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SEPARATION OF NATURALLY OCCURRING ACYLPHLOROGLUCINOLS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The separation of a series of naturally occurring *Dryopteris*, *Hagenia* and *Mallotus* compounds and several artefacts by reversed-phase high-performance liquid chromatography is reported. A particularly good separation of bicyclic *Dryopteris* phloroglucinols including side-shain homologues was achieved. Moreover, the easily occurring decomposition of polycyclic phloroglucinols during chromatography could be totally avoided using slightly acidic conditions.

INTRODUCTION

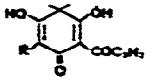
Several paper chromatographic (PC) and thin-layer chromatographic (TLC) methods for separation of the complex mixtures of naturally occurring polyclic *Dryopteris* phloroglucinols have been reported in the literature[1–19]. Also the analysis of those compounds by gas–liquid chromatography (GLC) has been studied under various experimental conditions[20]. However, it appears that the polycyclic phloroglucinols are very prone to decomposition reactions (see especially ref. 20) and thus cannot be directly analysed by GLC. Such decomposition reactions also occur in slightly alkaline conditions both in PC and TLC[9,21]. Moreover, several sensitive phloroglucinols are partly destroyed either by auto-oxidation or polymerization on silica gel layers on contact with air. These reactions can be totally avoided by TLC on polyamide but the separation is less successful[21].

In the present paper, almost all the known *Dryopteris* phloroglucinols, including some of their frequently occurring decomposition products (compounds 1–23 in Table I), were separated by reversed-phase high-performance liquid chromatography (HPLC). This method also proved suitable for separating the related kousso [*Hagenia abyssinica* (Bruce) Gmelin] phloroglucinols (compounds 24–29 in Table I) and kamala [*Mallotus philippinensis* (Lam.) Müll.-Arg.] phloroglucinols (compounds 30–35 in Table I) including some artefacts (*cf.* refs. 22 and 23).

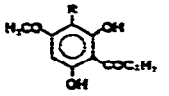
TABLE I

CHEMICAL STRUCTURES OF THE STUDIED COMPOUNDS

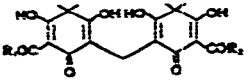
Compounds 1–23 are *Dryopteris* phloroglucinols, 24–29 are *Hagenia* phloroglucinols and 30–35 are *Mallotus* phloroglucinols. A = acetyl; P = propionyl; B = butyryl; iB = isobutyryl; iV = isovaleryl; 2MeB = 2-methylbutyryl. Compounds 1–4 are decomposition products which are readily formed by the action of alkali ($Ba(OH)_2$, MgO) on crude ether extracts in the isolation procedure (see refs. 9, 21 and 22). Aspidinol (4) can also be formed, *inter alia*, from *para*-aspidin (7a–c) and margaspidin (14) by chromatography on silica gel (ref. 21). 3-Methylbutyrylfilicinic acid (2) is less commonly found among the decomposition products of polycyclic phloroglucinols, but is also known as a natural compound in *Dryopteris fragrans* (L.) Schott (refs. 21 and 22). The albaspidins 5a–f are naturally occurring compounds, but they are also formed by rottlerone change from, *inter alia*, filixic acid (19a–f), flavaspidic acid (9a–c) and *para*-aspidin (7a–c) by the action of alkali, heat and also by chromatography on silica gel (see refs. 9, 21 and 25). Pseudo-aspidinol iB (iV, 2 MeB) (25) and α-kosin iBiB (iV, 2 MeB) (27) are artefacts that are formed by the action of alkali on 26, 28 and 29. Theoretically, 3-methylisobutyrylfilicinic acid (24) should also occur, although it has not been detected as yet (ref. 22). Rottleron (33) is an artefact that is readily formed from rottlerin (32). The probable natural occurrence of 30, 31, 34, and 35 is discussed in ref. 23.



