343. *The Isotopic Exchange Reaction between Deuterium Oxide and* cis- *and* trans-*Glutaconic Acids.*

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The exchange reactions between *cis-* and *trans*-glutaconic acids and deuterium oxide in the presence of an excess of alkali have been studied. The results clearly show that three hydrogen atoms are concerned, a result in accordance with modern views on tautomerism. It has been found possible to obtain an estimate of the mobility of this very simple " crypto-tautomeric " system, which is not susceptible to study by ordinary methods. A special mechanism, involving hydrogen-bond formation, is advanced to explain the observed greater velocity of isotopic exchange in the case of the *cis*-acid.

IN the field of organic chemistry deuterium has perhaps found its most successful application in the study of tautomerism. Experiments on isotopic exchange in the vinylacetic–crotonic acid system led to the first definite proof that three-carbon tautomerism could not be purely intramolecular in mechanism (Ives and Rydon, J., 1935, 1735). A further insight into the mechanism of this type of tautomerism was afforded by the exchange experiments of Ingold, de Salas, and Wilson (J., 1936, 1328), who showed that the tautomerism in the *cyclo*hexenylacetonitrile–*cyclo*hexylideneacetonitrile system was definitely bimolecular in mechanism. By the work of Ives (J., 1938, 91) this mechanism was later shown to hold also for the vinylacetic–crotonic acid system.

Isotopic exchange has thus proved to be a potent weapon for elucidating the intimate mechanism of tautomeric change. There exists, however, one type of tautomerism which cannot even be detected by the methods of organic chemistry. This type of tautomerism, for which the name " crypto-tautomerism " is suggested, arises when, owing to symmetry, the tautomeric individuals are identical. For instance, ordinary methods are unable to detect tautomerism in the symmetrical three-carbon system $X{\cdot}CH_2{\cdot}CH{:}CHX \rightleftharpoons X{\cdot}CH{:}CH{\cdot}CH_2X$, of which the simplest case is that of glutaconic acid ($X = CO_2H$).

It is known that isotopic exchange in methylene and methin groups is generally very slow in the absence of an ionising mechanism such as is provided by tautomerism (cf. Ives and Rydon, *loc. cit.*; Ives, J., 1938, 81) and it seemed to us that isotopic exchange provided a suitable method for detecting and studying such cases of crypto-tautomerism. Glutaconic acid was chosen for this purpose, and the opportunity was taken to confirm the existence of its *cis*-form, isolated by Malachowski (*Ber.*, 1929, **62**, 1323).

The exchange reactions of the glutaconic acids were first studied in 10% deuterium oxide solution in order that the kinetics could be suitably followed and the extent of the exchange found. At the same time it was considered desirable to determine exactly the number of hydrogen atoms taking part in the exchange so that no doubt could exist on this point and on the question of the partition ratio, a course which has not always been followed by other workers. Accordingly, the experiments were repeated in 92% deuterium oxide because, as can be ascertained by calculation, the effect of the partition ratio is reduced to a minimum in concentrated deuterium oxide solutions. In this manner it was possible to determine with certainty the number of exchanging hydrogen atoms.

The exchange reaction with glutaric acid was also studied in order to establish that methylene groups exchanged only slowly even when present in a molecule containing two activating groups such as carboxyl.

EXPERIMENTAL.

Preparation of Materials.—*trans*-Glutaconic acid was prepared by the method of Conrad and Guthzeit (*Annalen*, 1883, **222**, 249) and recrystallised several times from ethyl acetate containing a little light petroleum (b. p. 60—80°), m. p. 136° [Found : equiv. (by titration), 65·7. Calc. : equiv., 65]. Gidvani's method (J., 1932, 2666) did not give satisfactory results in our hands.

cis-Glutaconic acid was prepared by hydration of the hydroxy-anhydride by the following modification of Malachowski's method (*loc. cit.*) : 0·5 g. of the hydroxy-anhydride was added, with shaking, during 2 hours to 30 c.c. of water kept at 10—12°; the resultant solution was kept at 10° until it no longer gave a green ferric chloride reaction (about an hour). The solution was then decolorised with charcoal (in the cold), and the water removed by rapid distillation in a high vacuum into a receiver cooled to − 78° in alcohol and solid carbon dioxide. Eight such experiments gave 3·8 g. of crude *cis*-acid; several crystallisations from ether gave the pure acid, m. p. 135°, depressed on admixture with the *trans*-acid to 117°.

