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CIRCULAR DICHROISM OF SOME ARYL-2-PYRIDYLMETHANES

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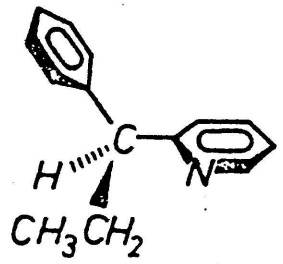
In continuation of our investigations on the circular dichroism of optically active 1,2-disubstituted-1,2-diarylethanes[1] we have recently undertaken studies with the aim to elucidate the application of CD in evaluation of the chirality of compounds with geminal aromatic carbo- or heterocyclic chromophores. The chirality is crucial from the viewpoint of biological activity of such compounds - there are many examples among this type with clearly observable biostereoselectivity[2].

Now we report briefly our preliminary results on the circular dichroism in the 270-215 nm range of some optically active aryl-2-pyridylmethanes, namely 1-9 in dilute cyclohexane (2.10^{-3}M/l) solution, where these compounds reveal relatively simple and distinctive chiroptical features (Fig. 1 and 2).

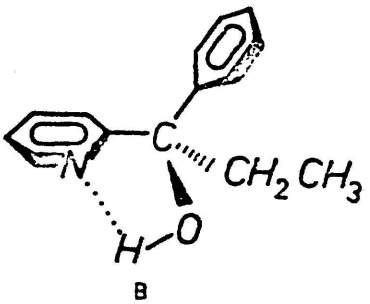
By chemical correlation with the known (-)-(αR,2S)-erythro-phenyl-2-piperidylmethanol and (+)-(1S,2'R)-erythro-1-phenyl-1-(2'-(1'-benzoyl)piperidyl)propane the absolute configurations of (+)-1[3], (-)-2[3] and resp. (-)-7[4], (+)-8[4] and (+)-9[4] were determined. The absolute configurations of (+)-4 and also (+)-8 (in agreement with the chemical correlation data) were recently determined by X-ray cryst

As it was mentioned already in most of the studied model compounds there is evidence for a relatively high conformational homogenity. One should expect therefore that the exibited Cotton effects reflect the absolute stereochemistry of both aromatic chromophores in the prefered conformation. When the torsional angle between the directions of the associated electric transition moments is negative, two split Cotton effects are to be expected: the first one of negative sign, followed by a positive one. This was observed in practice.

In the strongly prefered conformations of (+)-(S)-1, (+)-(S)-4 and (+)-(R)-8 with an intramolecular H-bond, the phenyl and the 2-pyridyl groups are situated in such a way that the angle between both interacting transition moments is positive. This leads to a positive couplet. The similar couplet is observed also in (+)-(S)-9. There are no data available about the conformational distribution in this last case. However the observed positive couplet could be considered as evidence for the prefered conformation A, shown on Fig. 5.



A



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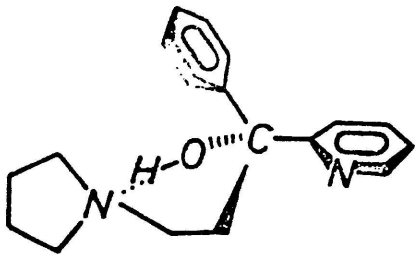

C

Figure 5

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ • BULGARIAN ACADEMY OF SCIENCES
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PREPARATION AND ABSOLUTE CONFIGURATION OF SOME 1-ARYL-1-(2-PYRIDYL)-PROPANES

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A series of optically active compounds described by the general formula ArC(X)(2-Py)—CH(Y)—CH_2Z has been prepared. The absolute configurations of five optically active compounds have been determined either by X-ray crystallographic analysis or by chemical correlation. It has been experimentally demonstrated that the Raney nickel catalyzed dehydroxylation of a benzylic chiral center with an additional geminal 2-pyridyl group also proceeds with retention of the configuration. The dependence of the optical rotation of the investigated compounds on the nature of the solvent and on N-protonation has been discussed in connection with the possibility of conformational redistribution.

INTRODUCTION

Chirality is often a crucial factor determining the biological activity of compounds with geminal aromatic groups at the chiral center. There are many examples among this type with clearly observable biostereoselectivity [1].

Our continuing interest in the application of circular dichroism (CD) in the evaluation of the chirality of compounds with geminal or vicinal aromatic carbo- and heterocyclic chromophores [2—4] is confirmed by the present report of our work dealing with the preparation and the determination of the absolute configuration of some chiral 1-aryl-1-(2-pyridyl)-propanes which represent appropriate models for subsequent chiroptical studies.

RESULTS AND DISCUSSION

The starting racemic compounds employed in our investigations: 1-(4-chlorophenyl)-1-(2-pyridyl)-3-(1-pyrrolidinyl)-propanol (**1**), 1-phenyl-1-(2-pyridyl)-3-(1-pyrrolidinyl)-propanol (**2**), 1-phenyl-1-(2-pyridyl)-propanol (**4**), and 1-phenyl-1-(2-pyridyl)-2-methyl-propanol (**7**) have been described earlier in the relevant literature [5—10]. Compounds (±)-**1** and (±)-**2** have been synthesized following the procedure described by Adamson [5] based on the reaction between 2-pyridyllithium and the appropriate Mannich ketones (Procedure A). There exist several different approaches [6—10] to the synthesis of compounds (±)-**4** and (±)-**7**. We have chosen as a more convenient

ПОЛУЧАВАНЕ И АБСОЛЮТНА КОНФИГУРАЦИЯ НА НЯКОИ 1-АРИЛ-1-(2-ПИРИДИЛ)-ПРОПАНИ

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(Р е з ю м е)

Получена е серия оптически активни съединения с обща формула ArC(X)(2-Py)—CH(Y)—CH_2Z. Чрез рентгеноструктурен анализ или чрез химична корелация е доказана абсолютната конфигурация на пет оптически активни съединения. Експериментално е доказано, че каталитичното дехидроксилиране върху Раней-никел на бензилен хирален център, който притежава допълнително геминална 2-пиридилова група, протича със запазване на конфигурацията. Изказани са съображения, че наблюдаваното изменение на оптичното въртене на изследваните съединения при смяна на разтворителя или при N-протониране е свързано със съществена промяна в конформационното разпределение.

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Preparation, Absolute Configuration and Conformation of Some α-Aryl-2-pyridylmethanols

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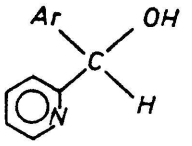
(Received December 6, 1985)

The syntheses of five optically active α-aryl-2-pyridylmethanols **1**—**5** are described. It is shown by means of chemical correlation with the known (−)-(α *R*,2*S*)-α-phenyl-2-piperidylmethanol **6** that all levo-rotatory isomers of **1**—**4** are of R configuration. It is also found via the relative integral intensities in the infrared spectra of the bands due to free and intramolecularly bonded hydroxyl groups in the compounds **1**—**4** and the free hydroxyl groups in the model compounds **7**—**10**, that the population of the conformers with an intramolecular OH···N bond in compounds **1**—**4** exceeds 80%.

In continuation of our investigations on the preparation, absolute stereochemistry and chiroptical properties of chiral 1,2-disubstituted 1,2-diarylethanes[1,2] were recently undertaken studies employing as models compounds containing two geminal aromatic carbo- or hetero-cyclic chromophores.

We presently wish to report the results from the preparation of a series of chiral α-aryl-2-pyridylmethanols **1**—**5** as well as data on their absolute configuration and preferred conformations obtained by means of chemical correlation and infrared spectroscopy. Our circular dichroism investigations require the stereochemical structure of the above mentioned compounds to be reliably known as the aim is the elucidation of some general relationships between molecular chirality and aromatic Cotton effects in compounds with geminal phenyl and pyridyl chromophores at the chiral center. It is known that such molecular moieties are present in important biologically active compounds with clearly observable biostereoselectivity.[3]



1 Ar = C_6H_5
2 Ar = 2-ClC_6H_4
3 Ar = 4-ClC_6H_4
4 Ar = 2,6-$Cl_2C_6H_3$
5 Ar = 4-$CH_3OC_6H_4$

Scheme 1.

Results and Discussion

Synthesis of the Racemic Methanols 1—5. Various approaches exist in the literature regarding the preparation of the racemic methanols **1**—**5**.[4-10] Among the most convenient ones is the procedure developed by Gilman and Spatz[10a] based on the interaction of 2-pyridyllithium with the corresponding aromatic aldehyde. In the present case the reaction was conducted at −78 °C, a temperature considerably lower than the recommended, leading to the racemic methanols **1**—**5** in yields significantly better than those reported for analogous compounds (see Experimental).

Preparation of the Optically Active Methanols. Among all presently considered methanols **1**—**5** only compound **1** was found described in an optically active form.[11-13] This compound resolved by Davies with (+)-tartaric acid[11] and considered as the pure levo-rotatory isomer[12-14] was found in the present investigations to be optically impure (see below).

The racemic methanols **2** and **4** were successfully resolved by means of (+)-tartaric acid, while the racemic **3** was resolved by means of (−)-*O*,*O*′-dibenzoyltartaric acid.

The optically active methanol **1** was obtained by dehalogenating samples of (+)-**2**, (−)-**3**, and (+)-**4** of highest purity by treatment acc. ref.[15] with hydrazine hydrate in the presence of palladium over charcoal. In all three cases the obtained samples of **1** showed at λ=589 nm in chloroform practically the same absolute value of the specific rotation i.e. +162.9°, −161.5°, and +162.8°. It can be seen that these values agree very well among themselves while on the other hand they considerably exceed the reported ones for **1** by other authors i.e. $[\alpha]_{589}=-86.2°$ [11] and $[\alpha]_{589}=-108°$.[12]

It can thus be concluded that in the present investigations the racemates of **2**, **3**, and **4** were completely resolved, while the previously reported resolution of (±)-**1** with (+)-tartaric acid[11,12] indeed afforded a product of 50—70% optical purity.

The resolution of (±)-**5** offered considerable difficulties, the best result being obtained with (+)-camphor-10-sulfonic acid. Repeated recrystallization of the diastereoisomeric sulfonates afforded insufficiently pure free base **5** which had to be subjected to additional fractional recrystallization in order to remove the less soluble (±)-**5** finally leading to the desired optically pure **5**.

Absolute Configuration. The possibilities for the application of chemical correlation are rather restricted since only a few chiral compounds with geminal aryl and pyridyl groups with reliable absolute configuration have hitherto been reported.[3a,16]

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CONFORMATIONAL ANALYSIS OF SOME SUBSTITUTED ARYL-2-PYRIDYLMETHANES

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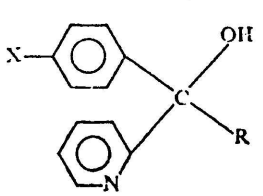
Dedicated to Academician B. Kurtev on the occasion of his 70th birthday

The conformations of two series of arylpyridylmethane derivatives have been studied by IR-spectroscopy and molecular mechanics calculations. The increase of the bulk of the alkyl substituent only slightly changes the conformational preferences. Differences, however, exist between the preferred conformations among the two series.

In continuation of our investigations on the preparation, absolute stereochemistry and chiroptical properties of chiral 1,2-disubstituted-1,2-diaryl ethanes [1, 2], studies, which employ as models compounds containing two geminal aromatic carbo- and hetero-cyclic chromophores, have been recently undertaken. The preparation of a series of chiral chloro-substituted at the phenyl ring α-aryl-2-pyridylmethanols as well as data on their absolute configuration and preferred conformations, obtained by means f chemical correlation and infrared spectroscopy, have been reported in a previous paper [3].

The purpose of this study was to determine the preferred conformations in two series of substituted aryl-2-pyridylmethanes which contain alkyl substituents of increasing size described by the general formulae.



1 X=H R=H
2 X=H R=CH_3
3 X=H R=C_2H_5
4 X=H R=$CH(CH_3)_2$
5 X=H R=$C(CH_3)_3$
6 X=H R=cyclo-C_6H_{11}

7 X=Cl R=CH_2CH_2N 

al helical conformer is strongly enhanced with increasing the bulk of the alkyl substituent (compound 13).

The present results support the expected differences in the conformational preference among the two series. In the case of the hydroxyl containing compounds (1—7) the preferred conformer has the alkyl group oriented towards the C(4)—C(5) bond of the pyridyl ring, while in the case of compounds 8—11 the alkyl substituent points to the less hindered site of the pyridyl ring, namely to the C(4)—N bond.

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КОНФОРМАЦИОНЕН АНАЛИЗ НА НЯКОИ ЗАМЕСТЕНИ АРИЛ-2-ПИРИДИЛМЕТАНИ

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Постъпила на 21. 9. 1987 г.

Посвещава се на акад. Б. Куртев по случай неговата 70-та годишнина

(Резюме)

Изучени са конформациите на две серии арилпиридилметанови производни посредством инфрачервена спектроскопия и метода на молекулната механика. Нарастването на обема на алкиловия заместител предизвиква слаба промяна в конформационната предпочетеност. Установени са обаче различия между предпочетените конформации на двете серии.

Дисертационният труд съдържа 209 стр., 57 фиг. и 11 таблици. Цитирани са 213 литературни източници.

Защитата на дисертационния труд ще се състои на 13.06.1988 г. от 16 ч. в аудиторията на ЕЦХ при БАН, кв."Гео Милев", ул. "Акад. Г.Бончев", бл. 11, на разширено заседание на СНС по органична химия при ВАК. Материалите по защитата са на разположение на интересуващите се и се намират в канцеларията на Института по органична химия с ЦФ - БАН, блок 9, стая 209, кв."Гео Милев", София 1113.

СПЕЦИАЛИЗИРАН НАУЧЕН СЪВЕТ ПО ОРГАНИЧНА ХИМИЯ ПРИ ВАК

Стефан Емилов Бояджиев

ПОЛУЧАВАНЕ, СТЕРЕОХИМИЯ И КРЪГОВ ДИХРОИЗЪМ НА ХИРАЛНИ АРИЛ-ПИРИДИЛМЕТАНИ

А В Т О Р Е Ф Е Р А Т

на

Д И С Е Р Т А Ц И Я

за присъждане на научна степен

"Кандидат на химическите науки"

Научен ръководител

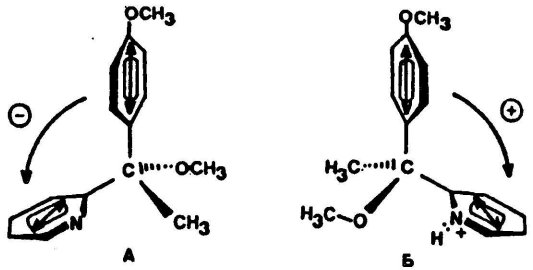
ст.н.с. кхн Николина Д. Берова

Рецензенти

проф. д-р хн Благой Благоев

ст.н.с. кхн Екатерина Симова

София, 1988 г.



Фиг. 53

Обръщането знака на куплета (и дори увеличаване на амплитудата му) при протониране в ацетонитрил и метанол очевидно отразява настъпила конформационна промяна, която води до положителна екситонна хиралност на хромофорната система (Фиг. 53, Б). Тази промяна може да се обясни ако се приеме предпочетеното приблизително син-перипланарно разположение на солватирания N^+-H и OCH_3 в сравнение с N^+-H и CH_3.

Твърде сходни до обсъдения по-подробно пример на (+)-(s)-22 са и КД спектри в различни разтворители и на останалите арил-2-пиридилметаноли и 1-арил-1-(2-пиридил)етаноли. Заместители в бензолното ядро в положение пара и мета не променят качествено картината при смяна на разтворителя или при протониране. Също така, нарастването на обема на алкиловия заместител при центъра на хиралност не води до съществена промяна на КД спектри при смяна на разтворителя.

Разгледаните по-горе няколко избрани примера показват, че полярността на разтворителя и (или) неговата способност да действува като водороден донор и (или) акцептор влияят извънредно много върху предпочетената конформация в разтвор на конформационно лабилните молекули от арил-2-пиридилметанов тип. Поради тази причина, определянето на абсолютната конфигурация въз основа на КД при тези съединения е най-надеждно в разтвор на неполярен разтворител, например циклохексан.

7. ИЗВОДИ

1) Синтезирани са серия рацемични съединения от арилпиридилметанов тип, от които 20 неописани в литературата.

2) Постигнато е успешно оптично разделяне през диастереоизомерни соли на 29 рацемични съединения от споменатия тип, в 26 от случаите - за първи път.

3) Доказано е опитно, че дехидроксилирането на 1-арил-1-(2-пиридил)алканоли в присъствие на Раней-никелов катализатор е стереоспецифична реакция, която протича със запазване на конфигурацията.

4) Установена е абсолютната конфигурация на 31 съединения от арил-2-пиридилметанов тип. В 17 случая това е постигнато посредством химична корелация със стандартни съединения, в 2 от случаите - чрез рентгеноструктурен анализ. Конфигурационният извод за останалите съединения се основава на кръговодихроичните спектри на in situ получени оптично активни молибденови комплекси.

5) Проведени са системни изследвания върху конформационното разпределение на серия 1-арил-1-(2-пиридил)алканоли, 1-арил-1-(2-пиридил)алкани и техни аналози посредством инфрачервена спектроскопия и молекулномеханични изчисления. И при двата типа съединения е установена висока конформационна предпочетеност, която при алканолите се изразява в преимуществено застъпване (над 80%) на конформацията с вътремолекулна водородна връзка $N_{Py}\cdots HO$, докато при алканите енергетично най-изгодната конформация се характеризира със син-перипланарно разположение на алкиловия заместител спрямо пиридиновия азотен атом.

6) Проведени са системни изследвания с метода на кръ-

говия дихроизъм на над 40 оптично активни арил-2-(3- или 4-)-пиридилметанови производни в различни разтворители. Установено е, че съединенията от арил-2-пиридилметанов тип и то главно в неполярен разтворител показват в областта 200-270 нм изненадващо прости и силно интензивни, бисигнатни ароматни Котонови ефекти. За разлика от тях, съответните аналози, съдържащи 3- или 4-пиридилов заместител, проявяват слабо интензивни и сложни по характер ефекти, сходни с тези на съединения с изолиран пиридилов или диарилметанов хромофор. Наблюдаваните специфични хироптични свойства на съединенията от арил-2-пиридилметанов тип са обяснени с две предпоставки: наличие на висока конформационна хомогенност в разтвор и възникване на екситонен тип взаимодействие между електричните моменти на най-дълговълновия $\pi \rightarrow \pi^*$ преход в пиридиновия хромофор ($^1A_1 \longrightarrow {}^1B_2$) и по-късовълновия $\pi \rightarrow \pi^*$ преход във фениловия хромофор ($^1B_{1u}$).

7) Установената зависимост между знака на кръговоди - хроичния екситонен куплет в разреден циклохексанов разтвор и абсолютната конформация на арил-2-пиридилметановата хромофорна система разкрива нови възможности за определяне по неемпиричен път абсолютната конфигурация на арил-2-пиридилметанови съединения, включително и на представители с ценно биологично действие, ако се познава тяхната конформационна предпочетеност в разтвор.

8) При проведените кръговодихроични изследвания е установено силно влияние на разтворителя върху интензивността и знака на наблюдаваните Котонови ефекти, което се свързва с промени в конформационното разпределение в резултат на солватационни ефекти.

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С П Р А В К А

относно приносите на дисертационния труд
на н.с. Стефан Емилов Бояджиев, озаглавен
"Получаване, стереохимия и кръгов дихроизъм на хирални арил-пиридилметани",
представен за присъждане на научната
степен "Кандидат на химическите науки".

Дисертационният труд има научно-фундаментален характер и представлява стереохимично и спектрално изследване с метода на кръговия дихроизъм на група оптично активни съединения от арил-пиридилметанов тип, с оглед да бъде изучена зависимостта между пространствен строеж и ароматни Котонови ефекти.

За тази цел са синтезирани серия модели рацемични арил-пиридилметанови производни, които притежават определени структурни особености (различно заместено бензолно ядро, изомерен пиридилов остатък и др.).

Постигнато е успешно оптично разделяне през диастереоизомерни соли на 29 рацемични съединения, в 26 от случаите - за първи път. Чрез стереоспецифично превръщане на част от тези съединения, кръгът на изследваните оптично активни съединения е разширен допълнително.

Доказано е опитно, че дехидроксилирането на 1-арил-1-(2-пиридил)алканоли в присъствие на Раней-никелов катализатор е стереоспецифична реакция, която протича със запазване на конфигурацията.

Установена е абсолютната конфигурация на 31 съединения от арил-2-пиридилметанов тип. Абсолютната конфигурация на 17 съединения от този тип е корелирана по химичен път със стандартни съединения, а на 2 - е доказана чрез рентгеноструктурен анализ. Изводът за конфигурацията на останалите съединения се основава на кръговодихроичните спектри на in situ получени оптично активни молибденови комплекси.

Въз основа на инфрачервена спектроскопия и молекулномеханични изчисления са направени заключения за конформационната предпочетеност на двата основни типа изследвани съединения: 1-арил-1-(2-пиридил)алканоли и 1-арил-1-(2-пиридил)алкани.

Проведени са системни изследвания с метода на кръговия дихроизъм на над 40 оптично активни арил-2-(3- или 4-)пиридилметанови производни в различни разтворители. Установено е, че съединенията от арил-2-пиридилметанов тип, и то главно в неполярен разтворител, показват в областта 200-270 нм изненадващо прости и силно интензивни, бисигнатни ароматни Котонови ефекти. За разлика от тях, съответните аналози, съдържащи 3- или 4-пиридилов заместител, проявяват слабо интензивни и сложни по характер ефекти, сходни с тези на съединения с изолиран пиридилов или диарилметанов хромофор. Наблюдаваните специфични хироптични свойства на съединенията от арил-2-пиридилметанов тип са обяснени с две предпоставки: наличие на висока конформационна хомогенност в разтвор и възникване на екситонен тип взаимодействие между електричните моменти на най-дълговълновия $\pi \longrightarrow \pi^*$ преход в пиридиновия хромофор ($^1A_1 \longrightarrow {}^1B_2$) и по-късовълновия $\pi \longrightarrow \pi^*$ преход във фениловия хромофор ($^1A_1 \longrightarrow {}^1B_{1u}$).

При проведените кръговодихроични изследвания е установено силно влияние на разтворителя върху интензивността и знака на наблюдаваните Котонови ефекти, което се свързва с промени в конформационното разпределение в резултат на солватационни ефекти.

Установената зависимост между знака на кръговодихроичния екситонен куплет в разреден циклохексанов разтвор и абсолютната конформация на арил-2-пиридилметановата хромофорна система разкрива нови възможности за определяне по неемпиричен път абсолютната конфигурация на арил-2-пиридилметанови съединения, включително и на нови представители от посочения тип притежаващи ценно биологично действие.

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Conformational analysis and circular dichroism of bilirubin, the yellow pigment of jaundice

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ABSTRACT

Conformational analysis of (4Z,15Z)-bilirubin-IXα by molecular mechanics computations reveals a global energy minimum folded conformation. Powerful added stabilization is achieved through intramolecular hydrogen bonding. Theoretical treatment of bilirubin as a molecular exciton predicts an intense bisignate circular dichroism spectrum for the folded conformation: $|\Delta\epsilon| \simeq 270$ L • mole^{-1} • cm^{-1} for the ~450 nm electronic transition(s). Synthesis of bilirubin analogs with propionic acid groups methylated at the α or β position introduces an allosteric effect that allows for an optical resolution of the pigments, with enantiomers exhibiting the theoretically predicted circular dichroism.

1. INTRODUCTION

(4Z,15Z)-Bilirubin-IXα (bilirubin), the hydrophobic and cytotoxic pigment of jaundice is a yellow-orange unsymmetrically substituted tetrapyrrole dicarboxylic acid produced in copious quantities by catabolism of heme (Fig. 1), principally from the hemoglobin of red blood cells.[1,2] Normal human metabolism, through the daily breakdown of about 10^{11} red blood cells, generates some 300 mg/day/individual of bilirubin, which is intrinsically unexcretable. Excretion into bile is accomplished following hepatic conjugation of bilirubin with glucuronic acid. What limits the facile excretability of bilirubin is poor solubility in water,[3] high lipid/water partition coefficient[1] and a proclivity to form complexes with serum albumin and other proteins[1-4] — three interrelated properties that dominate the transport and metabolism of bilirubin in vivo.[2,5] However, the interesting biologic and unusual solubility properties of the pigment do not correlate well with the porphyrin-like structure of Fig. 1 or with the more conventional linear representation (Fig. 2). If bilirubin adopted such conformations, which are sterically disfavored, as seen from an examination of CPK space-filled molecular models, it would be predictably polar, not lipophilic and probably excretable across the liver into bile.

The unusual properties of bilirubin, shared by certain analogs with methyl and vinyl groups differently located at C-2, C-3, C-17 and C-18 and by analogs with methyl and ethyl groups, as in mesobilirubin-XIIIα, can be ascribed, at least in part, to the location of propionic acid groups at C-8 and C-12. Transposing them with the adjacent methyl groups at C-7 and C-13 yields a pigment, as in mesobilirubin-IVα,[6] that is more polar and excretable without resort to glucuronidation or other structural modification.[7] Had Nature chosen to catabolize heme by oxidation at the β, γ or δ positions instead of α (Fig. 1), she would have produced bilirubins (IXβ, γ and δ) which do not have propionic acid groups at C-8 and C-12 and which are known to be more polar (and excretable) than the natural IXα isomer.[8]

However, propionic acid group location is only one necessary requirement. Other requirements include configuration and conformation of the tetrapyrrole skeleton — properties which are shared by all of the pigments mentioned above. In the following, we describe recent studies on conformational analysis of bilirubin and relate the results to a unique structural type. Circular dichroism (CD) and UV-visible spectra are computed for a wide range of conformations by treating bilirubin as a molecular exciton and using the coupled oscillator formalism. The computed spectra are compared with experimentally determined spectra and used to distinguish solution conformations. Experimental models are constructed in which allosteric effects allow for the separation of conformational enantiomers.

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5. SUMMARY

The preceding studies of bilirubin conformational analysis point to a folded conformation as the global energy minimum. This, together with the considerable additional stabilization attending intramolecular hydrogen bonding for this conformation, underscores the importance and relevance of intramolecular hydrogen bonding in bilirubins and their strong tendency to implement such hydrogen bonding. Intramolecular hydrogen bonding is found to be retained in a wide variety of solvents and plays a prominent role in even water at alkaline pH, where the carboxylic acid groups become ionized. The latter evidence is particularly relevant to on-going studies in aqueous solutions involving protein binding, conjugation and hepatic excretion of bilirubins. Bilirubin itself can be predicted to be resolvable into its intramolecularly hydrogen-bonded conformational enantiomers if the interconversion barrier is increased or the rates of interconversion retarded, as at sufficiently low temperatures. Although this has not yet been achieved, resolution has been accomplished for analogs where intramolecular steric repulsions render one conformational enantiomer destabilized relative to the other, e.g., through substitution of a propionic acid α-H or β-H by CH_3 to give enantiomeric α,α'- or β,β'-dimethylmesobilirubins-XIIIα. The *R,R* and *S,S* enantiomers have been synthesized, separated and shown to have **P** and **M** chirality of the component dipyrrinone long wavelength induced electric dipole transition moments.

6. ACKNOWLEDGEMENTS

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Absolute Configuration of Bilirubin Conformational Enantiomers

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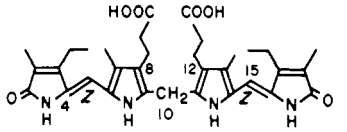
Abstract: Bilirubin, the yellow pigment of jaundice, is a bichromophoric tetrapyrrole formed in mammals by heme catabolism. It readily adopts either of two enantiomeric folded conformations which are shaped like ridge tiles and are stabilized by a network of intramolecular hydrogen bonds. Interconversion of the conformational enantiomers is rapid at room temperature and may be displaced toward either enantiomer by complexation with chiral agents. Intramolecular steric effects may also affect enantioselection. Thus, introduction of a methyl group at each of the β- and β'-carbons of the propionic acid chains on the symmetric bilirubin analog, mesobilirubin XIIIα, can shift the conformational equilibrium toward either the *M*- or the *P*-chirality intramolecularly hydrogen-bonded conformer, depending only on the *R* or *S* stereochemistry at β and β'. With the appropriate *R* or *S* configurations, intense bisignate circular dichroism (CD) may be detected for the ~430-nm transition(s) that is characteristic of exciton coupling between the component dipyrrinone chromophores. The absolute configuration of the β,β'-dimethylmesobilirubin XIIIα exhibiting a negative chirality CD exciton couplet ($\Delta\epsilon_{434}^{max}$ -337, $\Delta\epsilon_{390}^{max}$ +186 in $CHCl_3$) is firmly established as $\beta S,\beta' S$ by X-ray crystallography of the brucine salt of a monopyrrole synthetic precursor, (+)-(*S*)-3-[2,4-dimethyl-5-(ethoxycarbonyl)-1*H*-pyrrol-3-yl]butanoic acid (**5**). Molecular dynamics calculations on the $\beta S,\beta' S$ enantiomer confirm a strong preference (~20 kcal/mol stabilization) for intramolecularly hydrogen-bonded conformational enantiomers in which the *M*-chirality is favored over the *P*-chirality (by ~4 kcal/mol). For the first time, the absolute configuration of a bilirubin ridge-tile conformational enantiomer has been unequivocally established.

Introduction

Bilirubin (Figure 1), the cytotoxic yellow-orange tetrapyrrole pigment of jaundice[2,3] consists of two dipyrrinone units conjoined by a $-CH_2-$ group at C-10. As in structurally simpler molecular propellers, such as diphenylmethane,[4] rotation of the dipyrrinones about the bonds to the central $-CH_2-$ generates a large array of conformations, one of which is unique in being near or at the global energy minimum[5,6] and, particularly, in positioning the propionic acid -COOH group of one dipyrrinone unit in an ideal location for intramolecular hydrogen bonding with the pyrrole and lactam N—H and C=O residues of the other dipyrrinone. Stabilization of this conformation by a complementary network of intramolecular hydrogen bonds was first detected in the solid (by X-ray crystallography),[7] where bilirubin is folded into either of two ridge-tile-shaped enantiomers (Figure 1). Intramolecular hydrogen bonding has also been detected in solution by NMR,[8,9] where the bilirubin conformers are thought to persist as a pair of interconverting conformational enantiomers[2,10] in solvents that do not strongly perturb the matrix of intramolecular hydrogen bonds. Although the situation is less clear for solvents or other agents that may disrupt the folded pigment's hydrogen-bonding matrix, in the strong hydrogen bond acceptor $(CD_3)_2SO$, ^{13}C-NMR analysis of segmental motion in the propionic acid chains indicates that the -COOH residues are tethered to the dipyrrinones via bound solvent molecules.[11] Although it is probably folded,[12] the precise conformation of the pigment in $(CH_3)_2SO$ is uncertain.[13]

Molecular dynamics calculations on bilirubin confirm the importance of intramolecular hydrogen bonding. The conformational energy map (Figure 2) for rotations of the dipyrrinones about C-10 reveals a collection of isoenergetic global minima, which correspond to either identical or mirror image structures represented by the *M*- and *P*-chirality conformers of Figure 1. Interestingly, aside from differences due to enantiomerism, the global energy minimum conformation of bilirubin is essentially the same, whether hydrogen bonding is present or absent. Thus, with full hydrogen bonding, the global energy minimum for the *P*-chirality conformer lies at $\phi_1 = \phi_2 = 64°$; in the absence of hydrogen bonding, it lies at $\phi_1 = \phi_2 \simeq 70°$. However, the stabilization due to intramolecular hydrogen bonding is potentially considerable, which we estimate to be ~20 kcal/mol. This suggests that other conformers are essentially absent and that studies of bilirubin conformation should, as a starting point, focus on intramolecularly hydrogen-bonded structures.

