Reaction of 1,2,4,5-Tetrafluorobenzene with Butyllithium. Method A. Tetrahydrofuran Solvent.—1,2,4,5-Tetrafluorobenzene (15.0 g., 0.10 mole) in 20 ml. of tetrahydrofuran was added to a cooled (−70°), stirred solution of *n*-butyllithium[17] (0.20 mole, in 135 ml. of hexane) dissolved in 270 ml. of tetrahydrofuran. The addition took 10 min. and the temperature was not allowed to rise over −55°. After 20 min. Gilman color test IIA was negative. The mixture was then carbonated by bubbling carbon dioxide into the reaction. The mixture was allowed to warm to room temperature with continued carbonation. The reaction was then hydrolyzed with 300 ml. of 6 *N* hydrochloric acid. This two-phase mixture was then placed in a flask equipped with a short-path Vigreux column and distilled. The aqueous distillate, boiling between 100 and 108°, was extracted with diethyl ether, dried over magnesium sulfate, and aspirated on a water bath. The residue of 0.62 g. (3.2%) had m.p. 149–152° and was identified by mixture melting point and infrared analysis as 2,3,5,6-tetrafluorobenzoic acid. The solid pot residue from the above distillation, 19.9 g. (84%), m.p. 261–276°, was recrystallized from water to yield as the first crop 16.1 g. (67%), m.p. 283° (lit.[16] m.p. 283–285°), of tetrafluoroterephthalic acid. This material was characterized by infrared analysis and a mixture melting point with an authentic sample.

Method B. Diethyl Ether Solvent.—The above experiment was repeated except that diethyl ether was used in place of the tetrahydrofuran. Gilman color test IIA, however, was negative only after 2 hr. Carbonation and work-up as above yielded 6.5 g. (33%) of crude 2,3,5,6-tetrafluorobenzoic acid, m.p. 147–151°, 9.0 g. (37.7%) of crude tetrafluoroterephthalic acid, m.p. 276–278° (after one recrystallization from water), and 1.4 g. of an unidentified acidic material, m.p. 337.5–340°.

Reaction of 1,2,3,4-Tetrafluorobenzene with Butyllithium in Tetrahydrofuran.—1,2,3,4-Tetrafluorobenzene (15.0 g., 0.10 mole) in 20 ml. of tetrahydrofuran was added to a cooled (−70°), stirred solution of *n*-butyllithium[17] (0.20 mole in 135 ml. of hexane) dissolved in 270 ml. of tetrahydrofuran. The addition took 13 min. and the temperature was not allowed to rise over −55°. After 18 min. Gilman color test IIA was negative. The mixture was then carbonated by bubbling carbon dioxide into the reaction. The mixture was allowed to warm to room temperature with continued carbonation. The reaction was then hydrolyzed with 300 ml. of 6 *N* hydrochloric acid. This two-phase mixture was then placed into a flask equipped with short-path Vigreux column and distilled. The aqueous distillate boiling between 100 and 108° was extracted with diethyl ether. The diethyl ether was extracted with 5% sodium hydroxide solution. The extracted ether layer was dried and the ether was removed by distillation leaving 2.51 g. of a nonacidic liquid. Infrared analysis of this material suggested that it was an alkylated fluorobenzene. Vapor phase chromatographic analysis indicated that this material was a three- or four-component mixture.

The sodium hydroxide extract of the ether layer was acidified with 6 *N* hydrochloric acid and extracted with diethyl ether. The ether layer was dried and the solvent ether was removed by distillation to yield 6.04 g. of a semisolid material. This material was recrystallized from petroleum ether (b.p. 60–90°) and produced 4.14 g. of 2,3,4,5-tetrafluorobenzoic acid, m.p. 92–92.5°.

Anal. Calcd. for $C_7H_2F_4O_2$: C, 43.32; H, 1.04; F, 39.15. Found: C, 43.44; H, 1.19; F, 39.26.

