Constituents of the Roots of *Salvia prionitis*

Mai Li, Jin-Sheng Zhang,* Yong-Mao Ye, and Ji-Nian Fang

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 294 Tai-Yuan Road, Shanghai 200031, People's Republic of China

Received July 16, 1999

From the roots of *Salvia prionitis*, a novel alkaloid named prioline (**1**) and a new diterpene, prineoparaquinone (**2**), were isolated together with taxodione; microstegiol; 8,11,13-dehydroabietane; and 2-isopropyl-8-methyl-3,4-phenanthraquinone. The structures of the new compounds were established by spectroscopic means.

Salvia prionitis Hance (Labiatae), a herbaceous species distributed in the southern provinces of the People's Republic of China, is used in Chinese folk medicine for the treatment of tonsillitis, pharyngitis, pulmonary tuberculosis, and bacillary dysentery. More than 20 compounds have been previously isolated from the plant.[1−7] In our continuing investigation of its constituents, prioline (**1**), a novel alkaloid, and a new diterpene, prineoparaquinone (**2**), were isolated together with taxodione;[8] microstegiol;[9] 8,11,13-dehydroabietane;[10] and 2-isopropyl-8-methyl-3,4-phenanthraquinone,[11] which were isolated from this plant for the first time. The structures of **1** and **2** were determined by spectroscopic means.

Compound **1**, a novel alkaloid obtained as yellow needles from ethyl acetate, displayed a molecular ion at *m*/*z* 241.1097 (HRMS), corresponding to the formula $C_{15}H_{15}NO_2$. The UV spectrum of **1** was similar to that of sapriolactone,[2] suggesting that both compounds possess the same skeleton containing a highly conjugated naphthalene moiety. However, the polarities of the two compounds were quite different. The R_f values were 0.4 for **1** and 0.9 for sapriolactone by TLC (cyclohexane−EtOAc 1:1). The IR spectrum of **1** showed the presence of secondary amine (3400 cm^{-1}), phenolic hydroxyl (3280 cm^{-1}), amide carbonyl (1660 cm^{-1}), and conjugated naphthalene (1608, 1482, and 1358 cm^{-1}) moieties. The ^1H NMR spectrum of compound **1** indicated the existence of one aromatic isopropyl group at δ 1.37 (6H, d, J = 6.9 Hz) and 3.50 (1H, septet, J = 6.9 Hz), one aromatic methyl at δ 2.89 (3H, s), one aromatic proton singlet at δ 7.42, two aromatic proton doublets with an AB pattern at δ 7.30 (1H, J = 8.0 Hz) and 7.82 (1H, J = 8.0 Hz), and a secondary amine at δ 10.97 (1H, s, D_2O exchangeable). The ^1H NMR signal of H-5 was located further downfield because of the deshielding effect from the carbonyl group at C-2a. The ^{13}C NMR assignments of **1** were based on the analysis of ^{13}C, DEPT, and HMQC NMR experiments (Table 1), which showed signals of one methyl, one isopropyl, seven aromatic quaternary carbons, three aromatic tertiary carbons, and one amide carbonyl. The substituted positions were confirmed by the HMBC spectrum (Table 1). The HMBC correlations of δ_C 128.9 (C-4) to δ_H 2.89 (H-12), δ_C 131.3 (C-5) to δ_H 7.42 (H-6), and δ_C 126.7 (C-8b) to δ_H 7.82 (H-5) and 7.42 (H-6) demonstrated connectivities between C-3↔C-4↔C-5↔C-5a↔C-6, while the correlations of δ_C 119.1 (C-6) to δ_H 7.82 (H-5) and 3.50 (H-9), δ_C 141.8 (C-7) to δ_H 3.50 (H-9) and 1.37 (H-10, 11), and δ_C 139.9 (C-8) to δ_H 7.42 (H-6) and 3.50 (H-9), provided evidence of connectivities between C-6↔C-7↔C-8. Based on the above information, the structure of the new compound was established as **1**. Prioline (**1**) is the first alkaloid to have been isolated from a plant in the genus *Salvia*. We think it unlikely that prioline is an artificial product because no nitrogen-containing reagents were used in the process of extraction and isolation. It might be produced by the particular growth environment of the plant.

