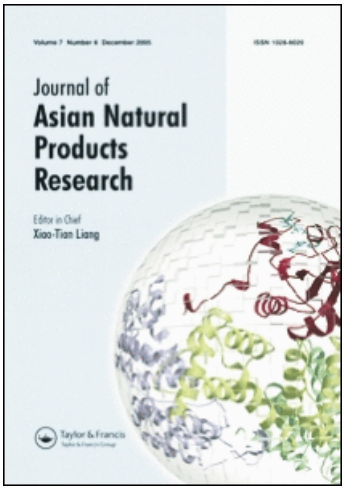

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Two new secoiridoid glycosides from the leaves of *Olea europaea* L.

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Two new secoiridoid glycosides, oleuricines A (**1**) and B (**2**), together with five known triterpenoids, β-amyrin, oleanolic acid, erythrodiol, urs-2β,3β-dihydroxy-12-en-28-oic acid, and β-maslinic acid, were isolated from the EtOAc-soluble part of EtOH extract of the leaves of *Olea europaea* L. The structures of these compounds were elucidated by various spectroscopic methods, including intensive 1D, 2D NMR, and HR-ESI-MS techniques.

Keywords: *Olea europaea* L.; secoiridoid glycosides; oleuricine A; oleuricine B

1. Introduction

Olea europaea L. has been widely cultivated in the Mediterranean area. The leaves of *O. europaea* L. have been used as a folk medicine by native people for the treatment of fever and other diseases, such as malaria. *O. europaea* L. has been transplanted into Sichuan and Gansu Provinces of China since last century. Secoiridoid glycosides are the most important chemical constituents of the genus *Olea* [1,2] and the biological activities of these compounds, such as antimicrobial, anti-inflammatory, hypotensive, and hypoglycemic activities, have been reported previously [3–5]. As a continuing investigation of bioactive constituents of the leaves of *O. europaea* L., two new secoiridoid glycosides, together with five known triterpenoids, β-amyrin, oleanolic acid, erythrodiol, urs-2β,3β-dihydroxy-12-en-28-oic acid, and β-maslinic acid, were isolated (Figure 1). Here, the isolation and structural elucidation of the new compounds are reported.

2. Results and discussion

Compound **1** was obtained as a yellow gum. Its molecular formula was established as $C_{31}H_{42}O_{18}$ on the basis of HR-ESI-MS at *m/z* 720.2719 $[M+NH_4]^+$. The IR spectrum showed the absorption bands for hydroxyl (3374 cm^{-1}), α,β-unsaturated carbonyl (1629 and 1702 cm^{-1}), and aromatic ring (1520 cm^{-1}). The structure of compound **1** could be elucidated on the basis of 1D and 2D NMR spectral data as well as comparison with the literature [6]. The ^{13}C NMR spectrum of **1** showed some signals for substructures of a secoiridoid, a hydroxytyrosol, and a β-glucopyranosyl, which were similar to that of the known oleuropein [6]. However, there was a set of additional signals (Table 1) due to a monosaccharide moiety, which was determined as a β-D-glucopyranosyl group by

