methosulfate and the effect of HOQNO in the absence and the presence of phenazine methosulfate. Similar effects as with HOQNO are obtained with antimycin A and dicoumarol. Geller [16] assumes that phenazine methosulfate serves as a fast "by-pass" around the site which is rate limiting in the system. Our data indicate that a site which is inhibited by antimycin A and HOQNO is "by-passed" by phenazine methosulfate. The point at which dicoumarol acts, which is also "by-passed" may or may not be the same as is acted upon by antimycin A and HOQNO.

Frenkel [17] has reported that an active ATP-ase is present in his "crude" preparation of *R. rubrum*. In our system both a Mg++-stimulated ATP-ase and a ^{32}P-ATP-exchange reaction are present. The ATP-ase activity was inhibited to more than 90 % and the ^{32}P-ATP-exchange activity to about 80 % by 7 mM atebrin, which Löw [18] has shown to inhibit respiration, ATP-ase and ^{32}P-ATP-exchange reactions in liver mitochondria. The possible relation between these reactions and LIP is under investigation.

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Chromatography of Tropolones on Paper Impregnated with Ethylenediaminetetraacetic Acid and Dimethyl Sulphoxide

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Tropolones have recently been found in a number of conifer heartwoods and their identification by paper chromatography is therefore a matter of considerable interest. Conventional methods for the identification of phenols have been tried but give bad results due to tailing of the spots. This disadvantage was successfully eliminated by Zavarin and Anderson [1] by using paper impregnated with phosphoric acid and toluene — *iso*octane as mobile phase. In a search for a chemically less agressive stationary phase, which would also be suitable for preparative work, the use of dimethyl sulphoxide was investigated. Although highly polar, this is a good solvent for most lipophilic compounds and has been used with advantage in paper chromatography of sugar acetates and related compounds [2].

Preliminary experiments using dimethyl sulphoxide impregnated paper with light petroleum as mobile phase indicated favourable R_F-values although the spots were still very elongated, extending from the starting line. The length of the spots was dependent on the amount of substance applied but the distance travelled by the lower edge of the spot reached a limit when the amount of tropolone was increased. These results point to an irreversible adsorption of the tropolones.

Tropolones are known to form stable chelates with a number of multivalent cations and an obvious explanation of their

tailing would be complex formation between the tropolone molecules and cations adsorbed on the cellulose surface. The ion exchange properties of cellulose due to its content of carboxyl groups is well known [3] and the influence of adsorbed metal ions on the tailing of ionisable compounds in paper chromatography has been discussed [3,4].

In view of these considerations it appeared possible to eliminate the tailing of the tropolone spots simply by adding an excess of a competing chelating agent. This was successfully achieved by chromatography on paper impregnated with partly neutralised solutions of ethylenediaminetetraacetic acid (EDTA) before impregnation with dimethyl sulphoxide. Under slightly acidic conditions which gave the best results, the tropolones investigated gave well-defined, almost circular spots. Light petroleum, *cyclo*-hexane and di-*iso*propylether were used as mobile phases, and the chromatograms were run in an atmosphere of low moisture content. The first two solvents gave a satisfactory separation of β- and γ-thujaplicins which have very similar properties. The R_F-values observed are given in Table 1. Within the range 5—200 μg these values were largely independent of the amount of substance applied. The R_F-values were fairly reproducible if care was taken to carry out the impregnation under identical conditions.

It is obvious that the use of paper impregnated with EDTA may be of a general value in eliminating tailing caused by adsorbed inorganic ions. The use of other chelating agents would also be possible; the phosphoric acid used by Zavarin and Anderson [2] is probably also an example of this.

Table 1. R_F-values of tropolones on paper impregnated with EDTA and dimethyl sulphoxide.

Mobile phase	Light petroleum B.p. 60—71°	*Cyclo*-hexane	Di-*iso*propyl ether
Tropolone	0.08	0.10	0.30
7-Hydroxy-4-*iso*propyl-tropolone	0.15	0.18	0.40
γ-Thujaplicin	0.32	0.38	0.55
β-Thujaplicin	0.40	0.45	0.60
α-Thujaplicin	0.62	0.65	0.75
Nootkatin	0.72	0.75	0.82

Experimental. The strips of paper (46 × 12 cm, Whatman No. 1) were soaked in an aqueous solution containing 0.015 moles of the disodium salt of EDTA and 0.005 moles of EDTA per litre. After drying in air, the strips were impregnated twice with a 25 % v/v solution of dimethyl sulphoxide in toluene as described previously [2]. The solvents were of reagent grade and were used without drying. The moisture content of the air in the tanks containing hydrocarbon solvents was kept low with anhydrous silica gel.

The tropolones (5—200 μg) were applied as 1—5 % solution in chloroform and the strips were protected from contact with air by covering with glass slides [2]. The chromatograms were developed by the descending technique, the solvent front reaching 30 cm from the starting line in 1—3 h. After short drying in air, the strips were sprayed with a 1 % solution of iron (III) chloride to give spots of red or, in case of 7-hydroxy-4-*iso*propyltropolone, violet colours. The tropolones could also be located under the ultra-violet lamp as dark spots which after spraying with alkali showed a greenish or yellowish fluorescence. They gave red or yellow spots on treatment of the chromatograms with a solution of *bis*-diazotised benzidine [5] followed by spraying with a sodium carbonate solution.

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