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Phloroglucinol Derivatives of *Hagenia abyssinica*. II.* The Structure Determination of Kosotoxin and Protokosin**

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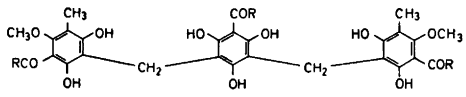
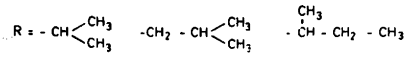
Phloroglucinol derivatives (kosins) from *Hagenia abyssinica* (Bruce) Gmel. have been reinvestigated. On the basis of reductive alkaline cleavages and spectroscopic evidence the structures (II) and (III) are proposed for kosotoxin and protokosin, respectively. The so-called β-kosin proved to consist of a mixture of isobutyryl (iB), isovaleryl (iV), and 2-methylbutyryl (2-MeB) side chain homologues of methylene-bis-pseudo-aspidinol (IV).

Les dérives phloroglucinoliques (kosines) isolés d'*Hagenia abyssinica* (Bruce) Gmel. ont été réexaminés. Sur la base des données des clivages réductifs alcalins et des preuves spectrales les structures (II) et (III) sont proposées pour la kosotoxine et la protokosine, respectivement. La prétendue β-kosine est en fait un mélange des homologues isobutyryles (iB), isovaléryles (iV) et méthyl-2 butyryles (2-MeB) des chaînes latérales du méthylène-bis-pseudo-aspidinol (IV).

In a recent publication [1] we reported the isolation of four phloroglucinol derivatives (kosins), designated K1 – 4, from kousso flowers (*Flos koso*).*** For one of these (K1), apparently identical with the earlier known kosidin,[2] we proposed the structure (I). Owing to its close structural similarity to the *Dryopteris* phloroglucinols, however, we preferred to give to kosidin the new name trispseudo-aspidinol.



(I)



We also isolated [1] substances with the properties recorded for kosotoxin (K2) [3,4] and protokosin (K3 and/or K4).[3,5,6] All kosins proved to be mixtures of isobutyryl (iB), isovaleryl (iV), and 2-methylbutyryl (2-MeB) side-chain homologues. Because of the small amounts isolated, the structures of kosotoxin (K2) and protokosin (K3 and/or K4) could not be fully elucidated. Through the kind cooperation of Professor T. Reichstein of Basle, however, we obtained a further commercial sample of Flos koso. As a result of extensive spectral analyses and examinations of degradative products, the structures (II) and (III) are now proposed for kosotoxin (K2) and protokosin (K4), respectively.†, ††

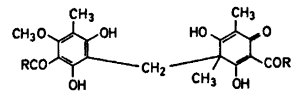
* Part I, Ref. 1.

** Part of this work was presented at the 4th Scandinavian Natural Products Chemistry Symposium held at Koli, Finland, June 12 – 17, 1973.

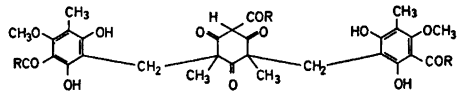
*** The dried female flowers (Flos koso) of *Hagenia abyssinica* (Bruce) Gmel. are known unde there names "kousso", "kosso" or "kusso" especially in Africa and the Near East. The kosins are presumably located in typical glandular hairs occurring on the epidermis.

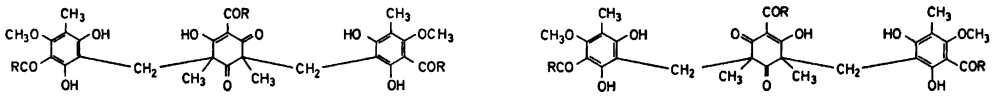
† It is plausible that even kosotoxin (II), for which only one formula is presented, can exist in several tautomeric forms.

†† K3, for which similar chemical and physical properties to those of K4 (protokosin) were reported,[1] proved to consist mainly of K4. The differences in their mass spectra turned out to be due to the fact that protokosin, at high temperatures, is transformed in the ionization chamber of the mass spectrometer to a mixture of compounds corresponding to $C_{41}H_{50}O_{11}$, $C_{40}H_{48}O_{11}$, $C_{39}H_{46}O_{11}$, and $C_{38}H_{44}O_{11}$.

