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A NEW FLAVAN GLUCOSIDE FROM *GLOCHIDION ERIOCARPUM*

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ABSTRACT

*From the aerial parts of Glochidion eriocarpum, a new flavan glucoside, 5,7,3',4'-tetra-O-methyl-ent-epicatechin 3-O-β-D-glucopyranoside (**1**), a known flavan, 5,7,3',4'-tetra-O-methylepicatechin (**2**), and three known triterpenes, lupeol (**3**), 3-epi-lupeol (**4**), and glochidonol (**5**), were isolated by using combined chromatographic separations. Their structures were elucidated on the basis of spectroscopic data.*

Key words: *Glochidion eriocarpum, Euphorbiaceae, flavan, ent-epicatechin.*

I - INTRODUCTION

Glochidion eriocarpum Champ. is shrub or treelet to 5 m high, monoecious; branchlets densely spreading yellowish or gray-yellow villous. This species is abundantly found throughout Vietnam. The roots and leaves are used in Vietnamese folk medicine to treat enteritis, indigestion, asthma, cholera, and rheumatism [1]. Previous investigations resulted in the isolation of eight lupanes from the roots and stem wood: lupeol; 3-*epi*-lupeol; lupenone; glochidol; glochidone; glochidonol; glochidiol; and lup-20(29)-ene-1β,3β-diol [2-3]. Recently, we have reported the isolation and determination of two triterpenoid saponins, glochieriosides A and B, along with three lupanes, glochidone, lup-20(29)-ene-3β,23-diol, and lup-20(29)-ene-1β,3β-diol, from the aerial parts. Among them, two saponins exhibited significant cytotoxic activity against various cancer cells [4].

In continuation of our investigation on this plant, we reported herein the isolation, structural elucidation, and cytotoxic evaluation of a new flavan glucoside, 5,7,3',4'-tetra-*O*-methyl-*ent*-epicatechin 3-*O*-β-D-glucopyranoside (**1**), a known flavan, 5,7,3',4'-tetra-*O*-methylepicatechin (**2**), and three known triterpenes, lupeol (**3**), 3-epi-lupeol (**4**) and glochidonol (**5**), from the aerial parts of *G. eriocarpum*.

II - EXPERIMENTAL

1. General

Optical rotations were determined on a Jasco DIP-370 digital polarimeter. The electrospray ionization (ESI) mass spectra were obtained using an AGILENT 1200 LC-MSD Trap spectrometer. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70 — 230 mesh and 230 — 400 mesh, Merck) and YMC RP-18 resins. Thin layer chromatography (TLC) was performed on DC-Alufolien 60 silica gel F_{254} (Merck

1.05554.0001) or DC Platen RP_{18} F_{254s} (Merck 1.15685.0001) plates. Spots were visualized by spraying 10% H_2SO_4 aqueous and heating for 5 min.

2. Plant material

The aerial parts of *G. eriocarpum* were collected at Tamdao National Botanical Park, Vinhphuc, Vietnam during December 2006 and identified by Dr Tran Huy Thai, Institute of Ecology and Biological Resources, VAST, Vietnam. An authentic sample (N° VP14) was deposited at the herbarium of the Institute of Natural Products Chemistry, VAST, Vietnam.

3. Extraction and isolation

Aerial parts of *G. eriocarpum* (7.0 kg) were powdered and extracted three times with hot MeOH (50°C) to give the methanol extract (500 g), which was then suspended in water and extracted in turn with *n*-hexane, chloroform, and ethyl acetate, giving corresponding extracts. The *n*-hexane (10 g) and chloroform (113 g) extracts were combined and crudely separated on a silica gel CC using stepwise gradient elution with *n*-hexane–acetone (20:1, 10:1, 5:1, 2.5:1, 1:1, and 0:1, v/v) to yield six sub-fractions, F1–F6. Sub-fraction F1 was further separated into five smaller fractions, F1A-F1E by a silica gel CC using stepwise gradient of *n*-hexane–ethyl acetate (50:1–1:1, v/v). Compounds **3** (35 mg) and **4** (15 mg) were purified from fraction F1A by a silica gel column using an eluent of *n*-hexane–ethyl acetate (35:1, v/v), followed by an YMC RP-18 column chromatography (acetone–water, 5:1, v/v). Fraction F1B was further separated using a silica gel CC eluting with *n*-hexane–ethyl acetate (5:1, v/v), followed by an YMC RP-18 CC (acetone–water, 2:1, v/v) to obtain compound **5** (30 mg). The ethyl acetate extract (25 g) was separated into seven fractions, E1-E7, by a silica gel CC using stepwise gradient elution with $CHCl_3$–MeOH (50:1, 20:1, 10:1, 5:1, 2.5:1, 1:1, and 0:1, v/v). Compound **2** (15 mg) was purified from fraction E2 (3 g) by a silica gel column with $CHCl_3$–MeOH (15:1, v/v). Fraction E4 (5 g) was further separated by a silica gel column using $CHCl_3$–MeOH–H_2O (10:1:0.1, v/v/v) to obtain compound **1** (25 mg).

