Three New Phthalides from *Gnaphalium adnatum*

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Three new phthalides, gnaphalides A – C (**1** – **3**, resp.), together with three known phthalides, were isolated from the aerial part of *Gnaphalium adnatum*. The structures of the new compounds were elucidated as 6-(1,1-dimethylprop-2-en-1-yl)-5,7-dihydroxy-2-benzofuran-1(3*H*)-one (**1**), 5-hydroxy-7-[(2-hydroxy-3-methylbut-3-en-1-yl)oxy]-2-benzofuran-1(3*H*)-one (**2**), and 1,3-dihydro-7-[(3-methylbut-2-en-1-yl)oxy]-1-oxo-2-benzofuran-5-yl *β*-D-glucopyranoside (**3**) on the basis of spectral analyses. The structure of **1** was also confirmed by X-ray crystallographic analysis. The three known phthalides, identified as 5,7-dihydroxyisobenzofuran-1(3*H*)-one (**4**), anaphatol (**5**), and 7-*O*-(*β*-glucopyranosyl)-5-hydroxyisobenzofuran-1(3*H*)-one (**6**), were isolated from the genus *Gnaphalium* for the first time.

Introduction. – The genus *Gnaphalium* (Asteraceae) is represented with 19 species in China, mostly growing in the southeastern part of the Yangtze River, and seven species have so far been found in Yunnan Province [1][2]. The chemical constituents reported so far from this genus include flavonoids, terpenoids, steroids, benzofuranones, and essential oils [3–6]. Some of these constituents showed antioxidant, antibacterial, anti-inflammation, and antitussive activities [7][8]. The aerial parts of *Gnaphalium adnatum* WALL. ex DC. in Xishuangbanna region have been used as Dai-nationality medicine for the treatment of cough, diarrhea, abdominal pain, and rheumatic pain. As a continuation of our studies on medicinal plants growing on the Yunnan-Tibet Plateau, *G. adnatum* was studied. To the best of our knowledge, no scientific study on this plant has hitherto been reported, except a general screening of Taiwanese plants for antibacterial activity against *Helicobacter pylori* [9].

From its aerial parts, three new phthalides, named gnaphalides A, B, and C (**1** – **3**, resp.), as well as three known phthalides, were isolated. The known phthalides, identified as 5,7-dihydroxyisobenzofuran-1(3*H*)-one (**4**) [10], anaphatol (**5**) [11], and 7-*O*-(*β*-glucopyranosyl)-5-hydroxyisobenzofuran-1(3*H*)-one (**6**) [12], were isolated from the genus *Gnaphalium* for the first time (*Fig. 1*). Herein, we report the isolation and structure elucidation of the new phthalides **1** – **3**.

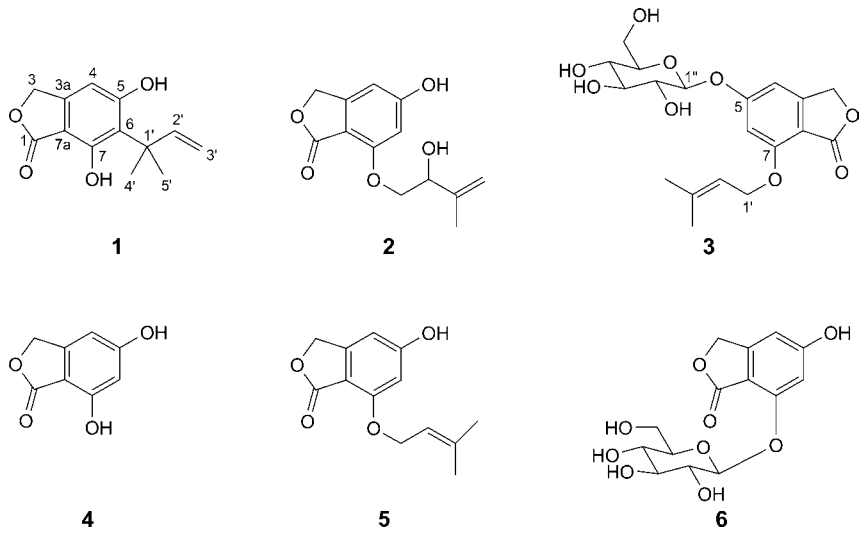

Fig. 1. *Structures of gnaphalides A – C* (**1** – **3**, resp.) *and* **4** – **6**

