The Structure of Ortho-desaspidin

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Condensation of 3-butyrylfilicinic acid (I) and o-desaspidinol (II) with formaldehyde yields a new compound called ortho-desaspidin, an isomer of the naturally occurring Dryopteris phloroglucinol derivative desaspidin (III). To establish which of the two possible structures (IV) and (V) was to be assigned to ortho-desaspidin, ultraviolet absorption measurements were carried out and ortho-desaspidin was found to have the structure (IV).

Ortho-desaspidin has also been shown to occur naturally in Dryopteris austriaca rhizomes, from which it has been isolated, and proved to be identical with the synthetic ortho-desaspidin.

Common among the naturally occurring phloroglucinol derivatives are the two-ring compounds A—CH_2—B, where A is butyrylfilicinic acid (I) and B is a methoxylated derivative of either butyrylphloroglucinol or butyryl-3-methylphloroglucinol. Of the three possible compounds having a methoxylated butyryl-3-methylphloroglucinol as B, two have been isolated from Dryopteris species, namely aspidin [1-3] (VI) and para-aspidin [4] (VII). The

Table 1. Physical, chemical and chromatographical properties of the phloroglucinol derivatives A-CH_2-B, where A is butyrylfilicinic acid and B is a methoxylated derivative of either butyrylphloroglucinol or butyryl-3-methylphloroglucinol.

Compound	Formula	Structure	Melting point	Ultraviolet absorption maxima in cyclohexane				R_F values on buffered papers[16,17]		Colour reaction (tetrazotized di-o-anisidine)
				mμ	ε	mμ	ε	pH 8.8	pH 9.1	
Desaspidin	$C_{24}H_{30}O_8$	III	151—153°	231	31 200	274	23 800	0.47	0.09	Purplish red
Ortho-desaspidin	»	IV	133—134°	230	24 900	293	23 800	0.77	0.33	Orange red
Para-aspidin	$C_{25}H_{32}O_8$	VII	123—125°	228	27 100	271	21 600	0.65	0.21	Red brown
Aspidin	»	VI	123—124°	230	27 100	292	20 000	0.74	0.25	Yellow
Isoaspidin	»	VIII	152—154°	228	30 300	292	21 600	0.48	0.09	Yellow (turns greyish on standing)

third compound, called isoaspidin (VIII), has never been found in *Dryopteris* but has now been synthesized from butyrylfilicinic acid and isoaspidinol [5] (IX) condensed with formaldehyde. It is a pale yellow compound with a melting point of 152—154° (Table 1).

Of the three possibilities in which B is a methoxylated butyrylphloro-glucinol, the 4-methoxy alternative called desaspidin [6],[7] (III) has been isolated from *Dryopteris* ferns. Since desaspidin, like para-aspidin and in contrast to aspidin, has the methoxy group in the *para* position in relation to the butyryl group, a more correct name for desaspidin would have been despara-aspidin. The name desaspidin, however, is so well-established that to change it is out of the question.
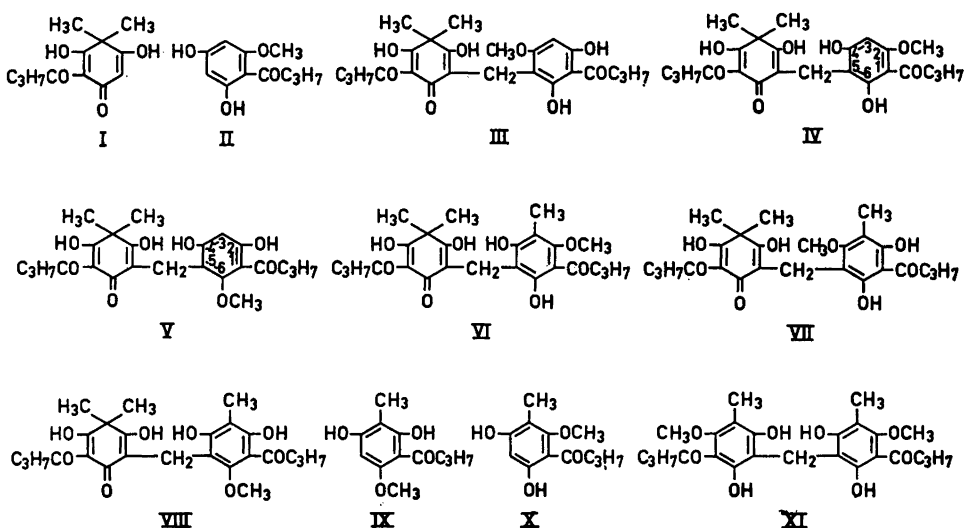
The two isomers of desaspidin with the methoxy group in the *ortho* position to the butyryl group are the compounds with the structures (IV) and (V). Theoretically, both of them might be expected to be formed when buty-rylfilicinic acid (I) and *o*-desaspidinol [8],[9] (butyrylphloroglucinol-2-methyl ether) (II) are condensed with formaldehyde. The synthesis yields only one of them, however, a pale yellow compound melting at 133—134°, which hereafter is called ortho-desaspidin (Table 1).

