Synthesis of Methyl DL-3-Phthalimido-5-oxo-6-diazohexanoate[1]

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Received February 19, 1965

By a series of reactions DL-aspartic acid (I) has been converted to methyl DL-3-phthalimido-4-oxo-5-diazopentanoate (VI). Wolff rearrangement of VI has led to DL-3-phthalimido-4-carbomethoxybutanoic acid (X) which has been converted to methyl DL-3-phthalimido-5-oxo-6-diazohexanoate (XII).

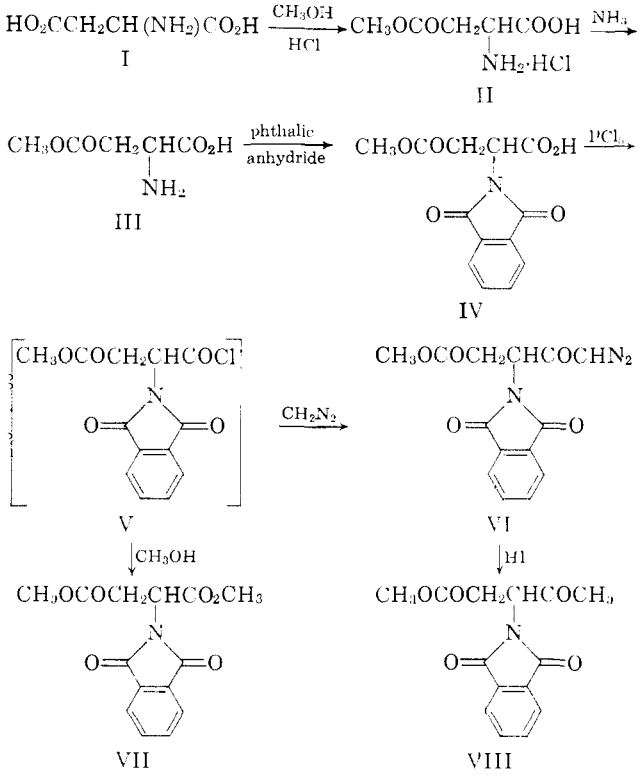
In view of the anticancer activity of azaserine and 6-diazo-5-oxo-L-norleucine it was considered desirable to attempt the preparation of other compounds having some of the structural features of these agents. Utilizing DL-aspartic acid as the starting material, a series of reactions has been run leading to compounds having the diazo, keto, ester, and phthalimido groups present. Attempts at converting the ester to the acid group and the phthalimido to the amino group have led only to unstable products.

DL-Aspartic acid β-methyl ester hydrochloride (II)[2] was prepared readily by simple esterification. The free amino acid ester (III) was obtained by treating II in methanolic solution with 1 equiv. of ammonia. Treatment of III with phthalic anhydride[3] yielded DL-2-phthalimido-3-carbomethoxypropanoic acid (IV). Phosphorus pentachloride converted IV to the corresponding acid chloride (V) which proved too unstable to isolate and analyze. On addition to ethereal diazomethane, however, the expected methyl DL-3-phthalimido-4-oxo-5-diazopentanoate (VI) was obtained. The acid chloride (V) also forms the dimethyl ester of DL-phthalimidobutanedioic acid (VII) on treatment with methanol and, hence, V is further characterized. The diazoketone VI (a compound with repeatedly high carbon analyses) was converted to methyl DL-3-phthalimido-4-oxopentanoate (VIII) on treatment with hydriodic acid (Scheme I). This derivative serves to further characterize VI.

When VI was subjected to the normal conditions of the Wolff rearrangement (a silver oxide slurry in water–dioxane) only the starting material could be recovered. A modification of the procedure introduced by Wilds and Meader,[4] however, led to formation of the benzyl ester of DL-3-phthalimido-4-carbomethoxybutanoic acid (IX). This intermediate IX was never isolated but was hydrogenated to yield the corresponding acid X. The acid X was then converted to the corresponding acid chloride XI which was not isolated but was treated with diazomethane to yield methyl DL-3-phthalimido-5-oxo-6-diazohexanoate (XII).

