Monatshefte für Chemie 137, 1463–1476 (2006)
DOI 10.1007/s00706-006-0543-8

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

Synthesis and Hepatic Metabolism of Xanthobilirubinic Acid Regioisomers

Stefan E. Boiadjiev[1], **Brian A. Conley**[1], **Justin O. Brower**[1], **Antony F. McDonagh**[2], and **David A. Lightner**[1,*]

[1] Department of Chemistry, University of Nevada, Reno, Nevada, USA
[2] Division of Gastroenterology and the Liver Center, University of California, San Francisco, California, USA

Received April 27, 2006; accepted May 11, 2006
Published online October 16, 2006

Summary. A set of four regioisomeric dipyrrinone propionic acids has been synthesized and their hepatic metabolism examined in rats: xanthobilirubinic acid and pseudo-xanthobilirubinic acid each with a propionic acid on a pyrrole ring; *exo*-ψ-xanthobilirubinic acid and *endo*-ψ-xanthobilirubinic acid, each with a propionic acid transposed to a lactam ring. After intravenous injection all four isomers were excreted to some degree in unchanged form in bile in normal rats. Xanthobilirubinic acid, the structurally closest dipyrrinone to bilirubin, and *exo*-ψ-xanthobilirubinic acid were excreted almost entirely in unchanged form. However, a small fraction of xanthobilirubinic acid acyl glucuronide was also detected. More extensive acyl glucuronidation was observed for pseudo-xanthobilirubinic acid, and *endo*-ψ-xanthobilirubinic acid underwent slow metabolism to unidentified more polar products that did not seem to be glucuronides.

Keywords. Pyrrole; Dipyrrinone; NMR.

Introduction

Dipyrrinones are yellow, non-fluorescent dipyrrolic pigments with an extensive system of conjugated double bonds, and they comprise the core chromophores of the yellow-orange tetrapyrrole pigment of jaundice and mammalian bile: bilirubin (Fig. 1A) [1, 2]. They have captured interest as simple analogs of bilirubin for use in model studies of chemical reactions [3], in the preparation of highly-fluorescent analogs [4], in photochemistry [5], and in hepatic metabolism [4, 6]. One dipyrrinone, xanthobilirubinic acid (*XBR*, Fig. 1B) has for the past three decades stood out as a bilirubin model compound and also as a standard for HPLC analysis of bilirubin glucuronides [7]. With respect to the location of a propionic acid at a

* Corresponding author. E-mail: lightner@unr.nevada.edu

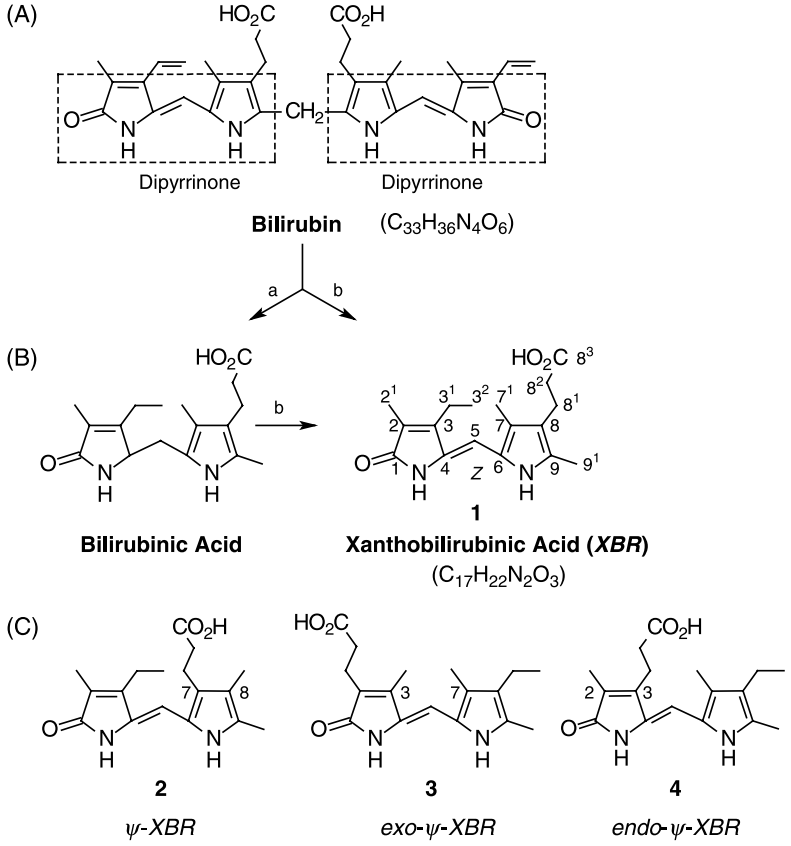


Fig. 1. (A) Bilirubin and (B) its dipyrrole cleavage products isolated in 1911: colorless "Bilirubinsäure" (*H. Fischer*) or "Bilinsäure" (*O. Piloty*), and yellow "Xanthobilirubinsäure" (*Fischer*) or "Dehydro-Bilinsäure" (*Piloty*): a: HI in CH_3CO_2H at 100°C followed by HI-PH_3; b: CH_3ONa/CH_3OH, 220–230°C or 0.1 *M* $KMnO_4$ at 7°C; (C) the target *XBR* regioisomers of the current work

"pyrrole" β-position, there are four regioisomers, including *XBR* (with its propionic acid at C(8)). One regioisomeric analog of *XBR* (**1**), with the C(7) methyl and C(8) propionic acid switched, we have named *pseudo-XBR* (**2**, ψ-*XBR*, Fig. 1C). Switching the C(3) ethyl and C(8) propionic acid of *XBR* gives a third regioisomer (**4**), which we call *endo-*ψ*-XBR*, and switching the C(2) methyl and C(3) propionic acid of the latter provides a fourth regioisomer, **3** (*exo-*ψ*-XBR*).

Dipyrrinones first appeared in the chemical literature in the form of bilinic acid [8a] or bilirubinic acid [9] and dehydrobilinic acid [8b] or *XBR* [9] nearly 100 years ago in *O. Piloty*'s and *H. Fischer*'s attempts to determine the structure of bilirubin. Bilirubinic acid and *XBR* are interconvertible by redox reactions. As C_{17} compounds, they represent one-half of the bilirubin structure, and their constitutional structures were characterized long ago. After several missteps originating from the degradative cleavage of mesobilirubin [9c], *Fischer*, *Bartholomäus*, and *Röse* postulated the hydroxypyrrole tautomer as the structure of bilirubinic acid and the correct (modern) lactam structure for *XBR* (Fig. 1B) in 1913 [10], albeit without designating its stereochemistry at C(4). Curiously, subsequent to

1914, *Fischer* drew only the lactim form of *XBR* through the end of his enormously prolific career (which ended with his death on March 31, 1945 in Munich). Although *Fischer*'s early interest (1911–1914) was in the structure of bile pigments, especially bilirubin, work on the structure of the latter appeared to wane in the *Fischer* labs after 1915 (while research on the structure of the heme porphyrin accelerated) with a few publications on the reduction of bilirubin in the mid-1920s. After having solved the structure of hemin, for which the 1930 Nobel Prize in Chemistry was awarded to *Fischer*, he resumed the structure determination of bilirubin and pursued the structure determination and synthesis of chlorophyll.

