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THE TOTAL SYNTHESIS OF BILIVERDINS OF BIOLOGICAL INTEREST

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Abstract.- The total synthesis of eight biliverdin isomers was achieved by oxidation of the corresponding 1,19-di-t-butyloxycarbonyl-b-bilenes. One of the biliverdin isomers is mesobiliverdin IXα – a dipropionate bilitriene –, one is a diacetate bilitriene, three isomers are mono-propionate-mono-acetate bilitrienes, and three are mono-propionate isomers. Their synthesis will allow a study of the substrate specificity of biliverdin reductase.

Biliverdins are natural products which are formed by the oxidative breakdown of heme IX[1]. The most common natural biliverdin is biliverdin IXα[2], although biliverdin IXβ was also isolated from biological media[3], and biliverdin IXδ (pterobilin) is a pigment of lepidopters[4]. Urobiliverdin I (which is not derived from heme IX) was recently isolated from prokaryotes and its structure was established by total synthesis[5,6]. Biliverdin IXα is formed in nature by the action of a heme oxygenase which selectively oxidizes the α-meso bridge of heme[7], and is excreted without further change in the lower vertebrates[1], while in algae and plants it is incorporated as the prosthetic group of biliproteins[8]. In mammals biliverdin IXα (an a,b,c-bilatriene) is reduced to bilirubin IXα (an a,c-biladiene) by a biliverdin reductase; an important step in the catabolism of heme to the urobilinoids[9]. The substrate specificity and properties of biliverdin reductase were little explored in the past, mainly due to the lack of suitable synthetic substrates of the enzyme. During our extensive studies on the specificity of the heme oxygenase-biliverdin reductase system we came to the conclusion that while the substrate specificity of heme oxygenase is confined to hemins carrying two vicinal propionic acid residues at C-6 and C-7[7], biliverdin reductase seems to have a broader substrate specificity and reduces a large variety of bilatrienes to biladienes[10]. A more accurate study of the specificity of biliverdin reductase required the synthesis of a fairly large number of biliverdins with different side chains, in order to establish the structural requirements for a substrate of biliverdin reductase.

The synthesis of biliverdins was based until recently on a stepwise approach using 3-pyrrolin-2-ones and 1(1OH)-dipyrrinones, which made possible the synthesis of a large number of biliverdins and its analogues[11]. More recently, a synthetic approach to biliverdins was developed based on the controlled oxidation of 1,19-di-t-butyloxycarbonyl-b-bilene and a,c-biladiene hydrobromides[12]. Although this oxidation gives only moderate to low yields of biliverdins, it has nevertheless the advantage of using many of the pyrrylmethane intermediates which are also used for porphyrin synthesis, thus enhancing their value as synthetic intermediates of both biliverdins and porphyrins. The oxidation of b-bilenes was recently used for the synthesis of urobiliverdins and coprobiliverdins[13]. In this report we show its usefulness to prepare eight biliverdin isomers to be used as potential substrates of biliverdin reductase.

Biliverdin 39 (Scheme 2) is mesobiliverdin IXα and carries two propionates at C-8 and C-12, the positions of the natural biliverdins of type IX. In biliverdin 38 the two propionates were replaced by two acetates, in 37 only one of the two propionates (at C-8) was replaced by an acetate, in 36 one propionate is at the C-2 exo-position (i.e., α to amide group), and one acetate is at the other exo-position (C-18), in 35 one propionate is at C-2 (exo) and one acetate is at an endo-position (C-7); while biliverdins 32-34 carry only one propionate.

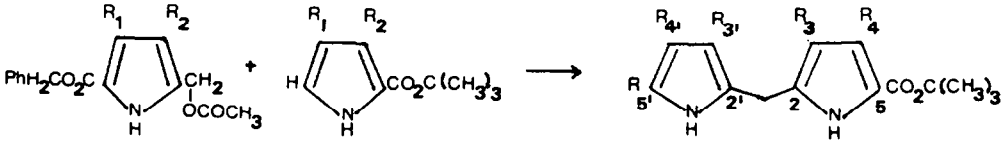
The synthesis of these biliverdins was achieved by condensation, following established procedures[14], of α-unsubstituted dipyrrylmethanes with α-formyldipyrrylmethanes (Scheme 1) which gave the corresponding b-bilene hydrobromides. The α-unsubstituted dipyrrylmethanes were obtained in situ by decarboxylation of the corresponding α-carboxydipyrrylmethanes (Scheme 1) with p-toluen-

sulfonic acid in an aprotic solvent. The b-bilenes (Scheme 2) were treated with trifluoroacetic acid to cleave and decarboxylate the t-butyl ester residues at C-1 and C-19, and were oxidized with bromine as described elsewhere[12]. The biliverdin esters were obtained after hydrolysis under mild alkaline conditions in average 15% yield from the two dipyrrylmethane halves. Biliverdin 32 was obtained from the condensation of the carboxydipyrrylmethane 15 and the formyldipyrrylmethane 22; biliverdin 33 was obtained by condensation of 13 and 22, biliverdin 34 by condensation of 17 and 22, biliverdin 35 by condensation of 19 and 22, biliverdin 36 by condensation of 17 and 25, biliverdin 37 by condensation of 11 and 28, biliverdin 38 by condensation of 11 and 31, and finally biliverdin 39 by condensation of 13 and 28.

The biliverdin esters were characterized by their ^{1}H NMR spectra, their mass spectra and their uv and vis spectra. The latter had the expected ε vis/ ε uv ratios of 0.20 to 0.30, which are typical of all the all-Z cyclic species[15]. In acid media the spectra changed to ε vis/ ε uv ratios of 0.50-1.00 typical of the stretched E-isomers were the spectra resemble those of an elongated polyene structure[13,15].

The synthesis of the α-benzyloxycarbonyl-dipyrrylmethanes 10, 12, 14, 16, 18, 20, 23, 26 and 29 (Scheme 1) was achieved by condensation of the 2-acetoxymethylpyrroles 1-5 with the 2-t-butyloxycarbonylpyrroles 6-9 (Scheme 1) following procedures which have been extensively discussed by us and by others[12,14]. Hydrogenolysis of the benzyl esters afforded the corresponding α-carboxydipyrrylmethanes. They were decarboxylated to α-unsubstituted dipyrrylmethanes by treatment with p-toluensylfonic acid in an aprotic solvent. When the obtention of α-formyldipyrrylmethanes were needed the α-unsubstituted dipyrrylmethane were formylated using dimethylformamide and benzoyl chloride in a modified Vilsmeier type reaction.

