Synthesis of Inhibitors of Bacterial Cell Wall Biogenesis. Analogs of D-Alanyl-D-alanine†

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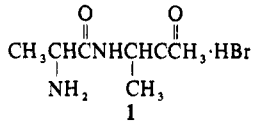
The syntheses of 2-(D-alanylamino)-3-butanone (1), DL-5-[α-(D-alanylamino)ethyl]-1(2)*H*-tetrazole (2), and D-alanyl-D-alaninehydroxamic acid (3) are described.

The primary site of action of the penicillins, cephalosporins, phosphonomycin, and oxamycin (cycloserine) is at the genesis of the bacterial cell wall. Phosphonomycin[1] inhibits the condensation of uridine diphospho-*N*-acetylglucosamine with phosphoenol pyruvate, a reaction mediated by a transferase, therefore blocking the synthesis of murein. Oxamycin[2] causes an inhibition of both alanine racemase and D-alanyl-D-alanine synthetase; the two enzymes are both involved in the formation of the specific dipeptide for the completion of the pentapeptide side chain attached to the polysaccharide backbone. The penicillins and cephalosporins act by inhibiting the enzymatic transpeptidation which causes crosslinking of the peptidoglycan chains via an exchange of the terminal D-alanine for the terminal amino group of the pentaglycyl moiety attached to the pentapeptide of a second peptidoglycan chain.[3]

We proposed that a D-alanyl-D-alanine analog which was devoid of the terminal carboxyl function could be incorporated into the peptide side chain of the peptidoglycan but would not allow the transpeptidation to occur. To test this hypothesis, it was desired to prepare 2-(D-alanylamino)-3-butanone (1), DL-5-[α-(D-alanylamino)ethyl]-1-(2)*H*-tetrazole (2), and D-alanyl-D-alaninehydroxamic acid (3).



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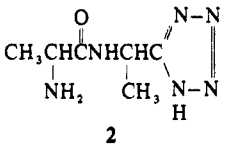

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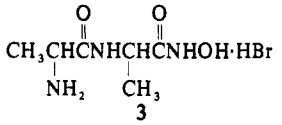

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The synthesis of 1 (Scheme I) was initiated utilizing *N*-carbobenzoxy-D-alanyl-DL-alanine (4) prepared by the method of Erlanger and Brand.[4] The Dakin–West reaction utilizing acetic anhydride and pyridine converted 4 to 2-(*N*-carbobenzoxy-D-alanylamino)-3-butanone (5). An attempt to remove the carbobenzoxy protecting group by hydrogenolysis gave 2-hydroxy-3,5,6-trimethylpyrazine (6) but the reaction of 5 with hydrobromic acid afforded the desired compound 1.

The preparation of the tetrazole 2 (Scheme II) started with the formation of phthaloyl-DL-alanine (7) which was converted to DL-α-(phthalimido)propionamide (8) by the procedure of Sheehan et al.[5] and then to the corresponding nitrile[6] 9. The tetrazole 10 was prepared by treating 9 with sodium azide and ammonium chloride after which the phthaloyl protecting group was removed by hydrazinolysis to give DL-[5-α-(amino)ethyl]-1(2)*H*-tetrazole (11). DL-5-[α-(*N*-Carbobenzoxy-D-alanylamino)ethyl]-1(2)*H*-tetrazole (12) was prepared by allowing 11 to react with *N*-carbobenzoxyalanyl azide which on treatment with hydrobromic acid in acetic acid, followed by neutralization with ammonium hydroxide, afforded the desired compound 2.

The hydroxamic acid 3 (Scheme III) was prepared from the ethyl ester of 4 by treatment with hydroxylamine to give DL- or D-α-(*N*-carbobenzoxy-D-alanylamino)propionhydroxamic acid (13). Hydrogenolysis of 13 produced the diketopiperazine 14 but cleavage of the protecting group with hydrobromic acid yielded 3.

Antimicrobial Testing. Compound 1, 2, 3 (D and DL), and 5 were tested for antibacterial activity in vitro by a twofold agar dilution test[7] using brain heart infusion agar, 10 ml/plate. The inoculum was one loopful of a 1:100 dilution 24-hr broth culture. The test was incubated at 37 for 24 hr. The test was carried against the following organisms: *Staphylococcus aureus* Smith, *Staphylococcus aureus* 209P, *Streptococcus pyogenes* C203, *Streptococcus faecalis* 10541, *Bacillus rubtilis* 10707, *Klebsiella pneumoniae* 10031, *Escherichia coli* Juhl, and *Salmonella typhimurium* Ed. No. 9. The minimum inhibitory concentration was >100 mg/ml for all the compounds tested. None of the compounds was found to possess significant antibacterial activity. The present investigation extended our knowledge of the structural requirements for transpeptidation. Despite the limited number of derivatives studied, the results might be interpreted as indicative of the importance of carboxylic group for transpeptidation to occur. Further studies on dipeptide analogs are being directed toward the development of such specific transpeptidation inhibitors.