The "internal" stereochemistry and nonbonded steric interactions in the intramolecularly bonded conformers are quite revealing. Close inspection of the steric environment of each of the diastereotopic hydrogens in the $-CH_2-CH_2-$ fragment of the intramolecularly hydrogen-bonded propionic acid groups suggests a way to displace the $M \rightleftarrows P$ equilibrium of Figure 1. Thus, when folded into the *M*-chirality ridge-tile enantiomer, the *pro-R* β-hydrogen (but not the *pro-S*) is brought into close nonbonded contact with the central $-CH_2-$ group at C-10, as illustrated in Figure 3 for the symmetric bilirubin analog, mesobilirubin XIIIα.



On the other hand, in the *P*-chirality enantiomer, it is the *pro-S* β-hydrogen that is buttressed against the C-10 $-CH_2-$ group. Consequently, when mesobilirubin or bilirubin adopts either of the thermodynamically preferred intramolecularly hydrogen-

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Table II. Circular Dichroism and Ultraviolet–Visible Spectral Data from 5×10^{-5} M ($\beta S,\beta' S$)-Dimethylmesobilirubin XIIIα (**1a**) and ($\beta R,\beta' R$)-Dimethylmesobilirubin XIIIα (**1b**) at 22 °C

			CD			UV	
enantiomer	solvent	dielectric constant[a]	$\Delta\epsilon^{max}$ (λ_1 in nm)	λ_2 at $\Delta\epsilon = 0$ (nm)	$\Delta\epsilon^{max}$ (λ_3, in nm)	ϵ^{max}	λ (nm)
1a	carbon tetrachloride	2.2	+179 (392)	406	−393 (434)	59 000	435
1b			−176 (392)	406	+385 (434)	60 800	435
1a	dioxane	2.2	+184 (389)	405	−336 (433)	56 600	431
1b			−180 (389)	405	+329 (433)	58 300	431
1a	benzene	2.3	+191 (390)	406	−362 (434)	55 400	433
1b			−204 (391)	407	+380 (434)	60 000	432
1a	toluene	2.4	+196 (391)	406	−375 (434)	55 800	433
1b			−192 (391)	406	+367 (434)	57 500	433
1a	diethyl ether	4.3	+183 (387)	402	−365 (429)	57 500	429
1b			−179 (387)	402	+357 (429)	59 200	429
1a	chloroform	4.7	+186 (389)	407	−337 (434)	55 500	431
1b			−188 (389)	407	+332 (435)	55 800	431
1a	tetrahydrofuran	7.3	+188 (390)	406	−338 (433)	56 200	431
1b			−184 (390)	406	+331 (434)	57 900	431
1a	dichloromethane	8.9	+180 (392)	407	−319 (433)	54 800	430
1b			−176 (392)	407	+312 (433)	56 400	430
1a	1,2-dichloroethane	10.4	+193 (389)	407	−332 (433)	55 400	430
1b			−189 (389)	407	+325 (433)	57 100	430
1a	1-butanol	17.1	+181 (391)	408	−293 (435)	55 800	427
1b			−177 (391)	408	+287 (435)	57 500	427
1a	acetonitrile	36.2	+181 (384)	403	−315 (429)	55 000	423
1b			−177 (384)	403	+308 (429)	56 700	423
1a	1-propanol	20.1	+169 (388)	406	−253 (431)	55 900	426
1b			−165 (388)	406	+248 (431)	57 600	426
1a	acetone	20.7	+182 (387)	404	−322 (430)	55 400	426
1b			−178 (387)	404	+315 (430)	57 100	427
1a	ethanol	24.3	+168 (389)	405	−284 (434)	55 900	426
1b			−164 (389)	405	+278 (434)	57 600	426
1a	methanol	32.6	+177 (386)	405	−285 (431)	56 600	425
1b			−175 (386)	405	+269 (431)	60 800	425
1a	*N*,*N*-dimethylformamide	36.7	+165 (386)	404	−246 (429)	53 100	421
1b			−169 (386)	404	+252 (429)	54 000	421
1a	dimethylsulfoxide	46.5	−5.8 (369)	385	+23.0 (425)	55 900	425
1b			+4.3 (368)	384	−24.2 (422)	56 700	425
1a	borate buffer, pH 8.5–10.0	(78)[b]	+107 (379)	397	−171 (424)	51 900	418
1b			−105 (379)	397	+167 (424)	53 500	418
1a	phosphate buffer, pH 7.4	(78)[b]	+95 (379)	398	−150 (423)	44 500	416
1b			−93 (379)	398	+147 (423)	45 800	416
1a	*N*-methylformamide	182.4	+200 (383)	400	−359 (427)	66 000	426
1b			−188 (383)	405	+337 (427)	68 300	427

[a] From: Gordon, A. J.; Ford, R. A. *The Chemist's Companion*; Wiley, New York, 1972; pp 4–8. [b] Pure water.

of bilirubin in alkaline aqueous solvents or in biological systems.[13]

Concluding Comments

Through intramolecular allosteric action, ($\beta S,\beta' S$)-dimethylmesobilirubin XIIIα (**1a**), with absolute stereochemistry at the β-positions of the propionic acid groups determined by X-ray crystallography, is forced to adopt the *M*-helicity ridge-tile conformation in $CHCl_3$, in other nonpolar solvents, and even in polar, hydroxylic solvents. Consistent with predictions, the *M*-helicity conformer exhibits intense bisignate negative exciton chirality circular dichroism for the long-wavelength transition(s), with the CD intensity dropping by only 15% in going from nonpolar solvents such as chloroform or benzene to more polar solvents such as acetonitrile or even polar, hydroxylic solvents such as methanol. Even in alkaline pH water, the CD intensities remain very high. These findings are important because (1) they establish the importance of intramolecular hydrogen bonding and how it can be used to control the handedness of conformations and (2) they firmly establish that the *M*-helicity conformational enantiomer of bilirubin will exhibit a negative exciton chirality CD.

Experimental Part

General Methods. Circular dichroism spectra were run on a JASCO J-600 spectropolarimeter, and UV–visible spectra were recorded on a Cary 219 or Perkin-Elmer 3840 diode array instrument. Rotations were determined on a Perkin-Elmer Model 141 polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded on a GE QE-300 or GN-300 300-MHz instrument, and infrared (IR) spectra were measured on a Perkin-Elmer Model 1610 FT instrument. HPLC analyses were carried out on a Perkin-Elmer high-pressure liquid chromatograph with an LC-95 UV–vis spectrophotometric detector (set at 410 nm) equipped with a Beckman-Altex ultrasphere-IP 5-μm C-18 ODS column (25 × 0.46 cm) and Beckman ODS precolumn (4.5 × 0.46 cm). The flow rate was 2.0 mL·min.$^{-1}$. The eluting solvent was 0.1 M di-*n*-octylamine acetate in 5% aqueous methanol (pH 7.7 at 31 °C). Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out at Desert Analytics, Tucson, Arizona.

Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Pentane-2,4-dione, ethyl acetoacetate, methyl crotonate, diethylene glycol, dimethyl sulfoxide, tetrahydrofuran, formic acid, and *p*-chloranil (tetrachloro-1,4-benzoquinone) were from Aldrich. Methanol, 2-propanol, dichloromethane, and chloroform were HPLC grade from Fisher; hydrogen peroxide and zinc dust were from Fisher. Tetrahydrofuran was dried by distillation from $LiAlH_4$; methanol was dried (Mg, reflux) and distilled. Brucine ($[\alpha]^{20}_D$ −79°) was used as obtained from Sigma.

X-Ray Crystallography. Diffraction measurements were made at 25 °C on a colorless crystal of the brucine salt of **5a**, grown from a solution in acetone and mounted on a Huber diffractometer constructed by C. E. Strouse. The crystal is monoclinic, space group $P2_1$. Precise cell dimensions were determined with Mo Kα radiation, from a least-squares fit to 23 reflections ($7.6° < 2\theta < 19.9°$): a = 8.170 (1), b = 12.383 (1), c = 18.945 (1) Å; β = 95.52 (1)°; V = 1908 Å^3; Z = 2. There were 5803 unique reflections (to $2\theta_{max} = 60°$), of which 2734 had $I > 2\sigma(I)$; the latter were used in solving the structure by direct methods (SHELX86[28a]) and refining by least squares (SHELX76[28b]). All calculations were made on a VAX3100 computer in the James D. McCullough X-Ray Crystallography Laboratory at UCLA. All non-H atoms were found by direct methods; H atoms were found on difference Fourier maps. The $\langle u^2 \rangle$ for

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Synthesis, Intramolecular Hydrogen Bonding and Conformation of Optically Active Bilirubin Amides. Analysis by Circular Dichroism and NMR

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Abstract: The conformation of the diamide and the bis-N-methylamide of an optically active analog of bilirubin ($\beta S,\beta' S$-dimethylmesobilirubin-XIIIα) is stabilized in a ridge-tile shape by intramolecular hydrogen bonding, as detected by ^{1}H-NMR and CD spectroscopy. The matrix of intramolecular hydrogen bonds resists disruption even in $(CH_3)_2SO$ solvent, where very strong exciton coupling CD is evident: $\Delta\epsilon_{427}^{max}$ -417, $\Delta\epsilon_{383}^{max}$ +234 (diamide).

INTRODUCTION

Bilirubin-IXα (Fig. 1), the yellow-orange neurotoxic pigment of jaundice, is formed in human metabolism by the normal turnover of hemoglobin and other heme proteins.[1-3] It is a conformationally mobile bichromophore with characteristics of a molecular propeller. Rotation of the dipyrrinone chromophores about the central -CH_2- unit at C_{10} generates a large number of conformational isomers, of which a ridge-tile shape conformer has a minimum number of non-bonded steric interactions.[3-5] This is also a conformation that brings the two propionic acid groups (located at C_8 and C_{12}) into close proximity with the pyrrole N-H and lactam —NH—C=O groups, with the result that a network of intramolecular hydrogen bonds can easily be established — thus rendering the ridge-tile conformation unusually stable.[6] This inward tucking of the CO_2H groups and tethering to opposing dipyrrinones through intramolecular hydrogen bonding also lowers the acidity and decreases the polarity of the pigment, leaving it unexcretable in normal metabolism (hepatic excretion), except *via* glucuronidation. The ridge-tile conformation is found in crystalline bilirubin and its salts,[7,8] and it is favored by bilirubin in non-polar organic solvents,[9-11] and

Dedicated to the late Professor Dr. Günther Snatzke (deceased January 14, 1992)

other solvents of Table 3, and the secondary amide **2a** exhibits only moderately reduced $\Delta\epsilon$ values. These data support the ^{1}H-NMR results and appear to confirm the importance of intramolecular hydrogen bonding and ridge-tile conformations (Fig. 5) in all solvents studied. Clearly, hydrogen bonding from the propionamide residues is not easily perturbed.

CONCLUDING COMMENTS

Intramolecular hydrogen bonding between propionic acid CO_2H and dipyrrinone groups is known to be a dominant, conformation stabilizing force in bilirubin and its analogs.[18] The current study shows that when the propionic acid residues are replaced by primary and secondary propionamide residues, intramolecular hydrogen bonding persists in all solvents. The amide hydrogen bonding is more stable than the acid hydrogen bonding, and the effect of such hydrogen bonding is to stabilize the typical folded ridge-tile conformations (Figs. 4 and 5). The extraordinarily large bisignate CD Cotton effects observed for the amides **1a** and **2a** are typical of excited state (electric dipole) interactions, or exciton coupling, between two proximal chromophores with little orbital overlap.[24,26] The component dipyrrinone chromophores of the bichromophoric mesobilirubin have strongly allowed long-wavelength electronic transitions (ϵ_{410}^{max} $\sim$37,000) but only a small interchromophoric orbital overlap in the folded conformation (where the dihedral angle $\simeq 100°$). They interact through resonance splitting, *i.e.*, by electrostatic interaction of the local transition moment dipoles, which are oriented along the long axis of each dipyrrinone.[4,25,26] The dipyrrinone-dipyrrinone intramolecular exciton splitting interaction produces two long wavelength transitions in the ordinary (UV-visible) spectrum and two corresponding bands in the CD spectrum.[4,25,26] One band is higher in energy and one is lower in energy, with the separation dependent on the strength and relative orientation of the dipyrrinone electric dipole transition moments.[27] When observed by UV-visible spectroscopy, the two electronic transitions overlap to give the broadened long wavelength absorption band characteristic of bilirubins. In the CD spectra, however, where the two exciton transitions are oppositely signed, bisignate CEs are typically seen as predicted by theory, Thus, in contrast to UV-visible absorption bands, which may show only slight broadening when the exciton splitting energy is small,[28] when two oppositely-signed curves overlap in the CD, there is considerable cancellation in the region between the band centers with the net result that the *observed* bisignate CE maxima are displaced from the actual locations of the (uncombined) CD transitions which typically flank the corresponding UV-visible band(s).

EXPERIMENTAL

General Procedures. All ultraviolet-visible spectra were recorded on a Perkin Elmer Model 3840 diode array or Cary 219 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a JASCO J-600 instrument. Nuclear magnetic resonance (NMR) spectra were determined on a GE QE-300 300-MHz spectrometer in $CDCl_3$ solvent (unless otherwise specified) and reported in δ ppm downfield from $(CH_3)_4Si$. Rotations were determined in $CHCl_3$ on a Perkin-Elmer model 141 polarimeter. Melting points

Synthetic Strategies for Understanding Bilirubin Stereochemistry

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Received 2 June 1994

Abstract: Synthetic approaches toward model tetrapyrrolic bilirubin-like analogs are described. The consequences of judiciously targeted remote variations of primary chemical structure on the three-dimensional shape of bilirubin analogs are discussed.

1. Introduction

Bilirubin-IXα, the hydrophobic and cytotoxic pigment of jaundice is a yellow-orange unsymmetrically substituted tetrapyrrole dicarboxylic acid produced in copious quantities by catabolism of heme (Fig. 1A), principally from the hemoglobin of red blood cells.[1,2] Judging only by the functional groups present in the molecule, the chemical properties of bilirubin are anomalous. It is poorly soluble in water at physiologic pH,[1] has a high lipid/water partition coefficient,[2] and a proclivity to form complexes with serum albumin and other proteins[1-3] — three interrelated properties that dominate the transport and metabolism of bilirubin in vivo.[1,4] These properties make bilirubin intrinsically unexcretable, *i.e.*, too lipophilic and protein bound to pass efficiently out of the circulation across either of the selective barriers, kidney and liver, into urine or bile. Nature circumvents this problem by hepatic conjugation with glucuronic acid. The glucuronides are much more polar than bilirubin, so polar that they are excreted readily in bile.[5]

Despite its implication in a large array of protein binding situations involved in its transport and metabolism,[1,4] the exact three-dimensional structure of bilirubin in solution or when bound to proteins, or in lipid matrices is not well understood. Recently it has become increasingly clear that under some circumstances bilirubin exhibits an extraordinary tendency to fold its shape and hydrogen bond *intra*molecularly,[6,7,8,9,10] as in Fig. 1B. Through an understanding of the stereochemistry of bilirubin and related synthetic analogs, one might expect to resolve long-standing questions about host binding forces and binding site topography in protein complexation. The compounds, whose syntheses are described in the following also have intrinsically fascinating stereochemically-controlled properties that are related to hydrogen bonding — such as solubility,[7] and optical activity.[11] In this role they provide useful insights into how stereochemistry controls amphiphilicity and how chiroptical properties become magnified and thus serve as exquisitely sensitive indicators of conformational equilibria.

The objective of this account is to describe the synthetic aspects of our recent research in providing useful molecular model tetrapyrrolic bilirubin-like compounds for assessing the stereochemistry of bilirubin in biological tissues.

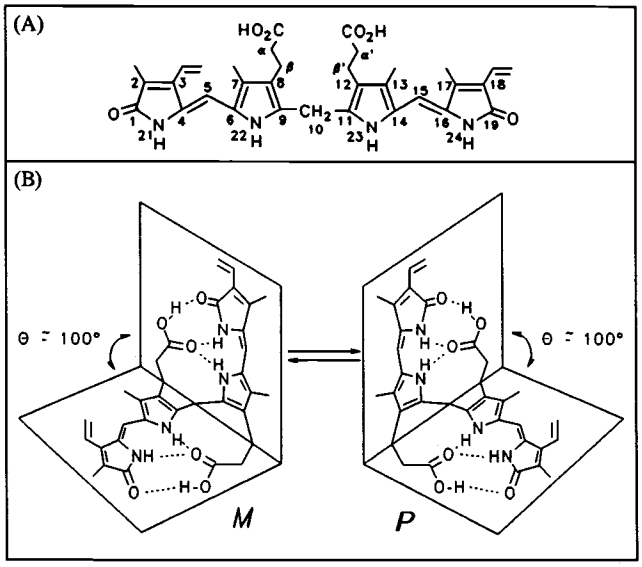

Figure 1. (A) Constitutional structure and numbering system of bilirubin-IXα. (B) Conformational representations for ridge-tile-shape ***M*** and ***P*** chirality, intramolecularly hydrogen-bonded, interconverting enantiomers of bilirubin-IXα.

2. Structural Features of Bilirubin

The constitutional structure of bilirubin was elucidated by Hans Fischer's group some 50 years ago through a combination of degradation, partial and total synthesis.[12] Aside from a few minor details, such as Fischer's preference for the lactim tautomer and an unspecified stereochemistry at the C_4 and C_{15} double bonds, the primary structure remains unchanged today (Fig. 1A). However, the frequently encumbered linear (Fig. 1A) or porphyrin-like representations of bilirubin are inconsistent with many of its properties.

The syntheses of pyrrolinone synthon **25** and central dipyrrylmethane **26** were elaborated. Pyrrolinone **25** was isolated following hydrogen peroxide oxidation of 3,4-dimethylpyrrole. Dialdehyde **26** was prepared via Vilsmeier-Haack reaction on the α,α'-unsubstituted dipyrrylmethane. Coupling two equivalents of **25** with **26** in strong basic condition led to low yield of desired tetrapyrrolic diacid **27** (R=H), while in presence of titanium(IV) chloride the corresponding diethyl ester **27** ($R=C_2H_5$) was obtained,[44] Fig. 13.

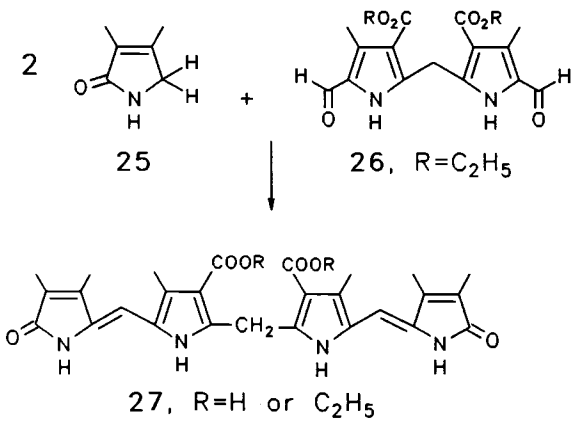

Figure 13. Synthesis of carboxyrubin (**27**) R=H.

Considering the solubility, polarity and ^{1}H-NMR properties of carboxyrubin **27** (R=H), it has been concluded that **27** does not adopt an intramolecularly hydrogen bonded conformation, and it exhibits properties more in line with exposed carboxylic acid groups.

8. Conclusion

Synthetic model compounds provide insight into bilirubin stereochemistry. The synthetic methods employed lead to a battery of structurally modified bilirubin analogs. They reveal a way to alter the three-dimensional shape of bilirubin and hence the solution properties of *intra*molecularly hydrogen-bonded bilirubin pigments by judiciously targeting remote changes of primary chemical structure. They also provide new bilirubin pigments for use in establishing the role of hydrogen bonding in protein binding, transport and metabolism studies.

Acknowledgement

Many graduate students and postdoctoral coworkers contributed to the body of the Nevada work cited in this account. Their efforts are much appreciated. We have been helped and stimulated by research collaboration with Dr. A.F. McDonagh (U.C. San Francisco) and Dr. F.R. Trull (Univ. Barcelona). Most of this work has been generously supported by the U.S. National Institutes of Health (HD 17779). Dr. Stefan Boiadjiev is on leave from the Institute of Organic Chemistry, Academy of Sciences, Sofia, Bulgaria.

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Conformational Analysis of an Optically Active Bilirubin Dimethyl Ester and Bis-*N*,*N*-Dimethyl Amide by Circular Dichroism, NMR and Molecular Dynamics

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Abstract: The dimethyl ester and bis-*N*,*N*-dimethylamide of an optically active analog of bilirubin ($\beta S,\beta' S$-dimethylmesobilirubin-XIIIα) is stabilized in a ridge-tile conformation by intramolecular hydrogen bonding, as detected by ^{1}H-NMR and CD spectroscopy. The ridge-tile shape is most evident in nonpolar solvents, where very strong exciton coupling CD is evident: *e.g.*, in benzene, $\Delta\epsilon^{max}_{412}$ -199, $\Delta\epsilon^{max}_{372}$ +87 for the dimethyl ester and $\Delta\epsilon^{max}_{421}$ -165, $\Delta\epsilon^{max}_{384}$ +46 for the bis-*N*,*N*-dimethylamide.

INTRODUCTION

Bilirubin, the lipophilic, water-insoluble and neurotoxic yellow pigment of jaundice, is biosynthesized in healthy adults at the rate of ~300 mg/day during the normal turnover of hemoglobin and other heme proteins and excreted across the liver into bile as water-soluble esters.[1,2] The linear structure (Fig. 1) represents only the constitutional structure of bilirubin, which is a conformationally mobile bichromophore with two dipyrrinones conjoined at their α-carbons to a -CH_2- group. Propeller-like rotations of the dipyrrinone chromophores about the central -CH_2- create a multitude of conformational isomers with widely differing shapes.[3,4,5] Of these, the most stable is shaped like a ridge-tile, where non-bonded steric interactions are minimized.[3-6] This unique conformation brings the pigment's propionic acid groups (located at C_8 and

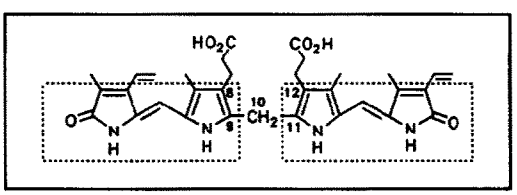

Figure 1. Linear representation for bilirubin, with its two dipyrrinone chromophores outlined in dashed boxes. Rotations of the dipyrrinone chromophores about the C_9-C_{10} and C_{10}-C_{11} bonds create a wide scope of conformational isomers.

CONCLUDING COMMENTS

Intramolecular hydrogen bonding between propionic acid CO_2H and dipyrrinone groups is known to be a dominant, conformation stabilizing force in bilirubin and its analogs.[3,6,11,12] The current study shows that when the propionic acid residues are replaced by methyl propionate or *N,N*-dimethylpropionamide residues, residual intramolecular hydrogen bonding persists in many solvents. In the ester, hydrogen bonding is somewhat more effective than in the *N,N*-dimethylamide, but both the ester and amide groups are less effective than the acid in hydrogen bonding. The effect of such hydrogen bonding is to stabilize folded ridge-tile conformations (Figs. 4 and 5), which are flatter in the ester and *N,N*-dimethylamide than in the parent acid.

EXPERIMENTAL

General Procedures. All ultraviolet-visible spectra were recorded on a Perkin Elmer Model 3840 diode array or Cary 219 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a JASCO J-600 instrument. Nuclear magnetic resonance (NMR) spectra were determined on a GE QE-300 300-MHz spectrometer in $CDCl_3$ solvent (unless otherwise specified) and reported in δ ppm downfield from $(CH_3)_4Si$. Rotations were determined in $CHCl_3$ on a Perkin-Elmer model 141 polarimeter. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Analytical thin layer chromatography was carried out on J.T. Baker silica gel IB-F plates (125 μ layer). Preparative layer chromatography was carried out using 1.0 mm thickness layers of Woelm silica gel F, thin layer chromatography grade. Radial chromatography was carried out on Merck Silica gel PF-254 with $CaSO_4$ preparative thin layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). HPLC analyses were carried out on a Perkin-Elmer Series 4 high performance liquid chromatograph with an LC-95 UV-visible spectrophotometric detector (set at 410 nm) equipped with a Beckman-Altex ultrasphere-IP 5 μm C-18 ODS column (25 x 0.46 cm) and a Beckman ODS precolumn (4.5 x 0.46 cm). The flow rate was 1.0 mL/minute, and the elution solvent was 0.1 *M* di-*n*-octylamine acetate in 5% aqueous methanol (pH 7.7, 31°C).

Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Dimethylformamide (DMF), chloroform, methanol, triethylamine (Et_3N), diphenylphosphoryl azide (DPPA), tetrachloro-1,4-benzoquinone (*p*-chloranil), acetic acid, tetrahydrofuran, 98% formic acid, dichloromethane, dimethylsulfoxide and sodium borohydride, were from Aldrich. Dimethylamine hydrochloride was from Matheson. Dimethylformamide was dried and distilled from barium oxide; triethylamine was dried and distilled from KOH; tetrahydrofuran was dried by distillation from lithium aluminum hydride; methanol was dried (Mg, reflux) and distilled.

(-)-3-[2,7,9-Trimethyl-3-ethyl-(10*H*)-dipyrrinone-8-yl]butanoic Acid [β(*S*)-Methylxanthobilirubic Acid] (7). (-)-(βS)-Methylxanthobilirubic acid (**7**) was prepared in 96% yield by saponification of the corresponding methyl ester **6**, described previously.[9]

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Synthesis and Stereochemistry of Optically Active Biliverdin Cyclic Esters

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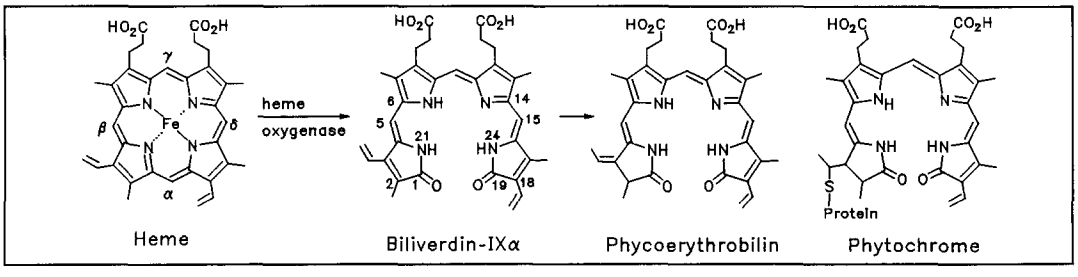
Abstract: ($\beta S,\beta' S$)-Dimethylmesobiliverdin-XIIIα dimethyl ester exhibits a positive long wavelength circular dichroism Cotton effect ($\Delta\epsilon_{644}^{max}$= +6.1) in $CHCl_3$, indicating an excess of the ***P***-helical conformation of the pigment. But when the pigment's propionic acids are linked together by -$(CH_2)_n$- chains in *cyclic esters*, the choice of cyclic ester conformation and pigment helicity is governed by the number (n) of CH_2 units, with $\Delta\epsilon$ near 640 nm varying from positive when n=1, 5 and 6, to negative when n=2, 3 and 4.

INTRODUCTION

Biliverdin-IXα is a blue-green pigment formed in plant and animal metabolism by oxidative cleavage of the porphyrin macrocycle of heme.[1,2,3] In mammals biliverdin is reduced rapidly and efficiently to bilirubin, the yellow pigment of jaundice, which is eliminated in normal metabolism following esterification in the liver by a glucuronosyl transferase and excretion into bile.[1,4] In other animals, biliverdin is excreted directly.[1] Verdins are distributed widely in nature. They serve as a colorant in egg shells, bones and butterfly wings, and are found, *mutatis mutandis*, in plants.[1-3] In blue-green algae, biliverdin is converted into photosynthetic pigments, phycocyanobilin and phycoerythrobilin.[2,3] In higher plants and some algae, it is converted into phytochrome, which is the photosensory pigment mediating photomorphogenesis.[2,3] Although four different biliverdins might be formed *via* oxidative cleavage of heme at the α, β, γ or δ site, typically the α-carbon is excised to give biliverdin-IXα, which is the most common biliverdin type.[1,2]



The constitutional structure of biliverdin was determined long ago by total synthesis,[5] and its stereochemistry is straightforward. In its most stable conformation, biliverdin adopts a helical, lock-washer shape

ring conformations are slightly preferred, but in **4**, the ***p*** is preferred. As with **1**, it is difficult to rationalize the CD data from the predicted energy differences of Table 5 — except perhaps in **4**, where the ***pM*** diastereomer is predicted to be 0.86-1.07 kcal/mole more stable than any others. Curiously, the Cotton effect signs, which reversed when the ester belt was expanded by one $-CH_2-$ unit (in going from **1** to **2**) and remain invariant as the number of $-CH_2-$ units increased from 2 to 4, are found to reverse again when the $(-CH_2-)_n$ belt is expanded by one more $-CH_2-$ (in going from $n=4$ (**4**) to $n=5$ (**5**).

SUMMARY

Linking the propionic acid groups of mesobiliverdin through $-(CH_2)_n-$ chains ($n=1$-6) imposes no unusual distortion in the verdin chromophore skeleton. Conformational analysis of the cyclic esters, which takes into account the intrinsic ***M*** and ***P*** verdin helicity as well as the helicity (***m*** and ***p***) of the cyclic ester suggests the presence of 4 diastereomers: ***mM***, ***mP***, ***pM*** and ***pP***. Molecular mechanics calculations indicate a low barrier (~ 10 kcal/mole) to $M \rightleftarrows P$ interconversion and a very high barrier ($\rightarrow \infty$) to $m \rightleftarrows p$ interconversion. Optically active ($\beta S,\beta' S$)-dimethylmesobiliverdin-XIIIα dimethyl ester (**7**) shows a weakly positive long wavelength CD Cotton effect, indicating a slight excess of the ***P***-helicity conformer — as predicted by molecular mechanics calculations. Verdin cyclic esters **1**, **5** and **6** also exhibit weakly positive long wavelength CD Cotton effects, suggesting a predominance of the ***mP*** or ***pP*** diastereomers. In contrast, verdin cyclic esters **2**, **3** and **4** show weakly negative long wavelength Cotton effects, indicating a slight predominance of ***mM*** and/or ***pM*** diastereomers. The energy differences are small, and molecular mechanics is less useful in rationalizing the observed CD data. The failure is probably due to neglect of solvent.

EXPERIMENTAL

General Procedures. All ultraviolet-visible spectra were recorded on a Perkin Elmer model 3840 diode array or Cary 219 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a Jasco J-600 instrument. NMR-spectra were obtained on a GE QE-300 or GN-300 300 MHz spectrometer in $CDCl_3$ solvent and reported in δ ppm upfield from $(CH_3)_4Si$. For ^{13}C-NMR spectra, the J-modulated spin-echo experiment (Attached Proton Test) was used. Optical rotations were measured in chloroform on a Perkin Elmer model 141 polarimeter. Mass spectra (EI) were measured on a Finnigan MAT SSQ 710 instrument. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Analytical thin layer chromatography was carried out on J.T. Baker silica gel IB-F plates (125μ layer). Radial chromatography was carried out on Merck silica gel 60 PF_{254} with $CaSO_4$ preparative thin layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA).

Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Dimethylsulfoxide (Fisher) was dried and distilled from calcium hydride. Methanol was dried and distilled from magnesium methoxide, and tetrahydrofuran (THF) was dried and distilled from lithium aluminum hydride. Diiodomethane, 1,2-dibromoethane, 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibromohexane, and cesium carbonate were from Aldrich.