SCHEME 1



1, $R_1 = A^{Me}$; R_2 = Me
2, $R_1 = P^{Me}$; R_2 = Me
3, R_1 = Me ; $R_2 = P^{Me}$
4, R_1 = Me ; R_2 = Et
5, R_1 = Me ; $R_2 = A^{Et}$

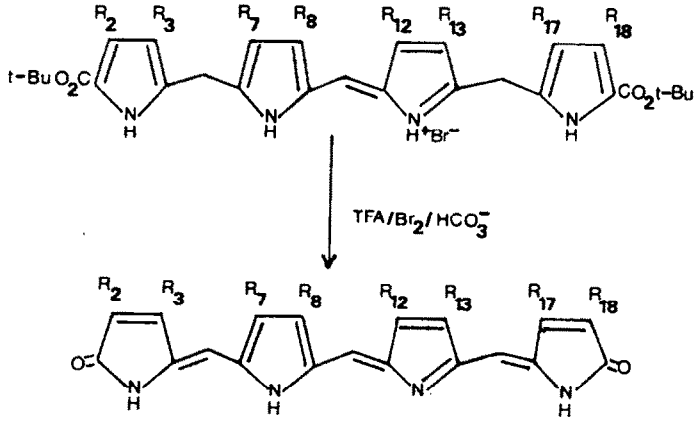
6, R_1 = Et; R_2 = Me
7, R_1 = Me; $R_2 = P^{Et}$
8, R_1 = Me; $R_2 = A^{Et}$
9, R_1 = Me; R_2 = Et

10, R = CO_2CH_2Ph; R_3 = R_4 = Me; R_3 = Et; $R_4 = A^{Me}$
11, R = CO_2H; R_3 = R_4 = Me; R_3 = Et; $R_4 = A^{Me}$
12, R = CO_2CH_2Ph; R_3 = R_4 = Me; R_3 = Et; $R_4 = P^{Me}$
13, R = CO_2H; R_3 = R_4 = Me; R_3 = Et; $R_4 = P^{Me}$
14, R = CO_2CH_2Ph; R_4 = R_4 = Me; R_3 = Et; $R_3 = P^{Et}$
15, R = CO_2H; R_4 = R_4 = Me; R_3 = Et; $R_3 = P^{Et}$
16, R = CO_2CH_2Ph; R_4 = R_3 = Me; R_3 = Et; $R_4 = P^{Et}$
17, R = CO_2H; R_4 = R_3 = Me; R_3 = Et; $R_4 = P^{Et}$
18, R = CO_2CH_2Ph; R_4 = R_3 = Me; $R_3 = A^{Et}$; $R_4 = P^{Et}$
19, R = CO_2H; R_4 = R_3 = Me; $R_3 = A^{Et}$; $R_4 = P^{Et}$
20, R = CO_2CH_2Ph; R_4 = R_4 = Me; R_3 = R_3 = Et
21, R = CO_2H; R_4 = R_4 = Me; R_3 = R_3 = Et
22, R = CHO; R_4 = R_4 = Me; R_3 = R_3 = Et
23, R = CO_2CH_2Ph; R_4 = R_3 = Me; R_3 = Et; $R_4 = A^{Et}$
24, R = CO_2H; R_4 = R_3 = Me; R_3 = Et; $R_4 = A^{Et}$
25, R = CHO; R_4 = R_3 = Me; R_3 = Et; $R_4 = A^{Et}$
26, R = CO_2CH_2Ph; $R_4 = P^{Me}$; R_3 = R_3 = Me; R_4 = Et
27, R = CO_2H; $R_4 = P^{Me}$; R_3 = R_3 = Me; R_4 = Et
28, R = CHO; $R_4 = P^{Me}$; R_3 = R_3 = Me; R_4 = Et
29, R = CO_2CH_2Ph; $R_4 = A^{Me}$; R_3 = R_3 = Me; R_4 = Et
30, R = CO_2H; $R_4 = A^{Me}$; R_3 = R_3 = Me; R_4 = Et
31, R = CHO; $R_4 = A^{Me}$; R_3 = R_3 = Me; R_4 = Et

Me = CH_3; Et = C_2H_5; $A^{Me(Et)} = CH_2CO_2CH_3(C_2H_5)$; $P^{Me(Et)} = CH_{a}CH_{b}CO_2CH_3(C_2H_5)$

Several of the pyrroles 1-19 were known from the pyrrole literature. The others were prepared by methods which have became usual in pyrrole chemistry. Pyrrole 40 (Chart 1) was prepared by a Knorr synthesis from benzyl acetoacetate and 2,4-pentanedione and was reduced with diborane to give 41 which was then acetoxylated with lead tetraacetate to 4. The preparation of the iodopyrrole 43 was recently reported[16] and by reduction with zinc and acetic acid it was transformed into 44, which by hydrogenolysis gave 45. Reesterification of 45 with t-butanol and dicyclohexycarbodiimide gave 6 (Scheme 1). Esterification of the known[17] carboxypyrrole 42 with isobutylene-sulfuric acid gave the ester 46, which was reduced with hydrogen to the acid 47 as described elsewhere[18], and the latter was decarboxylated with iodine to give 48, which was finally reduced with hydrogen to 9.

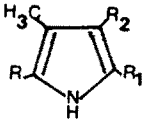
SCHEME 2



32, $R_2 = R_8 = R_{12} = R_{18} = CH_3$; $R_3 = R_{13} = R_{17} = C_2H_5$; $R_7 = P^{Et}$
33, $R_2 = R_7 = R_{12} = R_{18} = CH_3$; $R_3 = R_{13} = R_{17} = C_2H_5$; $R_8 = P^{Et}$
34, $R_3 = R_8 = R_{12} = R_{18} = CH_3$; $R_7 = R_{13} = R_{17} = C_2H_5$; $R_2 = P^{Et}$
35, $R_3 = R_8 = R_{12} = R_{18} = CH_3$; $R_{13} = R_{17} = C_2H_5$; $R_2 = P^{Et}$; $R_7 = A^{Et}$
36, $R_3 = R_8 = R_{12} = R_{17} = CH_3$; $R_7 = R_{13} = C_2H_5$; $R_2 = P^{Et}$; $R_{18} = A^{Et}$
37, $R_2 = R_7 = R_{13} = R_{17} = CH_3$; $R_3 = R_{18} = C_2H_5$; $R_8 = A^{Me}$; $R_{12} = P^{Me}$
38, $R_2 = R_7 = R_{13} = R_{17} = CH_3 = R_3 = R_{18} = C_2H_5$; $R_8 = R_{12} = A^{Me}$
39, $R_2 = R_7 = R_{13} = R_{17} = CH_3$; $R_3 = R_{18} = C_2H_5$; $R_8 = R_{12} = P^{Me}$

Me = CH_3; Et = C_2H_5; $A^{Me(Et)} = CH_2CO_2CH_3(C_2H_5)$; $P^{Me(Et)} = \underset{a}{CH_2}\underset{b}{CH_2}CO_2CH_3(C_2H_5)$

