ISSN 1070-3632, Russian Journal of General Chemistry, 2015, Vol. 85, No. 7, pp. 1677–1680. © Pleiades Publishing, Ltd., 2015.
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Chemical Transformations of the Condensation Products of Structurally Different Aminoacids with Pyridoxal as a Function of Ph, Solvent, and Temperature

F. V. Pishchugin and I. T. Tuleberdiev

Institute of Chemistry and Chemical Technology, National Academy of Sciences of Kyrgyzstan, Chui pr. 267, Bishkek, 720071 Kyrgyzstan
e-mail: pishugin@rambler.ru

Received November 17, 2014

Abstract—The kinetics and mechanism of chemical transformations of condensation products of structurally different aminoacids with pyridoxal as a function of pH of the medium, the solvent, and temperature were studied. The Schiff bases were found to be the most stable at pH close to neutral values. In acidic media the condensation products of α-aminoacids with pyridoxal decompose to the starting components. In alkaline media the elimination of the α-hydrogen atom from the aminoacid fragment and rearrangement of the Schiff base into the quinoid form occurs, the subsequent hydrolysis leading to the formation of pyridoxamine and ketoacids. The condensation products of the β- and ε-aminoacids with pyridoxal decompose to the starting components in acidic media but are stable in alkaline media.

Keywords: aminoacids, vitamins of B_6 group, Schiff bases, kinetics, acid-base catalysis

DOI: 10.1134/S107036321507018X

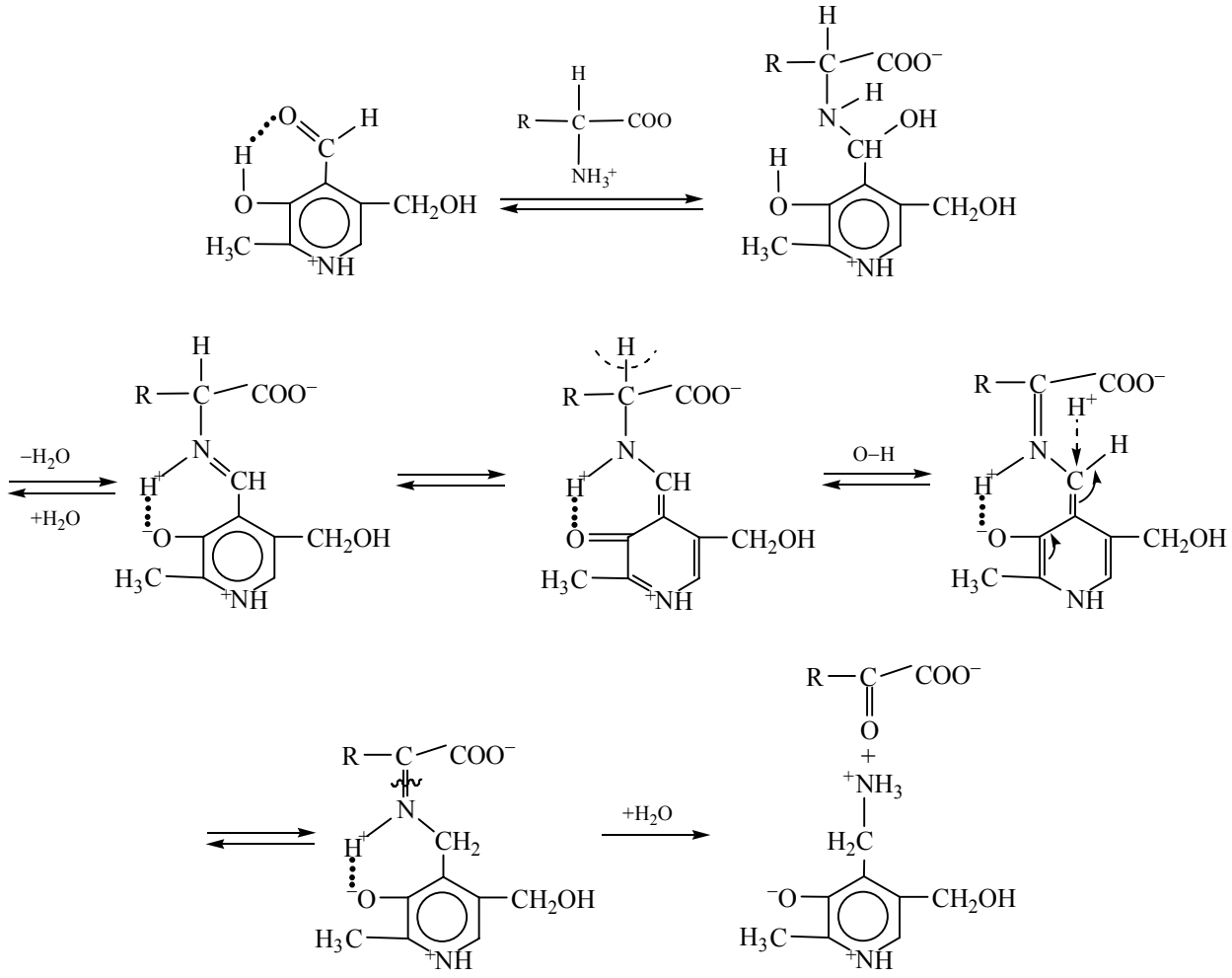
Vitamins of the B_6 group play the fundamental role in the nitrogen metabolism of living organisms participating in the enzymatic catalysis of various transformations of aminoacids and bioamines: transamination, decarboxylation, deamination, and amination of ketoacids. The main and intermediate products of these biochemical processes are Schiff bases. This conclusion was made on the basis of investigation of complex enzymatic or specific model systems. However, in view of complex character of enzymatic systems and fast, sometimes dubious mechanisms of the processes, the questions of chemical transformations of the Schiff bases as a function of their structure and reaction conditions (pH of the medium, solvent, temperature) remain open [1].

