Reaction of Biliverdins with Thiobarbituric Acid. A Novel Fragmentation Reaction of Bilin-1,19(21*H*,24*H*)-diones

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Summary Bilindiones are cleaved by reaction with thiobarbituric acid into two pyrromethenone fragments [*e.g.*, (**4a**) and (**5a**) from (**1a**)].

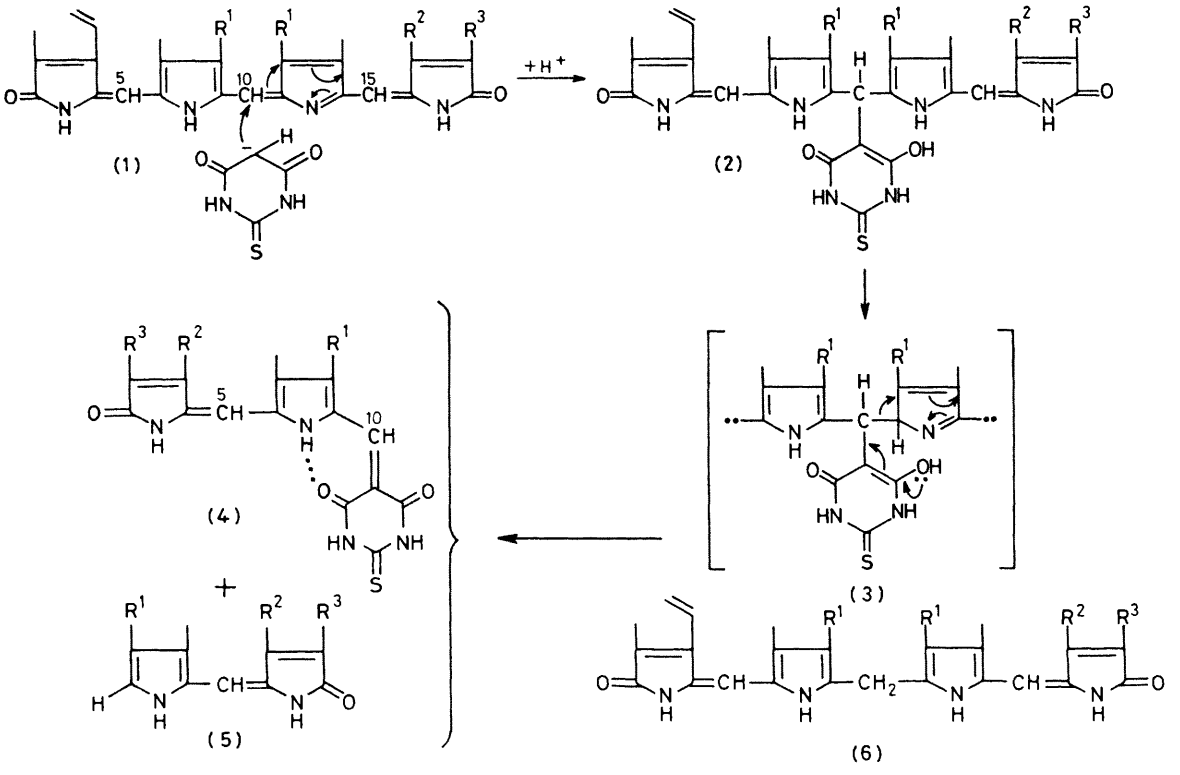
ALTHOUGH it is well known that nucleophiles add easily at the *meso*-position of pyrromethenes to yield *meso*-substituted dipyrrylmethanes,[1] there are few reports of analogous

additions to the central methine bridge of bilin-1,19(21*H*,24*H*)-diones.[2] We report here the first case of a carbanion addition to bilindiones of the biliverdin series[3] which is followed by a spontaneous break-down of the tetrapyrrole skeleton yielding two pyrromethenone fragments.

