# Synthesis of Unsymmetric Bile Pigments: Mesobilirubin-VIIIα, 17-Desvinyl-17-ethyl-bilirubin-VIIIα and 12-Despropionic Acid-12-ethyl-mesobilirubin-XIIIα

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The unnatural bile pigments 17-desvinyl-17-ethylbilirubin-VIIIα, mesobilirubin-VIIIα and 12-despropionic acid-12-ethyl mesobilirubin-XIIIα were synthesized *via* (1) "reverse scrambling" of bilirubin-XIIIα or mesobilirubin-IXα with mesobilirubin-IVα or etiobilirubin-IVγ or (2) following coupling of xanthobilirubic acid methyl ester with ψ-xanthobilirubic acid methyl ester, or coupling of xanthobilirubic acid methyl ester with kryptopyrromethenone.

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## Introduction.

(4*Z*,15*Z*)-Bilirubin-IXα (**BR-IX**), which is the yellow-orange cytotoxic pigment of jaundice, 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 encepthalopathy and other tissue damage [2]. Jaundice in the newborn may therefore lead to brain damage resulting in subtle intellectual or neurologic abnormalities, retarded motor development or even death. These adverse sequelae have prompted investigations directed toward understanding the relationship of the structure of BR-IX to its solution properties and biological function.

One of the most significant features of the structure of **BR-IX** is its unique 3-dimensional form, characterized by an ability and tendency to form *intra*molecular hydrogen bonds and theory control its conformation and polarity (Figure 1) [1]. This conformation is crucial for determining its (unusual) 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); and (ii) on $sp^3$ carbon at C-10, which constrains the molecule to bend in the middle and allow the two pyrromethanone 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, *e.g.*, **MBR-XIII**, on with methyl groups interchanged at C-2/C-3 or C-17/C-18, (*e.g.*, symmetrical bilirubins and mesobilirubins IIIα and XIIIα, all exhibit similar solubility properties, *e.g.* soluble in chloroform, insoluble in methanol; insoluble in dilute aqueous bicarbonate. However, isomers that do not have their propionic acid groups located at C-8 and C-12, *e.g.*, mesobilirubin-IVα have been shown to have very different solubility properties, *viz.* insoluble in chloroform, soluble in methanol;

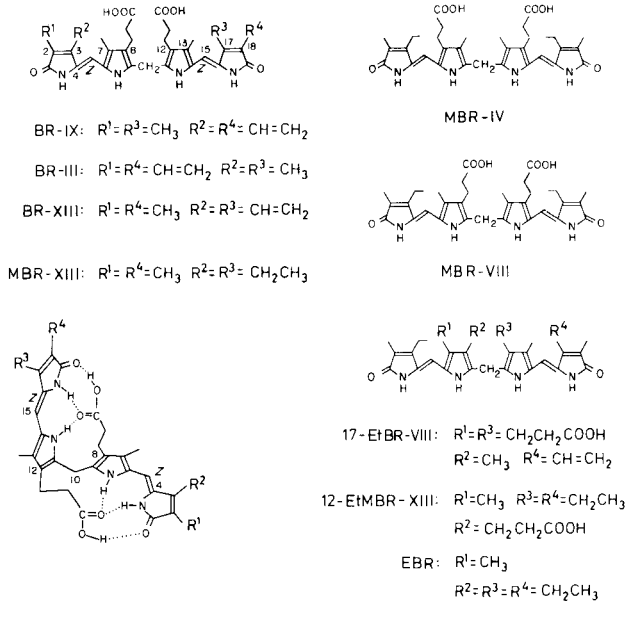


Figure 1. (Upper Left) Linear representations of bilirubin-IXα (**BR-IX**), bilirubin-IIIα (**BR-III**), bilirubin-XIIIα (**BR-XIII**) and mesobilirubin-XIIIα (**MBR-XIII**). (Lower Left) Folded, intramolecularly hydrogen-bonded conformation of **BR-IX, BR-IV, BR-XIII** and **MBR-XIII**. (Right) Linear representations of mesobilirubin-IVα (**MBR-IV**), mesobilirubin-VIIIα (**MBR-VIII**), 17-desvinyl-17-ethylmesobilirubin-VIIIα (17-**EtMBR-VIII**), 12-despropionic acid-12-ethylmesobilirubin-XIIIα (12-**EtMBR-XIII**) and etiobilirubin-IVγ (**EBR**).

soluble in dilute aqueous bicarbonate [3]. Because of their potential importance as comparison compounds in understanding the manifold events associated with bilirubin-IX$\alpha$ metabolism, *e.g.*, albumin and other protein binding, hepatic uptake, glucuronidation, excretion and toxicity, we synthesized the unsymmetric pigments with one propionic acid group located properly for intramolecular H-bonding, and the second propionic acid group either free and unable to participate (**MBR-VIII**) and 17-**EtBR-VIII**) or replaced by ethyl (12-**ETMBR-XIII**) (Figure 1).

