Xanthobilirubic Acid and Its Amides. Synthesis, Spectroscopy, and Solution Structures

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Xanthobilirubic acid, 5-[1,5-didehydro-3-ethyl-4-methyl-5-oxo-2*H*-pyrrol-2-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrol-3-propanoic acid, its methyl ester, amide, *N*-methylamide and dimethylamide, and kryptopyrromethenone have been synthesized and characterized spectroscopically. In d_6-DMSO solution all pyrromethenones were monomeric, with lactam and pyrrole N-Hs H-bonded to solvent. In deuteriochloroform, the pyrromethenones preferred a dimeric form, with intramolecular H-bonding between the lactam C=O of one unit and the lactam and pyrrole N-Hs of the second.

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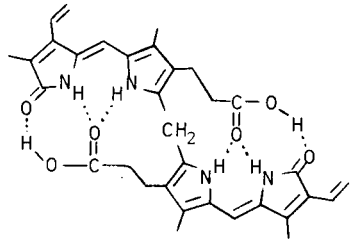
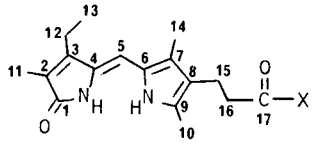
Introduction.

Considerable attention has been devoted to the properties of bilirubin (**BR**), especially its photochemistry, because of its central role in jaundice and neonatal phototherapy [1-3]. The conformation of **BR** in the solid was revealed by X-ray crystallography recently [5,6], but its solution structure is only now emerging, due largely to even more recent nmr investigations [6,7]. One of the most important and intriguing aspects of the structure of **BR** is its ability to exhibit intramolecular H-bonding (of the type shown in Figure 1) [1]. Indeed, the intramolecularly H-bonded conformation is probably the dominant species in non-polar organic solvents, such as chloroform, and may persist even in protein-bound **BR** [1,6]. On the other hand, the dimethyl ester of **BR** shows no tendency for intramolecular H-bonding of the type depicted in Figure 1 [6], an observation that illustrates the importance of the COO*H* to intramolecular H-bonding in **BR**. These findings raise the intriguing possibility that the primary and secondary bis-amides of **BR** might behave in H-bonding like the acid, with the tertiary bis-amide behaving like the ester. Our preliminary synthetic work aimed at exploring the conformation of **BR** amides, has involved the preparation and examination of pyrromethenone models for one-half of **BR** and its amides, namely xanthobilirubic acid (**XBR**) and its amide, *N*-methylamide and *N,N*-dimethylamide.



BR: BILIRUBIN-IXα



XBR: XANTHOBILIRUBIC ACID, X = OH

XBRME: XBR METHYL ESTER, X = OCH_3

XBRDMA: XBR DIMETHYL AMIDE, X = $N(CH_3)_2$

XBRMA: XBR METHYL AMIDE, X = $NHCH_3$

XBRA: XBR AMIDE, X = NH_2

KR: KRYPTOPYRROMETHENONE, C-17 = H

Figure 1. (Upper) Intramolecularly hydrogen bonded bilirubin-IXα. (Lower) Structures of pyrromethenone models for bilirubin.

Syntheses.

Xanthobilirubic acid (**XBR**, Figure 1) was prepared by modification of the Fischer-Grunewald [8] total synthesis. The key monopyrrole intermediates were 2,4-dimethyl-3-acetyl-5-carbethoxy-1*H*-pyrrole [9] and ethyl 2,4-dimethyl-5-(ethoxycarbonyl)-1*H*-pyrrole-3-propanoate. The former was prepared by the updated Fischer-Knorr synthesis [9] and converted in high yield to kryptopyrrole by an improved Huang-Minlon reduction-saponification-decarboxylation. Kryptopyrrole was oxidized to 3,5-dimethyl-4-ethyl-3-oxo-1*H*,5*H*-pyrrole by an improved oxidation, and this was then brominated to afford 5-bromomethylene-4-ethyl-3-methyl-3-oxo-1*H*-pyrrole (25% yield from ethyl 3-acetyl-2,4-dimethyl-1*H*-pyrrole-5-carboxylate), the important coupling partner in the **XBR** synthesis (it becomes ring A), and one useful in coupling to α-free pyrroles. The other key monopyrrole intermediate (*vide supra*), the pre-

cursor to ring B of **XBR**, was prepared *via* a Fischer-Knorr pyrrole synthesis from commercially available ethyl acetoacetate and previously less easily accessible ethyl 4-acetyl-5-hexanoate. The latter was prepared *via* an improved, simple and high yield reaction between ethyl acrylate and pentane-2,4-dione catalyzed by nickel acetonylacetate. In our hands, the coupling of 5-bromomethylene-4-ethyl-3-methyl-3-oxo-1*H*-pyrrole and 2,4-dimethyl-5-carboxy-1*H*-pyrrole-3-propanoic acid (35% yield from pentan-2,4-dione) went in high yield, (84%) to give xanthobilirubic acid methyl ester (**XBRME**), as did the saponification of **XBRME** to **XBR**.