Because bilirubinic acid and *XBR* represented only one-half of the bilirubin structure, *Fischer* sought to determine the other half, again by degradation [11]. These studies of the early 1930s led *Fischer* to corroborate the structure of *XBR* by synthesis [12] using a methodology that also allowed for the synthesis of an important regioisomer of *XBR* that differed only in switching the locations of the methyl and ethyl groups on the lactam ring (*iso-XBR*, Fig. 2A) [13]. Put into practice, the methodology also led to the equivalent regioisomer of ψ-*XBR*: *iso-*ψ*-XBR* (Fig. 2A), as well as **3**. The advances in synthesis of the late 1920s and early 1930s that made dipyrrinone synthesis possible involved condensation of an appropriate α-H pyrrole with a designed α-pyrrolealdehyde to afford a dipyrrylmethene as illustrated for the synthesis of *XBR* in Fig. 2B [12, 13].

Our interest in dipyrrinones **1**–**4** stems from their role as models for probing and elucidating the molecular requirements of substrate in the hepatic elimination of bilirubin. In particular, these bilirubin analogs serve as probes of the sensitivity of uptake transporters and glucuronosyl transferase isoforms to the location of the propionic acid group of the pigment. *XBR* (**1**) correlates structurally with natural bilirubin-IXα (Fig. 1A), while **3** correlates structurally with bilirubin-IXγ, and **2** and **4** correlate with bilirubin-IXβ and δ.

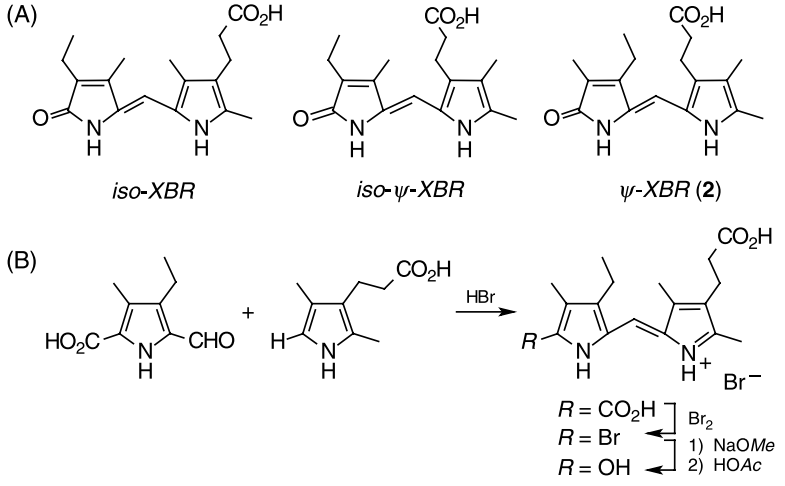
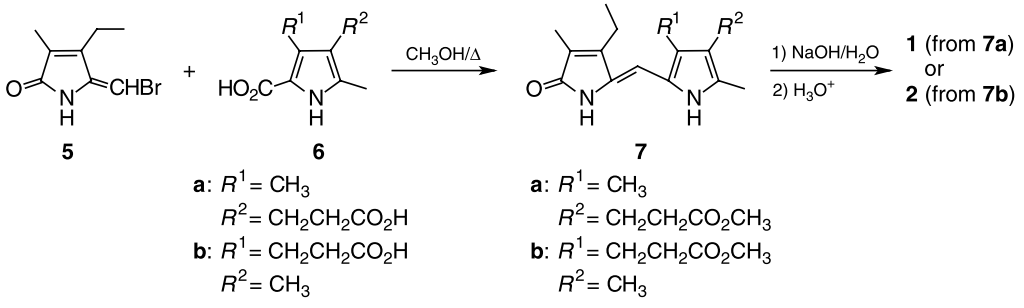


Fig. 2. (A) *Fischer* dipyrrinones synthesized, along with *XBR*, in the structure proof of mesobilirubin; (B) outline of the synthetic methodology developed by the *Fischer* group for the syntheses of *XBR* and the dipyrrinones of (A)

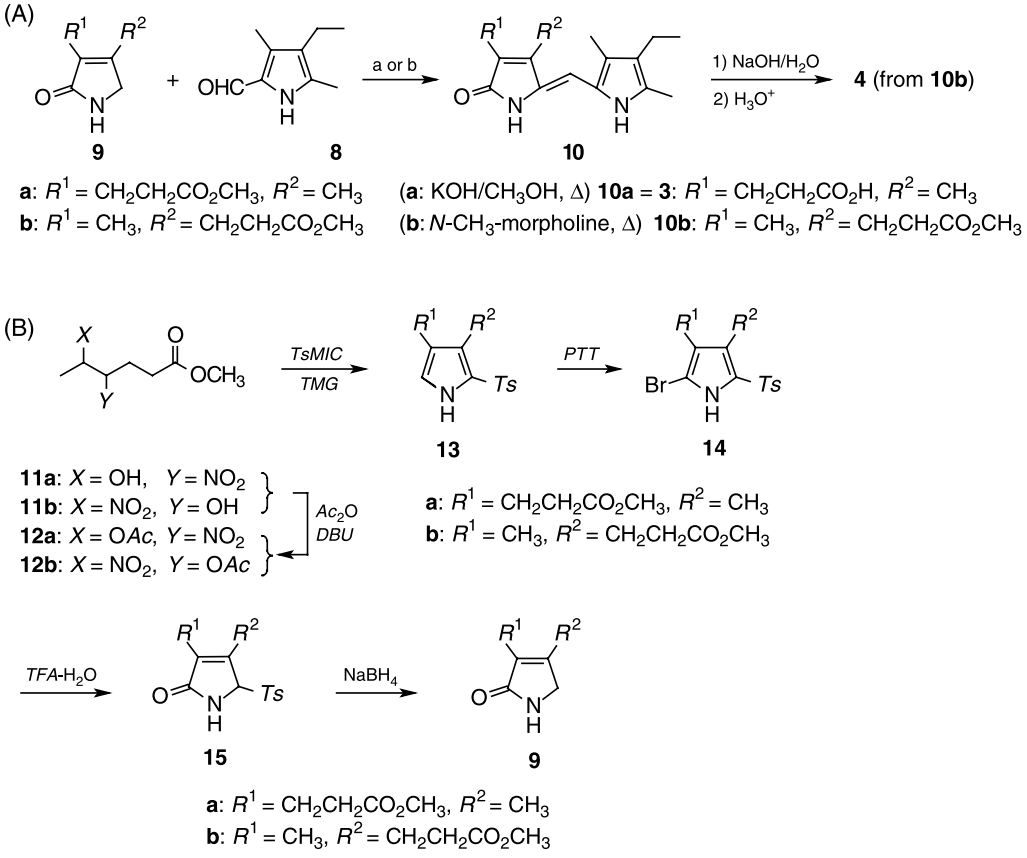
Results and Discussion

Synthesis Aspects

The synthesis of **1** has been reported previously and makes use of improved and modified preparations based on *Fischer*'s later work [14]. Thus, as outlined in Scheme 1, the bromomethylenepyrrolinone **5** was condensed with acid **6a** in refluxing, acidic CH_3OH to afford high yields of the methyl ester **7a** of **1** [15, 16]. Similarly, the methyl ester of **2** was prepared by condensing **5** with the isomeric acid



Scheme 1



Scheme 2

6b to afford the methyl ester **7b** of **2** [16]. This preparation of **7b** represents a new synthesis using the more modern methodology. The compound had previously been prepared only by *Fischer* in 1931 [12] using the method of Fig. 2B: condensation of hemopyrrole carboxylic acid (2,3-dimethylpyrrole-4-propionic acid) with 2-formyl-3-ethyl-4-methylpyrrole-5-carboxylic acid with CH_3OH-48% HBr to afford a dipyrrylmethene that was brominated by Br_2 in CH_3CO_2H to decarboxylate and replace with Br, then treated with AgO*Ac* or KO*Ac* to give **2**.