Some while ago, McDonagh and Assisi [3,4] showed that **BR-IX** underwent an acid-catalyzed disproportionation reaction, wherein the pyrromethenone units are scrambled, to afford a nearly 1:2:1 mixture of **BR-III**, **BR-IX** and **BR-XIII** isomers (eq. 1). With suitable modification, this reaction has proved to be the most convenient way to prepare **BR-III** and **BR-XIII** [5] and, after catalytic reduction of their vinyl groups, **MBR-III** and **MBR-XIII** (Figure 1) [4].

$$2\ \textbf{BR-IX} \overset{H^+}{\rightleftharpoons} \textbf{BR-XIII} + \textbf{BR-III} \qquad (1)$$

McDonagh also showed that acid treatment of a mixture of **BR-III** and **BR-XIII** gave **BR-IX**, as per the reverse of eq. 1 [4]. This "reverse disproportionation" (or "reverse scrambling") reaction offered the most attractive way for preparing unsymmetric isomers from known symmetrical precursors: **MBR-VIII** from **MBR-XIII** [6] and **MBR-IV** [6], 17-**EtBR-VIII** from **BR-XIII** [5] and **MBR-IV** [6], and 12-**EtMBR-XIII** from **MBR-XIII** [6] and **EBR** [6]. In each case the reactions proceeded smoothly and quickly in degassed, argon-saturated dimethylsulfoxide following the addition of a small quantity of concentrated hydrochloric acid, and, except with the synthesis of 12-**EtMBR-XIII**, the desired product was isolated by preparative tlc. Although **MBR-IV**, **MBR-VIII** and 17-**EtMBR-VIII** can be extracted into 0.1 *M* sodium bicarbonate from chloroform solvent and **MBR-XIII** and **BR-XIII** cannot, the extraction procedure offers no advantage since the desired rubins must still be separated by chromatography from co-extracted **MBR-IV**. (We could find no intermediate *p*H that would cleanly separate the bicarbonate-extractable rubins.) In the synthesis (above) of 12-**EtMBR-XIII** from **MBR-XIII** and **EBR**, we had expected the acid pigments to be co-extracted from chloroform into strong base (**MBR-XIII** is insoluble in 0.1 *M* sodium bicarbonate but soluble in 0.1 *M* sodium carbonate). Surprisingly, **MBR-VIII** would not even extract into 0.1 *M* sodium hydroxide and since the chromatographic separation of it from **EBR** proved difficult, a different synthetic procedure was developed - - one based on the coupling of pyrromethenones to tetrapyrrole pigments [6,7].

Falk, *et al* [7] had previously shown that 5'-methylpyrromethenones, *e.g.*, xanthobilirubic acid methyl ester [8] (**XBRME**) and kryptopyrromethenone [9] (**KRP**) are oxidized in degassed THF by DDQ to azafulvenes **1**, which undergo self-condensation in the presence of trifluoroacetic acid to the corresponding symmetrical mesobiliverdin-XIII$\alpha$ dimethyl ester (**MBV-XIII DME**) and etiobiliverdin (**EBV**), respectively. The reactive intermediate is proposed to be a carbocation **2** formed by protonation of the imino nitrogen of the azafulvene oxidation product **1**. The self-coupling synthetic pathway has been developed for the synthesis of mesobilirubins-XIII$\alpha$ and IV$\alpha$ and etiobilirubin [6]. It can also be used, as shown herein, for hetero-coupling, *e.g.*, **XBRME** with **KRP** to give 12-**EtMBV-XIII DME** (along with the self-condensation products **MBR-XIII DME** and **EBV**) and **XBR** with $\psi$-**XBRME** to give **MBV-VIII-13ME** (along with the self-condensation products **MBV-XIII** and **MBV-IV DME**). In the synthesis of 12-**EtMBR-XIII**, the verdin (ester) mixture derived from hetero-coupling of **XBRME** with **KRP** is first saponified then separated by column chromatography, and the isolated 12-**EtMBV-XIII** (34% yield, 68% of theoretical) is reduced to the desired rubin using sodium borohydride. Unlike their rubin counterparts, 12-**EtMBV-XIII** ($R_f \cong 0.25$) is well separated on silica gel chromatography from **EBV** ($R_f \cong 0.7$), with **MBV-XIII** ($R_f \cong 0.0$) slowest moving in chloroform-methanol, 10:1, vol/vol. The corresponding rubins show **MBR-XIII** ($R_f \cong 0.8$), 12-**EtMBR-XIII** ($R_f \cong 0.5$) and **EBR** ($R_f \cong 0.5$) with chloroform-methanol-acetic acid, 100:1:1, vol/vol/vol on silica tlc.

