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PHLOROGLUCINOL DERIVATIVES OF *HAGENIA ABYSSINICA*

MAURI LOUNASMAA
The State Institute for Technical Research, Chemical Laboratory, Otaniemi, Finland

and

CARL-JOHAN WIDÉN and AARRE HUHTIKANGAS
Department of Pharmacognosy, University of Helsinki, Helsinki, Finland

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Key Word Index—*Hagenia abyssinica*, Rosaceae, kousso, kosins, phloroglucinol derivatives, tripseudoaspidinol

Abstract—Four phloroglucinol derivatives have been isolated from *Hagenia abyssinica* (Bruce) Gmel The structure (IV) and partial structures (V), (VI) and (VII) are proposed All are mixtures of isobutyryl (iB), isovaleryl (iV) and 2-methylbutyryl (2-MeB) side chain homologues The two latter acyl groups have been detected for the first time in kousso

INTRODUCTION

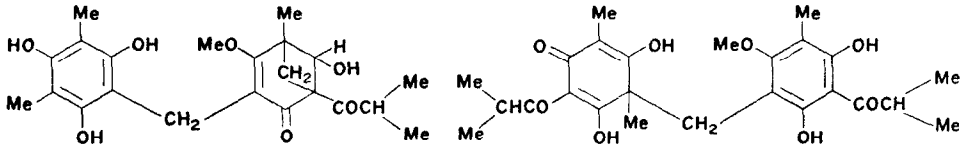
Hagenia abyssinica (Bruce) Gmel (Rosaceae) is a dioecious tree growing in East Africa and Ethiopia on mountains, attaining a height of up to 20 m [1] The dried female flowers or the entire panicles have long been used as an anthelmintic drug under the name 'kousso' or 'kosso' especially in Africa and in the Near East. The kousso flowers (Flos koso) were at one time included in most European Pharmacopoeias as an effective worm drug According to early chemical investigations,[2–5] kousso flowers contain phloroglucinol derivatives similar to those of *Dryopteris* ferns.

In 1937 Hems and Todd[6] isolated from commercial kousso a substance having the recorded properties of protokosin[3] together with some kosotoxin.[3,4] However, none of the earlier reported[5] kosidin was found Structure (I) was proposed for protokosin Later Birch and Todd[7] on the basis of studies of reduction products and spectral analyses, revised the structure of protokosin to (II) The latter workers also studied several additional samples of male and female flowers of *Hagenia abyssinica*. However, crystalline protokosin could be isolated only from one sample, the others yielding only amorphous products.

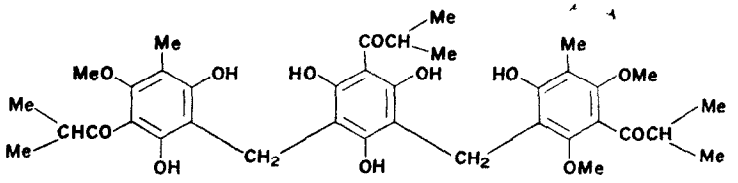
Later Riedl[8] and Orth and Riedl[9] on the basis of synthetic work rejected formula (II) for protokosin. A new tricyclic structure (III) was proposed, resembling more closely the

[1] TSCHIRCH, A (1923) *Handbuch der Pharmakognosie*, Vol III, p 19, Chr. Herm. Tauchnitz, Leipzig.
[2] FLÜCKIGER, F A and BURI, A (1874) *Arch Pharm.* **205**, 193
[3] LEICHSENRING, M (1894) *Arch Pharm* **232**, 51
[4] KONDAKOW, I and SCHATZ, N (1899) *Arch Pharm* **237**, 481
[5] LOBECK, A (1901) *Arch Pharm* **239**, 672
[6] HEMS, B A and TODD, A R (1937) *J Chem. Soc.* 562
[7] BIRCH, A J and TODD, A R (1952) *J Chem Soc* 3102.
[8] RIEDL, W (1956) *Chem Ber.* **89**, 2600
[9] ORTH, W A and RIEDL, W (1963) *Ann. Chem* **663**, 83.

