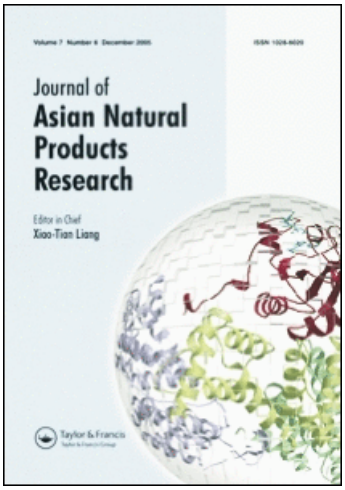

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Two new protoberberine quaternary alkaloids from *Corydalis yanhusuo*

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Two new protoberberine quaternary alkaloids, 13-methyl-palmatrubine (**1**) and 13-methyl-dehydrocorydalmine (**2**), were isolated from the tubers of *Corydalis yanhusuo* W.T. Wang. **1** was isolated as a natural product for the first time, and **2** a new compound. Their structures were elucidated on the basis of spectroscopic evidence, especially 1D- and 2D-NMR experiments.

Keywords: *Corydalis yanhusuo*; protoberberine alkaloids; 13-methyl-palmatrubine; 13-methyl-dehydrocorydalmine

1. Introduction

Corydalis yanhusuo W.T. Wang, called *Rhizoma corydalis* [1], has been employed in traditional Chinese medicine as an analgesic agent for treating spastic pain, abdominal pain, menstrual pain, and pain due to injuries [2]. It has also been widely used to promote blood circulation and treat coronary heart diseases [3,4]. Alkaloids are acknowledged to be the major active components in *C. yanhusuo* [5]. Among alkaloids, the main quaternary active principals of *C. yanhusuo* showed stronger activity than the tertiary ones [6,7]. However, up to now, very few quaternary alkaloids [8–10] have been isolated from it. In our search for new bioactive constituents of anti-myocardial ischemia, two protoberberine quaternary alkaloids, 13-methyl-palmatrubine (**1**) and 13-methyl-dehydrocorydalmine (**2**), were isolated from the ethanolic extract of the tubers of *C. yanhusuo*.

2 was a new compound, and **1** was isolated as a natural product for the first time. Although **1** was synthesized, no spectral data were reported in the literature. The chemical structures of compounds **1** and **2** were elucidated based on spectral analysis (^{1}H NMR, ^{13}C NMR, 2D-NMR, MS, UV, and IR). In this article, we present the isolation and structural determination of compounds **1** and **2**.

2. Results and discussion

13-Methyl-dehydrocorydalmine (**2**) (Figure 1), obtained as yellowish needles, was positive to Dragendorff's reagent, revealing that it was an alkaloid compound. The UV absorption maxima at 336 and 266 nm and bathochromic shifts on adding alkali indicated the presence of a typical phenolic berberine-type alkaloid [11,12]. The HR-ESI-MS (positive) showed the molecular ion peak at *m/z* 352.1546 $[M]^+$, supporting the molecular formula $C_{21}H_{22}NO_4$. The ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) indicated that the molecule contained 15 aromatic carbons, including 3 methoxyls, 1 hydroxyl, 2 methylenes, and 1 methyl. The chemical and spectral properties have made it possible to assume that the alkaloid had a protoberberine quaternary alkaloid skeleton

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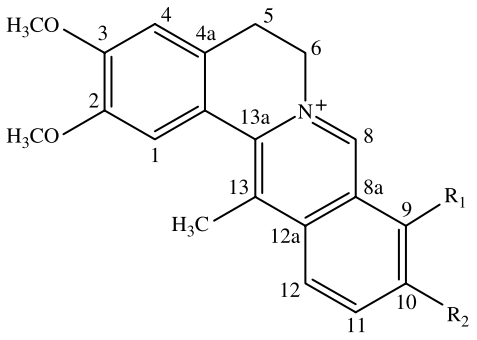

1 $R_1 = OH$, $R_2 = OCH_3$
2 $R_1 = OCH_3$, $R_2 = OH$
Dehydrocorydaline $R_1 = OCH_3$, $R_2 = OCH_3$

Figure 1. Structures of **1**, **2**, and dehydrocorydaline.

similar to that of dehydrocorydaline except for a different substituted group linked with C-10 [10,13].