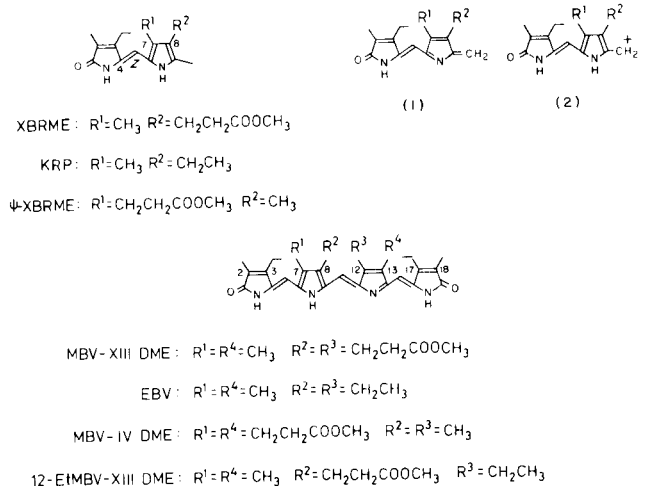


Figure 2. (Upper Left) Linear structures of xanthobilirubic acid methyl ester (**XBRME**) kryptopyrromethenone (**KRP**), pseudo-xanthobilirubic acid methyl ester ($\psi$-**XBRME**). (Upper Middle and Right) The azafluvenes from DDQ oxidation of the pyrromethenones, before (**1**) and after (**2**) protonation. (Lower) Linear structures of mesobiliverdin-XIII$\alpha$ dimethyl ester (**MBV-XIII DME**), etiobiliverdin (**EBV**), mesobiliverdin-IV$\alpha$ dimethyl ester (**MBV-IV DME**), 12-despropionic acid-12-ethyl-mesobiliverdin-XIII$\alpha$ methyl ester (12-**EtMBV-XIII ME**) and mesobiliverdin-VIII$\alpha$ 13-methyl ester (**MBV-VIII 13 ME**).

The pyrromethenone coupling pathway offers an alternative, useful procedure for synthesizing **MBR-VIII**. Here, the verdin esters are separated by column chromatography (**MBV-IV DME**) $R_f \cong 0.7$; **MBV-VIII-13ME**, $R_f \cong 0.4$; **MBV-XIII**, $R_f \cong 0.2$ on silica tlc using chloroform-methanol-acetic acid, (100:10:1, vol/vol/vol). Then the isolated **MBV-XIII-13ME** is saponified and reduced to the rubin with sodium borohydride.

In summary, the two conceptually different synthetic methods presented herein offer attractive, practical routes to a wide variety of bile pigments. The pigments **MBR-VIII**, 17-**Et-MBR-VIII** and 12-**EMBR-XIII** are all soluble in chloroform, unlike **MBR-IV**, but like **MBR-XIII** and **BR-IX**. Like **MBR-IV**, and unlike **MBR-XIII** and **BR-IX**, **MBR-VIII** and 17-**EtMBR-VIII** are soluble in 0.1 *M* sodium bicarbonate. 12-**EtMBR-XIII** is not soluble in 0.1 *M* sodium bicarbonate nor in 0.1 *M* sodium carbonate (in which **MBR-XIII** and **BR-IX** are soluble), nor in 0.1 *M* sodium hydroxide. The pigments are being examined for their hepatic excretability in experimental animals and for their potential as substrates for glucuronyl transferase.

