New Asparagine Analogs†

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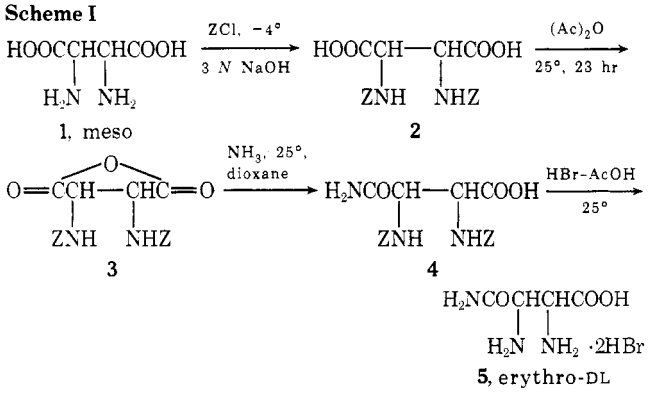
threo- and *erythro-β*-amino-DL-asparagine was prepared, respectively, from DL- and *meso*-2,3-diaminosuccinic acid by ammonolysis of the corresponding *N,N*-dicarbobenzoxydiaminosuccinic acid anhydride. *β*-Phenyl-DL-asparagine was synthesized from ethyl *α*-bromophenylacetate and diethyl acetylaminomalonate. *p*-Benzyloxyaniline with *α*-benzyl *N*-carbobenzoxy-L-aspartate were coupled in the presence of dicyclohexylcarbodiimide and the product was used in the preparation of *N*-(*β*-L-aspartyl)-4-hydroxyaniline. By applying different reaction conditions, benzyl *N*-carbobenzoxy-L-2-amino-4-oxo-5-diazopentanoate was converted to L-2-amino-4-oxopentanoic acid or its 5-bromo, 5-iodo, and 5-acetoxy derivatives. Reaction of phosphonium iodide with the 5-diazo intermediate gave an alkylated phosphonium oxide. The preliminary evaluation of some of these compounds as asparagine analogs showed that *erythro-β*-amino-L-asparagine approximated L-asparagine as a substrate for asparaginase, whereas the *β*-phenylasparagine and *N*-(*β*-L-aspartyl)-4-hydroxyaniline were inactive. The effect of some of these analogs as well as known compounds on the production of L-asparagine by intact P815Y tumor cells and the growth of asparagine-dependent L-5178Y lymphoblasts in culture were evaluated.

The enzyme L-asparaginase (L-asparagine amidohydrolase) inhibits the growth of certain transplantable and spontaneous tumors.[1] Subsequently, this finding led to the use of the enzyme from *Escherichia coli* in the treatment of human leukemia.[2] Since some tumor cells in culture require asparagine,[3] the availability of potential L-asparagine analogs might provide an alternate form of therapy of asparagine-requiring tumors. One of these analogs, 5-diazo-4-oxo-L-norvaline (DONV),[4] has been shown to inactivate L-asparaginase[5] and inhibit the growth of L-asparagine-dependent or L-asparaginase-sensitive tumor cells in culture.[6] The specificity of DONV as an asparagine analog parallels that of 6-diazo-5-oxo-L-norleucine, a glutamine analog, against the homologous enzyme glutaminase from *E. coli*; neither analog is active against the other homologous enzyme.[7-9] These findings with amidohydrolase enzymes suggested that new analogs should retain the 4-carbonyl function and that the group attached should be limited in size to permit access to receptor sites for asparagine. The validity of these hypotheses has been tested against asparagine synthesis in P-815Y leukemic cells[10] and L-asparaginase from *E. coli*.‡ To extend the structure–action relationship, we have synthesized *threo-* and erythro-*β*-amino-DL-asparagine, *β*-phenyl-DL-asparagine, *N*-(*β*-aspartyl)-4-hydroxyaniline, and several derivatives of L-2-amino-4-oxopentanic acid.

threo- and *erythro-β*-amino-DL-asparagine was prepared from DL- and *meso*-2,3-diaminosuccinic acid,[11] respectively, as shown for the erythro isomer in Scheme I. The infrared spectrum of *meso-N,N*-dicarbobenzoxydiaminosuccinic acid anhydride (**3**) is different from that of the corresponding DL derivative in the 7.8–8.5-, 9.2–11-, and 12.3–14-*μ* regions. Furthermore, *erythro-β*-aminoasparagine (**5**) is much more active than the threo isomer as a substrate for L-asparaginase. Attempts to prepare *β*-aminoasparagine by the reduction of the monoamide of dihydroxytartaric acid osazone[12] resulted only in the formation of racemic diaminosuccinic acid monohydrate.

