C3_C0045: CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Pseuduvaria monticola* (ANNONACEAE)

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Abstract: Phytochemical investigation of the leaves of *Pseuduvaria monticola*, collected from Trang Province, resulted in the isolation of eight known compounds including four sesquiterpenoids (**1-4**), one aporphine alkaloid (**5**), one flavonoid (**6**), one diterpenoid (**7**) and one benzopyran derivative (**8**). The structures were elucidated on the basis of spectroscopic methods including UV, IR and NMR and by comparison with reported literature data.

Introduction: The genus *Pseuduvaria* belonging to the family Annonaceae is distributed in Southeast Asia and extending from Indochina and the Philippines to New Guinea and Northeast Australia. This genus consists of 56 species[1] but 7 species are found in Thailand, *i.e. P. macrophylla* var. *sessilicarpa*, *P. monticola*, *P. multiovulata*, *P. rugosa*, *P. setosa*, *P. fragrans*, and *P. gardneri*. Previous phytochemical studies on *Pseuduvaria* species have revealed the presence of alkaloids,[2-4] diterpenoids[5] and benzopyran derivatives.[6] We now report the isolation and structure elucidation of eight known compounds (**1-8**) from the the leaves of *P. monticola*.

Methodology:

General experimental procedures: Vacuum liquid chromatography (VLC) and column chromatography (CC) were carried out on siliga gel 60 H (Merck) and silica gel 100 (Merck), respectively. Spots on TLC were detected under a UV lamp and by spraying with vanillin (1% in H_2SO_4). The melting points were determined on a Fisher-John melting point apparatus. The IR spectra were recorded as ATR on a Shimudzu FTIR-8900 IR spectrophotometer. The optical rotations were determined on a JASCO P-1020 polarimeter. ^1H and ^{13}C NMR spectra were measured on a 400 MHz Bruker FTNMR Ultra Shield^{TM} spectrometer using $CDCl_3$ as a solvent and tetramethylsilane as an internal reference.

Plant material: The leaves of *P. monticola* were collected in Trang Province, Thailand in 2010 and were identified by Dr. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU2010026) is deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

Extraction and Isolation: The dried and milled leaves *P. monticola* (2.6 kg) were extracted with EtOH at room temperature to give, after evaporation of the solvent under reduced pressure, a brown viscous residue (189.65 g) which was subjected to vacuum liquid chromatography (VLC) over silica gel using solvent of increasing polarity from n-hexane through EtOAc and MeOH. The eluates were collected and combined based on TLC characteristic to give nine fractions (1-9). Fraction 4 was further separated by VLC (hexane/CH_2Cl_2, 19:1, 9:1, 6:1, 4:1, 2:1, 1:1, 1:4, 1:6) to yield **2** (28.1 mg) and **4** (101.4 mg). Fraction 5 was purified by VLC (hexane/acetone, 99:1, 49:1, 33:1, 19:1, 14:1, 9:1, 6:1, 4:1) to give **1** (3.7 mg), **3** (3.0 mg), **6** (20.2 mg) and **7** (20.3 mg). Fraction 9 was further separated by VLC (hexane/Acetone, 99:1, 50:1, 33:1, 19:1, 9:1, 6:1, 4:1, 2:1, 1.5:1, 1:1) to afford **5** (97.8 mg) and **8** (7.0 mg).

1β-Hydroxy4(15),5*E*,10(14)-germacratriene (**1**)

Colorless oil; $[\alpha]_D$ -38.6 (*C* 0.2, $CHCl_3$); UV (MeOH) λ_{max} nm: 243 nm; IR ν_{max}: 3431, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.82 (3H, *d*, *J* = 6.8 Hz, H-13), 0.90 (3H, *d*, *J* = 6.8 Hz, H-12), 1.49 (1H, *m*, H-11), 1.58 (1H, *m*, H-9a), 1.66 (1H, *m*, H-8a), 1.79 (1H, *m*, H-7), 2.02 (1H, *m*, H-8b), 2.03 (2H, *m*, H-2), 2.19 (1H, *m*, H-3a), 2.43 (1H, *ddd*, *J* = 13.2, 12.8, 4.1 Hz, H-3b), 2.62 (1H, *m*, H-9b), 3.77 (1H, *m*, H-1), 4.84 (1H, *br s*, H-15b), 4.92 (1H, *br s*, H-15a), 5.00 (1H, *br s*, H-14b), 5.27 (1H, *br s*, H-14a), 5.43 (1H, *dd*, *J* = 16.0, 10.2 Hz, H-6), 6.00 (1H, *d*, *J* = 16.0 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4 (C-13), 20.7 (C-12), 29.9 (C-3), 31.8 (C-11), 34.5 (C-9), 36.1 (C-2), 36.2 (C-8), 52.5 (C-7), 76.0 (C-1), 110.5 (C-14), 112.8 (C-15), 129.6 (C-5), 137.9 (C-6), 146.7 (C-4), 153.5 (C-10). The spectral data for **1** were identical with those reported in the literature.[7]

