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Phloroglucinol Derivatives of *Hagenia abyssinica*. III.* Reductive Alkaline Cleavages of Kosotoxin and Protokosin, and of Aspidin BB (*Dryopteris assimilis*)

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Products formed from kosotoxin (II), protokosin (III), and aspidin BB (VII) by reductive alkaline cleavages in different experimental conditions have been investigated. In all three cases, pseudo-aspidinol (IV), 3-methylfilicinic acid (X), methylphloroglucinol-2-monomethyl ether (XV), and methylene-bis-pseudoaspidinol (XVIII) were detected among the reaction products. Moreover, in the case of aspidin BB (VII), butyrylfilicinic acid (VIIIb), filicinic acid (IX), 3-methylbutyrylfilicinic acid (Vd), and albaspidine BB (XVII) were found.

Les produits formés à partir de la kosotoxine (II), de la protokosine (III) et de l'aspidine BB (VII) par clivages réductifs alcalins dans des conditions différentes expérimentales, ont été examinés. Dans les trois cas, le pseudo-aspidinol (IV), l'acide méthyl-3 filicinique (X), l'éther monométhylique-2 du méthylphloroglucinol (XV) et le méthylène-bis-pseudo-aspidinol (XVIII) ont été détectés parmi les produits réactionnels. De plus, dans le cas de l'aspidine BB (VII) l'acide butyrylfilicinique (VIIIb), l'acide filicinique (IX), l'acide méthyl-3 butyrylfilicinique (V d) et l'albaspidine BB (XVII) ont été trouvés.

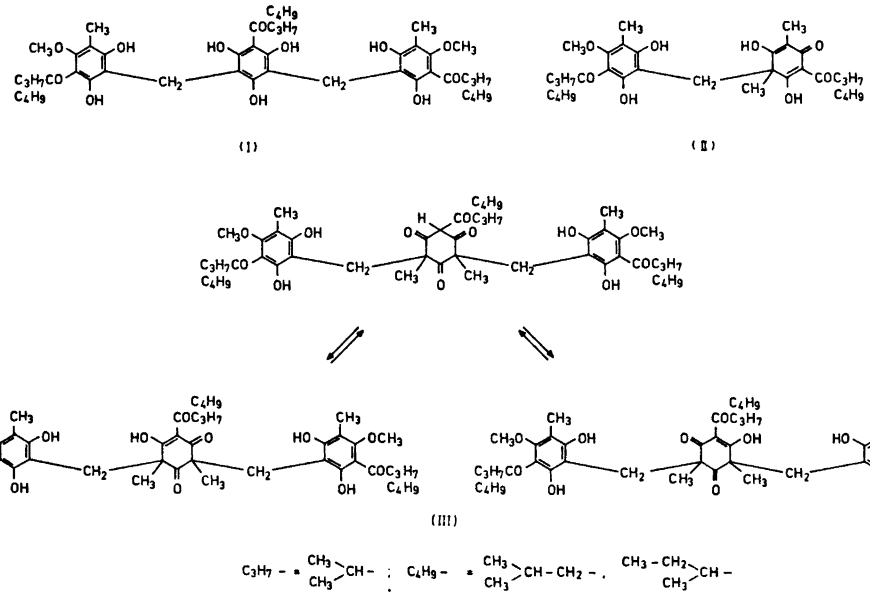
In previous papers [1,2] we have described the isolation and structure determination of three phloroglucinol derivatives (kosins) from female flowers of *Hagenia abyssinica* (Bruce) Gmelin. These are trispseudo-aspidinol (kosidin) (I), kosotoxin (II), and protokosin (III), each consisting of mixtures of isobutyryl (iB), isovaleryl (iV), and 2-methylbutyryl (2-MeB) acyl side chain homologues. The structure of trispseudo-aspidinol (I) was partly solved through an investigation of the monocyclic acylphloroglucinols formed by mild reductive alkaline cleavage of the molecule in the presence of metallic zinc.[1] This elegant cleavage method, originally reported by Boehm [3] and much used by him and later workers [4–6] for structure determinations of *Dryopteris* and *Hagenia* phloroglucinol derivatives, is here applied to the study of kosotoxin (II) and protokosin (III). Using the method in its original form, we were able to detect only the homologous pseudo-aspidinols iB, iV, and 2-MeB (IVa, b, and c) both from kosotoxin (II) and protokosin (III). No sign of 3-methylisobutyrylfilicinic acid (Va) or its iV (Vb) or 2-MeB (Vc) homologues was detected. Nor were 3,3-dimethylisobutyryl-filicinic acid (flavesone) (VIa), 3,3-dimethyl-isovalerylfilicinic acid (leptospermone) (VIb) or 3,3-dimethyl-(2-methylbutyryl)-filicinic acid (VIc) to be found among the products of protokosin (III).**

These results, as well as the absence of 3-methyl-butyrylfilicinic acid (fraginol ***) (Vd) from the decomposition products of *Dryopteris* phloroglucinol derivatives,[4,5,7] persuaded us to examine the products of reductive alkaline cleavage of kosotoxin (II) and protokosin (III) in more detail. Thereby, aspidin BB (VII)

* Part II, Ref. 2.