When VI was treated with 2 equiv. of hydrazine in methylene chloride[5] solution, an initial high-melting precipitate was formed which presumably was the hydrazine salt of 1,4-phthalizinediol. On removal of this salt by filtration and, on cooling the filtrate, a solid formed which could be collected by filtration but which began to show signs of decomposition on standing at

SCHEME I



room temperature. The product appeared stable under refrigeration. Diazo nitrogen analyses, however, showed only about half of the diazo nitrogen content expected for methyl DL-3-amino-4-oxo-5-diazopentanoate. Hence a mixture of this compound and the hydrazine salt of 1,4-phthalizinediol was probably obtained. All means attempted at the separation of the components failed.

In a similar run, the initial precipitate, weighing 137% of theoretical for the hydrazine salt of 1,4-phthalizinediol, and which partially melted on standing at room temperature, was treated with methanolic NaOH. Neutralization and removal of the solvent followed by shell-freezing, lyophilization, and refrigerated carbon chromatography in the manner of DeWald and Moore[5] resulted in the collection of material which melted and decomposed before reaching room temperature. The mother liquors of the product obtained from the hydrazine treatment were evaporated and the residue was subjected to the same treatment. Results, however, were similar.

An attempt to remove the phthaloyl group from XII appeared to result in the separation only of the 1,4-phthalizinediol salt. However, evaporation of the filtrate and treatment of the resulting residue in the

(1) This investigation was supported by a research grant (CA-04991) from the National Cancer Institute, Public Health Service.

(2) D. Coleman, *J. Chem. Soc.*, 2294 (1951).

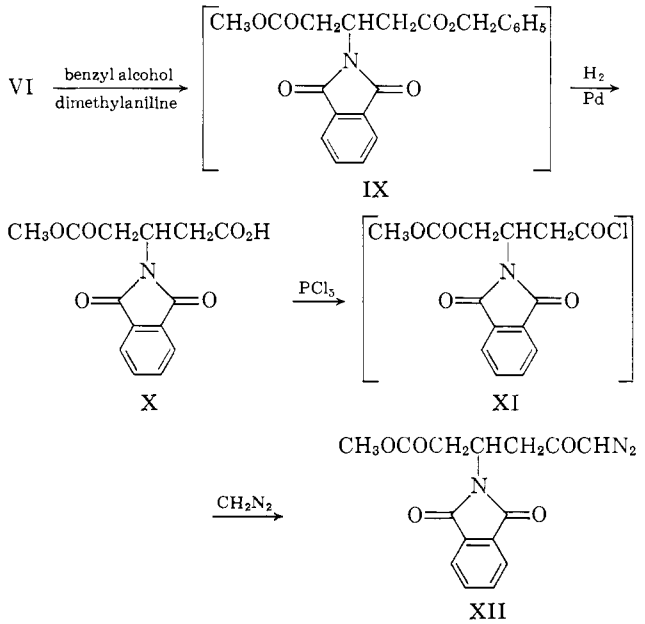
(3) J. H. Billman and W. F. Harting, *J. Am. Chem. Soc.*, **70**, 1473 (1948).

(4) A. L. Wilds and A. L. Meader, Jr., *J. Org. Chem.*, **13**, 763 (1948).

(5) H. DeWald and A. M. Moore, *J. Am. Chem. Soc.*, **80**, 3941 (1958).

manner described above resulted again in the formation of only a small amount of product which readily decomposed below room temperature. The attempt is described in the experimental portion of this paper.

Anticancer Screening.—In tests performed under the direction of the Cancer Chemotherapy National Service Center, VI, when administered intraperitoneally at dosages ranging from 0.03–25.60 mg./kg., showed little or no activity against Sarcoma 180 tumors in mice. Likewise, XII was found to be inactive in mice by the intraperitoneal route against Sarcoma 180 at a dosage of 500 mg./kg., against Lewis lung carcinoma at a dosage of 400 mg./kg., and against lymphoid leukemia L1210 at dosages of 200 and 400 mg./kg.



In our hands the acid chloride V did not yield crystalline products with higher diazoalkanes (diazoethane and diazopropane).

Protection of the amino group with the carbobenzoxy group was also attempted. DL-2-Amino-3-carbomethoxypropanoic acid (III) was readily converted to DL-2-N-carbobenzoxyamino-3-carbomethoxypropanoic acid on treatment with carbobenzoxy chloride. However, treatment with phosphorus pentachloride followed by addition of the resulting material to diazomethane yielded only a reddish oil which showed the gas evolution characteristic of a diazo compound on addition of an acid, but which resisted all efforts at crystallization.

