The results of the present work show that affinity adsorbents with primary amino groups can be considered as promising matrices for isolating and purifying the corresponding biologically active compounds, in particular, natural anionic phospholipids.

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STABILITY OF WATER-SOLUBLE VITAMINS AND COENZYMES.

VIII. KINETICS OF ACID HYDROLYSIS OF NICOTINOYL-γ-AMINOBUTYRIC ACID

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UDC 615.356:577.164].074

Nicotinoyl-γ-aminobutyric acid (I) and its salts are biologically active compounds with pronounced psychotropic and tranquilizing properties [4].

In the present work, we carried out a comparative study of the kinetics of hydrolysis in an acid medium of I and several of its analogs, derivatives of γ-aminobutyric acid (GABA): D-(+)-α,γ-dihydroxy-β,β-dimethylbutyryl-γ-aminobutyric acid (II); N-α- and N-γ-pyridylcarbonyl-γ-aminobutyric acids (III, IV). The principal kinetic parameters of the process, characterizing the stability of I, were determined, and the mechanism of the hydrolytic decomposition was discussed.

$$RCONH(CH_2)_3COOH$$

I—IV

R = β-Pyridyl (I), $HOCH_2C(CH_3)_2CH(OH)$ (II), α-Pyridyl (III), γ-Pyridyl (IV).

EXPERIMENTAL

In the investigation, commercial samples of I were used, mp 209-210°C, containing not less than 99% of the main compound (method of nonaqueous titration), GABA (the Aminalone preparation) and II (the Pantogam preparation) were Pharmacopoeia quality (State Pharmacopoeia (SP) 42-1903-82 and 42-2391-85). Compounds III and IV were synthesized at the Hetero-

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cyclic Compounds Laboratory of the Scientific Association "Vitaminy" [2]. The analytical reagents and the buffer solutions were prepared according to [3, 5]. The hydrolysis was studied in the temperature range of 70-100°C (±0.1°C). The course of the reaction was controlled from the change in the concentration of the hydrolysis product, GABA. This was determined spectrophotometrically according to a colored product obtained in the reaction with a sodium salt of 1,2-naphthquinone-4-sulfonic acid (V) on a "Specord M-40" apparatus; the method was accurate to within ±1.5%.

Method of Kinetic Investigations. A 0.1-1 g weighed sample of I is dissolved in 1N-10N hydrochloric or sulfuric acid, or in a buffer solution at pH 0.9-1.4, prepared from sodium acetate and hydrochloric acid. The solution obtained with an initial concentration of the compound of not less than 0.05-0.2 mole/liter is poured into 2 ml ampules, which are sealed and thermostated at 70-100°C. The ampules, withdrawn periodically (a total 8-10), are opened, and an aliquot portion of 1.2 ml is transferred into a 50 ml volumetric flask. The solution is neutralized with 5% NaOH to phenolphthalein. A 2 ml portion of the solution is withdrawn and transferred into a 25 ml volumetric flask. Then 1 ml of a 1% borax solution and 1 ml of a 0.5% solution of V in a mixture of methanol with water [3] are added, and the mixture is heated on a boiling water bath for 10 min. The mixture is cooled, and 1 ml of a formaldehyde solution in a mixture of hydrochloric and acetic acids [3] and 1 ml of 0.1N sodium thiosulfate solution are added into each flask. After 10 min, the optical density is measured on a spectrophotometer at the absorption maximum at a wavelength of 478 ± 1 nm. A control solution of the reagents is placed in a reference cuvette. The measured optical density D_t corresponds to the GABA content in the reaction mixture, while the difference $D_t - D_0$ (D_0 is the optical density of a solution obtained at the initial moment of time) corresponds to increase in the GABA content in the course of the reaction. The kinetic experiment is, in general, stopped at the stage of 50-80% conversion (the limiting degree of conversion is reached after 7-15 h in a solution of acids, and after 24-72 h in buffer solutions). A similar procedure is carried out with one of the reaction products, GABA, starting from a 0.01 g sample dissolved in water in a 50 ml volumetric flask.* A 2 ml portion of the solution obtained is transferred into a 25 ml volumetric flask, and the reaction of formation of a colored product with V is carried out as described above, and the value of the optical density D is obtained. The degree of transformation X is calculated from the formula

$$X = \frac{(D_t - D_0)C}{D \cdot C_0}. \quad (1)$$

where C_0 and C are the initial concentrations of I and GABA, respectively, in mole/liter.

