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CHEMICAL CONSTITUENTS WITH ANTISEPSIS ACTIVITY FROM THE RHIZOMES OF *Polygonatum odoratum*

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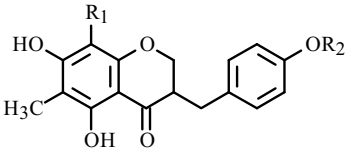
Polygonatum odoratum (Liliaceae) is widely distributed in the central and southwest areas of China. Its rhizome, also called "Yuzhu," is a famous traditional Chinese medicine and health food and is used as a nutritious tonic and remedy to treat lung disease and upset stomach and improve insulin resistance and diabetes [1, 2]. Previous studies on this plant only reported the isolation of the steroid saponins [3], furostanol glycosides [4], dipeptides [5], quercitol [6], and diosgenin [7]. But no other consituents have been separated from *P. odoratum*. In continuing our research on bioactive compounds from *Polygonatum* plants, we isolated four known methyl-homoisoflavanones **1–4** and one known 9,19-cyclolart triterpenoid (**5**) from the rhizomes extract of *P. odoratum*. This paper describes the isolation and structural characteristics of these compounds. The antisepsic activities of compounds **2–5** are also discussed.

This group of 3-benzylchroman-4-ones **1–4** is typically characterized by the appearance of two sets of doublet signals for the two protons each on C-2 and C-9, respectively, and a prominent complex multiplet signal for the proton at C-3. The δ_C values for C-2, C-3, and C-9 were also strikingly consistent in all these compounds.

Compound **1** was obtained as a white needle. EI-MS showed molecule ion peaks at *m/z* 328 $[M]^+$ and fragment ion peaks at *m/z* 121$[-CH_2-C_6H_4-OCH_3]^+$ (base peak), suggesting the existence of a methoxytropylium fragment in the molecule. The ^{13}C NMR (75 MHz, $CDCl_3$, Table 1) spectra showed 19 resonance lines, indicating four aromatic carbons (δ 160.80, 159.81, 158.40, 157.82), each bearing an oxygen function, four aromatic carbons (130.34, 114.32, 130.34, 114.32), each carrying a proton, two aromatic carbons (103.02, 102.38) linked to a methyl, one aromatic carbon (101.74) linked to a carbonyl, one alkyl-substituted aromatic carbon (130.34), one flavanone carbonyl carbon (198.72), one methylene carbon (69.13) having an oxygen function, one benzylic methylene carbon (32.20), one methyoxyl carbon (55.52), two methyl carbons (7.58, 7.09), and one methine carbon (47.07) having an oxygen function. The ^{1}H NMR (300 MHz, $CDCl_3$) spectrum showed 20 protons, indicating one methoxy (δ 3.80), two methyl (2.07, 2.03), two hydroxyl, two methylene (4.28, 4.12, and 3.19, 2.70), one methine (2.79), and four aromatic protons of the AA′BB′ system at δ 7.15 and 6.87. Compound **1** also showed the expected chelated hydroxyl at 12.38 (1H, s, disappears after addition of D_2O) and one exchangeable proton at 5.38 (1H, s, disappears after addition of D_2O).

The NOESY spectrum (Fig. 1) showed correlations of δ 2.79 (1H, m) to 4.28, 4.12, and 3.19, 2.70. The position of one methoxyl group was determined by correlations from the methoxyl protons at δ 3.80 (3H, s) to 6.87, showing this methoxyl proton to be at C-4′, and the two methyl groups were determined by correlations of 2.07 (3H, s) to 12.38 and 5.38, 2.03 (3H, s) to 5.38, showing the two methyls to be at C-6 and C-8. Compound **1** showed spectral data very similar to methylophiopogonanone B, which was isolated previously from *Ophiopogonis tuber* [8].



