Isolation, Structure, and Synthesis of Margaspidin, a New Dryopteris Phloroglucinol Derivative

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Investigation of *Dryopteris marginalis* has resulted in the isolation of a new phloroglucinol derivative, m.p. 178–180°, designated margaspidin. Characterization studies have indicated that it is a two-ring compound consisting of butyryl-3-methylphloroglucinol (II) and aspidinol (V) linked by a methylene bridge (I). The structure (I) has been confirmed by synthesis. Six other phloroglucinol derivatives have been identified and four of them isolated in pure form. These compounds are albaspidin, desaspidin, flavaspidic acid, *para*-aspidin, phloraspin, and phloraspidinol, of which the first three have been chromatographically detected by previous workers. Margaspidin has been found to be the major compound in *D. marginalis* extracts, the content being about ten times higher than that of the other phloroglucinol derivatives present in this *Dryopteris* species.

According to the official compendium (1), aspidium consists of the rhizomes and stipes of the two species of *Dryopteris* ferns—*viz.*, *D. filix-mas* (Linn.) Schott. (European aspidium or male fern) and *D. marginalis* (Linn.) Asa Gray (American aspidium or marginal fern). Although European aspidium has been chemically extensively studied over a century, American aspidium has been investigated only recently.

Hegnauer (2) and Fikenscher and Gibson (3) examined the phloroglucinol derivatives of some American *Dryopteris* species, including *D. marginalis*. They reported the occurrence of desaspidin, flavaspidic acid, and aspidinol as constituents of American aspidium. In addition, Hegnauer (2) detected the presence of albaspidin in some samples of *D. marginalis*. Furthermore, both papers reported indications of some unknown compounds.

The authors' studies on *D. marginalis* confirm the presence of desaspidin, albaspidin, and flavaspidic acid. Aspidinol, however, could not be detected in the extracts of the fern when alkaline treatment was omitted in the isolation procedure.

Recently, Penttilä and Sundman (4) have found that one-ring compounds such as aspidinol identified in *Dryopteris* species appear to be breakdown products of the original two or more ring phloroglucinol derivatives. Decomposition results either from alkaline treatment with magnesium oxide or other bases or application of excess of heat during the isolation procedure. This finding has been confirmed by the work performed in this laboratory (5) using the U.S.P. XVI method for the preparation of crude filicin and employing Ehrlich reagent for the detection.

In the isolation procedure that we followed the ether extract of the rhizome was treated as usual with magnesium oxide (6), but the decomposition of the phloroglucinol derivatives was reduced by a rapid workup of the base-treated extract so as to minimize as much as possible the exposure of the phenols to the basic conditions.

Received April 30, 1965, from the Department of Pharmacognosy and Natural Products, College of Pharmacy, Howard University, Washington, D. C., and the Laboratory of Metabolism, National Heart Institute, U. S. Public Health Service, Bethesda, Md.

Accepted for publication June 7, 1965.

The authors appreciate the cooperation of Dr. Henry M. Fales, Laboratory of Metabolism, Section of Chemistry, National Heart Institute, U. S. Public Health Service, Bethesda, Md.

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In the chemical study on the marginal fern albaspidin, desaspidin, and flavaspidic acid were identified chromatographically, and three other compounds, recently characterized by Penttilä and Sundman, also were identified. These three compounds are phloraspin (7), phloraspidinol (8), and para-aspidin (*p*-aspidin) (9). Identification of albaspidin, desaspidin, *p*-aspidin, and phloraspin was completed by isolating crystalline compounds and comparison with authentic samples.