Results and Discussion. – Gnaphalide A (**1**) was isolated as colorless flaky crystals. Its molecular formula was determined as $C_{13}H_{14}O_4$ by HR-EI-MS (234.0890 (M^+)). The IR spectrum displayed characteristic absorptions for OH (3398 cm^{-1}) and lactone (1694 cm^{-1}) moieties. The UV spectrum showed absorption at 304 nm. From ^{1}H- and ^{13}C-NMR (*Table*), HMBC, HMQC, NOESY, and ^{1}H,^{1}H-COSY data (*Fig. 2*), as well as the X-ray data, **1** was elucidated as 6-(1,1-dimethylprop-2-en-1-yl)-5,7-dihydroxy-2-benzofuran-1(3*H*)-one.

The ^{1}H- and ^{13}C-NMR (DEPT) spectra (*Table*) evidenced the presence of 13 C-atoms, including two Me groups (δ(H) 1.61 (*s*); δ(C) 27.2 and 27.2, resp.), a CH_2O group (δ(H) 5.19 (*s*); δ(C) 70.0), an olefinic CH_2 group (δ(H) 5.41 (*dd*, $J = 6.6, 10.5$); δ(C) 114.0), an olefinic CH group (δ(H) 6.35 (*t*, $J = 10.5$); δ(C) 148.7), an aromatic CH group (δ(H) 6.42 (*s*); δ(C) 103.2), and seven quaternary C-atoms (δ(C) 41.2, 104.1, 117.9, 145,8, 157.2, 163.4, and 173.6). In the HMBC spectrum (*Fig.* 2), the aromatic H-atom (δ(H) 6.42) showed ^3J-correlation with C(3) (δ(C) 70.0) and C(7a) (104.1), and the CH_2O group (δ(H) 5.19) showed ^3J-correlation with C(4) (δ(C) 103.2), C(7a) (104.1), and C(1) (173.6), suggesting that **1** was a 1(3*H*)-type phthalide. In addition, the olefinic CH_2 group (δ(H) 5.41) showed ^3J-correlation with C(1′) (δ(C) 41.2), and the olefinic CH group (δ(H) 6.35) showed ^3J-correlation with C(4′,5′) (δ(C) 27.2) and aromatic C(6) (117.9), indicating the presence of a 1,1-dimethylprop-2-en-1-yl group and revealing its attachment at C(6). Other HMBC, ^{1}H,^{1}H-COSY, and HSQC data, as well as the X-ray crystallographic analysis (*Fig.* 3), were in complete agreement with the assigned structure of **1** as 6-(1,1-dimethylprop-2-en-1-yl)-5,7-dihydroxy-2-benzofuran-1(3*H*)-one.

Gnaphalide B (**2**) was isolated as colorless needle crystals. The molecular formula was established as $C_{13}H_{14}O_4$ by HR-EI-MS (250.0839 (M^+)). The IR spectrum displayed characteristic absorptions for OH (3519 cm^{-1}) and lactone (1708 cm^{-1})

