PAPER CHROMATOGRAPHIC SEPARATION OF THE PHLOROGLUCINOL DERIVATIVES FROM *DRYOPTERIS* SPECIES

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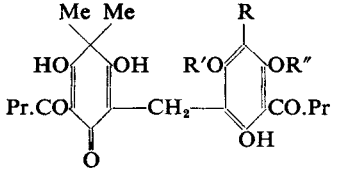
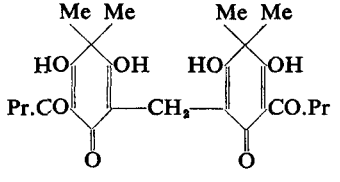
Received May 17, 1961

A paper chromatographic technique, using buffered filter papers, is described for separating from *Dryopteris* ferns the phloroglucinol derivatives and some of their decomposition products. The R_F values on papers buffered from pH 4·0 to pH 9·1 are given in the form of diagrams with the aid of which the appropriate buffer can be chosen depending on the mixture to be separated.

THE extracts of the dried rhizomes of different *Dryopteris* species have for long been used as anthelmintic drugs against tape-worm, especially *Diphyllobothrium latum* and *Taenia saginata*. The activity of these extracts is due to the phloroglucinol derivatives of which the following have been isolated in a pure state: aspidin (I), flavaspidic acid (II), desaspidin (III), albaspidin (IV), filixic acid (V), phloropyron (VI),* phloraspin (VII)* and aspidinol (VIII). These substances possess a



I. R = R″ = Me; R′ = H
II. R = Me; R′ = R″ = H
III. R = R″ = H; R′ = Me



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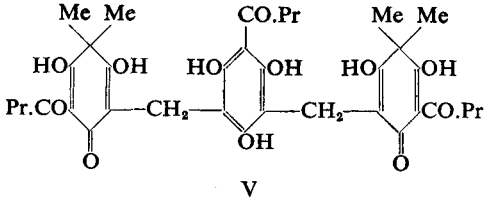


V

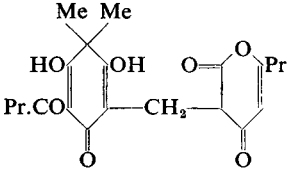
widely different anthelmintic activity as well as general toxicity. The composition of the extracts from different *Dryopteris* species varies considerably, and the same species differ according to habitat and season. The extracts also undergo changes during storage.

Because of the low therapeutic index of the extracts, serious complications have occurred in clinical use and the anthelmintic effect has

* The chemical structure of phloropyron and phloraspin has been resolved by the authors, and the data will be published in *Acta chem. scand.*, 1961.

sometimes proved unsatisfactory. A reliable analytical method is therefore required for the qualitative and quantitative determination of the phloroglucinol derivatives in *Dryopteris* ferns. Such a method would also be useful in taxonomic studies and in separating different *Dryopteris* species which are difficult to identify because of their numerous transition forms.

Chromatographic methods appeared to be the most suitable means of separating the closely related compounds contained in the extracts. Paper chromatography has been used by Klevstrand (1957) for separating some of the phloroglucinol derivatives from *Dryopteris* species. By his



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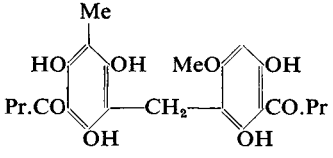
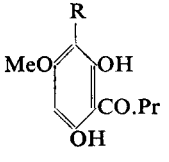


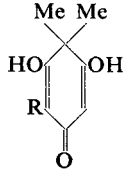
VII

technique flavaspidic acid and aspidinol can be separated from albaspidin and filixic acid, but the latter two only partly from each other. Büchi, Aebi and Kapoor (1957) have developed a reversed-phase-method for the separation of the crude aspidin and crude filicin phloroglucinol substances. Godin (1958) applied paper chromatography to the detection of substances isolated from *Dryopteris filix mas.* Klevstrand (1960) has reported a method using two-solvent-systems by which aspidin and phloraspin can be separated and a more satisfactory separation of albaspidin



VIII. R = Me

IX. R = H



X. R = H

XI. R = CO.Pr

and filixic acid obtained. Hegnauer (1961) also describes a two-solvent-system to run parallel to identify the phloroglucinol derivatives from *Dryopteris* species.