The amides of **XBR** were prepared by reaction of the free acid, **XBR**, with amine hydrochlorides using diphenylphosphoryl azide as the coupling reagent in dry dimethylformamide [10]. The reactions were simple to carry out and gave good to moderate yields of amide products (**XBRDMA, XBRMA, XBRA**, Figure 1). The amides exhibited large differences in solubility properties, akin to the difference between **XBR** and **XBRME**, with the dimethylamide (**XBRDMA**) being most soluble in organic solvents and the primary amide (**XBRA**) being least soluble.

Spectral Properties and Solution Conformation.

Symmetric bilirubins and pyrromethenones typically exhibit two N-H resonances in their nmr spectra, one from the lactam and one from the pyrrole ring (Table 1), and these signals are sensitive to solvent H-bonding characteristics. Kaplan and Navon [6,7], Schaffner *et al.* [11] and Lightner *et al.* [12] have assigned the lactam and pyrrole ^{1}H-nmr resonances of pyrromethenones and various symmetric and nonsymmetric bilirubins and their esters in deuteriochloroform and d_6-DMSO. Comparison of the lactam and pyrrole N-H signals of all the compounds of Table 1 run in d_6-DMSO shows only slight differences, with the lactam N-H resonances falling near 9.7 ppm and the pyrrole N-H near 10.2 ppm. Since DMSO is known to form H-bonds with amide [13] and pyrrole [14] N-H groups, we assume that all of the compounds of Table 1 are similarly and effectively H-bonded to DMSO (Figure 2A).

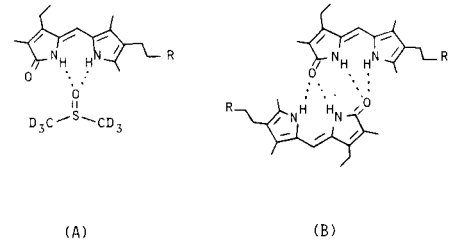
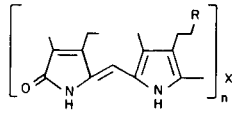


Figure 2. (Left) d_6-DMSO solvent hydrogen-bonded pyrromethenone structure. (Right) Intermolecularly hydrogen-bonded dimeric structure of pyrromethenones. R = H, CO_2H, CO_2CH_3, $CON(CH_3)_2$, $CONHCH_3$, $CONH_2$, see structures of Figure 1.

Table 1

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



Deuteriochloroform	10.57	9.15	[b] Mesobilirubin-XIIIα (**MBR**)
d_6-DMSO	9.72	10.27	R = CO_2H, X = CH_2, n = 2
Deuteriochloroform	10.54	10.27	[b] Mesobilirubin-XIIIα Dimethyl Ester (**MBRDME**)
d_6-DMSO	9.74	10.40	R = CO_2Me, X = CH_2, n = 2
Deuteriochloroform	11.3 [c]	10.2 [c]	Xanthobilirubic Acid (**XBR**)
d_6-DMSO	9.67	10.18	R = CO_2H, X = CH_3, n = 1
Deuteriochloroform	11.15	10.25	Xanthobilirubic Acid Methyl Ester (**XBRME**)
d_6-DMSO	9.72	10.26	R = CO_2CH_3, X = CH_3, n = 1
Deuteriochloroform	11.27	10.37	Xanthobilirubic Acid Dimethyl Amide (**XBRDMA**)
d_6-DMSO	9.70	10.25	R = $CON(CH_3)_2$, X = CH_3, n = 1
Deuteriochloroform	10.9 [c]	10.1 [c]	Xanthobilirubic Acid Methyl Amide (**XBRMA**)
d_6-DMSO	9.70	10.20	R = $CONHCH_3$, X = CH_3, n = 1
Deuteriochloroform	10.8 [c]	10.0 [c]	Xanthobilirubic Acid Amide (**XBRA**)
d_6-DMSO	9.73	10.23	R = $CONH_2$, X = CH_3, n = 1
Deuteriochloroform	11.25	10.30	Kryptopyrromethenone (**KR**)
d_6-DMSO	9.70	10.22	R = H, X = CH_3, n = 1

[a] Run at 25° in 2 × 10^{-2} *M* (d_6-DMSO) and 2 × 10^{-3} *M* (deuteriochloroform) solutions. [b] These known compounds were prepared in our laboratory by total synthesis from **XBRME** (R. W. Franklin, unpublished procedure). [c] Because the material is insoluble in deuteriochloroform, the N-*H* resonances for pure deuteriochloform, were obtained by extrapolation values obtained from d_6-DMSO-deuteriochloroform solutions. The extrapolation method, when applied to **XBRME**, accurately predicts the values (11.2 and 10.3) actually observed in pure deuteriochloroform.

