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The Absolute Configuration of Natural (−)-Stercobilin and Other Urobilinoid Compounds

(NMR/ORD/CD spectra/chromic acid degradation/chirality)

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ABSTRACT **Chromic acid degradation of natural (−)-stercobilin (1) yields 2(R)-methyl-3(R)-ethylsuccinimide (+2), whereby the absolute configuration of 1 at the chiral centers C-1, C-2, C-7, and C-8 is established. The substituted oxo-tetrahydrodipyrromethane precursor, 5, for the total synthesis of (−)-stercobilins 3 and 4, in which the relative configuration between the asymmetric centers is known, yields 2(S)-methyl-3(S)-ethylsuccinimide (−2) under the same conditions of degradation. Nuclear magnetic resonance studies of 1 and 3 show that in 1 the hydrogen atoms at C-2 and C-2′, as well as those at C-7 and C-7′, are *trans* relative to one another. Accordingly, natural (−)-stercobilin possesses the 2′(S), 7′(S) configuration, and has the configuration formula 6(1 (R), 2(R), 2′(S), 7′(S), 7(R), 8(R)). These results, coupled with those of earlier studies, also establish the absolute configuration of the (+)-urobilin 7 and of the phycobililn 8 at C-7′.**

The urobilinoid of human feces, natural (−)-stercobilin (1) (ref. 1), first obtained by Watson (2) in crystalline form, is optically active (3). Its constitutional formula 1 (4) contains six chiral centers. Only the relative configurations between pairs of these centers, e.g., the *trans* position of the beta substituents in the pyrrolidone rings, could be demonstrated by means of oxidative degradation from 1 to *threo*-2-methyl-3-ethylsuccinimide (5). In addition, Moscowitz *et al.* (6) concluded from the extraordinarily large amplitude of the 490-nm Cotton effect of 1 in nonpolar solvents that the carbon atoms 2′ and 7′ have the same configuration.

Recently it was possible to synthesize the (−)-stercobilins IXα 3 and 4 (7), in which the relative configurations of triads of chiral centers (C-1, C-2, C-2′, and C-7′, C-7, C-8) were unequivocally established on the basis of the precursors used in the syntheses. The high values of the optical rotation of both 3 and 4 further supported assignment of the same configurations of these compounds at C-2′ and C-7′. The thin-layer chromatographic (TLC) behavior, infrared spectra, and electronic spectra of 3 and 4 were indistinguishable from those of 1. However, only isomer 3 had the same Debye-Scherrer diagram as that of the natural product. It therefore appeared that 3 and 1 were identical, and that the relative configuration of natural (−)-stercobilin was thus established. However, the observation (C. J. Watson, unpublished data) that 3 did not yield the crystalline complex with iron (III) chloride characteristic of 1 (11, 12), but of no other urobilinoid thus far examined (12), caused us furthermore to compare the results of oxidative degradation of 5 and of natural (−)-stercobilin (1). Determination of the absolute configuration of 3 should be possible by oxidative degradation of the precursor 5 to 2-methyl-3-ethyl-succinimide (2), whose absolute configuration was established previously by Brockmann Jr. and coworkers (8–10).

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MATERIALS AND METHODS

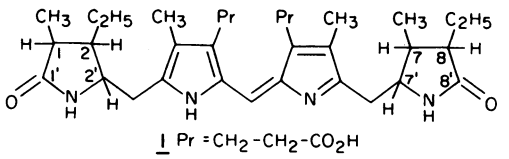
The NMR spectra were recorded with a Varian HA-100 nuclear magnetic resonance spectrometer, the ORD spectra with a Cary 60 recording spectropolarimeter, and the CD spectra with a Roussel–Jouan Dichrograph II.

