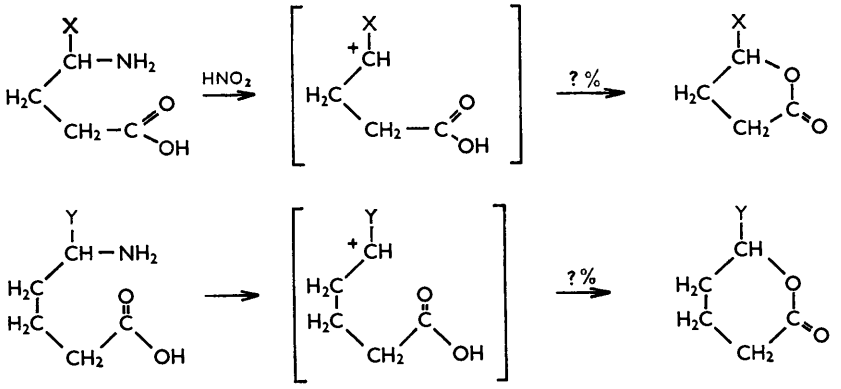
643. *The Deamination, by Nitrous Acid, of Amino-acids structurally Related to Glutamic Acid: γ-Aminobutyric, γ-Aminovaleric, α-Amino-adipic, and δ-Aminovaleric Acid, and Isoglutamine.*

By A. T. Austin and J. Howard.

The amount of lactone formed in the deamination of glutamic acid by nitrous acid decreases to one-third when the carboxyl group in the α-position to the amino-group is replaced by H or Me. This is attributed to a configuration-holding effect of the α-carboxylate group that minimises intermolecular attack on the intermediate carbonium ion. With amines that give, on deamination, five-membered ring lactones, the yield of lactone is nine times greater than from those amines that give six-membered ring lactones.

The evolution of two mol. of nitrogen instead of the one expected when glutamine reacts with nitrous acid (Van Slyke determination) has been shown to be related to the formation of a lactone which was postulated to arise because the carbonium ion (formed by the action of nitrous acid on the α-amino-group) underwent intramolecular cyclisation with the oxygen of the amido-group.[1] The same lactone is formed, to the same extent (93%), in the reaction



of glutamic acid with nitrous acid and the sequence of reactions was considered to be essentially the same for both glutamine and glutamic acid.

The compounds noted in the title were subjected to deamination by nitrous acid for the purpose of establishing whether the extent of the lactonisation is influenced by the presence of the carboxyl group in the α-position to the amino-group, and the size of the lactone ring. These items may be depicted in the generalised form as annexed, where X = CO_2H, H, Me, or $CO{\cdot}NH_2$, and Y = CO_2H or H.

Results

Deamination of γ-Aminobutyric Acid.—Fig. 1 shows the rate of disappearance of γ-aminobutyric acid from aqueous nitrous acid solutions of this compound and the rate of formation of

[1] Austin and Howard, *J.*, preceding paper.

lactone in the same solution. From the respective gradients it is seen that the initial rate of deamination is approximately three times that for the formation of the lactone. The broken line shows the calculated amount of lactone that would have been formed if the deamination led directly to γ-hydroxybutyric acid which then gave the lactone.

Deamination of γ-Aminovaleric Acid.—Similar plots for this acid are shown in Fig. 2. The gradients show that the initial rate of deamination of γ-aminovaleric acid is four times the initial rate of formation of γ-valerolactone.

Deamination of Isoglutamine, α-Aminoadipic Acid, and δ-Aminovaleric Acid.—Kinetic measurements were not carried out with these compounds. Instead solutions 0·02M with respect to the amino-compound and 0·04M in nitrous acid were prepared (as for the kinetic determinations) and the amount of deamination and lactone formation measured after ½ hr.