Experimental Section

Melting points were obtained on a calibrated Thomas-Hoover Unimelt and were corrected. Ir data were recorded on a Beckman IR-10 spectrophotometer, NMR data on Varian Associates Model A-60A and T-60 spectrometers (Me_4Si), uv spectra with a Cary Model 14, and optical rotation with a Carl Zeiss LEP A2. Microanalyses were conducted on the F & M Model 185 C, H, N analyzer, University of Kansas, Lawrence, Kan. Where analyses are indiciated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

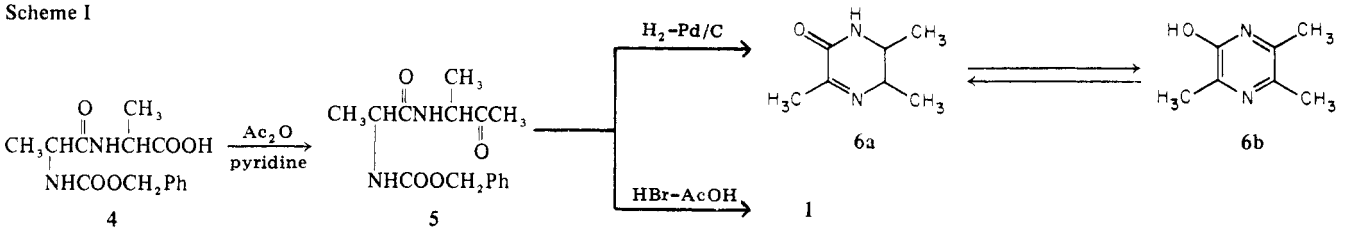
***N*-Carbobenzoxy-D-alanyl-DL-alanine (4).** This compound was prepared by the method of Erlanger and Brand.[3]

2-(*N*-Carbobenzoxy-D-alanylamino)-3-butanone (5). A mixture of *N*-carbobenzoxy-D-alanyl-DL-alanine (6.00 g, 20.4 mmol), pyridine (11.0 g), and acetic anhydride (18.0 g) was heated on a steam bath for 5 hr and steam-distilled until free of pyridine (90 ml); the residue was poured into excess aqueous $NaHCO_3$ and extracted with $CHCl_3$. The combined extracts were washed with a small amount of 3% HCl and a small amount of H_2O and dried (Na_2SO_4). The solvent was removed and the brown solid was recrystallized (EtOAc–*n*-hexane) to give pale brown needles: 3.80 g (64.4%); mp 118–118.5°; ir (KBr) 3318, 1714, 1673, 1636 cm^{-1}; NMR ($CDCl_3$) δ 1.31 (d, 3 H, J = 7.0 Hz, CH_3), 1.36 (d, 3 H, J = 7.0 Hz, CH_3), 2.19 (s, 3 H, $COCH_3$), 4.28 (q, 1 H, J = 7.0 Hz, CH), 4.48 (q, 1 H, J = 7.0 Hz, CH), 5.17 (s, 2 H, $PhCH_2$), 7.28 (s, 5 H, aromatic). Anal. ($C_{15}H_{20}N_2O_4$) C, H, N.

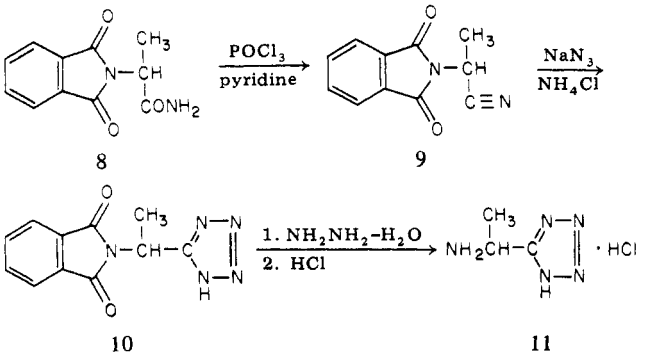
2-Hydroxy-3,5,6-trimethylpyrazine (6).[8,10] In 50 ml of MeOH containing 10 drops of acetic acid and 0.3 g of Pd/C (5%) was placed 1.16 g (4.00 mmol) of 2-(*N*-carbobenzoxy-D-alanyl-

† Dedicated to the memory of Professor Edward E. Smissman.