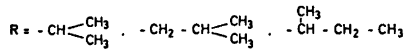


(II)

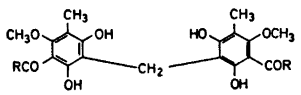




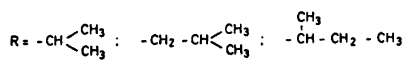
(III)



Moreover, we studied the so-called kosin, that is formed from kousso constituents by the action of alkali.[2–7] This "kosin", which was earlier considered to be a mixture of two isomeric dimethyl ethers of methylene-bis-methylphloroisobutyrophenone, assigned as "α-" and "β-kosin",[6,8] proved to be a mixture of compounds differing only in their acyl side chains. Thus, they all have the same "skeletal structure" as the earlier known "α-kosin" [IV, R = $-CH(CH_3)_2$]. Moreover, the acyl side chains proved to be the same as those found in the above mentioned naturally occurring phloroglucinol constituents of kousso: isobutyryl (iB), isovaleryl (iV), and 2-methylbutyryl (2-MeB). For the same reasons as advanced for trispseudo-aspidinol (I),[1] the name methylene-bis-pseudo-aspidinol is proposed for this mixture of "kosin" homologues (IV). Analogously, the earlier designated "α-kosin" [IV, R = $-CH(CH_3)_2$] should be called methylene-bis-pseudo-aspidinol iBiB.



(IV)



STRUCTURE DETERMINATIONS

Trispseudo-aspidinol (kosidin) (I). This tricyclic phloroglucinol derivative, for which the structure (I) was proposed earlier [1] and which was obtained as yellowish plates, m.p. 167 – 169 °C (methanol),[1] was not isolated in crystalline form in connection with the present work. According to semiquantitative TLC (*cf.* Ref. 1) it is a minor component (< 5 %) in the phloroglucinol mixture of kousso (crude kosin).

The mass spectrum of trispseudo-aspidinol (I) (Fig. 1) shows molecular peaks at *m/e* 710, 696, 682, and 668, corresponding to $C_{39}H_{50}O_{12}$, $C_{38}H_{48}O_{12}$, $C_{37}H_{46}O_{12}$, and $C_{36}H_{44}O_{12}$, respectively. These four peaks, as well as the peaks at *m/e* 653, 639, and 625, which can be assigned to the cleavages of C_4H_9- and C_3H_7- side chain units from the molecular ions, are in good agreement with the results of alkaline cleavages [1] and confirm that trispseudo-aspidinol (I) is a mixture of side chain homologues. The general fragmentation of trispseudo-aspidinol (I) is analogous to that found earlier for polycyclic *Dryopteris* phloroglucinols [9–11] and supports the proposed structure (I).

The NMR spectrum ($CDCl_3$) of trispseudo-aspidinol also supports the structure (I), in agreement with the NMR data reported earlier,[1]

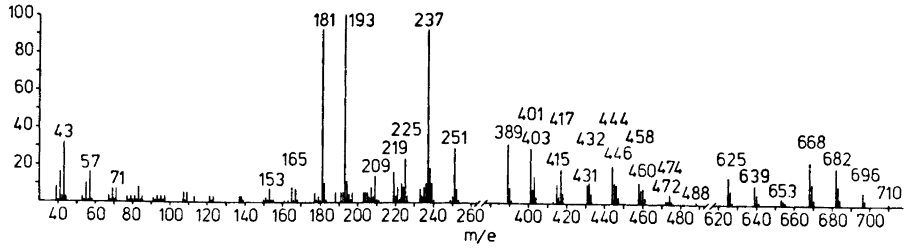


Fig. 1. Mass spectrum (70 eV) (*T* 200 °C) of trispseudoaspidinol (I).

showing the following signals:* δ 1.18 [18 H, d, *J* 7 Hz, mainly 3 $-CO-CH(CH_3)_2$], 2.12 (6 H, s, 2 CH_3-Ar), 3.72 (6 H, s, 2 CH_3O-Ar), 3.82 (4 H, s, 2 $-CH_2-$), ~3.9** [3 H, m, mainly 3 $-CO-CH(CH_3)_2$], 9.56 (2 H, s, 2 OH), 10.72 (2 H, s, 2 OH), 15.46 (1 H, s, OH), 15.56 (1 H, s, OH), 15.80 (1 H, br s, OH).

Kosotoxin (II). This bicyclic phloroglucinol derivative was isolated according to previously reported methods.[1] Repeated recrystallizations from hexane of the chromatographic fractions rich in kosotoxin gave yellowish plates, m.p. 119–122 °C (lit.[1] m.p. 110–112 °C). Kosotoxin is the main component (> 50 %) in the phloroglucinol mixture of kousso. The optical activity of kosotoxin (*cf.* Experimental Section) is in agreement with the asymmetric structure proposed (II). For the products obtained by reductive alkaline cleavage, which support well the structure (II); see Ref. 1 and Part III.

The mass spectrum of kosotoxin (II) (Fig. 2) shows molecular peaks at *m/e* 488, 474 and 460, corresponding to $C_{27}H_{36}O_8$, $C_{26}H_{34}O_8$ and $C_{25}H_{32}O_8$, respectively. The peaks at *m/e* 431 and 417 support the assumption that kosotoxin is a mixture of C_4H_9- and C_3H_7- side chain homologues and confirm the results of the alkaline cleavages. The general fragmentation pattern is similar to that found earlier for polycyclic *Dryopteris* phloroglucinols [9–11] and supports the proposed structure II.

