139. The Phloroglucinols of *Dryopteris aitoniana* PICHI SERM. (Dryopteridaceae, Pteridophyta)

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The phenolic compounds of the fern *Dryopteris aitoniana* were analyzed by improved methods avoiding alcaline reagents and contact with unbuffered SiO_2, which can cause deterioration. We confirmed the presence of five of the formerly (1971) reported six compounds, while the sixth, formerly incorrectly assumed to be aspidin (based on a spot in TLC), has now been isolated as cristals (TR-1579). It has the empirical formula $C_{27}H_{52}O_2$, but its structure is still unknown. Five other compounds were isolated in pure form, tetra-albaspidin BBBB (**25**-BBBB) a known but rare phloroglucinol, and four new compounds: penta-albaspidin BBBBB (**37**-BBBBB), hexa-albaspidin BBBBBB (**38**-BBBBBB), tetraflavaspidic acid BBBB (**26**-BBBB) and hexaflavaspidic acid BBBBBB (**39**-BBBBBB). The structures were established by degradation NMR and partly by field-desorption and fast-atom-bombardment (FAB) mass spectra. The oligoflavaspidic acids are very unstable compounds, deteriorating during isolation procedures using the older 'standard method'.

1. Introduction. – Nearly all members of the fern genus *Dryopteris* ADANS. contain in their rhizomes and stipe bases some characteristic phloroglucinol (1,3,5-benzenetriol) derivatives known as 'acyl-phloroglucinols' or just 'phloroglucinols'. They possess strong anthelmintic properties, and crude products (dried rhizome powder or extracts) of different fern species, particularly *Dryopteris filix-max* (L.) SCHOTT, have been used as cure against tape worm since antiquity. Although their medicinal use is dangerous and regarded as obsolete today, the pronounced biological activity was the reason that the isolation of the responsible compounds has been started already in the 19th century and that chemical structure of more than fifty such phloroglucinols is well-established today (see reviews of *Berti* and *Bottary* [1], *Penttilä* and *Sundman* [2], *Penttilä* [3], and *v. Euw et al.* [4]). Usually, a certain plant contains more than one of such phloroglucinols, and the composition is often constant within a particular species. Precise analysis of the actual 'spectrum' can sometimes be used as a valuable 'chemical marker' and a tool for solving some taxonomic problems in *Dryopteris* and related fern genera [4].

2. Analytical Methods. – As mentioned recently [4], the methods actually used so far for analysis were often not satisfactory. Most phloroglucinols are fairly stable to acids but many are very sensitive to alcaline conditions. The 'standard method' used by most workers during the last *ca.* 20 years [4] [5] includes two steps which can produce decompositions, losses and artefacts. These steps which, therefore, should be avoided are: *1.* 'purification' of crude extract by the 'MgO or $Ba(OH)_2$ procedure' to eliminate fatty acids and other non phenolic compounds, and *2.* chromatography on non-buffered SiO_2.

For analysis of crude mixtures and preparative isolation of pure phloroglucinols, we now use an 'improved method' [4] which involves the following steps.

2.1. Exhaustive extraction of the dried powdered rhizome with peroxide-free ether (as usual). The combined solution (containing all phloroglucinols) is first washed with aqueous HCl and H_2O to eliminate organic and inorganic cations and after evaporation giving the 'cation-free crude ether extract'. This can be analysed directly for the main compounds by TLC, paper chromatography (PC), or HPLC in appropriate buffered systems at pH of *ca.* 4 to 7. Compounds present only in trace amounts are best identified after partial preparative separation. *Table 1, 2,* and *3* give the systems we used for quick identifications in TLC. For preparative separation we used the following methods.

Table 1. R_f *Values in TLC on Silica Gel* 'HF (*Typ* 60) Merck' *Buffered for pH 4 and 6 in Three Different* Solvent Systems: *I: MeOH/(i-Pr)$_2$O/Cyclohexane 10:35:55; II: $CHCl_3$/Hexane/EtOH 45:45:10; III: $CHCl_3$/Hexane/EtOH/AcOH 45:35:16:4.* All *v/v.* (i-Pr)$_2$O decomposes quickly, freshly distilled product (b.p. 67–68°) is used. The given R_f values can differ even from plate to plate. Pure reference compounds must be used for identification. For the faster moving compound with lowest polarity (Compound TR-1579, **6**, **10** *etc.*) plates at pH 7 are useful (see *Table 2*) and for those with highest polarity a system with 3% AcOH (see *Table 3*) or using AcOH instead of $CHCl_3$ (see *Table 4*).

No. in [4] or this paper	Compound and colour after spraying with 'fast blue salt B' (0.1% in H_2O [18])		R_f Values I		II		III	
	Compound	Colour	pH 4	pH 6	pH 4	pH 6	pH 4	pH 6
2-B	Aspidinol B	violet	0.48	0.37	0.43	0.40	0.87	0.73
3-B	Fraginol B	yellow	0.52	0.25	0.49	0.47	0.89	0.74
4-AA	Norflavaspidic acid AA	brown-violet	0.33	0.03	0.54	0.17	1.00	0.65
4-AP	Norflavaspidic acid AB	brown-violet	0.37	0.03	0.54	0.17	1.00	0.70
5-AB	Flavaspidic acid AB	orange	0.07	0.00	0.19	0.02	0.70	0.31
5-BB	Flavaspidic acid BB	orange	0.12	0.00	0.27	0.02	0.82	0.45
	Compound TR-1579	yellow	0.91	0.86	0.80	0.72	1.00	1.00
6-AB	Aspidin-AB	yellow	0.83	0.55	0.72	0.73	1.00	1.00
6-BB	Aspidin-BB	yellow	0.85	0.70	0.78	0.72	1.00	1.00
7-AB	*para*-Aspidin AB	brown	0.75	0.31	0.67	0.66	1.00	1.00
7-BB	*para*-Aspidin BB	brown	0.81	0.47	0.69	0.67	1.00	1.00
8-AB	Desaspidin AB	orange-red	0.55	0.20	0.52	0.60	1.00	1.00
8-BB	Desaspidin BB	orange-red	0.57	0.23	0.54	0.52	1.00	1.00
9-AB	*ortho*-Desaspidin AB	orange	0.74	0.54	0.68	0.59	1.00	1.00
9-BB	*ortho*-Desaspidin BB	orange	0.77	0.69	0.74	0.63	1.00	1.00
10-AA	Albaspidin AA	red-brown	0.82	0.58	0.73	0.68	1.00	1.00
10-AB	Albaspidin AB	red-brown	0.84	0.69	0.78	0.70	1.00	1.00
10-BB	Albaspidin BB	red-brown	0.85	0.75	0.79	0.70	1.00	1.00
11-BB	Phloraspin BB	brown	0.32	0.26	0.45	0.43	0.81	0.71
12-BB	Phloraspidinol BB	violet	0.36	0.33	0.53	0.52	0.87	0.77
13-BB	Margaspidin BB	brown	0.55	0.45	0.52	0.50	0.89	0.80
14-BB	Methylene-bis-desaspidinol BB	violet	0.31	0.27	0.51	0.39	0.87	0.81
15-BB	Phloropyron BB	red	0.77	0.53	0.68	0.66	1.00	1.00
16-BB	Phloraspyron BB	brownish-orange	0.31	0.40	0.68	0.70	1.00	0.85
17-BB	Aemulin-BB	light brown	0.61	0.48	0.56	0.49	1.00	0.86
18-BB	Methylene-bis-aspidinol BB	violet	0.58	0.54	0.72	0.70	1.00	0.96
19-ABA	Filixic acid ABA	red-brown	0.64	0.11	0.70	0.53	1.00	1.00
19-ABB	Filixic acid ABB	red-brown	0.73	0.20	0.72	0.62	1.00	1.00
19-BBB	Filixic acid BBB	red-brown	0.81	0.40	0.72	0.73	1.00	1.00
20-BBB	Tris-*para*-aspidin BBB	brown	0.32	0.02	0.82	0.55	1.00	1.00
21-BBB	Trisdesaspidin BBB	light brown	0.25	0.02	0.68	0.37	1.00	1.00

Table 1 contd.

No. in [4] or this paper	Compound and colour after spraying with 'fast blue salt B' (0.1% in H_2O [18])		R_f Values I		II		III	
	Compound	Colour	pH 4	pH 6	pH 4	pH 6	pH 4	pH 6
23-ABB	Trisflavaspidic acid ABB	orange	0.00	0.00	0.11	0.00	0.43	0.16
23-BBB	Trisflavaspidic acid BBB	orange	0.01	0.00	0.14	0.01	0.53	0.22
24-BAB	Trisaemulin BAB	brown	0.72	0.23	0.81	0.81	1.00	1.00
24-BBB	Trisaemulin BBB	brown	0.77	0.39	0.85	0.82	1.00	1.00
25-ABBA	Tetra-albaspidin ABBA	red-brown	0.35	0.02	0.72	0.37	1.00	1.00
25-BBBB	Tetra-albaspidin BBBB	red-brown	0.61	0.05	0.91	0.73	1.00	1.00
26-ABBB	Tetraflavaspidic acid ABBB	orange	0.00	0.00	0.06	0.00	0.29	0.16
26-BBBB	Tetraflavaspidic acid BBBB	orange	0.00	0.00	0.07	0.00	0.34	0.18
37-BBBBB	Penta-albaspidin BBBBB	red-brown	0.16	0.03	0.89	0.43	1.00	1.00
38-BBBBBB	Hexa-albaspidin BBBBBB	red-brown	0.05	0.02	0.88	0.11	1.00	1.00
39-ABBBBB	Hexaflavaspidic acid ABBBBB	orange	0.00	0.00	0.01	0.00	0.16	0.15
39-BBBBBB	Hexaflavaspidic acid-BBBBBB	orange	0.00	0.00	0.01	0.00	0.18	0.16

Table 2. R_f *Values in TLC on Silica Gel Buffered for pH 7 with Solvent System Ia: MeOH/(i-Pr)$_2$O/Cyclohexane/AcOH 10:35:54.5:0.5.* Used to separate compound TR-1579 from other compounds of low polarity. Details as in *Table 1.*

Compound	Colour	R_f (System Ia)
TR-1579	yellow	0.86
Aspidin BB (**6**-BB)	yellow	0.41
Albaspidin BB (**10**-BB)	red-brown	0.96
Filixic acid BBB (**19**-BBB)	red-brown	0.34

Table 3. R_f *Values in TLC on Silica Gel Buffered for pH 4 with Solvent System IV: $CHCl_3$/Hexane/AcOH 45:31:16:8, Freshly Mixed, and System V: AcOEt/Cyclohexane/AcOH 60:35:5.* Details as in *Table 1.* System V has the advantage to avoid $CHCl_3$ which can sometimes cause decomposition of the sensitive compounds like oligoflavaspidic acids.

Compound	Colour	R_f	
		System IV	System V
Flavaspidic acid AB (**5**-AB)	orange	0.83	0.65
Flavaspidic acid BB (**5**-BB)	orange	0.90	0.76
Trisflavaspidic acid ABB (**23**-ABB)	orange	0.59	0.17
Trisflavaspidic acid BBB (**23**-BBB)	orange	0.73	0.34
Tetraflavaspidic acid ABBB (**26**-ABBB)	orange	0.47	0.03
Tetraflavaspidic acid BBBB (**26**-BBBB)	orange	0.59	0.08
Hexaflavaspidic acid ABBBBB (**39**-ABBBBB)	orange	0.38	0.02
Hexaflavaspidic acid BBBBBB (**39**-BBBBBB)	orange	0.47	0.03

2.2. Counter-current distribution between hexane and 95% aqueous MeOH. Fatty acids, steroids *etc.* remain completely in the hexane phase in this procedure, together with the bulk of less polar phloroglucinols (*e.g.* the albaspidins **10**, **19** *etc.*)[1]). The material from the MeOH phases is free of fatty acids *etc.* and contains the bulk of the more polar phloroglucinols (*e.g.* flavaspidic acids **5**, **23** *etc.*). Although the phloroglucinols are only

[1]) Compound numbers up to **36** are the same as in the review [4].

Table 4. *Semiquantitative Composition of Main Phloroglucinols (homologues not given)*

Species and No. of samples	Origin[b])	Ploidy level[c])	Reproduction[d])	Amount of dry rhizome used [g]	Crude (cation-free) extract [g] ([%])	2-B	5-BB	6-BB	7-BB	8-BB
D. aitoniana[e]) TR-2596	Md	(2×)	s.	11	0.51 (4.6)	–	++	+	(+)	–
D. aitoniana[f])	Md	(2×)	s.	200	13 (7.0)	–	++	–	(+)	–
D. filix-mas[e]) W. Gätzi s.n.	He	(4×)	s.	30	2.08 (6.9)	+	+++	–	+	+
D. filix-mas[f]) TR-4167	He	(4×)	s.	94.5	8.25 (8.75)	(+)	+++	–	+	+

[a]) Phloroglucinols from *D. filix-mas* as mixtures of A- and B-homologues. In *D. aitoniana* B-homologues dominate.
[b]) Md = Madeira, He = Switzerland.
[c]) 2× means diploid with 2n = 82 chromosomes in somatic cells of the sporophyte. If (2×) or (4×) are given in brackets, the chromosomes of the plants used in this work were not counted but the species is known to have this ploidy.

partly separated in this procedure, we found it helpful to obtain pure compounds in further steps.

2.3. Chromatography on columns of: *i*) microcrystalline cellulose (suitable for the most polar compounds); *ii*) polyamide (suitable for the least polar compounds), and *iii*) SiO_2 buffered at pH 4, 6, or 7, depending on polarity of material. It may have to be repeated in a different system.

Decomposition of sensitive phloroglucinols is avoided to a high degree by using this method. Its application to the analysis of *Dryopteris filix-mas* immediately revealed the presence of a new compound: tetraflavaspidic acid BBBB (**26**-BBBB). This compound is quickly destroyed by contact with unbuffered SiO_2 and was therefore so far obviously overlooked. It is nevertheless constantly present in *D. filix-mas*, a species rather often examined by different workers (see [4]). It is present in *D. aitoniana* together with related compounds of still higher molecular weight and is described with structure proof in this paper.

3. Plant Material. – *Dryopteris aitoniana* PICH. SERM. [6] is related to the group of ferns described by *Ching* [7] as the *D. filix-mas* group. It is endemic to the island of Madeira and known to be diploid (*Manton*, unpubl. quoted in [5]), *i.e.* an old ancestral species. Its correct identification poses no problems. Its rhizomes have already been analysed by *Widén et al.* [5], they reported the presence of six compounds (see *Table 4*) of which flavaspidic acid BB (**5**-BB), albaspidin BB (**10**-BB), and trisflavaspidic acid BBB (**23**-BBB) were isolated in crystals *(Scheme 1)*. For the present study, we used the dried rhizomes of 9 plants (CRFJ-9773-9781), total weight of 685 g collected by *C. R. Fraser-Jenkins* on 1.VIII.1979 (for details see the *Exper. Part*). Vouchers are deposited in the Herbarium of the British Museum (Natural History) (BM) and Herb. TR-5127 A–I.

