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# Amino Acids. I. DL-β-(Diazaphenyl)alanines (1)

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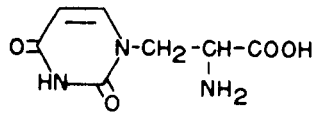
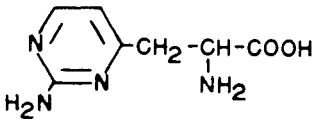
With the publication of this work, all six theoretically possible isomers of DL-diazaphenylalanines have now been reported. 2-Pyrazinyl- (VII), 3-pyridazinyl- (VIII), 4-pyridazinyl- (IX), 4-pyrimidinyl- (X), and 2-pyrimidinylalanine (XI) were synthesized by the catalytic (in the cases of VII-X) or stannous chloride (in the case of XI) reduction of the corresponding α-oximino-β-(diazaphenyl)propionic acids. These compounds were in turn prepared by the Claisen condensation of appropriate methyldiazines with diethyl oxalate followed by treatment of the resulting pyruvic ester with hydroxylamine.

Condensation of 5-bromomethylpyrimidine with diethyl N-carbobenzoxyaminomalonate yielded diethyl N-carbobenzoxyamino-(5-pyrimidinylmethyl)malonate. Hydrolysis and debenzylation of the condensation product readily gave the desired 5-pyrimidinylalanine (XII).

Natural products containing a β-substituted alanine moiety attached to heterocyclic ring systems have attracted considerable attention in recent years. Willardiine (2) (I), an amino acid isolated from *Acacia willardiana*, is a uracil derivative with the alanine moiety substituted through N-1 atom. Lathyrine (3) (II), isolated from seeds of *Lathyrus tingitanus*, is another pyrimidine amino acid with the alanine moiety attached to the ring through a C-C linkage. Noe and Fowden (4) reported that a new amino acid isolated from *Citrullus vulgaris*, β-1-pyrazolylalanine (III), is isomeric with histidine (IV). The structure of stizolobic acid (5), a constituent of the epicotyl tips of etiolated seedlings of *Stizolobium hassjou*, is believed to be β-(3-carboxy-γ-pyron-5-yl)alanine (V).



I



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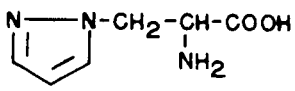

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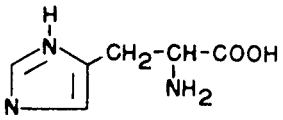

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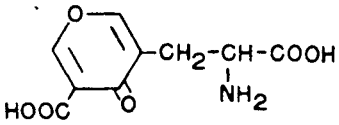

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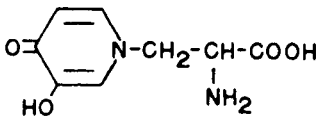

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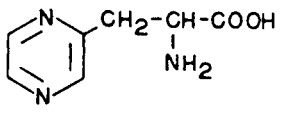

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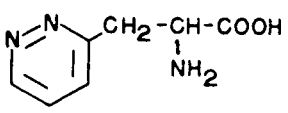

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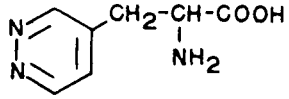

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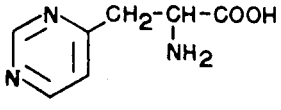

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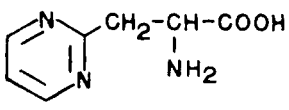
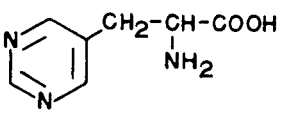