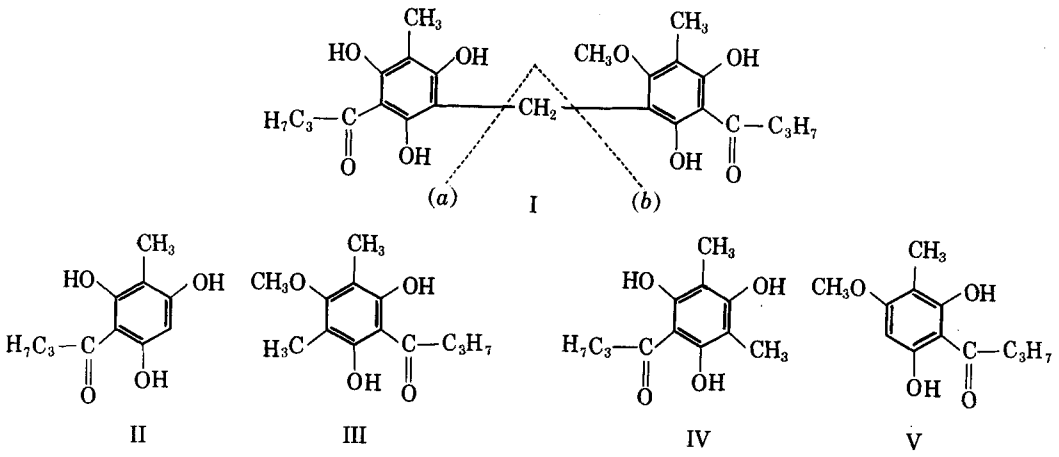
During chromatography of the marginal fern extract, an unidentified phenolic compound was detected on paper chromatograms. Isolation of this phenol was accomplished by chromatography of the purified ether extract on silicic acid column. Comparison with all known *Dryopteris* phloroglucinol derivatives revealed that this compound was hitherto not reported in *Dryopteris* ferns. We assign the structure I to it and propose the name margaspidin for this new phloroglucinol derivative.

Margaspidin, m.p. 178–180°, is a yellow-colored compound crystallizing from carbon tetrachloride. Its ultraviolet spectrum[1] in ethanol showed maximum absorption at 283–284 mμ (ε 20,310); on addition of dilute sodium hydroxide solution, the peak shifted to 290–292 mμ. Its cyclohexane solution showed a maximum absorption at 278 mμ (ε 21,080). Alcoholic solution of margaspidin gives green color with ferric chloride reagent.

As in the case of other *Dryopteris* phloroglucinol derivatives the alkaline cleavage of margaspidin is expected to occur at the methylene bridge as shown in Scheme I. Thus, the decomposition of I along path (*a*) would yield butyryl-3-methylphloroglucinol (II) and methylaspidinol (III), whereas along path (*b*) butyryl-3,5-dimethylphloroglucinol (IV) and aspidinol (V) would be formed. In the alkaline decomposition mixture from margaspidin all the four one-ring compounds were identified by paper chromatographic techniques.

The assigned structure I for margaspidin was confirmed by the following synthesis. Butyryl-3-methylphloroglucinol, aspidinol, and formaldehyde were reacted in dilute alkaline solution. Theoretically, under these conditions formation of three compounds is expected—the two symmetrical compounds—*viz.*, methylene-bis-butyryl-3-methylphloroglucinol and methylene-bis-aspidinol and the required unsymmetrical compound, margaspidin.

[1] U.V., I.R., and NMR spectra of all known *Dryopteris* phloroglucinol derivatives are being separately reported.



Scheme I

TABLE I.—TOXICITY OF *D. marginalis* PHLOROGLUCINOL DERIVATIVES

Compd.	LD_{50}, mg./Kg., Mouse	Confidence Limits, $p = 0.05$
Margaspidin	11.8	13.2–10.5
Phloraspin	11.8	13.8–10.1
Desaspidin	15.8	18.7–13.3
Para-Aspidin	17.0	18.7–15.5
Phloraspidinol	21.5	24.0–19.5
Albaspidin	27.7	30.6–25.0
Flavaspidic acid	94.00	110.0–80.3

Previous synthetic work (7, 8) has indicated that in an alkaline solution aspidinol does not easily condense with formaldehyde to form methylene-bis-aspidinol (10). This fact was utilized and by employing an excess of aspidinol, the yield of the undesirable condensation product—methylene-bis-butyryl-3-methylphloroglucinol—was suppressed, and the formation of the required unsymmetrical compound was increased. This was successfully realized when, from the reaction mixture, isolation of pure margaspidin was accomplished by repeated recrystallizations. The synthetic compound proved to be identical with the isolated margaspidin.

