Pyrimidines. XV. (Amino Acids. II.)

DL-β-(Dihydroxypyrimidinyl)alanines (1)

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The preparation of two uracil derivatives of alanine, DL-β-(5-uracilyl)alanine and DL-β-(6-uracilyl)alanine, are reported. A structural analog, DL-4,6-dihydroxy-β-(2-pyrimidinyl)alanine, which was reported to be labile to acid hydrolysis, has now been successfully prepared in our laboratories.

The relationship between the relative position of an alanine moiety on some nitrogen heterocyclic ring systems and their color formation with ninhydrin are discussed.

The striking structural similarity between willardiine (2) (I. an alanine-containing dioxopyrimidine) and mimosine (3,4) (II. an alanine-containing dioxopyridine) and their interesting biological activity illustrates the importance of compounds of this type and clearly indicates that other dioxoheterocyclic derivatives of β-substituted alanine should be investigated. In connection with our present study in the pyrimidine series, syntheses of three dioxopyrimidines with the alanine moiety attached to the 2-, 5-, and 6- positions of the pyrimidine ring were undertaken in our laboratory.

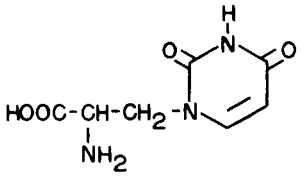
Reaction of 5-(chloromethyl)uracil (5) with diethyl acetamidomalonate (6) in basic medium gave the diethyl ester of acetamido[(2,4-dihydroxy-5-pyrimidinyl)methyl]malonic acid (III). Acid hydrolysis of III readily yielded DL-β-(5-uracilyl)alanine (IV) (7).

The isomeric DL-β-(6-uracilyl)alanine (VII) was prepared according to the method of Elliott, *et al.*, (8), for the synthesis of DL-β-(4-pyridyl)alanine: Claisen condensation of 2,4-diethoxy-6-methylpyrimidine (9) with diethyl oxalate produced ethyl 2,4-diethoxy-6-pyrimidinylpyruvate (V), which was converted to the oxime (VI) with hydroxylamine. Compound VI then underwent simultaneous reduction and hydrolysis with stannous chloride in concentrated hydrochloric acid to give DL-β-(6-uracilyl)alanine (VII) (10).

Shvachkin and Syrtsova (11) had attempted to prepare DL-4,6-dihydroxy-β-(2-pyrimidinyl)alanine (XII) by the acid hydrolysis of the dimethyl ester of amino[(4,6-dimethoxy-2-pyrimidinyl)methyl]malonic acid. Unexpected ring cleavage was noted by these authors and only aspartic acid was obtained (11). Compound XII has now been successfully prepared in our laboratory by the following procedure: 2-Methyl-4,6-dibenzyloxypyrimidine (VIII), prepared from 2-methyl-4,6-dichloropyrimidine (12) and benzyl alcohol, was condensed with diethyloxalate to give the ethyl ester of 4,6-dibenzyloxy-2-pyrimidinylpyruvic acid (IX). Compound IX was treated with hydroxylamine to form the oxime (Xa). Basic hydrolysis then converted Xa to the corresponding acid Xb. Debenzoylation of Xb was smoothly accomplished by hydrogenation in the presence of palladium-on-charcoal. The resulting intermediate, XI, was then converted to the desired product by Raney nickel reduction. Attempted direct conversion of Xb to XII by prolonged catalytic hydrogenation using palladium-on-charcoal yielded a dihydro-derivative of the desired product. Nuclear hydrogenation of the pyrimidine ring system has been discussed in our preceding paper (4).

Acid hydrolysis of XII readily yielded aspartic acid (11) as indicated by paper chromatographic comparison with an authentic sample of aspartic acid.

