ring after $(NH_4)_2SO_4$-H_2SO_4 spray.] *Anal.* ($C_9H_{16}ClN_3O_7$) C, H, N.

Nitrosation of *N*-(2-Chloroethyl)-*N'*-(*trans*-4-methylcyclohexyl)urea (6) in Ethanolic Hydrochloric Acid. N_2O_3 was bubbled into a cold (0–5°), stirred solution of 6[12] (125 mg, 0.570 mmol) in concentrated HCl (2 ml) and EtOH (1 ml) at a moderate rate for 15 min. Kept at 0–5°, the reaction mixture was stirred for 5 min before and after dilution with cold H_2O (20 ml). The light-yellow MeCCNU (7) that separated was collected and dried *in vacuo* over P_2O_5: yield 105 mg (74%); mp 70° (lit.[12] mp 70°); identity with an authentic sample established by ir (KBr) and pmr ($CDCl_3$-TMS).

Nitroso Group Migration in Ethanolic Hydrochloric Acid: CCNU (5a). An isomeric mixture[11] (200 mg), mp 69° dec, of **5a** (~65%) and **5b** (~35%) was stirred in suspension in a cold (0–5°) solution of EtOH (2 ml) in concentrated HCl (3 ml) for 1 hr. The mixture was diluted with cold H_2O (20 ml) and stirred at 0° for 20 min more. The light-yellow solid was collected, washed with cold H_2O (5 ml), and dried *in vacuo* over P_2O_5: yield 190 mg (95%); mp 89° (lit.[11] mp of CCNU 90°); pmr ($CDCl_3$-TMS) showed possibly a trace of **5b**.

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Synthesis and Antimicrobial Evaluation of *N*-D-Alanyl-1-aminoethylphosphonic Acid

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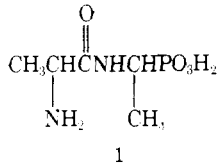
A number of antibiotic substances are known to exert antimicrobial activity by virtue of their ability to inhibit the synthesis of bacterial cell wall material.[1] Some of the compounds included in this class are vancomycin, ristocetin, penicillins, cephalosporins, phosphonomycin, and cycloserine (oxamycin). The mode of action of cycloserine is probably known in most detail and involves the inhibition of the enzyme that racemizes L-alanine and the enzyme that synthesizes D-alanyl-D-alanine.[2] Since the dipeptide D-alanyl-D-alanine is the terminal feature of the various peptides from which bacteria build their cell walls, it seemed to us that this would represent a possible point of attack on murein biosynthesis. It was with this purpose in mind that we undertook to prepare and evaluate the antimicrobial activity of the dipeptide *N*-D-alanyl-1-aminoethylphosphonic acid (1) which is a phosphonic acid analog of D-alanyl-D-alanine (2).



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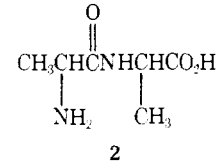

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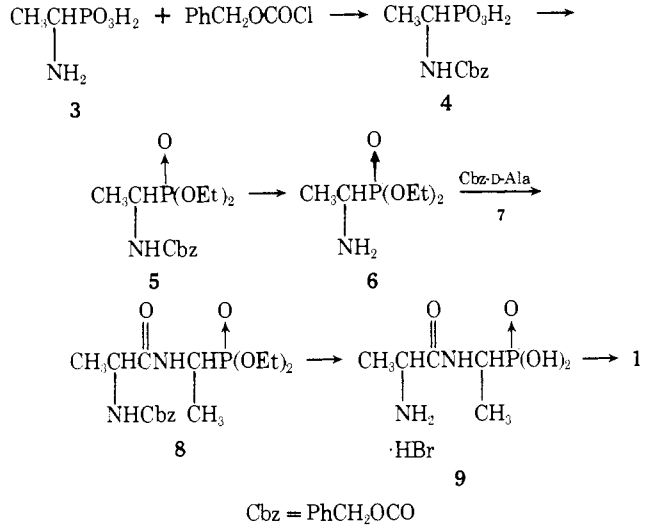
The dipeptide 1 was prepared as outlined in Scheme I using conventional procedures which have been shown to be applicable to the synthesis of peptides derived from α-aminobenzylphosphonic acid.[3-5] 1-Aminoethylphosphonic acid (3) was prepared by basically the same method as described by Chambers and Isbell[6] from triethyl 2-phosphonopropionate. In order to avoid any possible complications during the succeeding esterification step, the basic amine function of 3 was blocked by conversion to the carbobenzoxy derivative 4 using standard Schotten-Baumann conditions. In order to block the phosphonic acid portion, 4 was converted to the diethyl ester 5 by heating

