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TRITERPENOIDS OF *PAEONIA JAPONICA* CALLUS TISSUE

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Key Word Index—*Paeonia japonica*; Paeoniaceae; callus tissue; triterpenes.

Abstract—Six triterpenes were isolated from *Paeonia japonica* tissue culture. The structures were determined by spectral methods to be oleanolic acid, hederagenin, betulinic acid, 24-cycloartanol, 30-norhederagenin and 23-hydroxybetulinic acid.

INTRODUCTION

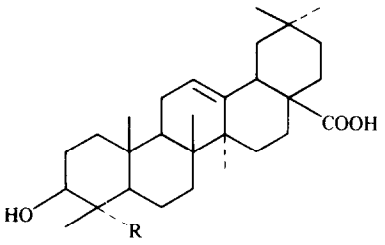
Paeonia japonica (Japanese name Yamashakuyaku) is the only species of this genus to be found in Japan and Korea, and occurs naturally in the mountain district. Its root is used in place of that of *P. lactiflora*, which is one of the most important crude drugs in traditional Chinese medicine and has been used as a circulatory tonic and diuretic, and is prescribed for diseases of women, weakness, night sweats and lumbar pain [1].

Paeoniflorine, the main monoterpene glucoside of *Paeonia*, has been reported to be present in the root of *P. japonica* [2]. We now report on the triterpene components of callus tissue of this plant.

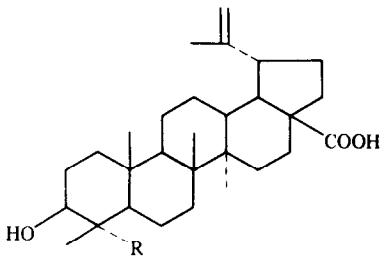
RESULTS AND DISCUSSION

Six triterpenes (**1**–**6**) were isolated from callus tissue derived from the stem of *P. japonica*. Compound **1** (1.95% of the dry wt) showed a typical oleanan skelton in the ^{1}H NMR spectrum and its properties were identical to those of oleanolic acid (**1**) (see Experimental). The structure of compound **2** (1.9% of the dry wt) was established by spectroscopic means and comparison with an authentic sample of hederagenin. (see Experimental) Compound **3** (0.5% of the dry wt) belonged to the lupan group of triterpenes and its spectral data were identical with those of an authentic sample of betulinic acid [4–6].

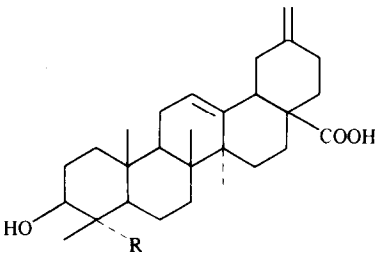
The ^{1}H NMR spectrum of compound **4** (3.0 mg, 0.08%



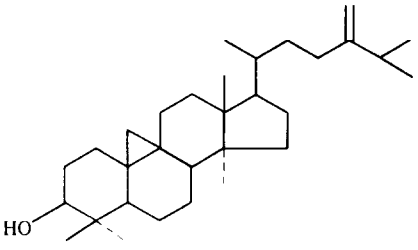
1 R = Me
2 R = CH_2OH



3 R = Me
4 R = CH_2OH



7 R = Me
5 R = CH_2OH



6

dry wt) showed one less methyl signal than **3** and two singlet protons were present at δ 4.76 and 4.91 ascribable to an endomethylene proton as in compound **3**. The signals attributable to the two carbinolic protons were shown at δ 3.71 (*d*) and 4.18 (*d*). The mass spectrum of **4** exhibited the molecular ion (M^+) at *m/z* 472, which was 16 mass units more than that of betulinic acid (**3**). In addition prominent peaks at *m/z* 248 and 203 were observed in the spectra of both **3** and **4**. From above the results, it was presumed that the D/E ring of **4** was the same as that of **3**. On comparison of the ^{13}C NMR chemical shifts of **4** and **3**, the data for the C/D/E rings showed the expected similarity. However, for C-3 and C-23 very large differences were apparent. The chemical shifts of the A/B rings were similar to those of hederagenin (**2**) (Table 1). Therefore, the D/E rings of **4** had the same skelton as **3** and the presence of a carbinol group in A ring was confirmed. The other assignments were supported by comparison of the ^{13}C NMR spectrum of **4** with those of betulinic acid (**3**) and hederagenin (**2**). Thus compound **4** was established to be 3β,23-dihydroxy lup-20(29)-en-28 oic acid and this is a new compound.

