Total Synthesis of Symmetric Bile Pigments:
Mesobilirubin-IVα, Mesobilirubin-XIIIα and Etiobilirubin-IVγ

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The unnatural bile pigments mesobilirubin-IVα, mesobilirubin-XIIIα and etiobilirubin-IVγ were synthesized following self-coupling of the pyrromethenones ψ-xanthobilirubic acid methyl ester, xanthobilirubic acid methyl ester and kryptopyrromethenone, respectively.

Introduction.

The natural bile pigment, (4Z,15Z)-bilirubin-IXα (BR-IX), is the yellow-orange, hydrophobic and cytotoxic pigment of jaundice. It is produced in abundant quantities by heme catabolism in mammals and transported as a non-covalent association complex with serum albumin to the liver for glucuronidation and subsequent excretion [1]. However, when the conjugation apparatus in the liver is not yet functional, as in the neonate, ready excretion of the toxic pigment is thwarted, and the albumin serves as a biologic buffer against bilirubin encephalopathy and other tissue damage [2]. Jaundice in the newborn may lead to neurologic dysfunction resulting in subtle intellectual or neurologic abnormalities, retarded motor development or even death. Consequently, a considerable effort has been made to understand the structure, properties and function of BR-IX and related pigments.

One of the significant aspects of BR-IX is its unusual 3-dimensional structure, characterized by an ability and tendency to form *intra*molecular hydrogen bonds and thereby control conformation and polarity (Figure 1) [1]. This largely determines its hydrophobic solubility properties and has important implications for biological function. The three features that together have a dominating effect on its shape include: (i) two pyrromethenone chromophores, each in a *syn*-periplanar conformation with Z-configuration C=C bonds (at C-4 or C-15); (ii) an sp³ carbon at C-10, which constrains the molecule to bend in the middle and allows the two pyrromethenone chromophores to rotate independently about the C-9, 10 and C-10, 11 single bonds; and (iii) two propionic acid groups, located at C-8 and C-12, which can form intramolecular hydrogen bonds with the pyrrole and lactam functions in the opposite half of the molecule.

The preference for intramolecularly hydrogen-bonded conformers in which polar groups are neutralized internally, explains why BR-IX exhibits lipophilic behavior and requires addition of (the polar) glucuronic acid for excretion. It also explains why analogs with vinyl groups reduced to ethyl (mesobilirubins), or with methyl groups interchanged at C-2/C-3 or C-17/C-18 (the symmetrical biliru-

bins and mesobilirubins (IIIα and XIIIα) all exhibit similar solubility properties, *e.g.* soluble in chloroform, insoluble in methanol, insoluble in dilute aqueous bicarbo-
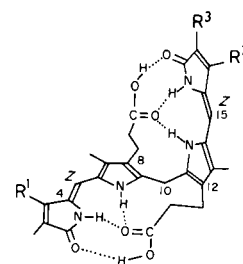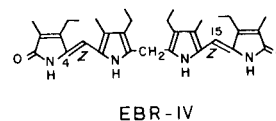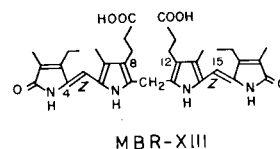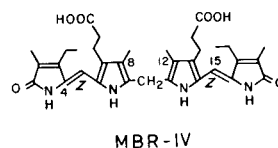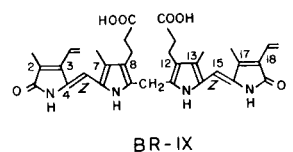

Figure 1. (Upper four) Linear representations for the structures of (4Z,15Z)-bilirubin-IXα (BR-IX), (4Z,15Z)-mesobilirubin-IVα (MBR-IV), (4Z,15Z)-mesobilirubin-XIIIα (MBR-XIII) and etiobilirubin-IVγ (EBR-IV). (Bottom) Intramolecularly hydrogen-bonded conformation of BR-IX (R¹ = R³ = CH=CH₂, R² = CH₃) and MBR-XIII (R¹ = R² = CH₂CH₃, R³ = CH₃).

nate. However, isomers that do not have their propionic acid groups located at C-8 and C-12, e.g. mesobilirubin-IVα, are predicted to have very different solubility properties, viz. insoluble in chloroform, soluble in methanol, soluble in dilute aqueous bicarbonate. Because of their potential importance as comparison compounds in understanding the manifold events associated with bilirubin-IXα, e.g. albumin and other protein binding, hepatic uptake, glucuronidation, excretion and toxicity, we synthesized the symmetric mesobilirubins-IVα and XIIIα, as well as the decarboxylated analog (etiobilirubin-IVγ) of the former.

Syntheses.

Mesobilirubin-IVα (MBR-IV), mesobilirubin-XIIIα (MBR-XIII) and etiobilirubin-IVγ (EBR-IV) were each prepared from the corresponding verdin (dimethyl ester), which was obtained by self-condensation of the relevant pyrromethenone using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the presence of trifluoroacetic acid [3]: (1) MBR-IV from pseudo-xanthobilirubic acid methyl ester (ψ-XBRME); (2) MBR-XIII from xanthobilirubic acid methyl ester (XBRME) and (3) EBR-IV from kryptopyrromethenone (KRP) (Scheme 1). The pyrromethenone intermediates used in this work were prepared by smooth, high yield reaction of 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1H-pyrrole (1) with the required pyrrole derivative: (2) to give ψ-XBRME, (3) to give XBRME, and (4) to give KRP. Syntheses of 1, 3 and 4 have been described previously in connection with the preparation of XBRME [4].

