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HOMORUBIN. A CENTRALLY HOMOLOGATED BILIRUBIN

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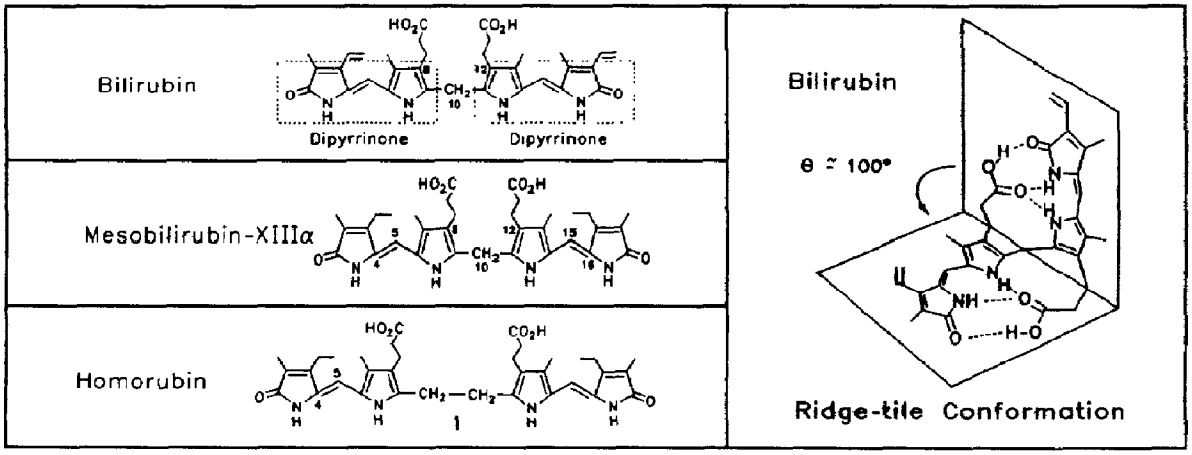
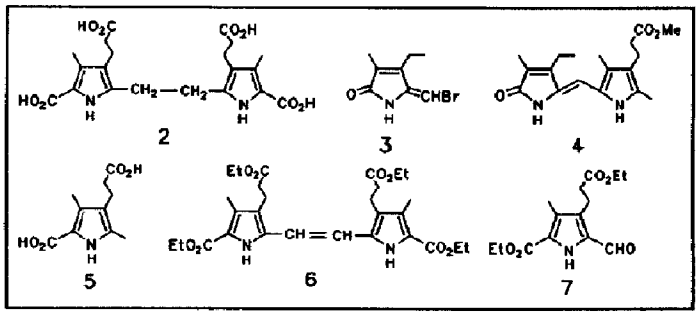
Abstract. The synthesis and spectroscopic properties are described for homorubin (**1**), a novel bilirubin analog with a central $-CH_2-CH_2-$ unit replacing the $-CH_2-$ bridge at C-10.

Bilirubin, the yellow-orange pigment of jaundice, is formed copiously in healthy humans and excreted as its glucuronide esters into bile.[1] Its structure consists of two dipyrrinones conjoined at a central $-CH_2-$ group, and its properties and metabolism are determined by the presence of two propionic acid groups, one in each dipyrrinone, located at C-8 and C-12. Through bond rotations about the central $-CH_2-$, the dipyrrinones and their attached propionic acid are brought into favorable juxtaposition for intramolecular hydrogen bonding, resulting in a highly stabilized conformation shaped like a ridge-tile.[2,3] Analogs such as mesobilirubin-XIIIα with propionic acids at C-8 and C-12 have very analogous properties and metabolism, as they also adopt the ridge-tile shape.[3,4] The shape is controlled by the presence of but a single central $-CH_2-$ group (C-10), about which nearly planar dipyrrone paddles may rotate until a stable conformation is adopted in which non-bonded steric interactions are minimized and intramolecular hydrogen bonding occurs.[3,5] If nature had made bilirubin with two central $-CH_2-$ units rather than one, another rotational degree of freedom would have been introduced that would enable the pigment to adopt new and different conformations. In the following we report the synthesis and properties of homorubin (**1**) an analog of mesobilirubin-XIIIα with a central $-CH_2-CH_2-$.



Homorubin (**1**)[6] was prepared following coupling of dipyrrole **2** with bromomethylenepyrrolinone **3**[7] in refluxing methanol to give homorubin dimethyl ester. The coupling step involves *in situ* α-decarboxylation followed by reaction of the α-free dipyrrole with **3**. It is entirely analogous to that used in the synthesis of methyl xanthobilirubinate (**4**) from **3** and monopyrrole **5**,[7] which resembles half of dipyrrole **2**. The yields of homorubin

dimethyl ester from **2** and **3** have so far been variable, ranging from 19 to 27% and are currently being optimized. Saponification to give **1** proceeded smoothly and in 80% yield in methanolic KOH. Synthesis of the colorless key dipyrrole intermediate (**2**) followed from the interesting conjugated analog (**6**), first by catalytic reduction (H_2/Pd(C)), 98% yield) then by saponification in methanolic KOH (94% yield). Yellow dipyrrole **6** was prepared in 38% yield by McMurry coupling (Zn, $TiCl_4$)[8] of monopyrrole aldehyde **7**, which was prepared from the diethyl ester of **5**[7] in 88% yield by conventional methods.[9]



