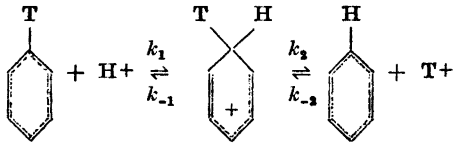
Kinetic Isotope Effect in Isotopic Exchange. Electrophilic Exchange of Hydrogen in Benzene and Toluene

LARS MELANDER and STIG OLSSON

Nobel Institute of Chemistry, Stockholm 50, Sweden

The existence of two heavy isotopes of hydrogen, deuterium and tritium, offers the possibility of determining the kinetic isotope effect in hydrogen exchange. As the reaction is symmetric except for the mass of the hydrogen, it is believed to be useful for examining the mechanism of electrophilic aromatic substitution [1]. If the reaction is represented by



and the intermediate is assumed to be present in a very small amount, the rate factor

$$\frac{k_2}{k_{-1} + k_2} = \frac{1}{k_{-1}/k_2 + 1}$$

will determine the isotope effect in the forward reaction. This holds irrespective of whether a hydrogen acceptor is involved in reactions -1 and 2 and also irrespective of whether the reaction proceeds *via* a low-stability intermediate of the π-complex type as assumed by Gold and Satchell [2]. If the σ-complex intermediate shown above behaves like an ordinary molecule, the ratio k_{-1}/k_2 could be supposed to be of the order of 7—10 for the tritium-protium and 4—6 for the deuterium-protium exchange. Consequently the following observable isotope effect could be expected

$$\frac{k_T}{k_D} = \frac{(k_{-1}/k_2)_D + 1}{(k_{-1}/k_2)_T + 1} \approx 0.6$$

This is indeed found.

As in previous experiments [3] 3 ml of the labelled hydrocarbon was shaken with 20 ml of aqueous sulphuric acid (this time

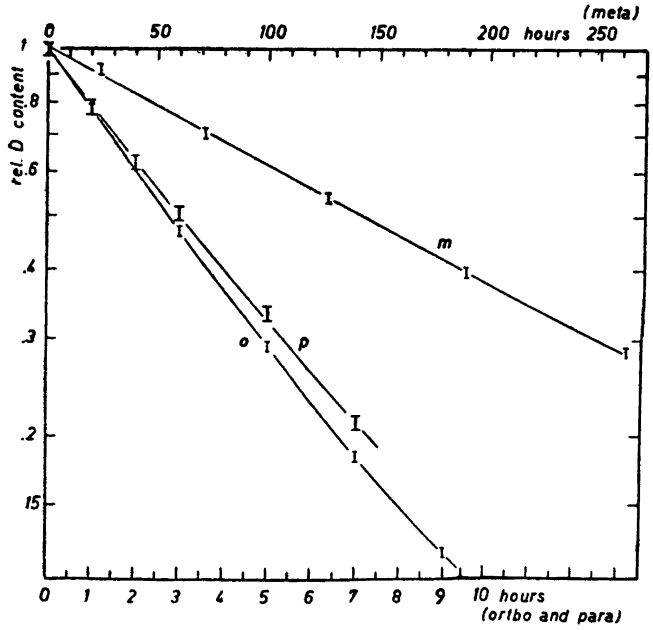


Fig. 1. Semilogarithmic plot of relative deuterium content of toluene-2-t (o), -3-t (m), and -4-t (p) as a function of time (note different time scales). For conditions, see the text.

80.8 %) at 25° C for different time intervals. In special experiments it was checked that the shaking frequency was without measurable influence on the observed rate, that the reaction took place in the acid phase, and that sulphonation was negligible. The deuterium derivatives were present in a few percent and the tritium derivatives in tracer concentrations, and the exchange of both isotopes was studied in the same experiments in each other's presence. In the analytical procedures (infrared spectrography and Geiger-Müller counting, respectively) the isotopes did not interfere. Fig. 1 shows the exchange curve for deuterium in the three nuclear positions of toluene. The limits of error were slightly less in the case of tritium. The exchange rates were determined from the initial slopes of the semilogarithmic curves [4].

The relative exchange rates for tritium in the individual positions of toluene are *o:m:p* = (47 ± 2):(1.00 ± 0.04):(41 ± 2) and for deuterium (51 ± 2):(1.00 ± 0.04):(46 ± 3). The former result differs from that obtained earlier [3] owing to insufficient shaking speed in the preliminary experiments. The results with deuterium are in agreement with similar and somewhat more approximate results obtained by Gold and Satchell in a homogeneous system (private communication).

Experiments on benzene have also been carried out in this laboratory, but the partial rate factors for the toluene positions cannot be obtained from these heterogeneous systems until the relative solubilities of the hydrocarbons have been determined.