To decide which of the two possible structures (IV) and (V) was to be as-signed to ortho-desaspidin, alkaline cleavage was first carried out. This method has often been used on purpose to break up methylene bridges so as to liberate and identify the one-ring compounds formed. It has turned out to be a valuable tool in resolving the structures of unknown *Dryopteris* phloroglucinol deriv-atives, *e.g.* phloropyron,[10] phloraspin,[9] para-aspidin,[4] and filixic acids.[11] If ortho-desaspidin has the structure (IV), the one-ring compound decisive of this structure, isoaspidinol (IX), should be identified in the decomposition

mixture, whereas if it has the structure (V) it should yield ψ-aspidinol (X). Unfortunately, this method was not applicable in the case of ortho-desaspidin, because the decomposition mixture was found to contain methylene-bis-*o*-desaspidinol as a by-product. The same compound can be obtained by condensing *o*-desaspidinol with formaldehyde and, at least theoretically, it may consist of a mixture of three isomeric compounds which in alkaline cleavage theoretically can yield both isoaspidinol and ψ-aspidinol.[8] Therefore, nothing could be concluded from the alkaline decomposition about the structure of ortho-desaspidin.

The formation of methylene-bis-*o*-desaspidinol is analogous to the formation of ψ-aspidin[1] (XI) from aspidin under similar conditions. ψ-Aspidin has been reported to be extremely resistant to alkali, and a similar alkali resistance could also be observed in the case of methylene-bis-*o*-desaspidinol. Because of this similarity it would appear that the structure (IV) in which the methoxy group is in the *para* position to the methylene bridge as it also is in aspidin, is the more probable for ortho-desaspidin. This interpretation could not be considered proof of the structure, however, chiefly because nothing is known about the reactions of isoaspidin under similar conditions.

Since attempts to determine the structure of ortho-desaspidin by chemical methods failed, comparative spectral studies were carried out. These turned out to be more successful in deciding which of the two possible structures (IV) and (V) was to be assigned to ortho-desaspidin.

According to previous reports the ultraviolet absorption spectra of the *Dryopteris* phloroglucinol derivatives depend on the polarity and pH of the solvents used.[12,13] This is a consequence of the β-diketonic character of these substances and indicates different degrees of enolization and dissociation in different solutions.[13,14] In order to obtain the most constant conditions, all measurements were made in ethanol solution containing small amounts of either hydrochloric acid or sodium hydroxide, by which a minimum or a maximum of dissociation, respectively, was aimed at. Generally, the *Dryopteris* phloroglucinol derivatives have maxima in two wave length regions, namely 215—240 mμ and 240—380 mμ.[13] Because the most characteristic changes induced by the keto-enol tautomerism fall within the longer wave length region, this region was used for the comparative absorption measurements.

The next higher homologues of the two possible ortho-desaspidin structures (IV) and (V) with a methyl group in position 3 are the two previously mentioned compounds aspidin (VI) and isoaspidin (VIII). In an ethanol solution containing small amounts of hydrochloric acid (0.01 N), aspidin and ortho-desaspidin have a maximum at 290 mμ, whereas the maximum of isoaspidin is at 286 mμ, all three being of about the same intensity ($\varepsilon = 21\,000-24\,000$).

In an ethanol-sodium hydroxide solution (0.01 N) aspidin and ortho-desaspidin absorb at 320 mμ ($\varepsilon = 15\,900$ and $18\,700$, respectively) while isoaspidin has a more intense maximum at 344 mμ ($\varepsilon = 29\,800$).

Because the absorption maxima of ortho-desaspidin in both acid and alkaline ethanol solutions occur at exactly the same wave lengths as the corresponding maxima of aspidin but differ from the maxima of isoaspidin, the structure (IV), in which the methoxy group is in the *para* position to the methylene

bridge, as it also is in aspidin, must be considered more probable than the structure (V).

Further, in ethanol solution containing hydrochloric acid the corresponding methoxy-containing one-ring compounds absorb at the following wave lengths: o-desaspidinol (II) at 286 mμ, ψ-aspidinol (X) at 282 mμ and isoaspidinol (IX) at 290 mμ. Thus, the substitution of a methyl group into the *ortho* position to the methoxy group of o-desaspidinol causes a hypsochromic shift, whereas the substitution of a methyl group into the *para* position gives a bathochromic shift.

This rule also applies to the next higher homologues of ψ-aspidinol and isoaspidinol. In ψ-aspidinol the substitution must occur into the *para* position to the methoxy group, whereas in isoaspidinol only the *ortho* position is available. The compounds obtained through these substitutions are identical (butyryl-3,5-dimethylphloroglucinol-2-methyl ether), and have an absorption maximum at 285 mμ, from which it follows that a *para* substitution in relation to the methoxy group has caused a bathochromic shift and an *ortho* substitution a hypsochromic one.

