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SYNTHESIS, ISOLATION, RELATIVE CONFIGURATIONS AND THIN-LAYER CHROMATOGRAPHIC BEHAVIOUR OF OPTICALLY ACTIVE 3-HYDROXY-2, 3-DIARYLPROPIONIC ACIDS (—) -MENTHYL ESTERS

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Посредством низкотемпературной Кляйзеновой реакции получены (—)-ментиловые эфиры оптически активных 3-гидрокси-2,3-диарилпропановых кислот. Суррогат, содержащий четыре диастереомера, разделен до индивидуальных изомеров путем комбинирования хроматографии на силикагеле и фракционной перекристаллизации. Относительные конфигурации соединений установлены путем использования ЯМР-спектров. Осуществлено тонкослойное хроматографическое разделение 16 соединений. При этом установлено, что трео-изомеры более подвижны, чем соответствующие эритро-изомеры. Эта реляция интерпретирована теоретически.

Optically active 3-hydroxy-2, 3-diarylpropionic acids (—)-menthyl esters have been prepared by low-temperature Claisen reaction. The crude products containing four diastereoisomers have been separated in a preparative scale to the individual isomers by combination of chromatography on silica gel and fractional recrystallization. The relative configurations of the compounds have been assigned on the basis of NMR-spectra. Thin-layer chromatographic separation of 16 compounds has been achieved. Thus, it has been established that the threo-isomers always move faster than the corresponding erythro-isomers. The relation is theoretically interpreted.

converted to **5a, b** and **5c, d** resp. Further, the diastereoisomers of **2**, **3** and **4** were transformed into **6**, **7** and **8** resp.

Thanks are due to Dr. M. Mladenova for a discussion concerning the preparation of 4 by other aldol-type reactions.

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СИНТЕЗ, ИЗОЛИРОВАНИЕ, ОТНОСИТЕЛЬНЫЕ КОНФИГУРАЦИИ И ТОНКОСЛОЙНОХРОМАТОГРАФИЧЕСКИЕ СВОЙСТВА (—)-МЕНТИЛОВЫХ ЭФИРОВ ОПТИЧЕСКИ АКТИВНЫХ 3-ГИДРОКСИ-2,3-ДИАРИЛПРОПАНОВЫХ КИСЛОТ

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(Резюме)

Низкотемпературная реакция Кляйзена была использована для синтеза (—)-ментиловых эфиров оптически активных 3-гидрокси-2,3-диарилпропановых кислот. Из сырого продукта изолировано по 4 оптически активных диастереомера путем хроматографического разделения на силикагеле с последующей фракционной перекристаллизацией. Относительные конфигурации соединений определены на основании ЯМР-спектров их О-ацетильных производных. С помощью тонкослойной хроматографии разделены 16 соединений и установлено, что трео-изомеры во всех случаях обладают большей хроматографической подвижностью, чем соответствующие *эритро*-изомеры. Указанная зависимость объяснена с точки зрения стереохимии и хроматографии, по аналогии с предыдущими исследованиями.

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ABSOLUTE CONFIGURATIONS OF SOME (—)-MENTHYL 2,3-DIARYL-3-HYDROXYPROPANOATES AND 1,2-DIARYL-1,3-PROPANEDIOLS

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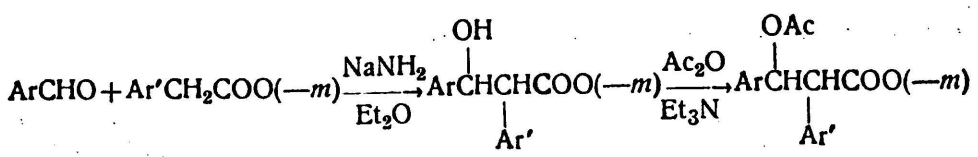
The absolute configurations of six diastereomeric (-)-menthyl 2,3-diaryl-3-hydroxypropanoates are determined via stereospecific conversions to the known (-)-menthyl *erythro* (2R, 3R)-2,3-diphenyl-3-hydroxypropanoate and *threo* (2R, 3S)-2,3-diphenyl-3-hydroxypropanoic acid. The absolute configurations of two other diastereomers is determined on the basis of CD spectra. The corresponding 1,2-diphenyl-1,3-propanediols and 0,0′-diacetyl derivatives are synthesized and correlated by stereospecific reduction of the esters.

It was shown earlier [1,2] that the thermodynamically controlled low-temperature Claisen reaction between (—)-menthyl arylacetates and aromatic aldehydes conducted in the presence of sodium amide in ether [3] leads to the hydroxy esters **1-5** (Fig. 1). The crude reaction product consists in all cases of the four theoretically possible diastereomers **a-d***.



1—5 a—d **6—10 a—d**

Ar=Ph, Ar′=Ph	1a—d,	6a—d
Ar=4-Cl-C_6H_4-, Ar′=Ph	2a—d,	7a—d
Ar=Ph, Ar′=4-Cl-C_6H_4—	3a—d,	8a—d
Ar=4-CH_3O-C_6H_4-, Ar′=Ph	4a—d,	9a—d
Ar=4-Br-C_6H_4-, Ar′=Ph	5a—d,	10a—d

Fig. 1

* The a-d designations are given in Table 1.

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АБСОЛЮТНИ КОНФИГУРАЦИИ НА НЯКОИ 2,3-ДИАРИЛ-3-ХИДРОКСИПРОПАНОВИ КИСЕЛИНИ (—)-МЕНТИЛОВИ ЕСТЕРИ И 1,2-ДИАРИЛ-1,3-ПРОПАНДИОЛИ

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(Резюме)

Посредством стереоспецифични превръщания до известните еритро-2R, 3R-2,3-дифенил-3-хидроксипропанова киселина (—)-ментилов естер и трео-(2R, 3S)-2,3-дифенил-3-хидроксипропанова киселина са доказани абсолютните конфигурации на (—)-ментиловите естери на шест диастереомерни 2,3-диарил-3-хидроксипропанови киселини. Абсолютната конфигурация на други два диастереомера е доказана въз основа на спектри на кръгов дихроизъм.

Чрез стереоспецифична редукция на естерите са синтезирани и корелирани съответните 1,2-дифенил-1,3-пропандиоли и 0,0′-диацетилните производни.

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PREPARATION, CIRCULAR DICHROISM AND ABSOLUTE CONFIGURATION OF SOME OPTICALLY ACTIVE 1,1,3-TRISUBSTITUTED PROPANOLS

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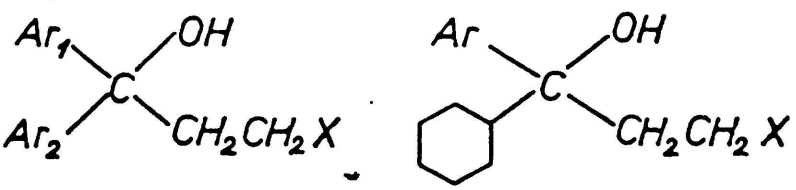
INTRODUCTION

As it's well known a number of 1,1,3-trisubstituted propanols represented by formulas I and II, where X is mainly a disubstituted amino group



I II

have already found a wide application as antiparkinson and antitussive drugs. That is the case of clofedanol [1] (I, Ar_1= Ph, Ar_2= o-Cl-C_6H_4, X= $N(CH_3)_2$; procyclidine [2] II, Ar= Ph, X= N⟨⟩ .HCl (for the optically active form see below (+)-9), benzhexol [2] (II, Ar= Ph, X= N⟨⟩ .HCl) and etc. Some of these compound exhibit a clearly observeble biostereoselectivity, i.e. the anti-muscarinic action of procyclidine and benzhexol [2] is almoust due to the laevorotatory forms.

In these cases elucidation of the absolute configuration of the biologically more active enantiomers could be of a conside-

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Synthesis of Some Optically Active α-Pyridyl-carbinols and Determination of Absolute Configuration from the CD of Their *in situ* Complexes with $[Mo_2(OAc)_4]$

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Four new α-pyridyl-aryl-carbinols have been synthesized, and twelve such racemates have been for the first time resolved by standard procedures. From the positive CD-couplet of the complex between (*S*)-1 and 23 an antiperiplanar arrangement of the H—C(OH, Ph)—C_α(Py)—N-moiety could be derived as the preferred conformation. From the CD-spectra above 300 nm of this and seven other compounds of known absolute configuration in the presence of *23* the following correlation with stereochemistry was derived, which was then applied to determining the absolute configuration of fourteen other α-pyridyl-aryl-carbinols: for (*R*)-configuration negative CD-bands appear between 500 to 510 (sometimes detectable only as shoulder) and at 408 to 470 nm, between 335 to 380 nm a positive Cotton effect is registered, and another one shows up below 320 nm, which has mostly a positive sign for secondary, but a negative one for tertiary carbinols of the mentioned absolute sense of chirality.

SYNTHESIS

Of the compounds used for these studies 4 racemates have been newly synthesized by known procedures (for details *cf.* EXPER.). The syntheses followed one of three pathways, *vic.* condensation of a (substituted) benzaldehyde or acetophenone with α-pyridyl lithium, or of α-acetyl-pyridine with an aryl magnesium bromide. By resolution *via* crystallization of diastereomeric salts prepared with different optically active acids, 12 of the 22 compounds have been prepared for the first time in optically active form.

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SAŽETAK

Sinteza optički aktivnih α-piridil-karbinola i određivanje apsolutne konfiguracije pomoću CD spektara njihovih *in situ* kompleksa sa $[Mo_2(OAc)_4]$

Nikolina Berova, Stefan Bojadziev, Rosiza Rakowska, Jadwiga Frelek i Günther Snatzke

Sintetizirana su četiri nova α-piridil-aril-karbinola, i po prvi puta je, koristeći standardni postupak, razlučeno dvanaest racemata tog tipa. Iz pozitivnog CD kupleta kompleksa između (*S*)-*1* i *23* može se zaključiti da ovaj u preferiranoj konformaciji posjeduje antiperiplanarni raspored strukturne jedinice H—C(OH, Ph)—C_α(Py)—N. Iz CD spektara iznad 230 nm ovog i sedam drugih spojeva poznate apsolutne konfiguracije u prisutnosti *23*, izvedena je korelacija s njihovom steričkom građom, koja je zatim primjenjena na određivanje apsolutne konfiguracije drugih četrnaest α-piridil karbinola: za (*R*)-konfiguraciju karakteristične su negativne CD vrpce između 500—510 nm (nekad opazive samo kao »rame«), i između 408—470 nm, dok se između 335—380 nm javlja jedan pozitivni Cottonov efekt. Još jedan Cottonov efekt javlja se ispod 320 nm, koji većinom ima pozitivan predznak za sekundarne, a negativan za tercijarne karbinole spomenutog apsolutnog smjera kiralnosti.

Synthese, Stereochemie und Antiulkusaktivität von 4-Phenyltetrahydroisochinolinen

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Es wurden die racemischen und optisch aktiven 4'- und 8-substituierten 4-Phenyltetrahydroisochinoline **2-5** synthetisiert und deren Effekt auf den Wasser-Immersions-Streßulkus bei Ratten untersucht. Alle Verbindungen inhibieren die durch Streß verursachten Geschwüre, wobei der Effekt bei **4** und seinem rechtsdrehenden Isomer (R^1=Cl, R^2=$NHCOOC_2H_5$) besonders deutlich ausgeprägt ist. Die Antiulkusaktivität von **4** ist 30 bis 50 mal höher als die der zum Vergleich herangezogenen Histamin-H_2-Antagonisten Cimetidin und Ranitidin. Mittels chemischer Korrelation wurde die absolute Konfiguration der optisch aktiven Verbindungen **2**, **3** und **4** bestimmt. Die Antiulkusaktivität bei **4** ist durch Enantioselektivität charakterisiert: S-(+)-**4** ist dreimal aktiver als R-(-)-**4**.

Synthesis, Stereochemistry, and Antiulcer Activity of 4-Phenyltetrahydroisoquinolines

The racemic and optically active 4'- and 8-substituted tetrahydroisoquinolines **2-5** have been synthesized and their effect on the water-immersion stress ulcer in rats has been studied. All compounds prevent the formation of stress ulcer, the strongest effect being exhibited by compound **4** and its dextrarotatory isomer (R^1=Cl, R^2=$NHCOOC_2H_5$). The antiulcer activity of **4** is 30 to 50 times higher than that of the H_2 receptor antagonists Cimetidin and Ranitidin used for comparison. The absolute configuration of the optically active compounds **2**, **3** and **4** has been determined by means of chemical correlation. The antiulcer activity of **4** is characterized by enantioselectivity, S-(+)-**4** is three times as active than R-(-)-**4**.

Es wird allgemein angenommen, daß die Magensalzsäure von wesentlicher pathologischer Bedeutung ist, obwohl die genaue Ethiologie des Magengeschwürs noch ungeklärt ist. Degeneration und Nekrose der durch Magensäuresekretion hervorgerufenen Magen-Darm-Mukosa führen zur Bildung peptischer Ulzera.

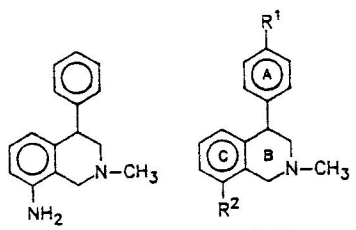
Die Magensäuresekretion wird von den Histamin H_2-Receptoren stimuliert. Antagonisten der H_2-Receptoren, z.B. Cimetidin und Ranitidin inhibieren die Magensäuresekretion bei Menschen und Tieren und werden zur Behandlung von Magengeschwüren peptischen Ursprungs angewendet. Gleichzeitig aber führen sie zu unerwünschten Nebeneffekten und sind bei der Prophylaxe und Behandlung von Streß-Ulkus schwach wirksam.

In den letzten Jahren wird der Rolle des Zentralnervensystems große Aufmerksamkeit in der Pathogenese peptischer Geschwüre gewidmet. Nach *Feldman* et al.[1] geht der emotionelle Streß oft der Entwicklung des peptischen Ulkus voraus. Anhaltender psychologischer oder physiologischer Streß führt zur Bildung von Magen- oder Zwölffingerdarmgeschwüren oder zu sog. Streß-Ulzera[2], die Hämorrhagie, Perforation und Tod bei 50% der Patienten hervorrufen[3].

In letzter Zeit herrscht die Meinung vor, daß peptischer Ulkus eine psychosomatische Krankheit ist. Nach *Guldahl*[4] äußern über 80% der Kranken mit peptischem Ulkus Symptome einer Scheindepression.

Vielzählige Untersuchungen mit Antidepressiva, Tranquilantien u.a. ergaben Antiulkuseffekte verschiedenen Grades. Die Antidepressiva Imipramin, Desmethylimipramin und Doxepin beeinflussen z.B. Streß-Ulkus und inhibieren das saure Magensekret[5,6,7], dagegen weisen Amitriptylin und Tandamin nur Antiulkusaktivität auf[8].

Das Antidepressivum Alival (**1**) mit einer von den tricyclischen Antidepressiva unterschiedlichen Struktur wirkt nur gegen durch Streß verursachte Effekte und hat keine antisekretorischen Eigenschaften[9].



1 **2-5**

2: R^1 = H; R^2 = $NHCOOC_2H_5$
3: R^1 = Cl; R^2 = NH_2
4: R^1 = Cl; R^2 = $NHCOOC_2H_5$
5: R^1 = H; R^2 = Cl

Schema 1

Wir stellten fest, daß Alival und sein rechtsdrehendes Isomer einen synergistischen Effekt mit den Histamin H_2-Antagonisten Cimetidin und Ranitidin im Wasser-Immersionsstreßulkus bei Ratten hervorrufen[10]. Dieser Effekt führt zu einer wesentlichen Erhöhung der Antiulkusaktivität der H_2-Antagonisten im Vergleich zu deren alleiniger Anwendung.

Daher erweiterten wir unsere Untersuchungen über den Antiulkuseffekt auf neue Vertreter der 4-Aryltetrahydroisochinoline. In der vorliegenden Arbeit wird der inhibierende Effekt der 4-Phenyl- und 8-substituierten Tetrahydroisochinoline **2-5** auf die Bildung von Streß-Ulkus besprochen.

Nach *Zara-Kaszian* et al.[11] weisen die racem. Alkoxycarbonylamino-tetrahydroisochinoline **2** und **4** sowie das 8-Aminoderivat **3** hohe antidepressive Aktivität auf und sind starke Inhibitoren des Dopamin- und Noradrenalin-Uptakes. Sie sind daher für unsere Untersuchungen von Interesse. Zur Untersuchung des Einflusses stereochemischer Faktoren auf die Antiulkusaktivität stellen wir die bisher nicht beschriebenen Antipoden der Verbindungen **2**, **3** und **4** her.

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168. Circular Dichroism of Some 2-(Phenylmethyl)pyridine Derivatives

by **Nikolina Berova**[a]), **Stefan Bojadziev**[a]), **Nevenka Bresciani-Pahor**[b]), **Petko M. Ivanov**[a]), **Biserka Kojić-Prodić**[c]), **Rositza Rakovska**[a]), **Živa Ružić-Toroš**[c]), and **Günther Snatzke**[d])*

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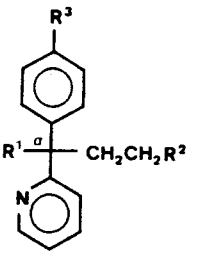
(20.VI.90)

The absolute configuration of the 2-(phenylmethyl)pyridine derivatives **1–9** had been established by X-ray diffraction and chemical correlation. Their CD spectra have been studied in different solvents for the free and protonated forms. It has now been found that, from the sign of the strong CD couplet between 270 and 220 nm, which was observable for all these compounds besides **7** and **9**, their absolute configuration can be determined much quicker.

1. Introduction. – Several 2-substituted pyridine derivatives show important pharmacological activities, and a few of them are used in human medicine. In Sofia, many compounds of this type have been synthesized [1] in enantiomerically pure form, but, of several, the absolute configuration could not be determined by chemical correlations alone. This could, however, be achieved by a combination of chemical correlation with the X-ray diffraction of two suitable derivatives [2]. Here, we report on the chiroptical properties of nine compounds related to each other, and compare the conformations in the crystal [2] and in solution.



	R^1	R^2	R^3
1	HO		Cl
2	HO		H
ent-**3**	H	H	H
ent-**4**	HO	H	H
5	H	NMe_2	Cl

Recently, we [3] described the chemical correlation between compounds **1–4**, including the catalytic replacement of an OH group by H. Although it is claimed [4] that this step can take place with retention, we nevertheless had to prove this independently for our compounds. For the sake of clarity, the same absolute configuration at the chiral centre has been assumed throughout, although in some cases actually the enantiomer had been investigated.

It is now proved unequivocally that the catalytic replacement of OH by H takes place with retention also in our case, as had been claimed for this reaction in general [4].

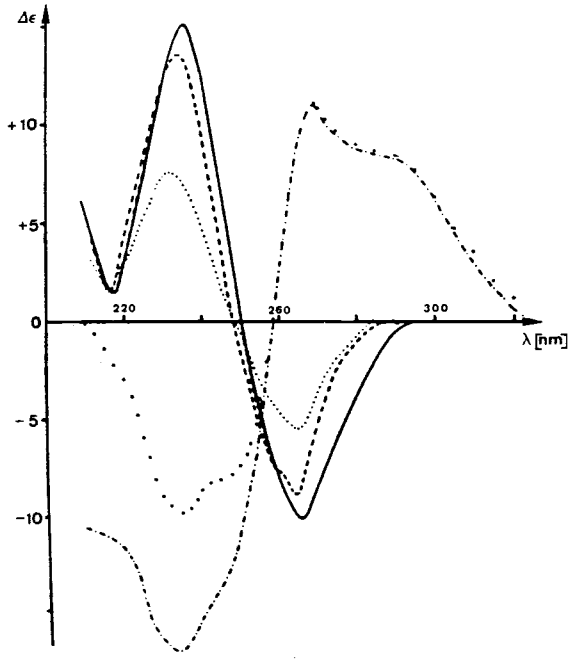


Fig. 2. *CD Spectrum of (+)*-ent-7 *in cyclohexane* (——), *MeCN* (– – – –) *MeCN + TFA* (– · – · – · –), *MeOH* (· · · · ·), *and MeOH + HCl* (· · · · · ·)

8. CD Spectra of (+)-8 and (+)-9. – The CD spectra of (+)-**8** in MeOH as well as in MeCN are very similar to each other and the *Cotton* effects around 272 and 229 nm have opposite signs, being characteristic for a CD couplet. Since, however, the $\Delta\varepsilon$ values are relatively small, they may be due to two individual *Cotton* effects which by chance have opposite signs and similar absolute magnitudes for the rotational strengths.

The most characteristic feature in the CD spectra of the *N*-oxide (+)-**9** is a very pronounced couplet-type CD at 220/197 nm, (MeOH or MeCN solution) with $\Delta\varepsilon$ values up to 74. In this wavelength range, several transitions may give rise to absorption bands, and we can, therefore, at present not discuss these CDs in another than purely empirical way. Since also other *N*-oxides of this type show this very intense CD couplet, we are now investigating such compounds in more detail.

9. Conclusions. – In the crystals of **1** [2], the torsional angle around the pivot bond to the pyridine is arranged in such a way, that the N-atom is synperiplanar to the substituted Et group. This is the same overall conformation as observed in solutions in unpolar solvents, as it could be deduced from the sign of the CD couplet. Its IR spectrum in very dilute solution in CCl_4 indicated, furthermore, an internal H-bridge between the OH group and the pyrrolidine-N-atom, which, on the basis of the models, seems easily possible for the discussed conformation of the pyridine ring. This synperiplanar conformation is retained even in more polar solvents like MeOH or EtOH, since a similar CD couplet is also found under these conditions.

After protonation, again a CD couplet can be found, but this time of opposite sign to the first mentioned one. This would not be expected, if the more basic pyrrolidine-N-atom were protonated. Accordingly, we can conclude that also the pyridine-N-atom is protonated under these conditions. Since the HN^+ moiety is larger than the free pyridine-N-atom alone, obviously the synperiplanar arrangement of the pyridine-N-atom and CH_2 is no more preferred, and the pyridine ring is rotated into another conformation. Molecular models show that several 'reasonable' conformations would be consistent with the CD, and, therefore, we are not able to deduce the new torsional angle from this CD.

Compound **2** differs from **1** only by the lack of the *p*-Cl substituent on the benzene chromophore. As we had proved in our CD studies of chiral 1,2-diphenylethanes, such a substituent has no essential influence upon the *p*- and *β,β'-Cotton* effects [9]; we expect, therefore, the CD behaviour of **2** to be very similar to that of **1**, which was indeed established. Furthermore, the absolute configuration of **2** is, thus, also determined by this comparison.