The ^{1}H NMR spectrum of compound **5** showed two protons at δ 4.75(*s*) and 4.80(*s*) ascribable to exomethylene protons and one proton at 3.24 (*m*) assigned to H-3α. Furthermore signals at δ 3.73 (*d*) and 4.19 (*d*) were ascribable to two carbinylic protons. The mass spectrum of **5** exhibited the molecular ion (M^+) at *m/z* 456 whilst peaks at *m/z* 232 (a) and 187 (a − 45) could be assigned to fragments of the D/E rings formed as a result of retro-Diels–Alder fragmentation of the C-ring of the β-amyrin Δ^{12}-skeleton [6]. These fragment ions were 16 mass units less than the corresponding fragments of hederagenin which indicated the presence of an exomethylene group in rings D/E of compound **5**. In addition the ^{13}C NMR spectrum showed 29 carbon signals which also suggested the nor-skeleton. Further, the low field carbon signals at δ 149.1 (*s*) and 107.0 (*t*) were assigned to the exomethylene C-20 and C-29 respectively, by comparison with the chemical shifts of akebonoic acid (**7**) recently obtained from *Akebia quinata* callus tissue [7]. The signals at δ 73.5 (*d*) and 68.1 (*t*) were further assigned to C-3 (with the β-configuration of the hydroxyl group) and to the C-23 hydroxymethyl carbon respectively. The remaining chemical shifts of **5** were also found to be coincident with the partial skeleton of authentic samples of hederagenin (**2**) (A/B ring) and akebonic acid (**7**) (C/D/E ring) respectively (Table 1).

Therefore, the structure of compound **5** was established as 3β-hydroxy-30-norhederagenin. [3β.23-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid]. This compound was identical with the genin afforded by acid hydrosysis of α-L-arabinopyranosyl-3β-hydroxy-30-norhederagenin obtained from *Akebia quinata* callus tissues [8]. This is first report of this genin from natural sources.

In its ^{1}H NMR spectrum, compound **6** (5.2 mg) ($M^+ = m/z$ 440) showed characteristic signals at δ 0.33 (*d*,*J* = 4.1 Hz) and 0.55 (*d*,*J* = 4.1 Hz) each integrating to one proton. This indicated the presence of a cyclopropane ring in the molecule. From the above results, compound **6** was determined as 24-methylenecycloartanol by the comparison of that of the published data [9, 10] and an authentic sample.

Paeonia japonica cultured cells produced triterpenes as the main constituents (*ca* 4.4% of the dry wt). The triterpenes isolated have not been reported from the intact plant. Furthermore, paeniflorine, the main component of the plant, was not detected in the cultured cells.

EXPERIMENTAL

Mps: uncorr; ^{1}H and ^{13}C NMR: 400 MHz and 100.6 MHz respectively, room temp., $CDCl_3$ and pyridine-d_5 with TMS as int. standard; MS: 70 eV, direct probe. Plant material *P. japonica* was collected in April 1981 from the Medicinal Plant Garden of this college.

Derivation and culture of callus tissue. The callus tissue from stalks was obtained in April 1981. Murashige and Skoog's medium (M & S) containing 2,4-D (1 mg/l; 3 mg/l) and kinetin (0.1 mg/l) as plant growth regulators were used for induction of callus tissues. The callus tissues were subcultured every 5 ~ 6 weeks on to fresh M & S medium (minus glycine) containing 2,4-D (1 mg/l) and kinetin (0.1 mg/l) at 26° ± 1 in the dark.