The NMR spectrum ($CDCl_3$) of kosotoxin also supports the structure II, the following signals are shown: δ 1.12 [6 H, d, *J* 7 Hz, mainly $-CO-CH(CH_3)_2$], 1.18 (6 H, d, *J* 7 Hz, mainly $-CO-CH(CH_3)_2$), 1.28 (3 H, s, $CH_3-C\lesssim$), 1.92 (3 H, s, $CH_3-C\lesssim$), 2.20 (3 H, s, CH_3-Ar), 3.04 (2 H, br, s, $-CH_2-$), 3.76 (3 H, s, CH_3O-Ar), ~ 3.9 [2 H, m, mainly 2 $-CO-CH(CH_3)_2$], 9.66 (1 H, s, OH), 11.06 (1 H, s, OH), 15.54 (1 H, s, OH) 19.18 (1 H, s, OH).

Protokosin (III). This tricyclic phloroglucinol derivative was isolated as described previously,[1] crystallized from acetone, and obtained in the form of colourless needles, m.p. 181–183 °C (lit. m.p. 174–176 °C,[1] 176 °C,[2,3] 182 °C,[2,5]). Protokosin proved to be a major component (*ca.* 30–40 %) in the phloroglucinol mixture of kousso. Its optical activity (*cf.* Experimental Section) supports the asymmetric structure proposed (III). For the products obtained by reductive alkaline cleavage, which are in agreement with the structure III, see Ref. 1 and Part III.

The mass spectrum of protokosin (III) (Fig. 3) presents molecular peaks at *m/e* 738 (weak), 724, 710, and 696, corresponding to $C_{41}H_{54}O_{12}$, $C_{40}H_{52}O_{12}$, $C_{39}H_{50}O_{12}$, and $C_{38}H_{48}O_{12}$, respectively. The peaks at *m/e* 681, 667, and 653 support the assumption that protokosin is a mixture of C_4H_9- and C_3H_7-side chain homologues and confirm the results of the alkaline cleavages. The general fragmentation pattern is similar to that found earlier for polycyclic *Dryopteris* phloroglucinols [9–11] and supports the proposed structure (III). However, the very

* The signals assigned to the OH groups in the NMR spectra of trispseudo-aspidinol (I), kosotoxin (II), protokosin (III), "kosin" (IV) (m.p. 148–150 °C), and pseudo-aspidinol (VIII) (m.p. 65–66 °C) disappear after treatment with D_2O. The intensities given for acyl side chain proton signals should be regarded as approximative since trispseudo-aspidinol, kosotoxin, protokosin, and in smaller amount "kosin" (m.p. 148–150 °C) and pseudo-aspidinol (m.p. 65–66 °C) are mixtures of isobutyryl, isovaleryl, and 2-methylbutyryl side chain homologues. The weak signals due to protons in the isovaleryl side chains and γ-protons and secondary β-protons in the 2-methylbutyryl side chains are omitted.

** Owing to a typing error this signal was earlier reported as 3.0.[1]

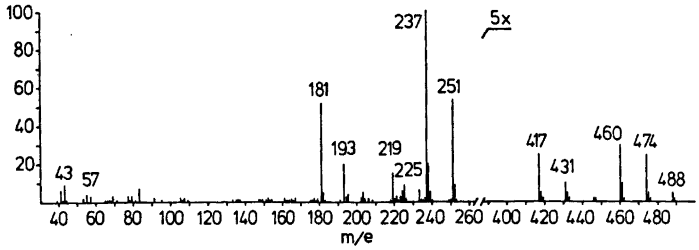


Fig. 2. Mass spectrum (70 eV) (*T* 150 °C) of kosotoxin (II).

weak intensities of the molecular peaks in the mass spectrum (Fig. 3) of protokosin are noteworthy.

The thermal rottlerone change, found earlier in connection with polycyclic *Dryopteris* phloroglucinols [9–11] and which takes place in the ionization chamber of the mass spectrometer, has to be taken into consideration in the interpretation of the fragmentation patterns of kousso constituents. In the case of protokosin, this phenomenon is clear, and among the compounds formed by this procedure, the "kosin" homologues are probably the most characteristic. They are partly responsible for example, for the formation of the ions assigned to *m/e* 488, 474, 460, 431, and 417.