** Flavesone (VIa) and leptospermone (VIb) are known as naturally occurring compounds in the essential oils of certain *Leptospermum*, *Xanthostemon*, and *Eucalyptus* species (Myrtaceae).[8,9]

*** Fraginol (Vd) is reported as a naturally occurring compound in *Dryopteris fragrans* (L.) Schott.[10,11]



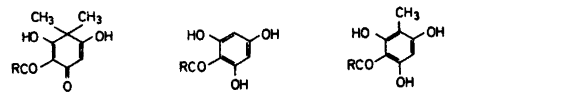
(I) (II)

(III)

(*Dryopteris assimilis* S. Walker) was used as a model compound. This phloroglucinol derivative, which frequently occurs in ferns of the *D. dilatata* complex,[11] is closely related to kosotoxin (II) in its chemical structure: *i.e.* the geminal dimethyl group found in aspidin BB (VII) is partly involved in the methylene bridge of kosotoxin (II).

Syntheses of isobutyrylfilicinic acid (VIIIa), 3-methylisobutyrylfilicinic acid (Va), and 3,3-dimethylisobutyrylfilicinic acid (VIa), and of their deacylated analogues (IX), (X), and (XI). For the examination of the decomposition products of kosotoxin (II) and protokosin (III) several synthetic model compounds were needed. Among these 3-methylisobutyrylfilicinic acid (Va), 3,3-dimethylisobutyrylfilicinic acid (VIa) and their deacylated analogues (X) and (XI) (syncarpic acid *) were of vital importance. The geminally substituted acylphloroglucinol derivatives were prepared in good yields by the C-methylation of isobutyrylphloroglucinol (XIIa), according to the methods of Riedl *et al.*[12,13] In all cases the resulting products were mixtures, containing isobutyrylfilicinic acid (VIIIa), 3-methylisobutyrylfilicinic acid (Va) and 3,3-dimethylisobutyrylfilicinic acid (VIa) in different ratios. In addition small amounts of substances such as unreacted isobutyrylphloroglucinol (XIIa), methylisobutyrylphloroglucinol (XIII), and their methyl ethers were also present. For the separation of isobutyrylfilicinic acid (VIIIa). 3-methylisobutyrylfilicinic acid (Va), and 3,3-dimethylisobutyrylfilicinic acid (VIa), obtained from the so-called carbonate fractions (see Refs. 12, 13), column chromatography on silica gel, with hexane – benzene in different ratios as eluent proved suitable. The first fractions consisted of mixtures of 3,3-dimethylisobutyrylfilicinic acid (VIa) and 3-methylisobutyrylfilicinic acid (Va),

* Syncarpic acid (XI) has been isolated from *Syncarpia laurifolia* Tenn. (Myrtaceae).[14]



(VIIIa) R = -CH(CH3)2 (XIIa) R = -CH(CH3)2 (XIII) R = -CH2-CH2-CH3

(XIIb) R = -CH2-CH2-CH3

Table 1. Products obtained by reductive alkaline cleavage of aspidin BB (VII) in different conditions (Cleavages A–C).

Compound	Cleavage A	B	C	R_F-values TLC [a]	PC [b]	Colour [c]
Aspidin BB (VII)	++	+	–	0.70	0.84 [d]	yellow
Albaspidin BB (XVII)	+	–	–	0.75	0.92 [d]	red
Methylene-bis-pseudo-aspidinol BB (XVIII)	+? [e]	+? [e]	+	0.73	0.92 [d]	pale brown
Pseudo-aspidinol B (IVd)	++	++	++	0.30	0.56 [d]	pale brown
Butyrylfilicinic acid (VIIIb)	++	++	++	0.23	0.41 [f]	red
3-Methylbutyrylfilicinic acid (Vd)	–	+	–	0.20	0.47 [f]	yellow
Filicinic acid (IX)	–	+	+	0.11	n.s.	blue
3-Methylfilicinic acid (X)	–	+	+	0.14	n.s.	orange yellow
Methylphloroglucinol-2-methyl ether (XV)	–	+	+	0.08	n.s.	dark brown

–, not detected; +, detected in traces or small amounts; ++, detected in large amounts; n.s., not studied. [a] At pH 6.0 in hexane–chloroform–ethanol 47.5:47.5:5.0.[1,5] [b] In cyclohexane–chloroform 1:1.[23] [c] Colour with Fast Blue Salt B. [d] At pH 8.6. [e] Could not be detected owing to overlapping of aspidin BB (VII) and albaspidin BB (XVII). [f] At pH 4.0.

from which the latter (Va) was separated by crystallization from ether. The subsequent fractions consisted of practically pure isobutyrylfilicinic acid (VIIIa).