β-Caryophyllene 8*R*, 9*R*-oxide (**2**)

Colorless oil; $[\alpha]_D$ -54.7 (*C* 0.6, $CHCl_3$); IR ν_{max}: 1693, 1461, 1379 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.99 (3H, *s*, H-14), 0.97 (1H, *m*, H-7a), 1.01 (3H, *s*, H-13), 1.20 (3H, *s*, H-15), 1.31 (1H, *m*, H-10a), 1.45 (1H, *m*, H-6a), 1.60-1.70 (2H, *m*, H-3), 1.64 (1H, *m*, H-6b), 1.77 (1H, *t*, *J* = 9.6 Hz, H-5), 2.11 (1H, *m*, H-7b), 2.12 (1H, *m*, H-11a), 2.25 (1H, *m*, H-10b), 2.35 (1H, *m*, H-11b), 2.62 (1H, *dt*, *J*=9.7, 9.3 Hz, H-2), 2.88 (1H, *dd*, *J* = 10.6, 4.2 Hz, H-9), 4.86 (1H, *br s*, H-12a), 4.99 (1H, *br s*, H-12b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.0 (C-15), 21.6 (C-13), 27.2 (C-6), 29.8 (C-11), 29.9 (C-14), 30.2 (C-10), 34.0 (C-4), 39.2 (C-7), 39.8 (C-3), 48.7 (C-2), 50.7 (C-5), 59.9 (C-8), 63.8 (C-9), 112.8 (C-12), 151.8 (C-1). The spectral data for **2** were identical with those reported in the literature.[8]

Kobusone (**3**)

Colorless oil; $[\alpha]_D$ -106.3 (*C* 0.7, $CHCl_3$); IR ν_{max}: 1742, 1452, 1121 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.95 (1H, *td*, H-7a), 2.15 (1H, *m*, H-7b), 1.035 (1H, *s*, H-14), 1.04 (1H, *s*, H-13), 1.31 (1H, *s*, H-15), 1.44 (1H, *m*, H-10a), 1.53 (1H, *m*, H-6a), 1.65 (1H, *m*, H-3a), 1.65 (1H, *m*, H-6b), 1.94 (1H, *m*, H-5), 2.07 (1H, *m*, H-3b), 2.41 (1H, *m*, H-10b), 2.54 (1H, *m*, H-11a), 2.57 (1H, *m*, H-11b), 2.70 (1H, *dd*, *J* = 10.0, 4.8 Hz, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.2 (C-15), 22.2 (C-13), 24.8 (C-10), 26.5 (C-6), 29.3 (C-14), 34.5 (C-4), 35.5 (C-3), 37.7 (C-11), 39.0 (C-7), 51.3 (C-5), 52.7 (C-2), 59.0 (C-8), 61.7 (C-9), 214.0 (C-1). The spectral data for **3** were identical with those reported in the literature.[8]

Spathulenol (**4**)

Colorless oil; $[\alpha]_D$ +14.6 (*C* 0.25, $CHCl_3$); IR ν_{max}: 3400, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.47 (1H, *dd*, *J* = 11.2, 9.6 Hz, H-2), 0.71 (1H, *m*, H-4), 1.04 (3H, *s*, H-12), 1.06 (3H, *s*, H-13), 1.28 (3H, *s*, H-15), 2.20 (1H, *m*, H-1), 1.31 (2H, *m*, H-5), 1.57 (1H, *m*, H-10b), 1.75 (1H, *m*, H-10a), 1.91 (1H, *m*, H-9b), 1.95 (2H, *m*, Hab-8), 2.07 (1H, *m*, H-9a), 2.42 (2H, *dd*, *J* = 13.3, 6.2 Hz, H-6), 4.66 (1H, *s*, H-15b), 4.69 (1H, *s*, H-15a); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.3 (C-14), 20.3 (C-3), 24.8 (C-5), 26.1 (C-12), 26.70 (C-9), 27.5 (C-4), 28.7 (C-13), 29.9 (C-2), 38.7 (C-6), 41.7 (C-10), 53.4 (C-8), 54.3 (C-1), 81.0 (C-11), 106.2 (C-15), 153.5 (C-7). The spectral data for **4** were identical with those reported in the literature.[9]