Like **1** and **2**, also *ent*-**4** contains an OH group at the chiral centre, but no other, more basic N-atom is present, which could act as a better acceptor for an H-bond than the pyridine-N-atom. One can, therefore, predict, that an internal H-bridge is now formed between OH and the pyridine-N-atom, and as already discussed, CD data support this view.

Compound **3** contains only one N-atom and no OH group. Since we observed a similar CD couplet in unpolar solvents for *ent*-**3** as for **1** and **2**, the arrangement of both rings of *ent*-**3** must also be similar to those of **1** and **2**. This conclusion from the CD data is supported by the MM-2 calculation [5]. Solvation by MeOH destabilizes this conformation, since in MeOH the CD couplet disappears, and one observes the usual small CD (with fine structure) within the α band.

For **6**, with Me instead of a bulkier substituent at the chiral centre, we also found great similarity between the crystal and the solution conformation. In unpolar solvents, most of the molecules have an internal H-bridge, being in accordance with the synperiplanar arrangement of the N–C–C–O moiety found in the crystal, even though there are several intermolecular H-bridges present to the tartrate unit. As a consequence of this preferred conformation in solution, the sign of the observed CD couplet in unpolar solvents is correctly predicted for **6**.

In view of the fact that, for other members of this series, an even more remote substitution may, however, cause changes of the conformation and, therefore, also of the CD, we still strongly advice, in any new set of even related compounds, to determine the absolute configuration for at least one member in an independent way.

In accord with these views are the chiroptical properties of *ent*-**7**. No internal H-bridge is possible, and its CD is in agreement with the one predicted for the preferred conformation (see above). Since no H-bridges are involved for this compound, MeOH weakens but does not completely eliminate the CD couplet.

In conclusion, we would like to emphasize that, for the determination of the absolute configuration of such conformationally mobile compounds of pharmacological importance, the quickly performed CD method, which, furthermore, does not destroy any material, can successfully be applied, provided a correct model compound of known configuration is available.

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SYNERGISM BETWEEN STEREOELECTRONIC AND STEREOPROTONIC EFFECTS IN THE ENZYMIC PEPTIDE BOND FORMATION

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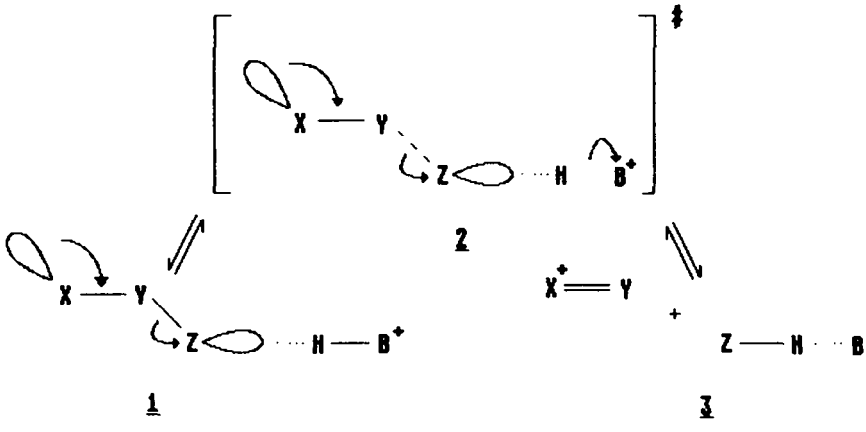
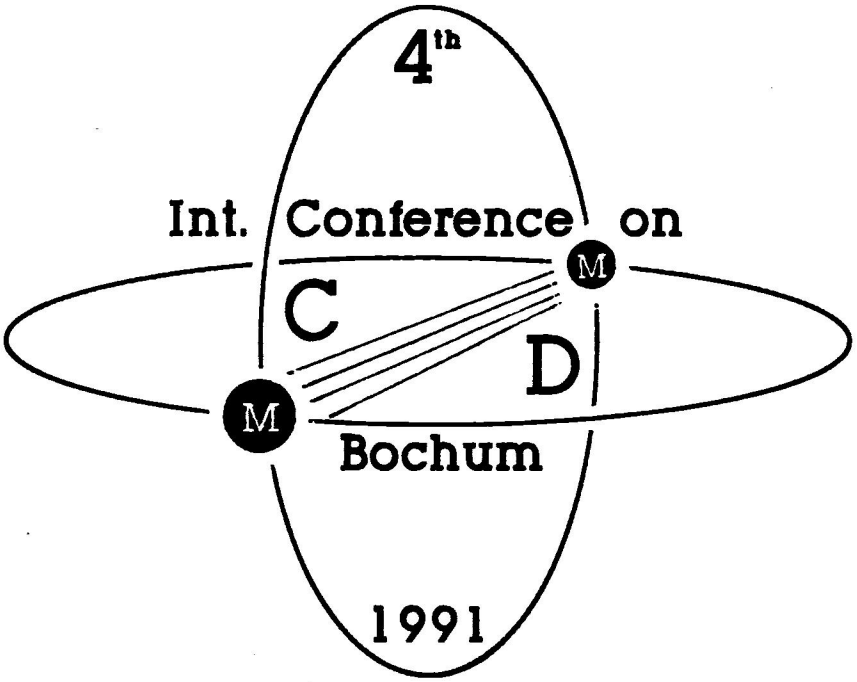
Abstract - *Synergism between stereoprotonic (an inherent preference for protonation/deprotonation in the lone pair direction) and stereoelectronic effects on the formation and breakdown of the tetrahedral intermediate has been demonstrated during the kinetically controlled enzymic aminolysis of specific esters by N-nucleophiles. While both N-methylated and N-unmethylated nucleophiles promote chymotrypsin anilide hydrolysis, the N-methylated nucleophiles do not aminolyse detectably acylchymotrypsin.*

The favourable interaction between a lone pair orbital *n* of the atom X (Scheme I) and the antibonding orbital σ^* of an antiperiplanar polar bond Y–Z (n-$\sigma^*_{Y\text{-}Z}$ interaction) proves to be essential for the stabilization of the ground state (thermodynamic effect) or transition state (kinetic effect) of the mo-



Scheme I

lecular conformation 1[1]. The thermodynamic effect is expressed by the conformational preferences of sugars (anomeric effect[1]) and nucleic acids (gauche effect[2]), the kinetic effect is observed in the stereoelectronic acceleration of the polar Y–Z bond cleavage/formation[3]. On the other hand, a protonation/



Lectures and Posters

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CIRCULAR DICHROISM FROM EXCITON COUPLING. CONFORMATIONAL ANALYSIS OF BILIRUBIN, THE NEUROTOXIC YELLOW PIGMENT OF JAUNDICE

Richard V. Person, Stefan E. Boiadjiev, Blake R. Peterson
Gisbert Puzicha and David A. Lightner*

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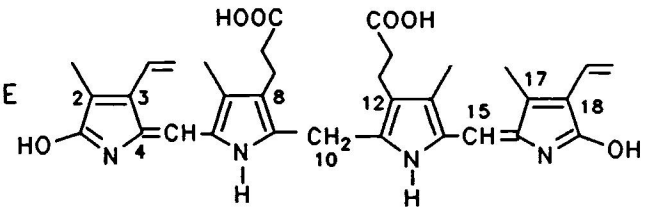
ABSTRACT

Conformational analysis of (4*Z*,15*Z*)-bilirubin-IXα (bilirubin) and its symmetric analog, mesobilirubin-XIIIα, using molecular mechanics computations indicates that a folded conformation falls at the global energy minimum. 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 optical resolution of the pigments, with enantiomers exhibiting the theoretically predicted circular dichroism.

INTRODUCTION

The constitutional structure of bilirubin was elucidated by Hans Fischer's group some fifty years ago through a combination of degradation, partial and total synthesis.[1] The molecule was shown to be an unsymmetrically substituted tetrapyrrole dicarboxylic acid consisting of two dipyrrole halves conjoined by a saturated -CH_2- group. The two halves are almost identical mirror images and, though covalently linked, can react more or less independently. Aside from a few minor details, such as Fischer's preference for the lactim tautomer (hydroxypyrrole) and an unspecified stereochemistry at the C-4 and C-15 carbon-carbon double

FISCHER'S STRUCTURE OF BILIRUBIN:



mations are predicted by exciton coupling theory to be similar. Significantly, in water at pH 9-11, where the propionic acid groups are ionized, the $\Delta\epsilon$ values are still ~50% of their maximum values. These data suggest that ionization of the propionic acid groups *per se* does not imply extensive breaking of the intramolecular hydrogen bonds, as discussed in other work.[16,22] Rather, the reduction in $\Delta\epsilon$ values is probably due more to the hydrogen-bonding solvation and polarity effects of water — just as for the pigment dissolved in (neutral) ethanol. These results are very important for and relevant to studies of bilirubin in aqueous solvents and when bound to albumins or other proteins in water. In the latter, they are consistent with a model in which the protein binds preferentially to one bilirubin enantiomer (Figure 7) through the action of salt formation between the pigment's propionic acid groups and specific amine residues on the protein.[21]

CALCULATED CIRCULAR DICHROISM

We have calculated the UV-visible and CD spectra,[33] using the coupled oscillator formalism, of mesobilirubin-XIIIα for all of the conformations represented in the bilirubin conformational energy map of Figure 4. Selected computed data (Table 2) illustrate the concepts and principles discussed above. The UV-vis transitions show the expected (1) blue shift for the porphyrin-like conformation (with parallel transition dipole moments) and (2) red shift for the linear conformation (with in-line transition dipole moments). The CD Cotton effect $|\Delta\epsilon|$ values reach a maximum for the folded conformation and decline to essentially zero in the porphyrin-like and the linear conformations. A full UV-CD-conformation map has been generated and will be published elsewhere.

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

TABLE 2. Selected Computed CD and UV-Visible Data[a,b] for Mesobilirubin-XIIIα: Porphyrin-like, Helical, Folded, Ridge-tile, Extended, Stretched and Linear Conformations.

Torsion Angle (°)		Conformation Type	Theoretical UV-Vis[c]				Summed Theor. UV-Vis		Computed CD[c]		
ϕ_1	ϕ_2		λ^β	ϵ_{max}	λ^α	ϵ_{max}	λ_{max}	ϵ_{max}	λ_1	λ_2	$\Delta\epsilon$ at λ_2
0	0	Porphyrin-like	392	75,400	475	~0	392	75,400	391	477	~0
20	20	Helical	397	67,700	469	7,800	398	68,115	395	472	+170
60	60	Folded or Ridge-tile[d]	414	34,600	447	44,000	434	65,251	401	463	+275
90	90	Gabled	415	16,700	446	58,700	441	68,829	401	463	+ 68
120	120	Extended	417	10,800	444	64,600	441	71,900	402	463	- 85
160	160	Stretched	422	2,000	439	73,400	438	75,000	402	463	-103
180	180	Linear	424	~0	436	75,400	436	75,400	402	462	~0

[a] Based on conformations in which the porphyrin-like conformation passes through P (Figure 7) on the way to linear.
[b] ϵ and $\Delta\epsilon$ in L • mole^{-1} • cm^{-1}.
[c] For the β-state and α-state of the molecular exciton.
[d] Folded, with intramolecular hydrogen bonding.

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 β-H (or α-H)[26] by CH_3 to give enantiomeric β,β'-dimethylmesobilirubins-XIIIα. The *S,S* and *R,R* enantiomers have been synthesized stereospecifically and shown to adopt the **M** and **P** chirality conformations, respectively, where **P** and **M** represents chirality of the component dipyrrinone long wavelength induced electric dipole transition moments with exciton model.

ACKNOWLEDGEMENTS

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Lecture

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YELLOW BICHROMOPHORES. CONFORMATIONAL ANALYSIS AND CIRCULAR DICHROISM

Stefan E. Boiadjiev, Richard V. Person, Darren L. Holmes, Meiqiang Xie, Blake R. Peterson, and David A. Lightner*

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Summary. Typical linear and porphyrin-like structure representations of bilirubin give an incorrect impression of its actual shape and expected solution properties. Molecular dynamics-assisted conformational analysis of bilirubin indicates that: (i) non-bonded intramolecular steric interactions are minimized in a ridge-tile shape conformation lying at a global energy minimum on the conformational energy map; and (ii) considerable additional stabilization is achieved through a network of intramolecular hydrogen bonds. The linear and porphyrin-like conformations are computed to lie some 37-48 kcal/mole above isoenergetic global minimum energy conformations, which correspond to superimposable (identical) or to nonsuperimposable (enantiomeric) mirror image intramolecularly hydrogen-bonded ridge-tile conformers separated by ~20 kcal/mole barriers. The conformation of bilirubin may be analyzed experimentally by UV-visible and, especially, circular dichroism (CD) spectroscopy. Such conformation-dependent spectra arise from exciton coupling between the two dipyrrinone chromophores of bilirubin. Theoretical analysis using the exciton coupled oscillator model allowed a mapping of CD $\Delta\epsilon$ for each bilirubin conformation of the conformational energy surface. Intense bisignate CD Cotton effects are predicted for the global energy minimum conformation, with $\Delta\epsilon \simeq \pm 200$ L mole^{-1} cm^{-1} for the long wavelength UV-visible absorption near 450 nm. Surprisingly, Cotton effect sign reversals without inversion of molecular absolute configuration are predicted when the ridge-tile conformations are flattened somewhat into higher energy structures.

Bilirubin, the cytotoxic yellow-orange pigment of jaundice,[1] is formed from heme in mammalian metabolism.[2,3] Although the structure of bilirubin is typically represented in linear form (Fig. 1A), the pigment is conformationally mobile like a two-blade propeller. The most important conformational changes follow from rotations about the C_9-C_{10} and C_{10}-C_{11} carbon-carbon single bonds, rotation angles ϕ_1 and ϕ_2 respectively. Rotations of the pyrrole β-substituents are much less important, except for carbon-carbon single bond rotations in the propionic acid chains at C_8 and C_{13}, as will become evident. Rotations about the C_5-C_6 and C_{14}-C_{15} carbon-carbon single bonds are relatively less important, and rotations about the C_4 and C_{15} carbon-carbon double bonds are high energy processes, that are relatively inaccessible, except through photoexcitation.[1,3]

Rotations about ϕ_1 and ϕ_2 are significant because the dipyrrinone propeller blades are forced to sweep out large spatial volumes, and the pigment can be transformed into a large number of very different shapes, which range from porphyrin-like ($\phi_1 \simeq \phi_2 \simeq 0°$) (Fig. 1B) to linear ($\phi_1 \simeq \phi_2 \simeq 180°$) (Fig. 1A). These two planar or near-planar conformations have

CORRELATION OF EXCITON CIRCULAR DICHROISM WITH ABSOLUTE CONFIGURATION

Exciton chirality theory allows for the prediction of the absolute configuration of a bichromophore molecule such as bilirubin if the relative orientation of the relevant electric transition dipole moments are known.[13] A typical exciton coupling-type CD curve is bisignate (Figs. 7-9). Enantiomers have mirror image CD curves — either a curve with a long wavelength positive-short wavelength negative or a curve with long wavelength negative-short wavelength positive series of Cotton effects. The two possible signed sequences of Cotton effects can be correlated with the relative orientation of the transition dipoles. When the dipoles are oriented with a positive torsion angle, corresponding to a positive helicity, the sequence of Cotton effect signs in the CD is long wavelength positive-short wavelength negative. When the torsion angle is negative, corresponding to a negative helicity, the sequence is long wavelength negative-short wavelength positive. The latter orientation clearly corresponds to the *M*-molecular chirality global minimum ridge-tile conformer of Fig. 1C; the former corresponds to its ***P***-molecular chirality enantiomer.

CONCLUDING COMMENTS

The preceding studies of bilirubin conformational analysis and circular dichroism point to a folded ridge-tile conformation as the global energy minimum. Conformational stabilization is due to two factors: (i) minimization of nonbonded steric interactions and (ii) powerful stabilization through intramolecular hydrogen bonding. The importance and relevance of intramolecular hydrogen bonding in bilirubins and their strong tendency to implement such hydrogen bonding is underscored by our studies. Intramolecular hydrogen bonding is, in fact, found to be retained in a wide variety of solvents, and it very likely 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 is predicted to be resolvable into its intramolecularly hydrogen-bonded conformational enantiomers if the interconversion barrier is increased and the rate of interconversion retarded, *viz.* at sufficiently low temperatures. Although such a resolution into conformational enantiomers has not yet been achieved, resolution has been accomplished for synthetic analogs where intramolecular steric repulsions render one conformational enantiomer destabilized relative to the other, *e.g.*, through substitution of a propionic acid β-H (or α-H) by CH_3 to give enantiomeric β,β'-dimethylmesobilirubins-XIIIα.

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Transannular Orbital Interaction in Diketones Detected by C-13 NMR Spectroscopy

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Abstract: ^{13}C-NMR spectra of a series of acyclic, monocyclic and polycyclic ketones and diketones serve as a basis for investigating transannular orbital interaction in diketones. The shielding of the ^{13}C=O resonance frequency of the diketone relative to the monoketone which can be observed in all cases, depends on factors such as the number of intervening σ-bonds, relative orientation of the carbonyl groups and intervening σ-bonds.

INTRODUCTION

Over 20 years ago Hoffmann described the phenomenon of electron delocalization through homoconjugation in terms of direct ("through-bond") and indirect ("through-space") interactions from localized chromophores.[2] Orbital interactions through space have been detected by photoelectron (PE)[3] and electron transmission spectroscopy,[3c,4] and by kinetics of solvolysis reactions.[5-7] For example, the large splittings of the π-orbitals (0.86 eV) and π^*-orbitals (1.52 eV) of norbornadiene, as detected by PE spectroscopy, are thought to originate mainly from through space interactions and to a lesser extent from through-bond coupling.[8] Transannular orbital interactions between two ketone carbonyl chromophores give large *n*-orbital splittings when overlap through skeletal σ-orbitals is possible, as in tetramethyl-1,3-cyclobutanedione (0.75 eV),[9] and smaller splittings arise when the overlap between the *n*-orbitals and the relevant σ-p ring orbitals is poor, as in 2,5-norbornanedione (0.16 eV).[10]

Although not as extensively investigated, transannular orbital interactions have also been detected by ^{13}C-NMR spectroscopy. With a favorable alignment of the C=O groups, $\delta_{C=O}$ of the dione may be strongly shielded relative to $\delta_{C=O}$ of the corresponding mono-ketone, *cf.* 2,5-norbornanedione ($\delta_{C=O}$ 212.3) and norbornanone ($\delta_{C=O}$ 218.1).[11] In the former, a 1,4-diketone, the chromophores are separated by three σ-bonds, but equivalently strong shieldings may be detected even in 1,5-diketones, where the

Dedicated to Professor Carl Djerassi on the occasion of his 70th birthday

that of (e), and the magnitude of $\Delta\delta$ is larger. Similarly, the magnitude of $\Delta\delta$ is larger in (b) than in (f), and larger in (c) than in (g). However, when the O=C···· C=O nonbonded distance becomes large, as in (d), $\Delta\delta$ is essentially the same as in its acyclic analog (h). Whether one can assign the difference in magnitudes of $\Delta\delta$ for the cyclic and acyclic analogs to through-space effects is unclear because of the difficulty in assessing the dependence of δ on the through-bond coupling path. However, on a qualitative basis, it seems clear that through-space effects play an important role in the ^{13}C=O shieldings of the cyclic diketones.

Through-space orbital interactions thus appear to be optimized in cyclic systems where the C=O groups have a favorable alignment. Compare $\Delta\delta$, for example, of the norbornanedione and adamantanedione [(b) and (d) of Table 1, respectively] to $\Delta\delta$ of cyclohexanedione and cyclooctanedione [(b) and (c) of Table 2, respectively]. In each case, the C=O groups are connected by three or four σ-bonds, but in the former set (Table 1) they are thought to be co-linear, and the magnitude of $\Delta\delta$ is larger. However, even with a favorable alignment, orbital interactions of diketones through the same three or four bonds do not necessarily give rise to very large $\Delta\delta$ magnitudes when there are intervening σ-bonds, even when the C=O are co-linear, as in (j), or when the C=O groups are not co-linear, as in (i).

CONCLUDING COMMENTS

The carbonyl resonances of all of the diones of this work (Table 2) are shielded relative to corresponding mono-ketones. This behavior is consistent with earlier observations in other bichromophoric systems with C=O chromophores aligned for transannular orbital interaction (Table 1).[11] The origin of this effect would appear to lie in orbital interaction through space as well as through σ-bonds. A more complete analysis of the relative contributions of through-space and through-bond orbital interaction awaits a theoretical analysis of ^{13}C=O shielding tensors[14] of the bichromophoric and monochromophoric substances of Tables 1 and 2.

EXPERIMENTAL

General. Nuclear magnetic resonance (NMR) spectra were determined in $CDCl_3$ on a GE QE-Plus spectrometer operating at 75.5 MHz for ^{13}C and are reported in parts per million downfield from tetramethylsilane, unless otherwise indicated. 1,3-Cyclobutanedione was a gift from LONZA Corp., 2-pentanone and 2,4-pentanedione, 1,4-cyclohexanedione and cyclohexanone, 2-hexanone and 2,5-hexanedione, cyclooctanone, 2-heptanone, 2-octanone and bicyclo[3•3•0]octane-3,7-dione were from Aldrich.

2,6-Heptanedione[15] was prepared according to the method of Cope and Overberger.[16,17]

2,7-Octanedione[18] was prepared according to the method of Januszkiewicz and Alper.[19]

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SYNTHESIS AND UNUSUAL PROPERTIES OF AN 8,12-BIS-PIVALIC ACID ANALOG OF BILIRUBIN

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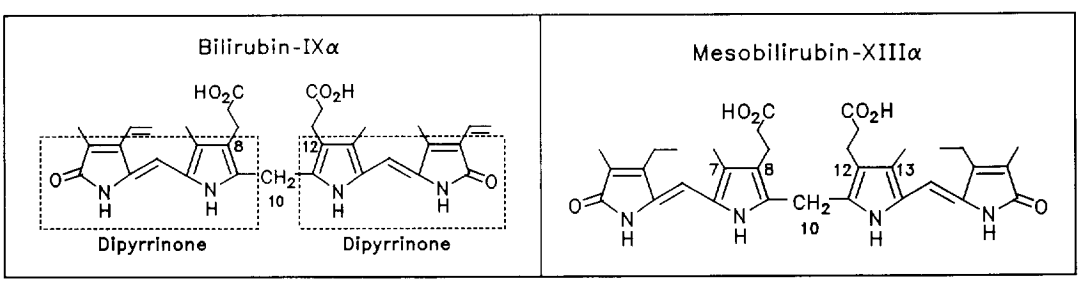
Abstract: A sterically congested analog of bilirubin with propionic acid groups replaced by pivalic acids (**1**) was synthesized from methyl 3-(2,4-dimethyl-5-ethoxycarbonyl-1*H*-pyrrol-3-yl)-2-methylpropionate (**8**). UV-visible and NMR spectroscopic analyses of **1** suggest intramolecular hydrogen-bonding and a preference for a ridge-tile conformation. The activation parameters for $M \rightleftarrows P$ conformational inversion of **1** were determined by dynamic NMR analysis to be $\Delta H^{\ddagger}$ 19.7 ± 1.4 kcal/mole and $\Delta S^{\ddagger}$ + 10.4 ± 4.5 eu. Molecular dynamics computations predict a global energy minimum for a somewhat more open ridge-tile conformation as compared with bilirubin. Circular dichroism of the pigment complex with human serum albumin gives a bisignate Cotton effect: $\Delta\epsilon_{431}^{max} = -51$, $\Delta\epsilon_{382}^{max} = +30$, with the opposite signed order as compared with that found for the parent mesobilirubin-XIIIα and bilirubin.

