Two Rare α-Pyrone (=2*H*-Pyran-2-one) Derivatives from *Gentiana rhodantha* FRANCHET

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Two new rare α-pyrone (=2*H*-pyran-2-one) derivatives, rhodanthpyrones A and B (**1** and **2**, resp.), together with fourteen known compounds, **3**–**16**, were isolated from the whole plants of *Gentiana rhodantha*. The structures of these compounds were elucidated by spectroscopic analyses. This is the first report on the occurrence of α-pyrone derivatives in the genus *Gentiana*.

Introduction. – *Gentiana rhodantha* FRANCHET (Gentianaceae), a perennial herb with a short rhizome and fleshy roots, is one of the twelve endemic Chinese species of section *Stenogyne* of *Gentiana*, and is mainly distributed in southwest China [1]. The dry herb of *G. rhodantha* is a common folk medicine for the people of Miao, Tujia, Han, and other nations used to clear heat, as antiphlogistic and antitussive remedy for treatment of lung, liver, and gallbladder diseases in the plant-distribution area [2]. A previous phytochemical study on *G. rhodantha* from Yunnan Province revealed the presence of various iridoid and secoiridoid glycosides, and phenolic compounds [3–6], and quality-standard evaluation on this crude drug for *Chinese Pharmacopeia* indicated mangiferin as the major constituent [7]. As part of our systematic studies on the plants of *Gentiana* [8–14], the presented work on *G. rhodantha* led to the isolations of two new α-pyrone (=2*H*-pyran-2-one) derivatives, rhodanthpyrones A and B (**1** and **2**, resp.), together with 14 known compounds. Their structures were elucidated by IR, HR-ESI-MS, and 1D- and 2D-NMR analyses.

Results and Discussion. – The EtOH extract of the whole plants of *G. rhodantha* was partitioned successively with petroleum ether, AcOEt, and MeOH. The latter two fractions were repeatedly subjected to column chromatography (CC; SiO_2, *MCI* gel, *Chromatorex ODS*, *Sephadex LH-20* gel), and HPLC to afford two new α-pyrone derivatives **1** and **2**, and 14 known compounds (*Fig. 1*). The known compounds were identified as lutonarin (**3**) [15], luteolin (**4**) [16], isoorientin (**5**) [17], 1,3,5,8-tetrahydroxyxanthone (**6**) [18], 1,3,8-trihydroxyxanthone 5-*O*-β-D-glucoside (**7**) [19], mangiferin (**8**) [20], 1,3,7-trihydroxyxanthone 2-*C*-β-D-glucoside (**9**) [21], norswertianin (**10**) [22], triptexanthoside A (**11**) [23], isovitexin (**12**) [24], naringenin (**13**) [25],

