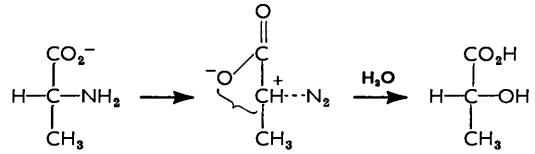
642. *The Steric Course of Lactonisation following the Deamination of Glutamic Acid, Glutamine, and γ-Aminovaleric Acid.*

By A. T. Austin and J. Howard.

The rear of the carbonium ion, formed by the action of nitrous acid on the α-amino-group of glutamine and glutamic acid, is protected against chemical attack by the α-carboxyl group and a similar interaction, by the γ-carboxyl or γ-carbamoyl group, protects the *front* of the carbonium ion. The two effects, operating together, in a spirocyclic transition state prevent other entities from reacting with the carbonium ion and account for the purity of the products formed in the two reactions.

The " front-side " interaction (based on the 80% retention of configuration of the lactone obtained when γ-aminovaleric acid was deaminated—with subsequent reaction by an S_Ni-mechanism—is derived from electrostatic interaction between the diazonium cation (the precursor of the carbonium ion) and the γ-carboxyl or γ-carbamoyl group, and constitutes a third factor conducive to the high yield of lactone when glutamine and glutamic acid are deaminated.

Hughes, Ingold, and their collaborators [1] have shown that the deamination of optically active alanine by nitrous acid gives an optically active lactic acid with retention of configuration. To explain this they postulate that the α-carboxylate group " holds " the configuration of the carbonium ion in much the same way as they suggested to account for retention in the unimolecular hydrolysis of optically active halogeno-acid ions. The α-carboxylate group is depicted as holding the carbonium ion in a pyramidal configuration until the incoming group enters the position originally occupied by the amino-group:



L-(+)-Alanine L-(+)-Lactic acid

If this happens it could influence the chemical course of the reaction because the carboxylate group should protect the rear of the carbonium ion against chemical attack. The purity of the products obtained in the deamination of glutamic acid and glutamine (nitrogen and 93% of lactone) indicates that the carbonium ion, which is formed by the action of nitrous acid on the α-amino-group, is strongly shielded against interaction with nitrite ions and solvent molecules. The present investigation has shown that in γ-amino-acids an interaction similar to that of the α-carboxylate group comes into play on the *other* side of the carbonium ion centre and shields the front of the carbonium ion.

In the absence of an α-carboxylate group the Hughes–Ingold school observed overall inversion of configuration (with much racemisation) in the deamination of simple optically active amines and explained this as due to a symmetrical shielding of the carbonium ion by the extruded nitrogen. It has been shown [2] that when the α-carboxyl group in αγ-dicarboxy-amines is replaced by the less polar hydrogen or methyl less lactone is formed on deamination and it was desirable to ascertain the steric course of lactonisation during deamination of γ-carboxy-amines where the configuration-holding effect of the α-carboxylate group is absent.

[1] Brewster, Hiron, Hughes, Ingold, and Rao, *Nature*, 1950, **166**, 179.
[2] Austin and Howard, *J.*, in the press.

Results

Deamination of L-(+)-*Glutamic Acid and* L-(+)-*Glutamine.*—Deamination of each of these compounds gave a lævorotatory hydroxy-acid. As a lactone is an isolable intermediate the results obtained may be depicted:

L-(+)-Glutamine ⟶ Lactone ⟶ (−)-α-Hydroxyglutarate
$[\alpha]_D^{21}$ + 6·75° (in H_2O) $[\alpha]_D^{21}$ −7·5° (in aq. NaOH)

L-(+)-Glutamic acid ⟶ Lactone ⟶ (−)-α-Hydroxyglutarate
$[\alpha]_D^{18}$ +29·6° (in 5% HCl) $[\alpha]_D^{21}$ −9·2° (in aq. NaOH)

Deamination of (+)-γ-*Aminovaleric Acid.*—The lactone formed in the deamination of this compound was extracted with ether and purified and its optical rotation measured. The lactone was then hydrolysed to the hydroxy-acid and the optical rotation measured.

