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CONCERNING THE MECHANISM OF FORMATION OF BILIVERDINS FROM b-BILENES

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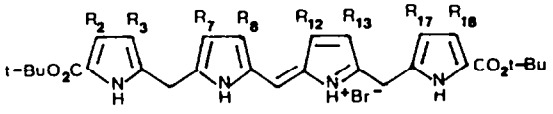
Abstract. The oxidation of 1,19-di-t-butoxycarbonyl-b-bilenes with bromine affords not only a major biliverdin, but also minor biliverdins. Elucidation of their structure revealed that cleavage of the b-bilene chain takes place during the oxidation, followed by dimerization of one dipyrrylmethene half. The synthesis of mesobiliverdins IXβ , XIβ , IVβ , IXδ and coprobiliverdin IIβ are described.

Biliverdins can be prepared by oxidation of b-bilenes using bromine in trifluoroacetic acid[1-5]. This is achieved by the controlled oxidation of 1,19-di-t-butoxycarbonyl-b-bilene hydrobromide, followed by an alkaline work-up. The oxidation of the b-bilene hydrobromide gives only moderate to low yields of biliverdins. Nevertheless, the synthesis of the b-bilene has the advantage of using pyrrylmethane intermediates which are also useful for porphyrin synthesis, thus enhancing their value as synthetic intermediates of both biliverdins and porphyrins.

When a 1,19-di-t-butoxycarbonyl-b-bilene hydrobromide is oxidized with bromine in trifluoroacetic acid, a major biliverdin is always obtained, together with minor green or blue products in negligible amounts that are usually observed in addition to the main biliverdin reaction product.

Attempts to obtain mesobiliverdins by oxidation of the appropriate 1,19-di-t-butoxycarbonyl-b-bilene hydrobromides afforded two biliverdins in each case. The main product was

SCHEME 1



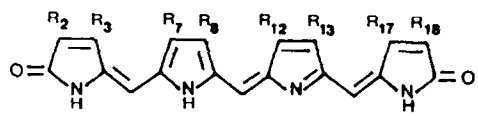
1. R_2=R_8=R_{12}=R_{17}=Me; R_{13}=R_{18}=Et; R_3=R_7=P^{Me}
4. R_3=R_8=R_{12}=R_{18}=Me; R_2=R_{17}=Et; R_7=R_{13}=P^{Me}
7. R_2=R_8=R_{13}=R_{18}=Me; R_{12}=R_{17}=Et; R_3=R_7=P^{Me}

Me=CH_3; Et=C_2H_5; P^{Me}=$CH_2CH_2CO_2CH_3$

the expected mesobiliverdin and the minor product was a biliverdin formed by the cleavage of the b-bilene followed by dimerization of one of the resulting dipyrrylmethene halves. The relation of the main to the minor biliverdin was usually 5:1.

Thus, when the b-bilene hydrobromide 1 (Scheme 1) was treated with trifluoroacetic acid to cleave and decarboxylate the t-butyl ester residues at C-1 and C-19 and this treatment was followed by oxidation with bromine as described elsewhere[1-5], *mesobiliverdin IX β* (2) and coprobiliverdin II β (3) were obtained (Scheme 2). The same procedure when applied to b-bilene hydrobromide 4, afforded the mesobiliverdin XI β (5) and the mesobiliverdin IV β (6). The obtention of mesobiliverdin IX δ (8) from the b-bilene hydrobromide 7 also gave coprobiliverdin II β (3) as the secondary product[6].

SCHEME 2



2. R_2=R_8=R_{12}=R_{17}=Me; R_{13}=R_{18}=Et; R_3=R_7=P^{Me}

3. R_2=R_8=R_{12}=R_{18}=Me; R_3=R_7=R_{13}=R_{17}=P^{Me}

5. R_3=R_8=R_{12}=R_{18}=Me; R_2=R_{17}=Et; R_7=R_{13}=P^{Me}

6. R_2=R_8=R_{12}=R_{18}=Me; R_3=R_{17}=Et; R_7=R_{13}=P^{Me}

8. R_2=R_8=R_{13}=R_{18}=Me; R_{12}=R_{17}=Et; R_3=R_7=P^{Me}

Me=CH_3; Et=C_2H_5; P^{Me}=$CH_2CH_2CO_2CH_3$

The formation of the minor biliverdin products can be rationalized as outlined in Scheme 3 for the case of b-bilene 1. Rearrangement in acid medium of the 1,19-dibromo-b-bilene 9 leads to the formation of the a-bilene 10[7], which could then be cleaved by attack of the trifluoroacetate anion to give the dipyrrylmethene 11. The latter dimerizes to give the a,c-biladiene 12 which is ultimately transformed into 3. It is known[1] that a,c-biladienes are oxidized by bromine to give biliverdins.