Chromic acid oxidation of 5

26 mg of 5 ($[\alpha]_D^{20} = -105°$C in methanol) dissolved in 3 ml of tetrahydrofuran were added dropwise to a solution of 12 g of CrO_3 in 80 ml of 15% H_2SO_4. The mixture was stirred for 3 hr at 80°C. After cooling, this solution was diluted with 150 ml of water and extracted eight times with 100-ml portions of ether. The combined ether phase was shaken with 400 ml of a saturated solution of $NaHCO_3$ and dried over Na_2SO_4. The ether was than removed in a flash evaporator. The degradation mixture (11.5 mg) was separated by means of preparative TLC on a 20 × 20 cm plate (silica gel $HF_{254 + 366}$, Merck, layer thickness 0.8 mm, petroleum ether (40–60°C)–ethyl acetate–isopropyl alcohol 44:5:1). The zone visible in ultraviolet light was removed, and the degradation imide was eluted with 20 ml of ethyl acetate. [For analytical TLC, the zone was detected by the chlorine benzidine reaction (13).] After removal of the solvent, the yield was 3.3 mg (31%) of (−)-2(S)-methyl-3(S)-ethylsuccinimide, as a colorless oil.

Chromic acid oxidation of 1

25 mg of 1 in 5 ml of tetrahydrofuran were added dropwise to a solution of 15 g of CrO_3 in 100 ml of 15% H_2SO_4. The mixture was stirred for 3 hr at 80°C. The workup and chromatographic purification were as above. The yield was 4.8 mg (42%) of (+)-2(R)-methyl-3(R)-ethylsuccinimide, as a colorless oil.



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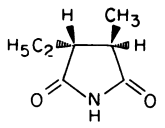

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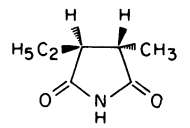

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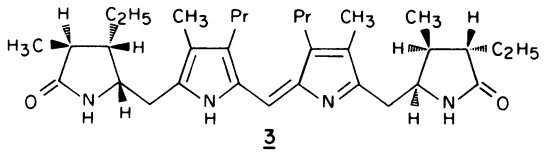

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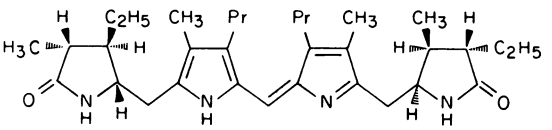

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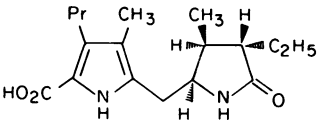

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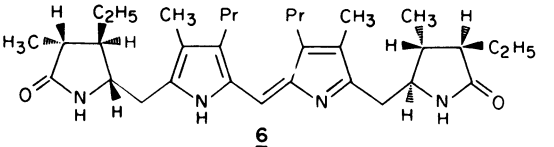

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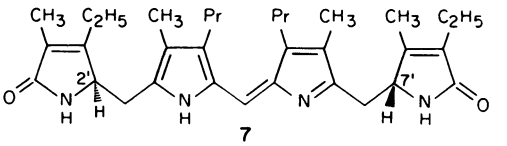

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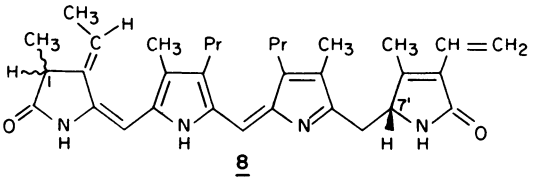

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NMR measurements

The proton NMR spectra of the hydrochlorides of **1** and **3** were determined on 0.05 M solutions in D_2O–[*U*-^{2}H]pyridine* 1:9. Through the exchange of all active hydrogen atoms with deuterium (D_2O), the spectra were somewhat simpler and, in addition, the formation of hydrogen bonding, which might perhaps have taken place in pyridine alone, was hindered by the addition of D_2O.

RESULTS AND DISCUSSION

In the neutral fraction of the chromic acid oxidation products of **5**, we found but one zone giving a positive color with the chlorine benzidine reaction (13) on TLC. The purified colorless oil from this zone had the following NMR spectrum: δ = 1.03 (*t*) 1.35 (*d*), 1.74 (*m*), 2.50 (*m*) ppm in $CDCl_3$. These data, together with the mass spectrum and the chromatographic behavior, characterized 2-methyl-3-ethylsuccinimide (**2**). The ORD and CD spectra (Fig. 1) revealed that it possessed a 2(S), 3(S)-configuration (−**2**). Hence, recalling the structure of the precursors (7), one concludes that the synthetic (−)-stercobilin **3** is 1(S), 2(S), 2′(S), 7′(S), 7(S), 8(S) in configuration. Correspondingly, isomer **4** has the configuration 1(R), 2(R), 2′(S), 7′(S), 7(S), and 8(S).