XI



XII

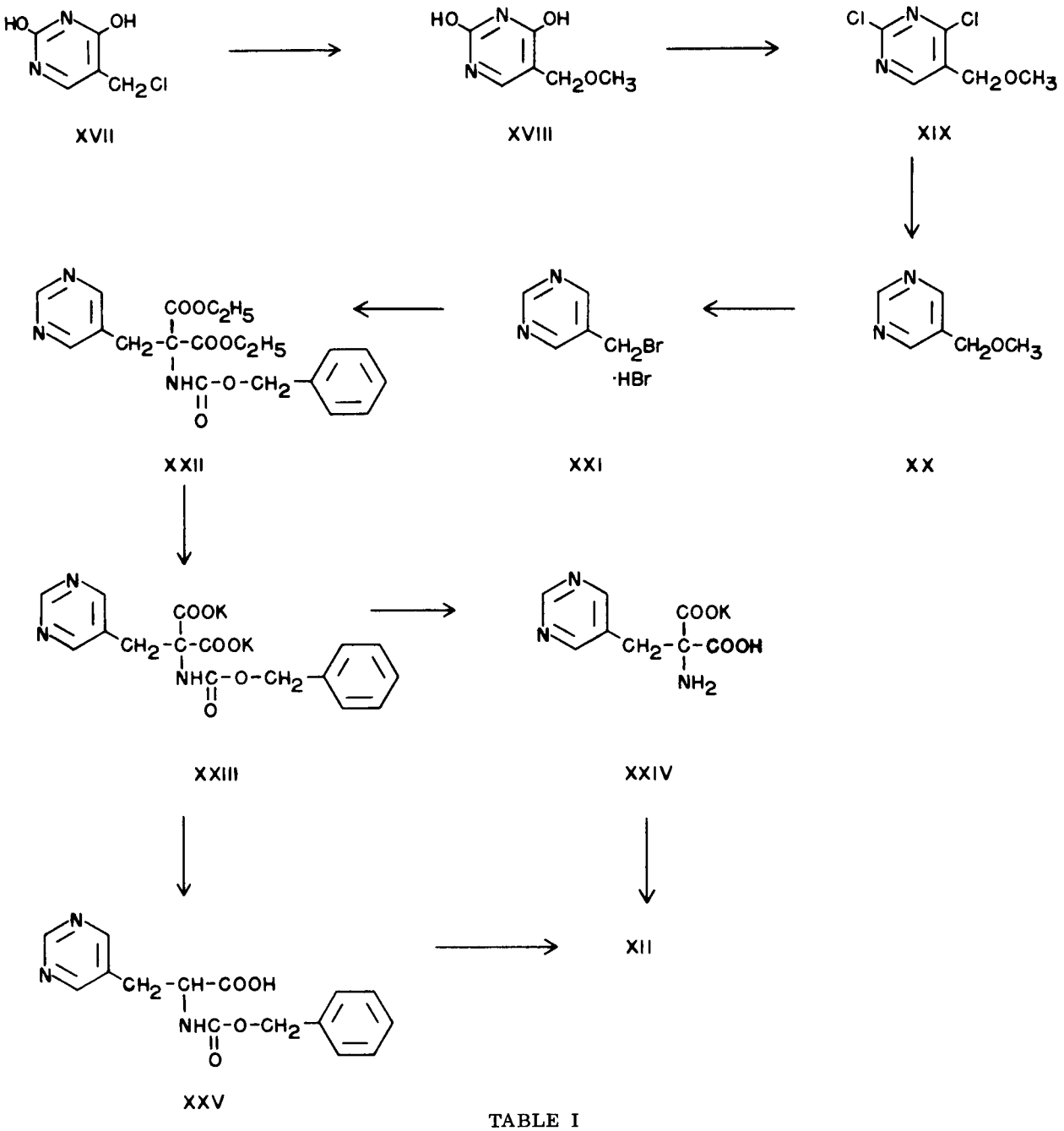



TABLE I

Observed Spectral Parameters for the Diazaphenylalanine $A_2B$ Systems

| Compound | Chemical Shift (a) (p.p.m.) | J/δ | J (c.p.s.) | Relative Chemical Shift (b) (c.p.s.) |
|---|---|---|---|---|
| 5-Pyrimidinyl (XII) | 3.98 | 0.136 | 6.3 | 46.2 |
| 4-Pyridazinyl (IX) | 4.08 | 0.136 | 6.5 | 47.8 |
| 2-Pyrazinyl (VII) | 4.19 | 0.142 | 6.3 | 44.3 |
| 3-Pyridazinyl (VIII) | 4.21 | 0.154 | 6.4 | 41.5 |
| 4-Pyrimidinyl (X) | 4.23 | 0.138 | 6.5 | 47.2 |
| 2-Pyrimidinyl (XI) | 4.62 | 0.107 | 5.8 | 54.3 |

(a) The measured shift of the α-proton. (b) The shift of the β-protons at 60 Mc., measured from the position of the α-protons.

A tyrosine analog, mimosine (6) (VI), an optically active form of leucenol isolated from the seeds and foliage of *Leucoena glauca* and *Mimosa pudica*, was found to inhibit tyrosine decarboxylase and competitively inhibit tyrosinase. Consequently, the compound inhibits the growth of hair in the anagen phase (the phase during which the rate of cell division is most rapid) in animals. This information suggests that certain compounds of this type may possess specific inhibitory properties against rapidly dividing cells without causing appreciable damage to the normal cells.

