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 dehydro-
genation to the stable pyrazine (7a). This ends a longstandig
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-por-
phobilinogen (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 syntheses[6]. 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 procedures[5], will now be described.

As had already been observed by J.J. Scott[7], 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
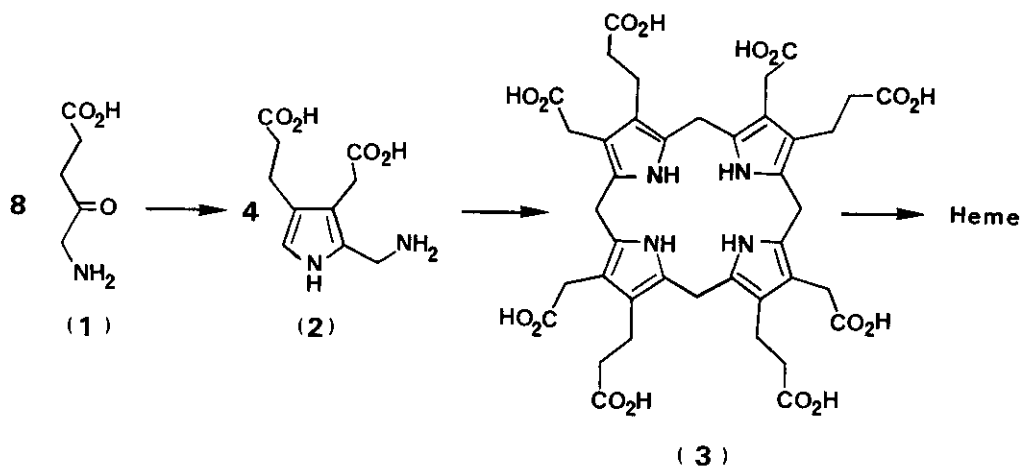
amount of an Ehrlich positive substance was isolated with 3 % yield and identified
as porphobilinogen (2). However the structure of the main product of this reaction
remained uncertain and an object of discussion. An information was given by
A.I. Scott[8] that in his laboratory the isomer (5) of porphobilinogen (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 (?) 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,
$NaHCO_3$, NH_4Cl, 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 $60^{o}C$ was most
favourable. The minor compound, moving slower in the PC (R = 1.00) and showing a
redviolet Ehrlich colour, was identified as porphobilinogen (2). The predominant
product with R_s = 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 CH_3OH/HCl
during 24 hours at $20^{o}C$, revealed the structure of a pyrazine dicarboxylic acid

