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SHORT COMMUNICATION

A chromatographic assay for male fern extract

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There is not yet a suitable assay for male fern extract. Variation in proportions of phloroglucinol derivatives are not reflected by the gravimetric determination for filicin (B.P. 1968). Neither the paper chromatographic method of Klevstrand (1961) nor the thin-layer method of von Schantz, Ivars & others (1962) are satisfactory. The present paper describes a two-dimensional thin-layer method for the separation of the two major constituents in *Dryopteris filix-mas* (L) Schott., namely flavaspidic acid and filicic acid, followed by their ultraviolet spectrophotometric determination.

Experimental

Silica gel G (Merck): washed with purified water, twice with dry ethanol and twice with dry chloroform, filtered off and dried in a current of air between washings. After final drying the material was powdered and then stored in an airtight container.

Method. The two-dimensional method of Fish & Kirk (1968) was used. Plates were prepared with the pre-treated silica gel (25 g) mixed with ascorbic acid (150 mg) in buffer solution, pH 6·0. Before spreading, the plates were soaked overnight in detergent solution, throughly rinsed and dried.

Commercial extract of male fern B.P. was dissolved in ether (100 mg/ml) and, by means of a Hamilton syringe fitted with a PB600 dispenser, 10 μl of the solution was spotted near one corner of a plate, using a spotting template (Brain & Hardman, 1968). Alongside each test plate, a control (blank) plate was developed two-dimensionally, then both plates were dried in the dark at 25° to remove all traces of solvent (about 2–3 h).

The male fern extract (1 g) was treated by the official assay process (B.P. 1968) and 10 μl of the chloroform solution of filicin obtained (75 ml) was spotted on to plates and chromatographed as above.

Samples of flavaspidic acid and filicic acid were obtained as described previously (Fish & Kirk, 1968) and fixed volumes (10 μl) of ethereal solutions containing known amounts of either of these compounds were also chromatographed. From the results calibration curves were constructed; they were linear over the range 0·001–0·004% w/v.

Elution and determination. The test plates were examined in ultraviolet light of 366 nm and the dark purple, fluorescence-quenched areas corresponding to flavaspidic acid and filicic acid were marked. The adsorbent from those areas, and from areas corresponding exactly in size and position on the blank plates, were separately removed using the glass transfer tool described by Bird, Brickley & others (1963).

Each portion of adsorbent was extracted repeatedly by mixing with dry chloroform (12 × 0·4 ml), the solutions being bulked and made up to volume (5 ml).

The absorbances (Aho, 1958) of the test solutions were measured at 290 nm for flavaspidic acid and at 283 nm for filicic acid using a path length of 1 cm. The chloroform extract of adsorbent from the control plate was used as a blank.

Results

The amounts of flavaspidic acid and filicic acid present in male fern extract and in the separated filicin, expressed as percentages by weight of the original extract, were: by direct determination—flavaspidic acid 19·8 (±1·7), filicic acid 10·8 (±2·1); calculated from figures obtained by assay of the filicin separated from the extract: 5·1 (±1·4) and 2·3 (±1·1) respectively. Each mean value was calculated from results of ten determinations.

Discussion

In the male fern used in Britain, *Dryopteris filix-mas*, the main activity can be ascribed to flavaspidic acid, and to traces of desaspidin usually present, and we suggest that assay for the former alone would give a good indication of potency in the crude drug and its extract. A similar method could be useful for the continental drug, derived from "*D. austriaca*". The method could also be extended to chemotaxonomic studies of various ferns.

The results show that the male fern extract we examined, which came from a 1968 batch of crude drug, and was of good quality, contained high proportions of the active flavaspidic acid and the inactive filicic acid, both of which must contribute largely to the weight of filicin determined in the official gravimetric assay. They also show that the alkali treatment during the isolation of filicin in that assay greatly reduces the contents of those compounds present. In the direct chromatographic assay proposed, undesirable breakdown of the labile compounds is avoided.

Use of pre-washed silica gel for chromatography is essential since impurities from adsorbents interfere with subsequent spectrophotometric determinations (Kirchner, Muller & Rice, 1954; Stanley & Vannier, 1957). The treatment described was successful in removing interfering substances.

For flavaspidic acid recoveries from chromatoplates were 72% and for filicic acid 84% of the amounts applied, and the method, applied directly to the extract, gave results which were reproducible over the range quoted and were as consistent as those given by Takki (1967) for the estimation of flavaspidic acid in filicin.

REFERENCES

AHO, E. (1958). "Über isolierung und tautomereformen der flavaspidsäure und anderer filix-phloroglucin abkömmlinge", *Annales Universitatis Turkuensis*, Turku.

BIRD, H. L., BRICKLEY, H. F., COMER, J. P., HARTSAW, P. E. & JOHNSON, M. L. (1963). *Analyt. Chem.*, **35**, 346–347.

BRAIN, K. R. & HARDMAN, R. (1968). *J. Chromat.*, **38**, 355–363.

FISH, F. & KIRK, W. R. (1968). *Pharm. Weekbl.*, **36**, 383–387 and references cited therein.

KIRCHNER, T. G., MULLER, T. M. & RICE, G. (1954). *J. Agr. Food Chem.*, **2**, 1031.

KLEVSTRAND, R. (1961). *Reports of Norwegian Pharmaceutical Society*, **11**, 191–199.

SCHANTZ, M. VON, IVARS, L., KUKKONEN, I. & RUUSKANEN, A. (1962). *Planta Med.*, **10**, 98–106.

STANLEY, W. C. & VANNIER, S. H. (1957). *J. Ass. off. agric. Chem.*, **40**, 282.

TAKKI, S. (1967). *Ann. Med. exp. Fenn.*, **45**, 333–340.