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Partition Coefficients of Ether-extractable Passionflower Alkaloids

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A simple countercurrent method, based upon the dependence of partition coefficients on pH, has been worked out to separate harman alkaloids. When applied to *Passiflora incarnata*, alkaloids were found which appear to be harman, harmol, harmalin, and harmalol, from their partition coefficients.

Some fifty of the about four hundred known species of *Passiflora* have been examined chemically or pharmacologically. Early investigators reported the presence of two alkaloids, only one of which could be extracted with chloroform and ether.[1-3] A later investigator of *Passiflora incarnata* L. found only one alkaloid to be present in this species and identified it as harman.[4,5] More recently, however, this species was reported to contain several alkaloids with R_F values corresponding to those of harman, harmin, and harmol, and two that were unidentified.[6,7]

A countercurrent procedure was found to be effective in separating curine and chondrocurine derivatives,[8-11] and it seemed likely that a similar method might be suitable for separating the harman alkaloids. Accordingly, partition coefficients of crystalline samples of harman and related alkaloids were measured, and the data were plotted against pH values so that suitable conditions for separation could be ascertained.

Thus, the ether-extractable alkaloid fraction of a *Passiflora incarnata* extract was divided into several fractions containing different alkaloids, and these were characterized by their different partition coefficients at various pH values and by their fluorescence in ultraviolet light.

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RESULTS

Partition coefficients for harman, harmine, harmol, harmaline, harmalol, methylharmine, and N-methylnorharman iodide between diethyl ether and buffer of different pH values are presented in Fig. 1. In Fig. 2, partition coefficients between chloroform and buffers are given for harmalol and for the pseudoquaternary compounds methylharmine and N-methylnorharman iodide.

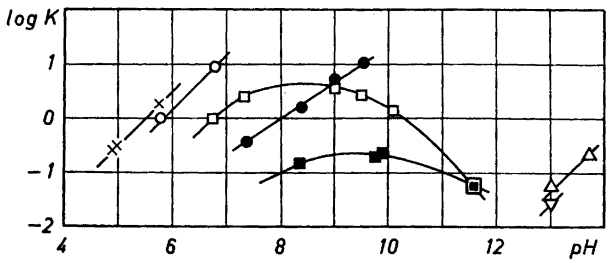

Fig. 1. Partition coefficients K between diethyl ether and water for harman ×, harmine O, harmol □, harmaline ●, harmalol ■, methylharmine △, and N-methylnorharman ▽.

It can be concluded from the figures that the alkaloids investigated can be separated efficiently from each other by simple countercurrent procedures using only a few separatory funnels, with the exception that harman and harmine cannot easily be separated from each other with the solvents used.[12, 13]

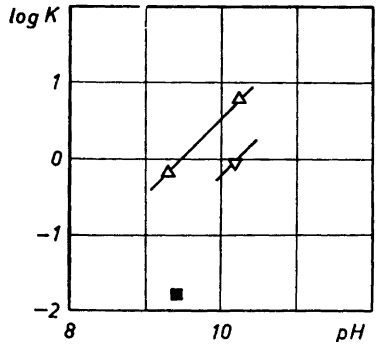

Fig. 2. Partition coefficients K between chloroform and water for harmalol ■, methylharmine △, and N-methylnorharman ▽.

Thus, for example, by using a buffer of pH 11.5 and ether in the volume ratio 4:1, harman, harmine, and harmaline appear in the ether phase, whereas harmol, harmalol, methylharmine, and N-methylnorharman are recovered in the aqueous phase. Using just one separatory funnel, over 97 % of these substances theoretically appear in appropriate phase; or by using two separatory funnels, over 99.7 %. Harmaline can be separated effectively from harman and harmine by using equal volumes of ether and buffer at pH 6.6. Harmol can be separated from harmalol, methylharmine, and N-methyl-

norharman by distribution between two volumes of ether and one volume of buffer at pH 8. Methylharmine and N-methylnorharman can be almost quantitatively (99 % theoretically) separated from harmalol by means of chloroform and alkaline buffer of pH 11 in the volume ratio 4:1, using two separatory funnels. Methylharmine and N-methylnorharman can be separated with equal volumes of chloroform and buffer at pH 9.8.

