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TRITERPENOIDS AND FLAVONOIDS FROM *PAEONIA LACTIFLORA*

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Key Word Index—*Paeonia lactiflora*; Paeoniaceae; plant; callus tissue; triterpenoid; monoterpenoid; flavonoid; paeoniflorin.

Abstract—Seven triterpenoids and two flavonoids were isolated from the roots and aerial parts of *Paeonia lactiflora*, respectively. This is the first report of triterpenoids, which is a new triterpene assigned as 11α, 12α-epoxy-3β, 23-dihydroxy-30-norolean-20(29)-en-28,13 β-olide, from this plant. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Paeonia lactiflora is one of the most important crude drugs in traditional Chinese medicine. Chemical studies on *P. lactiflora* have been carried out by many investigators, and the presense of the monoterpene glycoside, paeoniflorin and its related compounds has been reported [1-4].

In an earlier paper, we reported on the accumulation of 11 triterpenoids in the callus tissues of paeoniaceous plants (*P. lactiflora, P. japonica, P. suffruticosa*); however, paeoniflorin and related compounds were not produced [5]. The presence of triterpenoids in roots of the plant (*P. lactiflora*) has never been reported. The present paper reports on the presence of triterpenes together with flavonoid glycosides in the plant.

RESULT AND DISCUSSION

Seven triterpenes (**1**–**7**) were isolated from the roots of *P. lactiflora*. Triterpenes **1**–**5** were identified by comparsion of their spectral data with those of authentic samples as oleanolic acid (**1**), hederagenin (**2**), 11α, 12α-epoxy-3β,23-dihydroxyolean-28,13β-olide (**3**), 30-norhederagenin (**4**) and betulinic acid (**5**), which were isolated in a previous study [5]. Furthermore, two flavonoid glycosides were isolated from the aerial part of the plant. These were identified as kaempferol-3-*O*-β-D-glucoside (**8**) and kaempferol-3,7-di-*O*-β-D-glucoside (**9**).

Compound **6** was obtained as an amorphous powder. Its IR spectrum showed the presence of an aldehyde group (1724 cm^{-1}). The ^1H NMR spectrum was similar to that of compound **1**, except for a signal due to an aldehyde (δ 9.50), indicating it to be an oleanan type triterpene. The mass spectrum of compound **6** exhibited the $[M]^+$ peak at *m/z* 440 and prominent fragment ion peaks at *m/z* 232 and 207 were derived by a retro-Diels–Alder type fragmentation in ring C. In the ^{13}C NMR spectrum, a carbon signal at δ 208.2 revealed the presence of an aldehyde group at C-17 in compound **6** instead of the carboxyl group (δ 180.2) found in compound **1**. Thus, compound **6** was assigned as 3β-hydroxyolean-12-en-28-al, which was previously isolated from the galls of *Pistacia terebinthus* (Anacardiaceae) [6].

Compound **7** was obtained as an amorphous powder. Its molecular formula was determined as $C_{29}H_{42}O_5$ on the basis of its HR-mass spectrum (*m/z* 470.3040 $[M]^+$). Furthermore, the mass fragementation compound **7**, exhibited the characteristic pattern of a 11α,12α-epoxy-γ-lactone of the oleanane series, i.e. significant peaks at *m/z* 247 and 251 formed by decomposition accompanied with rearrangement together with the $[M]^+$ peak at *m/z* 470 [7] (Scheme 1). The IR spectrum showed absorption bands at 1652 and 894 cm^{-1}, suggesting the presence of an exomethylene in addition to the absorption bands of an epoxide ring (872 cm^{-1}) and a γ-lactone (1776 cm^{-1}). The presence of the exomethylene was supported also by proton signals at δ 4.70 (1H, *s*) and 4.66 (1H, *s*) and carbon signals at δ 147.7 (*s*) and 109.8 (*t*) in the NMR spectra. Furthermore, the ^{13}C NMR spectrum of compound **7** was analogous to that of compound **3**, except for the signals corresponding to ring E. The signals due to C-20 (δ 31.5) and the geminal dimethyl groups (δ 33.1 and δ 23.5) in compound **3** were displaced in the carbon signals at δ 147.7 (*s*) and δ 109.8 (*t*) ascribed to the exomethylene in compound **7**. Consequently, the location of the exomethylene in compound **7** was regarded at C-20. Thus, compound **7** was determined as