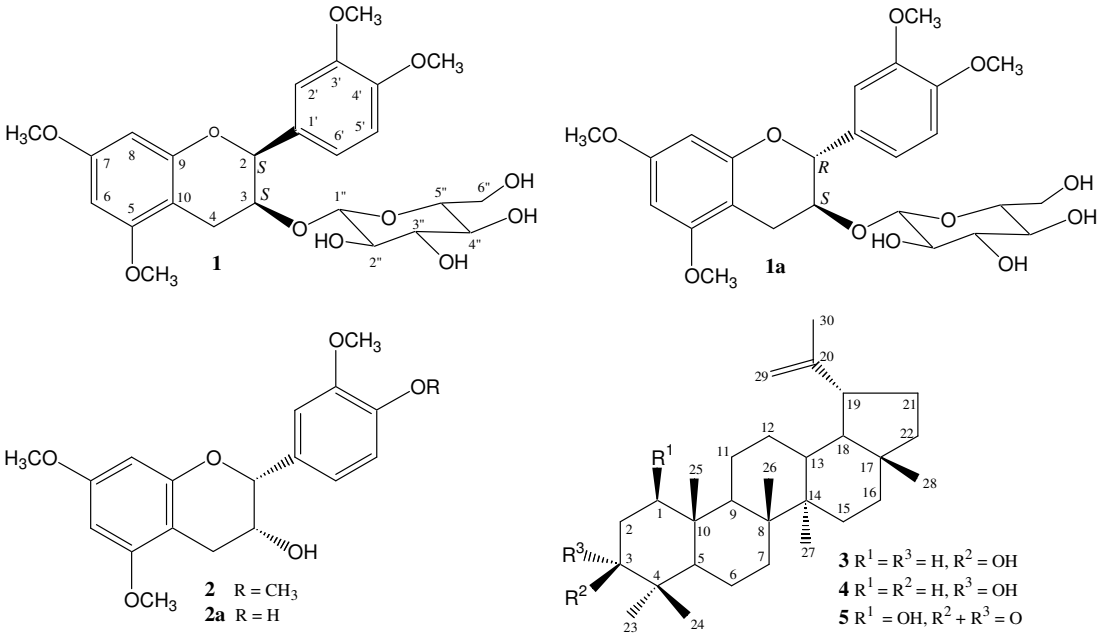

Figure 1: Structures of **1**-**5**, **1a,** and **2a**

5,7,3',4'-Tetra-*O*-methyl-*ent*-epicatechin 3-*O*-β-D-glucopyranoside (**1**): White powder; Positive *ESI-MS*: *m/z* 531 $[M+Na]^+$, 509 $[M+H]^+$, and 347 [M-glucose + H]$^+$, ($C_{25}H_{32}O_{11}$, M = 508); ^{1}*H-NMR* (500 MHz, CD_3OD) and ^{13}*C-NMR* (125 MHz, CD_3OD), see table 1.

5,7,3',4'-Tetra-*O*-methylepicatechin (**2**): White powder: Positive *ESI-MS*: *m/z* 347 [M + H]$^+$, ($C_{19}H_{22}O_6$, M=346); ^{1}*H-NMR* (500 MHz, CD_3OD) and ^{13}*C-NMR* (125 MHz, CD_3OD), see Table 2.

