in relation to the state of health of the individual. It is well known[8,9] that carotenoid and vitamin A levels in blood are directly related to diseases which affect the sites of carotene metabolism such as the small intestines and liver. It may become possible, therefore, to both detect and investigate very specific disease states by examination of blood plasma using the rather unusual technique of resonance Raman spectroscopy. Preliminary investigations of Rhesus monkey plasma in our laboratory gave spectra similar to the resonance Raman spectra of human blood plasma. That subject and the relationship of Raman spectra to further studies in human and animal health will be the topic of future communications. We thank Dr. D. CARLO, P. KNISKERN and J. JACKSON for providing the Rhesus monkey blood.

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Preparation of a Specific Ligand for the Purification of Arylsulfatases by Affinity Chromatography

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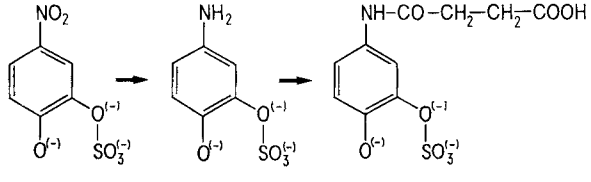
Summary. The reduction of *p*-nitrocatechol sulfate, the artificial substrate of arylsulfatases followed by a condensation of a succinic anhydride, gives a ligand for the purification of the enzyme by affinity chromatography.

To purify arylsulfatases (EC 3.1.6.1.), several methods were reported, using acetone precipitation[2], C.M. Sephadex, G200 Sephadex, etc. Unfortunately, they involved numerous steps, and active enzyme was generally obtained in poor yield.

Affinity chromatography seemed to be an attractive method to purify arylsulfatases from small quantities of tissues. It was first tried by SLOAN et al.[3] using psychosine sulphate as ligand, but during this step the enzyme was purified twice only. Moreover, psychosine sulphate is not readily available because the sulfate group is very sensitive to the acidic medium required for the cleavage of the fatty acid[4].

We have preferred to use the artificial substrate of the enzyme, *p*-nitrocatechol sulfate (I) (*p*-NCS). This compound cannot be used as a ligand as such because it has no amino or carboxyl group to bind to the Sepharose 4B CNBr activated matrix; the reduction of the nitro group allowed to reach the required amino group (*p*-aminocatechol sulfate: *p*-ACS). The well-known instability of such aminocatechols to oxydation led us to protect it by forming an amido group. This is the reason why we have condensed a succinyl chain on the *p*-ACS. The resultant amide should be much less oxidizable than the starting amine. The two steps of this synthesis are shown in the Formulae.



I II III

The instability of the starting product limited the choice of the methods used for the reduction of the nitro group. In acidic medium, sulfate group is hydrolyzed[3], while in basic medium this aminophenol is quickly oxidized; any increase in temperature favours this oxidization. The insolubility of the starting material in most of the organic solvents excluded the reduction in anhydrous medium: the use of mixed metal hydrids seemed hazardous. Hydrogenation over 5% Pd/C at 50 °C and 50 bars in aqueous ethanol 50:50 (v:v) gives tars. Finally we used 98% hydrazine hydrate over Raney Nickel in aqueous solution[5], which lead to the amino compound. The condensation of the succinyl chain on the *p*-ACS prevented effectively the oxydation of the ligand which must be kept dry. Indeed, a slow darkening was observed after several days in aqueous solution, corresponding to an hydrolysis of the sulfate group (as seen by IR).

Experimental. General procedure. *p*-nitrocatechol sulfate dipotassium salt was supplied from Sigma Co. UV-spectra (water) were recorded on a Varian 635 M Spectrophotometer. IR-spectra were performed as KBr pellets using a Perkin Elmer 457 Spectrophotometer and NMR-spectra on a Jeol C 60 HL apparatus using T.M.S. as an internal standard.

p-Aminocatechol sulfate. 98% hydrazine hydrate (0.9 ml) and Raney Nickel (Merck) (1 g) were added to an aqueous solution (20 ml) of *p*-nitrocatechol sulfate (500 mg). The mixture was stirred during 5 min until complete decolouration. After filtration, lyophilization gave a grey residue (II). UV (water): 245 nm; IR: 3380–3300 (NH), 1600 (aromatic C=C), 1245 and 1050 (sulfate group), 810 (aromatic C–H) cm^{-1}.

p-Succinylaminocatechol sulfate. *N*-ethyl, *N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (400 mg) and succinyl anhydride (300 mg) were added to an aqueous solution (20 ml) of the preceding crude *p*-aminocatechol sulfate. The mixture was stirred for 2 h at room temperature and lyophilized. The residue was washed with diethylether (4 × 20 ml) and dried with a stream of nitrogen. This compound (III) presented the following absorptions: UV (H_2O) λ_{max}: 235 nm. IR: 3500 (OH), 3480 (NH), 1725 (acid), 1675 (amid), 1245 and 1050 (sulfate group), 810 (aromatic C–H) cm^{-1}. NMR (D.M.S.O.d6): δ2,1 (m,4H CH2),6,5(m,3H aromatic H).

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