Scheme I



Z = carbobenzoxy

Although the synthesis of DL-2-amino-4-oxopentanoic acid from bromoacetone and diethyl acetylaminomalonate has been reported,[14] we have chosen to prepare the optically pure L-2-amino-4-oxopentanoic acid (**8**) and its 5-substituted derivatives as outlined in Scheme II. Attempts to replace the diazo function in compound **6**[4,13] with the fluoro function and to deblock the protecting groups in one step with anhydrous hydrogen fluoride produced only polymeric material instead of the desired 5-fluoro derivative. The preparation of the 5-chloro derivative from **6** has been reported.[13] In the absence of the catalytic action of copper powder, the 5-acetoxy derivative of **6** could not be formed even in a refluxing ethereal solution of acetic acid. The compounds in this series provide important model compounds in studies of the inactivation of asparaginase by DONV as well as the catalytic decomposition of this diazo ketone.[5]

In a separate attempt to obtain the 5-iodo derivative, **6** was allowed to react with phosphonium iodide; the reaction gave a product with the empirical formula $C_{10}H_{18}O_7N_2IP$. This compound is considered to be bis(2-amino-4-oxo-5-ylpentanoic acid)phosphine oxide hydriodide (**13**), since it is known that phosphines are rapidly oxidized in air to the oxides and that phosphine oxides form salts with acids.[15] It is interesting to note that phosphines or phosphine oxides may be alkylated by diazo compounds and are more basic than the *α*-amino function in amino acids.

Biological Studies. The asparagine analogs prepared in this report and related compounds have been evaluated in three systems: L-asparaginase from *E. coli*, production of asparagine by intact P815Y cells, and the growth of L5178Y leukemic cells (Table I). The kinetic properties of

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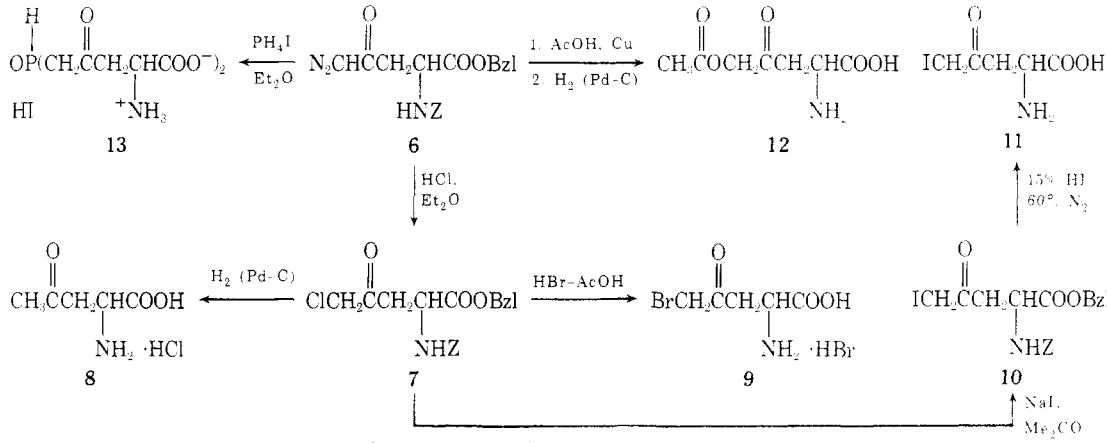
Scheme II



these analogs with L-asparaginase are briefly summarized. Of particular interest is the high stereospecificity of the β-aminoasparagine derivatives. Both the erythro and threo diasteromers of β-amino-DL-asparagine are substrates for this enzyme; however, release of ammonia occurs almost exclusively from one isomer of each of these racemic compounds. Thus, the enzyme rapidly releases only about 50% of the amide N as ammonia from each racemic pair as compared to 100% by hydrolysis with 1 *N* HCl at 100° for 3 hr. The excellent substrate properties of *erythro*-β-amino-DL-asparagine have been further studied by optical rotatory dispersion and circular dichroism measurements.[16] These have shown that only the erythro-L isomer serves as a substrate.