INTRODUCTION

Complicated, structurally interesting linear tetrapyrroles such as the natural products bilirubin and biliverdin are formed in animal metabolism from normal turnover of hemoglobin and other heme proteins.[1-3] Considerable effort has been devoted to understanding the properties and metabolism of bilirubin, with particular attention being focussed on its unique ability to fold into a conformation where the carboxylic acid groups embrace the opposing dipyrrinones in intramolecular hydrogen bonding.[4-6] This decreases the polarity of the pigment and renders it unexcretable in normal metabolism, except by glucuronidation.[2,3,7] Recently it has become evident that translocation of the pigment's propionic acid groups away from the natural locations at C(8) and C(12) leads to pigments which were more polar than bilirubin and do not require glucuronidation for hepatic excretion.[7,8] However, bilirubin analogs with propionic acid groups at C(8) and C(12), *e.g.*, mesobilirubin-XIIIα, typically exhibit the same unique polarity and excretability properties as bilirubin. These pigments, like bilirubin, tuck their carboxylic acid groups inward, where they are tethered to an opposing dipyrrinone by intramolecular hydrogen bonding shown in Figure 1.



Metabolism.

As with bilirubin and mesobilirubin-XIIIα, the tetramethyl analog (**1**) was not excreted in the (jaundiced) Gunn rat, which has a congenital deficiency of glucuronsyl transferase. Interestingly, when **1** was administered to a normal Sprague-Dawley rat, it was excreted into bile very slowly and only as its monoglucuronide. This contrasts markedly with the behavior of the parent, mesobilirubin-XIIIα, which is excreted promptly into bile, as mono and diglucuronides. Whether the longer excretion time and the exclusive formation of a monoglucuronide is due to ineffective uptake into the hepatocyte or to a rate decrease at the glucuronidation site is unclear.

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.[10] The current study shows that even when the steric bulk of the propionic acid chains is expanded to pivalic acid, intramolecular hydrogen bonding persists in non-polar solvents. And the effect of such hydrogen bonding is to stabilize predominantly a ridge tile conformation and to a lesser extent a flattened ridge-tile shape.

EXPERIMENTAL PART

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 GN-300 or Varian Unity Plus spectrometers operating at 300 and 500 MHz, respectively, in $CDCl_3$ solvent (unless otherwise noted). Chemical shifts were reported in δ ppm referenced to the residual $CHCl_3$ ^1H signal at 7.26 ppm and ^{13}C signal at 77.0 ppm. A J-modulated spin-echo experiment (*A*ttached *P*roton *T*est) was used to assign ^{13}C-NMR spectra. Mass spectra (EI) were measured on Finnigan MAT SSQ 710 instrument. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. 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). 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 ultrashere-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 3% aqueous methanol (pH 7.7, 31°C). Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Ethyl acetoacetate, pentane-2,4-dione, methyl methacrylate, diisopropyl amine, n-butyllithium in hexane, methyliodide, *p*-chloranil, and sodium borohydride were from Aldrich. Tetrahydrofuran, dichloromethane, chloroform, methanol, hexane, and dimethylsulfoxide were HPLC grade from Fisher. Tetrahydrofuran was dried by distillation from $LiAlH_4$; methanol was distilled from $Mg(OCH_3)_2$; dimethylsulfoxide was freshly distilled from CaH_2 under vacuum.

Methyl 3-(2,4-dimethyl-5-ethoxycarbonyl-1H-pyrrol-3-yl)-2-methylpropionate (**8**) was prepared from ethyl acetoacetate, pentane-2,4-dione, and methyl methacrylate in two steps as described previously.[11]

Observation of Vibrational Circular Dichroism for Overtone Transitions with Commercially Available CD Spectrometers

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It is demonstrated that some commercially available circular dichroism spectrometers can be used to gather vibrational circular dichroism data associated with overtone transitions. By way of specific example, the circular dichroism spectra of neat *S*-(−)-limonene and *R*-(+)-limonene are measured in the region 800–600 nm. The observed spectral features correspond to the overtone bands $\Delta v = 5$ and 6 for CH-stretching motions. A discussion of the data is also given.

Index Headings: **Vibrational circular dichroism; Overtone spectroscopy; Local modes.**

INTRODUCTION

The papers that comprise this issue of *Applied Spectroscopy* provide ample testimony that the measurement of vibrational circular dichroism (VCD) is now commonplace. This fact becomes more noteworthy when one realizes that there is still not commercially available an instrument dedicated to performing VCD measurements. All such instruments in current operation are home-built or involve commercial spectrometers that have been modified significantly. This situation has the consequence that the data gathering for almost all VCD studies is carried out in the laboratories of physical chemists, even when such studies are collaborative with organic, inorganic, or biochemically oriented chemists.

One purpose of this paper is to point out that the gathering of potentially interesting VCD data by, say, organic chemists is possible on instruments to which they frequently have current access. The type of VCD data just alluded to is that associated with the overtones of commonly occurring functional groups. For example, the higher overtones ($\Delta v > 2$) of CH-stretching modes often occur in "transparent" regions of the near-IR or visible. The associated VCD can be accessed readily with modern commercially available CD spectrometers, e.g., certain models made by JASCO, such as their Models J200D, J600, and J700.

By way of illustration, we shall present new VCD data taken on the JASCO Model J600 for *S*-(−)-limonene and *R*-(+)-limonene for $\Delta v = 5$ and 6. The corresponding transitions lie in the 800–600 nm range. We shall also include some previously reported data for $\Delta v = 3$ and 4 taken on the JASCO J200D, as well as the VCD data for the fundamentals.[1] We shall then compare and discuss all the VCD data, together with some relevant overtone absorption data. However, before doing so, we hasten to add that VCD overtone data have been and continue to be studied by others, most notably by Sugeta and coworkers for $\Delta v = 2$ in the 2000–1500 nm range.[2] We note also that Keiderling and Stephens reported VCD data in the same range some time ago.[3]

RESULTS AND DISCUSSION

In Fig. 1 (top) we show the superimposed VCD data for *S*-(−)-limonene and *R*-(+)-limonene in the 800–700 nm range ($\Delta v = 5$); in Fig. 1 (bottom) we show the analogous data for the range 685–645 nm ($\Delta v = 6$). All data were taken with neat samples contained in quartz cells 10 cm in pathlength. The scanning speed was always 10 nm/min with the time constant fixed at 4 s. Twenty-four accumulations of data were gathered for each sample, and these were averaged by a programmed routine of the instrument. The baseline was taken as the spectrum of racemic limonene, and subtraction of the baseline was always performed. The full-scale ellipticity was 2.0 mdeg in the 800–700 nm range, and was 0.4 mdeg in the 685–645 nm range. The enantiomeric excess was 75.1% in the case of the *S*-(−)-limonene sample, and 97.6% in the case of the *R*-(+)-limonene sample. Hence, multiplicative factors of 1.33 for *S*-(−)-limonene and 1.02 for *R*-(+)-limonene were applied to the experimental data to achieve the curves shown in Fig. 1. The corresponding observed spectra for the two enantiomers are virtually mirror images, thus lending credibility to the data.

In Table I we report for *R*-(+)-limonene the values for the bandcenter frequencies ν, the bandwidths $\Delta\nu$, and the rotational strengths R for $\Delta v = 1$, 3, 4, 5, and 6.[1] (We have yet to measure the data for $\Delta v = 2$.) The VCD bandwidths are observed to increase linearly with ν in much the same way as do absorption bandwidths.[4,5] Similarly, the rotational strengths are observed to decrease by an order of magnitude at each next higher overtone, as do absorption intensities.[4,5] Indeed, the overall intensities D, summed over all CH stretchings, are $D = 2.38 \times 10^{-41}$, 2.25×10^{-42}, and 1.74×10^{-43} esu^{2}cm^2 for the $\Delta v = 3$, 4, and 5 transitions, respectively (the spectra are reported in Ref. 1). These data further suggest that the anisotropy ratios are not heavily dependent on ν and are of the same order of magnitude as the ratio of the nuclear magneton over the molecular electric dipole moment.[6] Also, as in the case of absorption data, it can be seen from Fig. 2 that for $\Delta v = 1$ (the fundamental) through $\Delta v = 5$, the values of the observed frequencies ν divided by Δv for the set of positive VCD bands, and the corresponding values for the set of negative VCD bands, fall on separate straight lines. The values for the mechanical

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Linear and Circular Dichroism Spectroscopic Study of β,β′-Dimethylmesobilirubin-XIIIα Oriented in a Nematic Liquid Crystal

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(βR,β′R)-dimethylmesobilirubin-XIIIα, an optically active synthetic analogue of bilirubin, the yellow pigment of jaundice, and xanthobilirubinat, a dipyrrinone analogue for one-half of a rubin pigment, dissolved in the nematic liquid crystal ZLI 1695, have been studied by means of UV−vis polarized absorption and circular dichroism (CD) spectroscopy. The order parameters have been evaluated from the temperature dependence of the degree of anisotropy. The method of "vanishing spectral features" has also been taken into consideration. The reduced absorption spectra have been obtained. It has been found that the orientational properties as well as the polarization of the absorption bands of the two compounds are quite different. Moreover, the UV−vis absorption and CD spectra of β,β′-dimethylmesobilirubin-XIIIα have been interpreted on the basis of the exciton coupling model, assuming the point symmetry group C_2. The orientation of the principal axes of the orientational distribution tensor (order tensor) with respect to the molecular frame has been determined. That the anisotropic CD (ACD) spectra are not very different from the CD spectra can be understood from the spectroscopic analysis taking into account the orientational order.

1. Introduction

Bilirubin-IXα is the yellow-orange lipophilic cytotoxic pigment of jaundice, which plays an important role in life processes. In normal metabolism, an adult human generates and eliminates about 300 mg per day of this pigment, which is produced by catabolism of hemoglobin, mainly from red blood cells.[1,2] Bilirubin is produced in the spleen and transported to the liver, where it is transformed and secreted into the gall ducts as a component of bile. The main components of bile are water, phospholipids, cholesterol, and bile salts, which form a lyotropic liquid crystalline system.[3] Thus, in living organisms bilirubin resides in an anisotropic medium. Therefore, the study of bilirubin in any oriented matrix may provide information about the properties of this pigment in a natural environment.

Bilirubin-IXα and its analogues consist of two interacting dipyrrinone fragments covalently conjoined to the central methylene group at C10.[2] Both dipyrrinone groups are able to rotate independently about this central CH_2 (C10) group. These rotations allow the possibility of a large number of conformations. However, computations for bilirubin-IXα and its symmetric analogue in which the vinyl groups are replaced by ethyl (mesobilirubin-XIIIα) showed that the folded shape with the two dipyrrinone planes oriented to form a dihedral angle of about 100°[4-6] is energetically the most favorable conformation. Such a conformation of bilirubin was also found in the solid by X-ray crystallography.[7-9] It is stabilized through intramolecular hydrogen bonds and permits bilirubin to exist as a pair of enantiomeric conformers. When dissolved in achiral solvents, the pigment forms a racemic mixture of rapidly interconverting conformational enantiomers at room temperature. This equilibrium of enantiomers, however, can be shifted toward one or the other conformer either by addition of chiral agents,[10-12] complexing with protein,[13-15] or through methyl substitution in the propionic acid side chains,[5,6,16] which makes evident the optical activity of the pigment.

In this paper we study some properties of (βR,β′R)-dimethylmesobilirubin-XIIIα (**1**) dissolved in a nematic liquid crystal by means of UV−vis absorption and circular dichroism (CD) spectroscopy. The polarized absorption spectra of a solute in oriented anisotropic matrices provide information about alignment of molecules, order parameters, and polarization of electronic bands,[17] whereas the measurement of the CD of oriented molecules (ACD = CD of anisotropic samples) can be of further help for a quantitative analysis of molecular spectroscopic properties.[18,19]

2. Theoretical Background

2.1. Linear Dichroism. The molecular decadic absorption coefficients for light polarized parallel (ϵ_1) and perpendicular (ϵ_2) to the optical axis of a uniaxial sample are given by[18,20]

$$\epsilon_1 = \sum_{i,j} g_{ij33}\epsilon_{ij} = \sum_{i,j} a_{ij}^2 g_{jj33}^* \epsilon_{ii}^+ \quad (1a)$$

$$\epsilon_2 = \sum_{i,j} g_{ij11}\epsilon_{ij} = \sum_{i,j} a_{ij}^2 g_{jj11}^* \epsilon_{ii}^+ \quad (1b)$$

ϵ_{ii}^+ are the absorption coefficients for light beams polarized linearly parallel to the x_i^+ axes in a completely oriented system. The x_i^+ axes are the principal axes of the absorption tensor ϵ_{ij}.[20-22] a_{ij} are the elements of the orthogonal matrix which transforms the x_i^* coordinates into the x_i^+ coordinates. The coordinates x_i^* refer to the common principal axes of the order tensors g_{ijkk} (k = 1, 2, 3) and the following convention

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TABLE 5 (Continued)

compd	properties	our results[a]	Lightner et al.	other literature
	θ, θ', deg	calc: 128, 52 ($\varphi = 60°$)	130, 50[10]	
	ΔE_{NK}^{mn}, cm^{-1}	exp: 775	1074 (φ_1, $\varphi_2 = 60°$)[32]	
		calc: 950 ($\varphi = 60°$)		
	$R^{\alpha\beta}$(I), cgs	calc: $\pm 5.8 \times 10^{-38}$	calc: $\approx \pm 10 \times 10^{-38}$	
		fit: $\pm 13.3 \times 10^{-38}$		
	$R_f^{\alpha,\beta}$, cgs	$R_f^{\alpha} = 7.7 \times 10^{-38}$		
		$R_f^{\beta} = -4.5 \times 10^{-38}$		
	$\bar{\nu}_0$, cm^{-1}	$\bar{\nu}_0 = 23.9 \times 10^3$	$\bar{\nu}_0 = 23.5 \times 10^3$ [32]	
	$\epsilon^{\alpha}(\bar{\nu}_{max})$(I),	calc: 40.4×10^3	calc: 40.0×10^3	
	L mol^{-1} cm^{-1}	($\bar{\nu}_{max} = 23.10 \times 10^3$ cm^{-1})	($\bar{\nu}_{max} = 22.42 \times 10^3$ cm^{-1})	
	$\epsilon^{\beta}(\bar{\nu}_{max})$(I),	23.6×10^3	36.0×10^3	
	L mol^{-1} cm^{-1}	($\bar{\nu}_{max} = 24.68 \times 10^3$ cm^{-1})	($\bar{\nu}_{max} = 24.57 \times 10^3$ cm^{-1})[32]	
	$\Delta\epsilon^{\alpha}(\bar{\nu}_{max})$(I),	calc: 297	calc: 324	
	L mol^{-1} cm^{-1}	($\bar{\nu}_{max} = 22.65 \times 10^3$ cm^{-1})	($\bar{\nu}_{max} = 23.04 \times 10^3$ cm^{-1})	
	$\Delta\epsilon^{\beta}(\bar{\nu}_{min})$(I),	calc: −179	calc: −183	
	L mol^{-1} cm^{-1}	($\bar{\nu}_{min} = 25.10 \times 10^3$ cm^{-1})	($\bar{\nu}_{min} = 25.50 \times 10^3$ cm^{-1})[6]	

[a] In ZLI 1695.

where the upper and lower sign apply to the transition to the excited states α and β, respectively, and E_{NK}^{mn} is the excitation energy of the transition I taken here as 23.9×10^3 cm^{-1} from the center of the long-wavelength exciton bands of **1** (Figure 7) and $|\vec{R}^{mn}| = 6.0$ Å.[10] The rotational strength $R^{\alpha} = -R^{\beta}$ calculated with eq 43 then is 7.3×10^{-38} cgs. For the (βR,$\beta' R$) enantiomer of **1** there follows from eq 43 $R^{\alpha} > 0$ and $R^{\beta} < 0$.

The fitting of the CD band in isotropic phase of ZLI 1695 yields $R_f^{\alpha} = -R_f^{\beta} = 13.3 \times 10^{-38}$ cgs. From the CD experiment one gets $R_{exp}^{NK\alpha} = 5.1 \times 10^{-38}$ cgs for the positive band and $R_{exp}^{NK\beta} = -4.7 \times 10^{-38}$ cgs for the negative band which yields $R_{exp}^{NK\alpha} - R_{exp}^{NK\beta} = 9.8 \times 10^{-38}$ cgs and the amplitude $A_{iso} = 502$ L mol^{-1} cm^{-1}. The corresponding values from the curve fitting (Figure 9b) are $R_f^{NK\alpha} = 7.7 \times 10^{-38}$, $R_f^{NK\beta} = -4.5 \times 10^{-38}$, and $R_f^{NK\alpha} - R_f^{NK\beta} = 12.2 \times 10^{-38}$ cgs leading to an amplitude $A_f = 475$ L mol^{-1} cm^{-1}. $R_f^{NK\alpha}$ is too large because of the insufficent fit of the UV and CD band at the long-wavelength side of Iα and Iβ which can be seen also from the difference of the intensities of the two lobes of the couplet $R_f^{NK\alpha} - R_f^{NK\beta} > R_{exp}^{NK\alpha} - R_{exp}^{NK\beta}$.

In comparison to the value $R_f^{\alpha} = -R_f^{\beta} = 13.3 \times 10^{-38}$ cgs resulting from the curve fitting (Figure 9b) of the CD of **1** in the isotropic solution the value from the exciton theory (eq 43) is too small (55 % of the experimental result). Because $R(\gamma)$ with $\gamma = 121°$ is near its maximum, a false value of γ cannot be the reason for this discrepancy found. A variation of R as a function of φ has only a small influence on the calculated R^{α}. Therefore, a false value of φ cannot be the reason for a rotational strength too small. Furthermore, a variation of φ by more than 2−4 deg seems not to be realistic in view of the results found in literature.[32] A more critical point and thus a possible origin of a failure of the exciton theory may be the choice of the interchromophoric distance vector $\vec{R}^{mn}$. The direction of the vector $\vec{R}^{mn}$ is fixed if the origins of the multipole expansions are chosen symmetrically in the two fragments m, n. But its length $|\vec{R}^{mn}|$ then still depends on the position of these points which should be chosen in such a way that the dipole−dipole term outweighs the higher multipole terms. This remark concerns the exciton splitting energy as well as the factor $\vec{R}^{mn} \cdot \langle\vec{\mu}\rangle_{NK}^{m} \times \langle\vec{\mu}\rangle_{NK}^{n}$ in the expression given in eq 43a for the rotational strength R^{α}. A shift of these points will change the distance $|\vec{R}^{mn}|$. With $\gamma \approx 121°$ the experimental value of the rotational strength demands $|\vec{R}^{mn}| > 12$ Å which seems too large to be realistic. It should be mentioned here that the extension of the fragments is large compared to their distance which actually is a severe restriction for the applicability of the present approximation of the exciton theory. Furthermore, the change of the band structure gives an indication for a change of the potential curves for the excited fragment state involved in the exciton states.

In general, it should be possible by ACD spectroscopy to decompose the contributions of both exciton bands experimentally if the x_3^* orientation axis has a suitable orientation with respect to the transition moment directions of both bands Iα and Iβ. For **1** the difference between $\Delta\epsilon^A$ and $\Delta\epsilon$ (Figures 4 and 5) is very small and thus qualitatively consistent with the theory of exciton coupling for the case where the orientation axis is nearly perpendicular to both exciton transitions and, therefore, the ratio of the $\Delta\epsilon^A$ values of both bands is independent of the order parameters:[18]

$$\frac{\Delta\epsilon^A(A \rightarrow A)}{\Delta\epsilon^A(A \rightarrow B)} = -1 \qquad (44)$$

The amplitude A of a positive couplet can be calculated, neglecting the electric dipole−electric quadrupole term, from the expression[18]

$$A = [\Delta\epsilon^A(\bar{\nu}_{max};A \rightarrow B) - \Delta\epsilon^A(\bar{\nu}_{max};A \rightarrow A)] + [\Delta\epsilon^A(\bar{\nu}_{min};A \rightarrow B) - \Delta\epsilon^A(\bar{\nu}_{min};A \rightarrow A)] \approx A_{iso}(1 + S^*/2) \quad (45)$$

$\bar{\nu}_{max}$ and $\bar{\nu}_{min}$ are the wavenumbers of the positions of the maximum and the minimum of the couplet, respectively. A_{iso} is the amplitude of the CD measurement in the isotropic phase. Taking $S^* = 0.51$ for (βR,$\beta' R$)-dimethylmesobilirubin-XIIIα in ZLI 1695 at $T = 28$ °C, there results from eq 45 the value $A = 630$ L mol^{-1} cm^{-1}, which is in very good agreement with the value $A = 599$ L mol^{-1} cm^{-1} obtained from ACD measurement (Figures 4 and 5). The electric dipole−electric quadrupole contribution cannot be estimated because of the smallness of the ACD effect.

6. Conclusion

In Figure 10a,b the orientations of the transition moment directions of the transitions of symmetry A → B (C_2) of **1** and A → A (C_s) of **3** in the x_1, x_3 and in the molecular (x_2, x_3) plane, respectively, are placed together. The angles of the orientation axis of **3** against the CH_3-C1 direction and the transition moment vectors of the transitions I, II, and III are $\gamma^D = 94°$, $\gamma_I^D = 121°$, $\gamma_{II}^D = 43°$, and $\gamma_{III}^D = 38°$. The error of the absolute values of these angles is difficult to estimate. It is assumed to be about ±15°. Because the orientation axis divides the angle enclosed by the transition moment directions of the transitions I and II, the degrees of anisotropy of I and II are

comparable (that of II is about 15% smaller than that of I) whereas the R value of III is much smaller. This situation seems to be in contrast to the hierarchy of the R values of **1**. Here, R is about zero or even negative in the spectral region of the transition Iα whereas $R > 0$ and approximately equal for IIα and IIIα ($\beta_{II\alpha} \approx \beta_{III\alpha} \approx 44°$). The latter fact is a consequence of the exciton coupling with the transitions II and III which creates four exciton transitions in **1**. Two of them (IIα and IIIα) are polarized in the x_1, x_3 plane and the other two (IIβ and IIIβ) in the x_2 direction. From the direction of the transition moments of II and III in **3** there follows that IIα and IIIα gain almost all the intensity whereas IIβ and IIIβ are very weak and thus cannot be seen in the experimental spectra.