phloroglucinol derivatives in *Dryopteris* species This was in agreement with the earlier analytical data of Leichsenring [3]



(I) (II)



(III)

In connection with our recent studies on phloroglucinol derivatives in *Dryopteris* ferns,[10–12] we found it of interest to reinvestigate the structurally related kosins in *Hagenia abyssinica* We investigated a new sample of kousso collected from the Nyandarua district in Kenya in 1971 (sample 1), as well as another sample, over 30-year-old and of unknown origin from the Department of Pharmacognosy, University of Helsinki (sample 2)

RESULTS AND DISCUSSION

Isolation of the Kosins

The ether extracts and crude kosins (phloroglucinol mixtures) in kousso were made according to previously used methods [10,11] However, it appeared that the kosins could not be removed quantitatively, with MgO as base, from the fatty material and chlorophyll present in the extracts Therefore, the latter were subsequently treated with $Ba(OH)_2$, which is a stronger base When examined by TLC, the same phloroglucinol composition was observed in the crude extracts, Mg-kosins and Ba-kosins No detectable decomposition occurred under the slightly alkaline conditions during preparation of Ba-kosins Such decomposition reactions have, however, been observed in some *Dryopteris* phloroglucinols [10,11]

TABLE 1 YIELDS OF CRUDE KOSINS FROM *Hagenia abyssinica*

Organs investigated	Amount (g)	Material in ether extract (g)	(%)	Crude kosin MgO (g)	(%)	$Ba(OH)_2$ (g)	(%)
Sample 1							
Female flowers	436	41 5	9 5	7 61	1 74	4 08	0 94
Stems	62	1 8	2 9	0 33	0 52	0 31	0 50
Leaves	34	2 0	5 7	0 10	0 30	0 53	1 85
Sample 2							
Female flowers	150	9 1	6 1	1 3	0 87	Not studied	

[10] WIDÉN, C -J and BRITTON, D M (1971) *Can J Botany* **49**, 1589
[11] WIDÉN, C -J , VIDA, G , VON EUW, J and REICHSTEIN, T (1971) *Helv Chim Acta* **54**, 2824
[12] TRYON, R , WIDÉN, C -J , HUHTIKANGAS, A and LOUNASMAA, M (1973) *Phytochemistry* **12**, 683

The yields of the ether extracts and crude kosins from the investigated materials are listed in Table 1. TLC showed that the two samples had the same pattern of phloroglucinol derivatives. From sample 1, three different kosins, labelled K1–3 [the last being slightly contaminated by a fourth compound K4 (see sample 2)] were isolated in crystalline form by column chromatography on silica gel using the same eluents as for *Dryopteris* phloroglucinols [11,12].

TABLE 2. CHROMATOGRAPHIC AND PHYSICAL DATA OF THE ISOLATED KOSINS

Compounds	M.p.	R_fs on TLC at pH 6.0 solvent 1*	solvent 2†	Colour with fast blue salt B	Optical rotations	UV spectra (nm) in cyclohexane
K1 yellowish plates	167–169°	0.52	0.80	Orange red	$[\alpha]_D^{25}$ *ca* 0°‡ (*c* 0.0711, $CHCl_3$)	λ_{max} 229 (ε 39 000), 284 (ε 39 500) λ_{min} 250 (ε values calc on $C_{36}H_{44}O_{12}$)
K2 yellowish plates	110–112°	0.39	0.73	Pale yellow which disappears on standing	$[\alpha]_D^{25}$ + 8.1° ±0.2° (*c* 1.0498, $CHCl_3$)	λ_{max} 226 (ε 18 900), 283 (ε 19 300) λ_{min} 251 (ε values calc on $C_{25}H_{32}O_9$)
K3 colourless needles	177–178°	0.42	0.73	Pale brown	$[\alpha]_D^{27}$ +11.2° ±1° (*c* 0.8438, $CHCl_3$)	λ_{max} 224 (ε 26 100), 282 (ε 28 300) λ_{min} 241 (ε values calc on $C_{39}H_{46}O_{11}$)
K4 colourless needles	174–176°	0.42	0.73	Pale brown	$[\alpha]_D^{25}$ −14.1° ± 2° (*c* 0.2693, $CHCl_3$)	λ_{max} 224 (ε 34 900), 285 (ε 36 400) λ_{min} 249 (ε values calc on $C_{39}H_{50}O_{12}$)
Protokosin§ colourless needles	182°	—	—	—	$[\alpha]_D$ + 8.0° (*c* 10%, $CHCl_3$)	λ_{max} 223 (ε 25 860), 287 (ε 19 840) λ_{min} 253 (Solvent not given) (ε values calc on $C_{25}H_{32}O_8$)