Glutaric acid was prepared by Knoevenagel's method (*Ber.*, 1894, **27**, 2346) and crystallised from benzene to m. p. 98°.

Standard Solution.—A quantity of 10% deuterium oxide solution was prepared by dilution of a 99·6% solution, and its deuterium content determined. An accurately 1·050N-sodium hydroxide solution was then prepared from "AnalaR" sodium hydroxide and this 10% deuterium oxide, and standardised against sulphuric acid by a microvolumetric method.

Exchange Experiments.—The general procedure in the exchange reactions was to treat the acid with 5 mols. % excess of sodium hydroxide in N-solution at 20° (*i.e.*, in approximately 0·050N-alkaline solution). For this purpose 10 c.c. of the above standard solution were run into a tube containing 0·01 equiv. of the acid (*i.e.*, 0·6505 g. in the case of the glutaconic acids). The tube was then sealed, and placed in a thermostatic bath at 20·04° (± 0·01°). At the end of the given time the tube was opened, and solid silver nitrate added in quantity slightly greater than that calculated to react with the glutaconate and the excess alkali present. This was found to be a much more satisfactory method of removing hydroxyl ions from solution than the commonly used method of neutralisation with acid. Moreover, the glutaconate ions were also instantaneously removed from solution, so that the mixture of silver glutaconate and oxide could be quickly collected on a sintered-glass crucible. In a preliminary investigation 99·6% of the glutaconic acid was thus precipitated as silver glutaconate.

The precipitate, after several washings with 10% deuterium oxide, was dried at 130° for 5 hours, transferred to a porcelain boat, and kept overnight in a vacuum over phosphoric oxide in a vacuum desiccator. The dry mixture of silver glutaconate and silver oxide so obtained was in a very suitable form for combustion, whereas, when the sodium salts of acids are used for combustion, there is always the danger of errors being introduced owing to the hygroscopic nature of the salts.

In the experiments with glutaric acid, 0·6605 g. of the acid was treated with 10 c.c. of the standard solution at 20° (*i.e.*, under precisely the same conditions as with glutaconic acid). In this case, however, neutralisation at the end of the required time was effected by adding the calculated quantity of oxalic acid. The solvent was then removed by vacuum distillation, the residue dried at 100° in a vacuum for 6 hours, and then over phosphoric oxide in a vacuum desiccator overnight prior to combustion. This method, with modifications, was also used in the experiments with glutaconic acid and 92% deuterium oxide.

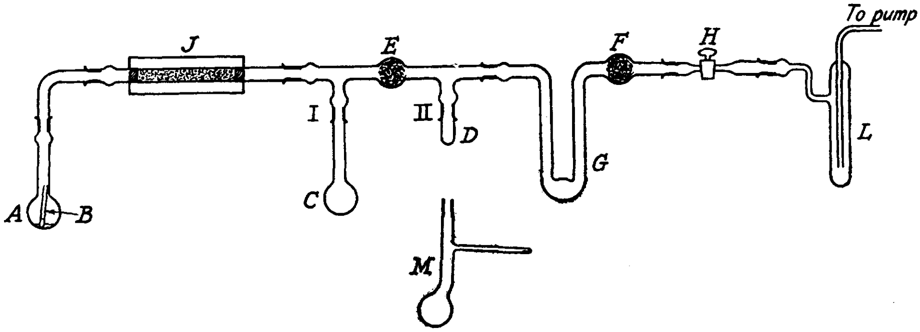
A clean piece of sodium, which had been weighed, was allowed to dissolve slowly in 10 g. of the 92% D_2O contained in a tube; this was then weighed, and a suitable weighed quantity of glutaconic acid added so that sodium hydroxide remained in excess. The solution was then heated at 100° and, at the end of the given time, the calculated quantity of oxalic acid required to neutralise the excess of hydroxide was added. The solvent was removed, and the residue heated at 200° in a vacuum for 4 hours in order to remove the last traces of water and, also, traces of excess oxalic acid if present. The residue was then divided into two portions, which were burnt and analysed separately.

