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Lanostane triterpene glycosides from the flowers of *Lyonia ovalifolia* var. *hebecarpa* and their antiproliferative activities

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ABSTRACT

Sixteen lanostane-type triterpene glycosides including eight new ones, named lyonicarposides A–H (**1**–**8**), were isolated from the flowers of *Lyonia ovalifolia* var. *hebecarpa* (Franch. ex F.B. Forbes & Hemsl.) Chun (Ericaceae). The chemical structures of the new compounds were elucidated by the comprehensive spectroscopic techniques and chemical methods. The $Mo_2(OAc)_4$–induced electronic circular dichroism method was used to determine the absolute configurations of C-24 in lyonicarposides A (**1**), C (**3**), and E (**5**). This is the first phytochemical study on the flowers of *L. ovalifolia* var. *hebecarpa*. All the isolates were evaluated for their antiproliferative activities against SMMC-7721, HL-60, SW480, MCF-7, and A-549 cell lines. Lyonicarposides A (**1**) and B (**2**) showed moderate antiproliferative activities against five cancer cell lines with IC_{50} values ranging from 12.39 to 28.71 μM. Lyonicarposides C (**3**) and G (**7**) and lyonifoloside M (**12**) selectively inhibited the proliferation of HL-60 and MCF-7 cell lines with IC_{50} values ranging from 13.03 to 17.71 μM. Interestingly, lyonifoloside L (**13**) selectively inhibited the proliferation of MCF-7 cell line with an IC_{50} value of 16.27 μM. Their structure-activity-relationships were discussed.

1. Introduction

Plants of the Ericaceae family, comprising of 103 genera and 3350 species, are widely distributed in temperate regions and sub-frigid zone. *Lyonia* is a small genus of the Ericaceae family, and there are only 35 species all over the world. In China, there are only six species and five varieties in the *Lyonia* genus. Previous phytochemical studies on the *Lyonia* genus mainly focused on *L. ovalifolia* and *L. ovalifolia* var. *elliptica*., leading to the isolation of flavonoids [1,2], phenylpropanoids [2–6], monoterpenoids [7], sesquiterpenoids [7,8], diterpenoids [9–13], and triterpenoids [2,14–16]. Some of them exhibited various biological activities, such as nervous system inhibitory [17], cAMP inhibitory [12], antibacterial [13], antiviral [16], and antihyperglycemic activities [18]. However, the phytochemical study on *Lyonia ovalifolia* var. *hebecarpa* (Franch. ex F.B. Forbes & Hemsl.) Chun is relatively rare.

Lyonia ovalifolia var. *hebecarpa* is a tree or shrub, 8–16 m height, and is mainly distributed in Southern China [19]. In a course of search for biological terpenoids from the Ericaceae plants in China [20–31], the leaves of *Lyonia. ovalifolia* var. *hebecarpa* have been phytochemically studied, leading to the isolation of 16 antiproliferative triterpene glycosides classified into lanostane, oleanane, and 9,10-*seco*-cycloarane types [32,33]. However, heretofore, there is no report of phytochemical studies on the flowers of *L. ovalifolia* var. *hebecarpa*. Expecting the other plant parts to be chemically distinct from the leaves, the flowers of *L. ovalifolia* var. *hebecarpa* were collected and phytochemically investigated, leading to the isolation of a total of 16 lanostane triterpene glycosides including eight new ones, named lyonicarposides A–H (**1**–**8**) (Fig. 1). This is the first phytochemical study on the flowers of *L. ovalifolia* var. *hebecarpa*. Herein, the extraction, isolation, structure elucidation, and the antiproliferative activities of 16 lanostane triterpene glycosides from the flowers of *L. ovalifolia* var. *hebecarpa* were described.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were measured with a Rudolph Autopol IV

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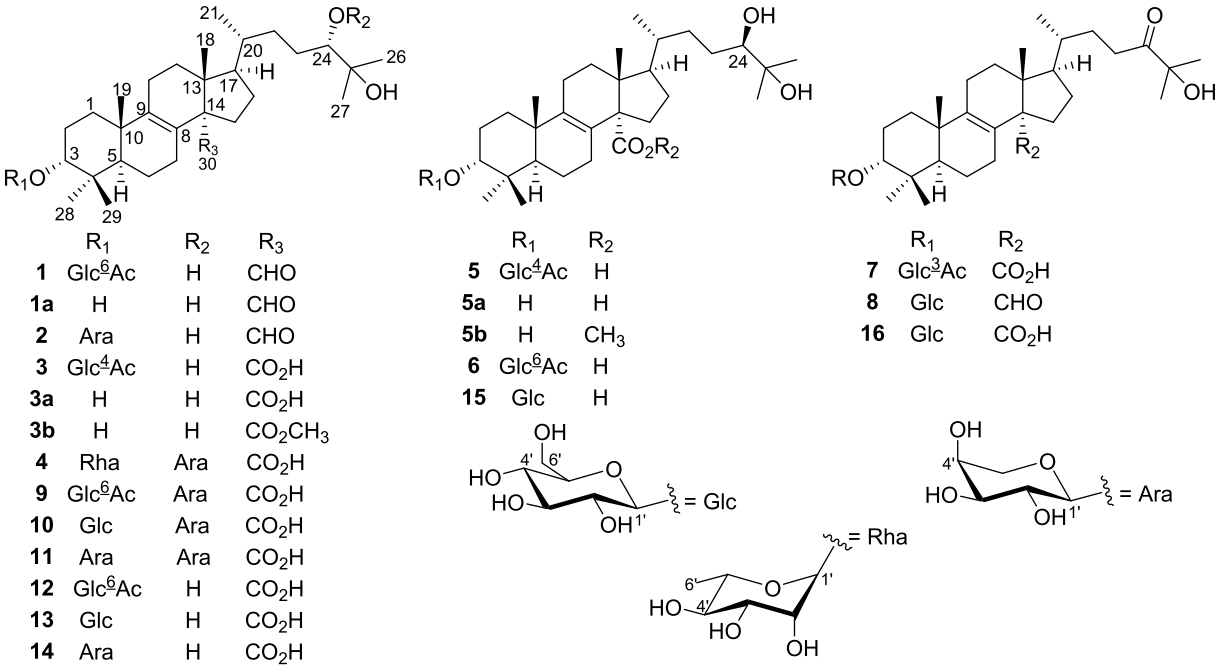

Fig. 1. Chemical structures of compounds **1–16**.

automatic polarimeter. The IR, UV, and ECD data were recorded by a Bruker Vertex 70 instrument, a Varian Cary 50 spectrophotometer, and a JASCO J–810 spectrometer, respectively. The NMR data were obtained through a Bruker AM–400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz with referencing to the residuals ^{1}H (δ_H 3.31 for methanol-d_4) or ^{13}C (δ_C 49.15 for methanol-d_4). HRESIMS were performed on a Bruker micrOTOF II spectrometer. Semi-preparative HPLC was accomplished on a Dionex 680 system with a UV detector or Agilent 1260 with a DAD detector using an RP C_{18} column (5 μm, 10 × 250 mm, Welch Ultimate XB–C_{18}). GC was analyzed using the Agilent 7820A gas chromatography and the capillary column (30 m × 0.25 mm × 0.5 μm, Welch WM-1). The cytotoxicity assay was performed using Multiskan FC multifunctional microplate reader.

2.2. Plant materials

The flowers of *Lyonia ovalifolia* var. *hebecarpa* (Franch. ex F.B. Forbes & Hemsl.) Chun (Ericaceae) were collected in Jinyun County (latitude 28°26′29″ North and longitude 120°8′59″ East), Zhejiang province, China, in June 2015, and authenticated by Professor Bing-Yang Ding at Wenzhou University. A voucher specimen (No. 2015-0617) has been deposited at School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology.

