

Young-Seok Byun and David A. Lightner\*

Department of Chemistry, University of Nevada,  
Reno, Nevada 89557-0020  
Received May 7, 1991

The unnatural bile pigment analog of bilirubin-IX $\alpha$ , 2,3,7,13,17,18-hexamethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione-8,12-dicarboxylic acid (**1**), was synthesized as its diethyl ester by coupling 4,4'-dimethyl-3,3'-dicarboethoxydipyrromethane-5,5'-dialdehyde with 3,4-dimethylpyrrolin-2-one.

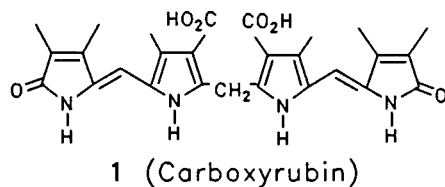
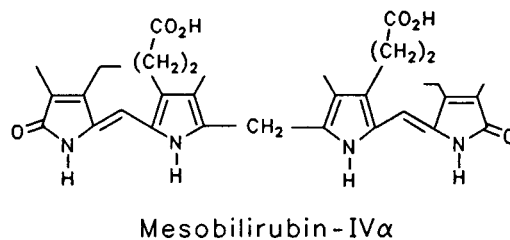
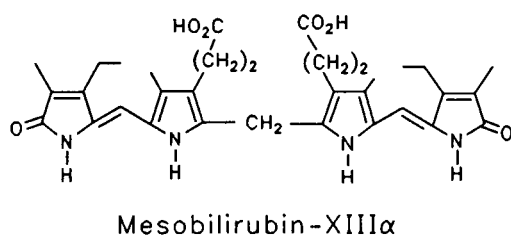
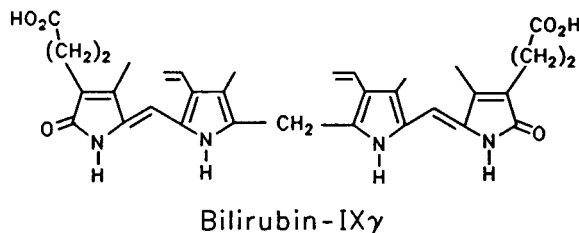
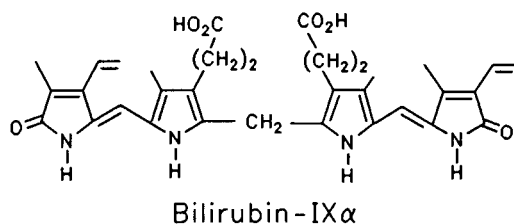
*J. Heterocyclic Chem.*, **28**, 1683 (1991).

### Introduction.

(4*Z*,15*Z*)-Bilirubin-IX $\alpha$  (bilirubin-IX $\alpha$ ), the hydrophobic and cytotoxic pigment of jaundice is a yellow-orange dicarboxylic acid produced in copious quantities by the normal catabolism of heme, principally from the hemoglobin of red blood cells [1]. Considerable effort has been devoted toward understanding the properties and metabolism of bilirubin with attention being focussed on the unique ability of the natural pigment to sequester its carboxylic acid groups and thereby lower its acidity and decrease its polarity [1,3]. In earlier work [2-4] it was shown that the location of the propionic acid groups on the tetrapyrrole carbon skeleton was the crucial determinant of sequestration ability. Thus, bilirubin analogs such as bilirubin-IX $\gamma$  and mesobilirubin-IV $\alpha$  are much more polar than bilirubin-IX $\alpha$  and in metabolism are also known to be secreted

hepatically without resort to further structural modification, such as glucuronidation. Mesobilirubin-XIII $\alpha$ , on the other hand, exhibits polarity and solubility properties rather similar to those of bilirubin-IX $\alpha$ , and like bilirubin-IX $\alpha$ , it cannot be excreted in mammalian metabolism without structural modification. The unusual properties of bilirubin-IX $\alpha$  and mesobilirubin can be ascribed, at least in part, to the location of their propionic acid groups at C-8 and C-12.

However, location is only one necessary requirement. Others include configuration and conformation of the tetrapyrrole framework [2,3,5] - properties which are, in fact, shared by all of the pigments shown above. Thus, in addition to having propionic acid groups at C-8 and C-12, the pigment must maintain the *Z* configuration at C-4 and C-15, with *syn*-periplanar or *syn*-clinal conformation



about the C-5/C-6 and C-14/C-15 single bonds, and it must be free to adopt the folded conformation obtained through rotations of the two pyrromethenone units about central  $-\text{CH}_2-$  unit at C-10. When these conditions are met, the C-8 and C-12 propionic acid  $\text{CO}_2\text{H}$  groups are brought into close proximity to the lactam  $\text{O}=\text{C}-\text{N}-\text{H}$  and pyrrole  $\text{N}-\text{H}$  functionalities to enable very effective, conformation-stabilizing intramolecular hydrogen bonding – effectively sequestering the carboxyl (and other polar groups) and rendering the pigment lipophilic. As can be seen from the drawings of Figure 1, intramolecular hydrogen-bonding is impossible for bilirubin-IX $\gamma$  and mesobilirubin-IV $\alpha$  (the former are soluble in methanol and insoluble in chloroform; the latter are insoluble in methanol and soluble in chloroform) and more excretable across the liver [6].

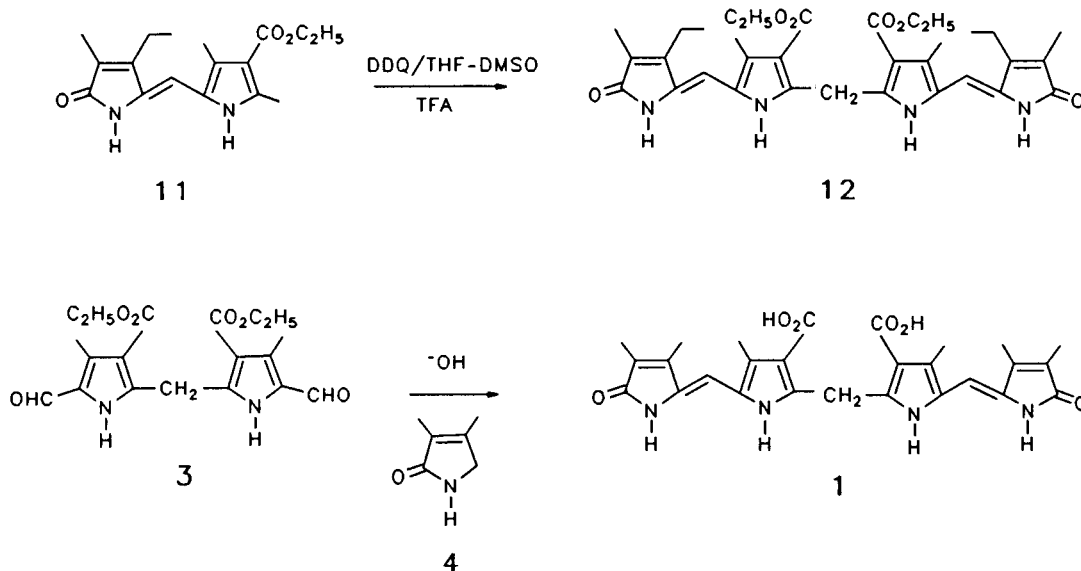
The influence of the location of the propionic acid chain on the properties of the bilirubin seems well-established [2-4]. However, the influence of the length of the alkanolic acid chains at C-8 and C-12 has not yet been called into question. Is hydrogen-bonding (and its attendant sequels) maintained with longer or shorter alkanolic acid chains? As part of a program to answer this question, we initiated a program to synthesize, analyze and perform metabolic studies on mesobilirubin-XIII $\alpha$  analogs with varying alkanolic acid chain lengths. The first such analog, **1** (“carboxyrubin”), with  $\text{CO}_2\text{H}$  groups attached directly to C-8 and C-12 and hence the analog with the shortest possible acid chains, is the subject of the present work.