Combustion of the Salts.—The combustion of the salts was carried out in a large combustion tube (13 mm. internal diameter), packed in the usual manner with coarse copper oxide which was maintained at a dull red heat, and through which a slow stream of pure dry oxygen passed. The combustion water was collected in a U-tube cooled to − 78°, followed by another similarly cooled U-tube to ensure complete recovery of the water. At the end of the combustion the apparatus was evacuated, and the combustion water distilled into a small capped tube for weighing.

Isotopic Analysis.—A modified micro-flotation method was used in the isotopic analysis. As the weight of combustion water available was only of the order of 0·1—0·2 g. a simple and accurate method was devised for diluting these samples and then estimating the density of the diluted solutions.

The dilution and purification apparatus, constructed in Pyrex glass, is shown in Fig. 1. Section *J* was of Jena glass packed with copper oxide which was maintained at a dull red heat by means of an electric furnace, and bulb *C* contained a small quantity of silver oxide which had previously been dried at 100° in a vacuum. The glass-wool plugs *E* and *F* served to prevent any possibility of dust (*e.g.*, copper oxide) being transported through the apparatus during distillations, and the flotation vessel *M* was of the type described by Anderson, Purcell, Pearson, King, James, Emeléus, and Briscoe (J., 1937, 1492), except that the float was considerably smaller, *viz.*, 13 mm. × 1 mm. The cleaning of the whole apparatus, except the furnace *J*, was also carried out as described by the above authors. Trap *L*, cooled to $-78°$, served to isolate the pump from the apparatus and to admit dry air into the latter. The procedure was as follows: The tube *B*, taken from the combustion apparatus and containing a weighed quantity of combustion water, was introduced into the tube *A* containing a suitable weighed quantity of "light" water. The apparatus was then evacuated by means of a "Hyvac" pump, the trap *G* having first been cooled to $-78°$ to collect the small head fraction distilling over during the evacuation. Tap *H* was closed, and the cooling bath removed from *G* to *C*, as a result of which the combustion water and dilution water were distilled together through the copper oxide furnace into the bulb *C* containing the silver oxide. The distillation proceeded smoothly without bumping, and when it was complete (4—5 hours) the bulb *C* was withdrawn and capped, the ice allowed to melt, and the solution thoroughly shaken with the silver oxide.

FIG. 1.



The plug *D* was then inserted in place of *C* in position I, the flotation vessel *M* in position II, and the apparatus once more evacuated to dry the flotation vessel. Dry air was admitted, bulb *C* again inserted in position I, and the diluted combustion water solution distilled into the flotation vessel *M* by evacuation and cooling. Dry air was again admitted, the flotation vessel removed and capped with tin foil, and the solution quickly poured into the side arm containing the dry float. The above precaution of inserting the flotation vessel in the apparatus only when the first distillation had been completed was observed because the float, when dry, is very sensitive to mechanical vibrations, more especially so as it is so small, and if it remained dry for 4—5 hours during a distillation the flotation temperature in standard water might be changed by as much as 0·01—0·02°.

The flotation measurements were carried out in the usual manner in a thermostatic bath, the temperature being read on a thermometer graduated to 0·05° which had previously been calibrated against a standard. The flotation temperatures so obtained had an error of less than ± 0·01°. The float itself was standardised at regular intervals in purified "light" water.

The densities of water were obtained by reference to the International Critical Tables, and the mol.-fractions of deuterium oxide were calculated by using Luten's formula (*Physical Rev.*, 1934, **45**, 161), *viz.*, $\Delta d^{25^\circ}_{4^\circ} = 0{\cdot}1078x - 0{\cdot}0012x(1 - x)$, the error due to the presence of ^{18}O in the heavy water being negligible. Allowance was made in the calculations for the change in volume of the float with temperature.

