transfers caffeine moves forward. When both alkaloids are ionized, the situation is reversed because benzene extracts ionic papaverine more readily than it does caffeine, and consequently papaverine superimposes on caffeine.

Conclusion

In spite of some limitations, such as the inability of some substances to change their degree of ionization with a change of pH and restriction of the mobile phase to non-polar solvents, the method can be employed for the separation of appreciable amounts of weak acids or bases and ampholites in short runs. The C.C.D. results may be of use for batch extraction at controlled pH.

Brucine and hydrastine can, for instance, be separated almost quantitatively by a single extraction with benzene by adjusting the pH of the buffer to 4.0.

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Chromatographic estimation of asarones in Indian *Acorus calamus* Linn. oil (tetraploid variety)

The roots of *Acorus calamus* Linn., growing in the plains of India, have been used for the treatment of various ailments[1,2] from very ancient times. Pharmacological studies have shown that the essential oil and asarone (*trans* and *cis*) possess relaxant, spasmolytic[3,4], and hypotensive[5] properties and have powerful insecticidal activity[6]. The above properties have been shown to be due to the presence of asarones (*trans* and *cis* forms) which are present to the extent of 82 %[7] in the essential oil of the Indian *Acorus calamus* Linn. (tetraploid variety). It has been shown that asarones (asarone and β-asarone) are the important constituents of the oil which determine its quality. At present there is no method available for the estimation of asarones in the oil. It was, therefore, considered worthwhile to develop a method for the quantitative estimation of the asarone content of the Indian calamus oil, which is obtained from the roots of *Acorus calamus* (tetraploid variety with chromosome number $2n = 36$ ($x = 9$)).

Investigation of the oil showed that the hydrocarbon part could be separated easily by adsorbing it on a column of alumina and then eluting it with petroleum ether; the asarone part could then be eluted from the column with a mixture of benzene and ether (9:1). On the basis of the above observations the following method was, therefore, developed.

Method

1 g of the oil of *A. calamus* (tetraploid variety) was chromatographed over 50 g of grade I (Brockmann) alumina packed in a column of 2 cm diameter. It may be observed from the elution curve (Fig. 1) that 35 ml of petroleum ether (b.p. 40–60°) elutes all the hydrocarbons and then 65 ml of mixtures of benzene and ether (9:1)

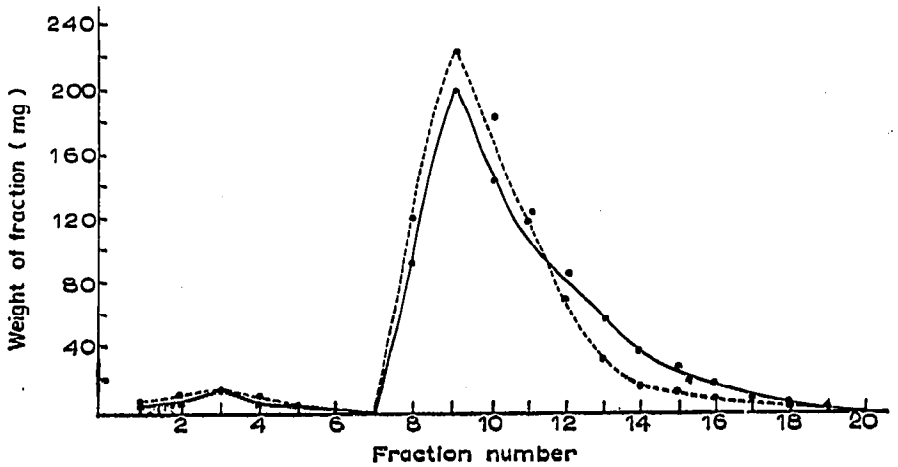

Fig. 1. Elution curve of asarone (*cis* and *trans*) from 1 g of *A. calamus* oil (tetraploid variety growing in the plains of India) over 50 g grade I alumina. Volume of each fraction: 5 ml. ——— oil distilled in 1963; – – – oil distilled in 1964.

elutes all the asarone present in the oil. The solvent is removed, the last traces being removed under vacuum and the residue weighed. The percentage of asarones can be calculated as follows:

$$\% \text{ Asarones} = \frac{\text{wt. of asarones}}{\text{wt. of oil}} \times 100$$

The method could not be verified by comparison with some conventional method. Nevertheless, we examined different samples of the oil distilled at various stages and found that the results were found to agree within 1 % (Fig. 1). The oils distilled in 1963 and 1964 gave 80.7 % and 79.9 %, respectively, as the asarone content.

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