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PHENOLIC GLUCOSIDES FROM *OLEA EUROPAEA* SUBSP. *AFRICANA*

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Key Word Index—*Olea europaea* subsp. *africana*; Oleaceae; lignans; (+)-1-acetoxypinoresinol 4′-β-D-glucoside; (+)-1-acetoxypinoresinol 4″-methyl ether 4′-β-D-glucoside; (+)-1-hydroxypinoresinol 4′-β-D-glucoside; esculin; secoiridoid; oleuropein.

Abstract—Two new lignan glucosides, (+)-1-acetoxypinoresinol 4″-methyl ether 4′-β-D-glucoside and (+)-1-hydroxypinoresinol 4′-β-D-glucoside, together with three known glucosides, (+)-1-acetoxypinoresinol 4′-β-D-glucoside, esculin and oleuropein, were isolated from the bark of *Olea europaea* subsp. *africana*.

INTRODUCTION

The bark of *Olea europaea* L. subsp. *africana* (Mill) Green (Oleaceae), known as the 'wild olive', has been used as an anti-febrile and an anti-rheumatic agent, and as a tonic in southern Africa [1]. In previous work, we reported the isolation of esculetin and scopoletin from this source [2], while Roux and co-workers isolated (+)-africanal, (−)-olivil and (+)-cyclo-olivil from the heartwood [3]. We also isolated a lignan glucoside, (+)-1-acetoxypinoresinol 4′-β-D-glucoside, two coumarins, esculetin and esculin, and a secoiridoid glucoside, oleuropein, from the bark of the cultivated olive of commerce, *O. europaea* L. [4, 5].

The wild olive, previously classified as *Olea africana* Mill, was recently reclassified as a subspecies of *O. europaea* [6], and our interest was accordingly directed at the phenolic glucosides from *O. europaea* subsp. *africana*.

RESULTS

(+)-1-Acetoxypinoresinol 4′-β-D-glucoside (**1**) and (+)-1-acetoxypinoresinol 4″-methyl ether 4′-β-D-glucoside (**2**) were isolated from the chloroform extract, the former being identified by direct comparison with an authentic sample [4]. The IR spectrum of **2** indicated the presence of a carboxy group (1730 cm^{-1}) and aromatic rings (1605, 1590 and 1510 cm^{-1}). The ^1H NMR spectrum of **2** exhibited resonances attributable to an aliphatic acetoxy group (δ 1.67) and three aromatic methoxy groups (δ 3.87). These data indicate a marked structural resemblance between **2** and **1**. In the ^{13}C NMR data of **2** (Table 1), $\Delta\delta$ + 1.6 (C-1″), + 1.2 (C-3″), + 2.0 (C-4″) and − 3.6 (C-5″) ppm shifts of aromatic carbons relative to those of the corresponding carbons of **1**, correlate with a veratryl unit in the C-6 position of the glucoside **2** [7]. The structure of **2** was confirmed as (+)-1-acetoxypinoresinol 4″-methyl ether 4′-β-D-glucoside by its identity with the compound obtained from methylation of **1** with excess diazomethane.

Table 1. ^{13}C NMR spectral data of compounds **1**–**3***

Carbon	1	2	3
C-1	97.0	96.9	91.1
C-5	58.2	58.2	60.8
C-4	69.7	69.7	70.2
C-8	73.8	73.8	74.7
C-2	86.2	86.1	86.8
C-6	84.6	84.3	85.4
C-1′	130.3	130.3	131.1
C-1″	131.2	132.8	132.3
C-2′	113.0	113.0	112.6
C-2″	110.7	110.1	110.8
C-3′	148.2	148.2	148.3
C-3″	147.5	148.7	147.5
C-4′	146.3	146.3	146.0
C-4″	146.3	148.3	146.0
C-5′	114.6	114.6	114.6
C-5″	115.3	111.7	115.1
C-6′	121.1	121.0	119.7
C-6″	119.0	118.5	118.8
$\underline{C}H_3CO$	20.6	20.6	
Me$\underline{C}$O	168.8	168.7	
MeO	55.6	55.4	55.6
	55.7	55.7	
Glc-1	99.9	99.8	100.4
Glc-2	73.2	73.2	73.2
Glc-3	76.9	76.9	76.8
Glc-4	69.7	69.7	69.7
Glc-5	76.9	76.9	76.8
Glc-6	60.7	60.6	60.8

*The solvent used in these experiments was DMSO-d_6.