#### Synthesis.

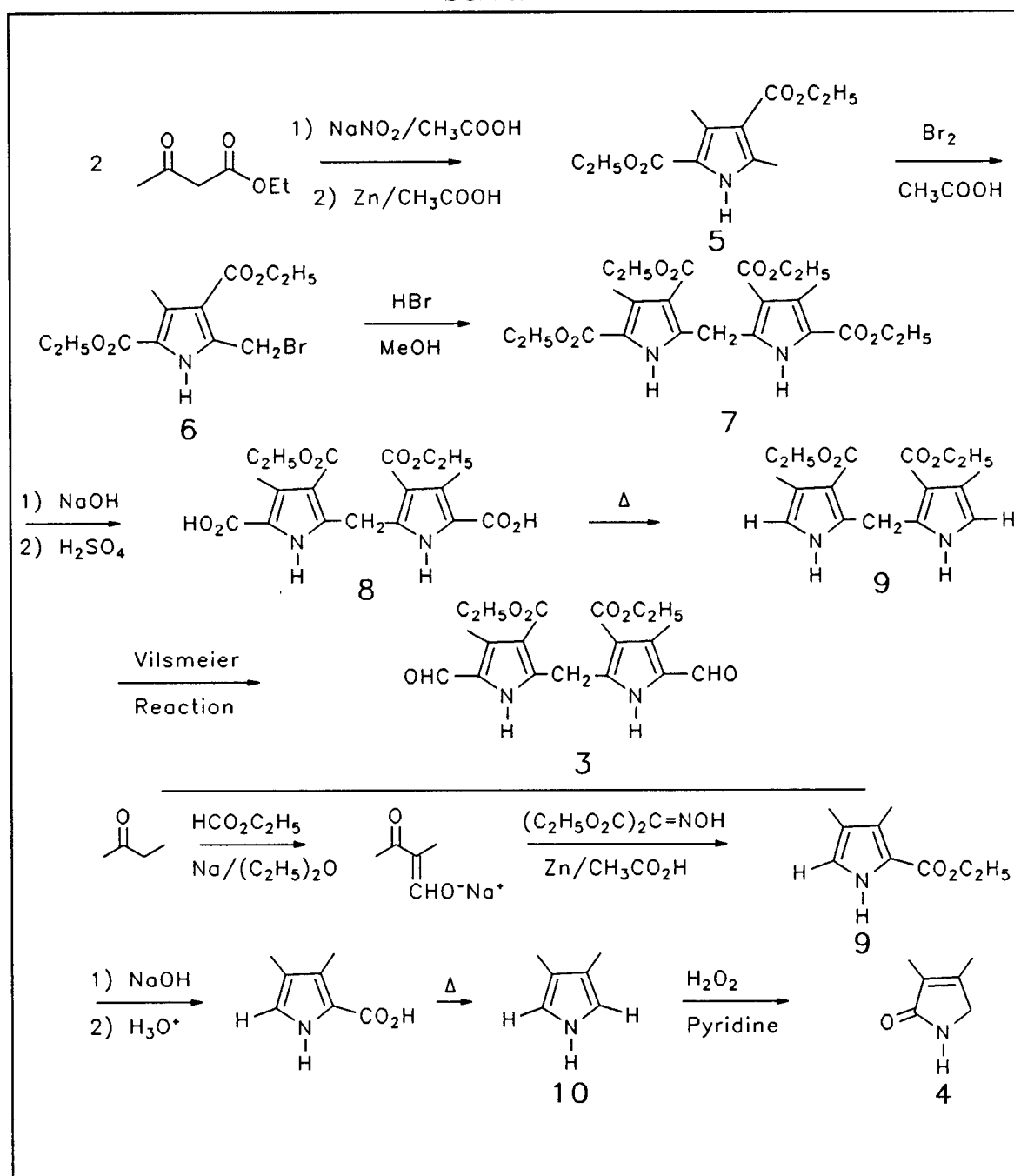
Our previously successful method [4,7] for synthesizing linear tetrapyrroles by oxidative self-coupling of dipyrroles was unsuccessful. Thus, although we could prepare pyrromethenone **11** [8], we could not achieve self-conden-

sation to verdin **12** either by reaction with dichlorodicyanoquinone in tetrahydrofuran and trifluoroacetic acid or by using our highly promising modification with chloranil in hot formic acid [9]. Possibly with direct attachment of the carboethoxy group to the pyrrole ring, the oxidation potential is raised above the limits of the oxidizing agents typically used. Consequently, we turned our attention to the possibility of preparing the desired bilirubin (**1**) by condensation of dipyrrole dialdehyde **3** with oxopyrrole **4**, a variation of the previously reported [6,10,11] successful syntheses of pyrromethenones similar to **11**. The question as to whether a methyl or ethyl group should be located at C-3 and C-17 was determined only by the greater ease of synthesizing oxopyrrole **4** from symmetric 3,4-dimethylpyrrole than in preparing the corresponding oxopyrrole with a 3-ethyl group.

The overall synthetic outline is shown in Schemes 1 and 2. In the first are presented the steps leading to the key synthetic intermediates, dipyrrole-dialdehyde **3** and oxopyrrole **4**. The dipyrrole is prepared in 6 steps from simple starting materials. Thus, ethyl acetoacetate was reacted with sodium nitrite in acetic acid to give the oxime, and this was reduced with zinc and reacted *in situ* with ethyl acetoacetate to give crystalline 2,4-dimethyl-3,5-dicarboethoxy-1*H*-pyrrole (**5**) in 57% yield. Reaction of **5** with bromine at 40° in glacial acetic acid produced shiny needles of 2-bromomethyl-4-methyl-3,5-dicarboethoxy-1*H*-pyrrole (**6**) in 42% yield. The bromomethylpyrrole **6** was self-condensed to afford dipyrrole **7** in 50% yield by heating at reflux in methanol and 47% aqueous hydrobromic acid. During heating the initially heterogeneous solution turned homogeneous (and brown), and a gas was evolved. The gas, sampled from the top of the condenser, was determined to be bromomethane by its 2.68 ppm peak in the



## SCHEME 1



$^1\text{H-nmr}$ . A white precipitate was isolated by filtration and was shown to be a by-product of the reaction, the methoxymethylpyrrole corresponding to the methanolysis product of **6**. When the reaction was carried out with an excess of methanol (1.2 g of bromo compound in 10 ml methanol), the methoxymethylpyrrole was the only product.

Saponification of dipyrrole **7** with 2 equivalents of sodium hydroxide gave its sodium salt. When the salt was acidified with 10% hydrochloric acid in an ice-bath, the

product was not pure; however, by acidifying the salt with 2*N* sulfuric acid at 70°, pure 4,4'-dimethyl-3,3'-dicarboethoxydipyrrolemethane-5,5'-dicarboxylic acid (**8**) could be obtained. The diacid was doubly decarboxylated, and the product **9** was removed by sublimation at about 210°, then reacted under Vilsmeier conditions to give 4,4'-dimethyl-3,3'-dicarboaldehydedipyrrolemethane (**3**) in quantitative yield. The other component is pyrroloinone **4**, which was prepared by oxidation of 3,4-dimethylpyrrole

