STUDIES ON PYRAZOLES

LXIII. Amino Acids of the Pyrazole Series*

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The possibility of the separation into optical antipodes of racemic amino acids of the pyrazole series has been investigated. The trans configuration of the acrylic acids obtained as by-products in the synthesis of β-amino acids of the pyrazole series by the Rodionov reaction has been shown by PMR and IR spectroscopy. The IR spectra of a series of amino acids are discussed.

In view of the fact that β-(pyrazol-1-yl)-α-L-alanine [2–9] has been found in some plant materials, we have previously carried out the synthesis of a number

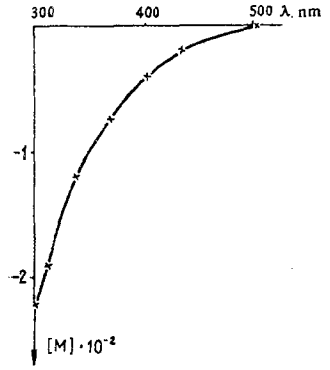


Fig. 1. Rotatory dispersion curve of β-(1-phenylpyrazol-4-yl)-α-alanine in 2 N HCl.

of amino acids containing a pyrazole ring with various substituents in positions 1, 3, and 5. Such pyrazole nuclei were attached to residues of α-alanine, β-alanine, or glycine [10–12]. We hoped that the amino acids obtained would prove to be antimetabolites of the natural amino acids. However, they showed no appreciable antibacterial and antifungal action in a dilution of 1 : 1000. On in vitro testing, a number of the pyrazolyl-α-alanines obtained proved to be extremely active against influenza virus. Unfortunately, on testing in vivo the activity fell sharply.†

We have made a number of attempts to separate the racemic amino acids into their optical antipodes.

For this purpose acetyl derivatives and esters of the amino acids were synthesized by the usual methods. It is important to note that the formation of esters was observed only when an equimolar amount of orthoformic ester was added to the reaction mixture.

Biological and chemical methods were used for the resolution of the amino acids into their optical antipodes.

The incubation of the acetyl derivative of DL-β-(1-phenylpyrazol-4-yl)-4-alanine with the crude acylase isolated from Aspergillus oryzae should have led to the L-amino acid as the result of the specific action of the acylase, which consists in the removal of the acetyl protection of only the L-amino acid. We obtained negative results, either because of the resistance of this acetyl derivative or because of the inadequate activity of the acylase available. This method has been used successfully by Japanese workers in the production of L-β-(pyrazol-1-yl)-α-alanine [6].

Making use of the esterase activity of chymotrypsin, we attempted to separate into optical antipodes the methyl ester of DL-β-(1-phenyl-pyrazol-4-yl)-α-alanine. However, in water—benzene solution with careful stirring, spontaneous hydrolysis of the ester took place as a result of which the racemic amino acid was obtained. Attempts at resolution with L-camphorsulfonic acid and with D-tartaric acid were also unsuccessful. It was possible to obtain the optical antipodes of β-(1-phenylpyrazol-4-yl)-α-alanine only by the fractional crystallization (from 50% aqueous ethanol) of the di-

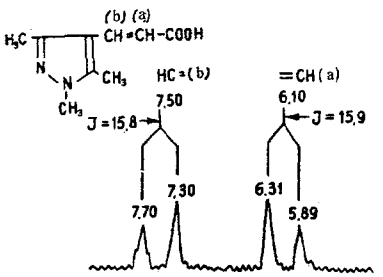


Fig. 2. Part of the PMR spectrum of β-(1,3,5-trimethylpyrazol-4-yl) acrylic acid.

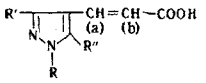
astereomeric salts of the isopropyl ester of this acid with dibenzoyl-D-tartaric acid (see Experimental).

The rotatory dispersion curves measured in the 589–300 nm region on a VNIEKIPRODMASh [All-Union Scientific Research Experimental Design Institute for Food Machinery] instrument has the smooth nature typical for the amino acids [13, 14] (Fig. 1).