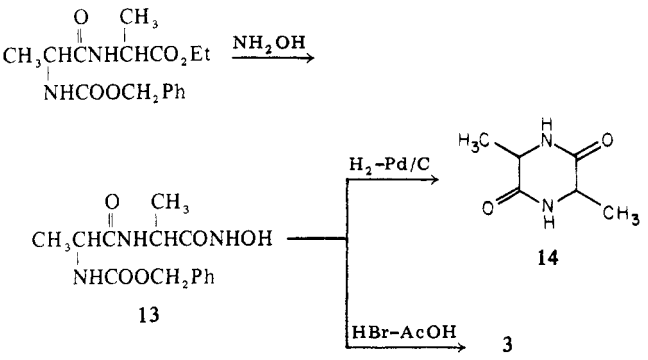
Scheme I



Scheme II



Scheme III



amino)-3-butanone (5) and the mixture was hydrogenated at atmospheric pressure for 2 hr. The mixture was filtered and the solvent removed to give a semisolid material. Recrystallization (ether–*n*-hexane) gave colorless needles: 2.20 g (36.2%); mp 195–196°; uv (MeOH) λ_{max} 228, 332 nm (ϵ 8550, 7380); uv (MeOH–5% HCl) λ_{max} 227, 326 nm (ϵ 9300, 5750); uv (MeOH–1 *N* NaOH) λ_{max} 233, 337 nm (ϵ 10,600, 7780); NMR ($CDCl_3$) δ 2.29 (s, 6 H, CH_3), 2.44 (s, 3 H, CH_3). Anal. ($C_7H_{10}N_2O$) C, H, N.

This reaction was also performed in aqueous acetic acid and gave identical results.

2-(D-Alanylamino)-3-butanone Hydrobromide (1). To 0.14 g (0.25 mmol) of 2-(*N*-carbobenzoxy-D-alanylamino)-3-butanone (5) was added 3 ml of a saturated solution of dry HBr in acetic acid. The reaction vessel was fitted with a $CaCl_2$ drying tube and allowed to stand overnight. To the mixture was added 10 ml of ether and the resulting oil was separated by decantation, after which it was washed several times with ether. This oil was dissolved in a small amount of EtOH and ether added to give a solid. Recrystallization (EtOH–ether) gave 0.02 g (33.8%) of the desired compound: mp 192–194°; ir (KBr) 3460, 3390, 1675, 1655 cm^{-1}. Anal. ($C_7H_{15}N_2O_2Br$) C, H, N.

DL-α-(Phthalimido)propionitrile (9). This compound was prepared by the method of Morley.[6]

DL-5-[α-(Phthalimido)ethyl]-1(2)*H*-tetrazole (10). A stirred mixture of DL-α-(phthalimido)propionitrile (9, 5.0 g, 25 mmol), sodium azide (1.80 g, 0.28 mol), ammonium chloride (1.50 g, 0.28 mol), and 20 ml of DMF was heated at 90–100° for 6 hr, filtered, and evaporated to dryness. The residue was stirred for 20 min with ice–H_2O (25 ml) and 5 *N* HCl (sufficient to give pH 1), and the solid was collected, washed with H_2O, and dried. Recrystallization from EtOH gave pale yellow prisms: 4.2 g (7%); mp 233–236° dec. Anal. ($C_{11}H_9N_5O_2$) C, H, N.

DL-5-[α-(Amino)ethyl]-1(2)*H*-tetrazole (11). A suspension of DL-5-[α-(phthalimido)ethyl]-1(2)*H*-tetrazole (10, 3.8 g, 15 mmol), EtOH (35 ml), and NH_2NH_2–H_2O (0.79 ml) was heated under reflux for 1 hr. After cooling, the resulting solid was collected, washed with EtOH, and digested at 15–20° with 1 *N* HCl (10 ml, three times). The digested solution was evaporated under reduced pressure to give an oil. This oil was used for the next reaction without further purification.