2.3. Extraction and isolation

The air-dried flowers of *L. ovalifolia* var. *hebecarpa* (1.5 Kg) were extracted (5 × 10 L) with MeOH at 40 °C. After the removal of the solvent under the reduced pressure, the crude extract (386.3 g) was suspended in H_2O (1 L) and then successively partitioned with chloroform (5 × 2 L) and *n*-BuOH (5 × 2 L). The chloroform fraction (186.7 g) was subjected to a silica gel (100–200 mesh) column and eluted with CH_2Cl_2–MeOH (50:1 to 0:1, v/v) to afford nine fractions Fr.1–Fr.9. Fr.4 (3.5 g) was isolated by RP C_{18} MPLC eluting with MeOH–H_2O (from 60:40 to 100:0, v/v) to yield the major fraction Fr.4D (1.2 g). Fr.4D was subjected to a silica gel (200–300 mesh) column and eluted with CH_2Cl_2–MeOH (50:1 to 0:1, v/v) to obtain three subfractions Fr.4D1–Fr.4D3. Compounds **7** (5.2 mg, t_R = 33.5 min) and **12** (3.6 mg, t_R = 36.1 min) were purified by semipreparative HPLC eluted with MeCN–H_2O (80:20, v/v, 1.5 mL/min) from Fr.4D1 (102 mg). Fraction Fr.4D2 (48.6 mg) was separated by a semipreparative HPLC eluting with MeCN–H_2O (84:16, v/v, 1.5 mL/min) to give compound **8** (12.2 mg, t_R = 28.6 min). Compounds **1** (3.0 mg, t_R = 49.6 min), **2** (7.0 mg, t_R = 45.5 min), and **5** (5.0 mg, t_R = 40.5 min) were obtained by semipreparative HPLC eluting with MeCN–H_2O (70:30, v/v, 1.5 mL/min) from the fraction Fr.4D3 (142 mg). Fr.5 (129.5 g) was chromatographed over a C_{18} MPLC eluting with MeOH–H_2O (from 50:50 to 100:0, v/v) to obtain the major fraction Fr.5C (2.7 g). Fr.5C was applied to a Sephadex LH-20 column to afford five subfractions (Fr.5C1–Fr.5C5). Compound **13** (15.1 mg, t_R = 33.3 min) was obtained from Fr.5C1 (148.7 mg) by a semipreparative HPLC eluting with MeCN–H_2O (80:20, v/v, 1.5 mL/min). Compounds **14** (12.6 mg, t_R = 24.3 min) and **16** (7.0 mg, t_R = 38.2 min) were isolated from Fr.5C2 (102.5 mg) by a semipreparative HPLC with MeCN–H_2O (82:18, v/v, 1.5 mL/min). Purification of Fr.5C3 (14.2 mg) by semipreparative HPLC with MeCN–H_2O (77:23, v/v, 1.5 mL/min) to yield compound **6** (2.6 mg, t_R = 45.3 min). Fraction Fr.5C4 (102.3 mg) was separated by a semipreparative HPLC eluting with MeCN–H_2O (80:20, v/v, 1.5 mL/min) to give compound **3** (3.6 mg, t_R = 33.3 min). Further purification of the fraction Fr.5C5 (248.6 mg) by a semipreparative HPLC eluting with MeCN–H_2O (78:22, v/v, 2.0 mL/min) afforded compounds **15** (7.0 mg, t_R = 20.6 min) and **6** (5.2 mg, t_R = 25.4 min). The *n*-BuOH fraction (49.3 g) was subjected to a silica gel (100–200 mesh) column and eluted with CH_2Cl_2–MeOH (30:1 to 0:1, v/v) to afford eight fractions Fr.1–Fr.8. Fr.6 (4.9 g) was isolated by an RP C_{18} MPLC eluting with MeOH–H_2O (from 50:50 to 100:0, v/v) to yield seven subfractions Fr.6A–Fr.6G. Fr.6E (0.8 g) was subjected to a silica gel (200–300 mesh) column and eluted with CH_2Cl_2–MeOH (40:1 to 0:1, v/v) to afford five subfractions Fr.6E1–Fr.6E5. Purification of Fr.6E3 (59.6 mg) by a semipreparative HPLC eluting with MeCN–H_2O (67:33, v/v, 1.5 mL/min) to yield compounds **4** (1.6 mg, t_R = 57.4 min), **9** (3.8 mg, t_R = 49.3 min), and **11** (3.6 mg, t_R = 53.3 min). Fraction Fr.6E4 (32.3 mg) was separated by semipreparative HPLC with MeCN–H_2O (65:35, v/v, 1.5 mL/min) to give compound **10** (5.6 mg, t_R = 46.8 min).

Table 1
^{1}H NMR spectroscopic data for compounds **1–4** in methanol-d_4 (δ in ppm, J in Hz, and 400 MHz).

No.	1	2	3	4
1α	1.48, m	1.40, m	1.42, m	1.29, m
1β	1.81, m	1.81, m	1.79, m	2.10, m
2α	1.83, m	1.84, m	1.78, m	1.78, m
2β	1.75, m	1.75, m	1.74, m	1.60, m
3β	3.39, br s	3.39, br s	3.49, br s	3.39, br s
5α	1.55, m	1.55, m	1.65, m	1.65, m
6α	1.55, m	1.56, m	1.55, m	1.55, m
6β	1.62, m	1.62, m	1.64, m	1.65, m
7α	1.88, m	1.89, m	1.71, m	1.95, m
7β	1.53, m	1.55, m	1.56, m	2.05, m
11α	2.12, m	2.12, m	2.14, m	2.03, m
11β	2.30, m	2.24, m	2.20, m	2.10, m
12α	1.93, m	1.93, m	1.71, m	1.70, m
12β	2.10, m	2.11, m	2.22, m	2.26, m
15α	2.07, m	2.05, m	2.00, m	1.56, m
15β	1.41, m	1.45, m	1.94, m	1.48, m
16α	2.10, m	1.92, m	2.10, m	1.40, m
16β	1.54, m	1.82, m	1.54, m	1.70, m
17α	1.05, m	1.06, m	1.54, m	1.57, m
18β	0.81, s	0.81, s	0.82, s	0.82, s
19β	1.10, s	1.11, s	1.07, s	1.06, s
20β	1.47, m	1.48, m	1.48, m	1.45, m
21α	0.96, d (6.5)	0.96, d (6.6)	0.96, d (6.2)	0.95, d (5.0)
22a	1.40, m	1.78, m	1.80, m	1.48, m
22b	1.40, m	1.75, m	1.73, m	1.48, m
23a	1.71, m	1.67, m	1.79, m	2.26, m
23b	1.71, m	1.62, m	1.70, m	2.26, m
24	3.14, dd (10.2, 1.6)	3.14, dd (10.3, 1.5)	3.14, dd (10.3, 1.8)	3.40, overlap
26	1.15, s	1.15, s	1.16, s	1.16, s
27	1.11, s	1.11, s	1.07, s	1.13, s
28α	0.92, s	0.97, s	0.99, s	0.97, s
29β	0.90, s	0.91, s	0.91, s	0.90, s
30	9.46, s	9.46, s		
1′	4.25, d (7.8)	4.21 d (6.4)	4.30, d (7.8)	4.18, br s
2′	3.17, dd (9.3, 7.8)	3.53, overlap	3.27, dd (9.3, 7.8)	3.57, overlap
3′	3.55, t (9.3)	3.52, overlap	3.55, t (9.3)	3.59, overlap
4′	3.27, t (9.3)	3.80, m	4.73, t (9.3)	3.54, overlap
5′a	3.39, ddd (9.3, 6.0, 2.4)	3.85 dd (12.4, 2.9)	3.39, ddd (9.3, 6.0, 2.8)	3.56, overlap
5′b		3.50, overlap		
6′a	4.37, dd (11.7, 2.4)		3.86, dd (11.7, 2.8)	1.25, d (6.3)
6′b	4.23 dd (11.7, 6.0)		3.66, dd (11.7, 6.0)	
1″				4.26, d (6.9)
2″				3.57, overlap
3″				3.46, overlap
4″				3.81, overlap
5″a				3.89, dd (12.5, 2.2)
5″b				3.56, overlap
OAc	2.09, s		2.09, s	

Table 2
^{13}C NMR spectroscopic data for compounds **1–8** in methanol-d_4 (δ in ppm and 100 MHz).

No.	1	2	3	4	5	6	7	8
1	31.6	31.6	31.7	31.8	31.1	31.1	31.1	31.6
2	22.7	22.3	22.7	22.5	22.3	22.6	22.4	22.4
3	82.5	82.2	82.6	82.3	82.3	82.6	82.6	82.0
4	38.3	38.4	38.3	38.3	38.3	38.2	38.3	38.4
5	46.1	46.0	46.1	47.5	45.7	45.8	45.7	46.0
6	19.3	19.3	19.3	19.4	19.4	19.4	19.1	19.4
7	27.4	27.4	27.4	28.5	28.9	28.5	29.1	28.5
8	123.5	123.5	128.9	128.9	128.9	128.8	129.0	123.5
9	146.2	146.2	141.7	141.7	141.8	142.0	141.7	146.3
10	39.1	39.2	39.1	38.7	38.7	38.7	38.7	39.2
11	23.1	23.1	23.2	23.4	23.4	23.4	23.4	23.4
12	32.0	32.0	32.0	32.7	32.7	32.7	32.7	32.7
13	47.3	47.3	47.3	48.1	48.3	48.3	48.2	47.3
14	68.5	68.5	64.1	64.0	64.0	64.0	64.2	68.5
15	28.8	28.8	28.8	29.6	28.5	29.2	28.5	29.2
16	29.1	29.1	30.0	31.1	30.5	30.5	30.4	30.5
17	54.5	54.5	52.2	52.2	52.4	52.3	52.2	54.3
18	17.5	17.5	18.3	18.4	18.3	18.3	18.4	17.4
19	20.1	20.1	20.3	20.3	20.3	20.3	20.3	20.1
20	37.7	37.7	37.7	37.0	37.8	37.8	37.0	36.8
21	19.4	19.4	19.4	19.2	19.4	19.2	19.1	19.0
22	34.6	34.6	34.6	34.3	34.9	34.9	30.1	34.9
23	28.8	28.8	29.2	32.7	29.2	31.1	34.0	31.1
24	80.6	80.6	80.6	89.4	79.9	79.9	218.0	217.8
25	74.0	74.0	74.0	73.7	74.0	74.0	78.1	78.5
26	25.9	25.9	25.9	26.7	25.7	25.8	26.9	26.9
27	24.9	24.9	24.9	25.0	25.0	25.1	26.9	26.9
28	29.1	29.2	29.1	29.3	29.1	29.1	29.1	29.1
29	23.1	23.1	23.1	23.1	23.0	23.1	23.0	23.0
30	200.8	200.8	180.3	180.2	181.0	180.3	181.7	200.8
1′	101.9	101.9	101.9	102.2	101.7	101.9	101.7	101.9
2′	75.1	72.6	74.1	72.9	75.3	75.1	73.6	75.1
3′	78.3	74.7	75.8	73.4	75.9	78.1	79.4	78.1
4′	72.1	69.9	73.2	74.4	73.3	72.2	70.2	72.2
5′	75.2	67.0	75.3	71.9	76.0	75.2	77.8	78.2
6′	64.9		62.8	19.6	62.9	65.0	62.8	62.5
1″				105.5				
2″				72.5				
3″				75.4				
4″				70.0				
5″				67.5				
OAc	20.9		20.9		21.1	20.9	21.3	
	172.8		172.4		172.4	172.9	172.8	