The angle enclosed by the transition moment vectors of the transition I, $\langle\vec{\mu}\rangle^{m}_{NK}$ and $\langle\vec{\mu}\rangle^{n}_{NK}$, of **1** has been determined to be $\vartheta = 106°$ from which $\gamma = 121°$ follows. From the fact that the exciton coupling does not lead to a measurable CD couplet, there follows $\gamma_{II} = \gamma_{III} = 54° \pm 15°$ and for the transition moment directions of IIα and IIIα the value $\beta = -44° \pm 10°$. The orientation axis of **1** is oriented approximately along the line connecting C2 and C8 (113.8°) at $\psi = 123°$. All data are summarized in Table 5.

Acknowledgment. This research was supported by the Deutsche Forschungsgemeinschaft and the Fonds der chemischen Industrie, Germany, and in part by the Poznań University of Technology Research Project. D.B. also thanks the Alexander von Humboldt Stiftung for financial support during the stays in Kaiserslautern. We thank Dr. H. Schulze for developing the programs of the fitting procedure for the evaluation of the spectroscopic constants and the order parameters. S.E.B. and D.A.L. thank the U.S. National Institutes of Health for support.

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p*H*-SENSITIVE EXCITON CHIRALITY CHROMOPHORE. SOLVATOCHROMIC EFFECTS ON CIRCULAR DICHROISM SPECTRA

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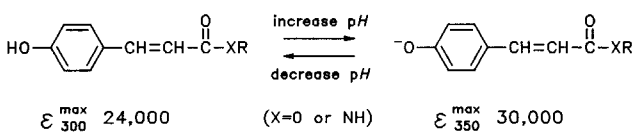
Abstract: Diesters (**1** and **3**) of (1*S*,2*S*) and (1*R*,2*R*)-cyclohexanediol and diamides (**2** and **4**) of (1*S*,2*S*) and (1*R*,2*R*)-diaminocyclohexane with *p*-hydroxycinnamic acid exhibit intense bisignate circular dichroism spectra in CH_3OH: **1** $\Delta\varepsilon$ +55 (323 nm), −34 (287 nm); **2** $\Delta\varepsilon$ +75 (318 nm), −55 (281 nm) and in $(CH_3)_2SO$: **1** $\Delta\varepsilon$ +53 (328 nm), −33 (292 nm); **2** $\Delta\varepsilon$ +65 (319 nm), −50 (280 nm). Added NaOH causes a bathochromic shift of ~50 nm in CH_3OH and ~80-90 nm in $(CH_3)_2SO$. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The search for new types of chromophores useful in forming derivatives of diols and diamines for exciton chirality[1] studies has uncovered a variety of carboxylic acids ranging from *para*-substituted benzoic and cinnamic acids to naphthoic and anthroic acids,[2] from dipyrrinone acids[3] to porphyrin acids.[4,5] The last are especially useful for long-range exciton coupling. In the current work, we focussed attention on the *p*-hydroxy-cinnamate chromophore for exploring a potential p*H* shift on exciton Cotton effects. Previously, *p*-methoxy and *p*-dimethyl-amino cinnamic acid esters have been used in exciton studies,[2] but to the best of our knowledge, the *p*-hydroxy has not. Yet, one can anticipate that its carboxylic acid esters and amides should exhibit large bathochromic ultraviolet (UV) and circular dichroism (CD) spectral shifts in the neutral to basic p*H* range. Consequently, we prepared *p*-acetoxycinnamic acid as the key chromophore to be used in our syntheses.

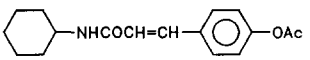
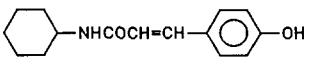


RESULTS AND DISCUSSION

Synthesis. As outlined in the Synthetic Scheme, *p*-hydroxycinnamic acid was acetylated in 89% yield using acetic anhydride in pyridine.[6] The product was converted with thionyl chloride to the corresponding acid chloride, which was reacted smoothly with (1*S*,2*S*) or (1*R*.2*R*)-*trans*-cyclohexanediol in dry dichloromethane

when deprotonated in dimethylsulfoxide (Table 2). Yet, again the UV spectra of the protonated forms are very similar in pure methanol and in dimethylsulfoxide.

TABLE 2. UV Spectral Data for Monoamides **9** and **10**.

	 **9**		 **10**			
Solvent	ε^{max}	λ (nm)	ε^{max}	λ (nm)	ε^{max}	λ (nm)
$CHCl_3$	24300	278	19900	307^{sh}	22300	291
CH_3OH	27500	277	22900	307	23600	292
$(CH_3)_2SO$	24600	276	20300	308^{sh}	23300	293
0.1 M NaOH/CH_3OH	—	—	29700	347	14200	313^{sh}
$(CH_3)_2SO$/NaOH[a]	—	—	31900	378	10800	327

[a] $(CH_3)_2SO$ containing 2% (vol) of a solution of 0.1 M NaOH in CH_3OH

CONCLUDING COMMENTS

The *p*-hydroxycinnamate chromophore has been shown to exhibit the expected excellent p*H*-sensitive spectral shifts in its exciton coupling CD and UV spectra of the diesters of (1*R*,2*R*) and (1*S*,2*S*)-*trans*-cyclohexanediol and the diamides of (1*R*,2*R*) and (1*S*,2*S*)-*trans*-diaminocyclohexane. An unusual solvatochromic effect on the phenoxide form in dimethylsulfoxide solvent leads to ~90 nm bathochromic shifts and 20-40% enhancements of $\Delta\varepsilon^{max}$. These findings indicate *p*-hydroxycinnamic acid may be useful for exciton chirality studies where red-shifted chromophores are important.[2]

EXPERIMENTAL

General. All circular dichroism spectra were recorded on a JASCO J-600 instrument, and all UV-vis spectra were recorded on a Cary 219 spectrophotometer. NMR spectra were obtained on a GE GN-300 spectrometer operating at 300 MHz. $CDCl_3$ 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. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. 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). Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ.

Spectral data were obtained in spectral grade solvents (Aldrich or Fischer). Enantiomerically pure (1*R*,2*R*) and (1*S*,2*S*)-*trans*-1,2-cyclohexanediol and 1,2-diaminocyclohexane were from Fluka; *trans*-*p*-hydroxycinnamic acid was from Acros.

Synthesis of the First Fluorinated Bilirubin

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Received August 5, 1996[⊗]

A symmetrical difluorinated bilirubin analog, 8,12-bis(2-carboxy-2-fluoroethyl)-3,17-diethyl-2,7,-13,18-tetramethyl-10*H*,21*H*,23*H*,24*H*-biline-1,19-dione (**9**), was synthesized from methyl 3-[2,4-dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl] propionate (**1**) in nine steps. Fluorine was introduced by reaction of an intermediate methyl 3-[1-(*tert*-butoxycarbonyl)-2,4-dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-hydroxypropionate (**5**), with (diethylamino)sulfur trifluoride (DAST). The fluorinated rubin exhibited the expected IR, UV−vis, and NMR spectroscopic properties, similar to those of the unfluorinated parent, mesobilirubin XIIIα. However, the solubility properties unexpectedly differed, with the fluorinated rubin being less soluble in organic solvents than its parent. While this phenomenon may be attributed to the much increased acidity of the carboxylic acid hydrogens in **9**, it probably also arises from less effective intramolecular hydrogen bonding due to a decreased basicity of the propionic acid carbonyl groups.

Introduction

Bilirubin IXα, the yellow-orange pigment of jaundice formed from heme during normal metabolism in humans and other mammals,[1,2] owes its peculiar solubility and solution properties to a stubborn tendency to tuck its polar carboxylic acid and amide groups inward, linking them up through hydrogen bonding (Figure 1).[3,4] The most stable and persistent conformation, shaped like a ridge-tile and maintained by six intramolecular hydrogen bonds, has been found in crystals of bilirubin[5,6] as well as in various solutions[7,8] and is thought to be important in its transport and metabolism.[2,9] Analogs that have the C-8 and C-12 propionic acid groups transposed to other sites on the pigment backbone (e.g., C-7 and C-13 in mesobilirubin IVα) are much more polar, behave completely differently, and do not engage in intramolecular hydrogen bonding.[9,10] But analogs with propionic acids at C-8 and C-12 (as in mesobilirubin XIIIα) or replaced by a wide range of differing alkanoic acid chain lengths apparently do retain the conformation-determining intramolecular hydrogen bonding motif, which determines their shapes and properties.[9,11]

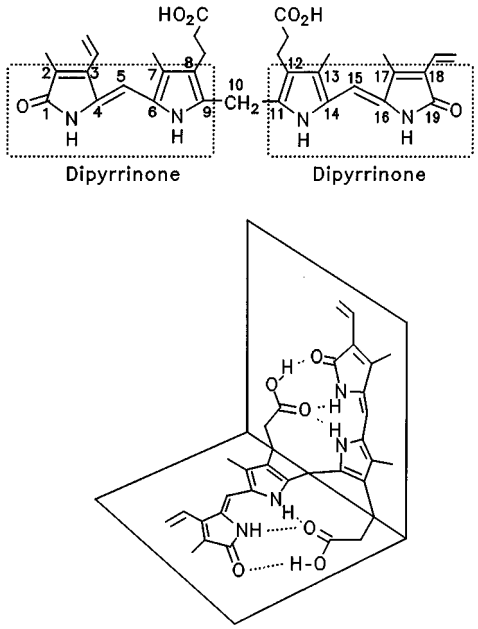

Figure 1. Bilirubin-IXα shown in a linear representation (upper) and in its most stable ridge-tile conformation (lower, only one of two enantiomers is shown). The ridge-tile is stabilized by a network of 6 intramolecular hydrogen bonds.

The key elements for intramolecular hydrogen bonding are thus a dipyrrinone receptor for the carboxylic acid group and a carboxylic acid tethered to ring carbons 8 and 12 (Figure 1). The carboxylic and dipyrrinone moieties form a complementary hydrogen bonding pair. Esterification disrupts the hydrogen bonding to some extent, as does amidation to give tertiary amides.[12] Even so, hydrogen bonding of the dipyrrinone to a carboxylate ion seems to be quite effective in retaining the pigment's folded, hydrogen-bonded conformation.[7,8] Since the carboxylic acid group is essential for effective hydrogen bonding to a dipyrrinone receptor, we wondered whether varying the acidity of the carboxylic acids might alter the effectiveness of the intramolecular hydrogen bonding. To achieve a profound alteration in carboxylic acid acidity, we determined that introduction of an α-fluorine, as in

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1996.

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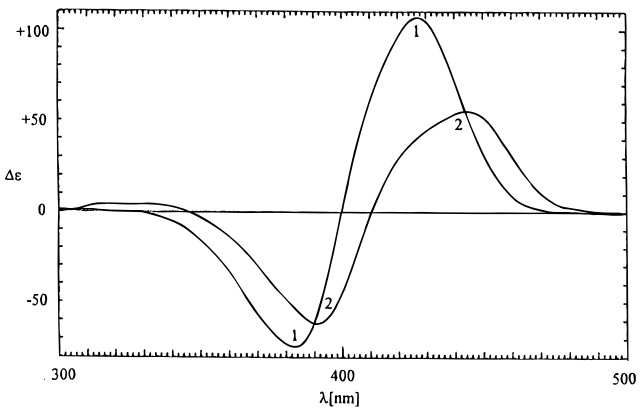

Figure 2. Circular dichroism (CD) spectra of 2×10^{-5} M solutions of α,α′-difluoromesobilirubin-XIIIα (**9**) (Spectrum 1) and mesobilirubin-XIIIα (Spectrum 2) in pH 7.4 aqueous phosphate buffer containing human serum albumin in 22 °C. The molar ratio of pigment to protein is 1:2. CD and UV-vis data for **9**: $\Delta\epsilon_{427}^{max} = +107$, $\Delta\epsilon_{383}^{max} -74.5$, and $\epsilon_{429}^{max} = 50{,}200$; and for mesobilirubin-XIIIα: $\Delta\epsilon_{444}^{max}$ +55.3, $\Delta\epsilon_{391}^{max}$ −62.0, and $\epsilon_{437}^{max} = 46{,}600$.

Cotton effect intensities are larger, suggesting a greater enantioselectivity—possibly due to the more acidic carboxylic acids of **9** forming a tighter salt linkage to an amine residue (lysine) on HSA.[22]

If the spectral data for **9** generally conform to expectations, the solubility and chromatographic data were surprising. The parent mesobilirubin XIIIα is soluble in chloroform (~1 mg/mL maximum), but **9** is very insoluble—so much so that ^{1}H-NMR could not be measured in this solvent. It also exhibits very limited solubility in most other organic solvents, although the solubility was sufficient for determining the UV−vis spectra, which were quite like that of mesobilirubin XIIIα. Dimethyl sulfoxide proved to be the best solvent for **9**, but it is not the solvent of choice for studying bilirubin conformation by NMR.[8] Comparison of TLC behavior on silica gel using 4% CH_3OH in CH_2Cl_2 as irrigant gave an R_f of 0.95 for mesobilirubin-XIIIα and R_F values of 0.39 and 0.36 for **9** (diastereomeric mixture). The data suggest that the two fluorines of **9** render it more polar than the parent. Comparison of HPLC behavior using a reversed-phase column and 0.1 M di-*n*-octylamine acetate in methanol as eluent[23] gave a retention time of 16.5 and 17.1 min for **9** *vs* 17.9 min for mesobilirubin XIIIα. Consistent with **9** being a mixture of diastereomers, it exhibits two peaks (1:1 ratio). These data suggest that **9** is only somewhat more polar than its parent.

Concluding Comments

The decreased solubility of α,α′-difluoromesobilirubin XIIIα (**9**) in organic solvents and its increased polarity, compared to the parent rubin, were unanticipated and suggest that intramolecular hydrogen bonding in **9** may be weaker or less influential. Other rubin acids that cannot hydrogen bond, e.g., mesobilirubin IVα, are also more polar and insoluble in solvents such as chloroform.[9] We surmise that the powerful inductive effect of the α-fluorine, in addition to rendering the carboxylic acid proton much more acidic than ordinary carboxylic acids,[13] also renders the nonbonded electrons of the carboxylic acid carbonyl much less basic (as reflected in the more shielded ^{13}COOH chemical shift, Table 1)—and thus possibly less effective in maintaining the hydrogen-bonded structure of Figure 1. Metabolism studies of **9** and the influence of the enhanced carboxylic acid acidity on hepatic glucuronidation and excretion are in progress.

Experimental Section

NMR spectra were obtained at 300 MHz and 500 MHz in $CDCl_3$ solvent (unless otherwise noted), and chemical shifts were reported in δ ppm. *J*-modulated spin-echo experiments (APT) were used to obtain ^{13}C-NMR spectra. ^{19}F-NMR spectra were referenced to external $CFCl_3$ standard at 0.00 ppm. GC−MS analyses were carried out on a capillary gas chromatograph (30 m DB-1 column) equipped with a mass selective detector. Analytical thin layer chromatography (TLC) was carried out on J. T. Baker silica gel IB-F plates (125 μm layer) using 4% methanol in dichloromethane. Radial chromatography was carried out on Merck silica gel PF_{254} with $CaSO_4$ preparative layer grade. HPLC analyses were carried out on a high-performance liquid chromatograph with a UV−vis spectrophotometric detector (set at 410 nm) and a Beckman-Altex ultrasphere-IP 5 μm C-18 ODS column (25 × 0.46 cm) with Beckman ODS precolumn (4.5 × 0.46 cm). The flow rate was 1.0 mL/min, and the elution solvent was 0.1 *M* di-*n*-octylamine acetate in 5% aqueous methanol (pH 7.7, 34 °C). Melting points are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. High-resolution mass spectra were run at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln. Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Ethyl acetoacetate, pentane-2,4-dione, methyl methacrylate, diisopropylamine, trimethylbromosilane, di-*tert*-butyl dicarbonate, tetra-*n*-butyl ammonium fluoride, *n*-butyllithium in hexane, (diethylamino)sulfur trifluoride (DAST), *p*-chloranil, and sodium borohydride were from Aldrich. Tetrahydrofuran, dichloromethane, chloroform, methanol, hexane, and dimethyl sulfoxide were HPLC grade from Fisher. Tetrahydrofuran was dried by distillation from $LiAlH_4$; methanol was distilled from $Mg(OCH_3)_2$; dimethyl sulfoxide was freshly distilled from CaH_2 under vacuum. Human serum albumin was defatted, from Sigma Chemical Co.

Methyl 3-[2,4-Dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]propionate (1). This pyrrole was prepared as reported previously in 45% yield from methyl acetoacetate and methyl 4-acetyl-5-oxohexanoate: mp 106−107 °C (lit.[24] mp 107−108 °C); ^{1}H-NMR δ 2.21 (3H, s), 2.26 (3H, s), 2.42 (2H, t, J = 7.4, 8.3 Hz), 2.70 (2H, t, J = 7.4, 8.3 Hz), 3.65 (3H, s), 3.81 (3H, s), 8.88 (1H, br s) ppm; ^{13}C-NMR δ 10.41, 11.13, 19.45, 34.77, 50.75, 51.31, 116.6, 119.8, 126.8, 130.4, 162.3, 173.4 ppm; MS *m*/*z* (rel intens) 239 ($M^{\bullet+}$; 35), 208 (6), 180 (4), 166 (82), 134 (100), 106 (8) amu.

Methyl 3-[2,4-Dimethyl-5-(methoxycarbonyl)-1*H*-pyrrol-3-yl]-2-hydroxypropionate (2).[25] To a solution of LDA (prepared from 50 mmol of diisopropylamine and 50 mmol of 1.6 M *n*-BuLi in hexane at −20 °C) in 60 mL of dry THF was added a solution of **1** (4.79 g, 20 mmol) in 60 mL of THF at −45 °C. After 1 h of stirring at −40 °C, an oxygen stream was bubbled through the solution for 45 min while the temperature reached −20 °C. The reaction was quenched with water, the product was extracted with $CHCl_3$, and the organic layer was washed with 3% HCl and water until neutral. Triethyl phosphite (3 mL) was added, the organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed under vacuum. After column chromatography on silica gel (hexane: ethyl acetate = 10:1−10:3.5) and recrystallization from EtOAc/

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Aqueous dissociation constants of bile pigments and sparingly soluble carboxylic acids by ^{13}C NMR in aqueous dimethyl sulfoxide: effects of hydrogen bonding

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Abstract pK_as for the acid dissociation of the carboxyl groups of bilirubin in water have been reported recently to be 8.1–8.4, or higher. These high values were attributed to intramolecular hydrogen bonding. They have led to suggestions that monoanions of bilirubin predominate at physiologic pH and are the species transported most readily into hepatocytes by carriers. Such high aqueous pK_as are inconsistent with recent ^{13}C nuclear magnetic resonance (NMR) measurements on mesobilirubin XIIIα, done on aqueous solutions containing dimethyl sulfoxide. To investigate whether the presence of dimethyl sulfoxide leads to unreliable values when using ^{13}C NMR spectroscopy to determine pK_as of carboxylic acids that can undergo intramolecular hydrogen bonding, we measured the pK_as of ^{13}C-labeled fumaric, maleic, and phthalic acids in solutions containing up to 27 vol % dimethyl sulfoxide. In addition, we used ^{13}C NMR to estimate the pK_as of 2,2′-methylenebis[5-carbomethoxy-4-methylpyrrole-3-[1-^{13}C]propanoic acid], a model for the two central rings of bilirubin. Our results show that ^{13}C NMR of aqueous dimethyl sulfoxide solutions can be used with confidence to measure pK_as of intramolecularly hydrogen-bonded carboxylic acids. They support our previous estimates for the pK_as of bilirubin and confirm that intramolecular hydrogen bonding has little effect on the acidity of bilirubins in water. Together with previous studies and chemical arguments they strongly suggest that reported aqueous pK_as of >8, or even >6, for the carboxyl groups of bilirubin are incorrect and that arguments used to rationalize them are questionable.—**Trull, F. R., S. Boiadjiev, D. A. Lightner, and A. F. McDonagh.** Aqueous dissociation constants of bile pigments and sparingly soluble carboxylic acids by ^{13}C NMR in aqueous dimethyl sulfoxide: effects of hydrogen bonding. *J. Lipid Res.* 1997. **38:** 1178–1188.

Supplementary key words bilirubin • biliverdin • fumaric acid • gallstones • jaundice • maleic acid • organic anions • phthalic acid • pK_a

Best-known as a colorful herald of hepatobiliary disease, bilirubin (BR) (**1, Fig. 1**) is the toxic agent in kernicterus, the major component of pigment gallstones, and an endogenous inhibitor of free-radical injury (1–3). Like its blue-green precursor biliverdin (**2**), it is a dicarboxylic acid. Accurate values for the acid dissociation constants (K_as) of biliverdin and BR have been difficult to ascertain because both pigments are only very sparingly soluble in water at physiologic pH and below (4–7). Until about 1980 most estimates and determinations of the K_as of BR indicated them to be about 5.0×10^{-5} M (pK_a 4.3)–1.3×10^{-6} M (pK_a 5.9) (7–13), which is within the range expected for aliphatic carboxylic acids (14). Recent studies (15–17), however, have concluded that they are about 10^{-8} M or less because of intramolecular hydrogen bonding (H-bonding). These extraordinarily low values are gradually becoming accepted in the literature (18–22).

Recently, we used ^{13}C nuclear magnetic resonance (NMR) spectroscopy to measure the K_as of several synthetic compounds related to BR (23–25). These included mesobilirubin (MBR) XIIIα (**3**), mesobiliverdin XIIIα (**4**), the two mono-pronionic bile pigments (**5**) and (**6**), and the di and monopyrrolic acids (**7**) and (**8**). For each compound we found K_as in the range expected for aliphatic carboxylic acids and no evidence for a large effect of intramolecular H-bonding. Since ^{13}C-NMR spectroscopy is a sensitive and accurate method for determining dissociation constants (26–29), our results led us to question the high pK_a values

Abbreviations: BR, bilirubin IXα; H-bond (ed, ing), hydrogen bond (ed, ing); HPLC, high pressure liquid chromatography; MBR, mesobilirubin; NMR, nuclear magnetic resonance.