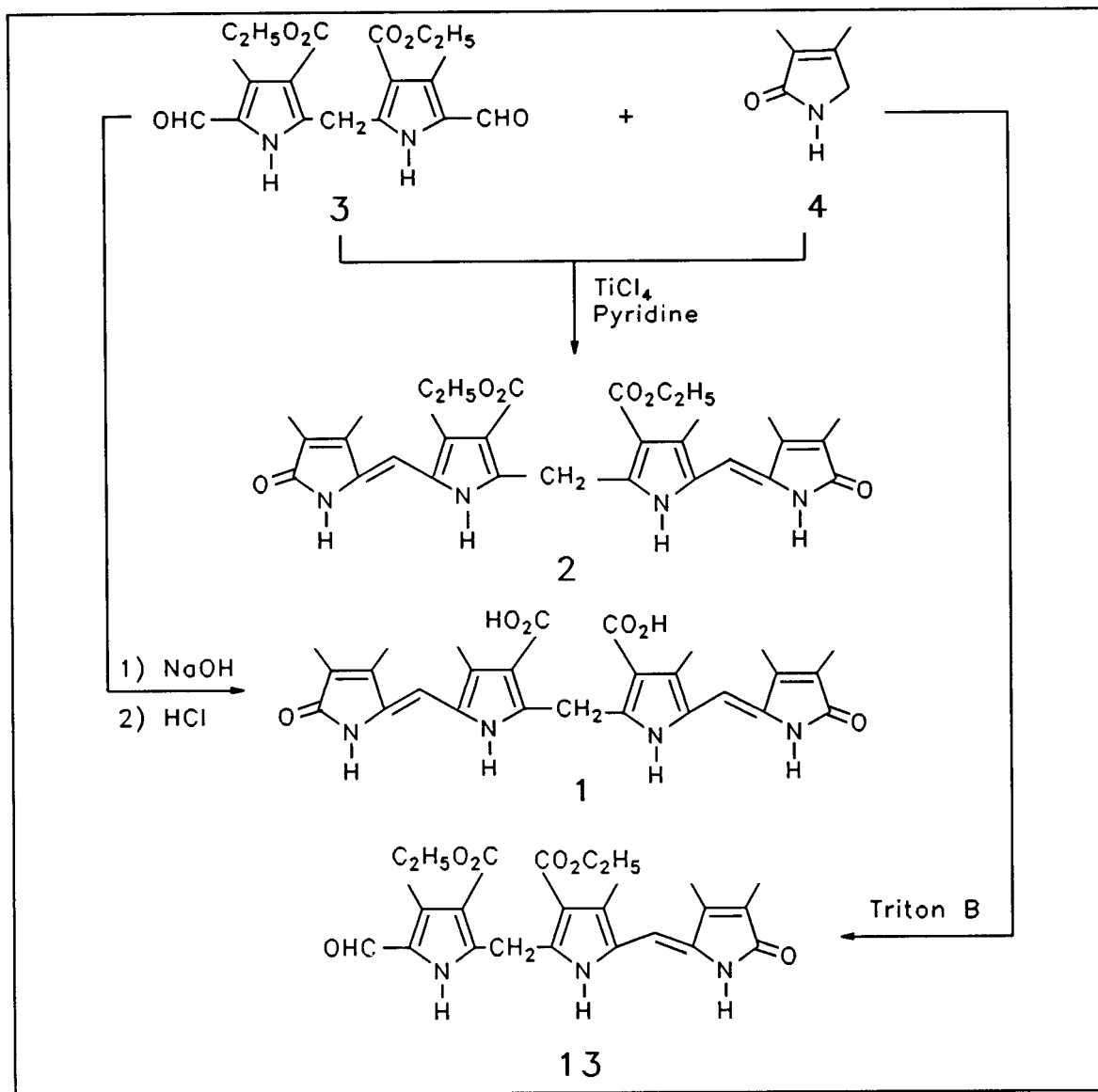
(10). The latter was prepared as shown in Scheme 1 in four steps from butanone, first by reaction with ethyl formate to give the sodium salt of 3-hydroxymethylene-2-butanone, which was condensed with *in situ* reduced diethyl oximomalonnate to afford pyrrole **9**, then saponification and decarboxylation of **9** (to give **10**).

We attempted several methods for coupling **3** and **4**, as outlined in Scheme 2. One was patterned after our previously successful [10] syntheses of pyrromethenones from pyrrole aldehydes and oxopyrroles. Thus, a mixture of **3** was heated with excess **4** in 4 *N* sodium hydroxide for 2 hours at reflux. After removing a brown precipitate formed during reflux (and shown to contain no desired product by <sup>1</sup>H-nmr), low yields of tetrapyrrole **1** were precipitated following the dark brown filtrate neutralization

with dilute hydrochloric acid. Other base-catalyzed procedures using weak bases such as cesium carbonate or potassium carbonate were unsuccessful; however, when Triton B (benzyltrimethylammonium hydroxide) was used [12] as a catalyst for the condensation reaction between oxopyrrole **4** and dipyrrole dialdehyde **3** in pyridine, tripyrrole **13**, and not the desired tetrapyrroles **1** or **2**, was isolated following tlc.

A successful synthesis of tetrapyrrole diester **2** was achieved using titanium tetrachloride [13] in pyridine to couple **3** and **4**, although the yield was very low (<5%). Saponification of **2** with 10% sodium hydroxide afforded diacid **1** in 60% yield following acidification with dilute acetic acid.

### SCHEME 2



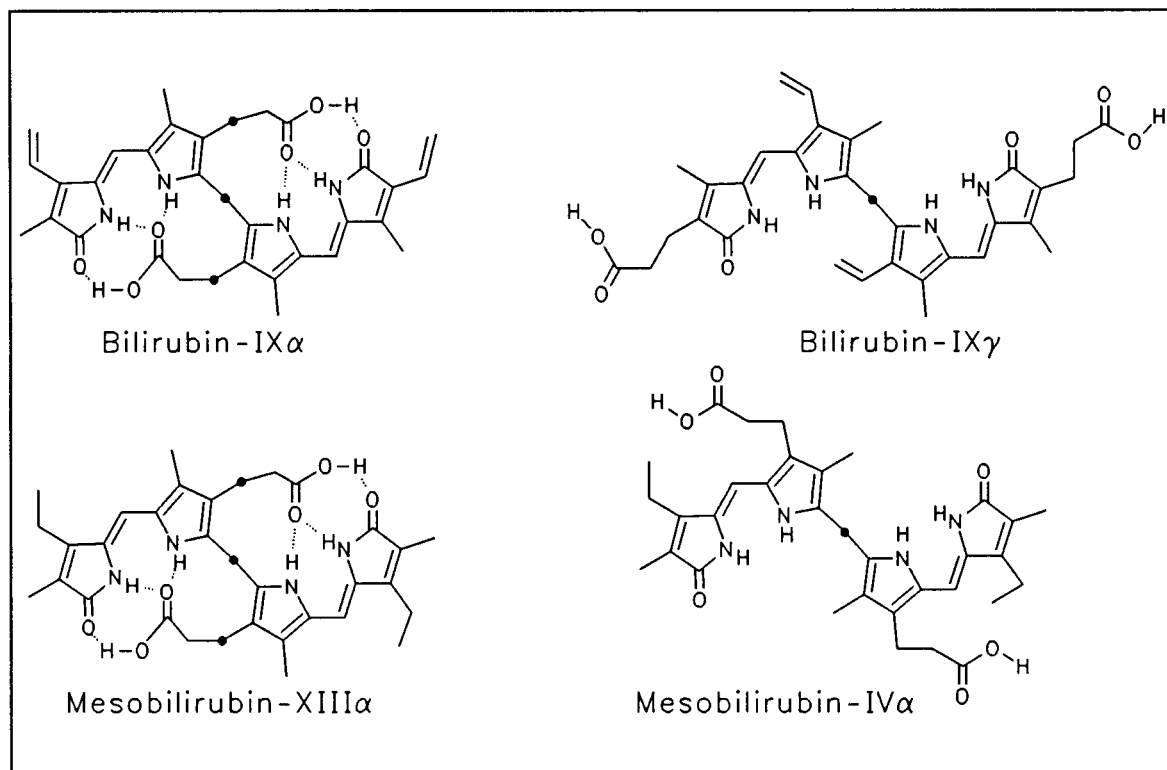
## Properties.

The properties of bilirubins are known to depend considerably on the ability of the pigment's polar groups to interact through intramolecular hydrogen bonding. Thus, sequestration of the propionic acid carboxyl groups of bilirubin-IX $\alpha$  and its symmetric analog mesobilirubin-XIII $\alpha$  by intramolecular hydrogen bonding with the pyromethenone lactam carbonyl and N-H and the pyrrole N-H make these pigments much less polar and more lipophilic than their counterparts with propionic acid groups anchored to other ring carbons, as in bilirubin-IX $\gamma$  and mesobilirubin-IV $\alpha$  (Figure 1). For example, bilirubin-IX $\gamma$  and mesobilirubin-IV $\alpha$ , in which the COOH groups cannot participate in intramolecular hydrogen bonding, can be extracted from chloroform into 5% aqueous sodium bicarbonate, but bilirubin-IX $\alpha$  and mesobilirubin-XIII $\alpha$  cannot, and the former pair run faster on reverse phase hplc and more slowly on silica tlc adsorption chromatography than the latter.