When thiobarbituric acid (25 mg) was added to a solution of biliverdin XIIIα dimethyl ester (**1a**) (70 mg)† in MeOAc (200 ml) a slow change of colour from greenish-blue to purple was observed. After 6 h at room temperature in the dark the solvent was evaporated and the residue redissolved in $CHCl_3$. Addition of hexane gave a violet precipitate (41 mg, isolated on crystallisation from $CHCl_3$–hexane, m.p. 138—139 °C) which was analysed‡ for $C_{22}H_{22}N_4O_5S$ [λ_{max} (MeOH) 553 (ε 30,600), 520 (sh), and 325 nm (17,200); (0·01 M NaOH in MeOH) 605 (31,300), 568 (30,400), and 520 nm (sh)]. The evidence for structure (**4a**) (or for a tautomeric form having an α-pyrrolenine ring and an enolic group in the thiobarbituric group)[4] came from the ^1H n.m.r. spectrum, which in $CDCl_3$ showed singlets corresponding to one proton each at δ 6·03 (5-H); 7·87 (10-H); 8·98, 10·14, 10·58, and 13·96 (NH or OH); in addition, it exhibited all the signals of the β-substituents of the pyrrole rings [strictly resembling those of the parent compound (**1a**)]. It has also been observed that only the lowest-frequency singlet of the four assigned to NH or OH protons did not exchange with D_2O; if this is due to the presence of a hydrogen-bonded, but not rapidly exchangeable, pyrrole NH,[5] structure (**4a**), rather than the pyrrolenine tautomer, must be assumed as being predominant in $CHCl_3$.

The mother liquors from precipitation of (**4a**), when evaporated, afforded a yellow compound (30 mg) which was shown to be (**5a**)[6] by ^1H n.m.r. spectroscopy [singlets at δ 6.30 (1H, methine bridge), 6·87 (1H, pyrrole α-H), 10·38 (1H, not exchangeable with D_2O, pyrrole NH), and 11·14 (1H, exchangeable with D_2O, lactam NH)]; visible spectroscopy [λ_{max} ($CHCl_3$) 409 nm (ε 49,700); ($CHCl_3$, 1% CF_3CO_2H) 434 nm (48,600)],[7] and also by reaction with 2-ethoxy-carbonylbenzenediazonium fluoroborate yielding an azo-pigment identical in all respects with that obtained by diazotisation of bilirubin XIIIα dimethyl ester (**6a**).[8]

By treatment of biliverdin XIIIα (**1b**) with thiobarbituric acid and separation of the reaction products by column



SCHEME

a;	$R^1 = CH_2CH_2CO_2Me$	$R^2 = CH{=}CH_2$	$R^3 = Me$
b;	$R^1 = CH_2CH_2CO_2H$	$R^2 = CH{=}CH_2$	$R^3 = Me$
c;	$R^1 = CH_2CH_2CO_2H$	$R^2 = Me$	$R^3 = CH{=}CH_2$

† Biliverdin XIIIα and its dimethyl ester were prepared through: (i) acid-catalysed isomeric scrambling of bilirubin IXα (**6c**) followed by t.l.c. separation of the XIIIα isomer (**6b**) (A. F. McDonagh and F. Assisi, *J.C.S. Chem. Comm.*, 1972, 117; P. Manitto and D. Monti, *Experientia*, 1973, **29**, 137); (ii) oxidation of bilirubin XIIIα with chloranil in $CHCl_3$–Me_2SO 8:2 (*cf.* ref. 9 for purification); (iii) esterification with methanolic 14% BF_3 (R. Bonnett and A. F. McDonagh, *J.C.S. Perkin I*, 1973, 881).

