The separation of sodium hypophosphite, phosphite and phosphate on a cellulose column

Although anion-exchange separations of salts of the lower phosphorus acids are well established, their partition chromatography with columns is undeveloped.

We have found that a separation obtained with a thin layer of cellulose may be fairly easily reproduced with a column of the same material. However, if the sample is introduced on to the column as an aqueous solution, it then passes down ahead of the solvent's mobile phase. To overcome this difficulty, the aqueous solution was adsorbed on to some cellulose. Drying removed excess water and a slurry was made with a little of the solvent to be used. In addition, the water content of the solvent was kept lower than that used to give an equivalent separation on a thin layer.

Experimental details

The column (35 cm long, 1.5 cm diameter) was carefully made up using a slurry of Whatman's Standard Grade Cellulose Powder in the starting solvent. The flow rate was approximately 20 ml/h at room temperature. The sample contained $NaH_2PO_2 \cdot H_2O$ (1200 μg of phosphorus), $Na_2HPO_3 \cdot 5H_2O$ (1300 μg P) and $NaH_2PO_4 \cdot 2H_2O$ (1000 μg P).

The solvent passed through under a linear gradient of increasing water concentration. The initial volume in both vessels was 500 ml with 10 % water in the mixing vessel and 47 % in the reservoir. Each contained 5 ml concentrated ammonia solution and the remainder was isopropanol and *tert.*-butanol in the ratio 2:1.

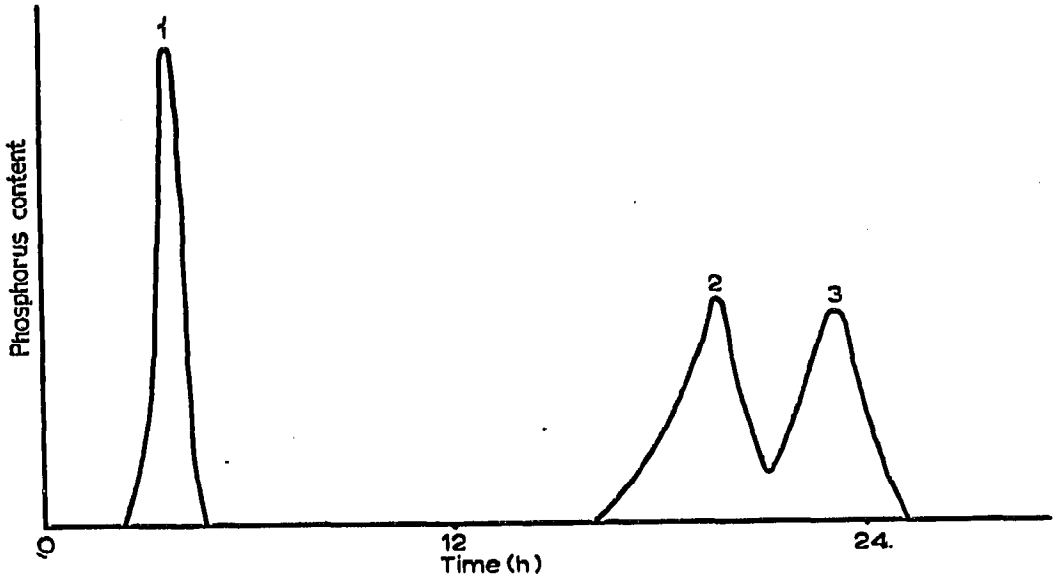

Fig. 1. Separation of (1) hypophosphite, (2) phosphite and (3) orthophosphate on a cellulose column.

The spread of the peaks was minimised by keeping the sample volume as small as possible (*e.g.* 0.1 ml aqueous sample solution) (Fig. 1).

Species were detected in the collected fractions by the nitric acid–vanadate–

molybdate method[1] after the organic components had been boiled off and the bromine/nitric acid oxidation performed.

