

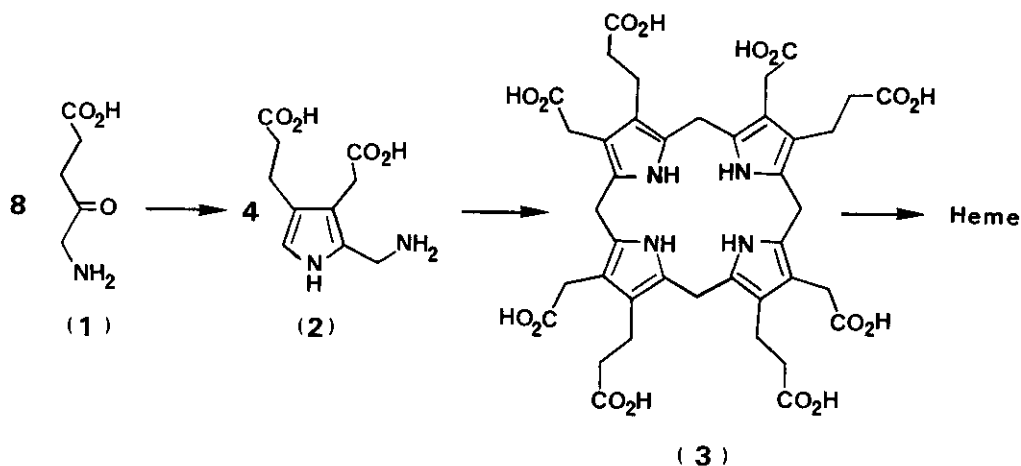
CONDENSATION PRODUCTS OF THE PORPHYRIN PRECURSOR
5-AMINOLEVULINIC ACID

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Abstract — Condensation of the biogenetic porphyrin precursor 5-aminolevulinic acid (1) in alkaline solution yields besides some porphobilinogen (2) a dihydropyrazine (6) as the predominant product, which was isolated and characterized after dehydrogenation to the stable pyrazine (7a). This ends a longstanding uncertainty and reveals that the azomethine reaction, as can be expected for α -aminoketones, is strongly preferred by 5-amino-levulinic acid (1) under nonenzymatic conditions. A nor-porphobilinogen (9) was formed by condensation of a protected aminoacetoacetic ester with (1).

5-Aminolevulinic acid (1) is the biogenetic precursor of the natural porphyrins and corrins^{1,2}. In this biosynthesis eight molecules of (1) condensate in pairs to form four molecules of the monopyrrole porphobilinogen (2), which then cyclo-tetramerize to give heme and other essential tetrapyrroles via uroporphyrinogen III (3). The elegance of this converging biosynthetic scheme stimulates its imitation in chemical procedures. 5-Aminolevulinic acid (1) and porphobilinogen (2), being key building blocks of the porphyrin biosynthesis provide promising possibilities for such biomimetic syntheses⁶. Recently we had reported on effective, few-step biomimetic porphyrin syntheses starting from derivatives of porphobilinogen (2)^{3,4}. Investigations on the condensation of 5-aminolevulinic acid (1), available by several excellent procedures⁵, will now be described.