The pot residue from the original distillation through the Vigreux column was extracted with diethyl ether. The ether was extracted with 5% sodium hydroxide solution. The basic solution was acidified with 6 *N* hydrochloric acid and again extracted with diethyl ether. The ether solution was dried over magnesium sulfate and distilled to remove the solvent leaving 8.96 g. of a dark brown semisolid material. This material was recrystallized from petroleum ether (b.p. 90–120°) several times to yield 2.23 g. of tetrafluorophthalic acid, m.p. 151–152° (lit.[16] m.p. 153–154°). In addition 4.25 g. of an unidentified material was obtained whose infrared spectrum showed both alkyl substitution as well as a carboxylic acid function.

Acknowledgment—The authors wish to thank J. V. Pustinger, Jr., of the Monsanto Research Corporation for the determination and interpretation of the n.m.r. spectra reported in this work. The F^{19} spectra were run on a Varian V-4300-2 D.P. spectrometer at 40.0 Mc./sec. Chemical shifts are reported in parts per million from trifluoroacetic acid.

Isomerization of the Ascorbic Acids

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The rare L-araboascorbic and D-xyloascorbic acids are herein shown to be formed from their well-known epimers, L-xyloascorbic and D-araboascorbic acids, respectively, when they are heated with excess base in aqueous methanol. Their formation is shown to proceed *via* racemization of the asymmetric ring carbon (C-4), resulting in an approximately equal mixture of C-4 epimers at equilibrium in each case. Methods are described for the separation and purification of isomers, thus providing a new, convenient route to these uncommon ascorbic acids.

It has been known for a long time that esters of L-*xylo*-hexulosonic (2-keto-L-gulonic) and D-*arabino*-hexulosonic (2-keto-D-gluconic) acids are converted by bases *via* internal alcoholysis to the corresponding ascorbic acids, L-xyloascorbic and D-araboascorbic acid, respectively.[1] We wish to report the previously unrecorded fact that these reactions, when conducted with excess base, lead to racemization at C-4 of the product and that the rare ascorbic acids, L-araboascorbic and D-xyloascorbic acid, are formed in each case in about equal amount with the common epimer, L-ascorbic and D-isoascorbic acid, respectively.

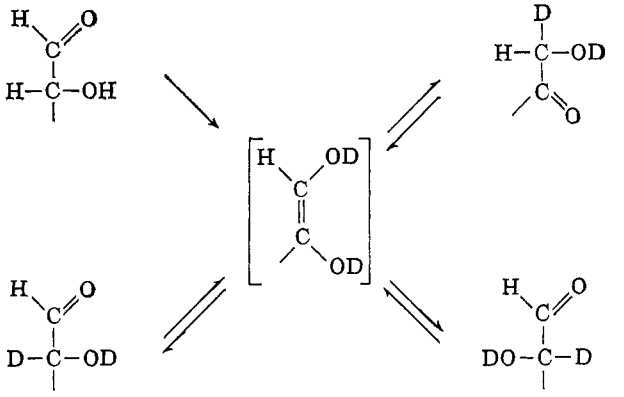
Surprisingly little degradation is involved, and our initial, chance chromatographic detection of the phenomenon has led to the development of a convenient synthetic method for the preparation of these uncommon ascorbic acids, heretofore obtainable only *via* rare sugars or cumbersome fragment condensation methods.[2] The racemizations involved present rather interesting and novel carbohydrate behavior and so will be discussed in some detail.

Reducing sugars are relatively stable in weakly acid solutions, but in alkaline solution they are subject to isomerizations, cleavages, and condensations. In fact, the classic Lobry de Bruyn–Alberda van Ekenstein transformation of aldoses by base to mixtures of C-2

(1) T. Reichstein and A. Grüssner, *Helv. Chim. Acta*, **17**, 311 (1934); U. S. Patent 2,265,121; U. S. Patent 2,301,811.