Pseudoxanthobilirubic acid methyl ester (ψ-XBRME) was prepared by modification of the XBRME total synthesis [4]. The key monopyrrole intermediates were 1 and ethyl 2,3-dimethyl-5-(ethoxycarbonyl)-1H-pyrrole-4-propanoate. The former was prepared from kryptopyrrole (2,4-dimethyl-3-ethyl-1H-pyrrole) as previously described [4]. The latter was prepared via Paal-Knorr type pyrrole synthesis from diethyloximinomalonate and ethyl 4,6-dioxo-5-methylheptonoate in the presence of zinc dust (Scheme 2). Its dicarboxylic acid 4 was obtained following careful saponification and acidification and was found to couple satisfactorily with 1 in refluxing methanol to afford ψ-XBRME.

Self-condensation of the pyrromethenones to the corresponding verdin was carried out in absolute tetrahydrofuran under Argon in the presence of trifluoroacetic acid using DDQ as oxidizing agent. The reactive intermediate is proposed [3] to be a carbocation 5 formed by protonation of the azafulvene oxidation product 6 [5]. Electrophilic addition of 5 to starting pyrromethenone affords the tetrapyrrole intermediate 7, which undergoes nucleophilic displacement of verdin product via attack at the quaternary CH$_3$ by trifluroacetate ion. The verdins were reduced to the corresponding mesobilirubins using sodium borohydride [6,7] in methanol or methanol-tetrahydrofuran solvent at 0° in nearly quantitative yield.
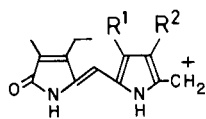
Since the diacids, MBR-IV and MBR-XIII have verdin diester precursors, a saponification step is necessary in their syntheses; however, in the preparation of EBR-IV no other modifications are necessary. In the synthesis of
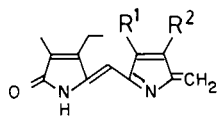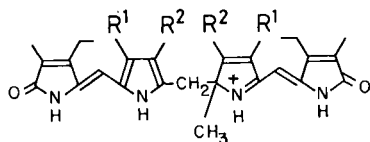
Scheme 1
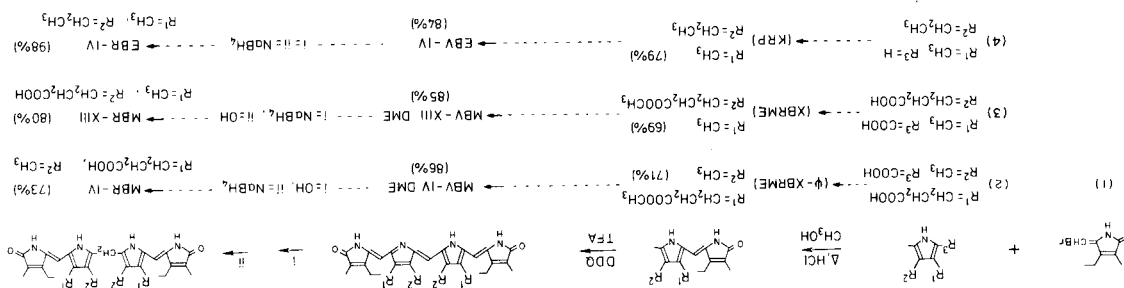
Scheme 2
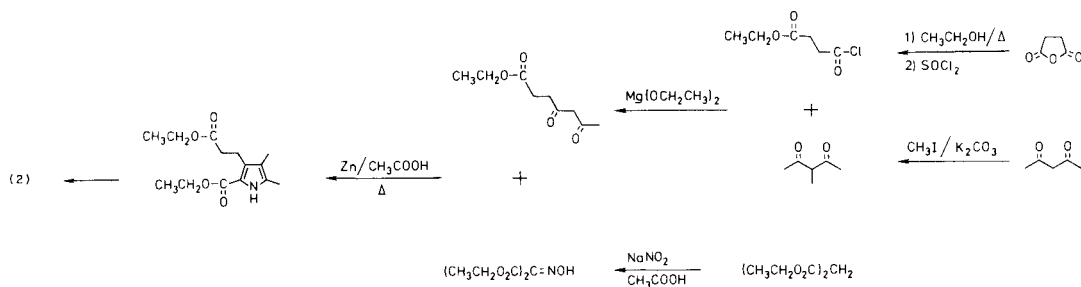
(5)

(6)

(7)

MBR-XIII, MBV-XIII DME is first reduced with sodium borohydride then the corresponding rubin dimethyl ester in saponified *in situ*. Because of its unique solubility properties, the product acid, MBR-XIII could be extracted into chloroform from the aqueous reaction solution after adjusting its *p*H to ~3. And the chloroform extract could be washed with dilute aqueous bicarbonate without extracting the desired pigment. Although MBV-IV DME and MBR-IV DME have solubility properties very similar to those of MBV-XIII DME and MBR-XIII DME (respectively), MBR-IV and MBR-XIII have very dissimilar solubility properties. Consequently, the workup described for MBR-XIII proved inappropriate for MBR-IV. Several approaches were attempted: (1) MBV-IV DME → MBR-IV

DME → MBR-IV, (2) ψ-XBR → MBV-IV → MBR-IV, (3) MBV-IV DME → MBV-IV →MBR-IV. Of these the third proved most successful in our hands. Thus, the MBV-IV DME formed from self-condensation of ψ-XBRME was saponified, and the product MBV-IV was reduced with sodium borohydride then precipitated from solution by the addition of 10% aqueous hydrochloric acid.

