oxidation of haemin gives four isomeric biliverdins, which have been isolated and characterised as their dimethyl esters.

THE oxidative cleavage of haemin (II) *in vitro* to give verdinoids has been extensively investigated because of its possible relevance to haem catabolism.[1,2] It has been implied (*e.g.* refs. 2 and 3) that the *in vitro* experiment is valid as a model only if it proceeds stereospecifically, as the natural breakdown apparently does,[4] with cleavage at the α-position (C-5) to give biliverdin IXα (I → III). While we do not accept this view, it is clearly important to know whether or not the model cleavage is stereoselective, and on this the literature is confused. Lemberg claimed (ref. 1, p. 460) that pure biliverdin IXα dimethyl ester (IIIa) was obtained following oxidative cleavage. However, Gray and his colleagues[5] concluded that cleavage of haemin ($N_2H_4$–$O_2$) gave a mixture of biliverdins IX. Very recently Rüdiger[6] and ÓCarra[3] have reported that all four isomers are formed, whereas Nichol and Morell[7] have concluded that the product is the β- (or δ-) isomer (IVa or VIa). In no instance have all four verdins been isolated in crystalline form, and characterised, and this we now report.

The oxidative cleavage was carried out with ascorbic acid–$O_2$ using an improved rapid method in which oxygen tension was kept high, and the reaction was complete after 3 min. at 37°. Treatment of the crude amorphous verdohaemochrome with methanolic KOH, followed by esterification with $BF_3$–MeOH and column chromatography on alumina gave the mixed biliverdin dimethyl esters (42%).

Separation of the biliverdin esters by careful t.l.c. (Merck Silica Gel G, 0·25 mm, 29°, 3% acetone in chloroform, 1·5 hr.) gave the four crystalline dimethyl esters (IIIa—VIa, Table). The separation could also be carried




*Properties of the isomeric biliverdin IX dimethyl esters*[a]

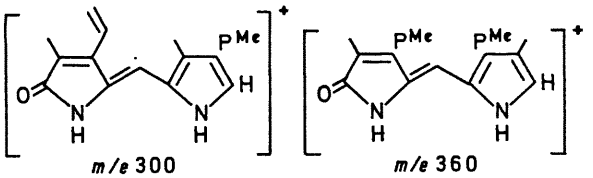
| Structure | M.p.[b] | $\lambda_{max}$ (nm) ($CHCl_3$) | $M^+$ | Bipyrrolic fragment ions | N.m.r.[c] (Ar*Me*), τ *endo* | *exo* |
|---|---|---|---|---|---|---|
| (IVa)β | 212—214° | 381, 648—654 | 610·280[d,e] | 360 | 7·71<br>7·80<br>7·86 | 8·26 |
| (IIIa)α | 205—207° | 379, 652—660 | 610·280[e] | 300, 311, 313 | 7·86<br>7·91<br>7·94 | 8·19 |
| (Va)γ | 205—207° | 376, 639 | 610·279 | 300[e], 311, 313 | 7·77<br>7·87<br>7·90(6H) | — |
| (VIa)δ | 172—174° | 379, 651—657 | 610·281[f] | 360[e] | 7·82<br>7·84 | 8·10<br>8·17 |

[a] Presented in order of decreasing mobility on the t.l.c. plate.
[b] Determined on Kofler–Reichert hot stage.
[c] Measured on Varian HA100 spectrometer. Insufficient amounts of the pure α- and β-isomers were available for this determination: the quoted values refer to the mixture of these obtained on "large scale" preparative t.l.c. The signals due to the β-isomer were ascertained by substracting those found in authentic biliverdin IXα dimethyl ester from the spectrum of the mixture.
[d] Calc. for $C_{35}H_{38}N_4O_6$, $M^+$ = 610·279. Measured on AEI Ltd. MS902 instrument.
[e] Base peak.
[f] A peak at *m/e* 612 was also observed.[10]

out with thicker layers (1·5 mm) but in this case the α- and β-isomers did not separate cleanly. The same four components were obtained when the coupled oxidation was carried out with $N_2H_4$–$O_2$ or with ascorbic acid–$H_2O_2$.

The identity of the α-isomer (IIIa) was established by comparison (mixed m.p., t.l.c. in 3 systems, electronic spectrum, mass spectrum) with a sample prepared from natural bilirubin (see following communication). The formulation of the other diesters rests on spectroscopic evidence as follows.

High-resolution mass spectrometry indicated that all four compounds were isomers of molecular composition $C_{35}H_{38}N_4O_6$. Two of the isomers gave bipyrrolic fragments at *m/e* 300, 311, and 313, whereas the other two gave fragments at *m/e* 360, 253 and 251. These fragment ions arise from cleavage about the central methine bridge.[8] The key fragments:




*m/e* 300 from IIIa; positional isomer from Va

*m/e* 360 from IVa and VIa

allowed division of the four isomers into two pairs. Since the α-isomer (IIIa) was already recognised, this allowed the γ-structure (Va) to be assigned, but did not distinguish between the remaining isomers (IVa, VIa).

We find that, for the biliverdin system, n.m.r. spectroscopy (for dilute solutions in $CDCl_3$) allows a distinction to be made between alkyl groups substituted on the two outer (*exo*) β-positions of the pyrrole rings and those on the six inner (*endo*) β-positions.[9] This difference presumably arises because the *endo*-β-positions are flanked, and deshielded, by two aromatic rings, whereas the *exo*-β-positions are markedly affected by only one (even for a macrocyclic conformation). Thus in octaethylbilatriene-*abc* the methylene groups appear at *ca.* τ 7·75 (4H, *exo*) and *ca.* τ 7·50 (12H, *endo*). In the n.m.r. spectra of various isomeric biliverdin dimethyl esters (including IIIα and XIIIα, following communication) the signals due to β-methyl groups fall into two ranges: *ca.* τ 8·1—8·3 (*exo*) and *ca.* τ 7·7—7·9 (*endo*). This allows the β-isomer (one *exo*-*C*-methyl at τ 8·26) to be distinguished from the δ-isomer (two-*C*-methyls at τ 8·10, 8·17). As shown in the Table the n.m.r. and mass spectrometric data reinforce each other and allow unique structural assignments to be made to the four isolated isomers, which has not been possible using oxidative degradations.[4–6]

The model cleavage is stereoselective, but to a minor degree only. The molar extinctions of the isomers (with the exception of β) have been measured. On the assumption that the β-isomer has the same extinction coefficient as the δ then the relative abundance of the four isomers is approximately α, 32%; β, 22%; γ, 18%; and δ, 28%. The implications of these results will be discussed elsewhere.

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