Experimental[6]

β-Methyl DL-aspartate hydrochloride (II) was prepared by a modification of the method of Coleman.[2] To 320 ml. of absolute methanol (commercial grade used without further drying) was added 29 g. (0.79 mole) of dry HCl. In the hot solution was dissolved 41 g. (0.31 mole) of DL-aspartic acid (I). The mixture was allowed to stand for 8 min., then was ice cooled for another 5 min. Ether (1450 ml.) was then added. On refrigeration, 46.7 g. (81.9% yield) of white crystals separated, m.p. 181–189° dec. The literature[2] gives m.p. 190°, but the product as described above is sufficiently pure for the following steps.

(6) Melting points of all analytical samples were taken in capillary tubes and obtained with a Drechsel melting point apparatus with thermometers calibrated against registered thermometers (U. S. Bureau of Standards) and, hence, are corrected.

β-Methyl DL-Aspartate (III).—A 43.6-g. (0.276-mole) quantity of II was dissolved in 375 ml. of boiling methanol. To the hot, stirred solution was added dropwise, 14.6 ml. (an equivalent amount) of concentrated NH_4OH. On refrigeration, white III precipitated and was collected by filtration; yield 25.5 g. (62.8%), m.p. 175–181° dec. An analytical sample from a similar run was prepared by recrystallization from methanol; m.p. 187–188° dec.

Anal. Calcd. for $C_5H_9NO_4$: C, 40.81; H, 6.17; N, 9.52. Found: C, 40.93; H, 6.02; N, 9.32.

Products obtained from various runs differed considerably in quality as determined by the melting point. Materials with melting ranges starting as low as 160° could be used satisfactorily for the next step, however.

DL-2-Phthalimido-3-carbomethoxypropanoic Acid (IV).—A 9.9-g. (0.067-mole) quantity of III was pulverized thoroughly with 10.5 g. (0.70 mole) of phthalic anhydride. The mixture was placed in an oil bath preheated to 180° and was stirred for 10 min. The mixture melted and a gas was given off. On cooling, the mixture became glassy. The glassy substance was dissolved by heating in an approximately equal volume of methanol. On refrigeration, a white solid formed. It was collected by filtration; yield 15.3 g., m.p. 140–153°. On recrystallization from water there was obtained 8.8 g. (47.4% yield) of flaky, white crystals, m.p. 147–158°. An analytical sample from a similar run melted at 152–159°.

Anal. Calcd. for $C_{13}H_{11}NO_6$: C, 56.32; H, 4.00; N, 5.05. Found: C, 56.54; H, 4.10; N, 4.98.

Methyl DL-3-Phthalimido-4-oxo-5-diazopentanoate (VI).—A 6.1-g. (0.022-mole) quantity of IV was placed in *ca.* 75 ml. of anhydrous ether with 4.8 g. (0.023 mole) of phosphorus pentachloride, and the mixture was shaken until solution took place. The solvent was removed under reduced pressure. Toluene (5 ml.) was added, and the mixture was placed under reduced pressure to remove both toluene and $POCl_3$. Any remaining traces of $POCl_3$ were then removed by lixiviating a few times with cold petroleum ether (b.p. 30–60°). The residue was then dissolved in anhydrous ether and added dropwise to an ether solution of diazomethane (undistilled). On refrigeration yellow crystals formed which were collected by filtration. One recrystallization from benzene–petroleum ether yielded 3.1 g. (46.8%) of product, m.p. 99–104° dec. An analytical sample was prepared from a similar run.

Anal. Calcd. for $C_{14}H_{11}N_3O_5$: N, 13.95. Found: N, 13.75.

Although N analyses were satisfactory, repeated C analyses were 2–3% high. However, satisfactory H analyses (*e.g.*, calcd., 3.68; found, 3.91), and a good analysis for the sequence compound (VIII, see below) as well as the method of synthesis establish the structure of this compound.