Dipyrrinone **4** had also been prepared in the early 1930s by *Fischer* and *Adler* [13] from the appropriate dipyrrylmethene, as above and in Fig. 2B. We preferred a more modern synthesis that would allow us to prepare both **3** and **4** and for these two dipyrrinones, one new (**3**) and the other (**4**) not synthesized since 1931 [13], we turned to the methodology developed by *Jayasundera*, *Inomata*, and *Kinoshita* for preparing dipyrrinones used in their syntheses of (tetrapyrrole) phycocyanobilin pigments [17]. As outlined in Scheme 2, this was accomplished by condensing the known kryptopyrrole aldehyde (**8**) with an appropriate pyrrolinone, **9a** or **9b**. The last two were synthesized by *Barton-Zard* pyrrole-forming reactions [18], borrowing from the methodology developed by *Kinoshita* and *Inomata* [17, 19].

The syntheses of **3** and **4** share two common intermediates, pyrrole aldehyde **8** and methyl 4-nitrobutanoate which is converted to pyrrolinones **9a** or **9b** (Scheme 2A). To synthesize **9a**, methyl 4-nitrobutanoate was converted to nitroalcohol **11a** (Scheme 2B) by condensation (*Henry* reaction) with acetaldehyde, catalyzed by KF in 2-propanol. Acetylation of **11a** to **12a** in 95% yield, followed by a *Barton-Zard* pyrrole-forming condensation with *p*-toluenesulfonylmethylisocyanide (*TsMIC*) [20] in the presence of tetramethylguanidine (*TMG*) afforded crystalline **13a** in 62% yield. Conversion of **13a** to **9a** was accomplished in 3 steps, as outlined in Scheme 2B. The unsubstituted α-position of tosylpyrrole **13a** is brominated using *N*,*N*,*N*-trimethylanilinium perbromide (*PTT*) to afford a 96% yield of **14a**, which was converted to **15a** in 46% yield in aqueous *TFA*. Detosylation of **15a** with $NaBH_4$ in ethanol smoothly gave an 80% yield of **9a**, which was condensed with **8** in hot aqueous-ethanolic KOH to give **3** in 71% yield. As is reported below, **9a** doubtless contains some ethyl ester due to trans-esterification, but the ester groups are saponified under the reaction conditions above.

To prepare **9b**, methyl 4-oxo-butanoate was condensed with nitroethane in the presence of *DBU* to afford methyl 4-hydroxy-5-nitrohexanoate (**11b**, Scheme 2B), which was acetylated (to **12b**), and the nitroacetate submitted to a standard *Barton-Zard* pyrrole forming reaction [18, 19] with *TsMIC* in the presence of *TMG* to give a 45% overall yield of tosylpyrrole **13b**. Conversion of **13b** to **9b** proceeded smoothly in two steps: (1) bromination at the vacant pyrrole α-position using *PTT* to afford a 67% isolated yield of **14b**, then (2) reaction of **14b** with *TFA* in aqueous *DMSO*, followed by Zn/I_2 to give the tosylpyrrolinone (**15b**) corresponding to **9b**, and finally detosylation with $NaBH_4$ in ethanol.

The latter (**9b**) was mainly the methyl ester (85%), with 15% ethyl ester due to trans-esterification in the $NaBH_4$/ethanol [19] reductive detosylation of **15b**. A change of solvent, from ethanol to methanol, avoided trans-esterification, but the reductive detosylation was much less effective and the yield of **9b** was low. Apparently in ethanol, the reducing agent is the more effective $NaBH_4$, which reacts more slowly with ethanol than methanol. In methanol, the $NaBH_4$ is rapidly converted to

$NaBH(OMe)_3$, a less effective reagent for cleaving the tosyl group. An acidic quench before ethanol removal and immediate chromatographic purification cleanly gave the methyl ester (**9b**). Condensing **9b** with **8** under a fairly standard coupling procedure (refluxing aqueous-ethanolic KOH) failed. However, after much experimentation, condensation using *N*-methylmorpholine in CH_3CN afforded methyl ester **10b** in 36% yield. A better yield (68%) of the free acid (**4**) was obtained instead of **10b** using *DBU* in CH_3OH to effect the condensation.

Structures and Spectroscopy

The structures of **1**–**4** follow logically from their synthetic precursors, the method of synthesis, and from characterization by NMR spectroscopy. *XBR* (**1**) and its dihydro analog, bilirubinic acid, are of historical interest as the first known dipyrrinones, both obtained by degradation of bilirubin (whose structure was unknown at the time). Their structures were proved by *Fischer*, first by degradation [9, 10] then by synthesis [12, 13]. The synthesis of **1** was improved from the original *Fischer* synthesis [12, 13], as outlined in Scheme 1. The regioisomeric *ψ-XBR* (**2**) was first synthesized by *Fischer* in 1931 [13] and more recently by the *XBR*-type procedure as outlined in Scheme 1 [16]. Like **1** and **2**, the synthesis of **4** was first achieved by *Fischer* [13], who condensed kryptopyrrole and 5-formyl-3-methyl-4-(2-carboxyethyl)pyrrole-2-carboxylic acid to produce an isomer of the dipyrrylmethene shown in Fig. 2B, with the C(3) ethyl and C(8) propionic acid interchanged. The resultant dipyrrylmethene was then converted to **4**. The new route to **4** (Scheme 2) has many advantages and gave a product with properties identical to *Fischer*'s **4**.

Table 1. ^{13}C NMR chemical shifts (δ, ppm) of four isomeric dipyrrinones (**1**–**4**) in $(CD_3)_2SO$ solvent

	Carbon[a]	**1** (*XBR*)[b]	**2** (*ψ-XBR*)	**3** (*exo-ψ-XBR*)	**4** (*endo-ψ-XBR*)
1	C=O	171.9	171.9	171.3	171.6
2	–C=	122.6	122.6	125.9	123.6
3	–C=	147.2	147.3	142.0	144.4
4	–C=	127.3	127.3	128.1	127.0
5	–CH=	97.6	97.8	98.5	98.2
6	–C=	121.7	121.4	121.9	121.9
7	–C=	122.3	126.2	122.4	122.4
8	–C=	118.7	114.5	121.5	121.6
9	–C=	129.4	129.1	128.9	128.8
2^1	CH_2/CH_3	8.1	8.0	19.1	8.2
2^2	CH_2	–	–	32.6	–
3^1	CH_2/CH_3	17.2	17.2	9.3	19.6
3^2	CH_2/CH_3	14.8	14.7	–	34.6
7^1	CH_2/CH_3	9.2	19.6	9.4	9.1
7^2	CH_2	–	35.4	–	–
8^1	CH_2/CH_3	19.5	8.6	16.9	16.9
8^2	CH_2/CH_3	35.0	–	15.5	15.5
9^1	CH_3	11.0	11.0	10.9	10.9
	CO_2H	174.0	173.9	173.7	173.6

[a] For carbon numbering system, see Fig. 1; [b] assignments are based on *g*HMBC experiments