Although homorubin (**1**) and mesobilirubin-XIIIα[7,10] are both yellow pigments with similar properties, they separate on TLC and HPLC. On silica gel TLC, **1** has a smaller R_f value (0.65) than mesobilirubin-XIIIα (0.92) with CH_2Cl_2-CH_3OH (95:5 vol/vol) irrigant. The data suggest that **1** is slightly more polar, and this is confirmed on reverse-phase HPLC,[11] where **1** has a shorter retention time (13.3 min.) as compared with mesobilirubin-XIIIα (15.6 min.) on an ODS-18 column eluting with 0.1 *M* di-*n*-octylamine acetate in methanol-5% H_2O at a flow rate of 1 mL/min. Consistent with homologation, the mass spectrometric molecular weight (602 amu) of homorubin (**1**) is found to be 14 mass units (one -CH_2-) higher than that of mesobilirubin-XIIIα (588 amu). As might be expected, their ^{13}C-NMR spectra (Fig. 1) are nearly identical, and due to symmetry, no new carbon or proton signals can be detected. The ^{13}C resonance of the central -CH_2-CH_2- unit in **1** is shifted downfield ~3 ppm relative to the C-10 -CH_2- unit in mesobilirubin-XIIIα. The C-4/C-16 carbon resonances are also deshielded by ~3 ppm, but the other carbon resonances are essentially identical, including those at C-5/C-15.

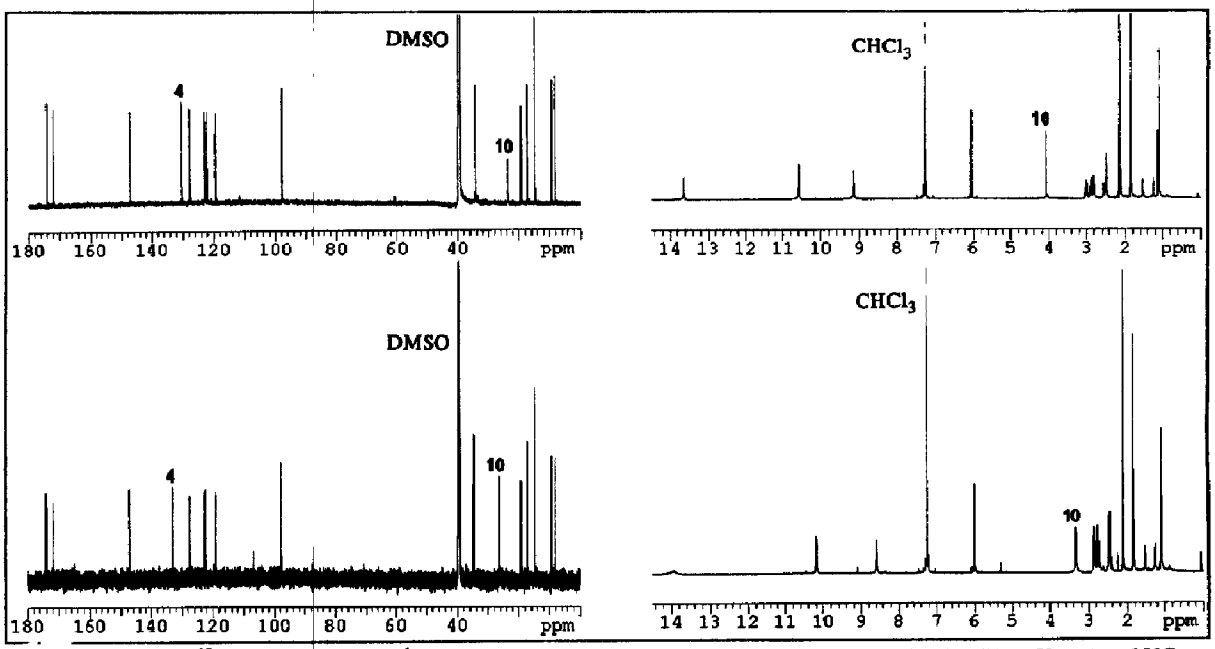


FIGURE 1. (Left) ^{13}C-NMR and (Right) ^{1}H-NMR spectra of homorubin (**1**) (Lower) and mesobilirubin-XIIIα (Upper) at 25°C. Data are reported in δ ppm downfield from $(CH_3)_4Si$ in $(CD_3)_2SO$ (^{13}C-NMR) and $CDCl_3$ (^{1}H-NMR).

