activity will also show whether these enzyme potencies belong to the group of enzymes responsible for the transfer of the glutamyl radical of glutathione to other amino-acids[4] and for the hydrolytic splitting of the tripeptide[6].

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[1] Waelsch, H., Borek, E., Grossowicz, N., Abst. Amer. Chem. Soc., 116th meeting, Atlantic City, 54C (1949).
[2] Grossowicz, N., Wainfan, E., Borek, E., and Waelsch, H., *J. Biol. Chem.*, **187**, 111 (1950).
[3] Waelsch, H., Owades, P., Borek, E., Grossowicz, N., and Schou, M., *Arch. Biochem.*, **27**, 237 (1950).
[4] Hanes, C. S., Hird, F. J. R., and Isherwood, F. A., *Nature*, **166**, 288 (1950).
[5] Stumpf, P. K., *Arch. Biochem.*, **25**, 451 (1950).
[6] Olson, C. K., and Binkley, F., *J. Biol. Chem.*, **186**, 731 (1950).

Mechanism of Hydrolysis of Benzoyl Chloride

THE following general conclusions emerge from the extensive work on the alcoholysis of substituted benzoyl chlorides[1]: (*a*) the changes in velocity constant with substituents are similar in all the various solvents used; (*b*) these changes fully support the contention that the reaction is a bimolecular S_N2 process.

A similar order in velocity constants is not observed in the hydrolyses in 50 per cent acetone[2] and, moreover, the order is difficult to interpret in terms of any one mechanism[3]. It has been suggested[3,4] that this is due to a change to a unimolecular S_N1 process, particularly when the chloride is substituted by methyl or methoxy groups. If this is true, this tendency should be largely repressed on changing to a solvent of low dielectric constant; and if the reaction then proceeds mainly by the S_N2 process, the rate-order should be the same as for the alcoholysis.

Substituent *p*-position	95 per cent aqueous acetone ($10^5\ k_{25}$)	40 per cent alcohol 60 per cent ether[5] ($10^5\ k_{35}$)	50 per cent aqueous acetone[2] ($10^4\ k_0$)
CH_3O	2·92 *(2·64)	5·3	—
CH_3	2·69 *(3·46)	6·43	12·5
H	5·10	10·8	4·33
Br	15·5	22·3	4·0
NO_2	169·0	203·7	50·0

* 95 per cent dioxan solution (refers to 5 ml. H_2O made up to 100 ml.). Velocity constants *k* at 0° and 25° expressed in sec.$^{-1}$ units.

The results in 95 per cent acetone show that, with the exception of anisoyl chloride, the predicted order is obtained. On replacing the acetone by dioxan, thus giving a solution with a much smaller dielectric constant, anisoyl chloride hydrolyses more slowly than toluoyl chloride, showing that a complete reversal in velocity order may be achieved. It is of interest to note that the bimolecular reaction with *p*-toluoyl chloride proceeds somewhat faster in the dioxan solution ($D \sim 6{\cdot}5$) than in the acetone solution ($D \sim 24{\cdot}5$), which would be difficult to explain in the case of a unimolecular ionization reaction.

On increasing the water content of the medium, the S_N1 mechanism readily preponderates in the most favourable cases, so that in 50 per cent aqueous acetone only the *p*-nitro compound is reacting entirely by the S_N2 mechanism. On this basis, the methyl-, methoxy- and bromo-compounds and the unsubstituted chloride react almost entirely by the unimolecular process.

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[1] Norris, J. F., *et al.*, *J. Amer. Chem. Soc.*, **57**, 1415 (1935); **61**, 1418 (1939).
[2] Olivier, S. C. J., *et al.*, *Rec. Trav. Chim.*, **46**, 609 (1927); **48**, 227 (1929), etc.
[3] Hughes, E. D., *Trans. Farad. Soc.*, **37**, 613 (1941). Baker, J. W., *Trans. Farad. Soc.*, **37**, 632 (1941).
[4] Hudson, R. F., and Wardill, J. E., *J. Chem. Soc.*, 1729 (1950).
[5] Branch, G. E. K., and Nixon, A. C., *J. Amer. Chem. Soc.*, **58**, 2499 (1936).

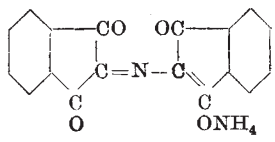
Possible Artefacts introduced by the Ninhydrin and Alloxan Reactions in Histochemical Applications

SINCE the work of Berg[1], the ninhydrin reaction has been commonly used as a histochemical test for the α-amino-acid groupings of proteins. The reaction of proteins with alloxan, giving rise to murexide, has already been criticized by Romieu[2] for lack of specificity and by Giroud[3] for the diffusion of the colour.

Our own experience shows that for two reasons caution is necessary when interpreting the results. The first is that the reaction products both of ninhydrin and alloxan with the α-amino-acid groups of proteins are to some extent soluble in water and thus may be adsorbed on cellular structures which are not the site of the reaction. The second is that other cellular structures, containing proteins but lacking affinity for the coloured reaction products, will fail to stain. In both cases the microscopical picture is misleading. This can be clearly demonstrated on granules of the blood eosinophiles of the horse and the frog. These structures give a very strong ninhydrin and a weaker alloxan test[4].

The chemical constitution of the reaction product of ninhydrin with amino-acids has been identified as the ammonium salt of 2-(1.3 diketoindanylidene-amino)-indandione[5] (I).

The reactions between proteins and alloxan give rise to the formation of murexide, the ammonium salt of purpuric acid, which has a very similar structure (II).



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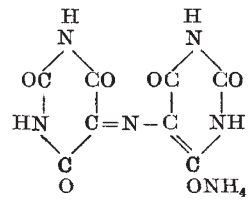

II