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# Para-aspidin, a New Phloroglucinol Derivative from Dryopteris Ferns

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Para-aspidin (I), isolated from *Dryopteris* species, has been shown to be an isomer of aspidin (II) differing from the same only through the position of the methoxy group. The structure of para-aspidin is confirmed by synthesis.

In our earlier reports concerning the phloroglucinol derivatives from *Dryopteris* ferns the structures of phloropyron [1] and phloraspin [2] are given. In addition to these substances we now have isolated and examined a new compound for which we suggest the name para-aspidin.

Para-aspidin is a yellow substance, melting point 123—125°, soluble in most organic solvents except in methanol and ethanol. It is a monobasic acid the analytical data of which agree with the formula $C_{28}H_{32}O_8$. The molecule was shown to contain one methoxy group. In alcoholic solution the ferric chloride reaction of para-aspidin is brownish red.

The ultraviolet absorption curves of aspidin and para-aspidin in cyclohexane solution are shown in Fig. 1. The maxima of aspidin occur at 230 mμ ($\varepsilon$ = 27 100) and at 292 mμ ($\varepsilon$ = 20 000), earlier reported 230 mμ ($\varepsilon$ = 25 500) and 290 mμ ($\varepsilon$ = 21 300) [3]. Para-aspidin has maxima at 228 mμ ($\varepsilon$ = 27 100) and at 271 mμ ($\varepsilon$ = 21 600).

From the similar solubilities of aspidin and para-aspidin it follows that the paper chromatographic separation of these substances is rather difficult. When the chromatography is performed with benzene-chloroform (1:1) solvent on buffered and formamide impregnated papers [4] para-aspidin appears to have at each pH somewhat lower $R_F$-values than aspidin, but the differences are too small for identifying the two substances. With tetrazotized di-*o*-anisidine [4] as reagent for detection of the spots they could, however, be identified. With this reagent aspidin yields a yellow spot whereas that of para-aspidin is red-brown.

Among the decomposition products obtained by an alkaline cleavage [5] of para-aspidin either along A - - - A or B - - - B, 3-butyrylfilicinic acid (III), aspidinol (IV) and methylaspidinol (V) could be identified. On the basis of

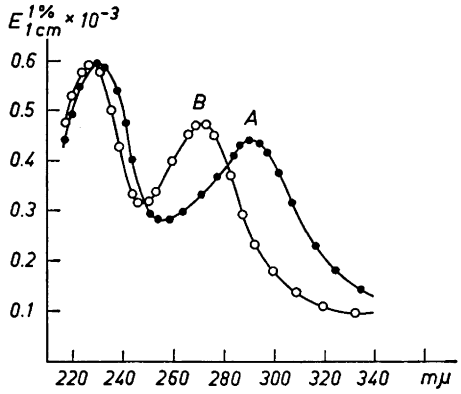


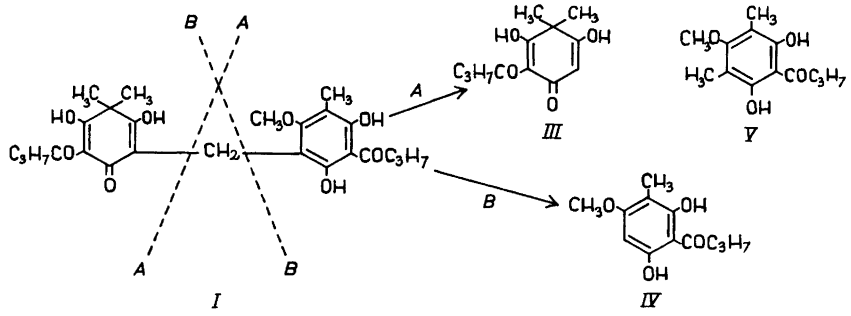

*Fig. 1.* Ultraviolet absorption spectra of aspidin (A) and para-aspidin (B) in cyclohexane.

these facts the structure of para-aspidin could be represented as (I). The structure is identical with that given by Boehm [6] for aspidin, m.p. 123—124°, which substance later has been shown actually to have the structure (II) [3,7]. Because both of these substances are included in the naturally occurring phloroglucinol derivatives of *Dryopteris* species there is every reason to question whether Boehm really dealt with aspidin or para-aspidin, both being yellow compounds with the formula $C_{25}H_{32}O_8$ and both having equal melting points as well as similar solubilities and ferric chloride reactions. Boehm's descriptions of aspidin include some derivatives of the same namely aniline and phenylhydrazine derivatives. These compounds were first prepared by Poulsson [8]; Boehm made use of them on purpose to prove his aspidin to be identical with the polystichin of Poulsson. Our efforts to carry out the same reactions resulted in an intensive yellow aniline derivative of aspidin, m.p. 132—134°, earlier reported 132° by Boehm [6] and Poulsson [8]. The aniline derivative of para-aspidin was pale yellow in colour and had a m.p. of 168—170° (decomp.). For the phenylhydrazone of aspidin a m.p. of 208—209° was observed, earlier reported 209° by Boehm [6] and 200—203° by Poulsson [8]. By the method of these authors we failed to obtain any crystalline phenylhydrazone of para-aspidin.

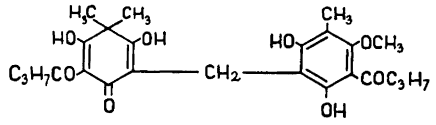
These experiments furnish positive proof of the substance examined by Boehm being aspidin (II) and not para-aspidin (I) despite the structural formula given by Boehm.

