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A SECOIRIDOID GLUCOSIDE FROM *OLEA EUROPAEA**

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Key Word Index—*Olea europaea*; Oleaceae; secoiridoid glucoside; oleuroside; structure elucidation.

Abstract—Besides two known glucosides, oleuropein and ligstroside, a new secoiridoid glucoside, oleuroside was isolated from *Olea europaea* and its structure was determined as secoxyloganin 3,4-dihydroxyphenethyl ester.

INTRODUCTION

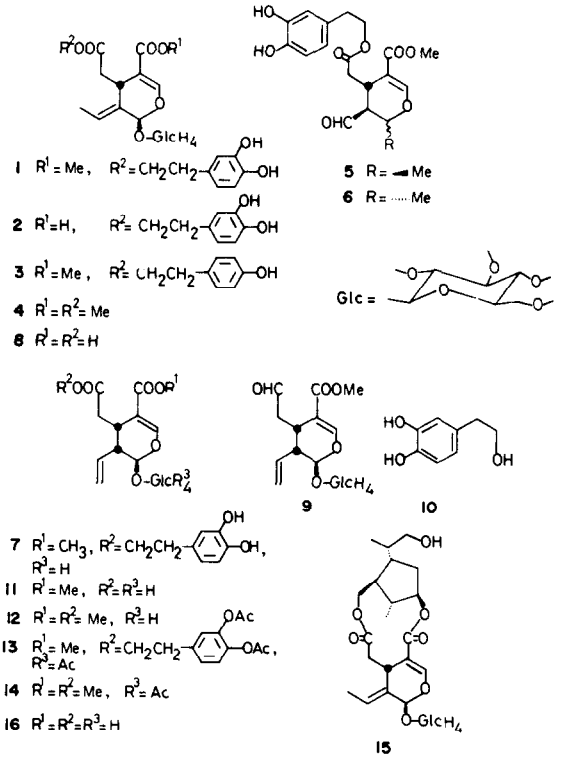
Along with the well known secoiridoid glucosides, oleuropein (**1**) [1,2] and demethyloleuropein (**2**) [3,4], ligstroside (**3**) [5, 6], oleoside dimethyl ester (**4**) [4, 6], and nonglycosidic secoiridoids **5** and **6** [6] have recently been isolated from olive (*Olea europaea*). This paper deals with the structure elucidation of a new secoiridoid glucoside oleuroside (**7**) which was isolated from this plant in the course of the preparatory studies on the biosynthesis of oleoside (**8**)-type secoiridoid gluosides‡.

RESULTS AND DISCUSSION

The water soluble part of the methanolic extract of fresh leaves of *O. europaea* gave on column chromatography on highly porous polymer and silica gel followed by further purification through preparative HPLC and preparative TLC, besides oleuropein (**1**) and ligstroside (**3**), a new glucoside, oleuroside (**7**), $C_{25}H_{32}O_{13}\cdot 1/2\,H_2O$, $[\alpha]_D^{17}$ −83.7° (MeOH) as a white powder. This glucoside showed UV absorption at 230, 283, 290 (inf.) nm (log ε 4.14, 3.47, 3.34) and IR bands at 3350, 1700, 1620, 1520 cm^{-1}. These spectral data suggested that oleuroside (**7**) is an iridoid glucoside related to oleuropein (**1**) and ligstroside (**3**), which possesses an aromatic portion and a chromophore –OOC–C=CH–O–. ^1H and ^{13}C NMR spectroscopy (see Experimental and Table 1) demonstrated that oleuroside had the structure shown, formula **7**, except that the positions of R^1 and R^2 remained to be determined.

As regards these position of attachment, confirmative evidence was provided by the long-range selective proton decoupling (LSPD) experiments as follows: Irradiation of the protons (δ 4.24) at C-1″ of phenethyl alcohol moiety caused sharpening of the signal of C-7 at δ 171.78, but did not influence the signal of C-11 at δ 166.77. By contrast, irradiation of the carbomethoxy proton (δ 3.65) caused a change of the C-11 signal into singlet, but did not affect the C-7 signal. Thus, 3,4-dihydroxyphenethyl alcohol (**10**) was proved to be attached to secoxyloganin (**11**) at the C-7 position. In fact, alkaline hydrolysis of **7** afforded **10** and a secoiridoid glucoside, the latter of which gave on methylation followed by acetylation secoxyloganin methyl ester tetraacetate (**14**). Thus, the structure of oleuroside was elucidated as **7**.

