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Phloraspyron and Phloraspidinol, New Phloroglucinol Derivatives from *Dryopteris* Ferns

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From the fern *Dryopteris austriaca* two new phloroglucinol derivatives, phloraspyron and phloraspidinol, have been isolated. Both are dimerous compounds with desaspidinol as one moiety. Phloraspyron (I) is composed of desaspidinol and 6-propyl-2,3-dihydropyran-2,4-dione bound by a methylene bridge. Phloraspidinol (II) is likewise a methylene compound consisting of desaspidinol and aspidinol. In addition to these two substances, the symmetrical methylene-bis-desaspidinol (III) has been shown to be a naturally occurring phloroglucinol derivative in *Dryopteris austriaca*.

The "raw aspidin" obtained by MgO [1] treatment of *Dryopteris austriaca* extract usually contains varying amounts of butyrylphloroglucinol-4-methyl ether, known as desaspidinol.[2,3] This compound can only be detected after alkali treatment of *Dryopteris* extracts, however, and must therefore be considered a decomposition product. The readiness with which free desaspidinol is formed implies the presence of compounds readily decomposing with liberation of desaspidinol. Such sources of desaspidinol are two previously isolated compounds, desaspidin [2,4] and phloraspin,[5] which may decompose in alkaline conditions to form free desaspidinol, as shown in Fig. 1. In this paper two new compounds of this type, called phloraspyron and phloraspidinol, are presented. In addition, a fifth compound containing desaspidinol, the symmetrical methylene-bis-desaspidinol previously synthesized by us [3,10] and hitherto known only as a synthetic compound, has now been found to be a naturally occurring phloroglucinol derivative in *Dryopteris austriaca* rhizomes.

By paper chromatography on buffered and formamide-impregnated papers phloraspyron and phloraspidinol were found to have the R_F-values shown in Fig. 2. The separation was carried out by the same method as before,[6] but instead of benzene and chloroform (1:1) a mixture of cyclohexane and chloroform (1:1) was used as eluent. The spots were detected with tetrazotized di-*o*-anisidine ("fast blue salt B", Merck) reagent, which gave a purple spot for phloraspyron and a red one for phloraspidinol.

By the technical method for isolation of phloroglucinol derivatives from *Dryopteris* extracts, a residue was obtained after the bulk of the aspidin,

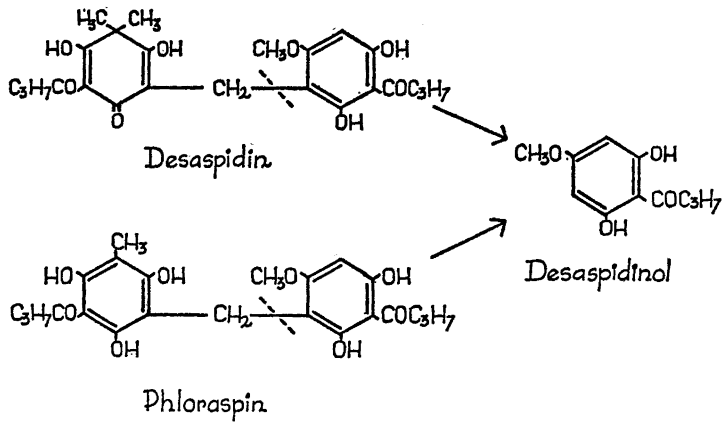


Fig. 1. Liberation of desaspidinol through decomposition of desaspidin and phloraspin in alkaline conditions.

albaspidin, phloropyron, desaspidin, and flavaspidic acid had been removed from the extract. Paper chromatographic analyses of the residue revealed the new phloroglucinol derivatives discussed in this paper. This residue was also used as source material for both phloraspyron and phloraspidinol. For isolation of phloraspyron it was further concentrated in respect of the compound sought and, finally, column chromatography was carried out on silica gel. Mixtures of benzene and hexane were used as eluents and the fractions rich in phloraspyron were combined. The phloraspyron obtained in this way was practically pure, containing only traces of desaspidin and albaspidin. After recrystallization from cyclohexane, phloraspyron was obtained as white crystals melting at 135—136°.