4. Results. – In the present work using the 'improved method', we could confirm the presence of five of the formerly [5] reported six compounds. These five compounds are: flavaspidic acid BB (**5**-BB), albaspidin BB (**10**-BB), tris-*para*-aspidin BBB (**20**-BBB) and trisflavaspidic acid BBB (**23**-BBB), and *para*-aspidin BB (**7**-BB)[2]). Except the last men-

[2]) The IUPAC names of these compounds are given in the *Exper. Part*.

of Dryopteris aitoniana *and, for Comparison,* D. filix-mas[a])

10-BB	19-BB	20-BBB	23-BBB	25-BBBB	26-BBBB	37-BBBBB	38-BBBBBB	39-BBBBBB	Literature	Comp. TR-1578
++	–	+	+++	–	–	–	–	–	[5]	
+++	–	+	++	+	+	+	+	+	[f])	(+)
–	+++	–	–	–	–	–	–	–	[5]	–
(+)	+++	–	(+)	–	(+)	–	–	–	[4]	–

[d]) s. = sexual, ap. = apomictic.
[e]) From [5], by the old 'standard method'.
[f]) Investigation by the 'improved method'.

tioned (present only in traces), all were isolated in pure crystals. On the other hand, we found no aspidin BB (**6**-BB). The spot in TLC which incorrectly was attributed to **6**-BB [5] turned out to be produced by a new compound TR-1579 of similar low polarity (see *Table 1* and *2*). It was now obtained in crystals with the empirical formula $C_{27}H_{52}O_2$ (408) (high-resolution MS) and characterized further by its IR spectrum *(Fig. 1)*, but its structure was not elucidated. Aside of this material the following five compounds could be isolated in crystals.

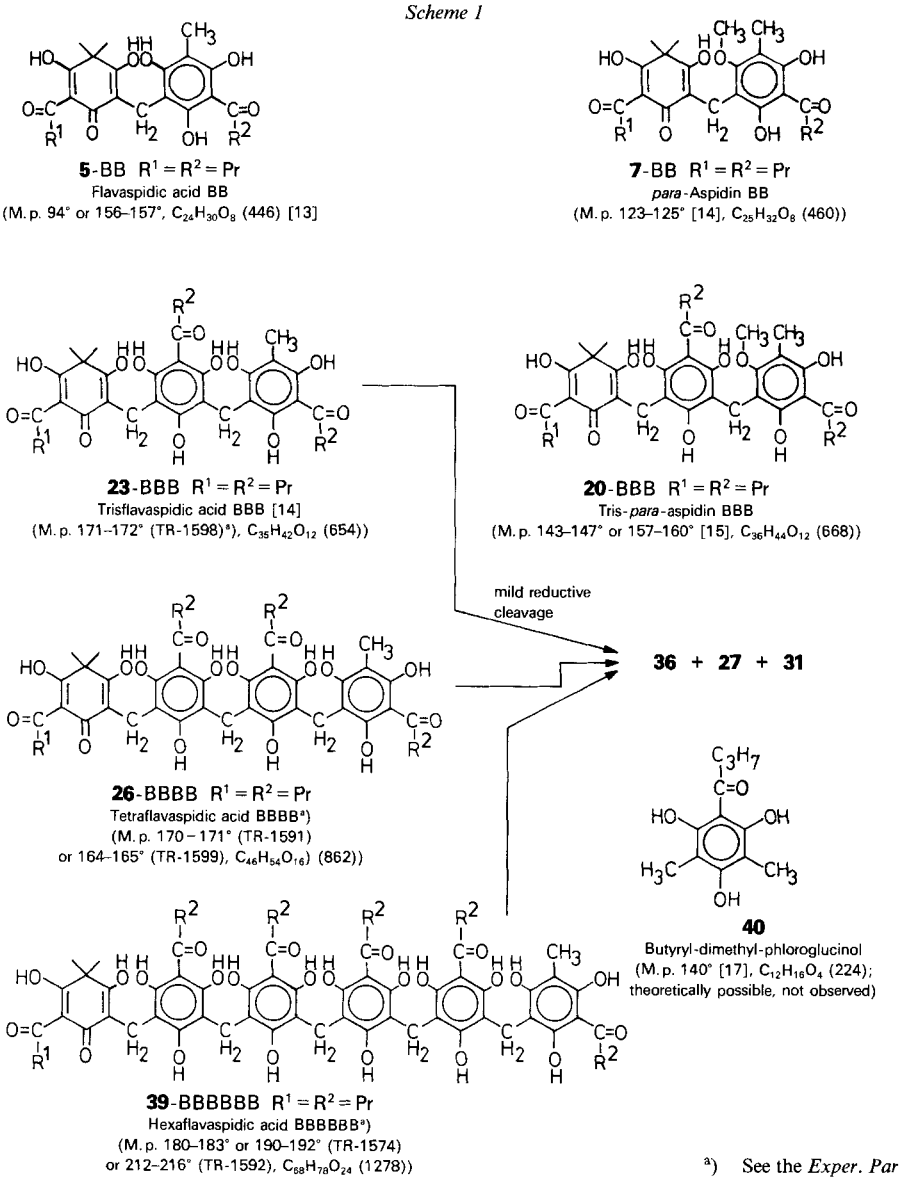
Tetra-albaspidin BBBB (= methylen-bis-norflavaspidic acid BBBB; **25**-BBBB), a known but rare compound, has been so far isolated only once from *'Dryopteris austriaca'* and also synthesized by *Penttilä* and *Sundman* [8]. The four following substances are new and their structures could be established: *penta-albaspidin BBBBB* (**37**-BBBBB), *hexa-albaspidin BBBBBB* (**38**-BBBBBB), *tetraflavaspidic acid BBBB* (**26**-BBBB), and *hexaflavaspidic acid BBBBBB* (**39**-BBBBBB)[3]) *(Scheme 2)*. The mentioned *tetraflavaspidic acid BBBB* (**26**-BBBB) has first been isolated from *D. filix-mas* and mentioned by *v. Euw et al.* [4] without details, it is described in the *Exper. Part.* Former and new results are summarized in *Table 4*.

From unidentified spots in TLC, we assume that small amounts of a *pentaflavaspidic acid* (between **26** and **39**) as well as oligo-albaspidins and oligoflavaspidic acids containing 7 and 8 rings may also be present in the plant, but these compounds were not isolated in pure form.

5. Remarks to the Formulae. – The letter B is the usual abbreviation for a butyryl side chain (–COR with R = *n* – C_3H_7). This is the most common acyl substituent in fern phloroglucinols. In *D. aitoniana* these B-homologues dominate. In other ferns, sometimes higher or lower homologues with valeryl (V), propionyl (P), or acetyl (A) side chains can be observed; sometimes (rarely) isomers, *e.g.* isobutyryl side chains *etc.* The type of side chain can best be estimated by vigorous reductive cleavage (see *Sect. 6.2.*). The crude extracts from *D. aitoniana* gave 91% butyric and *ca.* 9% acetic acid by this method [5], but the true content in A-homologues is probably even lower.

[3]) The IUPAC names of these new compounds are given in the *Exper. Part.*

Scheme 1



a) See the *Exper. Part.*

The formulae given are schematized for the compounds containing 2,5-cyclohexadienone rings and do not always represent the true state. Several tautomers (besides dimers or polymers, involving intermolecular H-bonding) are theoretically possible already in simple compounds with only one ring like butyrylfilicinic acid (**36**). *Äyräs et al.* [10a, b] deduced from ^{13}C-NMR studies that the tautomer **36b** with 2,4-cyclodienone structure is exclusively present in (D_6)acetone solution. In $CDCl_3$ solution, it is still the preferred

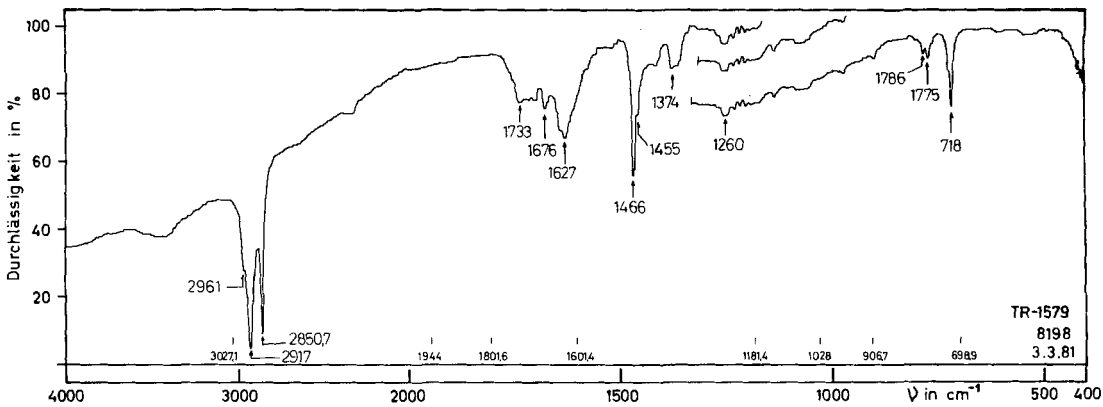


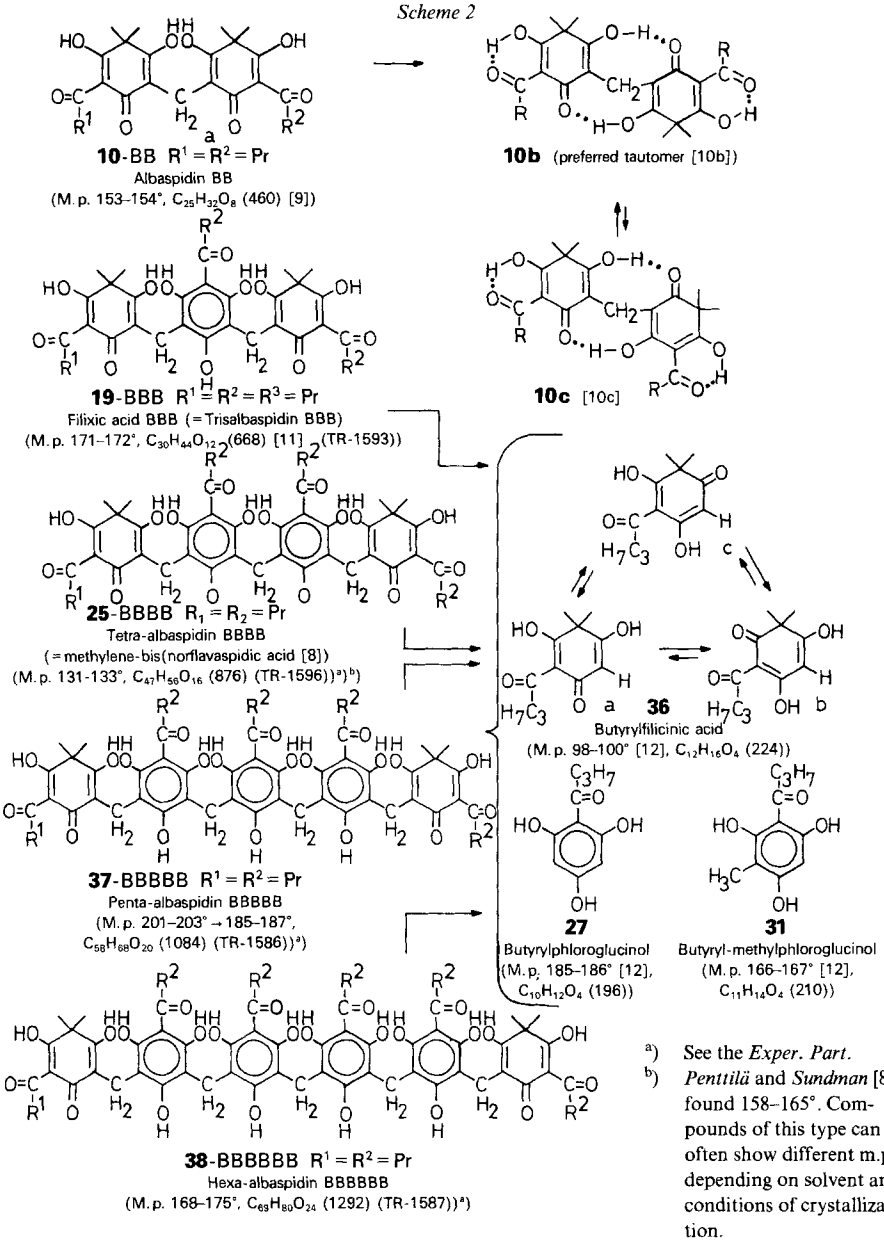
Fig. 1. *IR Spectrum of compound TR-1579* (m.p. 59–60°) *from* Dryopteris aitoniana (0.82 mg, solid in 200 mg KBr). Recorded by *K. Aegerter* in the Spectral Laboratory of the Institute of Organic. Chemistry, University Basel on a *Perkin-Elmer* spectrophotometer, model *125*.

tautomer, but other forms are present in smaller amounts, perhaps **36a** and **36c**, the spectra becoming complicated. But according to *Äyräs et al.* [10b], the preferred arrangement of albaspidin BB (**10**-BB) is **10b** with both rings as 2,5-cyclohexadienones in a particular rotamer stabilized by intramolecular H-bonds. No other tautomer could be observed in (D_6)acetone solution, but in $CDCl_3$ another unsymmetrical tautomer **10c** becomes visible in the spectra for which the tentative formula **10c** has been suggested by *Äyräs* [10c]. The presence of such a tautomer (and probably small amounts of a third one) is also visible in the ^{1}H-NMR spectrum (see *Fig. 2A* and *2B*) of a pure sample (freshly sublimed *in vacuo* to get it completely free of solvent traces). The other compounds with two, three, or more rings may behave in a similar way, but they have not yet been examined in detail. We nevertheless assume that the schematical formulae given for compounds with two or more rings with 2,5-hexadienones represent the closest approximation to the true state among other tautomers, while the actual orientation of the H-bonding is left undetermined.

6. Structure Determinations. – Possible structures could often be guessed from mobilities in TLC and colour reaction by assuming that groups of similar compounds may be present, formed in a common biosynthetic pathway [3], see also [1] [19]. Structure proof was obtained by combination of the four following methods:

6.1. *Reductive Cleavage under Mild Conditions, by the Method of* Boehm [20] (for details see [16]). Under these conditions only CH_2 bridges are opened, the so produced one-ring compounds are identified by PC and TLC [2] [21] and [22]. Even the presence of homologues can usually be detected [22]. Cleavage can, in principle, occur on both sides of the CH_2 bridge, but usually not all of the theoretically possible cleavage products are formed. From filixic acid BBB (**19**-BBB), only **36**, **27**, and **31** were obtained [11] [15]. From tetra-, penta-, and hexa-albaspidin (**25**-BBBB, **37**-BBBBB, **38**-BBBBBB), we now obtained the same cleavage products as expected. It must be pointed out that mild reductive cleavage of trisflavaspidic acid BBB (**23**-BBB) gives exactly the same three products **36**, **27**, and **31**. In this particular case, the ring at the right side in formula **23** produces the same compound **31** as partially obtained from the inner ring, just increas-

Scheme 2



a) See the *Exper. Part.*

b) *Penttilä* and *Sundman* [8] found 158–165°. Compounds of this type can often show different m.p. depending on solvent and conditions of crystallization.

ing the relative yield of **31**. As expected, we now got the same three cleavage products again from **26-BBBB** and **39-BBBBBB**. The theoretically possible dimethyl derivative **40** was not detected. At first sight, it could seem that the method is of limited value. This is partially true; nevertheless, it is an important step to show that the parent compounds really belong to this particular type of substances in which phloroglucinol rings (includ-

ing hexadienones) are linked by CH_2 bridges. In the particular case of *D. aitoniana*, the results obtained after mild reductive cleavage of the crude extracts showed that no or only traces of higher or lower homologues were present.