Though the distribution of oleoside (**8**) type secoiridoid glucosides such as oleuropein (**1**), ligstroside (**3**), jasminin



Formulae 1

*Part 60 in the series 'Monoterpene Glucosides and Related Natural Products'. For part 59, see Uesato, S., Xie, S., Inouye, H., Shingu, T., Inoue, M. and Doi, M. (1987) *Phytochemistry* **26**, 561.

‡Though oleoside 11-methyl ester or oleoside 7,11-dimethyl ester has erroneously been described as oleoside in some papers [e.g. refs 4, 6, 7], **8** represents the correct structure of the latter: cf. ref [2].

Table 1. ^{13}C NMR signals of compounds **1**, **7** and **12** in CD_3OD

C	7	1	12
1	97.47 *d*	95.25 *d*	97.35 *d*
3	153.68 *d*	155.10 *d*	153.64 *d*
4	110.00 *s*	109.32 *s*	109.85 *s*
5	29.01 *d*	31.76 *d*	28.96 *d*
6	35.54 *t**	41.23 *t*	35.27 *t*
7	174.31 *s*	173.21 *s*	174.67 *s*
8	134.35 *d*	124.85 *d*	134.43 *d*
9	45.27 *d*	130.75 *s*	45.26 *d*
10	120.58 *t*	13.55 *q*	120.42 *t*
11	168.84 *s*	168.65 *s*	168.65 *s*
7-OMe			52.03 *q*
11-OMe	51.74 *q*	51.97 *q*	51.68 *q*
1′	99.95 *d*	100.85 *d*	99.86 *d*
2′	74.63 *d*	74.69 *d*	74.45 *d*
3′	78.33 *d*	78.31 *d*	78.25 *d*
4′	71.49 *d*	71.42 *d*	71.36 *d*
5′	77.99 *d*	77.84 *d*	77.84 *d*
6′	62.73 *t*	62.72 *t*	62.66 *t*
1″	66.65 *t*	66.87 *t*	
2″	35.37 *t**	35.33 *t*	
3″	130.87 *s*	130.40 *s*	
4″	117.05 *d*	117.03 *d*	
5″	146.19 *s*	146.17 *s*	
6″	144.85 *s*	144.83 *s*	
7″	116.47 *d*	116.45 *d*	
8″	121.28 *d*	121.29 *d*	

Assignments were made by gated decouping mode and selective decoupling mode.

*Values are interchangeable.

(15) [8] in oleaceous plants is well known, this is the first example of a secologanoside **(16)** type secoiridoid glucoside to be isolated from this family. The occurrence of **7** in an oleaceous plant provides a supporting evidence for the intermediacy of secologanin **(9)** in the biosynthetic pathway of oleoside **(8)** type secoiridoids [9, 10].

EXPERIMENTAL

Mps: uncorr; NMR: ^1H, 200 MHz, ^{13}C, 50.10 MHz, TMS as inf. standard; TLC: silica gel GF_{254}, spots visualized by irradiation under UV light (254 nm) and by exposure to I_2 vapour; prep. TLC: silica gel PF_{254}, bands detected under UV light; CC: silica gel (Merck) and highly porous polymer HP-21 (Mitsubishi Kasei); medium pressure CC: silica gel PF_{254}; prep. HPLC: Hitachi Model 655, column dimension, 300 × 22 mm, packing, ODS, YMC-30 (Yamazen Co. Ltd.), flow rate, 5 ml/min.

Plant material. Leaves of *Olea europaea* L. grown in the Herbal Garden, Faculty of Pharmaceutical Sciences, Kinki University were collected in June 1986.

Isolation of Glucosides. Fresh leaves (492.4 g) of *O. europaea* were cut into small pieces and extracted with hot MeOH (1.5 l × 3). After concn *in vacuo*, the combined extracts were turbulated with H_2O (200 ml) and the insoluble material was filtered off through a Celite layer. After washing the Celite layer, combined filtrate and washings were concd *in vacuo* to *ca* 100 ml. The concd soln was subjected to CC on highly porous polymer HP-21 (900 ml), eluting successively with H_2O (1 l) and MeOH (2 l). The residue (23.2 g) of the MeOH eluate was chromatographed on a silica gel (710 g) column with $CHCl_3$–MeOH as eluant with an increasing MeOH content. The combined fractions eluted with $CHCl_3$–MeOH (19:1) were concd *in vacuo* to afford a residue (R-1, 1.66 g). Likewise, eluates with $CHCl_3$–MeOH (19:1), $CHCl_3$–MeOH (92.5:7.5 +9:1), (85:15) and (8:2) gave on concn *in vacuo* residues R-2 (3.10 g), R-3 (7.86 g) and R-4 (1.78 g), respectively.