Alkaline cleavage of phloraspyron yielded desaspidinol, aspidinol and 6-propyl-2,3-dihydropyran-2,4-dione, all identified by paper chromatography.[6] The structure (I) of phloraspyron was confirmed by synthesis, desaspidinol and 6-propyl-2,3-dihydropyran-2,4-dione being condensed with formaldehyde in a dilute alkaline solution. The synthesis yielded mainly phloraspyron and methylene-bis-desaspidinol; the latter was removed by treatment with 50 % methanol and by recrystallization from cyclohexane. The melting point and the mixed melting point with natural phloraspyron were 135—136°.

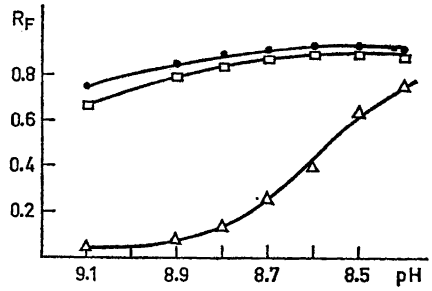
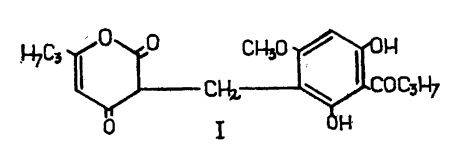
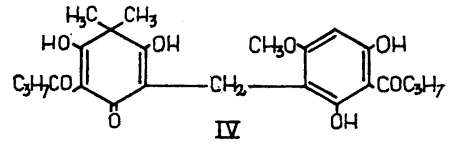


Fig. 2. Variation of the R_F-values with the pH of the papers. △ Phloraspyron; ● Phloraspidinol; ⊡ Methylene-bis-desaspidinol.



I



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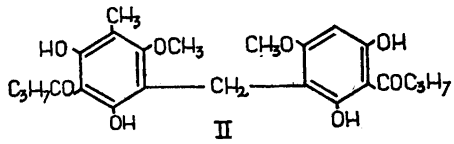
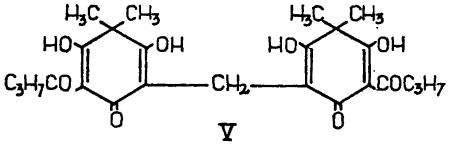


II



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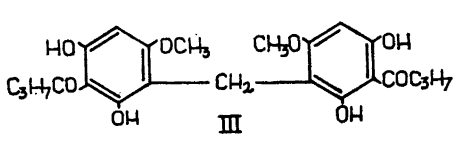
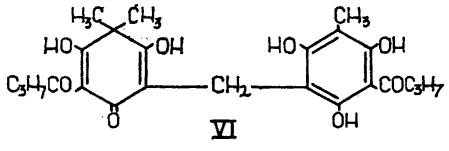
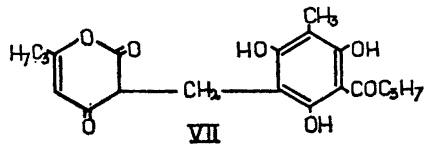


III



VI

Phloraspyron and phloropyron,[7] the latter previously isolated by us, can be derived from desaspidin (IV) or albaspidin (V), respectively, by replacing the 3-butyrylfilicinic acid moiety of these molecules with 6-propyl-2,3-dihydropyran-2,4-dione. The corresponding compound derived from flavaspidic acid (VI), for which the name flavaspyron is suggested, was similarly expected to be a naturally occurring phloroglucinol derivative in *Dryopteris*. Flavaspyron (VII) was synthesized from butyryl-3-methylphloroglucinol and 6-propyl-2,3-dihydropyran-2,4-dione condensed with formaldehyde, and was obtained as yellow crystals melting at 190—191°. At paper chromatography[6] performed on papers buffered to pH 4.0 and 5.0, flavaspyron showed the R_F-values 0.34 and 0.10, respectively. However, accurate chromatographic analyses of *Dryopteris* extracts and the phloroglucinol derivative concentrates prepared from the extracts resulted in a total absence of spots with such R_F-values. Thus, contrary to expectations, flavaspyron is not naturally occurring in *Dryopteris*. This observation was further confirmed by extremely sensitive thin-layer chromatographic methods,[8,9] by which no spots identical with the synthetic flavaspyron could ever be detected.



