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Abstract - The synthesis and some biological properties of the title compounds, beta-lactam antibiotics, are described.

Recent discoveries in the field of beta-lactam antibiotics show that the geometric relationship between the N1-C2 amide bond and the anionic binding site is likely to be an important determinant of antimicrobial potency.1 We sought to probe the limiting distance between these moieties by the synthesis of 1 and 2, wherein a tetrazole ring serves as a rigid spacer between the azetidin-2-one and carboxyl function. In addition to providing geometric definition to this relationship, the tetrazole function serves to withdraw electron density from the amide bond by induction, another effect usually correlated with bioactivity.

As in our previous report2 on N-(5-tetrazolyl)azetidin-2-ones, N-Boc-L-serine was protected as its tert-butyldimethylsilyl ether3 and coupled with 5-aminotetrazole to give 4. Alkylation with benzyl bromoacetate and triethylamine in acetonitrile afforded a 3.5/1 mixture of isomers. Different bases and solvents did not dramatically alter this ratio. Although the isomers could not be conclusively identified at this stage, literature precedent holds that 5-carbon-substituted tetrazoles alkylate predominantly at the 2-position and that 1- and 2-substituted tetrazole isomers exhibit consistent spectral and physical differences.4 The assignment of 5 and 6 was ultimately verified by a crystallographic study (vide infra). After separation by chromatography, 5 and 6 were desilylated by hydrogen fluoride in acetonitrile.5 Cyclization of 7 and 8 was achieved with diisopropylazodicarboxylate/triphenylphosphine6 to yield azetidin-2-ones 9 and 10. Deblocking with trifluoroacetic acid and acylation of the evaporated salts with phenylacetyl chloride afforded 11 and 12. Hydrogenolysis with palladium in the presence of potassium phosphate buffer gave 1 and 2.

To verify that the stereochemical integrity of intermediates was retained throughout the synthesis, 10 was deblocked to the free amine 13 and acylated with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(+)MTPACl]7 to give 14. Examination of the 200 MHz pmr spectrum showed 14 to be homogeneous with a single NH doublet at 7.46 δ (J = 7 Hz) and a single methoxyl quartet8 at 3.42 δ (J = 1.2 Hz). Therefore, any racemization taking place is undetectable by pmr. To firmly establish the identity of the tetrazolyl isomers, the structure of 10 was confirmed by X-ray crystallography.9
A 116-microgram sample of 1 on a 6-mm paper disc imparted a 14 mm zone of inhibition in a culture of Staph. aureus. Likewise, 59 micrograms of 2 gave a 21 mm zone. However, both compounds were inactive (>128 µg/ml 10^4 colony forming units) against a variety of more clinically representative bacteria, by a tube-dilution, minimum
inhibitory concentration assay. This result stands in marked contrast to the significant antimicrobial activity of the N-tetrazolylazetidin-2-ones 15. As the inductive effect upon the amide bond in 1 and 2 should be greater than that induced in 15 by the anionic tetrazole, we conclude that the distance between the electrophilic center and the anionic function is too large (5.08 Å) for efficient recognition by target enzyme binding sites.

Acknowledgment

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References

3) Selected physical data: 4: mp 152–55°C (D2O = +2.8° (c = 1, CH3OH); IR (CH3CN): 3300, 1720, 1600 cm⁻¹; 1H NMR (CDCl3) δ 6.19 (1 H, d, J = 7), 4.56 (1 H, m), 4.28 (1 H, dd, J = 3, 10.5), 3.97 (1 H, dd, J = 4, 10.5), 1.43 (9 H, s), 0.80 (9 H, s), 0.04 (6 H, s). 5: IR (CHCl3): 3410, 1755, 1715, 1550 cm⁻¹; 1H NMR (CDCl3) δ 7.40 (5 H, m), 5.38 (2 H, s), 5.26 (2 H, s), 4.40 (1 H, m), 4.10 (1 H, dd, J = 4, 10), 3.80 (1 H, dd, J = 6, 10), 1.46
4) IR (CHCl₃): 3405, 1760, 1715, 1555 cm⁻¹; ¹H NMR (CDCl₃) δ 7.40 (5 H, m), 5.46 (2 H, s), 5.24 (2 H, s), 4.40 (1 H, m), 4.18 (1 H, dd, J = 4, 10), 3.76 (1 H, dd, J = 7, 10), 1.50 (9 H, s), 0.88 (9 H, s), 0.10 (6 H, s).