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was, in fact 4Z,15Z-bilirubin IXα, as assumed; comparative control studies with biliverdin were not done; and chloroform, which has been shown to be unsuitable for such studies (69) was used as the organic phase. Earlier partitioning studies, not cited in (16), done with more suitable water-immiscible organic solvents gave data consistent with pK_as of ~4.3 and 5.3 (70), close to our measured values for MBR XIIIα (24). In principle, the solubility and partitioning methods are reliable. However, they require accurate measurement of extremely low concentrations of BR in water at acid pHs, which is difficult, and are prone to interference from traces of water-soluble diazo-reactive bilirubinoid impurities. The solubility method also requires knowledge of the intrinsic water solubility of unionized BR, which has proved difficult to measure accurately and is controversial (15, 16, 30). In contrast, the ^{13}C-NMR technique does not require measurement of concentrations, is insensitive to trace amounts of diamagnetic impurities, and unlike the partitioning procedure used recently (16) requires no evaporation or extensive manipulation and sampling of solutions.

CONCLUSIONS

In this and previous papers (23–25) we have shown systematically: *i*) that ^{13}C NMR in aqueous d_6-DMSO solutions is a reliable method for determining aqueous pK_as of mono- and dicarboxylic acids, even those that are intramolecularly H-bonded; *ii*) that mono-, di-, and tetrapyrroles containing propionic acid side-chains have pK_as similar to those of simple aliphatic acids; *iii*) that acids that can undergo the same type of intramolecular H-bonding as BR have pK_as similar to those that cannot; and *iv)* that the pK_a of BR analog **5,** with only one propionic acid, is 4.3. With that work as foundation we measured the pK_as of the COOH groups of MBR XIIIα and found that both are <6 with pK_{a1} ~4.2 and pK_{a2} ~4.9 (24). As the constitutional and three-dimensional structures of MBR XIIIα and BR are similar, we conclude that the two pK_as of BR also are likely to be in the range ~4.2–4.9. That conclusion is broadly consistent with widely overlooked solvent partitioning studies (69, 70) and all observations on the pK_as of BR made before 1985. Our studies reveal no peculiar effect of DMSO on H-bonding in BR and raise doubts about the accuracy of recent determinations by solubility (15, 26), spectrophotometric (26, 27), and solvent partitioning methods (16) and the significance of biological models based on them (17). Our data indicate that the predominant species present in simple aqueous solutions of BR at physiologic pH is the dianion, as previously pointed out by Brodersen (4, 5, 48) among others. This does not exclude the possibility that BR monoanions play a special role in phase transfer of BR in vivo, as proposed by Wennberg (71). The pK_as of BR will, of course, be dependent on the medium and values for water may differ from those for BR in other environments, such as bile or lipids. Studies on fatty acids and bile acids (27, 28) suggest that the pK of BR in a membrane might be some three units higher than in water. Our studies indicate that the aqueous ionization of BR is not abnormal or unusual or much influenced by intramolecular H-bonding, contrary to earlier unsubstantiated assumptions (15–17). What is odd about BR compared to other dicarboxylic acids such as biliverdin and protoporphyrin is its extraordinary lipophilicity (72) which seems to have a dominant effect on its transport and metabolism (32, 72). But for that, BR probably would not cross the placenta in fetal life (73) or require glucuronidation for hepatic excretion postnatally. Focusing only on ionization, neglecting three-dimensional structure and accepting improbably high aqueous pK_a values is liable to produce a jaundiced perspective of BR transport and metabolism.

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Analysis of Vibrational Circular Dichroism Data in the Near Infrared and Visible Range

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Since 1989 we have studied the near infrared and visible Vibration Circular Dichroism VCD of six monoterpenes of different absolute stereochemistry. The data, taken between 1500 and 600 nm, cover four successive CH-stretching overtones, namely those associated with differences in the vibrational quantum number v between 3 and 6. They are consistently composed of a bisignate couplet that correlates via a Birge–Sponer plot with the most prominent couplet in the infrared. We also measured data for (3*R*)-methylcyclopentanone between 2000 and 700 nm (from $\Delta v = 2$ to $\Delta v = 5$). Its spectrum is composed of one negative broad feature in each overtone region, all staying on a Birge–Sponer plot. In the following we report new data for (+) and (−)-fenchone, (+) and (−)-camphor, (*R*)-carvone, (*R*)-pulegone, terpinen-4-ol, (1*R*)-(+)-*trans*-isolimonene, and (+) and (−)-limonene oxide, to explore possible correlations between Near Infrared Circular Dichroism NIRCD and stereochemistry. We think the data show a modest but encouraging correlation with the distance of the C*H bond from the carbocyclic ring C=C bond or from the C=O bond and the configuration of the stereogenic centre C*. In conclusion, notwithstanding the paucity of data, we propose that NIRCD spectroscopy can be used to study configurational properties of isolated chiral C*H bonds in much the same way as local mode absorption spectroscopy is used to study isolated CH bonds.

Keywords: Near infrared circular dichroism (NIRCD), local modes, configuration, conformation, ketones, olefins

INTRODUCTION

The use of physical (in particular spectroscopic) methods in defining the absolute configuration of an optically active molecule, irrespective of its conformation, has been the goal of considerable research [1]. As pointed out by Djerassi [1] the specific rotation at the sodium D-line is insufficient, although commonly employed, as a possible definition of the absolute configuration. In the same article [1a], it was pointed out that a generalisation of the (+)/(−) definition of chirality may come from an Optical Rotatory Dispersion (ORD) curve with the possibility of perturbations from conformations being recognised, applications of the octant rule form the best examples for which both configuration

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Synthesis and Conformational Analysis of a Chiral Fluorinated Bilirubin Analog Lacking Carboxyl Groups

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An optically active analog **1** of etiobilirubin-IVγ with a single fluorine on each of the C(8) and C(12) alkyl groups has been synthesized in order to examine its potential for hydrogen bonding with fluorine. Circular dichroism spectroscopy reveals an unusually strong influence of 2,2,2-trifluoroethanol solvent on diastereo-selection of the *M*-helical conformation of (8^1S,12^1S)-**1**.

J. Heterocyclic Chem., **36**, 969 (1999).

Introduction.

The yellow pigment of jaundice, bilirubin (Figure 1) is an important and structurally interesting mammalian natural product that is produced copiously in normal human metabolism from hemoglobin and other heme proteins [1-3]. Much effort has been devoted toward understanding the properties and metabolism of bilirubin, with special focus on its unique ability to fold into a conformation where the carboxylic acids are joined by hydrogen bonding to the opposing dipyrrinones [4-6], which are known to be avid hydrogen bonding units [7]. Such hydrogen bonding decreases the polarity of the pigment, rendering it nonexcretable in normal metabolism, except following glucuronidation [3, 8]. Synthetic analogs with propionic acids at C(8) and C(12), such as mesobilirubin-XIIIα and **3** (Figure 1), exhibit properties very similar to that of biliru-

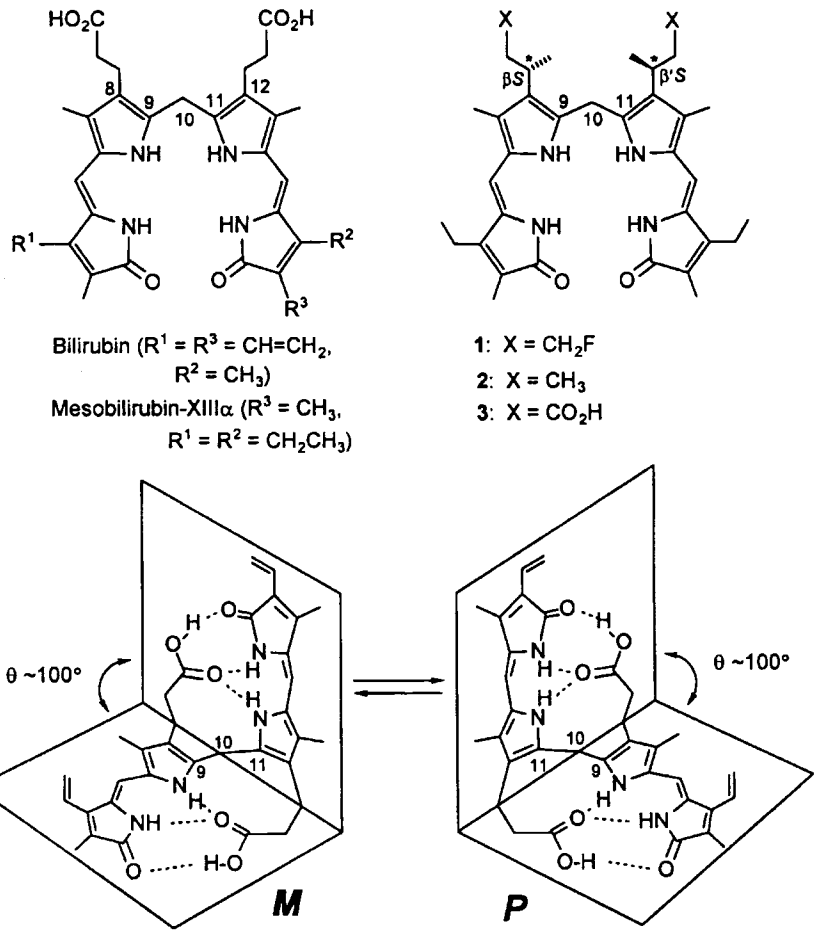

Figure 1. (Upper left) Bilirubin and its analog mesobilirubin-XIIIα in a porphyrin-like conformation. (Upper right) Chrial analogs of mesobilirubin-XIIIα, with stereogenic centers at *. (Lower) Interconverting enantiomeric conformaions of bilirubin, stabilized by intramolecular hydrogen bonds and shaped like ridge tiles. Hydrogen bonds are shown by dashed lines.

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Carboxylic acid ionization constants by ^{19}F NMR spectroscopy

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ABSTRACT: The ^{19}F NMR spectra of 26 simple fluorinated carboxylic acids were measured in aqueous solutions of pH 0.3–10.0. Analysis of the fluorine chemical shift dependence on pH allowed the determination of ionization constants from the titration curves; the values agreed with known pK_a values. The acidity of a fluorinated bilirubin precursor was established using this method. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: ^{19}F NMR; fluorinated carboxylic acids; ionization constants; pK_a

INTRODUCTION

The natural yellow–orange pigment bilirubin is the end product of heme catabolism in mammals.[1] Bilirubin contains two propionic acid groups, and their ionization behavior is implicated in the pigment's hepatic transport, neurotoxicity, formation of gallstones and protein and lipid membrane binding.[2] In a recent investigation of the inter-relationship between altered acidity of synthetic bilirubin analogs and their solution properties, such as stereochemistry, polarity, solubility and excretion, we introduced substituents with a strong electron-withdrawing effect in the vicinity of the carboxylic acid groups.[3,4] Methoxy and methylthio substitution at the α-carbon of each propionic acid of bilirubin was expected to decrease the acid pK_a by 1 unit, but this did not significantly change the pigments' overall properties.[3] In contrast, α-fluoro substitution, which was expected to decrease the pK_a by more than 2 units (CH_3CO_2H, pK_a = 4.76; FCH_2CO_2H, pK_a = 2.58[5]) led to drastically altered properties.[4] Synthetic α,α′-difluoromesobilirubin-XIIIα is polar and water soluble, whereas natural bilirubin is relatively non-polar, lipophilic and completely insoluble in water. Water solubility was attributed to complete ionization of the acid groups at pH ≈ 7. Therefore, we sought to obtain an independent quantitative estimate of acidity of α,α′-difluoromesobilirubin-XIIIα and some of its precursors.

The presence of a fluorine atom, with a nuclear spin I = 1/2, the natural isotopic abundance of 100% and high receptivity (a measure of the ease of detecting a nucleus; ^{19}F is 0.83 of that of protons) offer an opportunity to use ^{19}F NMR spectroscopy for the examination of ionization equilibria. The chemical shift (δ_F) range of a ^{19}F NMR signal is intrinsically very wide, and therefore the fluorine nucleus is an excellent, highly sensitive probe of its environment. This fact was used more than 35 years ago for pK_a determinations of *p*-fluoroacetophenone (C–H acidity) and *p*-fluorobenzamide (N–H acidity),[7] and as recently as 1998 for subtle changes of δ_F arising from solvent-induced isotope shifts due to enrichment of water with H_2 ^{18}O.[8]

The literature provides a number of examples in which ^{19}F NMR spectroscopy was used to study the ionization of protonated amines containing fluorine. Such amines were designed to have pK_as in the physiologically important range (pH 6.5–8.0) and were used in the development and application of NMR indicators for non-invasive and accurate intracellular pH measurement.[9–12] To the best of our knowledge, there have been no systematic reports on titrations of simple carboxylic acids and determination of their pK_as using ^{19}F NMR spectroscopy. In this paper we report on the changes of ^{19}F NMR chemical shifts associated with the ionization of carboxylic acids in the (strongly) acidic pH range, show how such changes can be used to measure their pK_as and apply the method to the pK_a determination of a fluorinated bilirubin precursor (**1**).

RESULTS AND DISCUSSION

A series of carboxylic acids bearing a single fluorine reporter atom on an sp^3-or sp^2-hybridized carbon atom or trifluoromethyl group attached to an aliphatic or aromatic carbon atom were studied. The structures of the acids investigated are shown in Scheme 1.

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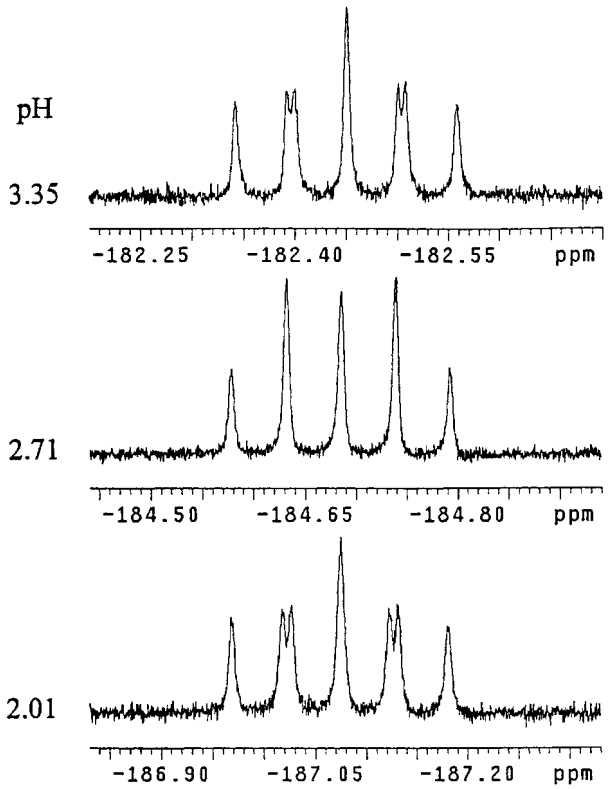

Figure 4. Changes of ^{19}F NMR chemical shift and $^{19}F^{1}H$ coupling pattern of α-fluoro(pyrrole)propionic acid **(1)** with pH

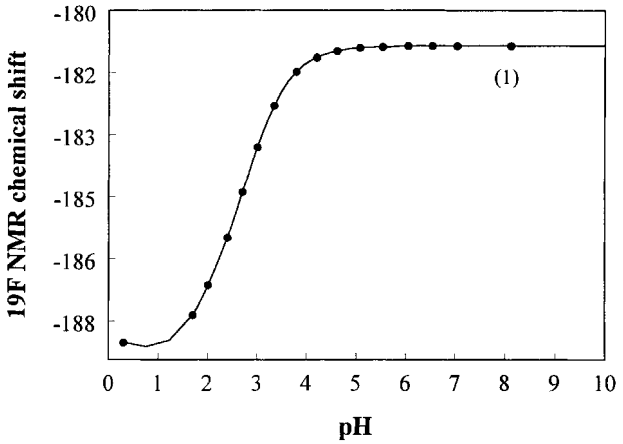

Figure 5. Variation of ^{19}F NMR chemical shift with pH for aqueous solutions of α-fluoro(pyrrole)propionic acid (**1**)

good agreement with literature data on the effect of one strongly electronegative fluorine substituent. The pK_a of **1** provides suggestive evidence that the acidity of the bilirubin synthesized from **1**[4] would also show a corresponding increase (~100-fold), and this doubtless contributes to its peculiar properties, such as its aqueous solubility.

Further work is in progress (P. B. Karadakov, University of Surrey, UK) to understand the observed ^{19}F NMR chemical shifts and $\Delta\delta_F$ by applying *ab initio* calculations.

CONCLUSIONS

The fluorine nucleus appears to be an excellent probe for monitoring by NMR ionization equilibria in acidic aqueous medium. Its wide chemical shift dispersion range allows accurate pK_a determinations for a variety of carboxylic acids. The method defines the expected high acidity of a fluorinated bilirubin precursor to be pK_a = 2.67.

EXPERIMENTAL

The ^{19}F NMR spectra were acquired at 25 ± 1 °C on a Varian Unity Plus spectrometer at 470.254 MHz in 5 mm tubes, and were referenced against external standards: hexafluorobenzene (5 ± 0.02 mM in $CHCl_3$) at δ − 162.90 ppm or $CFCl_3$ (10 ± 0.05 mM in $CHCl_3$) at δ 0.00 ppm, with shifts upfield from $CFCl_3$ being negative.[20] Typical experimental parameters were flip angle 55°, interpulse delay 3 s, collecting 128 transients, and spectral width 35 kHz using 70K data points. Each FID was zero filled to 262K and multiplied with an exponential function (line broadening 0.1 Hz) prior to Fourier transformation to give a 0.27 Hz digital resolution. Proton decoupling was not applied and consistently the chemical shift of a selected prominent line was followed. pH measurements were made using an Orion Model 811 pH meter with an Orion Model 91-02 combination electrode calibrated twice for the range 0.3–10.0 at pH 4.00, 7.41 and 10.00. Potassium tetraoxalate buffers (50 mM)[14] were used throughout, the pH being adjusted with HCl or KOH. The pH values reported in Table 1 refer to those of freshly prepared aqueous oxalic acid–oxalate containing solutions before dissolving the fluorinated acid. A stock solution of the each acid **1–26** (concentration 40 ± 1 mM) was prepared in DMSO-d_6. A 100 µl aliquot was diluted to a volume of 2 ml at each pH of the oxalate buffer, giving a total fluorinated acid concentration of 2.00 ± 0.05 mM and 5% (v/v) DMSO-d_6 in the NMR samples. The pH values of the NMR solutions were then rechecked and showed only small changes of 0.03–0.25 pH units, with larger variations occurring with the more acidic compounds. These findings are consistent with previous studies that showed that very low concentrations of DMSO exert only a very small effect on buffer pH.[22]

The small amount of added DMSO-d_6 served as an internal NMR deuterium lock and was used to maintain solution homogeneity. Whereas a number of the acids (*e.g.* **2, 4, 5** and **25**) used in this work are soluble in the

Pergamon

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TETRAHEDRON LETTERS

Dissociation Constants of Carboxylic Acids by ^{13}C-NMR in DMSO/Water

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Abstract: Measurements on eighteen ^{13}COOH-labeled acids, including hydrogen-bonded and non-hydrogen-bonded standards are self-consistent and suggest strongly that the aqueous pK_as of bilirubin are within the normal range for aliphatic carboxylic acids. Our empirical observations provide further evidence that the ^{13}C-NMR method is valid when suitable buffers, DMSO concentrations and extrapolations are used and refute recent suggestions to the contrary.

We recently used ^{13}C-NMR to estimate pK_as of simple mono- and dicarboxylic acids and several compounds related to bilirubin, a natural dicarboxylic acid. We made measurements in water and aqueous solutions containing not more than, and mostly very much less than, 31 mole% $(CD_3)_2SO$. With these methods we observed literature values for standard acids and were able to estimate the pK_as of bilirubin to be ~4.2–4.9, as expected for two non-interacting aliphatic COOH groups.[1] We undertook those studies because of our interest in applications of ^{13}C-NMR to ionization phenomena and our skepticism about recent reports[2] that had put the pK_as of bilirubin in the range 6.8–9.3, far from the typical aliphatic COOH value of ~5.

A subsequent Letter[3] has suggested that our estimates are unreliable. The criticisms appear to boil down to two main objections: (1) that we ignored effects of DMSO on COOH dissociation; and (2) that we failed to consider intramolecular interactions of carboxyl groups. Additionally, the Letter implied that our measurements of the pK_as of standard acids were inconsistent with accepted values. In the following we present additional validation of the ^{13}C-NMR method and show that it provides accurate estimates of the aqueous pK_as of aliphatic carboxylic acids, even those prone to intramolecular hydrogen bonding.

Fig. 1a shows ^{13}C-NMR titration data for [1-^{13}C]- phenylacetic acid in solutions containing from 0–31 mol% DMSO.[4] In these experiments, to avoid DMSO-buffer interactions, pH was adjusted only with HCl/NaOH and the pH plotted is the measured pH of the solution used for NMR runs. Textbook titration curves were obtained, even at 31 mole% DMSO, and apparent pK_a values for each solvent could be readily derived by standard methods. Plotting these apparent pK_as versus mole% DMSO (Fig. 1b) gives an acceptable linear relationship over the range of DMSO concentrations used and shows that apparent pK_as for DMSO-water solutions containing up to 31 mole% DMSO can be reliably extrapolated to give a good estimate of the value in DMSO-free solution (measured pK_a, 4.31; extrapolated, 4.35). For comparison, Fig. 1c shows ^{13}C-NMR titrations for ^{13}C-phenylacetic acid taken from an earlier paper.[1a] In this experiment, buffers were used to adjust pH and the pH used in plotting the data was

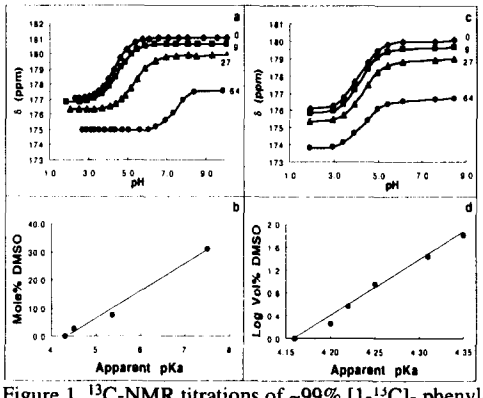

Figure 1. ^{13}C-NMR titrations of ~99% [1-^{13}C]- phenylacetic acid in DMSO/H_2O. Numbers beside the curves in (a) and (c) are vol% of DMSO. In (c) curves for 1.8 and 3.6 vol% DMSO have been omitted for clarity. For other details see the text.

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CIRCULAR DICHROISM AND CONFORMATIONAL ANALYSIS OF DIASTEREOMERIC BICAMPHORS

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Dedicated to Professor Otakar Červinka on the occasion of his 75th birthday in recognition of his outstanding contributions to the area of organic stereochemistry.

Stereospecific syntheses afforded *endo,endo*- (**1**) and *exo,exo*- (**2**) bicamphors, while the third possible diastereomeric *exo,endo*-bicamphor (**3**) originated from nonselective camphor radical dimerization. The stereochemistry of bicamphor linkage was confirmed by ^{1}H NMR analysis. Chiroptical and ultraviolet spectral data are presented for the three diastereomers **1**–**3** to show interchromophoric interaction. Conformational analysis to evaluate the relative orientation of each pair of carbonyl chromophores was accomplished by ^{1}H NMR spectroscopy and molecular mechanics calculations.