(4Z,10Z,15Z)-8,12-Bis(methoxycarbonyl-(βS)-methylethyl)-3,17-diethyl-2,7,13,18-tetramethyl-1,19,21,24-tetrahydrobilin-1,19-dione [($\beta S,\beta' S$)-Dimethylmesobiliverdin-XIIIα dimethyl ester] (**7**). Optically pure ester was prepared as described earlier,[14] and has mp 212-216°C and $[\alpha]_{436}^{20}$ -3480 (*c* 1.6 x10^{-3}, $CHCl_3$).

($\beta S,\beta' S$)-Dimethylmesobiliverdin-XIIIα (**8**). Optically pure **7** (482 mg, 0.75 mmol) was dissolved in a mixture of 225 mL of tetrahydrofuran:methanol (1:1 by vol), both N_2 saturated. To the solution was added ascorbic acid (225 mg) and 0.2 M aqueous NaOH (225 mL, N_2 saturated). The mixture was stirred under N_2 at 50°C for 5 h. After cooling to room temperature, 100 mL of water and 100 mL of chloroform were

Intermolecular Hydrogen Bonding in π Facial Dipyrrinone Dimers as Molecular Capsules

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Received March 10, 1995[⊗]

Abstract: (4*Z*)-Dipyrrinones, which are the component chromophores of yellow pigment of jaundice, are known to self-associate strongly in nonpolar solvents ($K_{assoc} \simeq 25\,000$ M at 25 °C in $CHCl_3$), forming *planar* dimers in which the monomers are linked tightly by four intermolecular hydrogen bonds. When the chromophore has an attached propionic or longer chain acid group, it forms a new type of *stacked* dimer through a network of six hydrogen bonds in which the carboxyl group of one dipyrrinone is tethered to the other dipyrrinone. Thus, xanthobilirubic acid and its homologs strongly self-associate as stacked dimers in contrast to its methyl ester, which forms planar dimers. The stacked dimers are recognized by large (1 ppm) shieldings of their N*H* resonances in their ^{1}H-NMR spectra, as compared with planar dimers. They are also recognized by unusually large optical rotations and exciton coupling in the circular dichroism spectra when a stereogenic center is present in the alkanoic acid chain. In $CHCl_3$, (β*S*)-methylxanthobilirubic acid (**1**) has $[\alpha]^{20}_D = -314°$ and $\Delta\epsilon^{max}_{434} = -10.9$, $\Delta\epsilon^{max}_{388} = +5.7$, whereas its methyl ester (**6**) has $[\alpha]^{20}_D = +62°$; $\Delta\epsilon^{max}_{370} < 1$.

Introduction

Dipyrrinones are typically bright yellow compounds exhibiting an intense UV−visible absorption near 400 nm ($\epsilon \simeq 30\,000$ L mol^{-1} cm^{-1}) associated with a long axis-polarized $\pi \rightarrow \pi^*$ excitation in the 14π-electron-conjugated chromophore (Figure 1).[1] The dipyrrinone chromophore is found in nature in tetrapyrrole bile pigments, especially in (4*Z*,15*Z*)-bilirubin-IXα, the yellow-orange pigment of jaundice.[1,2] Bilirubin is a dicarboxylic acid comprised of two dipyrrinones conjoined at and capable of independent rotations about a $-CH_2-$ group at C(10). It is through such rotations that the carboxyl group of one dipyrrinone is brought into sufficiently close proximity to engage the other dipyrrinone's lactam and pyrrole moieties in a matrix of intramolecular hydrogen bonds (Figure 1). Collectively, these six hydrogen bonds act as a potent stabilizer of the conformation of bilirubin, seen in three dimensions as a ridge-tile shape.[3] Whether in bilirubin or as independent units, dipyrrinones are known to be avid participators in hydrogen bonding.[1] They have also served as useful adjuncts in studies of jaundice phototherapy[4] and in bilirubin structure−biological function relationships.[5]

Dipyrrinones are known from X-ray crystallography[1,6] and molecular mechanics calculations[1,7] to prefer the lactam tautomer and the *Z*-configuration C=C at C(4) and to show substantial double-bond and single-bond character in the C(4)=C(5) and C(5)−C(6) bonds, respectively (Figure 2). They adopt essentially planar conformations ($\psi \simeq 0°$) in the crystal, where

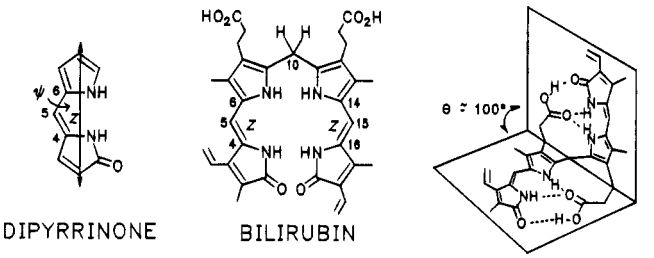

Figure 1. (left) Dipyrrinone chromophore. The double-headed arrow approximates the long axis polarization of the intense ~400 nm electronic transition. (center) Bilirubin in a porphyrin-like representation composed of two dipyrrinone chromophores. (right) Stable ridge-tile bilirubin conformation with hydrogen bonding between carboxylic acid groups and opposing dipyrrinones.

they are present as intermolecularly hydrogen-bonded planar dimers (Figure 2).[1,6] Most of these characteristics persist in nonpolar solutions. In $CHCl_3$, for example, dipyrrinones are strongly associated with dimerization constants of 1700 M (37 °C) for kryptopyrromethenone[8] and 25 000 M (22 °C) for methyl xanthobilirubinate[9] measured by vapor phase osmometry and ^{1}H-NMR spectroscopy, respectively. The dimers are held together by a matrix of four intermolecular hydrogen bonds, with a calculated stabilization enthalpy of 20−30 kcal/mol.[10] In methyl xanthobilirubinate, two intermolecularly hydrogen-

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1995.

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(5) (a) McDonagh, A. F.; Lightner, D. A. In *Hepatic Metabolism and Disposition of Endo and Xenobiotics*; Falk Symposium No. 57; Bock, K. W., Gerok, W., Matern, S., Eds.; Kluwer: Dordrecht, The Netherlands, 1991; Chapter 5, pp 47−59. (b) Lightner, D. A.; Reisinger, M.; Landen, G. L. *J. Biol. Chem.* **1986**, *261*, 6034−6038.

(6) (a) Cullen, D. L.; Black, P. S.; Meyer, E. F., Jr.; Lightner, D. A.; Quistad, G. B.; Pak, C.-S. *Tetrahedron* **1977**, *33*, 477−483. (b) Cullen, D. L.; Pèpe, G.; Meyer, E. F., Jr.; Falk, H.; Grubmayr, K. *J. Chem. Soc., Perkin Trans 2* **1979**, 999−1004. (c) Gossauer, A.; Blacha, M.; Sheldrick, W. S. *J. Chem. Soc., Chem. Commun.* **1976**, 764−765. (d) Sheldrick, W. S.; Borkenstein, A.; Blacha-Puller, M.; Gossauer, A. *Acta Crystallogr.* **1977**, *B33*, 3625−3635. (e) Sheldrick, W. S. *Isr. J. Chem.* **1983**, *23*, 155−166.

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(9) Nogales, D. F.; Ma, J.-S.; Lightner, D. A. *Tetrahedron* **1993**, *49*, 2361−2372.

(10) Molecular mechanics calculations and molecular modeling were carried out on an Evans and Sutherland ESV-10 workstation using version 6.0 of SYBYL (Tripos Assoc., St. Louis, MO) as described in ref 3. The ball and stick drawings were created from the atomic coordinates of the molecular dynamics structures using Müller and Falk's "Ball and Stick" program (Cherwell Scientific, Oxford, U.K.) for the Macintosh.

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Hydrogen Bonding and π-Stacking in Dipyrrinone Acid Dimers of Xanthobilirubic Acid and Chiral Analogs

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Abstract: Xanthobilirubic acid and its analogs self-associate strongly through intermolecular hydrogen bonding between their carboxylic acid and dipyrrinone components, forming π-stacked dimers. In contrast, their methyl esters form planar dimers conjoined by dipyrrinone to dipyrrinone intermolecular hydrogen bonding. When a stereogenic center is present in the propionic side acid chain, unusually large optical rotations and exciton coupling circular dichroism may be observed for the optically active acids: βS-methylxanthobilirubic acid (**1**) has $[\alpha]_D^{20} = -314°$ and $\Delta\epsilon_{434}^{max} = -10.9$, $\Delta\epsilon_{388}^{max} = +5.7$ ($CHCl_3$). In contrast, methyl esters show weaker rotations and vanishingly small CD: the methyl ester of (**1**) has $[\alpha]_D^{20}$ +62° and $\Delta\epsilon_{435} << 0.5$, $\Delta\epsilon_{390} << 0.5$.

INTRODUCTION

Bilirubin, the yellow pigment of jaundice[1,2] is a dicarboxylic acid composed of two dipyrrinone chromophores conjoined at a -CH_2- group (Fig. 1A). In the most stable bilirubin conformation, each dipyrrinone engages a carboxylic acid group in a matrix of intramolecular hydrogen bonds that collectively act as a potent stabilizing force.[3] Even simple dipyrrinones participate avidly in hydrogen bonding.[4] Kryptopyrromethenone[5] and methyl xanthobilirubinate,[6] for example, form planar dimers (Fig. 1B) in nonpolar solvents (K_{assoc} ≃ 25,000 M, $CHCl_3$, 25°C)[4] or engage in hydrogen bonding to solvents such as DMSO.[4,7] The types of hydrogen bonding shown in Figs. 1A and 1B have been found in crystals of bilirubin[8] and dipyrrinones,[4,9] and they have been detected in solution by ^{1}H-NMR N-*H* (and CO_2H) chemical shifts and NOEs.[4,6,7,10,11]

Two fundamentally different intermolecularly hydrogen bonded dimers, each with four hydrogen bonds, can be drawn for dipyrrinone esters (Figs. 1B and 1C). The planar dimer (Fig. 1B) is the most common form in the crystal and in nonpolar solvents. In methyl xanthobilirubinate, for example, the methyl group at C(2)

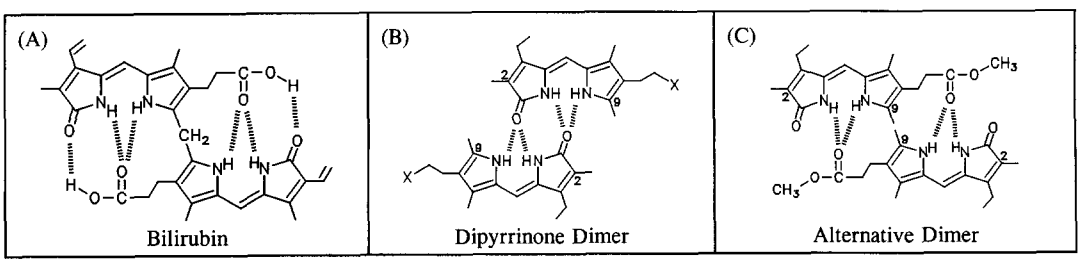
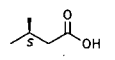
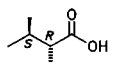
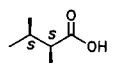
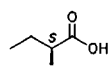
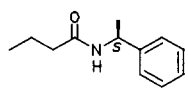
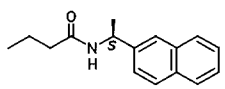


FIGURE 1. (A) Intramolecularly hydrogen bonded bilirubin with hydrogen bonding between carboxylic acids and dipyrrinones. (B) Intermolecularly hydrogen-bonded dipyrrinone dimer of kryptopyrromethenone (X=H) and methyl xanthobilirubinate ($X=CO_2CH_3$). (C) Alternative dimer representation for methyl xanthobilirubinate.

TABLE 6. Computed Energy Differences ($\Delta\Delta H_f$) Between *M* and *P*-Helicity Stacked Dimers of Dipyrrinone Acids **1-3** and the Observed Exciton Chirality CD Sign and Amplitude.

R =	Computed Stacked Dimers		Exciton Chirality CD		
	$\Delta\Delta H_f = \Delta H_f(M) - \Delta H_f(P)$ (kcal/mole)	Predominant Dimer	Observed Amplitude in: CCl_4	$CHCl_3$	Predominant Dimer
 **1**	-5.6	*M*	-37	-17	*M*
 **2**	-0.3	*M*	-13	-5	*M*
 **3**	-12.0	*M*	-50	-36	*M*
	-5.3	*M*	NA	NA	—
	-0.9	*M*	NA	-4.4	*M*
	-1.0	*M*	NA	-7.2	*M*

SUMMARY

Dipyrrinones with propionic acid groups at C(8) form a hitherto unrecognized stacked dimer in nonpolar solvents. The stacked dimer is held together in a chiral orientation with six hydrogen bonds linking dipyrrinone and acid groups. This is distinctly different for dipyrrinone esters and other dipyrrinones without acid groups. They form planar dimers with four hydrogen bonds. Because dipyrrinone acids form stacked dipyrrinone dimers, they exhibit more shielded N*H* chemical shifts in their ^{1}H-NMR spectra than do dipyrrinone esters, for example. In the stacked dimers, the dipyrrinones are held in either of two enantiomeric helical arrangements, *M* or *P*. This can be detected by circular dichroism spectroscopy of optically active dipyrrinone acids **1-3**, which prefer to adopt the *M*-helicity stacking arrangement. Evidence for interchromophoric interaction in the dimer is found in their exciton coupling bisignate CD spectra (Fig. 6), from which the absolute configuration (*M*) of the preferred dimer can be determined. This new understanding of how dipyrrinones may form dimers offers an explanation for previously reported bisignate CD spectra of the amides of xanthobilirubic acid with *R* and *S*-α-phenethylamine and *R* and *S* α-naphthylethylamine.[17]

EXPERIMENTAL

General Methods. All UV-visible spectra were recorded on a Perkin Elmer model 3840 diode array or Cary 219 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a JASCO J-600 instrument. NMR spectra were obtained on a GE QE-300 or GN-300 spectrometer operating at 300 MHz or Varian Unity Plus spectrometer operating at 500 MHz. $CDCl_3$ solvent (unless otherwise noted) was used and chemical

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STEREOCONTROL OF BILIRUBIN CONFORMATION

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Abstract: Optically active, diastereomeric synthetic bilirubin analogs (**1** and **2**) with an α-methyl and a β-methyl in each propionic acid side chain were synthesized. Both diastereomers adopt a folded, ridge-tile conformation. The methyls force the pigment to adopt either a left-handed (***M***) or right-handed (***P***) helicity. As evidenced by exciton-type circular dichroism spectra, in the ($\alpha S,\alpha' S,\beta S,\beta' S$) diastereomer (**1**) the ***M*** helicity ridge-tile enantiomer is strongly preferred; in the ($\alpha R,\alpha' R,\beta S,\beta' S$) diastereomer (**2**),the ***P*** helicity conformation is preferred.

INTRODUCTION

Bilirubin (Fig. 1) is a tetrapyrrole dicarboxylic acid formed in the normal metabolism of heme proteins.[1-3] In a healthy adult, it is produced at the rate of ~300 mg/day, principally from the breakdown of red blood cells. Bilirubin is intrinsically unexcretable but is efficiently eliminated by the liver following uptake and enzymic conversion to water-soluble glucuronides that are promptly secreted into bile. Impaired excretion of the glucuronides occurs in many types of hepatobiliary disease, but retention of native bilirubin is principally observed in newborn babies.[1-3] Accumulation of either native bilirubin or its glucuronides in the body is manifested in jaundice.

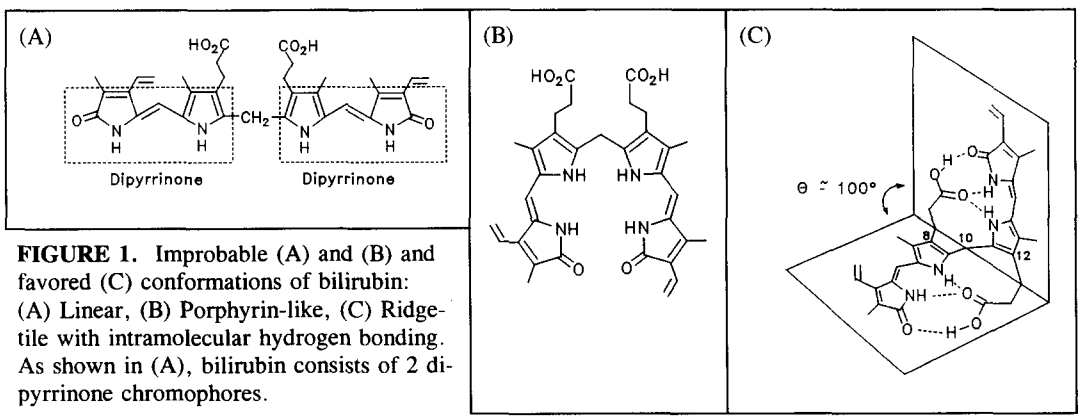

FIGURE 1. Improbable (A) and (B) and favored (C) conformations of bilirubin: (A) Linear, (B) Porphyrin-like, (C) Ridge-tile with intramolecular hydrogen bonding. As shown in (A), bilirubin consists of 2 dipyrrinone chromophores.

In all solvents except $(CH_3)_2SO$, **1** exhibits a negative exciton chirality, suggesting a predominance of the *M*-helicity conformer and confirming the predictions drawn above and based on nonbonded intramolecular steric interactions in the $\alpha S,\alpha' S,\beta S,\beta' S$ diastereomer. The CD intensities are 70-90% of those seen in $\beta S,\beta' S$-dimethylmesobilirubin-XIIIα,[8] thus indicating that its conformational equilibrium (Fig. 2) is already displaced completely toward *M*, and any conformational reinforcement due to the αS and $\alpha' S$ methyls is not only redundant but is probably offset by the introduction of a new *gauche* butane interaction between the α and β methyls. In $(CH_3)_2SO$ solvent, where the CE intensity drops to $<10\%$ of the maximum values — both in **1** and in $(\beta S,\beta' S)$-dimethylmesobilirubin-XIIIα,[8] it seems probable that the favored folded conformation has become somewhat more open to accommodate attachment of the solvent molecules.[16] As shown earlier,[3] flattening the ridge-tile leads to a reorientation of the dipyrrinone electric transition dipole moments to near parallelity (and hence to very weak bisignate CEs) and a change in torsion from (−) to (+) without a change in conformational chirality. In aqueous base the CEs remain strong, consistent with an *M* helicity dianion.

The CD data of $(\alpha R,\alpha' R,\beta S,\beta' S)$ diastereomer **2** contrast strongly with those of **1**. In nonpolar solvents such as cyclohexane and chloroform, **2** exhibits intense *positive* exciton chirality, consistent with the equilibrium of Fig. 2 being displaced toward *P*; whereas, the CD CEs of **1** are more intense with a *negative* exciton chirality due to a predominance of *M*. These data suggest that, on balance, the steric influence of the α-methyls counteracts and dominates that of the β-methyls when they work in opposition (in **2**). However, the dominance is not as effective in more polar solvents, where the CE intensities of **2** are far weaker than those of **1**. In solvents such as acetone and methanol, the β-methyls seem to dominate, but in most polar solvents the CD spectra of **2** is more complex than those of **1**, as if multiple species were present.

Dimethyl Esters. The UV spectra of **3** and **4** (Table 4) exhibit, like the parent acids **1** and **2**, characteristic exciton splitting of the long wavelength transition. The UV spectra of **3** and **4** differ, with the former being more like those of **1** and **2**, whose exciton bands are of nearly equal intensity. The latter, on the other hand have most of the intensity in the higher energy exciton component. Such data suggest that **3** and **4** adopt different conformations, that predominant conformation of **4** tends toward the porphyrin-like shape.

The CD spectra of **3** and **4** differ but are generally less intense than those of parent acids **1** and **2**. Diester **4** consistently exhibits negative exciton chirality CD; whereas, **3** exhibits variably negative and positive exciton chirality CD. The CD and UV data for **4** suggest a dominant positive helical conformation, probably in a dimer. On the other hand, the data for **3** are consistent with a folded conformation, similar to the ridge-tiles of Fig. 2, but with less hydrogen bonding and probably a more open conformation.

CONCLUDING COMMENTS

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin and its analogs, is known to be a dominant force in determining their conformation. The current study shows that when the propionic acid residues are substituted with methyl groups at the α and β positions, thus creating stereogenic centers, mesobilirubin-XIIIα is forced by nonbonded steric interactions to adopt either the *M* or *P* helicity ridge tile conformation. When all α,β centers have the *S* configuration, the *M*-helicity conformer is dominant. However, when the non-bonded interactions operate in conflict, as in the $\alpha R,\beta S$ configuration, a more open *P*-helicity ridge-tile is suggested for nonpolar solvents. In this more open conformation, the observed exciton chirality CD is inverted relative to that of the $\alpha S,\beta S$ isomer.

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Stereogenic competition in bilirubin conformational enantiomerism

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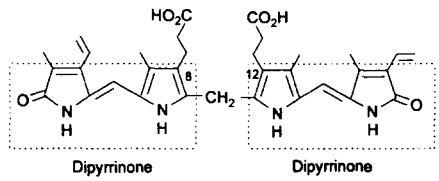
Abstract: Optically active bilirubin analogs (**1** and **2**) with a stereogenic center in each propionic acid side chain were synthesized and examined by CD and NMR spectroscopy. Introduction of an α-methyl and a β-methyl forces the pigment to adopt either a left-handed (***M***) or right-handed (***P***) helicity with a folded, ridge-tile conformation. As evidenced by exciton-type circular dichroism spectra, the (α*S*,α′*S*-**3**), (β*S*,β′*S*-**5**), (α*S*,β′*S*-**1**) stereoisomers strongly prefer the ***M***-helicity ridge-tile conformation, but the (α*R*,β′*S*) stereoisomer (**2**) prefers the ***P***-helicity. © 1997 Elsevier Science Ltd

Introduction

Bilirubin is a hydrophobic metabolite that is poorly excreted and can become toxic if it accumulates in the body. Normally, it does not accumulate to any extent because it is conjugated in the liver to glucuronide metabolites that are less hydrophobic and readily excreted across the liver into bile.[1,2] Optical spectroscopy of bilirubinoid pigments has long been important in the diagnosis of jaundice and in clinical measurements of bile pigments in body tissues. And circular dichroism (CD) spectroscopy has proven to be an excellent technique for elucidating the conformation of the pigment in solution,[3–6] especially in conjunction with NMR measurements.[7]

Bilirubin belongs to the class of pigments called "linear tetrapyrroles"; yet, its solution and biological properties do not correlate well with either the linear structure or a porphyrin-like structure. Its most stable conformation is now known to be bent in the middle, in the shape of a ridge-tile (Figure 1). In the ridge-tile conformation, which is flexible and not rigid, the propionic acid groups are engaged in intramolecular hydrogen bonding to the opposing dipyrrinone lactam and pyrrole components. In bilirubin and its mesobilirubin analogs, where vinyls are replaced by ethyl while retaining propionic acids at C(8) and C(12), considerable conformational stabilization of the ridge-tile shape is achieved through intramolecular hydrogen bonding,[8] leaving the polar groups tucked inward and thus rendering the pigment surprisingly lipophilic.[9]

BILIRUBIN



The ridge-tile conformations of Figure 1 with their near-C_2-symmetry are dissymmetric and enantiomeric. In an isotropic medium, bilirubin and its analog mesobilirubin-XIIIα consist of a 50:50 mixture of equilibrating conformational enantiomers. Displacement of the equilibrium toward one or the other of the enantiomers has been achieved by complexation with a chiral compound, such as quinine[6] or serum albumin,[10] and observed by CD of the pigment. Selective stabilization of one enantiomer can also be achieved through intramolecular nonbonded steric interactions, as has been observed when stereogenic centers are created by methyl substitution at either the α or the β carbons of

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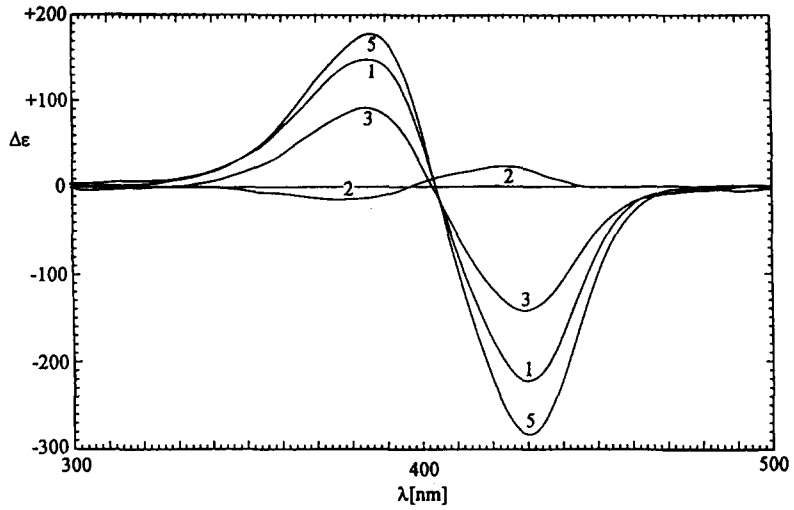

Figure 5. Circular dichroism of 1.5×10^{-5} M solutions of α,β′-dimethylmesobilirubins in methanol. Spectrum 1: **1** (α*S*,β′*S*), Spectrum 2: **2** (α*R*,β′*S*), Spectrum 3: **3** (α*S*,α′*S*), and Spectrum 5: **5** (β*S*,β′*S*) at 22°C.

The solvent $(CH_3)_2SO$ acts in an exceptional way on the spectra. In **1**, **3** and **5** the Cotton effect intensities drop to <10% of the values in *n*-hexane, as they reverse sign – in **1** and in **5**. The CD of **2** also undergoes sign inversion. It seems probable that the favored folded conformation has become somewhat more open (larger θ angle in Figure 1) to accommodate attachment of the solvent molecules.[24] As shown earlier, flattening the ridge-tile leads to a reorientation of the dipyrrinone electric transition dipole moments to near parallelity (and hence to very weak bisignate CEs) and a change in torsion from (−) to (+) without a change in conformational chirality.

Concluding comments

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin and its analogs, is known to be a dominant force in determining their conformation. The current study shows that when the propionic acid residues are substituted with methyl groups at the α and β positions, thus creating stereogenic centers, mesobilirubin-XIIIα is forced by nonbonded steric interactions to adopt either the ***M*** or ***P***-helicity ridge-tile conformation. With α*S*,β′*S* stereocenters, the ***M***-helicity conformer is dominant. However, when the non-bonded interactions operate in conflict, as in the α*R*,β′*S* configuration, a more open ***P***-helicity ridge-tile predominates. In the more open ridge-tile conformation of **2**, the observed exciton chirality CD is weaker and inverted relative to that of the α*S*,β′*S* isomer (**1**).

Experimental

General

All UV–vis spectra were recorded on a Perkin Elmer model 3840 diode array or Cary 219 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a JASCO J-600 instrument. NMR spectra were obtained on a GN-300 or Varian Unity Plus spectrometers operating at 300 MHz and 500 MHz, respectively. $CDCl_3$ solvent (unless otherwise noted) was used and chemical shifts were reported in δ ppm referenced to residual $CHCl_3$ ^1H signal at 7.26 ppm and ^{13}C signal at 77.00 ppm. J-modulated spin-echo experiment (*A*ttached *P*roton *T*est) was used to obtain ^{13}C-NMR spectra. The steady-state NOE enhancements were measured (300 MHz) by difference spectroscopy using 5 sec irradiation time which caused 80–95% saturation of the target signal. The acquisition time was 3.6 sec and the relaxation delay, 10–20 sec. One hundred twenty-eight (128) transients were

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SYNTHESIS AND STEREOCHEMISTRY OF BILIRUBIN ANALOGS LACKING CARBOXYLIC ACIDS

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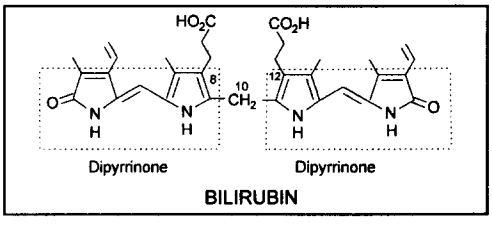
Abstract: Bilirubin analogs with propionic acid groups replaced by *sec*-butyl (**1**), 3-acetoxy-1-methylpropyl (**2**) or 3-acetamido-1-methylpropyl (**3**) were synthesized in enantiopure form and known absolute configuration. Although there are no propionic acid groups to act in stabilizing folded, ridge-tile conformations, the (*S*) stereogenic centers induce the pigments to adopt a left-handed (*M*) helicity — as evidenced by exciton circular dichroism spectra.

INTRODUCTION



Bilirubin is a bis-dipyrrinone dicarboxylic acid formed in normal human metabolism of heme proteins[1,2,3] at a rate of ~300 mg/day, mainly from the breakdown of red blood cells. High levels of serum bilirubin, which is lipophilic and intrinsically unexcretable, can cause irreversible neurologic damage. But the pigment is efficiently eliminated by the liver following uptake and enzymic conversion to water-soluble glucuronides that are promptly secreted into bile. Impaired excretion of the glucuronides occurs in many types of hepatobiliary disease, but retention of native bilirubin is principally observed in newborn babies.[1-3] Accumulation of either native bilirubin or its glucuronides in the body is manifested in jaundice.

Bilirubin is conformationally flexible in solution, but one conformation is significantly more stable than all the others: a ridge-tile structure with intramolecular hydrogen bonds between the pyrrole and lactam functions of the dipyrrinone halves and the propionic carboxyl (or carboxylate) groups (Fig. 1).[4,5] It can adopt porphyrin-shaped conformations, but these are disfavored and of relatively high energy. It might also adopt the linear conformation shown above, but it is especially high energy.[5,6] In fact, the ridge-tile shape is the only one that has been observed in crystals of bilirubin[4a,b] and its carboxylate salts.[4c] Spectroscopic studies, particularly NMR,[7,8,9] supported by energy calculations,[5a,6] strongly suggest that hydrogen-bonded ridge-tile conformers also prevail in solution. Individual ridge-tile conformers of bilirubin are conformational enantiomers that interconvert rapidly[7,8] in solution through a succession of non-planar intermediates in which the hydrogen bonding network is never completely broken.[5a,6] Yet, the energetically most favored ridge-tile conformation (interplanar angle, θ ~100°) is *not* rigid. It is flexible; and small, low energy rotations about the C(9)-C(10) and C(10)-C(11) bonds cause θ to close

dielectric solvents of Table 2. In nonpolar solvents a dimer is apparently dominant; so we modelled dimers of **1** in order to extract information on the stereochemistry of each pigment unit. These results are summarized in the dimeric structures of Figure 6. In the energy-minimum dimer, the two monomer units have essentially identical conformations, with $\phi_1 \sim -95°$, $\phi_2 \sim +13°$ and $\phi_1 \sim -95°$, $\phi_2 \sim +100°$ corresponding to an ***M***-helical rubin. These conformations are predicted[5a] to exhibit strong negative exciton coupling CD spectra. For comparison, the dimer formed of ***P***-helicity monomer units of **1** (Figure 6) lies 0.7 kcal/mole higher energy, and the two monomer units have essentially identical conformations, with $\phi_1 \sim +100°$, $\phi_2 \sim -21°$ and $\phi_1 \sim +100°$, $\phi_2 \sim -27°$. These conformations are predicted to exhibit strong positive exciton coupling Cotton effects. And the dimer formed from ***M*** and ***P***-helical components cannot adopt more than two hydrogen bonds and is higher energy. Since the ***M***-helical dimer is slightly favored over the ***P***, the observed moderate, negative exciton Cotton effects observed in nonpolar solvents (Table 2) are supported by this molecular mechanics analysis of conformation.