The corresponding butyrylderivatives butyrylfilicinic acid (VIIIb) and 3-methylbutyrylfilicinic acid (Vd) were prepared by analogous procedures.[12]

The geminally substituted deacylated phloroglucinol derivatives (IX), (X), and (XI) were prepared by reductive alkaline cleavage of the corresponding isobutyryl compounds (VIIIa), (Va), and (VIa), respectively.

The thin-layer and paper chromatographic properties of the synthetically prepared acylfilicinic acids as well as their deacylated homologues appear in Tables 1 and 2. All other compounds except 3-methylisobutyrylfilicinic acid (Va) and 3,3-dimethylisobutyrylfilicinic acid (VIa) were easily separated in the chromatographic systems used.

Reductive alkaline cleavage of aspidin BB (VII). Aspidin BB (VII) was subjected to three different cleavages, A, B, and C, all being modifications of the original method of Boehm [3] (for details, see the Experimental section). The products isolated by column chromatography or detected by TLC and PC are listed in Table 1. The decomposition of aspidin BB (VII) is further depicted in Scheme 1.

The first step in the decomposition of aspidin BB (VII) by the reductive alkaline treatment is the cleavage of the methylene bridge, resulting in large amounts of pseudo-aspidinol B (IVd) and butyrylfilicinic acid (VIIIb). These are the main reaction products in all three cleavages (Cleavage A, B, and C). Of the other possible products, 3-methylbutyrylfilicinic acid (Vd) was detected in trace amounts, but only after cleavage B. On the other hand, no 3-methyl-pseudoaspidinol B (XIVd) could be found.

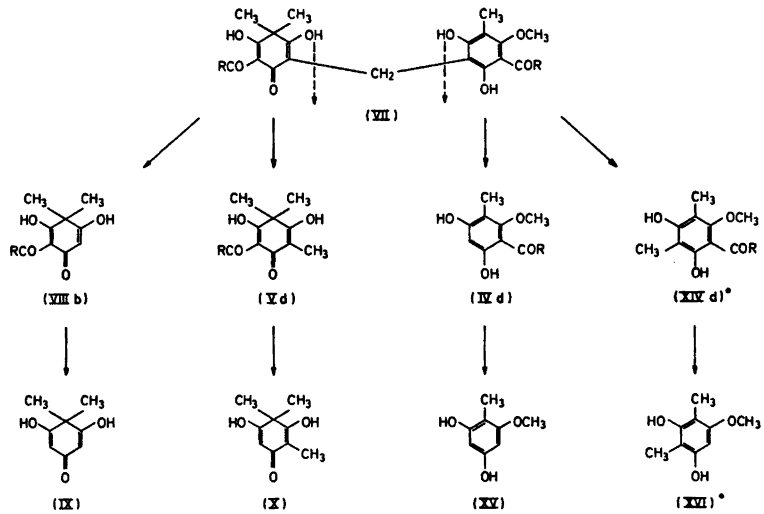
The second step in the decomposition of aspidin BB (VII) is the cleavage of the acyl side chains from the initially formed monocyclic compounds.* This second step, which leads mainly to the formation of filicinic acid (IX) and methylphloroglucinol-2-monomethyl ether (XV), is detectable after cleavage B and more clearly after cleavage C. In both cases, some 3-methylfilicinic acid (X) could also be de-

* To some extent, of course, the acyl side chain cleavage takes place directly from aspidin BB (VII).

Table 2. Products obtained by reductive alkaline cleavages of kosotoxin (II) and protokosin (III) in different conditions (Cleavages A – D).

Compound	Cleavage A	B	C	D	R_F-values in TLC [a]	in PC [b]	Colour [c]
Kosotoxin (II)	–	–	–	–	0.73	0.84 [e]	yellow
Protokosin (III)	–	–	–	–	0.73	0.84 [e]	pale brown
Methylene-bis-pseudo-aspidinol (XVIII)	+	+	+	+	0.75	0.92 [e]	pale brown
Pseudo-aspidinol (IV)	+ +	+ +	+ +	+ +	0.30	0.69 [e] 0.56	pale brown
Isobutyrylfilicinic acid (VIII a)	–	–	–	–	0.23	0.45 [f]	red
3-Methylisobutyrylfilicinic acid (Va)	–	–	–	–	0.20	0.50 [f]	yellow
3,3-Dimethylisobutyrylfilicinic acid (VIa)	–	–	–	–	0.20	0.50 [f]	yellow
3-Methylfilicinic acid (X)	–	–	–	+	0.14	n.s.	orange yellow
3,3-Dimethylfilicinic acid (XI)	–	–	–	+ ?[d]	0.20	n.s.	orange yellow
Methylphloroglucinol-2-methyl ether (XV) [g]	–	–	–	+	0.08	n.s.	dark brown

[a] At pH 6.0 in hexane – chloroform – ethanol 47.5:47.5:5.0.[1,5] [b] In cyclohexane – chloroform 1:1.[23] [c] Colour with Fast Blue Salt B. [d] From protokosin only. [e] At pH 8.6. The pseudo-aspidinol obtained from kousso constituents (see Experimental) separated in two spots, R_F 0.69 and 0.56. The latter was pseudo-aspidinol iB and the former a mixture of the pseudo-aspidinols iV and 2-MeB (see Ref. 1). [f] At pH 4.0. [g] Methylphloroglucinol-4-methyl ether (XXI) formed an orange spot with the R_F-value of 0.10.



