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## Four new alkaloids from *Polyalthia nemoralis* (Annonaceae)

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Four new alkaloids, polynemoralines A (**1**), B (**2**), C (**3**), and D (**4**), were isolated from the branches and leaves of *Polyalthia nemoralis* A DC. The structures of **1–4** were elucidated mainly on the basis of spectroscopic methods. The structure of **4** was confirmed by X-ray diffraction analysis.

**Keywords:** *Polyalthia nemoralis*; Annonaceae; aporphine alkaloids; polynemoralines A, B, C, D

### 1. Introduction

The genus *Polyalthia* (Annonaceae) comprises about 120 species that are distributed in tropical and semitropical zones of the Eastern hemisphere. *Polyalthia nemoralis* A DC is one of 17 species belonging to this genus found in China [1]. In folk medicine it has been used for the treatment of malaria, hepatitis, pneumonia, syphilis, and so on. The previous researches reported the isolation of five compounds including zincpolyanemine and cupric bis(pyridine-*N*-oxide-2-thiolate) with strong antimalarial activity from the root of this plant [2]. The purpose of searching bioactive compounds from Chinese plant resources and application of them prompted us to investigate the constituents of this plant. In this paper, we report the isolation and structure elucidation of four new alkaloids named polynemoralines A (**1**), B (**2**), C (**3**), and D (**4**) from the branches and leaves of *P. nemoralis* A DC.

### 2. Results and discussion

Polynemoraline A (**1**) was obtained as yellowish amorphous powder from acetone.

The positive Dragendorff reaction showed **1** to be an alkaloid. The molecular formula was deduced as  $C_{34}H_{20}N_2O_6$  from the HR-EI-MS at  $m/z$  552.1295  $[M]^+$ . The IR spectrum of **1** exhibited absorption bands at 3342 (NH), 2992, 2846 ( $CH_2$ ), 1697, 1655, 1587  $cm^{-1}$  (conjugated carbonyl and aryl groups), respectively. The UV spectrum of **1** showed intense absorption maxima at 219, 243, 261, 304, 317, 337, 396, and 440 nm. The information of HR-EI-MS, IR, and UV spectral data suggested that **1** could be an aporphine alkaloid [3].

In the  $^1H$ -NMR spectrum, two sets of disubstituted benzene proton signals were observed. By combining the analyses of its  $^{13}C$ -NMR, HMQC, and HMBC spectra, one set of signals at  $\delta_H$  9.17 (1H, d,  $J = 8.0$  Hz, H-11), 7.69 (1H, t,  $J = 8.0$  Hz, H-10), 7.48 (1H, t,  $J = 8.0$  Hz, H-9), 7.52 (1H, d,  $J = 8.0$  Hz, H-8), and the other set at  $\delta_H$  9.08 (1H, d,  $J = 8.0$  Hz, H-11'), 7.37 (1H, t,  $J = 8.0$  Hz, H-10'), 7.24 (1H, t,  $J = 8.0$  Hz, H-9'), 6.89 (1H, d,  $J = 8.0$  Hz, H-8') were assigned. Above data together with the molecular formula indicated **1** as an asymmetrical

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aporphine dimer. The proton singlet at  $\delta_{\text{H}}$  7.08 might be at position of 3 or 3' of one monomeric aporphine unit. The complex proton multiplets at  $\delta_{\text{H}}$  3.20 (2H) and 3.37 (2H) were assigned to be H<sub>2</sub>-5' and H<sub>2</sub>-4', respectively. In addition, three singlets at  $\delta_{\text{H}}$  6.54 (2H, s), 6.35 (1H, s), and 6.33 (1H, s) were due to the two methylenedioxy groups.

<sup>13</sup>C-NMR and HMQC spectra of **1** (Table 1) showed 34 carbon signals including 11 methines, 4 methylenes, and 19 quaternary carbons. Among them, a conjugated carbonyl resonance was found at  $\delta_{\text{C}}$  175.0. The two downfield proton signals at  $\delta_{\text{H}}$  8.24 and 8.61

in <sup>1</sup>H-NMR spectrum might be located near the carbonyl group, which was confirmed by HMBC correlations between H-3 ( $\delta_{\text{H}}$  8.24) and H-5 ( $\delta_{\text{H}}$  8.63) with C-4 ( $\delta_{\text{C}}$  175.0). Furthermore, the <sup>1</sup>H and <sup>13</sup>C-NMR signal assignments were obtained by HMQC and HMBC spectra analysis (Figure 1). The HMQC spectrum showed that C-7 ( $\delta_{\text{C}}$  120.4) and C-7' ( $\delta_{\text{C}}$  100.5) were quaternary carbons, thus the dehydroaporphine dimer was formed through the connection of C-7 and C-7' bonds.

The structural information obtained by 1D and 2D NMR showed a molecular formula of C<sub>34</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>, consistent with 26 degrees

Table 1. <sup>1</sup>H, <sup>13</sup>C NMR, and HMBC spectral data for polynemoraine A (**1**)\*.