‡ Satisfactory elemental analyses and mass spectra consistent with the assigned structures were obtained for all the compounds isolated.

chromatography (silica gel, $CHCl_3$ containing 4—6% MeOH) vinylneoxanthobilirubinic acid (**5b**) (81% yield)[6] and compound (**4b**) (73%) were isolated from the first and the second eluate, respectively In an analogous way biliverdin IXα (**1c**)[9] gave, as expected, two products, each being a mixture of isomers [*i e* , (**4b**)/**4**(**c**) and (**5b**)/(**5c**)] The isomeric ratio of both these mixtures was estimated *ca* 1 : 1 by n m r spectroscopy and by t l c separation of the ethyl anthranilate azo-pigments of (**5b**) and (**5c**) [8,10]

The cleavage of the bilatriene skeleton by reaction with thiobarbituric acid can be explained as shown in the Scheme, *i e* , assuming that intermediates such as (**2**) collapse by way of a retrograde Michael reaction This is supported by the following facts (i), the adduct (**2c**) was obtained as the only product when the reaction of biliverdin IXα (**1c**) with thiobarbituric acid was carried out in MeOAc containing 1% triethylamine [spectral data of the triethylamonium salt of (**2c**) λ_{max} ($CHCl_3$) 460 (sh), 427 nm, ^1H n m r ($CDCl_3$) singlets at δ 5 86 (1H, 10-H), 6 12 and 6·16 (1H each, 5-H and 15-H)], (ii) (**2c**) was found to undergo rapid conversion into (**4b**—**c**) and (**5b**—**c**) in acidic media (*e g* , 1% CF_3CO_2H in $CHCl_3$)

It must be pointed out that the fission of bilatrienes described here might offer a new approach to structural elucidations as well as to clinical analyses,[11] thus paralleling the well known diazo-reaction of biladienes-*a,c* [8,12] A more detailed and extended study of Michael additions to pyrromethene systems of bilins will be reported later

(*Received*, 16*th November* 1979, *Com.* 1202)

[1] Review A Gossauer and J Engel, 'Linear Polypyrrolic Compounds,' in 'The Porphyrins,' Vol 2 ed D Dolphin, Academic Press New York, 1978, ch 7, G M Gorter La Roy, C Erkelens, and J Lugtenburg *Rec Trav chim Pays-Bas* 1979, **98**, 140

[2] For addition of (a) borohydride ion A J Fathiadi and R Schaffer, *Experientia* 1971, **27**, 1139 (*cf* also ref 8), (b) cyanide H Falk and T Schlederer, *Monatsh Chem* , 1978, **109**, 1013, (c) alcohols and water A R Holzwarth H Lehner, S E Braslavsky, and K Schaffner, *Annalen*, 1978, 2002 (d) thiols and thioacids P Manitto and D Monti *Experientia* 1979, **35**, 1418

[3] For nomenclature see R Bonnett, 'Nomenclature' in 'The Porphyrins,' Vol 1, ed D Dolphin, Academic Press, New York, 1978, ch 1

[4] *Cf* D G Durham, C G Hughes and A H Rees, *Canad J Chem* 1972, **50** 3223

[5] It has been reported that the NH group of pyrrole even if hydrogen-bonded with proton acceptors, does not undergo rapid exchange processes (R J Abraham and H J Bernstein *Canad J Chem* 1959 **37**, 1056) An analogous behaviour of pyrrole rings has been observed in pyrromethenone compounds *e g* in bilirubin (**6c**) in $CDCl_3$ (P Manitto and D Monti, unpublished results)

[6] H Pfleninger, F El-Barkawi, K Ehl, R Kohler and A F McDonagh *Annalen* 1972, **758** 185, H Fischer and H Pfleninger, *Z Physiol Chem* 1942, **274**, 231

[7] *Cf* H von Dobeneck and E Brunner, *Z Physiol Chem* , 1965, **341**, 157

[8] F H Jansen and M S Stoll, *Biochem J* 1971, **125**, 585

[9] P Manitto and D Monti *Experientia*, 1979, **35**, 9

[10] P Manitto and D Monti, *Gazzetta*, 1977, **107**, 573

[11] J M C Gutteridge and T R Tickner, *Biochem Medicine*, 1978, **19**, 127, T R Tickner and J M C Gutteridge, *Clin Chim Acta*, 1978, **85**, 125

[12] D W Hutchinson, B Johnson, and A J Knell, *Biochem J* , 1972, **127**, 907