of KOH in 20 ml of water). The reaction mixture was allowed to stand at room temperature for 20 min and then poured into water. The resulting precipitate was chromatographed on silica gel (solvent: $CHCl_3$). The rearranged product (IVa) thus obtained was recrystallized from benzene to give colorless needles.

The similar treatment of IIIb and IIIc with KOH gave the rearranged products, IVb and IVc, respectively.

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Synthesis of β-(Pyrazolyl-N)-DL-alanine

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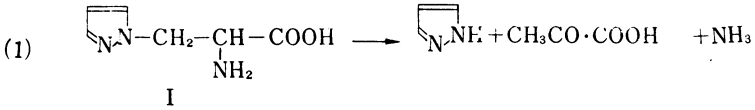
β-(Pyrazolyl-N)-L-alanine(I) is so far the only naturally-occurring amino acid containing a pyrazole ring; it is also unusual in possessing an alanine side chain directly to a nitrogen atom of the heterocyclic nucleus.

β-(Pyrazolyl-N)-L-alanine was first isolated from the pressed juice[2] and seed of watermelon(*Citrullus vulgaris*)[3] and has subsequently been found in several other plants.[3]

The correct chemical structure was proposed by Noe and Fowden[3] on the basis of its nuclear magnetic resonance spectrum and other properties. The structure has been confirmed as α-amino-β-(pyrazolyl-N)-propionic acid by comparison of the natural amino acid with the L-isomer obtained from synthetic material.[4,5] Additional supporting evidence was provided by the stoichiometric conversion of I to pyrazole, pyruvic acid and ammonia (reaction 1) by a pyrazolealaninase enzyme obtained from a strain of *Pseudomonas cruciviae*, grown in a medium in which β-(pyrazolyl-N)-L-alanine provided the carbon and nitrogen sources.[5]

(1) 

I

Murakoshi, Kuramoto, Haginiwa, and Fowden (1972) reported that β-(pyrazolyl-N)-alanine(I) was synthesized from pyrazole and O-acetylserine by an enzyme from watermelon seedlings (*Citrullus vulgaris*); no activity was detected when either serine or O-phosphoserine replaced serine as a substrate for the enzyme (serine was incorporated into β-(pyrazolyl-N)-alanine if acetyl-CoA was added to enzymic incubation mixture).[6] The synthesis was presumed to involve the formation of an enzyme-bound α-aminoacrylate moiety, following an intramolecular elimination of acetate: other reactions such as enzymic synthesis of tryptophan from indol and serine,[7] of mimosine from 3,4-dihydroxypyridine and O-acetylserine,[6] and

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of S-substituted cysteines from mimosine[8] or dichrostachinic acid[9] and a suitable thiol, probably involve the formation of a similar intermediary complex.

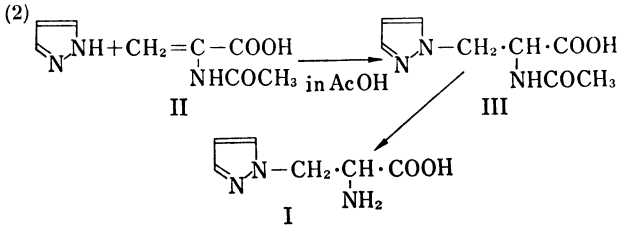
If this view is correct, α-acetamidoacrylic acid (II) might behave as a stable model compound of the enzyme-α-aminoacrylate complex and so react with pyrazole to give β-(pyrazolyl-N)-alanine, after a final acidic hydrolysis. This possibility was investigated under various conditions shown in Table I.

TABLE I. Reaction of α-Acetamidoacrylic Acid (II) with Pyrazole

Solvents	Reaction time (hr)	Temp. (°C)	Yield (%) of (I)
Acetate buffer[a]	20—25 days	room temp.	44
Acetic acid	1.5	reflux	94—96
Acetic acid	7	reflux	94—96
Dioxane	9	95—100	2
Triethylamine	4	reflux	trace
5% HCl–methanol	5	reflux	—
10% Na–methanol	5	reflux	trace

a) 0.1 M acetate buffer, pH 4.5: The mixture was allowed to stand, with occasionally stirring until α-acetamidoacrylic acid had dissolved in the buffer at room temperature.