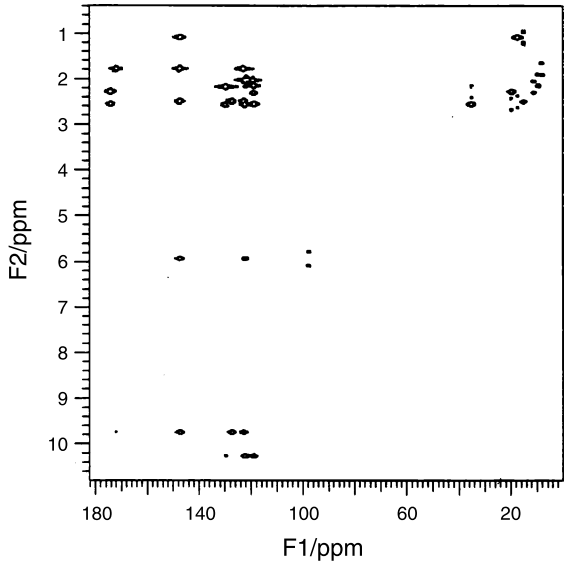


Fig. 3. HMBC of xanthobilirubinic acid (**1**) in $(CD_3)_2SO$

Regioisomeric dipyrrinone **3** was not prepared by *Fischer* and was apparently unknown before this work. All dipyrrinones **1**–**4** analyzed correctly for the basic formula, $C_{17}H_{22}N_2O_3$. Their ^{13}C NMR spectral data (Table 1) correlate well with each structure. For example, in Fig. 3 is shown the HMBC of **1**, from which one can make correlated assignments (Table 1) of ^1H and ^{13}C resonances. Thus, the lactam ring carbons of **1** and **2** show, as expected, the same set of structure-correlated resonances with essentially identical chemical shifts. The pyrrole ring carbon resonances of **3** and **4** similarly show the same structure-correlated chemical shifts, as expected. The regioisomeric differences between the pairs **1** and **2** and **3** and **4** show up in the pyrrole carbon signals of **1** and **2** and in the lactam carbon signals of **3** and **4**. Interestingly, having a propionic acid group on the pyrrole ring causes the C(5) resonance to appear 0.4–0.9 ppm more shielded than when the propionic acid is on the lactam ring (in **3** and **4**).

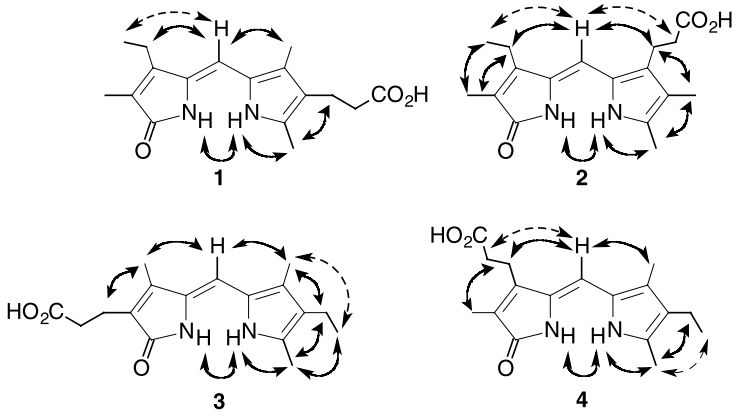


Fig. 4. Nuclear *Overhauser* effects (curved arrows: strong, solid curve; weak, dashed curve) of *XBR* (**1**), *ψ-XBR* (**2**), *exo-ψ-XBR* (**3**), and *endo-ψ-XBR* (**4**) in $(CD_3)_2SO$

Although its complete chemical structure is well-established in the literature, $^1H\{^1H\}$-Nuclear *Overhauser* effect spectroscopy (N*O*E) in $(CD_3)_2SO$ solvent reconfirmed the constitution and *syn-Z* stereochemistry at the exocyclic C(4)–C(5) double bond of *XBR* (**1**). Similarly, N*O*Es (Fig. 4) from **2**, **3**, and **4** in $(CD_3)_2SO$ clearly indicate the *syn-Z* conformation, as well as the spatial relationship of the methyl and propionic acid groups on the lactam ring (of **3** and **4**) and pyrrole ring (of **1** and **2**) – in accord with the expectation from directed synthesis. With the structures and stereochemistry of **1**–**4** firmly established, we began studies on their hepatic metabolism, initial results of which are reported below.

Metabolism

The hepatic metabolism and biliary excretion of **1**–**4** was studied by injecting small (∼0.25 mg) bolus doses, dissolved in ∼1 cm^3 of rat serum, intravenously in normal (*Sprague-Dawley*) adult male rats and homozygous adult male *Gunn* rats. *Gunn* rats are a mutant strain that is deficient in the *UGT1A* family of glucuronosyl transferases, including the specific isozyme, *UGT1A1*, that catalyzes bilirubin glucuronidation [21]. Samples of the injectate and of bile collected before and after injection of each dipyrrinone were analyzed by HPLC. Each compound was studied in at least two normal rats and two *Gunn* rats.

When injected into *Gunn* rats and normal rats, *XBR* (**1**) was excreted rapidly into bile in unchanged form. In normal rats, but not *Gunn* rats, the unchanged pigment in bile was accompanied by a very small proportion of a metabolite that

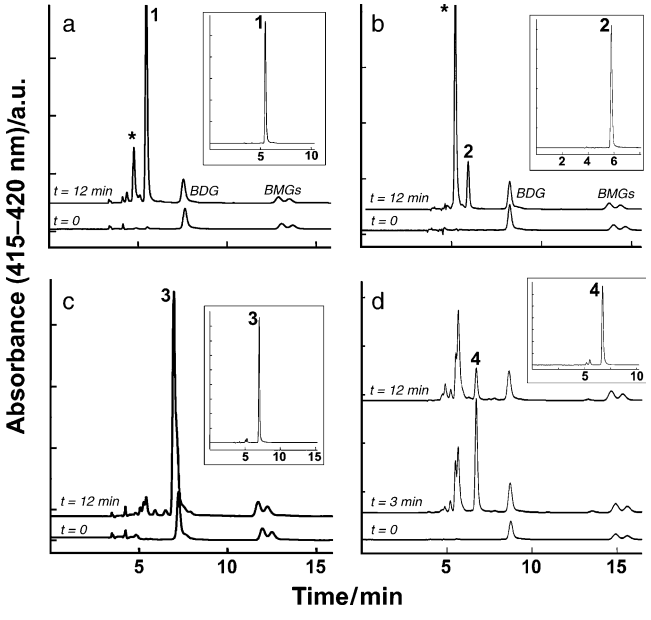


Fig. 5. Representative HPLC traces of bile collected just before ($t = 0$) and at the indicated times after injection of regioisomers **1**–**4** (panels **a**–**b**, respectively) in normal (*Sprague-Dawley*) rats; the insets show chromatograms of the solutions injected; peaks marked with an asterisk are presumptive acyl glucuronide metabolites; chromatographic conditions for each panel were similar, but not identical; *BDG* and *BMG* are the diglucuronide and monoglucuronides of bilirubin

eluted faster on HPLC (Fig. 5a). This metabolite disappeared on hydrolytic treatment of bile samples with sodium hydroxide and with β-glucuronidase. On this basis we conclude that the metabolite is *XBR* acyl glucuronide. Unchanged isomer **2** was also excreted promptly in bile in both *Gunn* and normal rats after intravenous injection. However, unexpectedly, in normal rats (Fig. 5b) a major fraction of the injected dose was converted to a more polar metabolite, identified as the acyl glucuronide of **2** by NaOH and β-glucuronidase hydrolysis. In the *Gunn* rat slow formation of unidentified more polar metabolites was also observed. As expected, isomer **3** was cleared from the circulation and excreted rapidly through the liver in unchanged form in normal rats (Fig. 5c). But surprisingly, in *Gunn* rats, the unchanged form was accompanied by a comparable amount of a more polar metabolite with an almost identical absorption spectrum. The identity of this metabolite remains to be determined. The final isomer **4** appeared rapidly in bile in unchanged form following injection in *Gunn* and normal rats. However, in both strains extensive metabolism to more polar products was also seen (Fig. 5d). These were stable to NaOH and β-glucuronidase hydrolysis and, therefore, are probably not glucuronides.

Thus, all four dipyrrinone regioisomers are cholephilic and are excreted rapidly in bile in unchanged form to different degrees following their intravenous injection in the rat. Isomers **1** and **2** also seem to form acyl glucuronides, the latter more so than the former. Isomer **4** seems to differ from the other three in its extensive conversion to unknown metabolites that do not appear to be acyl glucuronides.