6.2. *Reductive Cleavage under Vigorous Conditions* [22]. In this way the side chains are split off as free fatty acids and they can be identified and semiquantitatively estimated by GC, either directly [22] or as pentafluorobenzyl esters [23]. This method is particularly useful for checking for homologues and isomers. In *D. aitoniana*, only butyryl side chains are present with perhaps traces of Ac.

6.3. *Mass Spectra.* Electron-impact (EI) MS have extensively and successfully been used for fern phloroglucinols up to compounds with three rings [24–26], but besides extensive fragmentation unexpected thermic reactions, particularly the rottlerone rearrangement [4] [25a], often occur and they must carefully be considered to avoid misinterpretation of sometimes strong peaks, both for fragments as for rearrangement products in the region of the molecular ions. Peaks for true molecular ions are often rather weak already in phloroglucinols with only two rings [24c] [25b] [26], and very weak or absent in those with three rings [25a, b]. Knowledge of the precise empirical formula as deducible from the molecular ions in mass spectra is of particular value for structure proof of the compounds with four, five, and six rings like **25**, **32**, **38**, **26**, and **39**. It is impossible to achieve such information from EI-MS. Fortunately, both field-desorption (FD) and fast-atom-bombardement (FAB) MS could successfully be used for this purpose, the spectra will be published later [27].

6.4. *^{1}H-NMR Spectra*[4]). The ^{1}H-NMR spectra (in $CDCl_3$) of **10-BB**, **23-BBB**, **25-BBBB**, **26-BBBB**, **37-BBBBB**, **38-BBBBBB**, and **39-BBBBBB** are given in the *Fig. 2–8*. A spectrum of **10-BB** has already been published [28]. *Fig. 2* gives the spectrum obtained now with higher resolution and with a sample freshly sublimed *in vacuo*, to make sure that any trace of solvent is absent. The main signal of the CH_2 group at $\delta = 3.314$ ppm is well-resolved now from the double triplet centered at $\delta \approx 3.16$ ppm of the two CO–CH_2 groups of the side chains. The two main signals of the four geminal CH_2 groups at $\delta = 1.47$ and 1.54 ppm are now also well-separated from the multiplet of the two CO–CH_2–CH_2 groups centered at $\delta = 1.69$ ppm. The new spectrum shows that the former interpretation [28] was essentially correct and that the old spectrum was not falsified by residues of solvent. It also confirms that the position of the signal at $\delta = 3.314$ ppm is characteristic for the CH_2 group *between two* hexadienone rings. According to *Äyräs et al.* [10b], we assume that albaspidin BB (**10-BB**) is present in $CDCl_3$, mainly as the most

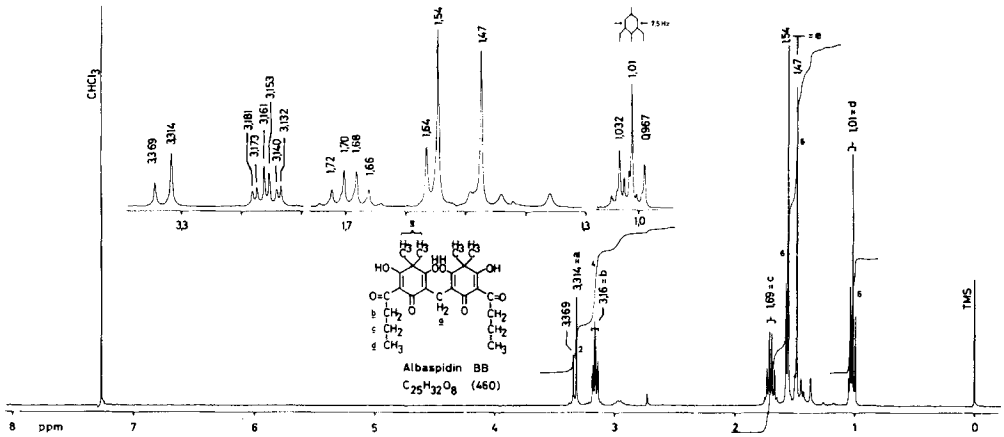


Fig. 2A. *360-MHz ^{1}H-NMR Spectrum of albaspidin BB* (**10-BB**; m.p. 151–152°) *freshly sublimed in a molecular still at 0.01 Torr and 135–150° bath temp.* Region of $\delta = 0$–8 ppm.

[4]) All ^{1}H-NMR spectra were taken in $CDCl_3$ solution. We express our gratitude to Dr. *H. Fuhrer* and Mr. *A. Borer*, physical laboratory of the *Ciba-Geigy AG*, Basel, for recording these spectra and giving their agreement to publish them. The 250-MHz spectra were recorded on a *Bruker WM-250* spectrometer, the 360-MHz spectra on as *Bruker HX-360* spectrometer, and the 400-MHz spectra on a *Bruker WM-400* spectrometer.

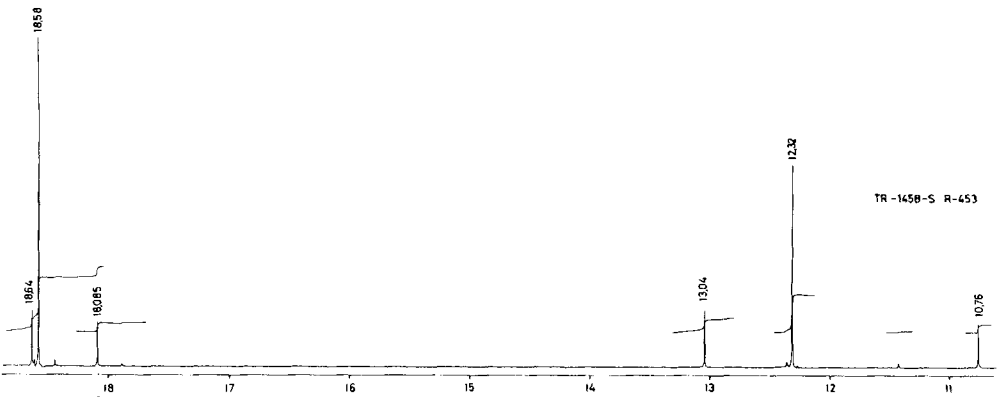


Fig. 2B. *^{1}H-NMR Spectrum of albaspidin* (**10-BB**; see *Fig. 2A*). *Region of δ = 10.76–18.64 ppm.* In the region between 8 and 10.76 ppm (partly missing here), three more OH signals were recorded formerly [27] at δ = 8.92, 9.49, and 10.16 ppm. All these signals disappear after shaking with D_2O.

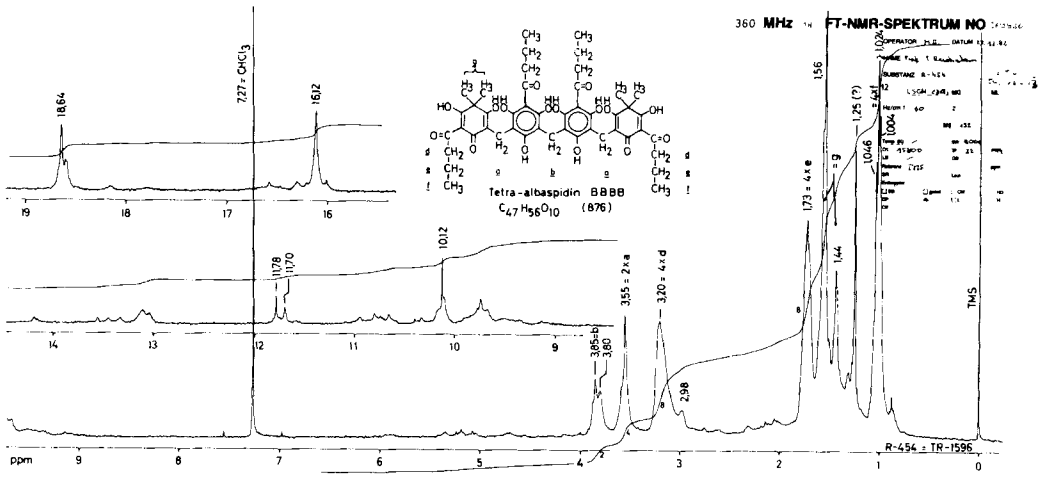


Fig. 3. *360-MHz ^{1}H-NMR spectrum of tetra-albaspidin BBBB* (**25-BBBB**; TR-1596; m.p. 131–133°) *isolated from* Dryopteris aitoniana. The signal at δ = 3.55 corresponds to the two CH_2 groups designated as 'a', while in the region of the middle group (designated as 'b'), two signals at 3.80 and 3.85 ppm are visible. They may be due to tautomeric forms. The integrated intensities of the signals 'a' and 'b' are well in agreement with 4:2. The signal at δ = 1.25 is due to residual solvent (insufficient drying).

stable tautomer **10b**. It is also well visible that the mentioned signal is accompanied by a weaker one at δ = 3.369 ppm which may be caused by the tautomer **10c**. Integration of intensities for both signals corresponds to two protons. A similar weak signal is visible at δ = 1.56 ppm, perhaps an analogous satellite of the main signal of two of the four geminal CH_2 groups at δ = 1.54 ppm. The presence of small amounts of a tautomer is particularly well visible in the region of H-bonded OH groups between 8 and 19 ppm *(Fig. 2B)*. As pointed out before [28], the intensity of some of these signals is less than corresponding to one proton, and they must, therefore, be caused by forms which are present in small quantities in the equilibrium.

In the ^{1}H-NMR spectrum of flavaspidic acid **BB** (**5-BB**) [5], the signal of the CH_2 group is at δ = 3.57 ppm, *i.e.* on distinctly lower field. This is the characteristic position for the CH_2 group situated between a hexadienone and an aromatic ring. It was recorded at similar values for *para*-aspidin **BB** (**7-BB**) [16], filixic acid **BBB** (**19-BBB**) [28] and other compounds with such partial structures. The signal of the CH_2 group between two aromatic rings was found at still lower field, $\delta \approx 3.70$–3.84 depending on substitution [29]. In tris-*para*-aspidin **BBB** (**20-BBB**) the two main signals at δ = 3.56 and 3.79 ppm are well separated [16].

These empirical rules were of great value in using ^{1}H-NMR spectra to establish or confirm the structures of compounds **19-BBB**, **25-BBBB**, **37-BBBBB**, and **39-BBBBBB** as well as **23-BBB**, **26-BBBB**, and **39-BBBBBB**. The relative intensities of the peaks at $\delta \approx 3.55$ and 3.70–3.85 ppm provide good proof for the number of rings present which is not deducible from the reductive cleavage. Absence or presence of the signal for the aromatic CH_3 group at $\delta \approx 2.10$ ppm allowed immediately to differentiate the oligo-albaspidins **19-BBB**, **25-BBBB**, **37-BBBBB**, and **38-BBBBBB** from the oligoflavaspidic acids **23-BBB**, **26-BBBB**, and **39-BBBBBB**.

6.5. *Combustion Analyses.* These were useful in this series mainly for excluding other elements as C, H, and O. They were not helpful for establishing the empirical formulae of the compounds described in this work, because it is usually impossible to isolate the sensitive compounds, free of solvent. FD- and FAD-MS turned out to be most helpful for this purpose.

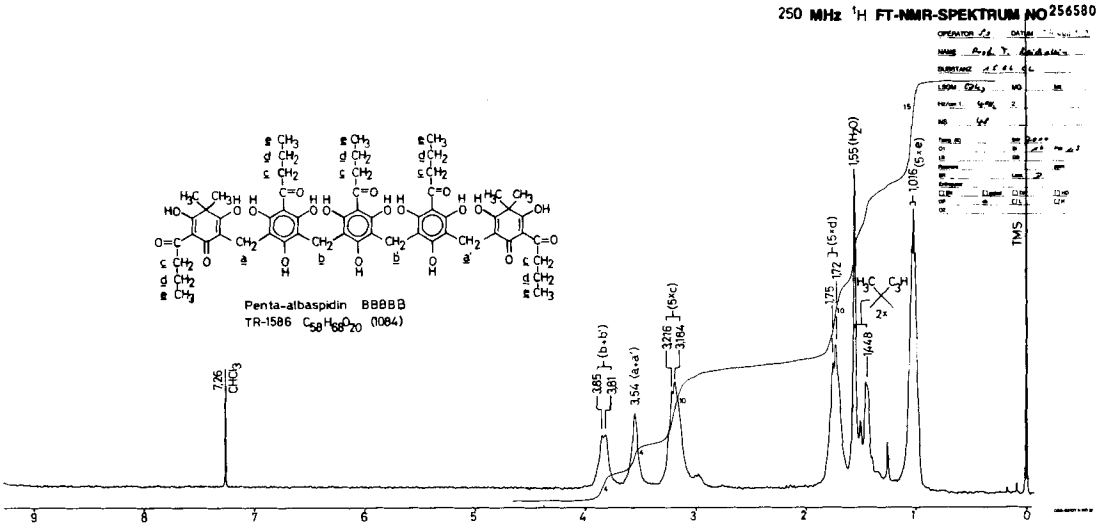


Fig. 4A. *250-MHz ^{1}H-NMR Spectrum of penta-albaspidin BBBBB* (**37-BBBBB**; m.p. 194–196°) *isolated from* Dryopteris aitoniana. The signal at $\delta = 3.54$ (a + a') is a wide *singlet*, while the signal corresponding to (b + b') is again split in two peaks at $\delta = 3.81$ and 3.85 ppm, perhaps again corresponding to two tautomeric forms. Splitting is also visible in the signals of the groups c and d.

