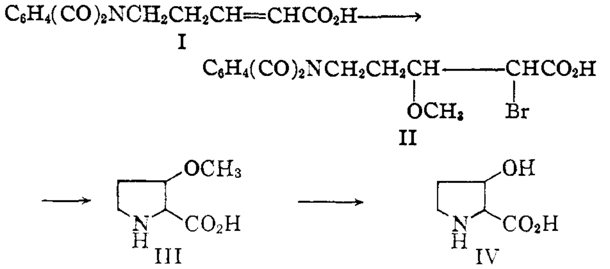
THE ISOLATION, CHARACTERIZATION AND SYNTHESIS OF 3-HYDROXYPROLINES

Sir:

Among the acidic hydrolysis products of the antibiotic Telomycin[1] we have found two amino acids not previously encountered in nature[2] or known synthetically. These two amino acids are designated as "slow moving" and "fast moving" hydroxyprolines, which relates to the difference in their behavior upon electrophoresis. Each amino acid has an analysis corresponding to $C_5H_9NO_3$ and affords proline upon reduction with red phosphorus and hydrogen iodide.[3] We also wish to report the synthesis of two racemates of 3-hydroxyproline, which correspond in chemical and physical properties to the two amino acids from Telomycin.


$C_6H_4(CO)_2NCH_2CH_2CH{=}CHCO_2H \longrightarrow$

I

$C_6H_4(CO)_2NCH_2CH_2\underset{OCH_3}{CH}-\underset{Br}{CHCO_2H}$

II

III

IV

5-Phthalimido-2-pentenoic acid (I)[4] was converted to 2-bromo-3-methoxy-5-phthalimidopentanoic acid (II) by a modification of the procedure of Carter and West[5a] for the preparation of 2-bromo-3-methoxybutyric acid from crotonic acid. The racemates of II were separated by fractional crystallization from toluene (IIA, 15%; m.p. 150–153°; *Anal.* Found: C, 47.44; H, 3.99; N, 4.26; Br, 23.25) (IIB, 13%; m.p. 112–114°; *Anal.* Found: C, 47.46; H, 3.95; N, 3.96; Br, 22.16).

The phthaloyl group was removed from IIA by titration with base at 0°,[6] then hydrolysis (1 hour in *N* hydrochloric acid at reflux). Phthalic acid, in almost quantitative yield, was separated by extraction into ether and, after concentration, the amine hydrochloride was treated with base to effect cyclization.[7] Neutralization followed by separation from sodium chloride on a sulfonic acid type cation exchange resin (ammonia elution) afforded one racemate of 3-methoxyproline (IIIA) in 34% over-all yield from IIA, m.p. 205.5–206.5°; *anal.* Found: C, 49.47; H, 7.54; N, 9.16.

Treatment of IIIA with constant-boiling hydrobromic acid, under reflux,[5a,b] afforded a single racemate of 3-hydroxyproline (IVA), 84%; m.p. 225–235° dec.; *anal.* Found: C, 45.76; H, 6.75; N, 10.73.

When the foregoing reaction sequence was carried out on IIB, a mixture of racemates of 3-methoxyproline (IIIA and IIIB) was obtained. These were separated by fractional crystallization of the copper salts (copper salt of IIIA is water-soluble; copper salt of IIIB is sparingly water-soluble) and regenerated on a cation exchange resin[8] (IIIA, 23%; m.p. 205.5–206.5°; mixture m.p. with IIIA (from IIA) undepressed) (IIIB, 13%; m.p. 184–185°; *anal.* Found: C, 49.65; H, 7.56; N, 9.54.) Compound IIIB afforded a single racemate of 3-hydroxyproline (IVB) in 84% yield, m.p. 223–230° dec.; *anal.* Found: C, 45.77; H, 6.85; N, 10.39.

The "fast moving" and "slow moving" hydroxyprolines from Telomycin were shown to correspond to IVA and IVB, respectively, by electrophoresis,[9a] paper chromatography[9b,c] and color reactions.

The relative mobilities (taking 4-hydroxyproline as unity) were: electrophoresis: 4-Hypro (1), *allo*-4-Hypro (1.15), IVA (1.15), IVB (0.83), "fast moving" Hypro (1.15), "slow moving" Hypro (0.83); paper chromatography (9b,9c): 4-Hypro (1,1), *allo*-4-Hypro (1.35, 1), IVA (1.80, 1.29), IVB (1.16, 1.08), "fast moving" Hypro (1.80, 1.29), "slow moving" Hypro (1.16, 1.08).

"Fast moving" hydroxyproline, "slow moving" hydroxyproline, IVA and IVB all gave a yellow color with ninhydrin spray but only "fast moving" hydroxyproline and IVA gave a color (blue) with isatin spray.

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(2) The isolation from bovine Achilles tendon collagen of an amino acid tentatively assigned the 3-hydroxyproline structure has been reported recently: R. B. Arlinghaus (Univ. Cincinnati, Ohio), *Dissertation Abstr.*, **22**, 2566 (1962).

(3) J. C. Sheehan, P. E. Drummond, J. N. Gardner, K. Maeda, D. Mania, A. K. Sen and J. A. Stock, in preparation.

(4) B. R. Baker, R. E. Schaub, M. V. Querry and J. H. Williams, *J. Org. Chem.*, **17**, 77 (1952).

(5) (a) H. E. Carter and H. D. West, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons., Inc., New York, N. Y., 1955, p. 813. (b) Refluxing hydrobromic acid converted O-methylthreonine to threonine.

(6) Reference 5a, p. 774, by a similar procedure methyl 2-bromo-3-methoxypropionate was saponified without loss of the bromine group.

(7) N. F. Albertson and J. L. Fillman, *J. Am. Chem. Soc.*, **71**, 2818 (1949). By this same procedure 2-chloro-5-aminopentanoic acid hydrochloride was cyclized to proline.

(8) M. Sato, K. Okawa and S. Akabori, *Bull. Chem. Soc. Japan*, **30**, 937 (1957).

(9) (a) pH 1.9; 3 kv.; 3 hours. (b) *n*-Butyl alcohol saturated with water with 10% v./v. of diethylamine; 120 hours; Whatman no. 1 paper; T. L. Hardy and D. O. Holland, *Chemistry and Industry*, 855 (1952). (c) *n*-Butyl alcohol–water–acetone–concentrated ammonium hydroxide (8:6:1:1); 116 hours; Whatman no. 1 paper; N. F. Shaw and S. W. Fox, *J. Am. Chem. Soc.*, **75**, 3421 (1953).

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ON THE STRAIN ENERGY IN CYCLOPROPENE AND THE HEAT OF FORMATION OF THE $C_3H_3^+$ ION

Sir:

The cyclopropenyl cation is predicted by the simple molecular orbital theory to have a significant delocalization energy.[1] For some time, we have been trying to obtain the ion by reactions such as the hydride abstraction from cyclopropene[2] *via* the

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(1) J. D. Roberts, A. Streitwieser, Jr., and C. M. Regan, *J. Am. Chem. Soc.*, **74**, 4579 (1952).

(2) K. B. Wiberg, Abstracts 131st Meeting of the Am. Chem. Soc., Miami, April 7–12, 1957, p. 39-O. Aryl and alkyl substituted cyclo-