VII

In order to isolate phloraspidinol, the residue mentioned above was treated with various cold solvents. After chromatographic analyses the fractions containing phloraspidinol could be recovered and combined. In this way phloras-

pidinol was obtained together with another very similar compound from which it could only be separated with difficulty. Repeated recrystallizations from methanol, in which phloraspidinol was somewhat less soluble, finally yielded pure phloraspidinol, which after further recrystallization from cyclohexane had a melting point of 193—194°.

The related compound was identified as methylene-bis-desaspidinol (III), which up to now has not been reported as a naturally occurring phloroglucinol derivative. The synthetic methylene-bis-desaspidinol was earlier obtained as a byproduct in the syntheses of phloraspin [5] and desaspidin [10] and was synthetically prepared from desaspidinol and formaldehyde.[10] In a pure state methylene-bis-desaspidinol has a melting point of 174—175°.

As a result of the very similar chemical properties of phloraspidinol and methylene-bis-desaspidinol the R_F-values of these two compounds differ only slightly from each other (Fig. 2). A satisfactory separation was obtained only on papers buffered to pH 9.1.

Alkaline decomposition of phloraspidinol yielded desaspidinol and aspidinol both identified by paper chromatography.[6] On this basis the structure of phloraspidinol could be given as (II) and was later confirmed by synthesis. Theoretically, desaspidinol and aspidinol condensed by means of formaldehyde should yield a mixture of phloraspidinol, methylene-bis-desaspidinol and methylene-bis-aspidinol. Because, however, aspidinol in an alkaline solution is known to condense only poorly with formaldehyde to form methylene-bis-aspidinol,[11,12] an excess of aspidinol was expected to increase the yield of phloraspidinol at the expense of methylene-bis-desaspidinol. In this way we intended to avoid the formation of the same mixture as that obtained from natural sources and which we knew to be difficult to separate. This intention was realized, and the synthetic product consisted mainly of phloraspidinol and unreacted aspidinol, with only traces of methylene-bis-desaspidinol. Pure phloraspidinol was obtained in the same way as the natural phloraspidinol and had a melting point and mixed melting point of 193—194°.

EXPERIMENTAL

Isolation of phloraspyron. In the technical procedure for isolation of aspidin, albaspidin, phloropyron, desaspidin and flavaspidic acid from *Dryopteris austriaca* extract, a residue remained as a methanolic solution. This solution was partially precipitated by addition of diluted methanol until the methanol concentration was about 50 %. The precipitate contained no phloraspyron and was discarded. The dilute methanolic solution was then quantitatively precipitated with dilute hydrochloric acid and the precipitate obtained was dried in a vacuum. This phloraspyron concentrate, consisting mainly of phloraspyron and aspidinol was dissolved in ether and extracted with 2 % sodium carbonate, the aspidinol remaining in the ethereal fraction. The alkaline solution was precipitated with hydrochloric acid, and the precipitate filtered off, washed with water and dried. The powder obtained was chromatographed through a silica gel column using hexane mixed with increasing amounts of benzene as eluent. The fractions were analyzed by paper chromatography.[6] The first fractions obtained with the hexane-benzene (1:1) mixture, which resulted in practically pure phloraspyron, were combined and recrystallized from cyclohexane. The melting point of pure phloraspyron was 135—136°. (Found: C 63.58; H 6.40; OCH_3 8.19. Calc. for $C_{20}H_{24}O_7$: C 63.83; H 6.38; OCH_3 8.25.)

