Counter current distribution of alkaloids with a pH gradient

This paper reports preliminary counter current distribution (C.C.D.) experiments, performed with a non-polar mobile phase and an aqueous stationary phase whose pH changes regularly from tube to tube.

The results show that substances whose ionization changes with pH can be separated in short runs if some physicochemical requirements are fulfilled.

Experimental

A 25 tube apparatus, 11.5 cc per phase, was employed. As mobile phase we utilized benzene or petroleum ether because more polar phases such as butyl alcohol gave strong gradient distortion during the run.

The aqueous layer consisted of the BRITTON AND ROBINSON universal buffer[1,2] distributed from the alkaline (first tube) to the acid zone with a gradient of about 0.3 pH unit per tube.

The alkaloids were dissolved in the organic solvent at concentrations of the order of 1 mg/cc.

After the run, they were located by measuring the absorbance of the aqueous layer at 270 mμ.

Results

Fig. 1 shows the results of the fractionation of a mixture of brucine and hydrastine, and Fig. 2, the separation of hydrastine and caffeine performed under similar conditions except for the limits of pH. The experiments show the presence of two components but the resolution is poor.

The C.C.D. carried out with a mixture of caffeine and papaverine (Fig. 3) showed the existence of an optimal transference number. In Fig. 4 we show the separation of a more complex mixture, ecgonine, strychnine, papaverine and caffeine.

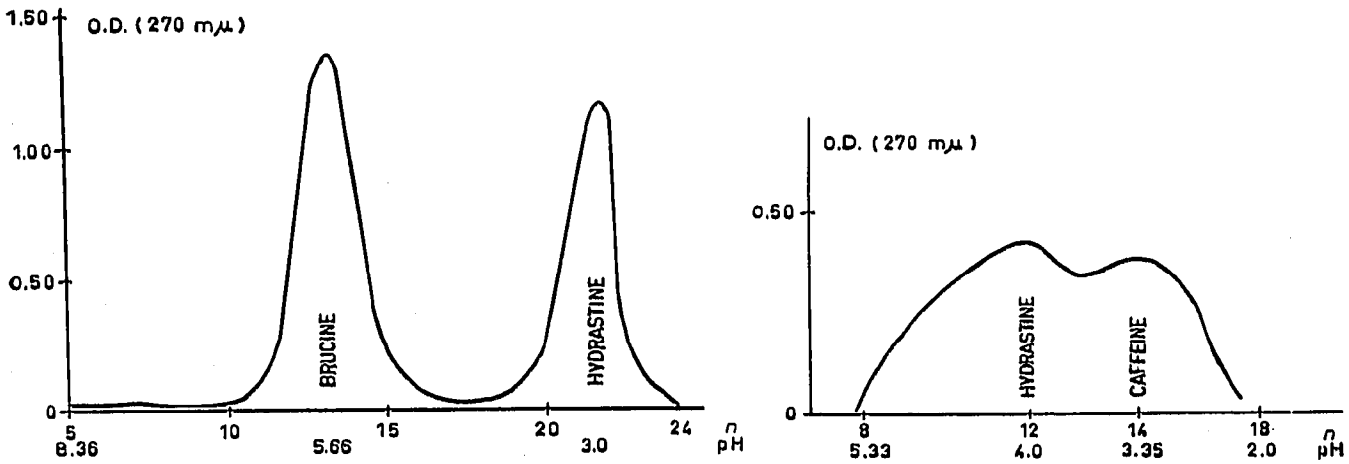

Fig. 1. C.C.D. of a mixture of brucine and hydrastine. Stationary phase BRITTON and ROBINSON universal buffer, pH range 9.0–2.5, 0.33 pH units per tube gradient. Mobile phase benzene. 25 transfers.

Fig. 2. C.C.D. of a mixture of hydrastine and caffeine.

