ditional pairs of isomeric N-alkylpyrazoles of unambiguous structure become available.

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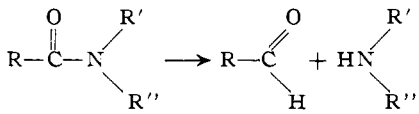
Chemical Cleavage of Proline Peptide Bonds

Sir:

We wish to report a reductive chemical cleavage of N-proline peptide bonds,[1a] utilizing lithium dissolved in methylamine.[1b] The reduction of tertiary amides is known to lead to the production of aldehydes[2-5] according to the following scheme.



N-proline peptide bonds are tertiary amides, therefore a similar cleavage would be expected to occur as follows.

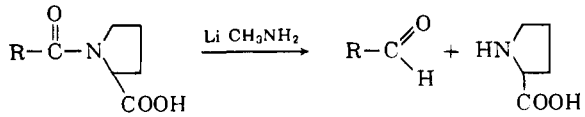


Table I lists the results obtained when a representative series of proline containing peptides were reduced by lithium dissolved in methylamine.

TABLE I

REDUCTIVE CLEAVAGE OF VARIOUS PROLINE PEPTIDES IN METHYLAMINE–LITHIUM SOLUTIONS

Peptide	Extent of cleavage, %	Method of determination
N-Acetyl-L-proline[c]	70	NIN[a]
L-Alanyl-L-proline[d]	66	NIN
Glycyl-L-proline[d]	71	NIN
Phenylpropionyl-L-proline[e]	70	NIN
Phenylpropionyl-L-prolyl-L-leucine[f]	62	PIP[b]
Val[5]-Hypertensin	51	PIP
Gramicidin "S,,	53	PIP
Glycyl-L-hydroxyproline	90	*p*-DAB[g]

[a] NIN = ninhydrin. [b] PIP = proline–imino peptidase treatment followed by colorimetric determination of proline. [c] D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, **193**, 81 (1951). [d] M. Bergmann, L. Zervas, H. Schleich, and F. Leinert, *Z. Physiol Chem.*, **212**, 72 (1932). [e] M.p. 107°. [f] M.p. 161–163°. [g] *p*-DAB = *p*-dimethylaminobenzaldehyde.

All the reductions were carried out under the following reaction conditions. A C-terminal proline dipeptide (0.5 mmole) was acetylated with acetic anhydride and dissolved in methylamine (30–40 ml.). N-methylacetamide (1 ml.) was added to minimize reduction of secondary amide–peptide bonds. The reaction mixture was cooled to −70° and an excess of metallic lithium was added. After 1 hr., a small amount of ammonium chloride was added to discharge the blue color of the mixture. The solvent was allowed to evaporate and the mixture was dissolved in water. Paper chromatography of the reduction mixture showed the presence of free proline which was determined colorimetrically.[6] The yield of proline was found to be 65–75% for all dipeptides treated.

Cleavage of the phenylpropionyl–L-proline bond in phenylpropionyl-L-prolyl-L-leucine was detected by paper chromatography using an authentic sample of L-prolyl-L-leucine as a marker and developing the paper chromatogram with acidic ninhydrin. The extent of this cleavage (62%) was determined using the specific exoenzyme L-proline imino peptidase[6] to cleave quantitatively the new N-terminal proline formed, followed by colorimetric determination of the free proline. The amount of C-terminal leucine (60%) found on enzymatic digestion was estimated by paper chromatography and quantitative ninhydrin assay.

The applicability of this method to larger molecules was tested by performing the cleavage on the synthetic decapeptide, Val[5]-Hypertensin, asp(NH_2)-arg-val-tyr-val-his-pro-phe-his-leu,[7] and on the cyclic peptide, Gramicidin "S,, -(val-orn-leu-phe-pro)$_2$-.[8]

Hydroxyproline peptides are cleaved similarly as shown by the high yield of hydroxyproline obtained by reductive cleavage of glycyl-L-hydroxyproline (Table I). The extent of cleavage was determined colorimetrically[9] after paper chromatography of the reaction mixture.

The use of this method for the detection of X–pro–Y sequences[10] (where X and Y represent amino acids) in cyclic peptides and proteins is now being studied.

Acknowledgment.—The authors thank Professor E. Katchalski for his interest in this work. This investigation was supported by grant No. AM-5098 from the National Institutes of Health, United States Public Health Service.

(1) (a) Preliminary attempts to cleave hydroxyproline bonds were reported by B. Witkop, "Advances in Protein Chemistry," Vol. 16, Academic Press, 1961, p. 235; (b) R. A. Benkeser, R. E. Robinson, D. M. Saure, and O. H. Thomas, *J. Am. Chem. Soc.*, **77**, 3230 (1955).

(2) F. Weygand and G. Eberhardt, *Angew. Chem.*, **64**, 458 (1952).

(3) A. J. Birch, J. Cymerman-Craig, and M. Slaytor, *Australian J. Chem.*, **8**, 512 (1955).

(4) H. C. Brown and B. C. Subba Rao, *J. Am. Chem. Soc.*, **80**, 5377 (1958).

(5) L. Birkofer and E. Frankus, *Chem. Ber.*, **94**, 216 (1961).

(6) S. Sarid, A. Berger, and E. Katchalski, *J. Biol. Chem.*, **234**, 1740 (1959); *ibid.*, **237**, 2207 (1962).

(7) This peptide was kindly supplied by Dr. R. Schwyzer of CIBA Ltd., Basle.

(8) This peptide was kindly supplied by Dr. M. M. Shemyakin, Institute for Chemistry of Natural Products, U.S.S.R. Academy of Science, Moscow.

(9) I. J. Bekhor and L. A. Bavetta, *Anal. Chem.*, **33**, 1807 (1961).

(10) S. Sarid and A. Patchornik, *Israel J. Chem.*, **1**, 63 (1963).

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Reduction of Acylated Aldono-γ-lactones to Aldofuranose Derivatives. A New Synthetic Pathway to Nucleosides[1]

Sir:

Since the majority of free sugars exist in the pyranose form, special methods must frequently be employed to

(1) This work is taken from a thesis submitted by Leon M. Lerner to the University of Illinois Graduate College in partial fulfillment of the requirements for the degree of Doctor of Philosophy. It was supported in part by Training Grant No. GM-471 from the Division of General Medical Sciences of the United States Public Health Service and by Grant P-161 from the American Cancer Society.