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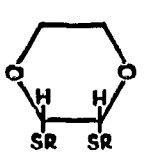
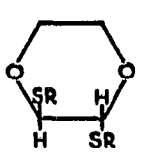
Separation of (^{32}P) dioxathion components on a silicic acid column

Technical dioxathion is a mixture of the *cis* (I) and *trans* (II) isomers of 2,3-*p*-dioxanedithiol S,S-bis(O,O-diethyl phosphorodithioate), the dioxene (III) derivative 2-*p*-dioxenethiol S-(O,O-diethyl phosphorodithioate), and several minor insecticidally active compounds. Although partial resolutions of the components are recorded[1,2] none was adequate for our purpose, which was the investigation of metabolism of the separate major acaricidal components of (^{32}P) dioxathion in strains of *Boophilus microplus* (Can.). A satisfactory separation was obtained in one operation on silicic acid columns using a gradient of benzene in *n*-hexane for elution. A pilot scale resolution of 1 mg of (^{32}P) dioxathion on 1 g of silicic acid yielded fractions which were tentatively identified by paper chromatography. A scaled up (× 100) column, giving similar separations, yielded enough of components for infrared identifications and metabolic studies. Resolution in the small column was superior to that in the large one mainly because of insufficient temperature control during the 21 h elution period with the latter.



I



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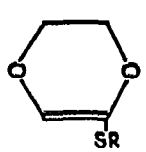
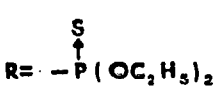


III



One g of solvent washed and activated silicic acid[3], partly deactivated with 15 % v/w water, was lightly ground to a slurry with starting solvent (10 % v/v benzene in *n*-hexane) and used to prepare a glass column 6 mm I.D. × 105 mm long fitted with a sintered glass supporting disc. A 1 mg sample of (^{32}P) dioxathion of

specific activity 14.9 mC/g* was applied to the column in a small volume of starting solvent and elution with 12 ml of solvent of this composition carried out. Then gradient elution was commenced using two vessels of 40 ml capacity to form the gradients. Benzene rich solvent was forced from the gradient vessel by nitrogen pressure through a teflon connecting tube to the magnetically stirred mixing vessel which was arranged to operate at constant volume (40 ml). The solvent mixture from the latter vessel was conducted to the top of the column through teflon tubing. A low pressure gas reservoir of 3 l capacity fitted with a mercury manometer was used to maintain solvent flow at 0.2 ml/min. Fractions, each 1 ml, were collected. All operations were carried out at approximately 24°. Radioactive monitoring was effected by leading the column effluent in teflon tubing (approx. 2.5 mm I.D., 3.5 mm O.D.) between the slits of a 4π paper chromatogram scanner and then to a fraction collector. A gradient was commenced after 12 ml was collected by slowly releasing pressure from the system to avoid upsetting the column packing, emptying the gradient vessel of starting solvent, and refilling with 75 % v/v benzene in *n*-hexane. Application of nitrogen pressure then produced the required gradient. These operations were repeated using 100 % benzene after 21 ml had been collected from the column. A final column elution

