Journal of Chromatography, 87 (1973) 433–436

CHROM. 6996

ANALYTICAL STUDIES ON ERGOT ALKALOIDS AND THEIR DERIVATIVES

I. SEPARATION OF ERGOT ALKALOIDS OF THE ERGOTOXINE AND ERGOTAMINE GROUPS BY THIN-LAYER CHROMATOGRAPHY

J. REICHELT and S. KUDRNÁČ
Research Institute for Pharmacy and Biochemistry, 194 04 Prague 9 (Czechoslovakia)
(Received August 13th, 1973)

SUMMARY

A method was developed for the separation of sixteen ergot alkaloids by thin-layer chromatography on silica gel impregnated with formamide, using solvent systems consisting of either diisopropyl ether–tetrahydrofuran–toluene–diethylamine (70:15:15:0.1) or diisopropyl ether–toluene–ethanol–diethylamine (75:20:5:0.1) as mobile phases. The method was applied to the analysis of raw mixtures of alkaloids and of extracts obtained from both ergotamine- and ergotoxine-type ergots. The method has been also found suitable for purity tests.

INTRODUCTION

A number of chromatographic methods have been developed for the analytical separation of natural ergot alkaloids, their isomers and their cleavage products. Originally, alkaloids belonging to the ergotoxine and ergotamine groups were separated by paper chromatography[1–9]. Later, it was found that their separation is much faster on suitable thin layers[10–20]. For this purpose silica gel G[13–17,20], aluminium oxide G[14–16,20] and formamide-impregnated cellulose[12,14,15,18], silica gel[10,11] or talc[19] have been used. Gibson[13] succeeded in complete separation of all L- and D-alkaloids of the ergotoxine group, using silica gel G thin layers and hexafluorobenzene as the mobile phase. This method has several disadvantages:

(1) Long lasting development (7 h on 40-cm plates).
(2) Application of only small amounts of samples.
(3) Relatively small differences between the R_F values (seven spots within a range of 0.23–0.43).

Moreover, Gibson determined the R_F values of ergotamine and ergosine, but not of their dextrorotatory-isomers.

When analyzing either ergot of the ergotoxine type or alkaloid complexes derived therefrom it is important to avoid any overlapping of the spots of the L-alkaloids by those of the D-isomers of the ergotamine and ergotoxine group. Repeating

some of the methods reported, we found that there is no separation either of ergocristinine from ergokryptine[11,12,19] or of ergosinine from ergocristine[10,12,18]. In the system of Agurell[14], ergocristinine and ergocorninine move into the region of L-alkaloids, and the methods described by other workers[16,17,20] are likewise not satisfactory. Consequently, these methods may result in considerably distorted findings.

In the present communication we report on results achieved with a new system, which allows a complete separation of all L- and D-alkaloids of the ergotoxine and ergotamine groups in a single development operation (routine ascending chromatography, flow time about 45 min). A model mixture, containing ergocristine, ergocornine, α- and β-ergokryptine, ergotamine, ergosine, ergostine*, ergometrine* and dextrorotatory isomers of these alkaloids, was completely resolved into its individual components. Apart from this model mixture, using our solvent system we succeeded in completely separating all the alkaloids mentioned above present in raw alkaloid mixtures and in extracts obtained from ergotamine- and ergotoxine-types of ergot. In tests for purity, the new system allows the application of as much as up to 50 μg of the substance tested.

EXPERIMENTAL

Silica gel for chromatography[21–23], 0.005–0.030 mm, was dried for 15 h at 120° before use. Glass plates, 20 cm × 20 cm, a Quickfit thin-layer spreader (Quickfit, Stone, Great Britain) and chromatographic chambers and UV lamp from Camag, Muttenz, Switzerland, were used.

Standard alkaloids were kindly supplied by Galena (Komárov u Opavy, Czechoslovakia); ergostine was obtained by courtesy of Dr. A. Hofmann (Sandoz AG, Basel, Switzerland).