*For part LXII, see [1].

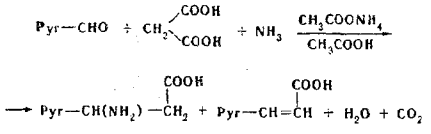
†The tests were carried out by N. S. Bogdanova in VNIKhFI [All-Union Chemical and Pharmaceutical Scientific Research Institute] (Division of the Chemotherapy of Infectious Diseases).

Chemical Shifts (δ) and Spin-Spin Coupling Constants of the Protons of the Double Bond of the Pyrazolylacrylic Acids

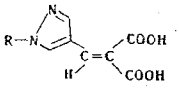


R	R′	R″	δ, ppm		J, Hz
			H_b	H_a	
C_6H_5	H	H	8.10	6.43	15.2
$CH_2C_6H_5$	CH_3	CH_3	7.73	6.06	16.0
C_6H_5	CH_3	Cl	7.44	6.25	15.4
C_6H_5	CH_3	CH_3	7.72	6.18	15.2
CH_3	CH_3	CH_3	7.50	6.10	15.9

We have previously reported that in the preparation of β-(pyrazol-4-yl)-β-alanines by the Rodionov method the corresponding acrylic acids (I) are formed as by-products; they were isolated and characterized without their configurations being established [12].



An analysis of the PMR spectra of the acrylic acids isolated gave the spin-spin coupling constants of the olefinic protons averaging 15 Hz for all the compounds, which showed their trans-configuration [15, 16] (table and Fig. 2). A confirmation of the trans-configuration of the acrylic acids is the presence of a distant strong band of the deformation vibrations of the hydrogen atoms at 973–975 cm^{-1} [17] in the IR spectra (Fig. 3a). Evidently the unambiguous course of the decarboxylation reaction in the system is due to the fact that the aromatic heterocycle is stabilized by the resonance effect of the trans configuration to a considerably greater extent than the cis configuration.



We recorded the IR spectra of the amino acids that we had obtained in KBr tablets on a Jasco IR-S instrument in the 600–4000 cm^{-1} region. In the spectrum of β-(3, 5-dimethylpyrazol-4-yl)-α-alanine (Fig. 3b) the two very intense amino acid absorption bands I (1620 cm^{-1}) and II (1518 cm^{-1}) due to the symmetrical and antisymmetrical vibrations of the NH_3^+ group are distinctly shown. In the 1590 cm^{-1} region there is a strong band of an ionized carboxyl group. The assignment of the 1405 cm^{-1} band to the symmetrical vibration of the COO^- group is also extremely probable.

The band in the 2100 cm^{-1} region belonging to the stretching vibrations of the NH_3^+ group which is typical for all amino acids can also be seen clearly in the 2120 cm^{-1} region. As usual, the stretching vibrations of the NH group of the pyrazole nucleus are in the 3570 cm^{-1} region. The stretching vibrations of the NH_3^+ group are shown by a broad band in the region around 3000 cm^{-1}. The majority of bands in the 1000–1590 cm^{-1} region relate to the vibrations of the pyrazole nucleus, but are difficult to identify. The typical bands retain their positions for the other pyrazolyl-α-alanines: β-(1-phenylpyrazol-4-yl)-α-alanine (3050, 2160, 1610, 1500, 1400 cm^{-1}); β-(5-chloro-3-methyl-1-phenylpyrazol-4-yl)-α-alanine (3030, 2100, 1610, 1520, 1407 cm^{-1}); and β-(1-benzyl-3, 5-dimethylpyrazol-4-yl)-α-alanine (3000, 2125, 1612, 1533, 1405 cm^{-1}).