Scheme 1. Reductive alkaline cleavage of aspidin BB (VII).

tected. 1,3-Dimethylphloroglucinol-4-monomethyl ether (XVI) was not found. We are the first to report the presence of 3-methylbutyrylfilicinic acid (Vd) among the decomposition products of aspidin BB (VII) (*cf.* Ref. 15–18). However, only traces were found, even here. 3-Methylfilicinic acid (X) on the other hand, was long ago isolated in small amounts by Boehm.[16] Earlier failures to detect 3-methylbutyrylfilicinic acid (Vd) or its acyl side chain homologues among the products of reductive alkaline cleavage of geminally disubstituted *Dryopteris* phloroglucinols, is possibly explained by the great instability of these products in the reaction conditions used (*cf.* also Riedl and Risse [7] and Aho [19]).

An alternative route in the decomposition of aspidin BB (VII) (*cf.* Ref. 17) leading to the formation of albaspidin BB (XVII) and methylene-bis-pseudo-aspidinol BB (XVIII $R = -CH_2-CH_2-CH_3$) is the so-called rottlerone change. This reaction, which proceeds according to the scheme

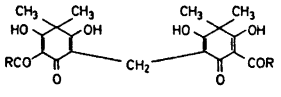
$$2R-CH_2-R' \rightleftharpoons R-CH_2-R + R'-CH_2-R'$$

is typical of the behaviour of methylene-bis-polyhydroxyphenols (polyhydroxydiphenylmethanes).[20] The formation of albaspidin BB (XVII) after cleavage A is clearly detected in its characteristic red spot (fast blue salt B) on TLC. The lightbrown spot corresponding to the other possible product, methylene-bis-pseudo-aspidinol BB (XVIII, $R = -CH_2-CH_2-CH_3$), cannot be detected with certainty because in the chromatographic systems used its R_F-values are very similar to those of the red spot of albaspidin BB (XVII) and the yellow spot of unreacted aspidin BB (VII). After more drastic reaction conditions, corresponding to cleavage B, there is no albaspidin BB (XVII) left, although the reaction mixture still contains some unreacted aspidin BB (VII). After cleavage C – the most drastic of the three variants used – the only of the bicyclic compounds present is methylene-bis-pseudo-aspidinol BB (XVIII, $R = -CH_2-CH_2-CH_3$), which is known to be a very alkali-stable substance.[18]

Reductive alkaline cleavage of kosotoxin (II) and protokosin (III). This was performed in the same three ways, as in the case of aspidin BB (VII). The products isolated by column chromatography or detected by TLC and PC are listed in Table 2, while decomposition is depicted in Schemes 2 and 3.

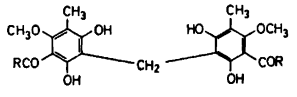
The first step in the decomposition of kosotoxin (II) and protokosin (III) is the cleavage of the methylene bridges, resulting in large amounts of pseudo-aspidinol iB, iV, and 2-MeB (IVa, b, c) in all three cases (cleavage A, B, and C). Although expected, no 3-methylisobutyrylfilicinic acid (Va), 1,3-dimethyl-5-isobutyrylphloroglucinol (XIX), or 3-methylpseudo-aspidinol iB (XIVa) or its isovaleryl or 2-methylbutyryl homologues were detected by TLC or PC. Neither were 3,3-dimethylisobutyrylfilicinic acid (VIa) or its isovaleryl and 2-methylbutyryl homologues observed in the case of protokosin (III).

The second step in the decomposition of both kosotoxin (II) and protokosin (III) could be expected to lead to 3-methylfilicinic acid (X), methylphloroglucinol-2-monomethyl ether (XV), 1,3-dimethylphloroglucinol-4-monomethyl ether (XVI), and 1,3-dimethylphloroglucinol (XX). Also some 3,3-dimethylfilicinic acid (XI) could be expected to be formed in the case of protokosin (III). However, none of these second step decomposition products were found after cleavage according to procedures A, B, or C. Rather, in all three cases, compounds of unknown structure were detected. Some of these showed R_F-values very similar to those of synthetical standards, but the colouration (fast blue salt B) was different. Owing to the presence of these unknown compounds in the reaction mixtures we tried a fourth, much more drastic variant of the reductive alkaline