2.4. Spectroscopic data

Lyonicarposide A ***(1)***: white amorphous powder; $[\alpha]_D^{25}$ −14 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 238.2 (2.92), 301.0 (2.28) nm; IR (KBr) ν_{max} 3401, 2940, 1744, 1703, 1630, 1453, 1384 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 701.4253 [M + Na]$^+$ (calcd for $C_{38}H_{62}O_{10}Na$, 701.4241).

Lyonicarposide B ***(2)***: white amorphous powder; $[\alpha]_D^{25}$ −69 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 239.0 (2.87), 302.0 (2.22) nm; IR (KBr) ν_{max} 3410, 2964, 1693, 1632, 1603, 1461, 1385 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 629.4025 [M + Na]$^+$ (calcd for $C_{35}H_{58}O_8Na$, 629.4029).

Lyonicarposide C ***(3)***: white amorphous powder; $[\alpha]_D^{25}$ −47 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230.7 (2.91) nm; IR (KBr) ν_{max} 3419, 2960, 1727, 1631, 1459, 1384, 1248 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 717.4248 [M + Na]$^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190).

Lyonicarposide D ***(4)***: white amorphous powder; $[\alpha]_D^{25}$ −33 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231.0 (2.74), 278.7 (1.94) nm; IR (KBr) ν_{max} 3418, 2936, 1633, 1539, 1455, 1384, 1067 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 791.4540 [M + Na]$^+$ (calcd for $C_{41}H_{68}O_{13}Na$, 791.4558).

Lyonicarposide E ***(5)***: white amorphous powder; $[\alpha]_D^{25}$ −46 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231.2 (2.83) nm; IR (KBr) ν_{max} 3414, 2942, 1728, 1630, 1596, 1376, 1250, 1030 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 717.4128 [M + Na]$^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190).

Lyonicarposide F ***(6)***: White amorphous powder; $[\alpha]_D^{25}$ −55 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231.2 (2.89) nm; IR (KBr) ν_{max} 3418, 2938, 1723, 1632, 1456, 1384, 1224, 1045 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 717.4182 [M + Na]$^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190).

Lyonicarposide G ***(7)***: White amorphous powder; $[\alpha]_D^{25}$ −30 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 234.7 (3.45), 285.0 (2.47) nm; IR (KBr) ν_{max} 3409, 2939, 1708, 1595, 1461, 1377, 1255, 1036 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; HRESIMS *m/z* 715.3979 [M + Na]$^+$ (calcd for $C_{38}H_{60}O_{11}Na$, 715.4033).

Lyonicarposide H ***(8)***: White amorphous powder; $[\alpha]_D^{25}$ −22 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 239.0 (3.11), 301.0 (2.50) nm; IR

Table 3
^{1}H NMR spectroscopic data for compounds **5–8** in methanol-d_4 (δ in ppm, J in Hz, and 400 MHz).

No.	5	6	7	8
1α	1.42, m	1.42, m	1.42, m	1.49, m
1β	1.79, m	1.78, m	1.79, m	1.93, m
2α	1.78, m	1.78, m	1.79, m	1.93, m
2β	1.74, m	1.72, m	1.74, m	1.81, m
3β	3.49, br s	3.38, br s	3.50, br s	3.50, br s
5α	1.65, m	1.62, m	1.63, m	1.57, m
6α	1.55, m	1.56, m	1.56, m	1.57, m
6β	1.64, m	1.62, m	1.64, m	1.65, m
7α	1.71, m	1.96, m	1.56, m	1.96, m
7β	1.56, m	2.08, m	1.62, m	2.08, m
11α	2.14, m	2.13, m	2.13, m	2.13, m
11β	2.20, m	2.24, m	2.23, m	2.24, m
12α	1.71, m	1.71, m	1.68, m	1.71, m
12β	2.22, m	2.20, m	2.26, m	2.20, m
15α	2.00, m	2.11, m	2.08, m	2.11, m
15β	1.94, m	1.71, m	1.98, m	1.71, m
16α	2.10, m	1.56, m	2.10, m	1.56, m
16β	1.54, m	2.11, m	1.54, m	2.11, m
17α	1.54, m	1.53, m	1.55, m	1.53, m
18β	0.82, s	0.82, s	0.81, s	0.81, s
19β	1.07, s	1.06, s	1.06, s	1.10, s
20β	1.48, m	1.47, m	1.46, m	1.47, m
21α	0.96, d (6.2)	0.96, d (6.2)	0.93, d (5.9)	0.93, d (6.6)
22a	1.80, m	1.75, m	1.42, m	1.75, m
22b	1.73, m	1.48, m	1.68, m	1.48, m
23a	1.79, m	1.42, m	2.02, m	1.42, m
23b	1.70, m	2.26, m	2.05, m	2.26, m
24	3.22, dd (10.3, 1.8)	3.21, dd (10.4, 1.9)		
26	1.16, s	1.16, s	1.29, s	1.29, s
27	1.07, s	1.13, s	1.29, s	1.29, s
28α	0.99, s	0.93, s	0.98, s	0.97, s
29β	0.91, s	0.89, s	0.91, s	0.92, s
30				9.47, s
1′	4.30, d (7.8)	4.24, d (7.6)	4.35, d (7.8)	4.30, d (7.8)
2′	3.27, dd (9.3, 7.8)	3.25, dd (9.1, 7.8)	3.29, dd (9.4, 7.8)	3.17, dd (9.0, 7.8)
3′	3.55, t (9.3)	3.55, t (9.1)	4.39, t (9.4)	3.34, t (9.0)
4′	4.73, t (9.3)	3.27, t (9.1)	3.34, t (9.4)	3.26, t (9.0)
5′	3.39, ddd (9.3, 6.0, 2.8)	3.38, ddd (9.1, 6.3, 2.6)	3.30, ddd (9.4, 5.5, 2.1)	3.22, ddd (9.0, 5.6, 2.0)
6′a	3.86, dd (11.7, 2.8)	4.37, dd (11.8, 2.6)	3.86, dd (11.8, 2.1)	3.86, dd (11.7, 2.0)
6′b	3.66, dd (11.7, 2.8)	4.23 dd (11.8, 6.3)	3.68, dd (11.8, 5.5)	3.56, dd (11.7, 5.6)
OAc	2.09, s	2.05, s	2.10, s	

(KBr) ν_{max} 3441, 2942, 1706, 1467, 1384, 1017 cm^{-1} ^{1}H NMR and ^{13}C NMR data, see Tables 2 and 3; HRESIMS m/z 657.3981 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_9Na$, 657.3979).

2.5. Acid hydrolysis and determination of the absolute configurations of sugar moieties in lyonicarposides A–H (1–8)

Lyonicarposide A (**1**) (0.5 mg) was hydrolyzed by 2 mM HCl (1.5 mL) for 6 h at 80 °C. After extraction with ethyl acetate (3 × 2.0 mL), the aqueous layer was evaporated to dryness and dissolved in anhydrous pyridine (0.2 mL). Then L-cysteine methyl ester hydrochloride (0.2 mg) was added to the solution. The mixture was kept at 60 °C for 1.5 h and subsequently was trimethylsilylated with *N*-trimethylsilylimidazole (0.1 mL) without any isolation at 60 °C for 1.5 h. The reaction mixture was suspended in H_2O (1.0 mL) and extracted with *n*-hexane (3 × 1.0 mL). The layer of *n*-hexane was directly analyzed by GC with WM-1 capacity column. Nitrogen was used as a carrier gas at a flow rate of 1.0 mL/min. Flame ionization detector (FID) and injection were set at 250 °C. The split ratio was 10:1 and the injection volume was 1 μL. The oven temperature was programmed as follows: the initial temperature at 220 °C for 5 min, increased to 260 °C at 2 °C/min. In the same way, the trimethylsilylthiazolidine derivatives of the standards D- and L-glucose were prepared and analyzed by GC. Retention times of trimethylsilylthiazolidine derivatives of the hydrate of **1**, D- and L-glucose were 15.946, 16.042, and 16.349 min, respectively.

