larger percentage of the nitronitrate fraction than it did in the *trans*-stilbene experiment, indicating that the *cis* addition process, though slow, was operative.

EXPERIMENTAL[5]

Reaction of trans-stilbene and dinitrogen pentoxide. A stirred solution of 3.0 g. (16.6 mmoles.) of stilbene and 3.5 g. (18 mmoles.) of tetraethylammonium nitrate in 100 ml. of methylene chloride was cooled to −20° while 16.6 mmoles. of dinitrogen pentoxide in 18.5 ml. of methylene chloride was added over 15 min. After addition of the dinitrogen pentoxide the mixture was stirred at −5° for 15 min. and at 3° for 45 min. Water (100 ml.) was then added to the reaction mixture and the organic layer was separated and washed with aqueous sodium bicarbonate and water and dried over magnesium sulfate. Removal of the methylene chloride left 4.0 g. of residue. The residue was taken up in methylene chloride and chromatographed on a 2.8 × 40 cm. silica gel column. Elution of the column with ligroin–methylene chloride 1:1 gave a fraction which after trituration with ligroin consisted of 3.84 g. (81%) of mixed *threo-* and *erythro-*α-nitrato-α′-nitrobibenzyls, m.p. 74–78°. A 2.00-g. portion of this mixture was recrystallized from ligroin four times to give *erythro*-α-nitrato-α′-nitrobibenzyl, 0.07 g., m.p. 157–160°. Further recrystallization from ligroin raised the m.p. to 165–166° dec.

Anal. Calcd. for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.80; H, 4.24; N, 9.28.

The ligroin filtrate[6] on standing deposited *threo*-α-nitrato-α′-nitrobibenzyl as a cluster of needles, m.p. 95.5–96.5°. Three recrystallizations from ligroin gave long needles, m.p. 96–97°.

Anal. Calcd. for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.67; H, 4.77; N, 9.65.

The amount of *threo* isomer present in the mixture isolated was determined by quantitative infrared analysis using dimethyl sulfoxide as solvent and the 11.37 μ band present only in the *threo* compound as a reference. The sample was found to be 81% the *threo* isomer; the remainder was assumed to be the *erythro*-nitronitrate.

Reaction of cis-stilbene and dinitrogen pentoxide. The procedure outlined above for the nitration of *trans*-stilbene was followed using 3.0 g. of *cis*-stilbene. The nitronitrate fraction isolated after chromatography weighed 0.51 g. When the nitration was allowed to proceed for 2 hr. at 3° and the residue handled as usual, there was obtained from the chromatographic column *trans*-stilbene, 0.88 g. (29%), identified by m.p. and infrared spectrum and a nitronitrate fraction of 1.13 g. Two recrystallizations of this material from ligroin gave DL-*erythro*-α-nitrato-α′-nitrobibenzyl, 0.41 g., 8.6%, m.p. 159–162°.

Nitration of threo-α-hydroxy-α′-nitrobibenzyl. A stirred solution of 0.78 g. (3.2 mmoles.) of *threo*-α-hydroxy-α′-nitrobibenzyl and 2.0 g. of tetraethylammonium nitrate in 50 ml. of methylene chloride was cooled to −20° while 3.6 mmoles. of dinitrogen pentoxide in 4 ml. of methylene chloride was added dropwise. After addition of the dinitrogen pentoxide the solution was allowed to warm to 0° over 1 hr. The organic layer was then washed with water, aqueous sodium bicarbonate, and water, and was dried over magnesium sulfate. The methylene chloride solution was concentrated to 25 ml. and then chromatographed on a 2.5 × 12 cm. silica gel column. The material eluted by 150 ml. of methylene chloride, 0.89 g. (97%), m.p. 87–93° was found to be 100% *threo*-α-nitrato-α′-nitrobibenzyl by analysis of its infrared spectrum. One recrystallization from ligroin gave needles, m.p. 96–97°.

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(5) All melting points are uncorrected.

(6) Except on this one occasion when the *threo*-nitronitrate crystallized in a clump and was separated mechanically, the *threo* compound could not be purified by recrystallization. Crystals melting at 85–96°, about 90% pure by infrared analysis, were always obtained.