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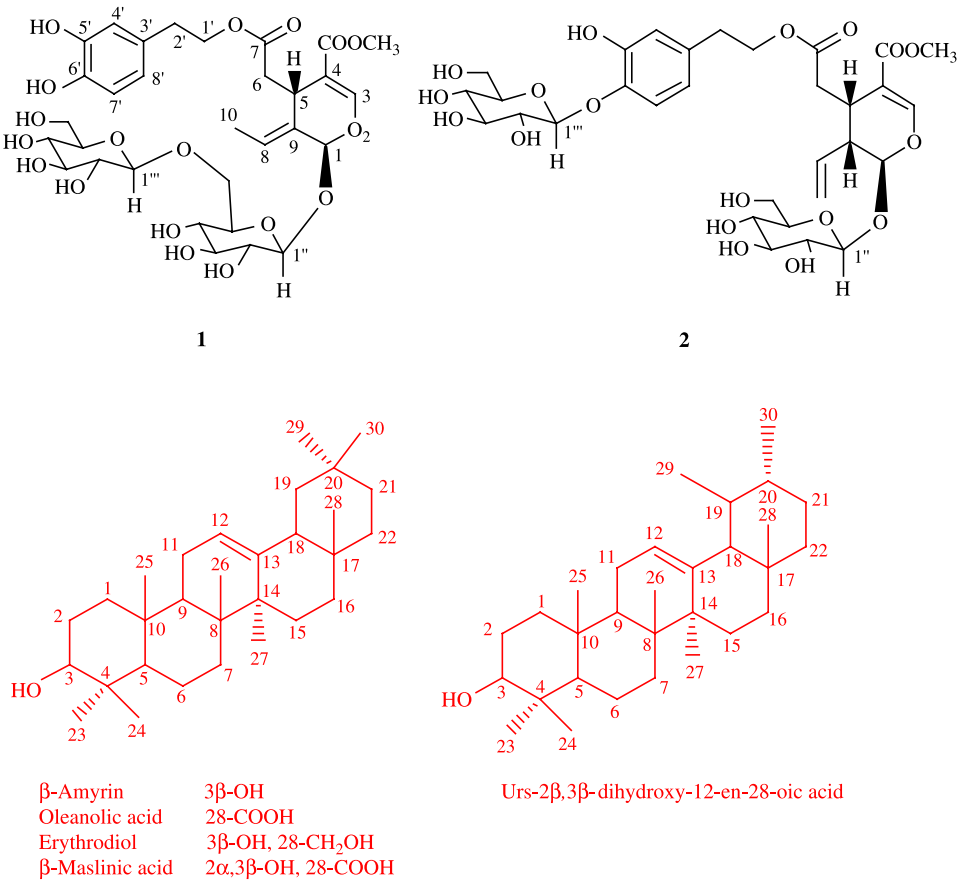


Figure 1. Chemical structures of isolated compounds.

comparing with that of methyl-β-D-glucopyranoside [7]. The result agreed with the acid hydrolysis assay. In this experimentation, D-glucopyranose was identified by paper chromatography (PC) through comparison with authentic sugars. Both D-glucopyranose moieties could be confirmed as the β-configuration on the basis of the anomeric proton signals at δ 4.19 (1H, d, $J = 7.6$ Hz) and 4.63 (1H, d, $J = 8.0$ Hz) in the ^{1}H NMR spectrum. The linkage between glucosyl groups was determined by the shift effects compared to oleuropein: C-6″ at downfield δ 69.9 and C-5″ at upfield δ 73.3 in **1**. This was confirmed by the key correlation between H-1‴ and C-6″ in the HMBC experiment (Figure 2). Thus, the structure of **1** was determined to be 6″-*O*-β-D-glucopyranosyloleuropein, named as oleuricine A.

Compound **2** was obtained as a yellow gum. Its molecular formula $C_{31}H_{42}O_{18}$ was deduced from HR-ESI-MS at *m/z* 725.2251 $[M+Na]^+$. The IR spectrum showed the absorption bands for hydroxyl (3361 cm^{-1}), α,β-unsaturated carbonyl (1629 and 1703 cm^{-1}), and aromatic ring (1509 cm^{-1}). The ^{13}C NMR spectrum of **2** was similar to that of oleuroside [6], except for the presence of another β-D-glucopyranosyl group (Table 1), which was deduced by comparing with methyl-β-D-glucopyranoside. This was confirmed by the acid hydrolysis assay. Acid hydrolysis of compound **2** gave D-glucopyranose, which was identified by PC comparison

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** and **2** in DMSO-d_6 (TMS, δ in ppm, J in Hz).