The carbon signal at δ_C 144.2 was assigned to C-9 for its HMBC correlations with H-8 at δ_H 9.68 and H-11 at δ_H 8.05.

Table 1. ^{13}C NMR spectral data for compounds **1** (in CD_3OD, 125 MHz), **2** (in CD_3OD, 125 MHz), and dehydrocorydaline (in $CDCl_3$, 75 MHz).

Position	**1**	**2**	Dehydrocorydaline [10]
1	116.6	116.5	113.9
2	149.5	149.9	147.6
3	152.5	153.4	150.5
4	112.6	112.6	110.7
4a	133.4	133.7	132.2
5	30.0	29.2	28.2
6	57.8	59.2	57.1
8	146.4	144.3	146.5
8a	121.3	123.8	121.7
9	163.9	144.2	151.3
10	151.2	150.8	146.3
11	124.1	132.1	125.4
12	107.2	122.7	119.7
12a	134.9	135.8	133.7
13	129.4	132.2	128.5
13a	134.7	138.3	136.3
13b	122.5	121.4	119.2
2-OMe	57.1*	57.2*	56.2*
3-OMe	57.3*	57.7*	56.5*
9-OMe		62.9	63.2
10-OMe	57.6		56.9*
13-Me	18.2	18.7	17.9

*To be interchangeable.

The connectivity of methoxyl group to C-9 was explained by the HMBC correlation from δ_H 4.16(3H, s, -OCH_3) to C-9, which was further confirmed by the NOESY correlation of H-8 with 9-OMe. The hydroxyl was indicated to be connected to C-10 (δ_C 150.8) because neither the HMBC correlation of C-10 with methoxyl group nor the NOESY cross-signal of methoxyl group with H-11 was observed (Figures 2 and 3). On the basis of these spectral data, **2** was determined to be 5,6-dihydro-10-hydroxy-2,3,9-trimethoxy-13-methyl-dibenzo[*a*,*g*] quinolizinium alkaloid, named 13-methyl-dehydrocorydalmine.

13-Methyl-palmatrubine (**1**) (Figure 1), obtained as red needles, was positive to Dragendorff's reagent, revealing that it was an alkaloid compound. The spectral data of **1** were almost identical to those of dehydrocorydaline, except for the absence of one methoxyl signal. The connectivity of methoxyl group to C-10 (δ_C 151.2) was explained by the HMBC correlations from the proton signal at δ_H 3.89 (3H, s, -OCH_3) to C-10 and verified by the NOESY correlation of the proton at δ_H 3.89 (3H, s, -OCH_3) with H-11 (δ_H 7.58, 1H, d). The hydroxyl was suggested to be connected to C-9 (δ_C 151.2) because neither the HMBC correlations of the methoxyl group with C-9 nor NOESY cross-signal of the methoxyl group with H-8 was observed. On the basis of these chemical and spectral properties, **1** was identified as 13-methyl-palmatrubine.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Fisher-Johns melting-point apparatus and are uncorrected. UV and IR spectra were obtained on a TU-1810 UV–Vis spectrophotometer and a Nicolet Impact 410 infrared instrument with KBr pellets. NMR spectra were obtained on a Bruker Avance DRX-500 spectrometer at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR, using CD_3OD as a solvent and TMS as the internal standard. Chemical shifts were reported in δ units (ppm) and coupling constants (J) in Hz. HR-ESI-MS were

Table 2. ^{1}H NMR spectral data for compounds **1** (in CD_3OD, 500 MHz), **2** (in CD_3OD, 500 MHz), and dehydrocorydaline (in $CDCl_3$, 300 MHz).