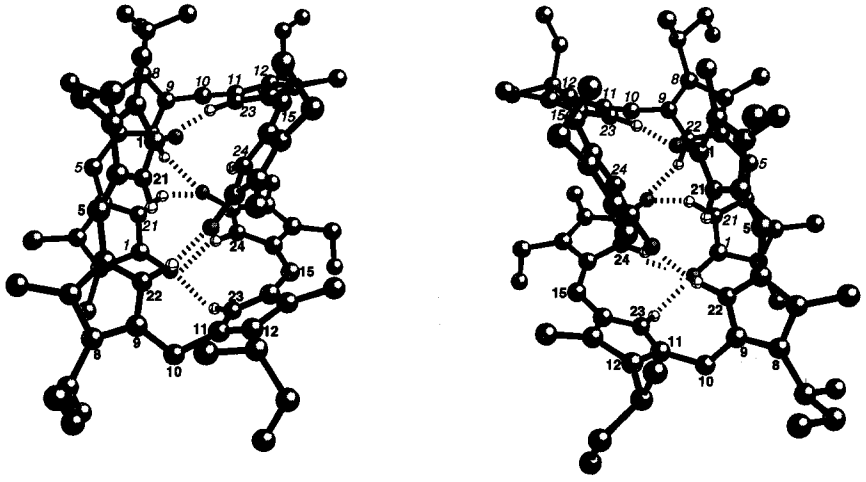


FIGURE 6. Ball and stick representations for low-energy dimers of **1**. (Left) Minimum energy dimer of **1** held by intermolecular hydrogen bonds between the component dipyrrinones. In each component of the dimer, $\phi_1 \sim -95°$, $\phi_2 \sim +13°$, corresponding to an ***M***-helicity. (Right) Higher energy dimer of **1** where each component adopts a ***P***-helicity with $\phi_1 \sim +100°$, $\phi_2 \sim -27°$. Hydrogens are removed from carbons for clarity of representation. Hydrogen bonds are shown by hatchmarks. In each dimer, bold numbering corresponds to one monomer unit; italics numbering corresponds to the second monomer unit.

CONCLUDING COMMENTS

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin, is known to be a dominant force in determining the pigment's conformation (Fig. 1). The current study shows that when the propionic acids at C(8) and C(12) are replaced by alkyl or ether groups having stereogenic centers at 8^1 and 12^1, **1** and **2** are forced by nonbonded steric interactions to adopt an ***M***-helicity conformation. When residual intramolecular hydrogen bonding is possible, as in amide **3**, the ***M***-helicity shaped ridge-tile conformer is dominant. Molecular dynamics computations support the interesting and surprising result derived from spectroscopy: the ***M***-helicity conformation is clearly favored, even in the absence of intramolecular hydrogen bonding.

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Enantioselection in bilirubin analogs with only one propionic acid group

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Abstract: Enantiopure synthetic bilirubin analogs (**1** and **2**) with only a single β-methyl propionic acid group adopt a folded, ridge-tile conformation stabilized by intramolecular hydrogen bonding. The β-methyl group forces the pigment to adopt a left-handed (*M*) helical conformation, as evidenced by exciton circular dichroism spectra and indicating that one propionic acid group is sufficient to control the pigment's conformation. © 1997 Elsevier Science Ltd

Introduction

Bilirubin (Figure 1), the yellow–orange neurotoxic pigment of jaundice, is a tetrapyrrole dicarboxylic acid formed in the normal metabolism of heme proteins.[1] In a healthy adult, it is produced at the rate of ~300 mg/day, principally from the breakdown of red blood cells. Bilirubin is a conformationally mobile bichromophore with characteristics of a molecular propeller.

Rotation of its two dipyrrinone chromophores about the central C(10) CH_2 unit generates a large number of conformational isomers, of which folded conformations, shaped like a ridge-tile have minimized non-bonded steric interactions.[2,3] The ridge-tile conformation, which is not rigid, brings the pigment's propionic acid groups into close proximity of the dipyrrinone NH and C=O groups, thus easily engaging a network of six intramolecular hydrogen bonds to make the ridge-tile conformation unusually stable.[2] This inward tucking of the CO_2H groups and tethering to opposing dipyrrinones through intramolecular hydrogen bonding decreases the polarity of the pigment, leaving it unexcretable in normal metabolism (hepatic excretion), except by glucuronidation.[1,4] The ridge-tile conformation is found in crystalline bilirubin and its salts,[5,6] and it is the favored conformation in solution.[7,8] However, when the propionic acid groups are translocated away from C(8) and C(12) [e.g., to C(7) and C(13)] the solution properties of the pigment undergo significant changes.[9] Such pigments are less lipophilic than bilirubin and much less soluble in non-polar organic solvents. They are also typically excretable (hepatic excretion) without glucuronidation.[10] However, analogs with propionic acid groups at C(8) and C(12), such as mesobilirubin-XIIIα, typically share bilirubin's unique lipophilic and hepatic excretion, because they also have their CO_2H groups sequestered by intramolecular hydrogen bonding (Figure 1).

Bilirubin intramolecular hydrogen bonding (Figure 1) is one of the most interesting and important facets of bilirubin conformation.[2–8] Although the two component dipyrrinone units of bilirubin-type molecules may rotate relatively freely and independently about the interconnecting central CH_2 group, two non-superimposable mirror image conformations are uniquely stabilized through an extensive network of intramolecular hydrogen bonds. These conformational enantiomers are known to interconvert fairly rapidly at room temperature over a barrier of ~20 kcal/mole.[7] Our interest in stabilization of pigment stereochemistry through the action of intramolecular hydrogen bonding led us to consider whether such hydrogen bonding might be retained in a bilirubin analog with only one propionic acid group.

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of the solvent molecules.[7] As shown earlier,[2] flattening the ridge-tile leads to a reorientation of the dipyrrinone electric transition dipole moments to near parallelity (and hence to very weak bisignate Cotton effects) and a change in torsion from (−) to (+) without a change in conformational chirality. In aqueous base the Cotton effects remain strong, consistent with an *M*-helicity dianion.

Concluding comments

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin and its analogs, is known to be a dominant force in determining their conformation. The current study shows that only a single propionic acid group at C(8) or C(12) is required for stabilization of the ridge-tile conformation and that when substituted with methyl groups at the β position to create a β(*S*) stereogenic center, **1** and **2** are forced by non-bonded steric interactions to adopt an *M*-helicity ridge-tile conformation.

Experimental

General

All circular dichroism spectra were recorded on a JASCO J-600 instrument, and all UV–Vis spectra were recorded on a Perkin–Elmer Lambda 12 or Cary 219 spectrophotometer. NMR spectra were obtained on GN-300 or Varian Unity Plus spectrometers operating at 300 MHz and 500 MHz, respectively. $CDCl_3$ solvent (unless otherwise noted) was used, and chemical shifts were reported in δ ppm referenced to residual $CHCl_3$ ^1H signal at 7.26 ppm and ^{13}C signal at 77.00 ppm. J-modulated spin–echo experiments (Attached Proton Test) were used to obtain ^{13}C-NMR spectra. Optical rotations were measured on a Perkin–Elmer model 141 polarimeter. HPLC analyses were carried out on a Perkin–Elmer Series 410 high-pressure liquid chromatograph with a Perkin–Elmer LC-95 UV–Vis spectrophotometric detector (set at 420 nm) equipped with a Beckman–Altex ultrasphere IP 5 μm C-18 ODS column (25×0.46 cm) kept at 34°C. The flow rate was 1.0 mL min^{-1}, and the mobile phase was 0.1 M di-*n*-octylamine acetate buffer in 5% aqueous methanol (pH 7.7 at 22°C). Radial chromatography was carried out on Merck Silica gel PF_{254} with $CaSO_4$ preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2 or 4 mm rotors. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ.

Commercial reagents were used as received from Aldrich or Acros. The spectral data were obtained in spectral grade solvents (Aldrich or Fisher). HPLC grade solvents (Fisher) were dried according to standard procedures[18] and distilled prior to use.

(1S,1′S)-3,17-Diethyl-8-(2-carboxy-1-methylethyl)-12-(2-methoxycarbonyl-1-methylethyl)-2,7,13,18-tetramethyl-(21H,24H)-bilin-1,19-dione (5, βS,β′S-dimethylmesobiliverdin-XIIIα methyl ester)

A mixture of optically pure dipyrrinones **8**[8a] (495 mg, 1.5 mmol) and **9**[11] (474 mg, 1.5 mmol), *p*-chloranil (1.85 g, 7.5 mmol), formic acid (33 mL), and CH_2Cl_2 (660 mL) was heated at reflux for 24 h. The volume was reduced by distillation to one-half and reflux was continued for 6 h. The mixture was then chilled overnight at −20°C. The separated solid was filtered, the blue filtrate was washed with H_2O (3×300 mL), dried (Na_2SO_4), filtered, and the solvent was removed under vacuum. The crude mixture of three verdins (**5, 6** and **7**) was separated by radial chromatography (4–6% CH_3OH/CH_2Cl_2) collecting the bright blue band with medium polarity. After removing the solvent, 324 mg (69%) of pure verdin monomethyl ester **5** was obtained. Mp 196–198°C; $[\alpha]_{436}^{20}$ −2680 (*c* 3.9×10^{-3}, $CHCl_3$); ^1H-NMR: δ 1.25 (6H, t, J=7.6 Hz), 1.27 (3H, d, J=7.1 Hz), 1.38 (3H, d, J=7.1 Hz), 1.84 (3H, s), 1.86 (3H, s), 2.15 (3H, s), 2.21 (3H, s), 2.52 (4H, q, J=7.6 Hz), 2.57 (2H, d, J=7.2 Hz), 2.65 (2H, d, J=7.2 Hz), 3.30 (1H, m), 3.54 (1H, m), 3.56 (3H, s), 6.01 (1H, s), 6.15 (1H, s), 6.92 (1H, s), 8.36 (1H, br. s), 9.31 (1H, br. s) ppm; ^{13}C-NMR: δ 8.08, 8.20, 10.43, 10.75, 14.46, 17.82, 17.90, 20.43, 20.64, 27.83, 27.89, 40.29, 41.19, 51.58, 96.76, 97.96, 115.01, 126.07, 127.48, 128.19, 128.46, 137.34, 137.94, 139.51, 140.70, 144.11, 146.85, 147.84, 171.92, 172.42, 174.64, 176.28 ppm.

CHIRALITY 9:604–615 (1997)

Exciton Chirality of Bilirubin Homologs

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Department of Chemistry, University of Nevada, Reno, Nevada

ABSTRACT Bilirubin, the yellow pigment of jaundice, is a bichromophoric tetrapyrrole that readily adopts either of two enantiomeric, folded conformations shaped like ridge-tiles and stabilized by a network of six intramolecular hydrogen bonds. Interconversion of these **M** and **P** helical chirality conformational enantiomers is rapid at room temperature but may be displaced toward either enantiomer by intramolecular nonbonded steric interactions. Introduction of a methyl group at the β and β′ carbons of the propionic acid chains on the symmetric bilirubin analog, mesobilirubin-XIIIα, shifts the conformational equilibrium toward the **M** or the **P**-chirality intramolecularly hydrogen-bonded conformer, depending only on the *S* or *R* stereochemistry at β and β′, resulting in pigments with intense exciton coupling circular dichroism (CD) for the ~430 nm transition(s). Optically active synthetic analogs of bilirubin with propionic acid groups lengthened systematically to heptanoic acid **(1–5)** were synthesized and examined by spectroscopy to explore the influence of alkanoic acid chain length on conformation and intramolecular hydrogen bonding. In these diacids and their dimethyl esters **(6–10),** strong exciton chirality CD spectra are observed, and the data are correlated with molecular helicity. *Chirality 9:604–615, 1997.*

KEY WORDS: circular dichroism; mesobilirubins; conformation; ridge-tile shape; molecular helicity

Bilirubin (Fig. 1) is a tetrapyrrole dicarboxylic acid produced in normal metabolism in mammals from heme proteins.[1–3] In a healthy adult, bilirubin is formed at the rate of ~300 mg/day, principally from the turnover of red blood cells. It is intrinsically unexcretable but is efficiently eliminated by the liver following uptake and enzymic conversion to water-soluble glucuronides that are promptly secreted into bile. Impaired excretion of the glucuronides occurs in many types of hepatobiliary disease, but retention of native bilirubin is principally observed in newborn babies.[1–3] Accumulation of either native bilirubin or its glucuronides in the body is manifested in jaundice.

Bilirubin belongs to the class of pigments called "linear tetrapyrroles"; yet, its solution and biological properties do not correlate well with either the linear (Fig. 1A) or a porphyrin-like (Fig. 1B) shape in which the polar carboxyl and lactam groups are freely solvated.[2,3] The preferred conformation, in organic solvents and in aqueous base, is now known to be neither linear nor porphyrin-like, which are sterically disfavored conformations.[4] Rather, bilirubin is bent in the middle and adopts the shape of a half-opened book or a ridge-tile. In this conformation, the propionic acid groups readily engage in intra-molecular hydrogen bonding to the opposing dipyrrinone lactam and pyrrole components. In bilirubin and its analogs with propionic acids at C-8 and C-12, hydrogen bonding provides considerable conformational stabilization of the ridge-tile shape, while tucking the polar groups inward and thereby rendering the pigment surprisingly lipophilic.[5]

The ridge-tile conformation is dissymmetric (with near C_2 symmetry); thus, bilirubin can adopt either of the two nonsuperimposable mirror image conformations shown in Figure 1C. Solutions of bilirubin in isotropic media can therefore be thought of as a 50:50 mixture of equilibrating conformational enantiomers. Displacement of the equilibrium toward one or the other of the enantiomers can be achieved by complexation with a chiral compound, such as quinine[6] or serum albumin,[7,8] and by the action of intramolecular nonbonded steric repulsions, as has been observed when stereogenic centers are created by methyl substitution at either the α or the β carbons of the propionic acid chains.[9,10] Such "intramolecular resolution" can be observed by circular dichroism (CD) spectroscopy. In the current work, we measure and analyze the CD spectra of new chiral analogs (Fig. 2) of bilirubin that are designed to probe how the length of the alkanoic acid chains influences the conformational equilibrium displayed in Figure 1C.

MATERIALS AND METHODS

Instruments

All circular dichroism spectra were recorded on a JASCO J-600 instrument, and all UV-vis spectra were recorded on a Perkin-Elmer (Norwalk, CT) Lambda 12 or

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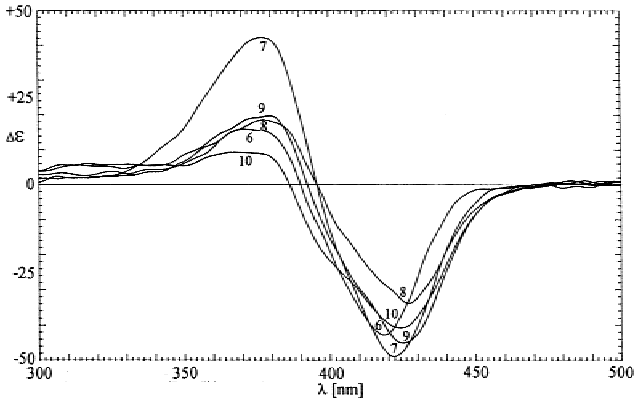


Fig. 5. Circular dichroism spectra of ~1 × 10^{-5} M solutions of (βS,β′S)-mesobilirubin-XIIIα dimethyl ester **(6)** and its homologs **7–10** in methanol at 22°C. The number on each curve corresponds to the pigment number.

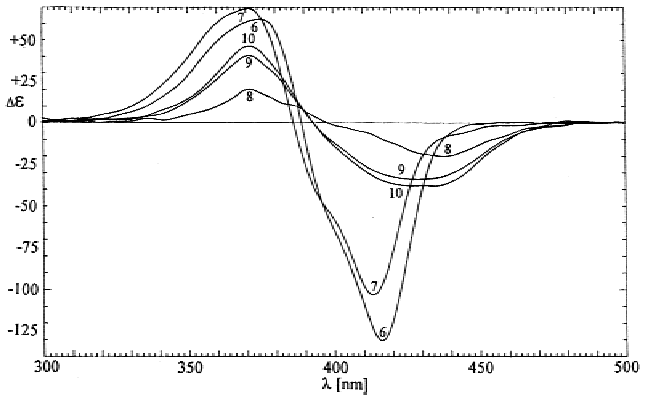


Fig. 6. Circular dichroism spectra of ~1 × 10^{-5} M solutions of (βS,β′S)-mesobilirubin-XIIIα dimethyl ester **(6)** and its homologs **7–10** in chloroform at 22°C. The number on each curve corresponds to the pigment number.

range of solvents (Table 4) and consistently indicate a negative exciton chirality, except for **6** in $(CH_3)_2SO$, $HCON(CH_3)_2$, and $HCONHCH_3$ solvents. The data suggest that the two dipyrrinone chromophores adopt a similar relative orientation, probably with **M**-helicity, governed only by the *S*-configuration of the stereogenic center in the alkanoic ester chain. The preferred helical orientation adopted by the dipyrrinone chromophores is probably forced by steric crowding operating on the dipyrrinones in cogwheel fashion.[22] Only when the alkanoic ester chains are short, as in β-methylpropionate and γ-methylbutyrate esters (**6** and **7**), may the behavior depart from the norm established by the longer chains. That is, in non-polar solvents promoting hydrogen bonding, the CE magnitudes are enhanced (compare Fig. 6). And in the unique β-methylpropionate ester analog a CE sign-reversal is observed in $(CH_3)_2SO$, $HCON(CH_3)_2$, and $HCONHCH_3$ solvents. The precise reason for the inversion occurring only with the β-methylpropionate ester chains is unclear.

The conclusions on the molecular shape of **6** and **7,** derived from the CD data, are further supported by UV-vis spectral data for the long wavelength exciton transitions near 400 nm (Table 5). The data indicate two exciton transitions, as expected, but unlike the spectra of rubin acids (Table 3), the shorter wavelength (higher energy) exciton transition is typically more intense than the lower energy in nonpolar solvents that preserve intermolecular hydrogen bonding. In hydrogen bonding solvents that interfere with dimerization, the longer wavelength exciton transition is usually somewhat more intense. As indicated previously,[4] these data correspond in the latter to ridge-tile shapes akin to those of Figure 1C, but in solvents that do not appreciably interfere with dimer formation through intermolecular hydrogen bonding, the preferred shape of **6** and **7**—and even more likely in **8–10**—tends toward helical porphyrin-like.

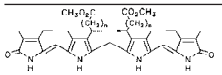
Given the strong bias toward negative exciton chirality in esters **6–10,** probably without recourse to intramolecular hydrogen bonding, the CD behavior of the corresponding rubin diacids becomes more understandable. The negative exciton chirality CEs observed for the propanoate ester **(6)** are greatly intensified in the propanoic acid **(1)** due to the influence of strong intramolecular hydrogen bonding defining an **M**-helicity pigment conformation with inherently strong CEs.[4,10,17] The peculiarity seen in the ester in $(CH_3)_2SO$ carries over to the acid, but not with the other solvents. These data are consistent with a more open ridge-tile angle.[4,10,17] In the example of the γ-methylbutyric acid chain **(2),** however, the inherent bias found in the γ-methylbutyrate ester **(7)** for a negative exciton chirality is largely overcome in nonpolar solvents, where intramolecular hydrogen bonding is expected to be strongest (compare data of Tables 2 and 4, and Figs. 3 and 6). If hydrogen bonding were not in force in the γ-methylbutyric acid rubin, then the CD spectra of the acid and ester would be expected to be essentially the same. In the rubin butanoic acid **(2),** the ridge-tile conformation of Figure 1C probably has a larger inter-planar angle—as suggested by earlier analysis[4,23] and imposed by intramolecular hydrogen bonding. However, the change from negative exciton chirality in **1** to positive exciton chirality in **2** does not necessarily imply a change in molecular helicity, as noted previously.[23] It requires simply that the relevant transition dipole moments change helicity, which happens as the ridge-tile conformation opens.[4]

Less dramatic influence of the carboxylic acid groups is seen when comparing the CD data for longer chain acids **3–5** (Table 2) to their ester analogs **8–10** (Table 4). A negative exciton chirality predominates, and the simplest interpretation is thus one of a predominance of negative molecular helicity. In nonpolar solvents, where hydrogen bonding forces can be expected to be more effective, the CE intensities are larger for the acids than for the corresponding esters; however, even in polar solvents there are noticeable large differences in CE intensities—all pointing to the influence of hydrogen bonding on the conformation of **3–5** and hence the exquisite sensitivity of the relative orientation of then component dipyrrinone chromophores to their CD spectra.[4,21]

CONCLUSIONS

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin and its analogs, is known to be a

TABLE 5. Solvent dependence of UV-vis spectra of (βS,β′S)-mesobilirubin-XIIIα dimethyl ester (6) and its homologs



		6 (n = 1)		**7** (n = 2)		**8** (n = 3)		**9** (n = 4)		**10** (n = 5)	
Solvent	ϵ^a	ϵ^{max}	λ (nm)	ϵ^{max}	λ (nm)	ϵ^{max}	λ (nm)	ϵ^{max}	λ (nm)	ϵ^{max}	λ (nm)
Hexane	1.9	72900	376	74400	372	79900	375	78700	376	78100	376
		sh 13100	430	sh 13800	428	sh 14600	428	sh 14100	433	sh 15500	433
Benzene	2.3	56300	388	65800	380	75600	380	74300	380	74000	380
		51200	415			sh 16200	428	sh 16300	433	sh 17600	430
$CHCl_3$	4.7	sh 55700	398	53000	386	68500	379	73300	380	73200	379
		59300	416	sh 45200	413	sh 22400	420	sh 17000	434	sh 17600	430
$CH_3CO_2C_2H_5$	6.0	60900	377	68700	372	75600	376	77500	375	77700	376
		sh 37600	408			sh 19300	420			sh 17600	428
THF	7.3	52500	387	62000	380	52300	381	69400	379	71100	386
		sh 42000	410	sh 31300	416					sh 25300	423
CH_2Cl_2	8.9	54400	391	64700	377	73500	378	74500	378	77800	378
		54100	412	sh 27000	417	sh 15400	428			sh 15500	434
$ClCH_2CH_2Cl$	10.4	53500	385	66600	378	71800	378	77000	379	77600	379
		48100	409			sh 13100	435	sh 17000	428	sh 16900	433
$CH_3(CH_2)_3OH$	17.1	55900	421	50500	394	49900	396	50000	379	49000	397
				49400	420	52300	425	52900	428	52700	430
$CH_3(CH_2)_2OH$	20.1	57400	420	50100	393	49000	397	49400	397	47700	397
				49500	419	52300	426	53700	429	53500	431
$(CH_3)_2CO$	20.7	56400	380	64300	377	67800	377	69100	378	71500	378
		sh 42000	410	sh 29900	414	sh 27000	422	sh 27400	421	sh 26700	422
C_2H_5OH	24.3	56800	421	50800	393	49800	398	49900	399	sh 48300	398
				51600	421	55200	428	56500	430	57300	433
CH_3OH	32.6	57800	421	50300	391	50400	397	sh 51000	400	sh 49400	400
				50600	418	54800	426	57400	430	58400	432
CH_3CN	36.2	62800	375	68500	374	73100	374	77200	375	75400	376
								sh 17000	427	sh 19000	422
$HCON(CH_3)_2$	36.7	50100	393	50200	387	sh 50900	395	sh 50300	397	sh 48500	394
		53500	420	51100	416	56500	423	58200	426	59800	426
$(CH_3)_2SO$	46.5	sh 47800	396	sh 48800	396	sh 48600	396	sh 46600	398	sh 45000	398
		59800	427	56800	427	63100	429	64900	433	66700	435
$HCONHCH_3$	182.4	47800	397	49500	394	48000	397	sh 48500	402	sh 47100	398
		51900	424	49700	422	53300	428	56200	430	58200	433

[a]Solvent dielectric constant.[24]

dominant force in determining their conformation. When the propanoic acid residues are substituted with methyl groups at the β positions to create *S*-configuration stereogenic centers, **1** is forced by nonbonded steric interactions to adopt the **M**-helicity ridge-tile conformation, as determined by CD spectroscopy and the exciton chirality rule.[10] The current study shows that even in the absence of intramolecular hydrogen bonding, non-bonded steric interaction alone are sufficient to lead to a preference for one helical enantiomer and to produce much weaker but still intense negative exciton chirality CD curves in the dimethyl ester **(6)** of **1**—and in its ester homologs **7–10**—probably corresponding to an **M** molecular helicity. When the propionic acids of (βS,β′S)-dimethylmesobilirubin-XIIIα **(1)** are lengthened to pentanoic **(3),** hexanoic **(4)** and heptanoic **(5)** acids the pigments still exhibit negative exciton chirality CD curves, but the γ-methylbutyric acid analog **(2)** exhibits mainly positive exciton chirality CD curves. These data emphasize the importance of hydrogen bonding on rubin CD spectra and the sensitivity of CD to small changes in the orientation of the exciton component chromophores.

ACKNOWLEDGEMENTS

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α-Halogenation of Ester Enolates Containing an N-Unprotected Pyrrole Nucleus

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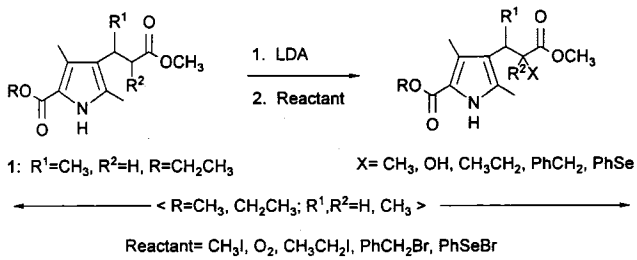
Abstract: The reaction of ester enolates derived from unprotected (1*H*-pyrrol-3-yl)propionates with CCl_4, CBr_4, and I_2 cleanly provides α-halogenated esters.

The 3-dimensional structure of bilirubin, the yellow pigment of jaundice, has been studied extensively by X-ray crystallography, NMR, UV-visible and CD spectroscopy.[1] Spectral studies were carried out on the natural pigment, as well as on specifically altered synthetic analogs. Such model compounds have intrinsically fascinating properties controlled by their stereochemistry and related to hydrogen bonding.[1-3] In our recent synthetic studies we focussed attention on enantiopure bilirubin analogs possessing methyl substituents in propionic acid chains.[3] These required the preparation of various pyrrole precursors from a common starting material, enantiomerically resolved monopyrrole **1**.[3a] The propionate ester chain was successfully α-alkylated, α-oxygenated or α-phenylselenated (Scheme 1) applying the rich chemistry of nucleophilic enolate anions[4] and appropriate alkyl halides,[3c,5a,c] oxygen,[5b] or phenylselenyl bromide.[5c]

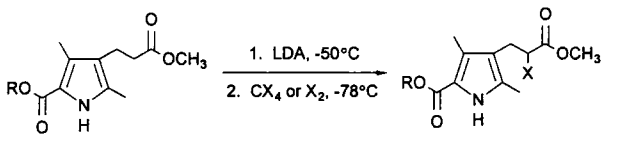


Scheme 1

We found that it was not necessary to protect the pyrrole NH group to prevent side reactions associated with deprotonation by LDA. In fact, alkylation of **1** with CH_3I (Scheme 1) went smoothly; whereas when the pyrrole NH was protected as a tBoc derivative alkylation diastereoselectivity decreased drastically,[5a] which suggests participation of the deprotonated pyrrole nitrogen in a chelation process. In this communication we present our results from α-halogenation reactions on ester enolates containing an *unprotected* pyrrole NH group.

Rathke and Lindert[6] described the α-halogenation of lithium ester enolates derived from simple alkanoates and LICA, using elemental bromine or iodine - a reaction that proceeds under mild conditions and in very high yield. Similar conditions were later applied for α-iodinating alkenoate ester enolates (using LDA as base),[7] as well as α-iodination of aldehydes (using KH as base)[8] and α-bromination of ketones (using LDA as base).[9] Arnold and Kulenovic[10] found that simple alkanoate ester enolates can be quenched at low temperature with CCl_4 or CBr_4 to afford α-chlorinated and α-brominated esters, respectively. α-Chlorination with CCl_4 was also achieved on the free alkenoic acid dianion.[11] More recently, dianions obtained from monosubstituted pyrroles containing acetate and α-dimethylaminoacetonitrile functionalities were selectively C-alkylated and acylated without resort to pyrrole nitrogen protection.[12]

Preliminary observations on the preparation and reactivity of the ester enolate derived from methyl 3,5-dimethyl-4-methoxycarbonylethyl-1*H*-pyrrole-2-carboxylate (Scheme 2) indicated that addition of CCl_4, either in THF solution or neat, to the enolate at -50°C led to complete conversion of the starting ester to α-chloroester **2** within 45 min. The latter could be isolated in 45-60% yield from the dark-brown crude reaction mixture after radial chromatography on silica gel, then recrystallization.



Scheme 2

Lowering the reaction temperature to -78°C and addition of neat CCl_4 gave a somewhat higher yield of **2**, but tarry material was still present. Rathke and Lindert[6] noticed similar further reactions of an α-iodoester when it was generated in presence of excess enolate. To overcome the side reaction, they recommended inverse addition of enolate to the halogen. We adopted this protocol, not only for iodination of our enolates but also for chlorination and bromination using CCl_4 or CBr_4, respectively (Scheme 2). Thus, we found that inverse addition of a cooled (-78°C) enolate solution to solutions of CCl_4 or CBr_4 at -78°C (see General procedure[13]) was superior to the earlier reported procedures.[10,11]

Our results from pyrrole enolate α-halogenation reactions and the spectral characteristics of the synthesized compounds **2-7** are shown in the Table.

Repeated experiments on a 50 mmol scale gave 72-76% of pure α-chloroesters **2** and **5** after a single recrystallization, without the chromatography purification step. α-Iodoesters **4** and **7** were obtained (on 5 mmol scale) in sufficient purity after chromatographic filtration (91-93% yield).

In conclusion, we have described a high yielding, smooth procedure to afford clean α-halogenation (Cl, Br, I) of ester enolates containing the N-unprotected pyrrole nucleus.

Acknowledgement

We thank the National Institutes of Health (HD 17779) for generous support of this work. Dr. Boiadjiev is on leave from the Institute of Organic Chemistry, Bulgarian Academy of Sciences.

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Altering the Acidity and Solution Properties of Bilirubin. Methoxy and Methylthio Substituents

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Substitution of electron-withdrawing groups at the α-position of an aliphatic carboxylic acid can be expected to increase the acidity of the acid. Methoxy and methylthio groups are especially effective; they increase the acidity of acetic acid by ∼1.1 pK_a units. Bilirubin, the water-insoluble pigment of jaundice, has two propionic acids, and an α-methoxy or α-methylthio substituent in each propionic acid can be expected to lower the pK_a similarly and thus alter its solubility properties. (A previously synthesized analogue, α,α′-difluororubin (**4**), is soluble in water.) Two new analogues of bilirubin, α,α′-dimethoxyrubin (**1**) and α,α′-bis(methylthio)rubin (**2**), have been synthesized, separated into diastereomers, and analyzed. The isomers are shown by NMR to adopt intramolecularly hydrogen-bonded ridge-tile-shaped conformations. Like bilirubin, both **1** and **2** are insoluble in water. Unlike bilirubin, **1** is soluble in dilute aqueous bicarbonate, but **2** is insoluble, which would not be predicted from the expectation that **1** and **2** have the same pK_a. The data hint at a much larger steric size of SCH_3 relative to OCH_3.

