of platinum oxide catalyst. The bomb was sealed and filled with hydrogen to a pressure of 650 p.s.i. and agitated by rocking for 1.5 hours. By that time pressure had dropped to 420 p.s.i. The reaction mixture was removed from the bomb, filtered and the filtrate was then distilled at a maximum temperature of 45° at 9 mm. to remove glacial acetic acid. The residual oil was poured onto 10 g. of ice where it solidified. To it 7 ml. of 50% potassium hydroxide solution was added drop by drop until the solution was basic to litmus. The doughy solid formed was separated from the aqueous solution and an oil was pressed from it. The oil weighed 1.7 g. and solidified to a light tan solid on standing. After recrystallizing twice from carbon tetrachloride, the solid was proved to be 2-keto-6,6-dihydroperfluorohexamethylenimine, m.p. 100–101°.

Anal. Calcd. for $C_6F_8H_3NO$: mol. wt., 257; N, 5.45. Found: mol. wt. (Signer method),[8] 263; N, 5.22, 5.17, 5.47.

Attempted Polymerization of 2-Keto-6,6-dihydroperfluorohexamethylenimine.[9] Sodium Catalyst.—Two-tenths gram of 2-keto-6,6-dihydroperfluorohexamethylenimine and 1 mg. of sodium were sealed in a glass tube after flushing six times with nitrogen. The tube was heated in an oil-bath at 60–124° for 30 minutes and then placed in a furnace at 250° for 1.5 hours. When the hot tube was removed from the furnace and tilted on its side, a white solid froze on its walls and a black residue was left at the bottom. The white solid was unchanged starting material, m.p. 100–101°.

Water as a Catalyst.—One-tenth gram of 2-keto-6,6-dihydroperfluorohexamethylenimine and 0.01 ml. of water were sealed in a glass tube after flushing five times with nitrogen. The tube was heated for three hours at 200°. It was then removed, cooled, opened and connected to a two-way stopcock which allowed access to a vacuum line or to a tank of nitrogen. After flushing the tube with nitrogen it was heated at 140–150° for one hour. Then the tube was evacuated with a water pump and the heating continued. This caused all but a very small amount of dark material to sublime from the bottom of the tube and to condense on the cooler portion of the tube walls. The heating was then discontinued. The solid which sublimed to the walls of the tube was unchanged starting material, m.p. 100–101°.

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The Reaction of Nitrous Acid with γ-Glutamyl Peptides

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The determination of amino nitrogen by the method of Van Slyke[2] gives satisfactory values with most α-amino acids and polypeptides, but high values have been observed with glycine and cystine.[2] Austin[3] has shown that in the case of glycine this anomaly is due to a complex series of reactions which ultimately lead to the formation of N_2O in addition to N_2.

Unusually high values have also been reported for glutathione, glutamine, and the γ-methyl and γ-ethyl amides of glutamic acid. Glutathione[4,5] yielded approximately 66% and the three amides[6,7] approximately 90% of their total nitrogen. These compounds all contain free α-amino and carboxyl groups and a γ-amide linkage. In a study of the action of nitrous acid upon a number of amides (including asparagine) Plimmer[8] showed that under the usual conditions of the Van Slyke procedure the amide group is inert, though it reacts in the presence of strong mineral acid. Lichtenstein[7] suggested that the α-amino group was first replaced by OH, that the resulting γ-hydroxy acid amide underwent ring-closure to yield a lactone with liberation of NH_3 or alkyl amine and that the latter

TABLE I

VAN SLYKE AMINO NITROGEN DETERMINATIONS[a]

Compound[b]	Equivalents of amino N per mole of compound — Reaction time, minutes — 3	10	30	60
H·Glu·OH (L)	1.0	1.0	1.0	1.0
H·Glu·Ala·OH (LL)	1.0	1.0	1.0	
Z·Glu·OBz (LL)	0.0	0.0		
H·Ala-Glu·OH (LLL)	1.0		1.0	1.0
H·Glu·OH (L)	1.7	1.8	1.9	
Glutathione	2.1	2.3	2.6	
S-Acetylglutathione	2.0	2.2		
H·Glu·OH (LL)	2.0	2.0	2.0	
H·Glu·OH (LL)	2.6	2.7	2.8	
H·Glu·OH (LLL)	2.6	2.8	2.8	
H·Glu·OH (LL)	1.9	2.0	2.0	
H·Glu·OH (L)	1.0	1.0	1.0	
H·Glu·OBz·HCl (L)	0.8	1.0	1.0	
H·Glu·Ala·OH [LL / L]	1.2	1.3	1.3	1.3
H·Glu-Ala·OH (LD)			1.4	
H·Glu-Ala·OH (LL)	1.1		1.1	1.2

