Tetrahedron Letters No.38, pp. 4615-4620, 1966. Pergamon Press Ltd.
Printed in Great Britain.

ACYLATED AZIRIDINE-2-CARBOXYLIC ACID PEPTIDES AS PENICILLIN ANALOGS

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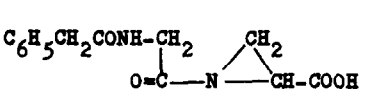
(Received 29 June 1966)

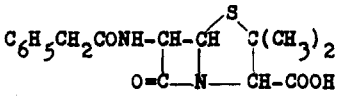
The antibiotic activity of penicillin is believed to be due to the acylation of an active site in an enzyme concerned with mucopeptide synthesis in the bacterial cell wall.[1] In turn, the acylating activity of penicillin has been associated with the reactive carbonyl group of the fused β-lactamthiazolidine ring system;[2] thus, the less reactive γ-lactam homologs of penicillin completely lack activity,[3] the more stable monocyclic dethiopenicillins have little or no activity[4] and acyclic analogs have a low order of activity.[5]

This communication reports the synthesis, characterization and microbiological assay of the dicyclohexylamine salt of N-phenaceturylaziridine-2-carboxylic acid (I), which may be considered as a structural analog (containing the correct "backbone") and perhaps also as a reactivity analog of peni-

cillin G (II) due to the presence of an "activated" amide[6] which occupies a position comparable to that of the β-lactam in the penicillin nucleus. N-Acylated aziridines are known to be good acylating agents[7] and to undergo hydrolysis with base very readily.[8]



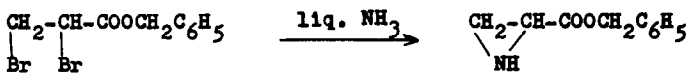
I



II

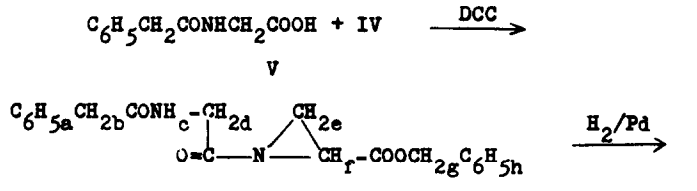
Treatment of benzyl acrylate[9] with one equivalent of bromine in benzyl alcohol, following a procedure of Marvel et al.,[10] afforded benzyl α,β-dibromopropionate (III) in 60% yield; b.p. 118° (0.025 mm), n_D^{22} 1.6385, ν_{max} 1745 cm^{-1}. Anal. Calcd for $C_{10}H_{10}Br_2O_2$: C, 37.30; H, 3.13; Br, 49.63. Found: C, 37.85; H, 3.30; Br, 49.08.

Dropwise addition of compound III to a large excess of liquid ammonia followed by ether extraction of the ammonia solution gave a mixture of benzyl aziridine-2-carboxylate (IV)[11] and benzyl alcohol; treatment with an ethereal solution of oxalic acid afforded the colorless monooxalate salt of benzyl aziridine-2-carboxylate; m.p. 75.5-77°, ν_{max}^{KBr} 1749 cm^{-1}. Anal. Calcd for $C_{12}H_{13}NO_6$: C, 53.93; H, 4.90; N, 5.24 Found: C, 54.24; H, 5.50; N, 5.50. The free base IV,[12] recovered by ether extraction of an aqueous solution of the oxalate salt made basic with sodium bicarbonate, was obtained in 34% overall yield from III; n_D^{25} 1.5420, ν_{max} 1730 cm^{-1}. Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.50; H, 6.00; N, 7.61.



III IV

Coupling of phenaceturic acid (V) and IV with N,N'-dicyclohexylcarbodiimide (DCC)[13] at -5° in methylene chloride gave crystalline VI in 50% crude yield; several recrystallizations from benzene —— Skelly B afforded an analytical sample of benzyl N-phenaceturylaziridine-2-carboxylate (VI)[12]; m.p. 84-85°, ν_{max}^{KBr} 1740, 1650 and 1540 cm^{-1}. Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.11; H, 5.60; N, 8.06. The nmr spectrum ($CDCl_3$ solvent, TMS internal standard) showed bands at τ 2.68 ($5H_a$, singlet), 2.75 ($5H_h$, singlet), 3.15 ($1H_c$, triplet), 4.87 ($2H_g$, singlet), 6.05 ($2H_d$, doublet), 6.52 ($2H_b$, singlet), 6.80 ($1H_f$, quartet) and 7.57 ($2H_e$, multiplet), assigned as indicated in structure VI.



