STUDIES ON PYRAZOLES

LIX. Synthesis of β-(4-Pyrazolyl)-β-alanines*

I. I. Grandberg, A. N. Kost, and L. F. Morozova

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A number of β-amino acids of the pyrazole series have been synthesized by the direct Rodionov reaction and have been characterized by their UV spectra, electrophoretic mobility, and paper chromatography.

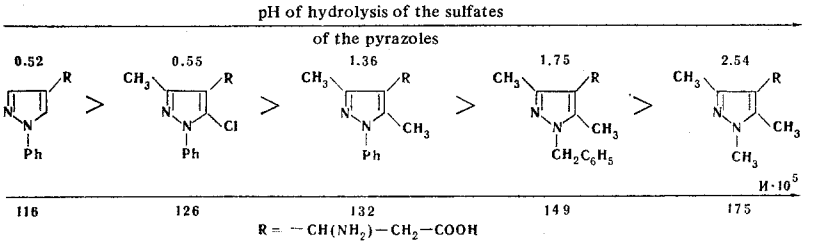
In view of the fact that β-(1-pyrazolyl)-α-alanine has been found in a number of plant materials [2–7], we have previously described α-amino acids containing a pyrazole ring of the type found in β-(4-pyrazolyl)-α-alanines and (4-pyrazolyl)-glycines [8, 9]. Their biological activity will be the subject of a separate communication. There is no information in the literature on β-amino acids containing the pyrazole ring.

In the present investigation we have carried out the synthesis of a number of substituted β-(4-pyrazolyl)-β-alanines using the Rodionov synthesis, i.e., the reaction of the corresponding 4-formylpyrazoles with malonic acid and ammonium acetate (Rodionov-Kravchenko modification) in acetic acid [10, 11]. The yields of amino acids were not always high (see Table 1), which is explained to a considerable extent by difficulties of isolation. If the reaction is carried out in an ethanolic medium (Johnson's modification) [10, 12] the yields are lower and the acids more difficult to purify.

The corresponding acrylic acids are formed as by-products in this reaction, and these were isolated and characterized (Table 2):

$$\mathrm{Pyr{-}COH + CH_2(COOH)_2 + NH_3 \xrightarrow[CH_3COOH]{CH_3COONH_4}}$$
$$\rightarrow \mathrm{Pyr{-}CH(NH_2){-}CH_2{-}COOH +}$$
$$+ \mathrm{[Pyr{-}CH{=}CH{-}COOH] + H_2O + CO_2}$$

The majority of the amino acids obtained are readily soluble in water and therefore only purification on ion-exchange resins Dowex 1×4 (100/200) in the OH^- form and Dowex 1×8 (100/200) in the HCO_3^- form proved successful. A check on the purity of the substances was carried out by paper chromatography and electrophoresis. Information on chromatographic and electrophoretic mobilities is given in Table 1. The UV spectra of these compounds have maxima in the 220–260 nm region, which is characteristic for the corresponding pyrazoles [13]. The introduction of an amino acid residue does not change the position of the maximum. The electrophoretic mobilities are in accordance with the basicity of the pyrazole nucleus (small deviations are observed for system I, pH 1.4) [14]. In acidic electrolytes, the amino acids move to the cathode (pH 1.4; 4.3) and in alkaline electrolytes to the anode (pH 11.3). The corresponding acrylic acids are formed as by-products in this synthesis and these were isolated and characterized (Table 2).



EXPERIMENTAL

3-methyl-1,5-diphenylpyrazole was obtained by the dehyrogenation of 3-methyl-1,5-diphenylpyrazoline [15]. 4-Formyl-1-phenyl-, 4-formyl-3,5-dimethyl-1-phenyl-, 4-formyl-3-methyl-1,5-diphenyl-, and 5-chloro-4-formyl-3-methyl-1-phenylpyrazoles were obtained by formylating the corresponding pyrazoles with N,N-dimethylformamide and phosphorus oxychloride [17]. 1-Benzyl-4-formyl-3,5-dimethyl- and 4-formyl-1,3,5-trimethylpyrazoles were synthesized by the procedure that we have described previously [8].

Synthesis of the β-amino acids. A flask with a reflux condenser was charged with 0.1 mole of the appropriate 4-formylpyrazole, 0.125 mole of malonic acid, 0.4 mole of ammonium acetate, and 1 mole of acetic acid. The mixture was heated in the boiling water bath for 10–14 hours. In some cases, the vigorous evolution of gas took place. After the end of the reaction, the excess of acetic acid was distilled off in vacuum in the water bath to dryness.