Table 2

^{13}C-NMR Assignments [a] for Xanthobilirubic Acid (**XBR**) and Its Methyl Ester, Amides and Kryptopyrromethenone Spectra are Run in d_6-DMSO and Reported in ppm Downfield from TMS

Carbon/X =	OH	OCH_3 [b]	OCH_3 [c,d]	$N(CH_3)_2$ [e]	$N(CH_3)_2$ [c,f]	$NHCH_3$ [g]	NH_2	H	H [c]
1	172.29	171.89	174.11	171.82	172.77	172.07	171.76	171.77	174.06
2	122.86	122.69	124.56	122.57	122.52	122.46	122.45	122.46	124.56
3	147.60	147.20	148.37	147.04	148.26	147.10	147.08	147.09	148.20
4	127.42	127.37	127.20	127.18	127.02	127.08	127.07	127.02	126.96
5	98.17 d	97.59 d	101.04 d	97.58 d	100.99 d	97.59 d	97.64 d	97.65 d	101.16 d
6	122.50	122.17	122.50	122.27	122.28	122.28	122.31	121.95	122.92
7	119.06	118.36	119.12	119.23	119.74	119.24	119.29	121.52	122.22
8	121.86	121.76	122.40	121.63	122.34	121.52	121.51	121.75	122.22
9	129.88	129.41	131.64	129.29	131.58	129.30	129.29	128.60	131.06
10	8.25 q	8.03 q	8.49 q	7.96 q	8.49 q	7.97 q	7.96 q	7.97 q	8.49 q
11	9.42 q	9.14 q	9.61 q	9.13 q	9.61 q	9.14 q	9.13 q	9.08 q	9.49 q
12	18.38 t	17.09 t	17.97 t	17.02 t	17.91 t	17.09 t	17.08 t	17.09 t	17.97 t
13	15.04 q	14.75 q	15.00 q	14.74 q	15.05 q	14.75 q	14.74 q	14.75 q	15.05 q
14	11.18 q	10.89 q	11.54 q	10.88 q	11.54 q	10.83 q	10.35 q	10.83 q	11.48 q
15	19.66 t	19.38 t	19.90 t	19.54 t	20.08 t	20.14 t	19.90 t	16.86 t	17.50 t
16	35.16 t	34.59 t	35.17 t	33.64 t	34.29 t	36.57 t	36.27 t	15.40 t	15.40 t
17	174.40	172.83	174.11	171.82	177.06	171.77	173.81		

[a] All resonances are singlets, unless otherwise designated, d = doublet, t = triplet, q = quartet. The carbon numbering system is found in Figure 1. [b] OCH_3, 51.08 q. [c] These spectra were run in deuteriochloroform. [d] OCH_3, 51.17q. [e] $N(CH_3)_2$, 34.69 q. [f] $N(CH_3)_2$, 35.40 q and 37.16 q. [g] $NHCH_3$, 25.34 q.

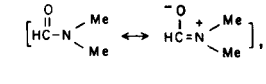
In deuteriochloroform, which is a less effective H-bonding solvent, a different situation obtains. For the pyrromethenones and mesobilirubin-XIIIα dimethyl ester (**MBRDME**), the lactam N-H resonances (all ~11.2 ppm) become more deshielded; whereas, the pyrrole N-H resonances (all ~10.2 ppm) are essentially unchanged. Falk *et al.* [15] have shown, through vapor phase osmometry studies, that pyrromethenones with lactam and pyrrole N-Hs, such as kryptopyrromethenone (**KR**), exist largely as *inter*molecularly H-bonded dimers (K_f = 1700 M^{-1} for **KR**) (Figure 2B). We therefore assume that the nearly invariant (through the pyrromethenones of Table 1) lactam and pyrrole N-H resonances in deuteriochloroform point to the predominance in that solvent of dimeric, intermolecularly H-bonded aggregates. Similarly, the data (Table 1) for **MBRDME** indicate a preference for intermolecularly H-bonded dimers, in keeping with the vapor phase osmometry molecular weight determination of **BR**-IXα dimethyl ester in chloroform [16].

In either d_6-DMSO or deuteriochloroform solvent, the role of the R group (Table 1) on the pyrrole β-ethano-R substituent is relatively unimportant--even where the R group contains an H-donor for purposes of intermolecular H-bonding, *e.g.* **XBR**, **XBRMA** and **XBRA**. As judged from the nearly identical lactam and pyrrole N-H resonances for **MBRDME**, **XBR**, **XBRME**, **XBRDMA**, **XBRMA**, **XBRA** and **KR**, polar carboxylic and, amide and ester functional groups play no significant role. Consequently, we suggest that the pyrromethenones of Table 1 exist largely as shown in Figure 2A for DMSO solutions and Figure 2B for deuteriochloroform solutions.