Extraction and isolation. Fresh callus tissue (947 g; 35 g dry wt) was extracted with cold MeOH and EtOAc in a Waring blender. The extracts were combined and concd under red. pres. to yield an extract which was partitioned between $CHCl_3$ and H_2O to

Table 1. ^{13}C NMR chemical shifts of compounds **2**–**5** and **7** (pyridine-d_5, TMS as int. standard)

C	2	5	7	3	4	C	2	5	7	3	4
1	38.9	38.8	39.0	39.3	39.2	16	23.8	23.8	23.8	32.9	32.9
2	27.5	27.7	28.1	28.3	27.9	17	46.7	47.1	47.1	56.7	56.7
3	73.5	73.5	78.2	78.2	73.6	18	46.5	48.0	48.0	47.8	47.8
4	42.8	42.9	39.4	39.5	42.9	19	42.0	42.0	42.0	49.7	49.7
5	48.7	48.7	55.9	55.9	48.9	20	30.9	148.5	148.5	151.4	151.4
6	18.6	18.6	18.8	18.8	18.6	21	34.2	38.4	38.4	31.3	31.8
7	33.0	33.0	33.3	34.9	34.6	22	33.2	30.4	30.4	37.6	37.6
8	39.8	39.8	39.8	41.2	41.2	23	68.0	68.1	28.8	28.7	68.2
9	48.1	48.1	48.1	49.8	49.8	24	13.0	13.1	16.5	16.4	12.9
10	37.8	37.2	37.4	37.4	37.6	25	15.9	16.0	15.6	16.3	16.5
11	23.8	23.8	23.8	21.3	21.3	26	17.5	17.5	17.4	19.3	19.5
12	122.6	122.6	123.3	26.2	26.2	27	26.2	26.2	26.2	14.9	14.9
13	144.8	144.9	143.5	38.7	38.7	28	180.2	179.4	177.3	178.9	178.9
14	42.2	42.0	42.1	42.9	42.9	29	33.2	107.0	107.1	19.5	19.5
15	28.3	28.3	28.3	30.3	30.3	30	23.7			109.9	109.9

obtain the organic—soluble fraction. The water phase was further partitioned with *n*-BuOH saturated with H_2O. The $CHCl_3$ soln was chromatographed over a column of silica gel (Merck 9385) and elution with $CHCl_3$ containing increasing proportion of MeOH afforded four fractions. The first fraction was purified by rechromatography over a silica gel column (HPLC, Kusano) to afford **6** from the *n*-hexane–AcOEt (5:1) eluate. The second fraction was further rechromatographed over a silica gel column (HPLC, Kusano) to afford **1** and **3**. The fourth fraction was purified by reversed-phase HPLC (Fuji gel R18-37) and elution with MeCN–H_2O (7:3) to afford **2**, **4** and **5**.

Oleanolic acid (**1**) (683.2 mg). Mp 196–198° (MeOH–$CHCl_3$) $[\alpha]_D^{24}$ +64.6° ($CHCl_3$, *c* 0.27); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705; ^1H NMR (C_5D_5N): δ 0.91 (3H, *s*), 0.96 (3H, *s*), 1.02 (3H, *s*), 1.04 (3H, *s*), 1.05 (3H, *s*), 1.24 (3H, *s*), 1.30 (3H, *s*), 3.32 (1H, *dd*, 4, 14 Hz), 3.45 (1H, *m*), 5.51 (1H, *m*); MS *m/z* (rel. int.): 456 $[M]^+$ (6), 248 (100), 207 (30), 204 (32), 203 (72), 189 (30).