An aliquot (1.95 g) of R-2 was subjected to prep. HPLC eluting with MeOH–H_2O (1:1) to give six fractions (R_t, 9.2, 12.4, 15.6, 34.5, 36.0 and 45.5 min), which on concn *in vacuo* gave respective residues Rp-1 (26.5 mg), Rp-2 (99.2 mg), Rp-3 (116.8 mg), Rp-4 (978.7 mg), Rp-5 (59.7 mg), Rp-6 (193.5 mg) in order of the retention time. An aliquot (150.5 mg) of Rp-4 was purified by prep. TLC with $CHCl_3$–MeOH (4:1, 2 developments) to give oleuropein **(1)** (140.2 mg) as a white powder. Rp-6 was subjected to prep. TLC ($CHCl_3$–MeOH, 4:1, 2 developments). Of two major bands, the more mobile one gave ligstroside **(3)** (10.1 mg) as a white powder, whereas the less mobile one furnished oleuroside **(7)** (90.9 mg) as a white powder. $[\alpha]_D^{17}$ −83.7° (MeOH; *c* 1.03). UV λ_{max}^{MeOH} nm (log ε): 230 (4.14), 283 (3.47), 290 (inf.) (3.34). IR ν_{max}^{KBr} cm^{-1}: 3350, 1700, 1620, 1520. FABMS *m/z*: 541 $(M+H)^+$. Found: C, 54.37; H, 5.84. $C_{25}H_{32}O_{13}\cdot 1/2\ H_2O$ requires: C, 54.62; H, 6.06. ^1H NMR (CD_3OD) δ: 2.30 (1H, *dd*, *J* = 16.1 and 8.8 Hz, H-6a), 2.70 (1H, *ddd*, *J* = 9.3, 4.4 and 4.2 Hz, H-9), 2.76 (2H, *t*, *J* = 6.8 Hz, H-2″), 2.87 (1H, *dd*, *J* = 16.1 and 5.4 Hz, H-6b), 3.22 (1H, *m*, H-5), 3.65 (3H, *s*, COOMe), 3.90 (1H, *br d*, *J* = 11.3 Hz, H-6′a), 4.18 (2H, *t*, *J* = 6.8 Hz, H-1″), 4.66 (1H, *d*, *J* = 7.6 Hz, H-1′), 5.14 (1H, *dd*, *J* = 18.2 and 1.5 Hz, H-10b), 5.15 (1H, *dd*, *J* = 9.3 and 1.5 Hz, H-10a), 5.45 (1H, *d*, *J* = 4.2 Hz, H-1), 5.57 (1H, *br dt*, *J* = 18.2 and 9.3 Hz, H-8), 6.55 (1H, *dd*, *J* = 7.8 and 2.0 Hz, H-8″), 6.67 (1H, *d*, *J* = 2.0 Hz, H-4″), 6.70 (1H, *d*, *J* = 7.8 Hz, H-7″), 7.46 (1H, *d*, *J* = 1.7 Hz, H-3).