Introduction

Bilirubin and biliverdin (Figure 1) are water-insoluble natural pigments produced in adult humans at a rate of ∼300 mg per day by heme catabolism.[1,2] Biliverdin in various forms is widely distributed in nature[3,4] but is not normally detectable in mammals because of its rapid enzymic reduction to bilirubin. Bilirubin has a much narrower distribution, occurring only in mammals. However, it is clinically important for several reasons:[1,2] its accumulation in blood and extravascular tissue is a useful sign of disease, usually liver disease; it can cause irreversible neurologic damage; it is involved in the formation of gallstones; and it may be an important radical-intercepting antioxidant.[5] In addition, bilirubin and its glucuronides have been extensively studied as paradigmatic models for hepatic glucuronidation and for carrier-mediated hepatic uptake and biliary excretion.[6]

Bilirubin is conformationally flexible in solution and preferentially adopts folded conformations shaped like ridge-tiles stabilized by a network of intramolecular hydrogen bonds between the propionic acid carboxyl groups and lactam/pyrrole functions of the neighboring dipyrrinones (Figure 1, inset).[3,7] In contrast, biliverdin and its naturally occurring analogues adopt nonplanar helical conformations resembling a porphyrin when viewed down the helix axis.[3,8] Although bilirubin can form helical conformers similar to those preferred by biliverdin, these are of relatively high energy.[7] The ridge-

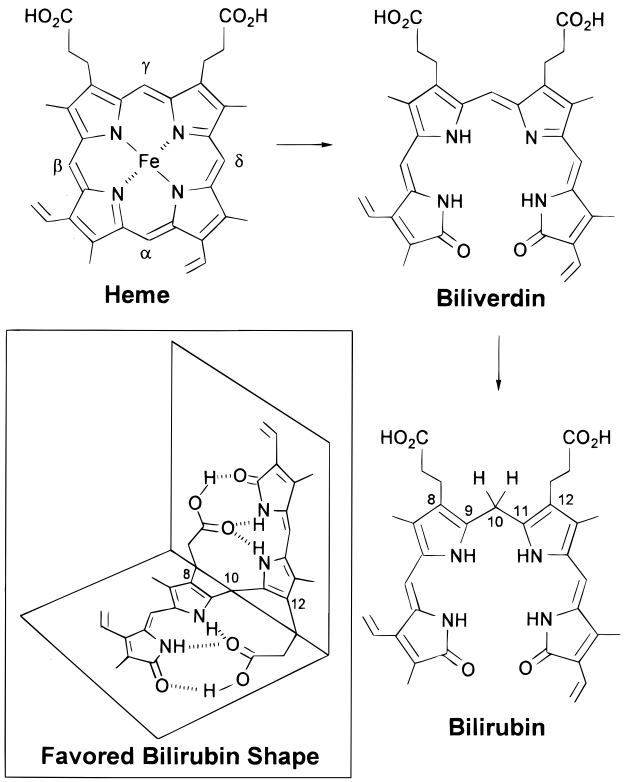

Figure 1. Conversion of heme to bilirubin shown in a prophyrin-like conformation. (Inset) Bilirubin in its energetically most stable, intramolecularly hydrogen-bonded ridge-tile conformation. Only one of two enantiomeric conformers is shown.

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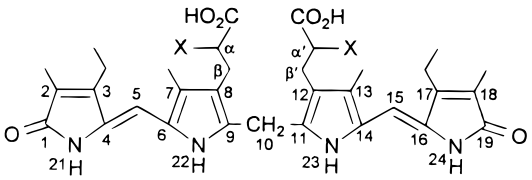
Table 4. Comparison of UV−Vis Spectral Data of α,α′-Dimethoxy (1), Bis(methylthio) (2), and Dimethyl (13) mesobilirubin-XIIIα Analogues and the Parent (3) at 22 °C[a]



pigment, X =	solvent	ϵ^{max} [b]	(λ, nm)	ϵ_{sh} [b,c]	(λ, nm)
rac-**1**, OCH_3	benzene	55 800	(433)	53 200	(418)
rac-**2**, SCH_3		54 200	(431)	51 700	(416)
meso-**2**, SCH_3		56 400	(429)	54 000	(411)
rac-**13**, CH_3		56 300	(431)	50 900	(417)
meso-**13**, CH_3		57 200	(431)	54 600	(412)
3, H		58 800	(435)	54 700	(417)
rac-**1**, OCH_3	$CHCl_3$	54 100	(425)		
rac-**2**, SCH_3		54 000	(431)		
meso-**2**, SCH_3		53 800	(426)	52 500	(410)
rac-**13**, CH_3		56 100	(433)		
meso-**13**, CH_3		55 900	(425)		
3, H		57 800	(431)		
rac-**1**, OCH_3	CH_3OH	58 800	(424)		
rac-**2**, SCH_3		55 800	(422)		
meso-**2**, SCH_3		62 300	(427)		
rac-**13**, CH_3		55 700	(426)		
meso-**13**, CH_3		57 400	(426)		
3, H		50 700	(426)	43 100	(401)
rac-**1**, OCH_3	CH_3CN	54 100	(417)		
rac-**2**, SCH_3		52 700	(417)		
meso-**2**, SCH_3		59 700	(420)	57 600	(402)
rac-**13**, CH_3		55 200	(425)		
meso-**13**, CH_3		58 300	(420)		
3, H		56 700	(422)		
rac-**1**, OCH_3	$(CH_3)_2SO$	58 700	(425)	53 300	(398)
rac-**2**, SCH_3		58 500	(421)	50 600	(396)
meso-**2**, SCH_3		66 800	(430)	60 500	(410)
rac-**13**, CH_3		52 800	(426)	47 300	(407)
meso-**13**, CH_3		61 300	(428)	50 000	(398)
3, H		57 000	(426)	49 100	(397)

[a] Conc ~1.5×10^{-5} M. [b] ϵ in L mol^{-1} cm^{-1}. [c] sh = shoulder.

Solution Properties. Like bilirubin and mesobilirubin-XIIIα (**3**), **1** and **2** are insoluble in water, whereas **4** is water-soluble. Apparently, solubility in neutral pH water can be achieved when the substituent causes the propionic acid pK_a to drop to ~2.6 but not to ~3.7. Whether the methyl groups in the OCH_3 and SCH_3 substituents play a role in determining water insolubility is unclear but might be resolved from an examination of a rubin with α-OH substituents. Like the parent, *rac*-**2** is insoluble in dilute aqueous sodium bicarbonate, but *rac*-**1** is soluble. This is odd, considering that the acidities of α-methoxy and α-(methylthio)acetic acid are nearly the same[26,27] (pK_a ~3.7). Also like bilirubin, the racemic diastereomers *rac*-**1** and *rac*-**2** are soluble in chloroform but insoluble in methanol. This again contrasts with the behavior of either diastereomer of the α,α′-difluororubin (**4**), which are insoluble in chloroform and soluble in methanol. Qualitatively, *rac*-**2** is more soluble in chloroform and less soluble in methanol than *rac*-**1**. Interestingly, the polarity difference between *rac*-**2** and *meso*-**2** is much greater than between *rac*-**1** and *meso*-**1**, so much so that while the diastereomers of **2** could be separated by adsorption chromatography, those of **1** could not. The R_f values for the rubins on silica gel TLC using 1% methanol in dichloromethane are *rac*-**1** (0.07), *rac*-**2** (0.87), *meso*-**2** (0.36), **3** (0.76), **4** (0.00), *rac*-**13** (0.84), and *meso*-**13** (0.46). Significantly, the racemic and meso diastereomers of **2** exhibit very different solubility properties: like bilirubin, *rac*-**2** is insoluble in methanol but freely soluble in chloroform, but *meso*-**2** is more soluble in methanol and much less soluble in chloroform. The solubility properties of **1** and **2** are consistent with intramolecularly hydrogen-bonded conformations (Figure 1 (inset)).

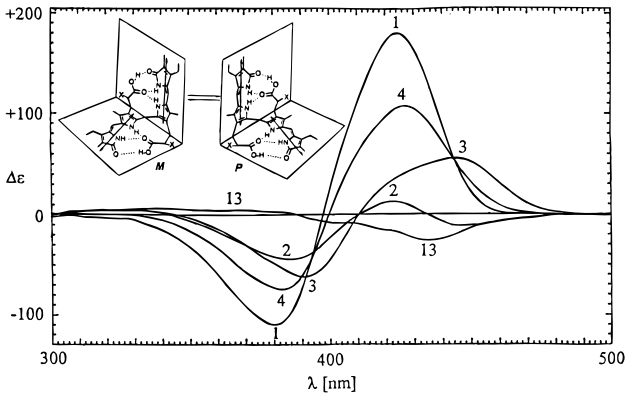


Figure 5. Circular dichroism of *rac*-**1** (X = OCH_3, curve 1), *rac*-**2** (X = SCH_3, curve 2), *rac*-**13** (X = CH_3, curve 13), **3** (X = H, curve 3), and **4** (X = F, curve 4) on human serum albumin (HSA) in pH 7.4 phosphate buffer at 22 °C. The concentration of pigment is ~2×10^{-5} M and that of HSA is 4×10^{-5} M. (Inset) Interconverting intramolecularly hydrogen-bonded enantiomeric conformations of mesobilirubins (**1**−**4**). The double-headed arrows represent the dipyrrinone long-wavelength electric dipole transition moments (vectors), and the relative helicities of the vectors are given as M, minus, or P, plus.

Concluding Comments

Substitution of methoxy and methylthio groups at the α-carbon of the propionic acid side chains of bilirubin is expected to lead to the same reduced pK_a and similar solution properties. Synthetic analogues **1** (α,α′-dimethoxymesobilirubin-XIIIα) and **2** (α,α′-bis(methylthio)mesobilirubin-XIIIα) behave differently, however. Although neither are soluble in water, like α,α′-difluoromesobilirubin-XIIIα (**4**), *rac*-**1** is soluble in dilute bicarbonate, while *rac*-**2** is insoluble. Like bilirubin, both **1** and **2** are soluble in chloroform and insoluble in methanol, and NMR spectroscopic analysis confirms that the pigments adopt intramolecularly hydrogen-bonded conformations shaped like ridge-tiles. Very large induced Cotton effects are found in the circular dichroism spectra of **1** in pH 7.4 phosphate buffered aqueous human serum albumin (HSA). Much weaker Cotton effects are found for **2**. Thus, although the same pK_a values are predicted for **1** and **2** and they adopt similar conformations, their solubility in dilute base and their complexation with HSA differ. The data, taken collectively, suggest a larger steric size of SCH_3 over OCH_3.

Experimental Section

NMR spectra were obtained at 300 and 500 MHz in $CDCl_3$ solvent (unless otherwise noted), and chemical shifts were reported in δ ppm. Analytical thin-layer chromatography (TLC), radial chromatography, and HPLC analyses were carried out as reported previously.[28] Melting points are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Dimethyl malonate, pentane-2,4-dione, methyl methacrylate, diisopropylamine, *n*-butyllithium in hexane, *p*-chloranil, and sodium borohydride were from Ald-

Pergamon

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Optical activity and stereochemistry of linear oligopyrroles and bile pigments †

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Contents

1. Introduction

Bile pigments belong to a class of pigments called linear tetrapyrroles,[1] although recent investigations of their stereochemistry suggest that most are not linear in shape.[1,2] Nonetheless, the term 'linear' satisfactorily distinguishes this class of oligopyrroles from those whose termini are covalently linked, as in the porphyrin macrocycles. Despite the fact that linear tetrapyrroles may assume many differing conformations, the linear representation seems to have been favored by Hans Fischer and is commonly found both in the literature and in texts.

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† Dedicated to Professors Albert Moscowitz (deceased September 1996) and Francesc R. Trull (deceased June 1997).

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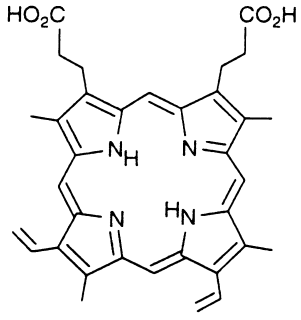


Protoporphyrin-IX

Linear oligopyrroles are widely found in nature, occurring during biosynthesis of cyclic tetrapyrroles and following their catabolism.[1] Bile pigments originate in porphyrin metabolism, usually by destruction of the iron complex of protoporphyrin-IX, called heme.[3] In mammals, an impressive array of bile pigments is produced (Fig. 1), including biliverdin and bilirubin and the fecal pigments arising from bacterial reduction of bilirubin in the gut: urobilin, stercobilin, etc.[4,5] In plants, the photoperiodicity pigment phytochrome, and the algal light-harvesting pigments phycoerythrin and phycocyanin all contain linear tetrapyrrole pigments related structurally to biliverdin and originate through protoporphyrin-IX.[1]

Cursory inspection of these pigments, derived from bilirubin and biliverdin, reveal the presence of stereogenic centers in and around the terminal lactam rings, suggesting the likelihood that these pigments might be found optically active from natural sources. Other, less obvious elements of stereochemistry concern the conformation of the pigments; and as it turns out, molecular conformation of bile pigments can have a dominating effect in their chiroptical measurements.

This was first shown by Moscowitz et al.[6] in their seminal study of the origin of optical activity in the urobilins, made almost 35 years ago without today's more sophisticated instrumentation and greater computational power. This study has, in our view, far wider implications and has profoundly influenced more recent stereochemical research in linear tetrapyrrole field. First, the authors pointed out that some linear tetrapyrroles, depending on their structure, were capable of exhibiting chiroptical phenomena originating not from the common one electron μ-m mechanism. Small alterations of structure, e.g., saturation of only one double bond, may lead to a switch from one optical activity mechanism to another — as will be seen in a comparison of biliverdin and bilirubin optical activity. Secondly, apparently for first time the importance of intramolecular hydrogen bonding was exquisitely and simply demonstrated as a conformation stabilizing force in the stereochemistry of tetrapyrroles. Naturally occurring linear tetrapyrroles bear up to 10 potential donor or acceptor sites for hydrogen bonding, and their mutual (intramolecular) interactions or interactions with the environment (intermolecular) are of the utmost importance for considerations of tetrapyrrole conformation.

In the following discussion of the optical activity of linear tetrapyrroles, we will refer to certain structural units found in them: (i) the dipyrrin (dipyrrylmethene or pyrromethene) chromophore of (blue-green) biliverdin and, especially, (red) urobilin and stercobilin, and (ii) the dipyrrinone (or pyrromethenone) chromophore found in (yellow-orange) bilirubin.

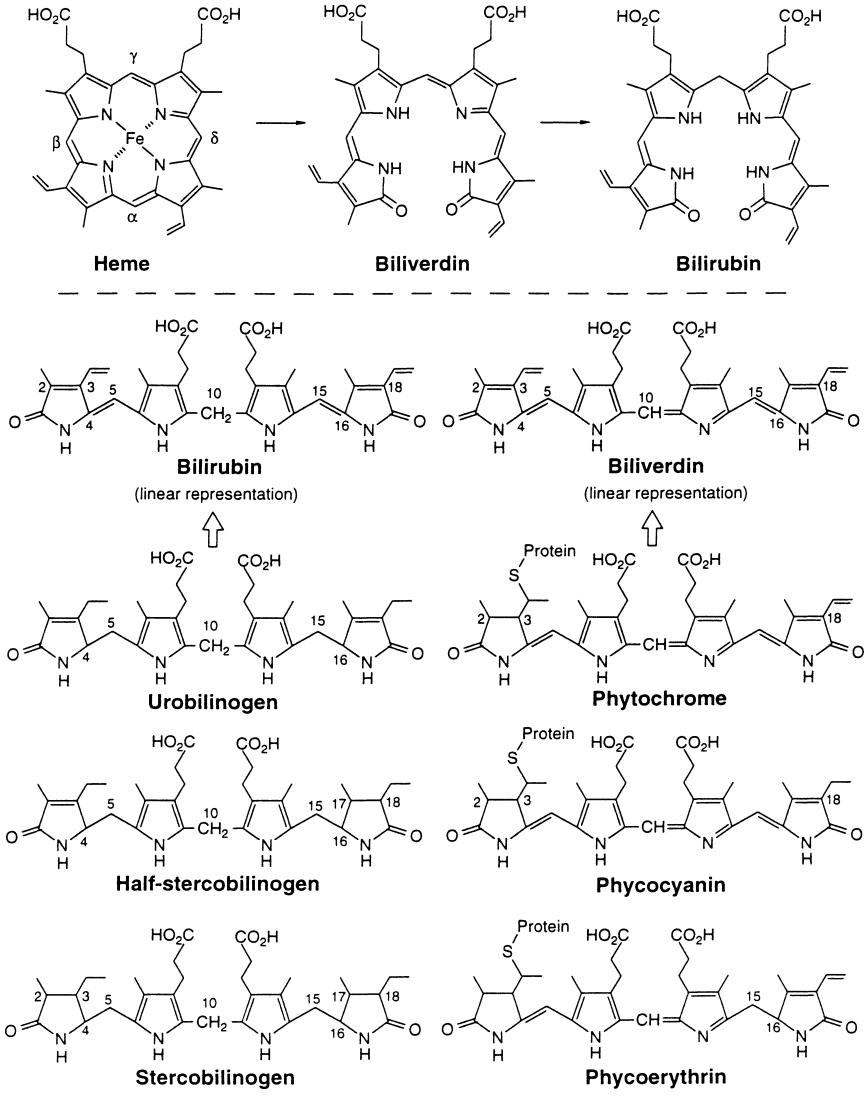
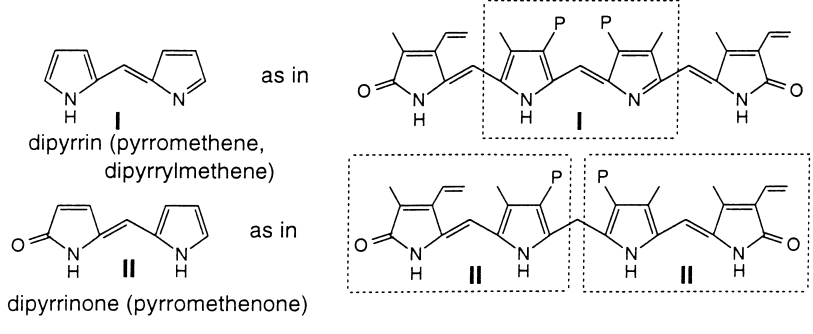


Figure 1. (Upper) Conversion of heme to biliverdin and bilirubin. (Lower left) Reduction of bilirubin to fecal bile pigment-chromogens: urobilinogen, half-stercobilinogen and stercobilinogen. The chromogens are easily oxidized at C(10) to give the corresponding red urobilin, half-stercobilin and stercobilin. (Lower right) Conversion of biliverdin to linear tetrapyrrole plant pigments



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TETRAHEDRON: *ASYMMETRY*

Intramolecular hydrogen bonding and its influence on conformation. Circular dichroism of chiral bilirubin analogs

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Abstract

Enantiopure synthetic bilirubin analogs with variously modified (e.g. alkyl for natural propionic acid or ester) C(8) and C(12) side chains and with but a single chiral center in either or both, exhibited exciton coupled circular dichroism (CD) spectra. The CD intensity is greater when the stereogenic center is in a propionic acid side chain than in an alkyl side chain. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Bilirubin (Fig. 1), the neurotoxic yellow-orange pigment of jaundice, is a tetrapyrrole dicarboxylic acid formed copiously from heme proteins (mainly the hemoglobin of red blood cells).[1–3] It is surprisingly water-insoluble at physiologic pH and does extract into aqueous bicarbonate,[3] peculiarities linked to its remarkable ability to fold into a bent shape, where the component dipyrrinone chromophores rotate about the central C(10) CH_2 to produce a stable conformation shaped like a half-opened book (or ridge-tile).[4–7] Non-bonded intramolecular steric interactions are minimized in such a conformation, which is stabilized by a network of intramolecular hydrogen bonds that bridge each propionic acid group to an opposing dipyrrinone lactam and pyrrole.[8–14] Since the conformation is determined only by the presence of the two dipyrrinones conjoined to a CH_2, and two propionic acids located at C(8) and C(12), a large number of bilirubin isomers (e.g. mesobilirubin-XIIIα, Fig. 1B) differing only in the lactam ring substituents have been found to exhibit the same solution properties as bilirubin and the same stabilized conformation.[4–10,15–18]

Recent studies have shown that stereogenic centers in the propionic acid chains of mesobilirubin-XIIIα, such as β,β′-, α,α′-, α,β′-dimethyl derivatives,[9,10,16,17] dimethoxy or di(methylthio),[19] can exert control over the conformational enantiomerism depicted in Fig. 1C. By minimizing nonbonded intramolecular steric compression interactions arising from such groups in the intramolecularly hydrogen bonded conformer, it was predicted and found that, for example, the (*S*,*S*) configuration methyls act to

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of the dipyrrinone electric transition dipole moments (Fig. 1C) to nearly parallel (and hence to very weak bisignate Cotton effects) due to a more open dihedral angle (θ) forced by intercalating molecules of $(CH_3)_2SO$.

3. Concluding comments

Intramolecular hydrogen bonding, which has a dominating influence on stabilizing the conformation of bilirubin as a ridge-tile shape, is present and very effective even when the rubin has only one propionic acid. The current study shows that a single propionic acid group at C(8) or C(12) is sufficient for conformational stabilization. When a stereogenic center is present in the lone propionic acid chain, the intramolecular nonbonded interactions are sufficient to displace the ***M*⇄*P*** conformational equilibrium in a major way toward either ***M*** (β*S* configuration) or ***P*** (β*R* configuration). When a stereogenic center is absent from the propionic acid chain, e.g. at C(12), but present in a methyl propionate chain at C(8), the unbalancing of the conformational equilibrium is much less effective. When the stereogenic center is in a C(8) alkyl chain, the influence is marginal.

4. Experimental

4.1. General

All circular dichroism spectra were recorded on a Jasco J-600 spectropolarimeter, and all UV–vis spectra were recorded on a Perkin–Elmer lambda 12 or a Cary 219 spectrophotometer. Optical rotations were measured on a Perkin–Elmer model 141 polarimeter. NMR spectra were obtained on a GN-300 or Varian Unity Plus spectrometers at 300 and 500 MHz ^{1}H frequency, respectively. Deuteriochloroform solvent was used throughout and chemical shifts were reported in δ ppm referenced to residual $CHCl_3$ ^{1}H signal at 7.26 ppm and $CDCl_3$ ^{13}C signal at 77.00 ppm. J-modulated spin-echo experiments (attached proton test) were used to obtain the ^{13}C NMR assignments. HPLC analyses were carried out on a Perkin–Elmer series 410 high-pressure liquid chromatograph with a Perkin–Elmer LC-95 UV–vis spectrophotometric detector (set at 420 nm for rubinoid compounds) equipped with a Beckman Altex ultrasphere IP 5 μm C-18 ODS column (25×0.46 cm) kept at 34°C. The flow rate was 1 mL per minute, and the mobile phase was 0.1 M di-*n*-octylamine acetate buffer in 5% H_2O in methanol (v/v) with pH 7.7 at 22°C. Radial chromatography was carried out on Merck silica gel PF_{254} with $CaSO_4$ binder preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2, or 4 mm thick rotors. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. High resolution FAB mass spectra were obtained at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, for samples which were >95% pure by HPLC and ^{13}C NMR.

The spectral data were obtained in spectral grade solvents (Aldrich or Fischer). HPLC grade solvents were dried and purified following standard procedures.[35]

4.2. The starting compounds

3-Ethyl-8-(2-(methoxycarbonyl)ethyl)-2,7,9-trimethyl-1,10-dihydro-(11*H*)-dipyrrin-1-one (**16**, xanthobilirubic acid methyl ester),[23] 3-ethyl-8-propyl-2,7,9-trimethyl-1,10-dihydro-(11*H*)-dipyrrin-1-one (**17**),[20] 8-(2-carboxyethyl)-3-ethyl-2,7,9-trimethyl-1,10-dihydro-(11*H*)-dipyrrin-1-one (**20**, xanthobilirubic acid),[23] and optically pure (+)-(*S*)-3-ethyl-8-(1-methylpropyl)-2,7,9-trimethyl-1,10-

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Dedicated to the memory of the late Professor Raymond N. Castle.

Bilirubin and its analogs are carboxylic acids that engage in intramolecular hydrogen bonding and are thus thought to be monomeric in solution, although the evidence for the molecularity in solution is indirect. Contrastingly, the dimethyl esters favor intermolecular hydrogen bonding and are thought to be dimeric, yet they, like the bilirubin (acids), exhibit essentially no concentration dependence of their NH nmr chemical shifts upon dilution from 10^{-2} to 10^{-5} (or even 10^{-6}) *M* in chloroform-d. Vapor phase osmometry (vpo) studies of chloroform solutions of eight bilirubins and their dimethyl esters clearly indicate that the former are monomeric, while the latter are dimeric — except when a β-methyl group (but not an α*-methyl) is present in each methyl propionate chain. Bilirubin mono-esters might be monomeric or dimeric in solution. Using vpo to study some seven mono-esters or mono acids, we found that the pigments were monomeric in chloroform.

J. Heterocyclic Chem., **37**, 863 (2000).

Introduction.

Bilirubin (Figure 1) is a tetrapyrrole dicarboxylic acid formed in the normal metabolism of heme proteins [1, 2]. In a healthy adult, it is produced at the rate of ~300 mg per day, principally from the breakdown of red blood cells. Bilirubin is intrinsically unexcretable but is efficiently eliminated by the liver, following uptake and enzymic conversion to water-soluble glucuronides that are promptly secreted into bile. Impaired excretion of the glucuronides occurs in many types of hepatobiliary disease, but retention of native bilirubin is principally observed in newborn babies [1-3]. Accumulation of either native bilirubin or its glucuronides in the body is manifested in jaundice.

Bilirubin is a conformationally mobile bichromophore with characteristics of a molecular propeller. Rotation of its two dipyrrinone chromophores about the central C(10) CH_2 unit generates a large number of conformational isomers, of which the folded conformations, shaped like a ridge-tile have their non-bonded steric interactions minimized [4]. The ridge-tile conformation, which is not rigid, brings the pigment's propionic acid groups into close proximity of the dipyrrinone NH and C = O groups, thus easily engaging a network of six intramolecular hydrogen bonds to make the ridge-tile conformation unusually stable [4]. This inward tucking of the CO_2H groups and tethering to opposing dipyrrinones through intramolecular hydrogen bonding decreases the polarity of the pigment, leaving it unexcretable in normal metabolism (hepatic excretion), except by glucuronidation [1-3]. The ridge-tile conformation is found in crystalline bilirubin and its salts [5,6], and it is the favored conformation in solution [4,7,8]. However, when the propionic acid groups are translocated away from C(8) and C(12), *e.g.*, to C(7) and C(13), the solution properties of the pigment undergo significant changes [9]. Such pigments are less lipophilic than bilirubin and much less soluble in non-polar organic solvents. Analogs with propionic acid groups at C(8) and C(12), such as mesobilirubin-XIIIα (Figure 1B), typically mimic bilirubin's unique lipophilic properties and hepatic excretability

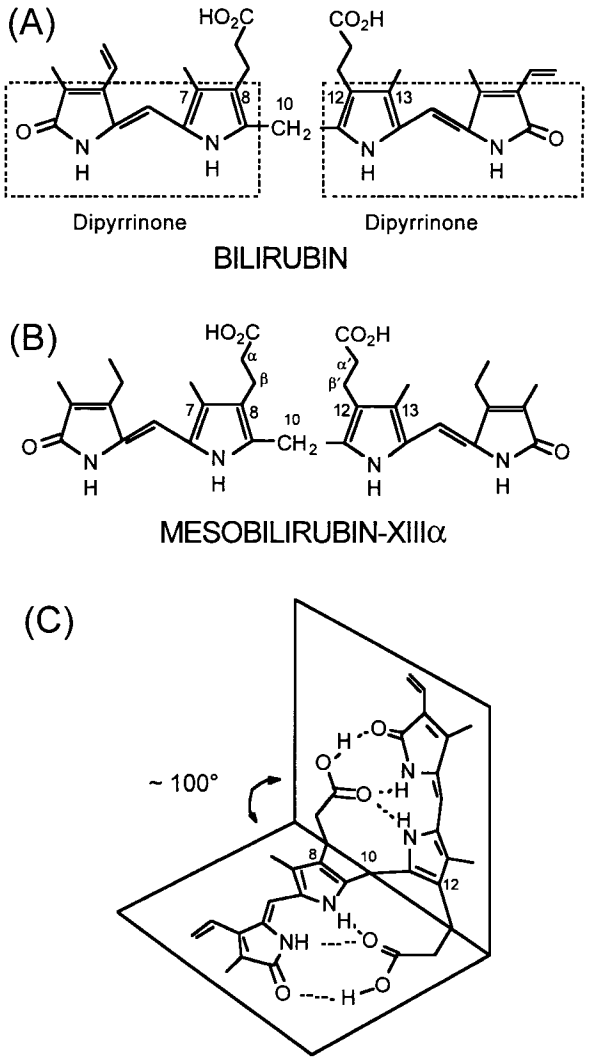

Figure 1. (A) Linear conformation of bilirubin; (B) linear conformation of mesobilirubin-XIIIα; (C) enantiomeric ridge-tile conformation of bilirubin stabilized by intramolecular hydrogen bonding. Mesobilirubin-XIIIα also preferentially adopts a very similar conformation. Hydrogen bonds are indicated by dashed lines.

Steric Size in Conformational Analysis. Steric Compression Analyzed by Circular Dichroism Spectroscopy

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Abstract: The relative steric size of methyl, ethyl, isopropyl, *tert*-butyl, phenyl, and benzyl groups has been determined from a sensitive tetrapyrrole model and exciton coupling circular dichroism (CD) measurements. Unlike the classical cyclohexane model, from which the relative steric demand of functional groups has been assessed quantitatively (*A*-values) and is based on the preference for equatorial vs axial orientations, the bilirubin model assesses substituent size from head-to-head steric compression. Thus, exciton CD amplitudes of a set of sensitive *anti*-chiral α(*R/S*)-substituted-*β′*(*S*)-methylmesobilirubins-XIIIα (**1**−**6**) suggests an apparent relative steric size: *tert*-butyl ∼ isopropyl > phenyl ∼ ethyl > benzyl > methyl. The order is somewhat different from that obtained by measuring equatorial vs axial configuration preferences on substituted cyclohexanes by NMR spectroscopy: *tert*-butyl ≫ phenyl > isopropyl > ethyl ∼ benzyl ∼ methyl.

Introduction

Some 45 years ago, Winstein and Holness[1] defined the "*A*-value" of a substituent group on chair cyclohexane as the thermodynamic preference for the equatorial conformation over the axial: *A*-value $= -\Delta G_{eq}^{\circ} = (RT \ln K_{eq})/1000$ for the axial ⇆ equatorial equilibrium (Figure 1). Over the years, conformational *A*-values have been determined and compiled[2,3] for a diverse array of groups. Such tabulations of *A*-values constitute an invaluable resource for quickly (and quantitatively) assessing the relative steric size of functional groups such as alkyl, hydroxyl, halogen, etc. For example, according to their *A*-values the steric demand of a methyl group ($A_{CH_3} \sim 1.74$) is significantly greater than that of a methoxyl ($A_{OCH_3} \sim 0.75$), which is smaller than that of a methylthio ($A_{SCH_3} \sim 1.00$).[2,3]

Most substituents prefer the equatorial conformation over the axial.[2,3] This may be attributed to the nonbonded steric repulsions between an axial group and the two gauche ring methylene groups at C(3) and C(5) of chair cyclohexane. Such steric repulsions are absent for equatorial groups (Figure 1). The cyclohexane model thus assesses steric size of functional groups on the basis of gauche interactions, and as in every steric model, it assumes a fixed template, i.e., chair conformations with no ring conformational distortion introduced by the functional groups−particularly the axial substituent.