1 - 4

1: $R_1 = R_2 = CH_3$; **2:** $R_1 = CH_3$, $R_2 = H$

3: $R_1 = OCH_3$, $R_2 = H$; **4:** $R_1 = R_2 = H$

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TABLE 1. ^{13}C NMR Data of Compound **1–4**

C atom	**1** ($CDCl_3$)	**2** (CD_3COCD_3)	**3** (CD_3OD)	**4** (DMSO-d_6)
2	69.13	69.91	73.60	68.75
3	47.07	47.38	51.38	45.67
4	198.72	199.41	202.83	197.84
4a	101.74	102.58	105.60	100.95
5	159.81	158.73	162.19	160.99
6	103.02	104.15	108.45	103.30
7	160.80	160.40	161.83	164.38
8	102.38	102.97	132.31	93.91
8a	158.40	156.96	156.16	160.16
9	32.20	32.34	36.38	31.26
1′	130.34	129.85	133.36	129.87
2′	130.34	130.91	134.37	128.06
3′	114.32	116.18	119.60	115.22
4′	157.82	155.2	160.41	155.84
5′	114.32	116.18	119.60	115.22
6′	130.34	131.24	134.37	128.60
4′-OCH_3	55.52			
6-CH_3	7.58	7.93	10.43	6.91
8-CH_3	7.09	7.45		
8-OCH_3			64.77	

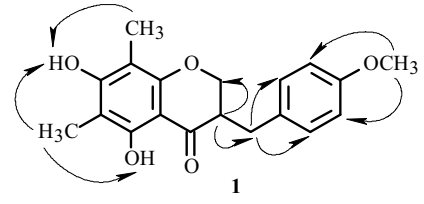


Fig. 1.

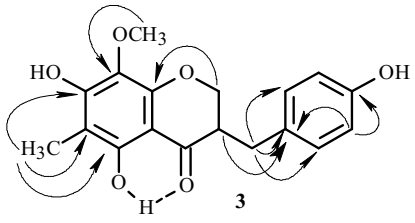


Fig. 2.

Fig. 1. ^1H–^1H NOESY of compound **1**.
Fig. 2. Key ^1H–^{13}C long-range correlation observed in HMBC spectra of compound **3**.

The ^1H NMR spectra of compounds **2–4** showed that these compounds both have a 4′-hydroxyl-substituted AA′BB′ system in ring B, contain a chelated OH-group at the C-5 position, and have a methyl group at the C-6. This was also observed from their ^{13}C NMR spectra (Table 1). Compound **2** was similar to **1** except that the 4′-methoxyl was substituted by an additional hydroxyl signal at δ_H 8.52 (1H, br.d). Compound **2** has been isolated previously from *Polygonatum alte-olbatum*, and the reported spectroscopic and physical data were in complete agreement with those determined for **2** [9]. Compound **3** was similar to **2** except that the 8-methyl was substituted by the methoxyl signal at δ_H 3.72 (3H, s). The position of this methoxyl group was determined by correlations from the methoxyl protons at δ 3.72 to C-8 (132.31) in the HMBC spectrum (Fig. 2). Compound **4** was similar to **3** except that the 8-methoxyl was substituted by an aromatic proton signal at δ 5.94 (1H, s) in ring A. Compounds **3, 4** have been isolated previously from *Disoporopsis aspera*, and the reported spectroscopic and physical data were in complete agreement with those determined for **3**, **4** [10].

Compound **5** was obtained as colorless needles, mp 180–186°C (petroleum–acetone). The Liebermann-burchard reaction was positive. Its molecular formula was determined to be $C_{30}H_{50}O_2$ based on HR-EI-MS, *m/z* 442.3801 $[M]^+$ (calcd 442.3811). EI-MS also showed molecule ion peaks at *m/z* 442 $[M]^+$. Its IR showed a hydroxy absorption band at 3406 cm^{-1}.