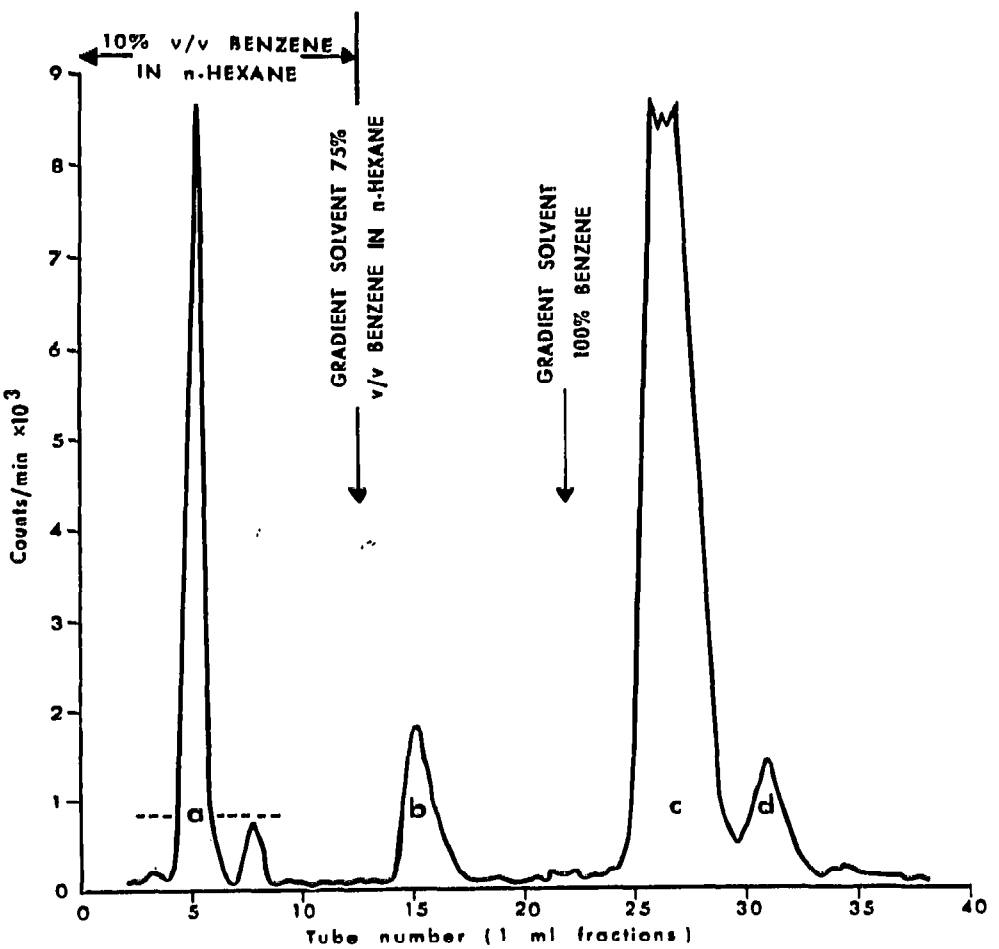


Fig. 1. Elution of (^{32}P) dioxathion components from a silicic acid column. a = Unidentified compounds (see text); b = dioxene derivative; c = *trans*-dioxathion; d = *cis*-dioxathion.

* Obtained from the Radiochemical Centre, Amersham, England.

with methanol indicated that more than 95 % of the applied material was eluted with benzene–*n*-hexane solvent.

Section (a) in Fig. 1 represents material readily eluted from the column and was considered similar to the early eluting fractions 1a, 1b, 1c obtained by ARTHUR AND CASIDA[2].Fraction (b) had the paper chromatographic behaviour of the dioxene derivative in the two aqueous acetonitrile–silicone reverse phase systems of CHAMBERLAIN *et al.*[4]. Fractions (c) and (d) had the R_F values of dioxathion isomers. Fractions corresponding to (a), (b), (c) and (d) which were eluted from the large scale column were examined by infrared spectroscopy using thin films of the liquids between sodium chloride plates. The components of section (a) (Fig. 1) were shown to be quite distinct from dioxathion type compounds and even more numerous than the elution profile indicates. Fraction (b) gave a spectrum (Fig. 2A), which has a strong distinguishing absorption at 1627 cm^{-1} assignable to the C=C stretching mode[5], and agrees with the partial spectrum and other spectral details published by ARTHUR AND CASIDA[2] for the dioxene derivative. The spectrum of fraction (c) (Fig. 2B) agrees with details of the above authors for *trans* dioxathion, whereas the spectrum of fraction (d) (Fig. 2C) corresponds to their details for the *cis* isomer. We obtained supporting evidence for the isomer structures in the stronger absorption of the *cis* com-