Solution Properties and Conformation.

The mesobilirubins are sensitive to air oxidation back to the verdins, with MBR-IV and EBR-IV being more sensitive than MBR-XIII. Hence all handling in solution is carried out in oxygen free, argon-saturated solvents. And because of the instability of these rubins in solution, it is important to purify completely at the final verdin stage. The verdins MBV-IV DME, MBV-XIII DME and EBV-IV are nicely crystalline substances, easily handled and purified. The mesobilirubins differ in their solubility properties, for example MBR-XIII and EBR-IV are soluble in chloroform but MBR-IV is insoluble; MBR-IV is soluble in 5% aqueous sodium bicarbonate but MBR-XIII and EBR-IV are insoluble; and MBR-IV and MBR-XIII are soluble in 0.1 *N* aqueous sodium carbonate but EBR-IV is insoluble.

The ¹H-nmr-spectra are especially revealing of the tendency, or lack thereof, toward the adoption of an intramolecularly hydrogen-bonded conformation (Figure 1). Symmetric bilirubins [8] and pyrromethenones [4] typically exhibit two N-*H*-resonances in their nmr spectra, one for the lactam and one for the pyrrole ring. The relative

Table 1

Assignments of Lactam and Pyrrole N-H Signals (δ, ppm) in the ¹H-NMR Spectra of Mesobilirubins [a]

Deuteriochloroform	10.85	9.65	[b] Mesobilirubin-IVα (MBR-IV)
Dimethylsulfoxide-d₆	9.82	10.36	R¹ = CH₂CH₂CO₂H, R² = CH₃
Deuteriochloroform	10.47	10.27	[c] MBR-IV DME
Dimethylsulfoxide-d₆	9.78	10.32	R¹ = CH₂CH₂CO₂CH₃, R² = CH₃
Deuteriochloroform	10.57	9.15	Mesobilirubin-XIIIα (MBR-XIII)
Dimethylsulfoxide-d₆	9.72	10.27	R¹ = CH₃, R² = CH₂CH₂CO₂H
Deuteriochloroform	10.54	10.27	[c] MBR-XIII DME
Dimethylsulfoxide-d₆	9.74	10.40	R¹ = CH₃, R² = CH₂CH₂CO₂CH₃
Deuteriochloroform	10.58	10.28	Etiobilirubin-IVγ (EBR-IV)
Dimethylsulfoxide-d₆	9.78	10.28	R¹ = CH₃, R² = CH₂CH₃

[a] Run at 21° in 10⁻² *M* dimethylsulfoxide-d₆ and 10⁻³ *M* deuteriochloroform solutions. [b] Because the material is insoluble in deuteriochloroform, the N-*H* resonances for pure deuteriochloroform were determined by extrapolation of the δ values in deuteriochloroform-dimethylsulfoxide-d₆ solutions to 0% dimethylsulfoxide-d₆. The extrapolation method, when applied in a test case for XBRME, correctly predicts (11.2 and 10.3) the values actually observed (11.15 and 10.25) in pure deuteriochloroform. [c] The esters were prepared by reaction of the corresponding acid with diazomethane.

shieldings of these resonances are sensitive to hydrogen-bonding, both *intra* and *inter*molecular. In hydrogen bonding solvents such as dimethylsulfoxide, the chemical shifts of all the lactam N-*H* resonances lie near 9.7-9.8 ppm; whereas, the pyrrole N-*H* resonances lie between 10.27-10.4 ppm (Table 1). However, in solvents that do not hydrogen-bond as strongly (chloroform) the pyrrole N-*H* resonances shift into two recognizable [4,8,9] patterns: those (near 9.2 ppm) associated with *intra*molecular hydrogen bonding of the type shown in Figure 1 and those (near 10.3 ppm) associated with pyrromethenone-to-pyrromethenone *inter*molecular hydrogen bonding [4,8-10]. Although all the lactam N-*H* signals have similar chemical shifts (10.47-10.58 ppm) in deuteriochloroform, the pyrrole ring N-*H* signals of MBR-XIII are nearly 1 ppm higher field than those of the other rubins of Table 1. This difference has been attributed to electron ring current shielding from the neighboring pyrrole ring held in the H-bonded conformation of Figure 1 [4]. The great similarity shown in the N-*H* chemical shifts by all other rubins of Table 1 is consistent with a different conformation, one not guided by *intra*molecular hydrogen bonding but probably of the pyrromethenone-to-pyrromethenone dimeric association [10] seen with bilirubin esters [4,11]. Thus, MBR-XIII and MBR-IV may serve as a potentially useful analogs of BR-IX, one with a similar solution conformation and properties, the other (MBR-IV) with very different solution conformation and properties.