Liriodenine (**5**)

Yellow needles; mp 281-282 °C ; UV (MeOH) λ_{max} nm: 338, 280, 260 nm; IR ν_{max}: 1737, 1418, 1228 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 6.33 (2H, *s*, O-CH_2-O), 7.12 (1H, *s*, H-3), 7.54 (1H, *td*, *J* = 8.0, 8.0, 1.2 Hz, H-9), 7.72 (1H, *td*, *J* = 7.2, 1.6 Hz, H-10), 7.74 (1H, *d*, *J* = 5.2 Hz, H-4), 8.53 (1H, *dd*, *J* = 7.8, 1.2 Hz, H-8), 8.56 (1H, *br d*, H-11), 8.86 (1H, *d*, *J* = 5.2 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 102.3 (O-CH_2-O), 103.2 (C-3), 108.0 (C-1b), 123.2 (C-1a), 124.3 (C-4), 127.3 (C-11), 128.6 (C-8), 128.7 (C-9), 131.2 (C-11a), 132.8 (C-7a), 133.9 (C-10), 135.7 (C-3a), 144.8 (C-5), 145.2 (C-6a), 147.9 (C-1), 151.8 (C-2), 182.4 (C-7). The spectral data for **5** were identical with those reported in the literature.[10]

5-Hydroxy-6,7,8-trimethoxyflavanone (**6**)

Yellow crystals; mp 99-100 °C; $[\alpha]_D$ -14.67 (*C* 0.12, MeOH); UV (MeOH) λ_{max} nm: 330, 275 nm; IR ν_{max}: 1662, 1600, 1485, 1430, 1360, 1310, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.76 (1H, *dd*, *J* = 17.2, 2.0 Hz, Ha-3), 2.98 (1H, *dd*, *J* = 17.2, 13.2 Hz, Hb-3), 3.99 (3H, *s*, 7-OCH_3), 3.71 (3H, *s*, 8-OCH_3), 3.76 (3H, *s*, 6-OCH_3), 5.35 (1H, *dd*, *J* = 13.2, 2.0 Hz, H-2), 7.32-7.40 (5H, *m*, H-2'-6'), 11.75 (1H, *s*, 5-OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 43.6 (C-3), 61.0 (6-OCH_3), 61.4 (7-OCH_3), 61.5 (8-OCH_3), 79.2 (C-2), 104.7 (C-4a), 126.0 (C-2', C-6'), 128.7 (C-3'-C-5'), 128.8 (C-4'), 133.2 (C-8), 134.1 (C-6), 138.5 (C-1'), 150.4 (C-5), 151.8 (C-8a), 155.5 (C-7), 197.0 (C-4). The spectral data for **6** were identical with those reported in the literature.[11]

Phytol (**7**)

Colorless oil; $[\alpha]_D$ +1.2 (*C* 1.0, $CHCl_3$); IR ν_{max}: 3373, 1463 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.84 (3H, *d*, *J* = 6.64 Hz, H-19), 0.86 (3H, *d*, *J* = 6.64 Hz, H-18), 0.87 (6H, *d*, *J* = 6.44 Hz, H-17, H-16), 1.67 (3H, *s*, H-20), 1.99 (2H, *t*, *J* = 7.3 Hz, H-4), 5.41 (1H, *t*, *J* = 6.9 Hz, H-2), 4.15 (2H, *d*, *J* = 6.9 Hz, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.2 (C-20), 19.7 (C-19), 19.8 (C-18), 22.6 (C-17), 22.7 (C-16), 24.5 (C-9), 24.8 (C-13), 25.1 (C-5), 27.9 (C-15), 32.7 (C-7), 32.8 (C-11), 36.7 (C-6), 37.4 (C-12), 37.4 (C-8), 37.4 (C-10), 39.4 (C-14), 39.9 (C-4), 59.4 (C-1), 123.1 (C-2), 140.3 (C-3). The spectral data for **7** were identical with those reported in the literature.[12]

Isopolycerasoidol (**8**)

