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A COUMARIN GLUCOSIDE FROM *XEROMPHIS OBOVATA**

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Key Word Index—*Xeromphis obovata*; Rubiaceae; coumarins; iridoids; xeroboside.

Abstract—From root bark of *Xeromphis obovata* three coumarins have been isolated: scopoletin, its 7-β-D-glucopyranoside (scopolin) and the new β-D-apiosyl-(1″→6′)-β-D-glucopyranoside of scopoletin, named xeroboside. Also two iridoids, deacetylasperulosidic acid methyl ester and gardenoside, have been isolated and identified as the corresponding acetyl derivatives.

INTRODUCTION

Xeromphis obovata (Hochst) Keay [2] is an arboreal plant of central and southern Africa utilized in traditional medicine. A decoction of the powdered root is applied directly on melanomas while the infusion is administered by mouth as an emetic and to relieve fever, nausea, general coughs, toothache, pains during pregnancy, dizziness, menorrhagia and depressed fontanelle. The pain relief is common to another *Xeromphis* species, *X. spinosa* (Thumb.) Keay [3]. Glucosides of oleanolic acid [4, 5] have been isolated from its pulp and leaves which are used as a piscicide [3].

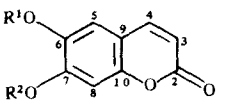
RESULTS AND DISCUSSION

CCD of the methanolic extract of root bark of *X. obovata* from Zimbabwe (vernacular name, ingwaqela) gave scopoletin **1**, scopolin (7-β-D-glucopyranoside of scopoletin) (**2**) [6], a new diglucocoumarin, named xeroboside (**3**), and a mixture of two iridoids. The latter was easily resolved through the corresponding acetyl derivatives upon previous verification by NMR of the absence of any acetyl groups. The three acetyl iridoids obtained were identified as deacetylasperulosidic acid methyl ester hexaacetate [7], gardenoside hexaacetate and pentaacetate [8].

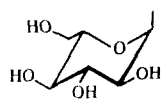
Xeroboside, **3**, has the molecular formula $C_{21}H_{26}O_{13}$. Its UV spectrum is similar to that of scopoletin [λ_{max}^{EtOH} nm (log ε): 338 (3.93), 287(3.75), 258 and 248 sh, 226 (4.22)]with a slight hypochromic effect. The ^{1}H NMR spectrum (DMSO-d_6) confirmed the presence of the scopoletin moiety and showed the presence of two anomeric protons (δ5.10, *d*, $J = 6$ Hz, and 4.80, *d*, $J = 2$ Hz) instead of one as in scopolin (δ5.10). On enzymatic hydrolysis with β-glucosidase, xeroboside gave glucose and apiose besides scopoletin. Both positive and negative ion FAB-mass spectra confirmed the M_r ($[M]^+ = m/z$ 486) and showed the loss of a pentose unit (132 mu) followed by a hexose (162 mu).

In the ^{13}C NMR spectra scopolin and xeroboside the resonances of the glucose unit are practically identical except for that of C-6′ which is at δ67.5 in the latter and δ60.7 in the former. This downfield shift for C-6 in **3** can be related to the attachment of the apiose unit. The downfield shifted C-1″ of apiose (δ109.4) confirms the β configuration of the anomeric linkage [9] cleaved by β-glucosidase albeit with difficulty.

The ^{1}H NMR spectrum of the pentaacetylxeroboside **4** confirms the 1″→6′ linkage of the apiose to the glucose unit. In fact whereas the resonances of H-2′, H-3′ and H-4′ of the glucose are shifted downfield by acetylation, the resonances of H_2-6′ remain upfield (δ3.70 and 3.84, AB part of the ABX system, X part at δ3.55, H-5′) as well as that of the AB system of the methylene group, H_2-4″, of apiose (δ3.82 and 3.98, $J_{gem} = 11$ Hz). The other two AB systems of apiose are at δ4.22 and 4.34 ($J_{gem} = 11.5$ Hz, H_2-5″) and at δ4.93 and 4.98 ($J = 2$ Hz, H-1″ and H-2″).



	R^1	R^2	R^3	R^4
1	Me	H	–	–
2	Me	Gl	–	–
3	Me	Ap-Gl	H	H
4	Me	Ap-Gl	Ac	H
5	Me	Ap-Gl	Ac	Ac
6	H	Ap-Gl	H	H



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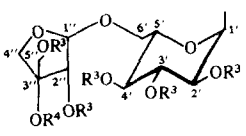

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*Part 15 in the series 'Research on African Medicinal Plants'. For Part 14 see ref. [1].