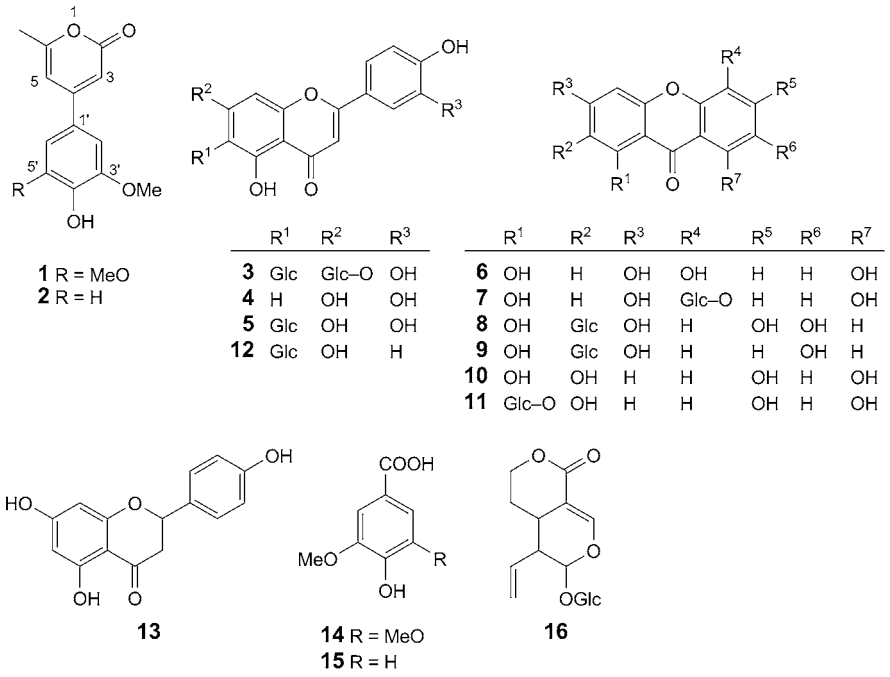


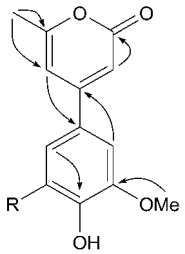
Fig. 1. *Compounds* **1**–**16** *isolated from* G. rhodantha

syringic acid (**14**) [26], vanillic acid (**15**) [26], and sweroside (**16**) [3], by comparison of their spectral and physical data with those of authentic samples or reported in literature.

Rhodanthpyrone A (**1**) was obtained as yellow cubic crystals. Its molecular formula, $C_{14}H_{14}O_5$, was determined on the basis of the HR-ESI-MS (*m*/*z* 263.0937 ($[M+H]^+$)). The IR spectrum displayed absorption bands for OH (3377 cm^{-1}), and C=O (characteristic of α-pyrone; 1690 cm^{-1}), and C=C moieties (1632 and 1548 cm^{-1}). The α-pyrone moiety was identified as 6-methyl-2*H*-pyran-2-one with a substituent at C(4) by analysis of the ^{1}H-NMR (δ(H) 6.41 (*d*, $J = 1.6$, H–C(3)), 6.68 (*d*, $J = 1.6$, H–C(5)), and δ(H) 2.35 (*s*, Me–C(6)), and ^{13}C-NMR signals (δ(C) 166.3 (C(2)), 106.3 (C(3)), 158.1 (C(4)), 104.7 (C(5)), 163.5 (C(6)), and 19.9 (*Me*–C(6)), with the aid of HMBCs Me–C(6) (δ(H) 2.35)/C(6) (δ(C) 163.5) and C(5) (δ(C) 104.7); and H–C(3)/C(2) (δ(C) 166.3) and C(5) (δ(C) 104.7). The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table*) also contained the signals for a benzene ring (δ(H) 7.02 (*s*, H–C(2′) and H–C(6′)); δ(C) 126.9 (C(1′)), 105.7 (C(2′), C(6′)), 149.7 (C(3′), C(5′)), and 140.0 (C(4′)), two MeO groups (δ(H) 3.94 (*s*) and δ(C) 57.0), indicating the presence of 3,5-dimethoxy-4-hydroxyphenyl moiety. In the HMBC spectrum of **1** (*Fig. 2*), the correlations H–C(5) (δ(H) 6.68)/C(1′) (δ(C) 126.9); and H–C(2′) (δ(H) 7.03)/C(4) (δ(C) 158.1) evidenced that the 4-hydroxy-3,5-dimethoxyphenyl moiety was attached to C(4) of the α-pyrone unit. Accordingly, compound **1** was established to be 6-methyl-4-(4-hydroxy-3,5-dimethoxyphenyl)-2*H*-pyran-2-one, named rhodanthpyrone A.

Table. *^{1}H- and ^{13}C-NMR Data* (CD_3OD, 400 and 100 MHz, resp.) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
2		166.3		166.4
3	6.41 (d, $J = 1.6$)	106.3	6.35 (s)	105.9
4		158.1		157.8
5	6.68 (d, $J = 1.6$)	104.7	6.63 (s)	104.6
6		163.5		163.4
1′		126.9		127.8
2′	7.03 (s)	105.7	7.25 (d, $J = 2.1$)	111.2
3′		149.7		149.6
4′		140.0		151.1
5′		149.7		
5′			6.88 (d, $J = 8.1$)	116.8
6′	7.03 (s)	105.7	7.23 (dd, $J = 8.1, 2.1$)	121.9
Me–C(6)	2.35 (s)	19.9	2.32 (s)	19.9
*Me*O–C(3′)	3.94 (s)	57.0	3.92 (s)	56.6
*Me*O–C(5′)	3.94 (s)	57.0		