[a] Temperature 22–26°. [b] The following abbreviations and symbols are used (*cf.* ref. 11); Z, carbobenzyloxy, $C_6H_5CH_2OCO$; Bz, $C_6H_5CH_2$; Ala, $NHCH(CH_3)CO$, C_3H_5ON; Glu, $NHCH(CH_2CH_2COOH)CO$, $C_5H_7O_3N$; peptide linkage indicated by dash, -; configuration follows compound in parentheses. When the γ-carboxyl group of glutamic acid is substituted, the substituent in the γ-position is indicated below the line: Glu; otherwise, a free γ-COOH group is implied, *e.g.*, α-L-glutamyl-D-glutamic acid: H·Glu-Glu·OH (LD); N-carbobenzyloxy-D-alanyl-α-benzyl γ-L-glutamyl-L-alanine; Z·Ala-Glu·OBz (DLL). [c] The stereoisomers gave almost identical amino N values (*cf.* ref. 9, 11).

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(2) D. D. Van Slyke, *J. Biol. Chem.*, **9**, 185 (1911); **12**, 275 (1912); **16**, 121 (1913).
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products then reacted with nitrous acid to yield the additional N_2.

We have observed[9] that γ-glutamylpeptides react with nitrous acid to yield all of their N as α-amino nitrogen under the usual conditions. In three minutes such a γ-dipeptide liberated a quantity of gas equivalent to approximately two moles of amino nitrogen; this value remained constant as determined in separate runs, for reaction times of 3, 10 or 30 minutes. The prompt completion of gas evolution and the observation that glutamic acid and α-glutamyl peptides yield no more than one mole (even after prolonged reaction times) strongly indicates that we are not dealing with a reaction analogous to that described for glycine.[3] It also seems unlikely that the behavior of the γ-peptides is due to rapid hydrolysis under the experimental conditions. Although the autohydrolysis of γ-glutamyl peptides in neutral solution has been reported,[10] this reaction occurs only on prolonged boiling and yields pyrrolidone carboxylic acid which is inert toward nitrous acid. Moreover, γ-L-glutamyl-L-glutamic acid in 0.5 *N* HCl showed no detectable hydrolysis after 24 hours at 25°. The possibility that the γ-peptide linkage as such was unstable specifically to nitrous acid was ruled out by the observation that the tripeptide H.Ala-Glu.OH[11] gave
└Ala.OH
the theoretical values for one amino N (3, 10 or 30 minutes reaction time).

It now appears that γ-peptides give rapid and complete reaction only when the α-amino and α-carboxyl groups of the glutamic acid residue are free. When the α-carboxyl group is substituted, as in the series H.Glu-Ala.OH,[11] the γ-peptide nitro-
└Ala.OH
gen reacts only partially; for instance, triethyl γ-glutamylglutamate has been reported[12] to yield a value of approximately 1.3 equivalents of amino N per mole.

Analysis of the reaction products of the γ-glutamyl peptides with nitrous acid indicated the presence of a lactone structure. The method used for the detection and quantitative estimation of the "lactone" was the colorimetric procedure[13] involving hydroxylamine. The quantitative relationships between the Van Slyke values and the amount of "lactone" formed are given in Table II. It can be seen that γ-glutamylglutamic acid, with nitrous acid, yielded two equivalents of amino N and 1.9 moles of "lactone"; γ-glutamyl-γ-glutamylglutamic acid, 2.8 equivalents amino N and 2.8 moles of "lactone." The γ-peptides, without prior treatment with nitrous acid, did not form hydroxamic acids under the usual conditions.[13]

On the basis of the work reported here no explanation or mechanism for this reaction consistent with all the observations can be proposed. However,

