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Depsides from *Prunella vulgaris*

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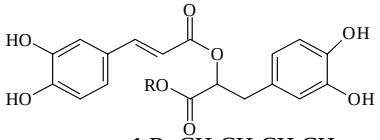
Abstract: A novel depsides **1**, together with three known ones **2**~**4** and two phenylpropanoids **5**~**6** were isolated from the ethanol extract of the spikes of *Prunella vulgaris*. On the basis of spectral and chemical evidence, their structures were determined as butyl rosmarinate **1**, ethyl rosmarinate **2**, methyl rosmarinate **3**, rosmarinic acid **4**, 3,4,α-trihydroxy-methyl phenylpropionate **5** and *p*-coumaric acid **6**, respectively.

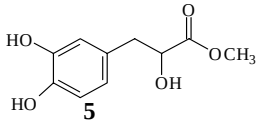
Keywords: *Prunella vulgaris*, Labitae, depsides, butyl rosmarinate.

Introduction

Prunella vulgaris L.(Labiatae) is a traditional Chinese drug and has hypotensive, antibacterial, anti-viral, anti-inflammatory, anti-tumor and hypoglycemic activities. Previously, the isolation and structural identification of a new triterpene saponin and five known compounds from the ethanol extracts of *P. vulgaris* were reported[1]. In this paper, we describe the isolation of four depsides and two phenylpropanoids from the same source. Their structures were established as butyl rosmarinate **1**, ethyl rosmarinate **2**, methyl rosmarinate **3**, rosmarinic acid **4**, 3,4,α-trihydroxy-methyl phenylpropionate **5** and *p*-coumaric acid **6** on the basis of spectroscopic analysis. Compound **1** was new and all known compounds were isolated from this plant for the first time.



1.R=$CH_2CH_2CH_2CH_3$
2.R=CH_2CH_3
3.R=CH_3
4.R=H



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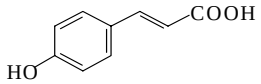

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Results and discussions

Compound **1** was obtained as a yellowish powder, gave positive reactions with ferric chloride and potassium ferricyanide, indicating that it was a phenol compound. UV absorption at 330, 222 nm showed the existence of conjugated unsaturated system. FAB-MS (*m/z*) gave pseudo-molecular ion peak at 455[M^++K]; Negative HRSI-MS exhibited a molecular formula $C_{22}H_{24}O_8$ [415.1403, calcd. 415.1393(M-1)]. ^{1}H-NMR

showed the presence of two aryl ABX systems at δ 7.20(1H, d, J=2.0Hz)，6.89(1H, d,J=8.5Hz), 7.06
(1H,dd, J=8.5,2.0Hz) and δ 6.83 (1H, d, J=2.0Hz)，6.77 (1H, d, J=8.5Hz), 6.64 (1H, dd, J=8.5, 2.0Hz). In addition, the signals of olefinic protons in pair were found at δ 7.59 (1H,d, J=16.0Hz), 6.32 (1H, d, J=16.0 Hz)，suggesting the existence of a *trans*-configuration olefinic bond. The chemical shifts of carbons connected with the two olefinic protons were observed at δ 146.3 and 112.9, respectively, in HMQC spectrum. In ^{13}C-NMR spectrum, there was a α, β-unsaturated carbonyl carbon signal (δ 165.9), which was correlated to the *trans*-olefinic protons. And the olefinic protons were correlated to aryl carbon signal (C-1 and C-2, see **Figure 1**). Those data suggested the existence of caffeoyl group.

Figure 1. main correlation HMBC for **1**

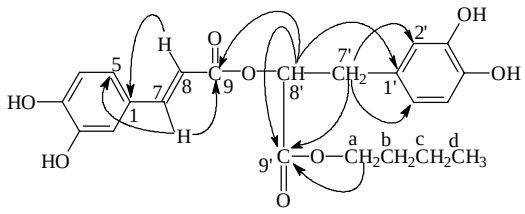

Table 1 NMR data for **1~6** (in DMSO-d_6)