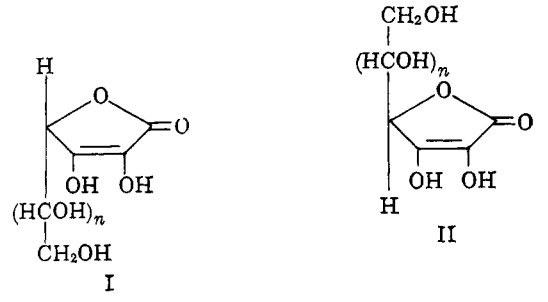
(2) B. Helferich and O. Peters, *Ber.*, **70**, 464 (1937); "The Vitamins," Vol. I, Sebrell and Harris, Ed., Academic Press, New York, N. Y., 1954, pp. 198–202.

epimers and ketoses[3] has become a significant synthetic tool in sugar chemistry. The mechanistic pathway for this isomerization involves an enediol intermediate according to Sowden and Schaffer.[4] Thus, D-glucose is isomerized by alkali in heavy water to products in which carbon-bonded deuterium is incorporated.



Similar configurational changes at C-2 upon treatment with alkaline reagents have been reported for aldonic lactones[5] and sugar acids.[6] However, in general, these latter substances are relatively stable and withstand prolonged heating with base before the onset of significant isomerization.

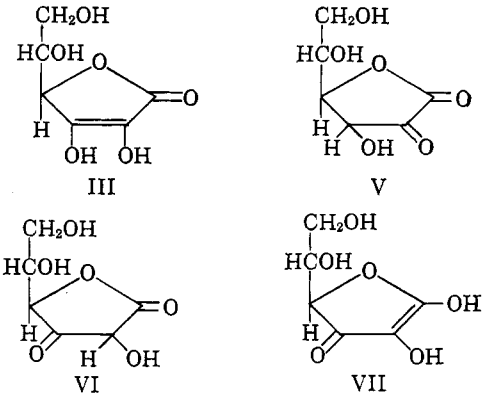
The present case concerns the ascorbic acids, a group of compounds similar in part to the sugar lactones. These acids, represented by formulas I and II, are derivable from both 2- and 3-keto sugar acids by lactonization and enolization.



The chemistry of ascorbic acid is usually interpreted on the basis of structure III, although it could theoretically react to some extent of course in any one of its various tautomeric modifications, V, VI, and VII.

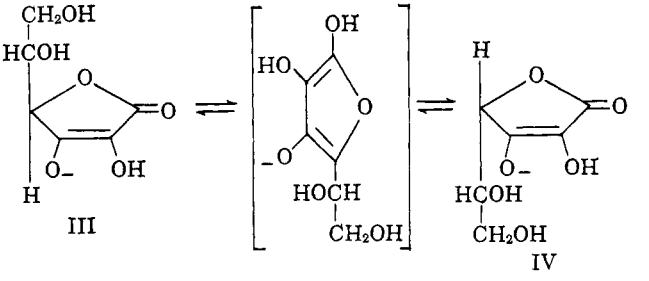
Although the ketonic structures V and VI can readily be drawn, the enediolic form III certainly predominates, as indicated by chemical evidence,[7] X-ray findings,[8] and high-intensity absorption in the ultraviolet (log ϵ 4.0 at λ_{max} 244 mμ), all indicating an α,β-unsaturated carbonyl structure.

As stated initially, we found chromatographic indication that the treatment of methyl L-*xylo*-hexulosonate

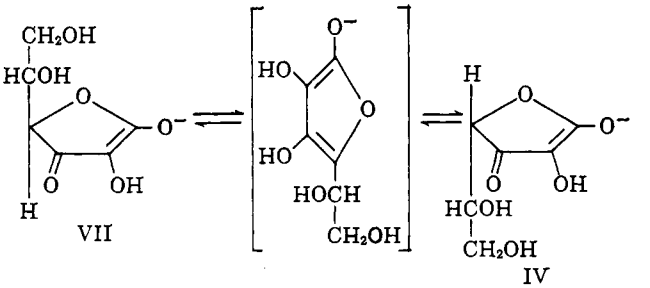


(methyl 2-keto-L-gulonate) in aqueous methanol with excess potassium hydroxide leads first to L-xyloascorbic acid and then, subsequently, to an araboascorbic acid. That the araboascorbic acid is derived from sequential isomerization rather than concomitant formation was evident from the identical behavior of pure xyloascorbic acid when subjected to the conditions of its own formation. The chromatographic evidence was then confirmed by driving the isomerization to equilibrium and separating the reaction mixture *via* fractional crystallization from acetonitrile to yield both L-xyloascorbic acid (III) and more soluble L-arabascorbic acid (IV.)