Scheme I



with triethyl orthoformate, following a recently reported procedure for the esterification of phosphonic acids.[7]

Hydrogenolysis of **5** produced the free amino ester **6**. Although removal of the carbobenzoxy group could also be achieved with HBr–HOAc, this method was found to give a low yield of impure product. Coupling of the amino ester **6** with carbobenzoxy-D-alanine (**7**) was accomplished in good yield with *N,N'*-dicyclohexylcarbodiimide (DCC) affording the carbobenzoxy dipeptide diester **8**.

Removal of the carbobenzoxy group from **8** with HBr–HOAc was accompanied by cleavage of the phosphonate ester groups. The resultant hydrobromide **9** was converted to the free base with moist Ag_2O and residual silver ion removed by an ion exchange resin giving the free dipeptide **1** as a mixture of diastereomers. Tlc of **1** on cellulose plates with a variety of solvent systems indicated the presence of the two isomers but a clean separation was not achieved. Further work aimed at the resolution of these isomers is planned.

Compound **1** was tested for antimicrobial activity against a variety of organisms on both complete and minimal media using D-cycloserine and 1-aminoethylphosphonic acid (**3**) as standards for comparison. The only activity observed was against *Pseudomonas aeruginosa* on minimal media and this activity was of the same order of magnitude as that exhibited by **3**. These results could mean that **1** is being incorporated into cell wall synthesis and that **3** instead of D-alanine is released during the cross-linking of the linear peptide strands. Another possibility is that **1** is a weak antimetabolite of D-alanyl-D-alanine. In order to gain additional information on this we are preparing the dipeptide similar to **1** but having 1-aminoethylphosphonic acid at the N-terminal part of the molecule.

Experimental Section

Melting points were taken on a Mel-Temp melting point apparatus and are corrected or uncorrected as indicated. Infrared spectra were taken on a Perkin-Elmer Model 257 spectrophotometer, nmr spectra on a Jeolco C-60HL spectrometer (Me_4Si, DSS), mass spectra on a Du Pont Model 21-492 spectrometer, and optical rotations on a Perkin-Elmer Model 141 polarimeter. Elemental analyses were performed by either Galbraith Laboratories, Inc., Knoxville, Tenn., or Chemalytics, Inc., Tempe, Ariz.

1-Aminoethylphosphonic Acid (3). 1-Aminoethylphosphonic acid (**3**) was prepared according to the procedure of Chambers and Isbell[6] using triethyl 2-phosphonopropionate[8] (119 g, 0.5 mol) to give 45.7 g (73%) of **3**: mp (uncor) 274–278° dec (lit. mp 283–284°,[6] mp 278°[9]).

***N*-Carbobenzoxy-1-aminoethylphosphonic Acid (4).** A well-stirred solution of 5 g (0.04 mol) of **3** in 30 ml of H_2O was adjusted to pH 9.5 with 4 *N* NaOH. To this was added 15 ml of Et_2O and 10.8 g (0.06 mol at 95%) of benzyl chloroformate and the mixture was cooled in an ice bath. Stirring was continued 11.5 hr and the pH maintained at 9–9.5 by intermittent addition of 4 *N* NaOH. The final mixture was extracted with Et_2O (discarded). The aqueous layer was poured slowly onto a mixture of 30 ml of H_2O, 30 ml of concentrated HCl, and 100 g of ice. This was extracted with Et_2O. The combined, dried ($MgSO_4$) Et_2O extracts were concentrated *in vacuo* and the residue was recrystallized from EtOAc–*n*-hexane giving 6.1 g (58.6%) of crystals of **4**: mp (cor) 111–113° dec; ir (Nujol) 1260 (P=O), 1690 (C=O), and 3295 cm^{-1} (NH); nmr (D_2O–NaOD) δ 7.34 (s, 5, C_6H_5), 5.1 (s, 2, $C_6H_5CH_2O$), 4.03–3.36 (m, 1, CH_3CHP), and 1.4 ppm (dd, 3, $J_{PH} = J_{HH} = 13$ Hz, CH_3CHP). *Anal.* ($C_{10}H_{14}NO_5P$) C, H, N, P.