Carbon	1			2	3	4	5	6
	^{13}C	DEPT	^{1}H					
1	125.1	C		125.3	125.3	125.3	128.2	125.2
2	115.4	CH	7.20(1H,d,J=2.0 Hz)	115.4	112.9	113.2	116.7	129.8
3	148.7	C		145.6	145.5	145.5	144.9	115.6
4	145.6	C		145.0	148.6	148.5	143.6	159.4
5	115.7	CH	6.89(1H,d,J=8.5 Hz)	115.8	115.4	115.3	115.2	115.6
6	121.7	CH	7.06(1H,dd,J=8.5/2.0 Hz)	121.7	121.8	121.5	119.9	129.8
7	146.3	CH	7.59(1H,d,J=16.0)	146.3	146.2	145.8	39.7	143.9
8	112.9	CH	6.32(1H,d,J=16.0)	112.9	114.8	114.8	71.6	115.3
9	165.9	C		165.9	165.8	165.8	173.9	167.7
1′	126.5	C		126.6	126.6	127.2		
2′	116.7	CH	6.83(1H,d,J=2.0)	116.7	116.6	116.6		
3′	145.0	C		148.7	144.9	144.8		
4′	144.1	C		144.1	144.0	143.9		
5′	114.9	CH	6.77(1H,d,J=8.5)	115.0	115.7	115.7		
6′	120.1	CH	6.64(1H,dd,J=8.5/2.0 Hz)	120.1	120.0	120.0		
7′	36.2	CH_2	3.05(2H,m)	36.2	36.1	36.1		
8′	72.9	CH	5.18(1H,m)	72.8	72.7	72.7		
9′	169.5	C		169.4	169.8	170.7		
a	64.3	CH_2	4.11(2H,t)	60.8			51.2	
b	30.0	CH_2	1.56(2H,m)	14.0	51.9			
c	18.5	CH_2	1.34(2H,m)					
d	13.5	CH_3	0.91(3H,t)					

In ^{13}C-NMR spectrum, there was another carbonyl carbon at δ 169.5.

The ^{1}H-NMR and ^{1}H-^{1}H COSY spectrum showed two groups of characteristic

signals at δ 3.05 (2H, m), 5.18 (1H, m) attributed to the group of —OCH(CO)CH_2—, and at δ 4.11 (2H, t), 1.56 (2H, m), 1.34 (2H, m) and 0.91 (3H, t) due to butoxy. HMQC spectrum revealed that the carbons at δ 36.2, 72.9 and 64.3, 30.0, 18.5, 13.5 (**Table 1**) linked with above protons, respectively. HMBC experiments gave the follow cross-peaks: δ 5.18 (H-8′) with two carbonyl signals, δ 165.9 and 169.5 (C-9 and C-9′) and aryl carbon δ 126.5 (C-1′); δ 3.05 (H-7′) with aryl carbons δ 116.7 and 120.1(C-2′ and C-6′); δ 4.11 (H-a) with carbonyl carbon δ 169.5 (**Figure 1**).

The comparison of the NMR data with those of a known compound, rosmarinic acid, with the aid of two-dimensional NMR techniques, permitted assigning all ^{13}C and ^{1}H NMR data for compound **1** (**Table 1**).

Therefore, the structure of compound **1** was determined as butyl rosmarinate.

Compound **2**, **3**, **4**, **5**, **6** were determined as ethyl rosmarinate, methyl rosmarinate, rosmarinic acid, 3,4,α-trihydroxy-butyl phenylpropionate and *p*-coumaric acid on the basis of physical property and the comparisons of spectra with known compounds.

Experimental

Plant Material

The dried spikes of *P. vulgaris* L. was purchased from ANGUO HEBEI herbal medicine market in August 1997 and identified by Professor Wang Xuan in Division of Pharmacognosy and a sample is deposited in Division of Natural Medicinal Chemistry, Beijing Medical University.

Extraction and Isolition

The dried spike powder (20kg) of *P. vulgaris* L. was extracted with 95% and 50% ethanol respectively. The 95% ethanolic extract was concentrated under reduced pressure and was extracted with petroleum ether, chloroform and n-butanol successively. The n-butanolic extract (510g) was dissolved in water to obtain water-insoluble substances 200g. This water insoluble part was chromatographed over silica gel column eluted with gradient solvent system of $CHCl_3$-MeOH (10:1 to 3:1) to afford 220 fractions (250ml in each fraction). Fraction 6~9 were subjected to a silica gel column chromatography, Sephadex LH-20 column chromatography, and finally preparative HPLC (70% MeOH as solvent system) to afford compound **1** (105mg), **2** (96mg), **6** (40mg). The 50% ethanol extract was partitioned between water and chloroform, n-butanol, succeessively. The n-butanol extract (700g) was fractioned by silica gel column chromatograph eluted with $CHCl_3$-MeOH-H_2O gradient system to obtained 1200 fractions (500ml/Fr). Fraction 241~320 were separated by silica gel column to give **5** (33mg), **3** (85mg) and **4** (98mg).

Identification

Compound **1**, a yellowish amorphous powder, gave positive ferric chloride and potassium ferricyanide reactions. UV λ_{max}^{MeOH} nm: 330, 222; EI-MS (*m/z*): 254(4.7), 236(5.3), 180(6.0), 162(2.0), 153(3.6), 135(2.5), 123(100), 110(23.5); FAB-MS (m/z): 455[M^++K]; negative HRSI-MS (415.1403, calcd. 415.1393) determined the molecular

formula to be $C_{22}H_{23}O_8$+H. ^{1}H- and ^{13}CNMR data see **Table 1**.