As the objects of investigation the Schiff bases synthesized by us, condensation products of pyridoxal hydrochloride with different aminoacids were chosen.

Earlier it was shown [2–7] that buffer solutions of all Schiff bases (except for the condensation product of pyridoxal with L-proline) in neutral media are of yellow color and are characterized by two absorption bands with λ_{max} = 350 and 420–430 nm. In acidic media, depending on the pH, the yellow color of the solutions gradually disappears and the intensity of the absorption maxima decreases. At pH > 6–7 the color of the solutions is gradually changed from yellow to orange and a new absorption maximum appears at 450 nm, whose intensity first reduces with time, and then increases (Fig. 1).

To investigate the effect of the structure of the Schiff bases on their stability and the mechanism of transformations, the Schiff bases with structurally different aminoacid fragments have been synthesized. Based on the calculated rate constants and determination of the routes of their chemical transformations it was found that the condensation products of pyridoxal hydrochloride with α-, β- and ε-aminoacids (glycine, L-α-alanine, β-alanine, lysine, L-glutamic acid, D,L-tryptophan) are stable in neutral media. In acidic media the condensation products of L-α-aminoacids with pyridoxal hydrochloride decompose to the starting compounds. In weakly acidic media, activation of the reaction centers of the Schiff bases occurs by protonation of the pyridine ring nitrogen atom (pK_a = 5.9 [1]), whereas in more acidic media it occurs by

Scheme 1.



protonation of the nitrogen atom of the C=N bond followed by destroying of the chelate structure (λ_{max} = 430 nm), the addition of water molecule, and the formation of the starting components, pyridoxal and aminoacids.

In alkaline media, the reaction proceeds in a different way. The elimination of the α-hydrogen from the aminoacid fragment leads to a new product with λ_{max} = 450 nm. The optical density of the solutions of pyridoxal with aminoacids first sharply decreases (λ_{max} = 450 nm) and then gradually increases. The color of the solution varies from yellow to orange being indicative of the formation of the quinoid structure. Then, the optical density is decreased due to hydrolysis of the quinoid structure with the formation of pyridoxamine and ketoacids. Proline, having the secondary NH group, does not form the Schiff base (Scheme 1).

The suggested scheme of decomposition of the condensation products of pyridoxal with aminoacids was proved by isolation and identification of the final products. In acidic hydrolysis, after the addition of excess alcohol to the reaction mixture, aminoacids were isolated, which are poorly soluble in ethanol. The isolated products give color reaction with ninhydrin. Pyridoxal remained in the solution was isolated and identified by the methods of UV and IR spectroscopy.

A more difficult problem was the isolation and identification of the products of alkaline hydrolysis of the Schiff bases. It was noted, that in some cases upon the treatment with alkali of the condensation products of pyridoxal with D,L-tryptophan or L-glutamic acid after some time the precipitates were formed, which were isolated and identified by the methods of elemental analysis, UV and IR spectroscopy. They turned out to be sodium salts of β-(3-indole)-α-