CHART 1



40, $R_1 = CH_3$; $R_2 = COCH_3$; $R = CO_2CH_2Ph$
41, $R_1 = CH_3$; $R_2 = CH_2CH_3$; $R = CO_2CH_2Ph$
42, $R_1 = CO_2H$; $R_2 = CH_2CH_3$; $R = CO_2CH_2Ph$
43, $R_1 = I$; $R_2 = CH_2CH_3$; $R = CO_2CH_2Ph$
44, $R_1 = H$; $R_2 = CH_2CH_3$; $R = CO_2CH_2Ph$
45, $R_1 = H$; $R_2 = CH_2CH_3$; $R = CO_2H$
46, $R_1 = CO_2C(CH_3)_3$; $R_2 = CH_2CH_3$; $R = CO_2CH_2Ph$
47, $R_1 = CO_2C(CH_3)_3$; $R_2 = CH_2CH_3$; $R = CO_2H$
48, $R_1 = CO_2C(CH_3)_3$; $R_2 = CH_2CH_3$; $R = I$

EXPERIMENTAL

Melting points were determined on a Kofler melting point apparatus and are uncorrected. ^{1}H 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 $CHCl_3$ 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). 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.

t-Butyl 4,3'-dimethyl-3-ethyl-4'-(methoxycarbonylmethyl)-5'-carboxy-dipyrrylmethane-5-carboxylate (11)

A solution of 1.08 g (3 mmol) of acetate 1[19], 0.63 g (3 mmol) of pyrrole 6, and 70 mg of p-toluensulfonic acid in 70 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, diluted with 70 ml of methylene chloride, 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. Dipyrrylmethane 10 was eluted from the column by using the same solvent and by applying a moderate pressure. The elution fractions containing 10 were monitored using TLC. The solvent was evaporated to dryness and the residue (1.37 g (90%); MS: m/e = 508 (M^+, 40)) was dissolved in 160 ml of tetrahydrofuran containing 0.2 ml of triethylamine, and was reduced with hydrogen at 50 psi over 0.7 g of 10% Pd on charcoal during 3 h. The catalyst was filtered, the solvent was evaporated to dryness in vacuo, and the residue dissolved in 5% methanol in chloroform was purified by filtration through a TLC silica gel column as described above; 0.89 g (80%) of 11 was obtained; mp 168-170° (ethanol-water). Found: C, 63.10; H, 7.20; N, 6.50. $C_{22}H_{30}N_2O_6$ requires: C, 63.16; H, 7.18; N, 6.70. ^{13}C NMR, ppm, 171.91 ($\underline{C}O_2CH_3$), 165.26 (CO_2H), 163.46 ($\underline{C}O_2$t-Bu), 81.36 ($\underline{C}(CH_3)_3$), 51.52 (OCH_3), 30.71 ($\underline{CH}_2CO$), 28.19 ($(CH_2)_3$), 22.22 (pyrr-$\underline{CH}_2$-pyrr), 17.15 ($\underline{CH}_2CH_3$), 15.62 ($\underline{CH}_3CH_2$), 10.69 (CH_3-4), 8.77 (CH_3-3').

t-Butyl 4,3'-dimethyl-3-ethyl-4'-(2-methoxycarbonylethyl)-5'-carboxy-dipyrrylmethane-5-carboxylate (13)

This dipyrrylmethane was prepared following the procedure described for the synthesis of 11 by condensation of the acetate 2[20] (1.12 g, 3 mmol) with pyrrole 6 (0.63 g, 3 mmol) which gave 0.78 g (50%) of dipyrrylmethane 12 (MS, m/e = 522 (M^+, 45)) which was reduced to give 13; 0.49 (76%); mp 136° (dec)(ethanol-water). Found: C, 63.79; H, 7.30; N, 6.50. $C_{23}H_{32}N_2O_6$ requires: C, 63.89; H, 7.41; N, 6.71. ^{13}C NMR, ppm, 173.39 ($\underline{C}O_2CH_3$), 165.34 (CO_2H), 163.39 ($\underline{C}O_2$t-Bu), 81.40 ($\underline{C}(CH_3)_3$), 51.28 (OCH_3), 34.70 ($\underline{CH}_2CO_2$), 28.32 ((CH_3)$_3$), 22.28 (pyrr-$\underline{CH}_2$-pyrr), 20.58 ($\underline{CH}_2CH_2CO_2$), 17.16 ($\underline{CH}_2CH_3$), 15.71 ($CH_2\underline{CH}_3$), 10.78 (CH_3-4), 8.67 (CH_3-3');MS: m/e = 432 (M^+, 18).

t-Butyl 4,4'-dimethyl-3-ethyl-3'-(2-ethoxycarbonylethyl)-5'-carboxy-dipyrrylmethane-5-carboxylate (15)

Condensation of the acetoxymethylpyrrole 3[21] (1.16 g, 3 mmol) with pyrrole 6 (0.63 g, 3 mmol) following the procedure described for the synthesis of 10 gave 1.4 g (90%) of dipyrrylmethane 14; MS: m/e = 536 (M^+, 41), which was reduced to give 15 as described for the obtention of 11. A 67% yield (0.77 g) of the acid 15 was obtained; mp 89° (ethanol-water). Found: 64.44; H, 7.50; N, 6.11. $C_{24}H_{34}N_2O_6$ requires: C, 64.57; H, 7.62; N, 6.28. ^{13}C NMR, ppm, 172.95 ($\underline{C}O_2$Et), 165.82 (CO_2H), 163.45 ($\underline{C}O_2$t-Bu), 81.36 ($\underline{C}(CH_3)_3$). 60.17 ($CO_2\underline{CH}_2$), 35.08 ($\underline{CH}_2CO_2$), 28.16 ((CH_3)$_3$), 22.12 (pyrr-$\underline{CH}_2$-pyrr), 19.53 (CH_2-3'a), 17.19 (CH_2-3a), 15.74 (CH_3-3b), 14.03 ($CH_2\underline{CH}_3$), 10.79 (CH_3-4'), 10.56 (CH_3-4).

t-Butyl 3,4'-dimethyl-3'-ethyl-4-(2-ethoxycarbonylethyl)-5'-carboxy-dipyrrylmethane-5-carboxylate (17)

Acetoxymethylpyrrole 4 (0.94, 3 mmol) and unsubstituted pyrrole 7 (0.84 g, 3 mmol) were condensed as described for the preparation of 10 and afforded the dipyrrylmethane 16 in 50% yield (0.72 g); MS: m/e = 536 (M^+, 54). The latter was reduced with hydrogen to give the acid 17 following the proced

ure described above; 0.39 g (65%) of 17 were obtained; mp 150° dec (ethanol-water). Found: C, 64.33; H, 7.52; N, 6.10. $C_{24}H_{34}N_2O_6$ requires: C, 64.57; H, 7.62; N, 6.28. ^{13}C NMR, ppm, 173.12 ($\underline{CO_2}Et$), 165.91 (CO_2H), 163.01 ($\underline{CO_2}t$-Bu), 81.83 ($\underline{C}(CH_3)_3$), 60.08 ($CO_2\underline{CH_2}$), 35.28 ($\underline{CH_2}CO_2$), 28.25 ($(CH_3)_3$), 22.38 (pyrr-$\underline{CH_2}$-pyrr), 21.22 (CH_2-4a), 17.28 (CH_2-3'), 15.29 (CH_3-3'), 14.07 ($CH_2\underline{CH_3}$), 10.54 (CH_3-4'), 8.69 (CH_3-3).