The NMR spectrum ($CDCl_3$) of protokosin also supports the structure III showing the following signals: δ 1.16 [18 H, m, mainly 3 $-CO-CH(CH_3)_2$], 1.66 (6 H, br s, 2 $CH_3-C\leqslant$), 2.20 (6 H, s, 2 CH_3-Ar), 3.64 (4 H, s, 2 $\geqslant C-CH_2-C\leqslant$), 3.76 (6 H, s, 2 CH_3O-Ar), ~3.9 [3 H, m, mainly 3 $-CO-CH(CH_3)_2$], 6.02 (1 H, br s, OH), 10.40 (1 H, s, OH), 13.14 (1 H, s, OH), 14.56 (1, H br s, OH), 19.02 (1 H, s, OH). The intensities given for the signals should be regarded as approximate, owing to the existence of protokosin in several tautomeric forms and to the fact that protokosin is a mixture of isobutyryl, isovaleryl, and 2-methylbutyryl side chain homologues. The spectrum shows a weak signal at δ 3.46 which is probably due to the proton attached to the central ring in the fully ketonic form.

Methylene-bis-pseudo-aspidinol ("kosin") (pseudo-aspidin) (IV). In 1901 Lobeck [8] claimed that the commercial "kosin" of E. Merck (Darmstadt), apparently the first crystalline product isolated from kousso flowers, was a mixture of two closely related compounds, which he called "α-" and "β-kosin". Each of these compounds proved to contain two methoxyl groups. In 1952 Birch and Todd [6] suggested the formulae V and VI for "α-" and "β-kosin", respectively. On the basis of extensive synthetic work, Orth and Riedl [8] later rejected the proposed structures. As their synthetic 5,5′-methylene-bis-(3-methyl-phloroisobutyrophenone-2-methylether) [IV R = $-CH(CH_3)_2$] proved to be identical with the "α-kosin" of Birch and Todd,[6] the structure of "α-kosin" was settled. Moreover, Orth and Riedl [8] showed that "β-kosin" was not identical either with compound (VI) or any other synthetical dimethyl ether of 5,5′-methylene-bis-(3-methyl-phloroisobutyropheno-

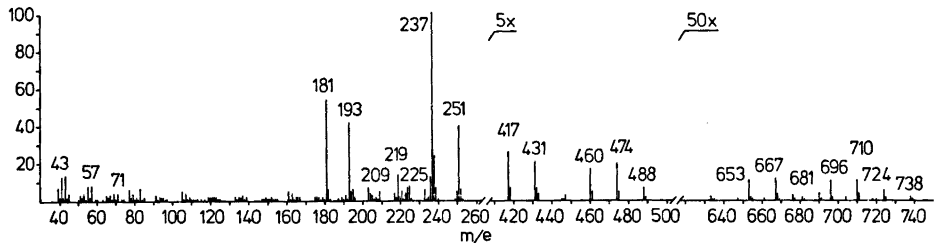
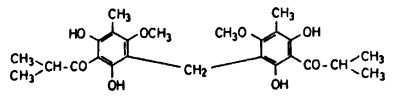
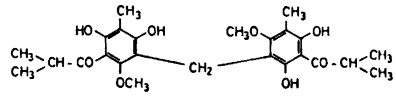


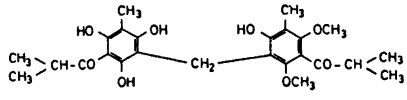
Fig. 3. Mass spectrum (70 eV) (*T* 220 °C) of protokosin (III).



(V)



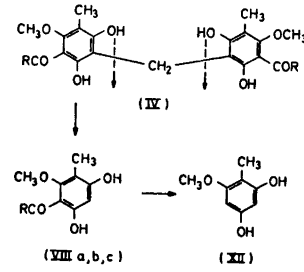
(VI)



(VII)

ne) containing the two methoxyl groups in different rings. Therefore, they proposed for "β-kosin" the tentative formula VII, where the two methoxyl groups are in the same ring.

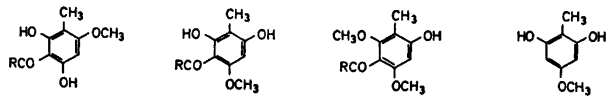
In the present work several "kosin" preparations with slightly varying melting points and crystalline forms * were isolated by column chromatography after treatment of crude kosin, kosotoxin (II), or protokosin (III) with alkali. In agreement with previous findings,[2-6] these "kosin" preparations proved to be very resistant against further break-down with alkali. "Kosin" was detected by TLC in the reaction mixture even after 24 h heating in 15 % NaOH on a water bath.** The sole decomposition products observed consisted of pseudo-aspidinols iB, iV, and 2-MeB (VIIIa, b, and c) (Scheme 1). As in our earlier work,[1] isomeric aspidinols such as (IX) and (X), were not detected by PC or TLC. Nor was there any sign of 3-methylphloroisobutyrophenone-2,6-dimethyl ether (XI) or other isomeric dimethyl ethers, which could be expected to appear among the decomposition products of (VII) or its isomer. On the other hand, some methylphloroglucinol-2-methyl ether (XII), resulting from the reductive alkaline cleavage of the acyl side chains of (VIIIa, b, and c), was recognized by TLC (Scheme 1). The same ether (XII) has previously been isolated by Lobeck [2] after strong reductive alkaline cleavage of his "α-kosin". Notably, no sign of methylphloroglucinol-4-monomethyl ether (XIII), which could be expected to be formed from compound X, was detected.