Mechanistically, formation of L-araboascorbic acid from L-xyloascorbic acid must involve racemization at C-4, probably *via* enolization of the anion of the unsaturated lactone of III with loss of configuration leading to a mixture of the epimeric anions of III and IV.



Alternatively, a similar but possibly less likely path can be drawn through the anion of the tautomeric enediol VII,[9] and of course this possibility cannot be excluded, *per se*.



In any case, both foregoing paths depend upon the lability of the proton at C-4. Although there is no literature evidence that the C-4 bonded hydrogen would

(3) Product composition and equilibrium point in the Lobry de Bruyn transformation [C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **14**, 195 (1895)] have been reported to be cation dependent in some but not all cases; see W. Pigman, "The Carbohydrates," Academic Press, New York, N. Y., 1957.

(4) J. C. Sowden and R. Schaffer, *J. Am. Chem. Soc.*, **74**, 505 (1952).

(5) W. N. Haworth and C. W. Long, *J. Chem. Soc.*, 345 (1929).

(6) E. Fischer, *Ber.*, **23**, 799 (1890); H. T. Bonnett and F. W. Upson, *J. Am. Chem. Soc.*, **55**, 1245 (1933).

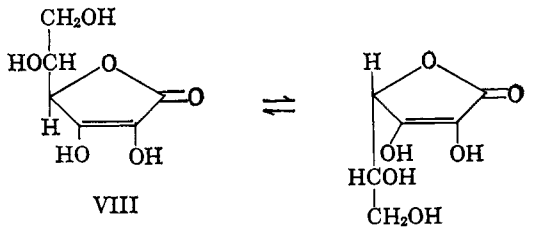
(7) R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. N. Reynolds, and F. Smith, *J. Chem. Soc.*, 1270 (1933).

(8) E. G. Cox, *Nature*, **130**, 205 (1932); **131**, 402 (1933); E. G. Cox and T. H. Goodwin, *J. Chem. Soc.*, 769 (1936).

(9) See W. N. Haworth, E. L. Hirst, F. Smith, and W. J. Wilson, *ibid.*, 829 (1937), for evidence which supports the existence of the tautomeric 3-keto structure (VII). The reaction of ascorbic acid with one molecular proportion of diazomethane yields mainly 3-O-methylascorbic acid and in small proportion the isomeric 1-methyl derivative.

be active, attention is called to Weigl's work on the infrared spectrum of deuterated ascorbic acid,[10] which he interpreted to indicate that at least one of the hydrogens attached to carbon was exchangeable. In addition, the kinetics of the system as revealed by polarimetry and thin layer chromatography lend support to a tautomeric interpretation, the equilibration character of the reaction being clearly evident. Aliquots after various reaction times were separated on silica gel coated plates, and comparison of optical densities in the ultraviolet at peak absorption (244 mμ) permitted quantitative evaluation of the changing ratio of species. In addition, the changing optical rotation of the reaction mixture was followed to constancy which indicated similarly that equilibration was nearly complete in about 17 hr. with the ratio of L-xyloascorbic to L-araboascorbic acid being approximately 1.3:1.0.

These findings suggested that D-araboascorbic acid (VIII) would equilibrate with D-xyloascorbic acid (IX) and, indeed, this was found to be the case.



VIII IX

Thus, identical treatment of the common D-araboascorbic acid in refluxing aqueous methanol with excess potassium hydroxide gave a mixture of starting material and D-xyloascorbic acid. Here, too, polarimetry and thin layer chromatography indicated near equilibration in about 16 hr., with D-xyloascorbic acid predominating over the D-araboascorbic acid isomer in a ratio of about 1.2:1.0.