(+)-1-Hydroxypinoresinol 4′-β-D-glucoside (**3**), esculin (**4**) and oleuropein (**5**) were isolated from the butano extract. Compounds **4** and **5** were identified by direc

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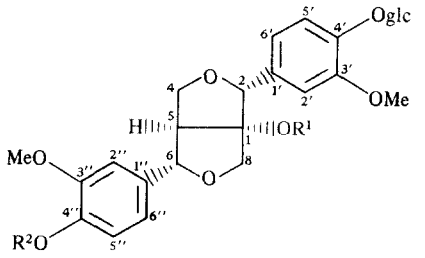

1 R^1 = Ac; R^2 = H
2 R^1 = Ac; R^2 = Me
3 R^1 = R^2 = H

comparison with authentic samples [5]. Both absorption maxima in the UV spectrum of **3** and their bathochromic shifts with base were very similar to those of **1**, while the ^{1}H NMR spectrum of **3** resembled that of **1** except for the disappearance of the signal assigned to an aliphatic acetoxy group. The ^{13}C NMR data indicated the absence of acetoxy resonances, but the presence of a hydroxy group at the C-1 position of **3**. The structure of **3** was determined as (+)-1-hydroxypinoresinol 4′-β-D-glucoside by its identity with the compound obtained by deacetylation of **1** with ammonia in methanol. Compounds **2** and **3** are new lignan glucosides of natural origin.

Recently, we have also isolated the lignan glucosides **2** and **3** from the bark of *O. europaea* [8]. Thus, both subspecies *africana* and *europaea* of *O. europaea* are identical in their bark chemistry and have the same lignans, coumarins and secoiridoids in common [2]. It is of interest that the bark of *Fraxinus japonica* Blume (Oleaceae), used since ancient times as an anti-febrile and an anti-rheumatic agent in Japan [9], contains the known active principles esculetin and esculin [10]. Their common distribution also in those barks of *Olea* spp. which have been used in similar therapeutic applications is noteworthy.

EXPERIMENTAL

All mps are uncorr. ^{1}H NMR spectra were obtained at 90 MHz using TMS as internal standard. ^{13}C NMR spectra were recorded at 15 MHz with Fourier transform using TMS as internal standard. Silica gel F_{254} (Merck) was used for TLC. The spots were detected by spraying the plates with 10% H_2SO_4 soln and heating.

Plant material. O. europaea subsp. *africana* was collected in Bloemfontein in October 1982. A voucher specimen has been deposited at the Herbarium of Higashi Nippon Gakuen University.

Isolation. Dry powdered bark (1.0 kg) of *O. europaea* subsp. *africana* was extracted 3 × with hot MeOH. The conc. extract plus H_2O was extracted successively with Et_2O, $CHCl_3$ and *n*-BuOH.

The $CHCl_3$ extract was chromatographed on a silica gel column using a $CHCl_3$–EtOH gradient. The fractions were monitored by TLC developing with $CHCl_3$–EtOH (4:1). The fractions showing a TLC spot at R_F 0.30 were concd to give **1** (546.6 mg). The fractions showing a TLC spot at R_F 0.43 were concd to give **2** (50.4 mg).

The *n*-BuOH extract was chromatographed on a silica gel column using a $CHCl_3$–EtOH gradient. The fractions were monitored by TLC developing with $CHCl_3$–MeOH–H_2O (65:35:10, lower layer). The fractions showing a TLC spot at R_F 0.53 were concd to give **3** (34.6 mg). The fractions showing a TLC spot at R_F 0.45 were concd to give **4** (132.4 mg). Those showing a TLC spot at R_F 0.60 were concd to give **5** (7.7 g).