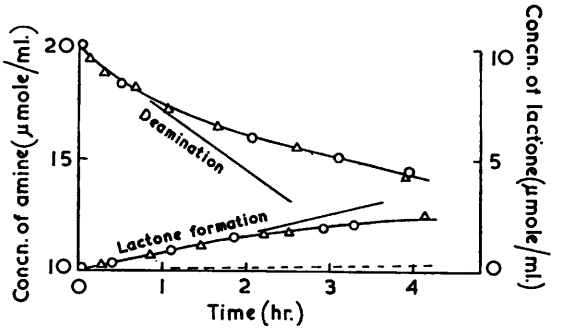


FIG. 1. *Reaction–time curves for* [γ-Aminobutyric Acid] = 0·02M + [HNO_2] = 0·04M *in aqueous solution at* 25·0°, pH = 2·8, *and* p_{NO} = 1 atm.

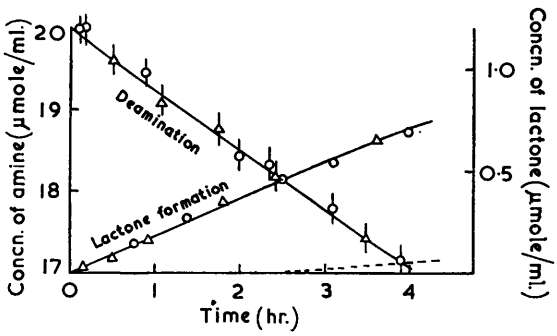


FIG. 2. *Reaction–time curves for* [γ-Aminovaleric acid] = 0·02M + [HNO_2] = 0·04M *in aqueous solution at* 25·0°, pH = 2·7, p_{NO} = 1 atm.

for the solution containing isoglutamine and after 2—3 hr. for solutions containing α-aminoadipic and δ-aminovaleric acid. 46% of the deaminated isoglutamine appeared as lactone; the corresponding figures for α-aminoadipic and δ-aminovaleric acid were 10% and 3% respectively.

Rate of Lactonisation of Hydroxy-acids.—Rates of lactonisation were determined at 25·0° in aqueous solution as follows: γ-hydroxybutyric, $k = 7{\cdot}2 \times 10^{-4}$; γ-hydroxyvaleric, $2{\cdot}8 \times 10^{-3}$; δ-hydroxyvaleric acid, $4{\cdot}5 \times 10^{-3}$ mole^{-1} l. sec.$^{-1}$.

DISCUSSION

As the rate of lactonisation of the hydroxy-acid is unable to account for any but a negligible amount of lactone one may assume that all the lactone formed in the initial stages is derived directly from the intramolecular cyclisation of the carbonium ion with the γ- or δ-carboxyl group. The following Table is drawn up from the ratio, initial rate of deamination/initial rate of lactone formation, to obtain the percentages of lactone formed in the deamination of γ-amino-butyric and -valeric acid; the entries for the last three compounds in the Table record the percentage of lactone obtained by direct measurement.

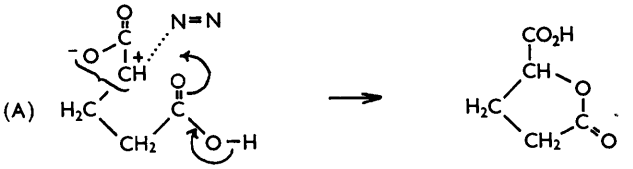
Effect of (a) *changing the group* α *to the amino-group and* (b) *size of lactone ring, on percentage of deamination leading to lactone.*

Acid	" α "-Group	Ring size of lactone	Lactone (%)
Glutamic	CO_2H	5	93 [1]
γ-Aminobutyric	H	5	33
γ-Aminovaleric	Me	5	25
Isoglutamine	$CO{\cdot}NH_2$	5	46
α-Aminoadipic	CO_2H	6	10
δ-Aminovaleric	H	6	3·5

(a) *Effect of changing the Group* α *to the Amino-group.*—When optically active halogeno-acids were solvolysed under unimolecular conditions retention of configuration at the asymmetric centre was observed and Hughes and Ingold [2] attributed the retention to the carboxylate group's holding a pyramidal transition state until the new group entered the position vacated by the outgoing group. Hughes, Ingold, and their collaborators [3] observed retention of configuration at the asymmetric centre also when optically active amino-acids were deaminated with nitrous acid, and a mechanistic interpretation similar to that given for the halogeno-acids was invoked.

