A SIMPLE CHEMICAL MODEL OF THE BIOSYNTHESIS OF PORPHOBILINOGEN

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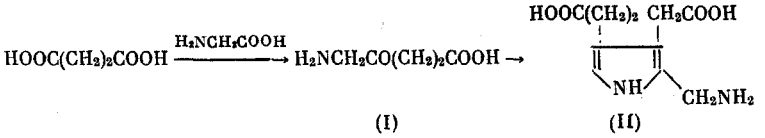
According to [1, 2], the biosynthesis of hem and vitamin B_{12} includes in the initial stages the C-acylation of glycine with succinic acid by the Dakin–West reaction, the autocondensation of δ-aminolevulinic acid (I) to the monopyrrole compound, namely porphobilinogen (II), and the subsequent transformation of (II) to porphyrins

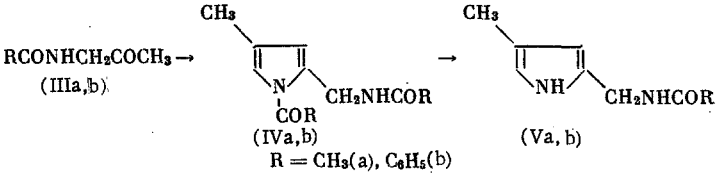


(I) (II)

The synthesis of (I) via the Dakin–West reaction can also be accomplished in a purely chemical manner, if hippuric acid is treated with the acid chloride of succinic acid monoester and the intermediate azlactone derivative is hydrolyzed with hydrochloric acid [3]. This method makes it possible to smoothly obtain the (I) hydrochloride in 51% yield. The second step in the biosynthesis of porphyrins, namely the conversion of (I) to (II), lends itself to chemical modeling with much greater difficulty: (I) is converted to (II) in only 3% yield under the influence of alkaline agents [4], while N-benzoyl-δ-aminolevulinic acid and other N-acyl-α-amino ketones react in a different direction, with the involvement of the more active methylene group to form 3-acylaminopyrroles [5].

In order to find routes for going from (I) to (II) we studied in the present paper the conditions for the autocondensation of the simpler N-acyl-α-amino ketones, namely N-acetyl- and N-benzoylaminoacetone (IIIa, b), to 2-(N-acylaminomethyl)pyrroles (IVa, b), which are related to (II).

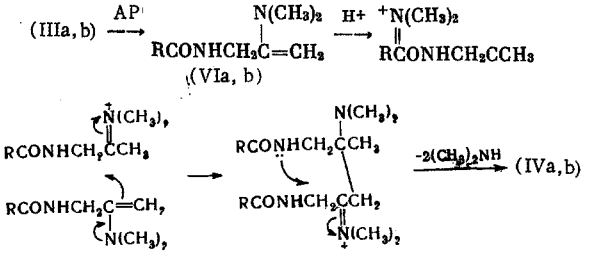
We were the first to establish that this reaction can be run to give the product in 40-60% yield if as condensing agents are used $P[N(CH_3)_2]_3$ (AP) in the presence of $(CH_3)_2NH \cdot HCl$ (DA), or an α-amino acid, in either refluxing benzene or xylene as the medium



(IIIa,b) (IVa,b) (Va, b)

R = CH_3(a), C_6H_5(b)

The conversion of (IIIa, b) to (IVa, b) probably proceeds via the intermediate step of forming enamines (IVa, b) and their autocondensation by the scheme:



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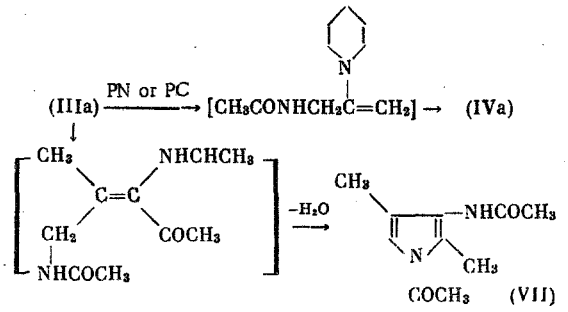
The autocondensation of (IIIa) to (IVa) proceeds less smoothly when piperidine (PN) or cyclohexanone piperidinenamine (PC) are used as the condensing agents, and at times is accompanied by crotonic condensation with the formation of the isomeric 1-acetyl-2,4-dimethyl-3-acetamidopyrrole (VII). The structure of (IVa, b) was proved by the Ehrlich tests, the elemental analyses, the alkaline hydrolysis of (IVa, b) to (Va, b), and also the UV, IR, and NMR spectra



