FIVE PHENOLIC COMPOUNDS IN THE UNDERGROUND PARTS OF *VANCOUVERIA HEXANDRA*

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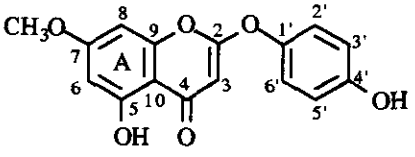
Abstract ----- From the underground parts of *Vancouveira hexanda*, a new 2-phenoxybenzochromone and four new prenylated flavones were isolated. Their structures were determined by the spectral analysis.

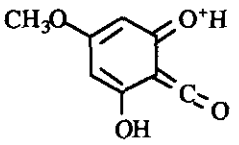
In our previous papers, the occurrence of new glycosides of flavonol with a γ,γ-dimethylallyl group in some *Epimedium* species,[1-5] and the presence of new cognate glycosides in *Vancouveria hexandra* [6-8] were described. In continuation of our study on the chemotaxonomy of Epimedieae (Berberdaceae), in particular, between *Epimedium* and *Vancouveria*, we tried to isolate of non-glycosidic phenolic compounds from a lesser polar fraction in the underground parts of *V. hexandra*, which resulted in the isolation of five new compounds. We deal with the structural elucidation of these compounds in this paper.

The underground parts of *Vancouveria hexandra* C. Morr. & Decne. were extracted with MeOH. After partition of the extract with EtOAc and *n*-BuOH, the *n*-BuOH soluble portion was subjected to silica gel column chromatography (Si CC). The fraction eluted with $CHCl_3$-MeOH (5 : 1) was further purified with Si CC and preparative tlc to give five new compounds (**1-5**).

Compound (**1**) *was obtained as a yellow amorphous, and gave* M^+ *at m/z* 300. *In the* ^1H *nmr spectrum, two* hydroxyl groups [δ 9.91 and 12.90 (chelated)] and a methoxyl group (δ 3.92) were observed. A set of *meta*

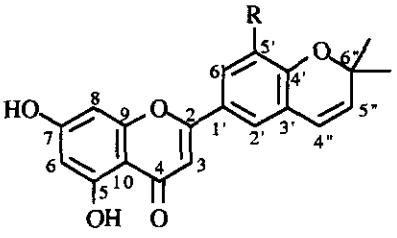


1

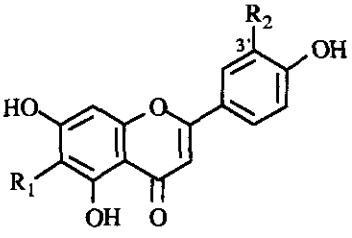


[A_1^+ + H] *m/z* 167

Figure 1



2: R = H 3: R = A



4: R1 = H, R2 = A 5: R1 = R2 = A

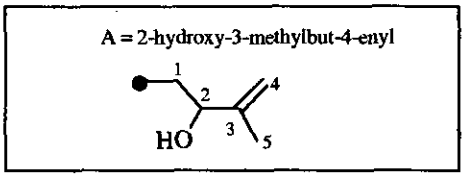


Table 1 ^{13}C nmr spectral data of **1**[a]

carbon No.	ppm
2	168.6
3	98.8
4	183.6
5	161.6
6	93.2
7	165.4
8	87.3
9	155.3
10	103.4
1'	143.5
2', 6'	122.3
3', 5'	117.0
4'	156.6
OMe	56.6

a : Measured in DMSO-*d6*, and assignment was referred to ref. 12.

coupled one-proton doublets [δ 6.47 and 6.71 (each *J*= 2 Hz)], a set of *ortho*-coupled two-proton doublet [6.95 and 7.26 (each *J*= 9 Hz)], and a one-proton singlet (δ 5.16) were further observed. Among them, the *ortho*-coupled protons were assinged to those on a *p*-oxygenated benzene ring. The singlet at δ 5.16 was assignable to H-3 in 2-phenoxybenzochromone.[9] These above data suggested that **1** was a 5,7,4'-trioxygenated 2-phenoxybenzochromone. The problem was the position of the methoxyl group, which was made clear as follows. In the eims, a fragment ion at *m/z* 167 (Figure 1) which corresponds to an [A_1^+ + H] fragment after retro Diels-Alder cleavage such as flavonoid compound suggested that the methoxyl group and one hydroxyl group were substituted on the A ring. Furthermore, the signals based on a chelated hydroxy