Conformational *A*-values have become the most readily available resource for predicting the relative steric demand of a variety of substituents even outside the cyclohexane framework, e.g., in situations such as molecular recognition studies, where steric demand is based more on a linear than a transverse or lateral buttressing.[4] To assess substituent steric demand in the linear or face-to-face orientation, we used circular dichroism (CD) spectroscopy to extract the steric demand of functional groups located on a very different stereochemical template from that provided by cyclohexanes, viz. the tetrapyrrole mesobilirubin-XIIIα (Figure 2). This tetrapyrrole, a synthetic analogue of bilirubin (the yellow pigment of jaundice[5]), offers a more sterically demanding molecular framework for assessing functional group steric size. Bilirubins are bichromophoric pigments, and the conformational equilibrium of interest involves interconverting conformational enantiomers where the two dipyrrinones pivot about a C(10) methylene connector (Figure 2).[6]

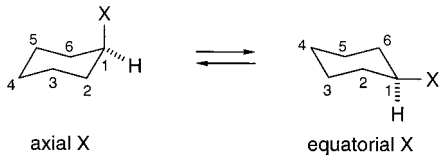

Figure 1. Cyclohexane chair−chair conformational equilibrium that interconverts axial and equatorial X groups. Gauche interactions between axial X and the C(3) and C(5) methylene group of the cyclohexane ring destabilize the axial conformer relative to the equatorial. The *A*-value for group X is defined in terms of the free energy for the equilibrium $A = -\Delta G^{\circ}_{eq} = (RT \ln K_{eq})/1000$.

Previous studies have shown that the most stable conformation of bilirubin and mesobilirubin is shaped like a ridge-tile, with the two dipyrrinones oriented nearly orthogonal. This conformation is further greatly stabilized by six intramolecular hydrogen bonds shared between each dipyrrinone and an opposing propionic acid carboxyl group (Figure 3).[6] Two enantiomeric intramolecularly hydrogen-bonded, ridge-tile conformations are possible, and they were found to interconvert at

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10.1021/ja002069c CCC: $19.00
Published on Web 11/03/2000

Table 4. Comparison of Exciton Chirality **A**-Values and Conformational *A*-Values as Indicators of Functional Group Steric Size in Compounds **1**−**6**

	exciton chirality CD **A**-values[a]				conformational *A*-values[b]	rel steric size from	
	n-hexane	CCl_4	$CHCl_3$	CH_2Cl_2		*A*-values	**A**-values[c]
$C(CH_3)_3$	+513	+497	+450	+441	4.9	2.80	1.81−1.85
C_6H_5	+418	+433	+347	+362	2.87	1.65	1.39−1.59
$CH(CH_3)_2$	+506	+488	+436	+453	2.21	1.27	1.74−1.86
CH_2CH_3	+427	+411	+367	+374	1.79	1.03	1.47−1.54
$CH_2C_6H_5$	+378	+370	+318	+351	1.76	1.01	1.27−1.44
CH_3	+277	+272	+250	+243	1.74	1.00	1.00

[a] From Table 3. [b] From ref 2 (Table 2.2) and ref 29 by NMR: CH_3, CH_2CH_3, $CH(CH_3)_2$ ($CFCl_3$–$CDCl_3$, 300 K), $CH_2C_6H_5$ (CD_2Cl_2, 202 K), $C(CH_3)_3$ (CD_2Cl_2, 153 K), C_6H_5 (CD_2Cl_2, 173 K). [c] Based on CD **A**-values, uncorrected for minor percents of ***M***-helical isomer in **1** (22%), **2** (9%), **5** (8%), and **6** (6%), as detected by ^{1}H NMR in $CDCl_3$. In this solvent, no ***M***-helical isomers could be detected in **3** and **4**.

solvents are consistently less than those of **3** and **4**, generally about the same as **2**, but more than those of **1** and **6**. The CD **A**-values of **1**−**6** thus indicate that phenyl and ethyl are about the same size, that ethyl is larger than benzyl, and both are significiantly larger than methyl. Conformational *A*-values indicate that ethyl, benzyl, and methyl are comparable in steric size. Thus, as determined by the more competitive *anti-chiral* systems **1**−**6**, their CD **A**-values suggest an apparent order for steric size: *tert*-butyl ∼ isopropyl > ethyl ∼ phenyl > benzyl > methyl.

While this ranking does not quite correspond to the relative steric size from conformational *A*-values, *tert*-butyl > phenyl > isopropyl > ethyl ∼ benzyl ∼ methyl,[29] the differences lie most significantly with the isopropyl, phenyl, and ethyl groups. Whether such differences can be attributed to entropy factors or to deformation of the molecular framework is at present unclear. Template mutability is always a concern. For example, the chair cyclohexane template (Figure 1) is not rigid but deformable within certain limits,[3] and any ring deformation caused by one axial group (e.g., *tert*-butyl) would not necessarily be the same as that caused by another (e.g., isopropyl). Recognition of the intrinsic quantitative aspects of steric size might also be compromised in the sterically congested bilirubin template of **1**−**6**, where potential deformation of the ridge-tile template (Figure 4) caused by a *tert*-butyl group might not be matched by an isopropyl, thus lead to a mismatch in the relative order of the group steric size.

Concluding Comments

The relative steric size of common functional groups, *tert*-butyl ∼ isopropyl > ethyl ∼ phenyl > benzyl > methyl, follows from exciton chiraltiy CD **A**-values using a novel conformational model based on the interconverting conformational enantiomers of bilirubin (Figure 3). In the bilirubin template, steric size is determined by head-to-head steric compression. The order differs somewhat from that (*tert*-butyl > phenyl > isopropyl > ethyl ∼ benzyl ∼ methyl) obtained from conformational *A*-values and the chair cyclohexane template, where steric interaction is dominated by gauche interactions.

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Supporting Information Available: Text providing the Experimental Section, including syntheses of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Relative Steric Size of SCH_3, OCH_3, and CH_3 Groups from Circular Dichroism Measurements

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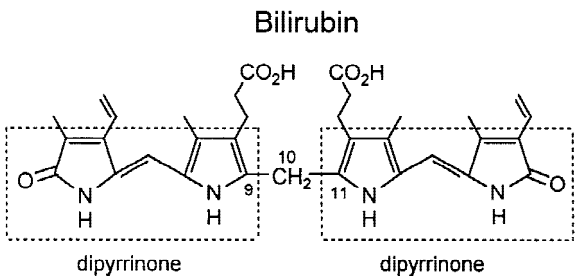
ABSTRACT The relative steric size of methyl, methoxy, and methylthio groups was determined from circular dichroism (CD) spectroscopy using a sensitive system based on the bilirubin model. In the cyclohexane model, equatorial vs. axial orientation and conformational analysis led to quantitative measurements of orientation preference or steric demand: conformational A-values $CH_3 > SCH_3 > OCH_3$. A more sterically demanding model for assessing group size has been found in bilirubin analogs, which are yellow pigments that adopt a ridge-tile shape stabilized by a matrix of intramolecular hydrogen bonds. Optically active bilirubins have been shown to exhibit intense bisignate CD Cotton effects from exciton coupling of their two dipyrrinone chromophores held in either of two enantiomeric ridge-tile conformations. Interconversion of these *M* and *P* conformational enantiomers of helical chirality is rapid at room temperature but may be displaced toward either enantiomer by intramolecular nonbonded steric interactions that arise when substituents are introduced at equivalent sterically demanding sites, *viz.,* the α or β carbons of the pigment's propionic acid chains. Such substituents shift the conformational equilibrium toward the *M* or the *P*-chirality conformer, depending only on the *S* or *R* stereochemistry at the α and β sites, and the resulting exciton CD for the ~430 nm transition(s) was used to evaluate the relative steric size, $SCH_3 > CH_3 > OCH_3$. *Chirality 12:204–215, 2000.*

KEY WORDS: conformational analysis; bilirubin; ridge-tile shape

Bilirubin (Scheme 1) is a water-insoluble yellow-orange tetrapyrrole pigment formed in normal human metabolism at a rate of about 300 mg per day by turnover of hemoglobin and other heme proteins.[1–4] It occurs only in vertebrates and is clinically important for several reasons:[2–4] its accumulation in blood and extravascular tissue (jaundice) is a useful sign of disease, usually liver disease; it can cause irreversible neurologic damage; it is involved in the formation of gallstones; and it may be an important radical-intercepting antioxidant.[5]



Scheme 1

Bilirubin is a bichromophoric pigment consisting of two dipyrrinones conjoined to a CH_2, about which they rotate like a molecular propeller to generate a wide array of conformers. Although conformationally flexible in solution, one conformation is significantly more stable than all the others: a folded, ridge-tile structure with intramolecular hydrogen bonds between the pyrrole and lactam functions of the dipyrrinone halves and the propionic carboxyl (or carboxylate) groups (Fig. 1a).[6] Bilirubin can form porphyrin-like helical conformers, but they are of relatively high energy and the linear conformation (above) is particularly high energy.[6,7] The ridge-tile conformation is the only one that has been observed in crystals of bilirubin[8,9] and its carboxylate salts.[10] Early spectroscopic studies, particularly NMR, have been supported by energy calculations and strongly suggest that hydrogen-bonded ridge-tile conformers also prevail in solution.[6,11,12] Individual ridge-tile conformers of bilirubin are chiral and both enantiomers occur in solution, interconverting rapidly[11,13] via a succession of nonplanar intermediates in which the hydrogen bonding network is never completely broken.[6] Even the energetically most stable conformation (ridge-tile, with interplanar angle, θ ~100°) is flexible. Small, low-energy internal rotations about the C(9)–C(10) and C(10)–C(11)

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TABLE 3. Comparison of CD and UV-vis spectral data of α-substituted-β′S-methylmesobilirubins-XIIIα at 22°C[a]

			CD			UV	
Pigment	Solvent	ϵ^b	$\Delta\epsilon^{max}(\lambda_3)$	λ_2 at $\Delta\epsilon = 0$	$\Delta\epsilon^{max}(\lambda_1)$	ϵ^{max}	λ(nm)
1	CCl_4	2.2	–355 (433)	406	+170 (391)	59,400	435
2			–244 (435)	408	+122 (390)	57,800	436
3			–375 (433)	406	+178 (391)	60,200	435
4			+326 (433)	406	–155 (390)	60,700	435
5			–331 (434)	407	+159 (393)	62,400	436
6			+181 (433)	405	–91 (390)	58,300	434
1	$CHCl_3$	4.7	–301 (432)	406	+167 (387)	56,100	431
2			–226 (433)	407	+126 (387)	55,800	430
3			–320 (432)	406	+181 (388)	57,200	432
4			+255 (431)	405	–151 (386)	55,700	428
5			–288 (434)	407	+167 (389)	58,200	432
6			+151 (428)	402	–99 (384)	56,900	424
1	$(CH_3)_2CO$	20.7	–287 (427)	402	+171 (382)	55,400	423
2			–205 (426)	399	+126 (380)	53,500	417
3			–311 (429)	403	+179 (386)	57,300	426
4			+149 (426)	401	–95 (381)	55,400	416
5			–269 (430)	404	+158 (386)	58,700	427
6			+92 (426)	402	–57 (383)	56,700	418
1	CH_3OH	32.6	–241 (426)	401	+162 (384)	58,000	422
2			–61 (434)	407	+38 (392)	57,500	422
3			–254 (428)	402	+166 (384)	59,000	424
4			+55 (423)	397	–37 (381)	57,000	420
5			–223 (430)	404	+147 (385)	58,900	425
6			+24 (424)	397	–15 (376)	57,700	422
1	CH_3CN	36.2	–272 (427)	401	+161 (382)	54,500	421
2			–209 (425)	399	+124 (379)	52,800	418
3			–296 (427)	401	+179 (383)	56,300	420
4			–8 (409)	395	+12 (382)	54,700	416
5			–258 (429)	403	+155 (384)	57,300	424
6			+91 (428)	403	–55 (385)	55,200	420
1	$(CH_3)_2SO$	46.5	–19 (423)	405	+16 (384)	59,000	427
2			–10 (438)	411	+11 (388)	60,300	427
3			–24 (422)	405	+21 (382)	59,900	426
4			+16 (420)	383	–2 (366)	61,700	427
5			+15 (425)	395	–11 (379)	60,000	428
6			–25 (425)	401	+31 (382)	62,500	428
1	CH_3NHCHO	182.4	–315 (425)	398	+190 (382)	64,500	426
2			–139 (428)	401	+80 (385)	64,500	427
3			–267 (425)	398	+153 (382)	66,700	425
4			+153 (425)	398	–97 (382)	65,900	425
5			–282 (427)	401	+178 (384)	63,500	427
6			+77 (427)	400	–51 (384)	62,800	427

[a]Conc. ~1.5×10^{-5} M.
[b]Dielectric constant from Gordon AJ, Ford RA, The Chemist's Companion. New York: Wiley; (1972). p 4–8.

able that the favored folded conformation has become somewhat more open (larger θ angle in Fig. 1) to accommodate attachment of the solvent molecules. This widens the angle made between the dipyrrinone electric dipole transition moments, a reorientation away from oblique and toward a more parallel in-line.[6] As shown earlier,[31] flattening of the ridge-tile conformation leads to a reorientation of the dipyrrinone electric transition dipole moments to near parallelity (and hence to very weak bisignate CEs) and a change in torsion from (–) to (+) without a change in conformational chirality.

CONCLUDING COMMENTS

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin and its analogs, is known to be a dominant force in determining their conformation. The current study shows that when the propionic acid residues are substituted with methoxy, methylthio, and methyl groups at the α and β positions, thus creating stereogenic centers, mesobilirubin-XIIIα is forced by nonbonded steric interactions to adopt either the *M* or *P*-helicity ridge-tile conformation. With α*S*,β′*S* stereocenters, the *M*-helicity conformer is dominant. However, when the nonbonded interactions operate in conflict, as in the α*S*,β′*S* configuration, this provides an opportunity to extract new information on the relative steric size of the group. The current studies show that an SCH_3 substituent has larger steric demand than an OCH_3, and, surprisingly, that an SCH_3 has a larger steric demand than the CH_3 in this bilirubin model.

Chirality Inversion in a Molecular Exciton

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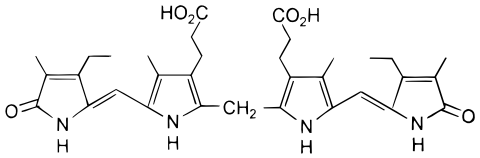
Abstract: The bichromophoric pigment bilirubin acts as a molecular exciton in its UV−visible and circular dichroism (CD) spectroscopy. The optically active analogue, (*βR*,*β′R*)-dimethylmesobilirubin-XIIIα exhibits intense bisignate CD Cotton effects in the region of its long wavelength UV−vis absorption near 400 nm, with $\Delta\epsilon_{434}^{max}$ +337, $\Delta\epsilon_{389}^{max}$ −186 in the nonpolar solvent $CHCl_3$, and nearly as intense Cotton effects in the polar, hydrogen bonding solvent CH_3OH: $\Delta\epsilon_{431}^{max}$ +285, $\Delta\epsilon_{386}^{max}$ −177. Addition of amines leads to Cotton effect sign inversions: in isopropylamine $\Delta\epsilon_{436}^{max}$ −605, $\Delta\epsilon_{392}^{max}$ +375, due to an inversion of molecular chirality.

Introduction

Bilirubin is a water-insoluble yellow-orange pigment, the end product of heme metabolism.[1,2]



Mesobilirubin-XIIIα

It occurs only in vertebrates (∼300 mg/day in healthy adult humans) and is clinically important for several reasons:[1−3] Its accumulation in blood and extravascular tissue is a useful sign of disease, usually liver disease; it can cause irreversible neurologic damage; it is involved in the formation of gallstones; and it is an endogenous inhibitor of free-radical injury.[4] In the form of its ester conjugates with sugars, it is the principal pigment in bile.

Bilirubin belongs to the class of pigments called "linear tetrapyrroles,"[2,5] but its solution and biological properties do not correlate well with either the linear (Figure 1A) or a porphyrin-like shape in which the polar carboxyl and lactam groups are freely solvated. Bilirubin is conformationally flexible in solution, but one conformation is significantly more stable than all of the others: a folded, ridge-tile structure with intramolecular hydrogen bonds linking the dipyrrinone pyrrole and lactam functions to the propionic carboxyl (or carboxylate) groups (Figure 1).[6−8] Although bilirubin can form helical conformers, they are of relatively high energy, and the linear (Figure 1A) and porphyrin-like conformations are especially high energy.[8,9] The ridge-tile conformation is the only one that has been observed in crystals of bilirubin[6,7] and its carboxylate salts.[10] Early spectroscopic studies, particularly by NMR, strongly suggested that hydrogen-bonded ridge-tile conformers also prevail in solution, even in the dipolar protophilic solvent dimethyl sulfoxide.[11] Such indications were supported recently by $^{13}C\{^1H\}$-heteronuclear Overhauser effect (NOE) measurements[12] and by energy calculations.[8,9] The ridge-tile conformation is dissymmetric, and bilirubin can adopt either of two nonsuperimposable mirror-image conformations (Figure 1B). Both enantiomers occur in the crystal[6,7] and in solution,[13] interconverting rapidly[11] in solution via a succession of nonplanar intermediates in which the hydrogen bonding network is never completely broken.[8] The energetically most favored ridge-tile conformation (with interplanar angle, $\theta \approx 100°$) is *not* rigid; however, it is flexible. Small, low-energy rotations about the C(9)−C(10) and C(10)−C(11) bonds cause θ to open or close somewhat, while maintaining hydrogen bonding.[8] Large rotations, however, break hydrogen bonds and lead to energetically unfavorable conformations. Such large bond rotations are associated with the interconversion of mirror image ridge-tile conformations—a dynamic process that occurs at an experimentally determined rate of ∼5.4 s^{-1} at 37 °C over an experimentally determined barrier of ∼18−20 kcal/mol.[14] The picture of the bilirubin structure is thus one of *flexible* enantiomeric *ridge-tile shapes* that *interconvert rapidly* at room temperature.

Solutions of bilirubin in isotropic media can be thought of as a 50:50 mixture of equilibrating ***M*** and ***P*** conformational enantiomers (Figure 1B). Displacement of the equilibrium toward the ***M*** or ***P*** can be achieved by enantioselective

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of selected nuclear Overhauser effects (NOEs). From an examination of molecular models of **1**, it becomes clear that in the energetically favored ***P***-helical conformation the βR methyl groups lie in a relatively nonhindered environment and near the methyls at C(7) and C(13), whereas the βR methine CH lies close to the C(10) methylene (Figure 9). On the other hand, in the less favored ***M***-helical conformation, the βR methyls lie in a sterically crowded environment buttressed against the C(10) methylene, whereas the βR methine CH lies in an unhindered environment and near the C(7) and C(13) methyls. From NOE measurements of **1** in $CDCl_3$ solvent it is thus not surprising to find an enhancement of the β,β'-CH signals upon irradiation of the C(10) CH_2 (Figure 9A) (but no enhancement of the β,β'-CH_3s) and an enhancement of the C(10) CH_2 upon irradiation of the β,β'-CHs (Figure 9B) (but no enhancement at the C(7) and C(13) CH_3s). Nor is it surprising to find an NOE between the β,β'-CH_3s and the C(7) and C(13) CH_3s (Figures 9C and D) but no NOE between the β,β'-CH_3s and the C(10) CH_2 (Figure 9C) or between the C(7) and C(13) CH_3s and the β,β'-CHs (Figure 9D).

With added amine sufficient to cause a strong exciton chirality inversion of **1** (Figures 4−6), the NOE results are characteristic of an ***M***-helicity conformation, signifying that the amine has caused an inversion of molecular chirality to match the sign inversion of the CD couplet. Now NOEs are found (i) between the C(10) CH_2 and the β,β'-CH_3s (Figures 9A and C), but not between the C(10) CH_2 and the β,β'-CHs (Figures 9A and B), and (ii) between the β,β'-CHs and the C(7) and C(13) CH_3s (Figures 9B and D).

Concluding Comments

We have found an unusual example of exciton chirality inversion with change of solvent. Strikingly, the characteristic positive exciton chirality found in typical organic solvents containing ($\beta R,\beta' R$)-dimethylmesobilirubin-XIIIα (**1**) (and the negative exciton chirality found in ($\alpha S,\alpha' S$)-dimethylmesobilirubin-XIIIα (**2**)) is inverted upon adding amines. In such amines studied, mainly alkylamines (primary, secondary, and tertiary), the Cotton effects magnitudes meet or exceed the largest seen in nonpolar solvents such as CCl_4, benzene, etc., but the signs are opposite. While the results of NOE analyses clearly indicate an inversion from the energetically favored ***P***-helical conformation to ***M*** attends the change of solvent to amine, it is not entirely clear how the amine acts to invert the conformation. It is clear, however, that the mechanism does not involve simply the expected acid−base reaction. Rather, the complex formed between amine and **1** (or **2**) is probably some sort of tight ion pair (carboxylate anion−ammonium cation) where the (protonated) amine is dragged into the matrix of intramolecular hydrogen bonds that preserves the ridge-tile conformation of the pigment. In the pigment−amine complex, apparently new nonbonded steric interactions are introduced, and these operate in opposition to the bias imposed by the α and β methyls on the propionic acids of **1** and **2**. These findings may have relevance to understanding the chirality of bilirubin in its complex with serum albumins (that are involved in transport of the pigment in the circulation in vivo).

Acknowledgment. We thank the National Institutes of Health (HD 17779) for generous support and the National Science Foundation (CHE-9214294) for assistance in purchasing the 500 MHz NMR spectrometer used in this work. Special thanks go to Mr. Lew Cary for his assistance in the NMR experiments. Dr. Stefan E. Boiadjiev is on leave from the Institute of Organic Chemistry of the Bulgarian Academy of Sciences, Sofia, Bulgaria.

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CHIRALITY 13:251–257 (2001)

Chirality Inversion in the Bilirubin Molecular Exciton

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Dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday

ABSTRACT The bichromophoric pigment bilirubin acts as a molecular exciton in its UV-visible and circular dichroism (CD) spectroscopy. In both polar and nonpolar solvents, an optically active analog, (β*R*,β′*R*)-dimethylmesobilirubin-XIIIα (**1**), exhibits intense bisignate CD Cotton effects in the region of its long wavelength UV-vis absorption near 400 nm: $\Delta\epsilon_{434}^{max}$ + 337, $\Delta\epsilon_{389}^{max}$ – 186 ($CHCl_3$), and $\Delta\epsilon_{431}^{max}$ + 285, $\Delta\epsilon_{386}^{max}$ – 177 (CH_3OH). However, introduction of an amine into a $CHCl_3$ solution of **1** causes the Cotton effect signs to become inverted, e.g., after addition of NH_3, $\Delta\epsilon_{433}^{max}$ – 345, $\Delta\epsilon_{389}^{max}$ + 243, and after addition of ethylene diamine, $\Delta\epsilon_{435}^{max}$ – 420, $\Delta\epsilon_{390}^{max}$ + 299. The sign inversions imply inversion of molecular chirality of the bilirubin and the phenomenon appears to be general for amines, including α,ω-diamines. 1,8-Diaminooctane was found to be more effective than longer or shorter chain analogs in producing CD sign inversion. *Chirality 13:251–257, 2001.*

KEY WORDS: conformational analysis; bilirubin, diamines; ridge-tile shape

Bilirubin (Fig. 1) is a lipophilic tetrapyrrole, the end product of heme metabolism and the yellow pigment of jaundice.[1,2] It occurs only in vertebrates (~300 mg/day in healthy adult humans) and is clinically important for several reasons.[1–3] Its accumulation in blood and extravascular tissue is a useful sign of disease, usually liver disease; it can cause irreversible neurologic damage; it is involved in the formation of gallstones; and it is an endogenous inhibitor of free-radical injury.[4] In the form of its ester conjugates with sugars, it is the principal pigment in bile.

Bilirubin belongs to the class of pigments called "linear tetrapyrroles,"[2,5] but its solution and biological properties do not correlate well with either the linear (Fig. 1, upper) or a porphyrin-like shape in which the polar carboxyl and lactam groups are freely solvated. Bilirubin is conformationally flexible in solution, but one conformation is significantly more stable than all the others: a folded, ridge-tile structure with intramolecular hydrogen bonds linking the dipyrrinone pyrrole and lactam functions to the propionic carboxyl (or carboxylate) groups (Fig. 1, lower).[6,7,8] The ridge-tile conformation is dissymmetric and bilirubin can adopt either of two nonsuperimposible mirror-image conformations. Both enantiomers occur both in the crystal[6,7] and in solution,[9] interconverting rapidly[10] in the latter via a succession of nonplanar intermediates in which the hydrogen bonding network is never completely broken.[8] The energetically most favored ridge-tile conformation (with interplanar angle, θ ~100°) is *not* rigid; however, it is flexible. Small, low-energy rotations about the C(9)–C(10) and C(10)–C(11) bonds cause θ to open or close somewhat, while maintaining hydrogen bonding,[8] while large rotations break hydrogen bonds and lead to energetically disfavored conformation. Large bond rotations are associated with the interconversion of mirror-image ridge-tile conformations—a dynamic process that occurs at an experimentally determined rate of ~5.4 sec^{-1} at 37°C over an experimentally determined barrier of ~18–20 kcal/mole.[11] The picture of bilirubin structure is thus one of *flexible* enantiomeric *ridge-tile shapes* that *interconvert rapidly* at room temperature.

Solutions of bilirubin in isotropic media can be thought of as a 50:50 mixture of equilibrating M and P conformational enantiomers (Fig. 1, lower). Displacement of the equilibrium toward the M or P can be achieved by enantioselective complexation with a chiral compound such as quinine,[12] serum albumin,[13,14] or by the action of intramolecular nonbonded steric repulsions, as has been observed when stereogenic centers are created by methyl substitution at either the α or the β carbons of the propionic acid chains.[9,15] It is this latter phenomenon that has attracted our interest because such "intramolecular resolution" can be observed by circular dichroism spectroscopy.[9,15–19]

The optically active compound of particular interest in the current work is an analog of bilirubin: (β*R*,β′*R*)-dimethylmesobilirubin-XIIIα (**1**).[9] Mesobilirubins differ from bilirubin in having ethyl rather than vinyl groups (*cf.* Figs. 1, 2). For the symmetric mesobilirubin-XIIIα, these small differences in structure in the substituents of lactam

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bonding distance (vs. six in mesobilirubin-XIIIα). This stabilization was clearly seen when two separate methylammonium cations replaced the dication: The resulting complex has the same characteristics as in alkyl-linked dication.

A significant dependence on the length of diamine chain was found when the dication salt bridges are formed on the convex surface of mesobilirubin-XIIIα dianion. In all of these complexes the ϕ_1 and ϕ_2 torsion angles increase up to −80° to −90°, which leads to opening of the ridge-tile (θ increases to 110° to 120°). Such diamine–pigment constructs suggest the possibility for inversion of the initially stable P helical conformation of **1** if (in $CHCl_3$) the diamine approaches at the convex face and converts it to concave. The energy necessary to overcome the steric repulsion between the β,β′-methyls and the 10-CH_2 (Fig. 3) might thus be supplied by favorable tight ion-pair attraction.

CONCLUSIONS

(β*R*,β′*R*)-Dimethylmesobilirubin-XIIIα **(1)** in $CHCl_3$ exhibits an intense positive exciton chirality CD near 400 nm, consistent with its strong preference to adopt a P-helical ridge-tile conformation (Figs. 1, 3). When ammonia gas or simple alkylamines are added to the pigment $CHCl_3$ solution, the Cotton effect intensities decrease to zero and invert to give even stronger negative chirality exciton CD spectra. α,ω-Diaminoalkanes are especially effective, with 1,8-diaminooctane being more effective than its shorter or longer chain analogs. It is likely that the complex formed between amine and **1** in $CHCl_3$ is some sort of tight ion pair (carboxylate anion–ammonium cation) where the (protonated) amine is dragged into the same matrix of intramolecular hydrogen bonds that preserves the ridge-tile conformation of the pigment. In so doing, the amine salt complex counters the intrinsic steric bias for a P-helicity imposed by the β*R*,β′*R* methyls through nonbonded steric interactions. These findings may have relevance to understanding the chirality of bilirubin in its complex with serum albumins, which transport bilirubin in blood.

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Dr. Stefan Boiadjiev is on leave from the Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia.

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TETRAHEDRON: *ASYMMETRY*

An enantiomerically pure bilirubin. Absolute configuration of ($\alpha R,\alpha' R$)-dimethylmesobilirubin-XIIIα

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Abstract—Enantiomerically pure (+)-($\alpha R,\alpha' R$)-dimethylmesobilirubin-XIIIα **1** and its ($\alpha S,\alpha' S$) enantiomer *ent*-**1** were synthesized in ten steps from simple precursors. Resolution was achieved at an early stage in the synthesis, with a racemic monopyrrole precursor *rac*-**6** being converted to its amides **8** with (1*S*)-camphor-2,10-sultam. Resolution of **8** to 99% d.e. was accomplished in three crystallizations, and the absolute configuration of the acid **6** was deduced by X-ray crystallography of the more crystalline, diastereomerically pure amide **7**. Circular dichroism spectroscopy of **1** showed intense bisignate Cotton effects: $\Delta\varepsilon_{435}^{max} = +344$, $\Delta\varepsilon_{391}^{max} = -193$ ($CHCl_3$), as expected for a molecular exciton, and consistent with a ***P***-helical intramolecularly hydrogen-bonded ridge-tile conformation. The Cotton effect magnitudes of **1** match almost exactly those found for (−)-($\beta S,\beta' S$)-dimethylmesobilirubin-XIIIα **11** and (+)-($\alpha R,\beta' R$)-dimethylmesobilirubin-XIIIα. However, the Cotton effect of the pseudo-*meso* diastereomer ($\alpha R,\beta' S$)-dimethylmesobilirubin-XIIIα **12** is not zero. Its large positive exciton couplet and ^{1}H NMR NOE analysis confirm that an α-CH_3 exerts a greater steric demand than a β-CH_3—by a factor of ∼3.

1. Introduction

Bilirubin (Fig. 1), the lipophilic yellow pigment of jaundice, belongs to the class of pigments called 'linear tetrapyrroles'[1] but its solution and biological properties[2] do not correlate well with either the linear structure (Fig. 1A) or a porphyrin-like structure (Fig. 1B), which are predicted to be rather polar. In bilirubin, and its mesobilirubin analogs, where vinyls are replaced by ethyl while retaining propionic acids at C(8) and C(12) (Fig. 1C), the most stable conformation is bent in the middle, in the shape of a ridge-tile.[3–6] In the ridge-tile conformation, which is flexible, not rigid, each propionic acid group is engaged in intramolecular hydrogen bonding to the opposing dipyrrinone lactam and pyrrole (Fig. 1D), thus affording considerable conformational stabilization and rendering the pigments surprisingly lipophilic.[7]

The ridge-tile conformations of Fig. 1D have near-C_2-symmetry. They are dissymmetric and enantiomeric. In an isotropic medium, bilirubin and its synthetic analog mesobilirubin-XIIIα[8] thus consist of a 50:50 mixture of equilibrating conformational enantiomers. Displacement of the equilibrium toward one or the other of the enantiomers has been achieved by complexation with a chiral compound, such as quinine[9] or serum albumin,[10] and observed by circular dichroism spectroscopy (CD).

