Spackman, Stein and Moore[21] and by microbiological assay[22] showed an amino acid composition consistent with the theoretically calculated values (chromatographic: $lys_{2.00}his_{1.00}arg_{2.88}ser_{1.76}glu_{1.00}pro_{1.05}gly_{0.97}met_{0.95}tyr_{0.99}phe_{1.02}$; microbiological: $lys_{1.97}his_{1.04}arg_{2.95}ser_{1.86}pro_{0.92}gly_{1.28}met_{0.98}tyr_{1.04}phe_{1.00}$). The intact pentadecapeptide was found to contain tyrosine and tryptophan in a molar ratio of one to one, as determined spectrophotometrically.[23]

(21) D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(22) The microbiological assay was carried out by the Shankman Laboratories, Los Angeles, Calif.

(23) T. W. Goodwin and R. A. Morton, *Biochem. J.*, **40**, 628 (1946).

(24) This work was supported in part by a grant (GM-2907) from the National Institutes of Health, United States Public Health Service, and a grant from the American Cancer Society.

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RECEIVED MARCH 9, 1963

A Further Example of Inversion of the Usual Antipodal Specificity of α-Chymotrypsin[1]

Sir:

In 1948 it was shown that the stereochemical course of the papain catalyzed synthesis of α-N-acylated α-amino acid phenylhydrazides, from certain α-N-acylated α-amino acids and phenylhydrazine, could be determined by the structure of the acyl component.[2] Subsequent studies[3,4] confirmed and extended these results but attempts to observe the same phenomenon with the comparable α-chymotrypsin catalyzed reaction were unsuccessful.[5]

In 1960 an inversion of the usual antipodal specificity of α-chymotrypsin was demonstrated when it was found that the rate of the α-chymotrypsin catalyzed hydrolysis of D-3-carbomethoxydihydroisocarbostyril, to the corresponding acid, was markedly greater than that of the L-antipode.[6] In providing an explanation for the preceding observations a theory was developed[7,8] which not only accounted for the above results but also forecast in general terms the existence of other examples of inversion of antipodal specificity as well as those involving diminished stereochemical preference in favor of the L-antipode for compounds of the type $R_1'CONHCHR_2COR_3$ and cognate structures.

In a recent communication Cohen, *et al.*,[9] describe an inversion of the usual antipodal specificity in the α-chymotrypsin catalyzed hydrolysis of ethyl α-acetoxypropionate. This behavior was explained in terms of a theory,[9] which was similar to that developed earlier,[7,8] and provided a needed example of inversion of antipodal specificity in a case where the structures were not conformationally constrained. However, there remained a need for a demonstration that the nature of the group R_1' in compounds of the type $R_1'CONHCHR_2COR_3$, with the nature of R_2 and R_3 remaining invariant, could determine the degree of stereochemical preference for a given antipode or cause an inversion of the antipodal specificity usually observed for α-chymotrypsin catalyzed reactions, *i.e.*, preference for the L-antipode.

An example of diminished stereochemical preference for the L-antipode in compounds of the type $R_1'CONHCHR_2COR_3$, and associated with the nature of R_1', became available when it was found that the kinetic constants for the α-chymotrypsin catalyzed hydrolysis of N-benzoylalanine methyl ester, in aqueous solutions at 25.0°, pH 7.90 and 0.20 M in sodium chloride, were $K_0 = 3.3 \pm 0.2$ mM and $k_0 = 0.0107 \pm 0.0002$ sec.$^{-1}$ for the D-antipode, and $K_0 = 9.8 \pm 0.9$ mM and $k_0 = 0.26 \pm 0.01$ sec.$^{-1}$ for the L-antipode.[8] For N-acetylalanine methyl ester, $K_0 = 611 \pm 10$ mM and $k_0 = 1.29 \pm 0.02$ sec.$^{-1}$ for the L antipode (in 0.50 M sodium chloride) with no detectable substrate activity being observable for the D-antipode.[10]

The sought for example of inversion of antipodal specificity for substrates of the type $R_1'CONHCHR_2COR_3$ arising from appropriate selection of the group R_1', with the nature of R_2 and R_3 remaining invariant, has now been found. In the α-chymotrypsin catalyzed hydrolysis of N-picolinylalanine methyl ester, in aqueous solutions at 25.0°, pH 7.90 and 0.10 M in sodium chloride, values of $K_0 = 18 \pm 1$ mM and $k_0 = 0.070 \pm 0.003$ sec.$^{-1}$ were obtained for the L-antipode (m.p. 59–60°, $[\alpha]^{25}D$ −15.3 ± 0.3° (c 3% in water)) and $K_0 = 17 \pm 1$ mM and $k_0 = 0.165 \pm 0.006$ sec.$^{-1}$ for the D-antipode (m.p. 59–60°, $[\alpha]^{25}D$ 15.3 ± 0.3° (c 3% in water)). The experiments were conducted with the aid of a pH-stat[11,12] under conditions where $[E] = 26$ μM[13] and $[S] = 2.3$–18.4 mM for the L-antipode and $[E] = 74$ μM and $[S] = 1.5$–12 mM for the D-antipode. The primary data were evaluated using a Datatron 220 digital computer programmed as described previously.[14]

With three examples of inversion of the usual antipodal specificity of α-chymotrypsin, involving both conformationally constrained and unconstrained substrates, two of which are α-N-acylated α-amino acid derivatives, it is evident that substantial support has now been provided for the explanation of this phenomenon given earlier.[7-9] It also follows that the more general theory[7,8] which envisions non-productive combination of substrate that is fully competitive with its productive combination with the active site of the enzyme has acquired added significance.