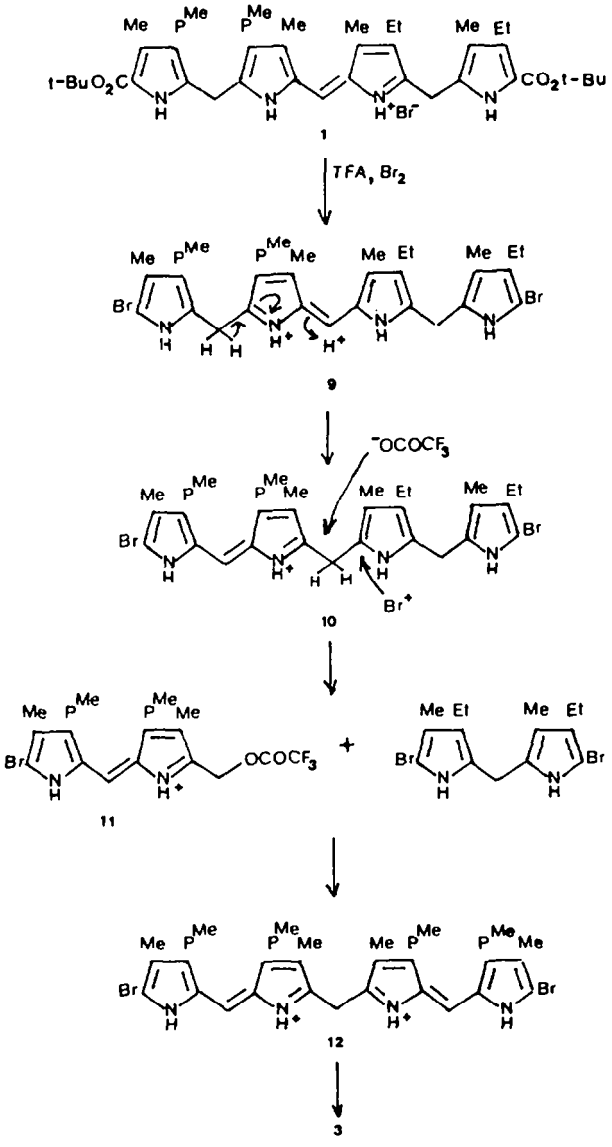
The synthesis of the 1,19-di-t-butoxycarbonyl-b-bilenes 1, 4 and 7 was achieved by condensation of the α-unsubstituted dipyrrylmethanes with α-formyldipyrrylmethanes. Five 5-t-butoxycarbonyl-5'-benzyloxycarbonyl dipyrrylmethanes 19, 21, 24, 26 and 29 were obtained by condensation of the corresponding 2-acetoxymethyl-pyrroles 13-15 with the α-unsubstituted pyrroles 16-18 (Scheme 4) following the procedures described by us[3,4] and others[1,2]. Hydrogenolysis of the benzyl residues of the dipyrrylmethanes 19, 21, 24, 26 and 29 over 10% Pd/C afforded the dipyrrylmethane-5'-carboxylic acids 20, 22, 25, 27 and 30. Decarboxylation of 22 and 27 with p-toluensulfonic acid in an aprotic solvent gave the α-unsaturated dipyrrylmethanes, which by formylation with the dimethylformamide-benzoyl chloride reagent afforded the 2-formyldipyrrylmethanes 23 and 28.

The pyrroles 13, 14, 16, 17 and 18 are known[4,8,9]. Pyrrole 31 (Chart 1) was prepared by a Knorr type synthesis from benzyl propionylacetate and t-butyl acetoacetate and was hydrolized with 33% hydrobromic acid in acetic acid to give 32. The reduction with diborane of 32 afforded the pyrrole 33[10], which was then transformed into its 2-acetoxymethyl derivate 15 with lead tetraacetate. The pyrrole 33 was prepared also by a Knorr synthesis from ethyl propionylacetate and t-butyl acetoacetate to give 34. Hydrolysis of 34 as described for 32

gave 35, which was reduced with diborane to 36 and transesterified with benzyl alcohol[10] to 33.

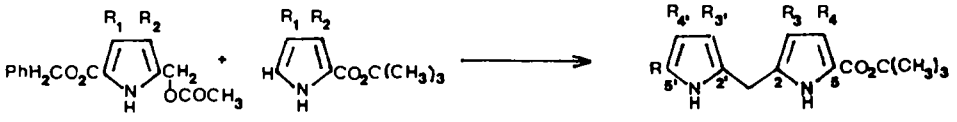
The biliverdin esters were charaterized by their NMR, their mass spectra and their UV and Vis spectra. ^{1}H NMR spectra were specially useful (see Experimental). The signals of the methyl residues were extremely useful in the characterization of the mesobiliverdins. In the mesobiliverdin IX β (2) the endo methyles at 8 and 12 were at lower field values (2.17

SCHEME 3



and 2.15 ppm) than the methyl protons of CH_3-17 and CH_3-2 (2.09 and 1.85 ppm). Mesobiliverdin IX δ (8) showed too peaks at 2.20 and 2.07 ppm for the protons of the CH_3-8 and CH_3-13 residues (endo) and two peaks at 1.85 and 1.80 ppm for the protons of the CH_3-2 and CH_3-18 residues (exo). The pattern of the methyl resonances was very different for mesobiliverdin XI β (5) and mesobiliverdin IV β (6) and allowed to distinguish between them. Mesobiliverdin XI β (5) showed three endo methyl groups at 2.2 ppm (CH_3-8 and 12) and 2.1 ppm (CH_3-3) and one methyl exo group at 1.82 ppm (CH_3-18). Mesobiliverdin IV β (6) showed one peak at