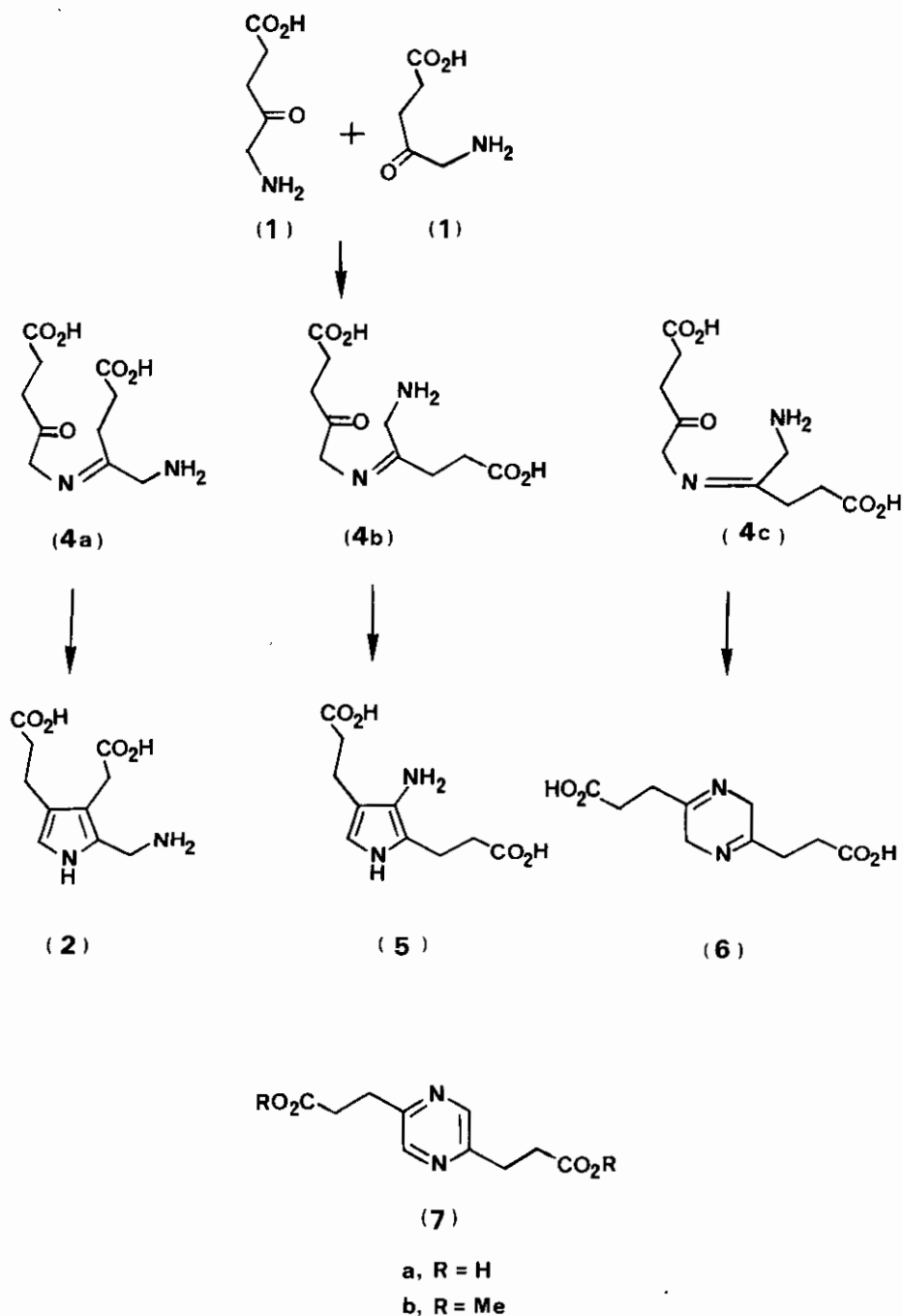
As had already been observed by J.J. Scott⁷, neutralized solutions of 5-amino-levulinic acid (1) develop a positive Ehrlich reaction after some time. After anaerobic treatment of (1) with aqueous alkali at 18°C for several days a small



amount of an Ehrlich positive substance was isolated with 3 % yield and identified as porphobilinogen (2). However the structure of the main product of this reaction remained uncertain and an object of discussion. An information was given by A.I. Scott⁸ that in his laboratory the isomer (5) of porphobilinogen (2) is the predominant one formed nonenzymatically from 5-aminolevulinic acid (1) dissolved in alkaline solution. The formation of (5) would differ from that of (2) by proceeding via the Z-form (4b) of the intermediary Schiff base (4), and not via the E-form (4a) as for porphobilinogen (2). As a third condensation, known for other α -aminoketones, a twofold azomethine reaction to give the dihydropyrazine (6) via (4c) had to be considered.