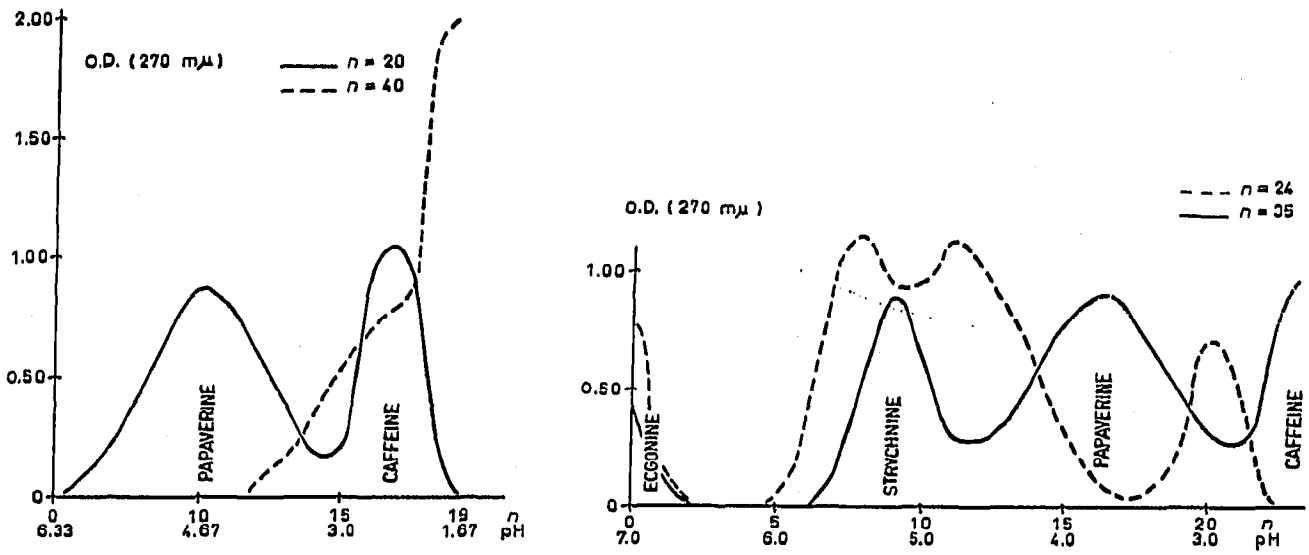

Fig. 3. Separation of the mixture papaverine and caffeine with BRITTON and ROBINSON universal buffer, pH range 8.0–2.0, 0.33 pH units per tube gradient. Mobile phase benzene. Full curve corresponds to the optimal number of transfers (20) and the dotted line to 40 transfers.

Fig. 4. Separation of a mixture of ecgonine, strychnine, papaverine and caffeine. pH range of the buffer 7.0–2.4, pH gradient 0.2 units per tube. Dotted line corresponds to 24 extractions and full line to 35 transfers.

Discussion

The separation of two alkaloids depends on their ionization and their extraction by the organic phase; the former is determined by the pK_b of the alkaloid and the latter by its partition coefficient.

When these properties serve to retard one of the substances with respect to the other, the separation is greatly facilitated. This occurs in the first example reported here, the constants for which are given in Table I.

TABLE I

IONIZATION CONSTANTS AND PARTITION COEFFICIENTS FOR VARIOUS ALKALOIDS

	pK_b	a*
Brucine	6.04	1.86/0.1
Caffeine	14.2	0.36/1.35
Hydrastine	7.8	8.89/0.025
Papaverine	8.07	$\geqslant$0.36/1.35

* a is given as organic phase/aqueous phase; values taken from SEIDELL[3].

The ionization tends to retard brucine because it occurs at a higher pH value than for hydrastine; at the same time extraction due to benzene is greater for hydrastine. It is clear that in this system brucine will be the slower solute.

The situation in the second example (see Table I) is the opposite, and the separation is very difficult.

In the third experiment, papaverine ionizes first and during the twenty former

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.