In the same way, lyonicarposides B–H (**2–8**) were hydrolyzed by 2 mM HCl, and the trimethylsilylthiazolidine derivatives of the hydrates of compounds **2–8** and the standards D- and L-glucoses, D- and L-arabinoses, and L-rhamnose were prepared. The trimethylsilylthiazolidine derivatives of the hydrates of compounds **3** and **5–8** gave GC retention times at 16.040, 15.978, 16.100, 15.999, and 15.913 min, respectively, suggesting the presence of D-glucose. Detection conditions of the derivatives of standards D- and L-arabinose, L-rhamnose, and hydrates of compounds **2** and **4** were different at column temperature: initially 220 °C for 5 min, raised to 270 °C at 5 °C/min. The retention times of the derivatives of standards D- and L-arabinoses and L-rhamnose were 11.255, 10.702, and 12.198 min, respectively. The trimethylsilylthiazolidine derivative of the hydrate of compound **2** was 10.628 min, indicating the presence of L-arabinose in **2**. The retention time of the derivative of the standard L-rhamnose was 12.198 min. Two sugars in compound **4** were determined to be L-arabinose and L-rhamnose by the GC retention times of their trimethylsilylthiazolidine derivatives at 10.626 and 12.298 min, respectively.

2.6. Preparation of the aglycone of lyonicarposides A (1), C (3), and E (5)

Lyonicarposide A (**1**) (3.0 mg) was dissolved in MeOH (0.5 mL) and then 0.6 mM TsOH·H_2O (40 μL) was added, and the mixture was stirred at 65 °C for 72 h. After the removal of MeOH by evaporation, the reaction mixture was diluted with H_2O (3.0 mL) and extracted with ethyl acetate (3 × 1.0 mL). The ethyl acetate fraction was subjected to a silica gel (200–300 mesh) CC eluting with a CH_2Cl_2–CH_3OH (from 50:1 to 20:1, v/v) gradient system to yield the aglycone **1a** (1.2 mg). In the same way, lyonicarposides C (**3**) (3.6 mg) and H (**5**) (5.0 mg) were hydrolyzed to give the aglycones **3a** and **5a**.

2.6.1. Lyonicarpol A (1a)

White amorphous powder; $[\alpha]_D^{25}$ −43 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ: 3.35 (1H, br s, H-3β); 0.81 (3H. s, H-18β), 1.10 (3H. s, H-19β), 0.96 (3H. d, J = 6.5 Hz, H-21α), 3.14 (1H. dd, J = 10.2, 1.6 Hz, H-24), 1.15 (3H. s, H-26), 1.11 (3H. s, H-27), 0.93 (3H. s, H-28α), 0.87 (3H. s, H-29β). HRESIMS m/z 497.3594 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3607).

2.6.2. Hebecarpolic acid A (3a)

The spectroscopic data of the aglycone **3a** were identical to those of hebecarpolic acid A [33].

2.6.3. Hebecarpolic acid E (5a)

The spectroscopic data of the aglycone **5a** were identical to those of hebecarpolic acid E [33].

2.7. Preparation of the methyl esters of the aglycones 3a and 5a

A mixture of **3a** and K_2CO_3 (1.0 mg) in anhydrous DMF (0.5 mL) was added to MeI (4.6 μL) and then the mixture was stirred at room temperature for 2 h. The reaction mixture was neutralized with Na_2SO_4 (0.5 M) and diluted in H_2O (1.0 mL), and extracted with ethyl acetate (3 × 1.0 mL). After concentration, the ethyl acetate fraction was subjected to silica gel CC (200–300 mesh) eluting with a CH_2Cl_2–CH_3OH (from 50:1 to 30:1, v/v) gradient system to yield the ester **3b**. In the same way, the ester **5b** was prepared from **5a**.

The spectroscopic data of the esters **3b** and **5b** were identical to those of hebecarpolic acid A methyl ester and hebecarpolic acid E methyl ester, respectively [33].

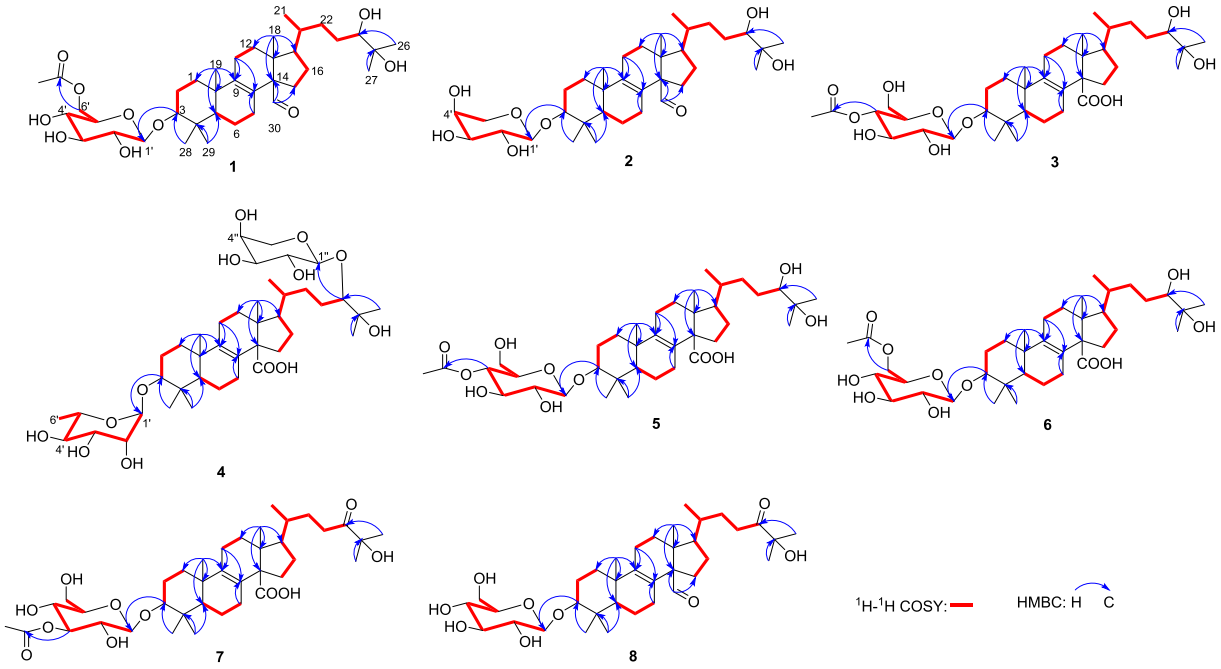

Fig. 2. ^{1}H − ^{1}H COSY and the key HMBC correlations of lyonicarposides A–H (**1–8**).

*2.8. Determination of the absolute configuration of the 24,25-diol moiety in compounds **1a**, **3b**, and **5b***

According to the method previously described [33], $Mo_2(OAc)_4$ was dissolved in dry DMSO to prepare a stock solution (1.0 mg/mL), then freshly prepared solution of $Mo_2(OAc)_4$ were added to compound **1a** (0.6 mg) so that the molar ratio of stock complex to ligand was about 1.2:1. The $Mo_2(OAc)_4$-induced ECD spectrum was immediately recorded every ten minutes until the IECD spectrum was constant. After subtracting the ECD spectrum of **1a**, the correlation between the absolute configuration of C-24 in **1a** and the Cotton effects in IECD spectrum were inferred using Snatzke's regulations [34]. Compounds **3b** and **5b** were treated in the same way.

2.9. Cytotoxicity assays

The cytotoxicity assays were performed according to the method reported previously [32,33] and the IC_{50} values of the compounds were calculated by Reed and Muench method.

3. Results and discussion

3.1. Structure elucidation

The air-dried flowers of *L. ovalifolia* var. *hebecarpa* (1.5 Kg) were powdered and extracted with MeOH. The crude extract was suspended in H_2O and then partitioned with chloroform and *n*-BuOH. The chloroform fraction was repeatedly subjected to silica gel, reversed phase (RP) C_{18} silica gel, and Sephadex LH–20 column chromatography, as well as HPLC to afford seven new lanostane-type triterpene glycosides (**1–3** and **5–8**), named lyonicarposides A–C and E–H, along with five known analogues (**12–16**). The *n*-BuOH fraction was treated in a similar procedure to give one new lanostane-type triterpene glycoside lyonicarposide D (**4**) and three known analogues (**9–11**). The known triterpene glycosides **9–16** were identified to be lyonifolosides P (**9**), J (**10**), R (**11**), M (**12**), and L (**13**) [16], hebecarposides A (**14**), E (**15**), and H (**16**) [33], respectively, by their NMR data analysis and comparison with literature data.