6) P. G. Mattingly, Suitable crystals effects. A multi-solution tangent formula approach reflections

9) IR (CHCl₃): 1790, 1760, 1715 cm⁻¹; ¹H NMR (CDCl₃) δ 7.36 (5 H, m), 5.70 (1 H, d, J = 17.5), 5.48 (1 H, d, J = 17.5), 5.24 (1 H, d, J = 12), 5.16 (1 H, d, J = 12), 4.79 (1 H, br s), 4.1 (2 H, m), 1.46 (9 H, s).

10) mp 188-90°; IR (CHCl₃): 3460, 1790, 1765, 1720, 1560 cm⁻¹; ¹H NMR (CDCl₃) δ 7.41, (5 H, br s), 5.41 (2 H, s), 5.27 (2 H, s), 5.06 (1 H, m), 4.22 (1 H, m), 4.02 (1 H, dd, J = 3.5, 6), 1.46 (9 H, s); MS: 347, 329.

11) IR (CHCl₃): 1795, 1770, 1685 cm⁻¹; ¹H NMR (CDCl₃) δ 7.34 (10 H, m), 5.98 (1 H, d, J = 6.5), 5.72 (1 H, d, J = 17.5), 5.48 (1 H, d, J = 17.5), 5.28 (1 H, d, J = 12), 5.14 (1 H, d, J = 12), 4.74 (1 H, ddd, J = 3.5, 6.5, 7.0), 4.12 (1 H, dd, J = 6.5, 6.5), 4.04 (1 H, dd, J = 3.5, 7), 3.66 (2 H, s). 12) mp 135° [D]D=+36.8° (c = 1, CHCl₃); IR (CHCl₃): 3940, 1790, 1765, 1680, 1560, 1560 cm⁻¹; ¹H NMR (CDCl₃) δ 7.40-7.30 (10 H, m), 6.08 (1 H, d, J = 7), 5.40 (2 H, s), 5.23 (2 H, s), 5.09 (1 H, ddd, J = 3, 6, 7), 4.18 (1 H, dd, J = 6, 6), 3.95 (1 H, dd, J = 3, 6), 3.67 (2 H, s). 14) IR (CH₂Cl₂): 3400, 1795, 1760, 1705 cm⁻¹; ¹H NMR (CDCl₃) δ 7.64 (1 H, d, J = 7), 7.5-7.3 (10 H, m), 5.41 (2 H, s), 5.27 (2 H, m), 4.26 (1 H, dd, J = 6, 6), 3.94 (1 H, dd, J = 3, 6), 3.42 (3 H, q, J = 1.2).

Co., Delft, Holland (1981); ORTEP-II, Oak Ridge National Laboratory, Oak Ridge, Tenn. (1970) with tangent formula recycling [J. Karle, Acta Cryst. B24, 182 (1968)] was used to find initial positions for all non-hydrogen atoms. Full matrix least squares and difference Fourier methods were used to find refined positions. The function minimized was \[ \sum (|F_o| - |F_c|)^2 \] with \( w = 1/\sigma^2_F \) to give an unweighted residual index of 0.053. Tables containing the final fractional coordinates, temperature parameters, bond distances and bond angles, have been deposited in the Cambridge Crystallographic Database.

10) E. coli, Sal. typhimurium, Ent. cloacae, Ent. aerogenes, K. pneumoniae, Prot. vulgaris, Prot. morganii, Prot. mirabilis, Ps. aeruginose, Ser. marcesana.

11) Calculated by rotation of N7-C10 in 10 to minimize the C11-O21 distance. This value is beyond the range of active compounds (<4.1 Å) cited by Cohen.1

12) It must be cautioned that any observed activity of 1 or 2 may not be due to the same mechanisms of action shared by "classical" beta-lactam antibiotics, i.e., the penicillin-binding proteins and cell-wall synthesis inhibition D. J. Waxman and J. L. Strominger, Annual Rev. Biochem. 52, 825 (1983).

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