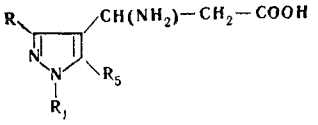
ISOLATION AND PURIFICATION OF THE AMINO ACIDS

β-(1-Phenyl-4-pyrazolyl)-β-alanine. The solid residue obtained was suspended in methanol and transferred to a glass filter where it was washed several times with methanol and with a small amount of water. This gave a white crystalline residue which was recrystallized from 30% aqueous dioxane (for constants, see Table 1).

β-(3-Methyl-1,5-diphenyl-4-pyrazolyl)-β-alanine. The residue after the evaporation of the acetic acid was suspended in hot methanol and washed several times on the filter with large amounts of hot methanol, under suction. This gave pure white crystals of the amino acid. To obtain additional amounts of the amino acid, the methanolic filtrates were evaporated to dryness and heated with 1 N hydrochloric acid. The corresponding acrylic acid was separated by filtration and crystallized from aqueous acetic acid. The passage of an aqueous methanolic solution of the amino acid hydrochloride contaminated

*For part LVIII, see [1].

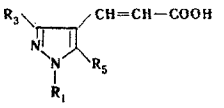
Table 1



R_1	R_3	R_5	Mp (in a sealed capillary), °C	R_f		UV spectrum (SF-4)		Electrophoretic mobility, u $\times 10^{-5}$		Empirical formula	Found, %		Calculated, %		Yield, %
				system I	system II	λ_{max}, nm	lg ε	system I	system II		C	H	C	H	
C_6H_5	H	H	234—235	0.66	0.29	251*	4.14	250	116	$C_{12}H_{13}N_3O_2$	62.14 62.07	5.55 5.67	62.32	5.66	71.4
C_6H_5	CH_3	C_6H_5	245—246	0.80	0,45	233**	4.40	233	130	$C_{19}H_{19}N_3O_2$	71.00 71.16	6.08 6.11	71.01	5.96	38.2
C_6H_5	CH_3	CH_3	206—207	0.72	0.42	240*	4.05	239	132	$C_{14}H_{17}N_3O_2 \cdot H_2O$	60.12 60.24	6.87 6.71	60.63	6.90	34.7
C_6H_5	CH_3	Cl	213—214	0.77	0.50	241***	4.07	242	126	$C_{13}H_{14}ClN_3O_2$	55.56 55.26	5.23 5.05	55.81	5.04	48.3
$CH_2C_6H_5$	CH_3	CH_3	210—212	0.73	0.40	252*** 258	2.22 2.30	286	149	$C_{15}H_{19}N_3O_2$	66.39 66.42	7.21 7.23	65.91	7.01	15
CH_3	CH_3	CH_3	283—285	0.38	0,20	225*	3.67	350	175	$C_9H_{15}N_3O_2 \cdot H_2O$	50.07 50.18	8.02 7.97	50.22	7.95	15

*In water.
**In 1 N hydrochloric acid.
***In methanol.

Table 2



R_1	R_3	R_5	Mp, °C	R_f in system II	Electrophoretic mobility, $cm^2/V \cdot sec \cdot 10^{-5}$, in system III	Empirical formula	Found, % C	Found, % H	Calculated, % C	Calculated, % H	Yield, %
C_6H_5	H	H	187[16]	0.47	318	—	—	—	—	—	—
C_6H_5	CH_3	C_6H_5	245—246[16]	0.74	382	—	—	—	—	—	32.5
C_6H_5	CH_3	CH_3	163—164[16]	0.60	424	—	—	—	—	—	31.0
C_6H_5	CH_3	Cl	201—203	0.73	404	$C_{13}H_{11}ClN_2O_2$	59.02 59.05	4.17 4.10	59.43	4.22	46.0
$CH_2C_6H_5$	CH_3	CH_3	229—230	0.72	447	$C_{15}H_{16}N_2O_2$	70.44 70.61	6.34 6.45	70.29	6.29	28.0
CH_3	CH_3	CH_3	201—202	0.44	531	$C_9H_{12}N_2O_2$	60.12 60.13	6.79 6.89	59.98	6.71	~20

with inorganic salts through a column of the anion-exchanger Dowex 1 × 4 (100/200) in the OH^- form and subsequent treatment of the hydrochloric acid eluates (1 N hydrochloric acid) with the anion-exchanger Dowex 1 × 8 (100/200) in the HCO_3^- form led to the pure amino acid [8]. (Two to three grams of amino acid can be purified on a column 30 cm high and 2.7 cm in diameter).

