A Halogen Exchange Reaction in a Diazonium Compound

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Some 60 years ago, Hantzsch [1,2] found that under suitable conditions, a halogen exchange reaction takes place in 2,4- and 2,6-dibromobenzenediazonium chloride, leading to mixtures of bromochlorobenzenediazonium bromides. An aromatic halogen atom, activated by electron-attracting groups in the positions *ortho* and *para* to the halogen, is known to be particularly reactive towards nucleophilic reagents [3]. 2,4-Dinitrohalobenzenes are well-known compounds of this kind. One might think that the diazonium group, $-N_2^+$, should have a considerably activating influence upon an *ortho*- or *para*-substituted halogen atom. In virtue of its formal positive charge, the diazonium group might be expected to attract the mobile π-electron cloud of the benzene ring even more than a nitro group would do. This idea has been emphasized by Bunnett and Zahler [3]. In a recent paper [4], Lewis and Johnson also give strong support to this concept.

Sihlbom [5,6] made a kinetic investigation of a rather unusual type of reaction occurring in some dinitroanilines, yielding corresponding dichloronitrobenzenes. The reaction was found to proceed with diazonium ions as intermediates.

Sihlbom's results suggested the present work, in which it has been found that 4-bromo-3-nitrobenzenediazonium chloride in dilute acetic acid containing excess hydrochloric acid exchanges its bromine atom for chlorine, thereby yielding 4-chloro-3-nitrobenzenediazonium chloride. This was shown by replacing the diazonium group with a chlorine atom in a Sandmeyer reaction with cuprous chloride. That the halogen exchange reaction did not occur during the Sandmeyer reaction was checked as described in the experimental part.

The reaction product from the Sandmeyer reaction would be 1,4-dichloro-2-nitrobenzene if the abovementioned halogen exchange had taken place, otherwise 1-bromo-4-chloro-2-nitrobenzene. In order to assay a mixture of these two compounds, IR-spectra (2—15 μ) of the pure components were run both in carbon disulphide and carbon tetrachloride solutions. The spectra, however, were too alike to make any quantitative analysis feasible. Therefore, a chemical method, previously used by Holleman [7], was employed. This involved a nucleophilic reaction with sodium methoxide converting the halogen atoms *ortho* to the nitro group into ionic form, thereby making it possible to precipitate them as silver salts after separation from the resulting 4-chloro-2-nitroanisole.

Experimental. 2.17 g (0.01 mole) of 4-bromo-3-nitroaniline, synthesized according to Blanksma [8], were dissolved in 25 ml of glacial acetic acid. This solution was carefully added to a solution of 0.7 g (0.01 mole) of sodium nitrite in 5 ml of conc. sulphuric acid while cooling in an ice-bath. The temperature of the reaction mixture was kept between + 10° and + 20°. 10 g of ice and 50 ml of conc. hydrochloric acid were added, and the resulting diazonium solution was left at + 25° for one week. It was then filtered from some brown sludge and poured into a cuprous chloride solution, prepared by dissolving 9.9 g of cuprous chloride in 35 ml of conc. hydrochloric acid (making 0.1 mole of cuprous chloride.) Nitrogen was evolved, and a yellowish substance was precipitated. By steam distillation, which was continued until no more organic material was carried over, this substance was purified and obtained as an almost colourless, crystalline mass. This, after washing and drying, was finally purified by sublimation *in vacuo*. Yield 1.15 g of colourless crystals, m.p. 53°, reported for 1,4-dichloro-2-nitrobenzene 53° [9]. Mixed with an authentic sample, m.p. found 53°. Hardly any material was left in the sublimation residue. Assuming the reaction product to be a mixture of 1,4-dichloro-2-nitrobenzene and 1-bromo-4-chloro-2-nitrobenzene, a halogen analysis was performed according to Holleman [7]. By this method, no bromine compound could be detected. The yield of 1,4-dichloro-2-nitrobenzene in the reaction series could then be calculated, being 60 %.

In order to prove that the halogen exchange had not taken place during the Sandmeyer reaction, the exchange experiment was repeated exactly as above, except that immediately after the conc. hydrochloric acid had been added to the diazonium solution, the Sandmeyer reaction was carried out. Steam distillation followed by vacuum sublimation yielded 1.8 g (76 %) of a colourless product melting at 68°, undepressed upon admixture with a sample of 1-bromo-4-chloro-2-nitrobenzene, synthesized according to Holleman [10], m.p. 69—70°. Thus it was concluded that halogen

exchange must have taken place in the diazonium compound at + 25°. The kinetics of this and similar reactions will be investigated.

All melting points were determined on the Kofler bank.

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Butyrylcholine Esterase: Influence of pH on Enzyme Activity and Irreversible Denaturation

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There is much confusion in the literature concerning the shape of the pH-activity curves of choline esterases. It is obvious that there are several factors which together form the shape of these curves. Assuming that the substrate used does not change its charge in the examined pH-area, the following factors have to be considered. Concerning the substrate: The concentration of the substrate is important, as the substrate may compete with hydrogen or hydroxyl ions for an active group in the enzyme. Concerning the medium: In using buffers for maintenance of different pH-values from, *e.g.*, 5—10, both the charge of the ions (*e.g.* phosphate), and thus the ionic strength, and the kind of substance present has to be changed. Concerning the protein: A change in enzyme activity from one pH to another may depend on true reversible changes in one or more of the active centres in the enzyme. On the other hand, the irreversible denaturation of the protein has to be considered. This depends on the temperature used in the experiment, the incubation time, the buffer ions, the ionic strength, the kind of enzyme and the state of purity of the enzyme used. Also, the procedure used during the experiment influences the enzyme activity; in this laboratory (unpublished work) it has thus been observed that the shaking rate in a Warburg apparatus influences the activity. Vigorous shaking causes a lowering of the activity.

During experiments on the purification of choline esterases we had reason to look into the stability of serum fraction IV-6-3 from human retroplacentar serum (BuChE). The studies were performed by means of an automatic recording titrator [1]. The sample containing the enzyme was kept, under stirring, in 0.10 M potassium chloride at 25°C during 5 — 120 min and at pH-values between 6.00 and 10.00. These pH-values were obtained by the addition of minute amounts of sodium hydroxide from the syringe of an automatic titrator. Afterwards, the pH was adjusted to 8.00 and the enzyme activity was determined. The substrate was a 10^{-2} M solution of bu-

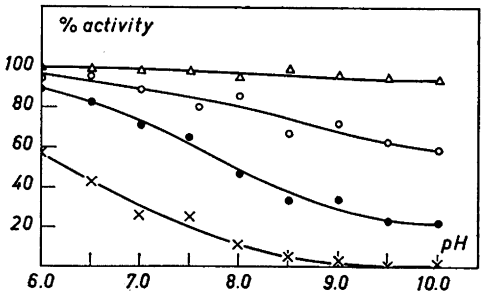

Fig. 1. Butyrylcholine esterase activity measured at pH 8.00 after incubation at different pH-values, expressed in percent of activity obtained at pH 8.00 with neglectible incubation time.
Incubation times: △ = 5 minutes, ○ = 30 minutes, ● = 60 minutes, × = 120 minutes. Temperature 25°C.