Diethyl *N*-Carbobenzoxy-1-aminoethylphosphonate (5). A stirred suspension of 5.2 g (0.02 mol) of **4** and 25 g (0.17 mol) of $(EtO)_3CH$ was slowly heated. The ethyl formate and EtOH byproducts were continuously removed by distillation thereby allowing the temperature to rise to 135° where it was held for 1 hr. The cooled mixture was filtered. The excess $(EtO)_3CH$ was removed from the filtrate under a high vacuum leaving 6 g (95.3%) of **5** as a viscous gum: ir (neat) 1170 (POEt), 1250 (P=O), 1725 (C=O), and 3250 cm^{-1} (NH); nmr (CCl_4) δ 7.1 (s, 5, C_6H_5), 4.94 (s, 2, $C_6H_5CH_2O$), 2.47–3.6 (m, 5, $POCH_2$ and CH_3CHP), and 1.48–0.99 ppm (m, 9, $POCH_2CH_3$ and CH_3CHP). The ester was used without further purification.

Diethyl 1-Aminoethylphosphonate (6). A mixture of 6 g (0.019 mol) of crude **5** and 1 g of 10% Pd/C in 75 ml of absolute MeOH was hydrogenated at 3.37 kg/cm^2 for 24 hr. The catalyst was filtered and the filtrate was concentrated *in vacuo* (room temperature) to give 2.4 g (70.6%) of the amino ester **6**:[10] ir (neat) 1120 (POEt), 1240 (P=O), and 3300, 3400 cm^{-1} (NH_2); nmr (CCl_4) δ 4.05 (m, 4, J = 7 Hz, $POCH_2$), 3.24–2.67 (m, unresolved, 1, CH_3CHP), 1.84 (s, 2, NH_2), and 1.43–1.03 ppm (m, 9, CH_3CHP and $POCH_2CH_3$); mass spectrum (70 eV) *m/e* (rel intensity) 181 (30), 138 (57), 44 (100).

Diethyl *N*-Carbobenzoxy-D-alanyl-1-aminoethylphosphonate (8). To a stirred solution of 4.89 g (0.027) of **6**, 5.3 g (0.027 mol) of DCC, and 50 ml of CH_2Cl_2 was added 6.02 g (0.027 mol) of Cbz-D-Ala (**7**),[11] prepared according to the procedure of Bergmann and Zervas,[12] in 30 ml of CH_2Cl_2. Stirring was continued for 24 hr, the suspension filtered, and the filtrate concentrated *in vacuo*. The residue was redissolved in 30 ml of CCl_4 and filtered, and the filtrate was concentrated to give 10.3 g (99%) of the carbobenzoxy peptide diethyl ester **8** as a glassy gum: ir (neat) 1170 (POEt), 1240 (P=O), 1675 (amide C=O), 1730 (urethane C=O), and 3300 cm^{-1} (NH); nmr ($CDCl_3$) δ 7.84–7.5 (broad, 1, CONH), 7.19 (s, 5, C_6H_5), 6.24–5.9 (broad, 1, $NHCO_2$), 5 (s, 2, $C_6H_5CH_2O$), 4.67–3.73 (m, 6, $POCH_2CH_3$, CHCON, and CHP), and 1.52–1.11 ppm (m, 12, $POCH_2CH_3$, CH_3CHCO, and CH_3CHP); mass spectrum (70 eV) *m/e* (rel intensity) 386 (100), 341 (9), 251 (27), 249 (89), 208 (95), 206 (85), 180 (77), 178 (50), 135 (36).

***N*-D-Alanyl-1-aminoethylphosphonic Acid (1).** To a stirred solution of 8.3 g (0.022 mol) of **8** in 50 ml of glacial HOAc was added 50 ml of 45% HBr–HOAc and stirring continued 2 hr. To this solution was added 750 ml of absolute Et_2O. After standing 4 hr, the solvents were decanted and last traces removed *in vacuo* leaving the HBr salt **9** as a gummy mass. This was taken up in 200 ml of H_2O and treated with 5 g of Ag_2O for 0.5 hr in a flask protected from light. The suspension was filtered through a pad of Celite, the filtrate passed through an Amberlite IRC-50 (2 × 45 cm), and the eluate concentrated *in vacuo* to give 4.2 g (99%) of a white solid. Trituration with several portions of hot MeOH afforded the dipeptide **1**: mp (uncor) 220–230° dec; ir (Nujol) 1260 (P=O) and 1670 cm^{-1} (C=O); nmr (D_2O) δ 4.34–3.66 (m, 2, CH_3CHCO and NCHP) and 1.6–1.13 ppm (m, 6, CH_3CHCO and CH_3CHP); $[\alpha]^{25}D$ −14.5° (c 2, H_2O). *Anal.* ($C_5H_{13}N_2O_4P$) C, H, N; P: calcd, 15.79; found, 15.31.