In summary, these reactions provide a convenient route to the C-4 epimer of any given ascorbic acid. However, no simple synthesis of L-ascorbic acid (D at C-4) *via* this route would be practical since the necessary (L at C-4) precursors (L-fructose, L-glucose, etc.) are not readily available from natural sources.

Experimental[11]

Separation of Isomers.—Resolution of the ascorbic acids by thin layer chromatography (t.l.c.) has not been reported previously.[12] Numerous paper chromatographic separations of greater or less efficacy have been reported,[13] but none lends itself to a rapid analysis or actual isolation of components. T.l.c. on silica gel coated plates gives a rapid and clean separation of xyloascorbic and araboascorbic acids, and this procedure could be used readily for the actual isolation of milligram quantities of the isomers. The plates are prepared by mixing silica gel G[14] (30 g.) with water (60 ml.) containing metaphosphoric acid (1.8 g.) and applying this slurry with an applicator to five 20 × 20 cm. glass plates to give a film 0.25 mm. thick. The plates are air-dried and then activated by drying overnight in an oven at 110°. Chromatoplates prepared in this manner are developed, after spotting with the mixture to be separated, in a system of acetonitrile–butyronitrile–water (66:33:2). The irrigation period is about 45 min., during which the solvent front rises about 17 cm. Spot or zone position is detected by spraying with 5% iodine in chloroform or 0.08% 2,6-dichlorophenolindophenol in ethanol. Under these conditions, the xyloascorbic acids have an R_f value of 0.26, and the araboascorbic acids, 0.38. Two- to 200-μg. mixtures can be separated in this manner; larger samples form diffuse spots.

Estimation of Ratio of Isomers.—The speed and sharpness of separation of the isomers by t.l.c. make this technique useful for semiquantitative analysis. Synthetic mixtures of L-xyloascorbic acid and D-araboascorbic acid were dissolved in water and chromatographed as described. Each mixture was spotted, together with an adjacent guide spot. After resolution, the plates were partially masked to permit selective spraying of the guide strips and the corresponding areas on the adjacent unsprayed strip were then scraped off into 15-ml. centrifuge tubes. The material obtained was eluted by adding 5 ml. of methanol, stirring thoroughly, and centrifuging. The clear, supernatant solution was analyzed directly for enediolic lactone content by measurement of the maximum optical density (244 mμ). Typical results are given in Table I.

TABLE I

EFFICACY OF SEPARATION AND RECOVERY OF MIXTURES OF THE ASCORBIC ACIDS *via* T.L.C. ON SILICA GEL

Mixture	Isomer	Applied, μg.	Recovered, μg.	Recovery, %
1	L-XAA[a]	41.0	41.6	101
	D-AAA[b]	63.5	61.0	96
2	L-XAA	82.0	75.0	92
	D-AAA	127.0	120.0	95

[a] L-Xyloascorbic acid. [b] D-Araboascorbic acid.

Polarimetry.—The isomerizations were also followed *via* the changing optical rotations of the reaction mixtures. Under the basic reaction conditions used, the observed rotations are those of the potassium salts of the acids. Zero-time readings are for the solutions in alkaline, aqueous methanol prior to heating. The solutions were then brought to reflux, and aliquots were removed with time, cooled to 27°, and their optical rotations measured. All values are the observed rotations in 2-dm. tubes.

Isomerization of L-Xyloascorbic Acid.—A solution of 17.6 g. (0.1 mole) of L-xyloascorbic acid in 200 ml. of 50% aqueous methanol containing 11.2 g. (0.2 mole) of potassium hydroxide was heated to refluxing. The composition of the reaction mixture was followed by t.l.c. and polarimetry. Observed rotational changes are shown in Table II.