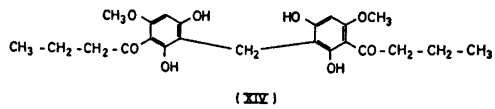
(IV) (VIII a,b,c) (XII)

R = -CH(CH3)2 (a), -CH2-CH(CH3)2 (b), -CH(CH3)-CH2-CH3 (c)

Scheme 1. Reductive alkaline cleavage of "kosin" (methylene-bis-pseudo-aspidinol) (IV)



(IX) (X) (XI) (XIII)



(XIV)

* According to Hems and Todd [5] "α-kosin" forms yellow needles, m.p. 158 °C, and "β-kosin" yellow prisms, m.p. 120 °C, when recrystallized from methanol.

** Even methylene-bis-pseudo-aspidinol BB (IV, R = $-CH_2-CH_2-CH_3$) (*cf.* Part III) and methylene-bis-*o*-desaspidinol BB (XIV), both of which contain methoxyl groups in *ortho* position to the acyl groups, are known to be very alkali stable compounds.[12,13]

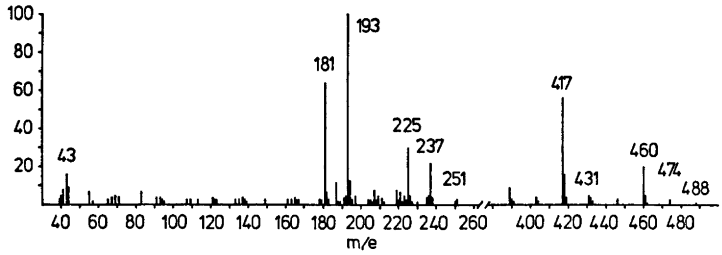


Fig. 4. Mass spectrum (70 eV) (*T* 130 °C) of "kosin" (methylene-bis-pseudo-aspidinol) (IV) (M.p. 148 – 150 °C).

In the light of these facts, it seems very probable that "α-" and "β-kosin" simply differ in their side chains, which consist of isobutyryl (iB), isovaleryl (iV), and 2-methylbutyryl (2-MeB) groups in different ratios.

The mass spectra of different "kosin" fractions (*cf.* Figs. 4 and 5) show molecular peaks at *m/e* 488, 474, and 460 corresponding to $C_{27}H_{36}O_8$, $C_{26}H_{34}O_8$, and $C_{25}H_{32}O_8$, respectively. The peaks at *m/e* 431 and 417 support the assumption that "kosin" fractions are mixtures of C_4H_9- and C_3H_7-side chain homologues and confirm the results of the alkaline cleavages. The general fragmentation pattern is similar to that found earlier for polycyclic *Dryopteris* phloroglucinols [9–11] and in agreement with the proposed structures (IV). The relative intensities of the molecular peaks in these spectra are in good agreement with the conclusion that "α-" and "β-kosin" differ only in their side chains. Thus the fraction melting at 148 – 150 °C shows a relatively strong molecular peak at *m/e* 460 and weak molecular peaks at *m/e* 474 and 488, supporting our conclusion that the "α-kosin" contains mainly isobutyryl side chains. On the other hand, the relative intensities of the molecular peaks at *m/e* 474 and 488 increase as the melting points of the different "kosin" fractions decrease, approaching the indicated melting point of "β-kosin" (m.p. 120 °C). Such changes point to the growing contribution of isovaleryl and 2-methylbutyryl side chains in the fractions.

The NMR spectrum (60 MHz) ($CDCl_3$) of the "kosin" fraction melting at 148 – 150 °C supports the structure (IV), showing the following signals: δ 1.18 [12 H, d, *J* 7 Hz, mainly 2 $-CO-CH(CH_3)_2$], 2.12 (6 H, s, 2 CH_3-Ar), 3.72 (6 H, s, 2 CH_3O-Ar), 3.84 (2 H, s, $-CH_2-$), ~3.9 [2 H, m, mainly 2 $-CO-CH(CH_3)_2$], 9.56 (2 H, s, 2 OH), 15.46 (1 H, s, OH) and 15.56 (1 H, s, OH).

The stereochemistry of both kosotoxin (II) and protokosin (III) has not yet been determined. However, it seems evident to us from the presence of optical activity, that the two chiral centres at positions 1 and 3 of the central ring of protokosin (III) (considered in the "triketonic" form) should have a like spatial arrangement of the groups. In this case the carbon atom at position 5 would not be asymmetric.