Table 1. NMR Data for Compound **1**[a]

position	δ_C (mult.)	δ_H (mult., J in Hz)	HMBC
2	170.4 (s)		
2a	120.9 (s)		H-4, H-12
3	140.3 (s)		H-5, H-12
4	128.9 (d)	7.30 (d, 8.0)	H-12
5	131.3 (d)	7.82 (d, 8.0)	H-6
5a	123.0 (s)		H-4
6	119.1 (d)	7.42 (s)	H-5, H-9
7	141.8 (s)		H-9, H-10 (11)
8	139.9 (s)		H-6, H-9
8a	117.1 (s)		
8b	126.7 (s)		H-5, H-6
9	28.3 (d)	3.50 (septet, 6.9)	H-6, H-10 (11)
10	22.9 (q)	1.37 (d, 6.9)	H-9, H-11
11	22.9 (q)	1.37 (d, 6.9)	H-9, H-10
12	18.7 (q)	2.89 (s)	H-4
NH		10.97 (br s)	

[a] Spectra were recorded in $CDCl_3$, with δ values expressed in ppm.

Prineoparaquinone (**2**) was isolated as dark yellow powder. The molecular formula, $C_{20}H_{22}O_5$, of **2** was established by the analysis of its HRMS, ^{13}C NMR, and DEPT data. Its IR spectrum displayed absorptions due to hydroxyl (3369 cm^{-1}) and carbonyl (1731, 1664, 1635 cm^{-1}) groups. The UV spectrum showed bands at 215, 254, 287, and 342 nm, indicating the presence of a 1,4-naphthaquinone moiety.[12] The ^1H NMR spectrum showed signals of one aromatic methyl at δ 2.25 (3H, s, Me-20), two methyl singlets at δ 1.34 and 1.31 (each 3H, s, Me-18, 19), two doublets for two ortho aromatic protons at δ 8.06 (1H, d, J = 7.9 Hz, H-7) and 7.58 (1H, d, J = 7.9 Hz, H-6), and two doublets at δ 6.56 (1H, d, J = 15.8 Hz, H-2) and 6.45 (1H, d, J = 15.8 Hz, H-3) due to two trans olefinic protons. The double bond was deduced as being located between C-2 and C-3 because, if it had been located between C-1 and C-2, the signal of the olefinic proton at C-1 would have been at much lower field (about δ 8.0) due to the significant deshielding effect of the carbonyl group at C-11.[13,14] However, no such evidence was observed in the ^1H NMR spectrum of **2**. The HMBC spectrum of its mono-acetyl derivative (**3**) (Table 2) revealed that the carbonyl carbon at δ 197.7 only correlated with two olefinic protons instead

* To whom correspondence should be addressed. Tel.: 0086-21-64311833. Fax: 0086-21-64370269. E-mail: jszhang@mail.shcnc.ac.cn.

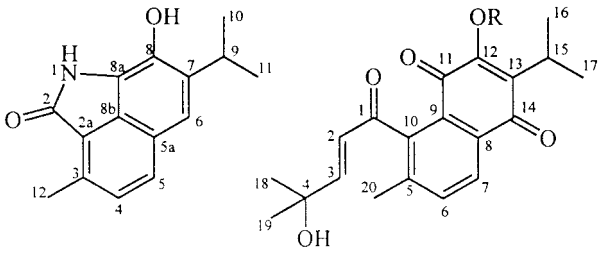
10.1021/np990357k CCC: $19.00
Published on Web 11/20/1999

Table 2. NMR Data for Compounds **2** and **3**[a]

	2		**3**		
position	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)	HMBC
1		198.3 (s)		197.7 (s)	H-2, H-3
2	6.56 (d, 15.8)	127.1 (d)	6.45 (d, 16.0)	127.6 (d)	H-3
3	6.45 (d, 15.8)	155.3 (d)	6.33 (d, 16.0)	154.3 (d)	H-2, H-18 (19)
4		70.6 (s)		70.6 (s)	H-2, H-3, H-18 (19)
5		139.6 (s)		141.9 (s)	H-7, H-20
6	7.58 (d, 7.9)	136.6 (d)	7.59 (d, 7.9)	136.2 (d)	H-20
7	8.06 (d, 7.9)	127.4 (d)	8.06 (d, 7.9)	127.7 (d)	
8		131.4 (s)		130.8 (s)	H-6
9		128.5 (s)		128.7 (s)	H-7
10		140.1 (s)		139.7 (s)	H-2, H-6, H-20
11		181.3 (s)		178.3 (s)	
12		152.8 (s)		150.9 (s)	H-15
13		126.4 (s)		142.9 (s)	H-15, H-16 (17)
14		183.9 (s)		183.8 (s)	H-7, H-15
15	3.36 (septet, 7.0)	24.6 (d)	3.29 (septet, 6.0)	25.7 (d)	H-16 (17)
16	1.26 (d, 7.0)	19.7 (q)	1.26 (d, 6.0)	20.5 (q)	H-15, H-17
17	1.27 (d, 7.0)	19.7 (q)	1.26 (d, 6.0)	20.5 (q)	H-15, H-16
18	1.34 (s)	28.9 (q)	1.32 (s)	28.7 (q)	H-3, H-19
19	1.31 (s)	29.1 (q)	1.32 (s)	29.0 (q)	H-3, H-18
20	2.25 (s)	19.1 (q)	2.28 (s)	19.4 (q)	H-6
21				168.3 (s)	H-22
22			2.31 (s)	20.6 (q)	