In the present investigation a sharp drop in the yield of lactone (93 to 33%) was observed when the α-carboxyl group in glutamic acid was replaced by hydrogen, and a similar drop (10% to 3·5%) from α-aminoadipic to δ-aminovaleric acid. These results suggest that the α-carboxyl group acts in the configuration-holding manner discussed in the preceding paper and so impedes intermolecular interaction at the carbonium ion site. It would then be expected that the γ-carboxyl group (or any other nucleophilic entity) would be repelled if it approached the rear of the carbonium ion. For example, interaction of glutamic acid would be restricted to the site vacated by the N_2^+ group in an S_Ni-type reaction (cf. A).

The carbonium ion would not be so effectively protected from rear attack if the α-carboxylate group were replaced by H, Me, or $CO{\cdot}NH_2$; other reactions (solvent, NO_2^- intervention) could compete with interaction of the γ-carboxyl group and the yield of lactone would be expected to fall.



This interpretation implies that the " holding " or " protecting " effect of the α-carboxylate group, invoked by the Hughes–Ingold school to explain the stereochemistry, also exerts a decisive influence on the nature of the reaction, and this, second, aspect is important in minimising " extraneous " reactions during the deamination of glutamine and of glutamic acid where the lactone yield is 93% and the evolved gas is pure nitrogen.

The interpretation is also consistent with the observation [4] that triethyl γ-glutamyl-glutamate evolved 59% of the total nitrogen in the Van Slyke determination, whereas γ-glutamylglutamic acid yielded two mol. of lactone and ~100% of the total nitrogen * under the same conditions.[5] Esterification of the α-carboxyl group reduced the amount of cyclisation and the yield of nitrogen.

* As with glutamine and γ-*N*-methylglutamine the " extra " gas is derived from the nitrosative breaking of the CO·NH bond of the original γ-amide group after this has been modified by intramolecular interaction with the α-carbonium ion.

[2] Ingold, " Structure and Mechanism in Organic Chemistry," Bell and Son, London, 1953, p. 381.
[3] Brewster, Hiron, Hughes, Ingold, and Rao, *Nature*, 1950, **166**, 179.
[4] Boothe, Mowat, Hutchings, Angier, Waller, Stokstad, Semb, Gazzola, and Subba Row, *J. Amer. Chem. Soc.*, 1948, **70**, 1099.
[5] Sachs and Brand, *J. Amer. Chem. Soc.*, 1954, **76**, 3601.

(b) *Effect of Ring-size of the Lactone.*—Although glutamic acid gave a 93% yield of the five-membered ring lactone, γ-carboxy-γ-butyrolactone, the next higher homologue, α-aminoadipic acid, gave only 10% of the six-membered ring lactone, δ-carboxy-δ-valerolactone. There was a similar nine-fold decrease in the amount of lactone when passing from γ-aminobutyric (giving a five-membered ring in γ-butyrolactone) to δ-aminovaleric acid (giving a six-membered ring in δ-valerolactone).

As it was not possible to detect any lactone after the deamination of aspartic acid [1] (which would have given a four-membered ring lactone) one can conclude that five-membered rings are much more easily formed in the deamination. One could cite many examples of other reactions where the formation of five-membered rings is preferred to that of six-membered rings and it is relevant van Tamelen and Shamma [6] found interaction of iodine with βγ- and γδ-unsaturated acids to give preferentially the five-membered iodo-lactones. The ease of formation of five-membered ring lactones is a general phenomenon and is understandable in terms of ring-angle strain and non-bonded interaction of the substituents; it is undoubtedly important in determining the high yield of lactone from glutamic acid and glutamine and, together with the " protecting " effect of the α-carboxyl group, underlies the marked anomaly when glutamine is determined in the Van Slyke procedure.