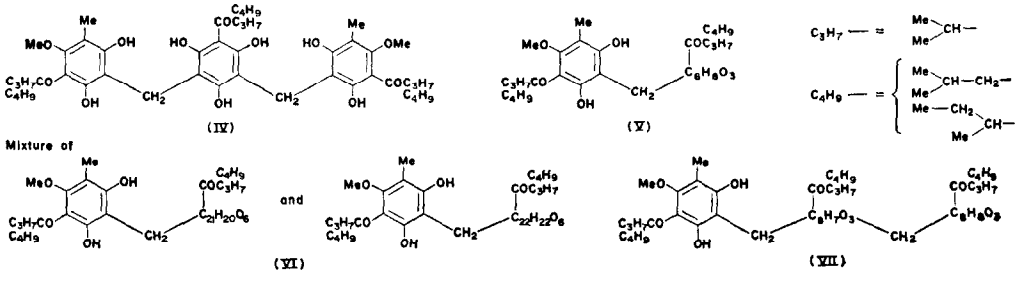
* *n*-Hexane–$CHCl_3$ (1:1)

† *n*-Hexane–$CHCl_3$–EtOH (19:19:2)

‡ The sample showed a very weak positive rotation. Due to the small amount of K1 available for the measurement, no exact value could be obtained. The 2-methylbutyryl group, which is one of the side chains present, has an asymmetric center and this should cause some rotation.

§ Taken from Ref. 7.

Sample 2 material was repeatedly crystallized from methanol to yield crystalline K4 slightly contaminated by K3. By TLC, K1 and K2 were detected in the original sample 2. In both samples, K2 was the main compound. Yields of all compounds were very low and although occurring in large amounts in both samples, K2 was only obtainable pure in small amounts. For TLC detection, the procedure used for *Dryopteris* phloroglucinols proved suitable [10,11]. When investigated by gradient technique on thin-layers buffered to pH 7–8 (see Ref. 13) tailing of the kosins was observed on the alkaline side of the plates. M.ps and TLC data of the isolated kosins are presented in Table 2. For the four kosins isolated, the structure (IV) (K1) and partial structures (V), (VI) and (VII) (K2–4, respectively) are proposed. Due to the very small amounts of isolated pure kosins, the analytical data were insufficient for elucidating all structural details.

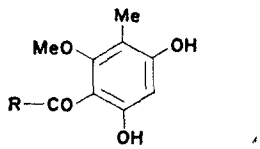


[13] HAAPALAINEN, L. and WIDÉN, C.-J. (1970) *Farm. Aikak.* **79**, 161.

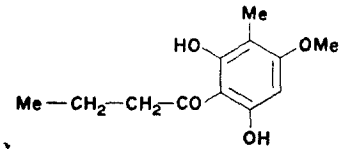
Structure Determinations

The acyl side chains of the four kosins were cleaved by strong alkali and the acids formed were identified by GLC Isobutyric acid was the main component, accompanied by isovaleric acid and 2-methylbutyric acid In previous investigations, only isobutyric acid was found The methylene bridges between the different ring systems were cleaved[14] and the products were compared by TLC and PC to synthetic standards (see Ref 13) From K1-4 one major component R_f 0 40, which formed a brown complex with fast blue salt-B, was detected on thin-layers buffered to pH 6 0 It had very similar R_fs to pseudo-aspidinol-B (VIIIa) On papers buffered to pH 8 6, however, it separated in two components, R_fs 0 30 and 0 38, with the same brown color after spraying with fast blue salt-B, the former being VIIIa has R_f 0 30 in this second system Thus, the alkaline cleavage products are mixtures of closely related homologous pseudo-aspidinols (acyl-3-methylphloroglucinol-2-methyl ethers) slightly differing from pseudo-aspidinol B (VIIIa) in their acyl side-chains [15]