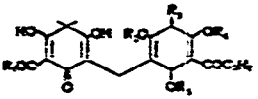
1 butyrylfilicinic acid; R = H
2 3-methylbutyrylfilicinic acid; R = CH_3



3 desaspidinol B; R = H
4 aspidinol B; R = CH_3

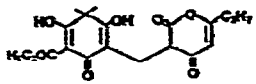


5a albaspidin PB; $R_1 = R_2 = C_3H_7$
5b albaspidin PB; $R_1 = C_2H_5$, $R_2 = C_3H_7$
5c albaspidin PP; $R_1 = R_2 = C_2H_5$
5d albaspidin AB; $R_1 = CH_3$, $R_2 = C_3H_7$
5e albaspidin AP; $R_1 = CH_3$, $R_2 = C_2H_5$
5f albaspidin AA; $R_1 = R_2 = CH_3$

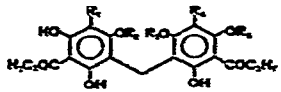


6a aspidin BB; $R_1 = C_3H_7$, $R_2 = R_5 = H$, $R_3 = R_4 = CH_3$
6b aspidin AB; $R_1 = CH_3$, $R_2 = R_5 = H$, $R_3 = R_4 = CH_3$
7a *para*-aspidin BB; $R_1 = C_3H_7$, $R_2 = R_3 = CH_3$, $R_4 = R_5 = H$
7b *para*-aspidin PB; $R_1 = C_2H_5$, $R_2 = R_3 = CH_3$, $R_4 = R_5 = H$
7c *para*-aspidin AB; $R_1 = CH_3$, $R_2 = R_3 = CH_3$, $R_4 = R_5 = H$
8a *iso*-aspidin BB; $R_1 = C_3H_7$, $R_2 = R_4 = H$, $R_3 = R_5 = CH_3$
8b *iso*-aspidin AB; $R_1 = CH_3$, $R_2 = R_4 = H$, $R_3 = R_5 = CH_3$
9a flavaspidic acid BB; $R_1 = C_3H_7$, $R_2 = R_4 = R_5 = H$, $R_3 = CH_3$
9b flavaspidic acid PB; $R_1 = C_2H_5$, $R_2 = R_4 = R_5 = H$, $R_3 = CH_3$
9c flavaspidic acid AB; $R_1 = CH_3$, $R_2 = R_4 = R_5 = H$, $R_3 = CH_3$
10a desaspidin BB; $R_1 = C_3H_7$, $R_2 = CH_3$, $R_3 = R_4 = R_5 = H$
10b desaspidin AB; $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = R_4 = R_5 = H$
11a *ortho*-desaspidin BB; $R_1 = C_3H_7$, $R_2 = R_3 = R_5 = H$, $R_4 = CH_3$
11b *ortho*-desaspidin AB; $R_1 = CH_3$, $R_2 = R_3 = R_5 = H$, $R_4 = CH_3$

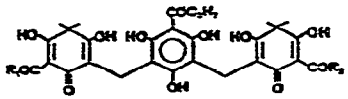
TABLE I *(continued)*



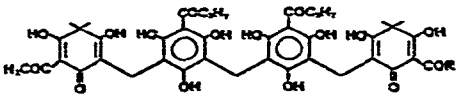
12 phloropyrone



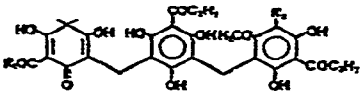
13 phloraspidinol BB; $R_1 = R_2 = R_3 = CH_3$, $R_4 = R_5 = H$
14 margaspidin BB; $R_1 = R_3 = R_4 = CH_3$, $R_2 = R5 = H$
15 aemulin BB; $R_1 = R_4 = R_5 = CH_3$, $R_2 = R_5 = H$
16 phloraspin BB; $R_1 = R_3 = CH_3$, $R_2 = R_4 = R_5 = H$
17 methylene-bis-aspidinol BB; $R_1 = R_2 = R_3 = R_4 = CH_3$, R_5=H
18 methylene-bis-desaspidinol BB;$R_1 = R_4 = R_5 = H$, $R_2 = R_3 = CH_3$