In the spectrum of β-(1, 3, 5-trimethylpyrazol-4-yl)-β-alanine (Fig. 3d) in addition to the typical amino acid bands at 1625 and 1540 cm^{-1}, distinct absorption can be seen in the 3400 cm^{-1} region which can only be ascribed to a nonionized amino group. This is obviously due to the partial ionization of the carboxyl group through the action of the nitrogen atoms of the pyrazole nucleus with a consequent decrease in the ionization of the amine group. In the spectra of the more weakly basic pyrazoles, the intensity of this band is considerably lower. Typical bands in the 2160 and 1390 cm^{-1} region are also clearly seen and are fairly strong.

The typical bands retain their position for the other pyrazolyl-β-alanines: β-(1-phenylpyrazol-4-yl)-β-alanine (3400 w, 3030, 1630–1510, 1385 cm^{-1}); and β-(3, 5-dimethyl-1-phenylpyrazol-4-yl)-β-alanine (3400 w, 3000, 2190, 1618, 1567, 1505 cm^{-1}).

In the spectrum of 1-phenylpyrazol-4-ylglycine (Fig. 3c), the amino acid band I can be seen clearly at 1655 cm^{-1}, while the amino acid band II is shifted considerably in the long-wave direction (1585 cm^{-1}) and probably overlaps the absorption of the phenyl nucleus. Typical amino acid bands can be seen at 2120 and 1394 cm^{-1}. The absorption bands for 5-chloro-3-methyl-1-phenylpyrazol-4-yl-glycine are at 3020, 2080, 1635, 1545, and 1385 cm^{-1}.

One of us [18] has described the acylation of pyrazoles with acetic anhydride in the presence of concentrated sulfuric acid. Attempts to use succinic anhydride in this reaction in order to obtain keto acids under these conditions proved unsuccessful. It was also impossible to obtain keto acids under the standard conditions of the Friedel-Crafts reaction [19–21].

Our attempts to obtain the 1-chloromethyl derivative of 3, 5-dimethylpyrazole by treating the 1-hydroxymeth-

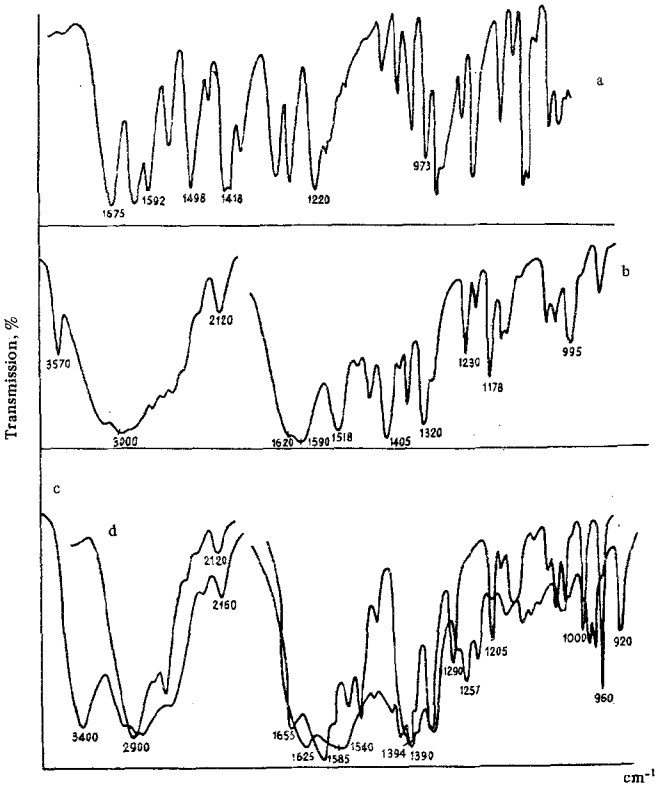


Fig. 3. IR spectra of amino acids and acrylic acids: a) β-(1-phenylpyrazol-4-yl)-acrylic acid; b) β-(3,5-dimethylpyrazoly-4-yl)-α-alanine; c) α-(1,3,5-trimethylpyrazol-4-yl)-β-alanine; d) 1-phenylpyrazol-4-ylglycine.

yl derivative with thionyl chloride were unsuccessful under varied conditions. This is the more surprising in that the reaction has been accomplished successfully for pyrazole itself [22].