Lupeol (**3**): White crystals; Mp. 215-216°C; Positive *ESI-MS*: *m/z* 427 $[M + H]^+$ ($C_{30}H_{50}O$, M = 426); ^{1}*H-NMR* (500 MHz, $CDCl_3$): δ (ppm) 3.19 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 2.38 (1H, ddd, *J* = 11, 11, 5.5 Hz, H-19), 0.97 (3H, s, H-23), 0.76 (3H, s, H-24), 0.83 (3H, s, H-25), 1.03 (3H, s, H-26), 0.95 (3H, s, H-27), 0.79 (3H, s, H-28), 4.69 (1H, d, *J* = 2.0 Hz, H_a-29), 4.56 (1H, d, *J* = 1.0 Hz, H_b-29), and 1.68 (3H, s, H-30); ^{13}*C-NMR* (125 MHz, $CDCl_3$): δ (ppm) 38.76 (C-1), 27.46 (C-2), 79.04 (C-3), 38.89 (C-4), 55.36 (C-5), 18.36 (C-6), 34.34 (C-7), 40.89 (C-8), 50.50 (C-9), 37.22 (C-10), 20.98 (C-11), 25.21 (C-12), 38.12 (C-13), 42.88 (C-14), 27.49 (C-15), 35.63 (C-16), 43.03 (C-17), 48.37 (C-18), 48.02 (C-19), 150.98 (C-20), 29.90 (C-21), 40.04 (C-22), 28.02 (C-23), 15.38 (C-24), 16.13 (C-25), 16.01 (C-26), 14.58 (C-27), 18.03 (C-28), 109.33 (C-29), and 19.34 (C-30).

3-*epi*-Lupeol (**4**): White crystals; Mp. 199-200°C; Positive *ESI-MS*: 427 $[M + H]^+$ ($C_{30}H_{50}O$, M = 426); ^{1}*H-NMR* (500 MHz, $CDCl_3$): δ (ppm) 3.38 (1H, br s, H-3), 2.38 (1H, ddd, *J* = 11.0, 11.0, 5.5 Hz, H-19), 0.82 (3H, s, H-23), 0.93 (3H, s, H-24), 0.84 (3H, s, H-25), 1.03 (3H, s, H-26), 0.96 (3H, s, H-27), 0.78 (3H, s, H-28), 4.68 (1H, br d, *J* = 1.5 Hz, H_a-29), 4.56 (1H, d, *J* = 1.0 Hz, H_b-29), and 1.68 (3H, br s, H-30); ^{13}*C-NMR* (125 MHz, $CDCl_3$): δ (ppm) 33.28 (C-1), 25.44 (C-2), 76.25 (C-3), 37.54 (C-4), 49.05 (C-5), 18.30 (C-6), 34.18 (C-7), 41.06 (C-8), 50.25 (C-9), 37.32 (C-10), 20.82 (C-11), 25.16 (C-12), 38.06 (C-13), 43.03 (C-14), 27.42 (C-15), 35.62 (C-16), 42.94 (C-17), 48.34 (C-18), 48.04 (C-19), 150.99 (C-20), 29.89 (C-21), 40.03 (C-22), 28.26 (C-23), 22.15 (C-24), 16.00 (C-25), 15.93 (C-26), 14.66 (C-27), 18.02 (C-28), 109.31 (C-29), and 19.31 (C-30).

