Acknowledgements

The authors are grateful to Prof. G. SHIMADA, Meijo University, for supplying aesculetin, Prof. N. MORITA, Toyama University, for xanthyletin, and Dr. K. HATA, Kyoto University, for seselin.

College of Pharmaceutical Sciences, Kitasato University, Shiba, Minato-ku, Tokyo (Japan)

TSUTOMU FURUYA
HISASHI KOJIMA

1 S. A. BROWN AND J. P. SHYLUK, *Anal. Chem.*, 34 (1962) 1058.
2 T. FURUYA, S. SHIBATA AND H. IIZUKA, *J. Chromatog.*, 21 (1966) 116.
3 A. B. SVENDSEN AND E. OTTESTAD, *Pharm. Acta Helv.*, 32 (1957) 457.
4 M. FUJITA AND T. FURUYA, *Yakugaku Zasshi*, 76 (1956) 535.

Received November 21st, 1966

J. Chromatog., 29 (1967) 382–388

Separation of the major alkaloids of *Peganum harmala* by high voltage ionophoresis

In the opinion of CROMWELL[1] none of the existing methods for the determination of the alkaloids of *Peganum harmala* are entirely satisfactory and for this reason he considers a new analytical study of these alkaloids to be necessary. CROMWELL's assertion continues to hold true ten years after it was first enunciated. The key point in all the determinations is the separation of harmine and harmaline. The technique is substantially the same as the fractional precipitation process followed when extracting these alkaloids from the plant. Here we shall deal with—among the different separation methods we have tried—the possibilities offered by ionophoresis on paper as the starting point for a micromethod which permits the evaluation of these tryptophan metabolism compounds in vegetables.

Several authors[2–8] have shown that a considerable number of alkaloids may be separated by ionophoresis on paper as long as they are sufficiently soluble in the buffer and do not remain adsorbed in the carrier. Most of such separations have been carried out at gradients not exceeding 10 V/cm. The advantage of using, in similar cases, fields of an intensity ten or twenty times higher is obvious. The speed of the separation will limit the broadening, distortion and overlapping of the bands due to diffusion.

But this economy of time and, above all, the desired increase of the resolving power will only be attained by either using a device capable of absorbing the heat produced by the Joule effect or adopting the necessary precautions so that the heat released is negligible. Both methods have been used in this work, with different results. We have used a heat exchanging device, cooled by brine circulation, capable of reaching temperatures of —18° for all the ionograms run in aqueous systems. When formamide or dimethylformamide have been used as the solvents the production of

heat has been so low that it has been possible to work at ordinary temperatures without the need to operate the cooling system.

1 % solutions of harmine and harmaline, in the buffer system corresponding to that with which the paper (Whatman 3MM) was impregnated, were used. In all the experiments the degree of impregnation of the paper was maintained uniform by passing the paper through a roller press, with the rollers set at the same pressure all the time so as to remove excess liquid.

As reference substance for measuring the electro-osmotic flow a solution (0.1 %) of methyl-umbelliferone was used.

The buffer system, formic acid–ammonium formate, which had yielded good results in our laboratory for the separation of the two alkaloids on an ion exchange column, was the first one tested for the ionophoresis. Since the ionization of the bases had to show the maximum difference at a pH of the buffer solution equal to half the sum of the pK's of the respective bases, the buffer pH was first set at 5.15, taking as a reference the values of the dissociation constants given by ORLOW[9]. Due to the fact that the pK of substances with ionizable groups capable of mutual interaction may be markedly affected by the ionic strength of the medium[10], experiments were carried out with the buffer values ranging from pH 5.9 to pH 3.6 within the concentration limits 0.02M to 0.1M. In no case was a total separation of the alkaloids achieved, tailing due to adsorption of the bases on the carrier occurred. Tests were then made with conventional buffer systems such as BRITTON's, diluted to ¼, and those of LORENZ-MÜLLER[11] ranging from pH 7 to pH 5, with ionic strengths ranging from 0.002 to 0.2 and potentials from 33 V/cm upwards.

In almost all these systems, it was possible to see, at the beginning, a difference in the migration velocity of the alkaloids, but as the operation goes on tails form and distortion occurs at the fronts. As a result the resolution is incomplete. Nevertheless with the 0.1M LORENZ-MÜLLER buffer 4 mm separations were achieved at a pH of 6.5 and a potential of 50 V/cm after 30 min. The dissipated heat was 240 W. The addition of ethyl and isopropyl alcohol and propylene glycol to the electrolyte, with a view to attenuating the adsorption effect did not substantially improve the result. On the other hand the addition of 25 % of formamide to the LORENZ-MÜLLER acetic acid–acetate buffer prevents the formation of tails and gives rise to larger displacements. The use of pure formamide as a solvent permits the rapid displacement of the alkaloids since there is very little heat evolution and higher potentials (200 V/cm) may be used. However, the migration velocity of both alkaloids is the same in this medium and there is no separation.