EXPERIMENTAL

N-Acetyl-β-(1-phenylpyrazol-4-yl)-α-alanine. In drops, 0.7 g of acetic anhydride (slight excess) was added to a solution of 1.15 g (0.005 mole) of the amino acid in 30 ml of glacial acetic acid, and the mixture was left overnight. Then the acetic acid was evaporated off in vacuum and the residual caramel-like mass was dissolved in boiling water. On cooling, colorless crystals of the acetyl derivative of the amino acid separated out [from aqueous acetic acid (5 : 1)]. Yield 0.9 g (66.2%). Mp 202.5–203° C. Found, %: C 61.28, 61.14; H 5.75, 5.86. Calculated for $C_{14}H_{15}N_3O_3$, %: C 61.52; H 5.53. EM × × 10^{-5} $cm^2/V \cdot sec$, 5.79 (pH 9).

N-Acetyl-β-(5-chloro-3-methyl-1-phenylpyrazol-4-yl)-α-alanine. This was obtained similarly to the above-described substance from 0.7 g (0.0025 mole) of the corresponding amino acid and 0.35 ml of acetic anhydride in glacial acetic acid. Yield 0.52 g (65%); caramel-like mass, readily disintegrating into a powder. It was purified by precipitation from heptane–benzene (5 : 1) with acetone. Mp 152–153° C. Found, %: C 56.23, 56.06; H 5.14, 5.30. Calculated for $C_{15}H_{16}ClN_3O_3$, %: C 56.06; H 5.00. EM (pH 9), 5.27 [12].

Methyl ester of β-(1-phenylpyrazol-4-yl)-α-alanine. A suspension of 6.93 g (0.03 mole) of β-(1-phenylpyrazol-4-yl)-α-alanine in 100 ml of absolute methanol was treated with 4.4 g (0.03 mole) of orthoformic ester, and a current of dry hydrogen chloride was passed through it. The temperature of the solution rose to boiling point, and the passage of hydrogen chloride was continued for 3–4 hr until saturation was complete. Then the methanol was evaporated off in vacuum. Another 100 ml of absolute methanol and 4.4 g of orthoformic ester were added to the viscous brown oil obtained. The resulting solution was again saturated with a current of hydrogen chloride. The end of the reaction was determined from the disappearance of the spot of the amino acid on a paper chromatogram and from the appearance of a strong spot of the ester of amino acid (in the butanol–water–acetic acid (4 : 5 : 1) system, R_f of the amino acid 0.60, R_f of the ester 0.66). The reaction mixture was evaporated in vacuum to dryness. This gave 7.2 g (75.4%) of the crystalline hydrochloride of the methyl ester of β-(1-phenylpyrazol-4-yl)-α-alanine.

With ice cooling, 28 ml of chloroform saturated with ammonia was gradually added to a suspension of 5 g (0.016 mole) of the dihydrochloride in 30 ml of dry chloroform. Another 5 ml of chloroform saturated with ammonia was added until the smell of ammonia ceased to disappear, and then the mixture was left overnight in the refrigerator at −10° C. The resulting precipitate of ammonium chloride was filtered off, and the chloroform was evaporated in vacuum at room temperature. This gave 2.9 g (75.3%) of the methyl ester of β-(1-phenylpyrazol-4-yl)-α-alanine in the form of a viscous yellowish oil. An ethanolic solution of picric acid was added to the chloroform solution of the ester. The yellow picrate that deposited was recrystallized twice from a mixture of ethanol and chloroform. Mp 181–182° C. Found, %: C 48.26, 48.18; H 3.90, 3.97. Calculated for $C_{13}H_{15}N_3O_2 \cdot C_6H_3N_3O_7$, %: C 48.10; H 3.82.

