Triterpenoid Constituents from the Roots of *Paeonia rockii ssp. rockii*

Teresa Mencherini, Patrizia Picerno, Michela Festa, Paola Russo, Anna Capasso, and Rita Aquino*

Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, Via Ponte Don Melillo, 84084, Fisciano (SA), Italy
S1. HPLC fingerprint of the plant crude extract (It has been obtained in the same conditions reported in the paper at p 13)
S2. Detailed Extraction and Isolation Procedures.

Powder dried roots of *P. rockii* (2.73 Kg) were defatted with *n*-hexane (17.17 g), and then extracted with chloroform and dried under vacuum giving 12.8 g of PR. A portion of PR (2.6 g) was chromatographed on a silica gel column (130 g) using a step gradient of CHCl₃ and MeOH (from 7:3 to 1:1). Fractions of 20 mL were collected, analyzed by TLC (silica 60 F₂₅₄ gel-coated glass sheets with CHCl₃ or mixtures CHCl₃-MeOH (95:5, 93:7, 9:1, 4:1) and combined to ten major fractions (I-X) based on TLC patterns. Fraction I was identified as pure paeonol. Fractions II-X were chromatographed by RP-HPLC on a 30 cm × 7.8 mm i.d. µ-Bondapack column. Fractions II and III were purified using MeOH-H₂O (8:2) as mobile phase (flow rate 2.0 mL min⁻¹). II (309.3 mg) yielded benzoic acid (37.2 mg, tₐ = 4.0 min), acetovanillone (14.3 mg, tₐ = 6.0 min), compounds 4 (11.9 mg, tₐ = 41.0 min), and 5 (49.3 mg, tₐ = 46.0 min; III (134.6 mg) afforded benzoic acid (14.3 mg, tₐ = 4.0 min), resacetophenone (26.2 mg, tₐ = 6.0 min), akebonic acid (19.8 mg, tₐ = 26.0 min), and compound 5 (24.9 mg, tₐ = 46.0 min). Fractions IV (487.8 mg), VII (65.5 mg), and VIII (33.3 mg) were purified using MeOH-H₂O (1:1) as the eluent, giving benzoic acid (22.2 mg, tₐ = 20.0 min) and paeoniflorigenone (41.8 mg, tₐ = 30.0 min) from IV; paeonenoide A (1.0 mg, tₐ = 12.0 min) from VII; and lawsonicin (2.1 mg, tₐ = 9.0 min) from VIII, respectively. Fraction V (106.9 mg) separated using MeOH-H₂O (6.5:3.5) consisted of benzoic acid (51.9 mg, tₐ = 5.0 min), compound 1 (1.6 mg, tₐ = 21.0 min), and 3β,23-dihydroxy-11α,12α-epoxy-30-nor-olean-20(29)-en-28,13β-olide (50.0 mg, tₐ = 27.0 min), respectively. Fraction VI (121.7 mg) was chromatographed using MeOH-H₂O (7:3) giving 30-nor-hederagenin (6.0 mg, tₐ = 23.0 min), compound 2 (1.7 mg, tₐ = 26.0 min), 7 (2.4 mg, tₐ = 32.0 min), paeonenoide C (0.6 mg, tₐ = 30.0 min), and 6 (55.2 mg, tₐ = 37.0 min). A portion of fraction IX (180.6 mg) was chromatographed with MeOH-H₂O (1:1) to give vanillic acid (5.2 mg, tₐ = 11.0 min), and benzoypaeoniflorin (56.8 mg, tₐ = 26.0 min); the remaining part of Fraction IX (128.0 mg) was separated using MeOH-H₂O (7.5:2.5) to obtain 3β,4β,23-trihydroxy-24,30-dinor-olean-12,20(29)-dien-28-oic acid (1.3 mg, tₐ =
14.0 min), and compound 3 (1.7 mg, \( t_R = 19.0 \) min). Finally, fraction X (95.7 mg), purified using MeOH-H\(_2\)O (3.7:6.3), yielded palbinone (2.0 mg, \( t_R = 10.0 \) min).

**Compound 1:** white powder; \([\alpha]^{21}_D +50.8 \) (c 0.13, MeOH); \(^1\)H (CD\(_3\)OD, 600 MHz) and \(^{13}\)C NMR (CD\(_3\)OD, 150.9 MHz), see Table 1 and Supporting Information; HRESIMS \( m/z \) 479.2800 [M + Na]\(^+\) (calcd for C\(_{28}\)H\(_{40}\)O\(_5\)Na, 479.2773).

**Compound 2:** white powder; \([\alpha]^{21}_D + 11.4 \) (c 0.14, MeOH); \(^1\)H (CD\(_3\)OD, 600 MHz) and \(^{13}\)C NMR (CD\(_3\)OD, 150.9 MHz) see Table 1 and Supporting Information; HRESIMS \( m/z \) 455.3186 [M - H]\(^-\) (calcd for C\(_{29}\)H\(_{43}\)O\(_4\), 455.3161).