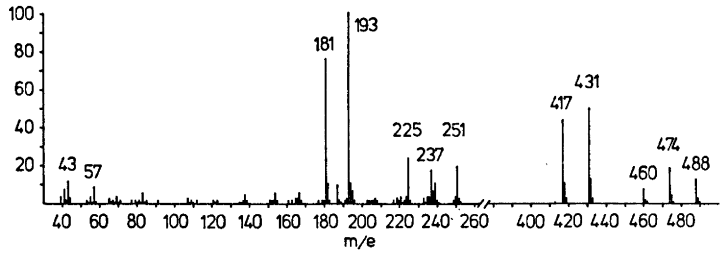
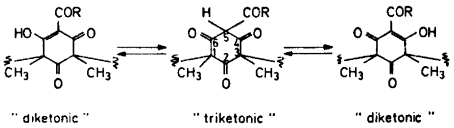


Fig. 5. Mass spectrum (70 eV) (*T* 130 °C) of "kosin" (methylene-bis-pseudo-aspidinol) (IV) (M.p. 125 – 127 °C).



If the two carbons at positions 1 and 3 should have an opposite spatial arrangement of the groups the whole molecule would have a plane of symmetry, the carbon atom at position 5 would be pseudo-asymmetric, and the compound would show no optical activity ("meso form").*

TLC of the kosins. The thin-layer chromatographic behaviour of the kosins was investigated on thin silica gel layers buffered to pH 4 – 8 using the gradient technique (Fig. 6).[14] Methylene-bis-pseudo-aspidinol (IV) and trispseudo-aspidinol (I) are easily separated at pH 4 – 7, but at pH 7 – 8 hardly at all. With increasing pH the R_F-value of methylene-bis-pseudo-aspidinol (IV) decreases, whereas that of trispseudo-aspidinol (I) increases. Protokosin (III) and kosotoxin (II) advance together at every pH examined, the former being a little faster than the latter. There is no distinct influence of the pH on the TLC-behaviour of these two substances. No separation of the different homologues of the different kosins was achieved by TLC.

EXPERIMENTAL

General. The UV spectra were measured with a Beckman DB-G grating spectrophotometer and the IR spectra 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 of the natural products (I, II, and III) have been recorded on an A.E.I. MS-9 double-focusing mass spectrometer (70 eV) at the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, through the courtesy of Dr. B. C. Das. The other mass spectra have been taken on a Perkin-Elmer 270 mass spectrometer. The optical rotations were measured on a Perkin-Elmer 141 Polarimeter. The melting points have been determined on a Reichert micro hot stage and are uncorrected. The thin-layer chromatographic methods were those previously reported.[1,14,15]

Material investigated. A 5 kg commercial sample of Flos koso "Siegfried" of unknown age and origin was investigated. By TLC it was shown to contain the same phloroglucinol derivatives as the material previously investigated (*cf.* Part I).[1]

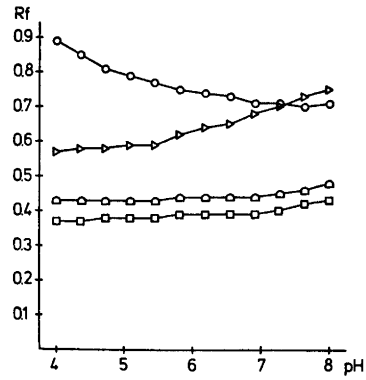


Fig. 6. Gradient TLC of trispseudo-aspidinol (I) (○, red), kosotoxin (II) (□, yellow), protokosin (III) (⌂, pale brown), and "kosin" (methylene-bis-pseudo-aspidinol) (IV) (△, pale brown) at pH 4 – 8. Chromatographed 3×. Solvent: hexane – chloroform 50:50. Colours with Fast Blue Salt B in parenthesis.

Preparation of ether extract and crude kosins. Preparations were made according to previously described methods.[1] The yields are listed in Table 1.

Column chromatography of crude Mg-kosin. Crude kosin (26.8 g) was suspended in benzene and chromatographed on 700 g of silica gel (Merck, particle size 0.05 – 0.2 mm) as previously described.[15] According to TLC, all fractions (10 ml each) were mixtures containing kosotoxin (II) and protokosin (III) in different ratios. The first fractions also contained some trispseudo-aspidinol (I). The four combined fractions (Table 2) were evaporated to dryness and separately dissolved in hot methanol, from which protokosin (III) started to crystallize as soon as cooling began. Kosotoxin (II), although present in large amounts (TLC), remained in solutions. After about one week, the mother liquors, containing only small amounts of protokosin (III),

Table 1. Yields of diethyl ether extract and crude kosins from Flos koso.