t-Butyl 3,4'-dimethyl-4-(2-ethoxycarbonylethyl)-3'-(ethoxycarbonylmethyl)-5'-carboxydipyrrylmethane-5-carboxylate (19)

Condensation of 5[22] (1.12 g, 3 mmol) and 7[23] (0.84, 3 mmol), following the procedure described above afforded 18 (0.96 g, 54%); MS: m/e = 594 (M^+, 12), which was reduced as described above to give 19; 0.65 g (80%); mp 117° (ethanol-water). Found: C, 57.24; H, 7.20; N, 5.60. $C_{26}H_{36}N_2O_8$ requires: C, 57.14; H, 7.14; N, 5.56. ^{13}C NMR, ppm, 173.03 ($\underline{CO_2}Et$-4), 171.48 ($\underline{CO_2}Et$-3'), 165.76 (CO_2H), 162.63 ($\underline{CO_2}t$-Bu), 81.60 ($\underline{C}(CH_3)_3$), 60.60 ($CO_2\underline{CH_2}$-4), 60.00 ($CO_2\underline{CH_2}$-3'), 35.19 ($\underline{CH_2}CO_2$-4), 29.48 ($\underline{CH_2}$-3'a), 28.18 ($(CH_3)_3$), 22.47 (pyrr-$\underline{CH_2}$-pyrr), 20.99 (CH_2-4a), 13.90 ($CH_2\underline{CH_3}$), 10.62 (CH_3-4'), 8.67 (CH_3-3).

t-Butyl 3,3'-diethyl-4,4'-dimethyl-5'-carboxydipyrrylmethane-5-carboxylate (21)

Condensation of the 2-acetoxymethylpyrrole 4 (0.94 g, 3 mmol) with the unsubstituted pyrrole 6 (0.63 g, 3 mmol) following the procedure described for the synthesis of 11 gave 85% of 20 (1.18 g), MS: m/e = 464 (M^+, 10), which was reduced to 21 as described above affording 0.86 g (90% yield) of the latter, mp 75° (ethanol-water). Found: C, 67.27; H, 8.10; N, 7.28. $C_{21}H_{30}NO_4$ requires: C, 67.38; H, 8.02; N, 7.49. ^{13}C NMR, ppm, 165.78 (CO_2H), 163.03 ($\underline{CO_2}t$-Bu), 80.87 ($\underline{C}(CH_3)_3$), 28.08 ($(CH_3)_3$), 22.10 (pyrr-$\underline{CH_2}$-pyrr), 17.04 (CH_2-3a), 15.42 (CH_2-3'a), 15.18 (CH_3-3b), 14.95 (CH_3-3'b), 10.57 (CH_3-4), 10.31 (CH_3-4').

t-Butyl 3,4'-dimethyl-3'-ethyl-4-(ethoxycarbonylmethyl)-5'-carboxy-dipyrrylmethane-5-carboxylate (24)

Condensation of the acetoxymethylpyrrole 4 (0.94 g, 3 mmol) with the unsubstituted pyrrole 8[24] (0.8 g, 3 mmol) following the described procedure afforded 1.08 g (69% yield) of the dipyrrylmethane 23; MS: m/e = 522 (M^+, 38), which was reduced to 24 following the usual procedure; 0.79 g (89% yield) mp 140° dec (ethanol-water). Found: C, 63.78; H, 7.30; N, 6.52. $C_{23}H_{32}N_2O_6$ requires: C, 63.89; H, 7.41; N, 6.71. ^{13}C NMR, ppm, 171.27 ($\underline{CO_2}Et$), 162.14 (CO_2H), 161.39 ($\underline{CO_2}t$-Bu), 80.74 ($\underline{C}(CH_3)_3$), 60.19 ($CO_2\underline{CH_2}$), 29.97 (CH_2-4a), 28.02 ($(CH_3)_3$), 22.34 (pyrr-$\underline{CH_2}$-pyrr), 17.10 (CH_2-3'a), 15.07 ($CH_2\underline{CH_3}$), 13.90 ($CO_2CH_2\underline{CH_3}$), 10.33 (CH_3-4'), 8.67 (CH_3-3).

t-Butyl 3,3'-dimethyl-4-ethyl-4'-(2-methoxycarbonylethyl)-5'-carboxy-dipyrrylmethane-5-carboxylate (27)

Condensation of pyrrole 2 (1.12 g, 3 mmol) and pyrrole 9 (0.8 g, 3 mmol) following the usual procedure afforded the dipyrrylmethane 26 (0.94 g, 60% yield), MS: m/e = 522 (M^+, 68), which was reduced to 27 by the usual procedure; 0.65 g (83% yield); mp 165° (ethanol-water). Found: C, 63.79; H, 7.30; N, 6.52. $C_{23}H_{32}N_2O_6$ requires: C, 63.89; H, 7.41; N, 6.71. ^{13}C NMR, ppm, 173.65 ($\underline{CO_2}CH_3$), 165.39 (CO_2H), 163.09 ($\underline{CO_2}t$-Bu), 81.17 ($\underline{C}(CH_3)_3$), 51.13 (OCH_3), 34.66 ($\underline{CH_2}CO_2$), 28.13 ($(CH_3)_3$), 22.44 (pyrr-$\underline{CH_2}$-pyrr), 20.59 (CH_2-4'a), 18.55 (CH_2-4a), 14.96 ($CH_2\underline{CH_3}$), 8.57 (CH_3-3), 8.46 (CH_3-3').