The relative polarities of pigments can often be detected and assessed by their chromatographic behavior [14]. Thus, it was interesting to note that bilirubin analog **1** had nearly the same retention time (16.9 minutes) as mesobilirubin-XIII $\alpha$  (16.3 minutes) on analytical hplc us-

ing as the eluent (0.1 M di-*n*-octylamine in methanol containing 5% water, 0.75 ml/minute flow rate). The hplc system is known to distinguish between those bile pigments which adopt intramolecular hydrogen bonding (as do bilirubin-IX $\alpha$  and mesobilirubin-XIII $\alpha$ ) and become lipid soluble and those that cannot (as in mesobilirubin-IV $\alpha$  and bilirubin glucuronides) and are more polar and more hydrophilic [4,7]. The latter pair move much faster than the former pair in the hplc system described, with retention times under 8 minutes. The data raise the initial expectation that **1** might exhibit significant intramolecular hydrogen bonding and thus show properties typical of bilirubin-IX $\alpha$  and mesobilirubin-XIII $\alpha$ . However, markedly contrasting chromatographic data may be seen in comparing the tlc behavior of **1** ( $R_f = 0$ ) and mesobilirubin-XIII $\alpha$  ( $R_f = 0.95$ ) on silica gel with dichloromethane-methanol 15:1, vol/vol irrigant. Pigment **1** travels like the very polar bile pigments, which is consistent with its solubility properties. We found **1** to be soluble only in dimethyl sulfoxide and aqueous base, and insoluble in chloroform, acetone, dimethylformamide and other solvents in which mesobilirubin-XIII $\alpha$  has some solubility.

A more direct way to detect intramolecular hydrogen bonding is through analysis of the  $^1\text{H-nmr}$  chemical shifts



**Figure 1.** Conformational structures of bilirubin-IX $\alpha$ , mesobilirubin-XIII $\alpha$ , bilirubin-IX $\gamma$  illustrating their ability to engage in intramolecular hydrogen bonding between the propionic acid and pyromethenone moieties.

of pyrrole and lactam N-H signals, particularly in deuteriochloroform and dimethyl sulfoxide- $d_6$  solvents [15]. For example, dipyrrole dialdehyde **3** showed interesting intramolecular hydrogen bonding as detected by  $^1\text{H}$ -nmr spectroscopy. Thus, comparison of the pyrrole N-H chemical shift of **3** with that of the monopyrrole aldehyde analog **14** (Table 1) revealed only a very small difference in dimethyl sulfoxide- $d_6$ , a solvent known [5,15,16] to hydrogen bond strongly to N-H hydrogens ( $>\text{S}^+-\text{O}^-\cdots\text{H}-\text{N}<$ ) and lead to strongly deshielded N-H signals. The carbonyl group of acetone hydrogen bonds ( $>\text{C}=\text{O}\cdots\text{H}-\text{N}<$ ) less well, apparently, and the N-H signals are more shielded. In deuteriochloroform, the N-H chemical shift of the dipyrrole **3** is relatively insensitive to dilution; whereas, the N-H of the monopyrrole **14** shows a marked sensitivity to dilution. These results suggest that **3** prefers to hydrogen bond intramolecularly and that **14** prefers to hydrogen bond intermolecularly, but the reasons for this selectivity are not clear.

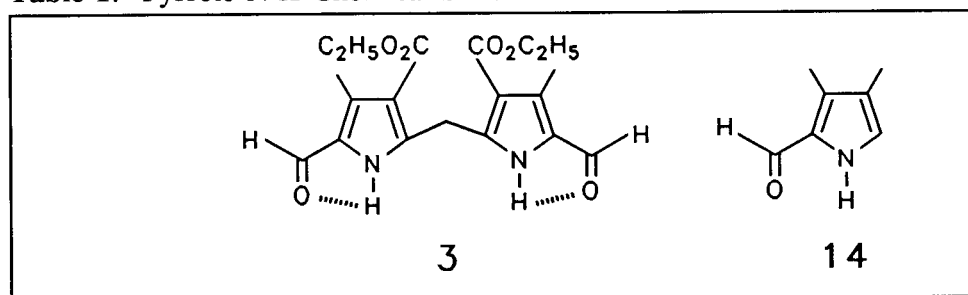
Intramolecular hydrogen bonding is especially pronounced in deuteriochloroform for bilirubin-IX $\alpha$  and mesobilirubin-XIII $\alpha$  (Figure 1) as detected by lactam N-H chemical shifts near 10.6  $\delta$  and pyrrole chemical shifts near 9.2  $\delta$  (Table 2) [15]. When intramolecular hydrogen bonding is not possible, as in etiobilirubin-IV $\gamma$  and mesobilirubin-IV $\alpha$  and its dimethyl ester, the pigment engages in intermolecular hydrogen bonding, as has been shown for mesobilirubin-XIII $\alpha$  dimethyl ester [5,15], and the pyrrole N-H moves downfield by  $\sim 1$  ppm. We could not measure either the pyrrole or lactam N-H chemical shift for carboxyrubin (**1**) in deuteriochloroform due to its ex-

treme insolubility. However, both could be measured for the diethyl ester. Whether this esterified pigment engages in intermolecular hydrogen bonding is unclear. The lactam N-H shift is approximately where expected, but the sensitive pyrrole N-H chemical shift is strongly shielded relative to all other N-H's in Table 2. How much of the shielding can be attributed to electronic effects due to the direct attachment of the carboethoxy group to the pyrrole ring is unclear, but the data suggest that the pyrrole N-H is not involved in either intermolecular or intramolecular hydrogen bonding.

In dimethyl sulfoxide- $d_6$ , a solvent which is known to hydrogen bond to the pyrrole and lactam N-H groups [15,16], the N-H chemical shifts are governed by association with the solvent. Thus, the lactam N-H chemical shifts are all comparable among the tetrapyrroles of Table 2, as are the pyrrole N-H chemical shifts, indicating that in this solvent, the pigments may all adopt the same conformation.

In summary, we show that carboxyrubin (**1**) does not adopt an intramolecularly hydrogen bonded conformation like that of bilirubin-IX $\alpha$  (Figure 1) and that it exhibits solubility properties more in line with an exposed carboxylic acid group. Molecular models indicate that any intramolecular hydrogen bonding is limited to the pyrrole N-H and the carboxyl carbonyl oxygen. The nonbonded distance between the carboxylic acid H and the lactam carbonyl is too long for effective intramolecular hydrogen bonding.

Table 1. Pyrrole N-H Chemical Shifts at 21°C



Solvent <sup>a</sup>	N-H Chemical Shift (ppm)	N-H Chemical Shift (ppm)
Dimethylsulfoxide- $d_6$	11.97	12.07
Acetone- $d_6$	10.79	11.08
Deuteriochloroform (conc.)	10.21	10.53
Deuteriochloroform (dil.) <sup>b</sup>	10.17	9.48

<sup>a</sup> Concentrations were  $10^{-2}$  M. <sup>b</sup> Concentrations were  $10^{-3}$  M.

**TABLE 2.** Solvent Dependence of Lactam and Pyrrole N-H Chemical Shifts<sup>a</sup>

Pigment	DMSO-d <sub>6</sub>		CDCl <sub>3</sub>	
	Lactam	Pyrrole	Lactam	Pyrrole
Bilirubin-IX $\alpha$	10.07, 9.93	10.48, 10.48	10.80, 10.70	9.27, 9.30
Mesobilirubin-XIII $\alpha$	9.72	10.27	10.57	9.15
Mesobilirubin-IV $\alpha$	9.82	10.36	10.85	9.65
Etiobilirubin-IV $\gamma$	9.78	10.28	10.58	10.28
Mesobilirubin-XIII $\alpha$ Dimethyl Ester	9.74	10.40	10.54	10.27
Mesobilirubin-IV $\gamma$ Dimethyl Ester	9.78	10.32	10.47	10.27
Carboxyrubin	9.93	10.60	b	b
Carboxyrubin Diethyl Ester	9.72	10.68	10.13	8.45

<sup>a</sup> Run on 10<sup>-2</sup> M dimethylsulfoxide-d<sub>6</sub> and 10<sup>-3</sup> M deuteriochloroform solutions at 21°C.