Before beginning the metabolic studies, we had expected that the metabolism and hepatobiliary excretion behavior of the four regioisomers would be essentially identical and that they would all be excreted rapidly in *Gunn* rats and normal rats, with little or no acyl glucuronidation in the latter. The subtle effects of propionate positioning are somewhat surprising. *Blanckaert et al.* [22] studied the metabolism of the α, β, γ, and δ isomers of bilirubin in *Gunn* rats and normal *Wistar* rats. They found that the IXα isomer, the only one in which the two carboxyl groups can undergo intramolecular hydrogen-bonding to contralateral lactam and NH groups, was excreted as glucuronides in normal rats and hardly at all as unchanged pigment in *Gunn* rats. In contrast, the three other regioisomers were excreted rapidly in bile in unchanged form in *Gunn* rats and partly as conjugates in normal rats. The γ isomer, which corresponds to regioisomer **3** of the *XBR* family, underwent the greatest amount of conjugation. However, we were unable to detect significant amounts of glucuronide in the bile of *Sprague-Dawley* rats following injection of **3**. Thus the metabolism of dipyrrinone carboxylates may not be predictive of the metabolism of the corresponding bilirubins, even those that cannot undergo intramolecular hydrogen bonding of the type shown by bilirubin IXα and mesobilirubin IXα.

Experimental

Nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ solvent on a GE QE-300 spectrometer operating at 300 MHz (for most of the ^{1}H NMR data, unless otherwise indicated) and at 125 MHz on a Varian Unity Plus 500 MHz spectrometer for most of the ^{13}C NMR data. N*O*E NMR spectra were obtained at 500 MHz. Chemical shifts were reported in δ/ppm referenced to the residual $CHCl_3$ ^{1}H signal at 7.26 ppm and ^{13}C signal at 77.23 ppm. Infrared spectra were recorded on a

Perkin-Elmer model 1610-FT IR instrument. Ultraviolet-visible spectra were recorded on a Perkin-Elmer λ-12 spectrophotometer. Melting points were taken on a Mel-Temp capillary apparatus. Satisfactory combustion analyses for carbon, hydrogen, and nitrogen were carried out by Desert Analytics, Tucson, AZ, and gave results within $\pm 0.4\%$ of the theoretical values. Analytical thin layer chromatography (TLC) was carried out on J.T. Baker silica gel IB-F plates (125 μm layers). Radial chromatography was carried out on Merck preparative layer grade silica gel PF_{254} with $CaSO_4$ binder using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2, or 4 mm thick rotors. Commercial reagents were used as received from Aldrich or Acros. Spectroscopic data were obtained in spectral grade solvents from Fisher and Acros. Deuterated chloroform and dimethylsulfoxide were from Cambridge Isotope Laboratories. Bromomethylenepyrrolinone **5** [15, 23]; pyrroles **6a** [15, 23], **6b** [16], and **8** [24] and dipyrrinone esters **7a** [15, 23] and **7b** [16] were synthesized according to previously published methods.

Methyl 4-nitro-5-hydroxyhexanoate (**11a**)
Acetaldehyde (30.0 g, 0.68 mol) and KF (0.80 g, 14 mmol) were stirred with 70 cm^3 2-propanal in an ice bath and had added to it dropwise a solution of methyl 4-nitrobutyrate (41.25 g, 0.28 mol) in 70 cm^3 2-propanol. The solution was allowed to warm to room temperature and stirred overnight. The light yellow solution was diluted with 200 cm^3 CH_2Cl_2, washed with H_2O (2×200 cm^3) and brine (100 cm^3), and then dried ($MgSO_4$). The solvent was removed (rotovap), yielding a clear yellow oil. Yield 50.87 g (95%) **11a** [25]; IR (thin film): $\bar{\nu} = 3463$, 2955, 1736, 1551, 1440, 1371, 1206, 1176, 1008, 736 cm^{-1}; ^{1}H NMR: $\delta = 1.28$ and 1.31 (d, $J = 10.5$ Hz, 3H), 2.18–2.55 (m, 4H), 3.70 (s, 3H), 4.12–4.28 (m, 1H), 4.51 (m, 1H) ppm; ^{13}C NMR: $\delta = 19.1$, 19.4, 23.4, 25.0, 29.8, 29.9, 51.9, 68.3, 91.8, 93.0, 172.4 ppm.

2-(p-Toluenesulfonyl)-3-methyl-4-(2-methoxycarbonylethyl)-1H-pyrrole (**13a**, $C_{16}H_{19}NO_4S$)
Methyl 4-nitro-5-hydroxyhexanoate from above (50.9 g, 0.22 mol) and *DMAP* (50 mg) were dissolved in 100 cm^3 acetic anhydride and stirred at room temperature overnight [25]. The solution was then cooled in an ice bath and water added to destroy the excess anhydride, and the mixture was then stirred for an additional 3 h. The green solution was then diluted with 200 cm^3 CH_2Cl_2, washed with H_2O (2×500 cm^3), aqueous saturated $NaHCO_3$ (5×500 cm^3), H_2O (1×500 cm^3), and brine (1×500 cm^3), and then dried ($MgSO_4$). The solvent was removed (rotovap) to afford a green oily product (**12a**). It was used directly in the next step. Yield 57 g (95%); IR (thin film): $\bar{\nu} = 2955$, 1825, 1745, 1555, 1439, 1372, 1234, 1020, 956, 854 cm^{-1}; ^{1}H NMR: $\delta = 1.31$, 2.18 (d, $J = 10.5$ Hz, 3H), 2.20 (s, 3H), 2.18–2.55 (m, 4H), 3.70 (s, 3H), 4.12–4.28 (m, 1H), 4.51 (m, 1H) ppm; ^{13}C NMR: $\delta = 15.6$, 16.2, 20.5, 20.6, 21.9, 23.7, 24.6, 29.4, 29.7, 51.7, 69.3, 69.7, 88.7, 89.6, 169.2, 171.7 ppm.

p-Toluenesulfonylmethylisocyanide (*TsMIC*) (0.78 g, 4.0 mmol) [20] and 1,1,3,3-tetramethylguanidine (1 cm^3, 2.2 mol eq) were stirred with 5 cm^3 *THF*/*i-Pr*OH (1/1 by volume) and had added to it a solution of methyl 4-nitro-5-acetoxyhexanoate (1.00 g, 3.6 mmol) in 5 cm^3 *THF*/*i-Pr*OH (1/1 by volume). The solution was then stirred at room temperature for 24 h. The solution was transferred to an *Erlenmeyer* flask and chilled in an ice bath. Water was slowly added, with stirring, precipitating the crude product. The precipitate was then collected by vacuum filtration, air dried, and recrystallized from CH_2Cl_2 and hexane to afford colorless crystals. Yield 0.72 g (62%); mp 140–141°C; IR (KBr): $\bar{\nu} = 3326$, 2951, 1734, 1437, 1316, 1301, 1137, 1087, 813, 707, 686 cm^{-1}; ^{1}H NMR: $\delta = 2.16$ (s, 3H), 2.40 (s, 3H), 2.49 (t, $J = 7$ Hz, 2H), 2.68 (t, $J = 7$ Hz, 2H), 3.65 (s, 3H), 6.71 (d, $J = 3$ Hz, 1H), 7.28 (d, $J = 8$ Hz, 2H), 7.75 (d, $J = 8$ Hz, 2H), 8.86 (br.s, 1H) ppm; ^{13}C NMR: $\delta = 9.3$, 20.6, 21.7, 34.6, 51.8, 120.5, 124.0, 124.2, 124.5, 126.7, 129.9, 140.0, 143.7, 173.4 ppm.