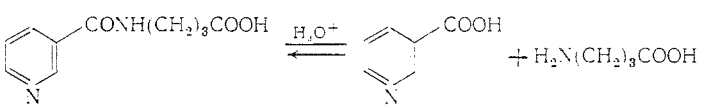
The effective rate constant K_1' is determined from a first order equation

$$K_1' = \frac{2.303}{t} \lg \frac{1}{1 - X} \quad (2)$$

RESULTS AND DISCUSSION

The solubility of I in water is largely dependent on the acidity of the medium: in the pH 1.5-6.0 range, it does not exceed 1 g/liter at 20°C. Therefore it is impossible to study the kinetics of its decomposition in this pH range by using the indirect method of determination, that requires a fairly high concentration of the starting material (4-5 g/liter). At pH ≤ 1.5, the solubility of I sharply increases because of the protonation of the nitrogen atom in the pyridine part of the molecules and formation of a readily soluble salt of the corresponding pyridinium base.

Stereochemistry and the Reaction Order. The presence in the hydrolysis products of I, or nicotinic acid (VI, according to UV spectrum, λ_{max} 261 nm [6]) and GABA (detected by the formation of a colored product with V [5, 7] and by TLC (SP 42-1903-82) indicates that in an acid medium the hydrolysis of I proceeds with rupture of the amide bond C–N according to the stoichiometric equation



(3)

*An aqueous solution is used in the determination of GABA in the reference sample, since there is practically no decomposition when a solution of GABA is heated in an acid medium.

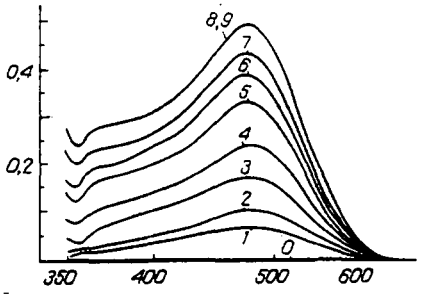


Fig. 1

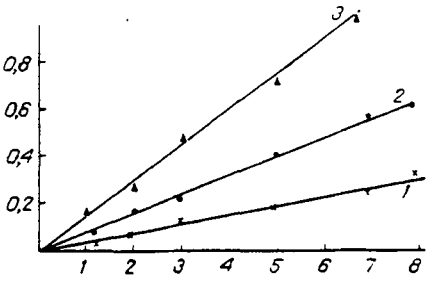


Fig. 2

Fig. 1. Absorption spectra of hydrolysis products of I (initial concentration in the ampule 0.096 mole/liter) in 6N HCl at 90°C treated by V. 0) At initial moment of time; 1, 2, 3, 4, 5, 6, 7, 8) after 1, 2, 3, 5, 8, 12, 18, 25 h from the beginning of the reaction, respectively; 9) absorption spectrum of GABA taken in an equimolar concentration. On abscissa) wavelength λ (in nm); on ordinate) optical density D.

Fig. 2. Semilogarithmic anamorphoses of kinetic curves of hydrolysis of I at 90°C in solutions of 1N HCl (1), 3N HCl (2), and 6N HCl (3). On abscissa) time t (in h); on ordinate) $\ln X/(1 - X)$.

TABLE 1. Rate Constants K_1' and K_2 in Acid Hydrolysis of I in Hydrochloric Acid Medium at 90°C

HCl concentration	$k_1' \cdot$ liter $\cdot$ mole^{-1}, h^{-1}	$k_2 \cdot 10^2$, liter $\cdot$ mole^{-1}h^{-1}
1 N.	2,95	2,95
2 N.	5,01	2,50
3 N.	7,85	2,62
6 N.	15,10	2,52

Note. The initial concentration of I is 0.05 M; $K_{2\,med} = 2.64 \pm 0.20$.

with the formation of 1 mole of GABA from 1 mole of I; GABA is quantitatively determined in the course of the kinetic experiment spectrophotometrically in the form of a Schiff base with the sodium salt of 1,2-naphthoquinone-4-sulfonic acid.

