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A new ellagic acid derivative from the fruits of *Eucalyptus globulus* Labill.

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Four ellagic acid derivatives have been isolated from the fruits of *Eucalyptus globulus* Labill., one of which is new compound, identified as 3-*O*-methylellagic acid 4′-*O*-α-L-2″-*O*-acetyl-rhamnopyranoside (**1**), the known compounds were identified as 3-*O*-methylellagic acid 4′-*O*-α-L-rhamnopyranoside (**2**), ellagic acid (**3**) and 3-*O*-methylellagic acid (**4**), on the basis of the analysis of ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, IR and MS spectral data. It is also assignment the ^{13}C NMR signals of 3-*O*-methylellagic acid 4′-*O*-α-L-rhamnopyranoside for the first.

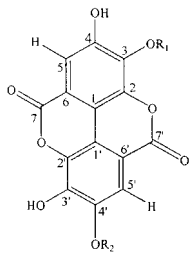
1. Introduction

Eucalyptus globulus Labill (Myrtaceae) is a medicinal plant widely distributed in southern China. *Eucalyptus* species have been used for medicinal purposes, for example, their leaves, barks and fruits have been used to treat colds, influenza, dysentery, enteritis, fevers, diarrhea, rheumatalgia and other complaints (Jiangsu New Medical College 1982; Xu et al. 1984). Besides volatile terpenoid constituents in the essential oil of leaves and fruits, several biologically active compounds were isolated mainly from the leaves of *Eucalyptus*, including triterpenoids (Begum et al. 2002), phloroglucinol derivatives (Ghisalberti 1996; Shibuya et al. 2001), flavonoids (Manguro et al. 1995) and tannins (Hou et al. 2000). Several ellagic acid rhamnosides were isolated from the stem bark (Kim et al. 2001) and wood (Yazaki and Hillis 1976). The present paper describes the isolation and structural elucidation of a new ellagic acid rhamnoside and first assignment the ^{13}C NMR of 3-*O*-methylellagic acid 4′-*O*-α-L-rhamnopyranoside.

2. Investigations, results and discussion

The 95% aqueous ethanolic extract of the fruit of *E. globulus* was successively partitioned with cyclohexane, EtOAc and *n*-BuOH. The EtOAc fraction was applied on silica gel column chromatography, and then further purified by Sephadex LH-20 column chromatography. A new ellagic acid acetylrhamnoside (**1**) was obtained, together with three known compounds, 3-*O*-methylellagic acid 4′-*O*-α-L-rhamnopyranoside (**2**), ellagic acid (**3**) and 3-*O*-methylellagic acid (**4**).

Compound **1** was obtained as a white needle crystal (MeOH), so its molecular formula was deduced to be $C_{23}H_{20}O_{13}$; the FT-ICR-HR-MS measured with negative ion mode gave a parent ion peak at m/z 503.0827 ($[M-H]^-$ calc. for $C_{23}H_{19}O_{13}$ requires 503.0826). The IR spectrum displayed characteristic absorptions for hydroxyl groups (3384 cm^{-1}), α,β-unsaturated lactone functions (1713 cm^{-1}) and aromatic rings (1605 cm^{-1}). In its ^{1}H NMR spectrum, ten proton signals including two aromatic singlets (δ 8.40 and 8.04), a methoxyl singlet signal (δ 4.17), and five oxygenated methine protons were observed, a doublet methyl signal (δ 1.21, J = 6.2 Hz) indicating the existence of one 6-deoxysugar, along with an acetyl group. The glycoside was easily determined as rhamnose through analysis of the chemical shifts and coupling patterns of its proton signals, the position of acetyl group were determined by the ^{1}H-^{13}C long-rang correlations in the HMBC spectra. The presence of an ellagic acid moiety in **1** was easily revealed by comparison of the ^{13}C NMR of **1** with that of **4**. Further analysis of the NMR spectra showed that **1** consisted of an ellagic acid moiety, a rhamnopyranosyl residue, and an acetyl group. These results suggested the structure of **1** was *O*-methylellagic acid correlated with acetylrhamnose. Compound **1** was very similar to 3′-*O*-(2″-*O*-acetyl-α-rhamnopyranosyl)-3-*O*-methylellagic acid (Kim et al. 2001) in its IR and ^{13}C NMR spectral data. However, the attachment position of sugar moiety to aglycone was different. The rhamnose attachment position and the structure of **1** were established through the interpretation of HSQC and HMBC spectral data. From the HSQC spectrum, aromatic carbons (δ 112.8 and 115.1) bearing singlet methine protons (δ 8.04 and 8.40, respectively) were correlated (C-5 and C-5′, respectively).