DL-5-[α-(*N*-Carbobenzoxy-D-alanylamino)ethyl]-1(2)*H*-tetrazole (12). To a mixture of 17 ml of AcOH, 7 ml of 5 *N* HCl, 70 ml of H_2O, and *N*-carbobenzoxy-D-alanine hydrazide[4] (3.08 g, 0.13 mmol) was added, in one portion, a cold aqueous solution of $NaNO_2$ (1.1 g, 15 mmol) at −5° to give a syrup. This syrup was taken up in 30 ml of Et_2O (cold). The Et_2O layer was kept cold while washing with H_2O, 3% $NaHCO_3$, and again with H_2O. After brief drying over Na_2SO_4, the azide solution was added in one portion to a mixture of crude DL-5-[α-(amino)ethyl]-1(2)-*H*-tetrazole hydrochloride (1.5 g, 13 mmol), DMF (10 ml), Et_3N (6.8 ml, 0.052 mol), and 1 ml of H_2O at 5°. After standing for 6 hr at 5°, the reaction mixture was stirred for 24 hr at room temperature, and then 2 ml of H_2O was added. The resulting solution was kept at 20–25° for 3 days and then acidified at 0° to pH 1 with 2 *N* HCl and added to a mixture of H_2O (160 ml) and Et_2O (100 ml). The Et_2O layer was separated and washed with H_2O and dried ($MgSO_4$). The solvent was evaporated to give a solid. Recrystallization from EtOAc–Et_2O gave a colorless solid: 0.23 g (6.8%); mp 183–184°. Anal. ($C_{14}H_{18}N_6O_3$) C, H, N.

DL-5-[α-(D-Alanylamino)ethyl]-1(2)*H*-tetrazole (2). A 30% HBr solution in AcOH (3.8 ml) was added to the DL-5-[α-(*N*-carbobenzoxy-D-alanylamino)ethyl]-1(2)*H*-tetrazole (12, 2 g, 6 mmol) in a reaction flask protected with a $CaCl_2$ tube under ice–water cooling. After standing overnight, 20 ml of absolute Et_2O was added and the resulting oil was separated by decantation. The oil was washed with Et_2O several times, dissolved in a small amount of H_2O, and adjusted to pH 4–5 with concentrated NH_4OH. The solvent was evaporated under reduced pressure to give a solid. Recrystallization from H_2O gave a colorless solid: 0.75 g (64.70%); mp 284° dec; ir (KBr) 3220, 1690 cm^{-1}. Anal. ($C_6H_{12}N_6O$) C, H, N.

DL-α-(*N*-Carbobenzoxy-D-alanylamino)propionhydroxamic Acid (13a). To a stirred suspension of NH_2OH–HCl (0.23 g, 3 mmol) in EtOH (10 ml) was added a solution of NaOEt (0.069 g of Na in 10 ml of EtOH below 20°); when neutral to litmus (about 45 min), the precipitated NaCl was filtered off. A solution of *N*-carbobenzoxy-D-alanyl-DL-alanine ethyl ester[4] (0.966 g, 3 mmol) in EtOH (5 ml) was added to the ethanolic solution of NH_2OH prepared above, followed by a solution of NaOEt (0.069 g) in EtOH (10 ml). The reaction mixture was allowed to stand for 20 hr at room temperature and then was acidified with AcOH. The solvent was evaporated and extracted with AcOEt, washed with a small amount of H_2O, $NaHCO_3$, and H_2O, and dried ($MgSO_4$). The solvent was evaporated to give a solid. Recrystallization from EtOAc gave a colorless solid: 0.2 g (22%); mp 174–175°; $[\alpha]_D$ 43.85° (*c* 2.0, EtOH); ir (KBr) 3300, 1690, 1670, 1630 cm^{-1}. Anal. ($C_{14}H_{19}N_3O_5$) C, H, N.

D-α-(*N*-Carbobenzoxy-D-alanylamino)propionhydroxamic Acid (13b). This compound was prepared by the same procedure described above: yield 59.5%; mp 185–185.5° dec; $[\alpha]_D$ 45.65°; ir (KBr) 3300, 1690, 1670, 1633 cm^{-1}. Anal. ($C_{14}H_{19}N_3O_5$) C, H, N.

3,4-Dimethyl-2,5-diketopiperazine (14). A mixture of MeOH (30 ml containing 5 drops of acetic acid), 0.13 g of Pd/C, and D-α-(*N*-carbobenzoxy-D-alanylamino)propionhydroxamic acid (13b, 0.3 g, 1 mmol) was hydrogenated at atmospheric pressure for 2 hr. The mixture was filtered and the solvent removed to give a solid. Recrystallization from EtOH gave colorless leaflets: 0.125 g (91%); mp 300–303°. Anal. ($C_6H_{10}N_2O_2$).

DL-α-(D-Alanylamino)propionhydroxamic Acid Hydro-

bromide (3a). A 30% HBr solution in AcOH (4 ml) was added to DL-α-(*N*-carbobenzoxy-D-alanylamino)propionhydroxamic acid (**13a**, 0.225 g, 00.73 mmol) in a reaction flask protected with a $CaCl_2$ tube under ice–water cooling. After standing 24 hr, 30 ml of absolute Et_2O was added and the resulting oil was separated by decantation. The oil was washed with Et_2O several times and treated with a small amount of EtOH to give a solid. Recrystallization from EtOH–Et_2O gave a pale yellow solid: 0.15 g (88.3%); mp 200–201°; $[\alpha]_D$ 33.0° (*c* 0.5, H_2O); ir (KBr) 3280, 3400, 1680, 1668 cm^{-1}. Anal. ($C_6H_{14}N_3O_3Br$) C, H, N.