Brown oil; $[\alpha]_D$ 14.67 (*C* 0.16, MeOH); UV (MeOH) λ_{max} nm: 294, 234 nm; IR ν_{max}: 3387, 1670, 1470 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.82 (3H, *s*, H-13), 1.60 (3H, *s*, H-14), 1.26 (3H, *s*, H-15), 2.12 (3H, *s*, H-7a), 5.16 (1H, *t*, *J* = 6.8 Hz, H-6), 6.38 (1H, *d*, *J* = 2.4 Hz, H-3a), 6.48 (1H, *d*, *J* = 2.4 Hz, H-5a), 6.87 (1H, *t*, *J* = 7.2 Hz, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.0 (C-13), 15.8 (C-14), 16.1 (C-7a), 22.1 (C-1), 22.5 (C-5), 24.0 (C-15), 27.4 (C-9), 31.4 (C-2), 38.0 (C-8), 39.4 (C-4), 75.3 (C-3), 112.7 (C-3a), 115.7 (C-5a), 121.2 (C-2a), 123.7 (C-6a), 125.4 (C-6), 127.0 (C-11), 133.8 (C-7), 144.9 (C-10), 145.9 (C-1a), 147.8 (C-4a), 173.2 (C-12). The spectral data for **8** were identical with those reported in the literature.[13]

Results and Discussion: The leaves of *P. monticola* were pulverized and extracted successively with EtOH. Vacuum liquid chromatography (VLC) of the residue from the methylene chloride extract furnished eight compounds **1-8**.

Compound **1** was analyzed for $C_{15}H_{24}O$. Its IR spectrum showed absorption bands at 3431cm^{-1} (hydroxyl) and 1454 cm^{-1} (double bond). The ^1H and ^{13}C NMR spectra of **1** indicated the presence of an isopropyl unit, a secondary alcohol moiety, a *trans* olefinic double bond and two exocyclic olefinic groups along with four methylene carbons and one methine carbon. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report,[7] compound **1** was identified as 1β-hydroxy-4(15),5*E*,10(14)-germacratriene.

Compound **2** was analyzed for $C_{15}H_{24}O$. Its IR spectrum showed absorption bands at 1693cm^{-1} (double bond) and 1461 cm^{-1} (C-O bond). The ^1H-NMR spectrum showed signals for three tertiary methyls, three signals of aliphatic methine groups and two geminal olefinic group. The ^{13}C-NMR spectrum showed three methyl carbons, five methylene carbons, three methine carbons and one terminal olefinic carbon. In addition, signals of one quaternary carbon, one oxyquaternary carbon and one olefinic quaternary carbon were observed. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report,[8] compound **2** was identified as β-caryophyllene 8*R*, 9*R*-oxide.

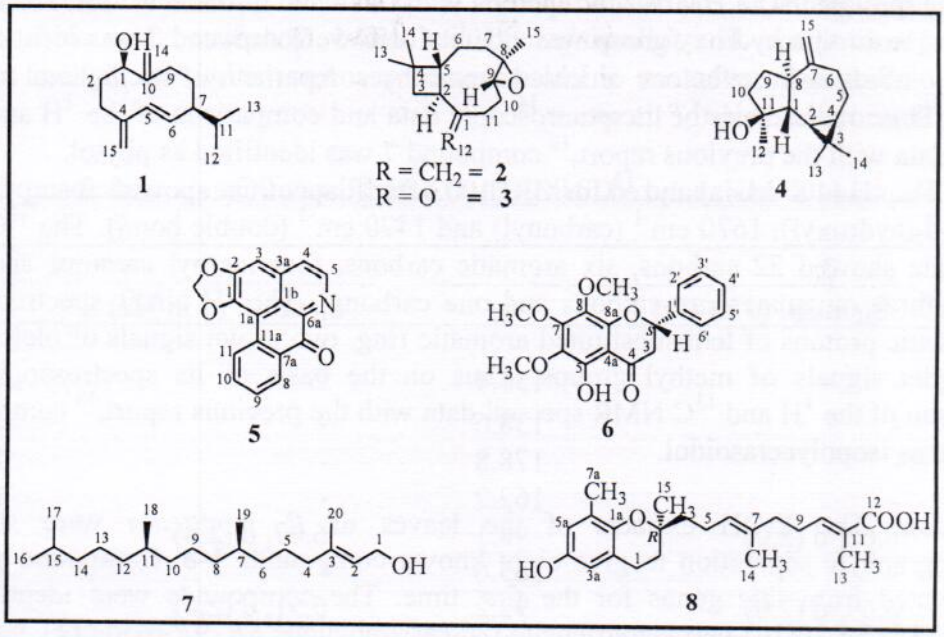

Figure 1. Compounds (**1-8**) isolated from *Pseuduvaria monticola.*

Compound **3** was analyzed for $C_{14}H_{22}O_2$. Its IR spectrum showed absorption band at 1742 cm^{-1} (carbonyl). The ^1H and ^{13}C NMR data of compounds **2** and **3** revealed that they differed only at C-12. While compound **2** had a C-12 exomethylene, compound **3** had a C-12 carbonyl group. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report,[8] compound **3** was identified as kobusone.