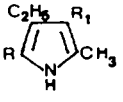
SCHEME 4



13. R_1=Me; R_2=P^{Et}
14. R_1=Me; R_2=Et
15. R_1=Et; R_2=Me
16. R_1=Et; R_2=Me
17. R_1=Me; R_2=Et
18. R_1=P^{Et}; R_2=Me
19. R=$CO_2CH_2C_6H_5$; R_3=R_4,=Me; R_3,=R_4=Et
20. R=CO_2H; R_3=R_4,=Me; R_3,=R_4=Et
21. R=$CO_2CH_2C_6H_5$; R_4,=R_4=Me; R_3,=R_3=P^{Et}
22. R=CO_2H; R_4,=R_4=Me; R_3,=R_3=P^{Et}
23. R=CHO; R_4,=R_4=Me; R_3,=R_3=P^{Et}
24. R=$CO_2CH_2C_6H_5$; R_4,=R_4=Me; R_3,=P^{Et}; R_3=Et
25. R=CO_2H; R_4,=R_4=Me; R_3,=P^{Et}; R_3=Et
26. R=$CO_2CH_2C_6H_5$; R_3=R_4,=Me; R_4=Et; R_3,=P^{Et}
27. R=CO_2H; R_3=R_4,=Me; R_4=Et; R_3,=P^{Et}
28. R=CHO; R_3=R_4,=Me; R_4=Et; R_3,=P^{Et}
29. R=$CO_2CH_2C_6H_5$; R_3,=R_4=Me; R_3=R_4,=Et
30. R=CO_2H; R_3,=R_4=Me; R_3=R_4,=Et

Me=CH_3; Et=C_2H_5; P^{Et}=$CH_2CH_2CO_2C_2H_5$

CHART 1



31. R=$CO_2CH_2C_6H_5$; R_1=$CO_2C(CH_3)_3$
32. R=$CO_2CH_2C_6H_5$; R_1=CO_2H
33. R=$CO_2CH_2C_6H_5$; R_1=CH_3
34. R=$CO_2C_2H_5$; R_1=$CO_2C(CH_3)_3$
35. R=$CO_2C_2H_5$; R_1=CO_2H
36. R=$CO_2C_2H_5$; R_1=CH_3

2.22 ppm which integrated for 6 H for the protons of the CH_3-8 and CH_3-12 and one peak at 1.83 ppm which integrated also for 6 H for the protons of CH_3-2 and CH_3-18.

EXPERIMENTAL

Melting points were determined on a Kofler melting point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian FT-80A spectrometer at a concentration of 10^{-2} M for biliverdin esters. Mass spectra were obtained with a Varian CH-7 spectrometer. Electronic absorption spectra were determined in CH_2Cl_2 using a Hitachi 110 A spectrophotometer at the concentration of 10^{-5} M. The silica gel used in column chromatography was TLC Kieselgel (Merck). TLC was performed on precoated silica gel F-254 plaques (Merck, 0.25 mm layer thickness). For biliverdin esters 10% acetone-chloroform was used as the TLC development system. The substances were spotted by spraying the plaques with Ehrlich's reagent (2% p-(dimethylamino)-benzaldehyde in 6N HCl), or by treatment with bromine vapour which gave orange or red colours with the dipyrrylmethanes.

Benzyl-5-t-butoxycarbonyl-3,4'-dimethyl-4-ethyl-3'-(2-ethoxycarbonylethyl)-dipyrrylmethane-5'-carboxylate (26)

A solution of 1.16 g (3 mmol) of acetate 13[8], 0.63 g (3 mmol) of pyrrole 17[4] and 72 mg of p-toluensulfonic acid in 72 ml of dry methylene chloride was heated at 40° during 4 h while stirred with a stream of N_2. The solution was then cooled, washed with water, then with a 5% sodium bicarbonate solution, again with water, dried (Na_2SO_4) and evaporated to dryness. The residue dissolved in a small volume of 2% methanol in benzene was applied on a TLC silica gel column (4 x 30 cm) which had been packed with the same solvent under pressure. The dipyrrylmethane was eluted from the column by using the same solvent and by applying a moderate pressure. The elution fractions were monitored using TLC. The solvent was evaporated to dryness affording an oil residue 1.45 g (90% yield); MS: m/e 536 (M^+, 12.5). ^{13}C NMR, ppm, 172.84 ($\underline{CO}_2C_2H_5$), 161.66 ($\underline{CO}_2CH_2C_6H_5$), 161.07 ($\underline{CO}_2C(CH_3)_3$), 79.75 ($\underline{C}(CH_3)_3$), 63.42 ($C_6H_5\underline{CH}_2$), 60.05 ($CO_2\underline{CH}_2CH_3$), 34.61 ($CH_2\underline{CH}_2CO_2$), 28.13 ($C(\underline{CH}_3)_3$), 22.68 (pyrr-$\underline{CH}_2$-pyrr), 19.14 ($\underline{CH}_2CH_2CO_2$), 18.25 ($\underline{CH}_2CH_3$), 14.88 ($CH_2\underline{CH}_3$), 13.83 ($CO_2CH_2\underline{CH}_3$), 10.42 ($CH_3$-4'), 8.27 ($CH_3$-3).