Glochidonol (**5**): White crystals; Mp. 228-230°C; Positive *ESI-MS*:441 $[M+H]^+$ ($C_{30}H_{48}O_2$, M = 440); ^{1}*H-NMR* (500 MHz, $CDCl_3$): δ (ppm) 3.89 (1H, m, H-1), 3.00 (1H, dd, *J* = 14.5; 8.0 Hz, H_a-2), 2.23 (1H, dd, *J* = 14.5, 4.0 Hz, H_b-2), 1.36 (1H, H-5), 1.31 (1H, H_a-7), 1.49 (1H, H_b-7), 1.51 (1H, H-9), 1.71 (1H, H-13), 1.41 (1H, H_a-16), 1.51 (1H, H_b-16), 1.41 (1H, H-18), 2.37 (1H, ddd, *J* = 11.0, 11.0, 5.5 Hz, H-19), 1.26 (1H, H_a-21), 1.92 (1H, H_b-21), 1.21 (1H, H_a-22), 1.42 (1H, H_b-22), 1.06 (3H, s, H-23), 1.06 (3H, s, H-24), 0.84 (3H, s, H-25), 1.05 (3H, s, H-26), 0.98 (3H, s, H-27), 0.80 (3H, s, H-28), 4.56 (1H, br s, H_a-29), 4.68 (1H, d, *J* = 1.5 Hz, H_b-29), and 1.68 (3H, s, H-30); ^{13}*C-NMR* (125 MHz, $CDCl_3$): δ (ppm) 79.69 (C-1), 45.19 (C-2), 215.51 (C-3), 47.17 (C-4), 51.53 (C-5), 19.66 (C-6), 33.07 (C-7), 43.04 (C-8), 50.81 (C-9), 43.01 (C-10), 23.17 (C-11), 25.31 (C-12), 38.09 (C-13), 41.26 (C-14), 27.59 (C-15), 35.61 (C-16), 43.04 (C-17), 48.38 (C-18), 47.99 (C-19), 150.73 (C-20), 29.90 (C-21), 40.04 (C-22), 27.91 (C-23), 19.91 (C-24), 11.77 (C-25), 16.02 (C-26), 14.50 (C-27), 18.08 (C-28), 109.49 (C-29), and 19.34 (C-30).

III - RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR spectra were typical for a flavan glycoside. The ^{1}H-NMR spectrum exhibited three ABX-type aromatic protons at δ_H 7.27 (1H, d, *J* = 1.5 Hz, H-2'), 6.94 (1H, d, *J* = 8.5 Hz, H-5'), and 7.07 (1H, dd, *J* = 8.5, 1.5 Hz, H-6'), indicating a 1',3',4'-trisubsituted B ring. Two *m*-coupled aromatic proton signals at δ_H 6.16 (2H, br s) were typical for proton H-6 and H-8 of the A ring. The presence of a sugar moiety was determined by an anomeric proton signal at δ_H 4.05 (1H, d, *J* = 7.5 Hz, H-1''). The large coupling constant of the anomeric proton indicated a β-glycosidic linkage. Moreover, the signals at δ_H 3.77, 3.80, 3.85, and 3.88 (each 3H, s) indicated four methoxyl groups.

Table 1: NMR data of **1** and **1a**

C	1a		1		
	δ_C [#]	δ_H [#] mult. (*J* = Hz)	δ_C [a,b]	DEPT	δ_H [a,c] mult. (*J* = Hz)
2	80.5	4.98 d (6.5)	79.05	CH	5.06 br s
3	75.4	4.31 m	74.82	CH	4.44 m
4	26.9	2.80 dd (16.5, 5.5) (α) 2.87 dd (16.5, 6.5) (β)	28.37	CH_2	2.92 m
5	160.2	-	161.08	C	-
6	92.5	6.13 d (2.5)	92.55	CH	6.16 s
7	161.3	-	160.36	C	-
8	95.43	6.11 d (2.5)	94.53	CH	6.16 s
9	156.5	-	156.86	C	-
10	102.9	-	102.7	C	-
1'	133.5	-	132.86	C	-
2'	112.8	7.01 d (1.6)	112.41	CH	7.27 d (1.5)
3'	150.4	-	150.17	C	-
4'	150.5	-	149.98	C	-
5'	112.0	6.93 d (8.5)	112.41	CH	6.94 d (8.5)
6'	120.7	6.96 dd (8.5, 1.5)	120.52	CH	7.07 dd (8.5, 1.5)
glc					
1''	103.6	4.05 d (7.5)	104.81	CH	4.05 d (7.5)
2''	75.3	3.1 dd (9.0, 7.5)	75.31	CH	3.11*
3''	77.9	3.18 dd (9.0, 9.0)	77.80	CH	3.18*
4''	71.7	3.23 dd (9.0, 9.0)	71.53	CH	3.24*
5''	78.3	3.14 m	77.99	CH	3.12*
6''	63.0	3.85 dd (12.0, 2.3) 3.64 dd (12.0, 6.0)	62.80	CH_2	3.83* 3.66 dd (12.0, 6.0)
4'-OMe	56.6	3.83 s	56.54	CH_3	3.85 s
3'-OMe	56.6	3.82 s	56.46	CH_3	3.88 s
7-OMe	56.0	3.79 s	55.93	CH_3	3.80 s
5-OMe	55.9	3.73 s	55.76	CH_3	3.77 s

[a]Measured in CD_3OD, [b]125 MHz, [c]500 MHz, [#]data of **1a** [5], *overlapped signals.