Flos koso (kg)	5.0
Material in diethyl ether extract (g)	324
(%)	6.5
Crude kosins MgO(g)	54
MgO(%)	1.1
$Ba(OH)_2$ (g)	70
$Ba(OH)_2$ (%)	1.4

* The 2-methylbutyryl group, which is one of the side chains present, has a chiral centre and this of course should cause some rotation.

Table 2. Melting points and yields of protokosin (III) from different fractions.

Fraction No. (10 ml each)	M.p. (°C)	Amount (mg)
1 – 32 (C_6H_6)	160 – 163	228
33 – 110 (C_6H_6)	166 – 171	1312
111 – 137 (C_6H_6)	171 – 175	593
138 – 410 (C_6H_6, C_6H_6-$CHCl_3$, 1:1)	175 – 178 [a]	870
Total		3003

[a] The melting point of the protokosin (III) of this fraction was raised to 181 – 183 °C by recrystallization from acetone.

were evaporated to dryness and the residues dissolved in hot hexane. The hexane solutions of the residues from fractions 33 – 410 (Table 3), which showed very similar TLC behaviour, were combined. On standing kosotoxin (II) slowly separated from the hexane solutions in several portions with slightly different melting points (Table 3). According to TLC the mother liquors still contained relatively large amounts of kosotoxin (II), which were not isolated in crystalline form.

Preparation of "kosins" (methylene-bis-pseudo-aspidinols) (IV), pseudo-aspidinols (VIIIa, b, c), and methylphloroglucinol-2-methyl ether (XII)

1. Mild reductive alkaline cleavage. Crude Ba-kosin (3.6 g) was mixed with 7.2 g of zinc powder, 50 ml of 5 % KOH was added, and the mixture heated on a water bath for 5 min. For details, see Part III, Cleavage A. The resulting product (3.6 g) was suspended in benzene and chromatographed on 100 g of silica gel. The fractions 1 – 7 (10 ml each) (benzene) gave 48.1 mg of "kosin" (IV) which, after crystallization from methanol, melted at 125 – 127 °C. The fractions 8 – 97 (benzene, benzene – chloroform, 1:1) gave another crop of "kosin" (IV) which, after crystallization from hexane, melted at 148 – 150 °C. The fractions 98 – 210 (chloroform) contained (TLC) pseudo-aspidinols (iB, IV, and 2-MeB acyl derivatives) (VIIIa, b, c) as well as some other compounds, the structures of which were not determined.

Table 3. Melting points and yields of kosotoxin (II) from the residues of different fractions.

Fraction No.	M.p. (°C)	Amount (mg)
1 – 32	100 – 110	315
	115 – 118	399
33 – 410	116 – 120	249
	119 – 122	121
	107 – 115	37
Total		1121

2. Strong reductive alkaline cleavage. Crude Ba-kosin (10 g) was mixed with 20 g of zinc powder, 100 ml of 15 % KOH was added and the mixture heated on a water bath for 24 h. For details, see Part III, Cleavage D. Part of the resulting product was used for determination of the organic acids formed. For details, see Part I. They consisted of isobutyric, isovaleric, and 2-methylbutyric acids in agreement with previous findings.[1] Another part of the resulting product (2.8 g) was used for column chromatography of the phloroglucinol derivatives formed. The fractions 1 – 12 (10 ml each) (benzene) gave 92 mg of "kosin" (IV), which, after crystallization from methanol, melted at 116 – 120 °C. The fractions 13 – 57 (benzene, benzene – chloroform, 1:1) contained only some unknown compounds, the structures of which were not determined. The fractions 58 – 112 (benzene – chloroform, 1:1, chloroform) contained some unknown compounds, as well as large amounts of pseudo-aspidinols (VIIIa, b, c). No crystalline compounds were obtained from hexane. Therefore, these fractions were rechromatographed on silica gel (hexane-benzene, 1:1). Seven fractions (50 ml each), containing pseudo-aspidinols (VIIIa, b, c), were collected and evaporated to dryness. These fractions, when combined and crystallized from hexane, gave three successive crystallizates of pseudo-aspidinols (VIIIa, b, c) (m.p. 59 – 61 °C, 275 mg), (m.p. 65 – 66 °C, 119 mg), (m.p. 62 – 65 °C, 98 mg). NMR ($CDCl_3$) (Fraction melting at 65 – 66 °C) : δ 1.18 [6 H, d, J 7 Hz, mainly $-CO-CH(CH_3)_2$], 2.12 (3 H, s, CH_3-Ar), 3.74 (3 H, s, CH_3-OAr), 3.86 [1 H, heptet, J 7 Hz, mainly $-CO-CH(CH_3)_2$] 6.22 (1 H, s, arom. H), 6.30 (1 H, s, OH), 13.06 and 13.12 (1 H, each s, OH). MS (fraction melting at 65 – 66 °C): M^+ at m/e 224 and 238 (weak) corresponding to $C_{12}H_{16}O_4$ and $C_{13}H_{18}O_4$. The fractions 113 – 127 (chloroform – ethanol, 95:5) gave 54 mg of methylphloroglucinol-2-methyl ether (XII), which, after crystallization from water, melted at 112 – 116 °C (lit.[12] 114 – 116 °C). MS: M^+ at m/e 154 corresponding to $C_8H_{10}O_3$.

