

New Phenolics from *Polygala fallax*

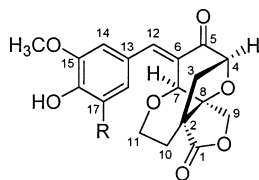
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Two new phenolic compounds, polygalolide A (**1**) and polygalolide B (**2**), together with three known xanthenes were isolated from the roots and stems of *Polygala fallax*. The structures of **1** and **2** were elucidated on the basis of spectroscopic evidence.

Polygala fallax Hemsl. (Polygalaceae) is a medicinal plant used as a tonic and antihepatitis drug in southern regions of the People's Republic of China.¹ The occurrence of both triterpenoid saponins² and oligosaccharide polyesters³ has been reported previously. In the course of our continuing investigation of bioactive natural products from folk medicinal plants used in the southern part of mainland China, we have investigated the phenolic constituents of *P. fallax*. Polygalolides A (**1**) and B (**2**), two new phenolic lactones with an unusual skeleton, have been isolated from this plant, along with three known xanthenes, 1,7-dihydroxy-2,3-methylenedioxyxanthone, 1,3,6-trihydroxy-2,7-dimethoxyxanthone, and 1,3-dihydroxy-2-methoxyxanthone. The structures of the new compounds **1** and **2** were established by means of spectral data interpretation.



1 R = H

2 R = OCH₃

The EtOH percolate of the powdered dried roots and stems of *P. fallax* was fractionated sequentially with petroleum ether, CHCl₃, and *n*-BuOH. The CHCl₃-soluble extract was separated by column chromatography over silica gel and Sephadex LH-20 to yield polygalolides A (**1**) and B (**2**) and the three known xanthenes listed above.

Polygalolide A (**1**) exhibited a [M + H]⁺ peak at *m/z* 359.1128 in the HRFABMS, indicating the molecular formula to be C₁₉H₁₈O₇. The IR spectrum showed absorptions for a hydroxyl group (3436 cm⁻¹), an olefinic bond (1640 cm⁻¹), an aromatic ring (1606, 1571, and 1512 cm⁻¹), and two carbonyl groups (1776 and 1683 cm⁻¹). The ¹³C NMR spectrum gave signals for a ketone carbonyl (δ_C 198.0 ppm), an ester carbonyl (δ_C 179.2), six aromatic carbons (δ_C 126.3, 114.4, 146.7, 148.9, 115.0, and 127.7), two olefinic carbons (δ_C 147.4 and 127.5), an oxygenated quaternary carbon (δ_C 82.8), two oxygenated methines (δ_C 81.2 and 67.6), two oxygenated methylenes (δ_C 69.1 and 56.0), one methoxy group carbon (δ_C 56.0), two aliphatic methylenes (δ_C 37.0 and 28.8), and an aliphatic quaternary carbon (δ_C 49.5). The ¹H NMR spectrum revealed the presence of a 1,2,4-trisubstituted phenyl group [δ 7.39 ppm (1H, dd, *J*

= 8.0 and 1.6 Hz), 7.43 (1H, d, *J* = 1.6 Hz), and 7.00 (1H, d, *J* = 8.0 Hz)], a trisubstituted olefinic proton [δ 7.96 (1H, br s)], an aryl methoxyl [δ 3.93 (3H, s)], two oxygenated methines [δ 4.89 (1H, br s); 4.59 (1H, br d, *J* = 8.0 Hz)], two oxygenated methylenes [δ 4.58, 4.50 (2H, d, *J* = 10.8 Hz); 3.72 (1H, dd, *J* = 12.8, 5.2 Hz), 3.43 (1H, br t, *J* = 12.8 Hz)], and two aliphatic methylenes [δ 3.06 (1H, dd, *J* = 14.2, 8.0 Hz), 2.03 (1H, dd, *J* = 14.2, 1.3 Hz), 2.13 (1H, br d, *J* = 14.0 Hz), 1.97 (1H, ddd, *J* = 12.8, 12.8, 5.2 Hz)]. All protons and carbons could be assigned as shown in Table 1 through the combined analysis of the ¹H–¹H COSY, ¹³C–¹H COSY, NOESY, and HMBC spectra of **1**.