In summary, we have synthesized three symmetric mesobilirubins: the isomeric MBR-IV and MBR-XII, whose solubility and three-dimensional structure differ according to the location of their propionic acid groups, and EBR-IV, a decarboxylated analog of MBR-XIII. MBR-XIII prefers to adopt on intramolecularly hydrogen bonded conformation (Figure 1); whereas, MBR-IV and EBR-IV cannot. MBR-IV, with its propionic acid groups not tied up in hydrogen bonding, is thus a very polar molecule, insoluble in chloroform but soluble in dilute bicarbonate. For MBR-XIII these properties are reversed, and only the chloroform solubility is shared by EBR-IV.

EXPERIMENTAL

General.

All nmr spectra were run on an IBM NR80/AF or JEOL FX-100 FT spectrometer in either deuteriochloroform (99.9% d_1) or dimethylsulfoxide-d_6 (99.9% d_6), both from Aldrich. All ir spectra were obtained from a Perkin-Elmer model 599 instrument, and all uv-visible absorption spectra were run on a Cary 219 instrument. Melting points were determined on a Mel-Temp capillary unit. Combustion microanalyses were obtained from Desert Analytics, Tucson, AZ. Thin layer chromatography (tlc) was carried out on J. T. Baker silica gel 1B-F plates (125 μ layer). Column chromatography was carried out on 32-63 μ activated silica gel for medium pressure chromatography (M. Woelm). High performance liquid chromatographic (hplc) analyses used a detector set at 420 nm and a Beckman-Altex Ultrasphere-IP 5 μm C-18 ODS column (25 x 0.46 cm),

with a Beckman ODS precolumn (4.5 x 0.46 cm) and a flow of 0.75 ml/minute of 0.1 *M* di-*n*-octylamine acetate in 5% aqueous methanol as eluent [12]. Tetrahydrofuran (distilled from lithium aluminum hydride, stored over sodium wire and filtered through activity 0 basic alumina (M. Woelm) before use), triethylamine and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), sodium borohydride, trifluoroacetic acid pentan-2,4-dione, diethylmalonate and succinic anhydride were from Aldrich. Chloroform and methanol (hplc grade) were from Fisher. Ascorbic acid and glycine were from Matheson, Coleman and Bell. Disodium EDTA, zinc powder, glacial acetic acid and benzene were from Mallinckrodt. The water used in this work, including aqueous solvents, and tetrahydrofuran, chloroform and methanol were rendered oxygen-free, argon-saturated by bringing to brief reflux under a stream of argon, cooling and storing under argon.

Diethyl Oximinomalonate.

To a one liter 3-neck flask equipped with a dropping funnel and mechanical stirrer were added diethyl malonate (107 g, 0.67 mole) and glacial acetic acid (120 g, 2.0 moles). To this solution was added sodium nitrite (127 g, 1.8 moles) in a minimal quantity of water over 4 hours at room temperature. After standing for 2 hours, two layers formed. The upper layer was separated, diluted with ether (300 ml), washed with dilute aqueous sodium bicarbonate (60 ml) and water (2 x 60 ml), and dried over anhydrous magnesium sulfate. The ether was removed (rotary evaporator) and the residue was distilled to give the desired product (104.6 g, 83%) as a viscous oil, bp 98-101°/0.05 mm (lit [13] bp 172°/12 mm).

β-Carboethoxypropionyl Chloride.

This half-ester acid chloride of succinic acid was prepared according to the method of Riegel and Lillienfield [14] from succinic anhydride by reacting it with ethanol to give the mono-ester, followed by reaction with thionyl chloride. It was obtained in 96% yield, bp 67-69°/3 mm (lit [14] bp 110-115°/30 mm).

3-Methyl-2,4-pentanedione.

This ketone was prepared in 72% yield according to Johnson *et al.* [15] by treating pentane-2,4-dione with anhydrous potassium carbonate and methyl iodide in acetone. It had bp 165-167° (lit [15] bp 170-172°).

Ethyl 4,6-Dioxo-5-methylheptanoate.

To a 2 liter round-bottom flask were added magnesium shavings (34.6 g, 1.44 g-atoms), carbon tetrachloride (7 ml) and absolute ethanol (375 ml). The mixture was heated at reflux on a steam bath for 4 hours to form magnesium ethoxide, and the excess ethanol was removed under vacuum. Anhydrous ether (830 ml) was added, and the mixture was cooled to 15°. With continued cooling to 15°, 3-methyl-2,4-pentanedione (158 g, 1.39 moles) in anhydrous ether (415 ml) was added to the magnesium ethoxide-ether mixture. After stirring at room temperature for 3 hours, the mixture was heated at reflux on a steam bath for one hour and transferred to a 3 liter 3-neck flask equipped with mechanical stirrer, dropping funnel and thermometer, then cooled to −10°. A solution of β-carboethoxypropionyl chloride (278 g, 1.68 moles) in anhydrous ether (278 ml) was added dropwise, with stirring at −10°. The solution was gradually warmed to room temperature with continuous stirring during 4 hours. It was then cooled in an ice bath and acidified with 25% aqueous sulfuric acid (278 ml). The liquid was decanted from the magnesium salts, which were washed with ether, and the combined ether phase was separated and stirred with 10% aqueous sodium hydroxide (2 x 150 ml). The ether was removed, dried over anhydrous magnesium sulfate and evaporated. Distillation of the residue gave the desired diketo-ester, 134.2 g, 48% yield, with bp 80-84°/0.02 mm (lit [16] bp 94-96°, 0.05 mm).

Ethyl 5-Carboethoxy-2,3-dimethyl-1-*H*-pyrrole-4-propanoate.

