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THE STEREOSPECIFIC SYNTHESIS OF CIS-CIS-STERCOBILINOGEN AND CIS-CIS STERCOBILIN*

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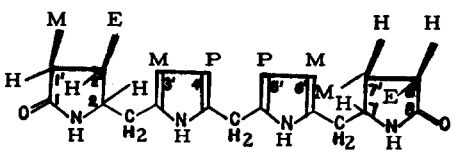
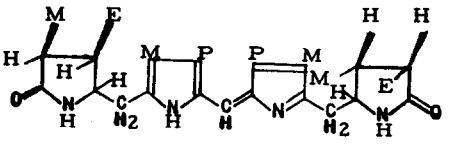
The methyl and ethyl substituents on a 2-pyrrolidone ring can be arranged in a cis or trans configuration. For cis substituted end rings in stercobilin or stercobilinogen IXα two pairs of stereoisomers are possible for a given configuration at the asymmetrical centers 2 and 7 (Fig. I).

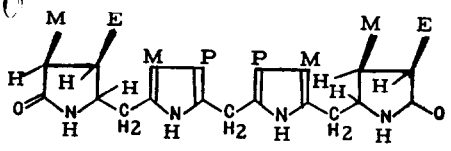
FIG. I



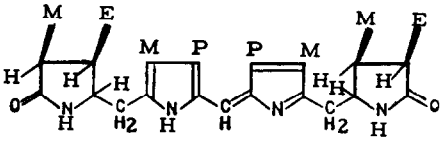
anti cis-cis stercobilinogen



anti cis-cis stercobilin



syn cis-cis stercobilinogen



syn cis-cis stercobilin

M = CH_3
E = $CH_2\ CH_3$
P = $CH_2\ CH_2\ COOH$

Gray and co-workers (1) have reported that natural stercobilin has a predominantly trans-trans configuration, synthetic stercobilin cis-cis. We have followed their nomenclature in the present paper.

Plieninger and Lerch (2) noted that catalytic hydrogenation with 5% palladium on charcoal in glacial acetic acid gave stereospecific cis addition for Δ^3-pyrrolin-2-ones, and thus we anticipated that stercobilinogen, which had previously been obtained by this method of catalytic hydro-

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genation of bilirubin (3), would be cis-cis, as turned out to be the case (see below). In the present work, we have in addition crystallized synthetic stercobilinogen for the first time. The crystals are tabular of monoclinic or triclinic symmetry, m.p. 119-122° C (uncorr.). Dissolved in $CHCl_3$ the substance shows no absorption from 350 to 900 mμ (Beckman DK spectrophotometer). The infrared spectrum was characteristic of a leuco- compound, lacking C = C stretch at 1610 cm^{-1} and the amino-HCl stretching band at 1515 cm^{-1} (examined in KBr pellet in a Perkin Elmer 21 infrared spectrophotometer). The x-ray crystal powder pattern was easily distinguishable from that of mesobilirubinogen or of other members of the urobilin group. The crystals exhibit an intense Ehrlich aldehyde reaction (4), $\varepsilon 560 = 2.05 \times 10^2$.

Chromic acid oxidation of bile pigments produces substituted imides (5); those for stercobilin IXα are represented by cis or trans methylethylsuccinimides (Fig. 2) (1) which can be identified by thin layer chromatography (6) and gas chromatography.

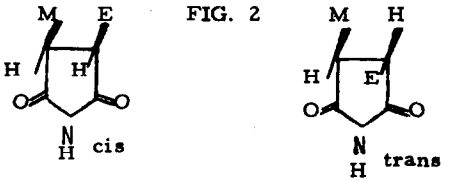

FIG. 2

The imides which were identified establish the configuration of the methyl and ethyl substituents on each end ring (but not the syn or anti configuration between rings I and IV). In the present study a 6 ft. gas chromatographic column of 3% Versamid 900 on Anakrom ABS 80-90 mesh was used in a Beckman GC-2A with a flame ionization detector. The temperature was 175° C, with helium as carrier (30 p.s.i.). Pure synthetic cis- and trans-methylethylsuccinimides on gas chromatography (GC) have retention times of 13 and 9 minutes, respectively. Synthetic stercobilinogen on CrO_3 oxidation yielded mainly cis-methylethylsuccinimide (Fig. 2) with 7-13% of trans-isomer in two runs, determined from the integrated areas from gas chromatography curves. This in part is derived from epimerization of the cis- to the more stable trans-isomer (1) at the elevated temperatures of the procedure, as was proven by lack of trans-ethylmethylsuccinimide on thin layer chromatography (TLC).

Catalytic oxidation of cis-cis stercobilinogen, using the same Pd/C with oxygen yielded stercobilin which on repeated recrystallization (6x) from methanol-ethyl acetate provided a

stercobilin hydrochloride of crystal habitus and melting point not previously observed. The crystals are tabular rhomboids melting at 215° C, as contrasted with 140° C for natural stercobilin. CrO_3 oxidation of these crystals yielded 95-96% of cis-methylethylsuccinimide. Synthetic stercobilin prepared by the method of Kay et al (3), without preliminary crystallization of stercobilinogen, has been found to yield 73-78% cis, 22-27% trans-succinimide isomers (1). The present crystalline stercobilin is effectively homogeneous cis-cis (syn or anti configuration is not determined). On CrO_3 oxidation it yields less than 5% trans-isomer, the same percentage of trans-isomer shown by chromatographically (TLC) (6) pure cis-ethylmethylsuccinimide run on GC. In confirmation of the results of Gray et al (1), natural crystalline stercobilin was found to yield only the trans- succinimide. Elementary analysis was not carried out on crystalline stercobilinogen because of its instability and rapid conversion to stercobilin. Analysis of crystalline cis-cis stercobilin prepared from stercobilinogen was as follows:

	Calc., %	Found, %
C	62.79	62.83
H	7.51	7.38
N	8.88	8.94

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