[Chem. Pharm. Bull. 33(1) 396—399 (1985)]

Coumarin and Secoiridoid Glucosides from Bark of *Olea africana* and *Olea capensis*

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(Received September 1, 1984)

Coumarin glucosides, esculin (**1**) and scopolin (**2**), were isolated from the bark of *Olea africana* MILL. (*O. europaea* L. subsp. *africana* (MILL.) GREEN) while isoscopoletin-β-D-glucoside (magnolioside) (**3**) was isolated from the bark of *Olea capensis* L. The secoiridoid glucoside oleuropein (**4**) was also isolated from both species.

Keywords——*Olea africana*; *Olea capensis*; Oleaceae; coumarin glucoside; esculin; scopolin; isoscopoletin-β-D-glucoside; secoiridoid glucoside; oleuropein; ^{13}C-NMR spectra

Olea africana MILL. (Oleaceae), known as the wild olive, is widely distributed in Southern Africa, whereas *Olea capensis* L., known as the ironwood, is only distributed in a restricted area of South Africa.[1,2] *O. africana* has recently been reclassified as a subspecies of *O. europaea* and is now known as *O. europaea* L. subsp. *africana* (MILL.) GREEN.[3] The bark of *O. africana* has been used as an antifebrile and an anti-rheumatic agent, and as a tonic in Southern Africa,[4] and the bark of *O. europaea* has been used similarly in Europe.[5] In previous papers,[6-8] we reported the isolation of lignans and coumarins from these barks. The results suggested a difference in the distribution pattern of phenolic compounds between these two species.

As a continuation of our studies on the constituents of *Olea* bark, this paper describes the isolation of three additional coumarin glucosides, esculin (**1**) and scopolin (**2**) from *O. africana* and isoscopoletin-β-D-glucoside (magnolioside) (**3**) from *O. capensis*, as well as a secoiridoid glucoside, oleuropein (**4**), from both species.

The extraction and separation were carried out as described in Experimental.

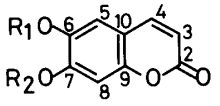

Chart 1

Glucoside **1**, a colorless crystalline powder, $C_{15}H_{16}O_9 \cdot 1/2H_2O$, mp 152—154 °C, showing pale blue fluorescence in ethanol solution, produced esculetin and D-glucose on acid hydrolysis. The infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data of **1** were in good agreement with those of authentic esculin from *O. europaea* L.[9]

Glucoside **2** was recrystallized from ethanol to give colorless needles, $C_{16}H_{18}O_9 \cdot H_2O$, mp 224—226 °C. The IR, ^{1}H-NMR and ^{13}C-NMR spectral data of **2** suggested that **2** is a coumarin glucoside. The acid hydrolysis of **2** gave scopoletin and D-glucose. Thus, glucoside **2** was identified as scopolin.

Glucoside **3** was recrystallized from ethanol to give colorless needles, $C_{16}H_{18}O_9 \cdot 2H_2O$,

Table I. ^{13}C-NMR Chemical Shifts[a]

	3	Isoscopoletin	$\Delta\delta$	1[b]	Esculetin[b]	$\Delta\delta$	2	Scopoletin	$\Delta\delta$
C-2	160.4	160.7	−0.3	160.7	160.8	−0.1	160.4	160.5	−0.1
C-3	112.7	112.5	+0.2	112.1	111.5	+0.6	113.2	111.6	+1.6
C-4	144.3	144.1	+0.2	144.5	144.3	+0.2	144.0	144.2	−0.2
C-5	113.1	111.9	+1.2	114.7	112.3	+2.4	109.7	109.5	+0.2
C-6	143.3	143.6	−0.3	142.7	142.9	−0.2	145.9	145.2	+0.7
C-7	152.8	151.8	+1.0	151.4	150.3	+1.1	149.9	151.1	−1.2
C-8	100.3	99.9	+0.4	103.2	102.7	+0.5	103.0	102.7	+0.3
C-9	150.0	148.4	+1.6	150.5	148.5	+2.0	148.9	149.6	−0.7
C-10	111.2	111.5	−0.3	110.9	110.8	+0.1	112.2	110.5	+1.7
OCH_3	56.2	56.0					56.0	55.9	
glc-1	100.3			102.3			99.6		
glc-2	73.1			73.4			73.0		
glc-3	77.0			77.3			77.0		
glc-4	69.6			69.8			69.6		
glc-5	76.8			76.1			76.7		
glc-6	60.6			60.8			60.6		