These analogs were prepared as potential therapeutic agents that might inhibit asparagine synthesis or the growth of asparagine-dependent cell lines. Production of L-asparagine by intact P815Y cells was inhibited by both the 5-bromo and 5-chloro derivatives[13] of L-2-amino-4-oxo-L-pentanoic acid. In both of these systems it was not possible to test L-2-amino-4-oxo-5-iodopentanoic acid because of its instability in protic solvents. Unlike the 5-chloro derivative, the 5-bromo analog at a concentration of 1×10^{-4} also caused 50% inhibition of the production of aspartic acid[10] by these cells and may therefore have been a less specific analog of asparagine. The effects of these analogs on the growth of L5178Y lymphoblasts in culture indicate the 5-bromo analog is less active as an inhibitor of the growth of this cell line which requires asparagine.

Experimental Section

Melting points were determined in capillary tubes using a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were performed by Baron Consulting Co., Orange, Conn., and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values.

***meso*-*N*,*N*-Dicarbobenzoxydiaminosuccinic Acid (2).** A solution of *meso*-diaminosuccinic acid[11] (3.3 g, 22.4 mmol) in 3 *N* NaOH (15 ml) was cooled to −4° with efficient stirring. Simultaneously, a solution of carbobenzoxy chloride (10.2 g) in C_6H_5Me (14 ml) and 3 *N* NaOH (20 ml) was added dropwise over 45 min at −4°. The reaction mixture was stirred vigorously at −4° for 2 hr, during which time an oily precipitate formed. H_2O (200 ml) was added and the mixture was extracted with Et_2O (3 × 70 ml). The aqueous layer was adjusted to pH 3 with concentrated HCl at 5–10° and refrigerated overnight. The precipitate was collected by filtration, dried, and dissolved in MeOH (100 ml) to remove the insoluble, unreacted *meso*-diaminosuccinic acid (0.34 g). The clear methanolic solution was concentrated *in vacuo* to yield the product (3.65 g, 39%). The analytical sample was recrystallized from H_2O: mp 178–178.5°. *Anal.* ($C_{20}H_{20}N_2O_8$) C, H, N.

***meso*-*N*,*N*-Dicarbobenzoxydiaminosuccinic Acid Anhydride (3).** A suspension of **2** (2.9 g, 7 mmol) in freshly distilled Ac_2O (35 ml) was stirred for 23 hr. The oily residue, after removal of the excess Ac_2O *in vacuo* at 32°, was dissolved in C_6H_5Me (10 ml) and the solution again concentrated *in vacuo*. This process was repeated twice to obtain a crystalline residue. The residue was dissolved in Me_2CO (15 ml) to remove traces of insoluble impurities. The anhydride crystallized slowly when Et_2O (15 ml) and light petroleum were added to the Me_2CO solution (2.5 g, 90%). The analytical sample was recrystallized again in the same manner: mp 144–146°. *Anal.* ($C_{20}H_{18}N_2O_7$) C, H, N.

***erythro*-DL-*N*,*N*-Dicarbobenzoxydiaminosuccinic Acid Monoamide (4).** Anhydrous NH_3 was introduced into a solution of **3** (4.05 g, 10.2 mmol) in dioxane (80 ml) for 30 min at 25°, during which time the monoamide began to separate as a gelatinous precipitate. The mixture was left at 25° overnight and, after filtration, the product was dissolved in hot H_2O (300 ml), filtered from traces of insoluble impurities. The filtrate was acidified with a few drops of AcOH and the amide separated at 4° as a crystalline solid (3.17 g, 75%): mp 174–175°. *Anal.* ($C_{20}H_{21}N_3O_7$) C, H, N.

***erythro*-β-Amino-DL-asparagine (5).**§ The monoamide **4** (3.13 g, 7.55 mmol) was dissolved in a 30% HBr–glacial AcOH solution (90 ml) and left overnight at 25°. β-Aminoasparagine dihydrobromide (**5**), a white, hygroscopic crystalline solid (1.15 g), was separated by centrifugation and washed several times with Et_2O with centrifugation. The HBr–AcOH supernatant solution was concentrated *in vacuo* at 50° to yield some additional dihydrobromide of lesser purity (1.27 g), making the total yield quantitative. The purer fraction, mp 180° dec, was analyzed. *Anal.* ($C_4H_{11}Br_2N_3O_3$) C, H, N. A solution of the dihydrobromide (1.17 g) in H_2O (4.5 ml) was carefully adjusted to pH 7 with 2 *N* NaOH and allowed to stand at 0°. *erythro*-β-Amino-DL-asparagine separated as a white crystalline solid (0.38 g, 67%). The analytical sample was recrystallized from H_2O: mp 210°. *Anal.* ($C_4H_9N_3O_3$) C, H; N: calcd, 28.56; found, 28.13.