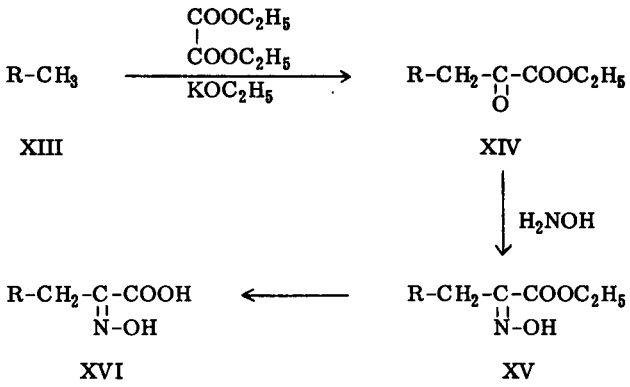
Many amino acids containing the alanine moiety, such as the widely known diazoacetate of L-serine (7) (azaserine, or O-diazoacetyl-L-serine) and 6-diazo-5-oxo-L-norleucine (8) (DON), possess significant antitumor activity by virtue of the inhibition of enzyme systems in the biosynthesis of purines.

More recently, Rennant and Anker (9) reported that when mice with different leukemias (especially with chronic lymphocytic leukemia) were treated with 5',5',5'-trifluoroleucine, the life span was appreciably extended. Although the mechanism of action of this compound is not yet clear, there are indications that this amino acid might interrupt the process of virus replication by interfering with the normal function of leucine and valine.

In accord with the preceding information, our initial consideration was focused upon the study of some alanine-containing heterocyclic ring systems (10,11). The present paper reports the synthesis of diaza analogs of phenylalanine. This important amino acid has been receiving much attention lately since (a) phenylalanine was found to be a precursor of tropic acid (12), (b) the closely related *p*-fluorophenylalanine inhibits the incorporation of DL-tryptophane into cell proteins (13), (c) phenylalanine is an essential metabolite for the influenza A and Newcastle Disease Viruses (14a), and (d) many ring-substituted phenylalanine derivatives have been prepared for virus inhibitory studies (14b,c).

Theoretically, replacement of the phenyl ring of phenylalanine by six-membered, diaza ring systems provides the following six isomers: 2-pyrazinyl- (VII), 3-pyridazinyl- (VIII), 4-pyridazinyl- (IX), 4-pyrimidinyl- (X), 2-pyrimidinyl- (XI), and 5-pyrimidinylalanine (XII).

Claisen condensation reactions of the methyl groups of 2-methylpyrazine (15), 3-methylpyridazine (16), 2-methylpyrimidine (17), and 4-methylpyrimidine (17b, 18) with diethyl oxalate have been reported (19,20). The 5-methylpyrimidine, as indicated by Pfleiderer and Mosthaf (19), does not undergo this type of reaction. Although a survey of the literature failed to reveal the same reaction for 4-methylpyridazine, it was readily accomplished in our laboratories. Using this approach compounds VII-X were synthesized by the following general reaction scheme: Reaction between the methylated diazines (XIII) with ethyl oxalate proceeded smoothly at room temperature in the presence of potassium ethoxide to yield the ethyl ester of the corresponding pyruvic acids (XIV). Hydroxylamine then converted XIV into the α-oximino derivatives XV. The latter were hydrolyzed (to yield XVI) and then catalytically reduced to the amino acids (21).




a. R = 2-pyrazinyl
b. R = 3-pyridazinyl
c. R = 4-pyridazinyl
d. R = 4-pyrimidinyl
e. R = 2-pyrimidinyl

α-Oximino-β-(2-pyrimidinyl)propionic acid (XVIe) was readily obtained from 2-methylpyrimidine (XIIIe) *via* the route XIIIe ⟶ XIVe ⟶ XVe⟶ XVIe. However, catalytic hydrogenation of XVIe under conditions similar to those for the preparation of the other amino acids gave unexpected results. It was found that the absorption of two molar-equivalents of hydrogen was complete within 5 minutes. Investigation on the resulting product furnished the following information: (a) the product failed to give a positive ninhydrin test; (b) only a weak ultraviolet absorption at *p*H 11 was observed, and none at *p*H 1 (22); (c) elementary analyses of carbon, hydrogen and nitrogen (empirical formula: $C_7H_{11}N_3O_3$; empirical formula for desired compound: $C_7H_9N_3O_2$) revealed the presence of two extra atoms of hydrogen and one atom of oxygen; Karl-Fischer determination ruled out the possibility of water of crystallization; (d) the out-of-plane bending infrared absorption spectrum for aromatic hydrogens (23), which were observed in the spectra of compounds VII-X, was absent; (e) the n.m.r. spectra also indicated the absence of aromatic hydrogen atoms. This information implies that the product underwent nuclear-hydrogenation rather than the desired side-chain reduction.

