The Isomeric Heterogeneity of Biliverdin Dimethyl Ester Derived from Bilirubin

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Summary Dehydrogenation of bilirubin with benzoquinone in acetic acid, followed by methylation, gives three isomeric biliverdin dimethyl esters, structures for which are suggested.

As described in the previous communication, we required a sample of pure biliverdin IXα dimethyl ester (II),[1] the preparation of which generally involves the dehydrogenation of natural bilirubin IXα (I) with ferric chloride [2–4] or benzoquinone[5] at *ca.* 95° in acidic media, followed by esterification. We find that the biliverdin ester formed under these conditions contains substantial amounts of biliverdin XIIIα dimethyl ester (III) and occasionally small amounts of biliverdin IIIα dimethyl ester (IV), which are not easily separated from the main component (II).

Thus, dehydrogenation (30 min., 100°, N_2) of bilirubin (I, 100 mg.) with benzoquinone in dimethyl sulphoxide–acetic acid (9 : 1 v/v), followed by esterification (BF_3–MeOH), and chromatography (alumina) gave biliverdin dimethyl esters (61 mg.), which were separated by preparative t.l.c. (Merck Silica Gel H, 1·5 mm., 5% acetone in chloroform, 7—8 hr.) into three components (upper, 3·4 mg.; middle, 26 mg.; lower, 10 mg.). Crystallisation ($CHCl_3$–60—80° light petroleum) gave, respectively, biliverdin IIIα dimethyl ester (IV), biliverdin IXα dimethyl ester (II), and biliverdin XIIIα dimethyl ester (III). When ferric chloride was used as oxidant (as above; in acetic acid;[6] or according to Nichol and Morell[4]) only the IXα and XIIIα isomers were detected. The procedure[3] described by Gray and his colleagues for the preparation of pure biliverdin IXα gave, in our hands, very little of that material: the main

Properties of the three isomeric biliverdin dimethyl esters

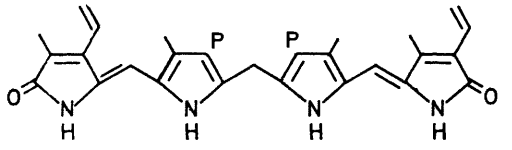
Structure	M.p.[a]	λ_{max} ($CHCl_3$) nm (ϵ)	M^+	Bipyrrolic fragments	N.m.r. (Ar*Me*)τ *endo*	*exo*
II (IXα)	208—209°	379 (51,800) 656—664 (15,100)	610·279[b]	300, 311, 313	7·84 7·90 7·92	8·16
III (XIIIα)	220—222°	378 (42,600) 652—654 (15,100)	610·278[b]	300, 311, 313	7·95(6H)	8·13(6H)
IV (IIIα)	198—202°(d)	381 (50,900) 653—661 (11,200)	610·278	300, 311, 313	7·81(6H) 7·89(6H)	

[a] Kofler–Reichert hot-stage. Literature[7] m.ps for *synthetic* samples: II, m.p. 206—209°; III, m.p. 226—228°, 230°, 245° all quoted; IV, m.p. 230° (d).
[b] Base peak.

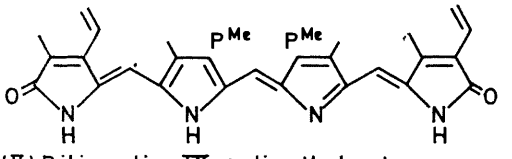
product was biliverdin dimethyl ester, and, again, this was a mixture of the IXα and XIIIα isomers.

The structures assigned to the products are based on their spectroscopic properties[8] (Table). High-resolution mass spectrometry indicated that the three diesters were isomers of composition $C_{35}H_{38}N_4O_6$. The occurrence in each spectrum of a bipyrrolic fragment ion at *m/e* 300 (but not at *m/e* 360) was ascribed to cleavage at the central methine bridge[2,8] to give fragments bearing two methyl groups, one vinyl group, and a methoxycarbonylethyl group. Whereas the n.m.r. spectrum of (II) showed four signals due to aryl methyl groups (three *endo*, one *exo*)[8], the n.m.r. spectra of (III) and (IV) suggested symmetrical substitution. One isomer had two signals (each 6H) due to Ar*Me* in the range suggesting *endo*-substitution,[8] and was assigned structure (IV): in the other isomer the n.m.r. spectrum indicated that two aryl methyl groups were *exo*, the other two *endo*, and this isomer was therefore assigned structure (III).

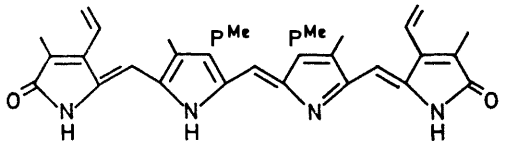
We suggest that the isomers (III, IV) arise from a reversible acid-catalysed cleavage of the bilirubin system about the central methylene bridge: recombination of the cleavage products would generate compounds of the IXα, XIIIα, and IIIα series. Such cleavage of the bilirubin system by electrophiles finds example in the diazo-reaction,[9] and in reactions in molten phenols:[10] the recombination process would be analogous to that leading to randomisation in porphyrinogens.[11] In accord with this, a mixture of bilirubin IXα and octaethylbiladiene-*a,c* on dehydrogenation gave, in addition to (II), (III), (IV), and octaethylbilatriene-*abc*, small amounts of two additional verdins (V, m.p. 184—188°; λ_{max} ($CHCl_3$) 376, 654—658 nm; VI, m.p. 202—204°; λ_{max}($CHCl_3$) 373, 650—656 nm). Mass spectrometry showed these to be isomers of molecular weight 582, and the fragmentation patterns were in accord with the structures shown.



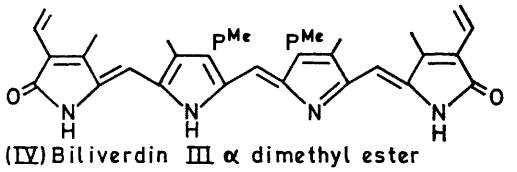
(I) Bilirubin IXα



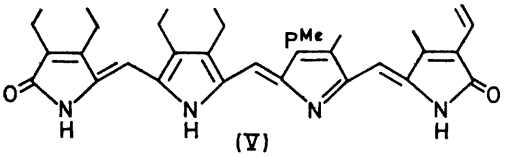
(II) Biliverdin IXα dimethyl ester



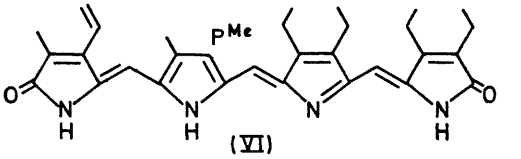
(III) Biliverdin XIIIα dimethyl ester



(IV) Biliverdin IIIα dimethyl ester



(V)



(VI)

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[1] Although biliverdin appeared in the lists of several chemical stockists up to about two years ago, it seems no longer to be commercially available.
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