2-(p-Toluenesulfonyl)-3-methyl-4-(2-methoxycarbonylethyl)-5-bromo-1H-pyrrole
(**14a**, $C_{16}H_{18}BrNO_4S$)
2-(*p*-Tosyl)-3-methyl-4-(methoxycarbonylethyl)-1*H*-pyrrole (10.19 g, 31.72 mmol) and *PTT* (13.2 g, 35.13 mmol) were dissolved in CH_2Cl_2 (200 cm^3) and stirred at 0°C for 30 min. The solution was

washed with aqueous sodium bisulfite ($2 \times 200\,cm^3$) and brine ($1 \times 200\,cm^3$), and then dried ($MgSO_4$). The solvent was removed (rotovap) to yield a tan powder. Yield 12 g (96%); mp 136–137°C; IR (KBr): $\bar{\nu} = 3290$, 2951, 1737, 1550, 1437, 1301, 1148, 1093, 1062, 911, 816, 712 cm^{-1}; ^1H NMR: $\delta = 2.18$ (s, 3H), 2.41 (s, 3H), 2.41 (t, $J = 7$ Hz, 2H), 2.66 (t, $J = 7$ Hz, 2H), 3.64 (s, 3H), 7.30 (d, $J = 8$ Hz, 2H), 7.77 (d, $J = 8$ Hz, 2H), 8.97 (br.s, 1H) ppm; ^{13}C NMR: $\delta = 9.7$, 20.3, 21.5, 33.8, 51.6, 105.3, 123.1, 124.8, 125.2, 126.6, 129.8, 139.4, 143.8, 172.9 ppm.

3-(2-Methoxycarbonylethyl)-4-methyl-5-(p-toluenesulfonyl)-3-pyrrolin-2-one (**15a**, $C_{16}H_{19}NO_5S$)
2-(*p*-Tosyl)-3-methyl-4-(2-methoxycarbonylethyl)-5-bromo-1*H*-pyrrole (**14a**) (0.29 g, 0.72 mmol) was dissolved in 6 cm^3 *TFA*, and 1.2 cm^3 H_2O were added. The solution was then stirred for two days. The solution was diluted with 50 cm^3 CH_2Cl_2 and washed with H_2O ($2 \times 50\,cm^3$), aqueous sodium bicarbonate ($2 \times 50\,cm^3$), and brine ($1 \times 50\,cm^3$). After drying ($MgSO_4$), the solvent was removed (rotovap) to afford the crude product. Recrystallization from methanol and water yielded light grey crystals. Yield 0.11 g (46%); mp 130–131°C; IR (KBr): $\bar{\nu} = 3333$, 2952, 1699, 1436, 1318, 1132, 1080, 814, 667, 627 cm^{-1}; ^1H NMR: $\delta = 2.18$ (s, 3H), 2.41 (s, 3H), 1.88–2.61 (m, 4H), 3.61 (s, 3H), 5.04 (s, 1H), 6.14 (s, 1H), 7.29 (d, $J = 8$ Hz, 2H), 7.64 (d, $J = 8$ Hz, 2H) ppm; ^{13}C NMR: $\delta = 13.3$, 19.0, 21.8, 31.4, 51.8, 79.3, 129.6, 129.8, 130.6, 136.2, 144.7, 146.2, 172.7, 172.7 ppm.

3-(2-Methoxycarbonylethyl)-4-methyl-3-pyrrolin-2-one (**9a**)
3-(2-Methoxycarbonylethyl)-4-methyl-5-(*p*-tosyl)-3-pyrrolin-2-one (**15a**) (0.39 g, 1.16 mmol) was dissolved in 35 cm^3 abs ethanol that had added to it a suspension of sodium borohydride (0.055 g, 1.45 mmol). The mixture was stirred at room temperature for 30 min, then diluted with 50 cm^3 CH_2Cl_2, and washed with dilute aqueous acetic acid ($1 \times 50\,cm^3$), H_2O ($2 \times 50\,cm^3$), and brine ($1 \times 50\,cm^3$). After drying ($MgSO_4$), the solvent was removed (rotovap) to afford a colorless powder. Yield 0.17 g (80%); mp 74–75°C (Ref. [26] 71–76°C); IR (KBr): $\bar{\nu} = 3233$, 2954, 1735, 1675, 1570, 1437, 1174, 1066, 969 cm^{-1}; ^1H NMR: $\delta = 2.01$ (s, 3H), 2.57 (s, 3H), 3.64 (s, 3H), 3.81 (s, 2H), 7.13 (s, 1H) ppm; ^{13}C NMR: $\delta = 13.1$, 19.0, 32.1, 50.3, 51.4, 130.4, 150.6, 173.2, 175.8 ppm.

2-(2-Carboxyethyl)-8-ethyl-3,7,9-trimethyl-(10H)-dipyrrin-1-one (**3**, $C_{17}H_{22}N_2O_3$)
3-(2-Methoxycarbonylethyl)-4-methyl-3-pyrrolin-2-one (**9a**) (0.16 g, 0.87 mmol) and 2-formylkryptopyrrole (**8**) (0.14 g, 0.96 mmol) were dissolved in 10 cm^3 methanol and 10 cm^3 6 *M* aqueous KOH. The solution was heated to reflux and stirred overnight. It was then diluted with H_2O (50 cm^3) and acidified with acetic acid. The desired product (**3**) was collected by vacuum filtration, affording a green/yellow powder. Yield 0.26 g (71%); mp 298–300°C; IR (KBr): $\bar{\nu} = 3356$, 2924, 1664, 1631, 1461, 1451, 1259, 1159, 964, 668 cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 0.98$ (t, $J = 7.3$ Hz, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.17 (s, 3H), 2.30 (q, $J = 7.3$ Hz, 2H), 2.40 (t, $J = 6.6$ Hz, 2H), 2.46 (t, $J = 6.6$ Hz, 2H), 5.95 (s, 1H), 9.78 (s, 1H), 10.24 (s, 1H), 12.09 (br.s, 1H) ppm; ^{13}C NMR in Table 1.

3-(2-Methoxycarbonylethyl)-4-methyl-2-p-toluenesulfonyl-1H-pyrrole (**13b**, $C_{16}H_{19}NO_4S$)
To a solution of 11.6 g (100 mmol) methyl 4-oxobutanoate and 8.3 cm^3 (115 mmol) nitroethane in 10 cm^3 anhydrous CH_3CN kept under N_2 at 0°C were added 1.5 cm^3 (10 mmol) *DBU* during 30 min, and the mixture was stirred at room temperature for 20 h. The mixture was diluted with 200 cm^3 ethyl acetate:diethyl ether (1:1) and washed with 2% aqueous HCl (50 cm^3) and H_2O ($3 \times 50\,cm^3$). After drying (Na_2SO_4) and filtration, the solvents were evaporated under vacuum, the residue (**11b**) was kept under 0.5 mm Hg vacuum at 40°C for 3 h, and then used immediately in the following step.

The crude nitroalcohol **11b** (17.6 g) was acetylated with 18.8 cm^3 (200 mmol) acetic anhydride in 60 cm^3 anhydrous CH_2Cl_2 in the presence of 25 mg *DMAP* for 18 h at room temperature. Excess acetic anhydride was destroyed with methanol (20 cm^3) added during 20 min, and after 1 h the mixture was diluted with 100 cm^3 CH_2Cl_2. The solution was poured carefully into 200 cm^3 5% aqueous $NaHCO_3$, then washed with H_2O ($3 \times 100\,cm^3$), dried (anhydrous $MgSO_4$), filtered, and the solvent was evaporated under vacuum. The product (**12b**) was used without further purification in the next step.