Key words: Camphor dimers; Molecular mechanics; Stereochemistry; Conformation analysis; CD spectroscopy; UV spectroscopy; NMR spectroscopy, ^{1}H and ^{13}C; Terpenoids.

The ketone carbonyl n→π* transition was among the first chromophores studied extensively by modern chiroptical methods: optical rotatory dispersion[1] (ORD) and circular dichroism[2] (CD) spectroscopy. Attractive factors include its accessibility ($\lambda_{max} \approx 300$ nm) and its spectroscopic nature (electric dipole forbidden – magnetic dipole allowed) which leads to a very large Kuhn's dissymmetry factor, $g = \Delta\varepsilon/\varepsilon$. Early investigations led to the first rationalization of optical activity imposed on a symmetric carbonyl group by its chiral environment, the octant rule[3–5]. Over the past fifty years a wealth of experimental data for the carbonyl n→π* transition CD and ORD have been accumulated, analyzed and reviewed[1–7]. Although the octant rule has been firmly established and extensively applied in the determination of absolute configuration or conformation of ketones, refinements (such as contributions from front octants[8]) have been made, and reservations have been expressed[6] when new perturbers or cyclic systems were investigated, *e.g.* cyclopropyl-containing tricyclic ketones[9]. The interpretation of CD Cotton

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A Water-Soluble Synthetic Bilirubin with Carboxyl Groups Replaced by Sulfonyl Moieties

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Summary. The first symmetrical bilirubin analog with CO_2H groups replaced by SO_3H, 8,12-*bis*-(2-sulfo-ethyl)-3,17-diethyl-2,7,13,18-tetramethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione, was synthesized from methyl (2,4-dimethyl-5-ethoxycarbonyl-1*H*-pyrrol-3-yl) acetate in nine steps *via* the sulfonic acid analog of xanthobilirubic acid (*XBR*) and isolated as its disodium salt. The sulfonic acid group was introduced at an early stage of the synthesis by reaction of an intermediate, ethyl 4-(2-bromoethyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate, with sodium sulfite. The disodium bilirubin disulfonate exhibits NMR spectroscopic properties rather similar to those of the parent carboxylic acid, mesobilirubin-XIIIα; however, its UV/Vis spectra are blue-shifted and broadened relative to those of the parent compound. Like mesobilirubin, the disulfonate displays a positive exciton chirality circular dichroism spectrum, albeit with weaker *Cotton* effects, in a buffered aqueous solution ($pH = 7.4$) containing a 2:1 molar ratio of human serum albumin.

Keywords. Pyrrole; Sulfonic acid; Spectroscopy; CD.

Introduction

Bilirubin-IXα (Fig. 1A), the yellow pigment of jaundice, is formed from heme during normal metabolism in humans and other mammals [1, 2] and owes its water insolubility and other properties to a persistent tendency to tuck its polar carboxylic acid and amide groups inward, linking them by hydrogen bonds [3, 4]. The most stable conformation, shaped like a ridge-tile and secured by six intramolecular hydrogen bonds (Fig. 1B), has been found in crystals of bilirubin [5] and its dicarboxylate dianion salt [6] as well as in solution [7, 8]. The ridge-tile conformation is believed to be important in the transport and metabolism of bilirubin [2, 9]. Analogs with vinyl groups reduced to ethyl (as in mesobilirubin-XIIIα), with alkyl substituents on the propionic acids [10], or even with electronegative substituents such as OCH_3 [11] or F [12] on the propionic acid chains all apparently retain the conformation-determining intramolecular hydrogen bonding motif, which determines the pigment's shape and properties. However, analogs with propionic acid groups transposed from ring carbons 8 and 12 to other sites on the pigment backbone (*e.g.* to 7 and 13 in mesobilirubin-IVα [13]) are much more polar. They cannot engage in intramolecular hydrogen bonding and behave completely differently in solution [13].

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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Hepatobiliary Excretion of Dipyrrinone Sulfonates in Mrp2-Deficient (TR^-) Rats

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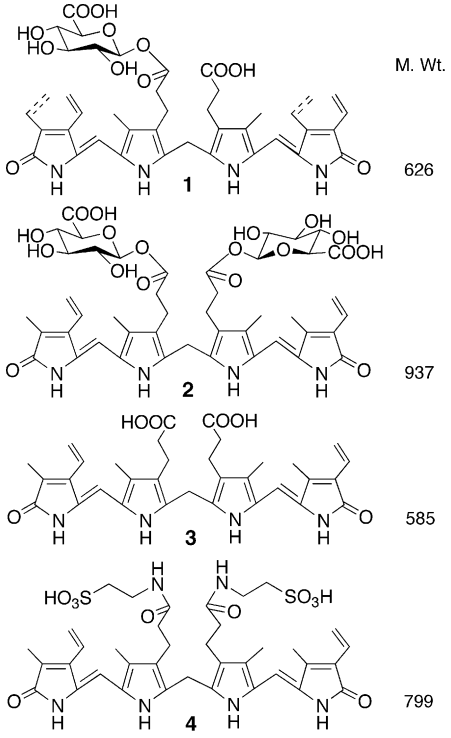
Abstract—The biliary excretion of the sodium salts of 8-(2-ethanesulfonic acid)-3-ethyl-2,7,9-trimethyl-1,10-dihydro-11*H*-dipyrrin-1-one (xanthosulfonic acid) and a fluorescent analogue (8-desethyl-*N*,*N*′-carbonyl-kryptopyrromethenone-8-sulfonic acid) was compared in Mrp2-deficient (TR^-) and normal rats. Both organic anions were excreted rapidly in bile in Mrp2-deficient rats, but the biliary excretion of the fluorescent sulfonate was impaired relative to normal controls. The rat clearly has efficient Mrp2-independent mechanisms for biliary efflux of these anions that are not used by bilirubin or its mono- and diglucuronides.

Efflux from liver to bile is a major process in the elimination of countless endogenous and exogenous chemicals from the body. Taurine and glycine amides of hydroxylated cholanic acids (bile salts), which are the major solutes of bile, are actively transported through the canalicular membrane of hepatocytes by an ATP-powered protein known as BSEP (bile salt export pump).[1] In contrast, the monoglucuronide (**1**) and diglucuronide (**2**) conjugates of bilirubin (**3**) (Scheme 1), which impart to bile its golden hue, are thought to be actively transported across the same membrane by the ATP-ase Mrp2 (multidrug resistance associated protein 2), also known as cMOAT (canalicular multispecific organic anion transporter).[2,3] Mutant rats (TR^- and EHBR rats) that do not express this protein are unable to excrete bilirubin glucuronides efficiently in bile and exhibit mild conjugated hyperbilirubinemia because of the accumulation of bilirubin glucuronides.[4,5] Similarly, people with Dubin–Johnson syndrome, caused by a rare genetic defect in Mrp2 synthesis, also develop hyperbilirubinemia. In addition to bilirubin glucuronides, the biliary excretion of a number of other chemicals has been shown to be impaired in Mrp2-deficient rats.[2,3] These include glucuronide and glutathione conjugates as well as glutathione itself. Such animal studies, along with isolated perfused liver experiments and many



Scheme 1. Linear representations of bilirubin monoglucuronides (**1**), bilirubin diglucuronide (**2**), bilirubin (**3**) and bilirubin ditaurine amide (**4**). (Only one of two possible isomeric monoglucuronides is depicted; in the other isomer, the positions of the methyl and vinyl groups on the end rings are reversed, placing vinyl groups as indicated by dotted lines in **1**.)

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On the Conformation of Bilirubin Ditaurate

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Summary. The first optically active taurine conjugate of a bilirubin was prepared by reaction of taurine sodium salt with the mixed anhydride formed from reaction of (βS,$\beta' S$)-dimethylmesobilirubin-XIIIα with isobutyl chloroformate. Analysis of the circular dichroism spectra of the conjugate in water and chloroform indicate a conformational preference for the (*M*)-helical ridge-tile conformation, thus providing the first spectroscopic evidence on the conformation of ditaurobilirubins.

Keywords. Taurine; Bilirubin; Dipyrrinone; CD.

Introduction

Taurine ($^{-}O_3SCH_2CH_2NH_3^{+}$), a conditionally essential nutrient important to mammalian development is found in plasma and milk, *inter alia*, and in bile as conjugates of bile acids, *e.g.*, taurocholic acid [1, 2]. It has also been found as a conjugate of the dicarboxylic acid bilirubin (the yellow pigment of jaundice) in the bile of certain fish (yellowtail, red sea bream, and flounder) [3]. It is not found in mammalian bile, however, where the principle bilirubin conjugates excreted into bile are mono and di-conjugates of glucuronic acid [4]. Bilirubin glucuronides are reactive, undergoing acyl migration and facile hydrolysis, and they are not readily available [5]. In contrast, the ditaurate does not undergo either and is far more stable, and it is available commercially. Consequently, it has been used as a surrogate for bilirubin diglucuronide *in vitro* and in animal studies, where it is smoothly excreted by the liver [6, 7]. Although its constitutional structure is known (Fig. 1), little is known of its conformation [8].

In the following, we describe the syntheses of the ditaurate of (βS,$\beta' S$)-dimethylmesobilirubin (**1**) and β-methylxanthobilirubic acid (**2**) (Fig. 2) and a spectroscopic study of **1** designed to provide new information on the three-dimensional structure of ditaurobilirubin.

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Conformation and Crystal Structure of Dipyrrinones with Oxindole Components

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Summary. Pyrrole α-aldehydes (2-formyl-4,5-dimethyl-1*H*-pyrrole and 2-formyl-*N*-methylpyrrole) condense readily at C(3) of indolin-2-ones to give dipyrrinone analogs, such as (3*Z*)-[(4,5-dimethyl-pyrrol-2-yl)-methylidenyl]-indolin-2-one and (3*E*)-[(1-methylpyrrol-2-yl)-methylidenyl]-indolin-2-one. ^{1}H-NMR N*O*E analyses and X-ray crystallography confirm the *syn*-(*Z*) configuration for the former and the *syn*-(*E*) configuration for the latter. The former is stabilized by intramolecular hydrogen bonding. Molecular mechanics calculations of the latter indicate no energy difference between the *syn* and *anti* conformations.

Keywords. Pyrrole; X-ray structure; Hydrogen bonding.

Introduction

Our interest in synthetic bile pigments with aromatic substituents [1–3] and annelated pyrrole rings led us to prepare and investigate potential dipyrrinone synthons for the latter. Two types of potential precursors are oxisoindole and oxindole, with the former leading to the conventional bile pigment types. The latter condenses easily with pyrrole α-aldehydes, reactions that formed the basis for a study of antiangiogenic agents targetting at receptor tyrosine kinases [4–6]. In the following we describe the synthesis of a new potential antiangiogenic agent **1** (Fig. 1) from 2-indolinone (**3**) and compare its conformation to that of a known analog **2** using N*O*E NMR spectroscopy and X-ray crystallography.

Results and Discussion

Synthesis

3-[(Pyrrol-2-yl)-methylidenyl]-indolin-2-ones **1** and **2** were prepared in excellent yields by piperidine-catalyzed condensation of **3** with 2-formyl-4,5-dimethylpyrrole

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2,3-Benzannelated dipyrrinone analogs (**1** and **2**) of xanthobilirubic acid (**3**) are prepared by base-catalyzed condensation of isoindolinone (**5**) and indolin-2-one (**6**) respectively, with methyl 3-(2-formyl-3,5-dimethyl-1*H*-pyrrol-4-yl)propanoate (**4**). Nuclear Overhauser effect H-nmr studies indicate that both **1** and **2** adopt preferentially a *syn-Z* configuration. The former forms a hydrogen-bonded homodimer in nonpolar solvents; the latter is intramolecularly hydrogen bonded.

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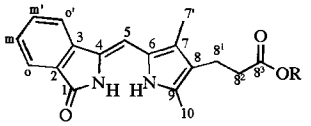
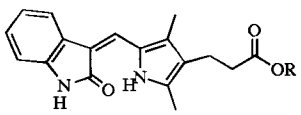
Introduction.

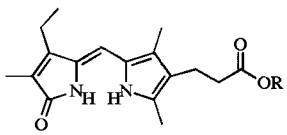
Porphyrins with aromatic rings fused at the β-positions have been a subject of recent syntheses and investigations [1]. Although linear tetrapyrroles have been prepared with aromatic rings attached [2], those with fused aromatic rings are largely unknown. In the following, we report the first 2,3-benzodipyrrinone analog (**1**, Figure 1) of methyl xanthobilirubinate (**3b**) [3], a synthetic precursor to mesobilirubin-XIIIα and its verdin [4] and related linear tetrapyrroles [5]. In addition, we report on **2**, a lactam moiety reversed constitutional isomer of **1**, and compare the properties of these two dipyrrinones.



1a: R = H **1b**: R = CH_3



2a: R = H **2b**: R = CH_3



3a: R = H **3b**: R = CH_3

Figure 1. Structures, numbering system and preferred conformations of benzo analogs **1** and **2** of xanthobilirubic acid **3a**.

Synthesis.

The common pyrrole reactant for the syntheses of **1** and **2** is pyrrole aldehyde **4b** [6] which was condensed with isoindolinone **5** (prepared from *o*-toluic acid, as reported previously [7]) to give **1b** and 2-indolinone (**6**) to give **2b** (Scheme 1). The condensation to give **2b** proceeded rapidly and smoothly in high yield (79%) from reaction of commercially available **6** with 1.1 equivalents of **4b** in methanol at reflux in the presence of 0.5 equivalents of piperidine. Condensation of the free propionic acid-aldehyde **4a** with **6** was conducted at a higher temperature in ethanol (reflux), using excess (2 equivalents) of piperidine. Condensation of **4b** with **5** proved to be unexpectedly difficult, suggesting that **5** was less reactive than 3,4-alkyl-substituted pyrrolinones in the same reaction. Although a literature report [8] that various salicylaldehydes condense in high yields with **6** in aqueous sodium hydroxide at reflux, reaction of **4b** or **4a** with **5** in the same type of condensation conditions afforded only a 6% yield of **1b** after treatment with diazomethane. Numerous combinations of bases (1,8-diazabicyclo[5.4.0]undec-7-ene, diethyl amine, piperidine) and solvents (*t*-butanol, 1,2-dimethoxyethane, dimethylsulfoxide, acetonitrile), with or without *t*-Boc protection on the nitrogen of **4b** or **5** were ineffective, affording only low yields of **1b** or inducing decomposition. The best yields (21-32%) of **1b** were obtained by carrying out the condensation of **4b** and **5** in dry, deoxygenated dimethylformamide with 2.5 equivalents of piperidine at 85-90 °C for 48 hours.

The constitutional structures of **1b** and **2b** follow from the structures of the reactants and the reaction mechanism, and they are confirmed by their carbon-13 nmr spectral assignments, assisted by HMQC and HMBC 2D experiments. Switching the orientation of the lactam (–NH–C=O) moiety in going from **1** to **2** strongly affects most of the carbon-13 nmr chemical shifts. Only a few remain constant: lactam C=O, 7′–CH_3, 10–CH_3, 8^1–CH_2, 8^2–CH_2, 8^3 C=O and OCH_3. All benzo ring carbons of **1b** are more deshielded than their counterparts in **2b**, except C(2), which is ~10 ppm more shielded. In contrast, the pyrrole ring carbons and C(5) are generally more shielded in **1b** than the corresponding carbons in **2b**.

Distinctions also appear in comparing the H-nmr spectra of **1b** and **2b**: the methine hydrogen at C(5) is ~0.8 ppm more shielded in **1b** than in **2b**, and the aromatic ring hydrogens of **1b** are all more deshielded than in **2b**. The pyrrole and lactam NHs of **1b** appear in the typical places for dipyrrinones that form hydrogen-bonded dimers in deuteriochloroform. However, in **2b**, the pyrrole NH is very

ON THE CONFORMATION OF GLYCOBILIRUBIN

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Dedicated to the memory of Professor Otakar Červinka.

The first optically active glycine conjugate **1** of a bilirubin was prepared in several steps from (*S*)-β-methylxanthobilirubic acid glycine conjugate **8**. The latter was synthesized by reaction of benzyl glycinate tosylate with the mixed anhydride formed in the reaction of (*S*)-β-methylxanthobilirubic acid **6** with isobutyl chloroformate. Spectroscopic analysis of the circular dichroism spectra of **1** in various solvents, including aqueous buffer, indicate a conformational preference for the ***M***-helical ridge-tile conformation, thus providing the first spectroscopic evidence on the conformation of glycobilirubins.

Keywords: Glycine conjugate; Bilirubin; Hydrogen bonding; Pyrroles; CD spectroscopy; NMR; Porphyrins; Oligopyrroles; Amino acids; Conformation analysis.

The essential amino acid glycine ($^{-}O_2CCH_2NH_3^{+}$) is found widely in nature, serving as a flexible link in proteins, recognition sites on enzymes and cell membranes, a component of molecular activity modifying conjugation, *etc.*[1] Together with taurine ($^{-}O_3SCH_2CH_2NH_3^{+}$), it is an important amide-linkage conjugate of bile acids[2–4], and both serve in detoxification mechanisms of xenobiotics *in vivo*[5]. Although taurine conjugates of the natural dicarboxylic acid bilirubin (the yellow pigment of jaundice) have been found in the bile of certain fish (yellowtail, red sea bream and flounder)[6], neither taurine nor glycine conjugates (Fig. 1) of bilirubin seem to be present in mammalian bile, where the principal bilirubin conjugates are mono- and diglucuronides[7]. Bilirubin glucuronides are reactive, undergoing acyl migration and facile hydrolysis, and pure conjugates are not readily available[8]. In contrast, bilirubin ditaurate (taurobilirubin) is very stable and commercially available, thus making it a useful surrogate for bilirubin diglucuronide *in vitro* and in animal studies, where it is smoothly excreted by the liver[9]. The bis-glycine amide of bilirubin (glycobilirubin[10]) is also predicted to be stable, but far less is known of its properties and conformation; few citations appear in the literature, and only one synthesis has been described[10].

pK_a and Aggregation of Bilirubin: Titrimetric and Ultracentrifugation Studies on Water-Soluble Pegylated Conjugates of Bilirubin and Fatty Acids†

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ABSTRACT: A water-soluble conjugate (**1**) with intact carboxyl groups was prepared by addition of poly(ethylene glycol) thiol (MPEG-SH) regiospecifically to the *exo* vinyl group of bilirubin. ^{1}H and ^{13}C NMR and absorbance spectroscopy in $CDCl_3$ and DMSO-d_6 confirmed the assigned structure and showed that pegylation did not disrupt the hydrogen-bonded ridge-tile conformation of the pigment moiety. Aqueous solutions of **1** were optically clear, but NMR signals were seen only from the MPEG portion and none from the tetrapyrrole, consistent with dissolved assemblies containing aggregated bilirubin cores within mobile polyether chains. On alkalinization (pH >12), signals from the pigment moiety reappeared. Titrimetric measurements on **1** in water showed the pK_a's of the two carboxyl groups to be similar (average 6.42). Control studies with pegylated half-esters of succinic, suberic, brassylic, thapsic, and 1,20-eicosanedioic acid showed that pegylation per se has little, if any, effect on carboxyl ionization. However, aggregation increases the apparent pK_a by ∼1−2 units. The molecularity of bilirubin in solution was further characterized by ultracentrifugation. Over the pH range 8.5−10 in buffer, bilirubin formed multimers with aggregation numbers ranging from ∼2−7. Bilirubin is monomeric in DMSO or $CHCl_3$ at $\sim 2 \times 10^{-5}$ M, but aggregation occurred when the $CHCl_3$ was contaminated with trace adventitious (perhaps lipoidal) impurities. These observations show that aggregation increases the pK_a's of aliphatic carboxylic acids relative to their monomer values in water. They are consistent with earlier ^{13}C NMR-based estimates of ∼4.2 and ∼4.9 for the aqueous pK_a's of bilirubin and similar studies of bilirubin in micellar bile-salt solutions. Together with earlier work, they confirm that the pK_a's of bilirubin are about normal for aliphatic carboxyls and suggest that the high (>7.5) values occasionally reported, including those based on $CHCl_3$ partitioning, are artifacts of aggregation or technique.

Bilirubin, a tetrapyrrolic dicarboxylic acid (Figure 1), is the cytotoxic yellow pigment of jaundice (*1*). Produced in healthy adults at about 300 mg/day by catabolism of heme, it is eliminated by the following series of poorly understood steps: delivery to the liver as a complex with serum albumin (SA);[1] dissociation from SA and uptake by hepatocytes; migration within hepatocytes to the endoplasmic reticulum where it is converted to mono- and diglucuronides by a bilirubin-specific glucuronosyl transferase (UGT1A1); passage of the glucuronides to the apical (canalicular) membrane; efflux of the glucuronides, but not the parent unconjugated pigment, into bile by the organic anion ABC transporter MRP2 (*2*). Even less well understood than these processes are the mechanisms of uptake of bilirubin into the brain and its neurological toxicity. Nevertheless, bilirubin has proved an informative model for the metabolism and transport of many xenobiotic carboxylic cholephilic organic anions, and considerable interest in the pigment has been rekindled recently by the rediscovery of its potent antioxidant properties and mounting evidence that it is a major physiologic cytoprotectant (*3*, *4*).

The structure, state of aggregation, and degree of ionization of bilirubin in vivo are clearly key determinants of its biochemical properties. Although bilirubins's chemical structure is frequently misrepresented or misinterpreted (*5*), its constitution and structure in the solid state and in solution in organic solvents are well-established. Its aggregation, which may be involved in its neurotoxicity and is a common, often overlooked, source of difficulty and error in experimental work (*6*), is less well characterized. And, although the two acidic side chains in bilirubin are simple aliphatic

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[1] Abbreviations: ABC, ATP binding cassette; CTAB, cetyltrimethylammonium bromide; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; EPPS, 4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid buffer; gHMBC, gradient heteronuclear multiple bond coherence; MPEG-OH (-SH, -OTs), poly(ethylene glycol) (thiol, tosylate) monomethyl ether; MRP2, multidrug resistance associated protein 2; NMR, nuclear magnetic resonance; PBLG, poly(γ-benzyl L-glutamate); PEG, poly(ethylene glycol); PEO, poly(ethylene oxide); SA, serum albumin; SDS, sodium dodecyl sulfate; UGT1A1, uridinediphosphoglucuronosyltransferase 1A1.