## 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 dimethyl sulfoxide-$d_6$ (99.9% $d_6$), both from Aldrich. All uv-visible absorption spectra were run on a Cary 219 instrument. Analytical thin layer chromatography (tlc) was carried out on J. T. Baker silica gel 1B-F plates (125 $\mu$ layer). Preparative layer chromatography (plc) was accomplished on 1000 $\mu$ layers of Woelm silica gel F. Column chromatography was carried out on 32-63 $\mu$ 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 $\mu$m 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 [10]. 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 and dimethylsulfoxide were from Aldrich. Chloroform and methanol (hplc grade) were from Fisher. Ascorbic acid and glycine were from Matheson, Coleman and Bell. Disodium EDTA, glacial acetic acid and benzene were from Mallinckrodt. All solvents and solutions used were rendered oxygen-free, argon-saturated by bringing to brief reflux under a stream of argon, cooling and storing under argon. Reactions were typically carried out under argon.

### Mesobilirubin-VIII$\alpha$ (**MBR-VIII**).

Mesobilirubin-XIII$\alpha$ (14 mg, 0.024 mmole) [6] and mesobilirubin-IV$\alpha$ [6] (14 mg, 0.024 mmole) in 6 ml of dimethyl sulfoxide was stirred magnetically for 1 minute following the addition of 0.6 ml of concentrated hydrochloric acid. Water (60 ml) was then added, and the yellow-green precipitate formed was collected by centrifugation, washed with water (2 x 20 ml) and dried in a vacuum dessicator for 24 hours. The product showed three spots on analytical tlc (chloroform-methanol-acetic acid, 100:2:1, vol/vol/vol), two of which correspond to the reactants, **MBR-XIII** ($R_f \cong 0.6$) and **MBR-IV** ($R_f < 0.1$) and the third, $R_f \cong 0.4$, to **MBR-VIII**. The products were separated by plc to afford 10 mg (70% theoretical yield) of **MBR-VIII**, which had uv-visible (dimethylsulfoxide): $\lambda$ max 400 nm, $\epsilon$, 24,888 and $\lambda$ max 428, $\epsilon$, 26,200 (double maxima) and $^1$H-nmr (deuteriochloroform containing a few drops of dimethylsulfoxide-$d_6$): $\delta$ 1.19 (t, 6H, J = 7 Hz), 1.77 (s, 6H, $CH_3$ at C-2, C-18), 1.94 (s, 3H, $CH_3$ at C-8), 2.09 (s, 3H, $CH_3$ at C-13), 2.39 (m, 12H, -$CH_2CH_2$-$CO_2$- and $CH_2$-$CH_3$), 3.99 (s, 2H, -$CH_2$- at C-10), 6.00 (s, 2H, =CH at C-5, C-15), 9.11 (br s, 1H, NH), 10.03 (br s, 2H, NH), 10.37 (br s, 1H, NH) ppm.

*Anal.* Calcd. for $C_{33}H_{40}N_4O_6$ (588.7): C, 67.33; H, 6.84; N, 9.52. Found: C, 67.02; H, 6.69; N, 9.56.

### 17-Desvinyl-17-ethylbilirubin-VIII$\alpha$ (17-**EtMBR-VIII**).

This rubin was prepared as **MBR-VIII** above, using 70 mg (0.12 mmole) of **BR-XIII** [6] and 70 mg (0.12 mmole) of **MBR-IV** [6]. The two rubins were dissolved together in 30 ml of dimethylsulfoxide and stirred for 5 minutes at room temperature following the addition of 3 ml of concentrated hydrochloric acid. The reaction was quenched by the addition of 250 ml of cold water, and the yellow-green precipitate collected by centrifugation, washed well with water and dried in a vacuum dessicator. Separation of the new rubin from the starting materials by plc using chloroform-methanol-acetic acid, 100:2:1, vol/vol/vol gave 29 mg (42% of theoretical) of 17-**EtMBR-VIII**. It had $^1$H-nmr (dimethylsulfoxide-$d_6$): $\delta$ 1.09 (t, 3H, J = 7, Hz), 1.69 (s, 3H, $CH_3$ at C-18), 1.77 (s, 3H, $CH_3$ at C-2), 1.91 (s, 3H, $CH_3$ at C-12), 1.99 (s, 3H, $CH_3$ at C-7), 2.37-2.72 (m, 10H, -$CH_2CH_2$-$CO_2$- and $CH_2$-$CH_3$), 3.93 (s, 2H, $CH_2$ at C-10), 5.48-5.69 (m, 2H, $C_{17}$ -CH=$CH_2$), 6.09 (s, 2H, =CH at C-5, C-15), 6.66-6.95 (m, 1H, -CH=$CH_2$), 9.77 (s, 1H, lactam NH), 10.03 (s, 1H, lactam NH), 10.32 (s, 1H, pyrrole NH), 10.43 (s, 1H, pyrrole NH) ppm.