When, instead of a methyl group, a butyrylfilicinic acid unit is substituted through a methylene bridge, aspidin is obtained from ψ-aspidinol and isoaspidin from isoaspidinol. Here again the *para* substitution results in a bathochromic shift, ψ-aspidinol and aspidin absorbing at 282 mμ and 290 mμ, respectively. The *ortho* substitution yields isoaspidin with a maximum at 286 mμ and, consequently, causes a hypsochromic shift, since isoaspidinol absorbs at 290 mμ.

Correspondingly, the butyrylfilicinic acid unit can be substituted into o-desaspidinol, the substitution occurring either at the *para* or the *ortho* position to the methoxy group. Ortho-desaspidin, the compound obtained through either of these substitutions, has a maximum at 290 mμ and o-desaspidinol a maximum at 286 mμ. Thus the substitution has resulted in a bathochromic shift and so must have taken place in the *para* position and the structure of ortho-desaspidin, therefore, is established as (IV).

In consideration of its structure, the natural occurrence of ortho-desaspidin was to be expected: its next higher homologue, aspidin, as well as their corresponding isomers desaspidin and para-aspidin, all occur naturally in *Dryopteris* species.

According to expectation ortho-desaspidin was actually found to be a natural phloroglucinol derivative and it was isolated from the "raw aspidin" obtained by MgO treatment of *Dryopteris austriaca* extract. Column chromatography on silica gel and treatments with various solvents yielded ortho-desaspidin in a pure state with a melting point of 131—133° and with synthetic ortho-desaspidin a mixed melting point of 132—134°.

The ortho-desaspidin content of *Dryopteris* extracts is extremely low; according to a semiquantitative paper chromatographic method [16] the amount of ortho-desaspidin was estimated as approximately 0.15 %, which is about 1/20 of the average content of para-aspidin previously reported by us.[4] In *Dryopteris filix mas* extract ortho-desaspidin could not be identified with certainty.

EXPERIMENTAL

Synthesis of isoaspidin. 3-Butyrylfilicinic acid (1.12 g) and isoaspidinol (1.12 g) were dissolved in aqueous potassium hydroxide (80 ml, 1 %) and formaldehyde (3.75 ml, 4 %) was added. The mixture was kept at room temperature for 1 min and made acid with hydrochloric acid (10 %). The precipitate was filtered off, washed with water and dried. The product contained isoaspidin, albaspidin, and unreacted isoaspidinol; the latter was easily removed as soluble in 50 % ethanol. To remove albaspidin, the product was treated with acetone and methanol and, finally, isoaspidin was recrystallized from ethanol. Pure isoaspidin had a m.p. of 152—154°. (Found: C 65.18; H 7.00; OCH_3 6.70. Calc. for $C_{25}H_{32}O_8$: C 65.22; H 6.96; OCH_3 6.74.).

Synthesis of ortho-desaspidin. 3-Butyrylfilicinic acid (224 mg) and o-desaspidinol (210 mg) were dissolved in aqueous potassium hydroxide (25 ml, 1 %) and formaldehyde (0.75 ml, 4 %) was added. The mixture was kept at room temperature for 1 min and made acid with hydrochloric acid (10 %). The precipitate was filtered off, washed with water and dried. The synthetic mixture contained about 60 % of ortho-desaspidin, the residue consisting of about equal amounts of the two symmetrical compounds albaspidin and methylene-bis-o-desaspidinol. Treatments with cold acetone resulted in practically pure ortho-desaspidin as soluble in acetone while the symmetrical compounds were very slightly soluble in this solvent. After recrystallizations from methanol ortho-desaspidin was obtained in a pure state, m.p. 133—135°. (Found: C 64.65; H 6.70; OCH_3 7.09. Calc. for $C_{24}H_{30}O_8$: C 64.57; H 6.73; OCH_3 6.95.).

Isolation of ortho-desaspidin. The finely ground rhizomes of *Dryopteris austriaca* (Jacq.) Woynar were extracted with ether for 8 h. The solvent was distilled off and the residue freed of fatty material by MgO treatment.[15] The "raw aspidin" so obtained was chromatographed through a column filled with silica gel using chloroform-cyclohexane (1:1) as solvent. Each fraction was analyzed by paper chromatography;[16] on papers buffered to pH 9.1 ortho-desaspidin has only slightly higher R_F values than aspidin but using tetrazotized di-o-anisidine as reagent the orange red colour of ortho-desaspidin was well distinguished from the yellow one of aspidin. The first fractions consisted of relatively pure albaspidin and were discarded. Ortho-desaspidin was obtained together with aspidin and small amounts of albaspidin. The best fractions were combined and the chromatographic procedure was repeated. Treatments with cold acetone removed the residual albaspidin and a mixture of aspidin and ortho-desaspidin was left behind. Repeated fractional crystallizations from hexane and ethanol yielded pure ortho-desaspidin, m.p. 131—133° and whith synthetic ortho-desaspidin mixed m.p. of 132—134°. (Found: C 64.63; H 6.85; OCH_3 7.21. Calc. for $C_{24}H_{30}O_8$: C 64.57; H 6.73; OCH_3 6.95.).

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