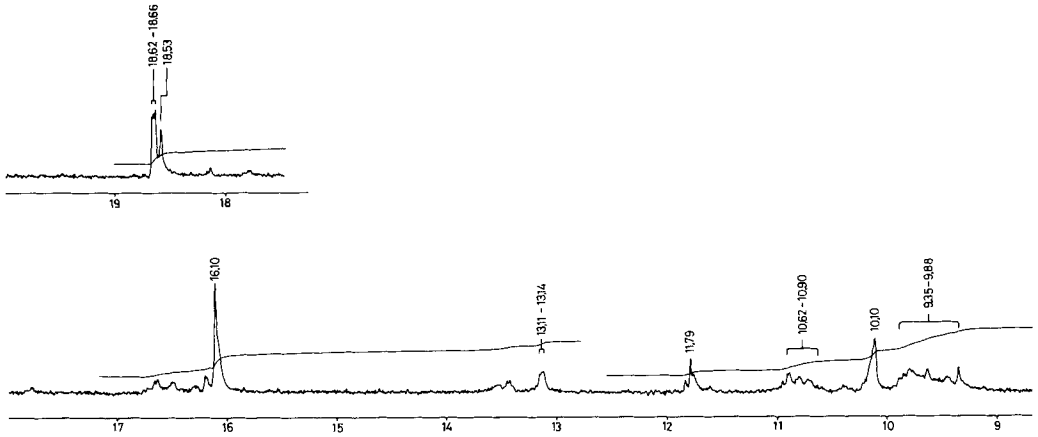


Fig. 4B. *250-MHz ^{1}H-NMR Spectrum of penta-albaspidin BBBBB* (**37-BBBBB**) *region of δ = 9–19 ppm, showing the signals of H-bonded OH groups*

Fig. 5A. 250-MHz ^{1}H-NMR Spectrum of hexa-albaspidin BBBBBB (**38**-BBBBBB; m.p. 168–175°) *isolated from* Dryopteris aitoniana. The signals at δ = 3.54 and 3.82 are visible here as wide *singlets*. The integrated intensities correspond to a relation of a:b as 4:6 in agreement with the deduced structure.

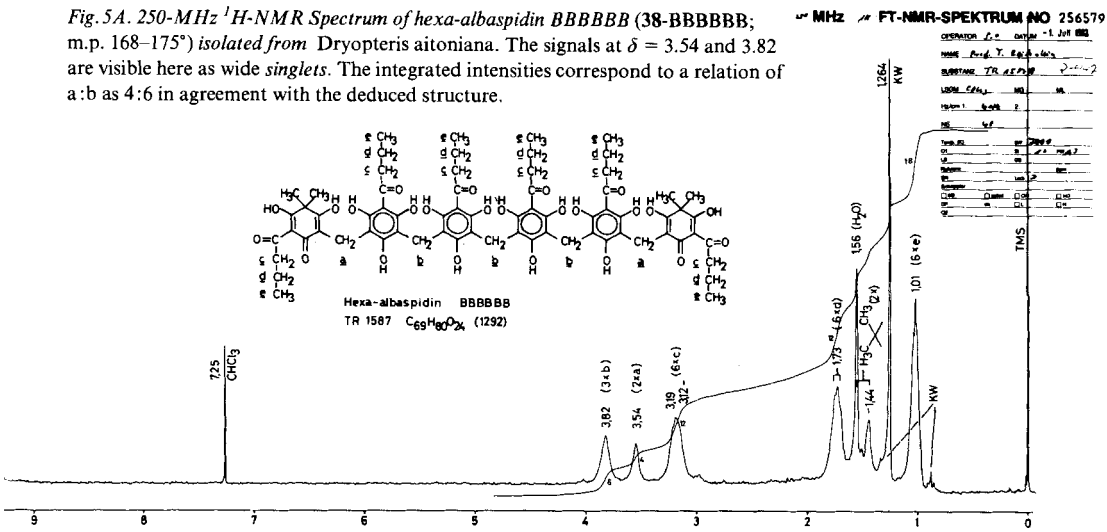
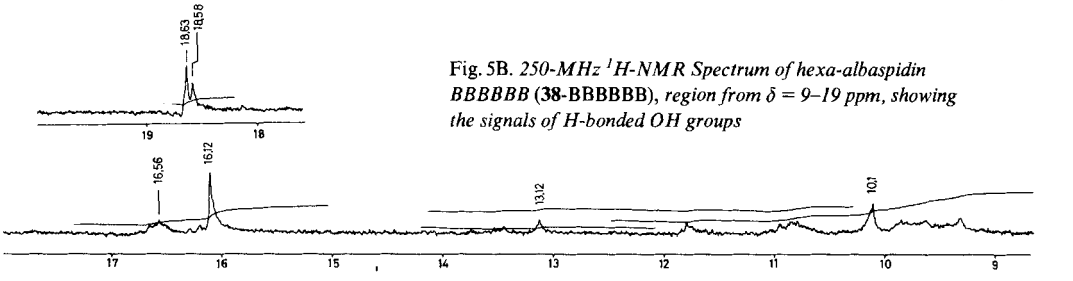


Fig. 5B. *250-MHz ^{1}H-NMR Spectrum of hexa-albaspidin BBBBBB* (**38**-BBBBBB), *region from δ = 9–19 ppm, showing the signals of H-bonded OH groups*



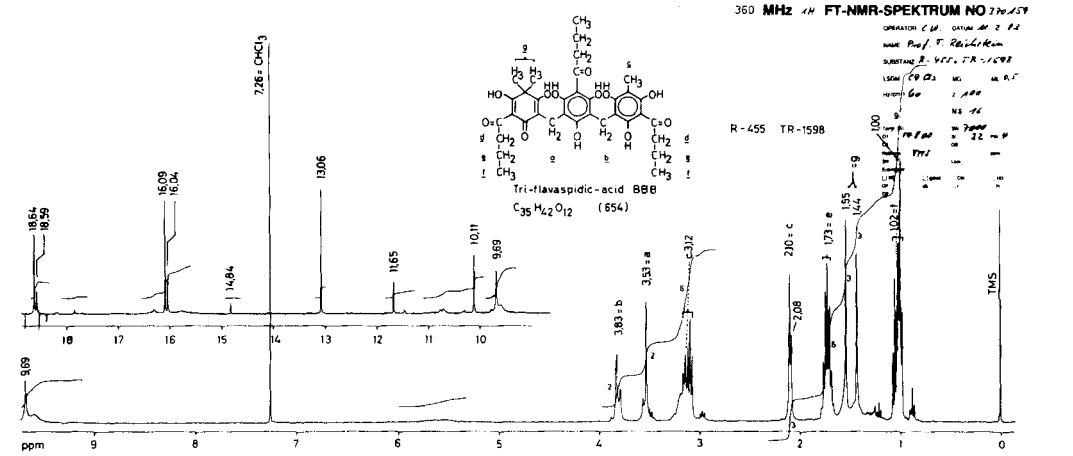


Fig. 6. *360-MHz ^{1}H-NMR Spectrum of trisflavaspidic acid BBB* (**23**-BBB; TR-1598; m.p. 171–172°) *isolated from* Dryopteris aitoniana. The signals of the two CH_2 groups a and b are on the expected position (main peaks at δ = 3.53 and 3.83 ppm), both accompanied by smaller satellites probably from another tautomeric form. Integration of intensities is well in agreement with the number of protons 2:2. The signal of the aromatic CH_3 group (c) is clearly visible at δ = 2.10 ppm with a satellite at 2.08 ppm, both together corresponding to 3 protons. In the region between 8 and 19 ppm many signals of H-bonded OH groups are visible, some again with intensities of less than one proton and probably corresponding again to minor amounts of other tautomers in the equilibrium.

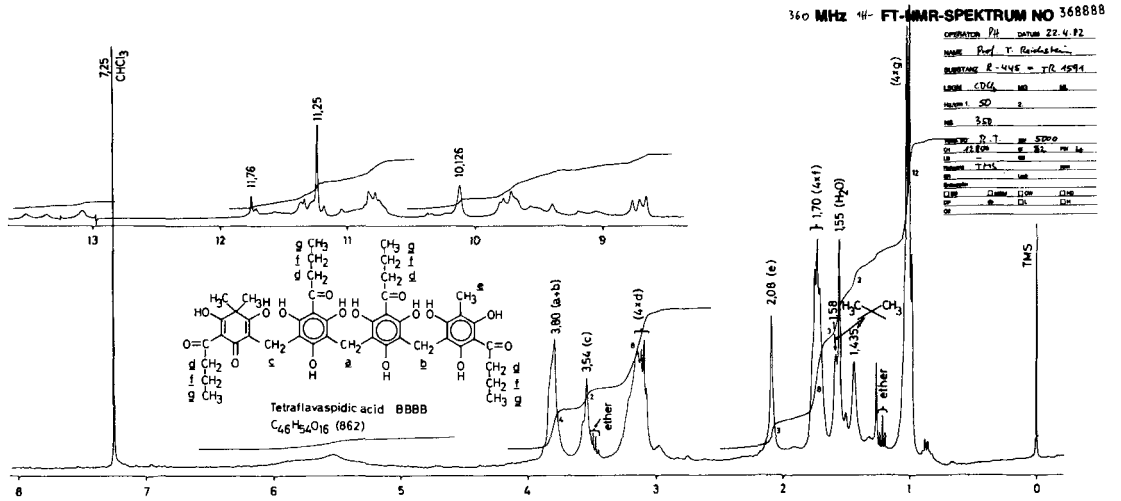


Fig. 7. *360-MHz ^{1}H-NMR Spectrum of tetraflavaspidic acid BBBB* (**26**-**BBBB**; TR-1591; m.p. 170–171°) *isolated from* D. aitoniana, *containing traces of solvent residues* (Et_2O, hexane and perhaps H_2O of crystallization). The signals of the CH_2, groups a and b are together, as expected at δ = 3.80 ppm (*s*, 4H), while the signal of group c is again at δ = 3.54 ppm (*s*, 2H), both seem to have minor satellites, perhaps due to other tautomers. The signal of the aromatic CH_3 group at δ = 2.08 ppm (*s*, 3H) no satellite is visible here.

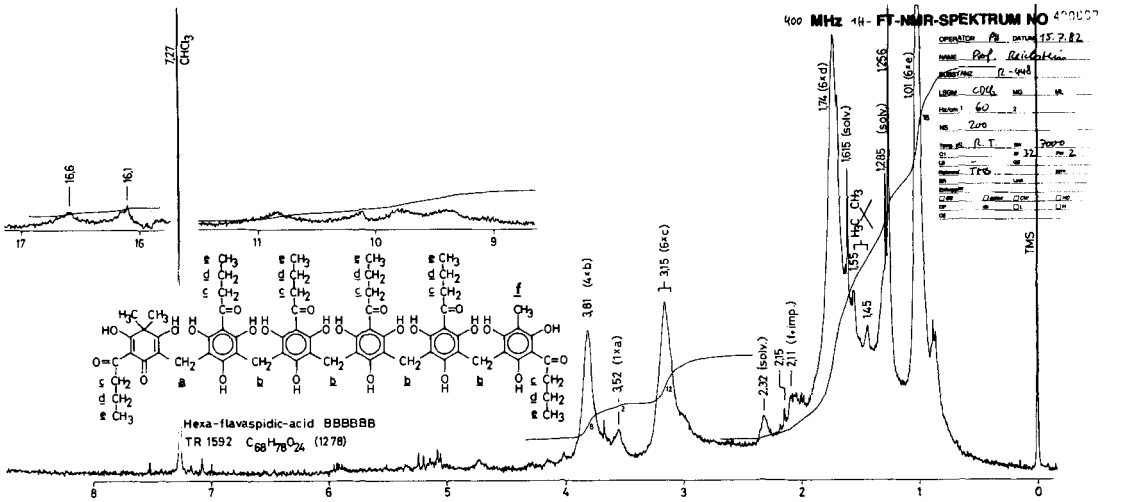


Fig. 8. *400-MHz ^{1}H-NMR Spectrum of hexaflavaspidic acid BBBBBB* (**39**-**BBBBBB**; TR-1592; m.p. 212–216°) *isolated from* Dryopteris aitoniana, *containing solvent residues* (signals at δ = 1.256; 1.285 and 1.615 ppm). The signals of the CH_2 group a and b are visible as wide *singlets* at ~ 3,52 and 3,81 ppm with intensities corresponding to 2:8, while the signal of the aromatic CH_3 group at $\delta \approx 2.11$ is partly covered by impurities (solvent residues?) and its intensity is not measurable with confidence.

6.6. *Volatility and Possible Association.* The finding that albaspidin BB (**10**-BB) in solution is essentially or exclusively present as the tautomer **10b** [10b], *i.e.* as a monomer, is in good agreement with the fact that it can well be sublimed *in vacuo* at *ca.* 130–150° without decomposition. It is impossible to do the same with flavaspidic acid BB (**5**-**BB**),

although of similar (even slightly lower) molecular weight. Appreciably higher temperatures are necessary to start volatilization and much decomposition products are obtained. We assume that this compound is mainly stabilized by strong intermolecular H-bonds as a dimer (or polymer). This may be the reason why it is also much more polar than albaspidin in TLC *(Table 1)*. Similar differences in association may be the reason for the big difference in polarity *(Table 1)* of the oligo-albaspidins **19**-BBB, **25**-BBBB, **37**-BBBBB, and **38**-BBBBBB and the oligo-flavaspidic acids **23**-BBB, **26**-BBBB, and **39**-BBBBBB.

7. Discussion. – In spite of some similarities the composition of the phloroglucinols in *D. aitoniana* is quite distinct from that found in *D. filix-mas*. The formation of the two series of oligo-albaspidins (**19**-BBB, **25**-BBBB, **37**-BBBBB, and **38**-BBBBBB) and oligo-flavaspidic acids (**23**-BBB, **26**-BBBB, and **39**-BBBBBB) is well understandable by assuming a similar biosynthetic pathway as found for the simpler compounds [3]. Similar, rather sensitive compounds of relatively high molecular weight may also be present in other ferns. Careful analytical methods avoiding destruction must be used for their detection.

We wish to express our gratitude to the following persons: Mr. *K. Aegerter*, Institute of organic chemistry, University of Basel, for drawing the formulae; Prof. *P. Äyräs*, Dept. of chemistry and biochemistry, University of Turku, Finland, for giving us information on unpublished ^{13}C-NMR spectra and preferred tautomers in some fern phloroglucinols; Mr. *C. R. Fraser-Jenkins*, Oxford, England, for collecting the plants; Dr. *H. Fuhrer* and Mr. *A. Borer*, physical laboratory of the *Ciba-Geigy AG*, Basel, for high-resolution ^{1}H-NMR, spectra, and Prof. *W. J. Richter*, physical laboratory of the *Ciba-Geigy AG*, Basel, and his coworkers for FD- and FAB-MS.

Experimental Part

General. – Melting points (m.p.) were recorded on a hot stage microscope and are corrected.

TLC. SiO_2 impregnated with citric-acid-phosphate buffer as recommended by *Schantz* and *Nikula* [30] was our preferred material, see *Widén et al.* [31].

Preparation of Buffers. Mixtures of two stock solutions (A and **B**) are used: A = 35.50 g of $Na_2HPO_4 \cdot 2H_2O$ ad 1000 ml of H_2O = 0.2M. B = 21.01 g of cryst. citric acid ($C_6H_8O_7 \cdot H_2O$) ad 1000 ml of H_2O = 0.1M. Buffer pH 4.0: 38.6 ml A + 61.4 ml **B**; pH 6.0: 63.2 ml A + 36.8 ml **B**; pH 7.0: 80.3 ml A + 19.8 ml **B**.