TABLE 2. Antibacterial Results of Several Compounds

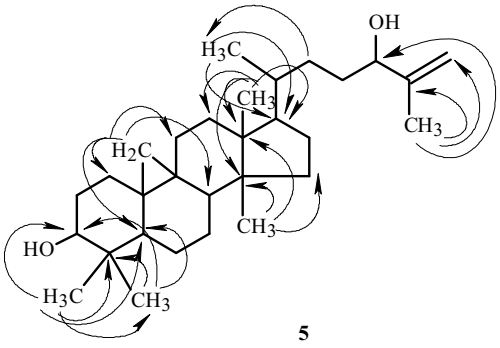
Compound	Diameter of inhibition circle, mm			
	E. coli	*B. cereus*	*B. prodigious*	*C. sepedonium*
2	7.00	6.80	5.00	9.64
3	13.00	9.24	6.64	10.50
4	5.00	6.56	4.24	2.56
5	5.00	13.00	13.63	4.28
Erythromycin	0.00	24.20	12.00	16.00

Note: concentration is 10 μg/mL.

TABLE 3. Antifungal Results of Several Compounds

Compound	Inhibition rate %					
	E. turcicum	*A .brassicae*	*V. dahliae*	*B. cinerea*	*C. lagenarium*	*F. graminearum*
2	38.64	25.06	29.52	29.26	40.83	22.35
3	58.24	51.67	44.92	34.80	50.00	30.64
4	31.20	21.67	26.67	28.40	38.00	18.82
5	0.00	0.00	14.76	11.64	100	40.59

Note: concentration is 10 μg/mL.



5

Fig 3. Key ^{1}H –^{13}C long-range correlation observed in HMBC spectra of compound **5**.

The ^{1}H NMR spectrum displayed two upfield shifted doublets at δ 0.56 and 0.33 (d, J = 3.86 Hz) assignable to a cyclopropyl methylene group (H_2-19) characteristic of cycloartane-type triterpenoids [11]. The spectrum also showed six methyl signals at δ 0.98 (3H, s, H-28), 0.81 (3H, s, H-29), 0.98 (3H, s, H-18), 0.88 (3H, d, J = 5.5 Hz, H-21), 0.88 (3H, s, H-30), and 1.75 (3H, s, H-27). The ^{13} C NMR (100 MHz, $CDCl_3$) and DEPT spectrum showed 30 carbon atoms at δ 147.51 (DEPT, C), 110.87 (DEPT, CH_2) and 1.75 (3H, s, H-27), and one double bond at C-25. The position of the double bond at C-25 was also confirmed by HMBC spectroscopy (Fig. 3), which showed the correlation of H-27 (δ 1.75) to 147.51 (C-25), 110.87 (C-26), and 76.37 (C-24). Compound **5** showed spectral data very similar to 9,19-cycloart-25-en-3β,24(*R*)-diol, which was isolated previously from *Fritillaria hupehensis* [12].

Antisepsic Activity of Compounds 2–5. Compounds **2–5** were tested for their antisepsis activity against 4 bacterials and 6 plant pathogens at concentration 10 μg/mL (Tables 2, 3). The results showed that the tested compounds both have activity. Compound **5** has a strong inhibition effect on the growth of *Colletotrichum lagenarium* with an inhibition rate of 100%, and its inhibition effect corresponds to erythromycin against *Bacterium prodigious* growth. Compound **3** showed strong inhibition against *Colletotrichum lagenarium*, *Alternaria brassicae*, *Verticillium dahliae*, *Exserohilum turcicum*, *Escherichia coli*, *Bacillus cereus*, and *Corynebacterium sepedonium* growth.

Melting points were deternined on an XT5-XMT apparatus. NMR spectra were recorded on Varian Unity 300 and Bruker DRX-400, using TMS as an internal standard. EI-MS data were obtained on Finnigan DSQ. HR-EI-MS data were obtained on Finnigan MAT 95 (70 ev). Chromatography was on Sephadex LH-20 (Amersham Biosciences).

The *Polygonatum odoratum* rhizomes were collected in Qinling mountains in September 2005. A voucher specimen (No. 065) has been deposited at the Herbarium of the Northwest Sci-Tech University of Agriculture and Forestry.