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

Condensation of pyrrole 1 (1.08 g, 3 mmol) and pyrrole 9 (0.8 g, 3 mmol) following the usual procedure afforded the dipyrrylmethane 29 (0.84 g, 55% yield), MS: m/e = 508 (M^+, 51) which was reduced to give 30; 0.63 g, 92% yield; mp 150-152° dec (ethanol-water). Found: C, 63.06; H, 7.10; N, 6.61. $C_{22}H_{30}N_2O_6$ requires: C, 63.16; H, 7.18; N, 6.70. ^{13}C NMR, ppm, 172.11 ($\underline{CO_2}CH_3$), 165.62 (CO_2H), 162.77 ($\underline{CO_2}t$-Bu), 80.85 ($\underline{C}(CH_3)_3$), 51.47 (OCH_3), 30.76 ($\underline{CH_2}CO_2$), 28.12 ($(CH_3)_3$), 22.46 (pyrr-$\underline{CH_2}$-pyrr), 18.54 (CH_2-4a), 14.99 ($CH_2\underline{CH_3}$), 8.80 (CH_3-3), 8.52 (CH_3-3').

t-Butyl 3,3'-diethyl-4,4'-dimethyl-5-formyldipyrrylmethane-5-carboxylate (22)

p-Toluensulfonic acid hydrate (0.6 g) dissolved in 12 ml of dry methanol and 60 ml of dry methylene chloride was added to a solution of 0.6 g of the dipyrrylmethane acid 21 in 10 ml of dry methylene chloride and the mixture was stirred during 2 hr at 20° under N_2. The solution was washed with water (50 ml), 5% aqueous sodium bicarbonate (2 x 50 ml) and water (50 ml). The organic phase was

dried (Na_2SO_4), evaporated to dryness in vacuo and the unsaturated dipyrrylmethane thus obtained (0.47 g, 91% yield) was dissolved in 14 ml of dry dimethylformamide and 0.67 ml of benzoyl chloride was added to the solution previously cooled at 5°. After 1.5 hr at 20°, the solution was diluted with ethyl ether (20 ml) and extracted with water (3 x 15 ml). The aqueous extracts were reextracted with ether (1 x 10 ml) and were then adjusted to pH 8 with a 10% sodium carbonate solution. After 18 hr at 20°, the solution was cooled at 5°, filtered and the solid residue was crystallized twice from methanol-water; 0.35 g (69% yield); mp 154°. Found: C, 70.42; H, 8.40; N, 7.69. $C_{21}H_{30}N_2O_3$ requires: C, 70.39; H, 8.38; N, 7.82. MS: m/e = 358 (M^+, 92). ^{13}C NMR, ppm, 176.46 (CHO), 161.26 ($\underline{CO}_2$t-Bu), 79.94 ($\underline{C}(CH_3)_3$), 28.48 ($(CH_3)_3$), 22.42 (pyrr-$\underline{CH}_2$-pyrr), 17.21 ($\underline{CH}_2CH_3$-3), 16.88 ($\underline{CH}_2CH_3$-3'), 15.42 (CH_3-3), 15.02 (CH_3-3'), 10.40 (CH_3-4), 8.63 (CH_3-4').

t-Butyl 3,4'-methyl-4-(ethoxycarbonylmethyl)-3'-ethyl-5'-formyldipyrrylmethane-5-carboxylate (25)

The carboxydipyrrylmethane 24 (0.7 g) was decarboxylated and then formylated as described for the synthesis of 22 and the formyldipyrrylmethane 25 was obtained in 43% overall yield (0.29 g); mp 98-99° (methanol-water). Found: C, 66.25; H, 7.58; N, 6.26. $C_{23}H_{32}N_2O_5$ requires: C, 66.35; H, 7.69; N, 6.37. MS: m/e = 416 (M^+, 4). ^{13}C NMR, ppm, 176.62 (CHO), 171.57 ($\underline{CO}_2$Et), 160.67 ($\underline{CO}_2$t-Bu), 80.29 ($\underline{C}(CH_3)_3$), 60.27 ($CO_2\underline{CH}_2$), 30.96 ($\underline{CH}_2CO_2$), 28.26 ($(CH_3)_3$), 22.56 (pyrr-$\underline{CH}_2$-pyrr), 16.87 (CH_2-3'), 14.99 ($CH_2\underline{CH}_3$-3'), 14.07 ($CO_2CH_2\underline{CH}_3$), 8.86 (CH_3-3), 8.60 (CH_3-4').

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

Dipyrrylmethane 27 (0.66 g) was decarboxylated with p-toluensulfonic acid as described for the synthesis of 22 and then formylated following the described procedure. The formylpyrrylmethane 26 was obtained in 26% overall yield (0.16 g); mp 78-79° (methanol-water). Found: C, 66.23; H, 7.58; N, 6.30. $C_{23}H_{32}N_2O_5$ requires: C, 66.35; H, 7.69; N, 6.37. MS: m/e = 416 (M^+, 74). ^{13}C NMR, ppm, 176.96 (CHO), 172.79 ($\underline{CO}_2CH_3$), 160.00 ($\underline{CO}_2$t-Bu), 79.90 ($\underline{C}(CH_3)_3$), 51.54 (OCH_3), 35.49 ($\underline{CH}_2CO_2$), 28.30 ($(CH_3)_3$), 22.77 (pyrr-$\underline{CH}_2$-pyrr), 19.23 (CH_2-4'a), 18.41 ($\underline{CH}_2CH_3$-4), 15.06 ($CH_2\underline{CH}_3$), 8.63 (CH_3-3), 8.53 (CH_3-3').

t-Butyl 3,3'-dimethyl-4-ethyl-4'-(methoxycarbonylmethyl)-5'-formyldipyrrylmethane-5-carboxylate (31)

The carboxydipyrrylmethane 30 (0.6 g) was decarboxylated following the procedure described for 21 except that decarboxylation time was 4.5 hr. The 5'-unsubstituted dipyrrylmethane (90% yield) was formylated as described for 22 and 31 was obtained in 61% yield (0.32 g); mp 64-65° (methanol-water). Found: C, 65.59; H, 7.39; N, 6.85. $C_{22}H_{30}N_2O_5$ requires: C, 65.67; H, 7.46; N, 6.97. MS: m/e = 402 (M^+, 45). ^{13}C NMR, ppm, 177.11 (CHO), 170.88 ($\underline{CO}_2CH_3$), 161.17 ($\underline{CO}_2$t-Bu), 79.89 ($\underline{C}(CH_3)_3$), 52.08 (OCH_3), 29.77 ($\underline{CH}_2CO_2$), 28.28 ($(CH_3)_3$), 22.77 (pyrr-$\underline{CH}_2$-pyrr), 18.39 ($\underline{CH}_2CH_3$-4), 15.05 ($CH_2\underline{CH}_3$), 8.59 (CH_3-3,3').