1 R = MeO
2 R = H

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **2**

Rhodanthpyrone B (**2**) was isolated as yellow powder. It exhibited a pseudomolecular-ion peak at m/z 233.0825 ($[M+H]^+$, calc. 233.0814) in the HR-ESI-MS corresponding to the molecular formula $C_{13}H_{13}O_4$. The UV spectrum showed the maximum absorbances at λ 326, 279, and 250 nm. The IR spectrum displayed absorption bands for OH (3380 cm^{-1}), C=O (1693 cm^{-1}), and C=C moieties (1631 and 1517 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of compound **2** were very similar to those of **1**, except for one aromatic H-atom signal (δ(H) 6.88 (d, $J = 8.1$)) of **2** replacing one MeO signal (δ(H) 3.94 (s)) of **1** on C(5′). A combination of NMR, IR, and MS data suggested that **2** was an analog of **1**. In the HMBC spectrum of **2**, the correlations H–C(5′) (δ(H) 6.88 (d, $J = 8.1$))/C(1′) (δ(C) 127.8) and C(3′) (δ(C) 149.6); H–C(6′) (δ(H) 7.23 (dd, $J = 8.1, 2.1$))/C(4) (δ(C) 157.9) and C(4′) (δ(C) 151.1), Me–C(6) (δ(H) 2.32)/C(6) (δ(C)163.4) and C(5) (δ(C) 104.6); H–C(3)/C(2) (δ(C) 166.4) and C(5) (δ(C)104.7); and H–C(5) (δ(H) 6.63)/C(1′) (δ(C) 127.8) indicated that the phenyl moiety was attached to C(4) of the α-pyrone unit. The HMBC MeO (δ(H) 3.92)/C(3′)

(δ(C) 149.6) indicated that the MeO group was attached to C(3′) of the benzene ring (*Fig. 2*). Based on these evidences, the structure of compound **2** was elucidated as 6-methyl-4-(4-hydroxy-3-methoxyphenyl)-2*H*-pyran-2-one, named rhodanthpyrone B.

In conclusion, 16 compounds were isolated and identified from the title plant, including two new α-pyrone derivatives, **1** and **2**, one secoiridoid, **16**, six xanthones, **6**–**11**, five flavonoids, **3**–**5**, **12**, and **13**, and two acid compounds, **14** and **15**. To the best of our knowledge, α-pyrone derivatives (except coumarins, *viz*. benzopyrone types) are rarely distributed in more than a dozen angiosperm families, such as Annonaceae [27], Lauraceae [28], Lamiaceae [29], Polygalaceae [30], Rosaceae [31], Cactaceae [32], Guttiferae [33]. α-Pyrone-type compounds were isolated from Gentianaceae for the first time. Our previous study showed that it was also very special for the appearance of the major constituent, *C*-glucoxanthone, mangiferin (**8**; average 2% (*w*/*w*)) in the whole plant of *G. rhodantha* [7]. This indicates the secondary metabolites of *G. rhodantha*, of the section *Stenogyne* are totally different from those found in species from the other sections of the genus *Gentiana*. Nuclear ribosomal ITS sequence data suggested that section *Stenogyne* would be better excluded from the genus *Gentiana* [34][35]. Further, the section *Stenogyne* was removed from *Gentiana* and was established as a new genus, *Metagentiana*, by *Ho et al.* on the basis of gross morphology, floral anatomy, chromosomes, palynology, and embryology [36]. Our results could offer a chemotaxonomic evidence for the above system. However, further systematic phytochemical research on other *Gentiana* species of section *Stenogyne* is necessary.