(1) Supported in part by a grant from the National Institutes of Health, U. S. Public Health Service.

(2) E. L. Bennett and C. Niemann, *J. Am. Chem. Soc.*, **70**, 2610 (1948).

(3) (a) H. B. Milne and C. M. Stevens, *ibid.*, **72**, 1742 (1950); (b) E. L. Bennett and C. Niemann, *ibid.*, **72**, 1798 (1950).

(4) W. H. Schuller and C. Niemann, *ibid.*, **73**, 1644 (1951).

(5) W. H. Schuller and C. Niemann, *ibid.*, **74**, 4630 (1952).

(6) G. E. Hein, R. B. McGriff and C. Niemann, *ibid.*, **82**, 1830 (1960).

(7) G. Hein and C. Niemann, *Proc. Natl. Acad. Sci.*, **47**, 1341 (1961).

(8) G. E. Hein and C. Niemann, *J. Am. Chem. Soc.*, **84**, 4487, 4495 (1962).

(9) S. G. Cohen, J. Crossley, E. Khedouri and R. Zand, *ibid.*, **84**, 4163 (1962).

(10) J. P. Wolf, III, and C. Niemann, *Biochemistry*, **2**, 493 (1963).

(11) T. H. Applewhite, R. B. Martin and C. Niemann, *J. Am. Chem. Soc.*, **80**, 1457 (1958).

(12) T. H. Applewhite, H. Waite and C. Niemann, *ibid.*, **80**, 1465 (1958).

(13) Based upon a molecular weight of 25,000 and a nitrogen content of 16.5%.

(14) H. I. Abrash, A. N. Kurtz and C. Niemann, *Biochim. Biophys. Acta*, **45**, 378 (1960).

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RECEIVED MARCH 18, 1963

Chemistry of Cephalosporin Antibiotics. III. Chemical Correlation of Penicillin and Cephalosporin Antibiotics

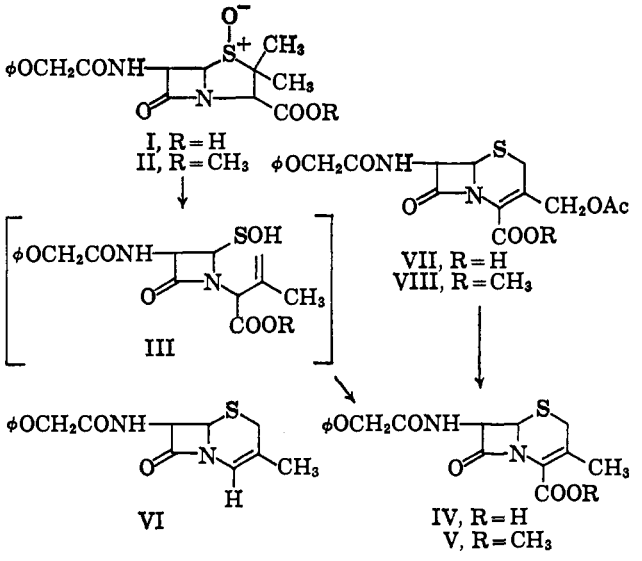
Sir:

Recent reports have shown that a series of new, potent, β-lactam-containing antibiotics can be synthesized from the naturally occurring substance, cephalosporin C.[1] These substances have the same carbon skeleton as penicillins but differ by the state of oxida-

(1) (a) B. Loder, G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961); (b) R. R. Chauvette, *et al.*, *J. Am. Chem. Soc.*, **84**, 3401 (1962).

tion and the size of the sulfur-containing ring. We now report the chemical conversion of penicillin to cephalosporin-type compounds.

Treatment of phenoxymethylpenicillin sulfoxide methyl ester (II), m.p. 121.5–122.5°, $[\alpha]_D$ +200° (periodate oxidation of phenoxymethyl penicillin[2] and esterification with diazomethane) with a trace of *p*-toluenesulfonic acid in xylene at reflux temperature gave a 15% yield of a less polar compound, m.p. 141–142°, $[\alpha]_D$ +94°, λ_{max}^{EtOH} 268 mμ, ε 7600, $\lambda_{max}^{CHCl_3}$ 5.62, 5.82, 5.92 and 6.12 μ, to which we have assigned structure V.[3] The survival of the β-lactam is indicated by the infrared band at 5.62 μ and the presence in the n.m.r. spectrum of a one proton quartet at 4.14 τ and a one proton doublet at 4.95 τ, characteristic of the two