β-Phenyl-DL-aspartic Acid. To a solution of acetylaminomalonic acid diethyl ester (6 g, 27.6 mmol) in EtOH (15 ml) was added a cold solution of Na (0.62 g, 31 mmol) in EtOH (13 ml). Ethyl α-bromophenylacetate (9.5 g, 39 mmol) was added slowly and the mixture was refluxed with stirring for 4 hr. After removal of the solvent *in vacuo*, the residue was dissolved in H_2O and the esters were extracted with Et_2O. The extracts, washed with H_2O and reduced *in vacuo*, gave a solid residue (11.9 g), which was hydrolyzed by refluxing in 9 *N* HCl (36 ml) for 3 hr. After removal of the excess acid from the hydrolysate *in vacuo*, the residue was dissolved in H_2O, made alkaline with NH_4OH, and treated with Norite. β-Phenylaspartic acid separated at 4° (1.8 g) after the filtrate was adjusted to pH 3 with AcOH. The mother liquor was adjusted to pH 9 with NH_4OH and applied to a column (35 × 2.5 cm) of DEAE-cellulose in the bicarbonate form. This was eluted with 0.5 *N* $Et_3NH{\cdot}HCO_3$ to yield an additional amount of the product (2.8 g, total yield 82%). The analytical sample was prepared by dissolving the product in dilute NH_4OH and reprecipitating with AcOH: mp 252–254°. *Anal.* ($C_{10}H_{11}NO_4$) C, H, N.

***N*-Carbobenzoxy-β-phenyl-DL-aspartic Acid.** To a stirred, ice-cooled mixture of β-phenylaspartic acid (2.2 g, 10.5 mmol) in H_2O (15 ml) was added MgO (0.8 g), Et_2O (10 ml), and carbobenzoxy chloride (2.36 g). After sitrring at 2° for 4 hr and at 25° for 17 hr, the excess MgO was removed by filtration. The filtrate was extracted with Et_2O, acidified with 6 *N* HCl, and then extracted with EtOAc. The dried EtOAc extracts (Na_2SO_4) were concentrated *in vacuo* to yield the crude product (1.4 g, 39%). Unreacted

§DL-2,3-Diaminosuccinic acid was carried through the same reaction sequence to yield *threo*-β-amino-DL-asparagine.

Table I. Enzymatic and Biological Properties of Asparagine Analogs

Analog	L-Asparaginase K_m	L-Asparaginase V_{max}[a]	P815Y whole cell asparagine production[b] Concn, *M*	P815Y whole cell asparagine production[b] % inhibition	Inhibition of L5178Y cell growth[c] Concn, *M*	Inhibition of L5178Y cell growth[c] % inhibition
L-$H_2NCOCH_2C(NH_2)HCOOH$	1×10^{-5}[d]	100			1×10^{-3}	0
L-$N_2CHCOCH_2C(NH_2)HCOOH$	1×10^{-5}[e]	2.7	1×10^{-4}	51	2×10^{-4}	17
			5×10^{-4}	88	8×10^{-4}	67
L-$ClCH_2COCH_2C(NH_2)HCOOH$	NA[f]		1×10^{-5}	62	3×10^{-6}	90
			5×10^{-5}	95		
L-$BrCH_2COCH_2C(NH_2)HCOOH$	NA		1×10^{-5}	23	3×10^{-6}	48
			1×10^{-4}	78		
L-$CH_3COCH_2C(NH_2)HCOOH$	NA		NA		3×10^{-5}	37
$H_2NCOC(NH_2)HC(NH_2)HCOOH$ (*erythro*-DL-)	7×10^{-5}[d]	85	NA		NA	
(*threo*-DL-)		0.1	NA		NA	
$H_2NCOCCH_3HC(NH_2)HCOOH$[g] (*threo*-DL-)	5×10^{-5}[d]	0.7	NA		NA	
$H_2NCOCH(C_6H_5)C(NH_2)HCOH$	NA		NA		NA	
L-HO-C_6H_4-$NHCOCH_2C(NH_2)HCOOH$	NA		NA		NA	