(+)-γ-Aminovaleric acid ⟶ (−)-γ-Valerolactone ⟶ (+)-Hydroxyvaleric acid
$[\alpha]_D^{22}$ +12° $[\alpha]_D^{22}$ −13·7° $[\alpha]_D^{22}$ +6°

The configuration of (+)-γ-aminovaleric acid was related to that of (+)-β-aminobutyric acid which was related to (+)-α-alanine by processes that did not involve breaking any of the bonds attached to the asymmetric carbon centre: when these amino-acids have the same sign of optical rotation they have the same configuration:

(+)-γ-aminovaleric acid ~ (+)-β-aminobutyric acid ~ (+)-α-alanine
(the symbol ~ means " corresponds in configuration to ")

During our work identical configurational relations have been reported for the second correlation by Fuks [3] and for the overall relation by Balenovic and Cerar.[4]

Discussion

The formation of the same lævorotatory hydroxy-acid * on the deamination of L-glutamic acid and L-glutamine shows that the steric course of the reaction leading to the lactone must also be the same. It has been established [6] that these acids give the same lactone (γ-carboxy-γ-butyrolactone) in the same amount, so the processes involved in their deamination must be very similar.

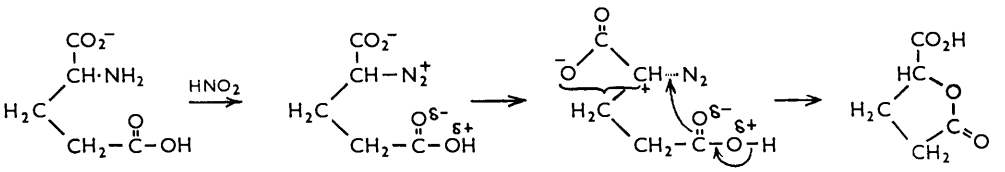
The 80% retention of configuration observed in the deamination of γ-aminovaleric acid (see below) shows that considerable interaction must occur between the carbonium ion (or a precursor of this ion) and the γ-carboxyl group at the " front " of the carbonium ion centre and accordingly any electrostatic interaction of the α-carboxylate group would have to be directed to the " rear " of the carbonium ion. The two interactions operating simultaneously in the deamination of glutamic acid and glutamine would afford very strong protection for the carbonium ion centre against attack by " outside " entities and this two-fold protection gives a reasonable explanation for the complete absence [6] of the expected intervention [7] by nitrite ion and for the high yield of lactone obtained when nitrous acid reacts with glutamic acid and glutamine under Van Slyke conditions.

The steric course of the cyclisation of the carbonium ion formed in the deamination of

* The difference in the observed optical rotations is probably related to slight variations in experimental procedure between the two sets of experiments and is not considered significant. α-Hydroxyglutaric acid has $[\alpha]_D^{19}$ ±8·65°.[5]

[3] Fuks, *Acta Pharm. Jugoslav.*, 1953, **3**, 35; *Chem. Abs.*, 1955, **49**, 8114.
[4] Balenovic and Cerar, *J.*, 1955, 1631.
[5] Fischer and Moreschi, *Ber.*, 1912, **45**, 2447.
[6] Austin and Howard, following paper.
[7] Austin, *J.*, 1950, 149.

glutamic acid is postulated to involve a spirocyclic transition state, the lactone being formed with retention of configuration at the asymmetric carbon centre:



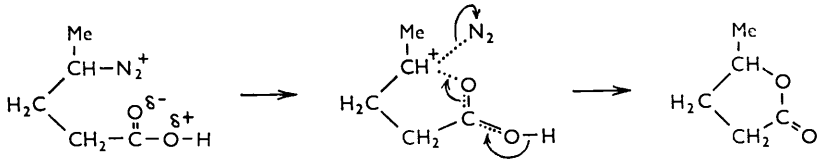
Deamination of (+)-γ-aminovaleric acid, $[\alpha]_D^{22}$ +12° (lit.,[4] +14°), gave unexpected results. The optical activity of the lactone, $[\alpha]_D^{22}$ −13·7° (lit.,[8] −27·75°), and of the hydroxy-acid obtained on hydrolysis, $[\alpha]_D^{22}$ +6° (lit.,[8] +14°), indicated that 80% of the reaction leading to the lactone had proceeded in a manner that retained the optical activity. It thus appeared that " free " carbonium ions are not involved in the formation of the lactone from γ-aminovaleric acid.