Lyonicarposide A (**1**) was obtained as a white amorphous powder. The molecular formula of $C_{38}H_{62}O_{10}$ was assigned to **1** based on the ^{13}C NMR data and HRESIMS ion at *m/z* 701.4253 $[M + Na]^+$ (calcd for $C_{38}H_{62}O_{10}Na$, 701.4241), indicating eight indices of hydrogen deficiency. Absorption bands at 1384 cm^{-1} and 1744 cm^{-1} in the IR spectrum suggested the presence of a double bond and a carbonyl group, respectively. ^{1}H NMR data of **1** (Table 1) showed resonances for eight methyl groups at δ_H 0.81 (3H, s, CH_3-18), 1.10 (3H, s, CH_3-19), 0.96 (3H, d, J = 6.5 Hz, CH_3-21), 1.15 (3H, s, CH_3-26), 1.11 (3H, s, CH_3-27), 0.92 (3H, s, CH_3-28), 0.90 (3H, s, CH_3-29), and an acetyl at δ_H 2.09 (3H, s, 6′-OAc), two oxymethines at δ_H 3.39 (1H, br s, H-3) and 3.14 (1H, dd, J = 10.2, 1.6 Hz, H-24), an aldehyde group at δ_H 9.46 (1H, s, H-30), and an glucopyranosyl unit at δ_H 4.25 (1H, d, J = 7.8 Hz, H-1′), 3.17 (1H, dd, J = 9.3, 7.8 Hz, H-2′), 3.55 (1H, t, J = 9.3 Hz, H-3′), 3.27 (1H, t, J = 9.3 Hz, H-4′), 3.39 (1H, ddd, J = 9.3, 6.0, 2.4 Hz, H-5′), 4.37 (1H, dd, J = 11.7, 2.4 Hz, H-6′a), and 4.23 (1H, dd, J = 11.7, 6.0 Hz, H-6′b) [16]. Combined with the DEPT and HSQC data, a total of 38 carbon resonances in the ^{13}C NMR data (Table 2) were assigned to be a glucopyranosyl unit at δ_C 101.9 (C-1′), 75.1 (C-2′), 78.3 (C-3′), 72.1 (C-4′), 75.2 (C-5′), and 64.9 (C-6′) [16], eight methyls, ten methylenes, five methines including two oxymethines at δ_C 82.5 (C-3) and 80.6 (C-24), four quaternary carbons, one oxygenated tertiary carbon at δ_C 74.0 (C-25), a tetrasubstituted double bond at δ_C 123.5 (C-8) and 146.2 (C-9), an ester carbonyl group at δ_C 172.8 (C-OAc), and an aldehyde group at δ_C 200.8 (C-30). A tetrasubstituted double bond, an aldehyde group, an acetyl group, and a glucopyranosyl unit account for four indices of hydrogen deficiency, and the remaining four indices of hydrogen deficiency suggested the presence of a tetracyclic system in **1**. Thus, lyonicarposide A (**1**) should be a tetracyclic triterpene glycoside.

A comparison of the ^{1}H and ^{13}C NMR data of lyonicarposide A (**1**) with those of lyonifoloside M (**12**) [16] revealed their structural similarity. The major difference was the presence of an aldehyde group (δ_H 9.46, s, H-30; δ_C 200.8, C-30) in **1**, replacing the carboxyl group (δ_C 180.5, C-30) in **12**. This was further confirmed by HMBC correlations from H-30 (δ_H 9.46, s) to C-14 (δ_C 68.5) and C-15 (δ_C 28.8) and from H_2-15 (δ_H 2.07, m; 1.41, m) to C-30. The glycosidation at C-3 was proved by the HMBC cross-peaks from H-1′ to C-3 and H-3 to C-1′. The planar structure of **1** was confirmed by the detailed 2D NMR analysis (Fig. 2).

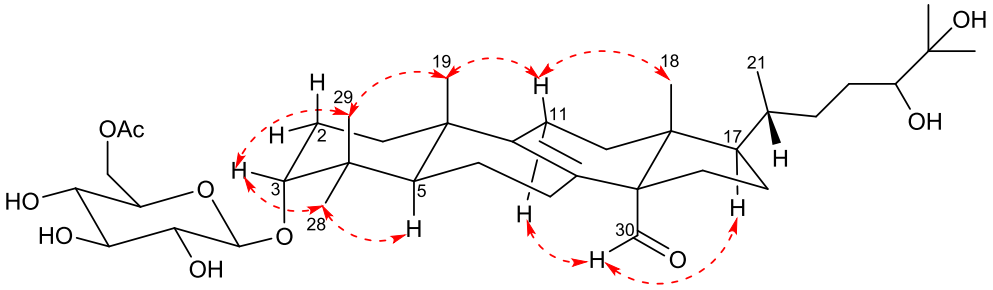

Fig. 3. Key NOESY correlations of lyonicarposide A (**1**).

Similar to **12**, H_3-19 in **1** was assigned as *β*-orientation. The broad single peak of H-3 (δ_H 3.39, br s) suggested the equatorial position of H-3 in chair conformation of the cylcohexane in ring A (Fig. 3), and a *β*-orientation of H-3 was assigned. The NOESY correlation between H_3-19*β* and H_3-29 indicated the *β*-orientation of H_3-29 and the *α*-orientation of H_3-28. The strong NOESY correlation between H_3-28*α* and H-5 and the lack of NOESY correlation between H_3-19*β* and H-5 suggested the *α*-orientation of H-5. The *β*-orientation of H_3-18 was determined by the NOESY correlations between H_3-19*β* and H-11*β* (δ_H 2.12, m) and between H-11*β* and H_3-18. The NOESY correlations between H-11*α* (δ_H 2.30, m) and CHO-30 and between CHO-30 and H-17 suggested the *α*-orientations of CHO-30 and H-17. The *α*-orientation of H-7 and the configuration of C-20 in **1** were established to be identical to that of **12** by their biogenetic relationships and the biosynthetic pathway of lanostane triterpenoids [35,36].

The absolute configuration of C-24 in **1** was determined by a $Mo_2(OAc)_4$–induced electronic circular dichroism (IECD) method [34]. According to the method previously described [32,33], **1** was hydrolyzed by TsOH to yield the aglycone **1a**. The $Mo_2(OAc)_4$-IECD spectrum of the **1a** (Fig. 4) showed the positive Cotton effect at 316 nm, suggesting the 24*S* absolute configuration of C-24 [34], which was identical to that of lyonifoloside M (**12**).

To determine the absolute configuration of the glucose moiety, lyonicarposide A (**1**) was hydrolyzed, and the trimethylsilylthiazolidine derivatives of the hydrolysate of **1** and the standards, D- and L-glucoses, were prepared. The GC retention time of the trimethylsilylthiazolidine derivative of the hydrolysate of **1** (t_R = 15.946 min) was almost same as that of D-glucose (t_R = 16.042 min), while different from that of L-glucose (t_R = 16.349 min), indicating the D-glucose in **1**. The large coupling constant of H-1′ (J = 7.8 Hz) revealed the *β*-glucopyranosyl linkage in **1**. Thus, the structure of **1** was defined as 3*α*-[(6′-*O*-acetyl-*β*-D-glucopyranosyl)-oxy]-24(*S*),25-dihydroxy-30-oxo-lanost-8-ene.

The molecular formula of lyonicarposide B (**2**) was determined to be $C_{35}H_{58}O_8$ based on the ^{13}C NMR data and HRESIMS ion at *m/z* 629.4025 $[M + Na]^+$ (calcd for $C_{35}H_{58}O_8Na$, 629.4029). Analysis of the ^1H and ^{13}C NMR data (Tables 1 and 2) revealed that the NMR data of **2** were similar to those of hebecarposide A (**14**) [33], and the major difference was that an aldehyde group (δ_H 9.46, s, H-30; δ_C 200.8, C-30) in **2** replaced the carboxyl group (δ_C 180.3, C-30) in **14**, which was supported by the HMBC correlations from H-30 (δ_H 9.46, s) to C-14 (δ_C 68.5) and C-15 (δ_C 28.8) and from H_2-15 (δ_H 2.05, m; 1.45, m) to C-30. Further 2D NMR data analysis (Fig. 2 and Supporting Information, Fig. S1) established the structure of lyonicarposide B (**2**). To determine the absolute configuration of the arabinose moiety, lyonicarposide B (**2**) was hydrolyzed, and the trimethylsilylthiazolidine derivatives of the hydrolysate of **2** and the standards, D- and L-arabinoses, were prepared. The GC retention time of the trimethylsilylthiazolidine derivative of the hydrolysate of **2** (t_R = 10.628 min) was close to that of L-arabinose (t_R = 10.702 min), instead of D-arabinose (t_R = 11.255 min), suggesting the L-configuration of the arabinose unit in **2**. The large coupling constant of H-1′ (J = 6.4 Hz) revealed the *α*-L-arabinopyranosyl linkage in **2**. Therefore, the structure of **2** was defined as 3*α*-[*α*-L-arabinopyranosyl)-oxy]-24(*S*),25-dihydroxy-30-oxo-lanost-8-ene.