The precision of this technique may be illustrated by results obtained in the initial standardisation of the 10% deuterium oxide, the solution being first weighed into the tube *B* and then diluted and purified as described above for the combustion water.

	Expt. I.	Expt. II.
Wt. of heavy water taken (g.)	0·2017	0·2439
Wt. of dilution water (g.)	0·5711	0·7947
Flotn. temp. in diluted heavy water	27·94°	27·02°
Flotn. temp. in standard water	16·44°	16·44°
Excess density (parts per million)	2600	2344
Mol.-fraction of D_2O in diluted heavy water	0·02439	0·02199
Mol.-fraction of D_2O in heavy water sample	0·09420	0·09438
D/H ratio for heavy water sample	0·1040	0·1042
Mean value for D/H ratio	0·1041	

Further, the technique is very adaptable, since it can be used for deuterium oxide solutions of any concentration whatever. In the case of the 90% D_2O solutions, quantities of only 0·01—0·05 g. were used in the analysis with quite accurate results.

Determination of the D/H *Ratio in the Equilibrium Solvent after Exchange.*—In the calculation of the partition ratio for the exchange, it was necessary to know the D/H ratio in the equilibrium solvent. The deuterium content of the initial 10% D_2O solution had been accurately determined before the addition of the sodium hydroxide in the preparation of the standard solution, so that it was possible to calculate an accurate value for the D/H ratio in the equilibrium solvent taking into account the following factors: (i) the weight of "light" hydrogen introduced into 10 c.c. of the solution in the hydroxyl group of the sodium hydroxide; (ii) the weight of "light" hydrogen introduced into 10 c.c. of the solution in the carboxyl groups of the glutaconic acid; (iii) the weight of deuterium lost from the solvent to the solute during exchange; (iv) the weight of "light" hydrogen gained by the solvent from the solute through exchange.

TABLE I.

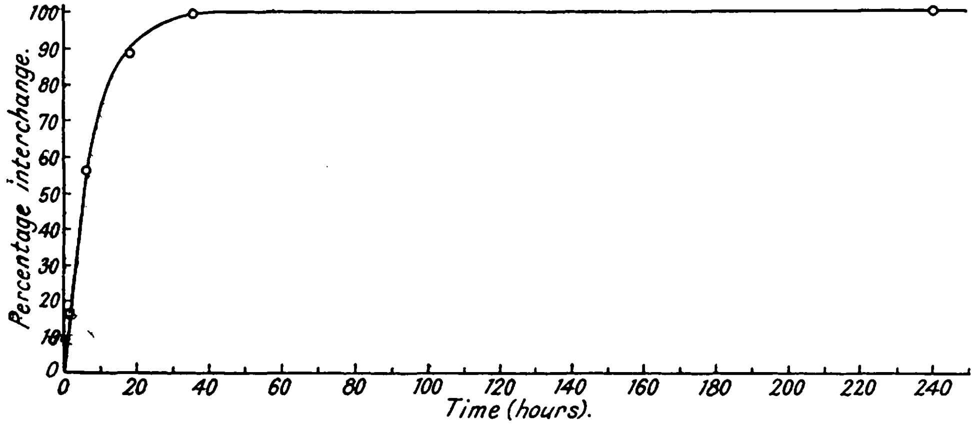
Exchange reactions between the trans-*glutaconate ion and* 10% *deuterium oxide.*

Conditions : 5 Mols. % excess of sodium hydroxide in N-solution at 20°;
D/H ratio of equilibrium solvent = 0·1007.