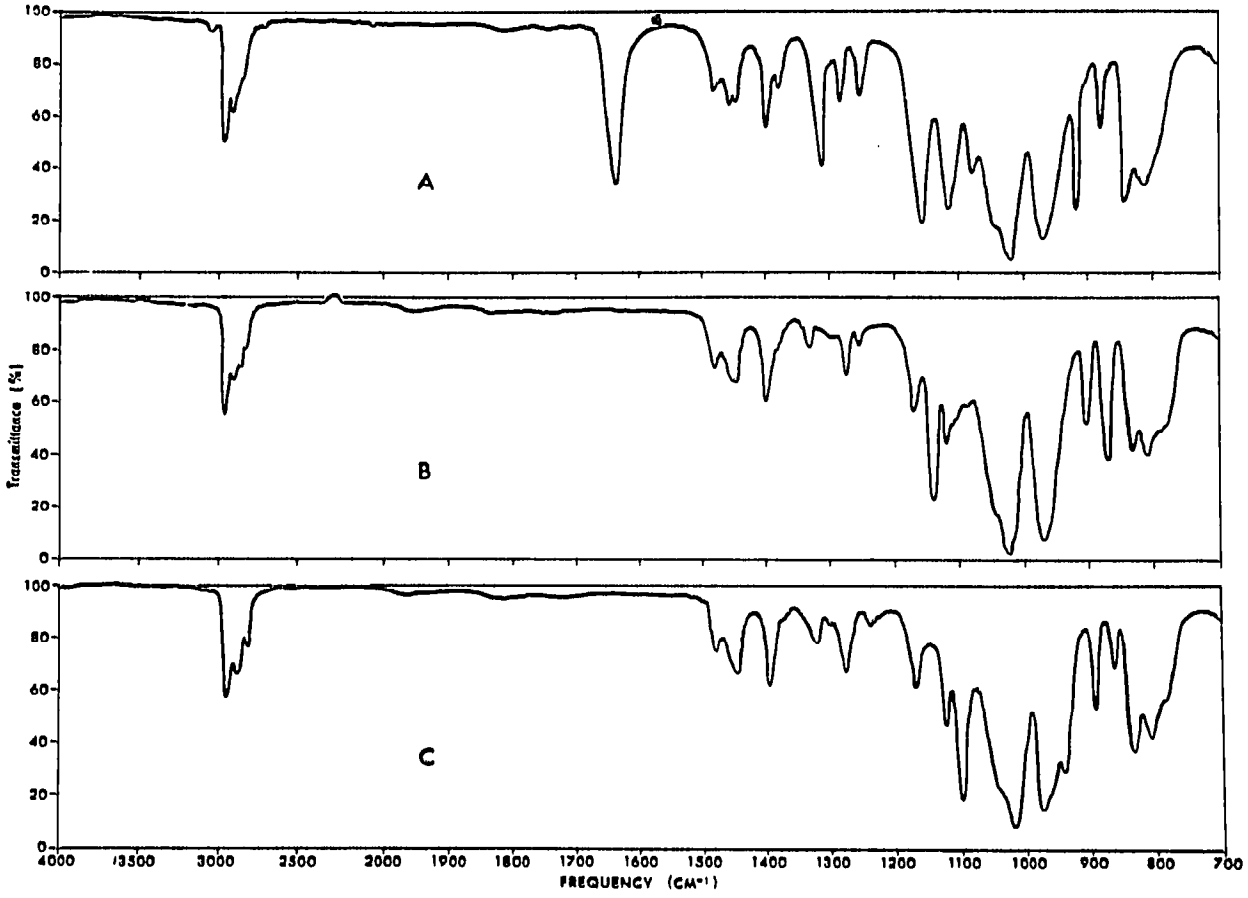


Fig. 2. Infrared spectra of thin films of (A) dioxene derivative, (B) *trans* isomer, and (C) *cis* isomer of (^{32}P) dioxathion.

pound on the column, greater toxicity to *B. microplus*, and apparent steric hindrance in comparative metabolic degradation, when compared to the *trans* isomer.

Spectra of samples obtained from successive 100 ml fractions from the large scale separation showed that sufficient resolution of *cis* and *trans* had occurred to yield most of each isomer uncontaminated by the other. Some minor components of dioxathion were also resolved by further elution of the column and were spectrally similar to the *cis* or *trans* isomer. Although commercial technical dioxathion usually contains about 20 % *cis* isomer our ^{32}P-labelled material contained only 4.6 %. This can be attributed to the variable composition obtained in laboratory scale synthesis of the material as found by DIVELEY *et al.*[1]. Although other complete* and partial spectra[2] have become available they have suffered from solvent absorbances or incompleteness. The spectra presented here are free of these defects and may simplify the task of others working with dioxathion components.

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