Levene and Heller [8] showed that (+)-γ-hydroxyvaleric acid belongs to the L-series and it has been shown in the present investigation and by others [4] that (+)-γ-aminovaleric acid has the same configuration as L-(+)-α-alanine.[9] Therefore, the hydroxy-acid obtained by hydrolysis of (−)-γ-valerolactone must have possessed the same configuration as the parent amine, the amino-compound being converted into the hydroxy-acid with predominant retention of configuration. This overall retention of configuration could have resulted from the γ-valerolactone's being formed from the amine with inversion and hydrolysed with inversion, but this is not so because γ-butyrolactone has been shown kinetically and by tracer studies with ^{18}O to undergo both basic and acidic hydrolysis with acyl–oxygen fission.[10] It is reasonable to assume that the same process would apply to γ-lactones in general. This being the case, hydrolysis of the acyl–oxygen bond of γ-valerolactone would yield the γ-hydroxy-acid with retention of configuration. Therefore the process leading to the lactone must also have occurred with retention of configuration.

This finding contrasts with the deamination of simple amines where, in the absence of an α-carboxyl group, net inversion of configuration was obtained.[1] It also differs from the stereochemical implication that the lactone formed in the solvolysis of γ-bromovaleric acid yielded, on hydrolysis, a hydroxy-acid with inverted configuration.[5,11,12,13] However, in this case it was apparent, from the rate of reaction, that the carboxylate group interacted with the γ-carbon atom in an internal bimolecular process and these bimolecular replacements have been shown to require linear transition states and always to lead to inversion at the centre undergoing replacement.

The S_N1 process for the formation of the lactone from γ-aminovaleric acid having been rejected because of the optical purity of the products, and the S_N2 process having been rejected because of the predominant retention of configuration of the products, the following mechanism is suggested: Electrostatic interaction between the diazonium cation and the γ-carboxyl group is postulated to lead to the formation of a quasi-six-membered ring. As the nitrogen breaks away the six-membered ring collapses to a fully covalent five-membered ring lactone with the oxygen of the γ-carboxyl group entering the site originally occupied by the amino-group by an S_Ni-mechanism: [14]

[8] Levene and Heller, *J. Biol. Chem.*, 1926, **69**, 165.
[9] Brewster, Hughes, Ingold, and Rao, *Nature*, 1950, **166**, 178.
[10] Long and Friedman, *J. Amer. Chem. Soc.*, 1950, **72**, 3692; Long, McDevit, and Dunkle, *J. Phys. Colloid Chem.*, 1951, **55**, 829.
[11] Caldin and Wolfenden, *J.*, 1936, 1239.
[12] Cowdrey, Hughes, Ingold, Masterman, and Scott, *J.*, 1937, 1252.
[13] Ingold, " Structure and Mechanism in Organic Chemistry," Bell and Sons Ltd., London, 1953, 384.
[14] Ref. 13, p. 392.



This mode of electrostatic orientation and formation of lactone would account for both the optical purity of the product and the retention of configuration, for the electrostatic interaction would be much more pronounced on that side of the asymmetric carbon atom leading to retention of configuration. The optical activity indicates that less than 20% of the lactone had been formed by intramolecular S_N2 interaction.