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CONCLUSION

For years, the aqueous dissociation constants of monomeric bilirubin have been described as uncertain. While highly accurate values are still unavailable, a substantial body of evidence now indicates that they are within the range ($pK_a = 4-5$) expected for aliphatic propionic acid groups, that they are not greatly influenced by intramolecular hydrogen bonding, as expected for this type of carboxylic acid, and that aggregation of the pigment leads to elevated apparent pK_a's. Indeed, unusually high pK_a values in the absence of any structure rationale should lead to a suspicion of some sort of aggregation phenomenon. Published values of >8.0 are no longer credible, and biological models (*15*) based on them may be misleading and erroneous. Estimates of the water solubility of bilirubin based on the same partitioning studies (*13*, *60*) and frequently cited in recent bilirubin literature (*25*, *99*−*102*) may also be incorrect.

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Intensely fluorescent dipyrrinones

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ABSTRACT: Intense fluorescence from a new, more highly conjugated (benzannelated) xanthoglow analog, 9,11-dimethyl-10-[2-(methoxycarbonyl)ethyl]-5*H*,7*H*-pyrrolo[2′,1′:6,1]pyrimidino[3,4-*a*]isoindole-5,7-dione (**1**), was determined accurately and compared with fluorescence from the parent xanthoglow, 1-ethyl-8-[2-(methoxycarbonyl) ethyl]-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (**2**). Benzoxanthoglow (**1**) gave a quantum yield for fluorescence (ϕ_F) of 0.78 in cyclohexane (λ_{exc} 412 nm, λ_{em} 487 nm), whereas xanthoglow methyl ester (**2**) gave $\phi_F = 0.80$ in cyclohexane (λ_{exc} 410 nm, λ_{em} 473 nm). In DMSO, **1** gave $\phi_F = 0.55$ (λ_{exc} 419 nm, λ_{em} 530 nm) and **2** gave $\phi_F = 0.65$ (λ_{exc} 419 nm, λ_{em} 508 nm), illustrating the large Stokes shifts and strong fluorescence properties of these easily synthesized yellow pigments. Copyright © 2004 John Wiley & Sons, Ltd.
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KEYWORDS: fluorescence; xanthoglow; dipyrrinones

INTRODUCTION

Dipyrrinones (Fig. 1) are typically yellow chromophores, with a strongly allowed absorption ($\varepsilon \approx 30\,000$) near 400 nm.[1] In solution, the singlet excited state from absorption of an ~400 nm photon is rapidly relaxed by $4Z \rightarrow 4E$ double-bond isomerization. For example, the quantum yield for $Z \rightarrow E$ photoisomerization of xanthobilirubic acid (Fig. 1), $\phi_{Z \rightarrow E}$, is ~0.22 ($\phi_{E \rightarrow Z}$ ~0.40) in EPA (diethylether–isopentane–ethanol, 5:5:2 v/v/v) at 20 °C[1] and ~0.2 in pH 7.4 aqueous buffer containing human serum albumin (HSA).[2] Radiative decay by fluorescence is correspondingly very weak, with minute fluorescence quantum yields: $\phi_F < 1 \times 10^{-3}$ in EPA[1] and $\phi_F < 3 \times 10^{-3}$ in aqueous buffered HSA at 22 °C.[2] At very low temperatures (77 K) in glasses, $\phi_{Z \rightarrow E}$ decreases to $< 5 \times 10^{-4}$ and ϕ_F increases to ~0.33 in EPA. When the $Z \rightarrow E$ photoisomerization is prevented by bonding constraints that link the two nitrogens by one-carbon[3–6] (or longer[6]) belts, ϕ_F measured at room temperature rises considerably.

In this work, we examined the influence of extended conjugation, through benzannelation of the dipyrrinone, measuring ϕ_F for **1** and making comparisons with the parent xanthoglow (**2**).[5] Because most general references[7,8] that describe methods for determining quantum yields do not outline a step-by-step experimentally detailed procedure for measuring relative ϕ_F, we describe a systematic method for relative ϕ_F measurements that should be useful to those learning fluorescence spectroscopy.

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RESULTS AND DISCUSSION

Synthesis

The parent, unbridged, methyl esters of xanthobilirubic acid (XBR, Fig. 1) and its 2,3-benzo analog, both known from earlier studies,[9,10] were dissolved in anhydrous methylene chloride solvent and reacted with 5 mol equiv. of 1,1′-carbonyldiimidazole in the presence of DBU base[5] to afford 90 and 93% isolated yields of **1** and **2**, respectively. Compounds **1** and **2**, solutions of which were intensely fluorescent to the naked eye, gave the expected ^{13}C and ^{1}H NMR spectra, with the latter showing the absence of the NH resonances and the former showing a new signal at 143–144 ppm for the imide carbonyl.

UV–visible absorption and fluorescence emission spectra

The UV–visible spectra of **1** and **2** (Table 1) reveal a long-wavelength maximum absorbance near 415–426 nm for **1** and 425–431 nm for **2** with a smaller associated ε, as has been noticed previously for planarized dipyrrinones.[3,4] In addition, a shorter wavelength UV band near 275–285 nm and a longer wavelength band lying close to 363–370 nm become evident in **1**. Only small wavelength shifts in the long-wavelength absorption

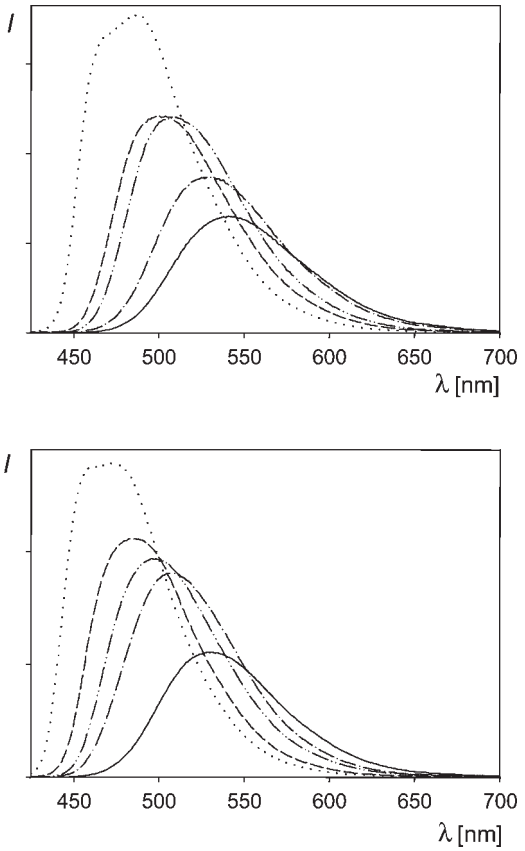

Figure 4. Solvent dependence of fluorescence emission from **1** (top) and **2** (bottom) in (• • •) cyclohexane, (-- -- --) benzene, (-- • • --) chloroform, (------) methanol and (-- • --) dimethyl sulfoxide

cyclohexane as 394.8 mg and that of **2** as 290.1 mg. From Eqn (1), and the available significant figures, then truncating at the final product, we found

$$\phi_F = (7.08_5 \times 10^{-2}/7.35_0 \times 10^{-2}) \times (290.1/394.8) \times (1.446^2/1.426^2) \times 0.90 = 0.66$$

Alternatively, using integral values from the fluorimeter:

$$\phi_F = (7.08_5 \times 10^{-2}/7.35_0 \times 10^{-2}) \times (1.57_2 \times 10^8/2.10_6 \times 10^8) \times (1.446^2/1.426^2) \times 0.90 = 0.67$$

The agreement between these two methods is excellent, and similarly excellent agreement was confirmed for three more samples in five different solvents. The disadvantage of the 'cut and weigh' method is obvious: the 'weight' of the emission band varies. Variations come from the different scaling factors along the *x*- and *y*-axes: in every sample and standard pair, the scaling must be adjusted to accommodate both band intensities. Thus, every pair (different solvent or sample) will give a different weight for the same standard (DPA). The numerical integral method avoids this complication, i.e. the intensity of DPA is always $2.10_6 \times 10^8$ (arbitrary units) and this value was used throughout the study. The various ϕ_F, λ_{exc} and λ_{em} values for **1** are summarized in Table 2. The fluorescence quantum yields of the parent xanthoglow methyl ester (**2**) were determined in the same manner and are shown in Table 2.

Clearly, the ϕ_F values of **1** and **2** are very large and approach unity in cyclohexane while tapering off in methanol. The latter solvent might have promoted self-association of these non-polar pigments; however, in the range of concentrations used, 10^{-5}–10^{-6} M, we found $\ll$2% deviation from Beer's law. The conjugation of **1** exhibits little effect on ϕ_F but a significant effect on λ_{em}, with the emission band showing a 10–22 nm bathochromic shift of **1** relative to **2**. Both **1** and **2** show large Stokes shifts, from 70 to 90 nm in non-polar solvents and from 110 to 120 nm in polar solvents.

CONCLUSION

Determinations of ϕ_F for **1** and **2** relative to three known standards reveal very high values of ϕ_F in a study that illustrates practical aspects, reproducibility and standardization of the method. The importance of **1** and **2** relates to the potential of those strongly blue–green-emitting yellow dyes in forming conjugates to proteins and nucleic acids, where they might serve as fluorophores in gene and protein profilings on DNA and protein chips.

EXPERIMENTAL

Fluorescence measurements were performed using a Jobin Yvon Fluorolog 3 Model FL3-22 instrument at

Table 2. Solvent dependence of the fluorescence quantum yields (ϕ_F) and excitation λ_{max} (λ_{exc}) and emission λ_{max} (λ_{em}) (nm) for **1** and **2**

	Cyclo-C_6H_{12}			C_6H_6			$CHCl_3$			CH_3OH			$(CH_3)_2SO$		
Compound	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F	λ_{exc}	λ_{em}	ϕ_F
1	412	487	0.78[a]	419	500	0.68	419	508	0.67	419	541	0.36	419	530	0.55
2	410	473	0.80	419	484	0.72	419	497	0.67[b]	419	531	0.35[c]	419	508	0.65

[a] $\phi_F = 0.75$ using perylene standard ($\phi_{st} = 0.91$) in air-free cyclohexane.
[b] $\phi_F = 0.63$ using anthracene standard.
[c] $\phi_F = 0.34$ using anthracene standard.

JOC*Note*

Readily Synthesized Novel Fluorescent Dipyrrinones

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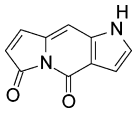

A new, highly fluorescent (ϕ_F up to 0.85) rigid *anti-Z*-dipyrrinone chromophore has been synthesized in high yield in a one-pot reaction by condensing two monopyrroles in the presence of DBU to form the pyrrolo[3,2-*f*]indolizine-4,6-dione nucleus.

Ordinary dipyrrinones, such as those elaborated from the *syn-Z* template of Figure 1A, are typically nonfluorescent because their ~420 nm excited states relax to a new ground state by rapid nonradiative decay involving $Z \rightarrow E$ isomerization of the C(4)−C(5) double bond.[1] When $Z \rightarrow E$ isomerization is prevented by bridging the two nitrogens with short carbon chains, strong dipyrrinone fluorescence (e.g., fluorescence quantum yield, ϕ_F ~0.85) may occur.[1−3] The very few examples of bridged dipyrrinones include the tricyclic ring system 3*H*,5*H*-dipyrrolo-[1,2-*c*:2′,1′-*f*]pyrimidine-3-one (Figure 1B), reported in 1986[2] and prepared from pyrrole-2-aldehyde in 3−4 steps. Analogues with β-substituents were prepared from the parent *syn-Z*-dipyrrinone by inserting a methano up to a 1,3-propano belt.[1,3] More recently we proposed a smoother, higher yield *N*,*N*′-bridging reaction in which a carbonyl group was inserted into the preformed *syn-Z*-dipyrrinone by treatment with 1,1′-carbonyldiimidazole (CDI) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the highly fluorescent 3,5-dione (Figure 1C).[4,5] In the following we describe a novel, high-yield condensation of two pyrroles to give directly the related (but unreported) pyrrolo[3,2-*f*]indolizine-4,6-dione chromophore (Figure 1D), based on the *anti-Z*-dipyrrinone skeleton. Such fluorescent pigments, with suitable polar groups attached, might be useful in fluorescence imaging of hepatic metabolism.

Dipyrrinones are typically synthesized by coupling two pyrrole precursors. Seeking a short, one-pot synthesis of new fluorescent dipyrrinones, we envisaged a reaction

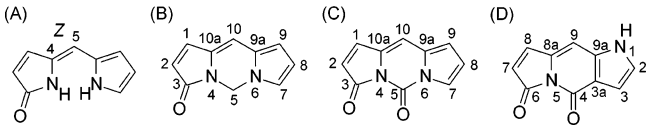

FIGURE 1. The *syn-Z*-dipyrrinone skeleton (A) and its methano-bridge analogue (B), 3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]-pyrimidine-3-one, (C) 3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione, and (D) pyrrolo[3,2-*f*]indolizine-4,6-dione. The dipyrrinone conformation of (A)−(C) is *syn-Z* and that in (D) is *anti-Z*.

sequence from two pyrrole precursors that would form a dipyrrinone in the first step and then proceed in a second step to form the desired tricyclic product. Thus, to prepare an *N*,*N*′-carbonyl-bridged *syn*-4*Z*-dipyrrinone (Figure 1C) might require a pyrrolinone and the *N*-methoxycarbonyl derivative of an α-formylpyrrole. However, the number of synthetic steps would not be reduced; so we considered an analogous reaction that might lead directly to a new type of fluorescent *Z*-dipyrrinone, rotated into the anti conformation and with the lactam nitrogen linked by a carbonyl group to a pyrrole β-carbon. Condensation−intramolecular cyclization was achieved by a highly successful route (outlined in Figure 2) involving base-catalyzed (ethanolic KOH) coupling of **8**[6] with **10**,[7,8] a readily available pyrrole α-aldehyde possessing a β-carboethoxy group. (Pyrrolinone **8** was obtained from 4-methyl-3-ethyl-2-*p*-toluenesulfonylpyrrole, prepared by a Barton−Zard reaction between *p*-toluenesulfonylmethyl isocyanide and 2-nitropentan-3-ol.[6]) The overall mechanism involves (i) standard formation of the dipyrrinone by aldol condensation followed by (ii) deprotonation of the lactam NH to promote nucleophilic attack on the C(7)−CO_2Et from the anti conformation of the dipyrrinone (Figure 2). From **8** + **10** in ethanolic KOH, a nearly quantitative yield of a very polar product was obtained that was shown to be a 73:27 mixture of the expected bridged dipyrrinone **1** and the unbridged dipyrrinone diacid **14**. Since the C(7) carboethoxy group is difficult to saponify (as with mesitoic acid esters[9]), we suggest that **14** might arise indirectly, from ring opening of **1** under the reaction conditions. Both **1** and **14** were suprisingly insoluble in most organic solvents and thus difficult to work with. Conversion of the mixture to methyl esters (using diazomethane) or isobutyl esters (using *i*-BuI-Cs_2CO_3) led to barely improved solubility, but sufficient for tedious chromatographic separation to give the methyl ester of **1** and its isobutyl analogue.

Interestingly, when the mixture of **1** + **14** was treated under more forcing conditions, with isobutyl iodide−Cs_2CO_3 in hot DMF, only the *N*-isobutyl isobutyl ester (**7**) was obtained. Apparently the C(7) CO_2H of **14** is esteri-

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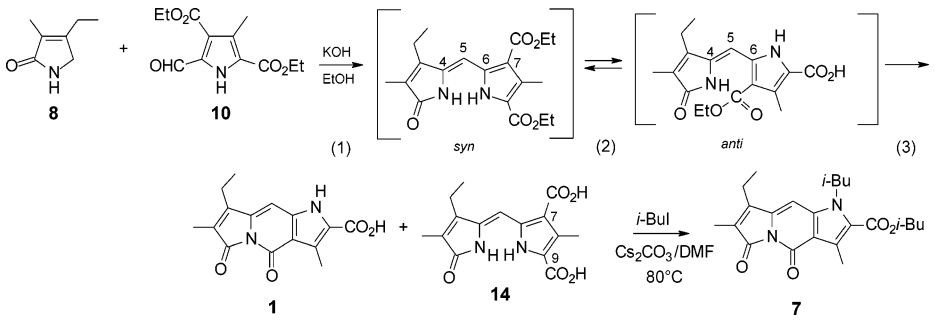
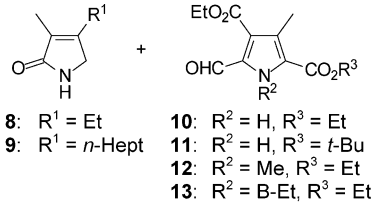
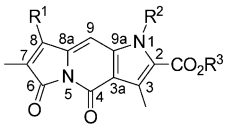


FIGURE 2. Condensation of pyrrolinone **8** and pyrrole aldehyde **10** to give bridged dipyrrinone **1** in three steps. A byproduct (**14**) is formed in the ratio 27:73, **14**:**1**, and the mixture can be converted to **7** as shown.



DBU | EtOH or *t*-BuOH



	R^1	R^2	R^3
2:	Et	H	Et
3:	Et	H	*t*-Bu
4:[a]	*n*-Hept	H	Me
5:[a]	Et	Me	Me
6:	Et	B-Et	Et

n-Hept = *n*-Heptyl
B-Et = $CH_2CH_2CH_2CO_2Et$

[a] Using KOH in place of DBU.

FIGURE 3. General reaction for synthesizing pyrrolo[3,2-*f*]-indolizine-4,6-diones (**2**-**6**) from simple monopyrrole precursors **8**−**13**.

fied, along with the C(9) CO_2H, and the C(7) ester is recycled through the intramolecular cyclization. Compound **7** had much better solubility properties than either the methyl ester of **1** or its isobutyl ester.

Exploring parameters that influence solubility in common organic solvents we prepared the *N*-methyl analogue (**5**) of **2** and the *n*-heptyl analogue (**4**) and found that the former had better solubility properties than the latter. To avoid the formation of the dipyrrinone diacid (**14**) byproduct, we eschewed ethanolic KOH, replacing it with DBU (Figure 3) to prepare **2** from **8** + **10** directly, smoothly, and in high yield. Similar treatment of **8** + **11** or **8** + **13** afforded the *tert*-butyl ester **3** or diester **6**, respectively.

The structures of **1**−**7** follow logically from their known precursors and their spectroscopic properties. In addition, X-ray quality crystals of **5** (triclinic space group $P\bar{1}$ with cell dimensions a = 8.3584 (17) Å, b = 11.6270 (18) Å, and c = 11.8366 (19) Å) and **7** (triclinic space group $P\bar{1}$ with cell dimensions a = 12.569 (2) Å, b = 14.698 (3) Å, and c = 15.133 (3) Å) were grown from DMSO (**5**) and

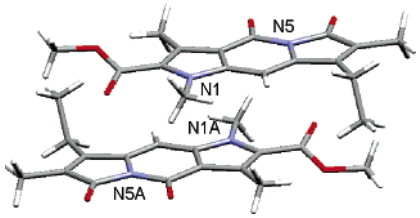


FIGURE 4. Crystal structure drawing of *N*-methyl methyl ester **5** molecules (in the unit cell). One DMSO molecule per molecule of **5** has been deleted for clarity of representation.

ethanol−water (**7**). Their crystal structures were determined, showing that both are planar, with C(3a)−C(9a)−C(9)−C(8a) torsion angles of 0.8° and 0.1°, respectively, and N(5)−C(8a)−C(9)−C(9a) torsion angles of −0.4° and −2.0°, respectively (see Figure 1 for the pyrrolo[3,2-*f*]-indolizine-4,6-dione numbering system). The N(5)−C(4)−C(3a) angles are 112.5° and 114.5°, respectively. The C(8a)−C(9) double bond lengths of **5** and **7** are 1.352 (7) and 1.322 (10) Å, respectively, and the C(9)−C(9a) single bond lengths are 1.415 (7) and 1.400 (11) Å, respectively: bond distances similar to those reported for unbridged dipyrrinones[10] and indicative of bond alternation in the six-membered ring. Crystals of **5** show two molecules in the unit cell in parallel planes (~3.5 Å apart) oriented head to tail (Figure 4) with one molecule of DMSO. The structure of **7** is less refined due to disorder in the isobutyls and significant thermal motion in the *N*-isobutyl. Crystals of **7** show two unique molecules in a 4-molecule unit cell along with one solvent molecule (modeled as EtOH) per pair of dipyrrinones. In contrast to **5**, **7** (with bulkier alkyls) packs not in parallel planes, but in planes inclined at 63° to each other.

As observed previously for *N*,*N*′-carbonyl-bridged *syn*-dipyrrinones, *N*,*C*-carbonyl-bridged *anti*-dipyrrinones **1**−**7** gave pronounced hypochromicity and a bathochromically shifted λ_{max} of long wavelength UV−vis transition (Figure 5) relative to unbridged dipyrrinones, with only a small influence due to changes in solvent type and polarity (Table S-1 of the Supporting Information). Solutions of **1**−**7** were strongly fluorescent to the eye. Excitation of the long wavelength band (392−399 nm) produced intense fluorescence between 435 and 505 nm (Table 1), with a large Stokes shift. The fluorescence quantum yields at room temperature in $CHCl_3$, CH_3OH, and DMSO determined versus 9,10-diphenylantracene stan-

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Exciton Chirality. (A) Origins of and (B) Applications from Strongly Fluorescent Dipyrrinone Chromophores

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Summary. (A) The origin of exciton interaction and examples of its application to organic stereochemistry are presented. (B) *N*,*N*′-Carbonyl-bridged dipyrrinones constitute a new class of highly fluorescent chromophores suitable for investigations of stereochemistry and absolute configuration. *N*,*N*′-Carbonylxanthobilirubic acid esters are strongly fluorescent, with a fluorescence quantum yield (ϕ_F) ~0.8, but produce only weak exciton CD from the *trans*-1,2-cyclohexanediol template. The ester of an analog with benzoic acid replacing propionic, *N*,*N*′-carbonyl-8-(4-carboxyphenyl)-3-ethyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one, exhibits strong fluorescence ($\phi_F = 0.68$, $\lambda_{em} = 493$ nm, $\lambda_{ex} = 422$ nm in $CHCl_3$) and UV-Vis absorption ($\varepsilon \sim 21000$ at 424 nm) in organic solvents. Its diester with (1*S*,2*S*)-cyclohexanediol is fluorescent and exhibits exciton circular dichroism ($\Delta\varepsilon = +15\,dm^3 \cdot mol^{-1} \cdot cm^{-1}$, $\lambda = 432$ nm; $\Delta\varepsilon = -4\,dm^3 \cdot mol^{-1}\,cm^{-1}$, $\lambda = 380$ nm) that correlates with the *Exciton Chirality Rule*.

Keywords. Circular Dichroism; Fluorescence; Exciton; Pyrroles.