Methyl DL-3-Phthalimido-4-oxopentanoate (VIII).—A 5.1-g. (0.017-mole) quantity of VI was dissolved in 75 ml. of $CHCl_3$. The solution was ice cooled and placed in a separatory funnel. Hydriodic acid (47–50%, 10 ml.) was added, and the mixture was shaken. There was a copious evolution of gas with the formation of iodine, and the solution rapidly reached room temperature. Shaking was continued until no more gas was evolved (1–2 min.). The mixture was diluted with water, and the chloroform layer was separated, washed with water, dilute sodium thiosulfate solution, and again with water, and dried ($MgSO_4$). On removal of the solvent a solid remained. On recrystallization (charcoal) once from 95% ethanol and once more from very dilute (*ca.* 10%) ethanol, there was obtained 1.32 g. (27.1% yield) of crystals, m.p. 95–100°. An analytical sample, prepared from a similar run, melted at 99–101°.

Anal. Calcd. for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76; N, 5.09. Found: C, 61.05; H, 4.93; N, 5.17.

DL-3-Phthalimido-4-carbomethoxybutanoic Acid (X).—A 1-g. (0.0033-mole) quantity of VI was placed in a solution of 5 ml. of N,N-dimethylaniline and 5 ml. of benzyl alcohol. The mixture was placed in an oil bath preheated to 190°. After an induction period of 3–4 min. there was a rapid evolution of gas. After another 8–10 min. at 190–194° gas evolution had subsided. The mixture was cooled in water and dissolved in ether. The ether solution was extracted with 100 ml. of 5% HCl and then with 5% $NaHCO_3$. It was washed with water and dried ($MgSO_4$). The solvent was removed under reduced pressure and the remaining oil was dissolved in 60 ml. of 95% ethanol. To the mixture was added 0.376 g. of 10% Pd on charcoal. It was then hydrogenated at 4.22 kg./cm.^{2} and 60° for 3–4 hr. The mixture was filtered and the solvent was removed under

reduced pressure. The remaining oil was dissolved in ether. The ether solution was extracted with 5% $NaHCO_3$ solution. The sodium bicarbonate extract was acidified with concentrated H_2SO_4. The cloudy mixture was extracted with ether, and the ether solution was washed with water and dried ($MgSO_4$). On filtration and removal of the solvent there remained an oil which crystallized from benzene–petroleum ether. One further recrystallization from the same solvent yielded 126 mg. (13.6%) of crystals, m.p. 114–116.5°. An analytical sample prepared from a similar run melted at 116–117°.

Anal. Calcd. for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81. Found: C, 57.56; H, 4.47; N, 4.42, 4.74.

Methyl DL-3-Phthalimido-5-oxo-6-diazohexanoate (XII).—This compound was prepared in the same manner as VI was prepared from its precursor. From 1 g. (0.0036 mole) of 3-phthalimido-4-carbomethoxybutanoic acid and 0.75 g. (0.0036 mole) of phosphorus pentachloride in 75 ml. of ether the intermediate acid chloride was obtained. After removing the $POCl_3$ in the usual manner, the remaining oil was dissolved in chloroform and added dropwise to a solution of diazomethane. After refrigeration there were obtained crystals which, after one recrystallization from benzene–petroleum ether, weighed 279 mg. (24.6%), m.p. 102.5–105°. An analytical sample melted at 103–106°.

Anal. Calcd. for $C_{15}H_{13}N_3O_5$: C, 57.14; H, 4.16; N, 13.33. Found: C, 57.14; H, 3.87; N, 13.24.

