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# SYNTHESIS AND CHARACTERIZATION OF COPROBILIVERDIN III, A NEW MODEL CHROMOPHORE

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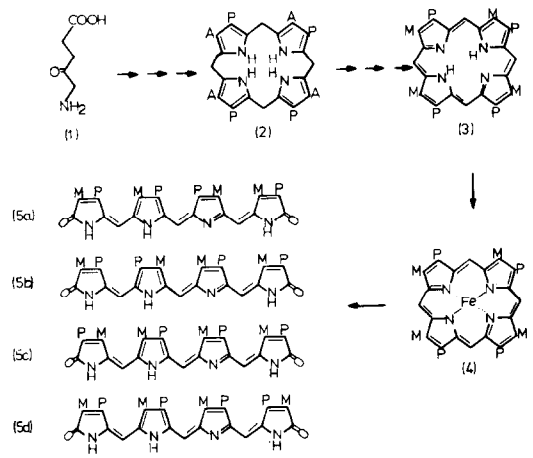
ABSTRACT: The synthesis of coprobiliverdin III, a new bile pigment with four conjugated pyrrole nuclei and four carboxylic acid side chains, is described. Coprobiliverdin is structurally characterized by chromic acid degradation, mass spectroscopy, UV vis and NMR spectroscopy.

Cyanobacteria, rhodophyta and cryptophyta contain special light-harvesting pigments, the phycocyanins and phycoerythrins which have been identified as biliproteins with thioether-linked bile pigment chromophores (1-5). For further structural investigations on this type of chromophore and its biosynthesis, model compounds are needed. Their synthesis should be convenient and include optional isotope-labeling, e.g. for NMR-experiments.

The synthesis started with 5-aminolevulinic acid (fig. 1, (1)) (6)(optionally isotope-labeled) which was incubated with a crude enzyme extract from Rhodopseudomonas spheroides, mutant R-26 (7). The resulting porphyrins were analyzed with the aid of high performance liquid chromatography (HPLC)(8). As main product coproporphyrin III (3) was identified besides coproporphyrin I and minute amounts of other porphyrins. As next step, iron was introduced according to the method of Warburg and Negelein (see 9).

For the subsequent chemical ring opening, the coprohemin (4) was subjected to a coupled oxidation with $O_2$/ascorbate (10) yielding coproverdohemochrome. The final ring cleavage was carried out by treatment with methanolic potassium hydroxide. After iron removal and esterification with methanolic boron trifluoride, a blue bile pigment, coprobiliverdin-III-tetramethyl ester (4 isomers)(5a-5d) resulted. The final separation and purification was carried out by liquid chromatography on a silica gel 60 column.

fig. 1
Synthetic route to coprobiliverdin III. -M = methyl side chain, -A = acetic acid side chain, -P = propionic acid side chain (2) (uroporphyrinogen) respective methyl ester thereof (3),(4),(5).




CHARACTERIZATION: Chromic acid degradation (11) yielded hematinic acid methyl ester as the only reacting product (detectable by thin layer chromatography (11)), proving the presence of one methyl- and one propionic acid methyl ester side chain at each of the four unsaturated rings, thus confirming structure (5a-5d).

A further structural confirmation arises from the mass spectrum which exhibits a mass peak at m/e = 730 (3.5 %). Two main fragments are found at m/e = 374 (10.4 %) and m/e = 360 (100 %). The fragments stem from a fragmentation at the middle methine bridge which is probably first reduced by disproportionation in the mass spectrometer. The methylene bridge is than fragmented (12).

The UV vis absorption spectrum of the new compound ($\lambda_{max}$: 368, 639 ($CHCl_3$) and $\lambda_{max}$: 357, 684 ($CH_3OH$/0.15 % HCl)) resembles very much that of mesobiliverdin dimethyl ester ($\lambda_{max}$: 379, 656-664 ($CHCl_3$)(14) and $\lambda_{max}$: 375, 695 ($CH_3OH$/5 % HCl)(15)), both as well with respect to the position of the long-wave absorption maxima as the intensity ratio of red/UV-transition. From this observation we conclude that the methyl- and propionic acid methyl ester side chains do not influence the $\pi$-electron system of the tetrapyrrole chromophore neither by electronic interaction nor by means of steric hindrance.

In the $^1H$ NMR spectrum, the resonance at -2,15 ppm is due to the methyl side chains. The signals of the propionic acid methyl ester side chains appear at -2.6 ppm (methylene-hydrogens) and -3.7 ppm (methyl-hydrogens). The chemical shift of the methin-hydrogens at C-5 and C-15 is -5.9 ppm, that at the C-10-hydrogen is -6.7 ppm.

The compound coprobiliverdin has to be regarded as a reference for the future study of natural bile pigments. It does not seem unlikely that the compound itself may turn out to be naturally occurring.

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REFERENCES:

1. Köst-Reyes, E., Köst, H.-P. and Rüdiger, W. (1975). Liebigs Ann. Chem. 1975, 1594.
2. Troxler, R.F., Kelly, P. and Brown, S.B. (1978). Biochem. J. 172, 569.
3. Köst-Reyes, E. and Köst, H.-P. (1979). Eur. J. Biochem. 102, 83.
4. Glazer, A.N., Hixson, C.S. and De Lange, R.J. (1979). Anal. Biochem. 92, 489.
5. Brown, A.S., Offner, G.D., Erhardt, M.M. and Troxler, R.F. (1979). J. Biol. Chem. 254, 7803.
6. Shemin, D. (1957). in "Methods in Enzymology", p. 648, Vol. 4, S.P. Colowick and N.O. Kaplan, eds., Academic Press, New York.
7. Details may be obtained from the authors.
8. Kushner, J.P. et al., in preparation.
9. Fuhrhop, J.-H. and Smith, K.M. (1975). in "Porphyrins and Metalloporphyrins", p. 804, K.M. Smith, eds., Elsevier Scientific Publishing Company, Amsterdam.
10. Bonnett, R. and McDonagh, A.F. (1973). J. Chem. Soc., Perkin Trans, I, 881.
11. Rüdiger, W. (1969). Hoppe-Seylers's Z. Physiol. Chem. 350,1291.
12. Rüdiger, W. (1971). Fortschr. Chem. Naturst., p. 61-139, W. Herz, H. Grisebach and G.W. Kriby, eds., Springer-Verlag, Wien, New York.
13. Stoll, M.S. and Gray, C.H. (1977). Biochem. J. 163, 59.
14. Bonnett, R. and McDonagh, A.F. (1970). Chem. Commun., 238.
15. Cole, W.J., Chapman, D.J. and Siegelman, H.W. (1968). Biochemistry 7, 2929.

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