

CHLOROACYL ESTERS AS HISTOCHEMICAL SUBSTRATES*

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Zeller (7); Zeller and Utz (8), and McNaughton and Zeller (3) have found that so-called true cholinesterases from snake venom and human erythrocytes hydrolyze chloroacetyl esters at a very high rate. Birnbaum and associates (1), and Rao and associates (6) report that the pattern of hydrolysis of N-chloroacetyl amino acids by hog kidney extract is different from that of the corresponding acetyl or glyceryl derivatives. These findings suggested the idea of trying chloroacetyl esters for histochemical use.

First of all, the chloroacetates of α -naphthol and of naphthol AS were prepared by esterifying the naphthols with chloroacetyl chloride in acetone-pyridine. The reaction mixture was poured into cold water. The precipitate was collected and dissolved in warm alcohol. The solutions were decolorized with charcoal and filtered. Enough water was added to make them opalescent. They were placed in the freezing compartment of the refrigerator. On cooling, copious crystalline precipitates of the esters were obtained. The chloroacetate of α -naphthol was obtained free from naphthol; it melted at 48.5°C. Naphthol AS chloroacetate always contained 7 to 12% of free naphthol; this impurity persisted in a practically undiminished amount in spite of repeated recrystallizations from dilute alcohol or acetone. On account of this gross impurity, the melting point of the crystals was not determined.

For use, about 10 mg. of the crystals was dissolved in about 1 ml. of acetone. The method was essentially the same as with the regular acetates (2), except that in the case of the α ester pH was kept at 7.4 because above this value the substrate was unstable. Even at pH 7.4, the substrate mixture darkened appreciably within 5 minutes and required repeated filtration in the course of incubation. The AS substrate mixture gave a copious red precipitate on addition of the diazonium salt (presence of free naphthol) and had to be filtered repeatedly over the same paper before it gave a clear yellow solution. This substrate mixture was reasonably stable and had to be refiltered only about every 10 to 15 minutes.

Paraffin sections of various tissues fixed in acetone or in neutral formalin were used. The pictures obtained were quite unexpected and entirely different from those seen with the regular acetates. With both chloroacetic substrates the mast cells in the tissues of all species examined (man, dog, rat, mouse, rabbit) became fairly intensely stained within one to two minutes, and by the end of five minutes they were quite dark. No other tissue element showed more than a trace of reaction at this time. In about 10 minutes, leucocytes began to stain intensely.

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The staining involved all stages of maturation from myelocytes to mature polymorphonuclears; however, not all of them. Both in leukemic infiltrates and in blood smears from cases of myeloid leukemia, a certain percentage (15 to 25) of the cells remained negative. Eosinophils stained poorly or not at all; monocytes, lymphocytes and stem cells remained entirely unstained. On prolonged incubation (20 minutes or more), many other elements began to react, and the final picture obtained was essentially the same as seen with the respective normal acetates, except for the additional extremely strong staining of mast cells and myeloid elements. It should be remarked that mast cells show a moderately intense reaction on prolonged incubation with naphthol AS acetate; they show a very faint activity or none at all with α -naphthol acetate. Myeloid elements do not hydrolyze either of the normal acetates.

Since there are data available that the toxicity of halogenated acids depends on the position of the halogen atom (4), two more substrates were prepared, the α - and β -chloropropionates of α -naphthol. These esters were synthesized by the thionyl chloride procedure as described by Nachlas and Seligman (5). They were obtained in the form of colorless or pale yellowish oils which did not crystallize even in the ice box. The histochemical pictures obtained with these substrates were quite interesting in that α -chloropropionate yielded distribution patterns identical with those given by chloroacetate while the results with β -chloropropionate were indistinguishable from those seen with normal acetate or propionate.

In test tube experiments, the chloroacetates were not noticeably hydrolyzed by even high concentrations of commercial heparin.

10^{-5} M eserine had no visible effect on the results; this finding indicates that the enzyme responsible for the hydrolysis of α -chloroacyl esters is not a cholinesterase.

SUMMARY

α -Chloroacyl esters of α -naphthol and of naphthol AS are hydrolyzed by mast cells and myeloid elements at a much faster rate than the corresponding regular acyl esters. The enzyme responsible for their hydrolysis is not a cholinesterase.

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