We now describe a method which, using buffered filter papers and a single solvent system, has proved successful in separating all known phloroglucinol derivatives from *Dryopteris* species. In addition to the naturally occurring substances of this class, some of their common decomposition products such as filicinic acid (X), 3-butyrylfilicinic acid (XI) and desaspidinol (= phlorobutyrophenone 4-methyl ether) (IX) are included in this investigation.

EXPERIMENTAL METHODS AND RESULTS

Materials and Solvents

Aspidin (I) m.p. 123–124°, yellow needles from ethanol. Flavaspidic acid (II) m.p. 156°, yellow crystals from benzene. Desaspidin (III) m.p. 152–154°, white crystals from ether. Albaspidin (IV) m.p. 148–150°, white crystals from methanol. Filixic acid (V) m.p. 180–181°, yellow crystals from ethyl acetate. Phloropyron (VI) m.p. 111–112°, white needles from ethanol. Aspidinol (VIII) m.p. 142–143°, yellowish prisms from benzene. Desaspidinol (IX) m.p. 127–128°, colourless needles from 50 per cent ethanol. Filicinic acid (X) m.p. 215–220° (decomp.), white crystals from ethanol. 3-Butyrylfilicinic acid (XI) m.p. 98°, white crystals from xylene.

Filixic acid is isolated from *Dryopteris filix mas* (L.) Schott, all the others from *Dryopteris austriaca* (Jacq.) Woynar with subsp. *dilatata* (Hoffm.) Sch. et Th. and subsp. *eu-spinulosa* (A. et G.) Hyl.

TABLE I

R_F VALUES OF PHLOROGLUCINOL DERIVATIVES ON PAPERS BUFFERED TO pH 5·0 AND 8·8 AND THE COLOURS OBTAINED WITH "FAST BLUE SALT B" MERCK REAGENT

Phloroglucinol compound	R_F values		Colours
	pH 5·0	pH 8·8	
Aspidin	—*	0·73	Yellow
Albaspidin	—*	0·56	Red orange
Desaspidin	—*	0·47	Purplish red
Phloropyron	—*	0·28	Yellow orange
Filixic acid	—*	0·10	Brown
Aspidinol	0·83	0·83	Purple
Desaspidinol	0·74	0·64	Purple
Flavaspidic acid	0·53	0·00	Greyish purple
Butyrylfilicinic acid	0·35	0·00	Carmine
Filicinic acid	0·00	0·00	Blue spot with red centre

* The substance moves with the solvent front.

Chloroform. DAB.6 grade chloroform is extracted with water, dried over Na_2SO_4 and distilled in subdued light. Benzene, pure. Formamide, "Merck" for chromatography. Acetone, pure.

Buffer solutions. pH 4·0–7·4 citrate-phosphate buffer (McIlvaine). pH 7·8–9·1 borate HCl buffer (Sörensen, Clark).

Buffered filter papers. Schleicher and Schüll 2043 b-papers are cut in 20 cm. strips across the machine direction. The papers are dipped in buffer solution, air dried, and stored in air-tight glass containers.

CHROMATOGRAPHIC PROCEDURE

The sheets of buffered papers are drawn quickly through the formamide: acetone 2:3 (v/v) solvent and the excess removed. The phloroglucinol substances 50–200 μg. in acetone or chloroform solution are immediately applied on freshly impregnated papers. With crude extracts amounts of up to 500 μg. can be used. The papers are developed by chloroform-benzene solvent 1:1 (v/v). During 2–2½ hr. at 20° the solvent front descends 25–30 cm. After drying, the spots are detected by spraying with tetrazotized di-*o*-anisidine, called "fast blue salt B", in 0·1 per cent

aqueous solution or by drawing them quickly through this solution. Immediately after, the phloroglucinol derivatives give spots of varying colours on a practically colourless background (Table I).