The situation with mesobilirubin-XIIIα (**MBR**), which is capable of intramolecular H-bonding, is different. In d_6-DMSO, the ^{1}H-nmr data (Table 1) are consistent with strongly solvent (DMSO) H-bonded forms, but deuteriochloroform the data point to the predominance in that solvent of the *intra*molecularly H-bonded conformation (Figure 1) previously assigned to bilirubin-IXα [1,6].

The ^{13}C-nmr data for the pyrromethenones of this work are assigned and summarized in Table 2. In d_6-DMSO the ring skeleton carbon resonances do not vary much from structure to structure; major differences appear only in the propionyl carbon, as might be expected. What little data can be obtained from deuteriochloroform solutions (here the limitation is one of major insolubility) hints at significant differences in the methine bridge (C-5) and a possible emerging correlation that the more deshielded C-5 resonance is to be associated with an intramolecularly H-bonded dimer (Figure 2B).

We note, further, in the ^{13}C-nmr spectrum of **XBRDMA** that the amide methyl groups are non-equivalent in deuteriochloroform (Table 2), as might be expected from the relatively high rotation barrier for the C-N bond of, *e.g.* dimethylformamide:



whose methyl resonances appear at 31.1 (*syn*-CH_3), and 36.2 (*anti*-CH_3) ppm [17]. In d_6-DMSO, the barrier is lowered; only one N-CH_3 resonance can be observed. Similarly, in the ^{1}H-nmr spectrum of **XBRDMA** in deuteriochloroform, the *N*-methyl groups are nonequivalent and appear at 2.89 (*syn*-CH_3) and 2.94 (*anti*-CH_3) ppm. These values are consistent with those of other *N*-methyl amides [18]. Upon warming to 42° in deuteriochloroform, the *N*-methyl resonances coalesce. From the coalescence temperature, we obtain an approximate activation barrier of 17 kcal/mole for amide carbonyl C-N rotation, value that falls nicely in the range of values determined for C-N rotation barriers in other amides [19].

In summary, we have been able to improve the synthesis of **XBR** and have prepared selected amide derivatives for the first time. The amides have been characterized spectroscopically and have also been shown to have the same solution conformation as **XBR** and its methyl ester: in d_6-DMSO, monomer units with lactam and pyrrole N-Hs H-bonded to solvent (Figure 2A); in deuteriochloroform, dimeric units held by intramolecular N-H to lactam C=O H-bonds (Figure 2B).

EXPERIMENTAL

General.

All nmr spectra were run on a JEOL FX-100 FT spectrophotometer in either d_6-dimethylsulfoxide (d_6-DMSO) (99.5% d_6) or deuteriochloroform (99.8% d_1), both from Stohler. All ir spectra were obtained on 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 micranalyses were obtained from MICANAL, Tucson, AZ. Thin layer chromatography (tlc) was carried out using silica gel F (M. Woelm) on analytical plates (125 μ) prepared from ethanol slurries with the plates being activated at 110-120° following ethanol evaporation. Column chromatography was carried out with silica gel, 70-230 mesh (M. Woelm). Dimethylformamide, ethyl acetoacetate, pentan-2,4-dione triethylamine, and the amine hydrochlorides were from Matheson; nickel acetonylacetate and diphenylphosphorylazide, were from Aldrich.

2,4-Dimethyl-3-ethyl-1*H*-pyrrole (Kryptopyrrole).

3-Acetyl-5-carboethoxy-2,4-dimethylpyrrole (335 g, 1.60 moles) [9], potassium hydroxide (234.2 g, 4.18 moles) hydrazine hydrate (233.4 g) and diethylene glycol (1300 ml) were placed in a three-neck 3-liter round-bottom flask equipped with a mechanical stirrer, nitrogen inlet and take-off condenser. A light flow of nitrogen gas was maintained and the mixture was stirred and heated slowly to boiling. The distillate which came over below 120° was discarded. The liquid which came over between 120° and 190° was collected and protected from atmospheric oxygen (total volume ~900 ml). When reaction was complete the distillate was placed in a separating funnel with an equal volume of distilled water. This mixture was then extracted with ether (1 × 250 ml, then 5 × 100 ml), the combined organic extracts dried (sodium sulfate), and the solvent removed. The remaining material, which was dark brown liquid, was distilled under reduced pressure to give pale yellow kryptopyrrole, 149 g, 75%, bp 60°/1-5 mm (lit [9] bp 104-105°/13 mm). It had ^{1}H-nmr (carbon tetrachloride): δ ppm 0.98 (t, 3H, J = 7 Hz), 1.90 (s, 3H), 2.30 (q, 2H, J = 7 Hz), 5.93 (broad s, 1H), 6.80 (broad s, 1H).

3,5-Dimethyl-4-ethyl-3-oxo-1*H*,5*H*-pyrrole.