*Anal.* Calcd. for $C_{33}H_{38}N_4O_6$ (586.7): C, 67.56; H, 6.53; N, 9.55. Found: C, 67.88; H, 6.96; N, 9.45.

### 12-Des-propionic Acid 12-Ethylmesobilirubin-XIII$\alpha$ (12-**EtMBR-XIII**).

All solvents and solutions were oxygen-free, argon-saturated, reactions and extractions were blanketed with argon. Xanthobilirubic acid methyl ester (**XBRME**) [8] (79 mg, 0.25 mmole) kryptopyrromethenone [6,9] (65 mg, 0.25 mmole) were 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 was added dropwise, during 90 minutes and with stirring, a solution containing 136 mg (0.6 mmoles) of DDQ dissolved in dry tetrahydrofuran (25 ml). The mixture was cooled in an ice bath for 5 minutes then diluted with an ice cold mixture of chloroform (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 *p*H. (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 (140 mg, 92%) 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 first blue band is collected and the solvent removed on a rotary evaporator to give 130 mg (86%) of a mixture of 12-**EtMBV-XIII**, **MBV-XIII DME** and **EBV**. Separation of the verdins was achieved following saponification, as follows. The verdin mixture (130 mg) was dissolved in 500 ml of methanol containing ascorbic acid (50 mg) and 1-2 mgs of disodium EDTA. To this solution was added 250 ml of 1 *M* aqueous sodium hydroxide, and the resultant solution was stirred for 1 hour at 37° under an argon atmosphere in the dark. Then the solution was acidified with acetic acid (32 ml) and extracted with a mixture of chloroform (250 ml) and *p*H 2.7 glycine·HCl buffer (1000 ml). The chloroform extract was washed with 0.1 *M* aqueous sodium bicarbonate (1 x 50 ml) then water (2 x 100 ml), filtered through chloroform-wetted filter paper and evaporated under vacuum at 40° to give 129 mg (100%) of blue solid. Analytical tlc on silica gel using chloroform-methanol, 10:1, vol/vol as eluent showed three spots corresponding to **EBV** ($R_f \cong 0.7$), 12-**EtMBV-XIII** ($R_f \cong 0.25$) and **MBV-XIII** ($R_f \cong 0$). The mixture was separated by column chromatography on silica gel (10 cm x 2.5 cm col-

umn). Chloroform-3% methanol eluted **EBV** (33 mg, 22%) followed by yellow and violet bands. A gradual increase of the % methanol elutes other impurities, and chloroform-10% methanol elutes 12-**EtMBV-XIII** (51 mg, 34%). Finally, chloroform-methanol-acetic acid, 7:3:0.03, vol/vol/vol eluted **MBV-XIII** (27 mg, 18%). The sample of 12-**EtMBV-VIII** was reduced directly to the rubin, as follows.

The verdin monoacid from above (51 mg, 0.094 mmole) was dissolved in 15 ml of methanol and cooled in an ice bath to 0° with magnetic stirring, then 510 mg of sodium borohydride was added all at once, with stirring. After 5 seconds the reaction solution had turned yellow. At this point water (35 ml) was added followed by dropwise addition of 1 ml of acetic acid to acidify the solution and precipitate the product, 12-**EtMBR-XIII**, which was removed by extraction into chloroform (3 x 10 ml). The combined chloroform extracts were washed with 0.1 *M* sodium bicarbonate (10 ml), water (10 ml) and filtered through chloroform-wetted filter paper. The solvent was removed on a rotary evaporator and the 12-**EtMBR-XIII** dried overnight in a vacuum dessicator. The yield of dried 12-**EtMBR-XIII** is 49 mg. It was pure by tlc on silica gel using chloroform-methanol-acetic acid (100:1:1, vol/vol/vol) or chloroform-ethanol (10:1) as eluent, and by hplc. It had uv-visible (dimethyl sulfoxide): λ max 395, ε, 22,900 and λ max 425, ε, 27,250 (double maximum) and $^1$H-nmr (dimethyl sulfoxide-$d_6$): δ 0.87 (t, 3H, J = 7 Hz, $CH_2$-$CH_3$ at C-12), 1.09 (t, 6H, J = 7 Hz), 1.77 (s, 6H, $CH_3$ at C-2, C-18), 1.99 (s, 6H, $CH_3$ at C-7, C-13), 2.15-2.65 (m, 14H, -$CH_2$- of -$CH_2CH_3$ and -$CH_2$-$CH_2$-$CO_2$-), 3.93 (s, 2H, -$CH_2$- at C-10), 5.95 (s, 2H, =CH at C-5, C-15), 9.82 (br s, 1H, lactam NH), 9.90 (br s, 1H, lactam NH), 10.36 (br s, 1H, pyrrole NH), 10.41 (br s, 1H, pyrrole NH) ppm.