It was surprising that the chromic acid oxidation of natural (−)-stercobilin (**1**) yielded 2(R)-methyl-3(R)-ethylsuccinimide (+**2**) in 42% yield. [Gray and Nicholson (14) mentioned that the mixture of imides obtained by degradation of **1** was weakly dextrorotatory.] Thus, it is clear that **1**, despite a Debye-Scherrer diagram indistinguishable from that of **3**, is not identical with **3**. Further, the high optical purity of +**2** obtained shows that **1** has the (R) configuration at C-1, C-2, C-7, and C-8. The similar values for optical rotation and the almost superimposable ORD spectra of **1** and **3** lead one to expect that **1**, like **3**, possesses the (S) configuration at C-2′ and C-7′, hence, that the hydrogens at C-2 and C-2′, as as well as C-7 and C-7′, are *trans* relative to one another, and, hence, that natural (−)-stercobilin has the configuration formula **6**. This expectation was confirmed by measurement of the NMR spectra of **1** and **3**.

Discussion of the NMR spectra

As might be anticipated, the NMR spectra are quite complicated, because of the unsymmetrical constitution of **1** and **3**. Thus, for example, only unresolved multiplets were observed for the resonances of the protons at C-2′ and C-7′, as well as those of the methylene bridges. Despite this, the spectra of **1** and **3** had distinguishing characteristics. For example, the 1- and 7-CH_3 groups of **3** exhibited the same chemical shift, while those in the spectrum of **1** evoked two signals separated from one another by 0.36 ppm. Correspondingly, the ethyl CH_2 protons of **3** absorbed in a narrow region, while in contrast the analogous resonances in the spectrum of **1** appeared as two multiplets, with centers of gravity of 1.4 and 1.7 ppm.

Because of the complexity of our spectra, the coupling constants $J_{2,2'}$ and $J_{7,7'}$ could not be inferred. Moreover, they appeared to be little suited to the determination of configuration of five-membered heterocycles (15, 16). However, the relative configurations of the chiral centers at 2 and 2′ and 7 and 7′ could be determined from comparison of the chemical shifts of hydrogen atoms and alkyl groups at these centers (7, 15–18). Extensive studies of 2,3-dialkyl-substituted succinic acid anhydrides and imides have shown that the alkyl groups of *cis*-substituted five-membered rings shield one another (Brockmann, H., Jr., and J. Bode, unpublished data). Consequently, alkyl hydrogen signals are produced at higher field strengths than with the isomeric *trans* compounds. On the other hand, in the spectra of the *trans* diastereomers, the resonances of the ring methine hydrogen atoms are displaced to higher field strengths by the alkyl

* This designation is equivalent to the also-used pyridine-d_5.

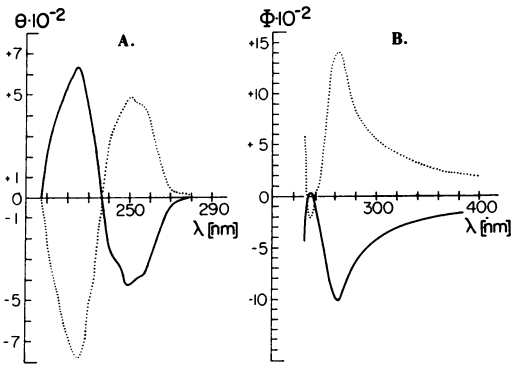

FIG. 1. CD-(*A*) and ORD-spectra (*B*) of +2 from 1(6) (· · · · ·) and of −2 from 5 (——).

groups on the same side of the ring plane. On this account, differences of 0.3–0.5 ppm for the position of the methine protons and of 0.08–0.15 ppm for the signals of the α-alkyl-hydrogens are found (Brockmann, H., Jr., and J. Bode, unpublished data). The chemical shifts of the 2′- and 7′-hydrogens in the NMR spectra of **1** and **3**, as well as those of the methylene bridge protons, are comprehensible only when **1** has the 2,2′-*trans* and the 7,7′-*trans* configurations. In **3**, the hydrogens at C-2 and C-2′, as well as C-7 and C-7′, as already postulated (7) on the basis of NMR spectra of the synthetic precursor **5**, are *cis* in relation to one another.