Position	**1**	**2**	Dehydrocorydaline [10]
1	7.28 (1H, s)	7.38 (1H, s)	7.16 (1H, s)
2			
3			
4	7.03 (1H, s)	7.12 (1H, s)	6.93 (1H, s)
4a			
5	3.08 (2H, t)	3.19 (2H, t)	3.25 (2H, t)
6	4.53 (2H, t)	4.83 (2H, t)	5.30 (2H, br s)
8	9.35 (1H, s)	9.68 (1H, s)	10.68 (1H, s)
8a			
9			
10			
11	7.58 (1H, d, $J = 8.5$ Hz)	8.05 (1H, d, $J = 8.5$ Hz)	7.87 (1H, d, $J = 9.1$ Hz)
12	7.02 (1H, d, $J = 8.5$ Hz)	7.84 (1H, d, $J = 8.5$ Hz)	7.92 (1H, d, $J = 9.1$ Hz)
12a			
13			
13a			
13b			
2-OMe	3.89 (3H, s)	3.91 (3H, s)	3.94 (3H, s)
3-OMe	3.91 (3H, s)	3.95 (3H, s)	4.00 (3H, s)
9-OMe		4.16 (3H, s)	4.08 (3H, s)
10-OMe	3.89 (3H, s)		4.35 (3H, s)
13-Me	2.78 (3H, s)	3.01 (3H, s)	2.97 (3H, s)

*To be interchangeable.

obtained on a VG-70SE mass spectrometer. D101 macroporous resin was purchased from the Chemical Plant of Nankai University (Tianjin, China). Silica gel (160–200 mesh; Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (GE Healthcare Bio-Sciences Co., Uppsala, Sweden), and RP-18 (YMC-GEL, ODS-A, 12 nm, S-50 μm; YMC Co., Kyoto, Japan) were used for column chromatography (CC), and silica gel GF_{254} (10–40 μm; Qingdao Marine Chemical Inc., Qingdao, China) for TLC.

3.2 Plant material

The dried tubers of *C. yanhusuo* W.T. Wang were collected from Hebei Province, China, in 2005, and were identified by Prof. Shi Yue.

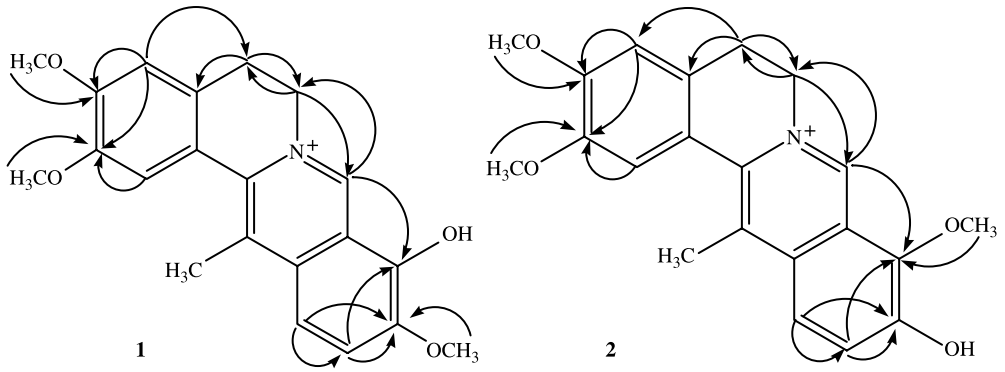

Figure 2. Key HMBC correlations of **1** and **2**.