To a solution of 16.6 g (85 mmol) *TsMIC* [20] and 21.1 cm^3 (170 mmol) 1,1,3,3-tetramethylguanidine in 70 cm^3 anhydrous *THF*/*i-Pr*OH (1/1 by volume) kept under N_2 at 0°C was added a solution of the crude nitroacetate **12b** in 15 cm^3 *THF*/*i-Pr*OH (1/1 by volume) during 45 min. Then the mixture was stirred for 20 h at room temperature. After dilution with 300 cm^3 CH_2Cl_2, it was washed with 2% aqueous HCl ($2 \times 80\ cm^3$), and H_2O ($3 \times 100\ cm^3$). The solution was dried over anhydrous $MgSO_4$, filtered, and the solvents were evaporated under vacuum. The residue was purified by radial chromatography and recrystallized from ethyl acetate-hexane to afford **13b**. Yield 12.3 g (45%); mp 108–109°C; ^{1}H NMR: $\delta = 1.98$ (d, $^4J = 0.8$ Hz, 3H), 2.40 (s, 3H), 2.42 (m, 2H), 2.87 (m, 2H), 3.68 (s, 3H), 6.70 (dq, $^3J = 1.2$ Hz, $^4J = 0.8$ Hz, 1H), 7.28 (d, $^3J = 8.3$ Hz, 2H), 7.76 (d, $^3J = 8.3$ Hz, 2H), 8.87 (br.s, 1H) ppm; ^{13}C NMR: $\delta = 9.8$, 19.7, 21.5, 34.6, 51.6, 120.8, 121.1, 123.7, 126.6, 127.1, 129.8, 139.8, 143.7, 173.3 ppm.

5-Bromo-3-(2-methoxycarbonylethyl)-4-methyl-2-p-toluenesulfonyl-1H-pyrrole (**14b**, $C_{16}H_{18}BrNO_4S$)

To a solution of 3.21 g (10.0 mmol) pyrrole **13b** in 80 cm^3 CH_2Cl_2 cooled in an ice bath was added a solution of 7.50 g (20.0 mmol) phenyl-*N*,*N*,*N*-trimethylammonium tribromide in 80 cm^3 CH_2Cl_2 during 15 min. After the addition was completed, stirring was continued for 15 min more. Then the mixture was diluted with 150 cm^3 CH_2Cl_2, washed with 3% aqueous sodium thiosulfate ($2 \times 100\ cm^3$) and H_2O ($3 \times 100\ cm^3$), dried (anhydrous $MgSO_4$), filtered, and the solvent was evaporated under vacuum. The residue was purified by radial chromatography and the combined fractions containing product were recrystallized from ethyl acetate-hexane. Yield 2.69 g (67%); mp 193–194°C (dec); ^{1}H NMR: $\delta = 1.92$ (s, 3H), 2.40 (s, 3H), 2.41 (m, 2H), 2.89 (m, 2H), 3.69 (s, 3H), 7.29 (d, $^3J = 8.3$ Hz, 2H), 7.78 (d, $^3J = 8.3$ Hz, 2H), 9.11 (br.s, 1H) ppm; ^{13}C NMR: $\delta = 9.7$, 20.3, 21.6, 34.4, 51.7, 105.1, 120.8, 124.9, 126.7, 128.0, 130.0 139.3, 144.1, 173.0 ppm.

4-(2-Methoxycarbonylethyl)-3-methyl-5-p-toluenesulfonyl-3-pyrrolin-2-one (**15b**, $C_{16}H_{19}NO_5S$)

To a solution of 2.0 g (5.0 mmol) bromopyrrole **14b** in 20 cm^3 *TFA* under N_2 was added a solution of 1.1 cm^3 (15.0 mmol) anhydrous dimethylsulfoxide in 12 cm^3 *TFA* during 10 min. After stirring for 2.5 h, iodine (127 mg, 0.5 mmol) followed by 1.31 g (20 mol) of zinc were added and stirring was continued for 2 h. The solids were removed by filtration, the filtrate was diluted with 150 cm^3 of H_2O, and the product was extracted with $CHCl_3$ ($4 \times 50\ cm^3$). The combined extracts were washed with 2% aqueous $NaHCO_3$ ($2 \times 50\ cm^3$), and then with H_2O ($3 \times 50\ cm^3$). After drying over anhydrous Na_2SO_4, filtration, and evaporation of solvent under vacuum, the residue was purified by radial chromatography and recrystallization from ethyl acetate-hexane. Yield 1.52 g (90%); mp 131–132°C; ^{1}H NMR: $\delta = 1.61$ (br.s, 3H), 2.43 (s, 3H), 2.61 (m, 1H), 2.71 (m, 1H), 2.87 (m, 1H), 2.98 (m, 1H), 3.68 (s, 3H), 5.21 (br.s, 1H), 6.42 (br.s, 1H), 7.31 (d, $^3J = 8.3$ Hz, 2H), 7.65 (d, $^3J = 8.3$ Hz, 2H) ppm; ^{13}C NMR: $\delta = 8.5$, 21.6, 22.2, 32.2, 51.8, 77.1, 129.3, 129.6, 130.6, 135.1, 145.0, 145.8, 172.3, 173.3 ppm.

4-(2-Methoxycarbonylethyl)-3-methyl-3-pyrrolin-2-one (**9b**)

To a solution of 1.01 g (3.0 mmol) tosylpyrrolinone **15b** in 50 cm^3 abs ethanol kept at 10°C was added 339 mg (9.0 mmol) sodium borohydride during 10 min. After 10 min stirring, the excess reagent was quenched with 2 cm^3 10% aqueous HCl and then the ethanol solvent was evaporated almost completely under vacuum. The residue was partitioned between 100 cm^3 H_2O and 100cm^3 $CHCl_3$. The organic layer was washed with H_2O ($2 \times 20\ cm^3$), dried over anhydrous $MgSO_4$, filtered, and the solvent was evaporated under vacuum. The residue was purified by radial chromatography and recrystallized from hexane-ethyl acetate. Yield 385 mg (70%); mp 85–86°C (Ref. [27] mp 82–83°C); ^{1}H NMR: $\delta = 1.79$ (m, 3H), 2.48 (t, $J = 7.5$ Hz, 2H), 2.68 (t, $J = 7.5$ Hz, 2H), 3.65 (s, 3H), 3.81 (br.s, 2H), 7.56 (br.s, 1H) ppm; ^{13}C NMR: $\delta = 8.3$, 23.0, 32.4, 48.3, 51.8, 129.6, 150.7, 172.6, 176.1 ppm.

8-Ethyl-3-(2-methoxycarbonylethyl)-2,7,9-trimethyl-(10H)-dipyrrin-1-one (**10b**, $C_{18}H_{24}N_2O_3$)

Pyrrolinone **9b** (366 mg, 2.0 mmol) was dissolved in 5 cm^3 anhydrous CH_3CN and transferred into a thick wall tube. Aldehyde **8** (907 mg, 6.0 mmol) and 2.3 cm^3 (20.0 mmol) *N*-methylmorpholine were

added under Ar, the tube was sealed and heated at 95–100°C for 84 h. After cooling, the mixture was diluted with 200 cm^3 $CHCl_3$, washed with 1% aqueous HCl (100 cm^3) and H_2O (3×100 cm^3). After drying over anhydrous Na_2SO_4, filtration, and evaporation of the solvent, the residue was purified by radial chromatography and recrystallization from CH_2Cl_2–CH_3OH. Yield 228 mg (36%); mp 224–226°C; ^{1}H NMR: $\delta = 1.07$ (t, $J = 7.6$ Hz, 3H), 1.97 (s, 3H), 2.15 (s, 3H), 2.40 (s, 3H), 2.41 (q, $J = 7.6$ Hz, 2H), 2.55 (t, $J = 7.8$ Hz, 2H), 2.89 (t, $J = 7.8$ Hz, 2H), 3.69 (s, 3H), 6.16 (s, 1H), 10.31 (br.s, 1H), 11.36 (br.s, 1H) ppm; ^{13}C NMR: $\delta = 8.7$, 9.5, 11.5, 15.4, 17.4, 20.0, 34.5, 51.8, 101.8, 122.1, 123.1, 123.5, 125.4, 126.4, 131.6, 144.2, 172.9, 173.5 ppm.