[a] Relative to asparagine. [b] Per cent inhibition of the net increase of L-asparagine was measured at the end of a 60-min incubation as described in Materials and Methods in ref 10. Uninhibited controls produced 1.3 nmol/mg of cells/hr. [c] Inhibition is expressed in relationship to growth of control cultures in Fischer medium with a generation time of 11 hr: G. A. Fischer and A. C. Sartorelli, *Methods Med. Res.*, **10,** 247 (1964). [d] Activity was measured by release of NH_3 by the Nessler's reaction: H. E. Wade and B. P. Philips, *Anal. Biochem.*, **44,** 189 (1971). [e] Activity was measured by decrease in the $A_{274\ nm}$ of the diazo ketone when the diazo N_2 was released (ref 5). [f] NA = not active as a substrate or inhibitor. [g] F. H. Brain, *J. Chem. Soc.*, 632 (1963).

starting material (0.8 g) was recovered in the aqueous phase. The analytical sample was prepared by acidifying a dilute NH_4OH solution of the product with 6 *N* HCl: mp 162–163°. *Anal.* ($C_{18}H_{17}NO_6$) C, H, N.

α-Benzyl *N*-Carbobenzoxy-β-phenyl-DL-aspartate. A mixture of *N*-carbobenzoxy-β-phenylaspartic acid (2.0 g, 5.9 mmol) and freshly distilled Ac_2O (10 ml) was stirred at 100° for 10 min. The excess Ac_2O was removed *in vacuo* to yield the crude *N*-carbobenzoxy-β-phenylaspartic anhydride (1.9 g). The purified anhydride [1.64 g, 5 mmol, mp 153–155° (Et_2O–petroleum ether)] and freshly distilled $PhCH_2OH$ (3.3 g) were heated together at 100° under anhydrous condition for 4 hr. The solution was taken up in Et_2O and extracted with 5% $NaHCO_3$. The bicarbonate extract was acidified at 4° with 6 *N* HCl and the ester was extracted with Et_2O. After removal of the Et_2O *in vacuo,* the ester was recrystallized from EtOAc–petroleum ether (2.04 g, 94%). The analytical sample was recrystallized from $C_6H_5CH_3$: mp 158–159°. *Anal.* ($C_{25}H_{23}NO_6$) C, H, N.

***N*-Carbobenzoxy-β-phenyl-DL-asparagine α-Benzyl Ester.** To a stirred, cooled solution of α-benzyl *N*-carbobenzoxy-β-phenylaspartate (0.68 g, 1.5 mmol) in Et_2O (5 ml) was added PCl_5 (0.43 g). When the reaction mixture became clear, it was poured into cold 6 *N* NH_4OH (7 ml). The amide, which separated as a crystalline solid (0.6 g, 80%), was washed with cold H_2O and recrystallized from MeOH–H_2O: mp 168–170°. *Anal.* ($C_{25}H_{24}N_2O_5$) C, H, N.

β-Phenyl-DL-asparagine. A solution of *N*-carbobenzoxy-β-phenylasparagine α-benzyl ester (0.63 g, 1.45 mmol) in 75% MeOH (25 ml) was hydrogenated over Pd black (0.3 g) for 2 hr. The catalyst was removed by filtration and washed with H_2O. The combined filtrates were concentrated *in vacuo* to yield the asparagine (0.29 g, 96%). The analytical sample was recrystallized from EtOH–H_2O: mp 228–230°. *Anal.* ($C_{10}H_{12}N_2O_3$) C, H, N.

***N*-(β-L-Aspartyl)-4-hydroxyaniline.** A solution of α-benzyl *N*-carbobenzoxy-L-aspartate[4,13] (0.71 g, 2 mmol), *p*-benzyloxyaniline (0.44 g, 2.2 mmol), and dicyclohexylcarbodiimide (0.46 g, 2.3 mmol) in dioxane (0.56 ml) was allowed to stand at 10° for 17 hr. The solution was filtered and the precipitate was washed thoroughly with EtOAc (200 ml). The combined filtrates were washed successively with 1 *N* HCl (3 × 30 ml), 1 *N* NaOH (3 × 30 ml), and H_2O (2 × 30 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield the crude *p*-benzyloxyanilide derivative (1.07 g). A solution of the recrystallized material [0.27 g, 0.5 mmol, mp 174–175° (EtOH)] in MeOH–AcOH–H_2O (15:5:1, 35 ml) was hydrogenated over 10% Pd-C (0.14 g) for 2 hr and the anilide was isolated in the usual manner and recrystallized from H_2O (65 mg, 54%): mp 195° dec. *Anal.* ($C_{10}H_{12}N_2O_4$) C, H, N.