Figure 1 shows the absorption spectra of samples of the reaction mixture treated by V in one of the experiments, during the course of the progressing reaction, up to total completion. The optical density of completely hydrolyzed I (curve 8) at the absorption maximum practically coincides with the optical density of GABA at the same concentration (curve 9). The limiting degree of conversion of I into GABA, calculated from equation (1), is generally 97-99%.

In an acid medium, the hydrolysis of I proceeds practically irreversibly and is described by a pseudo first-order equation

$$-\frac{dI}{dt} = K_1'[I]. \qquad (4)$$

Figure 2 shows examples of semilogarithmic anamorphoses of kinetic curves for certain experiments. The constant K_1' calculated from equation (2) is practically independent of the initial concentration of I (which confirms the first order of the reaction with respect to I): at an initial concentration of I of 0.030 M, K_1' is equal to 0.153 h^{-1}, at 0.048 M it is 0.157 h^{-1}, at 0.096 M it is 0.152 h^{-1}, at 0.192 M it is 0.146 h^{-1} (the reaction is carried out in 6N HCl at 90°C).

Dependence of K_1' on pH of Medium. The acid hydrolysis of I was studied under the conditions of excess acid sufficient to neglect its consumption during the hydrolysis and to

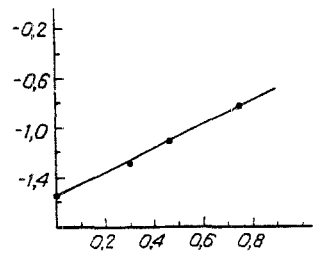


Fig. 3

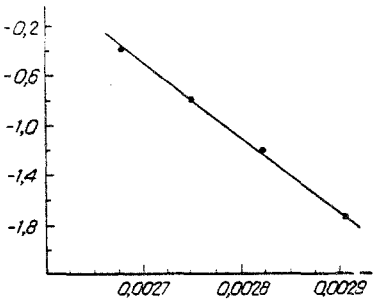


Fig. 4

Fig. 3. Dependence of $\log K_1'$ on $\log [H^+]$ for acid hydrolysis of I. On abscissa) $\log [H^+]$; on ordinate) $\log K_1'$.

Fig. 4. Dependence of effective rate constant of hydrolysis of I on temperature in 6N HCl (initial concentration of I 0.096 M). On abscissa) reciprocal temperature 1/T; on ordinate) $\log K_1'$

consider the H^+ concentration constant in the course of the experiment.

It was found that K_1' increases sharply with increase in pH. At pH 0.95, K_1' (in $h^{-1} \cdot 10^4$) is equal to 4.50, at pH 1.10, K_1' is 3.44, at pH 1.25 it is 2.68 and at pH 1.36 it is 1.42 (the reaction is carried out in a hydrochloric acid–sodium acetate buffer at 70°C; the initial concentration of I is 0.02 M. Thus, when the pH increases by 0.4 unit, there is more than a threehold increase in K_1'.

The order with respect to $[H^+]$ was determined from the dependences of $\log K_1$ on $\log [H^+]$ in a series of experiments, the results of which are given in Table 1. Figure 3 shows that the experimental points lie well on a line with tan of angle of slope ≈1, which corresponds to a first order with respect to H^+. Hence it follows that

$$K_1' = K_2 [H^+], \tag{5}$$

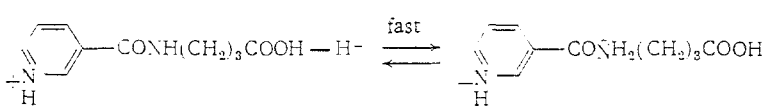
where K_2 is hydrolysis rate constant of a second order (first with respect to I and with respect to H^+, respectively). In this case, equation (4) acquires the form

$$-\frac{dI}{dt} = K_2 [I] [H^+]. \tag{6}$$

From equation (6), we can find the value of K_2. Table 2 shows that the convergence between the results obtained is most satisfactory.

Influence of Temperature. The dependence of the effective rate constant of hydrolysis of I in an acid medium on temperature is governed by the Arrhenius equation (Fig. 4). The mean value of the effective energy of activation is 25.8 ± 0.5 kcal/mole in 6N HCl, where the reaction rate is maximal.