<sup>b</sup> Insoluble

#### EXPERIMENTAL

##### General.

All nmr spectra were run on a GE QE-300 FT spectrometer in either deuteriochloroform (99.9% d<sub>1</sub>) or dimethyl sulfoxide-d<sub>6</sub> (99.9% d<sub>6</sub>), both from Aldrich. Infrared spectra were run on a Perkin-Elmer 1600 FT-IR spectrophotometer. All uv-visible absorption spectra were run on a Perkin-Elmer model 3840 diode array or Cary 219 instrument. Gas chromatography-mass spectrometry was accomplished on a Hewlett-Packard GC-MS model 5890A ion selective detector using a DB-1 (100% dimethyl polysiloxane) column. Analytical thin layer chromatography (tlc) was carried out on J. T. Baker silica gel 1B-F plates (125  $\mu$  layer). Preparative tlc was accomplished on Analtech silica gel G plates, 20 x 20 cm, 500  $\mu$  thickness, using dichloromethane-methanol 20:1, vol/vol. 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 410 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 [16]. Pentane-2,4-dione, ethyl acetoacetate, acetic acid, ethyl acetate, dimethylformamide, 1,2-dichloroethane, dichloromethane, 2-butanone, ethyl formate, diethyl malonate and pyridine were from Aldrich. Spectral grade methanol, chloroform and dimethyl sulfoxide were from Fisher. Methanol (hplc grade) was from Fisher. All combustion microanalyses were performed by Desert Analytics, Tucson, AZ.

##### 2,4-Dimethyl-3,5-dicarboethoxy-1H-pyrrole (5).

To a 3-liter four-neck round-bottom flask equipped with a mechanical stirrer, thermometer, condenser and dropping funnel were added 400 g of ethyl acetoacetate and 900 g of glacial acetic acid while the flask was cooled in an ice-salt bath. To this solution was then added, dropwise with stirring, a solution of 110 g of sodium nitrite in 180 ml of water. The temperature was kept below 5° and should not exceed 7°. After the sodium nitrite solution had been added, the mixture was stirred an additional hour. The solution changed color from pale-greenish yellow to colorless and clear. It was then allowed to warm to room temperature. After standing for about one hour, 200 g of zinc dust was added in portions with vigorous mechanical stirring while keeping the temperature between 55° and 65°, cooling with water bath. After the addition of zinc was complete, the reaction mixture was stirred for an additional hour. Then the solution was heated slowly to reflux. After heating at reflux for two hours the hot brown solution was poured into 15 liters of ice-water to give a white precipitate. The precipitate was filtered and dried in the air. The white precipitate (with a pale yellow tinge) was recrystallized from 95% ethanol (1800 ml) twice to give pure white product (200 g, 57% yield) mp 134.5-135° (lit [17] 136°). It had ir (nujol):  $\nu$  3264 (NH), 1691, 1671 cm<sup>-1</sup>; uv (acetonitrile):  $\lambda$  max 269 nm,  $\epsilon$ , 16,500;  $\lambda$  max 218 nm,  $\epsilon$ , 30,580; <sup>1</sup>H-nmr (deuteriochloroform):  $\delta$  1.31 (m, 6H, CH<sub>2</sub>CH<sub>3</sub>), 2.48 (s, 3H, CH<sub>3</sub>), 2.52 (s, 3H, CH<sub>3</sub>), 4.26 (m, 4H, OCH<sub>2</sub>), 9.95 (br s, 1H, NH) ppm; <sup>13</sup>C-nmr (deuteriochloroform):  $\delta$  11.94 (q), 14.04 (q), 14.30 (q, two carbons), 59.33 (t), 60.25 (t), 113.86 (s), 117.88 (s), 130.86 (s), 139.43 (s), 162.13 (s), 165.46 (s) ppm; ms: m/z (relative intensity) 239 (100%, M<sup>+</sup>), 210 (37%, M-C<sub>2</sub>H<sub>5</sub>), 194

(58%, M-OC<sub>2</sub>H<sub>5</sub>), 147 (79%) amu.

2-Bromomethyl-4-methyl-3,5-dicarboethoxy-1*H*-pyrrole (**6**).

2,4-Dimethyl-3,5-dicarboethoxy-1*H*-pyrrole (130 g, 0.544 mole) was dissolved in 500 ml of glacial acetic acid. The temperature of the solution was increased to 45° to dissolve all of the starting pyrrole. Bromine (312 g, 0.512 mole), dissolved in 52 ml of glacial acetic acid, was added to the solution adjusting temperature of the solution between 40 and 43° with air cooling or using an ice-water bath if necessary. After adding all of the bromine, the dark red solution was stirred for an additional half hour. The solution was cooled to room temperature. The precipitate that was obtained was filtered and washed with glacial acetic acid twice and then washed with 60% glacial acetic acid three times to give a white product. After concentrating the filtrate, more product was obtained. The combined product was recrystallized from benzene to give pure white shiny needles (75 g, 42% yield) with mp 150-150.5° (lit [18] 155-156°). It had ir (nujol):  $\nu$  3277 (NH), 1711, 1665, 1293, 1212, 1088, 792, 776, 754, 723; uv (acetonitrile):  $\lambda$  max 267 nm,  $\epsilon$ , 14,800;  $\lambda$  max 226 nm,  $\epsilon$ , 21,360; <sup>1</sup>H-nmr (deuteriochloroform):  $\delta$  1.39 (m, 6H, CH<sub>2</sub>CH<sub>3</sub>), 2.56 (s, 3H, CH<sub>3</sub>), 4.36 (m, 4H, OCH<sub>2</sub>), 4.85 (s, 2H, CH<sub>2</sub>Br), 9.78 (s, 1H, NH) ppm; <sup>13</sup>C-nmr (deuteriochloroform):  $\delta$  11.28 (q), 14.33 (q), 14.43 (q), 23.27 (t), 60.11 (t), 60.87 (t), 114.47 (s), 120.10 (s), 130.76 (s), 135.84 (s), 161.55 (s), 164.51 (s) ppm; ms: *m/z* (relative intensity) 317 (3%, M<sup>+</sup>), 316 (8%), 238 (100%, M-Br), 164 (41%) amu.

4,4'-Dimethyl-3,3',5,5'-tetracarboethoxydipyrrylmethane (**7**).

2-Bromomethyl-4-methyl-3,5-dicarboethoxy-1*H*-pyrrole (55.7 g, 0.175 mole) was suspended in 110 ml of methanol. Aqueous hydrobromic acid (concentrated, 46.5 ml) was added, and the heterogeneous solution was heated. The mixture became homogeneous and turned brown after heating for half an hour. After heating at reflux for 7 hours, a precipitate formed in the dark solution and was removed by filtration. This crude material was recrystallized from 95% ethanol to give 20.2 g (50% yield) of white product with a very pale pink tinge and mp 136-136.5° (lit [19] 134°). It had ir (nujol):  $\nu$  3208, 1731, 1697, 1663, 1563, 1276, 1204, 1123, 1072, 1015 cm<sup>-1</sup>; uv (acetonitrile):  $\lambda$  max 266 nm,  $\epsilon$ , 33,400;  $\lambda$  max 217 nm,  $\epsilon$ , 51,290; <sup>1</sup>H-nmr (deuteriochloroform):  $\delta$  1.35 (t, 6H, J = 7.2 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.42 (t, 6H, J = 7.2 Hz), 4.49 (s, 2H), 10.12 (s, 2H, NH) ppm; <sup>13</sup>C-nmr (deuteriochloroform):  $\delta$  11.05 (q), 14.41 (q), 24.51 (t), 51.23 (t), 60.17 (t), 60.24 (t), 113.08 (t), 118.68 (s), 129.73 (s), 138.91 (s), 161.03 (s), 161.51 (s) ppm; ms: *m/z* (relative intensity) 370 (100%, M-2 x C<sub>2</sub>H<sub>5</sub>OH), 343 (100%), 297 (99%) amu.