It was found earlier that selective stabilization of one enantiomer can also be achieved through intramolecular non-bonded steric interactions, e.g. when stereogenic centers are created by a single methyl substitution at either the α or the β carbons of both propionic acid chains.[11–13] Such forced 'intramolecular resolution' was first achieved in α,α′-dimethylmesobilirubin-XIIIα **1**[11] and its β,β′-dimethyl analog.[12] The absolute configuration at the β,β′ stereogenic centers of the latter was established unequivocally from an X-ray crystal structure of the resolved diastereomeric salt (with brucine) of a monopyrrole β-methylpropionic acid precursor to the central rings of the mesobilirubin. The absolute configuration of the former, resolved by partitioning into chloroform from its complex with human serum albumin in pH 9.0 solution, was deduced from the NMR ^{1}H{^{1}H}-NOE spectrum of its bis-amide from (−)-α-phenethylamine.[11] As such it does not have the same clarity as that from the crystallographic study of the β,β′-dimethylrubin. In addition the e.e. of the 'resolved' rubin was not apodictic, and that led recently to difficulties in quantitative determinations of functional group steric size using circular dichroism on the bilirubin platform.[14]

In the following, we present an improved synthesis and unequivocal determination of the absolute configura-

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>445 in CH_2Cl_2 solvent), in Et_2NH solvent the exciton chirality is found to be inverted from negative to positive (Table 3). Without exception, the CD **A**-values are intensified to the range +900 to +1000. Although it is unclear why the α-*i*Pr rubin exhibits a slightly smaller **A**-value, the consistently positive sign indicates an inversion of molecular chirality, from the dominant ***M*** in CH_2Cl_2 to the dominant ***P*** in Et_2NH.[25] Just why the rubin should adopt the conformation in which both the α and β′ groups are in the (apparently) more sterically compressed sites remains unclear. Here again, one is dealing with ammonium salts, and the conformational preference may in some way be associated with the inclusion of the cation in the ridge-tile structure.

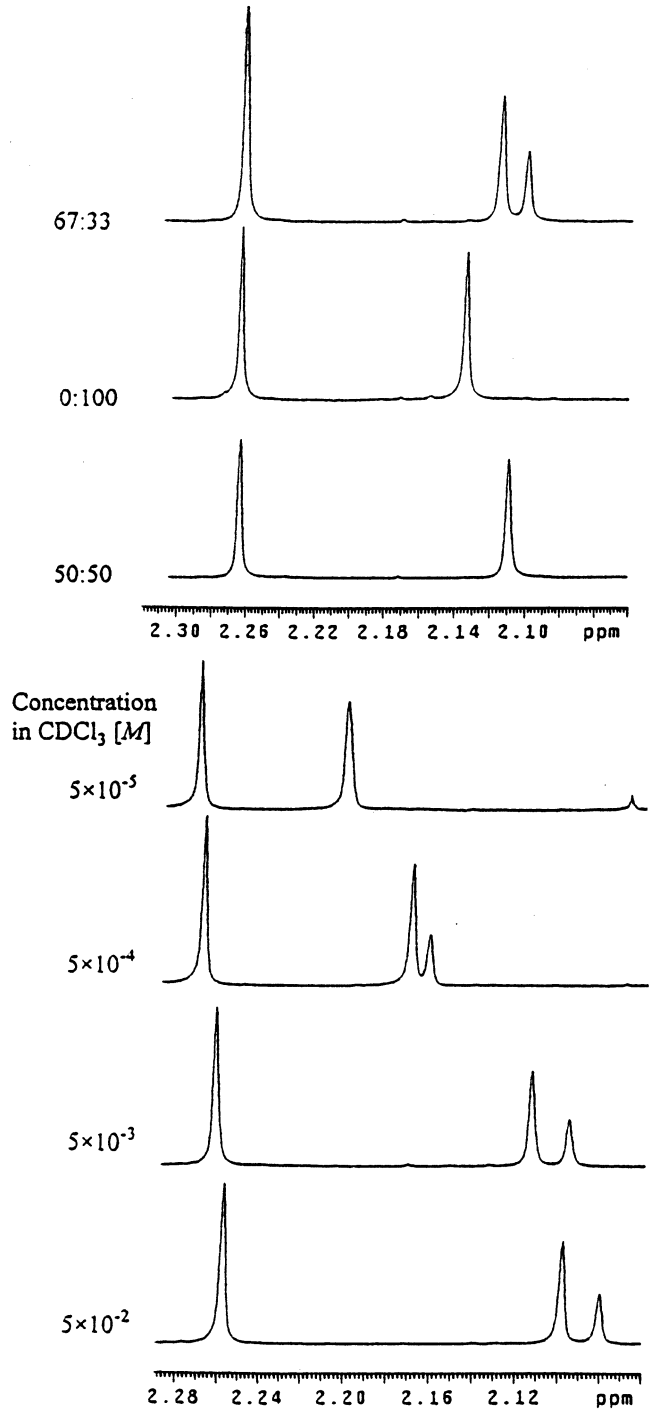

Figure 6. (A) Partial ^1H NMR spectra of the C(3) and C(5) methyl groups region in 5×10^{-3} M $CDCl_3$ solutions of pyrrole acid **6** with different enantiomeric excess. (B) Partial ^1H NMR spectra of pyrrole acid with 34% e.e. in $CDCl_3$ solutions showing the range of C(3)-CH_3 and C(5)-CH_3 signals at decreasing concentration.

2.6. Hydrogen-bonded dimers

^1H NMR studies of monopyrrole mono-acid **6** in $CDCl_3$ showed expected chemical shifts. Assignment of the C(5) methyl singlet signal as more shielded than the C(3) methyl singlet was based on NOE experiments from the NH. It was interesting to note, however, that although the C(3) methyl signal at 2.264 ppm remained invariant when comparing **6** to *rac*-**6** (Fig. 6), the C(5) methyl signal of the former was slightly more deshielded (2.135 ppm) than that of the latter (2.110 ppm) (all δ referred to strictly 5×10^{-3} M solutions). The NH signal was also slightly more deshielded in **6** (9.858 ppm) than in *rac*-**6** (9.821), but the α-methyl of **6** (1.164/1.178 ppm) was more shielded than in *rac*-**6** (1.180/1.194 ppm). These small differences in chemical shift might have escaped attention had not the 34% e.e. sample exhibited two signals for the C(5) methyl (2.102/2.117, Fig. 6A), with the C(3) methyl signal remaining at 2.264 ppm. Neither of the two C(5) signals lies at the same chemical shift as that seen in either **6** or *rac*-**6**, but the C(5) methyl of *rac*-**6** appears at the average of the two signals seen in 34% e.e. **6**.

It might be assumed that **6** forms dimers in $CDCl_3$ because in a dilution study in $CDCl_3$ (Fig. 6B), the C(5) methyl resonances of 34% e.e. **6** at 5×10^{-2} M shift upfield and coalesce at 5×10^{-5} M. Vapor pressure osmometry measurements of *rac*-**6** in $CHCl_3$ at 45°C gave an apparent molecular weight of 311 (versus that calculated for the monomer: 239), thus confirming some extent of dimer formation. In contrast, the dimethyl ester of 34% e.e. **6** shows only one signal for the C(5) methyl and no changes in chemical shift upon the 1000-fold dilution of Fig. 6B. For **6**, only one type of dimer is possible: **6**–**6** (or *R*,*R*). For *rac*-**6** there are two types: *R*,*R* (and *S*,*S*) and *R*,*S*, as shown in Fig. 7. Molecular mechanics (Sybyl) calculations indicate that the heterochiral (*R*,*S*) dimer is more stable than the homochiral (*R*,*R* or *S*,*S*) by ~2.5 kcal/mol. Thus, in *rac*-**6** one might expect a heterodimer mainly; whereas in **6**, only a homodimer is possible. The difference might account for the different chemical shifts, e.g. the C(5) methyl, seen in the ^1H NMR spectra of **6** and *rac*-**6** (Fig. 6A). In 34% e.e. **6**, one can expect to see signals for both the heterodimer (more deshielded) and homodimer (more shielded) in the ratio ~2:1, as observed.

3. Concluding comments

Methyl 4-(2′*R*-carboxypropyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate *rac*-**6** was synthesized and resolved to 100% e.e. as its amide **7** with (1*S*)-camphor-

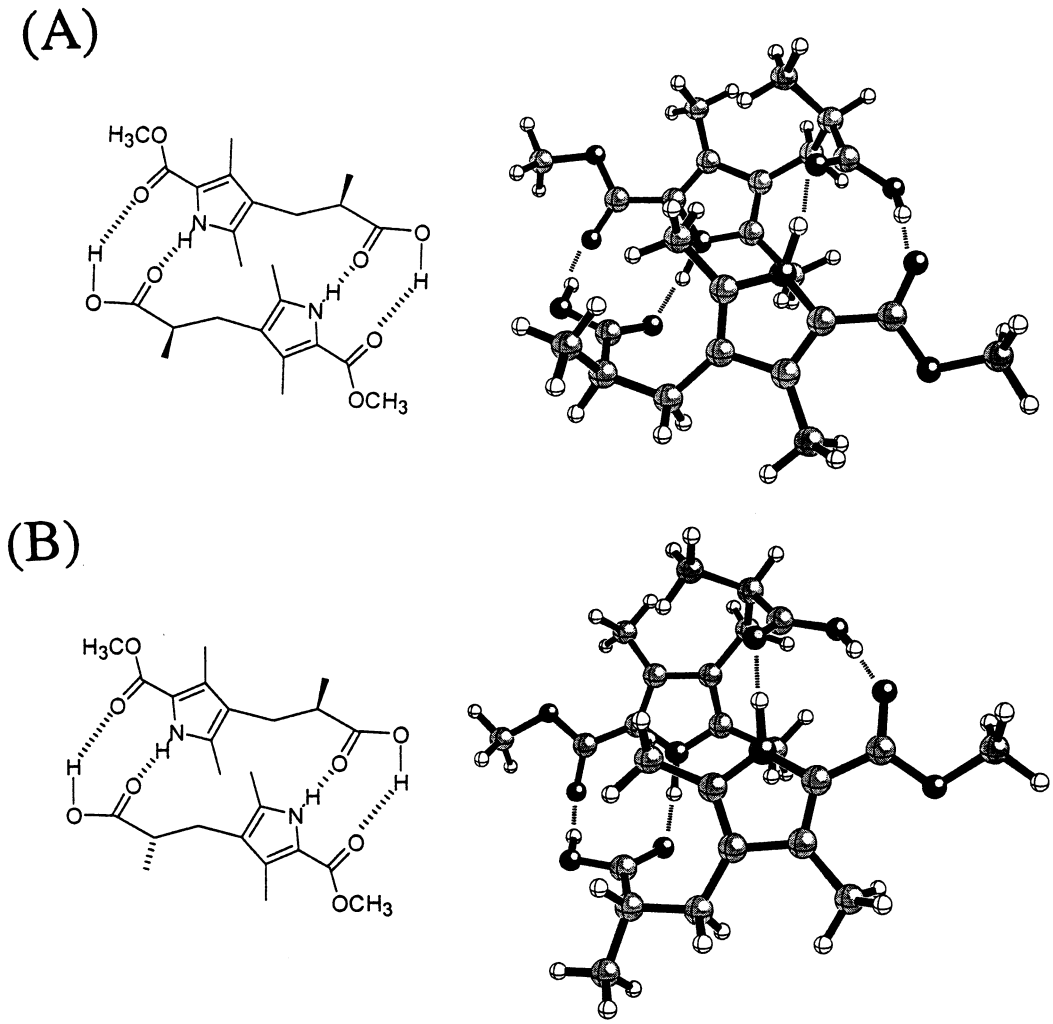

Figure 7. Line drawings (upper) and ball and stick representations of the hydrogen-bonded dimers of monopyrrole acid **6**: (A) homodimer, *R*,*R*, and (B) heterodimer *R*,*S*. The heterodimer is computed to be ~2.5 kcal/mol lower energy.

2,10-sultam. The absolute configuration of the acid component of the crystalline, resolved sultam amide was determined by X-ray crystallography, the absolute configuration of the camphor sultam being known. Both the racemic acid *rac*-**6** and resolved (αR) acid **6** form hydrogen-bonded dimers in $CDCl_3$, with the heterodimer (*R*,*S*) being favored over the homodimer. The resolved acid **6** was smoothly converted in three steps to (αR,$\alpha' R$)-dimethylmesobilirubin-XIIIα **1**, which exhibited a large positive exciton chirality CD spectrum in most non-polar solvents, but a large negative exciton chirality CD in diethylamine.

4. Experimental

All UV–vis spectra were recorded on a Perkin–Elmer Lambda-12 or Cary 219 spectrometer, and all circular dichroism (CD) spectra were recorded on a JASCO J-600 instrument. NMR spectra were obtained on Varian Unity Plus spectrometer operating at ^1H frequency of 500 MHz in $CDCl_3$ solvent (unless otherwise noted). Chemical shifts are reported in δ (ppm) referenced to the residual $CHCl_3$ ^1H signal at 7.26 ppm, and $CDCl_3$ ^{13}C signal at 77.00 ppm. A *J*-modulated spin–echo experiment (*A*ttached *P*roton *T*est), and HMBC and HMQC experiments were used to assign ^{13}C NMR spectra. Optical rotations were measured on a Perkin–Elmer model 141 polarimeter. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Gas chromatography–mass spectrometry analyses were carried out on a Hewlett–Packard 5890A capillary gas chromatograph (30 m DB-1 column) equipped with a Hewlett–Packard 5970 mass selective detector. Radial chromatography was carried out on Merck Silica Gel PF_{254} with gypsum preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). Vapor pressure osmometry measurements were taken on a Gonotec Osmomat 070 osmometer. Combustion analyses were carried out by Desert Analytics, Tucson, AZ.

Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Commercial reagents and HPLC grade solvents were dried and purified following standard procedures.[26]

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Atropisomerism in linear tetrapyrroles

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Abstract—Novel bilirubin and biliverdin congeners with propionic acids replaced by *o*-carboxyphenyl exhibit diastereomerism due to axial chirality about the carbon–carbon single bond linking the *o*-carboxyphenyl group to a pyrrole ring. Evidence for atropisomerism was found even in the monopyrrole precursor, ethyl 3,5-dimethyl-4-(*o*-carboxyphenyl)pyrrole-2-carboxylate. Like bilirubin, *o*-carboxyphenyl rubin **1a** adopts an intramolecularly hydrogen-bonded ridge-tile conformation in nonpolar solvents. In solutions containing optically active amines or human serum albumin **1a** exhibits intense bisignate exciton coupling-type induced circular dichroism for its long wavelength absorption near 400 nm.

1. Introduction

Atropisomerism has become synonymous with biphenyl or biaryl stereochemistry, where sufficiently high barriers to rotation about the interconnecting sp^2-sp^2 C–C bond may lead to isolatable stereoisomers.[1] Studies of atropisomerism about a pyrrole to phenyl sp^2-sp^2 C–C bond are few,[2] and in the following we describe one such example (the monopyrrole **4**) and show how it can be detected in a dipyrrole (**3c**) and in various new linear tetrapyrrole derivatives. Among the latter, we describe the syntheses and stereochemistry of novel bilirubin (**1**) and biliverdin (**2**) analogs whose two propionic acids are replaced by *o*-carboxyphenyls (Fig. 1).

2. Results and discussion

2.1. Synthesis

Pyrroles of the type illustrated by **4** (Fig. 1), whether with an *o*-carboethoxyphenyl, *o*-carbomethoxyphenyl or *o*-carboxyphenyl group on the pyrrole nucleus are unknown, as are variants with these same *o*-substituents located *m* or *p* on the benzene ring. In principle, they might be prepared[3] by a classical Fischer–Knorr synthesis from the appropriate 3-phenylpentane-2,4-dione, a reaction not yet reported, and a dione apparently unknown. An alternative synthesis might follow one developed by Chang and Bag[4] using a Suzuki coupling of a β-bromopyrrole with phenylboronic acid. Although *m* and *p*-carboxy (or carboalkoxy) phenylboronic acids have been described in the literature, the *o*-isomer had not. We therefore decided to reserve the pyrrole syntheses of *m* and *p*-carbo(alko)xyphenyl isomers to the Suzuki coupling method and pursue a more classical synthesis of **4** (Scheme 1). For the latter, we required 3-(*o*-carboxyphenyl)pentane-2,4-dione (**5a**), which could be produced in high yield by reaction of the sodium *o*-bromobenzoate with the sodium salt of pentane-2,4-dione.[5,6] The resulting acid (**5a**) was converted to its methyl ester (**5b**) by reaction with diazomethane, but Fischer–Knorr condensation of it with diethyl oximinomalonate using zinc in acetic acid led to only a 26% isolated yield of pyrrole methyl ester **4b**—along with substantial isocoumarin side-product, 4-acetyl-3-methyl-1*H*-2-benzopyran-1-one.[6] This mixture was separated only with considerably difficulty. The problems encountered in converting **5b** to **4b** were largely overcome by treating the acid (**5a**) under the same Fischer–Knorr pyrrole-forming condensation conditions to afford a 65% isolated yield of pyrrole acid **4a**.

Saponification of **4a** or **4b** to its diacid and condensation with 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole[7] afforded yellow dipyrrinone **3a** in 60% yield after treatment with CH_2N_2. Attempted oxidative coupling of dipyrrinone acid **3b** gave no verdin, apparently due to interference (by proton transfer) from the free CO_2H group; however, oxidative coupling of dipyrrinone ester **3a** gave a mixture of verdins (**2**) in 84% yield. Standard verdin reduction with $NaBH_4$ gave rubin ester **1b**, which was saponified to rubin diacid **1a** in 91% isolated yield.

2.2. Characterization

The structures of monopyrroles **4**, dipyrrole **3** and tetrapyrroles **1** and **2** follow logically from the method of synthesis (see Scheme 1) and the compounds were characterized by spectroscopy, especially ^{13}C NMR, which showed the expected characteristic carbon resonances for the pyrrole units and the *o*-carboxy (or

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3. Conclusions

Synthetic rubin **1a** is found to adopt preferentially a conformation shaped like a ridge-tile or half-opened book where the *o*-benzoic acids are engaged in intramolecular hydrogen bonding to the pigment's dipyrrinone components. As in natural bilirubin, the yellow pigment of jaundice, such intramolecular hydrogen bonding stabilizes the ridge-tile conformation. Unlike bilirubin the *o*-benzoic acid components present the opportunity for atropisomerism, for which a barrier of ~21 kcal/mol has been estimated by DNMR measurements.

4. Experimental

4.1. General procedures

Nuclear magnetic resonance spectra were obtained on a Varian Unity Plus spectrometer at 11.75 T magnetic field strength operating at ^{1}H frequency of 500 MHz and ^{13}C frequency of 125 MHz. $CDCl_3$ solvent was used throughout (unless otherwise specified), and chemical shifts are reported in δ ppm, referenced to the residual $CHCl_3$ ^{1}H signal at 7.26 ppm and $CDCl_3$ ^{13}C signal at 77.00 ppm. *J*-modulated spin-echo (Attached Proton Test) and gHMBC experiments were used to obtain the ^{13}C NMR assignments. The apparent multiplicities and values of spin–spin coupling constants (^{3}J, ^{4}J)[17] of all aromatic protons were confirmed by single-frequency homonuclear decoupling experiments and by the Varian simulation routine. All UV–Vis spectra were recorded on a Perkin–Elmer Lambda 12 spectrophotometer and the circular dichroism spectra were recorded on a JASCO J-600 dichrograph. Gas chromatography–mass spectrometry analyses were carried out on a Hewlett–Packard 5890A gas chromatograph (30 m DB-1 column) equipped with a Hewlett–Packard 5970 mass selective detector. HPLC analyses were carried out on a Perkin–Elmer series 410 high-pressure liquid chromatograph with a Perkin–Elmer LC-95 UV–Vis spectrophotometric detector (set at 420 nm for rubinoid compounds) equipped with a Beckman Altex ultrasphere IP 5 μm C-18 ODS column (25×0.46 cm) kept at 34°C. The flow rate was 1 mL per minute, and the mobile phase was 0.1 M di-*n*-octylamine acetate buffer in 5% H_2O in methanol (v/v) with pH 7.7 at 22°C. Radial chromatography was carried out on Merck silica gel PF_{254} with $CaSO_4$ binder preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2, or 4 mm thick rotors and analytical thin-layer chromatography was carried out on J. T. Baker silica gel IB-F plates (125 μm layer). Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. The combustion analyses were carried out by Desert Analytics, Tucson, AZ.

4-Dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) were from Aldrich. Di-*n*-octylamine was from Fluka. Methanol, chloroform, ethyl acetate and dichloromethane were HPLC grade from Fisher. Sodium borohydride, *p*-chloranil, sodium acetate, sodium hydroxide, anhydrous sodium sulfate, anhydrous magnesium sulfate, sodium nitrate, sodium bicarbonate, nitric acid, hydrochloric acid, 96% formic acid and ethanol were from Fisher. Argon and nitrogen were from Air Products. Solvents and reagents were used directly as provided by the vendor, except for the Fisher HPLC-grade solvents, which were further purified by standard procedures as described in detail in Ref. 18. The spectral data were obtained in spectral grade solvents, used as provided by Aldrich or by Fisher.

4.1.1. Ethyl 4-(*o*-carboxyphenyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (4a). To a preheated (~80°C) mixture of 9.81 g (150 mgA) of zinc, 10.3 g (125 mmol) of anhydrous sodium acetate and 40 mL of acetic acid were added simultaneously in small portions 11.01 g (50 mmol) of 3-(*o*-carboxyphenyl)-pentane-2,4-dione (**5a**)[5] and a solution of 18.92 g (100 mmol) of diethyl oximinomalonate[19] in 12 mL of acetic acid during 45 min while maintaining the temperature at 90–95°C. After the additions were complete, the mixture was heated at vigorous reflux for 4 h and then poured into 400 mL of ice and water. After stirring for 30 min, the aqueous layer was decanted, and the semisolid product was dissolved in a minimum volume of 45°C ethanol (~65 mL) and then precipitated by slow addition of ice-cold water over 1 h. After cooling for an additional hour in an ice bath, the crude product was collected by filtration, washed with water and dried. Purification of the product by radial chromatography (1.5–5.0% v/v CH_3OH in CH_2Cl_2) and recrystallization of the isolated solid from ethyl acetate–hexane afforded 9.33 g (65%) of pyrrole **4a**. It had mp 199–200°C; ^{1}H NMR: δ 1.35 (3H, t, J=7.1 Hz), 2.03 (3H, s), 2.14 (3H, s), 4.32 (1H, ABX_3, ^{3}J=7.1 Hz, ^{2}J=11.0 Hz), 4.34 (1H, ABX_3, ^{3}J=7.1 Hz, ^{2}J=11.0 Hz), 7.22 (1H, dd, ^{3}J=7.6 Hz, ^{4}J=1.0 Hz), 7.42 (1H, ddd, ^{3}J= 7.6, 7.5 Hz, ^{4}J=1.0 Hz), 7.55 (1H, ddd, ^{3}J=7.6, 7.5 Hz, ^{4}J=1.2 Hz), 8.04 (1H, dd, ^{3}J=7.6 Hz, ^{4}J=1.2 Hz), 9.50 (1H, brs), 10.34 (1H, very brs) ppm; ^{13}C NMR: δ 11.40, 11.73, 14.52, 60.11, 117.25, 123.12, 127.06, 127.33, 130.75, 130.92, 131.05, 131.98, 132.86, 135.77, 162.58, 171.22 ppm. MS *m*/*z* (rel. abund.): 287 ($M^{+\cdot}$, 100%), 270 (41%), 242 (55%), 214 (61%) amu. Anal. calcd for $C_{16}H_{17}NO_4$ (287.3): C, 66.88; H, 5.96; N, 4.88. Found: C, 66.79; H, 5.78; N, 4.79.

4.1.2. Ethyl 4-(*o*-methoxycarbonylphenyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (4b). Methyl ester **4b** was obtained by the same procedure as for **4a** by using 3-(*o*-methoxycarbonylphenyl)pentane-2,4-dione (**2**)[5]—except that after separating the crude product, it was dissolved in 150 mL of CH_2Cl_2 and treated with 100 mL of 1 M aq NaOH with vigorous stirring for 6 h. The organic layer was washed with water, dried (anh. $MgSO_4$), and filtered. The solvent was evaporated under vacuum. Radial chromatography of the residue (hexane–ethyl acetate, gradient 5.5:1 to 2.5:1 v/v) followed by recrystallization of the isolated solid from ethanol–water afforded 3.95 g (26%) of pyrrole **4b**. It had mp 135–136°C; ^{1}H NMR: δ 1.36 (3H, t, J=7.1 Hz), 2.09 (3H, s), 2.10 (3H, s), 3.72 (3H, s), 4.30 (1H, ABX_3, ^{3}J=7.1 Hz, ^{2}J=10.9 Hz), 4.32 (1H, ABX_3, ^{3}J= 7.1 Hz, ^{2}J=10.9 Hz), 7.21 (1H, dd, ^{3}J=7.6 Hz, ^{4}J=1.2 Hz), 7.39 (1H, ddd, ^{3}J=7.6, 7.4 Hz, ^{4}J=1.2 Hz), 7.52 (1H, ddd, ^{3}J=7.4, 7.6 Hz, ^{4}J=1.3 Hz), 7.90 (1H, dd, ^{3}J=7.6 Hz, ^{4}J= 1.3 Hz), 8.69 (1H, brs) ppm; ^{13}C NMR: δ 11.15, 11.84, 14.58, 52.02, 59.70, 117.13, 123.74, 126.91, 127.05, 129.69, 129.91, 131.31, 132.06, 132.67, 135.58, 161.81,

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Stable Monopyrrole Atropisomers

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Summary. Malonic ester derivatives of ethyl and methyl 3,5-dimethyl-4-(1′-iodoneopentyl)-1*H*-pyrrole-2-carboxylate exhibit restricted rotation about the pyrrole C(4)–C(1′) bond due to the bulky 1′-*tert*-butyl and malonic ester groups and the *ortho* effect at C(4) of the sterically crowded 3,5-dimethylpyrrole. The malonates belong to a rare class of atropisomers with restricted rotation about an sp^3–sp^2 C–C bond, and they undergo diastereomeric separation by TLC and crystallization: the diastereomers are stable in solution at room temperature. A crystal of one of the diastereomers, suitable for X-ray crystallography, gave the relative configuration of the chiral axis and stereogenic center at C(1′). Dynamic NMR studies of the purified diastereomers provide kinetic and thermodynamic parameters associated with the atropisomerism: $\Delta G^{\ddagger} = 132$–$134\,\mathrm{kJ/mol}$ ($\sim 32\,\mathrm{kcal/mol}$) at 383 K in $C_2D_2Cl_4$ solvent.

Keywords. Pyrrole; Diastereomers; NMR spectroscopy.

Introduction

Typically, atropisomerism is found in biaryls such as 1,1′-binaphthyls and certain 2,2′,6,6′-tetrasubstituted biphenyls, where bond rotation about the sp^2–sp^2 C–C bond is sufficiently restricted so as to lead to separable conformers [1, 2]. Separability here implies a rotational free energy barrier of $>110\,\mathrm{kJ/mol}$ at 300 K and half-life of 1000 s. In contrast, restricted rotation about an sp^2–sp^3 C–C bond has seldom been observed. Certain fluorene atropisomers have been isolated where sufficiently large substituents have been positioned judiciously to restrict bond rotation [1–4]. An even simpler example where atropisomers have been isolated has only one aromatic ring: α,α-di-*tert*-butyl benzyl alcohol (Figure 1A) [5]. Far fewer examples of atropisomerism have been recognized among bipyrroles [6], and none involving restricted rotation about an sp^2–sp^3 C–C bond has been reported for monopyrroles. In the following, we report what we believe to be the first examples (Figure 1B) of configurationally stable sp^2C–sp^3C atropisomers of a monopyrrole.

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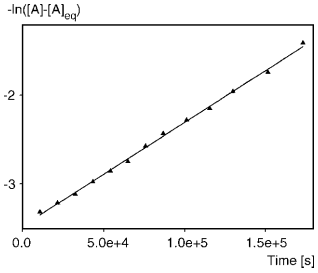

Fig. 5. Plots of $-\ln([A]-[A]_{eq})$ *vs.* time for trimethyl ester **1a** in $CDCl_2CDCl_2$ at 383 K based on changes in the α-$COOCH_3$ methyl signal intensity

Table 5. Summary of thermodynamic and kinetic parameters for the atropisomerism of **1a** at 383 K in $CDCl_2CDCl_2$

	From integration of ^{1}H-NMR signals at				Mean
	NH	1′-CH	One of α-$COOCH_3$	αCH_3	value
Integral ratio at $t \to \infty$	68.6 31.4	68.4 31.6	68.3 31.7	68.4 31.6	
K_{eq}	0.458	0.462	0.464	0.462	0.462
$k_1 + k_{-1}[s^{-1} \cdot 10^5]$	1.198	1.136	1.171	1.131	1.159
intercept	−3.4726	−3.4509	−3.4744	−3.4663	−3.4661
R	0.9983	0.9987	0.9991	0.9953	
k_1 $[s^{-1} \cdot 10^6]$	3.760	3.588	3.710	3.573	3.658
$\Delta G_1^{\ddagger}$ [kJ/mol]	134.4	134.5	134.4	134.6	134.5
k_{-1} $[s^{-1} \cdot 10^6]$	8.215	7.767	7.995	7.733	7.928
$\Delta G_{-1}^{\ddagger}$ [kJ/mol]	131.9	132.1	132.0	132.1	132.0

very similar to those measured for **1a**. From these rate constants the barriers to rotation were determined: for $\mathbf{1b} \rightarrow \mathbf{1b'}$ $\Delta G_1^{\ddagger} = 134.4\,\text{kJ/mol}$ and for $\mathbf{1b} \leftarrow \mathbf{1b'}$ $\Delta G_{-1}^{\ddagger} = 131.8\,\text{kJ/mol}$. The free energies of activation of **1b** are identical to those found for **1a** suggesting a lack of additional steric hindrance from the ethyl *vs.* methyl ester groups.

Experimental

All NMR spectra were obtained on a Varian Unity Plus spectrometer operating at ^{1}H frequency of 500 MHz in $CDCl_3$ solvent (unless otherwise noted). Chemical shifts are reported in δ (ppm) referenced to the residual $CHCl_3$ ^{1}H signal at 7.26 ppm, and $CDCl_3$ ^{13}C signal at 77.00 ppm. The ^{1}H-NMR spectra in $C_2D_2Cl_4$ were referenced to the residual ^{1}H signal at $\delta = 5.94$ ppm. For the dynamic NMR measurements, the probe temperature was controlled by a standard unit of Unity Plus system. A *J*-modulated spin-echo experiment (*A*ttached *P*roton *T*est) was used to assign ^{13}C-NMR spectra. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Gas chromatography-mass spectrometry analyses were carried out on a Hewlett-Packard 5890A capillary gas chromatograph (30 m DB-1 column) equipped with Hewlett-Packard 5970 mass selective detector. Combustion analyses were carried out by Desert Analytics, Tucson, AZ; these results were in favourable agreement with the calculated values. Commercial reagents and HPLC grade solvents were dried and purified following standard procedures [9]. Ethyl 3,5-dimethyl-1*H*-pyrrole-2-carboxylate (**3b**) was synthesized

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TETRAHEDRON: *ASYMMETRY*

Atropisomerism in monopyrroles

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Abstract—As observed by NMR, iodopyrroles **1a** and **1b** (ethyl and methyl 3,5-dimethyl-4-[(1′-iodo-2′,2′-dimethyl)propyl]pyrrole-2-carboxylate) and a variety of related derivatives with iodine replaced by methoxy **2**, thiomethyl **3**, acetic acid esters **4**, propionic acid ester **5** or malonic esters **6** exhibit restricted rotation about the C(4)–C(1′) bond due to the bulky *tert*-butyl group and an *ortho* effect from the sterically crowded 3,5-dimethylpyrrole. Most of the compounds, which are members of the rare class of atropisomers due to restricted rotation about an sp^3–sp^2 C–C bond, undergo diastereomeric enrichment by preparative TLC and crystallization. From dynamic NMR studies of the enriched diastereomers one can determine kinetic and thermodynamic parameters associated with the atropisomerism, e.g., $\Delta G^{\ddagger}$ ~24 kcal/mol for **1** and **5** (313 K), ~22 kcal/mol for **3** (273 K), and ~25 kcal/mol for **6** (313 K) in $C_2D_2Cl_4$ solvent.