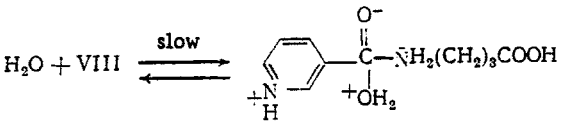
Reaction Mechanism. The results of the investigation of the conversion of I into GABA and VI in an acid medium, first order at a constant pH value, and the rectilinear dependence of the rate constant on the acidity of the medium in $\log K_1$ vs $\log [H^+]$ coordinates all indicate that, as in the case of another derivative of GABA, namely compound II, this reaction can be considered to be a specific acid catalysis reaction [5]. In analogy with the acid hydrolysis reactions of substituted amides, accompanied by splitting of the acyl carbon–nitrogen bond [1, 3, 5], it can be assumed that the decomposition of I proceeds by the mechanism of a bimolecular acid catalysis $A_{AC}2$. In this case, form VII, protonated at the pyridine ring nitrogen atom, reversibly adds a proton to form dication VIII, which slowly adds a molecule of water to form an intermediate form IX. The last compound decomposes rapidly to give reaction products.



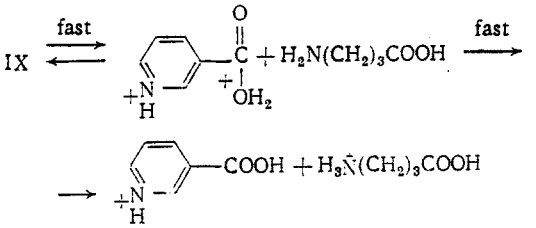
VII VIII

TABLE 2. Effective Rate Constants of Hydrolysis of Compounds of General Formula $RCONH(CH_2)_3COOH$ in 6N HCl

R	K_1' at 70°C, h^{-1}	K_1' at 100°C, h^{-1}
α-Pyridyl (III)	0.0049	0.13
β-Pyridyl (I)	0.0173	0.41
γ-Pyridyl (IV)	0.0282	0.46



IX



Because of its electron-acceptor properties, the protonated nitrogen atom of the pyridine part of the molecule has a strong polar influence on the stability of the C–N bond. Therefore the acid hydrolysis of I proceeds much more slowly than in the case of aliphatic N-acyl derivatives of GABA, to which compound II can be related. The rate constants of the acid hydrolysis of I and II differ by approximately 3 orders of magnitude. According to the data in [5], at pH 1.03 and at 70°C, K_1' for II is ≈0.36 h^{-1}. Under the same conditions, for I this value is equal to 0.00034 h^{-1} (see above). The higher reactivity of II is also confirmed by the lower value of the activation energy, which at pH 1.03 is equal to 20.2 ± 2 kcal/mole [5].

Table 2 shows not only K_1' for I, but also the values of the effective rate constants of hydrolysis of III and IV. Of the three substituted N-pyridylcarbonyl derivatives of GABA, the α-derivative is most slowly hydrolyzed, since the polar influence of the protonated nitrogen, in analogy with the influence of the polar substituents in the aromatic series, is stronger in the ortho-position, and then weakens. The small difference in the values of K_1' for I and IV is possibly due to the fact that the polar conjugation effect competes with the field effect, but in an opposite direction, and then decreases. Similar examples have been described in the literature. For example, it is known that the intramolecular conjugation effect that is transmitted in the benzene ring, is stronger in the more distant para-position than it is in the meta-position. The dependence of the rate constants of the acid hydrolysis of N-pyridylcarbonyl-γ-aminobutyric acids on the orientation of the pyridine ring in the molecule that we found is comparable with the dependence of the rate of hydrolysis of substituted aromatic amides on the position of the substituents in the aromatic ring. The relative rates of hydrolysis of ortho-, meta-, and para-aminobenzamides in 0.5N HCl at 100°C are 0.085, 0.81 and 0.85, respectively [1].

Thus, according to the ease of hydrolytic decomposition in an acid medium, the GABA derivatives that we studied can be arranged into the following series: II ≫ IV ≥ I > III.

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