Part A. Fundamentals of Exciton Chirality

Exciton Coupling

Exciton Spectra. Excitation of a molecule from its electronic *ground state* to an electronically *excited state* – promoted usually by the absorption of ultraviolet or visible light – involves movement of an electron, a process called an electronic transition. In near ultraviolet-visible light absorption, the electron typically moves from a high-energy occupied molecular orbital to a low-energy unoccupied molecular orbital, such as in $\pi \rightarrow \pi^*$ excitations. This movement of an electron creates an instantaneous dipole or polarization of charge (called an electric transition moment or electric transition dipole), a vector quantity with both a direction (orientation) and a magnitude (intensity) that vary according to the nature of the particular electronic transition and the chromophore involved. Two or more

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Exciton Chirality in *trans*-1,2-Diamidocyclohexanes: Fluorescent Chromophores

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Dedicated to Professor Piero Salvadori, Pisa, on the occasion of his 70th birthday.

ABSTRACT *N,N′*-Carbonyl-bridged dipyrrinones constitute a new class of highly fluorescent chromophores suitable for investigations of stereochemistry and absolute configuration. Xanthoglow (*N,N′*-carbonylxanthobilirubic acid) diamides of *trans*-1,2-diaminocyclohexane are strongly fluorescent (ϕ_F = 0.37, λ_{em} = 500 nm, λ_{ex} = 419 nm in $CHCl_3$) but exhibit only weak exciton circular dichroism (CD). In contrast, the diamide of (1*R*,2*R*)-diaminocyclohexane from the xanthoglow analogue whose propionic acid has been replaced by benzoic acid (*N,N′*-carbonyl-8-(4-carboxyphenyl)-3-ethyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one) exhibits even stronger fluorescence (ϕ_F = 0.62, λ_{em} = 495 nm, λ_{ex} = 422 nm in $CHCl_3$) and UV–visible absorption (ε = 41,600 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ at 424 nm) in organic solvents. Its exciton CD ($\Delta\varepsilon$ = −13 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$, λ = 432 nm; $\Delta\varepsilon$ = +2 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$, λ = 382 nm) correlates with the exciton Chirality rule. *Chirality 17:316–322, 2005.*

KEY WORDS: circular dichroism; dipyrrinones; diamines

A few years ago, we showed that dipyrrinone chromophore (Fig. 1), which is yellow with an intense, long wavelength $\pi-\pi^*$ absorption ($\varepsilon_{max} \sim$ 30,000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$, $\lambda_{max} \sim$ 410 nm) and an electric transition dipole moment oriented along the long axis of the molecule,[1,2] can be an effective chromophore for exciton chirality CD studies in the visible region.[3] An imaginary line connecting C(2) and C(8) of a dipyrrinone is approximately parallel to the long axis, long-wavelength electric transition moment, thereby suggesting that either C(8) or C(2) would be an appropriate site for attachment to chiral molecules. In the previous studies, we compared exciton CD spectra of *trans*-1,2-cyclohexanediol esters of dipyrrinones attached by their C(8) acetic acid or propionic acid chains. The acetic acid belt, with fewer degrees of motional freedom, gave the more intense CD, however, and showed an inverted CD couplet with respect to propionic derivatives, which correlated with the exciton chirality rule.[3] We had less success in preparing diesters from a dipyrrinone-8-carboxylic acid, which we expected would further reduce conformational mobility.

Subsequently, we showed that dipyrrinones with a carbonyl bridge connecting the pyrrole and lactam nitrogens have large fluorescence quantum yields $\phi_F > 0.5$, because excited state decay by 4*Z*→4*E* dipyrrinone carbon–carbon double-bond isomerization is prevented.[4] For those studies, we prepared a derivative of xanthobilirubic acid which we call xanthoglow (Fig. 2). To extend our dipyrrinone chromophore stereochemical investigations into exciton CD of *fluorescent* chromophores, we prepared a new fluorescent dipyrrinone derivative (xanthoglow benzoic acid) whose carboxylic acid linker is attached to a rigid benzene ring spacer at C(8). With a *para*-substitution pattern on the benzene ring and the orientation of the dipyrrinone long-wavelength transition dipole lying along the long axis of the chromophore,[1,3,5,6] the xanthoglow benzoic acid long wavelength transition moment is expected to lie approximately parallel to the long axis of the dipyrrinone pigment and the long axis of the benzoic acid.

There have been many studies of exciton chirality of diols, but fewer of diamines,[7–12] especially the benzamides of the simple enantiomeric *trans*-1,2-diaminocyclohexanes, whose amides have shown a strong tendency toward insolubility and gelation.[10] The stereochemistry of cyclic diamines has been studied by CD spectroscopy using nonamide chromophores, e.g., dicarboximide[11] and Schiff base or cyanine dye-type[12] chromophores. In the current work, we focus on the amides of *trans*-1,2-diaminocyclohexane with xanthoglow (**1**) and xanthoglow benzoic acid (**2**). We also include data from the amides of *p*-dimethylaminobenzoic acid (**3**), for comparison purposes. The CD spectral properties of the benzamides have been reported,[7] but there appear to be no literature data on the *p*-dimethylaminobenzamides. Although a fluorescent chromophore is not required for exciton chirality absorption CD studies, a strongly fluorescent chromophore might open up the possibility for complementary CD studies in the emission mode,[13,14] where increased sensitivity (from very low sample concentrations) could be an advantage.

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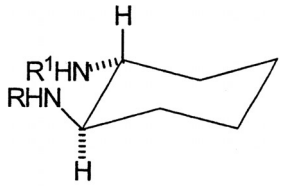
characteristics of this new CD reporter (as a monomeric chromophore corresponding to **2**) have been described.[19] The chromophore is an intense yellow pigment with ε_{425} = 20,700 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ in chloroform and high-fluorescence quantum yield ϕ_F = 0.78 in cyclohexane (green emitting solution, λ_{em} = 456 nm). Thus, the *N,N'*-carbonyl bridging causes an ~20 nm bathochromic shift and a significant hypochromic shift. In contrast, the unbridged dipyrrinone exhibits ε_{406} = 43,900 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ in chloroform and is not fluorescent.[16]

Consistent with **1** and **3**, **2** exhibited an exciton CD corresponding to the diamine absolute configuration (Fig. 5), as predicted by the exciton chirality rule.[8] Such consistency suggests that although the exact direction of the long-wavelength transition polarization in the chromophore of **2** is unknown, it is, most likely, similar to that of an unbridged dipyrrinone, namely parallel to an imaginary line connecting C(2) and C(8) of the dipyrrinone.[1,2,6] This line is almost parallel to the *para*-bonds on the benzene ring linker. Consequently, the mutual orientation of transition dipole moments in a bichromophore like **2** is independent of rotation around the *para*-bonds. Nevertheless, the benzene ring is not coplanar and conjugated to the fluorophore (compare in Table 1 the λ_{max} 423 nm of **1** with a saturated chain at C(8) vs. λ_{max} 424 nm of aryl-substituted **2**). Rotation about the dipyrrinone C(8) to C(4′)-benzoic acid bond should be fairly unrestricted. Molecular mechanics calculations[20] indicate that in the most stable conformation, the phenyl ring is rotated out of coplanarity with the dipyrrinone by ~85° due to steric buttressing by the C(9)–CH_3, and C(7)–CH_3. And X-ray crystallographic analysis of a monopyrrolic precursor of the fluorophore in **2,** as well as a *meta*-substituted analogue, revealed that the plane of the benzene ring is inclined at the pyrrole plane by 67–68° (unpublished results). From the absence of *ortho*-substituents on the benzene ring, we do not expect rotation about tricycle C(8)–*ipso*-benzene bond to be frozen in solution at ambient temperature. However, rotation about the single bond does not alter the orientation of the dipyrrinone long-wavelength transition dipole. As pointed out in the introduction, the advantage of *para*-substitution in **2** and **3** is in maintaining the directionality of the inherent transition dipole moment of the dipyrrinone chromophore. Regardless of the conformation around the *para*-bonds, the exciton interaction is governed by the conformation around the amide nitrogen atom.

TABLE 2. Fluorescence quantum yields[a] and emission wavelengths[b] (ϕ_F (λ_{em})) of diamides 1 and 2 and monoamide 4

Solvent[c]	(1*S*,2*S*)-**1**	(1*R*,2*R*)-**1**	(1*S*,2*S*)-**2**	(1*R*,2*R*)-**2**	(1*R*,2*R*)-**4**
Benzene	0.22 (478)	0.17 (488)	0.68 (482)	0.69 (481)	0.63 (479)
$CHCl_3$	0.37 (500)	0.36 (502)	0.61 (496)	0.62 (495)	0.52 (497)
CH_3OH	0.05 (529)	0.05 (529)	0.30 (529)	0.32 (529)	0.43 (528)
DMSO	0.17 (509)	0.18 (508)	0.56 (507)	0.58 (506)	0.64 (506)

[a]Relative quantum yield versus 9,10-diphenylanthracene standard: ϕ_F = 0.90 in cyclohexane (see Eaton, ref. 21).
[b]From excitation at 411–419 nm.
[c]Concentration, (3.0–7.7) × 10^{-7} M.



trans-(1*R*,2*R*)-Diamino-cyclohexane (R=R¹=H)

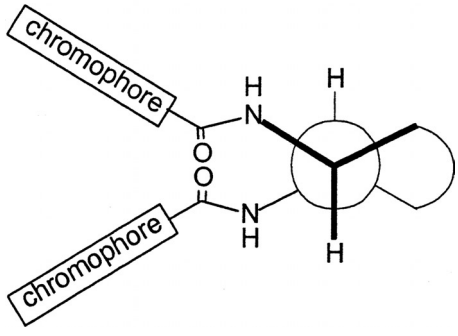


Fig. 5. (Upper) (1*R*,2*R*)-Diaminocyclohexane diamides (R = R¹), mono-amides (R¹ = H) with xanthoglow, xanthoglow benzoic acid, and *p*-dimethylaminobenzoic acid. (Lower) Newman projection looking down the 1,2 carbon–carbon bond of the diamine derivatized as amides with the relevant chromophores of Figure 2.

Although the CD intensities of bis-amide **2** were disappointingly low, enhanced ϕ_F values (Fig. 4) would suggest that this chromophore might be useful for FDCD.[13,14] A comparison of the ϕ_F values of Table 2 indicates strong fluorescence from the bis-amide (**2**) of the benzoic xanthoglow in aprotic solvents, mimicking the upward trend in ϕ_F observed for the xanthoglow bis-amide (**1**). The ϕ_F data for **2** are very similar to those of its mono-amide **4**, which indicates little if any intramolecular fluorescence quenching due to energy transfer in the bis-amide. All this suggests that FDCD might prove to be a sensitive method for determining the CD of these pigments.

A similar comparison of the solvent dependency of the CD $\Delta\varepsilon$ values of **2** shows little variation from nonpolar to polar or aprotic to protic. This behavior is rather different from the values for **1.** We suggest that the greater number of degrees of freedom between the chromophore and the diamine in **1** is responsible; whereas, in **2** and **3** the chromophores are constrained to lie along the stereogenic carbon–nitrogen axis designated in Figs. 1 and 5. Unlike the bis-amides with *p*-dimethylaminobenzoic acid, however, the chromophores are farther apart in **2**, with a concomitant weakening of the exciton interaction.

CONCLUSION

A new, highly fluorescent chromophore suitable for CD and potentially for FDCD has been prepared and used for exciton coupling CD of *trans*-1,2-diaminocyclohexane. The

bis-amides (**2**) follow the exciton chirality rule[8] but the CD $\Delta\varepsilon$ magnitudes are only ~1/4 as large as those from the bis-amides with *p*-dimethylaminobenzoic acid. However, the large ϕ_F values (0.3–0.7) might make the benzoic acid analogue of xanthoglow (Fig. 2) a very sensitive chromophore for FDCD studies.

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Synthesis and Hepatic Metabolism of Xanthobilirubinic Acid Regioisomers

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

Keywords. Pyrrole; Dipyrrinone; NMR.

Introduction

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

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Converting 9-methyldipyrrinones to 9-H and 9-CHO dipyrrinones

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Abstract—Yellow 9-methyldipyrrinones can be converted readily and in high yields to symmetric linear tetrapyrroles, blue biliverdinoids, which are cleaved in half, smoothly at room temperature to afford yellow 9-H dipyrrinones, and 9-CHO dipyrrinones as their violet to orange colored adducts with the carbon acid used for the scission: thiobarbituric acid (TBA), *N*,*N*′-diethylthiobarbituric acid, barbituric acid, *N*,*N*′-dimethylbarbituric acid, and Meldrum's acid. The adducts, usually only of passing interest, are formally Knövenagel condensation products of a 9-CHO dipyrrinone with TBA and other carbon acids of this work, and a reverse Knövenagel reaction of such adducts leads to 9-CHO dipyrrinones. Under a set of improved reaction conditions the sequence thus efficiently converts 9-CH_3 dipyrrinones to 9-H and 9-CHO dipyrrinones.

1. Introduction

Dipyrrinones[1] are the chromophores of bilirubin (Scheme 1), the yellow-orange pigment of mammalian bile and of jaundice, and they also constitute the two halves of biliverdin (Scheme 1), the blue-green biological precursor of bilirubin and the pigment of non-mammalian bile.[2] In both bilirubin and biliverdin, the dipyrrinone units are connected by a single carbon, C(10). Although these pigments are not biosynthesized in nature by conjoining two dipyrrinones, bilirubin and biliverdin analogs have been prepared synthetically by coupling two 9-H dipyrrinones with formaldehyde or its equivalent, or by coupling a 9-formyldipyrrinone with a 9-H dipyrrinone or even by oxidative coupling of 9-methyldipyrrinones.[1] The 9-H dipyrrinone precursors, as well as 9-formyldipyrrinones have been prepared by synthesis, typically from monopyrroles. 9-Methyldipyrrinones are likewise synthesized from monopyrroles, but direct conversion of these synthetically more accessible pigments to synthetically useful 9-H dipyrrinones has not been achieved.

In the mid 1920s Hans Fischer renewed his investigations of the constitutional structure of bilirubin and learned subsequently that bilirubins and biliverdins are cleaved to 9-H dipyrrinones in boiling resorcinol. Under brief reaction, bilirubin, its dimethyl ester and biliverdin dimethyl ester afforded only low yields of vinyl-neoxanthobilirubinic acid (Scheme 1) or its methyl ester—all with an *endo*-vinyl group.[3] The 'other half' of the tetrapyrrole, with the *exo*-vinyl group, was not recovered—an observation that led Fischer to first assume a symmetrically-substituted linear tetrapyrrole structure for bilirubin (which he subsequently disproved). In contrast, mesobilirubin cleaves to a good yield of a mixture of neo- and *iso*-neoxanthobilirubinic acids (Scheme 1) that proved difficult to separate at the time.

Some 50 years later, Manitto and Monti[4] demonstrated a novel, less vigorous, and high yield fragmentation of biliverdin and its symmetric analog, biliverdin-XIIIα dimethyl ester, a biliverdin analog with two *endo*-vinyl groups, to afford 9-H dipyrrinones from reaction with 1.5 equiv of thiobarbituric acid (TBA) (Scheme 2) in methyl acetate at room temperature. The green-blue color of the verdin changed gradually over 6 h to purple; and poorly-soluble, magenta-colored TBA adducts of dipyrrinones were precipitated from chloroform/hexane in ~80% yield. The pale yellow filtrates yielded 9-H dipyrrinones. The reaction has several advantages, the most significant are its simplicity and high yields, and the fact that the vinyl groups remain intact. Although unprecipitated TBA adducts render chromatographic separation of the 9-H dipyrrinones difficult, the cleavage reaction of biliverdin is probably the most convenient alternative to prepare not readily accessible 9-H dipyrrinones possessing vinyl groups, and it offers a convenient way to make other 9-H dipyrrinones,[5–7] given the verdin.

When a symmetrical verdin such as biliverdin-XIIIα dimethyl ester is treated with TBA, only one 9-H dipyrrinone product is possible: vinyl-neoxanthobilirubinic acid methyl ester. In this case, as with biliverdin, one half of the verdin is 'lost' to the TBA adduct, which might be viewed formally

Keywords: Pyrroles; Biliverdinoids; Retro-Knövenagel reaction; Carbon Acids.
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and **15** in $(CH_3)_2SO$ are yellow (somewhat deeper yellow than that found in a typical dipyrrinone), but **15** is a dark, red-orange solid and **14** is a yellow solid.

Anticipating an absorption band near 450 nm, we found it near 460 nm for **14** and near 430 nm for **15**. Strikingly, however, the ε value of this band in **14** exceeded 100,000, while that from **15** was close to 40,000 (Fig. 5). The 460 nm band of **14** is very sharp and very intense, e.g., **14a** in methanol ε=130,200 (456 nm). In contrast, **15a** in methanol has ε=44,000 (428 nm), an ε value that is still higher than from a typical dipyrrinone. The observed strong intensity of **14** is very much related to its narrow shape. In comparing the integrated intensities of **14** and **15**, and also those of the dipyrrinone adduct **4** and xanthobilirubinic acid methyl ester, one finds nearly identical values, with that of **14** being only ~10% higher. Thus, the observed difference in ε values due to TBA attachment to an α- versus a β-pyrrole position in **14** versus **15** is clarified; the dipole strengths are actually quite similar.

In contrast to the long wavelength band in **14** or **15**, which is shifted by ~100 nm from that of the dipyrrinone adducts of TBA, the shorter wavelength band lies (in **14**) at nearly the same wavelength (near 325 nm) but is shrunk considerably in intensity, down to ε ~6000. Apparently the intensity of the last is very much related to the presence of the dipyrrinone unit, as is the long wavelength band hypsochromic shift of ~100 nm.

3. Concluding comments

The current work elaborates on the mechanism and usefulness of the smooth cleavage of biliverdins by selected carbon acids to 9-H dipyrrinones and the carbon acid (Knövenagel) adducts of 9-CHO dipyrrinones. The reaction is improved by changes in solvent from the originally used ethyl acetate to methanol or DMSO and by choosing from among thiobarbituric acid (TBA), diethyl TBA, barbituric acid (BA), dimethyl BA, and Meldrum's acid—depending on the requirements of product isolation. For example, it is easy to isolate the adduct in high yield (>90%) when BA in CH_3OH is used to cleave the verdin, as the product precipitates almost entirely and in high purity (>95%) from the reaction mixture. From this carbon acid, the 9-H dipyrrinone may be isolated relatively easily and in high yield by chromatography of the mother liquor. The adduct, which shows tight intramolecular hydrogen bonding between the dipyrrinone pyrrole NH and a proximal C=O of the carbon acid, will undergo a retro-Knövenagel reaction, from which a 9-CHO dipyrrinone may be isolated in 26–66% yield.

4. Experimental section

4.1. General procedures

Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity Plus 500 MHz spectrometer in $CDCl_3$ solvent (unless otherwise specified) at 25 °C. Chemical shifts were reported in δ ppm referenced to the residual $CHCl_3$ ^1H signal at 7.26 ppm and ^{13}C signal at 77.0 ppm. A combination of heteronuclear multiple bond correlation (HMBC) spectra and $^1H\{^1H\}$ NOE data were used to assign ^1H and ^{13}C NMR spectra. UV–visible spectra were recorded on a Perkin–Elmer Lambda-12 spectrophotometer. Melting points were taken on a Mel Temp capillary apparatus and are corrected. 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 layers). Radial chromatography was carried out on Merck silica gel PF_{254} with gypsum preparative layer grade, using a Chromatotron (Harrison Research, Palo Alto, CA). Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Deuterated chloroform and dimethyl sulfoxide were from Cambridge Isotope Laboratories. (4*Z*)-3,8-Diethyl-2,7,9-trimethyl-10*H*-dipyrrin-1-one (kryptopyrromethenone)[8c,9] and etiobiliverdin-IVγ (**1**),[8] (4*Z*)-3-ethyl-8-(5-carbomethoxypentyl)-2,7,9-trimethyl-10*H*-dipyrrin-1-one,[8b] mesobiliverdin-XIIIα-8,12-dihexanoic acid dimethyl ester (**2**),[8b] 3,4-diethyl-5-methylpyrrole-2-carbaldehyde,[17] and 2,5-dimethylpyrrole-3-carbaldehyde[18] were prepared as described in the literature. Barbituric, 1,3-dimethylbarbituric, thiobarbituric, and 1,3-diethylthiobarbituric acids were from Aldrich and used as received, Meldrum's acid was synthesized according to a literature procedure,[28] and biliverdin-IXα dimethyl ester was obtained by esterification of bilirubin followed by oxidation.[2]

4.2. General procedure for biliverdin cleavage

To a solution of 249 mg (0.5 mmol) of etiobiliverdin-IVγ (**1**)[8] in 60 mL of methanol (obtained within 45–60 min) was added a solution of 1.2 mmol of carbon acid (thiobarbituric acid, TBA; diethyl TBA; barbituric acid, BA; dimethyl BA) in 60 mL of methanol, and the mixture was stirred at 25 °C for 1.5–2.5 h. Then the solvent was evaporated under vacuum (rotovap), and the residue was purified by radial chromatography. In reactions using TBA and diethyl TBA, radial chromatography was performed after filtration of the magenta-colored, partially insoluble adducts. The adduct from reaction of the verdin with BA was separated by filtration, and after evaporation the filtrate was chromatographed to yield 9-H dipyrrinones. Using the same stoichiometry, reaction conditions and work-up, verdin **2** and biliverdin dimethyl ester were reacted and worked up similarly.

4.2.1. 3,8-Diethyl-2,7-dimethyl-(10*H*)-dipyrrin-1-one (3). Neokryptopyrromethenone (**3**) was isolated (eluant CH_2Cl_2/AcOH/CH_3OH=100:2:1 to 100:2:3) in 76% yield following cleavage of etiobiliverdin-IVγ (**1**). It had mp 202–204 °C after crystallization from CH_3OH/CH_2Cl_2 (lit. mp 197 °C[11]); ^1H NMR ($(CD_3)_2SO$) δ: 1.08 (3H, t, J=7.6 Hz), 1.09 (3H, t, J=7.6 Hz), 1.77 (3H, s), 2.02 (3H, s), 2.33 (2H, q, J=7.6 Hz), 2.49 (2H, q, J=7.6 Hz), 5.95 (1H, s), 6.71 (1H, d, J=2.5 Hz), 9.69 (1H, s), 10.46 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 8.0, 9.0, 14.6, 14.8, 17.1, 18.0, 97.7, 118.8, 121.0, 123.4, 123.8, 125.3, 128.5, 147.3, 172.0 ppm; NMR data in $CDCl_3$ are in Table 1. Anal. Calcd for $C_{15}H_{20}N_2O$ (244.3): C, 73.73; H, 8.25; N, 11.47. Found: C, 73.45; H, 8.13; N, 11.64.

4.2.2. 3,8-Diethyl-2,7-dimethyl-9-(4′,6′-dioxo-2′-thioxo-(1′*H*,3′*H*,5′*H*)-pyrimidin-5′-ylidene)methyl-(10*H*)-dipyrrin-1-one (4). This pigment adduct was obtained (eluant