Experimental Part

General. Prep. HPLC: *Gilson 306* pump (16 ml/min) with a UV detector (210 nm) and an *Ultimate1 XB-18* (20 mm × 250 mm, 10 mm) column. Column chromatography (CC): silical gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Group Co., Ltd.* (=*QHCC*; P. R. China)), *Sephadex LH-20* (*GE-Healthcare Bio-Sciences AB*), *MCI* gel *CHP20P* (75–150 mm; *Mitsubishi Chemical Industry, Ltd.*), *HPD-600* (*Hebei Cangzhou Bao En Chemical Industry Co., Ltd.*), or *Chromatorex ODS* (100–200 mesh; *Fuji Silysia Chemical Co., Ltd.*). TLC: Precoated plates from *QHCC*; visualization under UV light or by heating after spraying with 10% H_2SO_4/EtOH soln. Optical rotations: *BXS07-WZZ-3A* automatic digital polarimeter. M.p.: *Büchi 540* apparatus. UV Spectra: *TU1901*, double-beam UV/VIS spectrophotometer. IR Spectra: *PerkinElmer* FT-IR spectrometer. 1D- and 2D-NMR spectra: *Bruker Advance III* instrument operating at 400 (^{1}H) and 100 MHz (^{13}C); chemical shifts (δ) in ppm; coupling constants *J* in Hz. HR-ESI-MS: *Synapt G2 Q-TOF* mass spectrometer; in *m*/*z*.

Plant Material. The dried whole plants of *Gentiana rhodantha* FRANCHET were collected from Weining, Guizhou Province, P. R. China, and identified by Dr. *Wu Li-Hong*. A plant specimen (wu2008002) was deposited with the Herbarium of Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine.

Extraction and Isolation. Dried plants of *Gentiana rhodantha* (7.5 kg) were extracted with 95% EtOH (3 × 80 l) at r.t. The soln. was evaporated *in vacuo* to afford a brownish residue. The residue (1.5 kg) was mixed with diatomite (3 kg), and then successively eluted and partitioned with petroleum ether (PE), AcOEt, and MeOH to yield extracts of 150, 350 and 450 g, resp.

The AcOEt extract (200 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH 100:1 → 0:1). Combination of similar fractions on the basis of TLC afforded nine fractions. *Fr. 4* (30 g) was submitted to repeated CC (*MCI* gel, H_2O/MeOH 2:8; SiO_2, PE/AcOEt 2:1; and *Sephadex LH-20*, MeOH) to afford **1** (20 mg), **2** (7 mg), **13** (10 mg), **14** (7 mg), **15** (20 mg). *Fr. 6* (20 g) was purified by CC (*MCI* gel; H_2O/MeOH 2:8; *Sephadex LH-20*, MeOH; and *Chromatorex ODS*, H_2O/MeOH 6:4), further separated by prep. HPLC, and yielded compounds **4** (10 mg) and **6** (36 mg).

The MeOH extract (100 g) was subjected to CC (*HPD-600*; MeOH/H_2O gradient) to furnish three extracts. The 30% MeOH extract (40 g) was separated by CC (*MCI*; H_2O/MeOH 1:0 to 0:1) to give five fractions. All fractions were rechromatographyed over *Sephadex LH-20* with MeOH and further recrystallizated, resp. *Fr. 1* (7 g) yielded compounds **3** (20 mg), **8** (50 mg), and **16** (40 mg), *Fr. 2* (4 g) afforded compounds **5** (34 mg) and **9** (9 mg), *Fr. 3* (5 g) gave compounds **7** (20 mg), **11** (5 mg), *Fr. 4* (3 g) yielded compound **12** (25 mg), and *Fr. 5* (2 g) furnished compound **10** (6 mg).

Rhodanthpyrone A (= *4-(4-Hydroxy-3,5-dimethoxyphenyl)-6-methyl-2*H-*pyran-2-one*; **1**). Yellow cubic crystals. M.p. 262–264°. $[\alpha]_D^{18} = 60.4$ ($c = 0.50$, MeOH). UV (MeOH): 326 (5.23), 279 (2.43), 250 (4.11). IR (KBr): 3377, 2993, 2973, 2948, 2839, 1690, 1632, 1548, 1517, 1454, 1324, 1215, 1120, 847, 719. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 263.0937 ($[M+H]^+$; calc. 263.0919).

Rhodanthpyrone B (= *4-(4-Hydroxy-3-methoxyphenyl)-6-methyl-2*H-*pyran-2-one*; **2**). Yellow amorphous powder. UV (MeOH): 330 (5.10), 277 (2.64), 255 (4.34). IR (KBr): 3380, 2938, 1693, 1631, 1592, 1517, 1326, 1288, 1208, 1129, 1032, 822, 770, 629, 635. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 233.0825 ($[M+H]^+$; calc. 233.0814).

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