Preparation of the Buffered SiO_2 Plates. 30 g of Silicagel *(Art. 7741, Kieselgel HF 254 + 366* (Typ *66) für Dünnschichtchromatographie Merck)* is well mixed with 60 ml of buffer and 20 ml of H_2O in a blender and left for 90 min; 0.10-mm thick layer of the paste is applied to glass plates 8 × 20 cm. These are dried at r.t. for 60 min, then activated at 105° for 60 min, and can be stored in a dry room for several weeks.

TLC (ascending) was performed mainly with one of the four following solvent systems: I: MeOH/$(i\text{-Pr})_2O$ (freshly distilled, b.p. 67–68°)/cyclohexane 10:35:55; II: $CHCl_3$/hexane/EtOH 45:45:10; III: $CHCl_3$/hexane/EtOH/AcOH 45:35:16:4; IV: $CHCl_3$/hexane/EtOH/AcOH 45:31:16:8; V: AcOEt/cyclohexane/AcOH 60:35:5. Normally 5 samples were run at the same plate. When the front had moved 175–180 mm, the plates were dried in air at *ca.* 20° for 5 min. Some workers recommend to run the same plate a second time, if resolution of slow moving compounds is insufficient. We had better results in using more polar systems in such cases and had them run only once. Spots were visualized first in the dark chamber under UV light (both short- and long-wave with maxima *ca.* 254 and 366 μm), and afterwards by spraying with a freshly prepared 0.1% soln. of 'fast blue salt' (*'Echtblausalz B'*, Merck; = tetrazotized di-*ortho*-anisidine in H_2O [32]).

Checking of Doubtful Spots. Spots located by UV (not sprayed) can be scratched out, the material slightly moistened with H_2O, eluted with AcOEt/Et_2O and rechromatographed in another system.

Checking Absorbing Material for Destructive Action. Polyamides, different types of silica gel, aluminium oxides *etc.* can be checked in the following way: on a plate prepared with one of such material, a drop of the soln. of the phloroglucinol is placed at the starting line and left there for 1 h. Another drop of the same soln. is applied on the second track and chromatography immediately started. If results on both tracks are the same the material is

harmless. Destruction is visible, if the material on the first track does not give any spot or at a different distance than the second. Checking can also be achieved as described in [31].

General Precaution and Preparative Isolation of Compounds. All commercial, pure solvents were distilled before use to eliminate traces of non-volatile impurities and particularly peroxides from Et_2O; this was kept in the dark for no longer than 2 d before use. $(i\text{-}Pr)_2O$ decomposes easily on standing to form i-PrOH and acetone, only the pure fraction of b.p. 67–68° was used. For liquid extraction and counter current distribution, selected separatory funnels were used in which the ground glass stoppers keep sufficiently tight to allow clean separation during the work with only a drop of H_2O or MeOH as lubricant, *i.e. without any fat, silicone, or other grease.* During storage in the dry state, the stoppers and stopcocks of such funnels must be protected with a strip of paper to prevent the stopcocks from getting stuck and assure their later removal.

Purification of Microcrystalline Cellulose. 'Art. 2331, Cellulose microcrystallin 'Avicel' *für die Säulenchromatographie, Merck'* was washed at r.t. (3× each) with H_2O, MeOH, and acetone, dried *in vacuo* (0.1 Torr, 70–80°) for 1 h.

Purification of Hydrophylic Polyamide for Column Chromatography. Commercial *'MN-Polyamid SC 6 (Polycaprolactam) für die Säulenchromatographie, Macherey Nagel & Co'* was washed (3× each) at r.t. with AcOEt, MeOH, acetone, and Et_2O and dried *in vacuo* at 30°.

Preparation of the Buffered Silica-Gel for Column Chromatography. Commercial fine grain silica gel [33] *(Art. 9385 Kieselgel, Korngrösse 0.040–0.063 mm (230–400 mesh ASTM) für Säulenchromatographie, Merck)* was washed with H_2O, MeOH, $CHCl_3$, acetone, and Et_2O, dried *in vacuo*, finally at 100°. 100 g of this material were well mixed with 50 ml of H_2O and 50 ml of buffer (see above), and dried *in vacuo* finally at 70° for 1 h. The later fractions eluted from columns prepared with this material contain citric acid. After evaporating *in vacuo*, they have to be dissolved in Et_2O (or Et_2O/AcOEt) and H_2O, and the org. layer washed with H_2O to get it free of citric acid. This is a complication as far unavoidable.

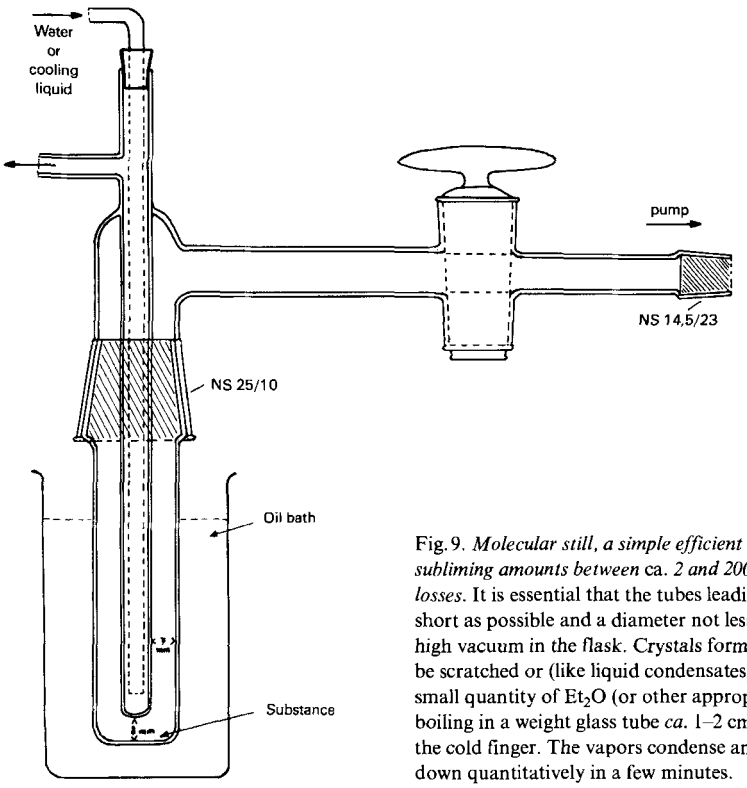


Fig. 9. *Molecular still, a simple efficient device for distilling or subliming amounts between* ca. *2 and 200 mg* in vacuo *without losses.* It is essential that the tubes leading to the pump are as short as possible and a diameter not less than 8 mm to secure high vacuum in the flask. Crystals formed on the cold finger can be scratched or (like liquid condensates) washed down with a small quantity of Et_2O (or other appropriate liquid), kept gently boiling in a weight glass tube *ca.* 1–2 cm below the lower end of the cold finger. The vapors condense and wash the material down quantitatively in a few minutes.

Plant Material. – 9 rhizomes collected in Madeira, *Eucalyptus* wood by stream *ca.* 2 km southwest of Portela, south of Porto da Cruz, on east-side of the Island at *ca.* 500 m alt., 1.VIII.1979 by *C.R. Fraser-Jenkins* No. 9773–9782. They were dried in the shade and obtained in Basel on 24.VIII.1979. Vouchers (pressed fronds) are deposited in the herbarium of the British Museum (Natural History), London (BM), and Herb. TR-5127 (A–I). Extraction and separation was done in two batches (with slight modifications).

Extraction of Batch 1. – The biggest rhizome (*ca.* 200 g, still green and fresh material) was cut in pieces, ground in a mill and 185 g of the dry powder warmed with 1000 ml Et_2O under reflux for 10 min and after cooling, filtered under slight pressure. The filter cake was further extracted 15× in the same manner. The last extract was virtually free from phenolics. The combined solns. were concentrated to 500 ml, and this soln. washed 3× with 20 ml 1N aq. HCl and 3× with H_2O. The aq. phases went through 3 more funnels with 100 ml of Et_2O each, to reextract traces of phenolic material. As a check the final acid aq. phases were combined, evaporated *in vacuo* and the residue dried over KOH at 0.1 Torr., leaving 2.04 g 'salts' (dark brown mass, soluble in MeOH but poorly soluble in H_2O or dil. HCl), which were not further investigated. The Et_2O layers were dried (Na_2SO_4) and evaporated giving 13 g (7%) of 'crude cation-free Et_2O extract'.

Counter-Current Distribution. This was performed by manual shaking in 5 separatory funnels. The 13 g of 'crude cation-free Et_2O extract' were transferred into the first funnel with MeOH/H_2O 95:5 (15 ml) and hexane (200 ml) and shaken (the material is not completely soluble, neither in MeOH alone nor in hexane alone). After separation, the MeOH layer was transferred to the second funnel where it was shaken with fresh hexane (100 ml), then to the third, fourth and fifth. Thereafter fresh 95% MeOH (15 ml) was again introduced into the first funnel and after shaking and separation transferred to the second and so on. The whole procedure was repeated until 45 MeOH phases have passed, they were worked up in 3 groups (see below).

The five remaining hexane layers (after 45 MeOH washings) were dried (Na_2SO_4) and evaporated giving 6.76 g 'hexane phase' (separation by chromatography, see below, *Chrom. 12* and *16*).

The first 15 MeOH layers were united, freed from MeOH *in vacuo* and the remaining aq. suspension extracted 3× with Et_2O and 2× with $CHCl_3$. After drying (Na_2SO_4), evaporation gave 2.03 g of Et_2O-soluble and 0.208 g of $CHCl_3$-soluble material. The MeOH layers No. 16–30 gave in the same way 0.960 g of Et_2O-soluble and 0.034 g of $CHCl_3$-soluble material. The MeOH layers 31–45 gave still 0.690 g of Et_2O-soluble and 0.024 g of $CHCl_3$-soluble material. These parts were chromatographed separately (*Chrom. 1* and *10*).

A total of 39 chromatographies were performed to separate the material and isolate pure compounds. Details are given only for a few to show how the different adsorbing materials were used.

Avicel was preferred for separating the highly polar material (MeOH layers of counter-current distribution) containing mainly flavaspidic acid (**5**) and the oligoflavaspidic acids **23**, **26**, and **39**, see *Chrom. 1, 28,* and *38*. With some care all these compounds can already be obtained in the first run. Less polar material (if present) is concentrated in the first fractions and can be rechromatographed on polyamide or buffered SiO_2. The material which may remain in the columns after elution with neutral solvents is best eluted with Et_2O or AcOEt containing 1–6% of AcOH and chromatographed. In *Chrom. 1* and *28*, we still used mixtures with $CHCl_3$ for this purpose before realizing that $CHCl_3$ is detrimental. Rechromatography of such very polar material can be done on *Avicel* or buffered SiO_2. Checking the precise weight of all fractions allows conclusion whether still some material is left on the column.

Polyamide was preferred for the least polar material (hexane layers of counter current distribution) including albaspidin (**10**) and the oligoalbaspidins **19**, **25**, **37**, and **38**. This material usually contains still small amounts of highly polar material which is hold back on polyamid rather firmly but can be washed down at the end by AcOEt containing 1–6% of AcOH. This part can also be separated on *Avicel* or buffered SiO_2.

Buffered SiO_2 was mainly used for separating mixtures (concentrates) obtained on one of the above mentioned columns, it is less advisable for separating the large amounts of crude extracts directly. It is more time-consuming, particularly for eliminating the citric acid from eluates. Processing large amounts cannot be finished in one day and the material suffers. We also realized only during the work that $CHCl_3$ as ingredient for elution mixtures is detrimental and lost much material by its use. The oligoflavaspidic acids **23**, **26**, and **39** in particular deteriorate quickly in $CHCl_3$ soln.

Chromatography 1. – The 1.65-g material of the MeOH layers 16–45 of counter-current distribution *(Batch 1)* representing the most polar material was chromatographed on a column of 200 g *Avicel* prepared with pentane. The 1.65-g material were first dissolved in MeOH (10 ml), the soln. adsorbed on 8 g of dry *Avicel* and dried *in vacuo*. The dry powder was packed on top of the columns. Pressure was applied in this and all following chromatograms to elute the fractions as fast as possible [33].

Fr. 1–2 (44 mg, eluted with pentane) showed in the TLC only the spot of albaspidin (**10**) and gave from pentane 4 mg of cryst. albaspidin, m.p. 145–154°.

Fr. 3–5 (359 mg, eluted with pentane) gave from benzene/pentane 91 mg of flavaspidic acid BB (**5-BB**), m.p. 159–162°, and 29 mg of a second crop slightly lower melting. The mother liquors gave in the TLC only a weak spot for albaspidin (**10**) aside of a strong one for **5**.

Fr. 6–14 (302 mg, eluted with pentane and pentane/benzene 9:1) did not crystallize, according to TLC, they contained flavaspidic acid (much) and some trisflavaspidic acid (**23**).

Fr. 15 (15 mg, eluted with pentane/benzene 9:1) gave from a trace $AcOH/H_2O$ 95:5 3 mg cryst. trisflavaspidic acid (**23**), m.p. 174–176° and 3 mg of a substance, m.p. 185–187°, both giving the same spot in TLC.

Fr. 16–36 all crystallized and the crystals of each fraction were checked in TLC. Those of similar composition were united as follows:

Fr. 16–20 (169 mg, eluted with pentane/benzene 9:1 and 8:2) gave from Et_2O/pentane 75 mg of a mixture (mainly tetraflavaspidic acid (**26**)). This was separated in *Chrom. 2*, together with the material from the mother liquor (94 mg)[5]).

Fr. 21–26 (133 mg, eluted with pentane/benzene 8:2 to 6:4) gave 67 mg crystals of which those from *Fr. 21–24* contained tetraflavaspidic acid (**26**) and hexaflavaspidic acid (**39**), while those from *Fr. 25–26* nearly pure **39**. These crystals (22 mg) were recrystallized from Et_2O/pentane giving pure hexaflavaspidic acid, prep. TR-1574, m.p. 190–192°. The material from the mother liquor (53 mg) was chromatographed on 11 g of SiO_2 buffered for pH 6, but no pure crystals could be obtained. Many fractions contained quickly moving compounds (albaspidin). Deterioration has obviously occurred perhaps by using $CHCl_3$ as solvent for elution.

Fr. 27–34 (196 mg, eluted with pentane/benzene and pure benzene) gave from Et_2O/pentane crystals (61 mg) containing mainly hexaflavaspidic acid (**39**), some showed m.p. *ca.* 185–187° and were quite pure. We unfortunately tried to separate them completely by chromatography on buffered SiO_2 *(Chrom. 5)*[5]), but the material deteriorated. The material from the mother liquor was united with analogous from *Fr. 35–36* (total 135 mg) and rechromatographed on SiO_2 buffered to pH 3 *(Chrom. 4)*[5]): only 2 mg of pure hexaflavaspidic acid (TR-1572), m.p. 210–215°, from Et_2O/MeOH in pure state and again rather much loss occurred ($CHCl_3$ was also used in this chromatography). After some time the m.p. of the crystals went down to 180–183°.