Benzyl *N*-Carbobenzoxy-L-2-amino-4-oxo-5-chloropentanoate (7).= Anhydrous HCl was introduced into a solution of benzyl *N*-carbobenzoxy-L-2-amino-4-oxo-5-diazopentanoate (6)[4,13] (3.1 g, 8.3 mmol) in Et_2O (500 ml) for 1 hr at room temperature. After removal of the solvent *in vacuo,* the residue was washed with MeOH (15 ml) to yield the crude product (2.2 g, 68%). The analytical sample was recrystallized twice from MeOH: mp 105–107°. *Anal.* ($C_{20}H_{20}ClNO_5$) C, H, Cl, N.

L-2-Amino-4-oxopentanoic Acid Hydrochloride (8). A solution of **7** (1.35 g, 3.36 mmol) in MeOH (200 ml) containing AcOH (0.5 ml) was hydrogenated over 10% Pd-C (0.5 g) for 1.5 hr. The catalyst was removed by filtration and washed with MeOH. The combined filtrates were evaporated to dryness *in vacuo* to give a quantitative yield of the hydrochloride of the product. The analytical sample was recrystallized three times more from a mixture of MeOH and EtOAc: mp 146° dec. *Anal.* ($C_5H_{10}ClNO_3$) C, H, N.

L-2-Amino-4-oxo-5-bromopentanoic Acid Hydrobromide (9). Compound **7** (1.17 g, 3 mmol) was added to a 35% solution of HBr in glacial AcOH (10 ml). After the vigorous evolution of CO_2 ceased, the reaction was stoppered and left overnight at 25°. Excess HBr was removed *in vacuo* and Et_2O (250 ml) was added; most of the crude hydrobromide of the bromo derivative separated as an oil (0.66 g). The Et_2O–AcOH supernatant solution was concentrated *in vacuo* to give additional product (0.1 g). The combined oily product was digested repeatedly with Et_2O (4 × 30 ml) until it solidified. The hygroscopic powder was dissolved in MeOH (17 ml) and treated with Norite. After removal of MeOH *in vacuo,* the oily residue was again digested with ether (4 × 30 ml) until it was rendered solid (0.72 g, 82%). Recrystallization of the product was accomplished by dissolving it in MeOH (1.2 ml), followed by slow addition of EtOAc (120 ml). The analytical sample was recrystallized several times more in the same manner: mp 161° dec. *Anal.* ($C_5H_9Br_2NO_3$) C, H, N, O; Br: calcd, 54.04; found, 54.74.

Benzyl *N*-Carbobenzoxy-L-2-amino-5-iodopentanoate (10). To a solution of **7** (2.53 g, 5.7 mmol) in Me_2CO (35 ml) was added a solution of NaI (2.5 g) in Me_2CO (15 ml) and the mixture was stirred at 25° for 3 hr. The filtrate from the NaCl was concentrated *in vacuo* to yield **10** (3.2 g, 100%). The analytical sample was recrystallized from MeOH: mp 111–112°. *Anal.* ($C_{20}H_{20}INO_5$) C, H, I, N.

L-2-Amino-4-oxo-5-iodopentanoic Acid (11). A stream of N_2 was bubbled through a suspension of **10** (0.1 g, 3 mmol) in 15%

= Although Khedouri, *et al.*,[13] employed this intermediate (7) in their preparation of L-amino-4-oxo-5-chloropentanoic acid, they did not isolate nor identify this compound; therefore, a procedure for its preparation is described herewith.

HI (30 ml) at 60° for 28 hr when no CO_2 was detected in the effluent gases. The clear solution was extracted with $CHCl_3$ (3 × 15 ml) and concentrated *in vacuo* to an oil, which solidified when digested with Et_2O (4 × 30 ml). The product (0.3 g, 39%) was washed with Et_2O several times to yield the analytical sample: mp 104° dec. *Anal.* ($C_5H_8INO_3$) C, N; H: calcd, 3.14; found, 3.62; I: calcd, 49.36; found, 51.75.

