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THE SYNTHESIS OF L-α-AMINO-β-(PYRAZOLYL-N)-PROPIONIC ACID IN *CITRULLUS VULGARIS*

N. SUGIMOTO, H. WATANABE and A. IDE
Osaka Research Laboratory, Tanabe Seiyaku Co. Ltd., Higashiyodogawa-ku Osaka, Japan

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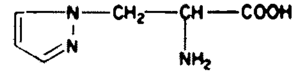
Abstract—The structure of the amino acid, L-α-amino-β-(pyrazolyl-N)-propionic acid, isolated from the semen of the water melon, *Citrullus vulgaris*, has been confirmed by synthesis.

α-AMINO-β-(PYRAZOLYL-N)-PROPIONIC acid, isolated from the semen of *Citrullus vulgaris*, is so far the only naturally occurring amino acid with a pyrazole structure and with the alanine group directly attached to the N of the heterocyclic ring.

Shinano *et al.*[1] isolated the amino acid (I) from the juice of *Citrullus vulgaris* and assumed the structure to be either α-amino-α-(imidazolyl-N)-propionic acid or α-amino-β-(imidazolyl-N)-propionic acid. Later, Fowden *et al.*[2] proposed the correct structure for I on partially convincing evidence and this hypothesis has now been confirmed by synthesis.

Pyrazole is readily synthesized in good yield by the reaction of tetramethoxypropane with hydrazine sulphate. The sodium salt of pyrazole was condensed with bromethylacetal in xylene yielding α-(pyrazolyl-N)-acetaldehyde. Addition of ammonium cyanide and subsequent hydrolysis according to the Strecker synthesis gave DL-α-amino-β-(pyrazolyl-N)-propionic acid. The latter was resolved after acetylation by addition of acylase from *Aspergillus oryzae* according to Chibata *et al.*[3] As only the L-acid is deacetylated, the L-amino acid may be separated from the mixture. This synthetic L-α-amino-β-(pyrazolyl-N)-propionic acid is identical in every respect with the naturally occurring compound isolated from the semen of *Citrullus vulgaris.*



I

EXPERIMENTAL

Pyrazole. A mixture of tetramethoxypropane (297 g) and hydrazine sulphate (214 g) was heated with stirring for 3 hr. The reaction mixture was concentrated to ½ volume under reduced press, neutralized with sodium carbonate, ether extracted, dried and evaporated. The residual oil was purified by vacuum distillation giving pyrazole (68 g; 62%) b.p. 108–110°/45 mm and crystallized from benzene in needles m.p. 70°.

*Diethyl-α-(pyrazolyl-*N*)-ethylacetal.* To sodium amide solution, prepared from sodium (23 g) in ammonium hydroxide (200 cc) and ferrous nitrate (0·02 g), pyrazole (70 g) was added and stirring continued for 30 min. Xylene (200 cc) was added and the solution warmed until the ammonia had

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[1] S. Shinano and T. Kaya, *Nippon Nogei Kagaku Kaishi* **31**, 759 (1957).

[2] F. F. Noe and L. Fowden, *Nature, Lond.* **193**, 69 (1959); L. Fowden, F. F. Noe, Ridd and White, *Proc. Chem. Soc.* 131 (1959).

[3] I. Chibata, T. Ishikawa and S. Yamada, *Bull. Agric. Chem. Soc. Japan* **21**, 304 (1957).

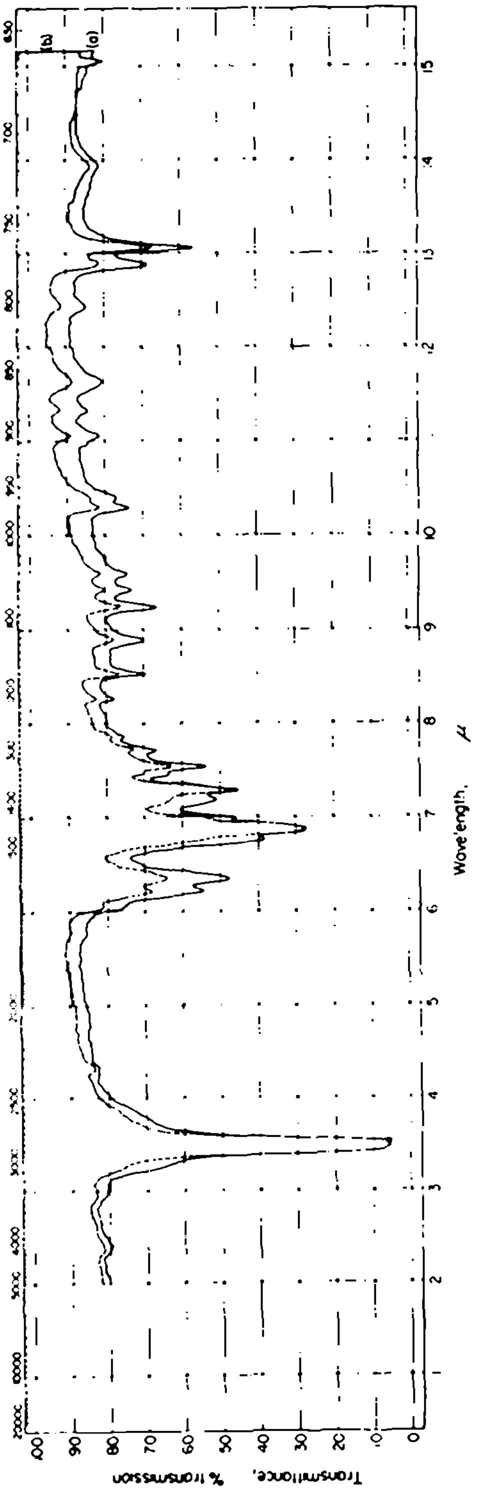

FIG. 1. I. R. spectra of synthesized and natural amino acids.
(a) Synthesized
(b) Natural