The ^{13}C-NMR spectrum of **1** revealed 25 carbon signals including four CH_3, two CH_2, twelve CH and seven quaternary carbons, detected by DEPT experiments. The sugar carbon signals at δ_C 104.81 (CH, C-1''), 75.31 (CH, C-2''), 77.80 (CH, C-3''), 71.53 (CH, C-4''), 77.99 (CH, C-5''), and 62.80 (CH_2, C-6'') indicated a *β*-D-glucopyranose [5]. The presence of four methoxyl groups were identified by carbon signals at δ_C 55.76, 55.93, 56.46, and 56.54. Besides twelve carbons of two aromatic rings, the glycol of **1** contained two oxygenated methines at δ_C 79.05 (C-2) and 74.82 (C-3) and one methylene at δ_C 28.37 (C-4), confirming a flavan skeleton structure. All carbons were assigned to relevant protons by an HSQC experiment.

The molecular formula of **1** was suggested

as $C_{25}H_{32}O_{11}$ by ESI-MS peaks at *m/z* 531 $[M+Na]^+$, 509 $[M+H]^+$, and 347 [M - glucose + $H]^+$. The NMR data of **1** resembled those of 5,7,3',4'-tetra-*O*-methylcatechin 3-*O*-β-D-glucose (**1a**) [5] as shown in Table 1. These evidence and H-C long-range correlations (*Fig. 2*) observed in the HMBC spectrum of **1** indicated that the two compounds had the same planar structure. Attachment of the glucosyl moiety at C-3 was confirmed by HMBC correlations from H-1" (δ_H 4.05) to C-3 (δ_C 74.82) and H-3 (δ_H 4.44) to C-1" (δ_C 104.81). Placements of four methoxyl groups at C-5, C-7, C-3' and C-4' were assigned by HMBC cross peaks from their protons at δ_H 3.77, 3.80, 3.88, and 3.85, to the corresponding carbons at δ_C 161.08 (C-5), 160.36 (C-7), 150.17 (C-3'), and 149.98 (C-4'), respectively.

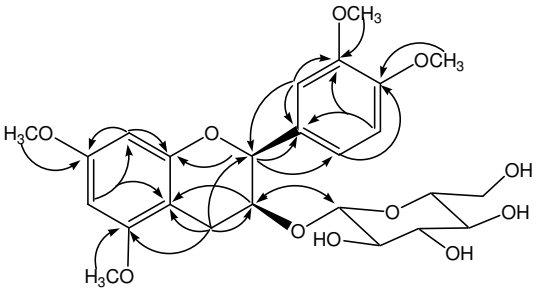

Fig. 2: Key HMBC correlations of **1**

The differences of NMR data between **1** and **1a** were only recognized from C-2 to C-4, confirming that the two compounds had different stereochemistry. Flavan 3-ol skeleton has four configurations at C-2 and C-3 as 2*R*,3*R*, 2*S*,3*S*, 2*S*,3*R*, and 2*R*,3*S*. To address the corrected configuration for **1**, its NMR data were carefully compared with similar reported compounds possessing all four configurations. As the results, the broad singlet peak (J ~ 0 Hz) of proton H-2 for **1** indicated *cis*-relationship between H-2 and H-3, versus *J* = 6.5 - 8.5 Hz for *trans* derivatives [6-9]. In addition, the proton chemical shift of H-3 (δ_H 4.44) was suitable for 3*S*-derivatives [6 - 8]. Thus the 2*S*,3*S* configuration was assigned for **1**. From all the above evidence, the structure of **1** was characterized as 5,7,3',4'-tetra-*O*-methyl-*ent*-epicatechin 3-*O*-β-D-glucopyranoside, a new compound from nature.