Compound **4** was analyzed for $C_{15}H_{24}O$. Its IR spectrum showed absorption bands at 3400 (hydroxyl) and 1640 cm^{-1} (double bond). The ^1H NMR spectrum exhibited signals for three singlet methyls, four methine protons, four methylene groups and exomethylene protons. The ^{13}C-NMR spectrum showed three methyl carbons, four methylene carbons, four methine carbons, one quaternary carbon, one oxyquaternary carbon and two olefinic carbons. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report,[9] compound **4** was identified as spathulenol.

Compound **5** was analyzed for $C_{17}H_9NO_3$. Its IR spectrum showed absorption band at 1737 cm^{-1} (carbonyl). The ^1H NMR spectral data displayed signals of seven aromatic and methylenedioxy protons. The ^{13}C NMR spectrum indicated the presence of 17 carbons with eight quaternary carbons, one carbonyl and two oxyaryl carbons. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report,[10] compound **5** was identified as liriodenine.

Compound **6** was analyzed for $C_{18}H_{18}O_6$. Its IR spectrum showed absorption bands at 1662, 1600 cm^{-1} (double bond) and 1485 cm^{-1} (C-O bond). The ^1H-NMR spectrum showed a multiplet of five aromatic protons, characteristic of a monosubstituted phenyl ring, three aromatic methoxy groups and one hydrogen bonded phenolic hydroxyl group. The ^{13}C-NMR spectrum exhibited signals of one monosubstituted aromatic ring, one hexasubstituted aromatic ring, three methoxy carbons, one oxymethine carbon, one methylene carbon and one carbonyl carbon. Thus on the basis of its spectroscopic data and comparison of the ^1H and ^{13}C NMR spectral data with the previous report,[11] compound **6** was identified as 5-hydroxy-6,7,8-trimethoxyflavanone.

Compound **7** was analyzed for $C_{20}H_{40}O$. Its IR spectrum showed absorption bands at 3373cm^{-1} (hydroxyl) and 1463 cm^{-1} (double bond). The ^1H NMR spectrum indicated resonances for an olefinic proton, four methyl groups, an allylic methyl singlet and allylic

methylene protons. The ^{13}C NMR spectral data showed resonances for twenty carbons, suggesting a diterpene. The following functionalities were deduced: two olefinic carbons, a carbinyl carbon and seventeen shielded resonances for methyl, methylene and methine carbons. Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report,[12] compound **7** was identified as phytol.

Compound **8** was analyzed for $C_{22}H_{30}O_4$. Its IR spectrum showed absorption bands at 3387 cm^{-1} (hydroxyl), 1670 cm^{-1} (carbonyl) and 1470 cm^{-1} (double bond). The ^{13}C NMR and DEPT data showed 22 carbons, six aromatic carbons, four methyl carbons, six methylene carbons, three oxyquaternary carbons and one carbonyl. The ^{1}H NMR spectrum displayed two aromatic protons of tetrasubstituted aromatic ring, two triplet signals of olefinic protons, four singlet signals of methyl groups. Thus on the basis of its spectroscopic data and comparison of the ^{1}H and ^{13}C NMR spectral data with the previous report,[13] compound **8** was identified as isopolycerasoidol.

Conclusion: The EtOH extracts of the leaves of *P. monticala* were subjected to chromatographyic separation to give eight known compounds **1-8**. Compound **1-4** and **6-8** were isolated from this genus for the first time. The compounds were identified as 1β-hydroxy4(15),5*E*,10(14)-germacratriene (**1**), β-caryophyllene 8*R*, 9*R*-oxide (**2**), kobusone (**3**), spathulenol (**4**), liriodenine (**5**), 5-hydroxy-6,7,8-trimethoxyflavanone (**6**), phytol (**7**) and isopolycerasoidol (**8**). Their structures were elucidated by spectral evidences and comparison with reported values.

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