Synthesis of DL-β-(5-Cytosinyl)alanine[1]

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With the exception of glycine, natural α-amino acids may be looked upon as β-substituted alanines. In a search for antimetabolites with possible activity against cancer, it seemed desirable to undertake the synthesis of unnatural α-amino acids in which the radical R of the formula, R—CH_2—$CH(NH_2)COOH$, would be a pyrimidine, purine, or substitution products thereof. This view was further supported by the fact that a review of the literature revealed no examples of compounds of such comparatively simple structures.

After a number of experiments using conventional methods for preparing various compounds of these types proved abortive in our hands, the procedures described below were tried and led to the successful synthesis of the first compound of this type, DL-β-(4-amino-2-hydroxy-5-pyrimidyl)alanine.

4-Amino-5-hydroxymethyl-2-methylthiopyrimidine (I) was used as a starting point for this series of reactions. The syntheses described by Ulbricht and Price[2] for I and for 4-amino-5-bromomethyl-2-methylthiopyrimidine hydrobromide (II) were modified and improved. These authors reported isolating II as a hygroscopic, noncharacterized solid.[3] In our hands, however, it was obtained as a white crystalline solid exhibiting the chemical and physical properties expected of such a substance. When II was allowed to react with diethyl acetamidomalonate in the presence of alkoxide ion, instead of the expected 2-acetamido-2-(4-amino-2-methylthio-5-pyrimidylmethyl)malonic acid, diethyl ester (III), a cyclic compound, 6-acetamido-5,6,7,8-tetrahydro-2-methylthio-7-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid, ethyl ester (IV), was isolated. Albertson and Archer[4] described a similar occurrence in their synthesis of ornithine

(1) Taken from a portion of the thesis submitted by B. Blank to the Temple University Graduate Council in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June 1958.

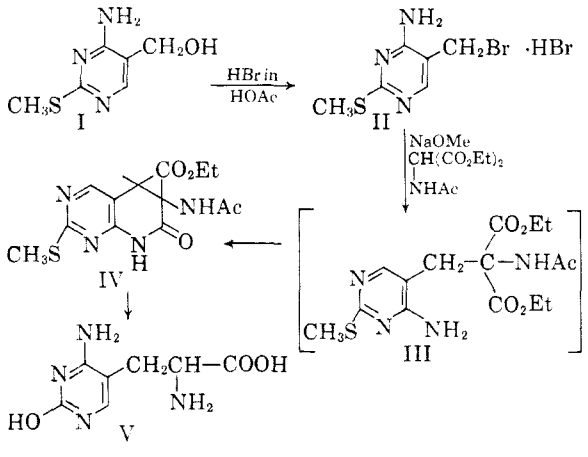
(2) T. L. V. Ulbricht and C. C. Price, *J. Org. Chem.*, **21**, 567 (1956).

(3) While this paper was being prepared, the synthesis of this compound (II) was described by T. Okuda and C. C. Price, *J. Org. Chem.*, **23**, 1738 (1958).

(4) N. F. Albertson and S. Archer, *J. Am. Chem. Soc.*, **67**, 2043 (1945).

from acrylonitrile and diethyl acetamidomalonate. In addition to elemental analysis, infrared spectral studies offered further evidence for the cyclic nature of IV as was shown by the presence of three distinct carbonyl bands at 5.7, 5.8, and 6.1 microns.

Subsequent hydrolysis of IV with concentrated hydrochloric acid followed by concentration and neutralization yielded the free amino acid, DL-β-(5-cytosinyl)alanine (V). This amino acid gave a strongly positive ninhydrin reaction. It was quite insoluble in organic solvents, but was readily soluble in dilute aqueous acids and bases. Its infrared spectra indicated the presence of a zwitterion with broad bands in the 3- and 6-micron regions. The ultraviolet spectra showed a single maximum at 283 millimicrons when determined in 0.1*N* hydrochloric acid.



EXPERIMENTAL[5]

4-Amino-5-hydroxymethyl-2-methylthiopyrimidine (I). The procedure used was a slight modification of the method described by Ulbricht and Price.[2] The use of a Soxhlet extraction apparatus was made unnecessary by the direct addition of portions of the solid 4-amino-5-carbethoxy-2-methylthiopyrimidine[2] to the stirred ethereal suspension of lithium aluminum hydride. The yields obtained from the use of this more rapid method were essentially the same as those reported.