Pure kryptopyrrole (149 g, 1.21 moles) was dissolved in pyridine (300 ml) previously saturated with nitrogen gas, and the mixture was placed in a three-neck round-bottom flask equipped with a reflux condenser, nitrogen inlet, thermometer, dropping funnel and magnetic stirrer and flushed with nitrogen. The solution was warmed to 55° and 30% hydrogen peroxide (150 ml) added dropwise with stirring at such a rate as to maintain the temperature below 55°, cooling with a water or ice bath, if necessary. When the addition was complete stirring was continued for an additional twenty minutes and then a further volume of 30% hydrogen peroxide (75 ml) added in one portion. The temperature of the reaction mixture was then increased to 95° and finally allowed to cool to room temperature. The bulk of the pyridine was removed on a rotary evaporator and the dark red oil remaining taken up in chloroform (250 ml). The chloroform solution was washed with 10% hydrochloric acid (2 × 50 ml), water (2 × 50 ml) and saturated sodium chloride solution (50 ml). After drying (sodium sulfate) the solvent was removed and the dark red oil remaining distilled under reduced pressure using a short-path distillation apparatus. The product was obtained as a light orange oil, sufficiently pure for the next synthetic step, which solidified on standing to give 94.2 g, 56%, bp 110-130°/1 mm. Bulb-to-bulb distillation gave a white solid mp 81-83° (lit [20] mp 83°). It had ^{1}H-nmr (carbon tetrachloride): δ ppm 1.08 (t, 3H, J = 7 Hz), 1.25 (d, 3H, J = 6 Hz), 1.70 (s, 3H), 2.30 (q, 3H, J = 7 Hz), 1.25 (d, 3H, J = 6 Hz), 1.70 (s, 3H), 2.30 (q, 2H, J = 7 Hz), 3.96 (q, 1H, J = 6 Hz), 7.65 (1H, broad s).

5-Bromomethylene-4-ethyl-3-methyl-3-oxo-1*H*-pyrrole.

A mixture of 4-ethyl-3,5-dimethyl-3-pyrrolin-2-one (93.7 g, 0.67 mole), absolute methanol (200 ml) and bromine (60 ml, 1.16 moles) dissolved in absolute methanol (300 ml) was placed in a round-bottom flask equipped with a reflux condenser and protected from moisture. The mixture was then heated at reflux for six hours. After cooling, the methanol was evaporated and the residue placed in a vacuum desiccator over sodium hydroxide pellets overnight. The residue was then taken up in hot carbon tetrachloride and treated with norite. On cooling, yellow crystals of the desired product separated from the solvent. Repetition of this procedure affords reasonably pure product, 79.8 g, 55%, mp 137-140° (138-140° after recrystallization 3×), (lit [8] mp 139-141°). It had ^{1}H-nmr (deuteriochloroform): δ ppm 1.15 (t, 3H, J = 7 Hz), 1.87 (s, 3H), 2.42 (q, 2H, J = 7 Hz), 5.80 (s, 1H), 7.65 (broad s, 1H).

Ethyl 4-Acetyl-5-oxo-hexanoate.

2,4-Pentanedione (62 ml, 0.6 mole), ethyl acrylate (66.5 ml, 0.64 mole) and nickel acetylacetonate (6.2 g, 22.5 mmoles) were placed in a sealed glass pressure flask. The flask was then placed in an oil bath at 120° and the temperature increased to 160°. **(N.B. As a safety precaution this reaction is carried out behind a safety screen.)** The flask and its contents were maintained at this temperature for two days, after which time the contents had turned a cloudy green color. Work-up was accomplished by filtering the crude product from the reaction through a sintered glass filter funnel packed with silica gel 30-70 mesh. The addition of chloroform to the crude material to reduce its viscosity and the use of a Buchner funnel connected to a water pump facilitated the filtration. The silica is washed thoroughly with chloroform. Evaporation of the solvent leaves a viscous oil which is then distilled under reduced pressure to yield a pale yellow oil with bp 110-120°/2 mm, (lit [21] bp 150-151°/10 mm). It had ^{1}H-nmr (carbon tetrachloride): δ ppm 1.20 (tc 3H, J = 7 Hz), 1.70-2.60 (10 H), 2.06 (s), 4.02 (q, 2H, J = 7 Hz).

2,4-Dimethyl-5-(ethoxycarbonyl)-1*H*-pyrrole-3-propanoic Acid Ethyl Ester.

Ethyl acetoacetate (143 g, 1.1 moles) and glacial acetic acid (440 ml) were placed in a 2-liter Erlenmeyer flask cooled in ice. A slution of sodium nitrite (81.4 g, 1.2 moles) in water (275 ml) was added slowly over a period of 3 hours, *i.e.* at such a rate as to maintain the temperature below 14°. The resulting mixture was left in the ice bath for three hours and then at room temperature overnight.