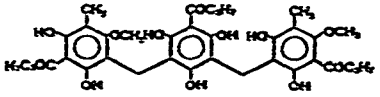
19a filixic acid BBB; $R_1 = R_2 = C_3H_7$
19b filixic acid PBB; $R_1 = C_2H_5$, $R_2 = C_3H_7$
19c filixic acid PBP; $R_1 = R_2 = C_2H_5$
19d filixic acid ABB; $R_1 = CH_3$, $R_2 = C_3H_7$
19e filixic acid ABP; $R_1 = CH_3$, $R_2 = C_2H_5$
19f $R_1 = R_2 = CH_3$



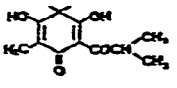
20a dryocrassin ABBA; $R = CH_3$
20b dryocrassin ABBP; $R = C_2H_5$

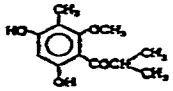


21a tris-*para*-aspidin BBB; $R_1 = C_3H_7$, $R_2 = CH_3$
21b tris-*para*-aspidin PBB; $R_1 = C_2H_5$, $R_2 = CH_3$
22 trisdesaspidin BBB; $R_1 = C_3H_7$, $R_2 = H$



23 trisaemulin BBB

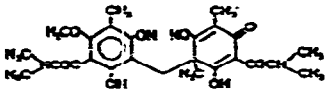




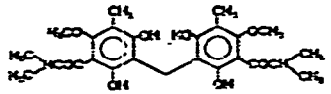
24 3-methylisobutyrylfilicinic acid
25 pseudo-aspidinol iB (iV, 2MeB)

(Continued on p. 216)

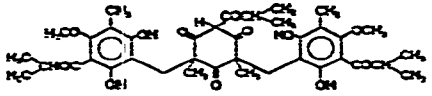
TABLE I *(continued)*



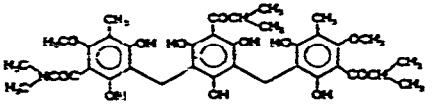
26 kosotoxin iBiB (iV, 2MeB)



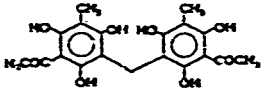
27 α-kosin iBiB (iV, 2MeB)



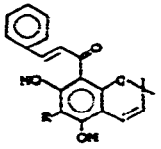
28 protokosin (iBiBiB (iV) 2MeB)



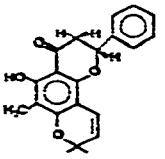
29 trispeudo-aspidinol iBiBiB (iV 2MeB)



30 methylene-bis-methylacetylphloroglucinol

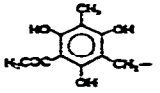


31 "red compound"; R = CH_3

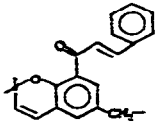


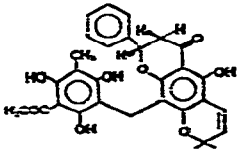
34 "yellow compound"

32 rottlerin, R =



33 rottlerone, R =





35 *isoallo*-rottlerin

EXPERIMENTAL

Compounds investigated

Table II lists the compounds studied. These were either prepared by synthesis or isolated from natural sources. Tetrahydrofuran (THF) solutions (0.02%) of the compounds were prepared.

HPLC experiments

A 10-μl volume was injected into a Waters M 6000 HPLC instrument provided with a 300 × 4 mm I.D. Bondapak C_{18} column. A Waters model 440 absorption detector was equipped with a 254 nm filter.

Several experiments were made to test acetonitrile, methanol and water in different combinations as solvent system in reverse-phase chromatography. However, THF–phosphoric acid–water (65:0.1:35) was found to be most suitable. The flow-rate was 2 ml/min, and the pressure drop 2000 p.s.i.