To a one liter 3-neck flask equipped with mechanical stirrer, dropping funnel and thermometer were added ethyl 4,6-dioxo-5-methylheptanoate (39.5 g, 0.20 mole) and glacial acetic acid (200 ml). The solution was warmed to 80° using a heating mantle, then anhydrous sodium acetate

(51.3 g, 0.63 mole) and zinc dust (43.5 g, 0.67 g-atom) were added with vigorous stirring. After heating the mixture to 95°, a solution of diethyl oximinomalonate (37.5 g, 0.20 moles) in glacial acetic acid (60 ml) and water (20 ml) was added slowly. The mixture was heated, with stirring on a steam bath for 2.5 hours. Then the hot liquid was poured from the zinc onto 800 ml of ice with stirring. On standing, the pyrrole crystallized and was removed by filtration, washed with water and dried to give 31.4 g, (59%) of product with mp 81-85°. Recrystallization from hot ethanol gave white crystals 22.4 g (44%) mp 83-84° (lit [16] mp 83-85). It had ^{1}H-nmr (deuteriochloroform): δ 1.20 (t, 3H, J = 7 Hz), 1.32 (t, 3H, J = 7 Hz), 1.87 (s, 3H), 2.14 (s, 3H), 2.4-3.21 (m, 4H), 4.05-4.50 (m, 4H), 9.48 (broad s, 1H, NH) ppm.

5-Carboxy-2,3-dimethyl-1H-pyrrole-4-propanoic Acid (2).

The pyrrole diester from above (6.48 g, 0.024 mole) was suspended in a little ethanol, then sodium hydroxide (4.5 g) dissolved in water (22.5 ml) was added. The mixture was made up to 60 ml by the addition of more ethanol and slowly brought to reflux. After 2 hours a light amber solution was obtained, which was cooled to room temperature. The ethanol was removed (rotary evaporator) at room temperature and the remaining solution was cooled in an ice-salt bath to −10°. On slow addition of 10% aqueous, nitric acid (72 ml) a pink-beige solid separated. It was collected by filtration, washed with a minimum quantity of ice water (< 40 ml) and dried in a dissicator over solid sodium hydroxide. It was too unstable to purify further and was used directly in the next step. The yield was 3.95 g (77%) of beige solid, mp dec above 118°. It had ^{1}H-nmr (dimethylsulf-oxide-d_6): 1.85 (s, 3H), 3.05 (s, 3H), 2.33-2.61 (m, 4H), 6.28 (s, 1H), 9.81 (broad s, 1H) ppm.

5-[1,5-Didehydro-3-ethyl-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-2,3-dimethyl-1H-pyrrol-4-propanoic Acid Methyl Ester (Pseudoxanthobilirubic Acid Methyl Ester, ψ-XBRME).

5-Bromomethylene-4-ethyl-3-methyl-2-oxo-1H-pyrrole (1) [4] (2.60 g, 12 mmoles) and 5-carboxy-2,3-dimethyl-1H-pyrrole-4-propanoic acid (2) (2.56 g, 12 mmoles) were placed in a 100 ml round-bottom flask together with 1 ml of water and 60 ml of methanol. The contents were blanketed with argon, protected from light and the mixture heated at reflux for 5 hours. After cooling to room temperature, the reaction vessel was placed in the freezer to complete the crystallization of the yellow product, which was removed by filtration. The solid was dissolved in chloroform (300 ml), washed with 10% aqueous sodium hydroxide solution (2 x 25 ml) then saturated aqueous sodium chloride (2 x 25 ml) and dried (anhydrous sodium sulfate). The solvent was evaporated (rotary evaporator) and the residue crystallized from benzene under argon to afford 2.68 g (68%) of yellow needles, mp 206.5-207° (lit [17] mp 205°). It had uv-visible (chloroform): λ max 405 nm, ϵ, 30,000; (methanol): λ max 410 nm, ϵ, 28,000; ir (nujol): ν 3330, 1735, 1680, 1617 cm⁻¹; ^{1}H-nmr (deuteriochloroform): δ 1.08 (t, 3H, J = 7 Hz), 1.84 (s, 3H), 1.86 (s, 3H), 2.27 (s, 3H), 2.30-2.95 (m, 6H), 3.55 (s, 3H), 6.07 (broad s, 1H), 10.2 (broad s, 1H), 11.2 (broad s, 1H) ppm; ^{13}C-nmr 8.54 (q), 8.89 (q), 11.58 (q), 15.04 (q), 18.02 (t), 20.36 (t), 35.69 (t), 51.66 (q), 100.98 (d), 115.48 (s), 121.98 (s), 122.50 (s), 127.42 (s), 127.83 (s), 131.63 (s), 148.54 (s), 173.64 (s), 174.16 (s) ppm. The nmr values correlate well with those of the isomer XBRME [4].

Mesobiliverdin-IVα Dimethyl Ester (MBV-IV DME).