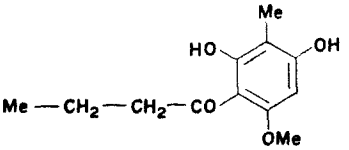
The composition of the acids obtained from the kosins indicates that it could be a mixture of the pseudo-aspidinols iB (VIIIb), iV (VIIIc), and 2-MeB (VIIId) It is noteworthy that the hop resins humulone and lupulone, which are phloroglucinol derivatives with an isovaleryl side chain, are accompanied by isobutyryl and 2-methylbutyryl analogues [16] No sign of the other isomeric aspidinols were found among the decomposition products of the isolated kosins



(VIIIa) R = Me—CH_2—CH_2
(VIIIb) R = $(Me)_2$CH
(VIIIc) R = $(Me)_2$CH—CH_2
(VIIId) R = Me—CH_2—CH(Me)



(IX)



(X)

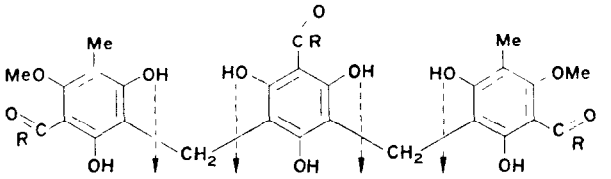
The existence of pseudo-aspidinol iB (VIIIb) (isobutyryl-3-methylphloroglucinol-2-methyl ether) unit in the molecule of protokosin has been previously postulated by Orth and Riedl [9] The phloroglucinols obtained after alkaline cleavage of K1 (IV) are shown in Scheme 1 In addition to pseudoaspidinol iB (VIIIb) (iV (VIIIc), 2-MeB (VIIId)) two more break-down products were identified as phloroglucinol iB (XIa) (iV (XIb), 2-MeB (XIc))

[14] WIDÉN, C-J, VON EUW, J and REICHSTEIN, T (1970) *Helv Chim Acta* **53**, 2176

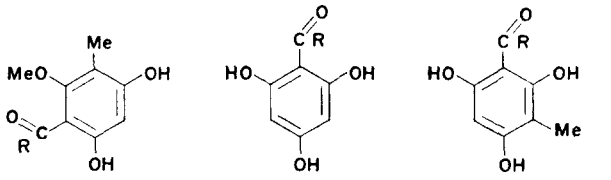
[15] The three different methylbutyrylphloroglucinol-monomethyl-ethers, pseudo-aspidinol-B (VIIIa) aspidinol-B (IX) and isoaspidinol-B (X) have been prepared by synthesis [13] On paper chromatograms at pH 8 6 they separate and give different colors with fast blue salt-B On thin-layers buffered to pH 6 0 the separation is less good However, even in the latter case the different aspidinols can be recognized by their characteristic colours with with above reagent

[16] ASHURTS, P R (1967) in *Fortschritte der Chemie organischer Naturstoffe* (ZECHMEISTER, L, ed), Vol XXV, p 65, Springer, Wien

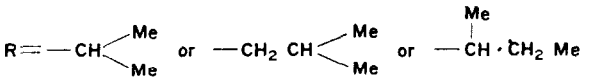
and methylphloroglucinol iB (XIIa) (iV (XIIb), 2-MeB (XIIc)) through comparison with authentic samples [17] These findings were also in agreement with those from MS (see below).