Table. *^{1}H- and ^{13}C-NMR* (300 and 75 MHz, resp.) *Data of Gnaphalides A – C* (**1** – **3**, resp.). δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		173.6		172.3		167.7
3	5.19 (*s*)	70.0	5.14 (*s*)	70.3	5.21 (*s*)	68.3
3a		145.8		153.9		151.6
4	6.42 (*s*)	103.2	6.48 (*s*)	101.9	6.76 (*s*)	101.1
5		163.4		167.3		163.9
6		117.9	6.44 (*s*)	101.0	6.70 (*s*)	101.0
7		157.2		160.6		158.2
7a		104.1		105.5		106.3
1′		41.2	3.96 (*d*, J = 7.8)	73.8	4.65 (*d*, J = 4.8)	65.2
2′	6.35 (*t*, J = 10.5)	148.7	4.30 – 4.28 (*m*)	74.0	5.44 (*d*, J = 4.8)	119.1
3′	5.41 (*dd*, J = 10.5, 6.6)	114.0		145.3		137.8
4′	1.61 (*s*)	27.2	5.04 (*s*), 4.87 (*s*)	113.0	1.76 (*s*)	18.1
5′	1.61 (*s*)	27.2	1.77 (*s*)	19.2	1.72 (*s*)	25.4
1″					5.01 (*d*, J = 6.6)	100.0
2″					3.31 – 3.27 (*m*)	73.1
3″					3.41 – 3.38 (*m*)	77.2
4″					3.21 – 3.17 (*m*)	69.6
5″					3.31 – 3.27 (*m*)	76.5
6″					3.74 – 3.69 (*m*), 3.45 – 3.42 (*m*)	60.6

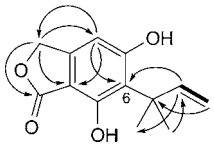

Fig. 2. *Significant ^{1}H,^{1}H-COSY* (—) *and HMB* (H → C) *correlations of* **1**

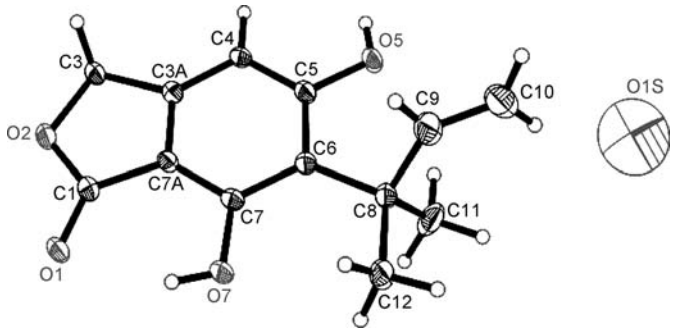

Fig. 3. *ORTEP Plot of X-ray crystal structure of* **1**

groups, and for an aromatic ring (1615 and 1482 cm^{-1}). The UV spectrum showed absorption at 286 nm. From the ^{1}H- and ^{13}C-NMR (*Table*), HMBC, HMQC, NOESY, and ^{1}H,^{1}H-COSY data (*Fig. 4*), **2** was elucidated as 5-hydroxy-7-[(2-hydroxy-3-methylbut-3-en-1-yl)oxy]-2-benzofuran-1(3*H*)-one.

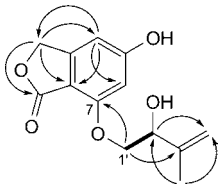

Fig. 4. *Significant $^1H,^1H$-COSY correlations* (—) *and HMBCs* (H → C) *of* **2**