a) The spectra were taken in micro cells with a JNM-FX 60 spectrometer (15.00 MHz) in DMSO-d_6 with TMS as an internal reference.
b) The same assignments were reported in the literature.[10]

mp 233—234 °C. The IR and ^{1}H-NMR spectral data of **3** suggested that **3** bears a marked structural resemblance to **2**. The ^{13}C-NMR spectrum of **3** was correlated with those of **1**, **2** and their aglycones. Table I presents the ^{13}C-NMR data and assignments. These data suggested that **3** is a glucoside of isoscopoletin. Both acid and enzymatic hydrolysis of **3** gave isoscopoletin and D-glucose. Consequently, the structure of **3** has been established as isoscopoletin-β-D-glucoside. This glucoside is already known as magnolioside from *Magnolia macrophylla* MICHX. (Magnoliaceae).[11]

Glucoside **4** was identified by direct comparison with authentic oleuropein from *O. europaea*;[9] it may have a common distribution in *Olea* species.

Glucosides **2** and **3** have been isolated for the first time from Oleaceae plants, though the occurrence of **1** is well known.[12] The distribution pattern of coumarins in *O. capensis*, as well as that of lignans, is clearly distinct from that in the type species, *O. europaea*. In addition, it is noteworthy from the medicinal viewpoint that bark of both *O. africana* and *O. europaea* commonly contains esculetin and esculin since these coumarins are known to be active principles[13] in the oriental medicine "shinpi (秦皮)" [the bark of *Fraxinus japonica* BLUME (Oleaceae)], which has been used since ancient times as an antifebrile and an anti-rheumatic agent in Japan.[14]

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The following instruments were used: optical rotation, Yanaco OR-50D; ultraviolet (UV) spectra, Shimadzu UV-210; IR spectra, Hitachi 270-30; ^{1}H-NMR spectra, Hitachi R-40 with tetramethylsilane (TMS) ($\delta = 0$) as an internal reference; ^{13}C-NMR spectra, JEOL JNM-FX 60, equipped with a JEC-980 computer. The abbreviations used are as follows: s, singlet; d, doublet; sh, shoulder.

Precoated thin-layer chromatography (TLC) plates, Silica gel $60F_{254}$ (Merck), were used for TLC. The spots were detected by spraying the plates with 10% H_2SO_4 soln. and heating. Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

Isolation——Dry powdered bark (1.0 kg) of *O. africana* collected in October 1982 at Bloemfontein, Republic of

South Africa, was extracted four times with hot MeOH. The MeOH solution was evaporated to a small volume under reduced pressure, diluted with water and filtered. The filtrate was extracted successively with ether, $CHCl_3$ and BuOH. The $CHCl_3$ extract (2.4 g) yielded esculetin and scopoletin, as did the ether extract.[6] The BuOH extract (33.4 g) was chromatographed on a silica gel column with a $CHCl_3$–EtOH gradient. The fractions were monitored by TLC developed with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, under layer). The fractions showing TLC spots at *Rf* 0.45, 0.59 and 0.60 gave 132.4 mg of **1**, 180.3 mg of **2** and 7.7 g of **4**, respectively.

Dry powdered bark (110 g) of *O. capensis* collected in November 1982 at Cape Town, Republic of South Africa, was treated in the same manner as described for *O. africana*. The $CHCl_3$ extract (0.3 g) gave isoscopoletin and scoparone, as did the ether extract.[6] The BuOH extract (20.4 g) gave 148.7 mg of **3** and 3.31 g of **4**.

Esculin (1)——Colorless crystalline powder, mp 152—154 °C. $[\alpha]_D^{22}$ −88.5° (*c* = 0.33 in MeOH). UV λ_{max}^{EtOH} nm (log ε): 224 (4.12), 250 (3.67), 298 (3.79), 336 (4.07). IR ν_{max}^{KBr} cm^{-1}: 3100—3550 (OH), 1700 (CO), 1680 (C=C), 1610, 1570 (arom. C=C). ^{1}H-NMR (in CD_3OD + DMSO-d_6) δ: 6.27 (1H, d, *J* = 10 Hz, C_3-H), 6.87, 7.47 (2H, each s, $C_{5,8}$-H), 7.87 (1H, d, *J* = 10 Hz, C_4-H). *Anal.* Calcd for $C_{15}H_{16}O_9 \cdot 1/2H_2O$: C, 51.58; H, 4.91. Found: C, 51.65; H, 4.80.

