Removal and elution of bands

Bands developed on thin-layer plates in isolation work were removed from the plates and the adsorbed material eluted by the method developed for quantitative thin-layer chromatography[2], with suitable changes in the apparatus to accommodate larger amounts of surface layer and volatile organic solvents. The vacuum-cleaner-type collection tube was made longer and sometimes of greater diameter; a 10 × 0.5 cm (O.D.) tube would hold the absorbent from a 10 × 1.5 to 2 cm band of 0.5 mm thick absorbent layer. The eluting apparatus (see Fig. 2) was extended and fitted with a sidearm for the pipette, and both open ends of the apparatus were loosely stoppered. By removing the lower stopper, micro drops of eluate were taken to spot on thin-layer plates in order to test for completeness of elution. Wicks were washed in the organic solvent and stored dry. More than one thread was used if elution did not start in $^1/_2$–1 h.

Sesquiterpene alcohols on alumina were eluted with methylene chloride; the materials were similarly recovered from potassium bromide pellets which had been used in the determination of their infrared spectra, the pellet being ground to a powder prior to elution. Approximately 1 mg was obtained from each 10 × 0.5 cm tube, 200 μl of solvent being required to elute it from alumina, much less from potassium bromide (compare JANAK's elution from a capillary tube with a few drops[9]). A *p*-coumaroyl ester was similarly eluted from silica with acetone.

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Column chromatography of tryptophan and some related indoles

A chromatographic separation of 5-hydroxytryptophan and tryptophan dissolved in water has been described by CONTRACTOR[1]. This note shows that the procedure can be extended to the quantitative separation of a greater range of indoles in water or in a complex biological system.

Experimental and results

The method was similar to that of CONTRACTOR[1], except that Amberlite CG-50 100 to 200 mesh resin was used instead of 400 mesh.

The results of two typical experiments are shown in Figs. 1 and 2.

Discussion

The preparative separation of indolic metabolites of tryptophan has mainly been achieved by extraction with organic solvents followed by paper chromatography (for a review, see SANDLER[2]). These procedures allow only small amounts of material to be processed at one time, and the recoveries of the final purified products tend to be poor. A group separation of indoles from urine has been described by SCHLOSSBERGER, KUCH AND BUHROW[3]. They used a Sephadex column but were unable to separate 5-hydroxytryptophan from 5-hydroxyindoleacetic acid.

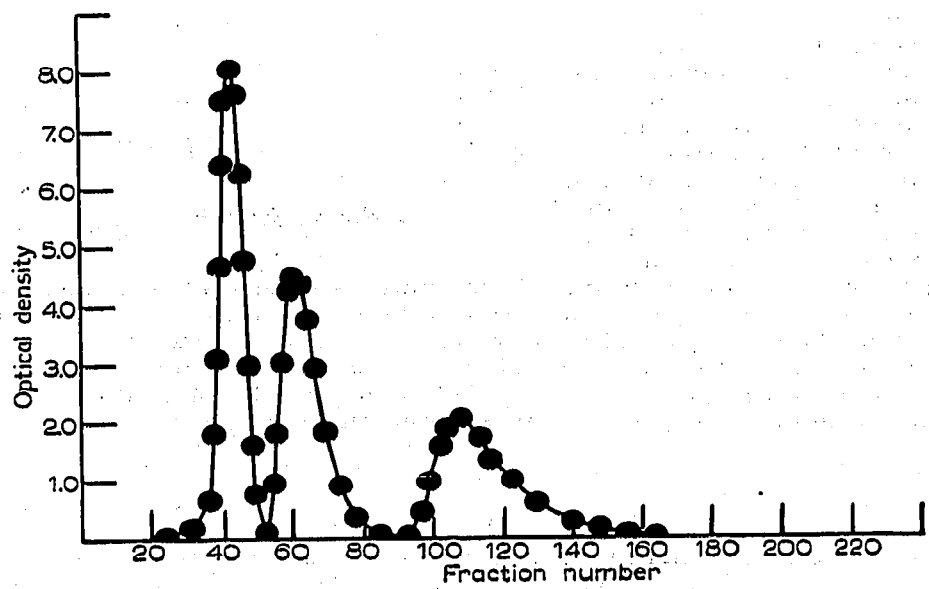

Fig. 1. Chromatographic separation on Amberlite CG-50 resin, 100–200 mesh, H^+ form, pH 7. Column dimensions = 57 × 1 cm. A mixture of 5.05 mg of 5-hydroxyindoleacetic acid, 5.1 mg of 5-hydroxytryptophan and 4.34 mg of tryptophan in 0.001 *N* HCl (0.5 ml) was quantitatively transferred to the column. Flow rate = 2 ml/min. 2-ml fractions were collected. Optical density of each fraction was determined at 275 mμ. Identity of the peaks was established by two-dimensional paper chromatography[4]. Recovery of 5-hydroxyindoleacetic acid (first peak) = 98%, 5-hydroxytryptophan (second peak) = 86% and tryptophan (third peak) = 92%.

