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## Note

### **Styrylphenyl tetrazolium chloride as a location reagent for thiolsteroids on paper chromatograms**

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In the course of studying the preparation and properties of thiolsteroids in this laboratory<sup>1</sup>, a specific location reagent was needed. Several location reagents for simple thiols were tried (including N-ethylmaleimide, chloroplatinic acid and nitroprusside reagents<sup>2</sup>) but were not successful for steroid thiols. Styryl tetrazolium reagent<sup>3</sup> (Styryl TZ) is widely used to locate reducing steroids<sup>4</sup>; for example, with the  $\alpha$ -ketol 21-hydroxy-pregn-4-ene-3,20-dione (DOC) it gives a dark blue spot on a pale pink background. On heating this colour changes to purple, a colour similar to that of the permanganate ion, with considerable intensification of background. Tetrazolium salts have been used with some success for simple thiols<sup>5-7</sup> which were shown to react similarly to  $\alpha$ -ketols in general.

With steroid thiols and thiolesters, Styryl TZ gave an orange or purple colour dependent on stereochemistry. The orange colour on prolonged heating (>1 min and 140° with an industrial air blower) suddenly changed to purple, being similar to L-cysteine and DOC in this respect. It is thought that the orange colour is indicative of a more complex reaction than simple reduction by the ester.

Free thiols reacted much more readily than thiol esters; the esters needed gently heating to fully develop the colour. Presumably, the hydrolysis to thiol is the rate-determining factor. Also the purple colour developed slightly faster than the orange.

The sensitivity for the orange colour was of the order 1-2  $\mu\text{g}/\text{cm}^2$ , whereas that for the purple colour was 0.1-1  $\mu\text{g}/\text{cm}^2$ . Roberts<sup>5</sup> noted a difference in sensitivities between various tetrazolium salts in reaction with simple thiols, so dianisole tetrazolium and triphenyl tetrazolium salts were tried. They both gave results similar to Styryl TZ but were less sensitive.

Seventeen thiolsteroids were investigated. An orange colour was obtained from: 5 $\alpha$ -androstan-17-one-3 $\alpha$ -thiol, 5 $\alpha$ -androstan-17-one-3 $\alpha$ -thiolacetate, 5 $\alpha$ -androstan-17-one-3 $\alpha$ -thiolpropionate, 5 $\alpha$ -androstan-3-one-17 $\alpha$ -thiolacetate, androst-5-ene-17-one-3 $\beta$ -thiolacetate, 5 $\beta$ -androstan-17-one-3 $\alpha$ -thiolacetate, 5 $\beta$ -androstan-17-one-3 $\beta$ -thiolacetate, 5 $\alpha$ -pregnan-20-one-3 $\alpha$ -thiol, 5 $\alpha$ -pregnan-20-one-3 $\alpha$ -thiolacetate, 5 $\alpha$ -cholestan-3 $\alpha$ -thiol, 5 $\alpha$ -cholestan-3 $\alpha$ -thiolacetate, 5 $\beta$ -cholestan-3 $\beta$ -thiolacetate. Bis-(5 $\alpha$ -androstan-17-one-3 $\alpha$ )-disulphide and 5 $\alpha$ -pregnan-3-one-20 $\alpha$ -thiolacetate gave a pale orange colour and were less sensitive.

The remaining three  $3\beta$ - $5\alpha$  conformers,  $5\alpha$ -androstan-17-one- $3\beta$ -thiol,  $5\alpha$ -androstan-17-one- $3\beta$ -thiolacetate and  $5\alpha$ -cholestan- $3\beta$ -thiolacetate, gave a purple colour.

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