Of the three ring systems investigated in our present work, the pyridazine ring was reported to be unaffected by hydrogen at three atmospheres pressure in the presence of palladium-on-charcoal (24). Hydrogenation of pyrazines has been achieved with platinum as a catalyst (25). When palladium-black was used, the pyrazine ring underwent nuclear hydrogenation at a very slow rate (25a). (This explains the fact that we were able to prepare DL-β-(2-pyrazinyl)alanine (VII) from XVIa, although in low yield (26)). Pyrimidine and its homologs, according to Smith and Christensen (27), could be

readily reduced catalytically in acid medium, but reduction was not effected under neutral or basic conditions (27). In order to further understand the nature of nuclear hydrogenation, 4-methylpyrimidine (17b,18) and 2-methylpyrimidine (17) were hydrogenated in the presence of palladium-on-charcoal. It was found that 4-methylpyrimidine was only partially reduced under neutral conditions to the corresponding dihydropyrimidine in 20 hours. On the other hand, the absorption of one mole of hydrogen per mole of 2-methylpyrimidine was essentially complete in 30 minutes (28). The uptake of hydrogen by 2-methylpyrimidine was also observed in basic medium with either palladium-on-charcoal or Raney nickel. The desired DL-β-(2-pyrimidinyl)alanine (XI) was finally prepared, in good yield, by stannous chloride reduction of XVIe.

The DL-β-(5-pyrimidinyl)alanine (XII) was synthesized as follows: 5-methoxymethyluracil (29) (XVIII), prepared in our laboratory by refluxing a mixture of 5-chloromethyluracil (30) (XVII) with excess sodium methoxide in methanol, was treated with phosphorus oxychloride to give 2,4-dichloro-5-methoxymethylpyrimidine (XIX). Catalytic hydrogenation of XIX yielded 5-methoxymethylpyrimidine (XX), which was converted *in situ* to 5-bromomethylpyrimidine hydrobromide (XXI). Condensation of XXI with the sodium salt of diethyl ester of N-(carbobenzoxyamino)malonic acid (31) gave the diethyl ester of 5-(pyrimidinylmethyl)-N-(carbobenzoxyamino)malonic acid (XXII). Ethanolic potassium hydroxide at room temperature converted XXII to the corresponding dipotassium salt XXIII. Hydrogenation of XXIII followed by neutralization gave the monopotassium salt of amino-5-(pyrimidinylmethyl)-malonic acid (XXIV). On the other hand, acidification of XXIII yielded 5-pyrimidinyl-N-carbobenzoxyalanine (XXV). The desired DL-β-(5-pyrimidinyl)-alanine (XII) was obtained from XXIV by acidification and from XXV by catalytic hydrogenation.

N.M.R. spectra (32) for the six diaza analogs of phenylalanine were generally similar, showing aromatic absorptions in the region of 6-10 p.p.m. and absorptions for the α- and β- protons (with respect to the carboxylic acid group) in the region of 3.5-5 p.p.m. Characteristic group formations in the aromatic portion of the spectrum were observed for the 2-, 4- and 5-pyrimidinyl-, and the 2-pyridazinyl-alanines. Unresolved aromatic group formations appeared for the 3-pyridazinyl- and 2-pyrazinyl-derivatives, but these were not inconsistent with the structural assignments.

The α- and β-alanine protons appeared in all spectra as $A_2B$ systems, showing weak to intermediate coupling (33). The spectral parameters of these systems are shown in Table I. The increasing chemical shift of the α-proton may be interpreted as the decreasing in electron density at the α-carbon.

## EXPERIMENTAL (34)

General Preparation of Diazaphenylpyruvates (XIV).

The following method can be used for either large or small scale preparation. A 2-l. three-necked flask equipped with mechanical stirrer, reflux condenser and dropping funnel was thoroughly purged with dry nitrogen. Twenty-one and one-half grams (0.55 g.-atom) of potassium was placed in the flask and, with caution, 100 ml. of absolute ethanol was introduced through the dropping funnel. Rapid stirring of the molten potassium dispersed the mass into small particles. In this manner the potassium reacted quickly and safely. After the solution was complete, 1 l. of absolute ether was added to the potassium ethoxide solution, followed by addition of 80.2 g. (0.55 mole) of purified diethyl oxalate with stirring. After 10 min. the appropriate methyl diazine was added. The reaction mixture was stirred at room temperature for 72 hr. under a slightly positive pressure of dry nitrogen. The resulting yellow precipitate was filtered, washed with ether, dried *in vacuo*, and then dissolved in a minimum amount of cold water. Acidification of the solution with glacial acetic acid produced a yellow precipitate, which was filtered, dried and recrystallized from ethanol.

In the case of the ethyl ester of 2-pyrimidinylpyruvic acid, which was found to be quite water soluble, the potassium salt was suspended in benzene and, with rapid stirring, acidified with glacial acetic acid. The resulting potassium acetate was filtered after 10 min., the filtrate evaporated to dryness *in vacuo*, and the residue recrystallized from ethanol.