Lyonicarposide C (**3**) was obtained as white amorphous powder, and a molecular formula of $C_{38}H_{62}O_{11}$ was deduced from the HRESIMS ion at *m/z* 717.4248 $[M + Na]^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190) and ^{13}C NMR data. Comparison of the NMR data of lyonicarposide C (**3**) (Tables 1 and 2) with those of lyonifoloside L (**13**) [16] revealed the presence of an additional acetyl group (δ_H 2.09, s; δ_C 172.8, 20.9) in **3**, suggesting that **3** was the acetyl derivative of **13**. The significant deshielding of H-4′ (δ_H 4.73, t, J = 9.3 Hz) and C-4′ (δ_C 73.2) in **3** compared to those (δ_H 3.28, t, J = 8.6 Hz, H-4′; δ_C 72.2) in **13** indicated that **3** should be a 4′-acetyl derivative of **13**, which was further supported by the HMBC correlation from H-4′ to the acetyl carbonyl carbon

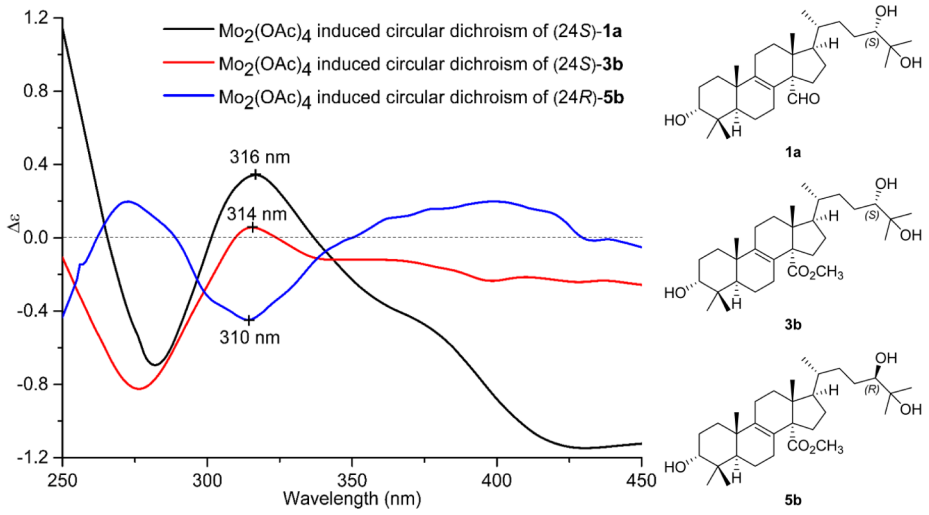

Fig. 4. $Mo_2(OAc)_4$ induced ECD spectra of compounds **1a**, **3b**, and **5b** in DMSO.

at δ_C 172.8 in **3**. To determine the absolute configuration of C-24, lyonicarposide C (**3**) was hydrolyzed by TsOH to get the aglycone **3a**, and then **3a** was methylated with CH_3I to afford the ester **3b** [33]. The positive Cotton effect at 314 nm in the $Mo_2(OAc)_4$–IECD spectrum of **3b** verified the *S* configuration of C-24 (Fig. 3). Similar to lyonicarposide A (**1**), a comparison of the GC retention time of the trimethylsilylthiazolidine derivative of the hydrolysate of **3** with those of the standards established the D-absolute configuration of the glucose moiety in **3**. The *β*- glucopyranosyl linkage in **3** was assigned by the large coupling constant (J = 7.8 Hz) of H-1′. Further analysis of the 2D NMR data (Fig. 2 and Supporting Information, Fig. S1) suggested that the aglycone of **3** was the same to that of **13**. Thus, the structure of **3** was identified as 3*α*-[(4′-*O*-acetyl-*β*-D-glucopyranosyl)-oxy]-24(*S*),25-dihydroxylanost-8-en-30-oic acid.

Lyonicarposide D (**4**) possessed a molecular formula of $C_{41}H_{68}O_{13}$ as established by the HRESIMS ion at m/z 791.4540 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{13}Na$, 791.4558) and ^{13}C NMR data. Comparison of the ^1H and ^{13}C NMR data of **4** (Tables 1 and 2) with those of lyonifolosides O (**10**) [16] suggested their similarities and the major difference was the presence of an additional rhamnopyranosyl unit (δ_H 4.18, br s, H-1′; 3.57, overlap, H-2′; 3.59, overlap, H-3′; 3.54, overlap, H-4′; 3.56, overlap, H-5′; 1.25, d, J = 6.3 Hz, H-6′;δ_C 102.2, C-1′; 72.9, C-2′; 73.4, C-3′; 74.4, C-4′; 71.9, C-5′; 19.6, C-6′) in **4**, replacing the glucopyranosyl unit in **10**. The HMBC correlation from the anomeric proton H-1′ (δ_H 4.18, br s) of the rhamnopyranosyl unit to C-3 (δ_C 82.3) suggested the location of the rhamnopyranosyl unit at C-3. Same to lyonicarposide B (**2**), The L-absolute configurations of the arabinose and rhamnose units in **4** were determined by comparison of the GC retention times of the trimethylsilylthiazolidine derivatives of the hydrolysate of **4** with those of the standards. Therefore, the structure of **4** was defined as 3*α*-[(*α*-L-rhamnopyranosyl)-oxy]-24(*S*)-[(*α*-L-arabinopyranosyl)-oxy]-25-hydroxylanost-8-en-30-oic acid by 2D NMR analysis (Fig. 2 and Supporting Information, Fig. S1) and chemical methods.

Lyonicarposide E (**5**) was found to possess the same molecular formula as lyonicarposide C (**3**) by the HRESIMS peak at m/z 717.4128 $[M + Na]^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190) and ^{13}C NMR data. The NMR data (Tables 2 and 3) of **5** also resemble to those of **3**, and the major differences were the deshielding of H-24 (δ_H 3.22, dd, J = 10.3, 1.8 Hz) and the shielding of C-24 (δ_C 79.9) in **5** compared to those (δ_H 3.14, dd, J = 10.3, 1.8 Hz, H-24; δ_C 80.6, C-24) in **3**. Thus, lyonicarposide E (**5**) should be the 24-epimer of lyonicarposide C (**3**) [33]. To determine the absolute configuration of C-24, lyonicarposide E (**5**) was hydrolyzed, and the resulted aglycone **5a** was methylated to afford the ester **5b** using the same method as **3**. The $Mo_2(OAc)_4$-IECD spectrum of **5b** displayed a negative Cotton effect at 310 nm (Fig. 3), instead of a positive Cotton effect in **3b**, proving the *R* configuration of C-24. The absolute configuration of the glucose unit in **5** was determined to be D by comparison of the GC retention times of the trimethylsilylthiazolidine derivative of the hydrolysate of **5** with those of the standards, D and L-glucoses, and the *β*-glucopyranosyl linkage in **5** was deduced from the large coupling constant (J = 7.8 Hz) of H-1′. Thus, the structure of **5** was established as 3*α*-[4′-*O*-acetyl-*β*-D-glucopyranosyl)-oxy]-24(*R*),25-dihydroxylanost-8-en-30-oic acid by 2D NMR analysis (Fig. 2 and Supporting Information, Fig. S1) and chemical methods.

Lyonicarposide F (**6**) possessed the same molecular formula as lyonicarposide E (**5**), as determined by the HRESIMS ion at m/z 717.4182 $[M + Na]^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190) and ^{13}C NMR data. The NMR data of **6** (Tables 2 and 3) were similar to **5**, and the major differences were the deshielding of H-6′a (δ_H 4.37, dd, J = 11.8, 2.6 Hz), H-6′b (δ_H 4.23, dd, J = 11.8, 6.3 Hz), and C-6′ (δ_C 65.0) in **6** compared to those (δ_H 3.86, dd, J = 11.8, 2.6 Hz, H-6′a; 3.66, overlap, H-6′b; δ_C 62.9) in **5**, indicating that the acetyl group was located at C-6′ in **6**. This deduction was supported by the HMBC correlation from H_2-6′ to acetyl carbonyl (δ_C 172.9). Further analysis of 2D NMR data (Fig. 2 and Supporting Information, Fig. S1) indicated the other parts of **6** to be the same to that of **5**. Accordingly, the structure of **6** was defined as 3*α*-[6′-*O*-acetyl-*β*-D-glucopyranosyl-oxy]-24(*R*),25-dihydroxylanost-8-en-30-oic acid by NMR data and chemical method.