Ethyl 4-acetyl-5-oxohexanoate (270 g, 1.35 moles) was added all at once to the above solution and then zinc dust (154 g) was added in small por-

tions to the vigorously stirred solution such that the temperature did not exceed 65° (Approximately 3-5 hours was required for this addition.) When the zinc has completely reacted, the mixture was heated to 100° for one hour and the resulting solution poured into ice water. The diester separated as an orange oil which solidified on standing. The diester was then filtered off and washed with copious volumes of water to remove zinc acetate which co-precipitated with the diester. The diester was then dissolved in chloroform, washed with water (2×) and with saturated sodium chloride solution (2×). After drying (sodium sulfate) the chloroform was removed and the residue recrystallized from 95% ethanol to give pale orange needles mp 72-73° (lit [22] mp 73°), 153 g, 46%. It had ^{1}H-nmr (deuteriochloroform): δ ppm 1.18 (t, 3H, J = 7 Hz), 1.30 (t, 3H, J = 7 Hz), 2.15 (s, 3H), 2.22 (s, 3H), 2.24-2.85 (m, 4H), 4.10 (q, 2H, J = 7 Hz), 8.88 (broad s, 1H).

2,4-Dimethyl-5-carboxy-1*H*-pyrrole-3-propanoic Acid.

Ethyl 4-(2-ethoxycarbonylethyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (2.16 g) was suspended in a few ml of ethanol, and a solution of sodium hydroxide (1.5 g) in water (7.5 ml) was added. The mixture was then made up to a total volume of 20 ml by addition of more ethanol, and the mixture was slowly heated to reflux. After two hours a light amber solution was left, which was cooled to room temperature. The ethanol was then removed on a rotary evaporator at room temperature. An off-white solid separated out from the remaining solution, which was cooled to −10° in an ice/salt bath. On addition of ice cold 10% nitric acid (24 ml) a pink solid formed immediately. **(N.B. It is vital to have the temperature below −8° and to add the nitric acid slowly, otherwise rapid decarboxylation begins and the product yield falls dramatically.)**

This was filtered off and washed with a minimal amount of water (40 ml) and dried over solid sodium hydroxide to give 1.45 g, 81.5%, of diacid. It had no mp but decomposed above 115° (lit [23]); ^{1}H-nmr (1:1 deuteriochloroform-d_6-DMSO): δ ppm 2.13 (s, 3H), 2.20 (s, 3H), 2.10-2.80 (m, 4H), 10 (broad s, 1H).

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

5-Bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole (1.30 g, 6.0 mmoles) and 2,4-dimethyl-5-carboxy-1*H*-pyrrole-3-propanoic acid (1.28 g, 6 mmoles) were placed in a 50 ml round bottom flask purged with nitrogen. Nitrogen purged methanol (30 ml) and water (1 ml) were then added and the mixture heated at reflux under nitrogen and in the dark for five hours. On cooling, the reaction mixture was refrigerated and the yellow precipitate obtained removed by filtration. Recrystallization from nitrogen purged benzene afforded 1.38 g, 71% of yellow needles mp 215-217° (lit [8] mp 217-220°). It had uv-vis (chloroform): λ max 403 nm, ε, 28,000, (methanol): λ max 411 nm, ε, 27,000; ir (nujol): ν 3355, 1735, 1670, 1630, cm^{-1}; ^{1}H-nmr (deuteriochloroform): δ 1.15 (t, 3H, J = 7 Hz), 1.92 (s, 3H), 2.10 (s, 3H), 2.25-2.87 (m, 6H), 3.53 (s, 3H), 6.07 (s, 1H), 10.25 (broad s, 1H), 11.15 (broad s, 1H) ppm; and ^{13}C-nmr in Table 2.

5-[1,5-Didehydro-3-ethyl-4-methyl-5-oxo-2*H*-pyrrol-2-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrol-3-propanoic Acid (Xanthobilirubic Acid, **XBR**).