TABLE II

ROTATIONAL CHANGES OF L-XYLOASCORBIC ACID IN BASE

Time, hr.	$[\alpha]^{27}D$, deg.	Time, hr.	$[\alpha]^{27}D$, deg.
0	24.90	12	8.01
1	22.60	14.5	6.58
2	20.28	15.5	6.14
4	16.55	17.25	5.47
6.25	13.24	24	3.56
7.5	11.51	34	2.53
9.5	9.92	40	2.31

T.l.c. after 1 hr. indicated, in addition to the L-xyloascorbic acid, a faster moving indophenol-reducing material with R_f of 0.38, consistent with an araboascorbic acid. With time the more mobile spot increased in intensity, as that of the less mobile decreased, until both became approximately equal. Quantitative analysis of another similar run, *via* t.l.c., indicated equilibration to be near completion between 16 and 24 hr., with a ratio of about 1.3:1.0 of xyloascorbic–araboascorbic species (Table III). After 15 hr. at reflux, the reaction solution was cooled and acidified

(10) J. W. Weigl, *Anal. Chem.*, **24**, 1483 (1952).

(11) Melting points were taken on a microscope hot stage and are not corrected. Infrared spectra were run on a Perkin-Elmer Infracord. Ultraviolet spectra were measured with a Cary 11 recording spectrophotometer. Optical rotations of reaction solution were determined at 27 ± 2° on a Hilger polarimeter, and of crystalline products on a Carl Zeiss photoelectric polarimeter at the same temperature by D. Williams.

(12) We are indebted to B. Singleton for the design of this system.

(13) W. I. Patterson and L. C. Mitchell, *J. Assoc. Offic. Agr. Chemists*, **36**, 1127 (1953); Y. Chen, F. A. Isherwood, and L. W. Mapson, *Biochem. J.*, **55**, 821 (1953); L. W. Mapson and S. M. Partridge, *Nature*, **164**, 479 (1949).

(14) E. Merck, A. G., Darmstadt.

TABLE III

CHANGING ISOMER RATIO WITH ISOMERIZATION OF L-XYLOASCORBIC ACID

Time, hr.	L-XAA/L-AAA	Time, hr.	L-XAA/L-AAA
0	100/0	8	70/30
1	91/9	12	63/37
4	77/23	16	60/40
6	74/26	24	57/43
		28	57/43

TABLE IV

ROTATIONAL CHANGES OF D-ARABOASCORBIC ACID IN BASE

Time, hr.	$[\alpha]^{27}D$	Time, hr.	$[\alpha]^{27}D$
0	24.72	11	6.00
1	22.16	13	4.41
2	19.59	15	3.18
5	13.95	16.75	2.43
7	11.25	24.5	−0.02
9	8.17	31	−1.09
		33	−1.42

with 95 g. (100% excess) of Amberlite IR-120 (H^+). The resin was removed and the filtrate concentrated *in vacuo* to remove the methanol. Lyophilization of the residual aqueous solution yielded an amorphous yellow solid which, on crystallization from acetonitrile, yielded three crops of recovered L-xyloascorbic acid and finally a fourth crop of crystals (3.9 g.). Infrared and ultraviolet spectra, m.p. 166–170° dec., and $[\alpha]^{27}D$ +13° identified the final material to be L-araboascorbic acid.[15]

(15) T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta*, **17**, 510 (1934).

TABLE V

CHANGING ISOMER RATIO WITH ISOMERIZATION OF D-ARABOASCORBIC ACID

Time, hr.	D-AAA/D-XAA	Time, hr.	D-AAA/D-XAA
0	100/0	9	58/42
1	90/10	11	56/44
3	83/17	17	48/52
5	74/26	24	47/53

Isomerization of D-Araboascorbic Acid.—Similar treatment of D-araboascorbic acid in an identical system yielded the optical rotation measurements shown in Table IV. T.l.c. here indicated formation of a less mobile species, R_f 0.26, comparable to that of a xyloascorbic acid, with equilibration near completion between 17 and 24 hr., with a ratio of about 1.0:1.1 of araboascorbic–xyloascorbic isomers (Table V).