The ^1H- and ^{13}C-NMR (DEPT) spectra (*Table*) revealed the presence of 13 C-atoms, including two aromatic CH groups (δ(H) 6.48 (*s*) and 6.44 (*s*); δ(C) 101.9 and 101.0), a CH_2O group (δ(H) 5.14 (*s*); δ(C) 70.3), an olefinic CH_2 group (δ(H) 5.04, 4.87 (*s*); δ(C) 113.0), a CHO group (δ(H) 4.30–4.28 (*m*); δ(C) 74.0), a CH_2O group (δ(H) 3.96 (*d*, $J = 7.8$); δ(C) 73.8), a Me group (δ(H) 1.77 (*s*); δ(C) 19.2), and six quaternary C-atoms (δ(C) 105.5, 145.3, 153.9, 160.6, 167.3, and 172.3). These data, together with seven degrees of unsaturation, suggested that **2** was also a phthalide. The ^{13}C-NMR (DEPT) spectra exhibited the following signals of phthalide C-atoms: δ(C) 172.3 (C(1)), 70.3 (C(3)), 153.9 (C(3a)), 101.9 (C(4)), 167.3 (C(5)), 101.0 (C(6)), 160.6 (C(7)), 105.5 (C(7a))). In the HMBC spectrum (*Fig. 4*), the olefinic CH_2 group (δ(H) 5.04 and 4.87) showed ^2J-correlation with C(3′) (δ(C) 145.3), and ^3J-correlations with C(2′) (74.0) and C(5′) (19.2), the CH–O H-atom (δ(H) 4.30–4.28) showed ^3J-correlation with C(4′) (δ(C) 113.0) and C(5′) (19.2), and the CH_2O H-atom (δ(H) 3.96) showed ^3J-correlations with C(7) (δ(C) 160.6) and C(3′) (145.3), indicating the presence of a 2-hydroxy-3-methylbut-3-enyl group and revealing its attachment at C(7). Other HMBC, $^1H,^1H$-COSY, and HSQC data were in complete agreement with the assigned structure of **2** as 5-hydroxy-7-[(2-hydroxy-3-methylbut-3-en-1-yl)oxy]-2-benzofuran-1(3*H*)-one.

Gnaphalide C (**3**) was isolated as colorless needle crystals. The molecular formula was deduced as $C_{19}H_{24}O_9$ from HR-EI-MS (396.1421 (M^+)). The IR spectrum displayed characteristic absorptions for OH (3550 cm^{-1}) and lactone (1749 cm^{-1}) groups, and for an aromatic ring (1615 and 1445 cm^{-1}). The UV spectrum showed an absorption at 304 nm. From the ^1H- and ^{13}C-NMR (*Table*), HMBC, HMQC, NOESY, and $^1H,^1H$-COSY data (*Fig. 5*), **3** was elucidated as 1,3-dihydro-7-[(3-methylbut-2-en-1-yl)oxy]-1-oxo-2-benzofuran-5-yl β-D-glucopyranoside.

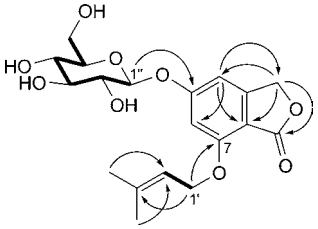

Fig. 5. *Significant $^1H,^1H$-COSY correlations* (—) *and HMBCs* (H → C) *of* **3**

The ^1H-NMR spectrum (*Table*) displayed signals of two aromatic H-atoms (δ(H) 6.76 (*s*, H–C(4)) and 6.70 (*s*, H–C(6))), a CH_2O group (5.21 (*s*, CH_2(3))), an olefinic CH group (5.44 (*d*, $J = 4.8$, H–C(2′))), a CH_2O group (4.65 (*d*, $J = 4.8$, CH_2(1′))), two

Me groups (1.76 (*s*, Me(4′)) and 1.72 (*s*, Me(5′))). These data were closely similar to those of **5** (anaphatol) [10]. The ^{13}C-NMR spectrum (*Table*) revealed the presence of phthalide C-atoms (δ(C) 167.7 (C(1)), 68.3 (C(3)), 151.6 (C(3a)), 101.1 (C(4)), 163.9 (C(5)), 101.0 (C(6)), 158.2 (C(7)), 106.3 (C(7a))), and a prenyl group (65.2 C(1′)), 119.1 C(2′)), 137.8 C(3′)), 18.1 C(4′)), and 25.4 C(5′))). The rest of the C-atom signals suggested the presence of a β-glucose (six signals at δ(C) 100.0 (C(1″)), 73.1 (C(2″)), 77.2 (C(3″)), 69.6 (C(4″)), 76.5 (C(5″)), and 60.6 (C(6″))), and the ^{1}H-NMR signal at δ(H) 5.01 (*d*, $J = 6.6$, H–C(1″)) evidenced the β-configuration of the glucose. Acid hydrolysis of **3** afforded glucose identified by co-TLC analysis with authentic sample. In the HMBC spectrum (*Fig. 4*), the CH_2O H-atom (δ(H) 4.65) showed ^3J-correlation with C(7) (δ(C) 158.2), evidencing the attachment of the prenyloxy moiety at C(7); the anomeric H-atom (δ(H) 5.01) showed ^3J-correlation with C(5) (δ(C) 163.9), confirming the presence of the β-glucose residue at C(5) of the phthalide. Therefore, the structure of **3** was identified as 1,3-dihydro-7-[(3-methylbut-2-en-1-yl)oxy]-1-oxo-2-benzofuran-5-yl β-D-glucopyranoside.