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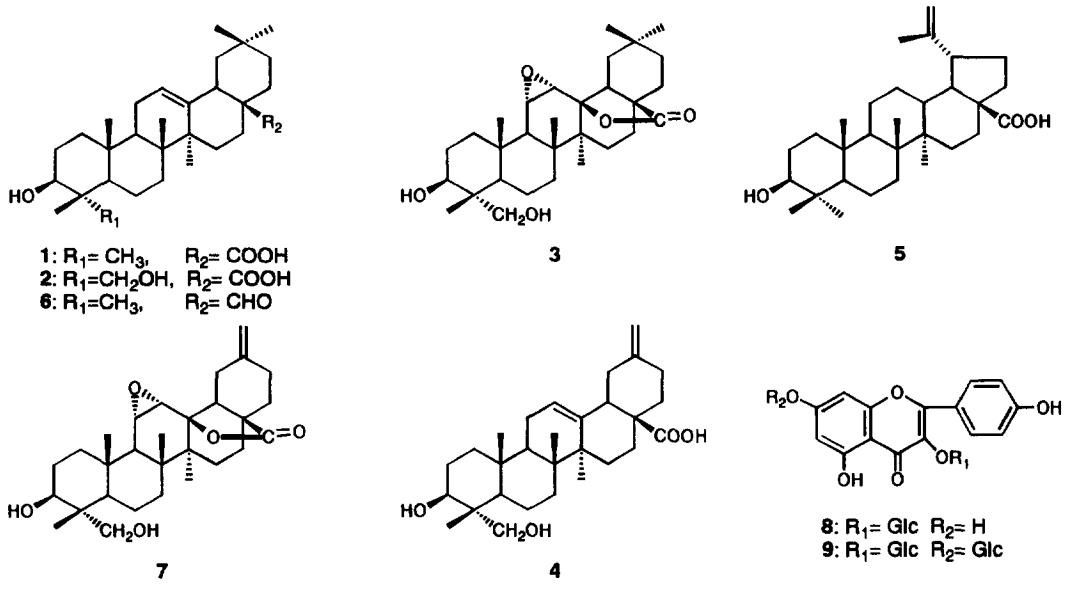


11α,12α-epoxy-3β,23-dihydroxy-30-norolean-20(29)-en-28,13β-olide. This is the first report of this compound from a natural source.

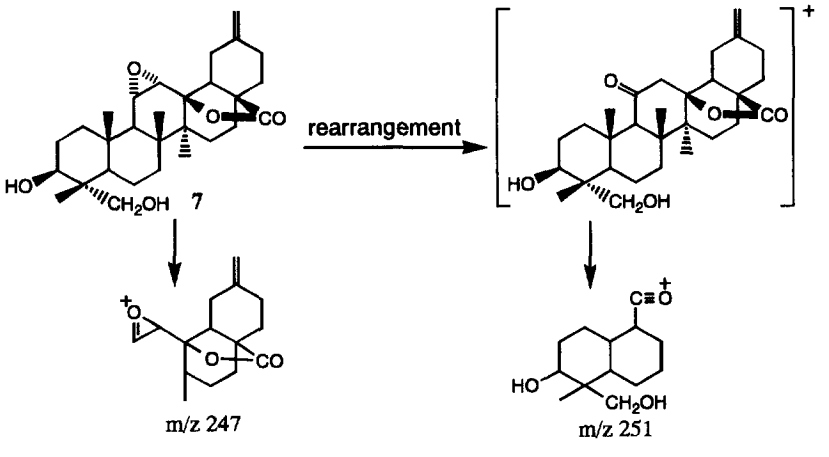
Seven triterpenes were isolated as minor constituents (0.025% dry wt) from the roots of the plant together with paeoniflorin (2.1% dry wt) as a main constituent. In addition, two flavonoids (1.06% dry wt) were isolated from the aerial parts of the plant. Oleanane and lupane type triterpenoids are biosynthesized in both of the callus tissues (5.94% dry wt) and the wild roots [5], but the former contained no paeoniflorin or other flavonoids [5].

EXPERIMENTAL

General procedures. Mp: uncorr.; ^1H and ^{13}C NMR: 400 MHz, 303 K, TMS as int. standard; IR. KBr. MS: Hitachi M-80A.