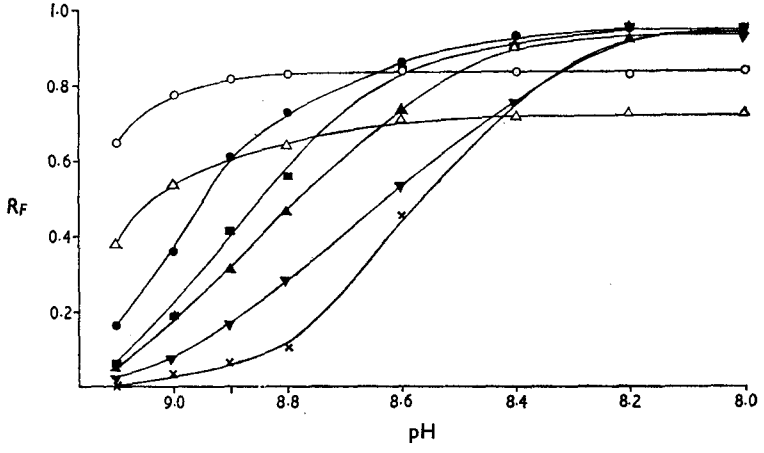


FIG. 1. Variation of R_F values with the pH of the papers.
● Aspidin. ■ Albaspidin. ▲ Desaspidin. ▼ Phloropyron.
X Filixic acid. ○ Aspidinol. △ Desaspidinol.

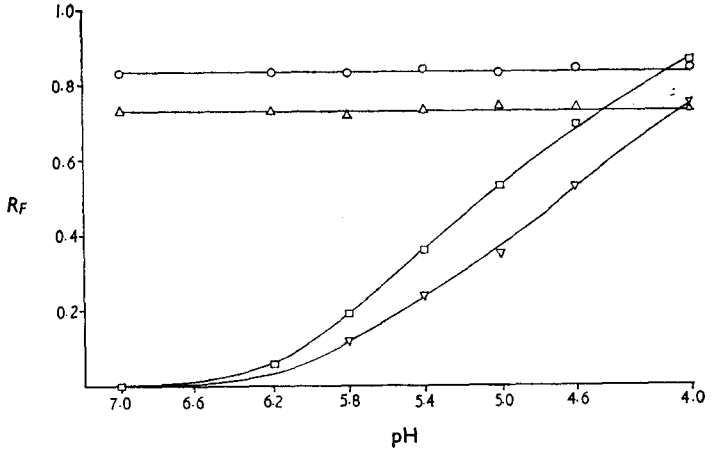


FIG. 2. Variation of the R_F values with the pH of the papers.
○ Aspidinol △ Desaspidinol □ Flavaspidic acid
▽ 3-butyrylfilicinic acid.

Aspidinol, however, first gives a nearly colourless or pale yellow spot which slowly changes to purple. For the detection of the phloroglucinol spots the "fast blue salt B" (Merck) has proved superior to all other reagents. [Ferric chloride - potassium ferricyanide (Klevstrand, 1957); Folin–Denis reagent (U.S.P. XV, 1955); ammonium vanadate - *p*-anisidine (Klinkhammer, 1958); Pauly reagent (Hais and Macek, 1958);

2,4-dinitrophenylhydrazine (Hais and Macek, 1958); vanillin - hydrochloric acid (Hais and Macek, 1958).] The many variations in colour which the different phloroglucinol derivatives give with this reagent, make it possible to identify even quite small quantities of phenolic constituents in raw materials and crude extracts, in which fatty or non-phenolic impurities dominate and may prevent a complete chromatographic separation. Also, with the "fast blue salt B" reagent the colour of the spots remains unchanged for over a year.

Figs. 1 and 2 show that by an appropriate buffering, any mixture of *Dryopteris* fern phloroglucinol derivatives can be separated satisfactorily. For most purposes papers buffered to pH 5·0 and 8·8 are adequate. The R_F values on these papers are listed in Table I. All R_F values were obtained by using 100 μg. amounts, but 2–5 μg. amounts of pure substances can easily be detected. The figures recorded are the average of several determinations giving slightly varying results, but the relative positions of the spots were always the same.

Discussion

On buffering the papers, the purity of the air should be ensured, because the wet papers easily absorb minute amounts of phenolic substances which often occur in laboratory atmosphere. This may later result in a dark coloured background or dark bands along the solvent front.

The paper buffered to pH 8·8 is recommended for the separation of all but the more acidic phloroglucinol substances—flavaspidic acid, filicinic acid and butyrylfilicinic acid. In some instances papers buffered to pH 8·6 or 8·4 give better and more distinct spots with less "tailing" than the paper at pH 8·8. Tailing has in no case badly impaired the results, and it can be avoided by using papers buffered to a lower pH than mentioned above. Aspidinol and desaspidinol always give very distinct spots, regardless of pH.

Separation on non-buffered papers resembles that on papers buffered to about pH 5·6, i.e., flavaspidic acid and butyrylfilicinic acid can be separated from aspidinol and desaspidinol, but all other substances move along the solvent front.

Preliminary attempts to extend the method to quantitative separations have shown promise.

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