RESULTS AND DISCUSSION

Table II lists the retention times of all compounds investigated, and Figs. 1–3 show liquid chromatograms of the homologous albaspidins (5a–f), aspidins (6a and b) and *ortho*-desaspidins (11a and b), and *Mallotus* phloroglucinols (30–35). In general, a good separation of most compounds investigated was achieved. The retention times can be adjusted by varying the THF–water ratio. In the solvent system used, all the compounds were eluted in less than 10 min. No decomposition reactions were observed during chromatography of the easily decomposing phloroglucinols in the slightly acidic conditions used (*cf.* Table I). The separation of the individual compounds studied is discussed below.

Dryopteris phloroglucinols (1–23)

Monocyclic compounds. These phloroglucinols (1–4) hardly separate in the HPLC system used. In TLC a slight separation is reported and in PC and GLC a very good separation is achieved[9,15,17,20].

Bicyclic compounds. The homologous albaspidins (5a–f), which differ only in the substitution of the acyl side-chain (–CH_3, –C_2H_5, –C_3H_7), can be well separated by HPLC (Fig. 1). In TLC a partial separation was achieved[15,26]. With PC a good separation has been reported, but under slightly alkaline conditions (pH 8.6–8.8) rottlerone change does occur:

albaspidin BB + albaspidin AA ⇌ albaspidin AB

This reaction can, however, be avoided by using acetic acid as solvent[9,25]. In our experiments no rottlerone change was observed when phosphoric acid was included in the solvent.

The different aspidins (6a–11b) and flavaspidic acids (9a–c) also separate well from each other. However, the retention times of albaspidin AP (5e) and aspidin AB (6b), and of albaspidin AA (5f), *para*-aspidin PB (7b) and *iso*-aspidin BB (8a) are very similar. In TLC the BB and PB homologues of the individual compounds move

TABLE II

SEPARATION OF NATURALLY OCCURRING *DRYOPTERIS*, *HAGENIA* AND *MALLOTUS* PHLOROGLUCINOLS AND SOME ARTEFACTS BY HPLC

Compound no.	*Source*	*Lit. ref.*	*Melting point (°C)*	*Relative retention time*
1	synth.	24	98–100	410
2	synth.	22	78– 79	425
3	synth.	24	121–123	395
4	synth.	24	140–141	410
5a	*D. assimilis*	27	153–154	965
5b	synth.	25	mixt.*	890
5c	synth.	26	125–128	835
5d	synth.	25	mixt.**	735
5e	synth.	25	mixt.***	690
5f	synth.	26	165–167	590
6a	*D. assimilis*	14	124–125	855
6b	*D. intermedia*	27	118–120	685
7a	*D. spinulosa*	27	123–125	660
7b	synth.	28	120–122	600
7c	synth.	28	137–140	485
8a	synth.	29	152–154	590
8b	synth.	29	126–127	445
9a	*D. abbreviata*	30	88– 89§	335
9b	*D. abbreviata*	30	88– 89§	310
9c	*D. abbreviata*	30	209–213	280
10a	*D. assimilis*	27	149–151	540
10b	synth.	28	142–145	435
11a	synth.	18	130–132	805
11b	synth.	18	149–150	640
12	*D. assimilis*	27	109–111	700
13	synth.	28	190–192	520
14	*D. marginalis*	28	174–176	630
15	*D. aemula*	18	90– 91	700
16	*D. marginalis*	28	206–208	515
17	*D. marginalis*	28	188–190	690
18	synth.	28	176–179	455
19a	*D. filix-mas*	14	mixt. 175–179§§	495 broad peak
19b	*D. filix-mas*	14		
19c	*D. filix-mas*	14		
19d	*D. arguta*	unpub.	mixt. 156§§§	515 broad peak
19e	*D. arguta*			
19f	*D. arguta*			
20a	*D. crassirhizoma*	31	208–210†	530
20b	*D. crassirhizoma*	31	208–210†	540
21a	*D. pallida*	unpubl.	mixt. 154††	425
21b	*D. pallida*			435
22	*D. assimilis*	27	135–137	425
23	*D aemula*	18	170	760
24	synth.	22	100–103	480
25	synth.	22	60– 61	445
26	*H. abyssinica*	22	119–122	540
27	synth.	22	148–150	795
28	*H. abyssinica*	22	181–183	990
29	*H. abyssinica*	22	167–169	685
30	synth.	23	280–282	410