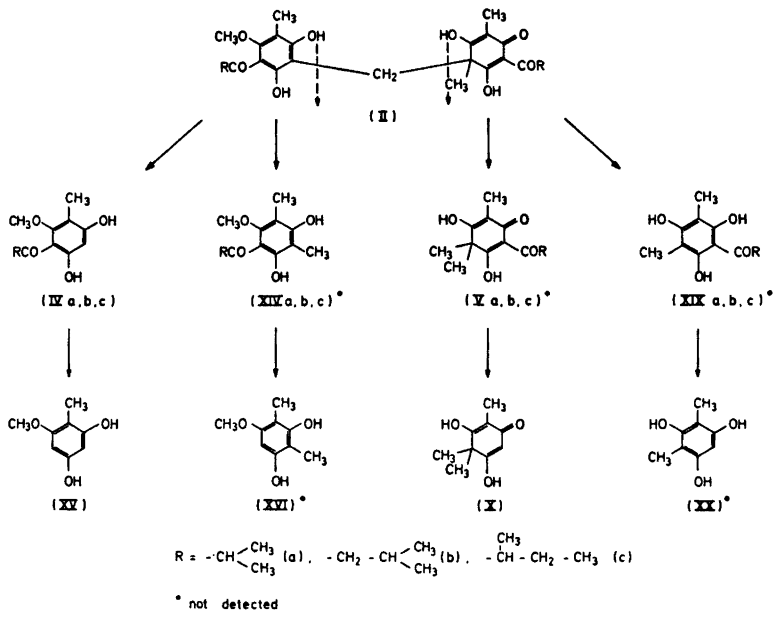


(XVII)

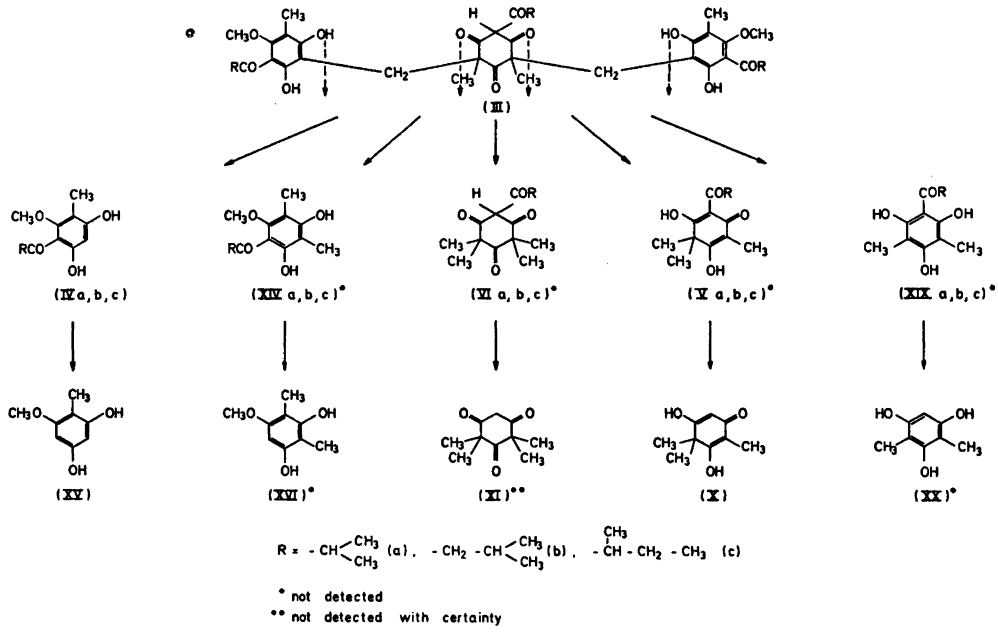
R = -CH2 - CH2 - CH3



(XVIII)

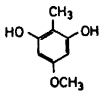


Scheme 2. Reductive alkaline cleavage of kosotoxin (II).



Scheme 3. Reductive alkaline cleavage of protokosin (III).

cleavage, called cleavage D (see the Experimental section). This time the unknown compounds were decomposed and large quantities of methylphloroglucinol-2-monomethyl ether (XV) * and trace amounts of 3-methylfilicinic acid (X) could be detected in the reaction mixture. The former was previously found by Lobeck [21] after reductive alkaline cleavage of kosotoxin (II) and "kosin" (XVIII).** However, the key degradation product expected from protokosin (III), 3,3-dimethylfilicinic acid (XI),*** could not be detected with certainty (*cf.* Table 2).



(XXI)

An alternative route in the decomposition of both kosotoxin (II) and protokosin (III) is the formation of iB, iV, and 2-MeB homologues of methylene-bis-pseudo-aspidinol ("kosin") (XVIII) ** by the rottlerone change. These products have been isolated earlier after reductive alkaline treatment of both kosotoxin (II) and protokosin (III), and they are known to be very alkali stable, as is the corresponding butyryl homologue (pseudo-aspidin BB) (XVIII R = $-CH_2-CH_2-CH_3$). Methylene-bis-pseudo-aspidinol ("kosin") (XVIII) ** could be detected after all three initial cleavage procedures (cleavage A, B, and C), but other products expected to be formed by the rottlerone change could not.

The structural similarity we have proposed here between aspidin BB (VII), kosotoxin (II), and protokosin (III) is supported by the fact that similar decomposition products, including methylene-bis-pseudo-aspidinol (XVIII),** pseudo-aspidinol (IV), methylphloroglucinol-2-monomethyl ether (XV), and 3-methylfilicinic acid (X), were obtained in all three cases.