Primary Antimicrobial Screening of 1. Antimicrobial screening of **1** was carried out by an agar well diffusion method utilizing both complete and minimal media. D-Cycloserine and 1-aminoethylphosphonic acid (**3**) were used as standards for comparison. Cultures employed for screening were obtained from the American Type Culture Collection (ATCC), Rockville, Md., and included the following organisms: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 11775), *Proteus vulgaris* (ATCC 9489), *Pseudomonas aeruginosa* (ATCC 15442), *Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763),

Aspergillus niger (ATCC 16888), and *Trichophyton mentagrophytes* (ATCC 9129).

The primary screening plates were prepared using Eugon agar (BBL) for bacteria and Mycophil agar (BBL) for fungi as complete media and Davis minimal agar (Difco) for bacteria and Czapek agar (Difco) for fungi as the minimal media.

The agar culture media were autoclaved, cooled to 45°, inoculated with bacterial or fungal suspensions, mixed thoroughly, and poured into Petri plates (100 × 15 mm) so that each plate contained 15 ml of agar culture media seeded with the test microorganism. Agar wells 10 mm in diameter were prepared in the plates and the samples (0.1 ml) were pipeted into the well. The solutions used were: D-cycloserine, 0.2 mg/ml; **3**, 20 mg/ml; 1, 20 mg/ml.

The plates were incubated at 37° for bacteria or 25° for fungi and read for activity at 24-hr intervals. The results were expressed as the distance from the edge of the well to the outer edge of the clear zone of inhibition.

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Centrally Acting Emetics. 8. Conformational Aspects of Certain Dihydrophenanthrene Congeners of Apomorphine[1],†

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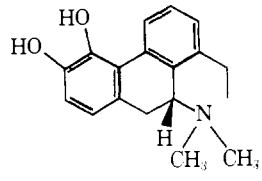
A prior communication[1] described the synthesis and biological effects of a ring-cleavage derivative (**1**) of apomorphine (**3**). The low order of emetic activity of **1**, which is



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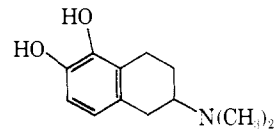

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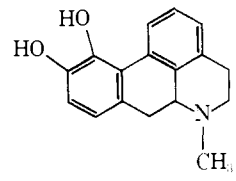

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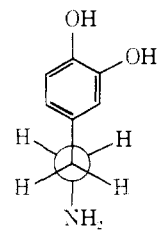
also closely related structurally to the potent emetic 5,6-dihydroxy-2-dimethylaminotetralin (**2**), was rationalized on conformational grounds. Rekker, *et al.*,[2] presented arguments that an anti disposition (**4**) of the catechol ring and the amino group of dopamine is the biologically active one. The dopamine portions of **2** and apomorphine exhibit this anti disposition.[1] It was proposed[1] that serious steric interaction between the dimethylamino group in a pseudo-equatorial conformation and the ethyl group at position 8 in compound **1** induces a conformational "flip" in the dihydrophenanthrene ring, to place the amino group in the less sterically crowded pseudo-axial arrangement. This conformation presents the dopamine portion of **1** in a gauche arrangement (**5**), and the molecule cannot interact optimally with the emetic receptor.

Inspection of molecular models led to the speculation that a phenanthrene system (**6**) lacking the ethyl group at position 8 would accommodate the dimethylamino group in the presumably more stable pseudo-equatorial disposition, in which the catechol ring and the amino group are in the biologically favorable anti conformation. Accordingly, it was predicted that **6** would exhibit potent emetic and peripheral apomorphine-like effects. Preparation of **6** was accomplished by a multistep sequence beginning with 3,4-dimethoxyphenanthrene-9-carboxylic acid. Spectral (ir, nmr) data were consistent with proposed structures of all compounds prepared.

Pharmacology. Compound **6** was evaluated in five dogs anesthetized with barbital sodium (200 mg/kg). The arterial pressure was measured using the right femoral artery and the compound was administered into the left femoral vein. In doses up to 2 mg/kg, **6** did not alter the blood



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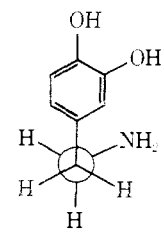
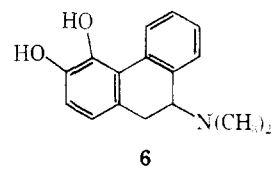


5, gauche



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