Further analysis of the ¹H–¹H COSY spectrum of **1** revealed spin-systems of H-4/H₂-3, H₂-11/H₂-10, and H-7/H-12 (allylic long-range coupling) in addition to a spin-system for the previously deduced 1,2,4-trisubstituted aromatic ring. The carbon skeleton of **1** was assembled using the results of the HMBC experiment. The HMBC correlations between the protons of the methoxyl group (δ_H 3.93) and C-15 (δ_C 146.7), between H-14 (δ_H 7.43) and C-16 (δ_C 148.9), C-18 (δ_C 127.7), between H-17 (δ_H 7.00) and C-15, C-13 (δ_C 126.3), and between H-18 (δ_H 7.39) and C-16, C-14 (δ_C 114.4) indicated that the aromatic ring was a 4-hydroxyl-3-methoxyphenyl group. The correlations between H-12 (δ_H 7.96) and C-13, C-14, C-18, C-6 (δ_C 127.5), and C-5 (δ_C 198.0) established the connectivity of the aromatic ring via the double bond (C-12 and C-6) to the ketone carbonyl (C-5). HMBC couplings from H-7 (δ_H 4.89) to C-11 (δ_C 56.0), C-8 (δ_C 82.8), C-2 (δ_C 49.5), C-5, C-6, and C-12 (δ_C 147.4), the couplings from H-4 to C-5, C-6, and C-8, and the coupling from H-10_{eq} to C-8 indicated the presence of a pyrano[3,2-*b*]pyran-3-one ring moiety. The couplings from H-4 (δ_H 4.59) to C-3 (δ_C 37.1) and C-2 revealed the connectivity of C-4 to C-2 through a methylene bridge (C-3). A γ-lactone ring was deduced from the correlations from H₂-9 (δ_H 4.58, 4.50) to C-1 (δ_C 179.2), C-2, and C-8. Further, the correlations from H₂-3 to C-1 and from H-7 to C-9 showed the connectivities of C-1 to C-2 and C-9 to C-8.

The relative stereochemistry of **1** was determined by a NOESY experiment (Figure 1). In this spectrum, a strong cross-peak between H-11_{ax} and H-3_β along with the cross-peaks between H-11_{ax} and H-10_{eq}, and between H-11_{eq} and H₂-10, indicated a chair (¹¹C₈) form of the pyran ring and a β-orientation of the methylene bridge between C-2 and C-4. The absence of a cross-peak between H-11_{ax} and H-7 showed an α-orientation and an equatorial configuration of H-7. In addition, the presence of correlations from H-7 to H-14 and H-18 suggested the *E* configuration of the double bond between C-6 and C-12. Therefore, the complete structure of **1** was determined as shown.

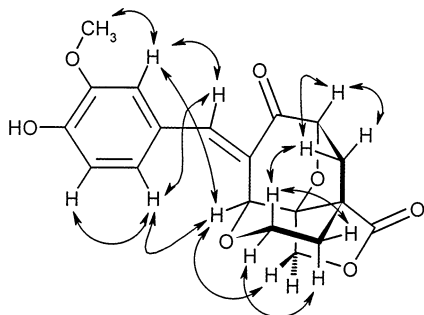
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Table 1. ^1H and ^{13}C NMR Data and HMBC Correlations of Compounds **1** and **2** (in CDCl_3)^a