N,N-Diacetylaminoacetone (VIII), which is formed by the Dakin–West reaction of glycine with Ac_2O [6], is also converted to (IVa) (with the cleavage of one acetyl group) under the influence of PN. The position adopted by us for the two acetyl groups on the nitrogen atom of (VIII) is in agreement with the data of the IR and NMR spectra

$$(CH_3CO)_2NCH_2COCH_3 \rightarrow \text{(IVa)}$$
(VIII)

The conversion of (IIIa, b) to (IVa, b) can be considered to be a simple model of the biosynthesis of (II) from (I).

EXPERIMENTAL

Autocondensation of N-Acetylaminoacetone (IIIa) to 1-Acetyl-2-(N-acetylaminomethyl)-4-methylpyrrole (IVa). A mixture of 0.5 g of (IIIa) and 0.58 g of L-aspartic acid in 5 ml of xylene was refluxed for 6 h, after which the hot xylene solution was decanted and evaporated in vacuo. The residue was rubbed with 15 ml of ether and 10 ml of water. The crystals were filtered, washed with ether, and dried in the air. We obtained 0.21 g (60%) of (IVa), mp 97-98° (from heptane). R_f 0.58 (here and subsequently TLC, Al_2O_3 (III activity), 1:2 acetone–benzene, detection in UV light). Ultraviolet spectrum (here and subsequently taken in alcohol on a Specord UV-VIS instrument): λ_{max} 244 nm (ε 9870). Infrared spectrum (here and subsequently taken on a UR-20 instrument in KBr; ν, cm^{-1}): 3270 (NH), 1710 (COH), 1640 (CONH). NMR spectrum (here and subsequently taken in pyridine on a DA-60-1L instrument, δ, ppm): 1.77 (CH_3, doublet), 1.88 and 2.15 ($2CH_3CON$, singlets), 4.72 (CH_2, doublet): (CD_3COCD_3), 5.94 (H_3, singlet), 6.92 (H_5, singlet), 6.00-6.50 (NH, multiplet). Found: C 61.86; H 7.14; N 14.63%. $C_{10}H_{14}N_2O_2$. Calculated: C 61.86; H 7.21; N 14.42%.

When glycine, the α- and γ-aminobutyric acids, asparagine, α-alanine, and leucine are used as the condensing agents the yield of (IVa) is respectively 52, 0, 40, 36, 24, and 0%. A mixture of 0.7 g of (IIIa), 1 g of AP, and 0.07 g of DA in 10 ml of benzene was refluxed for 1.5 h, after which it was evaporated and the residue was treated with a solution of 6 g of K_2CO_3 in 10 ml of water and 15 ml of ether. The precipitate was filtered. We obtained 0.24 g (41%) of (IVa), mp 97-98°. A solution of 3 g of (IIIa) and 3 g of PC in 20 ml of absolute benzene was kept at 20° for 15 h, after which it was evaporated and the residue was vacuum-distilled. We obtained 3.9 g of an oil with bp 195-100° (4 mm), the addition of ether to which gave 0.61 g (24%) of (IVa), mp 91-92° (from heptane).

A mixture of 1.77 g of (IIIa), 3.3 ml of PN, and 6 g of molecular sieves (4 Å) in 6 ml of absolute benzene was kept at 20° for 150 h, filtered, the filtrate was evaporated, after which the solvent and then the unreacted (IIIa) (~180°, 12 mm) were distilled off. The residue was rubbed with water and the obtained crystals were filtered. We obtained 0.5 g of (IVa).