V VI

Hydrogenolysis of VI over 10% palladium on carbon catalyst proceeded in ethyl acetate with the uptake of one equivalent of hydrogen and, after filtration and addition of one equivalent of dicyclohexylamine, yielded 72% of the crystalline dicyclohexylamine salt of N-phenaceturylaziridine-2-carboxylic acid (I);[12] m.p. 133.5-136.5°. ν_{max}^{KBr} 1725, 1675, 1650, 1560 and

1530 cm^{-1}. Anal. Calcd for $C_{25}H_{37}N_3O_4$: C, 67.69; H, 8.41; N, 9.47. Found: C, 67.75; H, 8.67; N, 9.29. The dicyclohexylamine salt of I responded to the quantitative hydroxylamine assay for penicillins[14] and gave 150% of the color intensity exhibited by the sodium salt of penicillin G (molar basis).

Professor Michael J. Pelczar, Jr., and Samir M. Badr El-Din of the Department of Microbiology have assayed the dicyclohexylamine salt of I for antibiotic activity. By a twofold serial dilution test using Staphylococcus aureus this salt showed an in vitro minimum inhibitory concentration at a level of 1000-2000 μg/ml. The antibiotic activity is of a very low order compared to the penicillins but approaches the bioactivity of the sulfonamides.

The present work demonstrates that specific structural requirements beyond that of an "activated" amide (and the correct backbone) are essential to attain the unusual antibiotic activity of the penicillins. Within a given series of molecules the relationship of biological activity and chemical structure has been summarized by the observation that "increase in flexibility usually decreases biological potency."[15] One important factor here may therefore be the greater number of conformations in the partial open-chain analog I compared with the rigid fused-ring system of penicillin (II). The requirements for bioactivity are being investigated by the synthesis of other penicillin analogs.

Acknowledgments. This investigation was supported in part

by Public Health Service Research Grant CA-07193 from the National Cancer Institute. We gratefully acknowledge assistance from an NSF Grant (GP-3756) for the purchase of a Varian A-60A nmr spectrometer.

REFERENCES

1. F. P. Doyle and J.H.C. Nayler, Advances in Drug Research, N. J. Harper and A. B. Simmonds, eds., Vol. I, p.62. Academic Press, New York and London (1964).

2. J. R. Johnson, R. B. Woodward and R. Robinson, The Chemistry of Penicillin, H. T. Clarke, J. R. Johnson and R. Robinson, eds., p. 440. Princeton University Press, Princeton, N.J. (1949).

3. V. du Vigneaud and F. H. Carpenter, The Chemistry of Penicillin, H. T. Clarke, J. R. Johnson and R. Robinson, eds., p. 1007. Princeton University Press, Princeton, N.J. (1949).

4. E. Kaczka and K. Folkers, The Chemistry of Penicillin, H. T. Clarke, J. R. Johnson and R. Robinson, eds., p. 256. Princeton University Press, Princeton, N.J. (1949).

5. F. W. Holly, E. W. Peel, E. L. Luz and K. Folders, J. Amer. Chem. Soc., 74 4539 (1952).

6. The designation "activated" amide is suggested by analogy to the widely used term "activated" ester. For a recent review of activated esters, see E. Schröder and F. Lübke, The Peptides, Vol. I, pp. 76, 97. Academic Press, New York and London (1965).

7. H. W. Heine, M. E. Fetter and E. M. Nicholson, J. Amer. Chem. Soc., 81, 2202 (1959).

8. The reaction at room temperature of methyl N-hippuryl-aziridine-2-carboxylate with one equivalent of lithium hydroxide gave an 80% yield of lithium hippurate. R. A. Clark, Ph.D. Thesis, University of Maryland, 1966.

9. C. Moureu, M. Murat and L. Tampier, Ann. Chim., 15, 221 (1921)

10. C. S. Marvel, J. Dec, H. G. Cooke, Jr., and J. C. Cowan, J. Amer. Chem. Soc., 62, 3495 (1940).

11. Based on the procedure for the preparation of methyl aziridine-2-carboxylate described by F. Hoffmann-La Roche & Co., Brit. Pat. 847,205, Sept. 7, 1960 (Chem. Abstr., 55, 7433 (1961)), as modified by R. A. Clark, Ph.D. Thesis, University of Maryland, 1966. See also E. Kyburz, H. Els,

St. Majnoni, G. Englert, C. von Planta, A. Furst and Pl. A. Plattner, Helv. Chim. Acta, 49, 359 (1966).

12. This compound gave an intense purple color when tested by the procedure described in the article entitled "Use of 4-(4'Nitrobenzyl)pyridine as Analytical Reagent for Ethylenimines and Alkylating Agents," by J. Epstein, R. W. Rosenthal and R. J. Ess, Anal. Chem., 27, 1435 (1955).

13. J. C. Sheehan and G. P. Hess, J. Amer. Chem. Soc., 77, 1067 (1955).

14. J. H. Ford, Anal. Chem., 19, 1004 (1947).

15. L. H. Sarett, 147th National Meeting of the American Chemical Society, Philadelphia, Penna., April 1964, Abstracts of Papers, p. 3-N.