A similar processing of the reaction mixture after 15 hr. of refluxing produced a crude yellow solid which, on fractional crystallization from acetonitrile, yielded a first crop (5.8 g.) of colorless crystals. Recrystallization from the same solvent gave material melting at 188–190° dec. with infrared and ultraviolet spectra identical with those for L-xyloascorbic acid. A mixture of this material with an equal amount of authentic L-xyloascorbic acid depressed the melting point to 170°, the value reported for the DL pair.[16] The optical rotation of the isolated material was levoratotory ($[\alpha]^{27}D$ −16°) thus identifying it as D-xyloascorbic acid.[16,17]

(16) T. Reichstein, A. Grüssner, and R. Oppenauer, *ibid.*, **16**, 1019 (1933).

(17) R. G. Ault, D. K. Baird, H. C. Carrington, W. N. Haworth, R. W. Herbert, E. L. Hirst, E. G. V. Percival, F. Smith, and M. Stacey, *J. Chem. Soc.*, **16**, 1019 (1933).

Selective Cleavage of Ornithyl and Diaminobutyryl Peptides[1]

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The selective cleavage of the N-α-naphthylamides of L-ornithylglycylglycine, L-2,4-diaminobutyrylglycylglycine, and L-ornithyl-L-leucylglycine leading to the lactams and dipeptide naphthylamides was studied. Although alkaline or acidic aqueous systems did not yield satisfactory results, selective cleavage could be demonstrated in absolute ethanol in the presence of triethylamine at 65°. Since glycylglycyl units are particularly prone to alkaline hydrolysis, the presence of this moiety in a peptide affords a very severe test for the selectivity of the cleavage. The scission of the leucylglycine derivative was slow but highly specific, a result attributable to steric hindrance.

Intramolecular nucleophilic attack of an amino group on a peptide can lead to lactam formation and scission of an amide bond under exceptionally mild conditions. Thus, Holley and Holley[3] found that if an aqueous solution of N-(2-amino-4-carbomethoxyphenyl)glycylglycylglycine is held at 25° for 5 hr. or at 70° for 15 min., the dihydroquinoxalone derivative and glycylglycine are obtained in very high yields. Another example of this type of facile peptide cleavage is the removal of the chloroacetyl group from N-chloroacetyl peptides with *o*-phenylenediamine, a reaction that is completed in aqueous solution at 100° after 1 hr.[4] Since in the above cases the attacking group is an aromatic amino group, it seemed of interest to ascertain whether lactam formation could also lead to selective peptide bond cleavage with aliphatic amines. An earlier study by Barrass and Elmore[5] on the cleavage of α-N-tosyl-DL-ornithylglycine and α-N-tosyl-L-2,4-diaminobutyrylglycine to yield the lactams and glycine gave no direct information on this point. With those substances there is no way of ascertaining the degree of cleavage specificity, nor do their results lend themselves readily to quantitative interpretation.

Most of the work reported here deals with the cleavage of the N-α-naphthylamides of L-ornithylglycylglycine, L-2,4-diaminobutyrylglycylglycine, and L-ornithyl-L-leucylglycine. The presence of the glycylglycine moiety affords a particularly stringent test for the specificity of the cleavage procedure since studies by Levene, *et al.*,[6] and by Synge[7] have shown this grouping to be the most susceptible to base- or acid-catalyzed hydrolysis. Information on the effects of steric hindrance was obtained from the leucylglycine derivative. The presence of the α-naphthylamide group permits

(1) Supported in part by Grant GM 05492, National Institutes of Health.

(2) Taken in part from the Ph.D. Dissertation of M. A. Lipson, State University College of Forestry, Syracuse University, July, 1963. National Science Foundation Predoctoral Cooperative Graduate Fellow, 1961–1963.

(3) R. W. Holley and A. D. Holley, *J. Am. Chem. Soc.*, **74**, 5445 (1952).

(4) R. W. Holley and A. D. Holley, *ibid.*, **74**, 3069 (1952).

(5) B. C. Barrass and D. T. Elmore, *J. Chem. Soc.*, 4830 (1957).

(6) P. A. Levene, R. C. Steiger, and A. Rothen, *J. Biol. Chem.*, **97**, 717 (1932).

(7) R. L. M. Synge, *Biochem. J.*, **39**, 351 (1945).