Some interesting observations in connection with the standard ninhydrin test for α-amino acids are worthy of note. In general, an α-amino acid reacts with ninhydrin to produce a violet coloration, which is believed to be the anion of diketohydrindylidene-diketohydrindamine (13). Cysteine, proline and hydroxyproline, on the other hand, form yellow colored intermediates with ninhydrin. Examination of the heterocyclic amino acids prepared in our laboratories revealed a correlation between structure and color developed with ninhydrin. Of the six amino acids previously reported (4), only DL-β-(4-pyridazinyl)-alanine and DL-β-(5-pyrimidinyl)alanine, in which the alanine moiety is separated from the ring nitrogen by two carbon atoms, gave the usual violet coloration with ninhydrin. When the alanine moiety is separated from the ring nitrogen by only one carbon atom, as in the case of DL-β-(2-pyrazinyl)-alanine, DL-β-(3-pyridazinyl)alanine, and DL-β-(4-pyrimidinyl)alanine, an orange-red coloration was noted with ninhydrin. When the carbon atom attached to the alanine moiety is situated between two nitrogen atoms, such as in the case of DL-β-(2-pyrimidinyl)alanine, only a brownish-yellow coloration was developed with ninhydrin. The same relationship was observed in the present series: DL-β-(5-Uracilyl)alanine (IV), violet; DL-β-(6-uracilyl)-alanine (VII), orange-red; and DL-4,6-dihydroxy-β-(2-pyrimidinyl)alanine (XII), brownish yellow. The naturally occurring amino acid lathyrine was also reported (14) to give an orange-red color with ninhydrin. Hydrogen bonding between the ring nitrogen and the alanine moiety probably plays an important role in the different color formation with ninhydrin. This assumption is further substantiated by the fact that both the dihydro derivative and the hydrolyzed



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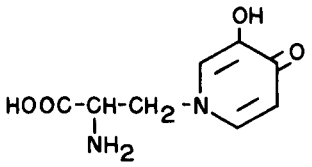

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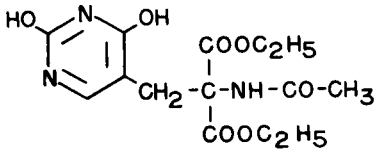

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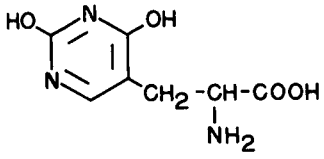

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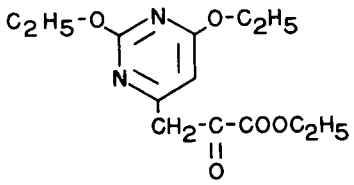

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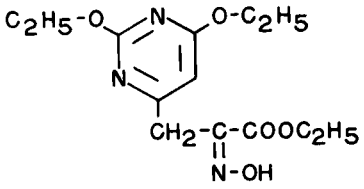

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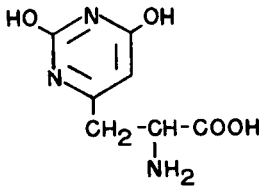
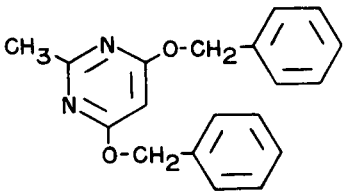


VII



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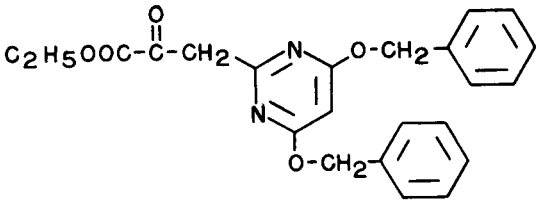

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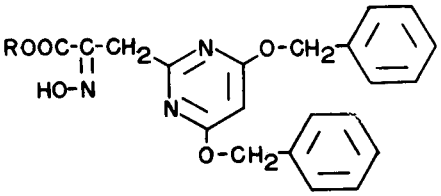
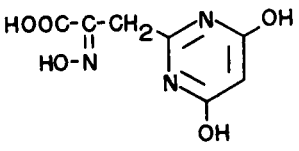
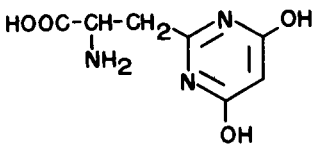


X a. R = C_2H_5

b. R = H



XI



XII

product of XII gave the "normal" violet coloration with ninhydrin.

Note added in proof. B. J. Whitlock, S. H. Lipton and F. M. Strong have recently reported [*J. Org. Chem.*, 30, 115 (1965)] that lathyrine produced a "red color", but the hydrogenated tetrahydrolathyrine produced a "normal lavender color" with ninhydrin. Their report, therefore, is in complete agreement with our observation.

EXPERIMENTAL (15)

Diethyl Acetamido[(2,4-dihydroxy-5-pyrimidinyl)methyl]malonate (III).