(IV)

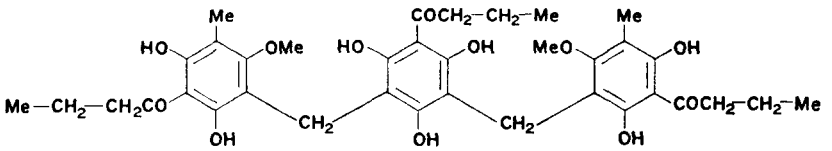


(VIII) b, c, d (XI) a, b, c (XII) a, b, c



SCHEME 1 ALKALINE CLEAVAGE OF K1

The structure of K1 (IV) is very similar to that proposed for protokosin (III) by Orth and Riedl [9] However, it contains one methoxyl group less From trisaspidinol-BBB (XIII), a compound recently isolated from *Dryopteris inaequalis* (Schlecht) O Kunze,[18] K1 differs only in the position of the methoxyl groups and in the substitution of the side chains Therefore, we propose the name trispseudo-aspidinol for K1 (IV) (mainly consisting of trispseudo-aspidinol iBiBiB)



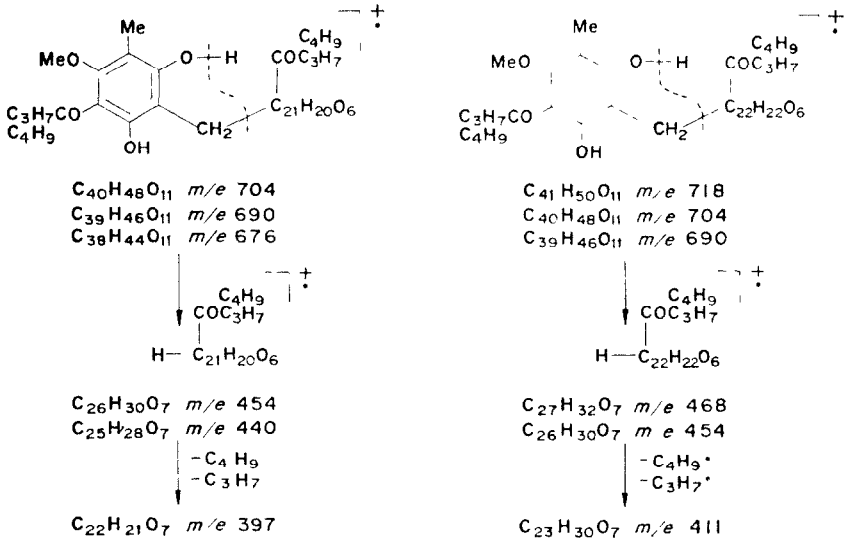
(XIII)

The MS of K1 presents four 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 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 and confirm that K1 is a mixture of side chain homologues The general

[17] All these compounds have been prepared by synthesis (unpubl) The different acylphloroglucinols give with fast blue salt-B one blue spot, R_f 0 20, which readily separates from that one of the acyl-3-methylphloroglucinols, R_f 0 16 The colour of the latter spot is first blue, then changing to brown (see Ref 13) The individual acylphloroglucinol homologues and acyl-3-methylphloroglucinol homologues, however, do not separate from each other with the methods used

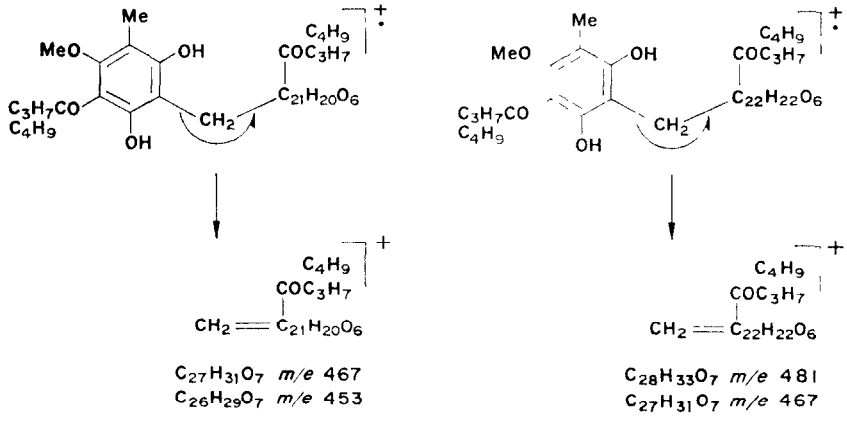
[18] WIDÉN, C -J , FADEN, R , LOUNASMAA, M , VIDA, G , VON EUW, J and REICHSTEIN, T (1973) *Helv Chim. Acta* **56**, in press