EXPERIMENTAL

General. The IR spectrum was measured with a Beckman IR-8 spectrophotometer. The NMR spectra have been taken with a Varian A-60 instrument using TMS as internal standard. The mass spectra have been recorded on a Perkin-Elmer 270 mass spectrometer. The melting points have been determined on a Reichert micro hot stage and are uncorrected. The thin-layer chromatographic methods were those previously reported.[1,5] The paper chromatographic method was that of Penttilä and Sundman.[23]

Reference substances

1. Isobutyrylphloroglucinol.$2H_2O$ (XIIa) was prepared from phloroglucinol by the method of Riedl.[24] Colourless plates, m.p. 71–74 °C/134–136 °C (water) (lit.[24] m.p. 70 °C/138 °C). MS: M+ at *m/e* 196 corresponding to $C_{10}H_{12}O_4$. IR: C=O 6.24 μ.

2. Isobutyrylfilicinic acid (VIIIa) was obtained as a by-product in the synthesis of 3-methylisobutyrylfilicinic acid (Va) according to Riedl and Risse.[12] Slightly brownish plates, m.p. 152–154 °C (hexane) (lit.[23] m.p. 153–154 °C). MS: M+ at *m/e* 224 corresponding to $C_{12}H_{16}O_4$. NMR ($CDCl_3$): δ 1.16 [6 H, d, *J* 7 Hz, $-CO-CH(CH_3)_2$], 1.40 and 1.50 (6 H, each s, gem. dimethyl group of the ring), 3.96 [1 H, heptet, *J* 7 Hz, $-CO-CH(CH_3)_2$] and 5.54 (1 H, s, vinyl H only partly). The signals of OH-groups are omitted.

3. 3-Methylisobutyrylfilicinic acid (Va) † was prepared from isobutyrylphloroglucinol (XIIa) by the method of Riedl and Risse.[12] Colourless plates, m.p. 100–103 °C (ether). MS: M+ at *m/e* 238 corresponding to $C_{13}H_{18}O_4$. NMR ($CDCl_3$): δ 1.14 [6 H, d, *J* 7 Hz, $-CO-CH(CH_3)_2$], 1.42 and 1.50 (6 H, each s, gem. dimethyl group of the ring), 1.90 (3 H, s, $CH_3-C\ll$), 3.96 [1 H, heptet, *J* 7 Hz, $-CO-CH(CH_3)_2$] 7.40 (1 H, s, OH), and 18.30 (1 H, s, OH).

4. 3,3-Dimethylisobutyrylfilicinic acid (flavesone) (VIa) † was prepared from isobutyrylphloroglucinol (XIIa) by the method of Riedl and Risse.[12] Pale yellow oil (lit.[9] b.p. 134 °C/10 mmHg). MS: M+ at *m/e* 252 corresponding to

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* Noteworthy, no methylphloroglucinol-4-monomethyl ether (XXI) could be found.

** R = $-CH(CH_3)_2$; $-CH_2-CH(CH_3)_2$; $-CH(CH_3)-CH_2-CH_3$.

*** Recently Kashman *et al.*[22] have isolated from *Myrtus communis* L. (Myrtaceae) two new acylphloroglucinol derivatives which they called myrtucummulone A and B, and from which they were able to prepare 3,3-dimethylfilicinic acid (syncarpic acid) (XI) by alkaline treatment. However, owing to the fact that all four methyl groups in 3,3-dimethylfilicinic acid (XI) are already present as such in myrtucummulone A and B, and not partly involved in the methylene bridges as in protokosin (III), the cases are not quite analogous.

† 3-Methylisobutyrylfilinic (Va), and 3-3-dimethylisobutyrylfilicinic acid (VIa) decomposing almost completely after few months storage, are unstable substances compared with isobutyrylfilicinic acid (VIIIa).

$C_{14}H_{20}O_4$. NMR ($CDCl_3$): δ 1.16 (6 H, d, J 7 Hz, $-CO-CH(CH_3)_2$, 1.36 and 1.46 (12 H, each s, 2 gem. dimethyl groups of the ring), 3.80 (1 H, heptet, J 7 Hz $-CO-CH(CH_3)_2$, and 17.80 (1 H, s, OH) (*cf.* Ref. 9).

5. Filicinic acid (IX) was obtained from isobutyrylfilicinic acid (VIIIa) by the method of Riedl and Risse.[12] Colourless plates m.p. 215 °C (ethanol) (lit.[12] m.p. 214–215 °C). MS: M+ at *m/e* 154 corresponding to $C_8H_{10}O_3$. NMR (DMSO-d_6): δ 1.20 and 1.26 (6 H, each s, gem. dimethyl group), 5.20 and 5.48 (weak) (< 2 H, each s, vinyl H's only partly). The signals of OH-groups are omitted.