Ethyl 2-pyrazinylpyruvate (XIVa) was obtained in 42% yield, m.p. 72-73° (15).

Ethyl 3-pyridazinylpyruvate (XIVb) was obtained in 42% yield, m.p. 104-105° (16).

Ethyl 4-pyridazinylpyruvate (XIVc) was obtained in 92% yield, m.p. 151-152°.

*Anal.* Calcd. for $C_9H_{10}N_2O_3$: C, 55.7; H, 5.2; N, 14.4. Found: C, 56.0; H, 5.5; N, 14.7.

Ethyl 4-pyrimidinylpyruvate (XIVd) was obtained in 78% yield, m.p. 133-134° (17b,18).

Ethyl 2-pyrimidinylpyruvate (XIVe) was obtained in 59% yield, m.p. 62-63° (17).

General Preparation of Ethyl α-Oximino-β-diazaphenylpropionates (XV).

The appropriate ethyl diazaphenylpyruvate (XIV) (19.4 g.; 0.1 mole) was refluxed 2 hr. with a mixture of 7.0 g. (0.1 mole) of hydroxylamine hydrochloride and 14 g. (0.1 mole) of sodium acetate trihydrate in 250 ml. of ethanol. The reaction mixture was filtered while hot and the filtrate reduced to dryness.

When excess hydroxylamine hydrochloride (and corresponding excess sodium acetate) was used, the yield of XV was drastically reduced.

Ethyl α-oximino-α-(2-pyrazinyl)propionate (XVa) was obtained, after recrystallization from ethanol, in 82% yield, m.p. 133-134°.

*Anal.* Calcd. for $C_9H_{11}N_3O_3$: C, 51.7; H, 5.3; N, 20.1. Found: C, 51.4; H, 5.5; N, 20.1.

Ethyl α-oximino-α-(3-pyridazinyl)propionate (XVb) was obtained, after recrystallization from ethanol, in 67% yield, m.p. 138-139°.

*Anal.* Calcd. for $C_9H_{11}N_3O_3$: C, 51.7; H, 5.3; N, 20.1. Found: C, 51.8; H, 5.7; N, 20.0.

Ethyl α-oximino-β-(4-pyridazinyl)propionate (XVc) was obtained, after recrystallization from ethanol, in 89% yield, m.p. 173-174°.

*Anal.* Calcd. for $C_9H_{11}N_3O_3$: C, 51.7; H, 5.3; N, 20.1. Found: C, 51.8; H, 5.7; N, 20.2.

Ethyl α-oximino-β-(4-pyrimidinyl)propionate (XVd) was obtained, after recrystallization from ethyl acetate, in 80% yield, m.p. 98-99° (21).

Ethyl α-oximino-β-(2-pyrimidinyl)propionate (XVe).

The crude product was purified by dissolving it in ethyl acetate, filtering, then adding petroleum ether (b.p. 35-60°) to the filtrate (ratio of ethyl acetate vs. petroleum ether, 2:1). On chilling the solution to -60° there was obtained a 58% yield of product, m.p. 81-82°.

*Anal.* Calcd. for $C_9H_{11}N_3O_3$: C, 51.7; H, 5.3; N, 20.1. Found: C, 51.6; H, 5.3; N, 20.2.

General Preparation of α-Oximino-β-(diazaphenyl)propionic Acids (XVI).

To 150 ml. of 5% sodium hydroxide heated to boiling was added 20.9 g. (0.1 mole) of XV. The mixture was refluxed for 5 min. and the hot solution acidified to *p*H 1 with concentrated hydrochloric acid. Upon cooling, long white needles of analytical purity were formed. They were collected by filtration and dried. Further recrystallization of the products was not necessary.

α-Oximino-β-(2-pyrazinyl)propionic acid (XVIa) was obtained in 63% yield, m.p. 188-189° dec. λ max (*p*H 1), 268 mμ (ε, 7,600); λ max (*p*H 11), 255 mμ (ε, 10,300).

*Anal.* Calcd. for $C_7H_7N_3O_3$: C, 46.4; H, 3.9; N, 23.2. Found: C, 46.2; H, 4.0; N, 23.1.

α-Oximino-β-(3-pyridazinyl)propionic acid (XVIb) was obtained in 66% yield, m.p. 182-183° dec. λ shoulder (*p*H 1), 243 mμ (ε, 4,200); λ max (*p*H 11), 245 mμ (ε, 10,500).

*Anal.* Calcd. for $C_7H_7N_3O_3$: C, 46.4; H, 3.9; N, 23.2. Found: C, 46.2; H, 4.1; N, 23.0.

α-Oximino-β-(4-pyridazinyl)propionic acid (XVIc) was obtained in 80% yield, m.p. 175-176° dec. λ max (*p*H 11), 245 mμ (ε, 10,800).