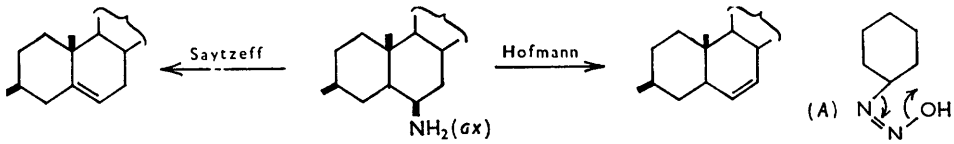
It is no criticism of the above mechanism that electrostatic effects, as depicted above, have been shown not to be important for Walden inversion [15] for no energetically important rate-determining interaction is attributed to the carboxyl group. The incorporation of this " front-side " interaction had been anticipated in the mechanism given on p. 3280 for the steric course of the lactonisation of glutamic acid. Essentially the same steps would apply for the reaction of glutamine. The effect must be strong because it can, in conjunction with the α-carboxylate group, prevent any detectable intervention by nitrite ion and it must be considered as a third factor, additional to the two already established,[2,6] that favours the high yield of lactone in the deamination of glutamic acid and glutamine.

A similar electrostatic orientation would have to be assumed during the deamination of asparagine, but the subsequent formation of a lactone would not be possible here: it would require the collapse of the quasi-five-membered ring to a four-membered ring lactone and this is known to be difficult on both theoretical and experimental grounds.

The possibility that the carboxyl group in γ-aminovaleric acid interacted with the diazonium cation to form a cyclic diazo-ester can be rejected. These compounds have been suggested as intermediates in the thermal decomposition of *N*-nitroso-derivatives [16] and their decomposition was postulated to account for the retention of configuration in the products. These reactions, however, were carried out under different conditions from ours. Moreover, if these diazo-esters were important intermediates in the deamination of γ-carboxy-amines it would be expected that asparagine, which would be able to form the six-membered ring required, would react with nitrous acid to evolve 100% of the nitrogen and this does not happen.

In view of the predominant " front-side " lactonisation observed in the deamination of γ-aminovaleric acid it is relevant that Shoppee *et al.*[17] deaminated saturated steroid equatorial amines in 50% aqueous acetic acid and obtained the corresponding alcohol (100% yield) with retention of configuration. Mills,[18] Streitwieser *et al.*,[19] and Dauben *et al.*[20] have obtained similar results with aminocyclohexanes and aminodecalins. From axial amines Mills and Dauben *et al.* obtained alcohols with predominant inversion together with much olefin. Shoppee *et al.*, however, observed retention of configuration in the deamination of the axial amines they studied (together with much olefin) and this was clearly inconsistent with the conclusions reached by the other workers that deamination of this type of compound is conformationally specific. Since there was no configuration-holding group present Shoppee *et al.* considered that carbonium ions were not formed and concluded that the reaction involved the interaction of water with the diazonium cation in a pyramidal transition state; but an immediate objection to this explanation became apparent when it was shown that the olefin formed conformed to the Saytzeff rule whereas elimination from the diazonium cation should have been governed by the Hofmann rule.

[15] Read and Walker, *J.*, 1934, 308.
[16] White, *J. Amer. Chem. Soc.*, 1955, **77**, 6014.
[17] Shoppee, Evans, and Summers, *J.*, 1957, 97.
[18] Mills, *J.*, 1953, 260.
[19] Streitwieser and Coverdale, *J. Amer. Chem. Soc.*, 1959, **81**, 4275.
[20] Dauben, Tweit, and Mannerskantz, *J. Amer. Chem. Soc.*, 1954, **76**, 4420.



Deamination of equatorial amines affords no inverted products and no olefin, hence it appears that free carbonium ions are not formed in the reaction. This is plausible because flattening of part of an alicyclic ring system is not easy and the formation of carbonium ions has been shown to require at least partial planarity.[21,22] The products of reaction have accordingly to be derived from something other than a carbonium ion. Bimolecular replacement is difficult in these systems because the other atoms in the ring system sterically hinder the formation of a linear transition state, and bimolecular elimination is difficult because a *trans*-planar transition state is difficult to realise. Another reaction path is brought into play and it is suggested that the diazohydroxide undergoes decomposition by an S_Ni mechanism (cf. *A*). S_Ni reactions preserve the essential features of unimolecular reactions [12,13] with the special feature that they result in retention of configuration in the products. Although Bartlett [23] has suggested a similar mechanism for the deamination of 1-apocamphylamine it is clear that he does not consider the process to be similar to the unimolecular ionisation process.