*Anal.* Calcd. for $C_{32}H_{40}N_4O_4$ (544.7): C, 70.56; H, 7.40; N, 10.29. Found: C, 70.86; H, 7.14; N, 10.18.

Mesobilirubin-VIIIα (**MBR-VIII**).

This rubin was prepared as above from xanthobilirubic acid (15 mg, 0.05 mmole) and ψ-xanthobilirubic acid methyl ester (16 mg, 0.05 mmole) were dissolved together in 25 ml of dry tetrahydrofuran with magnetic stirring. The resulting mixture was kept in the dark for 5 minutes at 23°, then 1 ml of trifluoroacetic acid was added during 30 seconds followed by the addition of a solution of 30 mg of DDQ in 10 ml of tetrahydrofuran during 60 minutes. The reaction mixture was kept in the dark for an additional hour with magnetic stirring after which it is cooled in an ice bath. Then the reaction mixture was diluted with a mixture of cold chloroform (50 ml) and 1% aqueous triethylamine in water (65 ml). The aqueous phase was extracted with chloroform (1 x 15 ml), and the combined organic phases were washed with 0.1 *M* sodium bicarbonate until the *p*H of the resulting aqueous phase is neutral. (This may require up to 5-10 extractions of 20 ml each. The process is very tedious but necessary in order to avoid the formation of red pigment. Tripyrrole aldehyde?). After a final wash with water, the blue organic phase is filtered through chloroform-wetted filter paper and the chloroform removed on a rotary evaporator. Analytical tlc on silica gel (chloroform-methanol-acetic acid, 100:10:1, vol/vol/vol) showed 3 spots corresponding to **MBV-IV DME** ($R_f \cong 0.7$), **MBV-VIII-13-ME** ($R_f \cong 0.4$) and **MBV-XIII** ($R_f < 0.1$).

The verdin mixture was separated by column chromatography (10 x 2.5 cm) on silica gel. The verdins were dissolved in 4 ml of chloroform and adsorbed on 1 g of silica during evaporation of the solvent. The dry, blue powder was added to the top of the column and eluted. Elution with chloroform-3% methanol gave some yellow and blue impurities. Elution with chloroform-5% methanol gave **MBV-IV DME**, with chloroform-15-20% methanol **MBV-VIII-13 ME** was eluted, and at 30-40% methanol the **MBV-XIII** band was collected. Analytical tlc showed that the **MBV-VIII-13-ME** required further purification, and this was accomplished on plc using chloroform-methanol-acetic acid, 100:10:1, vol/vol/vol. The isolated material was saponified and reduced (sodium borohydride) as follows.

**MBV-VIII-13 ME** (6 mg) was dissolved in methanol (50 ml) containing ascorbic acid (11 mg) and a trace of disodium, then 25 ml of 1 *M* sodium hydroade was added, and the solution was stirred for 90 minutes at 37° under argon in the dark. The solution was acidified with 10% hydrochloric acid and extracted with a mixture of chloroform (50 ml) and *p*H 2.7 glycine·HCl buffer (100 ml). The chloroform extract was washed with 0.1 *M* sodium bicarbonate (2 x 10 ml) and water (2 x 10 ml), filtered through chloroform-wetted filter paper and evaporated under vacuum at 40°. The isolated **MBV-VIII** (5 mg, 80%) is a blue solid, soluble in chloroform and very soluble in methanol. It was pure according to analytical tlc and used directly in the next step.

To **MBV-VIII** (5 mg) in methanol (5 ml), cooled in an ice bath, was added 50 mg of sodium borohydride. After one minute, the color became yellow. Then 4 ml of water was added, the solution was acidified with acetic acid and extracted with chloroform (3 x 5 ml). The combined organic layers were washed with 0.1 *M* sodium bicarbonate (5 ml) then water (5 ml) and the solvent was removed on a rotary evaporator after filtering through chloroform-wetted filter paper. The yellow product was slightly impure by tlc, containing traces of **MBR-IV** and **MBR-XIII**. Apparently, if the solution containing **MBR-VIII** is not completely neutralized, scrambling occurs. The product can be isolated by plc. It is quite insoluble in chloroform and has the $^1$H-nmr reported above.

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