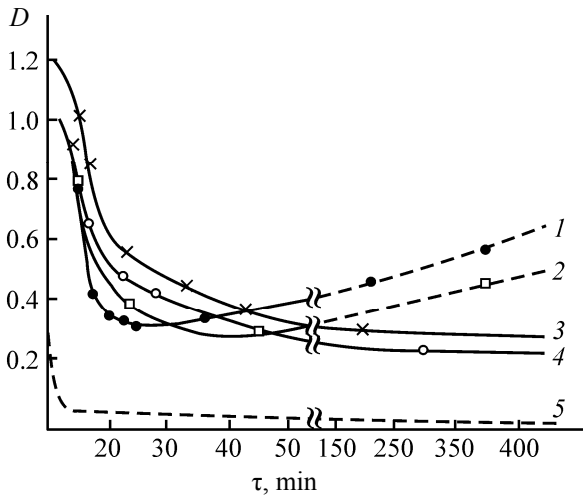


Fig. 1. Variation of optical density of 0.01 M solutions of (*1*) pyridoxalidene-α-L-alanine, (*2*) pyridoxalidene-L-arginine, (*3*) pyridoxalidene-β-alanine, (*4*) pyridoxalidene-L-lysine, and (*5*) pyridoxalideneproline with time (70% aqueous alcohol buffer solution, pH = 6.85; T = 20ºC).

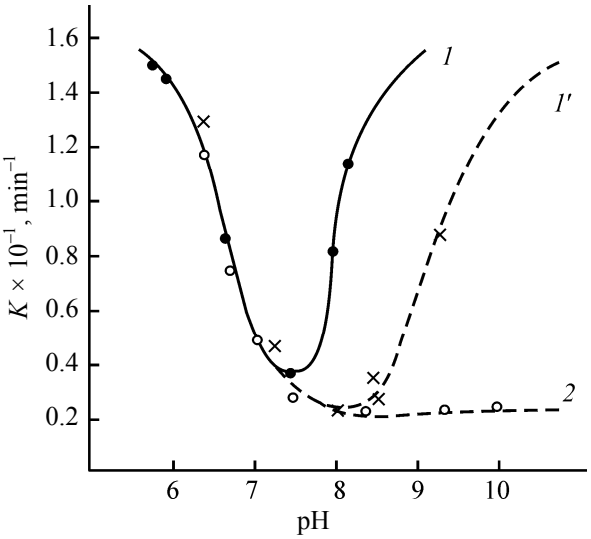


Fig. 2. Dependence of rate constants of the hydrolysis of (*1, 1'*) pyridoxalidene-L-arginine and (*2*) pyridoxalidene-β-alanine on pH of the solution (90% buffer solution, T = 20ºC). λ_{max} = 430 (solid line), 450 nm (dashed line) .

ketopropionic and α-ketoglutaric acid (identified by the quantitative reaction with 2,4-dinitrophenylhydrazine, UV, IR spectroscopy). The dependence of the reaction rates of the acidic and alkaline decomposition of the Schiff bases on the pH of the solution is illustrated in Fig. 2.

As can be seen from Fig. 2, with the increase in acidity the rate of hydrolysis to the initial components increases. The increase in the rate of decomposition of the Schiff bases in alkaline media has a more complex character since this route depends on the rates of several consecutive stages: (1) elimination of the α-hydrogen from the acidic component of the Schiff base; (2) rearrangement of the Schiff base to the quinoid structure; (3) hydrolysis of the quinoid structure under the action of water with the formation of pyridoxamine and ketoacid. The rates of alkaline decomposition depend on the degree of acidity of the α-hydrogen atom, pH of the medium, and the structure of the Schiff bases. The higher the acidity of the α-hydrogen and the pH of the medium, the more probable is the transformation of the Schiff base into the quinoid structure with its further hydrolysis. The suggested scheme is confirmed by the data of liquid chromatography of the products of the reaction between pyridoxal and D,L-tryptophan, as well as the isolation and determination of the structure of the intermediate and final products. The chromatographic analysis has shown that already at the beginning of the reaction in acidic media the intensity of the peaks of the Schiff bases decreases, while the intensity of the peaks of the final products increases. In low-alkaline media, apart from the starting compounds, the peaks of pyridoxamine and ketoacids appear.

The condensation products of pyridoxal hydrochloride with β-alanine and L-lysine are hydrolyzed in acidic media but are stable in the alkaline solutions. The condensation of pyridoxal hydrochloride with L-proline affords an unstable aminoalcohol, which could not be isolated as an individual substance because of its lability even in neutral media.