Deamination of axial amines always leads to a considerable proportion of olefin—it is usually the major product and in one of Shoppee's examples was the sole product in quantitative yield. The explanation for this is that the *trans*-planar transition state is easily achieved with axial amines as Mills [18] pointed out. Notwithstanding, the proportion of the olefin is always extremely high and this poses the question whether (*a*) olefin-formation is very easy or (*b*) substitution is very difficult. The question must be asked in the absolute sense as well as in the relative sense.

It seems that (*b*) contains the answer since the conditions obtaining in deamination (aqueous acetic acid) are not normally conducive to high yields of olefin. Bimolecular replacement would be difficult because extensive compression would arise in the formation of the linear transition state where four quasi-axially disposed groups would be on one side of the ring and three on the other, which is untenable in terms of conformational energy. Unimolecular replacement would also be difficult because flattening of part of a ring system is not easy and, as already noted, at least partial planarity is required for the formation of carbon ions. It is, therefore, clear that in these cases olefin formation, even in mild conditions of aqueous acetic acid, will not be in serious competition with substitution.

With the axial amino-derivatives of the smaller ring systems of cyclohexane and the decalins the experimental results require the assumption that distortion and buckling of the rings with concomitant conformational eclipsing of the hydrogen atoms is energetically realisable in order to account for an amount of equatorial product in excess of the axial product (9 : 1). It is rational to assume that buckling and distortion of the preferred conformation will extend from atom to atom over the whole molecule. If the ring system is extended (as in Shoppee's examples) it is only to be expected that the buckling and conformational eclipsing that must occur in the formation of the linear (or near-linear) S_N2 transition state at any part of the molecule will involve the spatial adjustments of many more atoms, and the energy required for that particular reaction would be correspondingly increased. Alternative reaction paths such as substitution by the S_Ni mechanism (cf. *B*) and elimination could then predominate.

This postulate that the diazohydroxide leads directly to the products gives a consistent explanation for Shoppee's observation that elimination is Saytzeff-controlled—the olefin

[21] Bartlett and Knox, *J. Amer. Chem. Soc.*, 1939, **61**, 3184.
[22] Doering, Levitz, Sayigh, Sprecher, and Whelan, *J. Amer. Chem. Soc.*, 1953, **75**, 1008.
[23] Bartlett in Gilman's "Organic Chemistry," Wiley and Sons, New York, 1953, Vol. III, p. 45.

is formed by fragmentation of the non-polar diazohydroxide and is not derived from the polar diazonium cation (cf. *C*).

Experimental

Deamination of L-(+)-*Glutamic Acid.*—Glutamic acid (0·7 g.), $[\alpha]_D^{19}$ +29·6° in 5% HCl, was added to saturated aqueous sodium nitrite (2 c.c.); then glacial acetic acid (0·5 c.c.) was added After 10 min. the solution was evaporated to dryness under a vacuum. The residue was hydrolysed for 1 hr. with 2N-sodium hydroxide (2 c.c.), and the optical rotation of the alkaline solution of sodium α-hydroxyglutarate was measured ($[\alpha]_D^{21}$ −9·2° ± 0·4°).

Deamination of L-(+)-*Glutamine.*—In the same conditions glutamine (0·3 g.), $[\alpha]_D^{21}$ +6·75° (in water), was converted into the hydroxy-glutaric acid and the alkaline solution gave $[\alpha]_D^{21}$ −7·5° ± 0·4°.

Deamination of (+)-γ-*Aminovaleric Acid.*—(+)-γ-Aminovaleric acid (1 g.), $[\alpha]_D^{22}$ +12°, was dissolved in 2N-acetic acid (5 c.c.), and 0·4N-aqueous sodium nitrite (25 c.c.) was added during 1 hr. This slow addition minimised the formation of nitro-compounds. The solution was extracted with ether (6 × 30 c.c.) and after removal of the ether (in a vacuum) the lactone residue was dissolved in water (10 c.c.) and shaken with charcoal. After filtration the optical rotation was measured in a 2-dm. tube and the lactone concentration was then determined colorimetrically: [6] $[\alpha]_D^{22}$ −13·8° (c = 0·85).