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
Secoiridoid				
1	5.85 (s)	92.9	5.38 (d, 4.4)	95.4
3	7.50 (s)	153.4	7.44 (s)	152.1
4		107.8		108.1
5	3.85 (dd, 4.0, 8.0)	30.1	3.11–3.19	27.6
6	2.37–2.43 (m)	39.9	2.65 (m)	33.8
7		170.7		171.7
8	5.94 (m)	123.1	5.49 (m)	133.2
9		129.3	2.72 (m)	43.2
10	1.66 (d, 6.0)	13.1	5.18 (m)	119.5
11		166.2		166.5
OMe	3.60 (s)	51.3	3.58	51.1
Hydroxytyrosol				
1′-a	4.01 (m)	64.1	4.08–4.15 (m)	64.6
1′-b	4.25 (m)		4.08–4.15 (m)	
2′	2.64 (m)	35.1	2.66 (m)	34.0
3′		129.3		129.7
4′	6.58 (s)	116.3	6.68 (s)	117.1
5′		145.0		144.1
6′		143.5		146.9
7′	6.59 (d, 8.0)	115.5	7.00 (d, 8.0)	116.5
8′	6.44 (d, 8.0)	119.5	6.58 (d, 8.4)	120.0
Glc				
1″	4.63 (d, 8.0)	99.0	4.48 (d, 8.0)	98.6
2″	3.35 (m)[a]	73.6	3.25 (m)[a]	73.3
3″	3.35 (m)[a]	76.4	3.24 (m)[a]	75.9
4″	3.07 (m)[a]	70.0	3.12 (m)[a]	70.0
5″	2.97 (m)[a]	73.3	3.11 (m)[a]	77.2
6″	3.31 (m)[a]	69.9	3.67 (m)[a]	61.1
Glc				
1‴	4.19 (d, 7.6)	102.9	4.58 (d, 7.2)	102.8
2‴	3.07 (m)[a]	73.3	2.95 (m)[a]	73.0
3‴	3.07 (m)[a]	76.5	3.10 (m)[a]	76.7
4‴	3.56 (m)[a]	70.0	3.02 (m)[a]	69.8
5‴	3.16 (m)[a]	77.4	3.07 (m)[a]	77.4
6‴	3.39 (m)[a]	61.2	3.34 (m)[a]	60.8

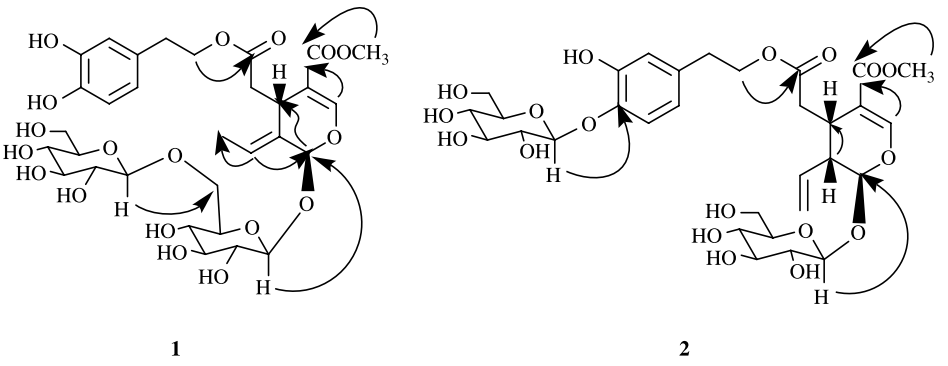
[a]Signal pattern unclear due to overlapping.

with authentic sugars. In addition, the anomeric proton signals at δ 4.48 (1H, d, $J = 8.0$ Hz) and 4.58 (1H, d, $J = 7.2$ Hz) were recognized in the ^{1}H NMR spectrum. The coupling constants indicated that both the D-glucopyranose moieties were of β-configuration. The β-glucopyranosyl group was linked at C-6′ of the hydroxytyrosol moiety, because of the C-6′ signal at downfield δ 146.9 compared with signals of oleuroside. This was also confirmed by the HMBC correlation between H-1‴ and C-6′ (Figure 2). Thus, the structure of compound **2** was determined to be 6′-*O*-β-D-glucopyranosyl oleuroside, named as oleuricine B.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Rudolph-Research Autopol-III automatic



1 2

Figure 2. Key HMBC correlations of **1** and **2**.

polarimeter. The UV spectra were measured on a T6 UV–vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd, Beijing, China). The IR spectra were obtained on a Nicolet-170SX FT-IR spectrometer (Madison, WI, USA); KBr pellets in cm^{-1}. The HR-ESI-MS spectra were recorded on a Bruker APEX-II mass spectrometer in *m/z*. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-400 FT-NMR spectrometer (Palo Alto, CA, USA), with TMS as an internal standard, δ in ppm, *J* in Hz. Silica gel (200–300 mesh) for column chromatography and silica gel GF_{254} for TLC were purchased from Qingdao Marine Chemical Factory, Qingdao, China. C_{18} reversed-phase (RP) silica gel (35–75 mm) was obtained from Analteck, Inc. (Newark, NJ, USA).

3.2 Plant material

The leaves of *O. europaea* L. were collected from Longnan County of Gansu Province in China on February 2008. The plant was identified by Prof. Huan-Yang Qi in Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, China, and a voucher specimen (No. ZY200803) has been deposited at Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, China.