**Compound 3:** white powder; \([\alpha]^{21}_D + 16.6 \) (c 0.14, MeOH); \(^1\)H (CD\(_3\)OD, 600 MHz) and \(^{13}\)C NMR (CD\(_3\)OD, 150.9 MHz), see Table 1 and Supporting Information; HRESIMS \( m/z \) 497.3300 [M + Na]\(^+\) (calcd for C\(_{29}\)H\(_{46}\)O\(_5\)Na, 497.3243).

**Paeonol:** \(^1\)H and \(^{13}\)C NMR data were consistent with those previously reported;\(^{17}\) ESIMS \( m/z \) 167 [M - H]\(^+\).

**Benzoic acid:** \(^{13}\)C NMR data were consistent with those previously reported;\(^{19}\) ESIMS \( m/z \) 123 [M - H]\(^+\).

**Acetovanillone:** \(^1\)H and \(^{13}\)C NMR data were consistent with those previously reported;\(^{16}\) ESIMS \( m/z \) 167 [M - H]\(^+\).

**Compound 4:** \(^1\)H and \(^{13}\)C NMR data were consistent with those previously reported;\(^{16}\) ESIMS \( m/z \) 457 [M - H]\(^+\).

**Compound 5:** \(^1\)H and \(^{13}\)C NMR data were consistent with those previously reported;\(^{16}\) ESIMS \( m/z \) 457 [M - H]\(^+\).

**Resacetophenone:** \(^1\)H and \(^{13}\)C NMR data were consistent with those previously reported;\(^{18}\) ESIMS \( m/z \) 153[M - H]\(^+\).

**Akebonic acid:** \(^1\)H and \(^{13}\)C NMR data were consistent with those previously reported;\(^{13}\) ESIMS \( m/z \) 441 [M - H]\(^+\).
Paeoniflorigenone: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{21}$ ESIMS $m/z$ 319 [M - H]$^+$.  

Paeonenoide A: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{13}$ ESIMS $m/z$ 473 [M - H]$^+$.  

Lawsonicin: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{20}$ ESIMS $m/z$ 361 [M - H]$^+$.  

$^{11}\alpha,12\alpha$-epoxy-$3\beta,23$-dihydroxy-$30$-nor-olean-20(29)-en-28,13$\beta$-olide: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{25}$ ESIMS $m/z$ 493 [M - Na]$^+$.  

30-Nor-ederagenin: $^{13}$C NMR data were consistent with those previously reported; $^{24}$ ESIMS $m/z$ 457 [M - H]$^+$.  

Compound 7: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{26}$ ESIMS $m/z$ 473 [M - H]$^+$.  

Paeonenoide C: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{13}$ ESIMS $m/z$ 465 [M - Na]$^+$.  

Compound 6: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{14}$ ESIMS $m/z$ 473 [M-H]$^+$.  

Vanillic acid: $^{13}$C NMR data were consistent with those previously reported; $^{19}$ ESIMS $m/z$ 169 [M - H]$^+$.  

Benzoylpaeoniflorin: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{17}$ ESIMS $m/z$ 585 [M - H]$^+$.  

$3\beta,4\beta,23$-Trihydroxy-$24,30$-dinor-olean-12,20(29)-dien-28-oic acid: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{23}$ ESIMS $m/z$ 459 [M - H]$^+$.  

Palbinone: $^1$H and $^{13}$C NMR data were consistent with those previously reported; $^{22}$ ESIMS $m/z$ 359 [M - H]$^+$.  


S3. $^1$H NMR (600 MHz, CD$_3$OD) of the new compound 1
S4. $^{13}$C NMR (600 MHz, CD$_3$OD) of the new compound 1
S5. Zoom $^{13}$C NMR (600 MHz, CD$_3$OD) of the new compound 1
S6. HSQC (600 MHz, CD$_3$OD) of the new compound 1
S7. HMBC (600 MHz, CD$_3$OD) of the new compound 1
S8. DQF-COSY (600 MHz, CD$_3$OD) of the new compound 1
S9. NOESY (600 MHz, CD$_3$OD) of the new compound 1
S10. $^1$H NMR (600 MHz, CD$_3$OD) of the new compound 2
S11. $^{13}$C NMR (600 MHz, CD$_3$OD) of the new compound 2
S12. Zoom $^{13}$C NMR (600 MHz, CD$_3$OD) of the new compound 2
S13. HSQC (600 MHz, CD$_3$OD) of the new compound 2

- CH$_2$OH at C-23
- Me-29/C-29

H-21/C-21
H-12/C-12
S14. HMBC (600 MHz, CD$_3$OD) of the new compound 2
S15. DQF COSY (600 MHz, CD$_3$OD) of the new compound 2
S16. NOESY (600 MHz, CD$_3$OD) of the new compound 2
S17. \(^1\)H NMR (600 MHz, CD\(_3\)OD) of the new compound 3
S18. $^{13}$C NMR (600 MHz, CD$_3$OD) of the new compound 3
S19. Zoom $^{13}$C NMR (600 MHz, CD$_3$OD) of the new compound 3
S20. HSQC (600 MHz, CD³OD) of the new compound 3
S21. HMBC (600 MHz, CD$_3$OD) of the new compound 3

H-23a and H-23b
C-5
C-3
C-4
S22. DQF COSY (600 MHz, CD$_3$OD) of the new compound 3
S23. NOESY (600 MHz, CD$_3$OD) of the new compound 3