Benzyl-5-t-butoxycarbonyl-3',4-dimethyl-3,4'-diethyl-dipyrrylmethane-5'-carboxylate (29)

This dipyrrylmethane was prepared following the procedure described for the synthesis of 26 by condensation of the acetate 15 (0.945 g, 3 mmol) with pyrrole 16[4] (0.63 g, 3 mmol) which gave 1.02 g (73.3%) of dipyrrylmethane 29, that was recrystallized from methanol-water mp 55-57°; MS: m/e 464 (M^+, 8.1). ^{13}C NMR, ppm, 161.49 ($\underline{CO}_2CH_2C_6H_5$), 161.31 ($\underline{CO}_2C(CH_3)_3$), 80.13 ($\underline{C}(CH_3)_3$), 65.54 ($C_6H_5\underline{CH}_2$), 28.38 ($C(\underline{CH}_3)_3$), 22.96 (pyrr-$\underline{CH}_2$-pyrr), 18.42 ($\underline{CH}_2CH_3$-4'), 17.10 ($\underline{CH}_2CH_3$-3), 15.23 ($\underline{CH}_3CH_2$-3), 14.94 ($\underline{CH}_3CH_2$-4'), 10.32 ($CH_3$-4), 8.38 ($CH_3$-3').

t-Butyl-3,4'-dimethyl-4-ethyl-3'-(2-ethoxycarbonylethyl)-5'-carboxydipyrrylmethane-5-carboxylate (27)

0.910 g of dipyrrylmethane 26 was dissolved in 70 ml of tetrahydrofuran containing 0.04 ml of triethylamine and was reduced with hydrogen at 50 psi over 0.450 g of 10% Pd on charcoal during 3 h. The catalyst was filtered, the solvent was evaporated to dryness in vacuo and the solid residue was crystallized from ethanol-water mp 133° (0.72 g, 95% yield); MS: m/e 446 (M^+, 39). ^{13}C NMR, ppm, 172.83 ($\underline{CO_2}CH_2CH_3$), 165.69 (CO_2H), 163.20 ($\underline{CO_2}C(CH_3)_3$), 81.24 ($\underline{C}(CH_3)_3$), 60.08 ($CO_2\underline{CH_2}CH_3$), 35.09 ($CH_2\underline{CH_2}CO_2$), 28.20 ($C(\underline{CH_3})_3$), 22.47 (pyrr-$\underline{CH_2}$-pyrr), 19.62 ($\underline{CH_2}CH_2CO_2$), 18.60 ($\underline{CH_2}CH_3$), 14.95 ($\underline{CH_3}CH_2$), 13.98 ($CO_2CH_2\underline{CH_3}$), 10.50 ($CH_3$-4'), 8.49 ($CH_3$-3).

t-Butyl-3',4-dimethyl-3,4'-diethyl-5'-carboxy-dipyrrylmethane-5-carboxylate (30)

The dipyrrylmethane 29 was reduced following the procedure described for 27 with 95% yield, mp 115° dec (ethanol-water). MS: m/e 374 (M^+, 7.4). ^{13}C NMR, ppm, 171.51 (CO_2H), 164.54 ($\underline{CO_2}C(CH_3)_3$), 81.18 ($\underline{C}(CH_3)_3$), 28.43 ($C(\underline{CH_3})_3$), 22.52 (pyrr-$\underline{CH_2}$-pyrr), 17.53 ($\underline{CH_2}CH_3$-4'), 17.27 ($\underline{CH_2}CH_3$-3), 15.48 ($\underline{CH_3}CH_2$-3), 14.48 ($\underline{CH_3}CH_2$-4'), 10.75 ($CH_3$-4), 8.74 ($CH_3$-3').

t-Butyl-3,4'-dimethyl-4-ethyl-3'-(2-ethoxycarbonylethyl)-5'-formyldipyrrylmethane-5-carboxylate (28)