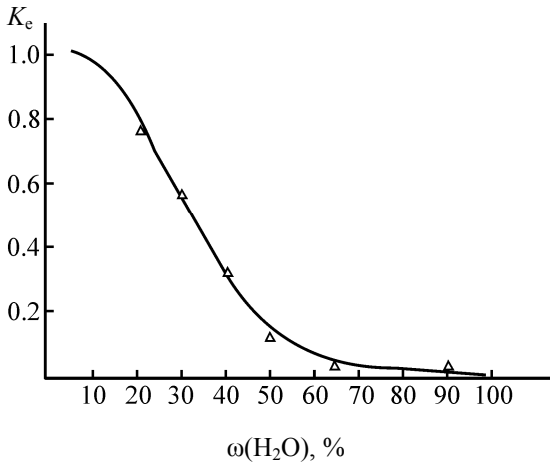


Fig. 3. Dependence of the equilibrium constant of the acidic hydrolysis of pyridoxalidene glycine on the water content in aqueous-alcohol buffer solutions (λ_{max} = 430 nm, T = 20ºC, pH = 6.6).

Investigation of stability of the Schiff bases as a function of solvent revealed the increase in the rates of acidic and alkaline hydrolysis with the increase in water content in aqueous alcohol buffer solutions. Apparently, this is due to the fact that the molecules of water are involved in the processes of decomposition of the Schiff bases to the starting components (pH < 7) or of the hydrolysis of the quinoid structures of the Schiff bases with the formation of ketoacids and pyridoxamine (pH > 7) (Fig. 3).

Therefore, the investigation of the rates of chemical transformations of the condensation products of pyridoxal hydrochloride with α-, β-, ε-aminoacids in neutral, acidic, and alkaline media at different temperatures showed that their stability decreases with increasing temperature.

EXPERIMENTAL

Pyridoxal hydrochloride of the "chemical pure" grade (Ferak Berlin), aminoacids, and their amides (Reanal, England) were used. Buffer solutions were prepared using standard procedures. The kinetics of the reactions were measured on a Spektonom-204 spectrophotometer. The reaction mixtures were kept at required temperature in a UH-8 thermostat with the accuracy of ±0.1°C. Specimens of the Schiff bases were dissolved in aqueous-alcohol buffer solutions, taking the moment of dissolution as the zero time. Kinetic measurements were carried out in cells 1.008 mm thick maintained at constant temperature. The pH of the solutions was measured on universal ionomer EV-74 with the accuracy of ±0.1 pH units. Since the UV spectra of the pyridoxal solutions vary depending on the pH and the solvent, as the reference cellss the solvents or solutions of pyridoxal were taken under the conditions similar to those of chemical transformations of the Schiff bases. With this approach, the changes in the optical density of starting components during their chemical transformations depend only on the formation of intermediate and final products of the reaction. The reaction rates were calculated from the first order equations for reversible and irreversible reactions using the program we developed. IR spectra were taken on a Nicolet Avatan 370 PGTS spectrophotometer. The products of decomposition of the Schiff bases were analyzed on a high-performance chromatograph PLC-20 Cole Parmer, eluent acetonitrile–water (20 : 80) (λ = 430 nm, V = 0.5 mL/min).

Pyridoxalidene-D,L-tryptophan. To a solution of 0.103 g of pyridoxal hydrochloride in 5 mL of 90% ethanol the solution of 0.102 g of D,L-tryptophan in the mixture of 72.5 mL of 90% ethanol and 2.5 mL of 0.2 M 90% acetate buffer solution was added. The obtained mixture was kept overnight at 5 °C. The formed precipitate of yellow-orange product was filtered and dried. Yield 0.197 g (94%), mp >350°C (decomp.). IR spectrum (KBr), ν, cm^{-1}: 3402 (NH^+), 1600–1616 (C=N, C=O, COO^-). UV spectrum, λ_{max}, nm: 350, 430. Found, %: C 53.1; H 6.0; N 8.9; $C_{19}H_{23}N_3O_4Cl_2Na$. Calculated, %: C 50.8; H 5.32; N 9.35.

Pyridoxalidene-L-lysine was prepared similarly from 0.103 g of pyridoxal hydrochloride and 0.102 g of L-lysine. Yield 0.168 g (88%), mp 240°C. UV spectrum, λ_{max}, nm: 350, 420. Found, %: C 43.8; H 6.23; N 10.15; ash 6.1. $C_{14}H_{22}O_4N_3Cl_2Na$. Calculated, %: C 43.0; H 6.23; N 10.7, ash 5.9.

Pyridoxalidene-L-arginine was prepared similarly from 20.6 mg of pyridoxal hydrochloride and 21 mg of L-arginine. The obtained solution was kept to constant optical density at λ_{max} 430 nm at room temperature, then evaporated at room temperature till the formation of yellow precipitate. Yield 35.8 mg (87.5%), mp >306°C (decomp.). UV spectrum, λ_{max}, nm: 350, 420. Found, %: C 39.15; H 6.78; N 15.7; Cl 9.9. $C_{19}H_{22}O_4N_3Cl_2Na$. Calculated, %: C 40.2; H 6.5; N 16.1; Cl 10.2.

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