One hundred and forty milliliters of 10% aqueous sodium hydroxide solution was purged with a stream of nitrogen while heating to reflux. Then 1.32 g (4.17 mmoles) of xanthobilirubic acid methyl ester were added with vigorous magnetic stirring to the refluxing sodium hydroxide solution. Vigorous reflux under nitrogen, was maintained for 4.5 hours. During the reflux period, solid yellow material was periodically flushed back into the aqueous phase. The reaction mixture was cooled, under nitrogen to room temperature and set in the freezer (0°) overnight. The resulting copious yellow precipitate was removed by filtration and washed with 3*N* sodium hydroxide to give bright yellow needles of sodium salt. Under nitrogen, the sodium salt was stirred with 80 ml of 10% hydrochloric acid for 15 minutes. The resulting mixture was filtered and the solid washed with water and dried under vacuum at 47° to afford 1.18 g (94%) of xanthobilirubic acid as a yellow powder with a pale green tinge, mp 290-295° dec. Upon combining and acidifying the two filtrates (above), an additional 0.08 g of yellow-green solid, mp 285-291° dec, was obtained. The xanthobilirubic acid could be further purified by recrystallization from acetone-DMSO as follows. In a 50 ml Erlenmeyer, 0.93 g of xanthobilirubic acid was covered with acetone and heated to boiling. Under gentle boil, a small amount of DMSO was added until all the solid dissolved. The hot, dark solution was filtered, washing the residues with hot acetone. The filtrate was diltuted with 1 volume of acetone to give approximately 40 ml total of solution. The solution was heated to boiling and 1.0 mml of hot water was added to give an immediate precipitate. After boiling the suspension for 30 sec, it was removed from heat, capped tightly and allowed to cool in the dark to room temperature. After several hours, filtration with acetone washing gave 520 mg of bright yellow microcrystals, mp 288-291°, (lit [24] mp 290-291°) rapid heating. A second crop, 120 mg, of dull yellow powder was obtained following addition of 2 ml of water to the filtrate and cooling to −20°. The remainder of the acid, 270 mg, could be recovered following addition of two volumes of water and two days standing at room temperature. The crystallization procedure removed green mesobiliverdin-XIIIα and yellow mesobilirubin-XIIIα and gave material with uv-vis (chloroform): λ max 408 nm, ε, 28,000, (methanol): λ max 416 nm, ε, 34,000; ir (nujol): ν 3360, 3200-2500, 1705, 1670, 1630 cm^{-1}; ^{1}H-nmr (d_6-DMSO): δ ppm 1.10 (t, 3H, J = 7 Hz), 1.81 (s, 3H), 2.07 (s, 3H), 2.22 (s, 3H), 2.25-2.80 (m, 6H), 5.93 (s, 1H), 9.67 (broad s, 1H), 10.18 (broad s, 1H), 11.83 (broad s, 1H); ^{13}C-nmr in Table 2.

Xanthobilirubic Acid Dimethylamide (**XBRDMA**).

Xanthobilirubic acid (**XBR**) (240.5 mg, 0.80 mmole) was mixed with 0.774 ml (990 mg, 3.6 mmoles) of diphenylphosphorylazide, 97 mg (1.2 mmoles) of dimethylammonium chloride and 0.227 ml, (202 mg, 2.0 mmoles) of triethylamine in 75 ml of dimethylformamide and stirred at ambient temperature under Argon for 48 hours. The solvents were then removed under vacuum using a rotary evaporator, and the residue was taken up in chloroform. The chloroform solution was washed with 1*N* aqueous sodium bicarbonate (2 × 25 ml) then water (2 × 40 ml) and dried (sodium sulfte). Evaporation of solvent gave the crude semisolid amide, which was purified by preparative tlc using 10:1 (v/v) benzene-ethanol, then crystallization (methanol-water) to give 161.7 mg (58%) of pure dimethylamide, mp 224.5-226°. A second crop of cyrstals (31.5 g, 11%) was obtained, mp 219.5-222.5°. It had uv-vis (chloroform): λ max 405 nm, ε, 36,500, (methanol): λ max 412 nm, ε, 33,200; ir (deuteriochloroform): ν 3345, 1662, 1028 cm^{-1}; ^{1}H-nmr (deuteriochloroform): δ ppm 1.15 (t, 3H, J = 8 Hz), 1.92 (s, 3H), 2.12 (s, 3H), 2.39 (s, 3H), 2.4-2.8 (m, 6H), 2.89 (s, *syn N*-Me, 3H), 2.94 (s, *anti N*-Me, 3H), 6.11 (s, 1H), 10.38 (s, 1H), 11.29 (s, 1H); ^{13}C-nmr in Table 2.

Anal. Calcd. for $C_{19}H_{27}N_3O_2$ (329): C, 69.30; H, 8.20; N, 12.77. Found: C, 69.04; H, 8.10; N, 12.76.

Xanthobilirubic Acid Methylamide (**XBRMA**).

Xanthobilirubic acid (**XBR**) (88 mg, 0.29 mmole) was mixed with 0.38 ml (466 mg, 1.69 mmoles) of diphenylphosphorylazide, 36.5 mg (0.5 mmole) of methylamine hydrochloride (previously sublimed and stored desiccated), 0.16 ml (116 mg, 1.15 mmoles) of triethylamine and 40 ml of dry dimethylformamide and stirred under Argon at room temperature for 43 hours. The solution was worked up as before (for **XBRDMA** above). Analytical tlc indicated only one spot (benzene-ethanol, 10:1, v/v or chloroform-methanol-acetic acid, 97:2:1, v/v/v), and the residue (79 mg, 87%) was crystallized from methanol-dimethyl formamide to give 44 mg (48%) of yellow crystalline *N*-methylamide. Second and third crops afforded 9 and 12.5 mg, respectively. The material melted with shrinking and decomposition, mp range 276-288°; uv-vis (chloroform): λ max 405 nm, ε, 33,000, (methanol): λ max 413 nm, ε, 35,500; ir (potassium bromide): ν 3340, 3290, 1670, 1632 cm^{-1}; ^{1}H-nmr (d_6-DMSO): δ ppm 1.05 (t, 3H, J = 8 Hz), 1.74 (s, 3H), 1.99 (s, 3H), 2.13 (s, 3H), 2.3-2.7 (m, 6H), 2.48 (s, *syn N*-Me, J = 11 Hz, 3H), 5.89 (s, 1H), 7.63 (broad s, 1H), 9.69 (broad s, 1H), 10.20 (broad s, 1H); ^{13}C-nmr in Table 2.