Extraction and isolation. Dried roots of *P. lactiflora* (5 kg) were extracted with MeOH under reflux. After removal of the solvent under red. pres., the MeOH extract was partitioned into a mixture of H_2O and $CHCl_3$ (1:1). The $CHCl_3$-soluble phase was chromatographed on silica gel (Fuji gel BW-300), using a gradient $CHCl_3$ and MeOH (100:0–70:30) and finally, MeOH, to give triterpenoids-containing frs. These frs were purified repeatedly by CC on silica gel (CIG column system, Kusano) using hexane–EtOAc–MeCN to give compounds **1**–**7**.

Dried aerial parts of *P. lactiflora* (500 g) were extracted with MeOH under reflux. The combined MeOH extracts were passed over activated charcoal packed in a column and were further eluted with MeOH and $CHCl_3$–MeOH (3:7). The combined MeOH soln was concentrated *in vacuo* and purified repeatedly by CC on silica gel using $CHCl_3$–MeOH to give compound **8**. The $CHCl_3$–MeOH (3:7) frs were purified



Scheme 1. Mass fragmentation of compound **1**.

Table 1. ^{13}C NMR spectral data for compounds **1–7** in pyridine-d_5

	1	2	3	4	5	6	7
1	38.9	38.9	38.7	38.8	39.3	39.4	38.6
2	28.1	28.1	27.4	27.7	28.3	27.5	27.4
3	78.1	73.5	73.0	73.5	78.1	78.9	72.9
4	39.4	42.8	43.1	42.9	39.5	39.7	43.1
5	55.8	48.7	48.0	48.7	55.9	56.2	48.0
6	18.8	18.6	17.9	18.6	18.8	19.1	17.9
7	33.2	33.0	31.2	33.0	34.8	33.5	31.2
8	39.8	39.8	41.0	39.8	41.1	40.3	41.0
9	48.1	48.2	49.8	48.1	50.9	49.3	51.3
10	37.4	37.8	36.7	37.2	37.5	37.6	36.7
11	23.1	23.8	52.9	23.8	21.2	24.1	53.0
12	122.6	122.6	57.4	122.6	26.1	122.6	57.3
13	144.8	144.8	87.6	144.9	38.6	142.6	87.1
14	42.2	42.2	41.2	42.0	42.8	42.6	41.7
15	28.3	28.3	27.1	28.3	31.2	27.6	27.1
16	23.7	23.8	21.7	23.8	32.9	22.7	22.0
17	46.7	46.7	44.1	47.1	56.6	49.9	44.1
18	46.5	46.5	51.4	48.0	47.8	48.3	54.8
19	42.0	42.0	38.1	42.0	49.8	46.3	34.7
20	31.0	30.9	31.5	148.5	151.3	31.1	147.7
21	34.2	34.2	34.5	38.4	30.2	33.7	32.4
22	33.3	33.2	27.7	30.4	37.6	28.4	30.2
23	28.3	68.0	67.7	68.1	28.6	29.2	67.1
24	16.5	13.0	12.7	13.1	16.3	17.0	12.7
25	15.6	15.9	17.7	16.0	16.4	15.9	17.7
26	17.4	17.5	18.9	17.5	16.4	17.8	18.9
27	26.2	26.2	20.5	26.2	14.9	26.1	20.4
28	180.2	180.2	178.9	179.4	178.9	209.2	178.2
29	33.3	33.2	33.1	107.0	109.9	33.6	109.8
30	23.8	23.7	23.5		19.4	24.1	

repeatedly by CC on silica gel using EtOAc–MeOH–H_2O to give compound **9**.

3β-Hydroxyolean-12-en-28-al (**6**). Amorphous powder. MS *m/z* (rel. int.): 440 $[M]^+$ (14), 232 (23), 207 (30), 204 (14), 203 (67), 189 (30); IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 1724 (aldehyde); ^1H NMR (pyridine-d_5): δ 9.50 (1H, *s*), 5.29 (1H, *t*-like), 3.30 (1H, *dd*, $J = 5,11$ Hz), 2.67 (1H, *dd*, $J = 4$, 14 Hz), 1.10, 1.07, 0.90, 0.83, 0.82, 0.75, 0.68 (each 3H, each *s*); ^{13}C NMR (pyridine-d_5): Table 1.