position	1			2		
	δ_{H}	δ_{C}	HMBC ^b	δ_{H}	δ_{C}	HMBC ^{b,c}
1		179.2	3 α , 3 β , 9 α , 9 β , 10ax		179.2	
2		49.5	3 α , 3 β , 7, 9 α , 9 β , 10ax, 10eq, 11ax, 11eq		49.5	
3 α	3.06 dd (14.2, 8.8)	37.1	4, 10ax, 10eq	3.04 dd (14.2, 8.8)	37.0	
3 β	2.03 dd (14.2, 1.3)			2.02 dd (14.2, 1.5)		
4	4.59 br d (8.0)	81.2	3 α , 3 β	4.56 br d (8.0)	81.2	
5		198.0	3 α , 3 β , 4, 7, 12		198.0	
6		127.5	4, 7, 12		127.9	
7	4.89 br s	67.6	4, 11ax, 11eq, 12	4.85 br s	67.7	
8		82.8	4, 7, 9 α , 9 β , 10eq		82.8	
9 α	4.58 d (10.8)	69.1	7	4.54 d (10.5)	69.2	
9 β	4.50 d (10.8)			4.47 d (10.5)		
10eq	2.13 br d (14.0)	28.8	3 α , 3 β , 11eq	2.11 br d (14.0)	28.9	
10ax	1.97 ddd (12.8, 12.8, 5.2)			1.95 ddd (12.8, 12.8, 5.2)		
11eq	3.72 dd (12.8, 5.2)	56.0	7, 10ax	3.71 dd (12.4, 5.2)	56.1	
11ax	3.43 br t (12.8)			3.42 br t (12.4)		
12	7.96 br s	147.4	7	7.91 br s	147.7	
13		126.3	12, 17		125.1	12
14	7.43 d (1.6)	114.4	12, 18	7.14 s	109.7	12
15		146.7	14, 17, OMe		147.1	14, OMe
16		148.9	14, 17, 18		138.2	14, 18
17	7.00 d (8.0)	115.0	18		147.1	18, OMe
18	7.39 dd (8.0, 1.6)	127.7	12, 14	7.14 s	109.7	12
MeO-15	3.93 s	56.0		3.90 s	56.4	
MeO-17				3.90 s	56.4	

^a The ^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively. Coupling constants (parentheses) are given in Hz.

^b ^1H signal correlating with ^{13}C resonance indicated. ^c HMBC correlations for the positions 1–12 of **2** are the same as those of **1**.

**Figure 1.** Selected NOESY correlations for **1**.

Polygalolide B (**2**) was obtained as a light-brown amorphous powder. Its molecular formula was determined as $\text{C}_{20}\text{H}_{20}\text{O}_8$ by the combined analysis of its HRFABMS, ^{13}C NMR, and DEPT NMR data. The ^1H and ^{13}C NMR spectra were very similar to those of **1** except for the signals corresponding to the phenyl group. The proton signals at δ_{H} 7.14 (2H, s) and 3.90 (6H, s) and the corresponding carbon signals at δ_{C} 109.7 and δ_{C} 56.4 indicated the phenyl group in **2** to be a 4-hydroxyl-3,5-dimethoxyphenyl unit. Thus, **2** was elucidated as the 17-methoxyl-substituted derivative of polygalolide A (**1**).

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto Seisakusho micro-hot stage melting apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 343 spectropolarimeter with MeOH as solvent. The UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 25 UV-vis spectrophotometer. The IR spectra were measured in KBr on a WQF-410 FT-IR spectrophotometer. The ^1H (400 MHz, CDCl_3), ^{13}C (100 MHz, CDCl_3), and 2D NMR spectra were recorded on a Bruker DRX-400 instrument using the signal of CDCl_3 as a reference (the singlet at δ 7.24 for the ^1H NMR data and a triplet centered at δ 77.0 for the ^{13}C NMR data). HRFABMS were recorded on a VG Auto Spec-3000 mass spectrometer in the positive-ion mode using glycerol as the matrix. EIMS were collected on a Micromass Platform EI 200 GC/MS instrument at 70 eV by

direct inlet. For column chromatography, Si gel 60 (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China) and Sephadex LH-20 were used. TLC was performed on precoated plates (Kieselgel 60GF₂₅₄, Merck) with detection effected by UV light (254 nm) and using I_2 vapor.