The information thus obtained about the partition coefficients of several harman alkaloids at various pH values was used for the separation of the alkaloids in *Passiflora incarnata*. Only those *Passiflora* alkaloids which can be extracted with ether from a slightly alkaline water solution are considered in this report. The partition coefficients of fluorescent substances in the several fractions were determined at various pH values, and the colour of the fluorescence was compared to that of known harman alkaloids. The results are given in Fig. 3.

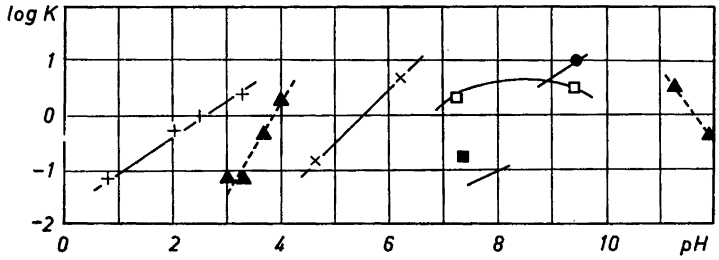

Fig. 3. Partition coefficients K between diethyl ether and water for fluorescent alkaloids in passionflower. Lines are drawn for harmane, harmaline, harmol, and harmalol to fit the corresponding points in Fig. 1. Not identified alkaloids: + and ▲.

It is evident that the fractions contained substances with the same partition coefficients as harman, harmol, and harmaline. In the fraction in which harmalol should appear, there was a substance with a partition coefficient close to that of harmalol and with the same fluorescence as harmalol. One fraction appeared to contain a phenolic alkaloid in which two nitrogen atoms may be involved in salt formation: the asymptote to a line fitted to the corresponding points in Fig. 3 appears to have an inclination of 2. The partition coefficient of this substance is 1 at a pH value of 4, and at pH 11.6. Still another unidentified alkaloid was present, the partition coefficient of which is 1 at pH about 2.5. The straight line in Fig. 3, fitted to the points corresponding to this substance, has an inclination of 2/3, as does the plot of harmalin in Fig. 1. This is not in agreement with the expected value, 1, for an alkaloid in which one nitrogen atom is involved in salt formation.

The partition coefficient of harmalol between ether or chloroform and buffer is less than 1 at all pH values. However, its value is about 11 in the pH range 9—10 between isoamyl alcohol and buffer, and about 7 when partitioning with ether, isopropyl alcohol and buffer of pH 10.25 (1:1:2).

In earlier studies it was found that some passionflower alkaloids may be extracted from water solution with ether and others with chloroform but not

with ether, and still others with amyl alcohol but neither with chloroform nor with ether.[1, 3] This is in agreement with the present findings.

It is apparent that the harman alkaloids may be separated by a simple countercurrent procedure.

EXPERIMENTAL

Material. Harman, harmine, and harmaline were obtained from Fluka AG, Buchs SG, Switzerland, and harmol, harmalol, methylharmine, and N-methylnorharmane iodide from L. Light & Co. Ltd., Colnbrook, England. An extract of *Passiflora incarnata* was obtained from S. B. Penick & Co., New York.

Buffer solutions. All buffer solutions were made up to the ionic strength 0.0625 in order to obtain the value for the activity factor from monovalent ions corresponding to a logarithm of -0.1.

Determination of partition coefficients. The fluorescence of solutions obtained by extracting a solution of the alkaloid in one phase with a series of equal volumes of the other phase of the various solvent mixtures was measured with a Beckman DU spectrophotometer. The coefficients were computed from the fluorescence values.[8, 14]

Passionflower extract. The portion of the ethanol extract of *Passiflora incarnata* which was soluble in dilute phosphoric acid was made slightly basic with sodium hydroxide and then extracted continuously with ether for several days. Only small amounts of alkaloids were extracted, sufficient for fluorescence measurements. (Further continuous extraction with a mixture of methylene chloride and methanol over a period of a week yielded an extract considerably higher in alkaloid content.)

Preliminary countercurrent separation of the passionflower extract. A modification of the procedure used for separating the pure harman alkaloids was used for the isolation of the passionflower alkaloids. Thus, phenolic alkaloids were separated with ether and buffer at pH 12.2; harman (and harmine if present) was separated at pH 4 (3:1) from an unidentified alkaloid; and the latter was separated from other fluorescent material at pH 1.2 (1:1 v/v).

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