Time (hours)	1	1	6	18	36	240
Wt. of combustion water (g.)	0·1650	0·1612	0·1513	0·1670	0·1586	0·1624
Wt. of dilution water (g.)	0·4905	0·4787	0·4586	0·4988	0·4748	0·4890
Flotn. temp. in diluted sample	17·91°	17·91°	20·96°	23·27°	24·00°	24·02°
Flotn. temp. in standard water	16·44°	16·44°	16·44°	16·46°	16·46°	16·46°
Excess density (p.p.m.)	256	256	861	1380	1556	1561
Mol.-fraction of D_2O in diluted sample	0·002401	0·002401	0·008077	0·01294	0·01459	0·01464
Mol.-fraction of D_2O in combustion water	0·00954	0·00955	0·03265	0·05183	0·05860	0·05902
Exchange number	0·42	0·42	1·43	2·267	2·563	2·581
Percentage of total exchange (calc. on the 10-day value)	16·1	16·1	55·33	87·83	99·28	100

Results.—The exchange numbers in Table I are the apparent numbers of hydrogen atoms per molecule exchanged on the assumption that no isotopic discrimination occurs. The graph (Fig. 2) obtained from these values by plotting percentage interchange against time was found to represent a pseudo-unimolecular reaction with a velocity constant of 22·5 × 10^{-4} min.$^{-1}$.

FIG. 2.



In the corresponding data for the *cis*-glutaconate ion (Table II), the excess of sodium hydroxide is given as only "approx. 5 mols. %" because the standard solution had been kept for several months in a glass container when these experiments were carried out, with the result that the alkalinity had diminished owing to attack on the glass. Since the results for *trans*-glutaconic acid

TABLE II.

Exchange reactions between the cis-*glutaconate ion and* 10% *deuterium oxide.*

Conditions : Approx. 5 mols. % excess of sodium hydroxide in N-solution at 20°;
D/H ratio of the equilibrium solvent = 0·1007.

	Expt. I.	Expt. II.
Time (hours)	1	120
Wt. of combustion water (g.)	0·1595	0·0859
Wt. of dilution water (g.)	0·4745	0·6018
Flotn. temp. in diluted sample	18·92°	20·60°
Flotn. temp. in standard water	16·49°	16·49°
Excess density of diluted water (p.p.m.)	439	776
Mol.-fraction of D_2O in diluted water	0·00413	0·00728
Mol.-fraction of D_2O in combustion water	0·0164	0·0588
Percentage of equilibrium exchange value	28	100

(Table I) had been obtained when the standard solution was fresh, it was decided that, in order to compare the rates of exchange of the *cis*- and the *trans*-glutaconate ion, the velocity constant for the exchange in the *trans*-glutaconate ion must be determined again in the aged solution. Accordingly, experiments were made immediately after the completion of the experiments with the *cis*-acid, and it was found that the velocity constant for the exchange with the *trans*-glutaconate ion had fallen to $12{\cdot}4 \times 10^{-4}$ min.$^{-1}$, *i.e.*, by almost 50% of the value obtained when the solution was fresh.

TABLE III.

Exchange reactions between the trans-*glutaconate ion and* 92% *deuterium oxide.*

Conditions : 0·1N-Alkaline solution at 100°; time, 4 hrs.; mol.-fraction of D_2O in the equilibrium solvent = 0·917.

	Expt. I.	Expt. II.
Wt. of combustion water (g.)	0·0671	0·0748
Wt. of dilution water (g.)	1·4145	1·4041
Flotn. temp. in diluted sample	29·40°	30·60°
Flotn. temp. in standard water	16·50°	16·50°
Excess density of diluted water (p.p.m.)	3012	3373
Mol.-fraction of D_2O in diluted water	0·02824	0·03165
Mol.-fraction of D_2O in combustion water	0·668	0·670
Mean value for the mol.-fraction of D_2O in the combustion water	0·669	

The results given in Table III enabled the number of exchanging hydrogen atoms to be determined with certainty. We can calculate the mol.-fraction of deuterium oxide to be expected in the combustion water from 1, 2, 3, or 4 atoms interchange, and these values are shown below, together with the observed value.

Atoms involved in interchange	1	2	3	4
Mol.-fraction of D_2O expected	0·24	0·47	0·677	0·88
,, ,, observed	—	—	0·669	—

There can clearly be no doubt that 3 hydrogen atoms only are exchanging, the results actually corresponding with an exchange of 2·96 hydrogen atoms per molecule.