fragmentation of K1 is analogous to that found earlier in connection of *Dryopteris* phloroglucinols[19,20] and supports the proposed structure (IV)



SCHEME 2 DETAILS OF THE MS FRAGMENTATION OF K3

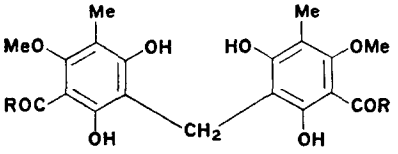
The MS of K2 presents three molecular peaks at *m/e* 488, 474, and 460 corresponding to $C_{27}H_{36}O_8$, $C_{26}H_{34}O_8$ and $C_{25}H_{32}O_8$, respectively The peaks at *m/e* 431 and 417 support the assumption that K2 is a mixture of side chain homologues and confirms the results of the alkaline cleavages In this case, too, the general fragmentation pattern is similar to that found for polycyclic *Dryopteris* phloroglucinols[19 20] in agreement with the proposed partial structure (V)



SCHEME 3 DETAILS OF THE MS FRAGMENTATION OF K3

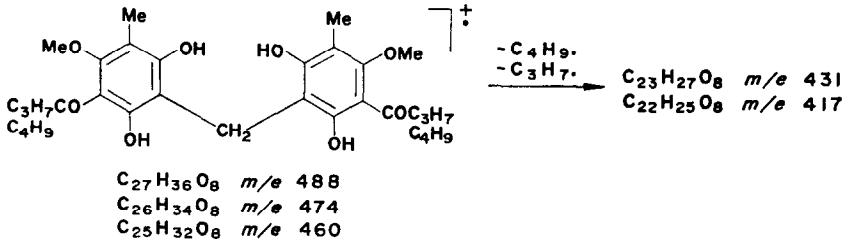
[19] LOUNASMAA, M , KARJALAINEN, A , WIDÉN, C -J and HUHTIKANGAS, A (1972) *Acta Chem Scand* **26**, 89
[20] LOUNASMAA, M , WIDÉN, C -J and REICHSTEIN, T (1971) *Helv Chim Acta* **54**, 2850

In the MS of K3 are four molecular peaks at *m/e* 718, 704, 690 and 676 corresponding to $C_{41}H_{50}O_{11}$, $C_{40}H_{48}O_{11}$, $C_{39}H_{46}O_{11}$ and $C_{38}H_{44}O_{11}$, respectively. Weak peaks at *m/e* 738 (very weak), 724, 710 and 696 indicate that the sample contains a little K4 homologues (see below) as impurities. The peaks at *m/e* 661, 647 and 633 are in good agreement with the assumption that K3 is also as mixture of C_4H_9 -and C_3H_7-side chain homologues, as indicated by the alkaline cleavages The general fragmentation pattern, of which some details are presented in Schemes 2 and 3, is similar to that of *Dryopteris* phloroglucinols and supports the proposed partial structure (VI)



(XIVa) R = $(Me)_2CH$
(XIVb) R = Me—CH_2—CH_2

The thermal rottlerone change, found earlier in connection with polycyclic *Dryopteris* phloroglucinols[20] 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 the kosins In the case of K3, this phenomenon is very evident and among the compounds formed by this procedure, probably the most characteristic ones are the α-kosin (pseudo-aspidin) homologues [21] These are responsible, e g for the formation of the ions of *m/e* 488, 474, 460, 431 and 417, as indicated below (Scheme 4)