The molecular formula of lyonicarposide G (**7**) was established as $C_{38}H_{60}O_{11}$ by the HRESIMS ion at m/z 715.3979 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_{11}Na$, 715.4033) and ^{13}C NMR data. The NMR data (Tables 2 and 3) of lyonicarposide G (**7**) were similar to those of hebecarposide H (**16**) [33], and the major difference was the presence of an additional acetyl group (δ_H 2.10, s; δ_C 21.3, 172.8) in **7**. In addition, C-3′ (δ_C 79.4) in **7** shifted to the downfield while C-2′ (δ_C 73.6) and C-4′ (δ_C 70.2) in **7** shifted to the upfield compared to C-3′ (δ_C 78.3), C-2′ (δ_C 75.2), and C-4′ (δ_C 72.2) in **16**, respectively, suggesting the location of the acetyl group at C-3′ in **7**. This deduction was supported by the HMBC correlation from H-3′ (δ_H 4.39, t, J = 9.4 Hz) to the acetyl carbonyl (δ_C 172.8). Detailed analysis of 2D NMR data demonstrated that the aglycone of **7** was the same to that of hebecarposide H (**16**) (Fig. 2 and Supporting Information, Fig. S1). Hence, the structure of **7** was defined as 3*α*-[3′-*O*-acetyl-*β*-D-glucopyranosyl)-oxy]-25-hydroxy-24-oxo-lanost-8-en-30-oic acid with chemical method.

Lyonicarposide H (**8**) was determined to have a molecular formula of $C_{36}H_{58}O_9$ by the HRESIMS ion at m/z 657.3981 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_9Na$, 657.3979) and ^{13}C NMR data. The NMR data (Tables 2 and 3) of lyonicarposide H (**8**) were similar to those of hebecarposide H (**16**) [33], except for an additional aldehyde (δ_H 9.47, s, H-30; δ_C 200.8, C-30) in **8**, replacing the carboxyl group (δ_C 181.2, C-30) in **16**, which was supported by the HMBC correlations from H-30 (δ_H 9.47, s) to C-14 (δ_C 68.5) and C-15 (δ_C 29.2) and from H_2-15 (δ_H 2.11, m; 1.71, m) to C-30. Finally, the structure of **8** was determined as 3*α*-[(*β*-D-glucopyranosyl)-oxy]-25-hydroxy-24,30-dioxo-lanost-8-ene by 2D NMR data analysis (Fig. 2 and Supporting Information, Fig. S1) and chemical methods.

In a previous phytochemical study, 11 lanostane triterpene glycosides were isolated from the leaves of *L. ovalifolia* var. *hebecarpa* [33]. In the current study, 16 lanostane triterpene glycosides including eight new ones were isolated from the flowers of *L. ovalifolia* var. *hebecarpa*. In Ericaceae family, lanostane-type triterpenoids were only found in the plants of *Lyonia* genera [16,33]. Therefore, lanostane triterpene glycosides could be considered as a chemotaxonomic marker for the *Lyonia* plants in the chemical classification of Ericaceae. Interestingly, seven lanostane triterpene glycosides from the flowers of *L. ovalifolia* var. *hebecarpa* were acetylated in the glucose units, which are different from the leaves parts and could be the characteristic constituents of the flowers of *L. ovalifolia* var. *hebecarpa*.

3.2. Antiproliferative activities in vitro

All the isolated 16 lanostane-type triterpene glycosides (**1–16**) were evaluated for their antiproliferative activities against five cancer cell lines (SMMC-7721, HL-60, SW480, MCF-7, and A-549) by the MTT method as described [32,33]. As shown in Table 4, lyonicarposides A (**1**) and B (**2**) with an aldehyde group at C-30 exhibited moderate antiproliferative activities against all the five cancer cell lines with IC_{50} values ranging from 12.3 to 28.7 μM. In contrast, lyonifoloside P (**9**) and hebecarposide A (**14**) without the 30-aldehyde group did not exhibit significant activity (IC_{50} > 40 μM), suggesting that the 30-aldehyde group is an important group to enhance the antiproliferative activity. Lyonicarposide C (**3**) and lyonifoloside M (**12**) with glycosidation at C-3 and a 24*α*-OH group selectively inhibited the proliferation of HL-60 and MCF-47 cell lines with IC_{50} values ranging from 13.0 to 17.7 μM. However, lyonicarposides E (**5**) and F (**6**) with glycosidation at C-3 but without 24*α*-OH did not exhibit significant antiproliferative activity, suggesting that the 24*α*-OH group may enhance the antiproliferative activity. This may explain why lyonifolosides P (**9**) and J (**10**) without 24*α*-OH did not show significant antiproliferative activity. Interestingly, lyonicarposide D (**4**) and lyonifolosides P (**9**), J (**10**), and R (**11**), did not exhibited significant antiproliferative activity, suggesting that

Table 4
Anti-proliferative activities of compounds **1–16** against five cancer cell lines (IC_{50} in μM).[a,b]

Compounds	HL-60	A-549	SMMC-7721	MCF-7	SW480
1	15.53 ± 0.38	12.39 ± 0.65	12.92 ± 0.30	13.80 ± 0.96	17.71 ± 0.22
2	15.19 ± 0.57	20.35 ± 0.46	20.43 ± 0.56	17.55 ± 0.22	28.71 ± 1.34
3	13.03 ± 0.27	> 40	> 40	17.53 ± 1.10	> 40
7	14.88 ± 0.84	> 40	> 40	13.84 ± 0.62	> 40
12	17.71 ± 0.44	> 40	> 40	13.71 ± 0.48	> 40
13	> 40	> 40	> 40	16.27 ± 1.45	> 40
cis-platin[c]	2.47 ± 0.12	13.84 ± 0.47	7.82 ± 0.62	13.46 ± 0.49	10.06 ± 0.30

[a] Other compounds: IC_{50} > 40 μM.
[b] Results were expressed as means ± SD (n = 3).
[c] Positive control.

diglycosidation of lanost-8-ene type triterpenoids may decrease the antiproliferative activity. Furthermore, Lyonicarposide G (**7**) with an acetyl group selectively inhibited the proliferation of HL-60 and A-549 cell lines with IC_{50} values of 14.9 and 13.8 μM, respectively, and showed more potent antiproliferative activity than hebecarposide H (**16**), suggesting the acetyl group in the glucose unit may enhance the antiproliferative activities of the lanostane triterpene glycosides. This deduction was supported by that lyonicarposide C (**3**) and lyonifoloside M (**12**) with an acetyl group exhibited more potent antiproliferative activities than lyonifoloside L (**13**) without an acetyl group. This preliminary structure-activity-relationships analysis provided a basis to optimize the lanost-8-ene type triterpene glycosides as antiproliferative agents.

4. Conclusions

The flowers of *L. ovalifolia* var. *hebecarpa* were phytochemically studied for the first time, leading to the isolation of a total of 16 lanostane-type triterpene glycosides including eight new ones, named lyonicarposides A–H (**1–8**). All the isolates were elucidated using comprehensive spectroscopic techniques and chemical methods. The antiproliferative activities of all compounds were assayed for their antiproliferative against SMMC-7721, HL-60, SW480, MCF-7, and A-549 cell lines. Lyonicarposides A (**1**) and B (**2**) showed anti-proliferative activities against five cancer cell lines with IC_{50} values from 12.39 to 28.71 μM. Lyonicarposides C (**3**) and G (**7**) and lyonifoloside M (**12**) selectively inhibited the proliferation of HL-60 and A-549 with IC_{50} values from 13.03 to 17.53 μM. In addition, lyonifoloside L (**13**) exhibited potent antiproliferative activities against MCF-7 with an IC_{50} value of 16.27 μM. This preliminary structure-activity-relationships analysis suggested that the 30-aldehyde group, 24α-OH group, and the acetyl group in the glucose moiety may be the active groups for the antiproliferative activities of the lanostane triterpene glycosides. This study not only enriches the chemical diversity of lanostane triterpene glycosides in Ericaceae plants, but also provided a basis to develop novel antiproliferative agents from the lanostane triterpene glycosides.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103598.