3,13,17-Triethyl-7-(2-ethoxycarbonylethyl)-2,8,12,18-tetramethyl-1,19-bilindione (32)

p-Toluensulfonic acid hydrate (216 mg, 1.2 mmol) was added to a solution of 100 mg (0.22 mmol) of the carboxydipyrrylmethane 15 and 178 mg (0.22 mmol) of the formyldipyrrylmethane 22 in 50 ml of dry methylene chloride and 5 ml of dry methanol. The solution was shaked until dissolution of the p-toluensulfonic acid and was kept at 20° during 14 hr. The solution was then poured over 100 ml of water, the organic phase was separated, it was washed first 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 a mixture of 10 ml of dry methylene chloride and 1 ml of methanol, 0.1 ml of a 48% hydrobromic acid solution was added, the solution was evaporated to dryness in vacuo and the residue dissolved in dry benzene and evaporated to dryness several times. The bright red crystalls of the b-bilene hydrobromide thus obtained (335 mg, 90% yield), were dissolved in 50 ml of previously degassed (N_2) trifluoracetic acid, the solution was cooled at 0-2° and 0.02 ml of bromine was added over a period of 1 hr while the solution was kept under N_2. After an additional hr at the same temperature, the solution was poured into 200 ml of degassed water, the aqueous solution was extracted with chloroform (3 x 50 ml), the organic layer was washed with water (50 ml), then with a 5% sodium bicarbonate solution (3 x 50 ml), with water again (50 ml), dried (Na_2SO_4) and evaporated to

dryness. The residue was dissolved in a small volume of 10% of acetone in chloroform and was filtered through a TLC silica gel column (2 x 20 cm) packed and prewashed with the same solvent. The blue band was eluted using the same solvent under slight pressure, the eluates were evaporated to dryness, and the residue was crystallized from methylene chloride-hexane; 40 mg (31% yield); mp 233-234° (dark blue prisms). Found: C, 71.38; H, 7.25; N, 9.78. $C_{34}H_{42}N_4O_4$ requires: C, 71.58; H, 7.36; N, 9.82. MS: m/e = 570 (M^+, 100). ^{1}H NMR, ppm, 6.67 (s, 1H, H-10), 5.96, 5.92 (s, s, 1H, 1H, H-5 and H-15), 4.17 (q, 2H, $CO_2\underline{CH_2}$), 2.82 (t, 2H, CH_2-7b), 2.48 (m, 8H, CH_2-3a, 7a, 13a, 17a). 2.19, 2.17 (s, s, 3H, 3H, CH_3-8 and 12), 1.83 (s, 6H, CH_3-2 and 18), 1.24 (m, 9H, CH_3-3b, 13b and 17b), 1.13 (t, 3H, $CO_2CH_2\underline{CH_3}$). λ_{max} 366 nm (ε 47,700), 641 (14,150); λ_{max} (H^+) 366 (55,700), 666 (59,000), 709 (63,000).

3,13,17-Triethyl-8-(2-methoxycarbonylethyl)-2,7,12,18-tetramethyl-1,19-bilindione (33)

By condensation of 124 mg (0.28 mmol) of dipyrrylmethane 13 and 100 mg (0.2 mmol) of formyldipyrrylmethane 22 following the procedure described for the obtention of 32, it was possible to prepare biliverdin 33 (dark blue prisms) in 16% yield (25 mg); mp 212° (methylene chloride-hexane). Found: C, 71.11; H, 7.09; N, 10.01. $C_{33}H_{40}N_4O_4$ requires: C, 71.22; H, 7.19; N, 10.07. MS: m/e = 556 (M^+, 100). ^{1}H NMR, ppm, 6.65 (s, 1H, H-10), 5.88 (s, 2H, H-5 and H-15), 3.65 (s, 3H, OCH_3), 2.85 (t, 2H, CH_2-8b), 2.50 (m, 8H, CH_2-3a, 8a, 13a and 17a), 2.17 (s, 3H, CH_3-12), 2.09 (s, 3H, CH_3-7), 1.81 (s, 6H, CH_3-2 and 18), 1.24 (m, 9H, CH_3-3b, 13b and 17b); λ_{max} 366 nm (ε 47,300), 634 (14,000); λ_{max} (H^+) 366 (55,000), 664 (24,000).

7,13,17-Triethyl-2-(2-ethoxycarbonylethyl)-3,8,12,18-tetramethyl-1,19-bilindione (34)

This bilindione was similarly prepared by condensation of 100 mg (0.22 mmol) of 17 and 78 mg (0.22 mmol) of 22. The product (21 mg, 17% yield) crystallized in dark blue crystalls from methylene chloride-hexane; mp 248-249°. Found: C, 71.39; H, 7.30; N, 9.72. $C_{34}H_{42}N_4O_4$ requires: C, 71.58; H, 7.36; N, 9.82. MS: m/e = 570 (M^+, 100). ^{1}H NMR, ppm, 6.65 (s, 1H, H-10), 5.80 (s, 2H, H-5 and H-15), 4.15 (q, 2H, $CO_2\underline{CH_2}$), 2.50 (t, 2H, CH_2-2b), 2.48 (m, 8H, CH_2-2a, 7a, 13a and 17a), 2.16 (s, 6H, CH_3-8 and 12), 2.13 (s, 3H, CH_3-3), 1.84 (s, 3H, CH_3-18), 1.25 (m, 9H, CH_3-7b, 13b and 17b), 1.15 (t, 3H, $CO_2CH_2\underline{CH_3}$). λ_{max} 368 nm (ε 51,800), 644 (14,900); λ_{max} (H^+) 366 (59,500), 664 (39,000).

13,17-Diethyl-2-(2-ethoxycarbonylethyl)-7-(ethoxycarbonylmethyl)-3,8,12,18-tetramethyl-1,19-bilindione (35)

This bilindione was likewise prepared by the condensation of the carboxydipyrrylmethane 19 (190 mg, 0.19 mmol) and the formyldipyrrylmethane 22 (130 mg, 0.19 mmol), and gave 42 mg (18% yield) of 35 as blue prisms; mp 233-234° (methylene chloride-hexane). Found: C, 68.68; H, 7.09; N, 8.89. $C_{36}H_{44}N_4O_6$ requires: C, 68.79; H, 7.01; N, 8.92. MS: m/e = 628 (M^+, 100). ^{1}H NMR, ppm, 6.63 (s, 1H, H-10), 5.95, 5.84 (s, s, 1H, 1H, H-5 and H-15), 4.15 (q, 2H, $CO_2\underline{CH_2}$), 3.47 (s, 2H, CH_2-7a), 2.57 (s, 2H, CH_2-2b), 2.47 (m, 6H, CH_2-2a, 13a and 17a), 2.20 (s, 3H, CH_3-8), 2.18 (s, 3H, CH_3-12), 2.17 (s, 3H, CH_3-3), 2.15 (s, 3H, CH_3-18), 1.20 (m, 12H, $CH_2\underline{CH_3}$); λ_{max} 366 (ε 62,700), 613 (22,900), λ_{max} (H^+) 366 (43,000), 660 (43,500).

7,13-Diethyl-2-(2-ethoxycarbonylethyl)-18-(ethoxycarbonylmethyl)-3,8,12,17-tetramethyl-1,19-bilindione (36)

This bilindione was similarly prepared by the condensation of the carboxydipyrrylmethane 17 (60 mg, 0.13 mmol) and the formyldipyrrylmethane 25 (55 mg, 0.13 mmol). The product (9 mg, 11% yield) gave blue-greenish prisms when crystallized from methylene chloride-hexane; mp 216°. Found: C, 68.69; H, 6.93; N, 8.80. $C_{36}H_{44}N_4O_6$ requires: C, 68.79; H, 7.01; N, 8.92. MS: m/e = 628 (M^+, 100). ^{1}H NMR, ppm, 6.65(s, 1H, H-10), 5.92, 5.85 (s, s, 1H, 1H, H-5 and H-15). 4.15 (q, 2H, $CO_2\underline{CH_2}$), 3.34 (s, 2H, CH_2-18a), 2.59 (s, 2H, CH_2-2b), 2.40 (m, 6H, CH_2-2a, 7a and 13a), 2.20 (s, 6H, CH_3-8 and 12), 2.15 (s, 6H, CH_3-3 and 17), 1.20 (m, 12H, $CH_2\underline{CH_3}$); λ_{max} 372 (ε 26,500), 651 (3,900); λ_{max} (H^+) 368 (26,800), 665 (16,100).