Physical data of the identified natural products, as well as those of methylene-bis-pseudo-aspidinol ("kosin") (IV) obtained by the alkaline treatment of the crude kosins

1. Trispseudo-aspidinol (kosidin) (I.). Yellowish plates, m.p. 167 – 169 °C (methanol).

UV (cyclohexane) (ε values calculated on $C_{36}H_{44}O_{12}$):λ_{max} 229 (ε 39 000), 284 (ε 39 500) nm. λ_{min} 250 nm. IR (KBr): λ 3.06 (m), 3.34 (m), 3.38 (m), 3.46 (w), 6.22 (s), 6.90 (s), 7.10 (s), 7.24 (m), 7.38 (m), 7.60 (sh), 7.88 (s), 8.44 (s), 8.68 (s), 9.02 (s), 9.42 (w), 9.70 (w), 10.02 (w), 10.18 (w), 10.36 (w), 10.64 (w), 10.80 (w), 11.06 (w), 12.50 (w) μ. NMR ($CDCl_3$): See theoretical section. MS: See theoretical section.

2. Kosotoxin (II). Yellowish plates, m.p. 119–122 °C (hexane). $[\alpha]_D^{25} + 11.9 \pm 0.2°$ (*c* 2.335, $CHCl_3$). UV (cyclohexane) (ε values calculated on $C_{25}H_{32}O_8$): λ_{max} 226 (ε 19 700), 283 (ε 24 000) nm. λ_{min} 251 nm. IR (KBr): λ 3.06 (m), 3.34 (m), 3.38 (m), 3.46 (w), 3.66 (w), 3.76 (w), 6.00 (m), 6.24 (s), 6.88 (s), 7.08 (s), 7.22 (s), 7.40 (m), 7.52 (sh), 7.80 (s), 8.00 (m), 8.12 (s), 8.42 (s), 8.60 (s), 8.82 (m), 9.04 (s), 9.52 (w). 9.80 (w), 9.94 (w), 10.08 (m), 10.40 (w), 10.62 (w), 10.92 (w), 11.16 (w), 12.56 (m), 13.30 (w) μ. NMR ($CDCl_3$): See theoretical section. MS: See theoretical section.

3. Protokosin (III). Colourless needles, m.p. 181–183 °C (acetone). $[\alpha]_D^{25} + 13.9 \pm 0.5°$ (*c* 0.610, $CHCl_3$). UV (cyclohexane) (ε values calculated on $C_{39}H_{50}O_{12}$): λ_{max} 224 (ε 28 200), 285 (ε 36 400) nm. λ_{min} 249 nm. IR (KBr): λ 2.92 (m), 3.14 (w), 3.34 (m), 3.38 (m), 3.46 (w), 6.20 (s), 6.48 (m), 6.90 (s), 7.10 (s), 7.22 (m), 7.38 (m), 7.52 (sh), 7.84 (m), 8.14 (w), 8.46 (s), 8.68 (s), 8.98 (s), 9.10 (s), 9.40 (sh), 9.68 (m), 10.02 (m), 10.36 (m), 10.64 (w), 10.80 (w), 11.04 (m), 12.40 (w), 13.80 (w) μ. NMR ($CDCl_3$): See theoretical section. MS: See theoretical section.

4. Methylene-bis-pseudo-aspidinol ("kosin") (IV). Yellow needles, m.p. 148–150 °C (hexane). UV (cyclohexane) (ε values calculated on $C_{25}H_{32}O_8$): λ_{max} 229 (ε16 000), 286 (ε 22 100) nm. λ_{min} 252 nm. IR (KBr): λ 3.06 (m), 3.34 (m), 3.38 (m), 3.44 (w), 6.24 (s), 6.88 (s), 7.10 (s), 7.24 (m), 7.36 (m), 7.62 (w), 7.88 (s), 8.12 (w), 8.46 (s), 8.66 (s), 8.80 (m), 9.02 (s), 9.38 (w), 9.84 (m), 10.08 (m), 10.16 (w), 10.34 (w), 10.80 (w), 11.08 (w), 12.52 (w) μ. NMR ($CDCl_3$): See theoretical section. MS: See theoretical section.

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