REACTIVE D-ALANYL-D-ALANINE PEPTIDE DERIVED FROM CYCLOSERINE

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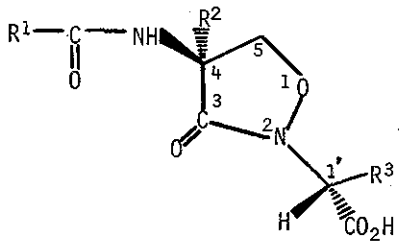
N-Phenylacetyl-2-(1'-carboxyethyl)cycloserine (1) was synthesized and its acylation was examined. Like penicillin and cephalosporin, the L-cycloserine derivative (1a) could be regarded as D-alanyl-D-alanine peptide containing a reactive peptide bond, but it displayed no antibacterial activity.

Tipper and Strominger[1] hypothesized that penicillin and cephalosporin resemble the D-alanyl-D-alanine end of the peptideglycan strand in a growing cell wall, and the transpeptidase presumably recognizes the antibiotic molecules as its substrate. When the β-lactams are cleaved, the transpeptidase becomes irreversibly acylated and inactive. There is an evidence suggesting that the antibacterial potency might pararell the acylating capability of β-lactam antibiotics.[2] From such a point of view, a compound having a conformation analogous to D-alanyl-D-alanine and high acylating ability similar to

penicillin and cephalosporin may also be expected to exhibit antibacterial properties. Such reasoning led us to synthesize the derivative (1) of cycloserine, which may be regarded as a new type of D-alanyl-D-alanine peptide.[3]



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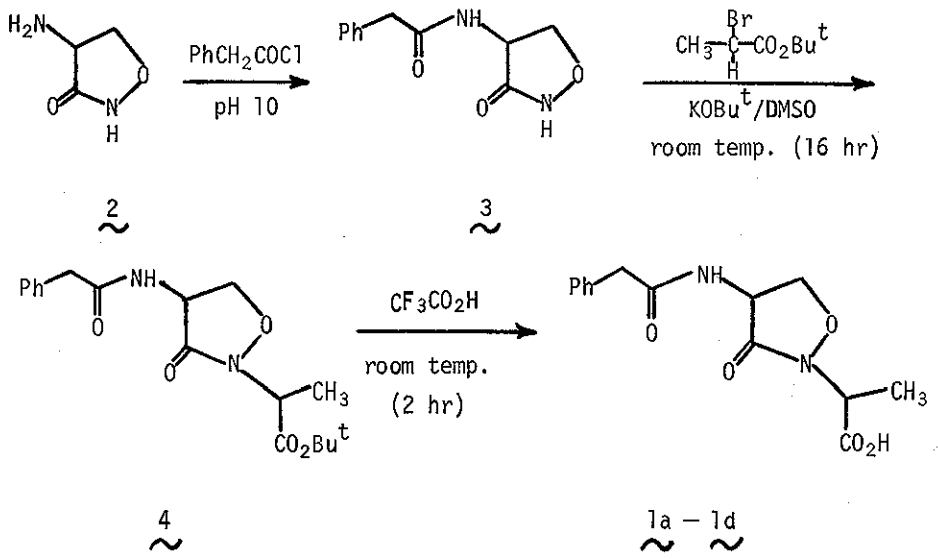
L-Cycloserine (2) (enantiomer of natural product), prepared by the method of Plattner, et al.,[4] was acylated with phenylacetyl chloride to afford phenylacetyl-L-cycloserine (3) [40% yield, mp 181–182°; $[\alpha]_D^{23}$ -25.4°; IR ν_{max} 3305, 1718, 1654, 1525 cm^{-1}].[5] Alkylation of 3 with t-butyl dl-α-bromopropionate gave the N-alkylated ester (4) [68% yield, viscous oil; IR $\nu_{max}^{CHCl_3}$ 3410, 1718, 1705, 1671 cm^{-1}] as a mixture of diastereoisomers. The t-butyl ester (4) was treated with CF_3CO_2H to give a 1:1.4 mixture of two diastereoisomers of the free acid (99% yield), which was separated into two isomeric compounds, 1a [mp 163–164°; $[\alpha]_D^{23}$ -88.5°; IR ν_{max} 3290, 1748, 1710, 1651, 1533 cm^{-1}; Rf 0.24] and 1b [mp 146–148°; $[\alpha]_D^{23}$ +44.2°; IR ν_{max} 3270, 1742, 1710, 1650, 1533 cm^{-1}; Rf 0.19] by fractional recrystallization from ethyl acetate.