Semiquantitative paper chromatographic analyses (11), performed on several batches of *D. marginalis* rhizomes, indicated that margaspidin is the major phloroglucinol derivative of this fern. The amount of margaspidin chromatographically identified and isolated from the rhizomes is about ten times the amount of any other phloroglucinol derivative present in this plant. It is known that butyryl-filicinic acid is the most common moiety of the two or more ring phloroglucinol derivatives present in all *Dryopteris* species investigated. However, from the exceptionally high amount of margaspidin in *D. marginalis* compared with other phloroglucinol derivatives it follows that butyrylfilicinic acid is only a minor moiety of the total phloroglucinols present in this species. This may be of some interest in the study of biogenesis of *Dryopteris* phloroglucinol derivatives.

Preliminary toxicity of margaspidin and other phloroglucinol derivatives identified in *D. marginalis* has been determined at the Pharmacological Laboratories, Medica Ltd., Helsinki, Finland. The LD_{50} values were obtained by intravenous injection in mice of freshly prepared buffered (pH 8.3–8.5) solutions. Results obtained by following the procedure of Litchfield and Wilcoxon (12) are recorded in Table I.

EXPERIMENTAL[2]

Dried powdered rhizome[3] of *D. marginalis* (31.6 Gm.) was macerated 8 hr. with 350 ml. of ether. The ether extract was filtered and the residue washed with small amounts of the solvent. The extract and washings were evaporated to dryness. The residue (3.4 Gm.) was treated with magnesium oxide according to Ackermann and Muehlemann (6). The raw filicin (0.69 Gm.) obtained was dissolved in chloroform–cyclohexane mixture (1:1) and chromatographed on a column of silicic acid (20 Gm.). The chromatogram was developed by elution with a chloroform–cyclohexane (1:1) mixture which had been previously washed with water to remove traces of alcohol from the chloroform and dried over sodium sulfate. Forty-milliliter fractions were collected, each fraction was evaporated to dryness, and a small amount of the residue of each fraction was dissolved in acetone and chromatographed on buffered papers according to Penttilä and Sundman (11). The phloroglucinol derivatives identified in each fraction were as follows.

Fractions 1 and 2 were combined and the residue purified by recrystallization from acetone, whereby 1.5 mg. of a colorless compound, m.p. 150–152°, was obtained. This compound was identified as albaspidin by mixed melting point and comparison of the infrared spectrum with that of an authentic sample.

The combined residues of fractions 3 to 7 yielded on repeated recrystallizations from methanol 13 mg.

[2] The U.V. and I.R. spectra were determined by Mrs. K. S. Warren, National Heart Institute, Bethesda, Md., to whom the authors are thankful. Microanalyses were performed by Mr. J. F. Alicino, Metuchen, N. J., and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Melting points were determined on a Kofler micromelting point apparatus and are corrected.

[3] The plant material used was obtained from *D. marginalis* which was collected from Maryland and kindly provided by Mrs. Margaret Donnald, Potomac, Md. The identity of the plant was confirmed by Professor R. Hegnauer, Laboratorium Voor Experimentale Plantensystematik, Leiden, Holland. The authors are grateful to Mrs. Donnald and Professor Hegnauer.

of a yellow compound, m.p. 123–124°. This phloroglucinol derivative was identified as *p*-aspidin by comparison (mixed melting point and infrared spectrum) with an authentic sample.

Paper chromatography of fractions 8 to 11 indicated that the phloroglucinol derivatives in these fractions consisted of a mixture of *p*-aspidin and desaspidin. Crystalline compounds were not obtained but examination of the subsequent fractions, 12 to 17, revealed that desaspidin was an almost exclusive compound present. The combined residue of these latter fractions yielded, on recrystallization from acetone, 21 mg. of the pure compound, m.p. 148–150°. Mixed melting point determination with an authentic sample of desaspidin showed no depression, and the infrared absorption spectra of the isolated and the authentic compounds were identical.