3,18-Diethyl-8-(ethoxycarbonylmethyl)-12-(2-ethoxycarbonylethyl)-2,7,13,17-tetramethyl-1,19-bilindione (37)

This biliverdin was prepared similarly by condensation of 90 mg (0.22 mmol) of 11 and 90 mg

0.22 mmol) of 28 which afforded 12 mg (9% yield) of 37; mp 213°(dark blue needles from methylene chloride-hexane). Found: C, 68.10; H, 6.78; N, 9.22. $C_{34}H_{40}N_4O_6$ requires: C, 68.00; H, 6.67; N, 9.33. MS: m/e = 600 (M^+, 100). ^{1}H NMR, ppm, 6.73 (s, 1H, H-10); 5.91, 5.86 (s, s, 1H, 1H, H-5 and H-15), 3.77, 3.74 (s, s, 3H, 3H, OCH_3); 3.65 (s, 2H, CH_2-8a), 2.90 (t, 2H, CH_2-12b), 2.40 (m, 8H, CH_2-3a, 8a, 12a and 18a), 2.15, 2.14 (s, s, 3H, 3H, CH_3-7 and 13), 2.12 (s, 3H, CH_3-17), 1.85 (s, 3H, CH_3-2), 1.25 (t, 3H, CH_3-3b), 1.11 (t, 3H, CH_3-18b); λ_{max} 366 nm (ε 58,800), 628 (17,800); λ_{max} (H^+), 367 (40,200), 664 (55,500).

3,18-Diethyl-8,12-di(methoxycarbonylmethyl)-2,7,13,17-tetramethyl-1,19-bilindione (38)

This biliverdin was prepared by condensation of 90 mg (0.22 mmol) of carboxydipyrrylmethane 11 and formyldipyrrylmethane 31, 87 mg (0.22 mmol) which gave 6.8 mg (6% yield) of 38 as deep blue greenish crystalls; mp 154-156° (methylene chloride-hexane). Found: C, 67.46; H, 6.39; N, 9.48. $C_{33}H_{38}N_4O_6$ requires: C, 67.58; H, 6.48; N, 9.56. MS: m/e = 586 (M^+, 27). ^{1}H NMR, ppm, 6.70 (s, 1H, H-10), 5.85 (s, 2H, H-5 and H-15), 3.70 (s, 6H, OCH_3), 3.57 (s, 4H, CH_2-8 and 12), 2.40 (m, 4H, CH_2-3a and 18a), 2.10 (b, 9H, CH_3-7, 13 and 17), 1.80 (s, 3H, CH_3-2), 1.15 (m, 6H, CH_3-3b and 18b); λ_{max} 360 nm (ε 23,000), 640 (5,100); λ_{max} (H^+), 366 (27,600), 673 (18,900).

3,18-Diethyl-8,12-di(2-methoxycarbonylethyl)-2,7,13,17-tetramethyl-1,19-bilindione (mesobiliverdin IXα) (39)

This bilindione was prepared by condensation of 86 mg (0.2 mmol) of carboxydipyrrylmethane 13 and 84 mg (0.2 mmol) of formyldipyrrylmethane 28 which gave 10 mg (9% yield) of 39 as deep blue crystalls; mp 208-210° (methylene chloride-hexane). Found: C, 68.30; H, 6.73; N, 9.02. $C_{35}H_{42}N_4O_6$ requires: C, 68.40; H, 6.84; N, 9.12. MS: m/e = 614 (M^+, 100); ^{1}H NMR, ppm, 6.72 (s, 1H, H-10), 5.90 (s, 2H, H-5 and H-15), 3.70 (b, 6H, OCH_3), 2.95 (m, 4H, CH_2-8b and 12b), 2.55 (m, 8H, CH_2-3a, 8a, 12a and 18a), 2.13 (b, 6H, CH_3-7 and 13), 2.12 (s, 3H, CH_3-17), 1.85 (s, 3H, CH_3-2), 1.25 (t, 3H, CH_3-3b), 1.10 (t, 3H, CH_3-18b); λ_{max} 367 nm (ε 39,500), 628 (11,000); λ_{max} (H^+) 366 (40,000), 660 (36,000).

Benzyl 3,5-dimethyl-4-acetyl-pyrrole-2-carboxylate (40)

A solution of 11 g of sodium nitrite in 40 ml of water was slowly added with stirring to a solution of 29 g (0.15 mol) of benzyl acetoacetate in 45 ml of acetic acid kept below 5°. The resulting solution was kept at 5° during 18 hr and was slowly added to a stirred solution of 16 g (0.16 mol) of 2.4-pentanedione and 28 g of sodium acetate in 30 ml of acetic acid, while zinc powder (28 g) was also simultaneously added. After the additions were completed, the resulting mixture was stirred and heated at 100° during 1 hr, the mixture was poured over 1 l of ice-water, the precipitate was filtered, dried and crystallized twice from methanol; 25 g (62% yield); mp 125°. Found: C, 70.92; H, 6.38; N, 5.40. $C_{16}H_{17}NO_3$ requires: C, 70.84; H, 6.27; N, 5.16. ^{13}C NMR, ppm, 195.34 (CO), 161.43 (CO_2), 138.88, 135.70, 128.85, 128.31, 127.97, 127.73, 123.28, 117.44 (arom), 65.81 (CH_2), 30.96 ($\underline{CH_2}CO$), 14.71 (CH_3-5), 12.56 (CH_3-3).

Benzyl 3,5-dimethyl-4-ethyl-pyrrole-2-carboxylate (41)

Boron trifluoride etherate (50 ml) was slowly added during 4 hr to stirred solution of 20 g of acetylpyrrole 40 and 7 g of sodium borohydride in 200 ml of dry tetrahydrofuran which was kept below 5° under a stream of N_2. After completing the addition the mixture was kept at 20° during 18 hr, it was then cooled below 5°, adjusted to pH 4 with 5% hydrochloric acid and extracted with chloroform (3 x 200 ml). The extracts were washed with water, dried (Na_2SO_4), evaporated to dryness and the residue was crystallized twice from methanol-water; 16.5 g (87% yield); mp 93°. Found: C, 74.52; H, 7.51; N, 5.26. $C_{16}H_{19}NO_2$ requires: C, 74.71; H, 7.39; N, 5.45. ^{13}C NMR, ppm, 161.46 (CO_2), 136.57, 129.75, 128.27, 127.94, 127.72, 127.10, 123.75 (arom), 65.16 (CH_2), 17.01 ($\underline{CH_2}CH_3$), 15.12 ($\underline{CH_3}CH_2$), 11.03 (CH_3-5), 10.44 (CH_3-2).