*Anal.* Calcd. for $C_7H_7N_3O_3$: C, 46.4; H, 3.9; N, 23.2. Found: C, 46.7; H, 4.0; N, 23.4.

α-Oximino-β-(4-pyrimidinyl)propionic acid (XVId) was obtained in 91% yield, m.p. 185-186° dec. λ max (*p*H 1), 242 mμ (ε, 6,200); λ max (*p*H 11), 245 mμ (ε, 11,000).

*Anal.* Calcd. for $C_7H_7N_3O_3$: C, 46.4; H, 3.9; N, 23.2. Found: C, 46.1; H, 4.1; N, 23.0.

α-Oximino-β-(2-pyrimidinyl)propionic acid (XVIe) was obtained in 62% yield, m.p. 177-178° dec. λ max (*p*H 1), 248 mμ (ε, 6,200); λ max (*p*H 11), 245 mμ (ε, 10,300).

*Anal.* Calcd. for $C_7H_7N_3O_3$: C, 46.4; H, 3.9; N, 23.2. Found: C, 46.1; H, 4.0; N, 22.9.

General Preparation of DL-β-(diazaphenyl)alanines (VII-X).

A solution of 4.5 g. (0.025 mole) of the appropriate α-oximino-β-diazaphenylpropionic acid (XVI) in 200 ml. of 5% aqueous ammonia was hydrogenated at 60 p.s.i.g. (4.22 kg/cm$^2$) in the presence of 2 g. of 10% palladium-on-charcoal. Theoretical uptake of hydrogen took place within 30 min. The reaction mixture was filtered and the filtrate reduced to *ca.* 30 ml. *in vacuo*. The *p*H of the resulting solution was adjusted to 4-5 with concentrated hydrochloric acid. After refrigerating overnight crystals of analytical purity separated from the solution (sometimes a small amount of ethanol was added to induce the crystallization).

In the reduction of α-oximino-β-(2-pyrimidinyl)propionic acid, 200 ml. of methanol was used in place of 5% aqueous ammonia.

Methanol could not be used in the reduction of the other α-oximino-β-(diazaphenyl)propionic acids due to their insolubility.

DL-β-(2-Pyrazinyl)alanine (VII), decomposed at 240-243°, was obtained in 10% yield. The product gave an orange-red coloration with ninhydrin. λ max (*p*H 1), 264 mμ (ε, 7,700); λ max (*p*H 11), 267 mμ (ε, 7,700). Attempted stannous chloride reduction of XVIa failed to yield VII.

*Anal.* Calcd. for $C_7H_9N_3O_2 \cdot {}^1/_4H_2O$: C, 49.0; H, 5.6; N, 24.5. Found: C, 49.1; H, 5.8; N, 24.6. Prolonged drying at 110° *in vacuo* did not remove the water of crystallization.

DL-β-(3-Pyridazinyl)alanine (VIII), decomposed at 224-226°, was obtained in 65% yield. The product gave an orange-red coloration with ninhydrin. λ max (*p*H 1), 244 mμ (ε, 2,000); λ max (*p*H 11), 251 mμ (ε, 2,000).

*Anal.* Calcd. for $C_7H_9N_3O_2$: C, 50.3; H, 5.4; N, 25.1. Found: C, 50.2; H, 5.5; N, 25.0.

DL-β-(4-Pyridazinyl)alanine (IX), decomposed at 235-236°, was obtained in 58% yield. The product gave a violet coloration with ninhydrin. λ max (*p*H 1), 239 mμ (ε, 3,000); λ max (*p*H 11), 246 mμ (ε, 2,700).

*Anal.* Calcd. for $C_7H_9N_3O_2$: C, 50.3; H, 5.4; N, 25.1. Found: C, 50.3; H, 5.7; N, 25.3.

DL-β-(4-Pyrimidinyl)alanine (X), decomposed at 208-210°, was obtained in 43% yield. The product gave an orange-red coloration with ninhydrin. λ max (*p*H 1), 244 mμ (ε, 7,000); λ max (*p*H 11), 245 mμ (ε, 7,000).

*Anal.* Calcd. for $C_7H_9N_3O_2$: C, 50.3; H, 5.4; N, 25.1. Found: C, 50.0; H, 5.6; N, 24.9.

Attempted Preparation of DL-β-(2-Pyrimidinyl)alanine (XI) by Catalytic Hydrogenation of α-Oximino-β-(2-pyrimidinyl)propionic Acid (XVIe).

A solution of 1.8 g. of analytically pure XVIe in 250 ml. of anhydrous methanol containing 0.9 g. of 5% palladium-on-charcoal was hydrogenated at 50° and 60 p.s.i.g. Two molar-equivalents of hydrogen were taken up within 20 min. The warm reaction mixture was quickly filtered and on cooling the filtrate yielded 1.5 g. of white solid which decomposed at 207-208° and gave a negative ninhydrin test. λ max (*p*H 11), 251 mμ (ε, 9,100).