The data obtained in the experiments on the glutarate ion are given in Table IV.

TABLE IV.

Exchange reaction between the glutarate ion and 10% *deuterium oxide.*

Conditions : Approx. 5 mols. % excess of sodium hydroxide in N-solution at 20°;
D/H ratio of the solvent = 0·101; time, 2½ days.

Wt. of combustion water (g.)	0·2283
Wt. of dilution water (g.)	0·6824
Flotn. temp. in diluted water	16·61°
Flotn. temp. in standard water	16·52°
ΔT	0·09°
Excess density of diluted water (p.p.m.)	15
Mol.-fraction of D_2O in the diluted sample	0·00014
Mol.-fraction of D_2O in the combustion water	0·00056

DISCUSSION.

In considering the experimental results, we note first that the very slight exchange observed with glutaric acid (Table IV) leaves no doubt that the very marked exchange observed under the same conditions with glutaconic acid must be due to some mechanism connected with the ionisation of the migrating hydrogen atom.

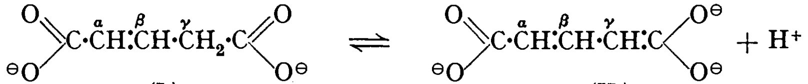
It is of interest to evaluate the partition ratio (the ratio of deuterium to hydrogen in the exchangeable hydrogens divided by the same ratio in the solvent) for the exchange reaction between 10% deuterium oxide and the glutaconic acids. The sodium salt submitted to combustion contains four hydrogen atoms and hence, since we have shown that three hydrogen atoms interchange, if the atomic fraction of deuterium in the three exchanging positions is x, the mol.-fraction of deuterium oxide in the combustion water will be

$$3x/[3(1 - x) + 3x + 1] = 3x/4$$

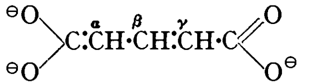
In the case of the experiments with 10% deuterium oxide and *trans*-glutaconic acid the mol.-fraction of D_2O in the combustion water was 0·05902. Hence $x = 0{\cdot}07869$. Therefore the D/H ratio in the interchangeable positions $= x/(1 - x) = 0{\cdot}08541$. Now, the D/H ratio in the equilibrium solvent was 0·1007. Hence the partition ratio $= 0{\cdot}08541/0{\cdot}1007 = 0{\cdot}848$. A similar calculation for the experiment with *cis*-glutaconic acid and 10% D_2O gives the partition ratio as 0·845. This is again a partition ratio for the *trans*-acid into which form the *cis*-acid in solution has isomerised. These values may be compared with the value, 0·89, found by Ives (*loc. cit.*) for vinylacetic acid, and the value, 0·67, found by Ingold, de Salas, and Wilson (*loc. cit.*) for the unsaturated nitriles. The closeness of our value to that of Ives and the large difference between this and the value of Ingold, de Salas, and Wilson seems to indicate some fundamental difference in the types of tautomerism. The difference may be due to the fact that our case and that of Ives are examples of extended keto–enol tautomerism, whereas the case studied by the other authors is an example of extended cyano–imino-tautomerism. It is, perhaps, hardly surprising that so fundamental a difference should reveal itself in this way.

It has been shown above that the isotopic exchange reaction with *trans*-glutaconic acid in the presence of 5 mols. % excess of sodium hydroxide in N-solution is pseudo-unimolecular with a velocity constant of $22{\cdot}5 \times 10^{-4}$ min.$^{-1}$. The time of half-interchange is 5 hours. Comparison of this value with the results of other workers shows that the glutaconate ion is an acid, in the Brönsted sense, of the same order of strength as acetone, and with a mobility greater than that of most tautomeric systems which have been investigated under like conditions. Cases of similar high mobility, apart from those of exchange with hydrogen in carboxyl and similar groups, are afforded by acetylacetone (Klar, *Z. physikal. Chem.*, 1934, *B*, **26**, 335) and a number of heterocyclic acids (Erlenmeyer and Weber, *Helv. Chim. Acta*, 1938, **21**, 863; Erlenmeyer, Weber, and Wiessmerr, *ibid.*, p. 1017) in alkaline solution and by pyrrole in acid solution (Harada and Titani, *Bull. Chem. Soc. Japan*, 1935, **10**, 554; 1936, **11**, 465). This high mobility leaves no doubt that the tautomerism exhibited by glutaconic acid is of the "bimolecular" class, *i.e.*, the proton and the anion have free, independent existence (cf. Wilson, *Trans. Faraday Soc.*, 1938, **34**, 183).