	R_1	R_2
1	CH_3	α-L-2″-*O*-acetylrhamnopyranoside
2	CH_3	α-L-rhamnopyranoside
3	H	H
4	CH_3	H

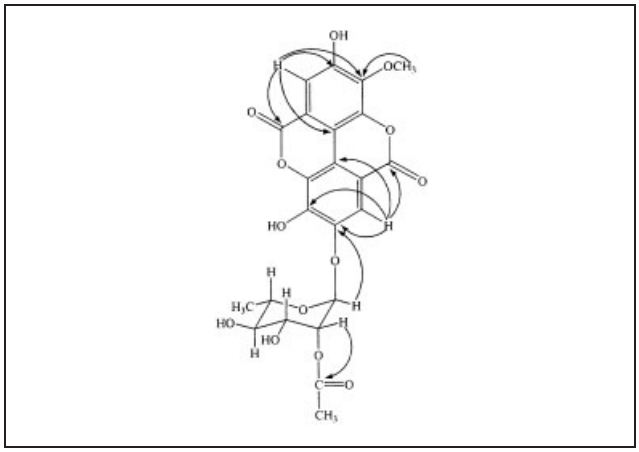


Fig.: Significant HMBC correlation of (H to C)

In the HMBC spectral data (Fig.), ^{1}H-^{13}C long range correlations were observed between the methoxyl protons (δ 4.17) to C-3 (δ 141.3), from the aromatic proton at δ 8.04 (H-5) to the lactone carbonyl carbon at δ 159.7 (C-7), and quaternary carbons at δ 112.1 (C-1), and oxygenated aromatic carbon 141.3 (C-3), 154.3 (C-4). Long-range correlations from the other aromatic proton at δ 8.40 (H-5′) to another lactone carbonyl carbon at δ 159.4 (C-7′), the quaternary carbon at δ 115.8 (C-1′) and oxygenated aromatic carbon at δ 144.5 (C-3′), 147.5 (C-4′) were also observed in the aglycone of **1**. In the sugar moiety, the ^{1}H-^{13}C long-range correlation from the anomeric proton at δ 6.25 to C-4′ (δ 147.5) ascertained that the sugar was attached to C-4′-*O* at the aglycone. The other ^{1}H-^{13}C long-range correlations from the down-field shifted methine proton at δ 6.14 (H-2″) to the carbonyl carbon of the acetyl group at δ 170.8 indicated that the sugar was 2″-*O*-acetylrhamnopyranoside. All of the ^{1}H NMR and most of the ^{13}C NMR chemical shifts were assigned unambiguously from the data obtained from HSQC and HMBC, except that a few quaternary carbons (C-2, C-6, C-2′, and C-6′) could not be unambiguously assigned. All of the NMR assignments agreed well with those of ellagic acid derivatives reported by Li et al. (Li et al. 1999).

Accordingly, the structure of **1** was established as 3-*O*-methylellagic acid 4′-*O*-α-L-2″-*O*-acetylrhamnopyranoside.

Compound **2** was similar as 3-*O*-methylellagic acid 4′-*O*-α-rhamnoside (Yazaki and Hillis 1976) in its IR and ^{1}H NMR spectral data. Compounds **2** was similar to **1** except short of the acetyl group. According to the ^{13}C NMR assignments of compound **1** and **4**, the ^{13}C NMR of compound **2** was first assigned (Table).