p-Toluensulfonic acid hydrate (0.4 g) dissolved in 8 ml of dry methanol and 20 ml of dry methylene chloride was added to a solution of 0.43 g of dipyrrylmethane acid 27 in 20 ml of dry methylene chloride and the mixture was stirred during 2.5 h under N_2. The solution was washed with water (20 ml), 5% aqueous sodium bicarbonate (2 x 20 ml) and water (20 ml). The organic phase was dried (Na_2SO_4), evaporated to dryness in vacuo and the unsaturated dipyrrylmethane thus obtained (0.370 g, 95.6% yield) was dissolved in 11 ml of dry dimethylformamide and 0.53 ml of benzoyl chloride was added to the cooled solution at 5° during 30 minutes. After 3 h at 20°, the solution was diluted with ethyl ether (20 ml) and extracted with water (3 x 10 ml). The aqueous extracts were reextracted with ether (1 x 10 ml) and were adjusted to pH 8 with 10% sodium carbonate solution. After 18 h at 20° the solution was cooled at 5°, filtered and the solid residue was recrystallized from ethanol-water, mp 104-106°, 0.170 g (43% yield). Found: C, 66.98; H, 5.59; N, 3.26. $C_{24}H_{24}NO_5$ requires: C, 66.75; H, 5.73; N, 3.45. MS: m/e 430 (M^+ 71.2). ^{13}C NMR, ppm, 176.69 (CHO), 172.81 ($\underline{CO_2}CH_2CH_3$), 161.23 ($\underline{CO_2}C(CH_3)_3$), 79.94 ($\underline{C}(CH_3)_3$), 60.34 ($CO_2\underline{CH_2}CH_3$), 34.71 ($CH_2\underline{CH_2}CO_2$), 28.37 ($C(\underline{CH_3})_3$), 22.88 (pyrr-$\underline{CH_2}$-pyrr), 19.15 ($\underline{CH_2}CH_2CO_2$), 18.42 ($\underline{CH_2}CH_3$), 15.04 ($CH_2\underline{CH_3}$), 14.07 ($CO_2CH_2\underline{CH_3}$), 8.62 ($CH_3$-4'), 8.23 ($CH_3$-3).

Benzyl 3-ethyl-4-t-butoxycarbonyl-5-methyl-pyrrole-2-carboxylate (31)

A solution of 7.2 g of sodium nitrite in 26 ml of water was slowly added with stirring to a solution of 20.6 g (0.1 mol) of benzyl propionylacetate in 30 ml of acetic acid kept below 5°. The resulting solution was kept at 5° during 18 h and was slowly added to a stirred solution of 15.82 g (0.1 mol) of t-butyl acetoacetate in 20 ml of acetic acid, while a mixture of zinc powder (18 g) and sodium acetate (18 g) was also simultaneously added. After the additions were completed, the resulting mixture was stirred and heated at 65° during 1 h. The mixture was poured over 1:1 of ice-water, the precipitate was filtered, dried and crystallized twice from methanol; 13.7 g (40% yield); mp 98-100°. Found: C, 69.82; H, 7.18; N, 4.20. $C_{20}H_{25}NO_4$ requires: C, 69.97; H, 7.28; N, 4.08. MS: m/e 343 (M^+, 26.5). ^{13}C NMR, ppm, 164.35 ($\underline{CO_2}C(CH_3)_3$), 161.35 ($\underline{CO_2}CH_2C_6H_5$), 79.76 ($\underline{C}(CH_3)_3$), 65.84 ($C_6H_5\underline{CH_2}$),

28.25 ($C(\underline{CH_3})_3$), 18.92 ($\underline{CH_2}CH_3$), 15.55 ($\underline{CH_3}CH_2$), 14.03 (CH_3).

2-Benzyloxycarbonyl-3-ethyl-5-methyl-pyrrole-4-carboxylic acid (32)

4 g of 31 were dissolved in 20 ml of acetic acid and 20 ml of a solution of 33% hydrobromic acid in acetic acid was added. The mixture kept during 4 h at room temperature, poured over 1:1 of ice-water and the precipitate filtered and washed with could water. Recrystallization from methanol-water afforded 3.2 g (95% yield), mp 242°. MS: m/e 287 (M^+, 31.9). ^{13}C NMR (NaOD), ppm, 176.26 (CO_2), 170.71 ($\underline{CO_2}CH_2$), 64.69 ($C_6H_5\underline{CH_2}$), 18.76 ($\underline{CH_2}CH_3$), 16.78 ($\underline{CH_3}CH_2$), 12.80 (CH_3).

Benzyl 3-ethyl-4,5-dimethyl-pyrrole-2-carboxylate (33)

Boron trifluoride etherate (20 ml) was slowly added to a cooled stirred solution of 1 g of the acid pyrrole 32 and 2 g of sodium borohydride in 80 ml of dry tetrahydrofuran. The mixture was kept at room temperature overnight, it was then cooled below 5°, adjusted to pH 4 with 5% hydrochloric acid and extracted with chloroform (3 x 100 ml). The extracts were washed with water, dried (Na_2SO_4), evaporated to dryness and the residue was crystallized twice from methanol-water; 0.892 (56% yield); mp 87° (lit[10] 89-90°). MS: m/e 257 (M^+ 42.6%). ^{13}C NMR, ppm, 161.18 ($\underline{CO_2}CH_2C_6H_5$), 65.24 ($C_6H_5\underline{CH_2}$), 18.42 ($\underline{CH_2}CH_3$), 15.00 ($\underline{CH_3}CH_2$), 11.21 ($CH_3$-4), 8.35 ($CH_3$-5).