SCHEME 4 FRAGMENTATION OF α-KOSIN HOMOLOGUES

The MS of K4 presents four 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 Peaks at *m/e* 718 (very weak), 704, 690 and 676 indicate that the sample contained some K3 homologues (see above) as impurities. The peaks at *m/e* 681, 667 and 653, 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 and confirm that K4 is a mixture of side chain homologues The general fragmentation pattern of K4, analogous to that of polycyclic *Dryopteris* phloroglucinols, supports the proposed partial structure (VII)

[21] α-Kosin iBiB (XIVa) has been synthesized by Orth and Riedl [9] It proved to be identical with α-kosin obtained by alkaline treatment of protokosin (see Ref 6) The corresponding *n*-butyryl derivative is called pseudo-aspidin-BB (XIVb)

The optical activities of K2–4 (Table 2), indicate that they are asymmetric, in contrast to the known *Dryopteris* phloroglucinols On the other hand K1 shows no optical activity in agreement with the proposed structure (IV) The NMR spectrum (240 MHz) ($CDCl_3$, internal standard TMS) of K1 supports the structure (IV) showing the following signals δ 1 16 (18 H, *m*, mainly three $-CO-CH(C\underline{H}_3)_2$ groups), δ 2 10 (6 H, *s*, two $C\underline{H}_3$-Ar groups), about δ 3 0 (3 H, *m*, mainly three $-CO-C\underline{H}(CH_3)_2$ groups), δ 3 70 (6 H, *s*, two $C\underline{H}_3O$-Ar groups) and δ 3 80 (4 H, *s*, two $\geqslant C-CH_2-C\leqslant$ groups) Due to the fact that K1 is a mixture of *i*-butyryl, *i*-valeryl and 2-methyl-butyryl side chain homologues, the intensities given for acyl side chain proton signals, have to be considered as approximate The OH-groups signals are omitted Moreover, the spectrum shows multiplets at about δ 0 90 (about 6 H) due to γ-protons in the *i*-valeryl and 2-methyl-butyryl side chains and between δ 1 6 and δ 1 9 (about 4 H) due to β-protons in the *i*-valeryl side chains and secondary β-protons in the 2-methylbutyryl side chains

Appropriate signals in the NMR spectra (60 MHz) ($CDCl_3$, internal standard TMS) of K2–4 at about δ 3 7 and δ 2 2 are in agreement with the presence of 'aromatic' MeO- and Me-groups, as indicated by the partial structures (V), (VI) and (VII) The small amounts of pure kosins available and the fact that the compounds were mixtures of acyl side chain homologues hampered the quantitative interpretation of the spectra

Comparison with Earlier Investigations

Protokosin, for which the melting points 176° and 182° have been given,[3,5,6] is apparently identical with either K3 (m p 177–178°) or K4 (m p 174–176°) or a mixture This identity is supported by the IR spectra, as well as by the crystal form (see Table 2 and Refs 3 and 5) Even the optical activities and the UV spectra (Table 2), although less specific, are relatively similar However, due to some differences in the UV spectra of K3 and protokosin, it seems that the protokosin of Birch and Todd[7] consisted mainly of K4

Lobeck[5] earlier mentioned the possible existence of anhydroprotokosin K3, which is very difficult to separate from K4, may theoretically arise from K4 by the loss of one molecule of water (probably forming an ether linkage between two rings) and two H-units (probably by a cyclization) However, the molecular formula ($C_{58}H_{74}O_{17}$), tentatively proposed by Lobeck[5] for anhydroprotokosin, is very different from that of K3 (see above)

Kosotoxin, which is anthelmintically active, is probably identical with K2 It has been isolated only in amorphous form by the earlier workers [3–6] The MW 476 given by Kondakow and Schatz[4] for their kosotoxin is in excellent agreement with the MW of K2 (see above) found by MS

Kosidin, for which the m p 178° has been given,[4] might be identical with K1 although the m p found for K1 is a little lower (167–169°) The identity is supported by similarities in the crystalline form If the identity of K1 with kosidin is accepted, the low concentration of K1 in kousso flowers might explain the reason why Hems and Todd[6] could not find it in their material