[a] Spectra were recorded in $CDCl_3$, with δ values expressed in ppm.

of with the methyl protons at δ 1.32, which further supported the fact that the trans double bond must be as proposed. The ^{1}H NMR spectrum of compound **2** also indicated the presence of an isopropyl group at δ 1.26, 1.27 (each 3H, d, J = 7.0 Hz, Me-16, 17), and 3.36 (1H, septet, J = 7.0 Hz, H-15). The ^{13}C NMR spectrum showed the presence of three carbonyl functions at δ 198.3, 183.9, and 181.3. The oxygenated quaternary carbon signal at δ 70.6 in the ^{13}C NMR spectrum and the lack of another isopropyl methine signal in the ^{1}H NMR spectrum indicated the presence of a hydroxyl group at C-4. The HREIMS showed fragment ions at *m/z* 281.1169 ($C_{18}H_{17}O_3$, 100%, $[M - CH_3 - CO - 2H]^+$), 324.1353 ($C_{20}H_{20}O_4$, 75%, $[M - H_2O]^+$), and 327.1209 ($C_{19}H_{19}O_5$, 5%, $[M - CH_3]^+$). Thus, the structure of compound **2** was deduced as 4,5-*seco*-5,10-*friedo*-4,12-dihydroxyabieta-2(*E*),5(10),6,8,12-pentaene-1,-11,14-trione, to which the trivial name prineoparaquinone has been accorded.



1

2 R = H
3 R = Ac

Experimental Section

General Experimental Procedures. The melting point was determined on a Kofler hot-stage apparatus and is uncorrected. UV spectra were recorded on a Beckman DU-600 spectrophotometer. IR spectra were measured on a Nicolet Magna 750 spectrophotometer. ^{1}H, ^{13}C, HMQC, and HMBC NMR spectrum were recorded on a Bruker AM-400 (^{1}H) or a Bruker AC-100 (^{13}C) spectrometer. Mass spectra were obtained on a MAT 711 mass spectrometer.

Plant Material. The roots of *Salvia prionitis* were collected in Nanchang City, Jiangxi Province, People's Republic of China, in May 1995, and authenticated by Professor Xiu-Lan Huang of our institute. A voucher specimen has been deposited in the Herbarium of Shanghai Institute of Materia Medica (SIMMP 95068).

Extraction and Isolation. Dried roots of *S. prionitis* (10 kg) were extracted with EtOAc for 6 days at room temperature. The solvent was removed in vacuo to yield 390 g of a gummy residue. The extract was subjected to repeated chromatography over Si gel eluted with solvent mixtures of cyclohexane−EtOAc of increasing polarity. The fractions eluted directly with cyclohexane afforded the colorless oil 8,11,13-dehydroabietane[10] (500 mg, 0.005%). The subsequent fractions were examined by TLC and further purified by preparative TLC (Si gel GF_{254}, 0.5 mm) to furnish 2-isopropyl-8-methyl-3,4-phenanthraquinone[11] (25 mg, 0.00025%), prioline (**1**, 15 mg, 0.00015%), taxodione[8] (40 mg, 0.0004%), microstegiol[9] (30 mg, 0.0003%), and prineoparaquinone (**2**, 15 mg, 0.00015%), respectively.