Fr. 35–36 (19 mg, eluted with benzene and benzene/Et_2O 85:15) gave from Et_2O/hexane 2 mg of cryst. hexaflavaspidic acid (**39**), m.p. 155–183°.

Fr. 37–44 (218 mg eluted with benzene/Et_2O, pure Et_2O and Et_2O + 5–20% 'mixture' of MeOH/$CHCl_3$/AcOEt 1:1:1[5])) did not crystallize. They were rechromatographed on buffered SiO_2 *(Chrom. 6)*[5]), but again without success and much loss.

Fr. 45–48 (121 mg, eluted with Et_2O + 40% 'mixture' and pure 'mixture') gave no crystals, and rechromatography on buffered SiO_2 (chrom. 7) gave no pure products.

Chromatography 2[5]). – The 94-mg material from mother liquor of *Fr. 16–20 (Chrom. 1)* was separated on a column of 12 g SiO_2, exactly like *Chrom. 3*. Only 2 mg of cryst. tetraflavaspidic acid (**26**) and 1 mg of cryst. hexaflavaspidic acid (**39**), m.p. 189–190°, were obtained.

Chromatography 3[5]). – The mixed crystals (75 mg) from *Fr. 16–20* of *Chrom. 1* were united with the residue (11 mg) of mother liquor of tetraflavaspidic acid (*Fr. 16–20* of *Chrom. 2*). This material (86 mg) was chromatographed on a column of 12 g SiO_2 buffered for pH 4 prepared in hexane. 'Mixture I' stands for EtOH/$CHCl_3$/hexane 20:40:40 'mixture II' for MeOH/$CHCl_3$/AcOEt 1:1:1.

Fr. 1–6 (0.9 mg, eluted with hexane + 10–65% 'mixture I') gave no spot in the TLC.

Fr. 7 (17 mg, eluted with pure 'mixture I') gave no crystals.

Fr. 8 (22 mg, eluted with pure 'mixture I') gave from Et_2O/pentane 13 mg of cryst. tetraflavaspidic acid, m.p. 167–169° (prep. TR-1575).

Fr. 9–12 (47 mg, eluted with 'mixture I' with addition of 2–3% 'mixture II') gave from Et_2O/pentane 18 mg of pure tetraflavaspidic acid, m.p. 170–171° (prep. TR-1571).

Fr. 13–14 (6 mg eluted with 'mixture I' + 4–7% 'mixture II') was, according to TLC a mixture of tetra- with a little hexaflavaspidic acid.

Fr. 15–19 (8 mg, eluted with 'mixture I' + 10–40% 'mixture II') was a similar mixture but about 1:1.

Fr. 20 (1.5 mg, eluted with 50% of each mixture) was a similar mixture in a ratio of *ca.* 1:2.

Fr. 21–22 (14 mg, eluted with 'mixture I' + 60 and 70% 'mixture II') gave from Et_2O/pentane 6 mg of hexaflavaspidic acid with double m.p. 110→220° (not quite pure).

Fr. 23–24 (7 mg, eluted with 80% and 100% 'mixture II') gave no spot in TLC.

This chromatography worked fairly satisfactory in spite of the use of $CHCl_3$, probably because it could be finished rather quickly.

[5]) This chromatography was performed before we realized that $CHCl_3$ is detrimental. $CHCl_3$ was avoided in all *Chrom. 12–39* (except *Chrom. 30*) and best systems given in *Chrom. 31–39*.

Chromatography 10. – The 2.03-g material of the MeOH layers 1–15 of the counter-current distribution was chromatographed on a column of 250 g *Avicel* prepared with pentane. Only 28 mg of cryst. flavaspidic acid (**5**) was obtained from the *Fr. 6–10* (70 mg, eluted with pentane) and rechromatography of *Fr. 20–32* (241 mg, eluted with hexane/benzene) on SiO_2 buffered for pH 4.5 *(Chrom. 11)* gave 1 mg crystals, m.p. 144–146° (TR-1576), showing only one spot in the TLC just between tetra- and hexaflavaspidic acid. The amount was insufficient to check for the suspected structure of pentaflavaspidic acid. A trace of hexaflavaspidic acid was also isolated in crystals, m.p. 117–120° (TR-1577). A better separation of the oligoflavaspidic acids, see *Chrom. 28, 30,* and *31* of *Batch 2.*

Chromatography 12. – Separation of 'hexane phase' on polyamide (cf. *Chrom. 25* of *Batch 2* and *Chrom. 16* on SiO_2): 1.78 g of the material (49 g rhizome) were chromatographed on a column of 100 g purified polyamide *SC 6* prepared with hexane. Each fraction was eluted with 150 ml of solvent.

Fr. 1–2 (13 mg, eluted with hexane) contained no phenolics.

Fr. 3–5 (778 mg, eluted with hexane) gave from hexane 70 mg of crude albaspidin crystals. The mother liquor contained more albaspidin (**10**), the compd. TR-1579, *para*-aspidin (**7**) and tris-*para*-aspidin (**20**). Used for *Chrom. 13.*

Fr. 6–9 (132 mg, eluted with hexane) gave from hexane 13 mg crude albaspidin crystals.

Fr. 10–22 (310 mg, eluted with hexane/benzene up to 60% benzene) contained flavaspidic acid and more polar compounds.

Fr. 23–32 (315 mg, eluted with hexane/benzene, pure benzene and benzene + AcOH up to 1%) gave 4 similar spots in TLC.

Fr. 33–44 (297 mg, eluted with benzene/AcOH to 4% acetic) gave spots of flavaspidic, tris-, tetra- and hexaflavaspidic acids. 7 mg of crude cryst. hexaflavaspidic acid, m.p. 152–162°, could be isolated from *Fr. 35,* and 2 mg from *Fr. 38.* The material from mother liquor (237 mg) was rechromatographed on SiO_2 *(Chrom. 24).*

Chromatography 13. – Isolation of fractions containing compound TR-1579 and of pure tris-*para*-aspidin (**20**): 700 mg amorphous material from *Fr. 3–5* of *Chrom. 12* were chromatographed on a column of 80 g SiO_2 buffered at pH 6 prepared with cyclohexane. Each fraction eluted with 70 ml of solvent. *Fr. 1* with cyclohexane, *Fr. 2–12* with 'mixture' (MeOH/(i-Pr)$_2$O/cyclohexane 10:35:55), from *Fr. 13* on with the same mixture after addition of 1–5% AcOH. Eluted material was distributed between AcOEt/Et_2O and H_2O to eliminate citric acids.

Fr. 1–3 (410 mg) contained palmitic acid, compd. TR-1579, albaspidin *etc.*, separation see below.

Fr. 4–5 (147 mg) gave 5 spots in TLC.

Fr. 6–10 (68 mg) gave similar spots.

Fr. 11–17 (23 mg) partly crystalline gave two spots, corresponding to *para*-aspidin (**7**) and tris-*para*-aspidin (**20**).

Fr. 18–25 (29 mg, crystalline), showed mainly the spots of tris-*para*-aspidin (**20**) and tetra-albaspidin (**25**). This was used for *Chrom. 23* (on SiO_2 buffered for pH 6), but no good separation could be achieved.

Fr. 26 (67 mg) gave from Et_2O/MeOH 22 mg pure cryst. of tris-*para*-aspidin **BB** (TR-1584), m.p. 124–126°, and 16 mg of second quality, m.p. 106–120°.

Fr. 27–32 (only 8 mg of material) contained only traces of highly polar phenolics.

Chromatography of the 'hexane phase' directly on buffered SiO *(Chrom. 16)* was less satisfactory, but with more experience, we got good separation of the oligo-albaspidins from the *Batch 2* on polyamide *(Chrom. 25).*

Separation of the Fr. 1–3 *(410 mg) from* Chrom. 13 *with* Na_2CO_3. This material was dissolved in Et_2O and shaken 2× with 3 ml of 2N Na_2CO_3 and 2× with H_2O. The Et_2O layer was washed with dil. HCl and H_2O and gave after drying (Na_2SO_4) and evaporation 320 mg of 'neutral' material (see *Chrom. 15*). The Na_2CO_3 solns. were acidified with HCl and extracted with Et_2O giving 33 mg of Na_2CO_3-soluble material and from hexane 5 mg of cryst. albaspidin (**10**). The H_2O washings treated in the same way gave 18 mg of material containing compd. TR-1579 used for *Chrom. 14.*

Chromatography 14. – The 18-mg material from H_2O washings (from *Fr. 1–3* of *Chrom. 13*) was chromatographed on a column of 4 g SiO_2 buffered for pH 7 prepared with pentane. Elution was done (4 ml each fraction) with pentane *(Fr. 1–4),* pentane + 'mixture' (MeOH/(i-Pr)$_2$O/cyclohexane 10:35:55) *(Fr. 5–10),* pure 'mixture' *(Fr. 10–14),* and 'mixture' + 0.1–7% AcOH *(Fr. 15–30).*

The *Fr. 1–15* (8.3 mg) gave only a faint spot in TLC. The *Fr. 16–18* (4.5 mg) contained compd. TR-1579 and were united with similar material. The *Fr. 19–30* gave together only 1.4 mg material, showing no spot in TLC.

Chromatography 15. – The 320-mg 'neutral' material from *Fr. 1–3* of *Chrom. 13* containing the main part of compound TR-1579 was chromatographed on 60 g SiO_2 buffered for pH 7 exactly in the same way with 50 ml per fraction.

Fr. 1–15 (233 mg) gave no spot in TLC. From *Fr. 9–11* (55 mg) 5 mg of crude palmitic acid, m.p. 50–60°, were crystallized from acetone/MeOH.

Fr. 16–21 (45 mg) gave the spot of compd. TR-1579.

Fr. 22–36 (11.5 mg) gave only a weak spot of highly polar material.

The 45-mg material from *Fr. 16–21* was warmed in the molecular still *(Fig. 9)* at 0.1 Torr from 140 to 180°. The distillate (10 mg) gave from acetone/MeOH 1 mg of compd. TR-1579, m.p. 62–65°. The crystalline material from mother liquor was used for *Chrom. 17*.

Chromatography 16. – Separation of the 'hexane phase' on SiO_2 *(cf. Chrom. 12)*: 4.98 g of this material (136 g dry rhizome) were chromatographed directly on 300 g of SiO_2 buffered for pH 7. 47 fractions (each 500 ml) were eluted with pentane *(Fr. 1–5)*, pentane + 'mixture' (MeOH/$(i\text{-Pr})_2O$/cyclohexane), 4–65% *(Fr. 6–13)*, pure 'mixture' *(Fr. 14–16)*, and 'mixture' + 0.1–5% AcOH *(Fr. 17–47)*.

Fr. 1–14 (294 mg) contained no phenolics but fatty acids *etc.*, discarded.

Fr. 15 (200 mg) showed the yellow spot of the compd. TR-1579 in TLC. It was directly treated in the molecular still at 0.05 Torr and 160–180° bath temp. The distillate (8 mg) gave from acetone/MeOH 4 mg of pale yellowish crystals, m.p. 55–60° (crude compd. TR-1579) united with similar material (see above). The non-distillable residue (171 mg) gave no spot in TLC.

Fr. 16–17 (1290 mg) gave from Et_2O/hexane 151 mg of crude albaspidin (**10**), m.p. 138–142°. The amorphous residues (1140 mg) were worked up for isolation of a little more compd. TR-1579 in two different ways (see below, *Chrom. 18*).

Fr. 18–19 (80 mg) did again contain albaspidin (no compound 1579) and did not crystallize.

Fr. 20–27 (252 mg) gave from Et_2O/hexane 42.5 mg of cryst. albaspidin (**10**).

Fr. 28–29 (550 mg) still contained some albaspidin and two other phenolics, one of them probably compd. TR-1583.

Fr. 30–31 (297 mg) again contained a little albaspidin (**10**), *para*-aspidin (**7**), tris-*para*-aspidin (**20**), tetra-albaspidin (**25**) and non-phenolics. It was rechromatographed *(Chrom. 37)* for isolation of **25** (31 mg of crude crystals).

Fr. 32–35 (596 mg): dark brown-green material contained **20**, **25**, **37** and **38**.

Fr. 36–42 (873 mg): dark-brown material showed only spots for **37** and **38**.

Fr. 43–47 (215 mg): dark-brown material showed only spots for **37** and **38**.

The following experiments including *Chrom. 17–22* were performed for isolation of the crude compd. TR-1579.

Chromatography 17. – The following material containing compd. TR-1579 was united (total 13.5 mg): Evaporated mother liquor from distilled *Fr. 16–21* of *Chrom. 15* (9 mg) and *Fr. 15* of *Chrom. 16* (4.5 mg). It was chromatographed on a column of 3 g polyamide *SC 6* prepared with pentane. The good material came down with *Fr. 1–4* (6.8 mg) giving 2 mg of cryst. compd. TR-1579. Further fractions eluted with benzene and benzene/AcOH mixtures gave a total of 7.7 mg material giving only a spot at the start in TLC.

Chromatography 18. – 100 mg of the amorphous material from *Fr. 16–17* of *Chrom. 16* were chromatographed on 4 g of polyamide *SC 6* exactly in the same way. The *Fr. 1–2* (20 mg) gave the spots of compd. TR-1579 and albaspidin. Distillation in the molecular still gave 5.5 mg of distillate (crude compd. TR-1579), it was united with similar material for *Chrom. 21*. 12 mg non-distillable residue gave no spot in the TLC (discarded).

The further *Fr. 3–28* gave 78.5 mg with only traces of phenolics (discarded).

Separation of the Further 970 mg Amorphous Material from Fr. 16–17 *of* Chrom. 16. This was separated with Na_2CO_3 soln. exactly like the *Fr. 1–3* from *Chrom. 13*. The Na_2CO_3-soluble material (53 mg) gave 14 mg of albaspidin (**10**), m.p. 148–150°, from Et_2O/pentane. The H_2O washing gave 66 mg of material showing the spot of compd. TR-1579 used for *Chrom. 20*. The 'neutral' (837 mg) material was used for *Chrom. 19*.

Chromatography 19. – The 837-mg 'neutral' material was chromatographed on 62 g of polyamide *SC 6*. Compd. TR-1579 was eluted in *Fr. 1–3* (110 mg, eluted with pentane) and used for *Chrom. 21*.

Fr. 4–8 (85 mg, eluted with pentane) gave from acetone/MeOH 24 mg of crystals, m.p. 62–65° (sample TR-1600), mixed m.p. with palmitic acid gave a depression (50–52°), no spot with 'fast blue salt' in TLC.

Fr. 9–12 (37 mg, eluted with pentane and pentane/benzene 95:5) gave in the same way 13 mg of crystals with double m.p. 56→110°, no colour with 'fast blue salt'.

Fr. 13–19 (75 mg, eluted with pentane/benzene up to 30% benzene) gave again 9 mg of similar crystals, m.p. 105–130°.

Fr. 20–42 (463 mg, eluted with benzene, Et_2O and Et_2O/AcOH up to 5%) gave also no spots in TLC.