Evidence of the tertiary hydroxy group of apiose in xeroboside (3) was obtained through the hexaacetyl derivative (5) which it gave together with the pentaacetate, 4, by acetylation with pyridine and acetic anhydride. This compound, besides showing the presence of an additional acetyl group, detectable in the ^1H and ^{13}C NMR spectra (see Experimental and Table 1) reveals the remarkable downfield shift of C-3″ (δ83.8 in 5 and 78.4 in 4). The structure of 6′-(β-D-apiosyl)-β-D-glucopyranosylscopoletin is thus unequivocally assigned to xeroboside (3).

The only coumarins containing apiose hitherto isolated are lariside, 2′-(β-D-apiosyl)-β-D-glucopyranosylscopoletin from *Salsola laricifolia* (Chenopodiaceae) [10], diospyroside, 6′-(β-D-apiosyl)-β-D-glucopyranosylaesculetin (6) from *Diospyros sapota* (Ebenaceae) [11], 6′-(β-D-apiosyl)-β-D-glucopyranosylcolumbianetin from *Lomatium dissectum* (Umbelliferae) [9] and adicardin, 7-apioglucoside of umbelliferone from *Adina cordifolia* (Rubiaceae) [12]. The co-occurrence of the two iridoids deacetylasperulosidic acid methyl ester and gardenoside has been reported in another Rubiacea, *Randia canthioides* [8].

EXPERIMENTAL

^1H and ^{13}C NMR spectra: TMS as int. ref. Separations were performed by CCD with a Craig Post apparatus (200 stages, 10 ml/10 ml, upper and lower phase).

Plant material. Root bark of *X. obovata* was collected from Luke McIlwaine Game Park near Harare (Zimbabwe). A voucher specimen is deposited with the National Herbarium.

Extraction and separation. Powdered dry bark (150 g) was extracted with MeOH and the dry extract (10.5 g) was submitted in two portions to CCD using the bi-phasic system H_2O–EtOH–EtOAc–cyclohexane (10:4:7:7). A pure substance (160 mg) (K_r 1.0) obtained in this step was identified as scopoletin (1) by direct comparison with an authentic sample. The more polar fraction ($K_r < 0.4$) was a complex mixture from which a pure compound (230 mg, K_r 3.0) identified as scopolin (2) was isolated by use of the solvent system H_2O–*n*-BuOH–EtOAc (10:7:3). The remaining mixture was partially resolved by CCD on recycling (1700 transfers) using H_2O–*n*-BuOH–EtOAc (10:9:1) and pure xeroboside (3, 170 mg, K_r 0.24) was thus obtained. The final mixture still containing xeroboside and two iridoids was acetylated overnight with pyridine and Ac_2O and after evaporation of the reagents under *vacuum* the acetyl derivatives obtained were easily resolved by CCD (430 transfers) with the mixture H_2O–EtOH–EtOAc–cyclohexane (8:4:3:9). The following compounds were obtained in the order: hexaacetylgardenoside (56 mg, K_r 4.3), hexaacetyl derivative of deacetyl asperulosidic acid methyl ester (48 mg, K_r 2.2), hexaacetylxeroboside (5, 41, mg, K_r 0.52), pentaacetylgardenoside (36 mg, K_r 0.34) and finally pentaacetylxeroboside (4, 108 mg, K_r 0.1).

Scopolin (2). Mp 202–204° from EtOAc, $[\alpha]_D^{20}$ −60 (MeOH; *c* 0.5); ^1H NMR (DMSO-d_6): δ3.1-3.8 (H-2′–H_2-6′), 3.80 (*s*, OMe), 5.10 (*d*, $J = 6$ Hz, H-1′), 6.32 (*d*, $J = 9.5$ Hz, H-3), 7.20 (*s*, H-8), 7.32 (*s*, H-5), 7.97 (*d*, H-4).