Hederagenin (**2**) (667.4 mg). Mp 292° (dec., MeOH–$CHCl_3$), $[\alpha]_D^{21}$ +64.5° (C_5H_5N, *c* 0.57); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1685; ^1H NMR (C_5D_5N): δ 0.94 (3H, *s*), 0.98 (3H, *s*), 1.00 (3H, *s*), 1.054 (3H, *s*), 1.059 (3H, *s*), 1.25 (3H, *s*), 3.20 (1H, *dd*), 3.72 (1H, *d*, 10 Hz), 4.17 (1H, *d*, 10 Hz), 4.20 (1H, *m*), 5.50 (1H, *t*-like); ^{13}C NMR (C_5D_5N), δ 13.02 (*q*), 15.94 (*q*), 17.46 (*q*), 18.58 (*t*), 23.68 (*q*), 23.77 (*t*), 23.82 (*q*), 26.15 (*q*), 27.54 (*t*). 28.30 (*t*), 30.92 (*s*), 32.95 (*t*), 33.18 (*q*), 33.24 (*t*), 34.22 (*t*), 37.78 (*s*), 38.90 (*t*), 39.75 (*s*), 41.98 (*t*), 42.18 (*s*), 42.81 (*s*), 46.47 (*d*), 46.65 (*s*), 48.13 (*d*), 48.65 (*d*), 68.01 (*d*), 73.52 (*d*), 122.55 (*d*), 144.81 (*s*), 180.17 (*s*); MS *m/z* (rel. int.): 472 $[M]^+$ (18), 248 (100), 203 (66).

Betulinic acid (**3**) (180.6 mg). Mp 282–285° (MeOH–$CHCl_3$), $[\alpha]_D^{21}$ +12.42° C_5H_5N *c* 0.61); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1700, 1620; ^1H NMR (C_5D_5N): δ 0.83 (3H, *s*), 1.02 (3H, *s*), 1.07 (3H, *s*), 1.08 (3H, *s*), 1.23 (3H, *s*), 1.80 (3H, *s*), 2.26 (2H, *m*), 2.64 (1H, *dd*), 2.74 (1H, *m*), 3.46 (1H, *t*, 8 Hz), 3.55 (1H, *m*), 4.78 (1H, *s*), 4.95 (1H, *d*, 2 Hz); ^{13}C NMR (C_5D_5N): δ 14.93 (*q*), 16.34 (*q*), 16.44 (*q*), 18.82 (*t*), 19.29 (*q*), 19.51 (*q*), 21.26 (*t*), 26.16 (*t*), 28.31 (*t*), 28.69 (*q*), 30.31 (*t*), 31.26 (*t*), 32.91 (*t*), 34.88 (*t*), 37.4 (*s*), 37.57 (*t*), 38.67 (*d*), 39.33 (*t*), 39.54 (*s*), 41.17 (*s*), 42.89 (*s*), 47.80 (*d*), 49.67 (*d*), 49.83 (*d*), 55.97 (*d*), 56.66 (*s*), 78.18 (*d*), 109.94 (*t*), 151.35 (*s*), 178.86 (*s*); MS *m/z* (rel. int.): 456 $[M]^+$ (30), 438 $[M-18]^+$ (12), 411 $[M-45]^+$ (6), 248 (45), 228 (58), 207 (66), 203 (38), 189 (100).

3β,23-Dihydroxy-30-norolean-12,20(29)-dien-28-oic acid (**5**). (12.5 mg). Mp 249–249° (MeOH–EtOAc), $[\alpha]_D^{21}$ +109.6° (C_5H_5N; *c* 0,31). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1690, 1630; ^1H NMR (C_5D_5N): δ 0.97 (3H, *s*), 1.03 (3H, *s*), 1.06 (3H, *d*, 2 Hz), 1.21 (3H, *s*), 2.62 (1H, *t*, 13.4 Hz), 3.24 (1H, *d*), 3.73 (1H, *d*, 10 Hz), 4.19 (1H, *d*, 10 Hz), 4.19 (1H, *m*), 4.75 (1H, *s*), 4.80 (1H, *s*), 5.51 (1H, *br s*); ^{13}C NMR (C_5D_5N); δ 13.05 (*q*), 15.95 (*q*), 17.45 (*q*), 18.59 (*t*), 23.80 (*t*), 26.15 (*q*), 27.67 (*t*), 28.33 (*t*), 30.39 (*t*), 32.97 (*t*), 37.24 (*s*), 38.37 (*t*), 38.83 (*t*), 39.81 (*s*), 41.99 (*t* × 2), 42.88 (*s*), 46.70 (*d*), 47.05 (*s*), 47.96 (*d*), 48.11 (*s*), 48.67 (*d*), 68.07 (*t*), 73.54 (*d*), 107.03 (*t*), 122.60 (*d*), 144.85 (*s*), 148.5 (*s*), 179.35 (*s*); MS *m/z* (rel. int.): 456 $[M]^+$ (10), 232 (71), 187 (100).