Ethyl 3-ethyl-4-t-butoxycarbonyl-5-methyl-pyrrole-2-carboxylate (34)

A solution of 9.2 g of sodium nitrite in 18 ml of water was slowly added with stirring to a solution of 18.4 g (12.7 ml) of ethyl propionylacetate in 38 ml of acetic acid, kept below 5°. The resulting solution was kept at 5° during 18 h and was slowly added to a stirred solution of 20.21 g (12.7 ml) of t-butyl acetoncetate in 25.5 ml of acetic acid, while a mixture of zinc powder (24 g) and sodium acetate (24 g) was also simultaneously added. After the additions were completed, the resulting mixture was stirred and heated at 70° during 1.5 h. The mixture was poured over 1:1 of ice-water, the precipitate was filtered, dried and crystallized from methanol; 12.56 g (35% yield); mp 98-99°. Found: C, 64.20; H, 8.05; N, 4.75. $C_{15}H_{23}NO_4$ requires: C, 64.05; H, 8.18; N, 4.98. MS: m/e 281 (M^+, 20). ^{13}C NMR, ppm, 164.42 ($\underline{CO_2}CH_2CH_3$); 161.91 ($\underline{CO_2}C(CH_3)_3$); 139.21, 137.02, 116.81, 113.64 (arom); 79.32 ($\underline{C}(CH_3)_3$); 59.99 ($CO_2\underline{CH_2}CH_3$); 28.14 ($C(\underline{CH_3})_3$); 18.81 ($\underline{CH_2}CH_3$); 15.33 ($CH_2\underline{CH_3}$) 14.02 ($\underline{CH_3}CH_2CO_2$); 13.84 (CH_3).

2-Ethyloxycarbonyl-3-ethyl-5-methyl-pyrrole-4-carboxylic acid (35)

The pyrrole 34 was hydrolized following the procedure described for 32. 3.09 g (96.6% yield) mp 230°. Found: C, 58.55; H, 6.57; N, 6.10. $C_{11}H_{15}NO_4$ requires: C, 58.67; H, 6.67; N, 6.22. ^{13}C NMR (NaOD), ppm, 175.26 (CO_2); 163.67 ($\underline{CO_2}CH_2CH_3$); 137.67, 137.25, 120.70, 116.19 (arom); 61.48 ($CO_2\underline{CH_2}CH_3$); 19.02 ($\underline{CH_2}CH_3$); 16.09 ($\underline{CH_3}CH_2$); 14.33 ($\underline{CH_3}CH_2CO_2$); 12.92 (CH_3).

Ethyl 3-ethyl-4,5-dimethyl-pyrrole-2-carboxylate (36)

Boron trifluoride etherate (40 ml) was slowly added to a cooled stirred solution of 2g of acid pyrrole 35 and 4 g of sodium borohydride in 180 ml of dry tetrahydrofuran. The mixture was kept at room temperature overnight, it was cooled below 5°, adjusted to pH 4 with 5% hydrochloric acid and extracted with chloroform (3 x 10 ml). The extracts were

washed with water, dried (Na_2SO_4), evaporated to dryness and the residue was crystallized from methanol-water. 1.37 g (79.2% yield); mp 80-81°. MS: m/e 195 (M^+ 100%). ^{13}C NMR, ppm, 162.07 ($\underline{CO_2}CH_2CH_3$), 134.12, 130.15, 116.35, 116.12 (arom), 59.69 ($CO_2\underline{CH_2}CH_3$), 18.73 ($\underline{CH_2}CH_3$), 15.29 ($\underline{CH_3}CH_2$), 14.64 ($\underline{CH_3}CH_2CO_2$), 11.44 (CH_3-4), 8.53 (CH_3-5).

Benzyl 3-ethyl-4-methyl-5-acetoxymethylpyrrole-2-carboxylate (15)

Lead tetraacetate (4 g) was added in small portions, over a period of 2 h, to a stirred solution of 33 (2 g) in 40 ml of glacial acetic acid and the solution was stirred for additional 4 h. The mixture was poured over ice-water, the precipitate was filtered, dried and crystallized twice from acetone water; 2.10 g (65% yield), mp 102°. Found: C, 68.65; H, 6.77; N, 4.22. $C_{18}H_{21}NO_4$ requires: C, 68.57; H, 6.66; N, 4.44. MS: m/e 315 (M^+, 39.7). ^{13}C NMR, ppm, 171.12 ($CH_3\underline{CO_2}$), 161.80 ($\underline{CO_2}CH_2C_6H_5$), 65.53 ($C_6H_5\underline{CH_2}$), 56.81 ($\underline{CH_2}O$), 20.56 ($\underline{CH_3}CO_2$), 18.14 ($\underline{CH_2}CH_3$), 14.85 ($CH_2\underline{CH_3}$), 8.16 ($\underline{CH_3}$).

13,18-diethyl-3,7-di(methoxycarbonylethyl)-2,8,12,17-tetramethyl-1,19-bilindione (mesobiliverdin IX β)(2) and 3,7,13,17-tetra(methoxycarbonylethyl)-2,8,12,18-tetramethyl-1,19-bilindione(coprobiliverdin II β)(3)