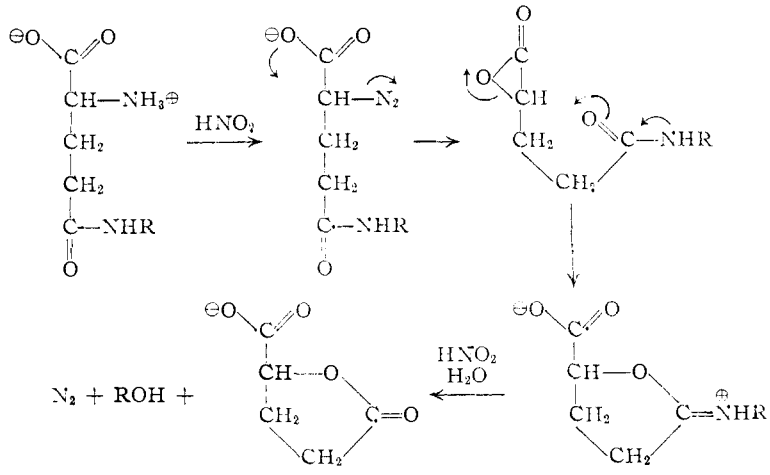
TABLE II

QUANTITATIVE RELATIONSHIPS BETWEEN AMINO N VALUES AND AMOUNT OF HYDROXAMIC ACID FORMED

Compound	Reaction time with nitrous acid,[a] min.	Moles Amino N per mole of compound	Moles of hydroxamic acid equivalent[b] per mole of compound
H·Ala·OH (DL)	10	1.0	0.03
H·Glu·OH (L) └NH_2	30	1.9	1.0
H·Glu·OH (LL) └Glu·OH	10	2.0	1.9
H·Glu·OH (LLL) └Glu·OH └Glu·OH	30	2.8	2.8
H·Glu·OH (LL) └Ala·OH	10	2.0	1.0
Glutathione	10	2.3	1.0
H·Glu-Ala·OH [LL] └Ala·OH [L]	30	1.3	0.3

[a] Temperature, 25°. [b] Using L-glutamic acid as standard.

the following scheme, analogous to that formulated by Craig[14] for an analogous case, is suggested as a working hypothesis.



This would account for the quantitative relationship between the amount of "lactone" formed and additional nitrogen evolved, the apparent retention of configuration of the α-carbon atom and the necessity for a free α-carboxyl group.

Experimental[15]

The synthesis and properties of most of the test substances have already been described.[9,11] The other starting materials were: L-glutamine (commercial sample); glutathione (Schwarz Laboratories); and S-acetylglutathione.[16] The synthesis of γ-L-glutamyl-L-glutamine is described below.

(1) Z·Glu·OBz (LL).—This was prepared by treating the
└Glu·OH
└NH_2
mixed ethyl carbonic acid anhydride of Z·Glu·OBz (L) (2.7 mmoles) with the potassium salt of L-glutamine (3.4 mmoles) as previously described.[11] It was recrystallized from 30% methanol. The yield of pure compound was 20–30%;

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(13) *E.g.*, S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).
(14) P. N. Craig, THIS JOURNAL, **74**, 129 (1952); *cf.* R. W. Maynert and E. Washburn, *ibid.*, **75**, 700 (1953).
(15) We are indebted for analytical work to T. Zelmenis (total and amino N).
(16) I am indebted to Dr. R. Dische for this material.

m.p. 157–159, $[\alpha]^{25}D$ +0.54° (1.9% in glacial acetic acid), $[\alpha]^{31}D$ −13.5° (1.9% in 95% alcohol).

Anal. Calcd. for $C_{25}H_{29}O_8N_3$ (499.5): N, 8.4. Found: N, 8.3.

(2) H·Glu·OH (LL).—This was obtained by the hydrogenation of compound I in 60% methanol in the usual manner.[9] It was recrystallized from water–ethanol; yield 80–90%, $[\alpha]^{23}D$ +8.7° (1.0% in 0.5 *N* HCl).

Anal. Calcd. for $C_{10}H_{17}O_6N_3$ (275.2): N, 15.3; amide N, 5.1; amino N, 5.1; carboxyl N, 5.1. Found: N, 15.2; amide N, 5.0; amino N, 14.2; carboxyl N, 4.4.

Stability of γ-L-Glutamyl-L-glutamic Acid in 0.5 *N* HCl.—The γ-peptide (0.0265 g.) was dissolved in 5 ml. of 0.5 *N* HCl at 25°. Aliquots (1 ml.) were then removed at suitable time intervals and carboxyl N analysis performed (ninhydrin, 7 minutes, *p*H 2.5).

Anal. Calcd. for $C_{10}H_{16}O_7N_2$ (276.2): carboxyl N, 5.1.