References

[1] Y. Kato, Phenolic constituents of *Lyonia ovalifolia*, Phytochemistry 12 (1973) 2302.
[2] D. Zhao, L. Su, R. Li, X. Chen, H. Li, Chemical constituents from the twigs and leaves of *Lyonia ovalifolia*, Biochem. Syst. Ecol. 78 (2018) 1–4.
[3] M. Yasue, Y. Kato, Constituents of Lyonia ovalifolia var. elliptica. II. Constituents of the wood, especially isolation of a new glycoside, lyoniside, Yakugaku Zasshi 81 (1961) 526–528.
[4] K. Kashima, K. Sano, Y. Yun, H. Ina, A. Kunugi, H. Inoue, Ovafolinins A-E, five new lignans from *Lyonia ovalifolia*, Chem. Pharm. Bull. 58 (2010) 191–194.
[5] M. Yasue, Y. Kato, Constituents of Lyonia ovalifolia var. elliptica, Yakugaku Zasshi 79 (1959) 403–405.
[6] M. Yasue, Studies on the constituents of Lyonia ovalifolia var. elliptica. V. On the difference of flavonoid components of the leaves supplied from various districts and isolation of isoengerletin, Yakugaku Zasshi 85 (1965) 1090–1092.
[7] X. Lv, Y. Li, S. Ma, J. Qu, Y. Liu, Y. Li, D. Zhang, L. Li, S. Yu, Bioactive megastigmane glucosides and monoterpenes from *Lyonia ovalifolia*, J. Asian Nat. Prod. Res. 21 (2019) 559–572.
[8] C. Wang, Y. Liu, C. Li, Study of chemical constituents from *lyonia ovalifolia* var *hebecarpa*, J. Henan Med. Univ. 36 (2001) 743–744.
[9] M. Yasue, Y. Kato, T. Kishida, H. Ota, Isolation of lyoniol-A, -B, and -C, the toxic principles of *Lyonia ovalifolia* var. *elliptica*, Curr. Org. Chem. 9 (1961) 171-171.
[10] T. Ohta, H. Hikino, Revised structure of lyoniatoxin, toxin of *Lyonia ovalifolia* var. *elliptica*, Chem. Pharm. Bull. 29 (1981) 280–282.
[11] J. Sakakibara, K. Ikai, M. Yasue, Structure of lyoniol-D, Chem. Pharm. Bull. 20 (1972) 861–862.
[12] Z. Wu, H. Li, W. Wang, H. Li, R. Chen, R. Li, H. Luo, Lyonin A, a new 9, 10-seco-grayanotoxin from *Lyonia ovalifolia*, Chem. Biodivers. 8 (2011) 1182–1187.
[13] X. Lv, Y. Li, S. Ma, J. Qu, Y. Liu, L. Li, R. Wang, S. Yu, Isopimarane and nor-diterpene glucosides from the twigs and leaves of *Lyonia ovalifolia*, Tetrahedron 73 (2017) 776–784.
[14] M. Yasue, Y. Kato, M. Sugimoto, J. Sakakibara, Constituents of *Lyonia ovalifolia* var. *elliptica*. VIII. Terpenoid constituents of the red shoots, Yakugaku Zasshi 89 (1969) 1736–1739.
[15] M. Yasue, J. Sakakibara, T. Kaiya, Studies on the constituents of *Lyonia ovalifolia* Drude var. *elliptica* Hand.-Mazz. XV. On the constituents of the flowers. (2) Terpenoid constituents, particularly on 3-O-p-hydroxy-trans-cinnamoylmaslinic acid and syntheses of the related compounds, Yakugaku Zasshi 91 (1971) 1200–1206.
[16] X. Lv, Y. Li, S. Ma, J. Qu, Y. Liu, Y. Li, D. Zhang, L. Li, S. Yu, Antiviral triterpenes from the twigs and leaves of *Lyonia ovalifolia*, J. Nat. Prod. 79 (2016) 2824–2837.
[17] H. Fukuda, K. Watanabe, T. Ito, Effect of lyoniol-A on decerebrate rigidity and electroencephalogram in rats, Jpn. J. Pharmacol. 22 (1972) 457–465.
[18] N. Hussain, A. Hameed, M.S. Ahmad, S. Yousuf, Z. Ali, M.H. Rahman, M.I. Choudhary, New iridoids from *Lyonia ovalifolia* and their anti-hyperglycemic effects in mice pancreatic islets, Fitoterapia 131 (2018) 168–173.
[19] Editorial Committee of the Flora of China of Chinese Academy of Sciences, Flora of China, Science Press, Beijing, 1991.
[20] M. Zhang, Y. Zhu, G. Zhan, P. Shu, R. Sa, L. Lei, M. Xiang, Y. Xue, Z. Luo, Q. Wan, G. Yao, Y. Zhang, Micranthanone A, a new diterpene with an unprecedented carbon skeleton from *Rhododendron micranthum*, Org. Lett. 15 (2013) 3094–3097.
[21] M. Zhang, Y. Xie, G. Zhan, L. Lei, P. Shu, Y. Chen, Y. Xue, Z. Luo, Q. Wan, G. Yao, Y. Zhang, Grayanane and leucothane diterpenoids from the leaves of *Rhododendron micranthum*, Phytochemistry 117 (2015) 107–115.
[22] J. Zhou, N. Sun, H. Zhang, G. Zheng, J. Liu, G. Yao, Rhodomollacetals A-C, PTP1B inhibitory diterpenoids with a 2,3:5,6-di-seco-grayanane skeleton from the leaves of *Rhododendron molle*, Org. Lett. 19 (2017) 5352–5355.
[23] J. Zhou, G. Zhan, H. Zhang, Q. Zhang, Y. Li, Y. Xue, G. Yao, Rhodomollanol A, a highly oxygenated diterpenoid with a 5/7/5/5 tetracyclic carbon skeleton from the leaves of *Rhododendron molle*, Org. Lett. 19 (2017) 3935–3938.

[24] N. Sun, Y. Zhu, H. Zhou, J. Zhou, H. Zhang, M. Zhang, H. Zeng, G. Yao, Grayanane Diterpenoid Glucosides from the Leaves of Rhododendron micranthum and Their Bioactivities Evaluation, J. Nat. Prod. 81 (2018) 2673–2681.
[25] J. Zhou, J. Liu, T. Dang, H. Zhou, H. Zhang, G. Yao, Mollebenzylanols A and B, highly modified and functionalized diterpenoids with a 9-benzyl-8,10-dioxatricyclo [5.2.1.0(1,5)]decane core from *Rhododendron molle*, Org. Lett. 20 (2018) 2063–2066.
[26] J. Zhou, T. Liu, H. Zhang, G. Zheng, Y. Qiu, M. Deng, C. Zhang, G. Yao, Anti-inflammatory Grayanane Diterpenoids from the Leaves of *Rhododendron molle*, J. Nat. Prod. 81 (2018) 151–161.
[27] N. Sun, Y. Feng, Q. Zhang, J. Liu, H. Zhou, H. Zhang, G. Zheng, J. Zhou, G. Yao, Analgesic diterpenoids with diverse carbon skeletons from the leaves of *Rhododendron auriculatum*, Phytochemistry 168 (2019), https://doi.org/10.1016/j.phytochem.2019.112113.
[28] N. Sun, Y. Qiu, Y. Zhu, J. Liu, H. Zhang, Q. Zhang, M. Zhang, G. Zheng, C. Zhang, G. Yao, Rhodomicranosides A-I, analgesic diterpene glucosides with diverse carbon skeletons from *Rhododendron micranthum*, Phytochemistry 158 (2019) 1–12.
[29] N. Sun, G. Zheng, M. He, Y. Feng, J. Liu, M. Wang, H. Zhang, J. Zhou, G. Yao, Grayanane diterpenoids from the leaves of *Rhododendron auriculatum* and their analgesic activities, J. Nat. Prod. 82 (2019) 1849–1860.
[30] G. Zheng, J. Zhou, L. Huang, H. Zhang, N. Sun, H. Zhang, P. Jin, M. Yue, L. Meng, G. Yao, Antinociceptive grayanane diterpenoids from the leaves of *Pieris japonica*, J. Nat. Prod. 82 (2019) 3330–3339.
[31] G. Zheng, P. Jin, L. Huang, N. Sun, H. Zhang, H. Zhang, M. Yue, L. Meng, G. Yao, Grayanane diterpenoid glucosides as potent analgesics from *Pieris japonica*, Phytochemistry 171 (2020) 112234.
[32] Y. Teng, H. Zhang, J. Zhou, Y. Li, G. Yao, Triterpenoid glycosides from the leaves of *Lyonia ovalifolia* var. *hebecarpa* and their antitumor activities, Chin. J. Org. Chem. 37 (2017) 2416–2422.
[33] Y. Teng, H. Zhang, J. Zhou, G. Zhan, G. Yao, Hebecarposides A − K, antiproliferative lanostane-type triterpene glycosides from the leaves of *Lyonia ovalifolia* var. *hebecarpa*, Phytochemistry 151 (2018) 32–41.
[34] G. Snatzke, U. Wagner, H. Wolff, Circulardichroism—LXXV: Cottonogenic derivatives of chiral bidentate ligands with the complex $[Mo_2(O_2CCH_3)_4]$, Tetrahedron 37 (1981) 349–361.
[35] S. Zhang, Z. Li, M. Stadler, H. Chen, Y. Huang, X. Gan, T. Feng, J. Liu, Lanostane triterpenoids from *Tricholoma pardinum* with NO production inhibitory and cytotoxic activities, Phytochemistry 152 (2018) 105–112.
[36] J. Zhao, Y. Yang, M. Yu, K. Yao, X. Luo, H. Qi, G. Zhang, Y. Luo, Lanostane-type C31 triterpenoid derivatives from the fruiting bodies of cultivated *Fomitopsis palustris*, Phytochemistry 152 (2018) 10–21.