3β-Hydroxy lup-20(29)-en-28 oic acid (**4**) (3.0 mg). Mp. 263–267° (MeOH–$CHCl_3$), $[\alpha]_D^{21}$ +88° (C_5H_5N; *c* 0.075). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1690, 1638; ^1H NMR (C_5D_5N): δ 0.91 (3H, *s*), 1.02 (3H, *s*), 1.04 (3H, *d*, 2.2 Hz), 1.08 (3H, *s*), 1.78 (3H, *s*) 2.19 (2H, *m*), 2.60 (1H, *d*, 12 Hz), 2.74 (1H, *t*, like), 3.54 (1H, *m*), 3.71 (1H, *d*, 10 Hz), 4.18 (1H, *d*, 10 Hz), 4.19 (1H, *m*), 4.76 (1H, *s*), 4.91 (1H, *s*); ^{13}C NMR (C_5D_5N): δ 12.89 (*q*), 14.93 (*q*), 16.50 (*q*), 18.64 (*t*), 19.49 (*q* × 2), 21.31 (*t*), 26.19 (*t*), 27.92 (*t*), 30.33 (*t*), 31.77 (*t*), 32.89 (*t*), 34.59 (*t*), 37.59 (*s* × 2), 38.68 (*s*), 39.18 (*t*), 41.18 (*s*), 42.92 (*s* × 2), 47.81 (*s*), 48.95 (*d*), 49.83 (*d*, *s*), 56.66 (*s*), 68.16 (*t*), 73.62 (*d*), 109.92 (*t*), 151.35 (*s*), 178.90 (*s*); MS *m/z* (rel. int.): 472 $[M]^+$ (15), 454 (14), 436 (23), 395 (16), 248 (83), 223 (33), 203 (75), 189 (100).

24-Methylene cycloartanol (**6**). Mp 115–117° (hexane–EtOAc), $[\alpha]_D^{21}$ +36.9° ($CHCl_3$; *c* 0.13). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640, 891; ^1H NMR ($CDCl_3$): δ 0.33 (1H, *d*, 4.2 Hz), 0.55 (1H, *d*, 4.1 Hz), 0.81 (3H, *s*), 0.90 (3H, *s*), 0.89 (3H, *d*, 6.4 Hz), 0.97 (6H, *s*), 1.025 (1H, *d*, 6.9 Hz), 1.030 (1H, *d*, 6.8 Hz), 3.29 (1H, *m*), 4.66 (1H, *d*, 1.4 Hz), 4.72 (1H, *s*); ^{13}C NMR ($CDCl_3$): δ 14.02 (*q*), 18.04 (*q*), 18.34 (*q*), 19.35 (*q*), 20.06 (*s*), 21.15 (*t*), 21.90 (*q*), 22.02 (*q*), 25.46 (*q*), 26.04 (*t*), 26.15 (*s*), 26.54 (*t*), 28.18 (*t*), 29.91 (*t*), 30.44 (*t*), 31.36 (*t*), 32.00 (*t*), 32.96 (*t*), 33.86 (*t*), 35.07 (*t*), 35.61 (*t*), 36.15 (*d*), 40.52 (*s*), 45.36 (*s*), 47.16 (*d*), 47.99 (*d*), 48.86 (*s*), 52.32 (*d*), 78.88 (*d*), 105.98 (*t*), 156.96 (*s*); MS *m/z* (rel. int.): 440 $[M]^+$ (38), 425 (54), 422 (53), 407 (61), 379 (35), 315 (28), 300 (52), 297 (32), 218 (100), 203 (77), 175 (74).

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