The NMR data of **2** were identical to those of **1**, except for the absence of the sugar moiety and this was supported by ESI-MS peak at *m/z* 347 $[M + H]^+$, corresponding to the molecular formula of $C_{19}H_{22}O_6$ (M=346). The ^{1}H-NMR spectrum of **2** also exhibited four methoxyl groups at δ_H 3.76, 3.79, 3.88, and 3.91; three ABX-type aromatic protons at δ_H 7.08 (d, *J* = 1.5 Hz), 7.05 (dd, *J* = 8.5, 1.5 Hz), 6.91 (d, *J* = 8.5 Hz); and two m-coupled aromatic protons at δ 6.11 and 6.19 (each d, *J* = 2.0 Hz). Nineteen carbons signals was observed in the ^{13}C-NMR spectrum of **2**, including four CH_3, one CH_2, seven CH and seven quaternary carbons, detected by DEPT experiments. An HSQC experiment allowed assignment of all carbons to relevant protons and the results were summarized in Table 2. The NMR data of **2** also resembled those of 4'-hydroxy-5,7,3'-trimethoxylflavan-3-ol (**2a**), except for the addition signals of a methoxyl group. The differences between the two compounds were only observed from C-2' to C-6', suggested attachment of the methoxyl group at C-4', which was confirmed by HMBC correlation between 4'-OMe proton (δ 3.88) to C-4' (149.02). The excellent agreement of ^{13}C-NMR data from C-2 to C-4 between **2** and **2a** (table 2) allowed assignment of the configuration at C-2 and C-3 to be 2*R*,3*R*-form. Consequently, **2** was elucidated as 5,7,3',4'-tetra-*O*-methylepicatechin. This is the first report of this compound from *Glochidion* species.

Compounds **3** and **4** were isolated as white crystals. Their NMR features indicated that they are lupane-type triterpenes, one typical constituent of *Glochidion* species. The ^{1}H- and ^{13}C-NMR exhibited typical signals for 50 protons and 30 carbons including seven tertiary methyls, one oximethine and one terminal di-substituted double bond. The NMR data of **3** resembled those of **4**, except for signals of the oximethine group. The NMR and ESI-MS data of **3** and **4** (see Experimental) were compared

with the corresponding reported values for lupeol [10] and 3-*epi*-lupeol [3], and found to match. Thus, **3** and **4** were identified as lupeol and 3-*epi*-lupeol, respectively.

Table 2: NMR data of **2** and **2a**

C	**2a**	**2**			
	δ_C [#]	δ_C [a,b]	DEPT	δ_H [a,c] mult. (J = Hz)	HMBC (H→C)
2	78.5	78.50	CH	4.94 (s)	3, 4, 1', 2', 6'
3	66.5	66.44	CH	4.27 (s)	2, 4
4	28.1	28.17	CH_2	2.87 dd (17.0, 4.0) 2.94 dd (17.0, 2.5)	2, 3, 5, 9, 10
5	159.3	159.32	-	-	-
6	93.3	92.25	CH	6.11 d (2.0)	5, 7, 8, 10
7	159.7	159.76	-	-	-
8	92.2	93.52	CH	6.19 d(2.0)	6, 7, 9, 10
9	155.2	155.31	-	-	-
10	100.3	100.4	-	-	-
1'	130.2	130.98	-	-	-
2'	114.4	110.01	CH	7.08 d (1.5)	2, 1', 3', 4', 6'
3'	145.5	149.29	-	-	-
4'	146.7	149.02	-	-	-
5'	109.2	111.48	CH	6.91 d (8.5)	1', 3', 4', 6'
6'	114.4	118.76	CH	7.05 dd (8.5; 1.5)	2, 2', 4', 5',
5-OMe	55.4	55.36	CH_3	3.79 s	5
7-OMe	55.4	55.45	CH_3	3.76 s	7
3'-OMe	56.0	56.02	CH_3	3.91 s	3'
4'-OMe	56.0	56.02	CH_3	3.88 s	4'

[a]Measured in CD_3OD, [b]125 MHz, [c]500 MHz, [#]δ_C data of **2a** [9].

The NMR data of **5** were similar to those of **3** and **4**, except for the presence of a carbonyl group at δ_C 215.51 (C-3). Compound **5** was elucidated as glochidonol by the good agreement of its NMR data with those reported for glochidonol [3] and the ESI-MS ion peak $[M+H]^+$ at *m/z* 441, corresponding to the molecular formula of $C_{30}H_{48}O_2$ (M = 440).

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