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## Note

# Separation of dihydroergotoxine (hydergine) into dihydroergocornine, dihydroergocryptine and dihydroergocristine by counter-current distribution

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Dihydroergotoxine, the methansulphonate salt of which is hydergine, is a mixture of equal amounts of dihydroergocornine, dihydroergocryptine and dihydroergocristine. It posesses adrenolytic activity and is able to inhibit the vasomotor centres, and is therefore used for its vasodilative, hypotensive and sedative effects in pharmacology. Dihydroergotoxine is obtained by hydrogenation of the ergotoxine fraction of the *Claviceps* alkaloids. This fraction is composed of ergocornine, ergocryptine and ergocristine, in various ratios depending on the species of ergot. These alkaloids were first isolated by Stoll and Hofmann[1] 30 years ago as the di-*p*-toluyl-L-tartrate salts. The isomers with a C–D *trans* junction, were obtained by hydrogenation of this mixture.

The separation and the quantitative analysis of the isomers is difficult because of the chemical and physical similarities between these substances. Up to now the separation of these alkaloids has been achieved only by paper[2–4] and thin-layer[5–7] chromatography.

In order to achieve the quantitative analysis of these compounds, we applied counter-current distribution, using as the mobile aqueous phase a buffer whose pH varied discontinously according to the method proposed for the separation of bases or acids[8,9]. The use of a mixture of chloroform and carbon tetrachloride (1:1) as the organic phase, instead of chloroform alone, allowed a less acidic pH to be used.

## EXPERIMENTAL

Hydergine (0.5 g) was added to water (19 ml) and to a 1:1 mixture of chloroform and carbon tetrachloride (20 ml). A phosphate buffer was added to the mixture with stirring until a pH of 4.8 was reached*. The two phases were loaded into tubes 1 and 2 of a Craig Model Post counter-current distribution apparatus (200 stages, volume of both upper and lower phase 10 ml). The partition system was as described above and the separation was monitored by thin-layer chromatography[5–7]. The amounts of alkaloids in every tube were determined by UV spectrophotometry ($\lambda$ 281 nm, upper phase).

The free alkaloids were obtained by adding sodium hydrogen carbonate to

* At pH 4.5 hydergine is precipitated.

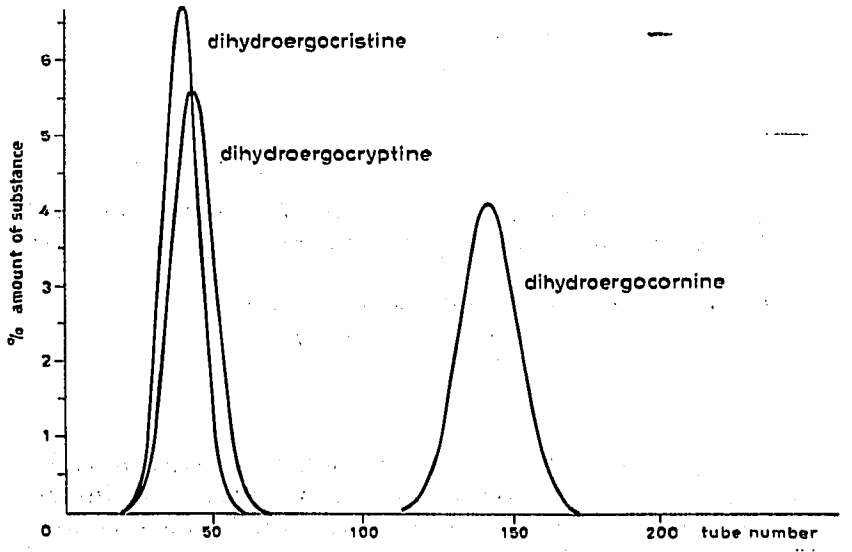


Fig. 1. Distribution curves of dihydroergocornine, dihydroergocryptine and dihydroergocristine after 270 transfers at pH 4.8 and 250 transfers at pH 4.5.

the aqueous phase and subsequent extraction with chloroform–carbon tetrachloride (1:1).

The expression that describes the counter-current partitition of a base between an organic and a mobile aqueous phase is[8,9]

$$\log \frac{r}{n-r} = -\mathrm{pH} + \log \frac{K_r \cdot K_b}{K_w}$$

This equation enables us to calculate the product $K_r \cdot K_b$ ($K_b$ is the dissociation constant, $K_r$ is the distribution coefficient between water and organic phase) when the following parameters are known: the pH of the buffer, the position of the maximum in the distribution curve ($r$) and the number of transfers ($n$).

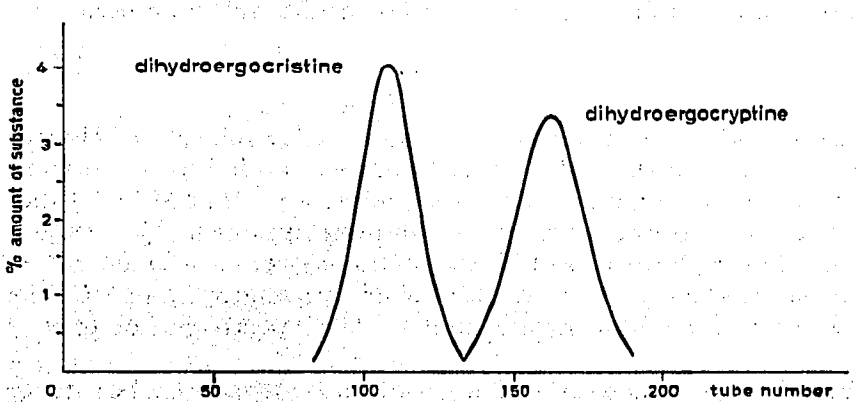


Fig. 2. Distribution curves of dihydroergocryptine and dihydroergocristine after 270 transfers at pH 4.8 and 1000 transfers at pH 4.5.

Dihydroergocornine was at a maximum at tube 60 after 270 transfers at pH 4.8; the $K_r \cdot K_b$ product was $1.6 \cdot 10^{-10}$. As $K_b$ is known[10], $K_r$ is $1.3 \cdot 10^{-3}$. Dihydroergocryptine and dihydroergocristine were at a maximum at tubes 162 and 108, respectively, after 270 transfers at pH 4.8 and 1000 transfers at pH 4.5. Their $K_r \cdot K_b$ product was $5.0 \cdot 10^{-11}$ ($K_r = 3.9 \cdot 10^{-4}$) and $3.2 \cdot 10^{-11}$ ($K_r = 2.5 \cdot 10^{-4}$), respectively. From the $K_r \cdot K_b$ products, the theoretical distribution curves of the three alkaloids were plotted and were found to overlap the experimental curves (Figs. 1 and 2).

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