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Chromatographic Separation of Œstriol, Œstrone and Œstradiol-17β

Non-ionic adsorption of aromatic substances on ion exchange resins discussed by D. E. Weiss[1] has been successfully utilized by me for the chromatographic separation of aromatic compounds, for example, alkylated phenols[2] and N-2 : 4-dinitrophenylamines[3], on the column of carboxylic acid type cation exchange resins, 'Amberlite *IRC*–50' and 'Duolite *CS*–101' (*H* form). The technique has now been extended to the chromatographic separation of œstrogens using partially esterified carboxylic acid type cation exchanger as adsorbent. About 30 ml. of 'Amberlite *IRC*–50' (*H* form: 200–300 mesh, screened wet in sodium ion form[4]) was boiled with 400 ml. of the mixture of ethanol and *N* hydrochloric acid (2 : 1 by vol.) for 40 hr. This partially esterified resin was washed with the mixture of ethanol and water (3 : 2 by vol.) and suspended in two volumes of the same mixture. The suspension was poured into a chromatographic tube and allowed to settle under gravity. A column 0·8 cm. in diameter and 62 cm. in height was used. Œstrogens were dissolved in the same solvent as that used for the packing of the column. 1 ml. of the solution was placed on the column and allowed to drain under gravity. Elution was performed with the same solvent and the effluent was collected in fractions of 40 drops. The ultraviolet absorption was measured at 280 mμ using a Beckman *DU* quartz spectrophotometer and plotted against fraction number (Fig. 1). The elution sequence was similar to that of reversed phase partition chromatography[5], the most polar component being eluted first. Recovery from the column was satisfactory and the elution pattern was reproducible. Since the condition of the column is unchanged after chromatography, the column can be used many times.

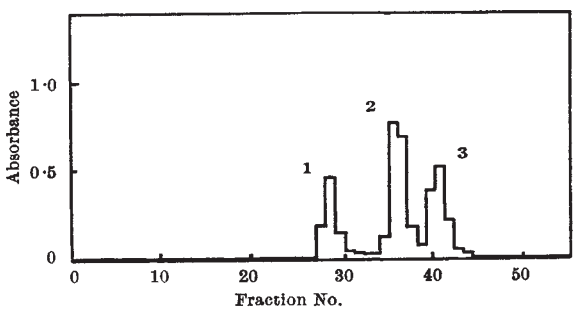

Fig. 1. Elution curve of œstrogens. 1, Œstriol; 2, œstradiol-17β; 3, œstrone. Flow-rate, 2 drops per min.; room temperature, 20° C.

Table 1. Recovery of Œstrogens from the Chromatographic Column

Œstrogens	Added (μgm.)	Recovered (μgm.)	Recovery (Per cent)
Œstriol	414	413	100
Œstradiol-17β	855	840	98
Œstrone	606	553	92

The application of this method for the quantitative analysis of urinary œstrogens is in progress and will be reported elsewhere.

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Determination of Epinephrine and Related Compounds on Paper Chromatograms

The most sensitive chemical method for determining epinephrine in solution is the reaction with ethylenediamine. The reaction yields a number of products, several of which are fluorescent. These substances can be used for quantitative measurement. Nor-epinephrine produces a fluorescence of different colour so that mixtures of the two compounds can be determined by the selection of appropriate filters in measuring the fluorescent light[1].

The number of products formed in this reaction was determined by chromatographing the products produced by the interaction of the diamine and various catechols. This was done on paper by using as solvent 5 per cent ammonium hydroxide and *n*-propanol (9 : 1). There are three to five fluorescent pigments (depending on the conditions of the reaction) and several coloured but not fluorescent ones. We have observed that epinephrine yields some products different from those given by nor-epinephrine, but under these conditions of chromatography the products from catechol, nor-epinephrine, hydroxytyramine and epinine migrate similarly. Also, adrenochrome produces the same products as adrenaline, supporting the contention[1] that the catechol–ethylenediamine reaction involves oxidation.

The addition of ferricyanide or iodine to the ethylenediamine does not change the qualitative nature of the fluorescent pigments, but does increase the amounts formed. Presumably by rapid oxidation of the catechol, one can prevent other side reactions which decrease the yield of fluorescent compounds. This was the reasoning used in making this reagent.

Potassium ferricyanide (0·1 per cent in 5 per cent aqueous ethylenediamine) or iodine (0·01 *N* in 5 per cent aqueous ethylenediamine) are most useful as oxidizing agents. The alkaline iodine solution is stable (room temperature) for only a few days, whereas the ferricyanide solution is considerably more stable (weeks). After spraying with either of these solutions, the chromatograms are placed in an oven at 50° C. for 5 min. We have used the light produced