Compounds **1**–**6** were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay *in vitro* against a panel of human tumor cell lines, including leukemia (HL-60), breast carcinoma (MCF-7), lung carcinoma (A549), colon carcinoma (SW480), and myeloid liver carcinoma (SMMC-7721). All compounds lacked activities against all tumor cell lines investigated at a concentration of 40 μM.

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Experimental Part

General. M.p.: *XT-4* melting-point apparatus; uncorrected. TLC: Silica gel GF_{254} (SiO_2; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Column chromatography (CC): SiO_2 (100–200 and 200–300 mesh; *Qingdao Marine Chemical Factory*). Optical rotations: *Jasco-20C* digital polarimeter. UV Spectra: *UV-210A* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AV-300* instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS and HR-EI-MS: *VG-autospec-3000* mass spectrometer; in *m*/*z* (rel. %). HR-ESI-MS: *AB QSTAR Pulsar* mass spectrometer; in *m*/*z*.

Plant Material. The aerial parts of *G. adnatum* were collected in Xishuangbanna, Yunnan Province, P. R. China, in May 2010. The identity of the plant material was verified by Prof. *Yong Tang*, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, P. R. China. A voucher specimen (No. 10-003) was deposited with the Key Laboratory of Medicinal Chemistry for Natural Resources, Yunnan University, Kunming, P. R. China.

Extraction and Isolation. The powdered, air-dried plant material (3.0 kg) was extracted with 95% aq. EtOH (5 × 20 l) at r.t. for 10 d. The EtOH extract was evaporated to yield a residue (490 g), which was suspended in H_2O and successively partitioned into petroleum ether- (PE; 49 g), AcOEt- (138 g), and BuOH-soluble (126 g) fractions. The PE-soluble fraction was subjected to repeated CC (SiO_2; PE/AcOEt 1:0 → 0:1) to yield five fractions, *Frs. 1–5*. *Fr. 3* (1.5 g) was further separated by CC (SiO_2; PE/AcOEt 20:1) and recrystallized (hexane/acetone 5:1) to afford **1** (12 mg). The AcOEt-soluble fraction was subjected to CC (SiO_2; PE/AcOEt, AcOEt, AcOEt/MeOH in increasing order of polarity). The fraction obtained with AcOEt/MeOH 1:1 (8.4 g) was resubjected to CC (SiO_2; CH_2Cl_2/AcOEt 30:1) to

furnish **5** (6.7 g). The fraction obtained with AcOEt/MeOH 1:5 (3.7 g) was further separated by CC (SiO_2; CH_2Cl_2/MeOH 25:1) to afford **2** (23 mg) and **4** (9 mg), from the head and tail fractions, resp. The BuOH-soluble fraction was subjected to CC (SiO_2; AcOEt/MeOH $1:0 \rightarrow 0:1$) to yield five fractions, *Frs. 1–5*. *Fr. 2* (1.6 g) was further purified by CC (SiO_2; CH_2Cl_2/MeOH 15:1) to give **6** (11 mg). *Fr. 3* (1.4 g) was repeatedly purified by CC (SiO_2; CH_2Cl_2/MeOH 10:1) to yield **3** (13 mg).