A sample of the lactone solution was hydrolysed with an excess of 0·2N-barium hydroxide and the optical rotation measured: $[\alpha]_D^{22}$ +6°. On acidification the solution became lævorotatory as the hydroxy-acid lactonised.

The partition coefficient for the distribution of γ-valerolactone between ether and water was found to be K (ether/water) = 0·525 at 20°. Experiments showed that less than 2% of the free hydroxy-acid was extracted by ether in 3—4 hr. from a neutral aqueous solution of γ-hydroxyvaleric acid.

Optical Resolution of γ-*Aminovaleric Acid.*—γ-Aminovaleric acid, prepared from lævulic acid, was resolved through the benzoyl derivative with quinine (cf. Fischer and Groh [24]): it had $[\alpha]_D^{22}$ +12° (lit.,[4] $[\alpha]_D^{22}$ +13·9°).

Preparation of (+)-β-*Aminobutyric Acid.*—Crotonic acid was treated with concentrated aqueous ammonia at 140°. The amino-acid was esterified, purified by distillation, and resolved with β-camphorsulphonic acid.[25] (The optically pure isomers were not prepared. It was sufficient for our purpose to have one isomer in sufficient excess.) The acid obtained on hydrolysis, $[\alpha]_D^{19}$ +5° in water (lit.,[25] $[\alpha]_D^{25}$ +35·3°), gave β-benzamidobutyric acid, m. p. 152°, $[\alpha]_D^{19}$ +6° in 2N-sodium hydroxide.

Conversion of Ethyl (+)-β-*Amino*-β-*methylbutyrate into Benzoyl*-α-*alanine* (*Barbier–Wieland*).—Ethyl β-amino-β-methylbutyrate was converted by conventional methods into 3-amino-1,1-diphenylbutan-1-ol, m. p. 154—155° (from light petroleum) (Found: C, 79·8; H, 7·9; N, 5·65. Calc. for $C_{16}H_{19}NO$: C, 79·5; H, 7·9; N, 5·8%), whose benzoyl derivative (prepared by benzoyl chloride–pyridine), m. p. 164° (Found: C, 79·65; H, 6·45; N, 4·35. Calc. for $C_{23}H_{23}NO_2$: C, 80·0; H, 6·66; N, 4·1%) (2 g.) was heated for 30 hr. with chloroform and iodine; the glassy residue obtained after removal of iodine was oxidised with potassium permanganate in acetone, giving benzoyl-α-alanine, m. p. 159°, $[\alpha]_D^{18}$ +6·8° in 2N-sodium hydroxide (Found: C, 61·8; H, 5·65; N, 7·45. Calc. for $C_{10}H_{11}NO_3$: C, 62·2; H, 5·7; N, 7·25%).

Conversion of (−)-γ-*Aminovaleric Acid into* β-*Benzamidobutyric Acid* (*Barbier–Wieland*).—The combined filtrates from the optical resolution of γ-aminovaleric acid (see above) afforded (−)-γ-benzamidovaleric acid, $[\alpha]_D^{20}$ +16° (lit.,[24] +21·9°). This was hydrolysed with 20%

[24] Fischer and Groh, *Annalen*, 1911, **383**, 370.
[25] Fischer and Scheibler, *Annalen*, 1911, **383**, 344.

hydrochloric acid and esterified with methanolic hydrogen chloride. The ester was then converted, via the tertiary alcohol and the derived olefin, into β-benzamidobutyric acid, m. p. 152°, $[\alpha]_D^{19}$ −6·65° in 2N-sodium hydroxide (Found: C, 63·6; H, 6·35; N, 6·7. Calc. for $C_{11}H_{13}NO_3$: C, 63·6; H, 6·3; N, 6·8%).

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