To a solution of 1.2 g. of sodium in 80 ml. of anhydrous methanol was added 10.8 g. (0.05 mole) of diethyl acetamidomalonate (6). After all the solid had dissolved 8.0 g. (0.05 mole) of 5-(chloromethyl)-uracil (5) was added in one portion to the stirred solution. The mixture was refluxed for 1 hr. and filtered while hot. The filtrate was then evaporated to dryness and the residue triturated with 100 ml. of ether. The solid was filtered and recrystallized from isopropyl alcohol to give 9.9 g. (59% yield) of product, m.p. 174-176°. λ max *p*H 1, 261 mμ (ε, 1,300); λ max *p*H 11, 286 mμ (ε, 11,600).

Anal. Calcd. for $C_{14}H_{19}N_3O_7$: C, 49.3; H, 5.6; N, 12.3. Found: C, 49.2; H, 5.6; N, 12.4.

DL-β-(5-Uracilyl)alanine (IV).

A mixture of 4.4 g. of III and 50 ml. of concentrated hydrochloric acid was refluxed for 4 hr. The reaction mixture was evaporated to dryness and to the residue there was added 15 ml. of water. The solution was neutralized with concentrated aqueous ammonia and chilled to precipitate the desired product. It was filtered, recrystallized from a small amount of water, dried at 110° and 15 mm., then allowed to equilibrate with moisture overnight to give 1.8 g. (64% yield) of white solid which did not melt below 360°. The product gave a violet coloration with ninhydrin. λ max *p*H 1, 262 mμ (ε, 7,000); λ max *p*H 11, 288 mμ (ε, 5,700).

Anal. Calcd. for $C_7H_9N_3O_4 \cdot H_2O$: C, 38.7; H, 5.1; N, 19.4. Found: C, 38.8; H, 4.9; N, 19.2.

Ethyl 2,4-Diethoxy-6-pyrimidinylpyruvate (V).

Seven grams (0.18 g.-atom) of potassium was carefully dissolved in the minimum amount of ethanol (see paper I (4) of this series for directions). The resulting solution was evaporated *in vacuo* almost to dryness and to the residue there was added 500 ml. of anhydrous ether followed by 26.3 g. (0.18 mole) of diethyl oxalate. A solution of 32.6 g. (0.179 mole) of 2,4-diethoxy-6-methylpyrimidine (9) in 90 ml. of pyridine was then added and the mixture was refluxed with stirring for 30 min. The solid product (the potassium salt) precipitated from the cooled reaction mixture. It was filtered, washed with ether, and air-dried to give 42 g. (73% yield) of product, m.p. 190-191° dec. A sample was converted to the free ester by acidification of the aqueous solution to *p*H 1 with dilute hydrochloric acid; recrystallization from a mixture of water and ethanol gave V as a white solid, m.p. 78-80°. λ max *p*H 1, 259 (ε, 10,200), 319 mμ (ε, 2,800); λ max *p*H 11, 244 (ε, 4,200); 285 (ε, 4,300), 348 mμ (ε, 21,200).

Anal. Calcd. for $C_{13}H_{18}N_2O_5$: C, 55.3; H, 6.4; N, 9.92. Found: C, 55.4; H, 6.6; N, 9.65.

Ethyl 2,4-Diethoxy-6-pyrimidinylpyruvate, Oxime (VI).

To a solution of 20 g. (0.0625 mole) of the potassium salt of ethyl 2,4-diethoxy-6-pyrimidinylpyruvate (V) in 165 ml. of 1-N hydrochloric acid and 150 ml. of ethanol at 50° were added 4.3 g. (0.0625 mole) of hydroxylamine hydrochloride and 6.1 g. (0.0625 mole) of sodium acetate. The mixture was stirred for 1 hr. without further heating. The volume of the resulting solution was reduced *in vacuo* to *ca.* 150 ml. The crude product, which deposited on cooling, was recrystallized from a mixture of water and methanol to give 14.1 g. (76% yield) of product, m.p. 118-120°. λ max *p*H 1, 259 mμ (ε, 12,500); λ max *p*H 11, 259 mμ (ε, 18,500).

Anal. Calcd. for $C_{13}H_{19}N_3O_5$: C, 52.5; H, 6.4; N, 14.1. Found: C, 52.7; H, 6.6; N, 13.9.

DL-β-(6-Uracilyl)alanine (VII).