β-(5-Chloro-3-methyl-1-phenyl-4-pyrazolyl)-β-alanine. The residue obtained after the evaporation of the reaction mixture readily dissolved in ethanol. Consequently, isolation was possible only in the form of the hydrochloride. The reaction mixture was extracted by heating with 1 N hydrochloric acid (4–5 times with 30 ml). The hydrochloric acid solution was treated with ether (4 × 50 ml). The yellow solid remaining after the treatment with 1 N hydrochloric acid consisted of the fairly pure substituted acrylic acid.

It was crystallized from aqueous ethanol.

The hydrochloric acid extracted was evaporated to dryness in vacuum. The free amino acid was isolated from a solution of the residue in 50% methanol on an ion-exchange resin. It was crystallized from aqueous methanol.

β-(3,5-Dimethyl-1-phenyl-4-pyrazolyl)-β-alanine. This was isolated and purified in a similar manner to β-(5-chloro-3-methyl-1-phenyl-4-pyrazolyl)-β-alanine. For analysis it was crystallized from water. White plates with a mother-of-pearl luster containing 1 mole of water of crystallization were obtained. The acrylic acid was recrystallized from aqueous acetic acid.

β-(1-Benzyl-3,5-dimethyl-4-pyrazolyl)-β-alanine. The solid residue was heated with 2 N hydrochloric acid. On cooling, the hydrochloric acid solution deposited crystals of the acrylic acid. When its hydrochloric acid solution was made alkaline with NH_4OH to pH 5, more crystals of the acrylic acid deposited, and these were recrystallized from aqueous acetic acid. The solution containing the amino acid was purified on an ion-exchange resin. For analysis it was reprecipitated from aqueous acetone with ether. The amino acid crystallized with one molecule of water of crystallization, from which it was freed by drying over P_2O_5 at 110° C for 4 hr.

β-(1,3,5-Trimethyl-4-pyrazolyl)-β-alanine. The residue obtained after the evaporation of the acetic acid dissolved completely in a small amount of 1 N hydrochloric acid. The hydrochloric acid solution was evaporated to dryness and the solid mixture was dissolved in aqueous methanol (1:3), passed through a column of resin in the OH^- form, and eluted with 2 N hydrochloric acid. The hydrochloric acid eluates were evaporated to dryness, the solid residue was dissolved in water, and the solution was made alkaline with NH_4OH to pH 4, whereupon a small amount of crystals of the acrylic acid deposited. These were filtered off and the filtrate was again purified on an ion-exchange resin by a method similar to that described above. This time, white crystals of the amino acid were obtained which were purified by two precipitations from methanol and ethanol with ether.

The yield of the acrylic acid could not be determined accurately because it partially deposited on the resin in the HCO_3^- form when the weakly acid eluates were treated. The acrylic acid was recrystallized from aqueous ethanol

Chromatography of the amino acids obtained. The R_f values of the amino acids were determined by ascending paper chromatography on "Khr. B" paper of the Leningrad Volodarskii mill in the following solvent systems: system I, n-butanol—water—acetic acid (4 : 5 : 1); system II, isopropanol—water—ammonia (10:1:1).

The spots were detected with ninhydrin. For all the amino acids except β-(1,3,5-trimethyl-4-pyrazolyl)-β-alanine, which appeared directly in the form of a bright blue-violet spot, treatment with ninhydrin gave yellow-brown colorations gradually changing to blue-violet.

The mobilities of the substituted acrylic acids were determined in system II. The spots were revealed with iodine.

Electrophoretic mobilities. These were determined under the conditions that we have described previously [8]. Electrolytes: I—pH 1.4, 30% CH_3COOH; the current was passed for 2 hr; u = 440 V; II—pH 4.3, pyridine—acetic acid—water (2:4:994); 4 hr; III—pH 11.3, 0.2 N NH_4OH; 2 hr; IV—borate buffer, pH 9.0; 1 hr.

In 0.2 N NH_4OH (pH 11.3) the spots obtained were unclear and diffuse, and therefore we did not measure the mobilities quantitatively. The electrophoretic mobilities were calculated from the formula $u = d/x \cdot t$, where

u is the electrophoretic mobility, $cm^2/V \cdot sec$;
x is the potential gradient—15.7 V/cm;
t is the time, sec; and
d is the distance from the start to the spot, cm.

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Moscow State University; Timiryazev Agricultural Academy, Moscow