(2)



α-Acetamidoacrylic acid (II), dissolved in acetic acid, reacted with pyrazole to form DL-α-acetamido-β-(pyrazolyl-N)-propionic acid (III) and subsequent hydrolysis by 10% hydrochloric acid gave β-(pyrazolyl-N)-DL-alanine (I) in 94—96% yield (reaction 2). β-(Pyrazolyl-N)-DL-alanine(I) formed was detected by paper chromatographic procedures, using ninhydrin as chromogenic reagent. The product co-chromatographed with authentic material in the following solvent systems: 1, butan-1-ol–acetic acid–water(90,10,29, by vol.); 2, butan-1-ol–ethanol–water (2,2,1, by vol.); 3, phenol–ethanol–water (3,1,1, by wt.); 4, butan-1-ol–pyridine–water(1,1,1, by vol.). *Rf* values determined for β-(pyrazolyl-N)-alanine in these solvents were 0.24, 0.15, 0.53, and 0.45, respectively. Further confirmation of the identity of the reaction product as β-(pyrazolyl-N)-alanine was obtained using an automatic amino acid analyzer (Shibata model AA-500, Tokyo). Under standard operating condition (150 cm column, 50°, 0.2N sodium citrate buffer, pH 3.25, flow rate 0.5 ml/min), β-(pyrazolyl-N)-alanine(I) eluted from column at about 156 ml, *i.e.*, at a position between the peaks of threonine and serine. The automatic amino acid analyzer was used to determine the percentage yield of β-(pyrazolyl-N)-alanine, based on the amount of pyrazole used.

Experimental

α-Acetamidoacrylic Acid (II)——II was prepared following the procedure of Arnstein and Clubb.[10] Pyruvic acid (44 g, 0.5 mole) was condensed with acetamide (59 g, 1.0 mole) by azeotropic distillation in toluene (150 ml) at a bath temperature of 160—170°. The toluene was decanted and the brown crystalline residue was washed with warm ethanol, yielding diacetamidopropionic acid (40—43 g, 40—45%), mp 197°.

A solution of diacetamidopropionic acid (10 g) in acetic acid (70 ml) containing 1 drop of conc. HCl was boiled gently for 10 min; on cooling, α-acetamidoacrylic acid crystallized as fine needles (yield 5 g, 73%), mp 204—205°.

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α-Acetamido-β-(pyrazolyl-N)-propionic Acid (III)——Pyrazole (70 mg, 1 mmole) and α-acetamidoacrylic acid (140 mg, 1.1 mmole), in 3 ml of 0.1M acetate buffer (pH 4.25), were allowed to stand at room temperature (18—20°) with occasionally stirring until all the α-acetamidoacrylic acid had dissolved. Alternatively, pyrazole (70 mg) and α-acetamidoacrylic acid (140 mg), in 3—4 ml of acetic acid were boiled gently for 1.5 hr. The residue, obtained after removal of acetic acid under reduced pressure, was dissolved in a small volume of ethanol, and then a little ether was added to the solution to precipitate α-acetamido-β-(pyrazolyl-N)-propionic acid (185 mg). Recrystallization from water gave colourless needles, mp 156—157°. *Anal.* Calcd. for $C_8H_{11}O_3N_3$: C, 48.72; H, 5.62; N, 21.31. Found: C, 48.53; H, 5.61; N, 21.45.

α-Amino-β-(pyrazoyl-N)-propionic Acid (I, β-(1-Pyrazolyl)alanine)——α-Acetamido-β-(pyrazolyl-N)-propionic acid (III) was hydrolyzed by treatment with 10% (w/v) HCl for 2 hr at 100°. Alternatively, the residue obtained in the previous step, after evaporation of acetic acid, was boiled directly with 10% HCl for 2 hr. After hydrolysis, HCl was removed by evaporation, and the resulting solution applied to a Amberlite IR-120 (H^+ form) column. After washing, the amino acid was eluted with 3% (w/v) ammonia; after decolorization and evaporation, the residue was recrystallized from 50% (v/v) ethanol to yield pure β-(1-pyrazolyl)alanine (I) in colourless plates, mp 243—246°. *Anal.* Calcd. for $C_6H_9O_2N_3$: C, 46.44; H, 5.85; N, 27.07. Found: C, 46.34; H, 5.81; N, 27.18.

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Reaction of Biguanides and Related Compounds. III.[1] Reaction of Biguanide and Amidinoisourea with Oxamate

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In the previous papers,[3-5] it was reported that the reaction of various 1-substituted biguanides with diethyl oxalate proceeded readily to afford 4-amino-6-substituted amino-*s*-triazine-2-carboxylate through the formation of intermediate five-membered ring compound. In this connection, the early studies[6,7] of the similar addition of 1-substituted biguanide to carboxylic ester or carboxamide to give 4,6-diamino-2-substituted *s*-triazine are of interest. We have now extended this addition to study the reaction of 1-substituted biguanide or N-amidinoisourea with oxamate. The following three compounds (I—III) are possible to form by this reaction, owing to the difference of reactivity of the carboxylic ester and carboxamide group in oxamate.

When 1-substituted biguanide was treated with an equivalent amount of ethyl N-alkyl-oxamate in methanol at room temperature, a product was obtained in fairly good yield. The

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