TABLE II *(continued)*

31	*M. philippinensis*	23	125–128	480
32	*M. philippinensis*	23	208–210	590
33	*M. philippinensis*	23	221–222	1000
34	*M. philippinensis*	23	143–145	640
35	*M. philippinensis*	23	178–180	555

* Obtained by heating 5a and 5c for 8 min at melting point.
** Obtained by heating 5a and 5f for 8 min at melting point.
*** Obtained by heating 5c and 5f for 8 min at melting point.
‡ A mixture of the homologues 9a and 9b.
‡‡ A mixture of 19a, 19b and 19c.
‡‡‡ A mixture of 19d, 19e and 19f.
† A mixture of 20a (main homologue) and 20b.
†† A mixture of 21a (main homologue) and 21b.

together but the AB homologues move slower and can thus be separated[15,18,29]. In PC a fairly good separation of most homologous aspidins and flavaspidic acids is reported[19,25]. This is the first time that separations of aspidin BB (6a) and *ortho*-desaspidin BB (11a) and of aspidin AB (6b) and *ortho*-desaspidin AB (11b) have been reported (Fig. 2, *cf.* ref. 18).

The fully aromatic phloroglucinols (13–18) can be well separated with our HPLC method. A relatively good separation of these compounds can be accomplished also by TLC[15,18,21] and PC[9,32].

Tricyclic and tetracyclic compounds. The homologous filixic acids (19a–f) and dryocrassins (20a and b) form broad peaks with poor resolution. Neither does the pair tris-*para*-aspidin (12a, b) and trisdesaspidin (22) separate. The retention time of trisaemulin (23) is much greater. For the separation of these compounds TLC and PC are much better[9,15,18,32].

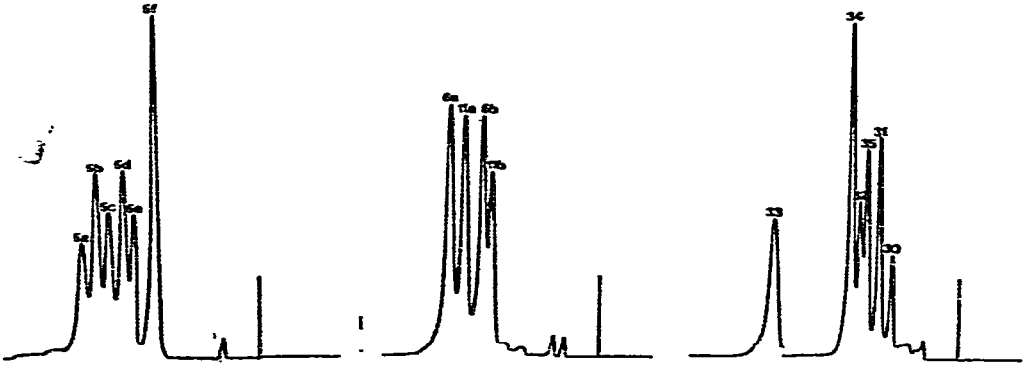


Fig. 1. HPLC chromatogram of the albaspidin homologues (5a–f).

Fig. 2. HPLC chromatogram of the homologous aspidins (6a and b) and *ortho*-desaspidins (11a and b).

Fig. 3. HPLC chromatogram of the *Mallotus* phloroglucinols (30–35).

Hagenia phloroglucinols (24–29)

These compounds can be well separated in the HPLC system used. However, owing to the existence of homologues (iB most prominent also some iV and 2MeB), the peaks of the naturally occurring kosotoxin (26) and protokosin (28) are broad and show minor side peaks. In TLC a slight separation of these two compounds has been achieved[22].

Mallotus phloroglucinols (30–35)

A very good separation of all these colouring matters can be observed (Fig. 3). In TLC only a partial separation of rottlerin (32) and the "red compound" (31), and of rottlerone (33) and the "yellow compound" (34) is reported[23].

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