In this study, we isolated a new ellagic acid rhamnoside, 3-*O*-methylellagic acid 4′-*O*-α-L-2″-*O*-acetylrhamnopyranoside (**1**). Until now, only eight ellagic acid rhamnosides have been isolated from plant sources, these are 4-(α-L-rhamnopyranosyl) ellagic acid (Yang et al. 1998), 4-*O*-(4″-*O*-acetyl-α-L-rhamnopyranosyl) ellagic (Yukihiro et al. 2003), 4-*O*-(α-L-rhamnopyranosyl)-3,3′-di-*O*-methylellagic acid (Malhorta and Misra 1981), 3-*O*-methylellagic acid 4′-*O*-α-L-rhamnoside (Yazaki and Hillis 1976), 3′-*O*-(2″-*O*-acetyl-α-L-rhamnopyranosyl)-3-*O*-methylellagic acid, 3′-*O*-(3″-*O*-acetyl-α-L-rhamnopyranosyl)-3-*O*-methylellagic acid, 3′-*O*-(4″-*O*-acetyl-α-L-rhamnophyranosyl)-3-*O*-methylellagic acid, and 3′-*O*-(α-L-rhamnopyranosyl)-3-*O*-methylellagic acid (Kim et al. 2001). The last five compounds were isolated from *E. globulus*. So this plant is a natural source of ellagic acid rhamnosides.

3. Experimental

3.1. General

CC: silica gel (Tsingtao Marine Chemistry Co. Ltd, 200–300 mesh) and Sephadex LH-20 (Pharmacia Co. Ltd.), the eluents were $CHCl_3$–MeOH (100 : 1 → 1 : 1) and MeOH, respectively. IR: KBr disc. EI-MS, ESI-TOF-MS and FT-ICR-HR-MS were performed on Finnigan TRACE 2000, MDS SCIEX API QSTAR and APEX II FT-ICR (Bruker Daltonics) mass spectrometer, respectively. NMR spectra were performed on a Varian INOVA

Table: ^{1}H and ^{13}C NMR spectral data for compounds 1 and 2 (δ_H, 500 MHz; δ_C, 125 MHz)

carbon	**1**		**2**		
	δ_H[a]	δ_C[a]	δ_H[b]	δ_H[a]	δ_C[a]
1		112.1			112.7
2		142.8			142.8
3		141.3			141.3
4		154.3			154.2
5	8.04 (1 H, s)	112.7	7.51 (1 H, s)	8.03 (1 H, s)	112.2
6		114.3			114.9
7		159.7			159.8
1′		115.8			115.4
2′		137.6			137.6
3′		144.5			144.3
4′		147.5			148.0
5′	8.40 (1 H, s)	115.1	7.70 (1 H, s)	8.48 (1 H, s)	114.3
6′		107.5			107.7
7′		159.4			159.5
1″	6.26 (1 H, s)	99.0	5.48 (1 H, br.s)	6.42 (1 H, br.s)	102.1
2″	6.14 (1 H, t-like, 2.0, 1.5)	73.4	4.01 (1 H, br.s)	4.90 (1 H, br.s)	71.9
3″	4.89 (1 H, dd, 9.5, 3.5)	70.2	3.85 (1 H, dd, 6.0)	4.76 (1 H, dd, 9.5, 3.5)	71.5
4″	4.28 (1 H, t, 9.5)	73.8	3.34 (1 H, t, 9.5)	4.40 (1 H, t, 9.5)	73.7
5″	4.60 (1 H, dq, 9.5, 6.5)	71.4	3.55 (1 H, m)	4.59 (1 H, dq, 9.5, 6.5)	72.6
6″	1.67 (3 H, d, 6.5)	18.4	1.14 (3 H, d, 6.0)	1.65 (3 H, d, 6.5)	18.6
OCH_3	4.17 (3 H, s)	61.3	4.05 (3 H, s)	4.17 (3 H, s)	61.3
COO		170.8			
CH_3	2.10 (3 H, s)	21.0			

[a] in pyridine-d_5; [b] in DMSO-d_6, J values (Hz) are in parentheses

500 spectrometer. All the NMR experiments were recorded at room temperature, operating at 499.89 MHz for ^{1}H and 125.71 MHz for ^{13}C with TMS as int. standard.