Compound **2**, a yellowish amorphous powder, and gave positive ferric chloride and potassium ferricyanide reactions. UVλ_{max}^{MeOH} nm：332，218; FAB-MS (*m/z*):389[M^++1]; ^{1}H-NMR (DMSO-d_6)δppm: 7.07 (1H,d,J=2.0Hz, H-2), 6.77 (1H,d,J=8.0Hz, H-5), 7.03 (1H, dd, J=8.0/ 2.0 Hz,H-6), 7.50 (1H, d, J=16.0Hz, H-7), 6.28 (1H, d, J=16.0Hz, H-8), 6.66(1H,d,J=2.0Hz,H-2′), 6.51 (1H, d, J=8.5Hz, H-5′), 6.64 (1H, dd, J=8.5/2.0Hz, H-6′), 2.96 (2H, m, H-7′), 5.07 (1H, m, H-8′), 4.09 (2H, q, H-a), 1.14 (3H, tr, H-b); ^{13}CNMR data see **Table 1**. The structure of **2** was determined as ethyl rosmarinic acid by comparing the NMR data with those of compound **1**.

Compound **3**, a yellowish amorphous powder, showed positive ferric chloride and potassium ferricyanide reactions. UVλ UVλ_{max}^{MeOH} nm：330, 217, 207; FAB-MS (*m/z*): 374[M^++1]; ^{1}H-NMR (DMSO-d_6) δppm: 7.06 (1H, d, J=2.0Hz, H-2), 6.77 (1H, d, J=8.0Hz, H-5), 7.01 (1H, d, J=8.0,2.0Hz, H-6), 7.48 (1H, d, J=16.0Hz, H-7), 6.26 (1H, d, J=16.0Hz, H-8), 6.61 (1H, d, J=2.0Hz, H-2′), 6.64 (1H, d, J=8.0Hz, H-5′), 6.49 (1H, dd, J=8.0/2.0Hz, H-6′), 2.95 (2H, m, H-7′), 5.12 (1H, dd, J=5.2 Hz, H-8′), 3.63 (3H, s, OCH_3); ^{13}C-NMR data see **Table 1**, above data were agreement with those of methyl rosmarinic acid[2].

Compound **4**, a yellowish amorphous powder, showed positive ferric chloride and potassium ferricyanide reactions. UVλ_{max}^{MeOH} nm：330, 221；^{1}H-NMR (DMSO-d_6) δppm: 7.05 (1H, d, J=2.0 Hz,H-2), 6.76 (1H, d, J=8.0Hz, H-5), 7.00 (1H, dd, J=8.0,2.0Hz, H-6), 7.46 (1H, d, J=16.0Hz, H-7), 6.23 (1H, d, J=16.0Hz, H-8), 6.67 (1H, d, J=2.0Hz, H-2′), 6.63 (1H, d, J=8.0Hz, H-5′), 6.52 (1H,dd, J=8.0,2.0Hz, H-6′), 2.90 (1H, m, H-7′a), 2.99 (1H, m, H-7′b), 5.02 (1H, m, H-8′); ^{13}C-NMR data see **Table 1**. The structure of **4** was determined as rosmarinic acid by a comparison of NMR data with those described in literature[2].

Compound **5**, a yellowish amorphous powder, gave positive ferric chloride and potassium ferricyanide reaction. UVλ_{max}^{MeOH} nm：282, 223；^{1}H-NMR (DMSO-d_6) δppm: 6.58 (1H, d, J=1.5Hz, H-2), 6.60 (1H, d, J=8.0Hz, H-5) ,6.42 (1H, dd, J=8.0,1.5Hz, H-6), 2.74 (1H,m, H-7a), 2.63 (1H, m, H-7b), 4.12 (1H, m, H-8). It was determined as 3,4,α-trihydroxyl-methyl phenylpropionate by comparison of NMR data with those of reported compound[3].

Compound **6**, a yellowish amorphous powder, gave positive ferric chloride and potassium ferricyanide reactions. UVλ_{max}^{MeOH} nm：290，225；^{1}H-NMR (DMSO-d_6) δppm:7.48 (2H, d, J=8.0Hz, H-2, H-6), 6.79 (2H, d, J=8.0Hz, H-2, H-5), 7.49 (1H, d, J=16.5Hz, H-7), 6.27 (1H, d, J=16.5Hz, H-8), the structure of **6** was determined as *p*-coumaric acid。

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