Prioline (1): yellow needles (EtOAc); mp 210−212 °C; UV (MeOH) λ_{max} (log ϵ) 215 (4.43), 256 (4.39), 354 (3.71) nm; IR (KBr) ν_{max} 3400, 3280, 2990, 1660, 1608, 1482, 1358, 1334, 1223, 1180, 681 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 241 $[M]^+$ (84), 226 (100), 198 (8), 170 (8), 154 (4), 98 (6); HREIMS *m/z* 241.1097 $[M]^+$ (calcd for $C_{15}H_{15}NO_2$, 241.1103).

Prineoparaquinone (2): dark yellow powder; UV (MeOH) λ_{max} (log ϵ) 215 (4.3), 254 (4.2), 287 (4.0), 342 (3.4); IR (KBr) ν_{max} 3369, 2960, 1732, 1665, 1635, 1589, 1569, 1460, 1373, 1259, 1126, 980, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HRMS *m/z* 327.1209 $[M - CH_3]^+$ (5), 324.1353 $[M - H_2O]^+$ (75), 296.1395 $[M - H_2O - CO]^+$ (17), 281 $[M - CH_3 - CO - 2H]^+$ (100), 257 (16), 227 (34), 149 (18), 77 (6).

12-Acetyl-prineoparaquinone (3). To the solution of prineoparaquinone (**2**, 10 mg) in 1 mL of acetic anhydride was added 3 drops of dry pyridine. After stirring at room temperature for 6 h with the exclusion of light, 5 g of crushed ice were added to destroy the excess acetic anhydride, and the solution was extracted with two 50-mL portions of ether. The organic layer was combined and dried over Na_2SO_4. The product was purified by preparative TLC to yield 9 mg of **3**: IR (KBr) ν_{max} 3381, 2990, 1774, 1668, 1371, 1290, 1180, 1082, 980, 850 cm^{-1}; ^{1}H NMR, ^{13}C NMR and HMBC data, see Table 2; EIMS *m/z* 384 $[M]^+$ (16), 369 (30), 366 (40), 324 (56), 281 (100), 257 (21), 213 (16), 141 (6); HREIMS *m/z* 384.1544 $[M]^+$ (calcd for $C_{22}H_{24}O_6$, 384.1574).

References and Notes

(1) Yang, B.-J.; Huang, X.-L.; But, P.-H.; Zhuang, G.-F.; Huang, Y.; Wang, X.-M.; Lin, L.-Z. *Acta Bot. Sin.* **1988**, *30*, 524−527.

(2) Huang, X.-L.; Wang, X.-M.; Zhang, J.-S.; Lin, L.-Z. *Acta Bot. Sin*. **1990**, *32*, 490−491.
(3) Zhang, J.-S.; Huang, Y. *Nat. Prod. Res. Develop*. **1995**, *7*, 1−4.
(4) Lin, L.-Z.; Wang, X.-M.; Huang, X.-L.; Huang, Y. *Acta Pharm. Sin*. **1990**, *25*, 154−156.
(5) Lin, L.-Z.; Wang, X.-M.; Huang, X.-L.; Huang, Y.; Yang, B.-J. *Planta Med*. **1988**, *54*, 443−444.
(6) Lin, L.-Z.; Blasko, G.; Cordell, G. A. *Phytochemistry* **1989**, *28*, 177−181.
(7) Lin, L.-Z.; Cordell, G. A.; Lin, P. *Phytochemistry* **1995**, *40*, 1469−1471.
(8) Kupchan, S. M.; Karim, A.; Marcks, C. *J. Org. Chem*. **1969**, *34*, 3912−3918, and references therein.
(9) Ulubelen, A.; Topcu, G.; Tan, N.; Lin, L.-J.; Cordell, G. A. *Phytochemistry* **1992**, *31*, 2419−2421.
(10) Kitadani, Masayuki; Yoshikoshi, Akira; Kitahara, Yoshio; de Paiya Campello, J.; McChesney, J. D.; Watts, D. J.; Wenkert, E. *Chem. Pharm. Bull.* **1970**, *18*, 402−405.
(11) Onitsuka, M.; Fujiu, M.; Shinma, N.; Maruyama, H. B. *Chem. Pharm. Bull.* **1983**, *31*, 1670−1675.
(12) Scott, A. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: London, 1964; p 123.
(13) Lee, H.-K.; Oh, S.-R.; Kim, J.-I.; Kim, J.-W.; Lee, C.-O. *J. Nat. Prod*. **1995**, *58*, 1718−1721.
(14) Topcu, G.; Eris, C.; Ulubelen, A. *Phytochemistry* **1996**, *41*, 1143−1147.

NP990357K