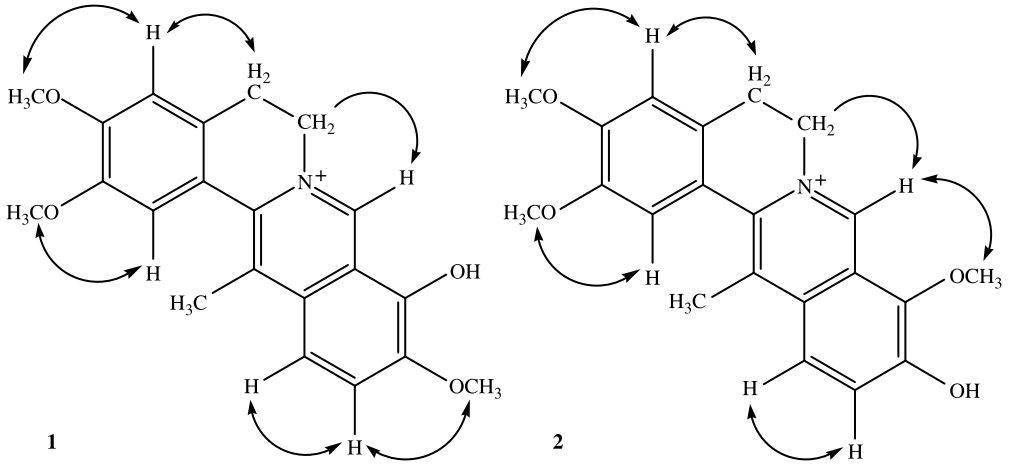

Figure 3. Key NOESY correlations of **1** and **2**.

A voucher specimen of the sample (no. Y050124) is kept in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

3.3 Extraction and isolation

The dried tubers of *C. yanhusuo* (5 kg) were extracted with 80% ethanol under reflux three times and filtered. The combined filtrate was concentrated and dried under reduced pressure. The extract (300 g) was chromatographed over a D101 macroporous resin column with H_2O and 50% and 95% EtOH as eluants (50 L of each eluant). The fraction (58 g) eluted with 50% EtOH was subjected to silica gel CC (160–200 mesh, 1500 g) eluted with a gradient of $CHCl_3$/MeOH (9:1, 5:1, 3:1, 1:1). The combined fractions (1 g) eluted with $CHCl_3$/MeOH (9:1) were isolated on a Sephadex LH-20 column and eluted with MeOH to afford **1** (100 mg). The combined fraction (7 g) eluted with $CHCl_3$/MeOH (5:1) was subjected to a reversed-phase CC (ODS-A, 12 nm, S-50 μm, 100 g) using MeOH/H_2O (including 1.6% HAc and 0.4% triethylamine) (30:70, V/V) as eluant. Fractions 3–7 were collected (200 mg in total) and further separated by preparative TLC (10 cm × 10 cm × 0.25 cm, silica gel F_{254}, *n*-butanol/HAc/H_2O (4:5:1, V/V/V)) to give **2** (20 mg).

*3.3.1 13-Methyl-palmatrubine (**1**)*

Red needles, mp 175–176°C. UV (MeOH) λ_{max} (nm, log ε): 232 (5.63), 272 (5.57), 338 (5.41); IR (KBr) ν_{max} (cm^{-1}): 1607, 1519, 1464, 1386, 1338, 1292, 1240, 1192, 1114; HR-ESI-MS (positive) *m/z*: 352.1558 $[M]^+$ (calcd for $C_{21}H_{22}NO_4$, 352.1549); ^1H and ^{13}C NMR spectral data are listed in Table 1.

*3.3.2 13-Methyl-dehydrocorydalmine (**2**)*

Yellowish needles, mp 193–194°C. UV (MeOH) λ_{max} (nm, log ε): 266 (5.49), 336 (5.39); IR (KBr) ν_{max} (cm^{-1}): 1604, 1521, 1459, 1382, 1346, 1281, 1210, 1173, 1104; HR-ESI-MS (positive) *m/z*: 352.1546 $[M]^+$ (calcd for $C_{21}H_{22}NO_4$, 352.1549); ^1H and ^{13}C NMR spectral data are listed in Table 2.

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