4,4'-Dimethyl-3,3'-dicarboethoxydipyrrylmethane (**9**).

4,4'-Dimethyl-3,3',5,5'-tetracarboethoxydipyrrylmethane (16.58 g, 0.036 mole) was suspended in 150 ml of 95% ethanol. Sodium hydroxide (2.87 g, 0.072 mole) in 25 ml water was added. The homogeneous brown solution was heated to give a homogeneous solution. After heating at reflux for 3 hours the solution was cooled to room temperature. Ethanol (95%, 300 ml) was added and the solution was neutralized with 2 *N* sulfuric acid at 70°. The white precipitate that formed was removed by filtration and dried in the air. The mp 245-250° (lit [20] 254°); ir (potassium bromide):  $\nu$  3400-2600 (br, COOH), 1677, 1570, 1508, 1269, 1125, 1082, 781 cm<sup>-1</sup>; <sup>1</sup>H-nmr (dimethyl sulfoxide-d<sub>6</sub>):  $\delta$  1.17 (t, 6H, J = 7.2 Hz, CH<sub>3</sub>), 2.42 (s, 6H, CH<sub>3</sub>), 4.11 (q, 4H, J = 7.2 Hz), 4.47 (s, 2H), 11.39 (s, 2H, NH), 12.53 (br s, 2H, COOH) ppm; <sup>13</sup>C-nmr

(dimethyl sulfoxide-d<sub>6</sub>):  $\delta$  11.55 (q), 14.07 (q), 25.05 (t), 59.03 (t), 112.75 (s), 118.94 (s), 128.41 (s), 137.56 (s), 162.15 (s), 164.41 (s) ppm.

The crude 4,4'-dimethyl-3,3'-dicarboethoxy-5,5'-dicarboxyldipyrrylmethane (12 g) was added to a sublimation apparatus and heated under vacuum at 210-220° using a Wood's metal bath. Sublimation of the desired product was very slow. After five days, the sublimed isolated product was found to have impurities by tlc (silica gel IB-F, dichloromethane/methanol, 10 ml/3 drops) and was purified by column chromatography (silica gel, dichloromethane/methanol, 20 ml/2 drops). The purified white solid melted at 177-177.5° (lit [21] 173°). It had ir (nujol):  $\nu$  3284, 1730, 1676, 1656, 1579, 1339, 1256, 1125, 1088 cm<sup>-1</sup>; uv (acetonitrile):  $\lambda$  max 270 nm,  $\epsilon$ , 9140; <sup>1</sup>H-nmr (deuteriochloroform):  $\delta$  1.38 (t, 6H, J = 7.2 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.16 (s, 6H, CH<sub>3</sub>), 9.38 (s, 2H, NH) ppm; <sup>13</sup>C-nmr (deuteriochloroform):  $\delta$  12.73 (q), 14.48 (q), 24.56 (t), 59.50 (t), 109.97 (s), 115.15 (s), 120.26 (s), 138.47 (s), 167.54 (s) ppm; ms: *m/z* (relative intensity) 318 (15%, M<sup>+</sup>), 243 (17%, M-OC<sub>2</sub>H<sub>5</sub>), 199 (100%, M-CO<sub>2</sub>Et-OC<sub>2</sub>H<sub>5</sub>), 184 (4%), 144 (3%), 108 (3%) amu.

4,4'-Dimethyl-3,3'-dicarboethoxydipyrrylmethane-5,5'-dialdehyde (**3**).

To a 50 ml 3-neck round-bottom flask with a condenser and dropping funnel was added *N,N*-dimethylformamide (dried over 4 Å molecular sieves) (0.90 g, 0.0123 mole). The flask was cooled in an ice bath to about 10°. Phosphorus oxychloride (1.90 g, 0.0123 mole) was added slowly. After warming to room temperature, 1,2-dichloroethane, 4 ml was added and the solution was stirred for 10 minutes. The solution was then cooled to about 5° and 4,4'-dimethyl-3,3'-dicarboethoxydipyrrylmethane (1.54 g, 4.84 mmoles) dissolved in a mixture of 1,2-dichloroethane (25 ml) and dichloromethane (1 ml) was dropped into the reaction mixture. When the addition was complete, the contents were heated at reflux for 15 minutes. The reaction mixture was then cooled to room temperature and sodium acetate (8 g) dissolved in 20 ml of water was introduced after the solution had been poured into a 100 ml round-bottom flask. The solution was heated at reflux for half an hour with vigorous mechanical stirring. After cooling to room temperature, the solution was transferred to 100 ml separatory funnel. The lower layer of 1,2-dichloroethane and dichloromethane was set aside and the upper layer of water was extracted with dichloromethane (2 x 5 ml). The combined brown organic solutions were washed with saturated sodium carbonate (2 x 10 ml) and dried over anhydrous potassium carbonate. The solvent was removed (roto-vap) to give an off-white product in quantitative yield. This material was crystallized to give beautiful white needles with mp 188-189° (lit [22] 188°). It had ir (nujol):  $\nu$  3209, 2922, 1733, 1700, 1653, 1277, 1134, 1074 cm<sup>-1</sup>; uv-visible (acetonitrile):  $\lambda$  max 295 nm,  $\epsilon$ , 33,500;  $\lambda$  max 235 nm,  $\epsilon$ , 25,850;  $\lambda$  max 219,  $\epsilon$ , 29,200; <sup>1</sup>H-nmr (deuteriochloroform):  $\delta$  1.41 (t, 6H, J = 7.2 Hz, CH<sub>3</sub>), 2.47 (s, 6H, CH<sub>3</sub>), 4.39 (q, 4H, J = 7.2 Hz, OCH<sub>2</sub>), 4.55 (s, 2H, CH<sub>2</sub>), 9.64 (s, 2H, CHO), 10.21 (s, 2H, NH) ppm; <sup>13</sup>C-nmr (deuteriochloroform):  $\delta$  10.67 (q), [14.31, 14.38] (q), 24.50 (t), 60.61 (t), 113.76 (s), 128.69 (s), 133.13 (s), 141.51 (s), 165.95 (s), [177.59, 177.73] (s) ppm; ms: *m/z* (relative intensity) 374 (33%, M<sup>+</sup>), 328 (50%, M-C<sub>2</sub>H<sub>5</sub>OH), 255 (100%, M-2 x C<sub>2</sub>H<sub>5</sub>OH-CO), 226 (22%, M-2 x C<sub>2</sub>H<sub>5</sub>OH-2 x CO) amu.

Sodium Salt of 2-Methyl-3-oxobutylaldehyde.

Crushed sodium (92 g) was added to a solution of 288 g of



dried 2-butanone and 296 g of ethyl formate in dry ether (4 liters) with vigorous mechanical stirring over a period of 6 hours, during which time the mixture was chilled in an ice-salt bath. The mixture was then stirred for 14 hours at room temperature (brown solution). After cooling the solution in a cold room (4°) for a few hours, the precipitate of sodium salt obtained was collected by filtration then washed thoroughly with cold, dry ether to afford 429 g (88% yield) of off-white product with <sup>1</sup>H-nmr (dimethyl sulfoxide-d<sub>6</sub>): δ 1.34 (s, 3H, CH<sub>3</sub>), 1.84 (s, 3H, CH<sub>3</sub>), 9.01 (s, 1H) ppm.