Isopropyl ester of β-(1-phenylpyrazol-4-yl)-α-alanine. This was obtained in a similar manner to the methyl ester from 5 g (0.021 mole) of the amino acid, 200 ml of isopropanol, and 8 ml of orthoformic ester. The yield of dihydrochloride was 4.8 g (64%); it was purified by precipitation from methanolic solution with a large amount of ether. The ester was liberated from 3.5 g (0.01 mole) of the dihydrochloride by treating its suspension in chloroform with 20 ml of chloroform saturated with ammonia. This gave 2.1 g (76%) of a viscous yellowish oil [in the butanol–water–acetic acid (4 : 5 : 1) system, R_f of the amino acid 0.60; R_f of the ester 0.84]. To obtain the picrate, a concentrated solution of picric acid in ethanol was added to a solution of the ester of the amino acid in ethanol was added to a solution of the ester of the amino acid in a small amount of chloroform. Ether was added to the mixture obtained and it was gently warmed, whereupon yellow crystals of the picrate deposited. Mp 173–174° C. Found, %: C 50.08, 49.82; H 4.44, 4.48. Calculated for $C_{15}H_{19}N_3O_2 \cdot C_6H_3N_3O_7$, %: C 50.11; H 4.41.

Resolution of the isopropyl ester of β-(1-phenylpyrazol-4-yl)-α-alanine using D-dibenzoyltartaric acid. 2.12 g (0.0077 mole) of the isopropyl ester of β-(1-phenylpyrazol-4-yl)-α-alanine in 15 ml of absolute methanol was added to a solution of 2.75 g (0.0077 mole) of D-dibenzoyltartaric acid [23] in 15 ml of absolute methanol, and the mixture was heated to the boil and left to crystallize. After 2 days, 8 g of salt "A" with mp 189–190° C (192–193° C after recrystallization from methanol), $[\alpha]_{302} = -958.1°$ [methanol–ethanol (1 : 1)] was filtered off, and the filtrate and the mother solution were evaporated in vacuum at a temperature not exceeding 40° C. This gave 3.3 g of salt "B" with mp 162–170° C. The salt "B" (3.3 g) was dissolved with heating in aqueous ethanol (5 : 1 by volume) and was left to crystallize at room temperature. At a temperature near to that of the room, thin acicular crystals of salt "A" with mp 188–190° C were filtered off, and at room temperature flocculent crystals with mp 177–179° C began to deposit; these were recrystallized twice from 50% aqueous ethanol. This gave crystals of salt "A" with mp 188–190° C. All the mother solutions were evaporated, giving a caramel-like mass with mp 160° C (salt "C").

A mixture of 0.19 g of salt "C" and 1.5 ml of 10% HCl was heated for 30 min. After cooling, oily crystals of dibenzoyl-D-tartaric acid separated out, and these were filtered off and the filtrate was extracted several times with ether and the extracts were evaporated in vacuum. The residue was dissolved in a small amount of water and the solution was carefully made alkaline with 15% ammonia to pH 4. Crystals of the (−)-amino acid deposited in an amount of 0.025 g (36.2%). Mp 240–242° C (decomp.). $[\alpha]_{436}$ −8.78°; $[\alpha]_{405}$ −17.5°; $[\alpha]_{365}$ −32.2°; $[\alpha]_{334}$ −52.7°; $[\alpha]_{313}$ −83.9°; $[\alpha]_{302}$ −95.6° (c, 0.32; 2N HCl; 2 days after dissolution). Similarly, salt "A" (mp 188–190° C yielded 20 mg of the (+)-amino acid with mp 240–243° C. $[\alpha]_{302}$ +141.0°; after 2 days $[\alpha]_{302}$ +89.6° (c, 0.24, 2 N HCl). The salt "A" with mp 192–193° C yielded an additional amount of the (+)-amino acid; $[\alpha]_{300}$ +174.9°; $[\alpha]^{20}$ +40.7°.

The PMR spectra of the acrylic acids were recorded on a JNM-2 instrument at a frequency of 39.56 MHz using dimethyl sulfoxide as solvent. The results are given in the δ scale, the signal from tetramethylsilane being taken as zero.

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