Extraction and Isolation. The air-dried rhizomes of *P. odoratum* (17 kg) were extracted with 95% EtOH at room temperature.The extract was concentrated under vacuum to give a brown waxy residue. The residue was partitioned between water and petroleum. The water fraction was further extracted with EtOAc. The evaporated EtOAc fraction (40.2 g) was chromatographed on a silica gel column eluted with a gradient petroleum–CH_3COCH_3 (98:2, 95:5, 90:10, 80:20, 1:1) to afford fractions 1–5. Further chromatography of fraction 1 (3.64 g) on a silica gel column eluted with a gradient petroleum–EtOAc (50:1, 25:1, 10:1, 5:1) yielded four subfractions. Subfraction 3 (0.89 g) was further chromatographed on silica gel and fractionated by permeation chromatography on Sephadex LH-20 in $CHCl_3$–MeOH (4:1) to afford homoisoflavanone **1** (4.4 mg). Subfraction 2 (0.26 g) was further chromatographed on silica gel to yield compound **5** (32.7 mg). Further chromatography of fractions 2 and 3 by silica gel and Sephadex LH-20 yielded three known homoisoflavanones **2** (50.2 mg), **3** (204.6 mg), and **4** (46.2 mg).

Compound 1: white needle, mp 156–160°C (CD_3COCD_3). $C_{19}H_{20}O_5$. EI-MS *m/z* (rel.int.): 328 $[M]^+$ (40), 209 (10), 208 (78), 207 (4), 121 (100), 77 (4), 57 (2). The ^1H NMR (300 MHz, δ, $CDCl_3$, J/Hz): 2.03 (3H, s, 8-CH_3), 2.07 (3H, s, 6-CH_3), 2.79 (1H, m, H-3), 2.70 (1H, dd, J = 10.2, 13.2, H-9a), 3.19 (1H, dd, J = 3.9, 13.2, H-9b), 3.80 (3H, s, 4′-OCH_3), 4.12 (1H, dd, J = 6.6, 11.1, H-2a), 4.28 (1H, dd, J = 4.5, 11.1, H-2b), 5.38 (1H, s, 7-OH), 6.87 (1H, d, J = 8.4, H-3′,5′), 7.15 (1H, d, J = 9.0, H-2′, 6′), 12.38 (1H, s, 5-OH). ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1.

Compound 2: pale yellow needle, mp 105–108°C ($CHCl_3$–MeOH). $C_{18}H_{18}O_5$. EI-MS *m/z* (rel. int.): 314 $[M]^+$ (96), 209 (10), 208 (78), 207 (100), 180 (17), 165 (11), 133 (6), 107 (42), 77 (10). ^1H NMR (300 MHz, δ, $CDCl_3$, J/Hz): 2.04 (3H, s, 8-CH_3), 2.06 (3H, s, 6-CH_3), 2.98 (1H, m, H-3), 2.69 (1H, dd, J = 10.2, 13.8, H-9a), 3.13 (1H, dd, J = 4.5, 13.8, H-9b), 4.14 (1H, dd, J = 8.1, 11.1, H-2a), 4.33 (1H, dd, J = 4.5, 11.1, H-2b), 6.80 (1H, d, J = 8.1, H-3′, 5′), 7.11 (1H, d, J = 8.4, H-2′, 6′), 8.52 (1H, br.d, 7-OH), 12.49 (1H, s, 5-OH). ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1.

Compound 3: pale yellow powder. $C_{18}H_{18}O_6$. EI-MS *m/z* (rel. int.): 330 $[M]^+$ (90), 315 (6), 224 (53), 209 (50), 181 (12), 153 (8), 107 (100), 77 (13), 55 (9). The ^1H NMR (300 MHz, δ, CD_3OD, J/Hz): 1.98 (3H, s, 6-CH_3), 2.78 (1H, m, H-3), 2.63 (1H, dd, J = 9.9, 13.5, H-9a), 3.09 (1H, dd, J = 3.9, 13.5, H-9b), 3.72 (3H, s, 8-OCH_3), 4.13 (1H, dd, J = 6.6, 11.1, H-2a), 4.28 (1H, dd, J = 4.2, 11.1, H-2b), 6.73 (1H, d, J = 8.7, H-3′, 5′), 7.05 (1H, d, J = 8.4, H-2′, 6′). The ^1H NMR (300 MHz, δ, CD_3OCD_3): 9.01 (1H, br.d, 7-OH), 12.33 (1H, s, 5-OH). ^{13}C NMR (75 MHz, CD_3OD): see Table 1.