#### 2-Carboethoxy-3,4-dimethyl-1*H*-pyrrole (9).

Glacial acetic acid (145 ml) was heated to 85° in a 500 ml three-neck round-bottom flask and 39.5 g of anhydrous sodium acetate was then added with mechanical stirring. Next, 36 g (0.29 mole) of the sodium salt of 2-methyl-3-oxobutyraldehyde, diethylximinomalonate (47.4 g, 0.25 mole) and a solution of 60 ml of glacial acetic acid in 25 ml of water were consecutively introduced, and the mixture was heated to 95°. Zinc dust (55 g) was then added slowly adjusting the temperature between 95° and 105°, after which the reaction mixture was heated and stirred for an additional 30 minutes. Pouring the reaction mixture into 900 ml of ice-water mixture caused the separation of a pale yellow oil, which solidified on standing in a cold room. The product obtained was removed by filtration and washed with water. The crude solid was dissolved in hot 95% ethanol (100 ml) and filtered to remove unreacted zinc. The hot solution was cooled to room temperature and kept in a cold room to give pale yellow crystals (10 g, 24% yield), mp 90-92° (lit [19] 94-95°). It had ftr (nujol): ν 3322 (NH), 2924, 1731, 1718, 1693, 1671, 1661, 1462 cm<sup>-1</sup>; uv (methanol): λ max 272 nm, ε, 14,420 (methanol); <sup>1</sup>H-nmr (deuteriochloroform): δ 1.33 (t, 3H, J = 7.2 Hz), 1.99 (s, 3H, CH<sub>3</sub>), 2.25 (s, 3H, CH<sub>3</sub>), 4.28 (q, CH<sub>2</sub>, J = 6.9 Hz), 6.63 (d, 1H, J = 2.7 Hz), 8.69 (s, 1H, NH) ppm; <sup>13</sup>C-nmr (deuteriochloroform): δ 9.84 (q), 10.19 (q), 14.51 (q), 59.73 (t), 119.30 (s), 120.02 (s), 120.51 (s), 126.54 (d), 161.72 (s) ppm.

#### 2-Carboxy-3,4-dimethyl-1*H*-pyrrole.

2-Carboethoxy-3,4-dimethyl-1*H*-pyrrole (3.6 g, 0.02 mole) was suspended in approximately 2 ml of 95% ethanol, then 30% potassium hydroxide (400 ml) was added. The mixture was heated at reflux for two hours, and the resulting brown solution was cooled to room temperature and placed in an ice-water bath. Concentrated hydrochloric acid was added dropwise to the cold brown solution to give white precipitate. The fine white solid was removed by filtration and washed with water. The solid was dried to give 2.99 g of product (quantitative yield). It decomposed at 205° and had ir (potassium bromide): 3359 (NH), 3200-2350 (COOH, hydrogen bonded), 1648 (C=O, conjugated with pyrrole) cm<sup>-1</sup>; uv (methanol): λ max 270 nm, ε, 12,870 (methanol); <sup>1</sup>H-nmr (acetone-d<sub>6</sub>): δ 1.93 (s, 3H, CH<sub>3</sub>), 2.20 (s, 3H, CH<sub>3</sub>), 6.71 (d, 1H, J = 3.0 Hz, =CH), 9.30 (br s, 1H, NH), 10.23 (s, 1H, COOH) ppm; <sup>13</sup>C-nmr (acetone-d<sub>6</sub>): δ 9.04 (q), 9.43 (q), 118.90 (s), 119.44 (s), 120.65 (s), 125.89 (d), 161.85 (s) ppm; ms: m/z (relative intensity) 95 (64%, M-CO<sub>2</sub>), 94 (100%, M-CO<sub>2</sub>H), 67 (15%) amu.

#### 3,4-Dimethyl-1*H*-pyrrole (10).

2-Carboxy-3,4-dimethyl-1*H*-pyrrole (1.5 g, 0.01 mole) was added to a 15 ml round-bottom flask and heated with a Wood's metal bath. The acid was decarboxylated at the temperature of the metal bath 180° and 190°. The pure decarboxylated product was

obtained by using a short path distillation apparatus. During the distillation the compound solidified. Pure product which smells like benzene, was obtained in 88% yield from the distillation, 900 mg, mp 30-31° (lit [23] mp 33°). It had uv (methanol): λ max 214 nm, ε, 4860; <sup>1</sup>H-nmr (deuteriochloroform): δ 2.03 (s, 6H, 2 CH<sub>3</sub>), 6.51 (d, 2H, J = 2.4 Hz), 7.75 (s, 1H, NH) ppm; <sup>13</sup>C-nmr: δ 9.93 (q), 115.51 (s), 118.16 (d) ppm; ms: m/z (relative intensity) 95 (61%, M<sup>+</sup>), 94 (100%), 80 (19%, M-NH) amu.

#### 3,4-Dimethyl-3-pyrrolin-2-one (4).

3,4-Dimethyl-1*H*-pyrrole (2.15 g, 0.023 mole) was dissolved in 6 ml of pyridine. Hydrogen peroxide (3 ml, 30%) was added slowly while the solution was stirred magnetically under argon, and the temperature was kept below 35°. After stirring 20 minutes, an additional 1.3 ml portion of hydrogen peroxide was added. The solution was then heated to 85° for 20 minutes. The solvent was removed on a roto-vap to give a light orange oil, which solidified on standing to give the desired product (1.5 g, 59% yield) [22]. It had ftr (nujol): ν 3186 (NH), 2921, 1733 (lactam), 1684 cm<sup>-1</sup>; <sup>1</sup>H-nmr (deuteriochloroform): δ 1.72 (s, 3H, CH<sub>3</sub>), 1.92 (s, 3H, CH<sub>3</sub>), 3.75 (s, 2H), 7.55 (br s, 1H) ppm; (dimethyl sulfoxide-d<sub>6</sub>): δ 1.61 (s, 3H), 1.90 (s, 3H), 3.70 (s, 2H), 7.85 (br s, 1H, NH) ppm; <sup>13</sup>C-nmr: δ 8.50 (q), 13.53 (q), 50.66 (t), 120.80 (s), 150.16 (s), 177.53 (s) ppm; ms: m/z (relative intensity) 111 (100%, M<sup>+</sup>), 96 (39%, M-CH<sub>3</sub>) amu.

#### 8,12-Bis-carboethoxy-2,3,7,13,17,18-hexamethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione (2).

4,4'-Dimethyl-3,3'-dicarboethoxydipyrromethane-5,5'-dialdehyde (3) (20.0 mg, 0.05 mmole) was dissolved in nitrogen-saturated pyridine; then 3,4-dimethyl-3-pyrrolin-2-one (4) (200.0 mg, 1.80 mmoles) was added. The mixture was stirred for 5 minutes, then one drop of titanium tetrachloride was added to the resulting brown solution. After heating at reflux for two hours under nitrogen in the dark, the solution was cooled to room temperature. Pyridine was removed by rotary evaporation, and the sticky brown product was dissolved in dichloromethane then washed with dilute acetic acid and water. After drying over anhydrous sodium sulfate, the dichloromethane was removed to give a crude brown product, which was purified by preparative tlc to give about 5 mg (16% yield) of pure product. It had mp 275-280°; ir (film): ν 3300, 2923, 2853, 1733, 1708, 1685, 1664, 1290, 1271, 1119, 1017, 723 cm<sup>-1</sup>; uv-visible (dichloromethane): λ max 384 nm, ε, 53,000; (methanol): λ max 385 nm, ε, 50,100; <sup>1</sup>H-nmr (deuteriochloroform): δ 1.37 (t, 6H, J = 7.2 Hz, CH<sub>3</sub>), 1.78 (s, 6H, C-2 and C-18, CH<sub>3</sub>), 2.02 (s, 6H, C-3 and C-17, CH<sub>3</sub>), 2.23 (s, 6H, C-7 and C-8, CH<sub>3</sub>), 4.45 (q, 4H, J = 7.2 Hz, OCH<sub>2</sub>), 4.56 (s, 2H, C-10, CH<sub>2</sub>), 5.90 (s, 2H, C-5 and C-15, =CH), 8.45 (s, 2H, pyrrole NH), 10.13 (s, 2H, lactam NH) ppm; (dimethyl sulfoxide-d<sub>6</sub>): δ 1.14 (2 x t, 6H, J = 6.9, 7.2 Hz, CH<sub>3</sub>), 1.74 (s, 6H, C-2 and C-18, CH<sub>3</sub>), 2.03 (s, 6H, C-3 and C-17, CH<sub>3</sub>), 2.20 (s, 6H, C-7 and C-8, CH<sub>3</sub>), 4.09 (2 x q, 4H, J = 6.9, 7.2 Hz, OCH<sub>2</sub>), 4.50 (s, 2H, C-10, CH<sub>2</sub>), 5.94 (s, 2H, C-5 and C-15, =CH), 9.72 (s, 2H, lactam NH), 10.68 (s, 2H, pyrrole NH) ppm; <sup>13</sup>C-nmr (deuteriochloroform): δ 8.49 (q), 9.89 (q), 11.45 (q), 14.47 (q), 25.33 (t), 60.57 (t), 97.57 (d), 123.18 (s), 123.67 (s), 126.57 (s), 133.93 (s), 137.03 (s), 138.36 (s), 141.66 (s), 167.11 (s), 173.47 (s) ppm; (dimethyl sulfoxide-d<sub>6</sub>): δ 9.41 (q), 10.66 (q), 12.43 (q), 15.28 (q), 27.36 (t), 59.97 (t), 97.97 (d), 112.65 (s), 123.75 (s), 124.29 (s), 126.67 (s), 134.13 (s), 138.99 (s), 142.59 (s), 165.88 (s), 172.27 (s) ppm; high resolution (FAB) ms: m/z (relative intensity) 561.2738 (70%, (M+1)<sup>+</sup>), Calcd. for C<sub>51</sub>H<sub>57</sub>N<sub>4</sub>O<sub>6</sub> =