Chromatography 20. – The 66-mg material from H_2O washing was chromatographed on 7 g of polyamide *SC 6* in the same way. The compd. TR-1579 came down with pure pentane in *Fr. 2–4* (8.5 mg).

Chromatography 21 and 22. – The 5-mg distilled material from *Fr. 1–2* of *Chrom. 18* was united with the 119-mg material from *Fr. 1–3* of *Chrom. 19* and chromatographed on 18 g of SiO_2 buffered for pH 7. Each fraction was eluted with 12 ml of solvent, *Fr. 1–5* with pentane, *Fr. 6–14* with pentane + 2–65% 'mixture' (MeOH/$(i\text{-}Pr)_2O$/cyclohexane 10:50:40), *Fr. 15–20* with pure 'mixture'.

Compd. TR-1579 came down with *Fr. 17–19* (97 mg) and could be concentrated to 70 mg in *Chrom. 22* (same conditions).

The 70-mg material gave in the molecular still 8 mg of distillate and from acetone/Et_2O 4 mg of crude crystals of compd. TR-1579.

Chromatography 24. – The 237-mg material *Fr. 33–34* from *Chrom. 12* was chromatographed on 26 g of SiO_2 buffered for pH 6 prepared in a 'mixture' (MeOH/$(i\text{-}Pr)_2O$/cyclohexane 15:60:25). Each fraction was eluted with 25 ml of the same 'mixture' and, from *Fr. 7* on, with addition of 0.1–6% AcOH. Weight of eluted material refers to material after elimination of citric acid.

Fr. 1–4 (62 mg) gave no spot in TLC, discarded.

Fr. 5–8 (77 mg, eluted with 'mixture' + 0.1–0.3% AcOH) gave the spots for albaspidin (**10**), trisflavaspidic acid (**23**), and tetraflavaspidic acid (**26**).

Fr. 9–16 (50 mg, eluted with 'mixture' + up to 2% AcOH) gave from Et_2O/MeOH 26 mg of cryst. hexaflavaspidic acid, m.p. 177–184° (not quite pure).

Fr. 17–23 (up to 4% AcOH) (17 mg), light brown material gave from Et_2O/MeOH 1:4 4 mg of cryst. sample TR-1585, m.p. 190–195°. This was even more polar than **39**, no MS could be recorded, neither under FD nor FAB conditions. It may be a salt or (more probably) a high molecular member of an oligo flavaspidic acid.

Fr. 24–28 (eluted with addition of 5 and 6% AcOH) gave only little material showing no spot in TLC.

Extraction of Batch 2. – The eight remaining rhizomes were rather hard, difficult to break and woody brown, not green. 500-g powder was extracted like *Batch 1* giving 28.5 g cation-free Et_2O extract. Counter-current distribution gave 14.88 g of hexane soluble material (3%) used for *Chrom. 25*. The MeOH phases were worked up in groups and processed further as follows: 1–5 (4.46 g) for *Chrom. 28*; 6–15 (2.225 g) for *Chrom. 30*; 16–25 (1.895 g) for *Chrom. 31*; 26–35 (1.325 g) and 36–45 (1.195 g) both together for *Chrom. 38*. The compd. TR-1579 was not observed in this second batch of rhizomes.

Chromatography 25. – The 14.88-g hexane-soluble material gave from hexane directly 930 mg of crude crystals of albaspidin. The remaining material (13.95 g) was chromatographed on 210 g of polyamide *SC 6* in a similar way as the first batch (see *Chrom. 12*).

Fr. 1–3 (6.312 g, eluted with pentane) gave 560 mg of cryst. albaspidin, m.p. 141–146°, and 207 mg of second and third crop crystals. Only very weak spots of other phenolics were visible in TCL and of the mother liquors.

Fr. 4–5 (777 mg, eluted with pentane) gave from acetone/MeOH 40 mg of colourless crystals, m.p. 62–65°, similar to crystals from *Fr. 4–8* of *Chrom. 19*, giving no colour with 'fast blue salt'.

Fr. 6 (218 mg, eluted with pentane) gave 30 mg of crystals, m.p. 100–135°, showing six spots in TLC, including tetra-albaspidin.

Fr. 7 + 8 (304 mg, eluted with pentane) gave from Et_2O/MeOH 38 mg of penta-albaspidin (TR-1586a), m.p. 167–170°.

Fr. 9 (107 mg, eluted with pentane) gave from $CHCl_3$/MeOH 12 mg of pure penta-albaspidin, m.p. 170–173°, and 7 mg second crop, m.p. 140–155°.

Fr. 10–14 (403 mg, eluted with pentane and pentane/benzene 95:5 for *Fr. 14*) gave from acetone/MeOH 58 mg of penta-albaspidin (TR-1586c), m.p. 150–174°.

Fr. 15–16 (93 mg, eluted with pentane/benzene containing 10–20% benzene) gave from acetone/MeOH 16 mg of penta-albaspidin (TR-1586d), m.p. 165–167°.

Fr. 17 (58 mg, eluted with pentane/benzene 6:4) gave in TLC still the main spot for penta-albaspidin with a weak one for hexa-albaspidin and from acetone/MeOH 13 mg of crystals (mixture), m.p. 100–105° (–150°), giving similar spots in TLC.

The united mother liquors of *Fr. 7–17* left 693 mg of dry material showing mainly the spot for penta-albaspidin in TLC.

Fr. 18–19 (295 mg, eluted with pentane/benzene 30:70 and pure benzene) gave from acetone/MeOH 72 mg of crystals, m.p. 80–105°, according to TLC a mixture of penta- and hexa-albaspidin.

Fr. 20 (180 mg, eluted with benzene/AcOH 99.5:0.5), gave 26 mg of crystals, m.p. 150–155°, containing mainly hexa- (with a little) penta-albaspidin.

Fr. 21–23 (462 mg, eluted with benzene + 0.7–1% AcOH) gave from acetone/MeOH 50 mg of crystals of hexa-albaspidin, m.p. 168–175° (sintering at *ca.* 155°, sample TR-1587).

Fr. 24–30 (825 mg, eluted with benzene + 1–2% AcOH) gave no crystals in TLC, *ca.* 8 spots, aside of five weak ones, including hexa- and penta-albaspidin, three fast-moving strong ones, the fastest corresponding to albaspidin (perhaps formed by rottlerone rearrangement?).

Fr. 31–33 (3.3 mg, eluted with benzene containing 2–3% AcOH) gave no crystals and in TLC 7 similar spots (the fastest for albaspidin missing).

Fr. 34–37 (1460 mg, eluted with benzene/Et_2O/AcOH 92:5:3) showing similar 6 spots (hexa-albaspidin missing).

Fr. 38–44 (665 mg, eluted with benzene/Et_2O/AcOH 87:10:3 – 75:20:5) gave 5 spots tailing from start.

Fr. 45–50 (890 mg, eluted with benzene/Et_2O/AcOH 65:30:5 – 55:40:5) consisted of mainly non-phenolics and a little fast-moving phenolics, only in *Fr. 47* a spot corresponding to trisflavaspidic acid.

Fr. 51–64 (570 mg, eluted with benzene/Et_2O/AcOH 45:50:5) gave several spots, one corresponding to hexaflavaspidic acid. This material was separated in *Chrom. 26* and *27*.

Fr. 65–70 (87 mg, eluted with benzene/Et_2O/AcOH 15:80:5 – 0:95:5), gave only one fast-moving spot in TLC.

Chromatography 26. – The 570-mg material from *Fr. 51–64* of *Chrom. 25* was chromatographed on a column of 100 g SiO_2 buffered for pH 6 prepared with a 'mixture' (MeOH/$(i\text{-Pr})_2O$/cyclohexane 20:60:20). Elution was done with the same 'mixture' and addition of 0.1–5% AcOH for *Fr. 8–25*. Each fraction eluted with 60 ml of solvent.

Fr. 1–4 (276 mg, eluted with 'mixture') gave only one fast-moving spot in TLC.

Fr. 5–7 (82 mg, eluted with 'mixture') gave still a strong, fast-moving spot and two spots corresponding to tetra- and hexaflavaspidic acid. 3.5 mg of the latter were obtained in crystals, m.p. 168–171°, and 2 mg from $CHCl_3/Et_2O$, m.p. 215–222°. The amorphous part was used for *Chrom. 27*.

Fr. 8–12 (85 mg, eluted with 'mixture' + 0.1–0.6% AcOH) gave from Et_2O 7.5 mg of hexaflavaspidic acid, m.p. 186–189°. The material from the mother liquors (giving a strong fast-moving spot) was used for *Chrom. 27*.

Fr. 13–17 (28 mg, eluted with 'mixture' + 0.8–1.5% AcOH). The dry amorphous residue was treated with pure Et_2O. The insoluble part washed with Et_2O/pentane and crystallized from $CHCl_3/Et_2O$ gave 1.8 mg of hexaflavaspidic acid, m.p. 186–190°. The material from the mother liquor gave besides a tailing, slow-moving spot also a fast moving (like albaspidin). It was used for *Chrom. 27*.

Fr. 18–25 (19 mg, eluted with 'mixture' + 2.5% AcOH) gave only a fast-moving, weak spot, discarded.

Chromatography 27. – The 162-mg material from mother liquors of *Fr. 5–17* from *Chrom. 26* was chromatographed on a column of 26 g SiO_2 buffered for pH 6 prepared with pentane and eluted with pentane and increasing amounts of 'mixture' (MeOH/$(i\text{-Pr})_2O$/cyclohexane 10:60:30) and from *Fr. 18* on addition of 0.1–6% AcOH.

Fr. 1–19 (7 mg, eluted with pentane, pentane/'mixture', pure 'mixture' and addition of 0.5–0.8% AcOH) gave no spot in TLC, discarded.

Fr. 19–27 (45 mg, eluted with 'mixture' + 1–4% AcOH) gave only one fast-moving spot in TLC.

Fr. 28–32 (27 mg, eluted with 'mixture' + 4.5–5% AcOH) gave a weak, slow-moving and a fast-moving spot, no crystals.

Fr. 33 (4 mg, eluted with 'mixture' + 5% AcOH) gave 3 tailing spots, one very-fast-moving.

Fr. 34–39 (23 mg, eluted with 'mixture' + 5.5–6% AcOH) gave tailing spots (one very fast) in TLC, from Et_2O/MeOH 3 mg of cryst. hexaflavaspidic acid, m.p. 176–180°.

Fr. 40–45 (26 mg, eluted with 'mixture' + 6% AcOH) gave from Et_2O/MeOH 7 mg of hexaflavaspidic acid, m.p. 210–215° and 6.5 mg of second crop, m.p. 179–185°.

This chromatography shows that the last fractions from *Chrom. 26* not only contained **39** (as expected), but also rather much material of very low polarity. The reason is still unknown.

Chromatography 28. – The 4.45-g material of the MeOH layers 1–5 of counter-current distribution *(Batch 2)* was chromatographed on a column of 400 g *Avicel* prepared in pentane in the same way as for *Chrom. 1*.

Small amounts of albaspidin and compd. TR-1579 could be traced by TLC in *Fr. 7–10*. Cryst. flavaspidic acid (13 mg) was isolated from *Fr. 16*. From the Fr. *20–22*, pure hexaflavaspidic acid (2 mg, m.p. 194–198°) was isolated and 24 mg more of this compound, m.p. 169–171°, by rechromatography of the material from mother liquor of *Fr. 17–23* (385 mg) on SiO_2 buffered for pH 6, avoiding $CHCl_3$ *(Chrom. 29)*.

Chromatography 30[5]). – The 2.2-g material from MeOH layers 6–15 of counter-current distribution was chromatographed directly on 200 g of SiO_2 buffered for pH 6, eluted with 200 ml of solvent for each fraction.

Only *Fr. 60–64* (69 mg, eluted with 'mixture' $CHCl_3$/AcOEt/hexane 40:30:30) did crystallize. 20 mg of hexaflavaspidic acid, m.p. 211–214°, were obtained from Et_2O.

Even the late *Fr. 65–66* (30 mg, eluted with the same mixture) gave a spot for tetraflavaspidic acid and a very fast-moving one.

Chromatography 31. – The 1.895-g material from MeOH layers 16–25 from counter-current distribution *(Batch 2)* was chromatographed on a column of 200 ml *Avicel* prepared in hexane. Processed similar to *Chrom. 1*, elution with 150 ml pro fraction.

Fr. 1–2 (1 mg, eluted with pentane) gave no colour.

Fr. 3–4 (221 mg, eluted with pentane) gave 64 mg of cryst. flavaspidic acid, m.p. 158–160°.

Fr. 5–6 (268 mg, eluted with pentane) gave mainly the spot of flavaspidic acid, as well as faster- and slower-moving spots in TLC. Even after chromatography on SiO_2 (*Chrom. 32*, *34*, and *35*) only small amounts of crystals could be obtained.

Fr. 7–13 (427 mg, eluted with pentane/benzene containing 5–15% benzene) contained fast-moving and slow-moving material but mainly trisflavaspidic acid (**23**) and was separated by *Chrom. 33*.

Fr. 14 (51 mg, eluted with pentane/benzene 8:2) gave only a trace of crystals.

Fr. 15–18 (158 mg, eluted with pentane/benzene 7:3 – 4:6) gave from Et_2O/MeOH 31 mg of cryst. tetraflavaspidic acid-BBBB (**26**-BBBB). Recrystallization from pure Et_2O gave 9 mg of anal. sample TR-1591, m.p. 170–171°. TLC gave only one spot, same as for TR-1571.

Fr. 19–23 (150 mg, eluted with pentane/benzene 3:7 and pure benzene) gave in TLC a main spot for **26** and two faster-moving, weak ones but no crystals.

Fr. 24–42 (458 mg, eluted with benzene/Et_2O and pure Et_2O) gave still the spot of **39**, the last *Fr. 36–42* contained very little material. This material was used for *Chrom. 39* to isolate hexaflavaspidic acid (**39**).

Fr. 43–56 (222 mg, eluted with Et_2O + increasing amounts of 'mixture' ($CHCl_3$/hexane/EtOH/AcOEt 45:35:16:4), from *Fr. 54* on pure 'mixture') still gave a very weak spot for **39**. This material was also used for *Chrom. 39*.

Chromatography 33. – The 432-mg material from *Fr. 7–13* of *Chrom. 31* was chromatographed on a column prepared of 45 g of SiO_2 buffered for pH 4 prepared with pentane. Elution (40 ml pro *Fr.*) was done with pentane with increasing amounts of 'mixture' (AcOEt/cyclohexane/AcOEt 48:48:4) and addition of extra 1–3% AcOEt to 'mixture' for *Fr. 29–42*.

Fr. 1–12 (1 mg, eluted with pentane with 0–40% 'mixture') gave no spot in TLC.

Fr. 13–16 (20 mg, eluted with pentane + 50% 'mixture') gave only the spot of albaspidin.

Fr. 17–20 (250 mg, TR-1593, eluted with pentane + 50% 'mixture') contained mainly trisflavaspidic acid. Purified in *Chrom. 36*.