*Anal.* Calcd. for $C_7H_{11}N_3O_3$: C, 45.4; H, 6.0; N, 22.7. Found: C, 45.5; H, 6.2; N, 22.7.

Preparation of DL-β-(2-pyrimidinyl)alanine (XI).

To a solution of 5.0 g. of stannous chloride (0.026 mole) in 20 ml. of concentrated hydrochloric acid was added 1.8 g. (0.01 mole) of α-oximino-β-(2-pyrimidinyl)propionic acid (XVIe) in one portion with stirring. There was an immediate heat response. After standing overnight at room temperature, the reaction mixture was diluted with 300 ml. of water, saturated with hydrogen sulfide and the resulting sulfide removed by filtration. The filtrate was evaporated *in vacuo* to an oily residue. The residual water in the product was removed by alternate azeotropic distillation with ethanol and benzene to give 1.6 g. (80% yield) of XI, isolated as a monohydrochloride salt. The crude product decomposed at 205-210°. An analytical sample was prepared by recrystallizing the product from boiling butanol in the presence of a few drops of water. The purified sample which decomposed at 210-212°, gave a brownish yellow coloration with ninhydrin. λ max (*p*H 1), 247 mμ (ε, 1,850); λ max (*p*H 11), 248 mμ (ε, 1,850).

*Anal.* Calcd. for $C_7H_9N_3O_2 \cdot HCl$: C, 41.3; H, 4.9; N, 20.6. Found: C, 41.5; H, 5.1; N, 20.6.

5-(Methoxymethyl)uracil (XVIII).

To a solution of 500 ml. of anhydrous methanol containing 7.3 g. (0.32 g.-atom) of sodium was added 48 g. (0.30 mole) of 5-(chloromethyl)uracil (30) (XVII). The mixture was refluxed for 30 min., the resulting sodium chloride removed by filtration, and the filtrate evaporated *in vacuo* to dryness. The solid residue was recrystallized from methanol to give 29.0 g. (62% yield) of XVIII, m.p. 203-204°. The product prepared by this method was found to be identical with that prepared by Cline, Fink and Fink (29).

2,4-Dichloro-5-(methoxymethyl)pyrimidine (XIX).

A mixture of 74 g. (0.474 mole) of XVIII, 74 g. of N,N-dimethylaniline and 350 ml. of phosphorus oxychloride was refluxed for 5 min. (prolonged reflux failed to give the desired product). Excess phosphorus oxychloride was removed *in vacuo* and the residue was poured onto ice flakes with vigorous stirring. The ice-cooled solution was then extracted with 3 x 300 ml. of ether. The ethereal extraction was washed with sodium bicarbonate solution, dried over anhydrous sodium sulfate, and distilled under reduced pressure. The product boiled at 95-97° at 1.5 mm. (38 g., 42% yield).

*Anal.* Calcd. for $C_6H_6Cl_2N_2O$: C, 37.3; H, 3.1; N, 14.5. Found: C, 36.9; H, 3.3; N, 14.6.

5-Methoxymethylpyrimidine (XX).

Thirty-five grams (0.18 mole) of XIX in 250 ml. of absolute ethanol was dehalogenated at 60 p.s.i.g. in a Parr hydrogenator in the presence of 1 g. (10%) of palladium-on-charcoal and 30 g. (0.36 mole) of anhydrous sodium acetate. The theoretical amount of hydrogen was consumed in 25 min. The reaction mixture was filtered and the filtrate concentrated to a syrup. Distillation of the syrup at 95-97° at 16 mm. gave 14.3 g. of XX, which was contaminated with a trace of acetic acid. The product was used for the next step without further purification.

5-Bromomethylpyrimidine Hydrobromide (XXI).

A solution of 12.4 g. of crude XX in 100 ml. of 32% of hydrobromic acid in 100 ml. of glacial acetic acid was heated at 100° for 3 hr. The resulting clear brown solution was concentrated at 35° *in vacuo* to a syrup (excessive heating of the residual syrup caused it to decompose). The syrup was triturated several times with anhydrous ether and the residue kept under 0.1 mm. for 18 hr. to remove the last traces of solvents. A light yellow solid (21.3 g. or 84% yield) was isolated which melted at 108-113°. (This product should be handled with care as it is extremely sternutatory and irritating to the eyes, nose and throat.)

*Anal.* Calcd. for $C_5H_5BrN_2 \cdot HBr$: N, 11.0; Br, 63.0. Found: N, 10.8; Br, 62.8.