3.3 Extraction and isolation

The air-dried leaves of *O. europaea* L. (2.5 kg) were extracted with EtOH (25 liters) at room temperature three times (each time for 5 days). The EtOH extract was evaporated under reduced pressure at 50°C to yield a residue (1 kg). The residue was suspended in H_2O (2000 ml) and then partitioned with petroleum ether (60–90°C, 6×1500 ml) and EtOAc (6×1500 ml) successively to give petroleum ether and EtOAc fractions. Then, the EtOAc fraction (300 g) was subjected to column chromatography eluted with a gradient of $CHCl_3$–CH_3OH. The fraction (100:5, v:v, 2500 mg) was separated to give four subfractions (A–D). On the basis of TLC analysis, subfraction A (600 mg) was further separated over a silica gel column eluting with petroleum ether–acetone (9:1) to yield β-amyrin (20 mg) and oleanolic acid (45 mg). Repeated silica gel column separation of subfraction D (800 mg) using the eluent petroleum ether–acetone (8:1) afforded erythrodiol (16 mg), urs-2β,3β-dihydroxy-12-en-28-oic acid (25 mg), and β-maslinic acid (30 mg). The fraction (100:20, v:v, 500 mg) was chromatographed over silica gel column eluting with EtOAc–CH_3OH (13:1) to yield three subfractions (E–G) on the basis of TLC analysis. Subfraction F (100 mg) was resubjected to column chromatography (C_{18} RP silica gel,

CH_3OH-H_2O 15:85) for several times to afford **1** (15 mg) and **2** (10 mg).

3.3.1 Oleuricine A (*1*)

A yellow gum, $C_{31}H_{42}O_{18}$; $[\alpha]_D^{20}$ −137 ($c = 1.00$, MeOH); UV (MeOH) λ_{max} (log ε): 282 (6.55), 244 (6.77) nm; IR (KBr) ν_{max} (cm^{-1}): 3374, 2922, 1702, 1629, 1521, 1439, 854; ^1H and ^{13}C NMR spectral data: see Table 1; HR-ESI-MS: *m/z* 720.2719 $[M+NH_4]^+$ (calcd for $C_{31}H_{46}O_{18}N^+$, 720.2709).

3.3.2 Oleuricine B (*2*)

A yellow gum, $C_{31}H_{42}O_{18}$; $[\alpha]_D^{20}$ −54 ($c = 1.00$, MeOH); UV (MeOH) λ_{max} (log ε): 276 (6.38), 248 (6.55), 234 (6.54) nm; IR (KBr) ν_{max} (cm^{-1}): 3361, 2921, 1705, 1630, 1509, 1436, 823; ^1H and ^{13}C NMR spectral data: see Table 1; and HR-ESI-MS: *m/z* 725.2251 $[M+Na]^+$ (calcd for $C_{31}H_{42}O_{18}Na^+$, 725.2263).

3.4 Acid hydrolysis

A solution of compound **1** or **2** (each *c*.5 mg) in H_2O (8 ml) was treated with 2 N HCl (2 ml) at 80°C for 10 h. The solution was evaporated under reduced pressure to yield a residue. The residue was dissolved in MeOH. The solvent system EtOAc–pyridine–H_2O (2:2:1) was used for PC identification of glucose.

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References

[1] G. Montedoro, M. Servili, M. Baldioli, R. Selvaggini, E. Miniati, and A. Macchioni, *J. Agric. Food Chem.* **41**, 2228 (1993).
[2] M. Brenes, A. Garcia, P. Garcia, J.J. Rios, and A. Garrido, *J. Agric. Food Chem.* **47**, 3535 (1999).
[3] G. Bisignano, A. Tomaino, R.L. Cascio, G. Crisafi, N. Uccella, and A. Saija, *J. Pharm. Pharmacol.* **51**, 971 (1999).
[4] C.M. Bitler, T.M. Viale, B. Damaj, and R. Crea, *J. Nutr.* **135**, 1475 (2005).
[5] F. Visioli, G. Bellomo, C. Montedoro, and C. Galli, *Atherosclerosis* **117**, 25 (1995).
[6] H. Kuwajima, T. Uemura, K. Takaishi, K. Inoue, and H. Inouyet, *Phytochemistry* **27**, 1757 (1988).
[7] P.A.J. Gorin and M. Mazurek, *Can. J. Chem.* **53**, 1212 (1976).