4-Amino-5-bromomethyl-2-methylthiopyrimidine hydrobromide (II). To a saturated solution of dry hydrogen bromide in 140 ml. of anhydrous acetic acid were added, with stirring, 9.6 g. (0.06 mole) of 4-amino-5-hydroxymethyl-2-methylthiothiopyrimidine and 520 ml. of acetic acid. The slightly cloudy solution was stirred and heated on a steam bath for 2 hr. whereupon a clear solution was obtained. This solution was concentrated to a small volume in a rotary vacuum still to avoid bumping caused by the precipitation of the hydrobromide salt as the solvent was removed. The pyrimidine salt was collected on a funnel and washed with dry ether to yield 13.5 g. (76.4%) of product. This was purified by dissolving it in methanol, adding ether until the solution became cloudy, and then cooling. II was thus obtained as white needles, m.p. about 300° dec.

Anal. Calcd. for $C_6H_8BrN_3S.HBr$: C, 22.87; H, 2.88; N, 13.34. Found: C, 23.38, 23.68; H, 2.91, 3.10; N, 13.57.

6-Acetamido-5,6,7,8-tetrahydro-2-methylthio-7-oxopyrido-[2,3-d]-pyrimidine-6-carboxylic acid, ethyl ester (IV). A solution of 2.2 g. (0.04 mole) of sodium methoxide and 4.4 g. (0.02 mole) of diethyl acetamidomalonate in 25 ml. of absolute alcohol was warmed and stirred for 15 min. Then 6.3 g. (0.02 mole) of II and 50 ml. of absolute alcohol were added to the stirred solution. There was an immediate precipitation of sodium bromide. The mixture was stirred under reflux for 3 hr. and poured into three volumes of ice and water. The alcohol was removed and the aqueous solution was extracted with chloroform. The combined chloroform extracts were dried over magnesium sulfate and evaporated. The residual pale yellow oil was diluted with ether and allowed to stand overnight whereupon a yellowish white solid was obtained. This was filtered and washed with ether to yield 2.9 g. (44.6%) of product, m.p. 165–170°. Recrystallization from absolute alcohol gave white crystals, m.p. 187–188°.

Anal. Calcd. for $C_{13}H_{16}N_4O_4S$: C, 48.13; H, 4.97; N, 17.28. Found: C, 48.10, 48.06; H, 4.69, 4.79; N, 17.26, 16.97.

DL-β-(*4-amino-2-hydroxy-5-pyrimidyl*)*alanine* (V). A solution of 2.5 g. (0.007 mole) of IV in 30 ml. of concentrated hydrochloric acid was refluxed for 4 hr. The solvent was removed at reduced pressure and the white solid obtained was evaporated twice with absolute alcohol. The amino acid hydrochloride was then dissolved in water, made slightly alkaline with aqueous ammonia, cooled, and filtered to give 1.1 g. (79.8%) of product. The crude amino acid was purified by dissolving it in 10% aqueous sodium hydroxide, treating it with Celite and Norit, and filtering. The filtrate was treated with dilute hydrochloric acid and the amino acid reprecipitated. This operation was repeated and then the amino acid was dissolved in dilute hydrochloric acid and reprecipitated with concentrated aqueous ammonia at *p*H 5. Finally V was again reprecipitated from dilute base with dilute hydrochloric acid to give 400 mg. of white solid, m.p. above 300°. The analyses obtained were quite dependent upon the degree of drying. When dried at 110° *in vacuo* to constant weight, the amino acid analyzed as the monohydrate.

Anal. Calcd. for $C_7H_{10}N_4O_3.H_2O$: C, 38.88; H, 5.60; N, 25.92. Found: C, 39.47, 39.27; H, 5.86, 5.65; N, 26.20.

A picrate was prepared from the crude hydrolysis concentrate by adding a saturated solution of picric acid in water to an aqueous solution of the crude amino acid hydrochlochloride.[6] Recrystallization from water yielded the monopicrate monohydrate, m.p. 217–219° dec.

Anal. Calcd. for $C_{13}H_{13}N_7O_{10}.H_2O$: C, 35.06; H, 3.95; N, 22.01. Found: C, 35.55, 35.52; H, 3.67, 3.70; N, 21.87, 21.86.

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(5) All melting points listed are uncorrected.

(6) Attempts to purify the hydrochloride by recrystallization were unsuccessful. On warming, the hydrochloride lost hydrogen chloride and was converted to the inner salt of the free amino acid (V) (the zwitterion).