Time, hours	0	6	24
Found: % carboxyl N	5.3	5.3	5.3

Estimation of Lactone.—L-Glutamic acid was used as a standard for the colorimetric estimation of the lactone formed. The procedure consisted of treating the test substance with nitrous acid under the usual Van Slyke conditions (with respect to *p*H and HNO_2 concentration) decomposing the excess HNO_2 with $NH_2OH·HCl$ and then preparing and estimating the hydroxamic acid–iron complex in the usual manner.[13]

Approximately 35–45 μmoles of glutamic acid (or amount of test substance which would yield 35–45 μmoles of lactone) was weighed into a 15-cc. volumetric flask. The following reagents were added in the order: 2.5 ml. of H_2O, 0.5 ml. of glacial acetic acid, 1.0 ml. of $NaNO_2$ solution (800 g. of $NaNO_2$ per liter of H_2O). After ten minutes at 25°, the solution was cooled (0°), and 5 ml. of 2 *M* $NH_2OH·HCl$ solution added dropwise. The solution was permitted to stand for 3 minutes at 25°, and enough H_2O was then added to bring the volume to 15 ml. Aliquots (3.0 ml.) of this solution were then added to a solution containing 1.0 ml. of 2 *M* $NH_2OH·HCl$ and 2 ml. of 3.5 *N* NaOH. The reaction mixture was allowed to stand for four minutes (25°), and then 1.65 ml. of HCl (HCl, sp. gr. 1.18 diluted with 2 parts by volume of H_2O) and enough $FeCl_3$ solution (0.37 *M* in 0.1 *N* HCl) were added to bring the final volume to 10 cc. (final *p*H 1.2). The optical density of the colored solution was determined with the aid of a Klett–Summerson photoelectric colorimeter (number 54 filter).

The glutamic acid standard showed an approximately linear relationship of concentration to optical density in the region 3–9 μmoles. Under these experimental conditions the absorption spectrum of the colored hydroxamic acid complex (measured in the Beckman model DU spectrophotometer) exhibited a broad maximum at about 540–500 mμ (using either glutamic acid or γ-glutamylglutamic acid as starting material).

Optical Configuration of the α-Hydroxy Acid Formed.—L-Glutamic acid (0.1225 g.), L-glutamine (0.1230 g.), γ-L-glutamylglutamic acid (0.114 g.) and L-alanine (0.1219 g.) were each treated with nitrous acid for ten minutes as described above. The solutions were then taken down to dryness in a stream of air, 5 ml. of H_2O added to each and the solvent was again evaporated. This was repeated twice more; then the residues were each taken up in 0.5 ml. of 2 *N* NaOH and the volume adjusted to 3 ml. with H_2O. The optical rotations were measured and the specific rotations calculated on the basis of complete conversion to the hydroxy acid.

Starting materials	$[\alpha]^{26}D$	Concn., %
H·Gl·OH (L)[17]	−11.1°	3.6
H·Glu·OH (LL) (L-Glu·OH)	−10.3	3.5
H·Glu·OH (L) (L-NH_2)	−10.8	3.6
H·Ala·OH (L)	− 9.2	4.1

(17) E. Fischer and A. Moreschi (*Ber.*, **45**, 2447 (1912)) prepared the disodium salt of L-α-hydroxyglutaric acid; $[\alpha]^{19}D$ −8.65° (1.6% in H_2O).

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Contribution of the Cyclopropyl Ring to Molar Refraction[1]

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In 1900, Tschugaeff[2] reported that the observed molar refractions of cyclopropane derivatives were higher than those calculated by summation of atomic and group refractivities. This difference between observed and calculated refractions was attributed by Tschugaeff to the cyclopropyl ring, and from the average difference for a number of compounds he concluded that the magnitude of this ring contribution was about 0.7. Östling[3] reported on a larger number of compounds, and his average difference agreed well with Tschugaeff's value. Consequently, the value 0.7 has generally been accepted as the contribution of the cyclopropyl ring to the molar refraction of cyclopropane derivatives.

More recently, Jeffery and Vogel[4] determined the contribution of the cyclopropyl ring by a different procedure: from the observed molar refraction of the cyclopropane derivative is subtracted the observed molar refraction of a structurally similar acyclic compound, two hydrogen atomic refractivities being added to the result to make up for the two additional hydrogens in the acyclic structure. From data obtained principally with alkyl cyclopropane mono- and dicarboxylates, the authors assigned the value 0.614 to the ring contribution.

In each of the previous investigations the number of individual cyclopropane compounds and also, the number of derivative types were limited; consequently, it was uncertain: (1) whether any one value adequately expresses for all cyclopropane derivatives the contribution of the ring to the molar refraction, and (2) which of the methods proposed for determining the ring contribution is most likely to give such a "constant" value. The physical properties of 30 cyclopropane derivatives, were available from previous work[5]; these data were used in the present effort to answer these questions.

Results and Discussion

In Table I are presented the observed molar refractions of the 30 cyclopropane derivatives, the dif-

(1) Presented before the Organic Division at the 124th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 6–11, 1953.

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