marized in Table I and show that either product can be made to predominate.[6]

TABLE I

Sensitizer[a]	Fluorescence maximum, mμ[b]	Total % conversion	Ratio III/II
Chlorin e₆	670	85	1:4.5
Hematoporphyrin	630	80	1:4.5
Rose Bengal	580	88	1:1.2
Erythrosin B	578	71	1.6:1
Eosin Y	565	82	3.1:1
Riboflavin[c]	510	61	30:1

[a] Photoöxygenations were conducted in pyridine for 80 hr. [b] Measured in pyridine with an Aminco-Bowman Spectrofluorometer. [c] Owing to low solubility this run was in pyridine–methanol (4:1) for 110 hr. About the same product ratio was observed in pyridine alone but the total conversion was only 35%.

This finding has practical value for synthetic work and raises questions on the precise nature of the intermediates in sensitized oxygenations.[7] Interestingly, there is a rough parallelism between the trend in product ratio and the trend in fluorescence emission maximum for the different sensitizers.

(6) Control experiments showed that both products are stable to the conditions of photoöxygenation.

(7) An example of sensitizer control of product composition in a photochemical reaction not involving oxygen was reported recently [G. S. Hammond, N. J. Turro and A. Fischer, J. Am. Chem. Soc., 83, 4674 (1961)].

(8) This work was supported by a U. S. Public Health Service Grant (RG-9693), by a Grant-in-Aid from the Hynson, Westcott and Dunning Fund, and by the Alfred P. Sloan Foundation.

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The Synthesis of a Biologically Active Pentadecapeptide Corresponding to an Altered Sequence in the Adrenocorticotropin (ACTH) Structure

Sir:

Since the synthesis[1] of a nonadecapeptide corresponding to the NH₂-terminal 19-amino acids in the 39-amino acid chain of ACTH structures, several investigators[2–6] have reported the synthesis of various chain lengths. We wish to describe herein the synthesis of a pentadecapeptide with a structure consisting of the first ten NH₂-terminal residues linked with a sequence of positions 15 to 19 in ACTH structures; namely, L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycyl-L-lysyl-L-lysyl-L-arginyl-L-arginyl-L-proline. This synthetic product, designated as $\alpha^{(1-10)+(15-19)}$-ACTH, was shown to have an ACTH potency of approximately 1 U.S.P. unit per mg., as estimated by in vitro[7] and in vivo[8] methods in the rat. The product was found to exhibit the full lipolytic potency of the natural[9] α_s-ACTH, when assayed[10] in vitro with peri-

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renal adipose tissues of the rabbit. By the in vivo hypophysectomized frog assay,[11] the synthetic peptide had only one hundredth of the melanocyte-stimulating activity of the native hormones. Recent studies[10] with various synthetic peptides related to ACTH appeared to show that the same amino acid sequence may be important for both lipolytic and melanocyte-stimulating activities. It is further noted that the decapeptide (α^{1-10}-ACTH) possesses approximately one tenth of the lipolytic[10] and the melanocyte-stimulating[12] activities of the natural hormone whereas the pentapeptide[13] (α^{15-19}-ACTH) has none. The synthetic pentadecapeptide described herein is the first instance where a separation of these two activities has been achieved. Moreover, as far as we are aware this report represents the first synthesis of a biologically active peptide in which the natural sequence of the ACTH structure has been altered.

N^α-Carbobenzoxy-N^G-tosyl-L-arginine[14] (I) was condensed with L-proline-t-butyl ester[15] by N-ethyl-5-phenyl isoxazolium-3'-sulfonate[16] (II) to give the crystalline protected dipeptide (III). III was hydrogenated and allowed to react with I, again with the aid of II. The protected tripeptide was converted to the pentapeptide N^α-carbobenzoxy-N^ϵ-t-butyloxycarbonyl-L-lysyl-N^ϵ-t-butyloxycarbonyl-L-lysyl-N^G-tosyl-L-arginyl-N^G-tosyl-L-arginyl-L-proline-t-butyl ester (IV) by stepwise reaction of the tripeptide base with N^α-carbobenzoxy-N^ϵ-t-butyloxycarbonyl-L-lysine p-nitrophenyl ester.[17] IV was purified by countercurrent distribution in the toluene system[1] ($K = 0.25$); m.p. 105–110°; $[\alpha]^{25}D$ −36° (c 1, methanol). Anal. Calcd.: C, 56.4; H, 7.22; N, 13.2; S, 4.64. Found: C, 56.2; H, 7.33; N, 13.0; S, 4.69.

IV was hydrogenated to yield the pentapeptide base (V) which was purified by countercurrent distribution in the toluene system[1] ($K = 0.73$); m.p. 112–117°; $[\alpha]^{25}D$ −33.7° (c 1, methanol). Anal. Calcd.: C, 54.8; H, 7.51; N, 14.6. Found: C, 54.5; H, 7.72; N, 14.4.

V was treated with crystalline carbobenzoxy-L-seryl-L-tyrosyl-L-seryl-L-methionyl-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophyl-glycine[5] in the presence of II to give the protected pentadecapeptide (VI). VI was purified by countercurrent distribution in the carbon tetrachloride system[1] followed by washing with methanol; m.p. 225–230° dec.; $[\alpha]^{25}D$ −51.5° (c 2.4, dimethylformamide). Anal. Calcd.: C, 57.0; H, 6.48; N, 14.0. Found: C, 56.6; H, 6.17; N, 13.8.

VI was treated with trifluoroacetic acid and then with sodium in liquid ammonia[18] to remove all the protecting groups. The crude pentadecapeptide was desalted and purified by chromatography in a carboxymethyl cellulose[19] column. The purified $\alpha^{(1-10)+(15-19)}$-ACTH was found to be homogeneous in paper and polyacrylamide gel[20] electrophoresis. Amino acid analysis of an acid hydrolysate of the synthetic pentadecapeptide both by the chromatographic method of

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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]

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(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-acetoxy-propionate. 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-chymo-

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trypsin 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 $R_1'CONHCHR_2$-COR_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 $-15.3 \pm 0.3°$ (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 $\pm 0.3°$ (c 3% in water)). The experiments were conducted with the aid of a pH-stat[11,12] under conditions where $[E] = 26 \mu M$[13] and $[S] = 2.3$–18.4 mM for the L-antipode and $[E] = 74 \mu M$ 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.

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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-

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