been completely eliminated. The sodium pyrazole in xylene was refluxed for 10 hr with diethyl-α-bromethylacetal (220 g) and after cooling the xylene layer washed with water, dried and evaporated under reduced press. The yield of diethyl-α-(pyrazolyl-N)-ethylacetal, b.p. 112–119°/23 mm was 56·3 g (32·4%) and bromacetal (43 g) was recovered. (Found: C, 58·27; H, 8·58; N, 13·94. $C_9H_{16}N_2O_2$ requires: C, 58·67; H, 8·75; N, 14·21%).

α-(*Pyrazolyl*-N)-*acetaldehyde*. Diethyl-α-(pyrazolyl-N)-ethylacetal (20 g) was added to 5% HCl (100 cc) at 90–95° and the temp maintained for 5 min. The solution was cooled rapidly with ice water, the pH adjusted to 7·0 with sodium bicarbonate, extracted with chloroform, dried, evaporated and distilled under reduced press in an atmosphere of nitrogen, yielding α-(pyrazolyl-N)-acetaldehyde 7·4 g (59%) b.p. 66–70°/0·7 mm. The 2,4-dinitrophenylhydrazone crystallized from ethanol in yellow needles m.p. 173–173·5°. (Found: C, 45·56; H, 3·85; N, 28·97. $C_{11}H_{10}O_4N_6$ requires: C, 45·52; H, 3·47; N, 28·96%).

R_f VALUES OF SYNTHESIZED AND NATURAL AMINO ACIDS

Solvent used	R_f Values	
	Synthesized	Natural
BuOH : AcOH : H_2O (4 : 2 : 1)	0·42	0·41
BuOH : AcOH : H_2O (2 : 2 : 1)	0·45	0·45
PhOH : 0·1% NH_3 aq. sol. (4 : 1)	0·74	0·73

DL-α-*Amino*-β-(*pyrazolyl*-N)-*propionic acid*. α-(Pyrazolyl-N)-acetaldehyde (22·8 g) in ether (100 cc) was saturated with dry ammonia at 0° and the resulting solution allowed to stand overnight. The residue, after evaporation of the ether, was dissolved in a small portion of water, 12% HCN solution (48·3 cc) added and stirring continued at room temp for 10 hr. The aminonitrile was hydrolysed by refluxing with 25% HCl (210 cc) for 2 hr, the solution was evaporated to dryness under reduced press, the residue dissolved in water and decolourized with animal charcoal. Ammonium hydroxide was added to the filtrate till pH 7·0 and an equal volume of ethanol added. The product, (2·1 g; 9·5%) crystallized from water in colourless plates m.p. 243–244°. (Found: C, 46·58; H, 6·01; N, 26·87. $CH_9O_2N_3$ requires: C, 46·44; H, 5·85; N, 27·07%).

DL-α-*Acetamino*-β-(*pyrazolyl*-N)-*propionic acid*. The DL-amino acid (1·7 g) dissolved in 10% NaOH (4·4 cc) was acetylated with acetic anhydride (1·34 g) and 10% NaOH (5·2 cc) in the usual manner yielding the acetyl derivative as colourless needles (1·4 g) from water m.p. 153–153·5°. (Found C, 48·76; H, 5·54; N, 20·94. $C_8H_{11}O_3N_3$ requires: C, 48·72; H, 5·62; N, 21·31%).

L-α-*Amino*-β-(*pyrazolyl*-N)-*propionic acid*. The DL-acetamino acid (1·2 g) was dissolved in water (6 cc) and the pH adjusted to 7·2 with 2N NaOH. To this solution, 5×10^{-4} mole cobaltous chloride (0·6 cc), crude acylase (32 mg; extracted from *Aspergillus oryzae* with water and precipitated by addition of an equal volume of acetone) and a drop of toluene was added. After incubation at 37° for 50 hr, the pH was adjusted to 5 with dil HCl and the mixture boiled for 5 min to coagulate the protein, filtered and concentrated to 1/3 volume under reduced press. Ethanol (10 cc) was added and the solution allowed to stand overnight, yielding L-α-amino-β-(pyrazolyl-N)-propionic acid (200 mg) which crystallized from water in colourless plates m.p. 243–244° (decomp). (Found: C, 45·86; H, 5·52; N, 26·78. $C_6H_9O_2N_3$ requires: C, 46·44; H, 5·85; N, 27·07%). The synthetic L-α-amino-β-(pyrazolyl-N)-propionic acid is identical in all respects with the natural product.

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