6. Butyrylfilicinic acid (VIIIb) was prepared by acylation of filicinic acid (IX).[7] Slightly brownish plates, m.p. 98–100 °C (hexane) (lit.[28] m.p. 98–99 °C). MS: M+ at *m/e* 224 corresponding to $C_{12}H_{16}O_4$. NMR ($CDCl_3$): δ 1.01 (3 H, t, J 7 Hz, $-CO-CH_2-CH_2-CH_3$), 1.40 and 1.50 (6 H, each s, gem. dimethyl group), 1.74 (2 H, m, $-CO-CH_2-CH_2-CH_2-CH_3$), 3.02 (2 H, t, J 7 Hz, $-CO-CH_2-CH_2-CH_3$) and 5.54 (< 1 H, s, vinyl H only partly). The signals of OH-groups are omitted (*cf.* Ref. 10).

7. 3-Methylbutyrylfilicinic acid (Vd) ** was prepared from butyrylphloroglucinol (XIIb) by the method of Riedl and Risse.[12] Colourless plates, m.p. 78–79 °C (ether) (lit.[7] m.p. 87 °C). MS: M+ at *m/e* 238 corresponding to $C_{13}H_{18}O_4$. NMR ($CDCl_3$): δ 0.98 (3 H, t, J 7 Hz, $-CO-CH_2-CH_2-CH_3$), 1.40 and 1.50 (weak) (6 H, each s, gem. dimethyl group), 1.68 (2 H, m, $-CO-CH_2-CH_2-CH_2-CH_3$), 1.90 (3 H, s. $CH_3-C\langle$) and 3.00 (2 H, t, J 7 Hz, $-CO-CH_2-CH_2-CH_3$). The signals of OH-groups are omitted (*cf.* Ref. 10).

8. 3-Methylfilicinic acid (X) was obtained by reductive alkaline cleavage of 3-methylisobutyrylfilicinic acid (Va).[12] Yellowish plates, m.p. 178 °C (benzene) (lit.[12] m.p. 179–180 °C). MS: M+ at *m/e* 168 corresponding to $C_9H_{12}O_3$. NMR: (DMSO-d_6): δ 1.22 and 1.30 (6 H, each s, gem. dimethyl group), 1.68 (3 H, s, $CH_3-C\langle$) and 5.36 (< 1 H, s, vinyl H only partly). The signals of OH-groups are omitted.

9. 3,3-Dimethylfilicinic acid (XI) was prepared by reductive alkaline cleavage of 3,3-dimethylisobutyrylfilicinic acid (VIa).[12] Colourless needles, m.p. 186–189 °C (benzene (lit.[12] m.p. 187–190 °C). MS: M+ at *m/e* 182 corresponding to $C_{10}H_{14}O_3$. NMR: (DMSO-d_6). δ 1.20 (weak) and 1.30 (12 H, each s, 2 gem. dimethyl groups) and 5.28 (1 H, s, vinyl H). The signal of OH-group is omitted.

10. Pseudo-aspidinol B (IVd) was prepared by alkaline treatment (Cleavage A) of aspidin BB (VII) according to the method of Aebi *et al.*[17] Colourless plates, m.p. 70–72 °C (hexane) (lit.[17] m.p. 70–72 °C). MS: M+ at *m/e* 224 corresponding to $C_8H_{10}O_3$. NMR ($CDCl_3$): δ 0.98 (3 H, t, J 7 Hz, $-CO-CH_2-CH_2-CH_3$), 1.74 (2 H, m, $-CO-CH_2-CH_2-CH_2-CH_3$) 2.10 (3 H, s, CH_3-Ar), 3.04 (2 H, t, J 7 Hz, $-CO-CH_2-CH_2-CH_3$), 3.74 (3 H, s, CH_3O-), 6.20 (2 H, two superposable s, OH and aromatic H) and 13.20 (1 H, s, OH) (*cf.* Ref. 25).

11. Methylphloroglucinol-4-monomethyl ether (XXI) was prepared by the method of Weidel.[26] Slightly brownish needles, m.p. 124 °C (xylene) (lit.[26] m.p. 124 °C). MS: M+ at *m/e* 154 corresponding to $C_8H_{10}O_3$.

12. Methylene-bis-pseudo-aspidinol BB (XVIII R = $-CH_2-CH_2-CH_2$) was prepared by alkaline treatment (Cleavage C) of aspidin BB (VII) according to the method of Aebi *et al.*[18] Colourless needles, m.p. 140–141 °C (acetone) (lit.[18] m.p. 141–143 °C) MS: M+ at *m/e* 460 corresponding to $C_{25}H_{32}O_8$.