dimethylester (7b) obviuosly formed by dehydrogenation and methylation of a
tautomeric mixture of the dihydropyrazine (6). Accordingly it shows a molecular
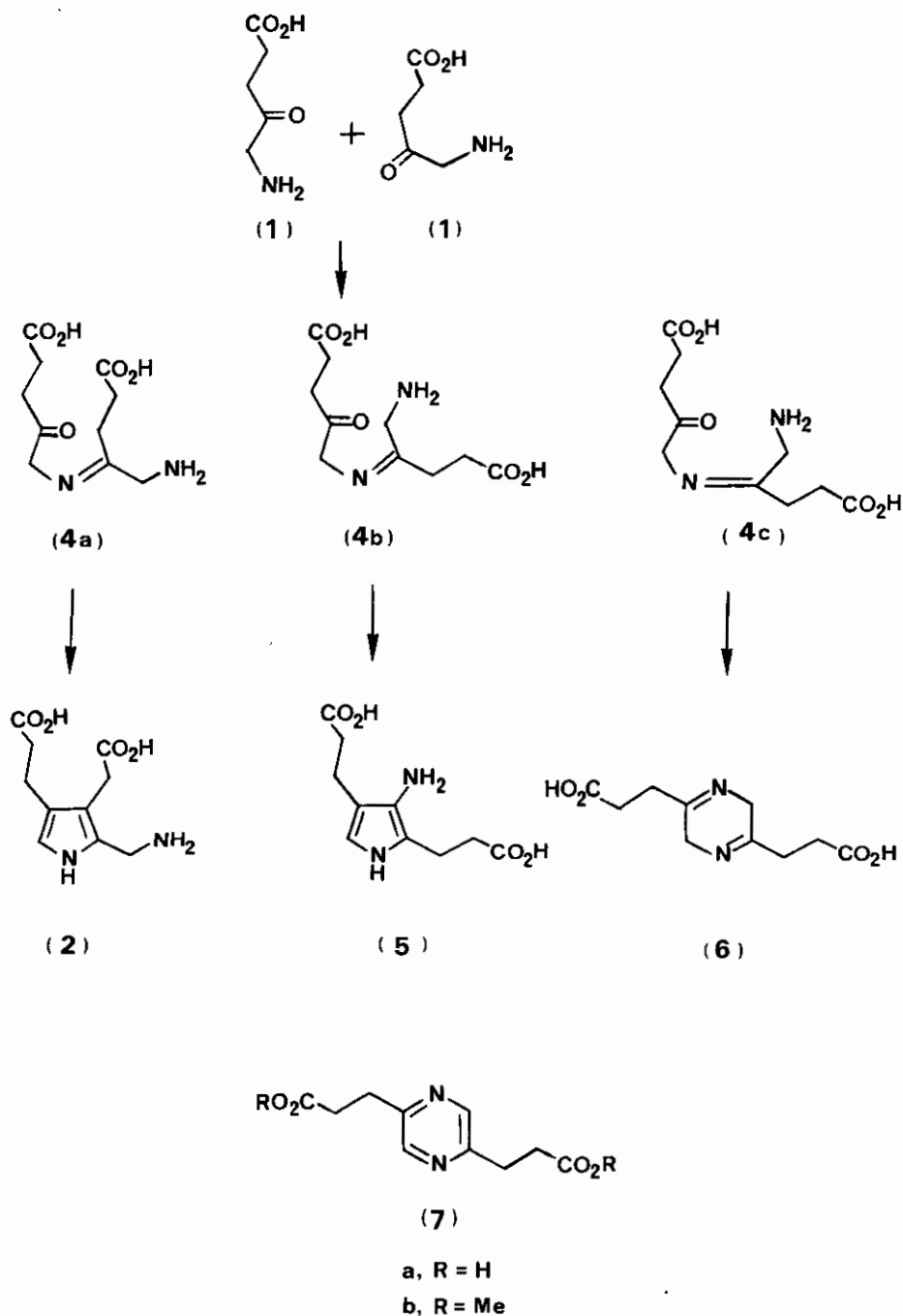
ion at m/e 252 and two intensive fragment ions 221 and 193, derived from it by loss of OCH_3 and CO_2CH_3, while analogous ions of the pyrrol (5)[8] should appear two mass units higher. In order to confirm the assumption that the unstable dihydropyrazine (6) is the main condensation product of 5-aminolevulinic acid (1), its direct iso- lation, 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; $C_{10}H_{12}N_2O_4$, IR (KBr): 1710 (Carboxyl-CO), 1490, 1420 (pyrazine ring streching), 820 cm^{-1} (pyrazine out of plane); MS: m/e = 224 (37 %, M^+), 179 (100 %, $M-CO_2H$), 133 (51 %, $M - 2 CO_2H - H$), 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 explained[1] 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 conden- sation 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, $C_{19}H_{18}N_2O_6$; IR (KBr): 3580 (Pyrrol-NH), 1725 cm^{-1} (Carboxyl-CO); MS: m/e = 370 (39 %, M^+), 324 (42 %, $M - C_2H_5OH$), 297 (62 %, $M - CO_2C_2H_5$), 251 (21 %, $M - CO_2C_2H_5 - CO_2H - H$), 160 (100 %, Phthalimidomethyl).
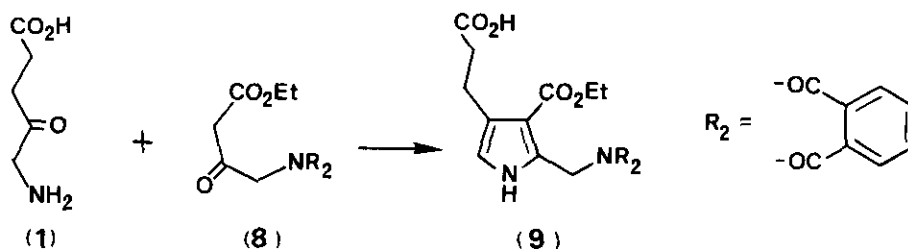
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6. According to Breslow's definition, Q. Rev., Chem. Soc. 1, 553 (1972), biomimetic Syntheses must not correspond exactly to the conditions of biosynthesis: 'Biomimetic chemistry is the branch of organic chemistry, which attempts to imitate natural reactions and enzymatic processes as a way to improve the power of organic chemistry'.

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