Diethyl N-Carbobenzoxyamino-(5-pyrimidinylmethyl)malonate (XXII).

To a warm solution of 250 ml. of anhydrous methanol containing 2.7 g. (0.117 g.-atom) of sodium was added 19.4 g. (0.063 mole) of diethyl N-carbobenzoxyaminomalonate (24), with stirring. After 5 min., 14.6 g. (0.058 mole) of 5-bromomethylpyrimidine hydrobromide (XXI) was added in one portion whereupon a vigorous reaction started immediately. After the reaction had subdued the mixture was refluxed for 3 hr. (The reactions were run under anhydrous conditions.) The precipitated salt was removed by filtration and the filtrate evaporated *in vacuo*. The resulting residue was then extracted with ether and the ethereal extract washed twice with 5% sodium carbonate solution to remove the unreacted diethyl N-carbobenzoxyaminomalonate. The $Na_2SO_4$ dried ethereal extract was evaporated under reduced pressure to give 20.2 g. of brown oil. This oil, which resisted all attempts at crystallization, was used for the next step without further purification.

Dipotassium Salt of N-Carbobenzoxyamino-(5-pyrimidinylmethyl)malonic Acid (XXIII).

To a solution of 20 g. of potassium hydroxide in 80 ml. of absolute ethanol was added 20.2 g. of XXII. The mixture was allowed to stir at room temperature overnight. The solid which had precipitated overnight was filtered, washed thoroughly with absolute ethanol, and

dry ether. After drying, 13.0 g. (62% yield) of white crystalline compound was obtained, which decomposed at 225°. This compound, which was still slightly impure (*Anal.* Calcd. for $C_{16}H_{13}K_2N_3O_6$: N, 10.0. Found: N, 10.8), was used directly for the preparation of the following two compounds, XXIV and XXV.

Monopotassium Salt of Amino-5-(pyrimidinylmethyl)malonic Acid (XXIV).

A solution of 2.0 g. of XXIII in 50 ml. of water containing 0.2 g. of 10% palladium-on-charcoal was hydrogenated at 60 p.s.i.g. for 1 hr. The catalyst was removed (the odor of toluene was detected), the filtrate, after being adjusted to *p*H 6 with hydrochloric acid, was concentrated to 5 ml. *in vacuo*. The solution was then diluted with 10 ml. of ethanol. On cooling, white crystals deposited from the solution. The solid product was filtered and recrystallized from a small amount of water and ethanol to give 0.7 g. (57% yield) of long white needles, m.p. 189-196° dec.

*Anal.* Calcd. for $C_8H_8KN_3O_4 \cdot {}^1/_2H_2O$: C, 37.2; H, 3.5; N, 16.3. Found: C, 37.1; H, 3.8; N, 16.6.

DL-β-[N-Carbobenzoxy-5-(pyrimidinylmethyl)]alanine (XXV).

To a solution of 4.2 g. of XXIII in 15 ml. of water was added dropwise 4 ml. of concentrated hydrochloric acid. There was an immediate and vigorous evolution of carbon dioxide. The mixture was warmed carefully on the steam bath for 15 min. to completely eliminate carbon dioxide. On cooling, 1.9 g. (63% yield) of analytically pure product was gradually formed, m.p. 183-185° dec. λ max (*p*H 1), 248 mμ (ε, 3,100); λ max (*p*H 11), 249 mμ (ε, 3,000).

*Anal.* Calcd. for $C_{15}H_{15}N_3O_4$: C, 59.8; H, 5.0; N, 14.0. Found: C, 59.5; H, 5.1; N, 14.0.

DL-β-(5-Pyrimidinyl)alanine (XII).

In a hydrogenation bottle containing 300 ml. of warm methanol was added 1.5 g. of XXV. After flushing the bottle with dry nitrogen 0.3 g. of 10% palladium-on-charcoal was added and the mixture was hydrogenated at 30 p.s.i.g. for 45 min. After removal of the catalyst, the filtrate was evaporated to *ca.* 20 ml. *in vacuo*. On standing, 0.4 g. (47% yield) of white crystalline product precipitated. The product gave a violet coloration with ninhydrin. λ max (*p*H 1), 248 mμ (ε, 2,300); λ max (*p*H 11), 250 mμ (ε, 2,100). This compound was also prepared by acidification of XXIV in water with concentrated hydrochloric acid. Paper chromatographic measurement (25°, descending) of both products developed under a system of methanol-formic acid-water (15:3:1) gave identical $R_f$ values: 0.82.

*Anal.* Calcd. for $C_7H_9N_3O_2 \cdot {}^1/_4H_2O$: C, 49.0; H, 5.6; N, 24.5. Found: C, 48.6, 49.2; H, 5.7, 5.6; N, 24.7, 24.4.

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