560.2713), 560 (95%, M<sup>+</sup>), 515 (15%, M-OCH<sub>2</sub>CH<sub>3</sub>), 485 (40%), 441 (15%), 392 (12%), 287 (100%), 154 (92%) amu.

Anal. Calcd. for C<sub>35</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub> (560.6): C, 66.43; H, 6.43; N, 10.00. Found: C, 66.14; H, 6.27; N, 9.73.

2,3,7,13,17,18-Hexamethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione-8,12-dicarboxylic Acid (**1**) (Carboxyrubin).

#### Method 1.

To a solution of 3,4-dimethyl-3-pyrrolin-2-one (277.5 mg, 2.5 mmoles) in 4 *N* sodium solution (5 ml) and methanol (1.5 ml) at 70° under argon was added 4,4'-dimethyl-3,3'-dicarboethoxydipyrrylmethane-5,5'-dialdehyde (**3**) (93.5 mg, 0.25 mmole) in methanol (5 ml). The mixture was heated at reflux for four hours under nitrogen in the dark. The resulting dark solution was cooled to room temperature. The brown precipitate that formed was removed by filtration and washed with a small amount of 4 *N* aqueous sodium hydroxide. The filtrate was set aside, and the brown solid was triturated with dilute aqueous acetic acid, and the yellowish-orange precipitate that resulted was isolated by centrifugation, after washing with water. However, this material had many extraneous peaks in the <sup>1</sup>H-nmr and did not correspond with the desired product (**1**). The dark brown filtrate from above was acidified carefully with 5% aqueous hydrochloric acid to give a brown precipitate, which was isolated by centrifugation. The dark brown solid (15 mg) obtained had decomposition point 180° and ir (potassium bromide):  $\nu$  3600-2500 (br, COOH), 1680, 1665, 1634, 1475, 1441, 1402, 1382, 1348, 1277, 1166, 1132, 1109 cm<sup>-1</sup>; uv-visible (methanol):  $\lambda$  max 396 nm,  $\epsilon$ , 40,700; (dimethyl sulfoxide)  $\lambda$  max 398,  $\epsilon$ , 44,800; <sup>1</sup>H-nmr (dimethyl sulfoxide-d<sub>6</sub>):  $\delta$  1.77 (s, 6H, C-2 and C-18, CH<sub>3</sub>), 2.05 (s, 6H, C-3 and C-17, CH<sub>3</sub>), 2.22 (s, 6H, C-7 and C-13, CH<sub>3</sub>), 4.52 (s, 2H, C-10, CH<sub>2</sub>), 5.95 (s, 2H, C-5 and C-15, =CH), 9.93 (s, 2H, lactam NH), 10.60 (s, 2H, pyrrole NH), 11.94 (s, 2H, COOH) ppm. The acid was analyzed as its diethyl ester.

#### Method 2.

Diethyl ester **2** (5 mg, 1 x 10<sup>-5</sup> mole) was suspended in methanol (0.1 ml), then 0.5 ml of 10% aqueous sodium hydroxide was added. The heterogeneous solution was heated at reflux under nitrogen in the dark for one hour to give homogeneous solution. The solution was then cooled to room temperature, diluted with nitrogen-saturated water (10 ml) and filtered. The dark brown filtrate was neutralized with glacial acetic acid, and through

vacuum, the desired dark brown dicarboxylic acid was obtained (3 mg, 60% yield) and had spectroscopic properties identical to those reported above.

#### Acknowledgement.

We thank the National Institutes of Health (HD-17779) for generous support of this work.

#### REFERENCES AND NOTES

- [1] For leading references see J. D. Ostrow, ed, *Bile Pigments and Jaundice*, Marcel Dekker, Inc., New York, 1986.
- [2] D. A. Lightner and A. F. McDonagh, *Acc. Chem. Res.*, **17**, 417 (1984).
- [3] A. F. McDonagh and D. A. Lightner, *Pediatrics*, **75**, 443 (1985).
- [4] F. R. Trull, R. W. Franklin and D. A. Lightner, *J. Heterocyclic Chem.*, **24**, 1573 (1987).
- [5] H. Falk, *The Chemistry of Linear Oligopyrroles and Bile Pigments*, Springer-Verlag, New York and Vienna, 1989.
- [6] A. F. McDonagh and D. A. Lightner, In *Hepatic Metabolism and Disposition of Endo and Xenobiotics*, (Falk Symposium, No. 57, K. W. Bock, W. Gerok and S. Mattern, eds) Kluwer, Dordrecht, The Netherlands, 1991, Chapter 5, pp 47-57.
- [7] F. R. Trull and D. A. Lightner, *J. Heterocyclic Chem.*, **25**, 1227 (1988).
- [8] G. Puzicha, D. P. ShROUT and D. A. Lightner, *J. Heterocyclic Chem.*, **27**, 2117 (1990).
- [9] D. P. ShROUT, G. Puzicha and D. A. Lightner, *Synthesis*, 1991, in press.
- [10] D. A. Lightner and Y.-T. Park, *Tetrahedron*, **35**, 463 (1979).
- [11] H. Plieninger and P. Decker, *Liebigs Ann. Chem.*, **598**, 198 (1956).
- [12] E. Ghera and Y. Sprinzak, *J. Am. Chem. Soc.*, **82**, 4949 (1960).
- [13] A. Gossauer and J. P. Weller, *Chem. Ber.*, **111**, 486 (1978).
- [14] A. F. McDonagh, L. A. Palma, F. R. Trull and D. A. Lightner, *J. Am. Chem. Soc.*, **104**, 6865 (1982).
- [15] F. R. Hull, J. S. Ma, G. L. Landen and D. A. Lightner, *Israel J. Chem.*, **23**, 211 (1983).
- [16] J. K. Gawronski, T. Polonski and D. A. Lightner, *Tetrahedron*, **46**, 8053 (1990).
- [17] H. Fischer and H. Orth, *Die Chemie des Pyrrols*, Vol **1**, Akademische, Verlagsgesellschaft M. B. H., Leipzig, 1934, p 255.
- [18] Reference 17, p 43.
- [19] G. C. Kleinspehn, *J. Am. Chem. Soc.*, **77**, 1546 (1955).
- [20] Reference 17, p 343.
- [21] Reference 17, p 341.
- [22] J. A. De Groot, PhD Thesis, Univ. Leiden, *Synthesis and Properties of Bile Pigment Model Compounds*, 82 (1982).
- [23] Reference 17, p 43.