It is seen that the directive influence of the methyl group is not exactly the same for the two exchange reactions, *i. e.* the isotope effect is not the same in the three positions. In Fig. 2 the initial slopes are represented on a reduced time scale such that all deuterium curves coincide. The results for benzene have also been included. The slopes of the tritium curves relative to the single deuterium curve give the isotope effects. The results are given in Table 1, and an idea of the accuracy could be obtained from a comparison of Fig. 2 with the typical experimental curve in

Table 1. Isotope effects in hydrogen exchange for different aromatic positions. For conditions, see the text.

Position	k_T/k_D
Toluene, *ortho*	0.53
Toluene, *meta*	0.59
Toluene, *para*	0.52
Benzene	0.65

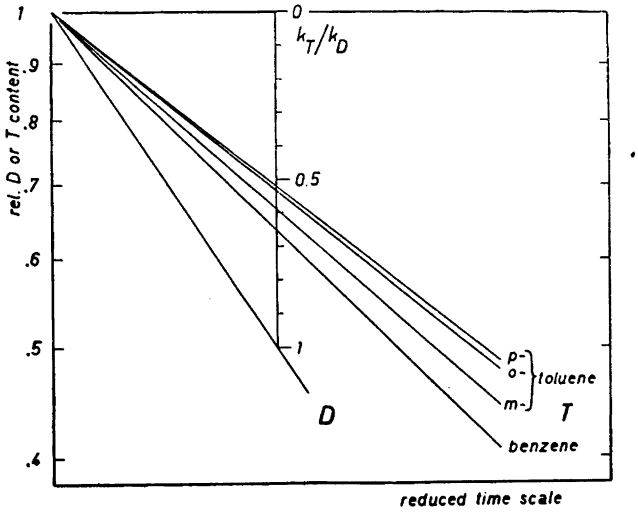


Fig. 2. Isotope effect in hydrogen exchange for different aromatic positions, cf. the text.

Fig. 1. All differences between the isotope effects except that between the *ortho* and *para* positions in toluene are believed to be significant. Differences in solubility between isotopic molecules are believed to be negligible and without importance for the present conclusions.

From the work privately communicated by Gold and Satchell it is evident that the *meta* position in toluene is somewhat more reactive than a single position in benzene, just as with the common electrophilic substitutions. Thus, it appears that the ratio k_T/k_D increases with decreasing reactivity, but whether this is a general rule cannot, of course, be inferred from the present very limited experimental material.

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Open Peptide Chains in Chymotrypsinogen and α-Chymotrypsin

BODIL MEEDOM

Carlsberg Laboratorium, Copenhagen, Denmark

The principle of breaking S—S linkages in proteins by oxidation has been used extensively and with considerable success in the study of the chemical constitution of the peptide chains in these substances [1].

Upon the suggestion of M. Ottesen the author has applied this method to the study of α-chymotrypsin and chymotrypsinogen. Part of this work was reported in a paper read at a meeting in Uppsala in January 1956 [2] and in the following the further development of these investigations is briefly described.

The opening of the disulfide bridges by oxidation [3] causes the α-chymotrypsin molecule to fall apart essentially into three peptides which were separated as follows:

The oxidized enzyme was dissolved in alkali and pH brought down to 6 whereby a precipitate (fraction B) was formed. The supernatant was placed on a small Dowex 50 column (H^+ form). By elution with water, fraction A was collected, and by subsequent elution with NH_4OH, fraction C was obtained.

Fractions A, B, and C could also be separated by dialysis (removal of A) followed by free electrophoresis at pH-values higher than 7.5 (separation of B and C).

Amino acid analysis and end-group determinations were carried out on the fractions using the Sanger method [4] for amino-endgroup determination and digestion by carboxypeptidase [5] for the carboxyl-end-group estimation. The results were as follows:

Fraction A is a peptide composed of 13 amino acids, *viz.* 1 cysteic acid, 1 serine, 1 glutamic acid, 1 alanine, 2 glycine, 2 valine, 1 *iso*leucine, 2 leucine, and 2 proline. The N-terminal amino acid is cysteic acid and the C-terminal leucine.

Fraction C is a larger peptide containing about 50 amino acids. Of the species present in chymotrypsin only histidine and arginine are missing in C. Alanine is the N-terminal amino acid and tyrosine the C-terminal.

Fraction B seems to be a long peptide chain with an N-terminal *iso*leucine and a C-terminal tyrosine.

It is rather surprising that all three separated peptides are attacked by carboxypeptidase inasmuch as native α-chymotrypsin only contains two C-terminal end-groups as estimated by degradation with this enzyme [5].

The higher susceptibility of oxidized α-chymotrypsin to the action of carboxypeptidase may be due to an "unmasking" of a group which, although it conforms to the specific requirements of the enzyme, is inaccessible to it in native α-chymotrypsin under normal conditions.

The same effect would naturally be observed if a peptide bond had been broken during the oxidation procedure, but we feel justified in ruling out this possibility since no new N-terminal group seems to be formed.

The presence of a hidden C-terminal group in α-chymotrypsin suggests that of an identical group in chymotrypsinogen.

Oxidized, dialyzed chymotrypsinogen was therefore incubated with carboxypeptidase and the reaction mixture analyzed for free amino acids in the usual way.