To a solution of carboxydipirrylmethane 20[1] 0.0748 g (0.2 mmol) and the formyldipyrrylmethane 23[3] 0.1004 (0.2 mmol) in 40 ml of methylene chloride was added a solution of 0.215 g (0.176 mmol) of anhyd p-toluensulfonic acid in 4.5 ml of dry methanol and the mixture was stirred at room temperature overnight. The solution was then poured over 100 ml of water, the organic phase was separated, washed with 5% aqueous sodium bicarbonate (2 x 50 ml), then with water (2 x 50 ml) dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in 10 ml of dry methylene chloride and 1 ml of methanol containing 0.01 ml of 48% hydrobromic solution was added. The solution was evaporated to dryness in vacuo and the residue dissolved in dry benzene and evaporated to dryness (twice). The red residue was dissolved in anhyd methylene chloride and poured over a column (1.5 x 15 cm) of deactivated alumina (prepared by suspending Merck grade I alumina in methanol, filtering and drying in air), prewashed with methylene chloride. The bilene (yellow band) was eluted with the same solvent and was collected in a mixture of 1 ml of methanol and a drop of 48% hydrobromide acid. The eluates were evaporated to dryness and the red residue was redissolved in dry ethyl ether and the solution was again evaporated to dryness. Bright red crystals of b-bilene hydrobromide was obtained (0.161 g, 90% yield) λ_{max} 510 nm. ^1H NMR, ppm, 7.15 (b, 1H, CH=), 4.4 (s, 4H, pyrr-$\underline{CH_2}$-pyrr), 4.1 (m, 4H, $CO_2\underline{CH_2}$), 2.65 (m, 12H, $\underline{CH_2}CH_2CO$ and $\underline{CH_2}CH_3$), 2.30 (s, 6H, $2CH_3$), 2.25 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 1.65 (b, 18H, $(CH_3)_3$), 1.35 (m, 12H, $\underline{CH_3}CH_2$ and $\underline{CH_3}CH_2CO_2$).

The b-bilene hydrobromide were dissolved in 20 ml of previously degassed (N_2) trifluoracetic acid, the solution was cooled at 5° and 0.02 ml of bromine was added over a period of 40 minutes while the solution was kept under N_2. The solution was poured over solid sodium bicarbonate, cooled degassed water was added (200 ml) and extracted with nitrogen purged chloroform (3 x 50 ml). The organic layer washed with water, dried (Na_2SO_4) and evaporated. The residue was dissolved in a small volume of 10% acetone in chloroform and was filtered through a TLC silica gel column (3.5 x 24 cm) packed and prewashed with the

same solvent. The blue bands were eluted using the same solvent under slight pressure, the eluates were evaporated to dryness and each residue was dissolved in 5 ml of 5% sulfuric acid in methanol and kept at 5° overnight. Each solution was diluted with chloroform and then washed with 5% sodium bicarbonate solution, water, dried (Na_2SO_4) and evaporated to dryness. Mesobiliverdin IX β (Rf 0.5) was recrystallized from methylene chloride-hexane, mp 218° (lit[11] mp 220–221°) gave blue crystals (18 mg, 14.6% yield). Found: C, 68.20; H, 6.75; N, 9.03. $C_{35}H_{42}N_4O_6$ requires: C, 68.40; H, 6.84; N, 9.12. MS: m/e 614 (M^+, 100%). ^{1}H NMR, ppm, 6.65 (s, 1H, H-10), 5.95 (s, 1H, H-5), 5.85 (s, 1H, H-15), 3.67 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 2.55 (m, 12H, $\underline{CH_2}\underline{CH_2}CO$ and $\underline{CH_2}CH_3$), 2.17 (s, 3H, CH_3-8), 2.15 (s, 3H, CH_3-12), 2.09 (s, 3H, CH_3-17), 1.85 (s, 3H, CH_3-2), 1.15 (m, 6H, CH_3-13b and 18b); λ_{max} 363 nm (ε 43,659), 655 nm (ε 14,430).

Coprobiliverdin II β (Rf 0.26) was recrystallized from methylene chloride-hexane mp 115° gave deep blue crystals (5.1 mg, 4% yield). Found: C, 64.20; H, 7.16; N, 7.48. $C_{39}H_{53}N_4O_{10}$ requires: C, 64.11; H, 7.26; N, 7.67. MS: m/e 730 (M^+, 20.3), 360 (100). ^{1}H NMR, ppm, 6.72 (s, 1H, H-10), 6 (s, 2H, H-5 and H-15), 3.71 (s, 6H, $2OCH_3$), 3.67 (s, 6H, $2OCH_3$), 2.6 (m, 16H, CH_2CH_2CO), 2.2 (s, 6H, CH_3-8 and CH_3-12), 1.85 (s, 6H, CH_3-2 and CH_3-18); λ_{max} 363 nm (ε 62,796), 650 nm (ε 21,431).

2,17-diethyl-7,13-di(2-methoxycarbonylmethyl)-3,8,12,18-tetramethyl-1,19-bilindione (mesobiliverdin XI β)(5) and 3,17-diethyl-7,13-di(2-methoxycarbonylmethyl)-2,8,12,18-tetramethyl-1,19-bilindione (mesobiliverdin IV β)(6)