EXPERIMENTAL

Collection data for sample 1 *of* Hagenia abyssinica Flora of Kenya, K_3, Nyandara District, Aberdele Mts, Kimakia Forest Station, Alt 2460 m Tree cultivated at the forest station—very common in the surrounding forest Collection date 21 Sept, 1971 Coll R B Faden, C H S Kabuye and P S Green 71/852 This sample consisted of the entire panicles

Origin of the sample 2 *of* Hagenia abyssinica Sample 2 consisted of an old sample of separated female flowers from the drug collection of the department of Pharmacognosy, University of Helsinki, Finland

Preparation of the ether extracts The female flowers, stems and leaves were macerated separately four times at 20° with peroxide free—Et_2O After removal of the Et_2O, dark green oily extracts remained

Preparation of the crude Mg- *and* Ba-*kosins* The preparation of crude Mg- and Ba-kosins were made according to the previously described methods [10,11]

Column chromatography of crude Mg-*kosins from sample* 1 7 5 g of crude kosin were suspended in C_6H_6 and chromatographed on 187 g of silica gel (Merck, particle size 0 05–0 2 mm) as previously described [10,11] The fractions 1–50 (10 ml each) (C_6H_6) gave after cryst from MeOH 3 9 mg K1, m p 167–169° The fractions 51–355 (C_6H_6–$CHCl_3$, 1 1) gave when cryst from MeOH 6 9 mg of K2, m p 110–112°, as well as 46 1 mg of a pale yellow oil, which according to TLC consisted of fairly pure K2 Fractions 356–532 ($CHCl_3$–EtOH, 97 3) gave after several recryst from MeOH 15 1 mg chromatographically pure K3 (see theoretical part), m p 177–178°

Isolation of kosins from crude Mg-*kosins of sample* 2 The crude kosins, 1 3 g, were repeatedly cryst from MeOH to give 17 1 mg of chromatographically pure K4 (see theoretical part), m p 174–176°

Cleavage of the acyl side chains in the kosins 50 mg crude kosin, 100 mg Zn-powder and 20 ml 15% NaOH were mixed and heated for 24 hr at 100° After acidification, the organic acids formed were taken into Et_2O and then analysed by GLC

Cleavage of the methylene bridges of the isolated kosins This was performed by heating the kosins 5 min on H_2O bath in 5% NaOH as described for trispara-aspidin [14]

GLC of the organic acids The organic acids were identified by GLC using authentic acids as standards The free acids were studied using a 2-m steel column, i d 0 32 cm with 5% FFAP on Chromosorb AW HMDS 80–100 mesh, in a Perkin–Elmer F11 gas chromatograph fitted with FID N_2 was used as carrier gas (32 ml/min) Injection block temp 270°, oven temp programmed from 70 to 180° with 2°/min Due to overlapping of peaks of free 2-methylbutyric acid and isovaleric acid, the corresponding butyl esters were prepared by the method of Jones and Davidson [22] These esters were compared with synthetic samples by GLC using a 50-m capillary column coated with FFAP Injection block temp 250°, oven temp programmed from 60 to 180° with 2°/min Carrier gas N_2 (4 ml/min)

MS The MS of kosins were recorded on an A E I MS-9 double-focusing mass spectrometer (70 eV) (source temps utilized for K1–4 were 200°, 150°, 260° and 220°, respect) at the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, through the courtesy of Dr B C Das

Optical rotations The optical rotations of kosins were measured on a Perkin–Elmer 141 Polarimeter at the Institut fur Organische Chemie, Universitat Basel, Switzerland, through the courtesy of Prof T Reichstein and Dr J von Euw

NMR The NMR spectrum ($CDCl_3$) of K1 was recorded on an I E F 240 B spectrometer (240 MHz) at the Institut d'Electronique Fondamentale, Faculté des Sciences d'Orsay, France, through the courtesy of Dr S K Kan

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[22] JONES, E P and DAVIDON, V L (1965) *J Am Oil Chemists' Soc* **42**, 121