Benzyl 3-methyl-4-ethyl-5-acetoxymethylpyrrol-2-carboxylate (4)

Lead tetraacetate (4 g) was added in small portions, over a period of 2 hr, to a stirred solution of 41 (2 g) in 40 ml of glacial acetic acid and the solution was stirred for additional 3 hr. The mixture was poured over ice-water, the precipitate was filtered, dried, and crystallized twice

from acetone-water; 1 g (40% yield); mp 109°. Found: C, 68.75; H, 6.53; N, 4.24. $C_{18}H_{21}NO_4$ requires: C, 68.57; H, 6.66; N, 4.44. ^{13}C NMR, ppm, 171.08 ($\underline{O}O\underline{C}CH_3$), 161.16 ($CO_2$), 136.16, 128.24, 127.79, 126.81, 126.72, 126.21, 118.65 (arom), 65.54 (CH_2Ph), 56.75 (CH_2O), 20.57 ($\underline{CH_3}CO$), 16.84 ($\underline{CH_2}CH_3$), 15.67 ($\underline{CH_3}CH_2$), 10.12 (CH_3).

Benzyl 3-methyl-4-ethyl-pyrrole-2-carboxylate (44)

A solution of 4 g of the iodopyrrole 43 in 80 ml of acetic acid was heated at refluxed under a stream of N_2 while 8 g of zinc powder were added over a period of 3 hr. The mixture was then cooled, filtered, poured over ice-water (500 ml), extracted with chloroform (3 x 100 ml), the extracts were dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in a small volume of 2% methanol in benzene and was filtered through a short column of TLC silica gel packed and prewashed with the same solvent. Evaporation of the eluates containing 44 left behind a residue which was crystallized from methanol-water; 21 g (80% yield); mp 31-32° (lit.[16] 31-32°).

3-Methyl-4-ethyl-pyrrole-2-carboxylic acid (45)

A solution of 2 g of the benzyl ester 42 in 80 ml of tetrahydrofuran and 0.5 ml of triethylamine were reduced with hydrogen over 0.5 g of 10% Pd on charcoal at 50 psi during 3 hr. The catalyst was filtered, the filtrates were evaporated to dryness, and the residue was crystallized from benzene-hexane; 1.13 (90%); mp 154-155°. Found: C, 62.56; H, 7.07; N, 9.28. $C_8H_{11}NO_2$ requires: C, 62.74; H, 7.19; N, 9.15.

t-Butyl 3-methyl-4-ethyl-pyrrole-2-carboxylate (6)

A solution of 1 g of the carboxypyrrole 45 and 0.8 g of dicyclohexylcarbodiimide in 40 ml of dry tetrahydrofuran and 30 ml of dry t-butanol was stirred during 18 hr at 20°. The precipitate was filtered, the filtrate was evaporated to dryness, the residue was resuspended in 20 ml of dry benzene, the precipitate filtered again, the filtrate was evaporated to dryness, the residue was dissolved in a small volume of 3% methanol in benzene and filtered through a column (2 x 20 cm) of TLC silica gel packed and eluted with the same solvent. The eluates containing 6 (monitored by TLC) were pooled, evaporated to dryness and the residue was crystallized from ethanol-water; 0.73 g (54% yield); mp 70-71° (lit.[12b] 95°, from methylene chloride-hexane). Found: C, 68.78; H, 9.15; N, 6.59. $C_{12}H_{19}NO_{12}$ requires: C, 68.89; H, 9.09; N, 6.70. ^{13}C NMR, ppm, 161.50 (CO_2), 127.09 (C-5), 80.17 ($\underline{C}(CH_3)_3$), 28.33($(CH_3)_3$), 18.09 ($\underline{CH_2}CH_3$), 14.47 ($\underline{CH_3}CH_2$), 10.09 (CH_3).

Benzyl 3-methyl-4-ethyl-5-t-butyloxycarbonyl-pyrrole-2-carboxylate (46)

Liquid isobutylene (30 ml, obtained by cooling isobutylene to -70°) was added to a suspension of 3 g of the pyrrolecarboxylic acid 42[18] in 60 ml of methylene chloride containing 0.6 ml of concentrated sulfuric acid. The flask was tightly stoppered and the mixture was stirred at 20° during 18 hr. The flask was cooled below 5°, the solution was washed with a 5% sodium bicarbonate solution (2 x 100 ml), then with a 10% sodium carbonate solution (2 x 100 ml), water (1 x 100 ml), dried (Na_2SO_4), evaporated to dryness and the residue was crystallized from methanol-water; 3.24 g (90% yield); mp 88° (lit.[18] mp 89°). Found: C, 69.85; H, 7.09; N, 4.18. $C_{20}H_{25}NO_4$ requires: C, 69.97; H, 7.28; N, 4.08.

t-Butyl 3-ethyl-4-methyl-5-iodo-pyrrole-2-carboxylate (48)

A solution of 3 g of iodine in 15 ml of ethanol was added dropwise during 45 min to a stirred solution of 1.5 g of decarboxypyrrole 47[18] in 60 ml of water containing 5 g of sodium bicarbonate, while the mixture was kept below 5°. The mixture was further stirred for 2 hr, filtered and the residue was crystallized from methanol-water; 1.45 g (73% yield); mp 113°. Found: C, 43.39; H, 5.33; N, 7.30. $C_{12}H_{18}NO_2I$ requires: C, 43.50; H, 5.43; N, 7.25. ^{13}C NMR, ppm, 72.49 (C-I).

t-Butyl 3-ethyl-4-methyl-pyrrole-2-carboxylate (9)

A solution of 1.3 g of the iodopyrrole 48 and 2 g of sodium acetate in 100 ml of ethanol was reduced with hydrogen at 50 psi during 3 hr over 200 mg of 10% Pd on charcoal. The catalyst was filtered, the solution was evaporated to dryness, the residue was partitioned between chloroform (100 ml) and water (50 ml), the organic layer was dried (Na_2SO_4), evaporated to dryness and the residue cryst

allized twice from methanol-water; 830 mg (90% yield); mp 103-105°. Found: C, 68.80; H, 9.12; N, 6.60. $C_{12}H_9NO_2$ requires: C, 68.90; H, 9.09; N, 6.70. ^{13}C NMR, ppm, 161.54 (CO_2), 131.69 (C-5), 80.12 ($\underline{C}(CH_3)_3$), 28.23 ($(CH_3)_3$), 18.11 ($\underline{CH_2}CH_3$), 15.06 ($\underline{CH_3}CH_2$), 9.50 (CH_3).

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