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Note

Separation of radioiodine-labelled 2,3,5-triiodobenzoic acid on Sephadex LH-20

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2,3,5-Triiodobenzoic acid (TIBA) is frequently used as a plant growth regulator[1]. Radioiodine-labelled TIBA may be used to reveal the metabolism of TIBA and its incorporation into different plants. Since the method of labelling based on the isotopic exchange of iodine at the 2 position does not yield only radioiodine-labelled TIBA but also by-products, such as radioiodine-labelled 2-iodo and 2,3-diiodobenzoic acid (MIBA and DIBA), the production of radioiodine-labelled TIBA of high radiochemical purity requires the separation of the target molecule from the by-products, from the so-called free iodine and from the inactive reagents used for the labelling procedure.

In this paper it is shown that radioiodine-labelled TIBA can be separated from the other labelled and inactive substances by means of adsorption chromatography on Sephadex LH-20 dextran gel with aqueous ethanol as eluent.

EXPERIMENTAL

The labelling procedure was a slightly modified version of that described by Westera and Gijlwijk[2]. TIBA (0.1–0.3 mg) was dissolved in acetone in a glass ampoule and 1–5 mCi ^{131}I or ^{125}I in dilute sodium carbonate solution were then added. After removal of acetone and water by evaporation, the ampoule was sealed and placed into an electric oven at 160° for 2–4 min. The ampoule was then opened and the reaction mixture dissolved in water. An aliquot of this solution was chromatographed.

Sephadex LH-20 dextran gel, swollen for 12–24 h in distilled water, was poured into a glass tube (430 × 10 mm I.D.) the bottom of which was equipped with a porous disc. A 0.1–0.3-ml volume of the reaction mixture from the labelling procedure was placed on the top of the gel and was allowed to soak in it. After adsorption equilibrium had been attained (10–20 min) the eluent was delivered by a peristaltic pump at a flow-rate of 22–24 ml/h. The eluent was an aqueous solution of ethanol the pH of which was adjusted either with hydrochloric acid or with citrate buffer.

The effluent was passed over a sodium iodide–thallium scintillation crystal and the count rate was recorded by an x–y plotter. The distribution coefficient, k', was calculated according to eqn. 1:

$$k' = \frac{V_e - V_0}{W} \quad (1)$$

where V_e is the elution volume, V_0 the dead volume and W the weight of the adsorbent.

RESULTS

Fig. 1 shows an elution pattern obtained when the reaction mixture from the labelling procedure was chromatographed by the use of 50% ethanol eluent, the pH of which was adjusted to pH = 2 with hydrochloric acid. It can be concluded that the elution volume of TIBA, DIBA and MIBA increases with increasing number of iodine atoms per molecule, *i.e.*, the higher the iodine content of the molecule the higher the elution volume.

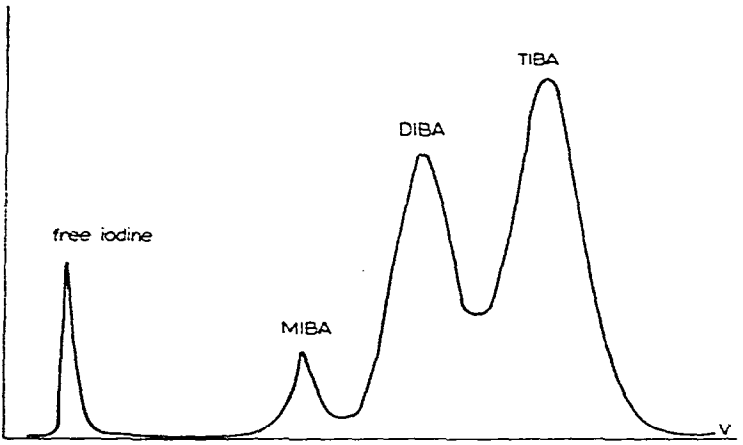

Fig. 1. Elution pattern obtained when the reaction mixture from the labelling procedure was chromatographed with 50% aqueous ethanol (pH = 2).