Semiquantitative paper chromatographic analysis [4] showed that the amounts of para-aspidin in both *Dryopteris austriaca* and *Dr. filix mas* varied considerably, the average content being about 0.2—0.4 % of the air dried rhizomes. The quantities actually isolated from the material were always considerably smaller which is due to the easily occurring decomposition of para-aspidin especially under alkaline conditions. Probably this also is the reason why para-aspidin so long has escaped detection in spite of thorough investigations in this field.

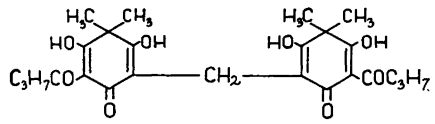
For synthesizing para-aspidin (I) 3-butyrylfilicinic acid (III) and aspidinol (IV) were condensed with formaldehyde in dilute alkaline solution. The yield



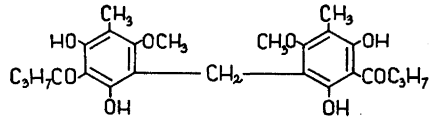
*I* *III* *V* *IV*



*II*



*VI*



*VII*

of para-aspidin hereby was remarkably high which was due to the fact that of the two possible symmetrical compounds, albaspidin (VI) and methylene-bis-aspidinol (VII), only small amounts of albaspidin were formed whereas not even traces of methylene-bis-aspidinol could be detected. The synthesis of aspidin is reported to proceed similarly: the reaction yields mainly aspidin at the cost of the two possible symmetrical compounds [7]. Para-aspidin was purified by recrystallizing from methanol and as pure had a melting point of 124—125° showing no depression on mixing with natural para-aspidin.

## EXPERIMENTAL

*Isolation of para-aspidin.* The finely ground rhizomes of *Dryopteris austriaca* (Jacq.) Woynar were extracted with ether for 8 h. (*Subsp. eu-spinulosa* (A. et G.) Hyl. is preferably chosen as source for para-aspidin because of its low content of flavaspidic acid.) The solvent was distilled off and the residue freed of fatty materials by MgO treatment [9]. The "raw aspidin"so obtained was dissolved in methanol and kept for fractional crystal-

lization at 4°. By the help of paper chromatographic analysis [4] the fraction of para-aspidin together with small amounts of albaspidin, aspidin and desaspidin could be separated. Repeated recrystallizations from methanol yielded pure para-aspidin, m.p. 123—125°. (Found: C 64.62; H 7.01; $OCH_3$ 7.16. Calc. for $C_{25}H_{32}O_8$: C 65.22; H 6.95; $OCH_3$ 6.75.)

*Alkaline cleavage of para-aspidin.* Para-aspidin (100 mg) was dissolved in aqueous sodium hydroxide (5 %, 20 ml), zinc dust (200 mg) was added and the mixture was heated on a steam bath for 5 min. The cooled solution was diluted with water, acidified with sulphuric acid (10 %) 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 and 5.0 [4]. On the paper pH 8.8 two main spots were obtained, the $R_F$-values and colours of which were identical with those obtained by chromatography of authentic specimens of aspidinol, m.p. 142—143°, $R_F$ 0.83, purple spot and methylaspidinol, m.p. 111°, $R_F$ 0.90, yellow spot. On the paper pH 5.0 a carmine red spot with $R_F$ 0.35 was obtained and it was identical with that of 3-butyrylfilicinic acid, m.p. 98°.

*Aniline and phenylhydrazine derivatives of aspidin and para-aspidin.* According to Boehm [6] the aniline derivatives of aspidin and para-aspidin were prepared as follows: aspidin (460 mg), respectively para-aspidin (460 mg), was mixed with aniline (400 mg, b.p. 184°) and the mixture heated on a steam bath for 5 min and then kept over sulphuric acid over night. After adding abs. ethanol (4 ml) a crystalline precipitate was obtained in a few hours. Recrystallizations from abs. ethanol yielded the aniline derivatives in a pure state showing the melting points mentioned before. An elementary analysis of the aniline derivative of para-aspidin was performed. (Found: C 69.50; H 7.16; N 2.78. Calc. for $C_{31}H_{37}NO_7$: C 69.53; H 6.91; N 2.61.)

The phenylhydrazone of aspidin was prepared according to Boehm [6] whereby a crystalline compound melting at 208—209° was obtained. When para-aspidin was similarly treated with phenylhydrazine only dark coloured amorphous compounds were formed. All attempts to modificate the method in order to obtain crystalline products were unsuccessful.

*Synthesis of para-aspidin.* 3-Butyrylfilicinic acid (224 mg) and aspidinol (224 mg) were dissolved in aqueous potassium hydroxide (10 ml, 1 %) and formaldehyde (0.75 ml, 4 %) was added. The mixture was kept at room temperature for 15 min and made acidic with hydrochloric acid (10 %). The precipitate was filtered off, washed with water and dried. After recrystallizations from methanol the product had a m.p. of 124—125° and with natural para-aspidin a mixed m.p. of 123—125°. (Found: C 65.72; H 6.90; $OCH_3$ 7.08. Calc. for $C_{25}H_{32}O_8$ C 65.22; H 6.95; $OCH_3$ 6.75.)

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