Acid Hydrolysis of Esculin (1)——**1** was treated with 1% H_2SO_4 soln. to give esculetin, mp 272—274 °C, which was identified by direct comparison with an authentic sample. The presence of D-glucose in the hydrolyzate was shown on TLC developed with BuOH–AcOH–H_2O (4 : 1 : 1).

Scopolin (2)——Colorless needles from EtOH, mp 224—226 °C. $[\alpha]_D^{25}$ −89.8° (*c* = 0.12 in MeOH). UV λ_{max}^{EtOH} nm (log ε): 227.8 (4.24), 248.3 (3.69) sh, 254.5 (3.65) sh, 289.3 (3.81), 340.2 (3.99). IR ν_{max}^{KBr} cm^{-1}: 3670 (OH), 1705 (CO), 1620 (C=C), 1570, 1515 (arom. C=C). ^{1}H-NMR (in DMSO-d_6) δ: 3.78 (3H, s, OCH_3), 6.25 (1H, d, *J* = 9.5 Hz, C_3-H), 7.07, 7.20 (2H, each s, $C_{5,8}$-H), 7.87 (1H, d, *J* = 9.5 Hz, C_4-H). *Anal.* Calcd for $C_{16}H_{18}O_9 \cdot H_2O$: C, 51.61; H, 5.41. Found: C, 51.72; H, 4.90.

Acid Hydrolysis of Scopolin (2)——**2** was treated with 1% H_2SO_4 soln. to give scopoletin, mp 205—207 °C, which was identified by direct comparison with an authentic sample. The presence of D-glucose in the hydrolyzate was shown on TLC.

Isoscopoletin-β-D-glucoside (3)——Colorless needles from EtOH, mp 233—234 °C. $[\alpha]_D^{22}$ −74.7° (*c* = 0.2 in MeOH). UV λ_{max}^{EtOH} nm (log ε): 226.2 (4.10), 249.4 (3.69) sh, 293.4 (3.73), 335.3 (3.95). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1710 (CO), 1630 (C=C), 1580, 1530 (arom. C=C). ^{1}H-NMR (in DMSO-d_6) δ: 3.84 (3H, s, OCH_3), 6.23 (1H, d, *J* = 10 Hz, C_3-H), 7.01, 7.32 (2H, each s, $C_{5,8}$-H), 7.83 (1H, d, *J* = 10 Hz, C_4-H). *Anal.* Calcd for $C_{16}H_{18}O_9 \cdot 2H_2O$: C, 49.23; H, 5.68. Found: C, 49.04; H, 5.64.

Acid Hydrolysis of Isoscopoletin-β-D-glucoside (3)——**3** was treated with 1% H_2SO_4 soln. to give isoscopoletin, mp 187—190 °C, which was identified by direct comparison with an authentic sample. The presence of D-glucose in the hydrolyzate was shown on TLC.

Enzymatic Hydrolysis of Isoscopoletin-β-D-glucoside (3)——**3** was treated with β-glucosidase (Miles Laboratories) at room temperature for a week. Isoscopoletin was identified by direct comparison with an authentic sample. D-glucose was identified by TLC.

Oleuropein (4)——Amorphous powder. $[\alpha]_D^{22}$ −128.4° (*c* = 0.61 in EtOH). UV λ_{max}^{MeOH} nm (log ε): 228 (4.25), 280 (3.74). IR ν_{max}^{KBr} cm^{-1}: 3200—3550 (OH), 1700 (CO), 1620 (C=C), 1590, 1510 (arom. C=C). ^{13}C-NMR (in CD_3OD) δ: 95.0 (C-1), 154.9 (C-3), 109.1 (C-4), 31.5 (C-5), 41.0 (C-6), 172.9 (C-7), 124.6 (C-8), 130.3 (C-9), 13.2 (C-10), 168.5 (C-11), 100.7 (C-1′), 74.5 (C-2′), 78.1 (C-3′), 71.2 (C-4′), 77.7 (C-5′), 62.5 (C-6′), 130.5 (C-1″), 116.2 (C-2″), 146.0 (C-3″), 144.7 (C-4″), 116.8 (C-5″), 121.0 (C-6″), 68.6 (C-α), 35.1 (C-β), 53.0 ($\underline{C}H_3O$). *Anal.* Calcd for $C_{25}H_{32}O_{13} \cdot 2H_2O$: C, 52.08; H, 6.29. Found: C, 52.21; H, 6.04.

Acknowledgement We thank Miss S. Kitagawa of our University for obtaining the ^{1}H- and ^{13}C-NMR spectral data.

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