Formation of 1-Acetyl-2,4-dimethyl-3-acetylaminopyrrole (VII) from (IIIa). A solution of 9 g of (IIIa) and 1.68 ml of PN in 27 ml of absolute benzene was kept at 20° for 150 h, and then the solvent and unreacted (IIIa) were distilled off. The residue was rubbed with water and the precipitate was filtered. We obtained 0.16 g of (VII), mp 182-183° (from toluene and then from water). R_f 0.38 (Silufol UV-254, 1:1 acetone–benzene). NMR spectrum (δ, ppm): 1.93 (CH_3CONH, here and subsequently singlets), 2.14 and 2.20 ($2CH_3$), 2.38 ($CH_3CON<$). Found: C 61.76; H 7.17; N 14.45%. $C_{10}H_{14}N_2O_2$. Calculated: C 61.75; H 7.21; N 14.43%. From the mother liquor (after the recrystallization of (VII) from toluene) we isolated 0.12 g of (IVa). The saponification of (VII) with a solution of KOH in CH_3OH (12 h, 20°) gave 2,4-dimethyl-3-acetylaminopyrrole [5].

Saponification of (IVa) to 2-(N-Acetylaminomethyl)-4-methylpyrrole (Va). A mixture of 0.1 g of (IVa) and 0.4 g of NaOH in 5 ml of alcohol was kept at 20° for 4 h, after which it was evaporated in vacuo, and the residue was treated with water and extracted with ether. Removal of the solvent gave 0.07 g (90%) of (Va), mp 81-83° (washed with heptane). R_f 0.35 (Al_2O_3 (III activity), 1:2 acetone–benzene). Ultraviolet spectrum: λ_{max} 216 nm (ε 7350). NMR spectrum (δ, ppm): 1.84 (CH_3, singlet), 2.00 (CH_3CONH, doublet), 4.45 (CH_2, doublet). Found: N 18.03%. $C_8H_{11}N_2O$. Calculated: N 18.40%.

Autocondensation of N-Benzoylaminoacetone (IIIb) to 1-Benzoyl-2-(N-benzoylaminomethyl)-4-methylpyrrole (IVb). A mixture of 0.55 g of (IIIb) [6], 0.5 g of AP, and 0.05 g of DA in 5 ml of absolute benzene was refluxed for 3.5 h, evaporated, and the residue was treated with K_2CO_3 solution and ether. The precipitate was filtered, washed with water, then with ether, and dried in the air. We obtained 0.24 g (50%) of (IVb), mp 145-146° (from heptane). R_f 0.69 (Silufol UV-254, 1:1 acetone–benzene). Ultraviolet spectrum λ_{max} 230 nm (ε 25,600). Infrared spectrum (ν, cm^{-1}): 3270 (NH), 1690 ($CON<$), 1635 (CONH). NMR spectrum (δ, ppm): 1.80 (CH_3, singlet), 5.10 (CH_2, doublet), 6.30 and 6.48 (H_3 and H_5, singlets). Found: C 74.92; H 5.79; N 8.80%. $C_{20}H_{18}N_2O_2$. Calculated: C 75.40; H 5.67; N 8.81%.

Saponification of (IVb) to 2-(N-Benzoylaminomethyl)-4-methylpyrrole (Vb). A mixture of 0.37 g of (IVb) and 0.1 g of KOH in 10 ml of alcohol was kept at 20° for 12 h, evaporated, the residue was treated with water, and the crystals were filtered. We obtained 0.18 g (72%) of (Vb), mp 144-145° (from heptane). NMR spectrum (DMSO, δ, ppm): 4.47 (CH_2, doublet), 5.87 and 6.48 (H_3 and H_5, here and subsequently singlets), 8.82 (CONH), 10.22 ($HN<$). Found: C 72.65; H 6.56; N 13.19%. $C_{13}H_{14}N_2O$. Calculated: C 72.80; H 6.53; N 13.08%.

Conversion of N,N-Diacetylaminoacetone (VIII) to (IVa). N,N-Diacetylaminoacetone (VIII) was obtained as described in [6]. Infrared spectrum (ν, cm^{-1}): 1730, 1720, 1700 (CO). NMR spectrum (CCl_4, δ, ppm): 2.10 (CH_3CO, here and subsequently singlets), 2.20 ($2CH_3CON$), 4.39 (CH_2).

We obtained 0.3 g of (IVa) from 2 g of (VIII) by treatment with PN in the presence of molecular sieves, as described above.

CONCLUSIONS

The autocondensation of the N-acyl derivatives of aminoacetone to 1-acyl-2-(N-acylaminomethyl)-4-methylpyrroles was accomplished under the influence of either hexamethylphosphorous triamide or aspartic acids.

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