Formamide supplied by Carlo Erba, Milan, Italy, was used, all other solvents being of reagent grade.

Preparation of plates

To 20 g of dried silica gel was added a mixture of 18 ml of formamide and 4 ml of 5% aqueous ammonia and 45 ml of anhydrous ethanol; the suspension was homogenized for 5 min by rotation and then spread on to five plates of 20 cm × 20 cm (layer thickness, 0.25 mm). The plates were allowed to stand for 30 min, and then heated for 5 min at 75° in a thermostat-controlled oven. After cooling, the plates were immediately ready for chromatography. If kept in a closed vessel over formamide, the plates remain satisfactory for use for 5 h.

Sample application

Alkaloid separation studies were done with a model mixture containing 1 μg of each alkaloid in each 5-μl portion of solution. The alkaloids were dissolved in a chloroform–benzene mixture (1:1) saturated with formamide. Purity tests were done with 5 μl of 1% sample solutions in the same solvent mixture. Alkaloid extracts of ergot (a new extraction process is the subject of another communication) were applied in amounts corresponding to 5–50 μg of alkaloid bases. Routine ascending de-

* Both belong to a different group of ergot alkaloids.

velopment was performed in non-saturated chambers (length of run, 17.5 cm) and the spots were detected at a wavelength of 366 nm.

Mobile phases

Diisopropyl ether–tetrahydrofuran–toluene–diethylamine (70:15:15:0.1) and diisopropyl ether–toluene–anhydrous ethanol–diethylamine (75:20:5:0.1) were used as mobile phases.

RESULTS AND DISCUSSION

The result of alkaloid separation of the model mixture is presented in Fig. 1. Out of more than 50 mobile phases tested, those mentioned above yielded the best results. Both systems produced almost equivalent results, the separation being better in a non-saturated than in a saturated chamber. Our method removes the imperfections of previously described procedures for chromatographic separation of ergot alkaloids, and allows a complete and clean separation of all the alkaloid bases mentioned above. Since it separates sharply ergot alkaloids from the accompanying coloured and other ballast matter, it is very suitable for the analysis of raw alkaloid mixtures

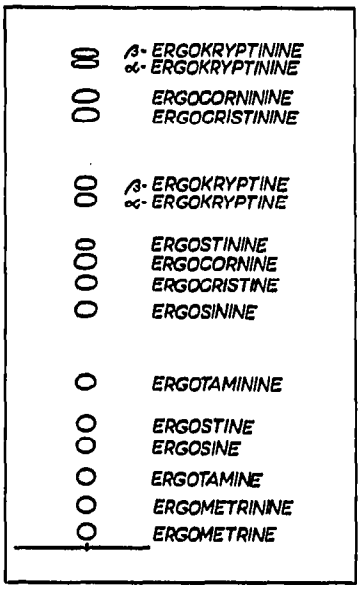

Fig. 1. Thin-layer chromatography of a model mixture of 1-μg amounts of ergot alkaloids on silica gel impregnated with formamide. Mobile phase, diisopropyl ether–tetrahydrofuran–toluene–diethylamine (70:15:15:0.1).

and of extracts prepared from both ergotamine- and ergotoxine-type ergots. Furthermore, amounts as high as up to 50 μg of alkaloid bases per spot may be applied. If necessary, the identification of individual compounds can be verified by mixed chromatograms using reference samples. In the solvent systems described, both D-lysergic and D-isolysergic acids remain at the starting-line; their amides (ergine and isoergine) have R_F values identical with those of ergometrine and ergometrinine, respectively. The separation of these substances will be reported elsewhere.