All solvents and solutions were oxygen-free, argon-saturated; reactions and extractions were blanketed with argon. ψ-Xanthobilirubic acid dimethyl ester (ψ-XBRME) (158 mg, 0.5 mmole) was dissolved in dry, tetrahydrofuran (50 ml) containing trifluoroacetic acid 2.5 ml) and maintained at 20° in the dark with magnetic stirring. To this solution is added dropwise, during 90 minutes and with stirring, a solution containing 136 mg (0.6 mmole) of DDQ dissolved in dry tetrahydrofuran (25 ml). The mixture is cooled in and ice bath for 5 minutes then diluted with an ice cold mixture of chlorform (50 ml) and 1% triethylamine in water (75 ml). The aqueous phase was washed with chloroform (2 x 15 ml), and the combined organic phases were washed with 0.1 M aqueous sodium bicarbonate until the aqueous phase shows a neutral pH. (This required 5-10 (x

15 ml) washings, then with water (2 x 15 ml). The organic phase was filtered through chloroform-wetted filter paper, and the solvent was removed on a rotary evaporator. The resultant blue product was purified by column chromatography on silica gel (10 cm x 2.5 cm) using chloroform-methanol 50:1 as eluent. A fast moving pink band is removed and discarded. The blue band is collected and the solvent removed on a rotary evaporator to give 118 mg (82%) of pure product, with purity determined by tlc using chloroform-methanol 50:1 as eluent. It had mp 240-244°; uv-vis (methanol): λ max 645 nm, ϵ, 10,500; λ max 365 nm, ϵ, 40,000; ir (chloroform): ν 1720, 1675, 1617 cm⁻¹; ^{1}H-nmr (deuteriochloroform): δ 1.18 (t, 6H, J = 8 Hz), 1.75 (s, 6H, CH$_3$ at C-2, C-18), 2.13 (s, 6H, CH$_3$ at C-8, C-12), 2.42-2.49, 2.77-2.70 (overlapping t, 12H, -CH$_2$- at C-3, C-7, C-13, C-17, -CH$_2$- CO$_2$-), 3.63 (s, 6H, -CO$_2$CH$_3$), 5.87 (s, 2H, =CH at C-5, C-15), 6.64 (s, 1H, =CH at C-10), 8.44 (broad s, 3H, NH) ppm; (dimethylsulfoxide-d_6): δ 1.11 (t, 6H, J = 8 Hz), 1.78 (s, 6H), 2.27 (s, 6H), 2.45-2.60, 2.76-2.84 (m, 12H), 3.66 (s, 6H), 6.14 (s, 2H), 7.16 (s, 1H) ppm.

Anal. Calcd. for C$_{35}$H$_{42}$N$_4$O$_6$ (614.75): C, 68.38; H, 6.89; N, 9.11. Found: C, 68.23; H, 6.79; N, 8.70.

Mesobilirubin-IVα (MBR-IV).

All solvents and solutions were oxygen-degassed, argon-saturated, and all reactions and extractions were blanketed with argon. Mesobiliverdin-IVα dimethyl ester (MBR-IV DME) (120 mg, 0.20 mmole) was dissolved in 125 ml of a 1:1 (vol/vol) mixture of methanol-tetrahydrofuran containing ascorbic acid (100 mg) and a few mgs of disodium EDTA. To this solution was added 125 ml of 0.1 M aqueous sodium hydroxide, and the resultant solution was stirred 90 minutes at 37° under an argon atmosphere in the dark. Then the solution was acidified with 10% aqueous hydrochloric acid and extracted with an mixture of chloroform (250 ml) and pH 2.7 glycine-HCl buffer (500 ml). The chloroform extract was washed with 0.1 M aqueous sodium bicarbonate (2 x 50 ml) then water (2 x 200 ml), filtered through chloroform-wetted filter paper and evaporated under vacuum at 40° to give 114 mg (100%) of diacid. The material is pure by tlc (silica gel, chloroform-methanol-acetic acid, 100:10:1 eluent) and used directly in the reduction step.

The verdin diacid from above (114 mg, 0.19 mmole) was dissolved in a 20 ml of a 1:1 (vol/vol) mixture of methanol and tetrahydrofuran and cooled in an ice bath at 0° with magnetic stirring; then, 1.0 g of sodium borohydride was added all at once, with stirring. After 10 seconds the reaction solution had turned yellow-brown. At this point water (40 ml) was added followed by dropwise addition of 10% aqueous hydrochloric acid to acidify the solution and precipitate the product, MBR-IV, which was removed by centrifugation and the mother liquors filtered. The recovered precipitates were washed with water (2 x 20 ml) and dried overnight in a vacuum dessicator. The yield of dried MBR-IV is 73%. It was pure by tlc on silica gel using chloroform-methanol-acetic acid (100:2:1, vol/vol/vol) or chloroform-ethanol (10:1) as eluent, and by hplc. It did not melt but decomposed above 180°. It had uv-visible (dimethylsulfoxide): λ max 392 nm, ϵ, 29,250 and λ max 423 nm, ϵ, 29,500 (double maxima); ^{1}H-nmr (dimethylsulfoxide-d_6): δ 1.09 (t, 6H, J = 7 Hz), 1.71 (s, 6H, CH$_3$ at C-2, C-18), 1.77 (s, 6H, CH$_3$ at C-8, C-12), 2.15-2.70 (m, 12H, -CH$_2$- of -CH$_2$CH$_3$ and -CH$_2$CH$_2$-CO$_2$-), 3.89 (s, 2H, -CH$_2$- at C-10), 6.04 (s, 2H, =CH at C-5, C-15), 9.82 (s, 2H, lactam NH), 10.36 (s, 2H, pyrrole NH) ppm.

Anal. Calcd. for C$_{33}$H$_{40}$N$_4$O$_6$·3H$_2$O (642.80): C, 61.67; H, 7.21; N, 8.72. Found: C, 61.88; H, 6.96; N, 8.56.