Harmine and harmaline bases are soluble in the dimethylformamide–acetic acid system at ordinary temperatures. As it was known that the pH of this system did not coincide with the value it has for aqueous solutions, the pH of the different dimethylformamide–acetic acid systems was taken as that given by the pH meter when a saturated calomel electrode and a glass electrode were inserted in the system.

Fig. 1 shows the ionophoretic behaviour of the bases in each of the systems within the pH range 8–5.5. In all cases the liquid at the electrodes was the same as that with which the paper was impregnated. Ionophoresis took 30 min, the potential was 200 V/cm, the current less than 1 mA and the process could be completed without using the cooling device. Curve 1 shows the *difference* between the distances travelled by harmine and harmaline after 30 min. Curve 2 shows the distance travelled

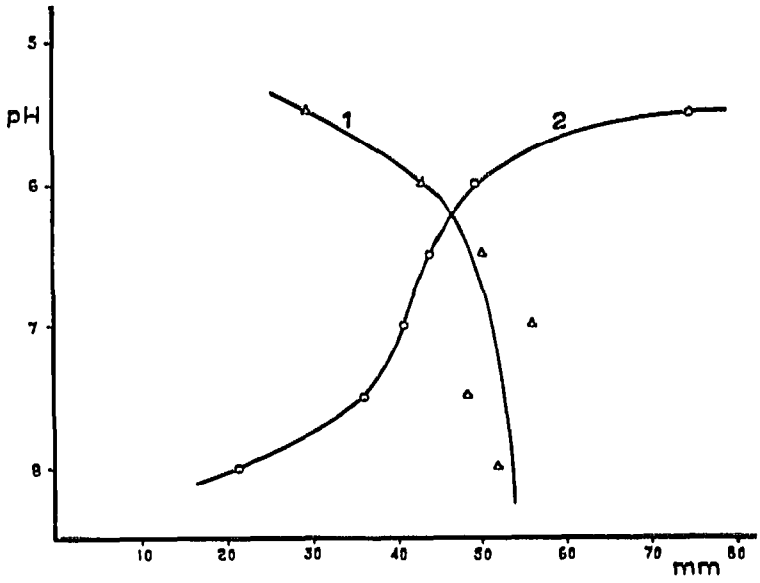

Fig. 1. Curve 1: Difference between the distances travelled by harmaline and harmine at 200 V/cm in DMF–acetic acid at different pH values. Curve 2: Distance travelled by harmaline *vs.* pH of medium.

by harmine during the same time. A close dependence between the migration velocity of the bases and acidity of the medium is observed. It can also be deduced that, at any point within the pH range investigated, migration velocities sufficient to resolve the mixture in about 10 minutes are obtained and that, in general, the optimum working range is located between pH 6.5–7.5.

Instituto "J. C. Mutis" de Farmacognosia del C.S.I.C., Serrano 113, Madrid (Spain)

L. CARRERAS
M. GONZÁLEZ ALONSO

1 B. T. CROMWELL, in K. PAECH AND M. V. TRACEY (Editors), *Moderne Methoden der Pflanzenanalyse*, Band IV, Springer, Berlin, 1955, p. 470.
2 G. B. MARINI-BETTÒLO AND J. A. COCH-FRUGONI, *Gazz. Chim. Ital.*, 86 (1956) 1324.
3 G. B. MARINI-BETTÒLO AND M. LEDERER, *Nature*, 174 (1954) 133.
4 G. B. MARINI-BETTÒLO, M. LEDERER, M. A. JORIO AND A. PIMENTA, *Rend. Ist. Super. Sanità*, 18 (1955) 898.
5 H. MICHL AND F. HARBERLER, *Monatsh.*, 85 (1954) 779.
6 I. SANO AND H. KAJITA, *Klin. Wochschr.*, 33 (1955) 956.
7 J. DE WAEL, *Chem. Weekblad*, 49 (1953) 229.
8 W. DECKER AND J. SCHREIBER, *Naturwiss.*, 40 (1963) 553.
9 J. E. ORLOW, *Chem. Zentr.*, II (1932) 70.
10 A. A. GREEN, *J. Am. Chem. Soc.*, 55 (1933) 2331.
11 G. LORENZ MILLER AND R. H. GOLDER, *Arch. Biochem.*, 29 (1950) 420.

Received January 31st, 1967