The ability of remote parts of a molecule to approach each other (by rotation) and in so doing to modify the chemistry when one of the parts is chemically attacked underlies the anomalous behaviour of a number of organic reactions.[7] It has been invoked [8] to explain Birkinshaw's observation that palitantin readily takes up one atom of iodine from ethanolic iodine to form a mono-iodo-compound in 80% yield.[9] The cyclic intermediate postulated here to account for the anomalous behaviour of glutamine is also involved in the selective splitting of tyrosyl [10] and tryptophyl [11] peptides and in the explanation [10] for the unexpected degradation of oxytocin by bromine water.[12]

The anomalous result created is most pronounced when the internal interactions can reach the reaction site by formation of five- or six-membered rings and the term " chelate interaction " is suggested to denote these modifications in chemical behaviour caused by intervention of a part of the molecule normally remote from the site of reaction.

Experimental

γ-Aminobutyric Acid.—γ-Chlorobutyryl chloride [13] was converted into ethyl chlorobutyrate by addition to absolute ethanol, and the fraction of b. p. 186—188° was used to prepare the γ-phthalimido-ester by Sheehan and Bolhofer's method.[14] The phthalimido-group was removed by hot 50% sulphuric acid, and the amino-acid obtained on neutralisation with barium carbonate crystallised from water on addition of ethanol; it had m. p. 199—201° (decomp.) (Found: C, 46·5; H, 8·5; N, 13·4; Van Slyke determination, 99·2. Calc. for $C_4H_9NO_2$: C, 46·6; H, 8·7; N, 13·4%).

γ-Aminovaleric Acid.—Lævulic acid was converted into the phenylhydrazone which was reduced to γ-aminovaleric acid by Fischer and Groh's method.[15] Crystallised twice from water by addition of ethanol, it had m. p. 162° (lit.,[9] 162°). A Van Slyke determination gave 100·0% of nitrogen.

[6] Van Tamelen and Shamma, *J. Amer. Chem. Soc.*, 1954, **76**, 2315.
[7] Arnold, de Moura Campos, and Lindsay, *J. Amer. Chem. Soc.*, 1953, **75**, 1044; Goodman and Winstein, *J. Amer. Chem. Soc.*, 1957, **79**, 4788.
[8] Bowden, Lythgoe, and Marsden, *J.*, 1959, 1662.
[9] Birkinshaw, *Biochem. J.*, 1952, **51**, 271.
[10] Schmir, Cohen, and Witkop, *J. Amer. Chem. Soc.*, 1959, **81**, 2228; Corey and Haefele, *ibid.*, p. 2225.
[11] Patchornik, Lawson, and Witkop, *J. Amer. Chem. Soc.*, 1958, **80**, 4748, 4747.
[12] Mueller, Pierce, and du Vigneaud, *J. Biol. Chem.*, 1953, **204**, 857; Ressler, Trippett, and du Vigneaud, *ibid.*, p. 816; Popenoe and du Vigneaud, *ibid.*, 1953, **205**, 133; Ressler and du Vigneaud, *ibid.*, 1954, **211**, 809.
[13] Loftfield, *J. Amer. Chem. Soc.*, 1951, **73**, 1365.
[14] Sheehan and Bolhofer, *J. Amer. Chem. Soc.*, 1950, **72**, 2786.
[15] Fischer and Groh, *Annalen*, 1911, **383**, 370.

α-Aminoadipic Acid.—The method of Waalkes, Fones, and White,[16] starting with adipic acid, was modified by using dimethylformamide as the solvent for the reaction between the α-bromoadipic ester and potassium phthalimide. The phthalimido-group was removed by hydrochloric acid,[17] and the free amino-acid obtained by passing the hydrochloride through Zeo-Karb 216 (20—60 mesh) [1] (Found: C, 44·5; H, 6·9; N, 8·9; Van Slyke determination, 98. Calc. for $C_6H_{11}NO_4$: C, 44·75; H, 6·8; N, 8·7%).

δ-Aminovaleric Acid.—Cyclopentanone [18] was converted into the oxime which was heated with 30N-sulphuric acid.[19] The α-piperidone was hydrolysed with 2N-sulphuric acid and after neutralisation (barium carbonate) was purified by passage through Zeo-Karb 216 (20—60 mesh).[1] Recrystallisation from water by addition of ethanol (twice) gave an acid, m. p. 158° (lit.,[20] 157—158°) (Found: C, 50·45; H, 9·35; N, 11·6; Van Slyke determination, 95. Calc. for $C_5H_{11}NO_2$: C, 51·2; H, 9·4; N, 12·0%).