The ^{1}H-NMR spectra (Fig. 1) are also very similar, with significant differences occurring only in the more shielded central -CH_2-CH_2- proton resonances at 3.34 ppm in **1** compared with the C-10 -CH_2- resonance (4.08 ppm) in mesobilirubin-XIIIα, and in the N-*H* resonance region between 8 and 11 ppm. Most interestingly, the lactam and pyrrole N-*H* chemical shifts differ by only ~0.5 ppm from the corresponding resonances in mesobilirubin-XIIIα.[10] These particular results provide an important structural and stereochemical insights because in bilirubin and mesobilirubin-XIIIα N-*H* chemical shifts have been used to detect carboxylic acid to dipyrrinone intramolecular hydrogen bonding and distinguish it from dipyrrinone to dipyrrinone intermolecular hydrogen bonding found in their dimethyl esters and in dipyrrinones.[12] With *intra*molecular hydrogen bonding (see previous page), the lactam and pyrrole N-*H* resonances in $CDCl_3$ are typically ~10.6 and ~9.2 ppm, respectively; in *inter*molecular hydrogen bonding, they are typically ~10.5 and ~10.3 ppm, respectively.[12,13] The observed N-*H* resonances of **1** at 10.2 and 8.6 ppm in $CDCl_3$ are more characteristic of *intra*molecular hydrogen bonding. Although in mesobilirubin-XIIIα intramolecular hydrogen bonding translates into the ridge-tile structure,[14] it seems intuitively unlikely that **1** could adopt a ridge-tile shape, considering that two C-C-C bond angles must be accommodated in the center of the molecule as opposed to one in mesobilirubin-XIIIα.

Like mesobilirubin-XIIIα, homorubin **1** gives yellow solutions in organic solvents, and their UV-visible spectra (Fig. 2) are nearly identical. This is surprising, as the spectrum of mesobilirubin is determined by exciton coupling between its two dipyrrinone chromophores, with the shape and position of the component exciton bands being dependent on the relative orientation of the dipyrrinone long wavelength electric dipole transition moments.[3] The UV-visible spectrum of mesobilirubin-XIIIα corresponds to the ridge-tile structure shown earlier. Although homorubin is also expected to be an exciton system, it would not be expected that the relative orientation of its dipyrrinones would be the same as in mesobilirubin. In simpler analogs: diphenylmethane for mesobilirubin-XIIIα and bibenzyl for homorubin, it is easy to imagine a much wider variety of conformers in the latter than in the former. Only a few such possibilities are diagrammed in Fig. 3, where the gabled conformation of diphenylmethane corresponds roughly to the ridge-tile in bilirubin or mesobilirubin-XIIIα, and homorubin might adopt conformations akin to the limiting *cis* and *trans* conformers shown for bibenzyl. Although the preferred conformation of **1** is as yet not completely clear and awaits the results of extensive molecular dynamics calculations currently underway, preliminary results indicate a probable preference for a conformation resembling the *cis* of Fig. 3.

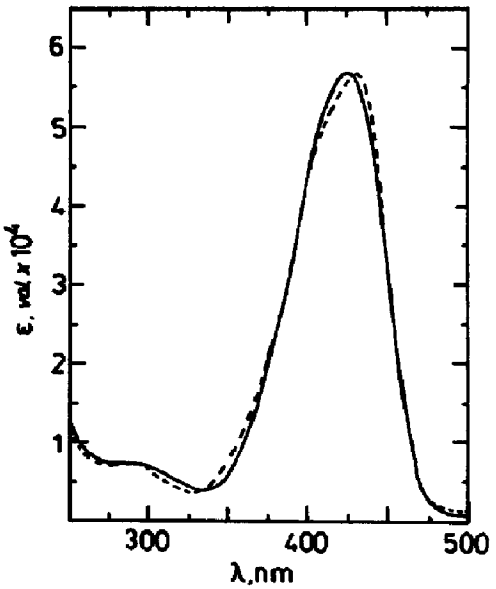


FIGURE 2. UV-vis spectra of 10^{-5} *M* homorubin (———) and mesobilirubin-XIIIα (- - -) in CH_2Cl_2 solvent at 22°C. For the former, ϵ^{max}_{424} = 57,000; for the latter, ϵ^{max}_{430} = 57,000.

Synthetic investigations in progress include oxidation of the -CH_2-CH_2- unit of **1** to -CH=CH- and =CH—CH= and the preparation of new expanded bilirubin analogs[15] with other 2-carbon and $(CH_2)_n$ extensions. Molecular modelling and metabolic studies of homorubin are currently underway.

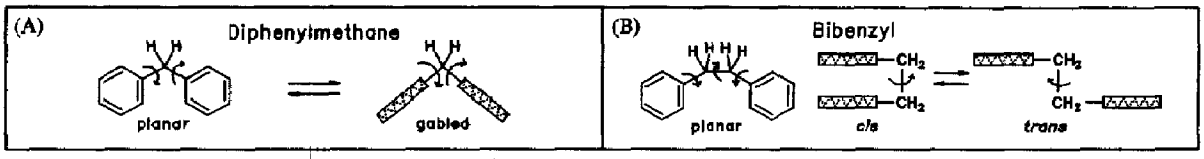


FIGURE 3. (A) Planar and gabled conformations of diphenylmethane — a rotational model for mesobilirubin-XIIIα. The gabled conformation, with phenyl rings face-to-face correspond approximately to the ridge-tile conformation of mesobilirubin-XIIIα. (B) *Cis* and *trans* rotational conformers of bibenzyl, a rotational model for homorubin (1), which is thought to favor the *cis*.

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