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

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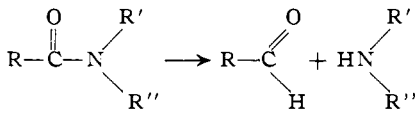
RECEIVED FEBRUARY 3, 1964

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.

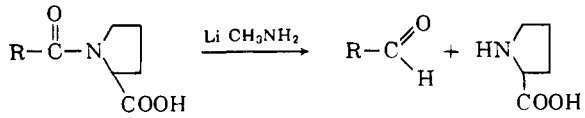


Table I lists the results obtained when a representative series of proline containing peptides were reduced by lithium dissolved in methylamine.

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.

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RECEIVED JANUARY 24, 1964

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.

convert them to furanose derivatives, the choice of method being dictated by the nature of the individual sugar. The aldonolactones, on the other hand, are normally isolated as γ-lactones. In this communication we describe a procedure whereby esters of aldono-γ-lactones may be converted to esters of aldofuranoses.

Bis-3-methyl-2-butylborane (disiamylborane) has been used in this laboratory as an agent to reduce tetra-O-acyl-D-hexono-γ-lactones to 2,3,5,6-tetra-O-acylhexofuranoses. The procedure was based on the reaction reported in a communication by Brown and Bigley[2] in which the reduction of γ-butyro- and γ-valerolactone to their respective hydroxy aldehydes was described. These investigators also reported that ester groups were not affected by the reagent, and our results have verified this. Classical methods of reducing lactones to hemiacetals with sodium amalgam or sodium borohydride resulted in very poor yields when applied to the esterified lactones.

The extent of reduction was determined on an aliquot from a chloroform solution. After treatment with methanolic sodium methoxide at 0°, the sugar was extracted into water, salts were removed by stirring with a mixture of cation- and anion-exchange resins, and the amount of reducing sugar was determined by the anthrone test.[3]

To prepare hexofuranosyl nucleosides, which was our primary interest, the anomeric hydroxyl group of the tetraacyl furanose was esterified, and a glycosyl halide was prepared from it. This was coupled with chloromercuri-6-benzamidopurine, which, after removal of the blocking groups, gave the desired product.

2,3,5,6-Tetrabenzoyl-D-gulono-γ-lactone (I) was prepared in good yield from D-gulono-γ-lactone by adaptations of previously reported methods,[4] m.p. 155–156°, $[\alpha]^{21}D$ −89.8° (*c* 4.1, $CHCl_3$), infrared spectrum: λ_{max}^{film} 5.5 (γ-lactone), 5.8 μ (benzoate carbonyl). *Anal.* Calcd. for $C_{34}H_{26}O_{10}$: C, 68.67; H, 4.41. Found: C, 68.62; H, 4.40.

This material (17.8 g., 30 mmoles) in 50 ml. of tetrahydrofuran was slowly added in a nitrogen atmosphere to 75 ml. of tetrahydrofuran containing 0.125 mole of disiamylborane.[5] After standing overnight at room temperature, 10 ml. of water was slowly added and the mixture was refluxed for 0.5 hr. The solution was cooled to 0° and 20 ml. of 30% hydrogen peroxide was very slowly added while the pH was maintained between 7 and 8 with 3 *N* sodium hydroxide.[6] The mixture was concentrated *in vacuo* to a small volume and extracted several times with chloroform which was dried over calcium chloride. Determination of the reducing sugar content indicated a 100% yield. Paper chromatography after de-esterification showed only one spot, corresponding to D-gulose. The chloroform was evaporated *in vacuo* and the sirup obtained was dissolved in warm absolute ethanol from which the product, 2,3,5,6-tetra-O-benzoyl-D-gulofuranose (II), crystallized. The yield of combined crops was 17.2 g. (97%), m.p. 156–157°, $[\alpha]^{21}D$ −55.3° (*c* 4.03, $CHCl_3$), infrared spectrum: λ_{max}^{film} 2.85 μ (hydroxyl); no lactone peak at 5.5 μ. *Anal.* Calcd. for $C_{34}H_{28}O_{10}$: C, 68.40; H, 4.73. Found: C, 67.91; H, 4.60.

The anomeric hydroxyl group of II was acetylated for 18 hr. with acetic anhydride in pyridine. The product crystallized as feathery white needles from warm absolute ethanol to give an 85% yield of 1-O-acetyl-2,3,5,6-tetra-O-benzoyl-D-gulofuranose (III). Recrystallization from ethanol afforded the analytical material, m.p. 131–132°, $[\alpha]^{19}D$ −56.8° (*c* 3.73, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{30}O_{11}$: C, 67. 70; H, 4.74. Found: C, 67.93; H, 4.97.

From III (4.52 g., 7.1 mmoles) the glycosyl chloride (IV) was synthesized[7] and condensed with chloromercuri-6-benzamidopurine.[8] Removal of the blocking groups with hot methanolic sodium methoxide gave the nucleoside, 9-β-D-gulofuranosyladenine (150 mg., 6% from III). Recrystallization from water gave fluffy white needles, m.p. 229–230°, $[\alpha]^{19}D$ −56.2° (*c* 2.03 1 *N* HCl). Ultraviolet and infrared spectra showed: $\lambda_{max}^{H_2O}$ 259 mμ (ε 14,400); λ_{max}^{KBr} 2.85 (OH, NH), 6.05, 6.25, 6.70 (NH and purine ring), 9.1, 9.25–9.45, 9.70 μ (C–O–C, C–O–H). A dimedone test after periodate treatment showed the presence of 0.95 mole of formaldehyde per mole of the nucleoside and indicated the presence of the furanose ring structure. *Anal.* Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.45; H, 5.09; N, 23.56. Found: C, 44.20; H, 5.19; N, 23.50.

In a similar manner, D-galactono-γ-lactone tetraacetate (VI) was reduced in 83% yield, but the product, 2,3,5,6-tetra-O-acetyl-D-galactofuranose (VII), did not crystallize readily.

The sirup [infrared spectrum: λ_{max}^{film} 2.85 μ (OH), no γ-lactone at 5.6 μ] was acetylated to yield β-D, galactofuranose pentaacetate (VIII) (60%), m.p. 99–100°, $[\alpha]^{21}D$ −40.5° (*c* 4, $CHCl_3$), (lit.[9] m.p. 98°, $[\alpha]^{20}D$ −41.6°). The glycosyl chloride (IX) was prepared[7] and coupled with chloromercuri-6-benzamidopurine.[8] Removal of the blocking groups and purification of the nucleoside from the picrate[10] gave 9-β-D-galactofuranosyladenine (21% from VIII), m.p. 226–227°. Admixture with an authentic sample[11] gave no depression of the melting point. The infrared and ultraviolet spectra were identical with those of the authentic material.

We are in the process of applying this procedure to the reduction of other esterified hexono-γ-lactones to be used for the preparation of aldofuranosyl nucleosides and other aldofuranosyl derivatives. An important application of the method lies in its use for the preparation of C′-1 labeled furanosyl nucleosides. These could be obtained from C-1 labeled lactones prepared by the use of $NaC^{14}N$ in the Fischer–Kiliani cyanohydrin synthesis.

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(11) The authors are grateful to Dr. M. L. Wolfrom for providing a sample of this substance, prepared by another route.

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RECEIVED FEBRUARY 10, 1964