We investigated the condensation of 5-aminolevulinic acid (1) in aqueous NaOH, NaHCO₃, NH₄Cl, and HCl under variation of reaction time and temperature and chromatographic analysis (n-butanol/acetic acid/water = 4 : 1 : 5, on paper Schleicher and Schüll 2043 b)⁶. It turned out that only in alkaline solutions two condensation products are formed. The ratio of about 1 : 10 is largely independent from the reaction conditions. 5 N NaOH during eight hours at 60°C was most favourable. The minor compound, moving slower in the PC ($R_f = 1.00$) and showing a redviolet Ehrlich colour, was identified as porphobilinogen (2). The predominant product with $R_f = 1.25$ and bluegray Ehrlich colour proved to be instable and difficult to isolate. Methylation gave mixtures of products. The mass spectrum of a methylation product separated from the reaction mixture, obtained with CH₃OH/HCl during 24 hours at 20°C, revealed the structure of a pyrazine dicarboxylic acid

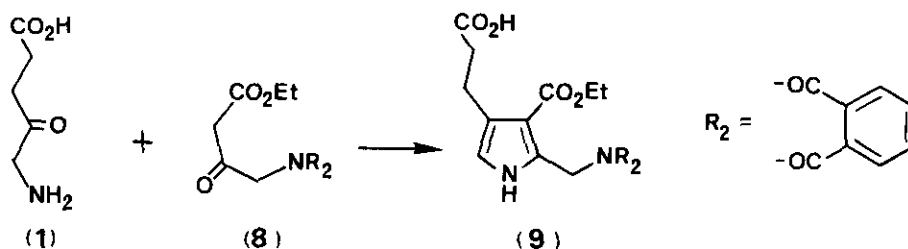
dimethylester (7b) obviously formed by dehydrogenation and methylation of a tautomeric mixture of the dihydropyrazine (6). Accordingly it shows a molecular



ion at m/e 252 and two intensive fragment ions 221 and 193, derived from it by loss of OCH_3 and CO_2CH_3 , while analogous ions of the pyrrol (5)⁸ should appear two mass units higher. In order to confirm the assumption that the unstable dihydropyrazine (6) is the main condensation product of 5-aminolevulinic acid (1), its direct isolation, was achieved by a condensation of 167 mg (1 mmol) (1) in 5 N KOH under addition of 600 mg (2.2 mmol) HgCl_2 . Afterwards the filtrate was neutralised, and on concentration 30 mg (27 %) pyrazinedipropionic acid (7a) separated in colourless prisms, mp 219-221°C; $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_4$, IR (KBr): 1710 (Carboxyl-CO), 1490, 1420 (pyrazine ring stretching), 820 cm^{-1} (pyrazine out of plane); MS: m/e = 224 (37 %, M^+), 179 (100 %, $\text{M} - \text{CO}_2\text{H}$), 133 (51 %, $\text{M} - 2 \text{CO}_2\text{H} - \text{H}$), 119 (12 %, 133 - CH_2).

As it has thus been shown that the pyrazine derivative (6) is the predominant condensation product of 5-aminolevulinic acid (1), its azomethine condensation appears to be strongly preferred in comparison to the aldol reaction. The enzymatic condensation of (1) on the other hand gives porphobilinogen (2) by aldol and azomethine reactions in high yield. This is explained¹ by binding one molecule 5-aminolevulinic acid (1) to the enzyme as a Schiff base, and subsequent aldol condensation with the second 5-aminolevulinic acid molecule.

It should be possible to favour the pyrrole ring closure in the chemical condensation by changing the reactivity of one of the 5-aminolevulinic acid molecules in the following way. Protection of the aminogroup against Schiff base formation by reversible acylation, and activation of the methylene group for aldol reaction. Such a derivative of (1) would be the phthalimidoacetoacetic ester (8). As expected, (8) reacted with 5-aminolevulinic acid under mild conditions, similar to those of the Knorr condensation to form pyrrol (9), which is a N-protected nor-porphobilinogen, mp 196°C, $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}_6$; IR (KBr): 3580 (Pyrrol-NH), 1725 cm^{-1} (Carboxyl-CO); MS: m/e = 370 (39 %, M^+), 324 (42 %, $\text{M} - \text{C}_2\text{H}_5\text{OH}$), 297 (62 %, $\text{M} - \text{CO}_2\text{C}_2\text{H}_5$), 251 (21 %, $\text{M} - \text{CO}_2\text{C}_2\text{H}_5 - \text{CO}_2\text{H} - \text{H}$), 160 (100 %, Phthalimidomethyl).



ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We thank Mr. Manfred Küpper for his subtle mass-spectrometric measurements.

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Received, 2nd September, 1980