Table 2 ^{13}C nmr spectral data of compouds 2-5

carbon No.	2*	3**	carbon No.	4***	5***
2	162.5	163.5	2	165.5	164.7
3	104.0	103.3	3	104.2	103.6
4	182.4	181.6	4	183.2	182.7
5	162.3	161.4	5	160.9	160.3
6	99.5	98.8	6	99.9	109.7
7	164.1	164.1	7	165.5	160.4
8	94.2	93.8	8	95.6	94.2
9	157.8	157.2	9	158.0	156.7
10	105.3	103.7	10	105.6	104.7
1'	123.5	122.7a	1'	123.2	122.3
2'	124.5	122.0	2'	128.0	127.0
3'	121.5	121.4a	3'	131.1	130.5
4'	156.5	153.7	4'	163.4	162.9
5'	117.0	129.3	5'	117.7	117.3
6'	127.6	127.3	6'	127.5	127.4
4"	121.4	120.6	1", 1"'	38.9	38.8b
5"	131.7	131.6	2", 2"'	77.0	76.3, 76.4
6"	76.5	77.2	3", 3"'	148.4	147.6, 147.7
Me	28.3 (x 2)	28.6	4", 4"'	111.2	110.2, 110.7
1"'		35.6	5", 5"'	18.6	18.3, 18.4
2"'		73.4			
3"'		148.0			
4"'		109.8			
5"'		17.7			

*$CDCl_3$, **DMSO-d_6, ***acetone-d_6 a: Maybe interchangable, b: Another carbon was buried in solvent signals.

group and on a typical set of one-proton *meta*-coupled doublets in the ^1H nmr spectrum indicated that the A ring was oxygenated at C-5 and C-7. When **1** was compared with another known 2-phenoxybenzochromone possessing a 5,7-dihydroxyl A ring isolated from *Artemisia capillaris*,[9] the protons at C-6 and C-8 of **1** were shifted in a lower field than those of known one. These data suggesed that the A ring was a 5-hydroxy-7-methoxyl substitution, which was finally estimated by an NOE experiment. On irradiation of the methoxyl group, NOEs were observed at both H-6 (19%) and H-8 (20%), respectively. The structure of **1** was, consequently, concluded to be 2-(*p*-hydroxyphenoxy)-5-hydroxy-7-methoxybenzochromone. To the best of our knowledge, 2-phenoxy-benzochromones were rare phenolics in plant kingdom and a few instances have been confirmed in Compositae[9-11] and Rosaceae.[12] 2-Phenoxybenzochromone with a 5-hydroxy-7-methoxy moiety has been synthetically derived, however, it is a first isolation of such a derivative as natural product. The ^{13}C nmr spectral data are shown in Table 1.

Compounds (2-5) were suggested to be a flavone compound by their uv spectral data. Compound (2) was obtained as a yellow amorphous, and gave M^+ at *m/z* 336. In the ^1H nmr spectrum, a set of *ortho*-coupled olefinic protons [δ 5.71, 6.38 (each *J*= 10 Hz)] and two methyls (δ 1.48) were observed, indicating the presence of a dimethylpyran ring. The base peak at *m/z* 321 in the Eims showed the ring was fused on the ring B. In the ^1H nmr spectrum, two hydroxyl groups [δ 10.20 and 13.00 (chelated)], a set of *meta*-coupled one-proton doublets were supported the A ring of **2** to be a 5,7-dihydroxyl substitution. Furthermore, a typical ABX system at δ 6.83 (1H, d, *J*= 9 Hz), 7.48 (1H, d, *J*= 2 Hz) and 7.63 (1H, dd, *J*= 9, 2 Hz) was observed in the ^1H nmr spectrum. These data indicated that the dimethylchromene ring was fused on the B ring and formed the ring between C-3' and 4'-OH. Consequently, the structure of **2** was determined to be 5,7-dihydroxy-6",6"-dimethylpyrano(2",3" : 4',3')flavone.