Xanthobilirubic Acid Methyl Ester (XBRME).

This pyrromethenone was prepared from **1** and **3** exactly as described previously [4,10].

Mesobiliverdin-XIIIα Dimethyl Ester (MBV-XIII DME).

This verdin was prepared as above but can also be prepared [3], as can MBV-IV DME as shown in the following scaled up reaction.

All solutions and solvents used in this preparation were oxygen-degassed, argon-saturated. Reactions and extractions were carried out with an argon blanket. Xanthobilirubic acid methyl ester (XBRME) [4] (945 mg, 3.0 mmoles) was placed in a one liter, 3-neck round-bottom flask

equipped with dropping funnel, argon purge and magnetic stirring. Dry tetrahydrofuran (200 ml) was added with stirring. (The pyrromethenone only partially dissolves). Argon-saturated trifluoroacetic acid (15 ml) was added slowly, upon which the pyrromethenone dissolves completely and the color changes from yellow to orange. (The orange color may become masked by a green tinge to the solution). Then a solution of DDQ (840 mg, 3.7 mmoles) dissolved in dry tetrahydrofuran (80 ml) was added dropwise to the stirred pyrromethenone solution over a period of one hour. The solution changes from orange to green to blue-green to blue. The solution was allowed to stir for an additional hour, after which it was cooled in an ice bath. Then ice-cold chloroform (ethanol free) was added and the resulting two-phase mixture was transferred to a 3-liter separatory funnel containing a mixture of ice-cold argon-saturated aqueous triethylamine solution (228 g, 312 ml of triethylamine + 1200 ml of water), ascorbic acid (1.5 g), 350 ml of chloroform. The separatory funnel was shaken for several minutes, and the bluish chloroform layer separated. The aqueous phase was extracted a second time with chloroform (100 ml), and the combined chloroform extracts were washed with cold water (4 x 300 ml), saturated aqueous sodium chloride solution (1 x 300 ml) and dried over anhydrous sodium sulfate. The organic solvent was removed (rotary evaporator), redissolved in a minimum of chloroform and filtered through a 4 cm thick pad of silica gel (80-200 mesh, M. Woelm) on a fitted glass funnel. The yield is 90% of dark blue iridescent solid of sufficient purity for conversion to the mesobilirubin.

Further purification may be achieved by column chromatography, as above (for MBV-IV DME) using silica gel and chloroform-methanol (50:1) or chloroform-pyridine (100:1) as eluent. Thus, chromatography of one-third of the material on 150 g of silica gel (70-230 mesh) using chloroform-pyridine (100:1) as eluent afforded 275 mg (85%) of blue pigment that could be recrystallized from chloroform-hexane to give crystals with mp 244-245° (lit [3,18] mp 246-247°). It had uv-visible (methanol): λ max 632, ϵ, 9,800; λ max 367, ϵ, 40,000; ir (chloroform): ν 1718, 1675, 1615 cm^{-1}; ^{1}H-nmr (deuteriochloroform): δ 1.21 (t, 6H, J = 8 Hz), 1.81 (s, 6H, CH$_3$ at C-2, C-18), 2.09 (s, 6H, CH$_3$ at C-7, C-13), 2.56-2.93 (overlapping t, 12H, -CH$_2$- at C-3, C-8, C-12, C-17, -CH$_2$CO$_2$-), 3.67 (s, 6H, CO$_2$CH$_3$), 5.92 (s, 2H, =CH at C-5, C-15), 7.19 (s, 1H, =CH at C-10), 8.48 (broad s, 3H, NH) ppm.

Mesobilirubin-XIIIα (MBR-XIII).

All solutions and solvents used in this preparation were oxygen-free, argon-saturated; reactions and extractions were carried out under an argon atomosphere. Sodium borohydride (1 g) was added slowly to ice-cold, methanol (100 ml) in an ice bath. When evolution of hydrogen had ceased, the apparatus was flushed with argon and sealed. A solution of MBV-XIII DME (110 mg, 0.18 mmole) in 100 ml of purified tetrahydrofuran was added with stirring during 20 minutes. The resulting green solution was allowed to warm up to room temperature, with stirring maintained for 30 minutes (or until it had turned yellow). Then, ice cold argon-saturated water (5 ml) was added slowly, and when hydrogen evolution (if any) had ceased a 1 M solution of sodium hydroxide (50 ml) containing ascorbic acid (50 mg) and a few mgs of disodium EDTA was added all at once. This mixture was protected from light, warmed to 40° on a water bath then stirred at 40° for 2 hours. At the end of this period, an orange-brown solution remained. It was transferred to a separatory funnel, washed with chloroform (25 ml) then acidified to pH 2.7 with argon-saturated glycine-HCl buffer. The resulting mixture was extracted with chloroform (50 ml + 2 x 25 ml), and the combined organic extracts were washed with water (100 ml), saturated aqueous sodium bicarbonate solution (2 x 50 ml) and saturated aqueous sodium chloride solution (100 ml). The chloroform solution was then dried over anhydrous sodium sulfate and filtered through a thin pad (0.5 cm) of silica gel. Evaporation of the chloroform gave 53 mg (48%) of MBR-XIII.