3-(2-Carboxyethyl)-8-ethyl-2,7,9-trimethyl-(10H)-dipyrrin-1-one (**4**, $C_{17}H_{22}N_2O_3$)
A mixture of 158 mg (0.5 mmol) **10b**, 12 cm^3 ethanol, and 4 cm^3 10% aqueous NaOH was heated at reflux for 3 h. After cooling, the ethanol solvent was removed under vacuum. The residue was diluted with 5 cm^3 H_2O and slowly acidified at 0°C with 10% aqueous HCl. After stirring for 30 min, the precipitated product was collected by filtration, washed with H_2O (3×5 cm^3), and dried at 80°C under vacuum for 24 h (P_2O_5) to give **4**. Yield 123 mg (81%); mp 271–273°C (dec); ^{1}H NMR ($(CD_3)_2SO$): $\delta = 0.97$ (t, $J = 7.5$ Hz, 3H), 1.77 (s, 3H), 2.01 (s, 3H), 2.16 (s, 3H), 2.29 (q, $J = 7.5$ Hz, 2H), 2.37 (t, $J = 7.6$ Hz, 2H), 2.73 (t, $J = 7.6$ Hz, 2H), 5.97 (s, 1H), 9.79 (s, 1H), 10.25 (s, 1H), 12.46 (v.br.s, 1H) ppm; ^{13}C NMR in Table 1.

Metabolism Studies
The methods used have been described in detail elsewhere [4, 28]. For β-glucuronidase hydrolysis of glucuronides in bile 40 mm^3 glucuronidase solution (prepared by adding 1 cm^3 water to 1000 units Sigma bacterial *E. coli* Type II or VIIa glucuronidase) was mixed with 20 mm^3 bile, incubated at 37°C in the dark for 60 min, diluted with 140 mm^3 HPLC eluent and microfuged to remove precipitated protein. The supernate (20 mm^3) was chromatographed at once. For NaOH hydrolysis, 20 mm^3 samples of bile were mixed with 10 mm^3 1.0 *M* NaOH, followed after 3 min by 10 mm^3 1.0 *M* HCl and 80 mm^3 of HPLC eluent; the final mixture was microfuged and of the supernate an HPLC was carried out without delay. The isocratic HPLC eluent was 0.1 *M* di-*n*-octylammonium acetate (prepared from Aldrich di-*n*-octylamine and glacial acetic acid) in *Me*OH containing from 2–8% water and the column an Ultrasphere-IP 5 μm C-18 ODS column (25 cm, ID 0.46 cm) fitted with a similar precolumn (4.5 cm, ID 0.46 cm). The column flow rate was 0.75–1.0 cm^3/min and the detector was set at the absorption maximum of the injected regioisomer in the HPLC solvent.

Acknowledgements

This work was supported by the U.S. National Institutes of Health (HD17779 and DK26307). *BAC* was an *NSF REU* Undergraduate Research Fellow. *JOB* was an R.C. Fuson Graduate Research Fellow. *SEB* is on leave from the Institute of Organic Chemistry, Sofia, Bulgaria.

References

[1] a) Falk H (1989) The Chemistry of Linear Oligopyrroles and Bile Pigments. Springer-Verlag, Wien; b) Lightner DA (1982) Structure, Photochemistry and Organic Chemistry of Bilirubin. In: Heirwegh KPM, Brown SB (eds) Bilirubin, Vol 1. CRC Press, Boca Raton, FL, pp 1–58
[2] Chowdhury JR, Wolkoff AW, Chowdhury NR, Arias IM (2001) Hereditary Jaundice and Disorders of Bilirubin Metabolism. In: Sly AL, Valle D (eds) The Metabolic and Molecular Bases of Inherited Disease, Vol II. McGraw-Hill Inc, NY, chap 125, pp 3063–3101
[3] Bobál P, Lightner DA (2000) Synthesis, p 1835
[4] Woydziak ZR, Boiadjiev SE, Norona WS, McDonagh AF, Lightner DA (2005) J Org Chem **70**: 8417

[5] a) McDonagh AF, Lightner DA, Agati G (1998) Monatsh Chem **129**: 649; b) Landen GL, Park Y-T, Lightner DA (1983) Tetrahedron (Symposium-in-Print on Linear Tetrapyrroles) **39**: 1893; c) Lamola AA, Braslavsky SE, Schaffner K, Lightner DA (1983) Photochem Photobiol **37**: 263
[6] McDonagh AF, Lightner DA, Boiadjiev SE, Brower JO, Norona WS (2002) Bioorg Med Chem Lett **12**: 2483
[7] Blanckaert N, Kabra PM, Farina FA, Stafford BE, Marton LJ, Schmid R (1980) J Lab Clin Med **96**: 198
[8] a) Piloty O, Thannhauser SJ (1912) Liebig's Ann Chem **390**: 191; (b) Piloty O, Thannhauser SJ (1913) Chem Ber **45**: 2393
[9] a) Fischer H, Röse H (1912) Chem Ber **45**: 1579; b) Fischer H, Röse H (1912) Chem Ber **45**: 3274; c) Fischer H, Röse H (1913) Hoppe Seyler's Z Physiol Chem **82**: 391; d) Fischer H, Röse H (1913) Chem Ber **46**: 439
[10] a) Fischer H, Bartholomäus E, Röse H (1913) Hoppe Seyler's Z Physiol Chem **84**: 262; b) Fischer H, Röse H (1914) Hoppe Seyler's Z Physiol Chem **89**: 255
[11] Fischer H, Hess R (1931) Hoppe Seyler's Z Physiol Chem **194**: 193
[12] Fischer H, Adler E (1931) Hoppe Seyler's Z Physiol Chem **197**: 237
[13] Fischer H, Adler E (1931) Hoppe Seyler's Z Physiol Chem **200**: 209
[14] Fischer H, Hartmann P (1934) Hoppe Seyler's Z Physiol Chem **226**: 116
[15] Grunewald JO, Cullen R, Bredfeldt J, Strope ER (1975) Org Prep Proc Intl **7**: 103
[16] Trull FR, Franklin RW, Lightner DA (1987) J Heterocyclic Chem **24**: 1573
[17] Jayasundera KP, Kinoshita H, Inomata K (2000) Bull Chem Soc Jpn **73**: 497
[18] Barton DHR, Kervagoret J, Zard SZ (1990) Tetrahedron **46**: 7587
[19] Kinoshita H, Hayeshi Y, Murata Y, Inomata K (1993) Chem Lett, p 1437
[20] Chen Q, Huggins MT, Lightner DA, Norona W, McDonagh AF (1999) J Am Chem Soc **121**: 9253
[21] Clarke DJ, Keen JN, Burchell B (1992) FEBS Lett **299**: 183
[22] Blanckaert N, Heirwegh PM, Zaman Z (1977) Biochem J **164**: 229–236
[23] Shrout DP, Lightner DA (1990) Synthesis, p 1062
[24] Lightner DA, Quistad GB (1973) J Heterocyclic Chem **10**: 273
[25] Bobál P, Lightner DA (2001) J Heterocyclic Chem **38**(2): 527
[26] Battersby AR, Dutton CJ, Fookes CJ (1988) J Chem Soc Perkin Trans 1: 1569
[27] Plieninger H, Kurze J (1964) Liebig's Ann Chem **680**: 60
[28] Ghosh B, Lightner DA, McDonagh AF (2004) Monatsh Chem **135**: 1189