Compound 4: white powder. $C_{17}H_{16}O_5$. EI-MS *m/z* (rel. int.): 300 $[M]^+$ (100), 193 (96), 167 (22), 107 (45), 77 (8). The ^1H NMR (300 MHz, DMSO-d_6, δ, J/Hz): 1.85 (3H, s, 6-CH_3), 2.89 (1H, m, H-3), 2.56 (1H, dd, J = 9.9, 13.8, H-9a), 2.98 (1H, dd, J = 4.8, 13.8, H-9b), 4.01 (1H, dd, J = 7.8, 11.4, H-2a), 4.20 (1H, dd, J = 3.9, 11.4, H-2b), 5.94 (1H, s, H-8), 6.67 (1H, d, J = 6.9, H-3′, 5′), 7.00 (1H, d, J = 7.8, H-2′, 6′), 12.42 (1H, s, 5-OH). ^{13}C NMR (75 MHz, DMSO-d_6): see Table 1.

Compound 5: colorless needles (petroleum–aceton), mp 180–186°C. $C_{30}H_{50}O_2$. HR-EI-MS *m/z* 442.3801 $[M]^+$ (calcd 442.3811). IR (KBr, ν_{max}, cm^{-1}): 3406, 2967, 2937, 2853, 1380, 1466, 1443, 1373, 1319, 1287, 1096, 1047, 1028, 1007, 989. EI-MS *m/z* (rel.int.): 442 $[M]^+$ (11), 424 (41), 409 (51), 381 (30), 355 (9), 315 (20), 302 (59), 297 (28), 203 (51), 175 (72), 135 (80), 121 (100), 71 (88). The ^1H NMR (400 MHz, δ, $CDCl_3$, J/Hz): 0.33 (1H, d, J = 3.86, H-19a), 0.56 (1H, d, J = 3.86, H-19b), 0.81 (3H, s, H-29), 0.88 (3H, s, H-30), 0.88 (3H, d, J = 5.5, H-21), 1.75 (3H, s, H-27), 3.28 (1H, m, H-3), 4.02 (1H, t, H-24), 4.93 (1H, s, H-26). ^{13}C NMR (100 MHz, δ, $CDCl_3$): 31.98 (C-1), 29.70 (C-2), 78.86 (C-3), 40.50 (C-4), 47.13 (C-5), 21.23 (C-6), 28.09 (C-7), 47.98 (C-8), 20.02 (C-9), 26.12 (C-10), 26.49 (C-11), 32.91 (C-12), 45.31 (C-13), 48.83 (C-14), 31.92 (C-15), 28.15 (C-16), 52.20 (C-17), 18.34 (C-18), 30.40 (C-19), 35.93 (C-20), 19.33 (C-21), 35.57 (C-22), 29.89 (C-23), 76.37 (C-24), 147.51 (C-25), 110.87 (C-26), 17.62 (C-27), 25.45 (C-28), 14.01 (C-29), 14.01 (C-29), 18.03 (C-30).

Antisepsic Activity. Four bacteria (*Escherichia coli*, *Bacillus.cereus, Bacterium prodigious, Corynebacterium sepedonium)* and six plant pathogens *(Exserohilum turcicum*, *Alternaria brassicae*, *Verticillium dahliae*, *Botrytis cinerea*, *Colletotrichum lagenarium*, *Fusarium graminearum*) were collected in the microbe laboratory of Northwest Sci-Tech University of Agriculture and Forestry and were identified by Li Xiao-Ming, senior laboratorian. The hyphae growth method was used to study the plant fungicidal activity, and the filter paper-diffuse method was used to study the bacterial activity [13]. Each treatment was repeated three times, and cultivation was for 96 h at 28.8°C in a constant temperature culture box.

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