As for the role of the ethanol concentration of the eluent, the same relationship was found as in case of iodothyronines[3,4] *i.e.*, the experimental data can be fitted by eqn. 2:

$$\log k' = \log k_0' - n \cdot \log S \qquad (2)$$

where k' is the the distribution coefficient, k_0' the distribution coefficient at 1 mole/dm^3 ethanol, S the ethanol concentration and n a constant for a given organic solvent. Fig. 2 shows a plot of log elution volume *vs.* log solvent concentration for TIBA.

As can be seen from Fig. 1, the separation of the mono-, di- and triiodo-substituted benzoic acids at pH = 2 is governed by the number of iodine atoms per molecule. At constant ethanol concentration, the elution volume is a function of the pH of the eluent, in agreement with the finding of Brook and Housley[5], *i.e.*, the ionized form of the substrate is partially excluded from the gel, while the non-ionized form is considerably adsorbed. The dependence of the distribution coefficient on the pH of the eluent is demonstrated by the data of Table I.

In the case of the chromatographic separation in the production of radioiodine-

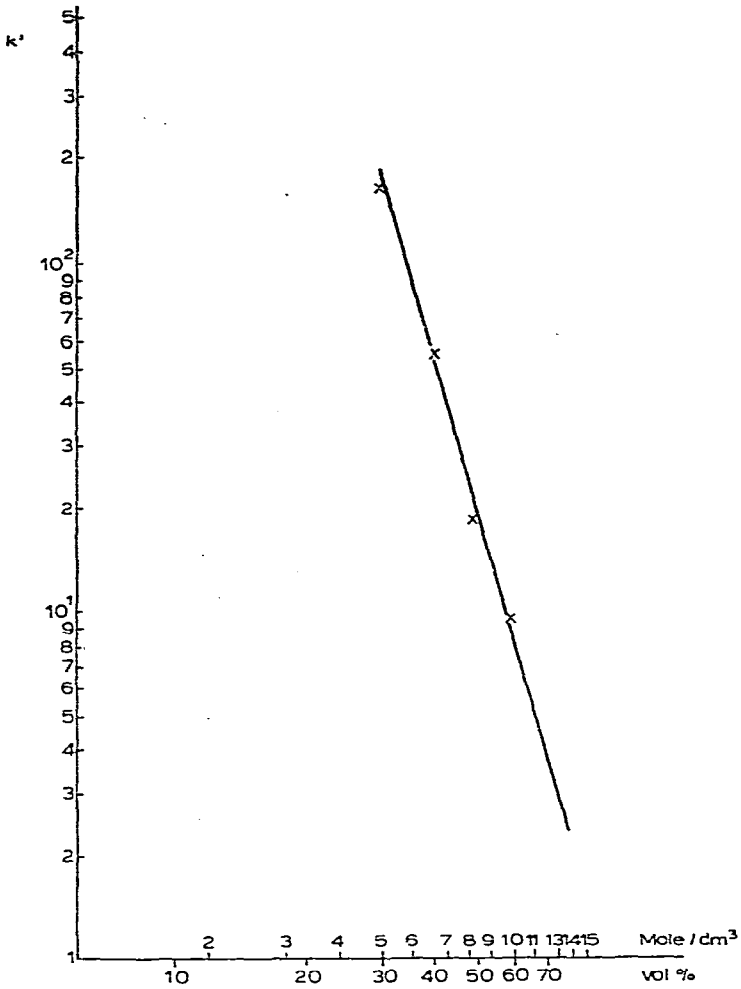

Fig. 2. The log k' *versus* log S relationship obtained for TIBA.

TABLE I

ELUTION VOLUME AND THE DISTRIBUTION COEFFICIENT OF RADIOIODINE LABELLED TIBA AS A FUNCTION OF THE pH OF THE 50% ETHANOL–WATER ELUENT

pH of the eluent	*Elution volume (cm^3)*	*Distribution coefficient*
1	33.5	18.8
2	33.5	18.8
3	27.4	14.7
4	20.0	9.8
6	17.0	7.8
8.5	13.4	5.4

labelled TIBA of high chemical and radiochemical purity, it is recommended that free iodine is first removed with aqueous 0.1 *N* HCl as eluent followed by the elution of TIBA with 40% aqueous ethanol.

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