Fr. 21–22 (4 mg, eluted with pentane + 50–70% 'mixture'), *Fr. 23–28* (13.5 mg, eluted with pure 'mixture') and *Fr. 29–32* (2.5 mg, eluted with 'mixture' + 1% AcOH) did not crystallize.

Fr. 33–42 (15 mg, eluted with 'mixture' + 2–3% AcOH) gave 4 mg of pure hexaflavaspidic acid (**39**) in crystals from Et_2O (TR-1592e), m.p. 206–210°.

Chromatography 36. – 200 mg of sample TR-1593 (amorphous material from *Fr. 17–20* of *Chrom. 33*) were chromatographed on a column of 25 g SiO_2 buffered for pH 4 prepared in pentane. Elution (each fraction with 20 ml solvent) was done with mixtures of AcOEt/cyclohexane/AcOH 65:35:0 – 60:35:5.

Fr. 1 (21 mg, eluted with 'mixture' (65:35:0)) gave spots for trisflavaspidic acid, flavaspidic acid and faster ones.

Fr. 2 (106 mg, eluted with 'mixture' (64:35:1)) gave a very strong spot for trisflavaspidic acid and faster ones as *Fr. 1*. 24 mg of pure trisflavaspidic acid could be crystallized as described in *Chrom. 38*. It is difficult to crystallize if no material for nucleation is available.

Fr. 3–6 (50 mg, eluted with 'mixture' (63:35:2 – 61:35:4)) gave spots for **5**, **10**, **23**, and **26**. 12.7 mg of cryst. tetraflavaspidic acid (**26**), m.p. 168–169°, were obtained from Et_2O.

Fr. 7–9 (14 mg, eluted with 'mixture' (60:35:5)) gave from Et_2O, washed with pentane, 8 mg of crystalline material which, according to TLC, was a mixture of **26** and **39**.

Chromatography 37. – The 297-mg material from *Fr. 30* and *31* of *Chrom. 16* was chromatographed on a column of 40 g SiO_2 buffered to pH 4. Each fraction eluted with 20 ml of MeOH/$(i\text{-}Pr)_2O$/cyclohexane/AcOH 10:55:35:0 – 10:55:34:1.

Fr. 1–3 (192 mg, eluted without AcOH) gave no crystals, 4 spots in TLC corresponding to **7**, **10** and **20**, and **25**.

Fr. 4 (37 mg, eluted without AcOH) gave from Et_2O/MeOH 22 mg of crude tetra-albaspidin **25**, m.p. 123–126°, and after recrystallization 19 mg of anal. sample TR-1596, m.p. 131–133°.

Fr. 5 (17 mg, eluted with addition of 0.5% AcOH) gave from Et_2O/MeOH 9 mg of crude tetra-albaspidin, m.p. 130–132°.

Fr. 6–7 (24 mg, eluted with addition of 0.5% AcOH) gave 12 mg of crystals, m.p. 150–153°, which turned out to be a mixture.

Fr. 8–13 (11 mg, eluted with 1% AcOH) gave only a spot at the start, discarded.

Chromatography 38. – *Isolation of Cryst. Trisflavaspidic Acid* (**23**). The 2.5-g material of the MeOH layers 26–45 of counter-current distribution *(Batch 2)* was chromatographed on a column of 240 g *Avicel* prepared in pentane with 150 mg per fraction (see *Chrom. 1*).

Fr. 1–2 (6.1 mg, eluted with pentane) gave no spot in TLC, discarded.

Fr. 3–7 (357 mg, eluted with pentane) gave spots for **10** and **5** in TLC, it was not further separated.

Fr. 8–17 (464 mg, eluted with pentane) contained mainly trisflavaspidic acid BBB (**23**-BBB). Crystals were first obtained when the dry material of *Fr. 8* (60 mg) was dissolved at 20° in *ca.* 0.6 ml of AcOH/H_2O 95:5 and left for 2 h at 0°. The other fractions gave crystals in the same way rather quickly after nucleation: 93 mg (m.p. *ca.* 167–170°) were obtained from Fr. 8–17. After drying *in vacuo* over KOH, they could easily be recrystallized from Et_2O/pentane. The anal. sample TR-1598 showed m.p. 171–172°. The mixed m.p. with tetraflavaspidic acid-BBBB (**26**-BBBB, TR-1591 having the same m.p.) gave a depression (m.p. 153–163°).

Fr. 18–25 (175 mg, eluted with pentane) gave 37 mg of crystals which were a mixture of tris- and tetraflavaspidic acid.

Fr. 25–26 (70 mg, eluted with pentane/benzene 95:5 and 90:10) gave from Et_2O/pentane 23 mg of crude crystals, m.p. 145–150°, and after recrystallization from Et_2O/pentane 5 mg of pure tetraflavaspidic acid (**26**), m.p. 164–165°, and 6 mg of a second crop, m.p. 155–157°.

Fr. 27–31 (206 mg, eluted with pentane/benzene 90:10 – 70:30) gave from Et_2O/pentane (after nucleation) 48 mg of tetraflavaspidic acid BBBB (**26**-BBBB), m.p. 164–166°. Recrystallization from Et_2O/pentane gave the anal. sample (TR-1599), m.p. 164–165°.

Fr. 32–35 (139 mg, eluted with pentane/benzene 65:35 – 40:60) gave in the same way 15 mg of crystals, m.p. 164–166°, and from the less Et_2O-soluble part 11 mg of crystals, m.p. 182–185°. Both gave in TLC only the spot for tetraflavaspidic acid (**26**).

Fr. 36–46 (490 mg, eluted with benzene, benzene/Et_2O, pure Et_2O, and Et_2O + 1% AcOH) containing higher polar material were not separated.

Fr. 47–54 (500 mg, eluted with Et_2O + 1% AcOEt), also not separated. Amount checked to make sure that all material was eluted.

Chromatography 39. – The 600-mg material from *Fr. 24–56* of *Chrom. 31* was chromatographed on a column of 60 g SiO_2 buffered for pH 4 prepared with cyclohexane. Each fraction eluted with 50 ml of solvent 'mixture' (MeOH/(i-Pr)$_2$O/cyclohexane/AcOH from 0:0:100:0 up to 10:0:84:6).

Fr. 1–3 (334 mg, eluted with cyclohexane and 'mixture' (10:35:55:0)): brown residue.

Fr. 4–6 (20 mg, eluted with 'mixture' (10:35:55:0 – 10:45:45:0)): brown residue.

Fr. 7–8 (27 mg, eluted with 'mixture' (10:55:35:0)): brown residue.

Fr. 9–15 (40 mg, eluted with 'mixture' (10:70:20:0 – 10:85:4:1)) gave from acetone 10 mg of crystals of hexaflavaspidic acid (**39**), m.p. 205–212°, and 0.8 mg second crop, m.p. 180–190°.

Fr. 16–23 (31 mg, eluted with 'mixture' (10:80:8:2 – 10:50:36:4)) gave from AcOH + 5% H_2O 4 mg of cryst. hexaflavaspidic acid (**39**).

Fr. 24–27 (22 mg, eluted with 'mixture' (10:40:46:4 – 10:0:85:5)) gave in the same way 5 mg of crystals, m.p. 144–146°, according to TLC a mixture of **39**, **26**, and a little of a faster-moving compound.

The Isolated Compounds. – *Compound TR-1579.* The same compound (4.5 mg) was isolated from another fern, *Dryopteris pulvinifera* (BEDD.) O. KZE. They were united with the 16 mg of crude material we obtained from the several chromatograms after distillation *in vacuo*. These 20.5 mg were freshly distilled in the molecular still at 0.01 Torr and 120–130° bath temp. leaving 4.5 mg residue. The pale yellow distillate (13.5 mg) gave from Et_2O/MeOH 4.9 mg of anal. sample, m.p. 59–60°, very pale yellowish crystals. Optically inactive $[\alpha]_D^{20} < 0.5°$ ($c = 0.6$ in $CHCl_3$). The regenerated crystals were used for IR *(Fig. 1)*, NMR and HR-MS: M^+ found 408.3976 ± 0,0041 corresponding to $C_{27}H_{52}O_2$ (calc. 408.3967) and $M^+ - H_2O$ found 390.3853 corresponding to $C_{27}H_{50}O$ (calc. 390.3851). It has not been identified.

Tris-para-*aspidin BBB* (*2-[[3-[[2,4-Dihydroxy-6-methoxy-5-methyl-3-(1-oxobutyl)phenyl]methyl]-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl]-3,5-dihydroxy-4,4-dimethyl-6-(1-oxobutyl)-2,5-cyclohexadien-1-one*, **20**-BBB) from Et_2O/MeOH and a trace of H_2O, m.p. 124–126°. *Widén et al.* [15] recorded m.p. 143–147° or 157–160° for the material from *Dryopteris remota* (A. BR.) DRUCE when crystallized from acetone. According to TLC in several systems, they were identical. Our material was optically inactive (as expected) and it was not possible to sublime it unchanged in the molecular still.

Albaspidin BB (*2,2'-Methylene bis[3,5-dihydroxy-4,4-dimethyl-6-(1-oxobutyl)-2,5-cyclohexadien-1-one]*, **10**-BB), crystals, m.p. 151–152° from Et_2O/hexane, identical with authentic material [28]. It can be sublimed in the molecular still at 0.01 Torr and *ca.* 135–140° bath temp. without any decomposition. This is also the best method to purify crude crystals. The HR-NMR spectrum is given in *Fig. 2A*.

Tetra-albaspidin BBBB (*2,2'-Methylene bis[2,4,6-trihydroxy-5-(1-oxobutyl)-1,3-phenylene]bis[(methylene)-[3,5-dihydroxy-4,4-dimethyl-6-(1-oxobutyl)-2,5-cyclohexadien-1-one]]*) (*methylen-bis-norflavaspidic acid*, **25**-BBBB). Our anal. sample TR-1596, cryst. from Et_2O/MeOH with a trace of H_2O showed m.p. 131–133°. *Penttilä* and *Sundman* [8] recorded 158–165° for synthetic material. Melting points of this type of compounds can vary considerably depending on method of crystallization. Our material gave the 3 compounds **36**, **27**, and **31** after mild alcaline cleavage. In FD-MS a M^+ peak at m/z 876 corresponds to $C_{47}H_{56}O_{16}$ [27]. The NMR spectrum *(Fig. 3)* is in agreement with the suggested structure (**25**-BBBB).

Penta-albaspidin BBBBB (*2-[3-[3-[3-[2,4-Dihydroxy-3,3-dimethyl-5-(1-oxobutyl)-6-oxo-1,4-cyclohexadienyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-3,5-dihydroxy-4,4-dimethyl-6-(1-oxobutyl)-2,5-cyclohexadien-1-one*, **37**-BBBBB). The anal. sample TR-1586 crystallized from benzene/Et_2O and showed m.p. 167–170°, from Et_2O/MeOH m.p. 165–167°, from acetone/Et_2O and from Et_2O/pentane crystals of m.p. 194–196°, and from pure Et_2O of m.p. 201–203° were obtained. The high-melting form showed after some weeks m.p. 185–187°. Reductive cleavage gave the same three compounds **26**, **27**, and **31** as obtained from **25**. In the FD-MS M^+ peak at m/z 1084 and $M\mathrm{H}^+$ peak at m/z 1085 were recorded [27]. The NMR spectrum *(Fig. 4)* is in good agreement with the given structure.

Hexa-albaspidin BBBBBB (*2-[3-[3-[3-[3-[2,4-Dihydroxy-3,3-dimethyl-5-(1-oxobutyl)-6-oxo-1,4-cyclohexadienyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-3,5-dihydroxy-4,4-dimethyl-2,5-cyclohexadien-1-one*, **38**-BBBBBB). The anal. sample TR-1587 crystallized from acetone/MeOH (nearly insoluble in MeOH), m.p. 168–175° (with sintering at *ca.* 155°). Higher-melting crystals could be obtained from Et_2O. Reductive cleavage gave again only **36**, **27**, and **31**. The FD-MS showed peaks for M^+ at m/z 1292 and $M\mathrm{H}^+$ at m/z 1293 [27]. The NMR spectrum *(Fig. 5)* is in good agreement with the given formula.

Flavaspidic Acid BB (*3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl-6-{[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)phenyl]methyl}-2,5-cyclohexadien-1-one*, **5**-BB) was obtained as the high-melting form from Et_2O, m.p. 152–153°, identical with authentic material [5]. It was not possible to sublime it in the molecular still. After 40 min at 0.1 Torr and 170° bath temp., only 2.6 mg of material were distilled. These still gave the spot of flavaspidic acid in TLC but did not crystallize.

Trisflavaspidic Acid BBB (*3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[[2,4,6-trihydroxy-3-(1-oxobutyl)-5-[[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)phenyl]methyl]phenyl]methyl]-2,5-cyclohexadien-1-one*, **23**-BBB). This compound is known and has been synthesized by *Penttilä* and *Sundman* [15]. It is known to be difficult to crystallize it (see best procedure under *Chrom. 38*). The anal. sample (TR-1599) cryst. from Et_2O/pentane showed m.p. 164–165° ([15]: 168–174° (dec.)). An EI-MS was published [25a], but only with a very faint M^+. The FD-MS on the other hand shows very distinct peaks for M^+ at m/z 668 and $M\mathrm{H}^+$ at m/z 669 [27]. The ^{1}H-NMR spectrum has never been recorded, we give it in *Fig. 6*.

Tetraflavaspidic Acid BBBB (*3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[3-[3-[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,5-cyclohexadien-1-one*, **26**-BBBB). As mentioned, this compound has first been obtained recently from *Dryopteris filix-mas* by *v. Euw et al.* [4] but not described. Our anal. sample crystallized from Et_2O/pentane showed m.p. 164–165°. Reductive cleavage gave again the three compounds **26**, **27**, and **31** as expected. In the FD-MS, M^+ at m/z 862 and $M\mathrm{H}^+$ at m/z 863 were well visible [27]. Depending on conditions, peaks for rottlerone rearrangement at m/z 876 can also occur. The ^{1}H-NMR spectrum *(Fig. 7)* is in good agreement with the suggested structure.

Hexaflavaspidic Acid BBBBBB (*3,5-Dihydroxy-4,4-dimethyl-2-(1-oxobutyl)-6-[3-[3-[3-[3-[2,4,6-trihydroxy-3-methyl-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,4,6-trihydroxy-5-(1-oxobutyl)phenyl]methyl-2,5-cyclohexadien-1-one*, **39**-BBBBBB). The anal. sample (TR-1574) crystallized from Et_2O/pentane showed m.p. 190–192°, but occasionally crystals of m.p. 216–218° (TR-1592) could be obtained. From acetone/MeOH, crystals had m.p. 180–183°. They showed no optical activity $[\alpha]_D^{20} < 2°$. Reductive cleavage gave the compounds **26**, **27**, and **31** as expected. In the FD-MS no M^+ peak was visible in the region of m/z 1278 ($C_{68}H_{78}O_{24}$). The ^{1}H-NMR spectrum *(Fig. 8)* is in good agreement with the suggested structure.

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