δ-Valerolactone.—This was prepared by Schniepp and Geller's method [21] from pentane-1,5-diol. Saponification showed 99% purity.

γ-Butyrolactone and γ-Valerolactone.—Commercial material was purified by distillation. Fractions, b. p. 203—204° and 207°, respectively, were used.

Estimation of Lactones.—These lactones were used to prepare the calibration between optical density and lactone concentration in colorimetric estimations of the lactones formed in the deaminations: the ferric–hydroxamic acid colour complex was used with the procedure already described.[1] γ-Carboxy-γ-butyrolactone, γ-butyrolactone, and γ-valerolactone gave the same optical density for the same concentration of lactone. δ-Valerolactone required 20 min. for quantitative reaction with the hydroxylamine and a separate calibration chart as the calibration was different from that for the five-membered ring lactones. As the other three lactones gave identical calibration charts it was assumed that the calibration chart for δ-carboxy-δ-valerolactone would be the same as for δ-valerolactone and the one chart was used for estimating these two lactones.

Estimation of Amino-acids.—(a) *γ-Aminobutyric acid.* This acid was estimated by Sörensen's formol titration, the copper complex and the ninhydrin method previously [1] used being restricted to α-amino-acids. The determinations were readily reproducible but in our hands the first pink colour of phenolphthalein in the titrations always corresponded to only 92% reaction. Simultaneous potentiometric titrations also gave the end-points at 92% reaction and pH 8·5. At pH 9, the pH usually quoted for the end-point, the titrations corresponded to 100% reaction but at this pH the maximum gradient in the potentiometric titration had been passed and the colour of the phenolphthalein was so intense that comparison with a colour standard was impossible. Identical behaviour was obtained when several different samples of carefully purified glycine were tested. As, however, the end-point to the first pink of phenolphthalein was sharp and reproducible the method was used to estimate γ-aminobutyric acid and the concentration so obtained was " corrected " by use of the factor 100/92.

(b) *γ-Aminovaleric acid.* The Sörensen formol titration gave unsatisfactory results, and potentiometric titration was also unsatisfactory as the $\Delta pH/\Delta V$ gradient was quite small at the end-point. The acid was therefore determined by the Van Slyke procedure. During the runs samples were removed from the reaction vessel and plunged into 0·01N-sodium hydroxide to stop the reaction. The amino-nitrogen was then determined. The results were reproducible to ±1%.

(c) *α-Aminoadipic and δ-aminovaleric acid.* These were most conveniently determined by the Van Slyke procedure. Although there is some uncertainty surrounding the method the results are reproducible when the stated conditions are adhered to and the results obtained had the accuracy required. The Van Slyke apparatus was the manometric type supplied by Messrs. Gallenkamp & Co., Ltd., London.

Determination of Rate Constants for the Lactonisation of γ-Hydroxybutyric, γ-Hydroxyvaleric, and δ-Hydroxyvaleric Acid.—These were determined as described for γ-hydroxyglutaric acid.[1] The results were calculated on the basis of initial rates and the determinations were made over

[16] Waalkes, Fones, and White, *J. Amer. Chem. Soc.*, 1950, **72**, 5760.
[17] Gaudry, *Canad. J. Res.*, 1949, **27**, *B*, 21.
[18] Vogel, " Textbook of Practical Organic Chemistry," Longmans, Green & Co., London, 1956, 3rd edn., p. 340.
[19] Fox, Dunn, and Stoddard, *J. Org. Chem.*, 1941, **6**, 410.
[20] Wallach, *Annalen*, 1900, **312**, 180.
[21] Schniepp and Geller, *J. Amer. Chem. Soc.*, 1947, **69**, 1545.

the pH range 1·3—2·3. The rates were all of the same order of magnitude and it was assumed that for α-hydroxyadipic acid it would also be of the same order.

Run Procedure.—The experimental procedure was as described for the kinetic investigations with glutamine and glutamic acid.[1] Samples were withdrawn at timed intervals and run into the appropriate solutions for the estimation of the unchanged amino-compound, and the lactone was determined by the method already described.[1]

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