The product was purified by low pressure column chromatography (40 x 1 cm column) using chloroform as eluent. The first yellow band afforded pure MBR-XIII (48 mg), with purity checked by tlc or silica gel (chloroform-ethanol, 10:1, vol/vol as eluent) and hplc. A second yellow product (2 mg) of unascertained structure was eluted with chloroform

containing 1% methanol. The MBR-XIII melted with decomposition at 310° (lit [19] mp 312-315°, dec). It had uv-visible (chloroform): λ max 431 nm, ϵ, 60,000; ^{1}H-nmr (deuteriochloroform): δ 1.12 (t, 6H, J = 7 Hz), 1.85 (s, 6H, CH$_3$ at C-2, C-18), 1.85 (s, 6H, CH$_3$ at C-7, C-13), 2.48 (q, 4H, J = 7 Hz), 2.78 (m, 8H, -CH$_2$CH$_2$-CO$_2$-), 4.06 (s, 2H, -CH$_2$- at C-10), 6.04 (s, 2H, =CH at C-5, C-15), 9.15 (s, 2H, pyrrole NH), 10.52 (s, 2H, lactam NH), 13.31 (broad s, 2H, COOH) ppm; ^{1}H-nmr (dimethylsulfoxide-d$_6$): δ 1.10 (t, 6H, J = 8 Hz), 1.70 (s, 6H, CH$_3$ at C-2, C-18), 2.06 (s, 6H, CH$_3$ at C-7, C-13), 2.5-3.0 (masked by solvent), 3.97 (s, 2H, -CH$_2$- at C-10) , 5.95 (s, 2H, =CH at C-5, C-15), 9.72 (s, 2H, lactam NH), 10.27 (s, 2H, pyrrole NH), 11.88 (broad s, 2H, COOH) ppm.

5-[1,5-Didehydro-3-ethyl-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-2,4-dimethyl-3-ethyl-1H-pyrrole (Kryptopyrromethenone, KRP).

The solvents and reagents used were oxygen-degassed, argon-saturated. Reactions and extractions were carried out under an argon atmosphere. 5-Bromomethylene-4-ethyl-3-methyl-2-oxo-1H-pyrrole (1) [4] (2.5 g, 11.5 mmoles) and kryptopyrrole (4) [4] (1.45 g, 11.5 mmoles) were placed in a 250 ml round-bottom flask equipped with a condenser and charged with 100 ml of methanol under an argon atmosphere. Three drops of concentrated hydrochloric acid were added, and the solution was heated at reflux for 4 hours then allowed to cool. The yellow-orange precipitate obtained was removed by filtration and washed with ice cold methanol-water (1:1). A second crop of crystals was obtained by concentrating the combined filtrate to one-half its volume and cooling in a refrigerator. The filtered and washed second crop was combined with the first and recrystallized from benzene (under argon) to afford 2.25 g (79%) of yellow needles, mp 250-251° (lit [10] mp 248-250°). It had spectral properties matching those reported previously [4,10].

Etiobiliverdin-IVγ (EBV-IV).

This verdin was prepared exactly as for MBV-IV DME, using 129 mg (0.5 mmole) of kryptopyrromethenone, to afford 104 mg (84%) of the desired product, mp 262-265° (lit [3] mp 263-266°) and spectroscopic properties matching those reported previously [20,21].

Etiobilirubin-IVγ (EBR-IV).

All solutions and solvents were oxygen-degassed, argon-saturated, and all reactions and extractions were blanketed with argon. Etiobiliverdin (70 mg, 0.12 mmole) in 20 ml of a 1:1 mixture of methanol and purified tetrahydrofuran was cooled in an ice bath, then sodium borohydride was added at once with stirring. Stirring was continued for 5 minutes at ice temperature, followed by 5 minutes at room temperature. (The latter was necessary since the reaction had failed to go to completion at ice temperature). At this time, the reaction color is yellow-brown. The reaction is quenched by the addition of 40 ml of water, and the rubin was extracted into chloroform (3 x 10 ml). The combined chloroform extracts were washed with water (2 x 5 ml), filtered through chloroform-wetted filter paper and evaporated (rotary evaporator). The product EBR, 70 mg, 98%, is purified by column chromatography (10 x 2.5 cm column) on silica gel using chloroform-methanol, 100:2 as eluent. It had mp 216-218° (lit [22] mp 218-220°) and ^{1}H-nmr (deuteriochloroform): δ 1.00, 1.10 (t, 12H, CH$_3$), 1.53 (s, 6H, CH$_3$ at C-2, C-18), 2.08 (s, 6H, CH$_3$ at C-7, C-13), 2.30-2.64 (m, 8H, -CH$_2$-), 4.09 (s, 2H, CH$_2$ at C-10), 5.93 (s, 2H, =CH at C-5, C-15), 10.28 (s, 2H, pyrrole NH), 10.58 (s, 2H, lactam NH) ppm; ^{1}H-nmr (dimethylsulfoxide-d$_6$): δ 0.78 (t, 6H, J = 7 Hz), 1.11 (t, 6H, J = 7 Hz), 1.60 (s, 6H, CH$_3$ at C-7, C-13), 2.03 (s, 6H, CH$_3$, at C-2, C-18), 2.19-2.58 (m, 8H, -CH$_2$-), 3.95 (s, 2H, CH$_2$ at C-10), 5.99 (s, 2H, =CH at C-5, C-15), 9.78 (2H, lactam NH), 10.28 (s, 2H, pyrrole NH) ppm.

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