13. For the preparation of methylphloroglucinol-2-monomethyl ether (XV) and for the isolation of kosotoxin (II) and protokosin (III), see Part II.[2]

14. Aspidin BB (VII), m.p. 124–125 °C, consisted of an old sample isolated from *D. assimilis* S. Walker.[27]

** This is an unstable substance, like its corresponding isobutyryl isomer (Va).

Reductive alkaline cleavages

1. Aspidin BB (VII). Cleavage A. 1.2 g of aspidin BB (VII) was thoroughly mixed with 2.4 g of zinc dust, and 100 ml of 5 % NaOH solution was added. The mixture was kept on a boiling water bath for 5 min. The warm green-yellow solution was then separated from Zn by filtering, and the filter paper was washed with water. After cooling, the solution was acidified (pH 2) with 33 % H_2SO_4, whereupon a yellow precipitate was formed. The acidic solution was shaken three times with 50 ml ether and the combined ether solutions were shaken twice with 50 ml of distilled water and dried over Na_2SO_4. The filtered ether solution was evaporated *in vacuo*, whereupon 1.2 g of an oil was obtained. The phloroglucinols contained in this oil were separated by column chromatography on 30 g of silica gel. The fractions 1–14 (10 ml each) (benzene-light petroleum 1:1) contained, according to TLC, a mixture of albaspidin BB (XVII) and aspidin BB (VII), that did not separate. The fractions 15–40 (benzene-light petroleum 1:1) gave on crystallization from hexane 44 mg of aspidin BB (VII), m.p. 121–123 °C. The fractions 41–67 (benzene) contained only traces of aspidin BB (VII) and the fractions 68–83 gave by crystallization from hexane 40 mg of pseudo-aspidinol B (IVd), m.p. 70–72 °C. The fractions 84–140 (benzene–ether 9:1) contained only butyrylfilicinic acid (VIIIb), which was obtained as an oil.

Cleavage B. A 100 mg sample of aspidin BB (VII) was mixed with 200 mg of zinc dust and 20 ml of 5 % NaOH solution was added. The mixture was boiled for 5 min over a bunsen flame and the phloroglucinols were

separated as explained above for cleavage A and investigated by TLC (*cf.* theoretical section).

Cleavage C. A 1.0 g sample of aspidin BB (VII) was mixed with 2.0 g of zinc dust and 100 ml of a 15 % solution of NaOH was added. The mixture was boiled for 5 min on a Bunsen flame and processed according to Cleavage A. Crystallization of the resulting oil (1.0 g) from acetone gave 20 mg of methylene-bis-pseudo-aspidinol BB (XVIII R= $-CH_2-CH_2-CH_3$), m.p. 140–141 °C. No further uniform compounds were obtained.

2. *Kosotoxin (II). Cleavage A.* 1.0 g of kosotoxin (II) was subjected to reductive alkaline cleavage in the same way as described for aspidin BB (VII). The phloroglucinols of the reaction mixture (1.0 g) were separated by column chromatography on 30 g of silica gel. The fractions 1–12 (10 ml each) (benzene) were crystallized from methanol to give 41 mg of "kosin" (XVIII),* m.p. 128–132 °C. The melting point of these crystals was raised to 148–150 °C by recrystallization from hexane. The fractions 13–32 (benzene, benzene–chloroform 1:1) failed to give crystalline compounds from methanol. The fractions 33–43 (benzene–chloroform 1:1) contained (TLC) pseudo-aspidinols (IVa, b, c) as well as one compound of unknown structure. No crystals were obtained from hexane. The fractions 44–52 (benzene-chloroform 1:1), which contained the same compounds as the fractions 33–43, were dissolved in ether and extracted 4 times with 10 % $NaHCO_3$ solution. The ether solution was evaporated, and the residue dissolved in hexane, whereupon 20.4 mg of pseudo-aspidinol (IVa, b, c,) m.p. 60–61 °C was obtained.

Cleavages B and C were performed as described for aspidin BB (VII) and the resulting products investigated by TLC.

Cleavages D. 100 mg of kosotoxin (II) was mixed with 200 mg of zinc dust and added to 20 ml of a 15 % solution of NaOH. The mixture was warmed for 24 h on a water bath and processed as described for aspidin BB (VII), and then investigated by TLC.

3. Protokosin (III). Cleavage A. 1.06 g of protokosin (III) was subjected to reductive alkaline cleavage as described for aspidin BB (VII). The resulting phloroglucinols (1.06 g) were chromatographed on 32 g of silica gel. The fractions 1–22 (10 ml each) benzene) gave 77.9 mg of "kosin" (XVIII),* m.p. 127–129 °C, when crystallized from hexane. Fractions 23–32 (benzene) contained an unknown compound, the structure of which was not determined.

Fractions 33–92 (benzene, benzene–chloroform 1:1) contained pseudo-aspidinols (IVa, b, c). The residue after evaporation of the solvents was dissolved in ether and extracted four times with a solution of $NaHCO_3$. The evaporated ether solution was dissolved in hexane, giving 27.6 mg of pseudo-aspidinols (IVa, b, c), m.p. 58–61 °C. Fractions 93–190 (benzene-chloroform 1:1, chloroform) gave only mixed crystals of pseudo-aspidinols (IVa, b, c) and unknown compounds which were not further separated.

Cleavages B, C and D were performed as described for aspidin BB (VII) and kosotoxin (II), and resulting products were investigated by TLC.

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* R= $-CH(CH_3)_2$; $-CH_2-CH(CH_3)_2$; $-CH(CH_3)-CH_2-CH_3$.

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