D-α-(D-Alanylamino)propionhydroxamic Acid Hydrobromide (3b). This compound was prepared by the same method described above: yield 72.6%; mp 200–202° dec; $[\alpha]_D$ 27.4° (*c* 0.5, H_2O); ir (KBr) 3300, 3400, 1680 cm^{-1}. Anal. ($C_6H_{14}N_3O_3Br$) C, H, N.

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Synthesis and in Vitro Evaluation of 8-Hydroxyquinoline Analogs as Inhibitors of Dental Plaque†,[1]

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A group of 5-substituted 8-hydroxyquinolines with predicted log *P* values in the 1–4 range has been prepared from either 8-hydroxyquinoline or its appropriate derivative. 5-Formyl-, 5-iodo-, 5-fluoro-, 5-acetyl-, and 5-methoxymethyl-8-hydroxyquinoline in addition to methyl 5-(8-hydroxyquinolyl)acetate and ethyl 5-(8-hydroxyquinolyl)acetate displayed greater in vitro antiplaque activity than 8-hydroxyquinoline.

Dental plaque adheres to teeth and soft tissue and consists primarily of oral bacteria and inorganic salts in a matrix of proteins and polysaccharides. The presence of plaque has been linked to gingivitis, periodontal disease, and caries. It is thought that a principle event in plaque formation is the synthesis of polysaccharides by certain oral bacteria. These polysaccharides have been shown to induce precipitation of *Streptococcus mutans* which facilitate colonization of this bacteria and, in turn, serve as a point of attachment for the other components of plaque. Since a bacterial by-product is felt to initiate plaque formation, many antibacterials have been tested for their ability to inhibit the bacteria responsible for the formation of plaque. The most promising development in this area was the discovery that chlorhexidine was a very effective inhibitor of this organism. Clinical studies revealed that this agent caused soreness of the oral mucosa and discoloration of tooth surfaces, the tongue, and gingiva. During an initial screening of antibacterial agents for antiplaque activity, the 8-hydroxyquinolines were shown to have good activity.[1] Utilizing commercially available 8-hydroxyquinolines we recently demonstrated a correlation between antiplaque activity and partition coefficient (log *P*) values.[2] It was observed that only compounds having log *P* values between 1 to 4 displayed antiplaque activity. Based on this information we synthesized a group of 5-substituted 8-hydroxyquinolines with predicted log *P* values in the 1–4 range in an attempt to optimize antiplaque activity.

† This is dedicated to Professor Edward Smissman who will always be remembered by V. D. W. and D. B. M. as an outstanding medicinal chemist, educator, and friend.

Chemistry. 5-Cyanomethyl-8-hydroxyquinoline (**7**) was prepared by an entirely different method than that previously reported by Pujari and Rout.[8] 5-Chloromethyl-8-hydroxyquinoline hydrochloride was allowed to react with sodium cyanide in Me_2SO at 90° for 45 min to give a good yield of 5-cyanomethyl-8-hydroxyquinoline (**7**). Acidic hydrolysis of the cyano compound **7** gave the corresponding acid analog. Methyl 5-(8-hydroxyquinolyl)acetate (**9**) and ethyl 5-(8-hydroxyquinolyl)acetate (**10**) were obtained in good yields by refluxing 5-(8-hydroxyquinolyl)acetic acid with either 3% methanolic HCl or 3% ethanolic HCl. 5-Amino-8-hydroxyquinoline which was used in the synthesis of 5-fluoro-8-hydroxyquinoline (**12**)[10] was obtained by hydrogenation of 5-nitro-8-hydroxyquinoline using PtO_2 as the catalyst and THF as the solvent. The literature method[9] uses ethanol as the solvent but that gave a dark brown or black product which was very difficult to purify. The hydrogenation product was isolated as the dihydrochloride since the free base is very unstable and decomposes on standing (Table I).

Biological Results. Antiplaque activity as displayed by 8-hydroxyquinoline requires that a compound be an antibacterial agent. Therefore the analogs were first evaluated for their in vitro antibacterial activity against *Strept. mutans* 6715, a pure strain of plaque-forming bacteria (see Experimental Section). 8-Hydroxyquinoline and solvent control were tested concurrently. As shown in Table II, except for compounds **2**, **5**, and **8** all com-