The oximino ester (VI) (23.8 g., 0.08 mole) was added with stirring, to a solution of 250 ml. of concentrated hydrochloric acid and 59 g. of stannous chloride. The mixture heated rapidly and a solution resulted. After the initial reaction had subsided, the resulting solution was refluxed for 30 min. and diluted with 500 ml. of water. Hydrogen sulfide was then bubbled through the solution after which the precipitated stannic sulfide was removed by filtration. The volume of the filtrate was reduced to 200 ml. and neutralized with aqueous ammonia. The precipitated product was collected by filtration and recrystallized from dilute hydrochloric acid to give 10.3 g. (51% yield) of the alanine, isolated as a monohydrochloride monohydrate. The product, which gave an orange-red coloration with ninhydrin, did not melt below 360°. λ max *p*H 1, 260 mμ (ε, 7,100); λ max *p*H 11, 281 mμ (ε, 7,000).

Anal. Calcd. for $C_7H_9N_3O_4 \cdot HCl \cdot H_2O$: C, 33.2; H, 4.8; N, 16.6. Found: C, 33.4; H, 4.8; N, 16.3.

2-Methyl-4,6-dibenzyloxypyrimidine (VIII).

Fifty-two grams (2.3 g.-atom) of sodium was dissolved in 600 ml. of boiling benzyl alcohol. The solution was cooled at 80-100° while 163 g. (1 mole) of 2-methyl-4,6-dichloropyrimidine in 200 ml. of warm (40-50°) benzyl alcohol was added. After the addition was complete, the mixture was stirred at room temperature for 1 hr. and then poured into 1 l. of water. The reaction mixture was extracted with 4 x 200 ml. of ether. The ethereal extract was washed twice with water and dried over anhydrous sodium sulfate, and then distilled under reduced pressure. The desired product, 283 g. (93% yield), was collected at 193-195°/0.7 mm.

Anal. Calcd. for $C_{19}H_{18}N_2O_2$: C, 74.5; H, 5.9; N, 9.15. Found: C, 74.4; H, 6.0; N, 8.90.

Ethyl 4,6-Dibenzyloxy-2-pyrimidinylpyruvate (IX).

Four grams (0.1 g.-atom) of potassium was dissolved in 100 ml. of absolute ethanol and the excess ethanol was removed *in vacuo*. To the residue was added 250 ml. of anhydrous ether followed by 14.6 g. (0.1 mole) of diethyl oxalate. The mixture was heated on a steam bath until all the solid had dissolved. To the solution was added 30.6 g. (0.1 mole) of freshly distilled 2-methyl-4,6-dibenzyloxypyrimidine (VIII) and 50 ml. of dry pyridine. The mixture was refluxed for 3 hr. (longer refluxing time reduces the yield). The reaction mixture was cooled overnight, and the yellow solid filtered. The solid, which is the potassium salt of the desired product, was added to 300 ml. of water containing 6 ml. of glacial acetic acid. The suspension was manually stirred for 10 min. and the resulting pasty product gradually solidified upon refrigeration. It was then filtered to give 16 g. (38% yield) of product, m.p. 99-101°. Recrystallization from a mixture of water and ethanol raised the melting point to 103-105°.

Anal. Calcd. for $C_{23}H_{22}N_2O_5$: C, 68.0; H, 5.4; N, 6.89. Found: C, 68.0; H, 5.8; N, 6.69.

Ethyl 4,6-Dibenzyloxy-2-pyrimidinylpyruvate, Oxime (Xa).

To 370 ml. of boiling methanol was added 4.4 g. (0.063 mole) of hydroxylamine hydrochloride, 5.9 g. of sodium acetate and 19.1 g. (0.047 mole) of finely ground IX. After all the solid had dissolved (prolonged heating should be avoided), the reaction mixture was allowed to stir at room temperature overnight and then chilled. The resulting solid was filtered and dried to give 16.5 g. (84% yield) of the desired product of analytical purity, m.p. 121-123°.

Anal. Calcd. for $C_{23}H_{23}N_3O_5$: C, 65.5; H, 5.5; N, 9.97. Found: C, 65.5; H, 5.8; N, 9.68.

4,6-Dibenzyloxy-2-pyrimidinylpyruvic Acid, Oxime (Xb).

A mixture of 8.3 g. of the ester Xa, 1.6 g. of sodium hydroxide and 70 ml. of water was refluxed for 5 min. The resulting solution was cooled and acidified to *p*H 1 with hydrochloric acid. The precipitated product was recrystallized from methanol to give 6.3 g. (81% yield) of product, m.p. 133-135°.

