Paper Chromatographic Separation of some Phloroglucinal Derivatives from *Dryopteris filix mas*

From crude filicin, obtained via the oleoresin, filicic acid, flavaspidic acid and albaspidin were isolated, while filicinic acid was obtained by heating filicic acid in the waterbath with zinc dust and a 10% sodium hydroxyde solution for 6 h.

Paper. Use was made of Whatman No. 1 filter paper. The length of the employed strip was 270 mm and cut in the machine direction; the width was 70 mm.

Solvent. A 2 *N* sodium carbonate solution in which, immediately before chromatographing, 0·2 w/v% sodium sulfite had been dissolved.

Reagent. 1·5 ml of a solution of 1% sulfanilamide in 10% hydrochloric acid are mixed with 1·5 ml of a 5% sodium nitrite solution and gently shaken for 1 min. Next the whole partly neutralized with 1 ml 2 *N* sodium carbonate solution and water is added up to 50 ml. The reagent is always freshly prepared.

Ascending chromatography. 2 mg of the isolated substances are dissolved in 1 ml chloroform. By means of a pipette 3 μl of these solutions, are brought on the paper at 40 mm from the lower end of the strip at a mutual distance of 17·5 mm. In order to prevent difficulty in the spots being wetted by the solvent, first 1·5 μl is brought on the paper and after the chloroform has evaporated, 1 μl and finally 0·5 μl, instead of 3 times 1 μl. This is done to prevent the substance accumulating in the boundary of the spot.

When the spots are dry, the chromatogram is run at a temperature of 20°C. The paper is taken out of the cylinder as soon as the liquid front has reached a distance of 200 mm from the starting line, which takes about $3^1/_4$ h. Next the paper is dried for about 10 min in an oven at 80°C, sprayed with the reagent and dried again. Directly after the spraying the spots become visible and show some difference in colour for the different substances[1].

Result. See Table.

Substance	Colour	Average R_f value
Flavaspidic acid . . .	brownish-yellow	0·28
Filicic acid	brown	0·53
Albaspidin	violent-yellow	0·74
Filicinic acid.	orange-yellow	0·84

A mixture of these 4 substances shows the same average R_f values. The crude filicin itself, which is brought onto the paper in a concentration of 10 mg per ml chloroform, shows the R_f values of filicic acid and flavaspidic acid. In this concentration albaspidin is not visible. The crude filicin of some other fern species is being investigated.

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Résumé

L'acide filicique, l'acide flavaspidique, l'albaspidine et l'acide filicinique ont pu être isolés par chromatographie sur papier, suivant la méthode ascendante, avec comme phase mobile une solution de carbonate de sodium 2 *N*, dans laquelle on a dissous 2 g de sulfite de sodium par litre. Les spots étaient rendus visibles en pulvérisant une solution de sulfanilamide diazotée. En même temps se manifestait aussi une différence de couleur pour les dites substances phlorogluciniques.

[1] The spraying with reagent is done in the fume-chamber.

The Influence of Oestrogen and Thyroid on the Pituitary and Blood Content of FSH and LH

Hyperthyroidism when experimentally induced, increases the duration of the oestrous cycle, and this is attributed to prolongation of the luteal phase (Hayashi[1] and Weichert[2]). Thyroid administration produced an increase in the number and size of corpora lutea (Sidki[3]; Weichert and Boyd[4] and Sidki and Soliman[5]). Such observations indicate that thyroid-active materials possess an oestrogen like effect by favouring the production of LH (Stein and Lisle[6]; Chu[7] and Okans and Takana[8]).

Under natural conditions, the increased oestrogen level during the follicular phase of the oestrous cycle was revealed to be concomitantly associated with increased thyroid activity and increased level of thyrotrophic hormone in the blood (Soliman and Reineke[9] and Soliman and Badawi[10]).

The present investigation was thus devised to ascertain the influence exerted by oestrogen and thyroid on the FSH and LH content of the blood and hypophyseal glands of ovariectomized rats.

Ovariectomy was performed on 24 mature female rats and these were divided into four groups with six animals in each. Group I was kept as control. To the rats of group II, desiccated thyroid was administered at the level of 0·1% in the diet for 30 days. Parenteral administration of 1 μg of oestradiol benzoate (B.D.H.) daily for 30 days was conducted to the animals of group III. The animals of group IV were treated with oestradiol and, in addition, desiccated thyroid was fed for the same duration, after which all the animals were killed by decapitation under light ether anesthesia, and the blood of the animals of each group was pooled together and allowed to coagulate. The sera were procured by centrifugalization at a rate of 3,000 r.p.m. for 15 min and subsequently treated with acetone and ethyl alcohol in order to precipitate the proteins and the precipitate that arose was redissolved in saline so as to bring the sera back to their original volume. Such a procedure was adopted to annul any error that might arise from the presence of oestrogen in the serum of the rats. The hypophyseal glands of each group were cleanly dissected, weighed in a torsion balance, pooled together and thoroughly ground. The resulting suspension was so adjusted that every 0·1 ml of saline contained 1 mg of fresh hypophysis.

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