In order to determine the absolute configuration of the above isomers, their antipodes were prepared from D-cycloserine (natural antibiotic) under the same sequence as described above. The enantiomers obtained were 1c [mp 165–166°; $[\alpha]_D^{21}$ +87.1°; Rf 0.24; NMR (pyridine-d_5) δ 1.67 (3H, d, J = 8.0 Hz, C-CH_3), 3.80 (2H, s, C_6H_5-CH_2), 4.35, 4.73 (each 1H, AB part of ABX, J_{AB} = 8.0 Hz, J_{AX} = 7.2 Hz, J_{BX} = 10.4 Hz, CH_2 at C_5), 5.12 (1H, q, J = 8.0 Hz, CH_3-CH)] and 1d [mp 148–149°; $[\alpha]_D^{21}$ -46.4°; Rf 0.19; NMR (pyridine-d_5) δ 1.64 (3H, d, J = 8.0 Hz), 3.81 (2H, s, C_6H_5-CH_2), 4.22, 4.77 (each 1H, J_{AB} = 8.3

Hz, J_{AX} = 7.7 Hz, J_{BX} = 10.1 Hz), 5.18 (1H, q, J = 8.0 Hz)]. The IR and NMR spectra of the two enantiomers (1a and 1c, and 1b and 1d) were identical.

Catalytic hydrogenolysis (PtO_2 in AcOH) of the lower melting isomer 1d afforded N-phenylacetylserylalanine, which was then hydrolyzed with 6 N HCl at 110° to give S(+)-alanine and R(+)-serine. Thus, the absolute configuration of 1d should be expressed as 4R-1'S and, therefore, those of 1a, 1b, and 1c should be shown as 4S-1'S, 4S-1'R, and 4R-1'R, respectively.



2 3 4 1a – 1d

The antibacterial activity of 1b which has the same configuration as D-alanyl-D-alanine dipeptide was examined together with those of 1a, 1c, and 1d, but unfortunately, they displayed no antibacterial activities against S. aureus and E. coli at 1 mg/ml in vitro (paper disk diffusion method). In order to evaluate the acylating capability of these cycloserine derivatives, we measured the hydroxide ion-catalyzed hydrolysis rates, which are known to be correlated

with antibacterial activity of penicillins and cephalosporins.[2c,2g] The kinetic experiments were carried out in aqueous sodium hydroxide solution (μ = 0.5) at 35°. The rate of the hydrolysis of 1 obeyed the first-order rate law. The pseudo-first-order rate constant (k_{obs}) was found to be directly proportional to the hydroxide ion activity, indicating the validity of the equation, $k_{obs} = k_{OH} \times a_{OH}$. The values of k_{OH} for all compounds synthesized were approximately 1.6 $M^{-1}hr^{-1}$, and revealed that the acylating ability of 1 was reasonably high compared with those determined under similar conditions for simple and ring-fused β-lactams,[2b,6] but one-fiftieth to one-third of that of the active D-alanyl-D-alanine compounds such as penicillins[6,7] and Δ^3-cephalosporins,[2d-2f] and was similar to inactive Δ^2-deacetoxycephalosporins.[2d]

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5 Satisfactory elemental analyses were obtained for all compounds reported; IR spectra were recorded in Nujol mull, unless otherwise stated; Optical rotations were measured in methanol solution (c = 1); Rf values were obtained by the TLC on silica gel with a solvent system of $CHCl_3$: MeOH: H_2O = 2:2:1 (organic layer).

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