3.2. Plant material

Fruits of *Eucalyptus globulus* were obtained from the Jinggangshan region in Jiangxi Province of CHINA, in August 2002 and identified by Professor Chen Hubiao. A voucher specimen of the plant is deposited at Herbarium of School of Pharmaceutical Sciences, Peking University.

3.3. Extraction and isolation

Powdered fruits of *E. globulus* (4.5 kg) were refluxed with 95% EtOH for 3 times (3 h/times). The ethanolic extract was filtered and concentrated *in vacuo*. The residue was suspended in 95% EtOH and partitioned with cyclohexane, to give a cyclohexane extract. The 95% EtOH layer was concentrated *in vacuo* and redissolved in water and partitioned successively with ethyl acetate (EtOAc) and BuOH to afford corresponding extracts. The EtOAc-soluble part was concentrated *in vacuo*, and the residue (93.4 g) was applied on silica gel column chromatography eluted with a $CHCl_3$–MeOH gradient from (100 : 1 → 1 : 1). Six major fractions (Fr. A–Fr. F) were obtained from concentrated eluates. Fr. A was subjected to further silica gel column chromatography using $CHCl_3$–MeOH from 30 : 1 to 1 : 1 as eluent to give 4 fractions (Fr. A-1–Fr. A-4). Compound **1** (10 mg) was crystallized from the Fr. A-3. Compound **2** (67.3 mg) was crystallized from the Fr. C Fr. D was subjected to further silica gel column chromatography with $CHCl_3$–MeOH and the Sephadex LH-20 chromatography with MeOH as eluant to afford **3** (1861.6 mg) and **4** (84.9 mg).

3.4. 3-O-Methylellagic acid 4′-O-α-L-2″-O-acetylrhamnopyranoside (1)

White needly crystal (MeOH), IR ν_{max} (KBr) cm^{-1}: 3384, 2936, 1713, 1605, 1495, 1430, 1370, 1260, 1127, 1057. The FT-ICR-HR-MS: m/z 503.0827 ([M−H]$^-$ calc. for $C_{23}H_{19}O_{13}$ requires 503.0826), 443.0613 [M−$OCOCH_3$]$^-$, 315.0141 [M−$COCH_3$−Rha]$^-$, 299.9911 [M−$COCH_3$−Rha−CH_3]$^-$. ESI-TOF-MS m/z 503.0574 [M−M]$^-$ ($C_{23}H_{19}O_{13}$) requires 503.0825). ^{1}H NMR, ^{13}C NMR: Table.

3.5. 3-O-Methylellagic acid 4′-O-α-L-rhamnopyranoside (2)

White needly crystal (MeOH), IR ν_{max} (KBr) cm^{-1}: 3421, 2920, 1741, 1607, 1493, 1439, 1353, 1268, 1208, 1116, 1106, 1057. ESI-TOF-MS m/z 461.0423 [M−H]$^-$ ($C_{23}H_{19}O_{13}$ requires 461.0720). ^{1}H NMR, ^{13}C NMR: Table.

3.6. Ellagic acid (3)

Yellow powder, IR ν_{max} (KBr) cm^{-1}: 3356, 3073, 1698, 1613, 1583, 1509, 1448, 1396, 1340, 1259, 1195, 1111, 1057. EI-MS m/z 302.2. NMR data were in agreement with the reported data for ellagic acid (Li et al. 1999).

3.7. 3-O-Methylellagic acid (4)

Yellow powder, IR ν_{max} (KBr) cm^{-1}: 342, 3073, 1723, 1606, 1583, 1496, 1427, 1346, 1194, 1111, 1062. ESI-TOF-MS m/z 315.0053 [M−M]$^-$. NMR data were in agreement with the reported data for 3-O-methylellagic acid (Tanaka et al. 1998).

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