Attempted Preparation of 3-Amino-5-oxo-6-diazohexanoic Acid. A 2-g. quantity of XII was dissolved in 25 ml. of CH_2Cl_2 and cooled in an ice-salt bath. The mixture was stirred and a solution of 0.46 g. (2 equiv.) of 95+% hydrazine in 7 ml. of CH_2Cl_2 was added from a dropping funnel. After stirring and cooling for 2 hr. the mixture was kept at −10° overnight. It was then stirred and cooled in an ice-salt bath for another 5 hr. and kept overnight again at −10°. The resulting solid (0.754 g.) was collected by filtration. The filtrate was reduced to about 8 ml. under reduced pressure. An ice-cold solution of 70 ml. of methanol and 14 ml. of 1 *N* NaOH was added. The solution was then stored at −10° overnight. The pH was then very carefully adjusted to 6.5 (Beckman zeromatic pH meter) with 2 *N* HCl. Most of the methanol was removed under reduced pressure. The remaining solution was shell-frozen and lyophilized. There remained about 10 ml. of an orange-yellow oil. The oil was placed on a refrigerated chromatographic column 10 mm. in diameter and 7 cm. in length packed with equal weights of N.F. activated charcoal and Celite 545. It was eluted with 1% acetone. Fractions (10 ml.) were collected and checked for a diazo compound by adding HI solution to samples. The two fractions that gave an iodine color with HI were shell-frozen and lyophilized. There remained in each fraction a white solid. In an attempt to purify the material from a typical fraction, 0.5 ml. of water was added leaving a sticky mass; it was decanted. Ethanol (3 ml.) was added to the decantate. This yielded a white solid with the characteristics of NaCl. The mother liquor, after filtration, yielded an oil which decomposed at room temperature.

The sticky mass referred to above likewise yielded only NaCl when treated with absolute ethanol, and the mother liquor gave an oil which decomposed at room temperature.

Methyl DL-Phthalimidobutanedioate (VII).—Two grams (0.0072 mole) of IV was converted to the corresponding acid chloride as described in the preparation of VI. After the $POCl_3$ had been removed, 13 ml. of absolute methanol were added and the mixture was heated for a few minutes. On refrigeration white crystals were formed which were collected by filtration. One recrystallization from methanol yielded 1.1 g. (52.2%) of product melting at 95–99°. An analytical sample melted at 97.5–100°.

Anal. Calcd. for $C_{13}H_{11}NO_6$: C, 57.73; H, 4.50; N, 4.81. Found: C, 57.92; H, 4.25; N, 4.60.

Acknowledgment.—We acknowledge the assistance of Charles O. Garrison, Thomas H. Henscheid, and Boyd D. Nash.

Enzyme Inhibitors. VIII. Studies on the Mode of Binding of Some 6-Substituted 9-(Hydroxyalkyl)purines to Adenosine Deaminase[1,2]

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Received January 7, 1965

The syntheses of some 6-substituted 9-(2-hydroxypropyl)purines and some 6-substituted 9-(2,3-dihydroxypropyl)purines have been accomplished by the condensation of 5-amino-4,6-dichloropyrimidine with the appropriate amino alcohols, followed by ring closure of the resultant substituted pyrimidines to give the desired 6-chloro-9-substituted purines. Displacement of the 6-chloro group by certain nucleophilic reagents gave a variety of 6-substituted derivatives. Enzymatic evaluation of these compounds established that the 6-amino and the 6-methylamino derivatives inhibited adenosine deaminase. Comparison of the 6-aminopurines which were substituted at the 9-position by *n*-propyl, 3-hydroxypropyl, 2-hydroxypropyl, and 2,3-dihydroxypropyl groups established that there is only one hydroxyl binding site on adenosine deaminase in the area two to three carbons removed from the 9-position of the purine nucleus.

Several previous studies on the determination of the sites on the substrate which are important for binding to adenosine deaminase have revealed that rather large changes in the substituent at the 9-position of the purine nucleus can be made without markedly altering the capacity of the compound to bind to the enzyme.[3,4] Thus, it would appear that the 9-position of a 6-aminopurine would be a suitable area for attempting the preparation of active-site-directed irreversible inhibitors.[5] Before such active-site-directed irreversible inhibitors can be designed in a rational manner, it is necessary to know as much as possible about the binding sites, as well as the areas on the enzyme which have a large bulk tolerance. In an attempt to learn more about the nature of the binding by the substituent at the 9-position of the purine nucleus, we have synthesized and studied, as potential reversible inhibitors of adenosine deaminase, those purines which have at the

(1) This investigation was supported by Public Health Research Grant CA-06388-03 from the National Cancer Institute, by a Public Health Service research career program Award 5-K3-CA-18718-03 from the National Cancer Institute, by training Grant 5-T1-GM-575 from the Division of Medical Sciences, and by Grant T-337 from the American Cancer Society.

(2) For the previous paper of this series, see H. J. Schaeffer and E. Odin, *J. Pharm. Sci.*, **54**, 421 (1965).

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