Compound (**3**) was obtained as a yellow amorphous, and gave M^+ at *m/z* 420. All spectral data suggested that **3** had a same A ring and such a dimethylchromene ring systm as **2**. The presence of another 2-hydroxy-3-methylbut-4-enyl group was indicated by the following signals in the ^1H nmr spectrum : δ 1.77 (3H, s, Me), 2.70 (1H, dd, *J*= 14, 9 Hz), 3.02 (1H, dd, *J*= 14, 5 Hz), 4.18 (1H, m), 4.70 and 4.80 (1H each, br s, $=CH_2$). Further protons of the ring B were observed as two *meta*-coupled doublets [δ 7.66 and 7.70 (*J*= 2 Hz)]. These data suggested another 2-hydroxy-3-methylbut-4-enyl group was attached at C-5' of **2**. Consequently, the structure of **3** was confirmed to be 5,7-dihydroxy-5'-(2-hydroxy-3-methylbut-4-enyl)-6",6"-dimethylpyrano(2",3" : 4',3')flavone.

Compounds (**4**) and (**5**) were obtained as a yellow amorphous. Compound (**4**) gave M^+ at *m/z* 354, and the uv and ^1H nmr spectra indicated that **4** was an apigenin with a substituent at C-3'. In the ^1H nmr spectrum, the substituent was regarded as a 2-hydroxy-3-methylbut-4-enyl group. The structure of **4** was then determined to be 5,7,4'-trihydroxy-3'-(2-hydroxy-3-methylbut-4-enyl)flavone. Compound (**5**) was a flavone substituted further with another 2-hydroxy-3-methylbut-4-enyl group on the A ring compared with **4**. The position of the group was determined to be at C-6 on the A ring, because the chemical shift of the chelated hydroxyl group at C-5 was appeared at δ 13.47 which is in a lower field than those of **2-4**.[13] The strucure of **5** was, therefore, characterized as 5,7,4'-trihydroxy-6,3'-di(2-hydroxy-3-methylbut-4-enyl)flavone.

EXPERIMENTAL

Plant material and extraction were described in a preceding paper.[7]

Isolation of compounds 1-5. The $CHCl_3$-MeOH (5:1) fraction was further chromatographed on silica gel using some $CHCl_3$-MeOH solvent systems and finally purified by use of preparative tlc to give **1** (14 mg), **2** (22 mg), **3** (8 mg), **4** (12 mg) and **5** (10mg) as pure form.

Compound 1 [2-(p-hydroxyphenoxy)-5-hydroxy-7-methoxybenzochromone]: A pale yellow amorphous; Hrms Calcd for $C_{16}H_{12}O_6$: 300.0634. Found: 300.0612; Eims *m/z* (rel. int.): 300 (M^+, 100), 271 (20), 208 (2), 179 (2), 167 (27); uv (nm, MeOH): 229, 283; ^1H nmr (DMSO-*d*6) δ: 3.92 (3H, s, OMe), 5.16 (1H, s, H-3), 6.47 (1H, d, *J*= 2 Hz, H-6), 6.71 (1H, d, *J*= 2 Hz, H-8), 6.95 (2H, d, *J*= 9 Hz, H-3' and 5'), 7.26 (2H, d, *J*= 9 Hz, H-2' and 6'), 9.91 (1H, s, $C_{4'}$-OH), 12.90 (1H, s, C_5-OH).

Compound 2 [5,7-dihydroxy-6",6"-dimethylpyrano(2",3" : 4',3')flavone]: A yellow amorphous; Hrms Calcd for $C_{20}H_{16}O_5$: 336.1004. Found: 336.1025; Eims *m/z* (rel. int): 336 (M^+, 27), 321 (100), 300 (11), 272 (84), 169 (8), 152 (7); uv (nm, MeOH): 268, 342, +NaOMe: 226, 275, 312, 362, +$AlCl_3$: 279, 300sh, 362, 389, +$AlCl_3$/HCl: 279, 300sh, 357, 388, +NaOAc: 264, 274sh, 310, 369, +NaOAc/H_3BO_3: 269, 345; ^1H nmr (DMSO-*d*6) δ: 1.48 (6H, s, 2 x Me), 5.71 (1H, d, *J*= 10 Hz, H-5"), 6.31 (1H, d, *J*= 2 Hz, H-6), 6.38 (1H, d, *J*= 10 Hz, H-4"), 6.45 (1H, d, *J*= 2 Hz, H-8), 6.54 (1H, s, H-3), 6.83 (1H, d, *J*= 9 Hz, H-5'), 7.48 (1H, d, *J*= 2 Hz, H-2'), 7.63 (1H, dd, *J*= 9, 2 Hz, H-6'), 10.20 (1H, s, C_7-OH), 13.00 (1H, s, C_5-OH).