Acetylation of oleuroside **(7)**. **7** (43.1 mg) was acetylated with Py–Ac_2O (each 0.43 ml) for 15 hr at room temp. The product was purified by prep. TLC ($CHCl_3$–MeOH, 50:1) and recrystallized from EtOH to give oleuroside hexaacetate **(13)** (36.2 mg) as colourless needles, mp 122–123°. $[\alpha]_D^{17}$ −82.8° ($CHCl_3$; *c* 1.00), FABMS *m/z*: 793 $(M+H)^+$. Found: C, 56.36; H, 5.71. $C_{37}H_{44}O_{19}$ requires: C, 56.06; H, 5.59. ^1H NMR ($CDCl_3$) δ: 1.89, 1.99, 2.01, 2.08 (2H, each *s*, 4 × OAc), 2.25, 2.27 (6H, each *s*, 2 × phenolic OCOMe), 2.28 (1H, *dd*, *J* = 16.3 and 9.5 Hz, H-6a), 2.76 (1H, *ddd*, *J* = 10.0, 6.0 and 3.0 Hz, H-9), 2.91 (2H, *t*, *J* = 7.0 Hz, H-2″), 2.96 (1H, *dd*, *J* = 16.3 and 5.0 Hz, H-6b), 3.15 (1H, *dddd*, *J* = 10.0, 6.0, 5.0, and 2.0 Hz, H-5), 3.65 (3H, *s*, COOMe), 3.72 (1H, *ddd*, *J* = 9.5, 4.5 and 2.0 Hz, H-5′), 4.11 (1H, *dd*, *J* = 12.5 and 2.0 Hz, H-6′a), 4.24 (2H, *t*, *J* = 7.0 Hz, H-1″), 4.28 (1H, *dd*, *J* = 12.5 and 4.5 Hz, H-6′b), 4.84 (1H, *d*, *J* = 8.0 Hz, H-1′), 5.00 (1H, *dd*, *J* = 9.5 and 8.0 Hz, H-2′), 5.08 (1H, *t*, *J* = 9.5 Hz, H-4′), 5.11 (1H, *d*, *J* = 10.5 Hz, H-10a), 5.16 (1H, *d*, *J* = 16.4 Hz, H-10b), 5.20 (1H, *t*, *J* = 9.5 Hz, H-3′), 5.23 (1H, *d*, *J* = 3.0 Hz, H-1), 5.45 (1H, *ddd*, *J* = 16.4, 10.5 and 10.0 Hz, H-8), 7.01 (1H, *br s*, H-4″), 7.06 (1H, *br d*, *J* = 8.0 Hz, H-8″), 7.10 (1H, *d*, *J* = 8.0 Hz, H-7″), 7.33 (1H, *d*, *J* = 2.0 Hz, H-3).

Alkaline hydrolysis of oleuroside **(7)**. A soln of **7** (20.4 mg) in 0.5 N NaOH (1 ml) was stirred for 6 hr at room temp. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) and extracted with EtOAc (4 ml × 4). The dried EtOAc layer was evapd *in vacuo* to give a syrupy substance (2.4 mg), which was identified with an authentic sample of 3,4-dihydroxyphenethyl alcohol **(10)** (^1H NMR, IR). The aq. layer was evapd *in vacuo* and the residue (17.4 mg) was dissolved in MeOH and subjected to methylation with ethereal CH_2N_2 followed by acetylation in the usual way. The product was purified by prep. TLC (C_6H_6–Et_2O, 3:7) and recrystallized from EtOH to furnish secoxyloganin

methyl ester tetraacetate (**14**) (14.0 mg) as colourless needless, mp 149° (lit. mp 140.5° [11], 145° [12]), $[\alpha]_D^{16}$ −91.6° ($CHCl_3$; *c* 0.69) (lit. $[\alpha]_D$ −99° ($CHCl_3$; *c* 1) [11], $[\alpha]_D$ −107° ($CHCl_3$) [12]). ^{1}H NMR ($CDCl_3$) δ: 1.90, 2.00, 2.03, 2.10 (12H, each *s*, 4 × OCOMe), 2.28 (1H, *dd*, *J* = 16.9 and 9.4 Hz, H-6a), 2.85 (1H, *ddd*, *J* = 9.6, 5.6 and 3.0 Hz,, H-9), 2.96 (1H, *dd*, *J* = 16.9 and 5.0 Hz, H-6b), 3.18 (1H, *m*, H-5), 3.65 (3H, *s*, 7-OMe), 3.68 (3H, *s*, 11-OMe), 3.73 (1H, *ddd*, *J* = 9.5, 4.5 and 2.5 Hz, H-5′), 4.14 (1H, *dd*, *J* = 12.5 and 2.5 Hz, H-6′a), 4.29 (1H, *dd*, *J* = 12.5 and 4.5 Hz, H-6′b), 4.88(1H, *d*, *J* = 8.0 Hz, H-1′), 5.02 (1H, *dd*, *J* = 9.5 and 8.0 Hz, H-2′), 5.11 (1H, *t*, *J* = 9.5 Hz, H-4′), 5.23 (2H, *d*, *J* = 9.6 Hz, H-10a and d, *J* = 17.7 Hz, H-10b), 5.27 (1H, *d*, *I* = 3.0 Hz, H-1), 5.53 (1H, *dt*, *J* = 17.7 and 9.6 Hz, H-8), 7.38 (1H, *d*, *J* = 2.0 Hz, H-3).

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