11α,12α-Epoxy-3β,23-dihydroxy-30-norolean-20(29)-en-28,13β-olide (**7**). Amorphous powder, $[\alpha]_D^{d27}$ + 78.4° (*c* = 0.89, $CHCl_3$). MS *m/z* (rel. int.: 470$[M]^+$ (16), 251 (67), 247 (58), 232 (36), 219 (38), 201 (44), 189 (59), IR ν_{max}^{KBr} cm^{-1}: 3416 (OH), 1776 (γ-lactone), 1650 (exomethylene), 872 (epoxy), ^1H NMR (pyridine-d_5) δ: 4.70 (1H, *s*), 4.66 (1H, *s*), 4.23 (1H, *dd*, $J = 5.2$, 11.2 Hz), 4.19 (1H, *d*, $J = 10.5$ Hz), 3.69 (1H, *d*, $J = 10.5$ Hz), 3.18 (1H, *d*, $J = 3.8$ Hz), 3.13 (1H, *dd*, $J = 1.6$, 3.8 Hz), 2.60 (1H, *dd*, $J = 3.9$, 12.9 Hz), 2.34 (1H, *dd*, $J = 3.7$, 13.7 Hz), 1.02, 1.03, 1.10, 1.15 (each 3H, each *s*); ^{13}C NMR (pyridine-d_5): Table 1.

Kaempferol-3-O-β-D-glucoside (**8**). Crystallization from $CHCl_3$-MeOH yielded yellow needles, mp 177–180°. $UV\lambda_{max}^{MeOH}$ nm (log ε): 205.4 (4.44), 265.4 (4.18), 349.9 (4.11); ^1H NMR (pyridine-d_5) δ: 8.44 (2H, *d*, $J = 8.4$ Hz, H-2′, 6′), 7.19 (2H, *d*, $J = 8.4$ Hz, H3′, 5′), 6.70 (2H, *br s*, H-6,8), 6.33 (1H, *d*, $J = 7.2$ Hz, anomeric-H), 4.24–4.38 (5H, *m*, sugar moiety); ^{13}C NMR (pyridine-d_5) δ: 177.4 (C-4), 164.1 (C-7), 161.1 (C-5), 159.8 (C-4′), 156.3 (C-2, 9), 133.0 (C-3), 130.7 (C-2′, 6′), 121.0 (C-1′), 115.0 (C-3′, 5′), 104.1 (C-10), 101.4 (C-1″), 98.7 (C-6), 93.6 (C-8), 77.2 (C-3″), 76.5 (C-5″), 74.2 (C-2″), 70.1 (C-4″), 61.0 (C-6″).

Kaempferol-3,7-di-O-β-D-glucoside (**9**). Crystallization from MeOH yield yellow needles, mp 191–193°. $UV\lambda_{max}^{MeOH}$ nm (log ε): 203.4 (4.32), 265.4 (4.13), 350.7 (4.06); ^1H NMR (pyridine-d_5) δ: 8.36 (2H, *d*, $J = 8.8$ Hz, H-2′, 6′), 7.14 (2H, *d*, $J = 8.8$ Hz, H3′, 5′), 6.96 (1H, *d*, $J = 2.4$ Hz, H-8), 6.76 (1H, *d*, $J = 2.4$ Hz, H-6), 6.36 (1H, *d*, $J = 7.2$ Hz, anomeric-H), 6.74 (1H, *d*, $J = 7.2$ Hz, anomeric-H), 4.21–4.38 (10H, *m*, sugar moiety); ^{13}C NMR (pyridine-d_5) δ: 177.7 (C-4), 163.0 (C-7), 160.9 (C-5), 160.1 (C-4′), 157.0 (C-9), 156.1 (C-2), 133.8 (C-3), 130.9 (C-2′, 6′), 120.9 (C-1′), 115.2 (C-3′, 5′), 105.9 (C-10), 101.3 (C-1‴), 100.3 (C-1″), 99.6 (C-6), 94.7 (C-8), 77.4 (C-3‴), 77.3 (C-3″), 76.6 (C-5″, 5‴), 74.4 (C-2‴), 73.3 (C-2″), 70.2 (C-4‴), 70.0 (C-4″), 61.1 (C-6″, 6‴).

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