Was obtained by condensation of 0.892 g (0.2 mmol) of carboxydipyrrylmethane 25[4] and 0.860 g (0.2 mmol) of formyldipyrrylmethane 28 to give the b-bilene hydrobromide which was oxidized as described above, affording the mesobiliverdin XI β and the mesobiliverdin IV β. Mesobiliverdin XI β (Rf 0.45) was crystallized from methylene chloride-hexane mp 205–206° gave blue prisms (20 mg, 16.3% yield). Found: C, 68.31; H, 6.70; N, 9.05. $C_{35}H_{42}N_4O_6$ requires: C, 68.40; H, 6.84; N, 9.12. MS: m/e 614 (M^+, 100). ^{1}H NMR, ppm, 6.65 (s, 1H, H-10), 5.9, 5.92 (s, s, 1H, 1H, H-5 and H-15), 3.66 (s, 6H, OCH_3), 2.8 (d, 4H, CH_2CH_2CO), 2.55 (d, 8H, $\underline{CH_2}CH_2CO$ and $\underline{CH_2}CH_3$), 2.2 (s, 6H, CH_3-8 and 12), 2.1 (s, 3H, CH_3-3), 1.82 (s, 3H, CH_3-18), 1.2 (m, 6H, $\underline{CH_3}CH_2$). λ_{max} 360 nm (ε 41,066), 649 nm (ε 13,689).

Mesobiliverdin IV β was crystallized from methylene chloride-hexane mp 220°(Rf 0.35), gave blue prisms (4 mg, 3.3% yield). Found: C, 68.52, H, 6.90; N, 9.15. $C_{35}H_{42}N_4O_6$ requires: C, 68.40; H, 6.84; N, 9.12. MS: m/e 614 (M^+, 100). ^{1}H NMR, ppm, 6.7 (s, 1H, H-10), 5.95 (s, 2H, H-5 and 15), 3.65 (s, 6H, OCH_3), 2.8 (d, 4H, $CH_2\underline{CH_2}CO$), 2.5 (d, 8H, $\underline{CH_2}CH_2CO$ and $\underline{CH_2}CH_3$), 2.22 (s, 6H, CH_3-8 and 12), 1.83 (s, 6H, CH_3-2 and 18), 1.25 (m, 6H, $CH_2\underline{CH_3}$). λ_{max} 361 nm (ε 29,600), 652 nm (ε 10,930).

12,17-diethyl-3,7-di(methoxycarbonylethyl)-2,8,13,18-tetramethyl-1,19-bilindione(mesobiliverdin IX δ)(8)

Was obtained by condensation of 0.0748 g (0.2 mmol) of carboxylic dipyrrylmethane 30 and 0.1004 g (0.2 mmol) of formyldipyrrylmethane 23[3] to give the b-bilene hydrobromide which was oxidized as described above to give mesobiliverdin IX δ and coprobiliverdin II β.

Mesobiliverdin IX δ (Rf 0.6) was crystallized from methylene chloride-hexane mp 188–

190° gave dark blue prisms (25 mg, 20% yield). Found: C, 68.20; H, 6.50; N, 9.07. $C_{35}H_{42}N_4O_6$ requires: C, 68.40; H, 6.84; N, 9.12. MS: m/e 614 (M^+, 100%). ^{1}H NMR, ppm, 6.7 (s, 1H, H-10) 6 (s, 1H, H-5), 5.92 (s, 1H, H-15), 3.7, 3.65 (s, s, 3H, 3H, OCH_3), 2.65 (m, 12H, CH_2CH_2CO and $\underline{CH}_2CH_3$), 2.20 (s, 3H, CH_3-8), 2.07 (s, 3H, CH_3-13), 1.85 (s, 3H, CH_3-2), 1.80 (s, 3H, CH_3-18), 1.1 (m, 6H, $\underline{CH}_3CH_2$), λ_{max} 362 nm (ε 37,940), 650 nm (ε 13,671).

Coprobiliverdin II β (Rf 0.22) was recrystallized from methylene chloride-hexane, mp 115° as deep blue crystals (4 mg, 3.2% yield).

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REFERENCES

1. Smith, K.M. and Kishore, D. Tetrahedron. 1983, 39, 1841-1847.
2. Smith, K.M. and Pandey, R.K. Tetrahedron. 1984, 40, 1749-1755.
3. Valasinas, A.; Sambrotta, L.; Díaz, L.E. and Frydman, B. J.Org.Chem. 1986, 51, 3001-3006.
4. Awruch, J. and Frydman, B. Tetrahedron. 1986, 42, 4137-4146.
5. Iturraspe, J.B.; Bari, S. and Frydman, B. J.Am.Chem.Soc. 1989, 111, 1525-1527.
6. For nomenclature of bilene pigments see: R.Bonnett. In The Porphyrins (Edited by D. Dolphin)(loc.cit.) Vol.1, p.1, Academic Press, New York (1979).
7. Clezy, P.S. and Fookes, C.J.R. Aus.J.Chem. 1974, 27, 371-382.
8. Díaz, L.; Buldain, G. and Frydman, B. J.Org.Chem. 1979, 44, 973-977.
9. Cavaleiro, J.A.S.; Kenner, G.W. and Smith, K.M. J.Chem.Soc. Perkin I. 1973, 2478-2485.
10. Ellis, J.; Jackson, A.H.; Jain, A.C. and Kenner, G.W. J.Chem.Soc. 1964, 1935-1949.
11. Jackson, A.H.; Kenner, G.W. and Smith, K.M. J.Chem.Soc. C. 1968, 302-310.