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

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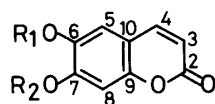
Coumarin glucosides, esculin (**1**) and scopolin (**2**), were isolated from the bark of *Olea africana* MILL. (*O. europaea* L. subsp. *africana* (MILL.) GREEN) while isoscopoletin- β -D-glucoside (magnolioside) (**3**) was isolated from the bark of *Olea capensis* L. The secoiridoid glucoside oleuropein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



- 1: R₁ = glucose, R₂ = H
2: R₁ = CH₃, R₂ = glucose
3: R₁ = glucose, R₂ = CH₃

Chart 1

Glucoside **1**, a colorless crystalline powder, C₁₅H₁₆O₉·1/2H₂O, 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 (^1H -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, C₁₆H₁₈O₉·H₂O, mp 224–226 °C. The IR, ^1H -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, C₁₆H₁₈O₉·2H₂O,

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 ^1H -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 principles¹³⁾ 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; ^1H -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 60F₂₅₄ (Merck), were used for TLC. The spots were detected by spraying the plates with 10% H₂SO₄ 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 R_f 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 isoscapoletin 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. $[\alpha]_D^{22} -88.5^\circ$ ($c=0.33$ in MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 224 (4.12), 250 (3.67), 298 (3.79), 336 (4.07). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3100–3550 (OH), 1700 (CO), 1680 (C=C), 1610, 1570 (arom. C=C). $^1\text{H-NMR}$ (in $\text{CD}_3\text{OD} + \text{DMSO}-d_6$) δ : 6.27 (1H, d, $J=10$ Hz, $\text{C}_3\text{-H}$), 6.87, 7.47 (2H, each s, $\text{C}_{5,8}\text{-H}$), 7.87 (1H, d, $J=10$ Hz, $\text{C}_4\text{-H}$). Anal. Calcd for $\text{C}_{15}\text{H}_{16}\text{O}_9 \cdot 1/2\text{H}_2\text{O}$: 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. $[\alpha]_D^{25} -89.8^\circ$ ($c=0.12$ in MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3670 (OH), 1705 (CO), 1620 (C=C), 1570, 1515 (arom. C=C). $^1\text{H-NMR}$ (in DMSO- d_6) δ : 3.78 (3H, s, OCH_3), 6.25 (1H, d, $J=9.5$ Hz, $\text{C}_3\text{-H}$), 7.07, 7.20 (2H, each s, $\text{C}_{5,8}\text{-H}$), 7.87 (1H, d, $J=9.5$ Hz, $\text{C}_4\text{-H}$). Anal. Calcd for $\text{C}_{16}\text{H}_{18}\text{O}_9 \cdot \text{H}_2\text{O}$: 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.

Isoscapoletin- β -D-glucoside (3)—Colorless needles from EtOH, mp 233–234 °C. $[\alpha]_D^{22} -74.7^\circ$ ($c=0.2$ in MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 226.2 (4.10), 249.4 (3.69) sh, 293.4 (3.73), 335.3 (3.95). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400 (OH), 1710 (CO), 1630 (C=C), 1580, 1530 (arom. C=C). $^1\text{H-NMR}$ (in DMSO- d_6) δ : 3.84 (3H, s, OCH_3), 6.23 (1H, d, $J=10$ Hz, $\text{C}_3\text{-H}$), 7.01, 7.32 (2H, each s, $\text{C}_{5,8}\text{-H}$), 7.83 (1H, d, $J=10$ Hz, $\text{C}_4\text{-H}$). Anal. Calcd for $\text{C}_{16}\text{H}_{18}\text{O}_9 \cdot 2\text{H}_2\text{O}$: C, 49.23; H, 5.68. Found: C, 49.04; H, 5.64.

Acid Hydrolysis of Isoscapoletin- β -D-glucoside (3)—**3** was treated with 1% H_2SO_4 soln. to give isoscapoletin, 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 Isoscapoletin- β -D-glucoside (3)—**3** was treated with β -glucosidase (Miles Laboratories) at room temperature for a week. Isoscapoletin was identified by direct comparison with an authentic sample. D-glucose was identified by TLC.

Oleuropein (4)—Amorphous powder. $[\alpha]_D^{22} -128.4^\circ$ ($c=0.61$ in EtOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 228 (4.25), 280 (3.74). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3200–3550 (OH), 1700 (CO), 1620 (C=C), 1590, 1510 (arom. C=C). $^{13}\text{C-NMR}$ (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 (CH_3O). Anal. Calcd for $\text{C}_{25}\text{H}_{32}\text{O}_{13} \cdot 2\text{H}_2\text{O}$: C, 52.08; H, 6.29. Found: C, 52.21; H, 6.04.

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