Anal. Calcd. for $C_{21}H_{19}N_3O_5$: C, 64.1; H, 4.8; N, 10.7. Found: C, 63.8; H, 4.9; N, 10.7.

4,6-Dihydroxy-2-pyrimidinylpyruvic Acid, Oxime (XI).

To 200 ml. of anhydrous methanol at 40° was added 44 g. (0.112 mole) of Xb. One gram of 10% palladium-on-charcoal was added to the solution and the mixture was hydrogenated at 60 p.s.i.g. During 15 min. two molar-equivalents of hydrogen were consumed after which the reaction mixture was evaporated to dryness under reduced pressure. To the residue was added 200 ml. of water followed by concentrated aqueous ammonia until all the white solid dissolved. The catalyst was then filtered and the filtrate was acidified with glacial acetic acid whereupon a solid separated. The product was collected by filtration from the chilled suspension. Recrystallization of the product from water gave 15.4 g. (60% yield) of XI, isolated as an ammonium salt. The

compound decomposed with melting at 226° (rapid heating with melting point bath temperature preheated to 200°). λ max *p*H 1, 254 mμ (ε, 9,000); λ max *p*H 11, 250 mμ (ε, 14,000).

Anal. Calcd. for $C_7H_{10}N_4O_5$: C, 36.5; H, 4.4; N, 24.4. Found: C, 36.5; H, 4.1; N, 24.3.

DL-4,6-Dihydroxy-β-(2-pyrimidinyl)alanine (XII).

A mixture of 2.1 g. (0.009 mole) of the preceding oximino acid (XI), 10 ml. of concentrated aqueous ammonia, 150 ml. of water and 4 g. of Raney nickel was hydrogenated at 60 p.s.i.g. until the theoretical amount of hydrogen (two molar-equivalents) was consumed (18 hr.). The catalyst was filtered and the volume of the filtrate was reduced to *ca.* 80 ml. The solution was cooled to 0° and adjusted to *p*H 4 with 10% hydrochloric acid. After refrigeration overnight an off-white solid separated from the solution. It was collected by filtration to give 1.2 g. (66% yield) of product which decomposed at 250° without melting. λ max *p*H 1, 257 mμ (ε, 5,400); λ max *p*H 11, 252 mμ (ε, 8,200). The product gave a brownish-yellow coloration with ninhydrin.

Anal. Calcd. for $C_7H_9N_3O_4$: C, 42.2; H, 4.5; N, 21.1. Found: C, 42.1; H, 4.6; N, 20.8.

Attempted Preparation of XII from Xb by Catalytic Hydrogenation with Palladium-on-charcoal.

A mixture of 6.8 g. of Xb, 4 g. of 10% palladium-on-charcoal in 200 ml. of methanol was hydrogenated at 60 p.s.i.g. overnight. Five molar-equivalents of hydrogen (four molar-equivalents were required theoretically) were consumed. The reaction mixture was evaporated to dryness and the resulting white solid recrystallized from water to give 2.3 g. of white solid, m.p. 276-278° dec. The solid, which gave a positive test (purple color) with ninhydrin, failed to show the characteristic pyrimidine absorption in the ultraviolet region. The product analyzed correctly as the dihydro-derivative of the desired compound Xb.

Anal. Calcd. for $C_7H_{11}N_3O_4$: C, 41.8; H, 5.5; N, 20.9. Found: C, 42.1; H, 5.5; N, 20.6.

In a second run, the hydrogenation was terminated as soon as four molar-equivalents of hydrogen were consumed. From the resulting reaction mixture the same dihydro-derivative was isolated, however in lower yield.

Hydrolysis of DL-4,6-Dihydroxy-β-(2-pyrimidinyl)alanine.

A mixture of 250 mg. of XII was refluxed with 20 ml. of 20% hydrochloric acid for 50 min. The resulting reaction mixture had no absorption in ultraviolet region and gave a positive (purple coloration) ninhydrin test. Paper chromatographic study indicated the presence of aspartic acid: R_f of the hydrolyzed product = 0.65; R_f of aspartic acid = 0.65. Both were measured at 25° (descending) on Whatman No. 1 paper. Solvent used: 95 parts of methanol and 5 parts of 5% hydrochloric acid.

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