Chromatography on CG-50 resin offers many advantages. Indoles can be separated quantitatively from a water solution in a single chromatogram (Fig. 1), or for example in the presence of the contaminants of an enzyme reaction mixture, by successive chromatograms (Fig. 2). Comparatively large amounts of indoles can be obtained in a pure state using this technique.

The 100–200 mesh resin used in the present study is easier to prepare and has a faster flow rate than the 400 mesh resin used previously (CONTRACTOR[1]), whilst possessing similar properties of resolution. The mild conditions provided by using water as an eluant for these labil ecompounds lead to high recoveries.

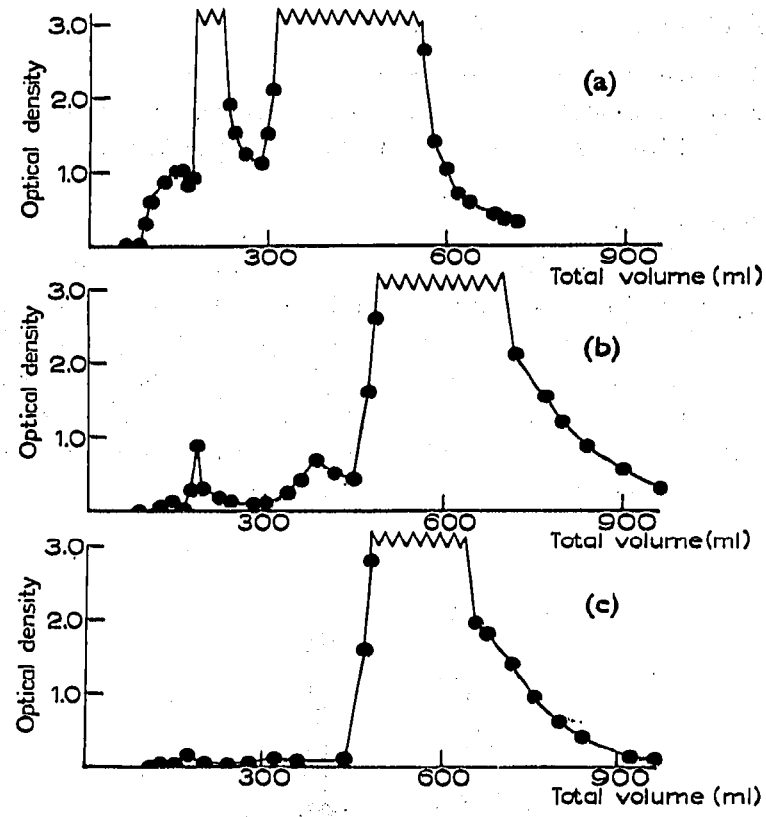

Fig. 2. Successive chromatographic separation of D-5-hydroxytryptophan after incubation of DL-5-hydroxytryptophan (103 mg) with snake venom L-amino acid oxidase[5]. Amberlite CG-50 resin 100–200 mesh, H^+ form, pH 7. Column dimensions = 57 × 2.5 cm. Optical density of each fraction was determined at 275 mμ. Identity of the peaks was established by two-dimensional paper chromatography[4]. (a) Lyophilised reaction mixture was dissolved in a minimal volume of 0.001 *N* HCl and was quantitatively transferred to the column. Flow rate = 2 ml/min. 3-ml fractions were collected. D-5-Hydroxytryptophan and 5-hydroxyindoleacetic acid were present in peak 3 only. (b) Peak 3 (vol. 300–660 ml) of chromatogram (a) was lyophilised and transferred to a fresh column. Flow rate = 2 ml/min. 2-ml fractions were collected. 5-Hydroxyindoleacetic acid and D-5-hydroxytryptophan were present in peaks 2 and 3, respectively. (c) Peak 3 (vol. 460–1000 ml) of chromatogram (b) was lyophilised and transferred to a fresh column. Flow rate = 4 ml/min. 2-ml fractions were collected. Only D-5-hydroxytryptophan was present in the peak. Overall recovery of D-5-hydroxytryptophan at this stage = 68%. For final purification, traces of L-5-hydroxytryptophan were removed by incubating with guinea-pig kidney L-aromatic amino-acid decarboxylase[6], and after lyophilisation, passing through a further column as in (a). Any 5-hydroxytryptamine thus formed is retained on the column.

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