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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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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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).