Gnaphalide A (=*6-(1,1-Dimethylprop-2-en-1-yl)-5,7-dihydroxy-2-benzofuran-1(3*H*)-one*; **1**). Colorless flaky crystals ($CDCl_3$). M.p. 155–157°. UV (MeOH): 304 (3.80). IR (KBr): 3398, 3184, 1694, 1618, 1434. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Table*. EI-MS (pos.): 234 (100, M^+), 219 (41), 201 (26), 191 (22), 175 (24), 161 (13), 147 (11), 115 (18), 91 (25), 77 (28), 65 (19). HR-EI-MS: 234.0890 (M^+, $C_{13}H_{14}O_4^+$; calc. 234.0892).

Gnaphalide B (=*5-Hydroxy-7-[(2-hydroxy-3-methylbut-3-en-1-yl)oxy]-2-benzofuran-1(3*H*)-one*; **2**). Colorless needles (MeOH). M.p. 212–214°. $[\alpha]_D^{20} = -12.4$ ($c = 0.12$, MeOH). UV (MeOH): 286 (3.82). IR (KBr): 3519, 3124, 2954, 1708, 1615, 1482, 1447. ^{1}H- and ^{13}C-NMR ((D_6)DMSO): *Table*. EI-MS (pos.): 250 (3, M^+), 205 (9), 180 (100), 179 (51), 162 (60), 151 (34), 137 (85), 134 (96), 121 (67), 105 (34), 92 (12), 77 (18), 71 (51), 65 (30). ESI-MS (pos.): 273 ($[M + Na]^+$), 233, 217, 205, 167. HR-EI-MS: 250.0839 (M^+, $C_{13}H_{14}O_5^+$; calc. 250.0841).

Gnaphalide C (=*1,3-Dihydro-7-[(3-methylbut-2-en-1-yl)oxy]-1-oxo-2-benzofuran-5-yl β-*D*-glucopyranoside*; **3**). Colorless needles (MeOH). M.p. 122–124°. $[\alpha]_D^{20} = -55.6$ ($c = 0.12$, MeOH). UV (MeOH): 304 (3.90). IR (KBr): 3550, 2970, 1749, 1612, 1445. ^{1}H- and ^{13}C-NMR ((D_6)DMSO): *Table*. EI-MS (pos.): 396 (7, M^+), 329 (25), 234 (100), 219 (92), 216 (80), 179 (94), 167 (99), 145 (63), 137 (78), 127 (49), 115 (32), 91 (40), 85 (99), 77 (26), 69 (98). ESI-MS (pos.): 419 ($[M + Na]^+$), 351, 329, 273, 257, 189, 167. HR-EI-MS: 396.1421 (M^+, $C_{19}H_{24}O_9^+$; calc. 396.1420).

Acid Hydrolysis of **3**. A soln. of **3** (4 mg) in 2N HCl (5 ml) was heated for 2 h. After removing HCl by evaporation *in vacuo*, the mixture was diluted with H_2O (5 ml) and extracted with AcOEt (5 ml). The aq. layer was neutralized with 1N NaOH and subjected to TLC with standard β-glucose.

X-Ray Crystal-Structure Analysis of **1**. $C_{13}H_{14}O_4 \cdot H_2O$, M_r 252.26, $T = 100(2)$ K, orthorhombic, space group *Iba*2, $Z = 8$, $a = 16.1370(5)$ Å, $b = 24.5821(8)$ Å, $c = 6.4534(2)$ Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, $V = 2559.94(14)$ Å^3, $\mu(CuK_\alpha) = 0.844$ mm^{-1}, 6062 reflections measured; 2010 independent reflections ($R_{int} = 0.0266$). The final R indices ($I > 2\sigma(I)$) were $R^1 = 0.0536$ and $wR(F^2) = 0.1585$. The final R indices (all data) were $R^1 = 0.0540$ and $wR(F^2) = 0.1590$. The goodness-of-fit on F^2 was 1.051. *Flack* parameter, 0.4(3).

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