Chromatographic examination of the residues from fractions 18 to 25 revealed a mixture of phloroglucinol derivatives. Flavaspidic acid and phloraspidinol were identified in this mixture by paper chromatographic comparison with authentic samples. The amounts of these compounds were, however, too small for completing the isolation.

Paper chromatography of the fractions 26 to 30 and comparison with all known *Dryopteris* phloroglucinol derivatives suggested that these fractions contained the new phenol, margaspidin. The R_f value on papers buffered to pH 8.8 was 0.08, pH 8.4 was 0.69, and pH 8.2 was 0.86. Repeated recrystallizations yielded 119 mg. of yellow crystals, m.p. 178–180°, of pure margaspidin [$\nu_{max.}^{CHCl_3}$ 3200 (broad), 1610 (broad), 1154, and 1128 cm.$^{-1}$].

Anal.—Calcd. for $C_{24}H_{30}O_8$: C, 64.57; H, 6.72. Found: C, 64.51; H, 6.81.

After fraction 30 was collected, the silicic acid column was eluted with chloroform to yield fractions 31 and 32. Paper chromatography of these fractions indicated phloraspin to be an almost exclusive compound in these fractions. Repeated recrystallizations of the residues of 31 and 32 furnished 3 mg. of the pure compound, m.p. 212–213°, identified by mixed melting point with an authentic sample of phloraspin and identical infrared absorption spectra of the two samples.

Alkaline Cleavage of Margaspidin.—Margaspidin (25 mg.) was dissolved in 30 ml. of 5% aqueous sodium hydroxide solution, 300 mg. of zinc dust was added, and the mixture heated on a steam bath for 5 min. Thirty milliliters of water was added, and the cooled mixture was filtered, acidified with 10% sulfuric acid, and extracted with ether. The ethereal solution was evaporated to dryness and the residue dissolved in acetone. The acetone solution was used for paper chromatography employing two different solvent systems.

Papers buffered to pH 4.0 and impregnated with formamide (11) were developed with cyclohexane–chloroform (1:1) mixture and tetrazotized di-*o*-anisidine (fast blue salt B, E. Merck) reagent was employed as a chromogen. Three compounds which could be chromatographed under these conditions were identified by identical R_f values and coloration with those of authentic samples of aspidinol (0.59, purple), methylaspidinol (0.91, light pink), and butyryl-3,5-dimethylphloroglucinol (0.04, yellow). The fourth compound, which could not be moved under these conditions from the starting point, yielded a purple coloration with the fast blue salt B. An authentic sample of butyryl-3-methylphloroglucinol was found to behave similarly. Further confirmation of the presence of all these four compounds was accomplished by using unbuffered papers and tetraline–acetic acid–water (10:10:1) as solvent mixture. The R_f values of the four spots which were found to be identical with authentic samples were: butyryl-3-methylphloroglucinol, 0.21; butyryl-3,5-dimethylphloroglucinol, 0.66; aspidinol, 0.70; and methylaspidinol, 0.85.

Synthesis of Margaspidin.—Butyryl-3-methylphloroglucinol (224 mg.) and aspidinol (448 mg.) were dissolved in 50 ml. of 1% aqueous sodium hydroxide, and 0.75 ml. of 4% formaldehyde was added. The mixture was kept at room temperature for 5 min., then acidified with 10% hydrochloric acid. The precipitate was filtered, washed with water, and dried. To remove the excess of aspidinol, the precipitated product was dissolved in 50% alcohol and allowed to stand at room temperature overnight. A precipitate obtained on standing was filtered and further purified by repeated recrystallizations from carbon tetrachloride to furnish yellow crystals, m.p. 178–180°. Mixed melting point determination of this crystalline compound and the isolated margaspidin showed no depression, and the ultraviolet and infrared absorption spectra of the synthetic and the natural specimens were identical.

Anal.—Calcd. for $C_{24}H_{30}O_8$: C, 64.57; H, 6.72. Found: C, 64.60; H, 6.92.

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