One would naturally anticipate the same effects for the signals of the methine protons and alkyl groups at C-2 and C-7. In the spectra, however, only the methyl doublets and ethyl triplets are to be located with reasonable certainty. Since it cannot be decided *a priori* which doublets are related to the C-1 and C-7 methyl groups (and the same is true for the triplets of the C-2 and C-8 ethyl groups) these signals cannot be employed in the determination of configuration. Nevertheless, if one accepts the assignments of the signals given in Table 1, which are in accord with the above discussion, one notices that the alkyl groups at C-2 and C-7 absorb at different field strengths in **1** and **3**. This is true in even greater degree for the alkyl groups at C-1 and C-8. This finding may be related to the fact that, as considerations of CPK† models show, only compound **3** can assume conformations in which the C-1 and C-8 alkyl groups prove to be in the unshielded region of 8′- or 1′-lactamcarbonyl groups.

The absolute configurations of other urobilinoids

An important result of the findings just described is that the chiroptical behavior of the stercobilins is dependent to a large degree on the chirality at C-2′ and C-7′. Even the complete reversal of configuration at carbon atoms 1, 2, 7, and 8 has no significant influence on the ORD and CD spectra, and as the identical Debye-Scherrer spectra of **1** and **3** show, does not influence the crystal powder pattern. This appears to be in agreement with a model developed previously (6), according to which the chiral sense of the inherently dissymmetric dipyrromethene chromophore is responsible for the large amplitude of the 490-nm Cotton effect of stercobilins in non-polar solvents, and this chiral sense is determined principally through the chirality of the carbon atoms 2′ and 7′.

† CPK, Corey-Pauling space-filling models with Koltun connectors.

TABLE 1. *NMR spectra of natural* (−)-*stercobilin* (**1**) *and synthetic* (−)-*stercobilin* (**3**) *in* [*U*-^2H]*pyridine*-D_2O

Protons	1 (6)	3	Relative intensity	Multiplicity
Methine	7.70*	7.68	1	Singlet
2′-H, 7′-H	3.76	4.20	2	Multiplet
Bridge-CH_2	3.15	3.04	4	Multiplet
Propionic acid-β-CH_2	3.15	3.12	4	Double triplet
Propionic acid-α-CH_2	2.82	2.82	4	Double triplet
3-CH_3, 6-CH_3	2.10	2.15	6	Singlet
1-H, 2-H, 7-H, 8-H	1.9-2.4	2.1-2.6	4	Multiplet
Ethyl CH_2	1.2-1.9	1.5-1.8	4	Multiplet
7-CH_3†	1.28	1.21	3	Doublet
1-CH_3†	0.92	1.21	3	Doublet
2-Ethyl-CH_3†	1.02	0.96	2	Triplet
8-Ethyl-CH_3†	0.86	1.08	2	Triplet

* The chemical shifts are given in δ[ppm], with tetramethylsilane as internal standard.

† Although not certain, assignment of doublets and triplets is in best accord with the interpretation given in the text.

Since (+)-urobilin (**7**) from feces (18) has a visible absorption spectrum very similar to that of (−)-stercobilin, while the ORD spectra of the two compounds are mirror image-like, it was concluded that **1** and **7** had opposite configurations at C-2′ and C-7′ (6). Recently (+) and (−)-urobilin IXα have been synthesized (19). As expected, both compounds show mirror-image ORD curves, and those of (−)-urobilin and (−)-stercobilin are very similar. Therefore, from our results, (+)-urobilin (**7**) must possess the 2′(R), 7′(R)-configuration.

The 7′ configuration of the phycobilin **8** has previousyl been identified with that of **7** by chemical and ORD-spectroscopic investigation (20, 21). It follows, therefore, that **8** has the 7′(R) configuration.

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