Anal. Calcd. for $C_{16}H_{25}N_3O_2 \cdot 1/4H_2O$ (319.5): C, 67.60; H, 7.98; N, 13.15. Found: C, 67.68; H, 8.05; N, 13.04.

Xanthobilirubic Acid Amide (**XBRA**).

Compound **XBR** (120 mg, 0.397 mmole), 0.51 ml (651 mg, 2.37 mmoles) of diphenylphosphorylazide, 55.5 mg (1.04 mmoles) of ammonium chloride and 0.168 ml (121 mg, 1.20 mmoles) of triethylamine were mixed and stirred in 40 ml of dry dimethylformamide under Argon for 42 hours. The reaction mixture was evaporated to dryness (vacuum, rotary evaporator) and the residue was washed with chloroform and filtered. The yellow solid left behind one major yellow spot by tlc (benzene-ethanol, 10:2, v/v or chloroform-methanol-acetic acid, 97:2:1, v/v/v) and two small faster moving spots. Crystallization from methanol-dimethylformamide gave 32.5 mg, first crop, and 8.7 mg second crop, both pure by tlc, 35% yield. The substance melted with shrinking and darkening at 265-295°. It had uv-vis (chloroform): λ max 407 nm, ε, 28,500, (methanol); λ max 414 nm, ε, 27,000; ir (potassium bromide): ν 3400, 3355, 3190, 1672, 1630, cm^{-1}; ^{1}H-nmr (d_6-DMSO): δ ppm 1.09 (t, 3H, J = 8 Hz), 1.78 (s, 3H), 2.04 (s, 3H), 2.18 (s, 3H), 2.4-2.8 (m, 6H), 5.93 (s, 1H), 6.65 (broad s, 1H), 7.19 (broad s, 1H), 9.73 (broad s, 1H), 10.23 (broad s, 1H) ppm; ^{13}C-nmr in Table 2.

Anal. Calcd. for $C_{17}H_{23}N_3O_2 \cdot 1/4H_2O$ (305.5): C, 66.78; H, 7.69; N, 13.74. Found: C, 66.53; H, 7.67; N, 13.54.

4-Ethyl-3-methyl-5-(4-ethyl-3,5-dimethylpyrrolyl-2-methylidene)-3-pyrrolin-2-one (Kryptopyrromethenone, **KR**).

5-Bromomethylene-4-ethyl-3-methyl-3-oxo-1*H*-pyrrole (2.16 g, 10.0 mmoles) was placed in a 100 ml round-bottom flask with reflux condenser. The flask was purged with nitrogen, then 1.38 ml (1.23 g, 10.0 mmoles) of kryptopyrrole, 50 ml of methanol (previously distilled from sodium methoxide and dimethyl phthalate under nitrogen) and 1.0 ml of distilled water were added. After 3.25 hours at reflux under nitrogen a copious precipitate of yellow needles was evident. The mixture was cooled to −20° overnight and filtered. The yellow crystals were washed with cold methanol and air-dried to give 1.65 g (64%) of needles, mp 245-248° dec (lit [15] mp 248-250°). The filtrate and washings were combined and evaporated, and the residual solid was chromatographed on silica gel (using hexane-chloroform, 1:1, v/v) to give an additional 270 mg (10.5%) of **KR**. The material could be recrystallized from benzene (under nitrogen) to give bright yellow needles, mp 242° dec. Pure **KR** had uv-vis (chloroform): λ max 409 m, ε, 33,900, (methanol): λ max 416 nm, ε, 39,400; ir (nujol): ν 3356, 1735, 1660, 1630 cm^{-1}; ^{1}H-nmr (deuteriochloroform): δ ppm 1.06 (t, 3H, J = 7 Hz), 1.17 (t, 3H, J = 7 Hz), 1.92 (s, 3H), 2.11 (s, 3H), 2.37 (s, 3H), 2.38 (q, 4H, J = 7 Hz), 6.37 (s, 1H), 10.30 (broad s, 1H), 11.25 (broad s, 1H); ^{13}C-nmr in Table 2.

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