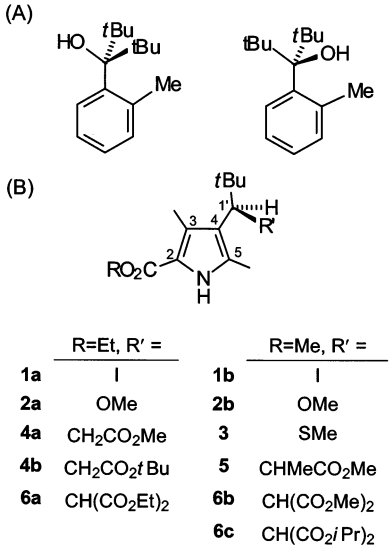
1. Introduction

Atropisomerism is usually associated with biaryl systems, where bond rotation about the sp^2–sp^2 C–C bond is sufficiently restricted so as to lead to separable conformers.[1,2] Typically, this means a free energy barrier of 26.2 kcal/mol at 300 K and a half-life of 1000 s. Restricted rotation about an sp^2–sp^3 C–C bond has been observed less often.[3] In systems involving fluorenes, with sufficiently large substituents judiciously placed to interfere with bond rotation, atropisomers have been isolated.[1,2,4] One of the simplest examples has only one aromatic ring: the di-*tert*-butyl compound of Fig. 1A, whose atropisomers have been isolated.[5] There are far fewer examples of atropisomerism of the biaryl type among pyrrole compounds,[6] a research area of current interest to us. In the following, we report what we believe to be the first examples (Fig. 1B) of sp^2C–sp^3C atropisomerism in a monopyrrole.

2. Results and discussion

2.1. Iodopyrroles 1a and 1b

Over 25 years ago, Khan and Plieninger[7] reported on the synthesis of **1a** from a β-free pyrrole **7a** by reaction with pivaldehyde in the presence of HI (Scheme 1). Although **1a** was characterized, only its melting point and combustion analysis data were published. NMR data were absent and not mentioned. We repeated the synthesis and isolation of pure **1a** and also prepared methyl ester **1b**, both of which are useful compounds in the synthesis of highly hindered bilirubins and biliverdins. Interestingly, the ^{1}H NMR spectrum of **1a** was



	R=Et, R′ =		R=Me, R′ =
1a	I	**1b**	I
2a	OMe	**2b**	OMe
4a	CH_2CO_2Me	**3**	SMe
4b	CH_2CO_2tBu	**5**	$CHMeCO_2Me$
6a	$CH(CO_2Et)_2$	**6b**	$CH(CO_2Me)_2$
		6c	$CH(CO_2iPr)_2$

Figure 1. (A) Isolable atropisomers with one aromatic ring. (B) Atropisomeric monopyrroles with restricted rotation about the C(4)–C(1′) bond. The absolute configuration at C(1′) is arbitrary.

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Table 2. Values of $[A]-[A]_{eq}$ ($=\Delta$) and $\ln([A]-[A]_{eq})$ ($=\ln$) for the dominant diastereomer of **1b** during the course of its equilibration in $CDCl_2CDCl_2$ at 313 K.

Measurement	^{1}H NMR signals							
	N–H		C(1′)–H		C(3)–CH_3		C(5)–CH_3	
	Δ	ln	Δ	ln	Δ	ln	Δ	ln
1	19.5	2.9704	22.4	3.1091	22.9	3.1311	23.1	3.1398
2	13.2	2.5802	15.3	2.7279	15.7	2.7537	15.6	2.7473
3	8.9	2.1861	10.2	2.3224	10.7	2.3702	10.5	2.3514
4	6.1	1.8083	6.8	1.9169	7.3	1.9879	7.0	1.9459
5	4.2	1.4351	4.7	1.5476	4.9	1.5892	4.7	1.5476
6	2.7	0.9933	3.1	1.1314	3.3	1.1939	3.1	1.1314
7	1.8	0.5878	2.0	0.6931	2.2	0.7885	2.0	0.6931
8	1.3	0.2624	1.4	0.3365	1.5	0.4055	1.3	0.2624
9	0.8	−0.2231	0.9	−0.1054	1.0	0.0000	0.8	−0.2231
10	0.5	−0.6931	0.6	−0.5108	0.7	−0.3567	0.6	−0.5108

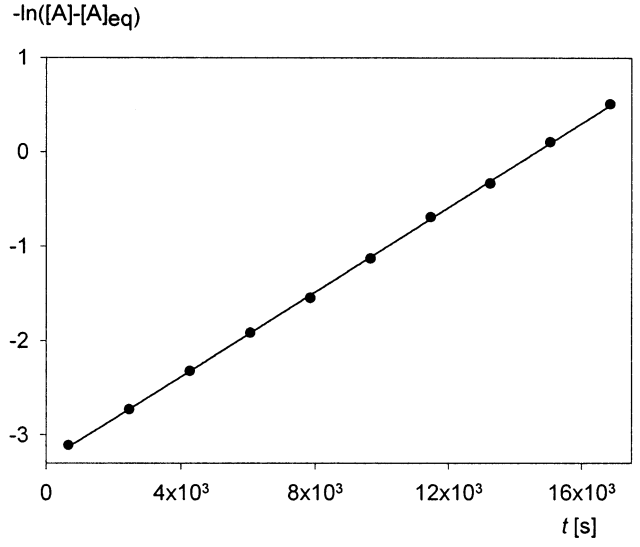


Figure 7. Plot of $-\ln([A]-[A]_{eq})$ versus time for iodide **1b** in $C_2D_2Cl_4$ at 313 K from the ^{1}H NMR data for the C(1′)–H signal of Table 2.

A more complete study of the atropisomerism of **6a** was conducted: dynamic NMR study at four different temperatures (293, 313, 333 and 353 K) in order to compute $\Delta H^{\ddagger}$ and E_a. As above, at each temperature, k_1 and k_2 were determined experimentally and from these data $\Delta G_1^{\ddagger}$ and $\Delta G_2^{\ddagger}$ were calculated (Table 4). Using $\ln(k/T)=23.76-\Delta H^{\ddagger}/RT+\Delta S^{\ddagger}/R$, Eyring plots of $\ln(k/T)$ versus $1/T$ gave parallel straight lines for k_1 and k_2, and from the slope, one finds $\Delta H^{\ddagger}=20.95$ and 20.23 kcal/mol for the forward and back isomerizations, respectively (Fig. 8). From the intercepts, one finds $\Delta S^{\ddagger}=-13.59$ and -14.53 cal/deg/mol for the forward and reverse reactions, respectively. Similarly, using $\ln k=-E_a/RT+\ln A$, Arrhenius plots of $\ln k$ versus $1/T$ gave $E_a=21.58$ and 20.86 kcal/mol for the forward and reverse reactions. The A factors, computed from the intercepts, are 1.95×10^{10} and 1.21×10^{10} for the forward and back reactions, respectively (Fig. 8). Finally, a plot of $R\ln K_{eq}$ versus $1/T$ gave $\Delta H^{\circ}=0.86$ kcal/mol and $\Delta S^{\circ}=1.40$ cal/deg/mol (Fig. 9). The ΔG° values from $\Delta G^{\circ}=\Delta H^{\circ}-T\Delta S^{\circ}$ match up well with those determined from $\ln(k_1/k_2)$: 0.45 versus 0.45 (293 K), 0.42 versus 0.42 (313 K), 0.39 versus 0.38 (333 K) and 0.37 versus 0.37 (353 K), respectively.

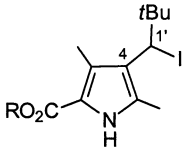
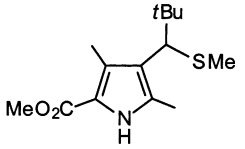
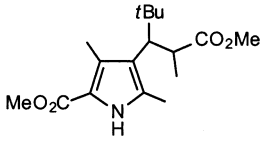
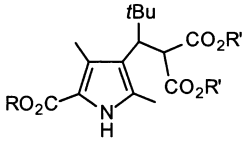
3. Concluding comments

A new class of atropisomeric pyrroles with restricted rotation about an sp^2–sp^3 carbon–carbon bond has been prepared and analyzed. Kinetic studies indicate that the ease of rotational isomerism[11] about the C(4)–C(1′) bond of **1**–**6** correlates with the size of the R′ group and bond lengths at C(1′) of Scheme 1: $CH(CO_2R)_2>CHMeCO_2Me\sim I>SCH_3>CH_2CO_2R>OCH_3$. Atropisomers **1**–**6** slowly reach a $\sim$7:3 equilibrium at room temperature. For the isolation of atropisomers that are stable at room temperature, the size of the R′ group must be enlarged, such as when the malonic ester tertiary hydrogen of **6a** or **6b** is replaced by methyl. That raises the activation barrier to atropisomerism to $\sim$32 kcal/mol at 382 K in $C_2D_2Cl_4$.[12]

4. Experimental

All NMR spectra were obtained on a Varian Unity Plus spectrometer operating at the ^{1}H frequency of 500 MHz in $CDCl_3$ solvent (unless otherwise noted). Chemical shifts are reported in δ (ppm) referenced to the residual $CHCl_3$ ^{1}H signal at 7.26 ppm, and $CDCl_3$ ^{13}C signal at 77.00 ppm. The ^{1}H NMR spectra in $C_2D_2Cl_4$ were referenced to the residual ^{1}H signal at $\delta=5.94$ ppm. For the dynamic NMR measurements, the probe temperature was controlled by a standard unit of the Unity Plus system. A J-modulated spin-echo experiment (Attached Proton Test) was used to assign ^{13}C NMR spectra. The underlined NMR signals belong to the dominant diastereomer throughout. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Gas chromatography–mass spectrometry analyses were carried out on a Hewlett–Packard 5890A

Table 3. Kinetic and thermodynamic parameters calculated for the atropisomerism of pyrroles at 313 K

		k_1 x 10^5	k_2 x 10^5	$\Delta G_1^{\ddagger}$	$\Delta G_2^{\ddagger}$	$\Delta G°$
	**1a**: R = Et	9.388	17.777	24.13	23.73	0.40
	1b: R = Me	7.213	15.135	24.28	23.82	0.46
	**3**: [b]	1.576	3.780	21.93	21.46	0.47
	**5**:	10.162	10.750	24.08	24.05	0.03
	**6a**: R = R′ = Et	1.741	3.448	25.17	24.74	0.43
	6b: R = R′ = Me	2.263	4.160	25.01	24.63	0.38
	6c: R = Me, R′ = *i*Pr	1.599	3.181	25.22	24.79	0.43

[a] $\Delta G_1^{\ddagger}$ (free energy of activation for the forward reaction), $\Delta G_2^{\ddagger}$ (free energy of activation for the reverse reaction) and $\Delta G°$ (equilibrium free energy) in kcal/mole; k in s^{-1}. [b] T = 273 K.

Table 4. Kinetic and thermodynamic parameters for **6a** from variable temperature dynamic NMR experiments

	Temperature (K)			
Experimental[a]	293	313	333	353
k_1	1.505×10^{-6}	1.741×10^{-5}	1.361×10^{-4}	8.232×10^{-4}
k_2	3.261×10^{-6}	3.448×10^{-5}	2.433×10^{-4}	1.465×10^{-3}
K_{eq}	0.4615	0.5056	0.5596	0.5619
$\Delta G_1^{\ddagger}$	24.95	25.17	25.46	25.77
$\Delta G_2^{\ddagger}$	24.50	24.75	25.07	25.36
$\Delta\Delta G^{\ddagger}$	0.45	0.42	0.39	0.41
ΔG°[b]	0.45	0.42	0.38	0.40

[a] k in s^{-1}; $\Delta G_1^{\ddagger}$ (free energy of activation for the forward reaction), $\Delta G_2^{\ddagger}$ (free energy of activation for the reverse reaction) and $\Delta G°$ (equilibrium free energy) in kcal/mol.
[b] Calculated from $\ln K_{eq} = -\Delta G°/RT$.

capillary gas chromatograph (30 m DB-1 column) equipped with Hewlett–Packard 5970 mass selective detector. Radial chromatography was carried out on Merck silica gel PF_{254} with gypsum preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). The same type of silica gel was used for preparative TLC on 20×20 cm glass plates with layer thickness of 0.75 mm. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Commercial reagents and HPLC grade solvents were dried and purified following standard procedures.[13] Ethyl 3,5-dimethyl-1*H*-pyrrole-2-carboxylate was synthesized according to a literature procedure.[14]

4.1. Methyl 3,5-dimethyl-1*H*-pyrrole-2-carboxylate, 7b

To an acetate buffer [NaOH (36 g, 0.9 mol) and glacial acetic acid (375 mL)] was added a solution of dimethyl malonate (160 mL, 1.40 mol) in acetic acid (80 mL), and the mixture was cooled in an ice bath. A solution of $NaNO_2$ (203 g, 2.94 mol) in H_2O (320 mL) was added over 3 h with gentle stirring, then the mixture was allowed to warm overnight to ambient temperature. Sodium chloride (250 g) was added, and after stirring for 45 min, the mixture was extracted with diethyl ether (4×250 mL). After evaporation of the ether solvent, the resulting crude dimethyl oximinomalonate solution[15] was used immediately in the following step.

To a mechanically-stirred mixture of pentane-2,4-dione (103 mL, 1.00 mol), zinc (196.2 g, 3.00 g), acetic acid (950 mL) and anh. sodium acetate (205 g, 2.50 mol), preheated to 80°C, was added a solution of the above oxime in acetic acid (30 mL). The rate of addition was such as to maintain the internal temperature at 80–85°C (3 h). Then the mixture was heated under reflux for 3 h

JOC Article

Novel Benzoic Acid Congeners of Bilirubin

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Three regioisomeric bilirubins and biliverdins with propionic acids replaced by benzoic acids were synthesized from the corresponding xanthobilirubic acids by oxidative coupling. The rubins were found to exhibit widely varying polarity, spectroscopic properties, and stereochemistry. The isomer with *ortho* benzoic acids (**1*o***) was much less polar than either the *meta* (**1*m***) or *para* (**1*p***) because **1*o*** (but not **1*m*** and **1*p***) can adopt a folded conformation with both carboxylic acids intramolecularly hydrogen bonded to the opposing dipyrrinones. The consequences of such conformational differentiation are found in the varying ^{1}H NMR, UV−vis, and circular dichroism spectral properties.

Introduction

Jaundice appears in humans who cannot efficiently eliminate bilirubin (Figure 1), which is a yellow tetrapyrrole dicarboxylic acid formed in normal adult metabolism, principally from the heme of red cells, at the rate of 250−300 mg per day.[1,2] Perhaps the most important aspect of the bilirubin molecular structure, concluded from numerous investigations, is its strong propensity to adopt a ridge-tile shape[3] with both carboxylic acid groups firmly engaged in intramolecular hydrogen bonding, each to a dipyrrinone.[3−8] Such a structure (Figure 1) explains many of the chemical properties of the pigment, especially its lipophilicity, e.g., soluble in $CHCl_3$ but insoluble in CH_3OH.[9] It also helps to understand why bilirubin is not excreted intact by the liver (in contrast to biliverdin or more polar bilirubin isomers where the propionic acids are displaced from C(8) and C(12), e.g., to C(7) and C(13)).[10,11] For excretion, a specific glucuronosyl transferase enzyme (UGT1A1) converts one or both of bilirubin's propionic acids to glucuronide esters in the livers of both humans and rats.[1,12−14]

Effective intramolecular hydrogen bonding between the bilirubin dipyrrinones and its carboxylic acid groups requires that the propionic acids (i) stem from C(8) and C(12) and (ii) be capable of orienting their CO_2H termini toward the dipyrrinones.[3,4] Mesobilirubin-XIIIα (Figure 2) has these essential components and orientation, and its most stable conformation is the intramolecularly hydrogen-bonded ridge-tile (as in Figure 1).[4] Molecular modeling studies indicate that a bilirubin with propionic acids replaced by acrylic acids with *cis* (but not *trans*) carbon−carbon double bonds should adopt an intramolecularly hydrogen-bonded ridge tile conformation. Bilirubins with *ortho* benzoic acid groups replacing propionic acids have their CO_2H groups locked more firmly into the stereochemistry required for intramolecular hydrogen bonding[15] and are predicted to behave similarly. In contrast, the *meta* (**1*m***) and *para* (**1*p***) regioisomers have longer carbon chain paths connecting C(8)/C(12) to the CO_2H groups, longer carbon chain paths that have their counterparts in (i) the lipophilic rubin with butyric acids replacing propionic, and (ii) in the more polar pentanoic acid rubin.[16] Unlike butyric acid and pentanoic acid rubins, the carbon chain paths of **1*m*** and **1*p*** are very inflexible and no more flexible than that of **1*o***. In the following we describe the syntheses of **1*o***, **1*m***, and **1*p*** and compare their polarity and spectroscopic properties and stereochemistry.

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or phosphate) solutions of **1*o*** and **1*m*** exhibit strong positive exciton chirality bisignate ICD Cotton effects, effectively the same as observed for bilirubin. In contrast, **1*p*** exhibits a much weaker negative chirality Cotton effect that is broadly bisignate (Figure 10). The CD intensities of **1** are comparable in magnitude to those of bilirubin, apparently due to a similar enantioselective chiral complexation by HSA. Interestingly, for reasons yet unclear, a change in pH, from 7.4 to 8.0 leads to more intense Cotton effects for both **1*o*** and **1*m*** but not for **1*p***. The ICD data for **1*o*** and **1*m*** suggest rather similar conformations on HSA, whereas the contrasting ICD data for **1*p*** suggest a different conformation or a mixture of conformations.

Conclusions

Novel analogues (**1**) of bilirubin with propionic acids replaced by benzoic acids offer three different isomers and three different fixed orientations of the carboxylic acid groups relative to the two dipyrrinone components of the pigment. In only one isomer, the *ortho* (**1*o***), can intramolecular hydrogen bonding (Figure 3) effectively stabilize the ridge-tile conformation. Consequently, **1*o*** is less polar than either the *meta* (**1*m***) or *para* (**1*p***) isomers and exhibits uniquely different chromatographic and spectroscopic properties.

Experimental Section

General Procedures. Nuclear magnetic resonance spectra were acquired at 11.75 T magnetic field strength instrument operating at ^{1}H frequency of 500 MHz and ^{13}C frequency of 125 MHz in solutions of $CDCl_3$ (referenced at 7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C) or $(CD_3)_2SO$ (referenced at 2.49 ppm for ^{1}H and 39.50 ppm for ^{13}C). *J*-modulated spin−echo (*A*ttached *P*roton *T*est) and gHMBC experiments were used to assign the ^{13}C NMR spectra. Gas chromatography−mass spectrometry analyses were carried out on a gas chromatograph (30 m DB-1 column) equipped with a mass selective detector. Radial chromatography was carried out on silica gel PF_{254} with $CaSO_4$ binder preparative layer grade, using 1-, 2-, or 4-mm thick rotors, and analytical thin-layer chromatography was carried out on silica gel IB-F plates (125-μm layer). Melting points are uncorrected. The combustion analyses were carried out by Desert Analytics, Tucson, AZ. High-resolution FAB mass spectra were obtained at the Nebraska Center for Mass Spectrometry, University of Nebraska, Lincoln, for samples which were >98% pure by NMR.

The spectral data were obtained in spectral grade solvents, used as provided, and dimethylformamide was purified by a standard procedure.[41]

Methyl 3,5-Dimethyl-4-iodo-1*H*-pyrrole-2-carboxylate (11). According to Treibs and Kolm[26] for iodination of the ethyl ester of **10**, to a solution of 15.32 g (100 mmol) of methyl 3,5-dimethyl-1*H*-pyrrole-2-carboxylate (**10**)[25] in 150 mL of ethanol, 6 mL (105 mmol) of acetic acid, and 22 mL (200 mmol) of 30% aqueous hydrogen peroxide was added a solution of 18.26 g (110 mmol) of potassium iodide in 25 mL of water during 10 min at 75−80 °C. The mixture was stirred at 75 °C for 10 min. Then it was cooled slowly while adding 60 mL of water and chilled at −15 °C for 2 h. The product was collected by filtration, washed with cold aqueous ethanol, and dried under vacuum. Recrystallization from ethyl acetate−hexane afforded 26.12 g (94%) of iodopyrrole **11**: mp 188−189 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (3H, s), 2.29 (3H, s), 3.85 (3H, s), 9.14 (1H, br. s) ppm; ^{13}C NMR ($CDCl_3$) δ 14.3, 14.6, 51.3, 72.1, 118.1, 130.9, 134.6, 161.6 ppm; MS *m*/*z* (relative abund.) 279 ($M^{+\bullet}$, 100%), 247 (85%), 220 (9%), 120 (8%). Anal. Calcd for $C_8H_{10}INO_2$ (279.1): C, 34.43; H, 3.61; N, 5.02. Found: C, 34.38; H, 3.69; N, 4.94.

General Procedure for Syntheses of Arylpyrroles 8*m* and 8*p*. To an argon-protected solution of 2.79 g (10 mmol) of iodopyrrole **11** and 2.70 g (15 mmol) of boronic acid **12*m***[23] or **12*p***[24] in 70 mL of purified and deoxygenated dimethylformamide was added 462 mg (0.4 mmol) of $Pd(PPh_3)_4$[27] followed by a hot solution of 2.12 g (20 mmol) of anh. Na_2CO_3 in 13 mL of deoxygenated water. The mixture was placed in a preheated oil bath at 100−105 °C and stirred under Ar for 20−25 min (until sudden color change from yellow to dark brown-black). After cooling with an ice bath, the mixture was diluted with 350 mL of diethyl ether and washed with 10% aqueous NH_4OH (3 × 50 mL) and then with water (4 × 50 mL). The organic layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated under vacuum. The residue was purified by radial chromatography (eluent hexane−ethyl acetate = 93:7 to 85:15) collecting the bright fluorescent band of arylpyrroles **8*m*** or **8*p***. After solvent evaporation, the material was recrystallized from hexanes−ethyl acetate.

Methyl 3,5-Dimethyl-4-(*m*-methoxycarbonylphenyl)-1*H*-pyrrole-2-carboxylate (8*m*). The *meta*-isomer was obtained in 77% yield: mp 140−141 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (3H, s), 2.28 (3H, s), 3.87 (3H, s), 3.93 (3H, s), 7.43 (1H, m), 7.47 (1H, m), 7.93 (1H, m), 7.97 (1H, m), 8.78 (1H, br. s) ppm; ^{13}C NMR ($CDCl_3$) δ 11.3, 12.1, 51.1, 52.1, 117.4, 123.7, 126.6, 127.4, 128.3, 130.16, 130.21, 131.1, 134.5, 135.4, 162.2, 167.2 ppm; MS *m*/*z* (relative abund.) 287 ($M^{+\bullet}$, 81%), 256 (44%), 255 (100%), 224 (9%), 195 (12%), 168 (31%). Anal. Calcd for $C_{16}H_{17}NO_4$ (287.3): C, 66.88; H, 5.96; N, 4.88. Found: C, 66.78; H, 5.74; N, 4.96.

Methyl 3,5-Dimethyl-4-(*p*-methoxycarbonylphenyl)-1*H*-pyrrole-2-carboxylate (8*p*). Following the procedure above, the *para*-isomer was synthesized in 78% yield: mp 155−156 °C; ^{1}H NMR ($CDCl_3$) δ 2.27 (3H, s), 2.30 (3H, s), 3.87 (3H, s), 3.94 (3H, s), 7.31 (2H, m, ^3J = 8.5 Hz), 8.07 (2H, m, ^3J = 8.5 Hz), 8.83 (1H, br. s) ppm; ^{13}C NMR ($CDCl_3$) δ 11.3, 12.2, 51.1, 52.0, 117.7, 123.7, 126.5, 127.8, 129.5, 129.8, 130.3, 140.1, 162.1, 167.1 ppm; MS *m*/*z* (relative abund.) 287 ($M^{+\bullet}$, 94%), 255 (100%), 224 (25%), 196 (11%), 168 (21%). Anal. Calcd for $C_{16}H_{17}NO_4$ (287.3): C, 66.88; H, 5.96; N, 4.88. Found: C, 67.20; H, 6.02; N, 4.92.

3-Ethyl-8-(*o*-methoxycarbonylphenyl)-2,7,9-trimethyl-1,10-dihydro-11*H*-dipyrrin-1-one (5*o*) was prepared as reported previously[15] from reaction of **6** and **7*o***: mp 289−291°C (lit.[15] mp 289−291°C); UV−vis ϵ_{435}^{sh} 27,700, ϵ_{410}^{max} 45,900 (benzene), ϵ_{407}^{max} 40,500 ($CHCl_3$), ϵ_{413}^{max} 42,000 (CH_3OH), ϵ_{400}^{max} 38,100 (CH_3CN), ϵ_{412}^{max} 39,900, ϵ_{399}^{max} 38,100 (DMSO); ^{13}C NMR and ^{1}H NMR in Tables 1 and 2.

3-Ethyl-8-(*m*-methoxycarbonylphenyl)-2,7,9-trimethyl-1,10-dihydro-11*H*-dipyrrin-1-one (5*m*). A mixture of 1.44 g (5 mmol) of monopyrrole **8*m***, 2.00 g (50 mmol) of sodium hydroxide, 30 mL of ethanol, and 9 mL of water was heated at reflux for 4 h. After cooling, the ethanol solvent was evaporated under vacuum, and the residue was diluted with 10 mL of 50% aqueous $NaNO_3$. The solution was cooled at −20 °C and acidified by slow addition of a solution of concentrated HNO_3 in 50% aqueous $NaNO_3$ (1:5 v/v). The precipitated product was collected by filtration, washed with cold water and dried overnight under vacuum. This crude diacid (**7*m***) was obtained in nearly quantitative yield and was used immediately in the following step without further characterization.

A mixture of diacid from above, 1.17 g (5.4 mmol) of 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole (**6**),[28] 35 mL of anhydrous methanol, and 1 drop of 48% aqueous HBr was heated at reflux for 9 h. Then the mixture was chilled overnight at −20 °C. The precipitated crude dipyrrinone acid **4*m*** was collected by filtration, suspended in 50 mL of CH_3OH

(41) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: England, 1988.

МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛЕВЕН

Стефан Емилов Бояджиев

СТЕРЕОХИМИЯ НА ЛИНЕЙНИ ТЕТРАПИРОЛИ, ПРОИЗВОДНИ ОТ ЖЛЪЧНИТЕ ПИГМЕНТИ И ТЕХНИТЕ СЪСТАВНИ ЕЛЕМЕНТИ

АВТОРЕФЕРАТ

НА ДИСЕРТАЦИЯ ЗА ПРИДОБИВАНЕ НА НАУЧНАТА СТЕПЕН
″ДОКТОР НА ХИМИЧЕСКИТЕ НАУКИ″

Научна специалност
01.05.03 Органична химия

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Плевен, 2011 г.

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Дисертационният труд е обсъден и насрочен за защита от разширен катедрен съвет на Катедра „Химия и биохимия & физика и биофизика" на Медицински университет – Плевен.

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Автор: Стефан Емилов Бояджиев
Заглавие: Стереохимия на линейни тетрапироли, производни от жлъчните пигменти и техните съставни елементи.
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ИЗВОДИ

Получените при разработването на дисертационния труд резултати имат научно-фундаментален характер. Те могат да се използват при следващи стереохимични изследвания върху олигопироли с приложение на конформационен анализ и електронен кръгов дихроизъм.

1. Предложен е конформационен модел за мезобилирубинови производни съдържащи хирални центрове, с който се предсказва изместване на динамичното равновесие. Цялостната 3D молекулна форма в тези производни се контролира от вътрешномолекулни стерични въздействия от разстояние. Първостепенно значение в този модел се отрежда на конформационно-стабилизиращата роля на вътрешномолекулните водородни връзки.

2. Открити са синтетични методи за получаване на два възлови монопирола в енантиомерно чиста форма. Прилагането на подходящи химични модификации в тези две съединения води до редица предшественици на хомохирални ди- и тетрапироли. Разработени са нови и са усъвършенствани синтетични превръщания в химията на пироли, дипиринони и тетрапироли. Съществените приноси сред тях са реакциите на естерни енолати от N-незащитени пироли с разнообразни електрофили и окислителната кондензация на два различни дипиринона.

3. Абсолютната конфигурация на четири монопирола е установена с рентгеноструктурен анализ. От него следва абсолютната конфигурация на голям брой потомствени на монопиролите оптично активни аналози на ксантобилирубиновата киселина, мезобиливердина и мезобилирубина.

4. Проведен е детайлен конформационен анализ на хирални мезобилирубини, мезобиливердини и дипиринони чрез изчисления с метода на молекулната механика и динамика. Резултатите от тях съответстват на основните изводи от предложения модел за конформационен контрол чрез алостерично въздействие. Конформационното предпочитание на изследваните съединения е доказано експериментално с резултатите от модерни ЯМР методи и от спектроскопия на кръгов дихроизъм.

- Доказано е, че две метилови групи на α, α'- или на β, β'-хирални центрове в мезобилирубин XIIIα изместват в значителна степен конформационното равновесие $\boldsymbol{M} \rightleftharpoons \boldsymbol{P}$. Струпване на повече конформационно-направляващи групи не е съпроводено с повишена диастереоселекция. Доказано е, че ***M***-хиралният билирубинов конформер

се характеризира с отрицателна екситонна хиралност в кръговия дихроизъм.

- Доказана е важността на вътрешномолекулното водородно свързване при контрола на хиралността на билирубинови конформери. Мезобилирубини без значителна конформационна стабилизация от вътрешномолекулни водородни връзки са конформационно хетерогенни, от което следва ниска интензивност на кръговия им дихроизъм.

- Установено е, че заместител на α-хирален център в остатъка от пропионова киселина има по-високи стерични изисквания от същия заместител на β-хирален център. Използвайки кръгов дихроизъм е оценен относителният стеричен размер на групи, които насочват в противоположни посоки конформационното равновесие в α, β'-заместени мезобилирубини.

- Установено е, че екситонната хиралност се обръща в присъствие на амини.

5. Изведена е корелация между абсолютна конфигурация, предпочетена молекулна хиралност (екситонна хиралност) и знаци на кръгово-дихроичните ефекти в хирални мезобилирубини.

6. Определена е връзката между стереохимия и кръгово-дихроични ефекти в циклични естери на мезобиливердин XIIIα.

7. Предложен е нов тип 3D димерни структури при хомолози на ксантобилирубиновата киселина. Хиралните представители от този клас се характеризират с интензивен кръгов дихроизъм вследствие на екситонно взаимодействие от междумолекулен произход.

8. Установена е атропизомерия в мезобилирубини съдържащи остатъци от *орто*-бензоена киселина. Доказана е атропизомерия в пиролови съединения, която възниква поради затруднено въртене около $sp^3 - sp^2$ въглерод-въглеродна връзка. С подходящи методи са изолирани индивидуални атропизомери от този тип, чиито кинетични параметри на изомеризация са установени.