Of the types of silica gel available for chromatography, that prepared according to Pitra and co-workers[21–23] gave the best results: the alkaloids are separated sharply, the flow of the mobile phase is satisfactorily fast (the elution takes about 45 min with both systems) and the background of the thin layer does not interfere with 254–366 nm UV detection. Silica gel H, Types 60 and 150 (E. Merck, Darmstadt, G.F.R.), yield somewhat inferior results. Moreover, Type 60 has its own fluorescence which lowers the detection sensitivity, as the fluorescence of trace alkaloids is overlapped by that of the substrate itself. A similar problem occurs with silica gel L 5/40 (Lachema, Brno, Czechoslovakia). The flow-rate of the mobile phase through silica gel H from Woelm (Eschwege, G.F.R.) is about one quarter of that through the other silica gel products mentioned; consequently, the spots expand by diffusion, and the separation deteriorates.

The drying of the silica gel (15 h at 120°) prior to the preparation of the thin layer standardizes the amount of water added in the form of the 5% aqueous solution of ammonia to the silica gel during its impregnation with formamide. Ethanol, owing to its higher viscosity, is better for the slurrying of the silica gel than is the acetone used formerly[10,11]. Such thin layers have a higher degree of homogeneity.

The quality of the alkaloid separation depends on the purity of the formamide used; for chromatography, reagent grade formamide is indispensable. Formamide supplied by Carlo Erba produced the best results. Various amounts of formamide (0.6–1.0 ml/g of dried silica gel) were tested for the impregnation; 0.9 ml/g was found to be optimal.

REFERENCES

1 I. M. Hais and K. Macek, *Paper Chromatography*, Czechoslovak Academy of Sciences, Prague, 2nd ed., 1959, pp. 563–567.
2 K. Macek, J. Hacaperková and B. Kakáč, *Pharmazie*, 11 (1956) 533.
3 J. Kolšek, *Mikrochim. Acta*, (1956) 1379, 1662.
4 P. Horák and S. Kudrnáč, *Cesk. Farm.*, 5 (1956) 595.
5 A. Stoll and A. Rüegger, *Helv. Chim. Acta*, 37 (1954) 1725.
6 R. Schindler and A. Bürgin, *Helv. Chim. Acta*, 39 (1956) 2132.
7 M. Pöhm, *Arch. Pharm. (Weinheim)*, 291 (1958) 468.
8 A. Hofmann, *Pharm. Weekbl.*, 100 (1965) 1261.
9 W. Schlienz, R. Brunner, A. Rüegger, B. Berde, E. Stürmer and A. Hofmann, *Pharm. Acta Helv.*, 43 (1968) 497.
10 V. Procházka, F. Kavka, M. Průcha and J. Pitra, *Cesk. Farm.*, 13 (1964) 493; 14 (1965) 154; 15 (1966) 363.
11 P. Horák, *Cesk. Farm.*, 17 (1968) 37.
12 T. Hohmann and M. Rochelmeyer, *Arch. Pharm. (Weinheim)*, 297 (1964) 186.
13 M. S. Gibson, *J. Chromatogr.*, 44 (1969) 631.
14 S. Agurell, *Acta Pharm. Suecica*, 2 (1965) 357.
15 M. C. Guven and L. Eroglu, *Eczacilik Bul.*, 10 (1968) 53; *C.A.* 69 (1968) 46080d.
16 J. L. McLaughlin, J. E. Goyan and A. G. Paul, *J. Pharm. Sci.*, 53 (1964) 306.
17 M. Zinser and C. Baumgärtel, *Arch. Pharm. (Weinheim)*, 297 (1964) 158.
18 K. Teichert, E. Mutschler and H. Rochelmeyer, *Deut. Apoth.-Ztg.*, 100 (1960) 283.
19 I. Zarebska and A. Ożarowski, *Farm. Polska*, 22 (1966) 518.
20 R. Fowler, P. J. Gomm and D. A. Patterson, *J. Chromatogr.*, 72 (1972) 351.
21 J. Pitra, H. Kolářová and V. Krtilová, *Czech. Pat.*, 101,425 (1961).
22 J. Pitra and J. Štěrba, *Chem. Listy*, 56 (1962) 544.
23 J. Reichelt and J. Pitra, *Collect. Czech. Chem. Commun.*, 27 (1962) 1709.