(+)-1-*Acetoxypinoresinol* 4′-β-D-*glucoside* (**1**). Colourless needles, mp 183.5–185°, $[\alpha]_D^{22}$ + 7.9° (*c* 1.0; EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.31), 279.5 (3.83). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 254, 280, 292. IR ν_{max}^{KBr} cm^{-1}: 3325 (OH), 1735 (CO), 1600, 1590, 1520 (aromatic C=C). ^{1}H NMR (CD_3OD): δ1.67 (3H, *s*, alcoholic OAc), 2.77–3.03 (1H, *m*, H-5), 3.87 (6H, *s*, 2 × MeO), 3.24–4.44 (4H, *m*, H-4, 8), 5.05 (1H, *s*, H-2), 6.67–7.30 (6H, *m*, Ar-H). (Found: C, 57.68; H, 5.93. $C_{28}H_{34}O_{13} \cdot 1/2H_2O$ requires: C, 57.23; H, 6.00%.)

(+)-1-*Acetoxypinoresinol* 4″-*methyl ether* 4′-β-D-*glucoside* (**2**). Amorphous powder, $[\alpha]_D^{20}$ + 9.1° (*c* 1.6; EtOH). UV λ_{max}^{EtOH} nm (log ε): 231 (4.22), 279 (3.72). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (CO), 1605, 1590, 1510 (aromatic C=C). ^{1}H NMR (CD_3OD): δ1.67 (3H, *s*, alcoholic OAc), 2.75–3.08 (1H, *m*, H-5), 3.87 (9H, *s*, 3 × MeOH), 3.22–4.44 (4H, *m*, H-4, 8), 5.05 (1H, *s*, H-2), 6.77–7.28 (6H, *m*, Ar-H). (Found: C, 57.53; H, 5.97. $C_{29}H_{36}O_{13} \cdot H_2O$ requires: C, 57.04; H, 6.27%.)

Compound **1** in MeOH was methylated with excess CH_2N_2 to give (+)-1-acetoxypinoresinol 4″-methyl ether 4′-β-D-glucoside, which was identical to **2** in all respects.

(+)-1-*Hydroxypinoresinol* 4′-β-D-*glucoside* (**3**). Colourless plates, mp 127–129°, $[\alpha]_D^{23}$ − 9.3° (*c* 0.42; MeOH). UV λ_{max}^{EtOH} nm (log ε): 228 (4.11), 279.8 (3.66). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 254, 280, 292. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1600, 1515 (aromatic C=C). ^{1}H NMR (CD_3OH): δ 2.96–3.14 (1H, *m*, H-5), 3.80, 3.83 (6H, each *s*, 2 × MeO), 3.47–4.52 (4H, *m*, H-4, 8), 4.80 (1H, *s*, H-2), 6.60–6.80 (6H, *m*, Ar–H). FDMS *m/z*: 536 $[M]^+$ ($C_{26}H_{32}O_{12}$).

Compound **1** was deacetylated with NH_3 in MeOH to give (+)-1-hydroxypinoresinol 4′-β-D-glucoside, which was identical to **3** in all respects.

Esculin (**4**). Colourless crystalline powder, mp 152–154°. 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 (aromatic C=C). ^{1}H NMR (CD_3OD + DMSO-d_6): δ6.27 (1H, *d*, *J* = 10 Hz, H-3), 6.87, 7.47 (2H, each *s*, Ar-H), 7.87 (1H, *d*, *J* = 10 Hz, H-4). ^{13}C NMR (DMSO-d_6): δ160.7 (C-2), 112.1 (C-3), 144.5 (C-4), 114.7 (C-5), 142.7 (C-6), 151.4 (C-7), 103.2 (C-8), 150.5 (C-9), 110.9 (C-10), 102.3 (C-1′), 73.4 (C-2′), 77.3 (C-3′), 69.9 (C-4′), 76.1 (C-5′), 60.8 (C-6′). (Found: C, 51.65; H, 4.80. $C_{15}H_{16}O_9 \cdot 1/2H_2O$ requires: C, 51.58, H, 4.91%.)

Oleuropein (**5**). Amorphous powder, $[\alpha]_D^{22}$ − 128.4° (*c* 0.61; 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 (aromatic C=C). ^{13}C NMR (CD_3OD): δ95.0 (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 (MeO). (Found: C, 52.21; H, 6.04. $C_{25}H_{32}O_{13} \cdot 2H_2O$ requires: C, 52.08; H, 6.29%.)

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