Benzyl *N*-Carbobenzoxy-L-2-amino-4-oxo-5-acetoxypentanoate. To a solution of **6** (1.3 g, 3.4 mmol) in Et_2O (80 ml) were added AcOH (4 ml) and Cu powder (0.5 g). The mixture was stirred at 25° for 17 hr. The filtrate from the solids was concentrated *in vacuo* to an oil, which, when triturated with cold Et_2O, yielded the crude 5-acetoxy derivative (0.5 g, 35%). The analytical sample was recrystallized from EtOH–H_2O after treatment with Norit: mp 100–102°. *Anal.* ($C_{22}H_{23}NO_7$) C, H, N.

L-2-Amino-4-oxo-5-acetoxypentanoate (12). A solution of benzyl *N*-carbobenzoxy-L-2-amino-4-oxo-5-acetoxypentanoate (314 mg, 0.76 mmol) in MeOH (50 ml) containing AcOH (0.13 ml) was hydrogenated over 10% Pd–C (0.13 g) for 1.5 hr. The filtrate from the catalyst was concentrated *in vacuo* to give a quantitative yield of **12** (150 mg). The analytical sample was recrystallized from MeOH: mp 137° dec. *Anal.* ($C_7H_{11}NO_5$) C, H, N.

Bis(L-2-amino-4-oxo-5-ylpentanoic acid)phosphine Oxide Hydroiodide (13). A mixture of **6** (2 g, 5.4 mmol), PH_4I (1.7 g), and Et_2O (150 ml) was stirred at 25° under anhydrous conditions for 17 hr. The oily residue after removal of Et_2O *in vacuo* was dissolved in glacial AcOH (60 ml), and PH_4I (2 g) was added. The mixture was stirred at 45–50° for 5 hr and then at 25° for 2 days. The product separated as an orange hygroscopic precipitate and the AcOH supernatant solution was concentrated *in vacuo* to yield additional compound. The product (0.34 g, 29%) was washed successively with small amounts of glacial AcOH and EtOAc to yield the analytical sample: mp 145° dec. *Anal.* ($C_{10}H_{18}IN_2O_7P$) C, H, I, N; P: calcd, 6.67; found, 7.10.

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Synthesis and Antitumor and Antibacterial Activity of Benzoquinones Related to the Mitomycins

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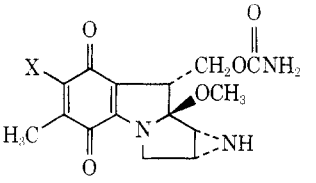
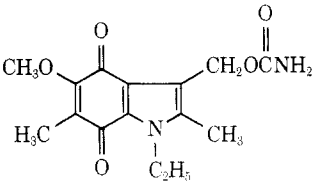
In an attempt to determine the minimum structural requirements for antibacterial and antitumor activity in mitomycin analogs, five different hydroxymethylbenzoquinones were synthesized and converted into their methyl carbamates. The hydroxymethylbenzoquinones were somewhat more active than either their carbamates or simple benzoquinone analogs against certain gram (+) and gram (−) bacteria. In standard NCI screens the carbamates possessed ED_{50} values of 12–26 μg/ml in the KB cell culture and were inactive against L-1210 lymphoid leukemia.

The mitomycins comprise a group of antibiotics whose potent action against both gram-negative and gram-positive bacteria as well as certain tumors is well known. Their usefulness is limited, however, by their relatively high toxicity. In search of less toxic compounds hundreds of derivatives and analogs have been prepared. Although none of these derivatives has proven more useful than mitomycin C (**1a**), both synthetic[1,2] and degradative[3,4] studies have shown certain desaziridinomitosenes to retain some antitumor and antibacterial properties. Related indoloquinones (*e.g.*, **2**) seem to retain only the antibacterial properties.[2,5] Carbazilquinone (**3**), a benzoquinone related to the mitomycins in that it contains the same three carcinostatic functional groups, has been reported to possess a better therapeutic index and higher maximum effectiveness than mitomycin C against lymphoid leukemia L-1210 (see Chart I).[6] It also is effective against both transplantable and primary tumors in mice.[7] The high activity of these partial structures suggested to us that the minimum structural requirements for activity in mitomy-

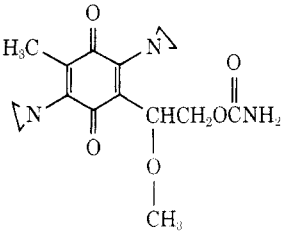
Chart I



1a, X = NH_2 (mitomycin C)
b, X = OCH_3 (mitomycin A)



2



3 (carbazilquinone)