Plant Material. Roots and stems of *P. fallax* were collected from Guangning, Guangdong, People's Republic of China, in October 1998. A voucher specimen (No. 648611) has been deposited at the herbarium of South China Institute of Botany, Chinese Academy of Sciences, Guangzhou, People's Republic of China.

Extraction and Isolation. The powdered air-dried roots and stems (3.70 kg) of *P. fallax* were extracted by percolation with 90% EtOH three times at room temperature. The EtOH extracts were concentrated in vacuo to obtain a deep brown syrup (1.08 kg). This syrup was suspended in H_2O , and the aqueous suspension was sequentially extracted three times each with petroleum ether, CHCl_3 , and *n*-BuOH. The combined CHCl_3 extract, upon evaporation, yielded a light brown syrup (21 g). This syrup was subjected to silica gel column chromatography, eluted with CHCl_3 –MeOH mixtures of increasing polarities, to obtain five fractions (1–5). Fraction 1 was further separated by silica gel chromatography eluted with petroleum ether–acetone (9:1) to give three further fractions, 1A, 1B, and 1C. Fraction 1A was purified on a Sephadex LH-20 column, by elution with acetone, to afford polygalolide A (**1**, 20 mg, 0.00054% yield). Using the same method, fraction 1B afforded polygalolide B (**2**, 18 mg, 0.00049% yield), and fraction 1C afforded 1,3-dihydroxy-2-methoxyxanthone (16 mg, 0.00043% yield) (mp 174–176 °C) (lit.⁴ 176–178 °C). Fraction 2 was rechromatographed on a silica gel column using petroleum ether–acetone (8:2) as the eluent to give 1,7-dihydroxy-2,3-methylenedioxyxanthone (50 mg, 0.00135% yield) (mp 245–247 °C) (lit.⁵ 243–245 °C) and 1,3,6-trihydroxy-2,7-dimethoxyxanthone (15 mg, 0.00041% yield) (mp 228–230 °C) (lit.⁶ 231–233 °C). The three known xanthenes above were identified by physical and spectral data comparison to literature values.

Polygalolide A (1): light brown amorphous powder, $[\alpha]_{\text{D}}^{24} -14.4^\circ$ (*c* 0.018, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.28), 251 (3.96), 360 (4.16) nm; IR (KBr) ν_{max} 3436 (OH), 1776 (C=O), 1683 (C=O), 1640 (C=C), 1606, 1571, 1512, (aromatic ring), 1115, 1084 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) and ^{13}C NMR (100 MHz, CDCl_3), see Table 1; EIMS m/z 358 $[\text{M}]^+$ (100), 341 $[\text{M} - \text{OH}]^+$ (15), 330 $[\text{M} - \text{CO}]^+$ (18), 315 $[\text{M} - \text{CO} - \text{Me}]^+$

(10), 287 (14), 182 (14), 181 (27), 176 (28), 161 (48), 151 (27), 139 (17), 137 (23), 133 (25), 109 (25), 105 (28), 81 (23); HRFABMS m/z 359.1128 $[M + H]^+$ (calcd for $C_{19}H_{19}O_7$, 359.1131).

Polygalolide B (2): light brown amorphous powder; $[\alpha]^{24}_D$ -21.3° (c 0.015, MeOH); UV (MeOH) λ_{max} ($\log \epsilon$) 204 (4.11), 247 (3.79), 354 (4.10) nm; IR (KBr) ν_{max} 3436 (OH), 1776 (C=O), 1683 (C=O), 1640 (C=C), 1606, 1571, 1512, (aromatic ring), 1115, 1084 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS m/z 388 $[M]^+$ (100), 360 $[M - CO]^+$ (18), 345 $[M - CO - Me]^+$ (17), 317 (15), 285 (5), 207 (31), 206 (18), 191 (17), 182 (20), 181 (47), 167 (92), 163 (43), 151 (40), 149 (43), 139 (41), 137 (25), 135 (40), 109 (67), 81 (43); HRFABMS m/z 389.1225 $[M + H]^+$ (calcd for $C_{20}H_{21}O_8$, 389.1236).

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