Isolation of phloraspidinol. The methanolic solution mentioned above was dried in a vacuum and repeatedly treated with cold hexane, cyclohexane, benzene and methanol.

After every treatment the portions containing phloraspidinol and methylene-bis-desaspidinol, discovered by paper chromatography,[6] were recovered. The final separation of these two compounds was achieved by recrystallization from methanol. Phloraspidinol in a pure state showed a melting point of 193—194°. (Found: C 63.75; H 6.62; OCH_3 14.01. Calc. for $C_{24}H_{30}O_8$: C 64.57; H 6.73; OCH_3 13.90.)

Alkaline cleavage of phloraspyron. Phloraspyron (20 mg) was dissolved in aqueous sodium hydroxide (5 %, 20 ml), zinc dust (100 mg) was added and the mixture was heated on a steam bath for 5 min. The cooled solution was filtered and diluted with water, acidified with dilute sulphuric acid and extracted with ether. The ethereal solution was evaporated to dryness and the residue dissolved in acetone and chromatographed on papers buffered to pH 8.8.[6] Two main spots were obtained, the R_F-values and colours of which were identical with those obtained by chromatography of authentic specimens of desaspidinol, m.p. 127—128°, and aspidinol, m.p. 142—143°.

For detection of 6-propyl-2,3-dihydropyran-2,4-dione, phloraspyron was dissolved in sodium carbonate (2 %) and boiled for some minutes.[7] The cooled solution was made acidic with hydrochloric acid and kept at room temperature overnight. The clear liquid was poured off and extracted with chloroform. The concentrated solution was paper-chromatographed on unbuffered paper. The main spot was identical with that obtained by chromatography of an authentic specimen of 6-propyl-2,3-dihydropyran-2,4-dione, m.p. 93—94°.[7]

Alkaline cleavage of phloraspidinol. The alkaline cleavage of phloraspidinol was performed analogously with the alkaline cleavage of phloraspyron. At chromatography of the decomposition mixture only aspidinol and desaspidinol were identified.

Synthesis of phloraspyron. Desaspidinol (210 mg) and 6-propyl-2,3-dihydropyran-2,4-dione (154 mg) were dissolved in potassium hydroxide (20 ml, 1 %), and formaldehyde (0.75 ml, 4 %) was added. The mixture was kept at room temperature for 1 min and acidified with dilute hydrochloric acid. The precipitate was filtered off, washed with water and dried. The product was dissolved in a minimum of methanol and an excess of 50% methanol was added. The precipitate formed contained the bulk of the methylene-bis-desaspidinol; phloraspyron was precipitated from the filtrate by dilution with water. After several recrystallizations from cyclohexane the product had a m.p. of 135—136° and with natural phloraspyron a mixed m.p. of 135—136°. (Found: C 63.63; H 6.26; OCH_3 7.89. Calc. for $C_{20}H_{24}O_7$: C 63.83; H 6.38; OCH_3 8.25.)

Synthesis of phloraspidinol. The synthesis was carried out as above, starting from desaspidinol (210 mg) and aspidinol (448 mg). The dried product was recrystallized from methanol and yielded pure phloraspidinol, m.p. and mixed m.p. with natural phloraspidinol 193—194°. (Found: C 63.75; H 6.63; OCH_3 13.73. Calc. for $C_{24}H_{30}O_8$: C 64.57; H 6.73; OCH_3 13.90.)

Synthesis of flavaspyron. Butyryl-3-methylphloroglucinol (210 mg) and 6-propyl-2,3-dihydropyran-2,4-dione (154 mg) were condensed with formaldehyde as described above. The purification of the raw product was achieved by recrystallization from cyclohexane and carbon tetrachloride. The m.p. of pure flavaspyron is 190—191°. (Found: C 62.05; H 6.26. Calc. for $C_{20}H_{24}O_7$: C 63.83; H 6.38.)

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