hydrogen atoms on the four-membered ring in cephalosporins and certain penicillin derivatives. Also, the n.m.r. spectrum shows a single methyl group attached to carbon (7.84 τ) and non-equivalent methylene signals (6.73 and 6.44 τ, J = 18 c.p.s.) which can be assigned to the hydrogens attached to the carbon adjacent to sulfur. The presence of the double bond is indicated by the infrared band at 6.12 μ and the ultraviolet absorption spectrum which is similar to that of the corresponding cephalosporin derivative (VIII). A plausible pathway for the formation of V is *via* a sulfoxide elimination[4] to the intermediate (III), subsequent addition of the sulfenic acid to the double bond with the sulfur becoming attached to the primary carbon[5] and the loss of a proton to yield V.

Thermal treatment of phenoxymethylpenicillin sulfoxide (I)[2,6] with and without added mineral acid yielded not the desired product (IV) but a neutral substance (VI), m.p. 173.5–174.5°, $[\alpha]_D$ −35.3°, λ_{max}^{EtOH} 256 mμ, ε 9150, $\lambda_{max}^{CHCl_3}$ 5.65, 5.92 and 6.03 μ (sh) which possessed an olefinic proton as shown by a peak at 3.52 τ in the n.m.r. spectrum. High pressure catalytic (Pd–C) hydrogenolysis of VII[1b] provided an acid assigned structure IV since its methyl ester, also obtained by reduction of VIII, m.p. 149–150°, $[\alpha]_D$ +53°, was identical with V made by the sulfoxide rearrangement.

The isolation of the same derivative (V) from both a cephalosporin and a penicillin provides a direct chemical correlation of the two types of compounds. The sulfoxide rearrangement involved in this correlation is not peculiar to penicillin sulfoxide but can be applied to simpler cyclic sulfoxides.[7]

(2) We wish to thank Dr. E. H. Flynn of these Laboratories for this procedure.

(3) Rotations were determined in dioxane, n.m.r. spectra were recorded on Varian HR-60 in $CDCl_3$ with tetramethylsilane as internal standard and all compounds for which melting points are reported have given satisfactory elemental analyses.

(4) This initial step is analogous to the thermal *cis*-elimination of 1,2-diphenyl-1-propyl-phenyl sulfoxides to α-methylstilbenes: C. A. Kingsbury and D. J. Cram, *J. Am. Chem. Soc.*, **82**, 1810 (1960). In our case a trace quantity of acid was necessary to cause a reaction.

(5) The compound arising from addition of the sulfenic acid to the double bond in the opposite sense apparently is unstable in the described experiment. Under other conditions this is the predominant product isolated. Details will be reported in future publications.

(6) A. W. Chow, N. M. Hall and J. R. E. Hoover, *J. Org. Chem.*, **27**, 1381 (1962).

(7) R. A. Mueller and R. B. Morin, unpublished results from these Laboratories.

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RECEIVED MARCH 28, 1963

BOOK REVIEWS

Gas Chromatography. Principles, Techniques and Applications. By A. B. LITTLEWOOD, King's College, University of Durham, Newcastle upon Tyne 1, England. Academic Press Inc., 111 Fifth Avenue, New York 3, N. Y. 1962. xi + 514 pp. 16 × 23.5 cm. Price, $15.00.

Dr. Littlewood's book represents an excellent addition to the suddenly proliferating number of general texts on the theory and practice of gas chromatography. The text is highly recommended for graduate level and as an introductory work for research people. No previous familarity with gas chromatography is required. The first chapter supplies the reader with an introduction to the mechanism, apparatus, history and applicability of the gas chromatography technique in a notably clear exposition distinguished by the author's ability to avoid the obscuring use of chromatographic jargon and equations. Succeeding chapters deal with theory of chromatographic separations, column performance and, finally, the practical selection and preparation of column construction materials. The author treats difficult theoretical discussions in a pedagogical progression, proceeding from the simple to the complex and even giving illustrative calculations where necessary. Chapters are also devoted to theory, construction and operation of most of the detectors used in gas chromatography, injection systems, quantitative analysis principles and techniques, and temperature programming. Finally, a major portion of the text (pp. 372–471) is devoted to a discussion of the important applications of gas chromatography. The organization of this chapter is based on the chemical type of sample material; *i.e.*, permanent gases, aromatic hydrocarbons, nitrogen compounds. The book makes excellent use of diagrams and includes many prints of applications, illustrations. Here the author faces what must be a common problem. The illustrations chosen are generally chromatograms taken from the "original" publication on a particular subject. While this is of historical value and gives proper credit to the scientist who first performed the separation, the results shown are often primitive compared to what may be obtainable in current practice. It might be more appropriate in a text which features applications guidance to obtain more representative illustration from current rather than "classical" sources.

The nomenclature used generally follows IUPAC recommendations, but occasionally employs unique symbols where they aid in the orderly development of a theoretical principle. Adequate references are cited, including publications as recent as 1961.