Table 1. ^{13}C NMR spectral data for compounds 1–5*

C	1	2	3	4	5
2	160.2	160.5	160.6	160.7	160.6
3	112.5	112.3	112.4	115.1	115.0
4	142.3	144.2	144.2	142.8	142.7
5	107.0	113.3	113.4	109.8	109.7
6	143.2	146.0	146.0	147.3	147.3
7	149.2†	149.8†	149.8†	149.1	148.9
8	102.5	103.1	103.2	107.1	105.8
9	110.5	109.7	109.8	114.0	114.1
10	149.8†	148.9†	149.0†	149.5	149.2
OMe	55.2	56.0	56.1	56.7	56.5
1′		99.6	99.6	99.6	99.5
2′		73.1	73.4	70.9	70.9
3′		77.1	76.7	72.3	72.3
4′		69.6	69.5	68.6	68.5
5′		76.7	76.1‡	73.5	72.4
6′		60.7	67.5	66.7†	66.1
1″			109.4	106.4	106.6
2″			75.5‡	74.2	73.1
3″			78.8	78.4	83.8
4″			73.1	75.6	75.7
5″			63.6	67.1†	62.8
Me-CO				20.2; 20.5	20.5; 21.0
				20.6	
Me-CO				169.1; 169.3	168.9; 169.0
				169.7; 170.1	169.2; 169.5
				171.2	170.0; 170.4

*1, 4 and 5 in $CDCl_3$; 2 and 3 in DMSO-d_6.

† and ‡ These values may be interchanged in the same column.

Xeroboside (**3**). Crystals from EtOH and EtOAc, mp 195–197°, $[\alpha]_D^{20}$ −179 (EtOH; *c* 0.6), FAB-MS: *m/z* 486 $[M]^+$, 345, 292; UV λ_{max}^{EtOH} nm (log ε): 338 (3.93), 287 (3.75), 258 and 248 sh, 226 (4.22); ^{1}H NMR (DMSO-d_6): δ3.81 (*s*, OMe), 4.80 (*d*, *J* = 2 Hz, H-1″), 5.10 (*d*, *J* = 6 Hz, H-1′), 6.30 (*d*, *J* = 9.5 Hz, H-3), 7.14 (*s*, H-8), 7.29 (*s*, H-5), 7.94 (*d*, H-4). (Found: C, 52.01; H, 5.17. $C_{21}H_{26}O_{13}$ requires: C, 51.85; H, 5.39%).

Hydrolysis of xeroboside. β-Glucosidase (5 mg) was added to a soln of xeroboside (20 mg) in H_2O (10 ml) and acetate buffer at pH 5.5 (10 ml). The mixture was covered with toluene and kept at 36° for 7 days. The same amount of enzyme was added on the third and fifth days. At the end the turbid soln was extracted with EtOAc and from the organic phase, using CCD with H_2O–EtOH–EtOAc–cyclohexane (10:4:7:7), scopoletin (**1**) was isolated and identified by direct comparison. In the aq. phase, after extraction with *n*-BuOH and percolation through a column of Dowex 50 W(H^+), apiose and glucose were identified by TLC (Kieselgel 60 F_{254}, H_2O–MeOH–HOAc–ethylene chloride 2:3:5:10).

Pentaacetylxeroboside (**4**). Mp 201–203° from *n*-hexane. ^{1}H NMR ($CDCl_3$): δ2.0–2.1 (5 *s*, 5 Ac), 2.45 (*bs*, exchangeable with D_2O, HO-3″), 3.55 (*br*, *dd*, *J* = 5.5 and 11 Hz, H-5′), 3.70–3.84 (*m*, partially overlapped, H_2-6′), 3.79 (*s*, OMe), 3.82 and 3.98 (2 *d*, J_{gem} = 11 Hz, H_2-4″), 4.22 and 4.34 (2 *d*, J_{gem} = 11.5 Hz, H_2-5″), 4.93 (*d*, *J* = 2 Hz, H-1″), 4.98 (*d*, *J* = 2 Hz, H-2″), 5.1–5.3 (H-1′–H-4′), 6.25 (*d*, *J* = 9.5 Hz, H-3), 6.82 (*s*, H-5), 7.10 (*s*, H-8), 7.55 (*d*, H-4).

Hexaacetylxeroboside (**5**). Mp 77–79° from *n*-hexane; $[\alpha]_D^{20}$ −54.2 (MeOH; *c* 0.4); ^{1}H NMR ($CDCl_3$): δ1.9–2.1 (6*s*, 6 Ac), 3.60 (*br dd*, *J* = 5.5 and 11 Hz, H-5′), 3.75–3.90 (*m*, partially overlapped, H_2-6′), 3.81 (*s*, OMe), 4.08 and 4.13 (2*d*, J_{gem} = 11 Hz, H_2-4″), 4.45 and 4.70 (2*d*, J_{gem} = 11.5, H_2-5″), 4.90 and 5.25 (2*d*, *J* = 2 Hz, H-1″ and H-2″, respectively), 5.05–5.30 (H-1′–H-4′), 6.28 (*d*, *J* = 9.5 Hz, H-3), 6.86 (*s*, H-5), 7.05 (*s*, H-8), 7.58 (*d*, H-4).

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