Compound 3 [5,7-dihydroxy-5'-(2-hydroxy-3-methylbut-4-enyl)-6",6"-dimethylpyrano(2",3" : 4',3')flavone]: A yellow amorphous; Hrms Calcd for $C_{25}H_{24}O_6$: 420.1550. Found: 420.1578; Eims *m/z* (rel. int.): 420 (M^+, 47), 405 (51), 350 (100), 335 (64), 153 (24); uv (nm, MeOH): 242, 269, 347, +NaOMe: 269, 315, 372, +$AlCl_3$: 277, 360, 387, +$AlCl_3$/HCl: 278, 360, 387, +NaOAc: 274sh, 318, 361, +NaOAc/H_3BO_3: 269, 351; ^1H nmr (DMSO-*d*6) δ: 1.43 (6H, s, 2 x Me), 1.77 (3H, s, H-5"), 2.70 (1H, dd, *J*= 14, 9 Hz, H-1'"), 3.02 (1H, dd, *J*= 14, 5 Hz, H-1'"), 4.18 (1H, m, H-2'"), 4.70 (1H, br s, H-4'"), 4.80 (2H, br s, H-4'" and OH), 5.84 (1H, d, *J*= 10 Hz, H-5"), 6.19 (1H, d, *J*= 2 Hz, H-6), 6.47 (1H, d, *J*= 2 Hz, H-8), 6.49 (1H, d, *J*= 10 Hz, H-4"), 6.76 (1H, s, H-3), 7.66, 7.70 (1H each, d, *J*= 2 Hz, H-2' and 6'), 10.80 (1H, s, C_7-OH), 12.92 (1H, s, C_5-OH).

Compound 4 [5,7,4'-trihydroxy-3'-(2-hydroxy-3-methylbut-4-enyl)flavone]: A yellow amorphous; Hrms Calcd for $C_{20}H_{18}O_6$: 354.1103. Found: 354.1124; Eims *m/z* (rel. int.): 354 (M^+, 36), 336 (9), 321 (22), 284 (100), 155 (16), 153 (22); uv (nm, MeOH): 268, 340, +NaOMe: 256, 323, 397, +$AlCl_3$: 251, 276, 301,

352, 400, +$AlCl_3$/HCl: 276, 300, 351, 400, +NaOAc: 275, 301, 379; ^{1}H nmr (acetone-*d*$_6$) δ: 1.20 (3H, br s, H-5"), 2.70 (1H, dd, *J*= 15, 9 Hz, H-1"), 3.02 (1H, dd, *J*= 15, 4 Hz, H-1"), 4.48 (1H, m, H-2"), 4.80, 5.00 (1H each, br s, H-4"), 6.25 (1H, d, *J*= 2 Hz, H-6), 6.53 (1H, d, *J*= 2 Hz, H-8), 6.63 (1H, s, H-3), 7.00 (1H, d, *J*= 9 Hz, H-5'), 7.82 (1H, dd, *J*= 9, 2 Hz, H-6'), 7.88 (1H, d, *J*= 2 Hz, H-2'), 9.80 (1H, s, C_7-OH), 13.00 (1H, s, C_5-OH).

Compound 5 [5,7,4'-trihydroxy-6,3'-di(2-hydroxy-3-methylbut-4-enyl)flavone]: A yellow amorphous ; Hrms Calcd for $C_{25}H_{26}O_7$: 438.1678. Found: 438.1648; Eims *m/z* (rel. int.): 438 (M^+, 7), 420 (11), 405 (6), 387 (367 (100), 350 (27), 206 (18), 286 (18), 165 (22), 123 (13); uv (nm, MeOH): 274, 336, +NaOMe: 275, 330, 395, +$AlCl_3$: 285, 310, 370, +$AlCl_3$/HCl: 276, 310, 370, +NaOAc: 270, 309, 370, +NaOAc/H_3BO_3: 271, 336 ; ^{1}H nmr (acetone-*d*$_6$) δ: 1.19 (6H, br s, 2 x Me, H-5" and 5'"), 2.85-3.05 (4H, m, H-1" and 1'"), 4.42 (2H, m, H-2" and 2'"), 4.77, 4.79, 4.95, 4.99 (1H each, br s, H-4" and 4'"), 6.50 (1H, s, H-8), 6.58 (1H, s, H-3), 6.96 (1H, d, *J*= 9 Hz, H-5'), 7.78 (1H, dd, *J*= 9, 2 Hz, H-6'), 7.82 (1H, d, *J*= 2 Hz, H-2'), 13.47 (1H, s, C_5-OH).

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