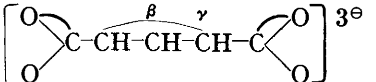
This tautomeric ionisation of the glutaconate ion occurs in the following manner:



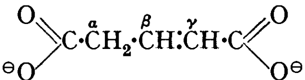
(I.) (II.)



(III.)



(IV.)

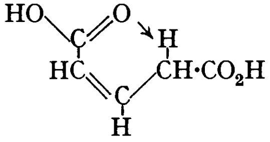


(V.)

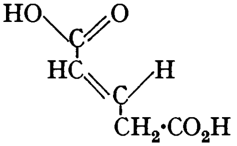
The ion (II) so produced is obviously only one of two possible canonical structures, of which the other is (III), the two together making up the mesomeric ion (IV). Now the symmetry of the system requires that (II) and (III) contribute equally to the structure of the mesomeric ion. It follows from this that recombination of the proton with the kinetically free mesomeric anion (IV) must take place with equal readiness at C_α and C_γ, *i.e.*, half of the re-associations will occur at C_α and half at C_γ. Now, if we start with the undissociated ion (I), reassociation at C_α gives rise to "isomerisation," leading to (V), whereas reassociation at C_γ causes the re-formation of (I) without " isomerisation "; in other words, one-half of the reassociations brings about isomerisation. It may be deduced from this that the rate of " isomerisation " will be one-half of the rate of ionisation (of the ion), since this will be the rate-determining step.

Now the exchange rate in 10% deuterium oxide is $22{\cdot}5 \times 10^{-4}$ min.$^{-1}$, which is an approximate measure of the rate of ionisation; hence an approximate measure of the rate of " isomerisation " is one-half of this, *i.e.*, 11×10^{-4} min.$^{-1}$. We wish to emphasise that this rate is, of course, only an approximation, and, in fact, the correction introduced above will be more than compensated by the difference in rates of transfer of protons and deuterons. Only experiments with pure deuterium oxide could give an accurate value. For this reason, and also because the experimental conditions are different, comparison with the observed rate of isomerisation of other three-carbon systems is not profitable; we may, however, say that this system is very much more mobile than any of the others. This case is interesting as being the first in which it has been possible to obtain an experimental assessment of the mobility of a crypto-tautomeric system.

The results with *cis*-glutaconic acid showed that, at equilibrium, the interchange was identical with that found for the *trans*-acid; the same three hydrogens thus take part in the exchange. In one hour at 20° the exchange with the *cis*-acid was four times that found for the *trans*-acid under the same conditions; this confirms the real difference between the two isomers. This greater mobility with the *cis*-acid is no doubt due to the instability and higher energy content of the *cis*-ion; steric inversion to the *trans*-ion will bring about exchange of one hydrogen. The results show that this inversion must be a faster process than the isomerisation process; this seems to be unlikely if the mechanism of steric inversion involves the mesomeric ion. It is suggested that ionisation of hydrogen of the methylene group is responsible, and that this occurs through the agency of hydrogen-bond formation which is sterically possible only in the case of the *cis*-acid :



cis



trans

To sum up, our experiments are in complete accord with the modern view of the chemistry of the glutaconic acids. Isomerisation takes place through an intermediate common mesomeric ion (IV) which is the modern successor of the old " normal " form (cf. Thorpe, J., 1931, 1014). The resonance energy set free in the formation of this ion is no doubt responsible for the reversion to type which was for long so puzzling a feature of the chemistry of the glutaconic acids (cf. Ingold, Oliver, and Thorpe, J., 1924, **125**, 2128).

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