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC correlations
1	151.2		
1'	142.3		
1a	115.2		
1a'	118.0		
1b	120.2		
1b'	117.3		
2	148.4		
2'	146.5		
3	109.6	8.24 (1H, s)	C-1, 1b, 2, 4
3'	108.4	7.08 (1H, s)	C-1', 1b', 2', 4'
3a	128.7		
3a'	128.4		
4	175.0		
4'	30.4	3.37 (2H, m)	
5	155.9	8.63 (1H, s)	C-4, 6a
5'	41.0	3.20 (2H, m)	
6a	124.1		
6a'	140.4		
7	120.4		
7'	100.5		
7a	131.2		
7a'	132.2		
8	126.7	7.52 (1H, d, $J = 8.0$ Hz)	C-7, 10, 11a
8'	122.8	6.89 (1H, d, $J = 8.0$ Hz)	C-7', 10', 11a'
9	128.9	7.48 (1H, t, $J = 8.0$ Hz)	C-7a, 10
9'	127.8	7.24 (1H, t, $J = 8.0$ Hz)	C-7a', 8'
10	127.6	7.69 (1H, t, $J = 8.0$ Hz)	C-8, 9, 11a
10'	123.1	7.37 (1H, t, $J = 8.0$ Hz)	C-8', 11', 11a'
11	127.3	9.17 (1H, d, $J = 8.0$ Hz)	C-1a, 7a
11'	127.6	9.08 (1H, d, $J = 8.0$ Hz)	C-1a', 7a', 9', 10'
11a	126.4		
11a'	123.9		
1-OCH <sub>2</sub> O-	103.2	6.54 (2H, s)	C-1, 2
1'-OCH <sub>2</sub> O-	101.3	6.35 (1H, s), 6.33 (1H, s)	C-1', 2'

\*Measured in CDCl<sub>3</sub> at 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C NMR, respectively, with assignments confirmed by HMQC and HMBC spectra.

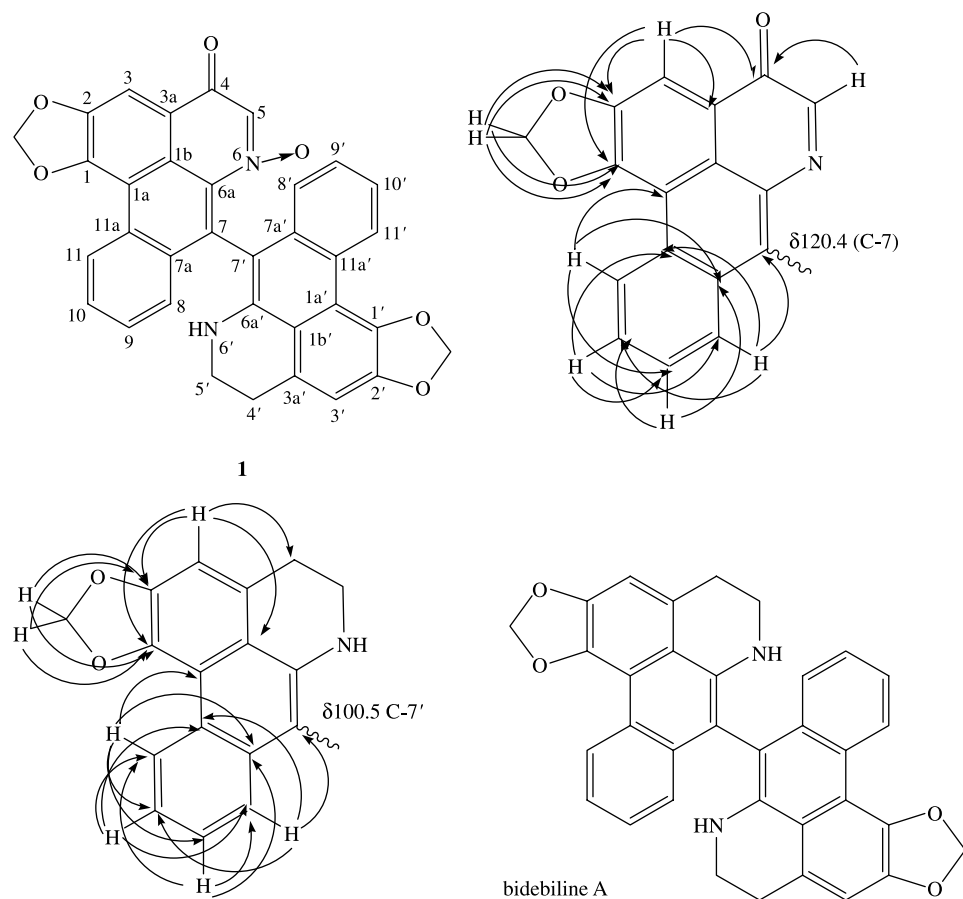


Figure 1. Structures and key HMBC correlations of **1** and bidebiline A.

of unsaturation, which indicated that there was an extra oxygen atom in the molecule. The EI-MS showed two characteristic fragments at  $m/z$  552 ( $M^+$ , 100) and 536 ( $M-16$ , 35), which supported that **1** was an *N*-oxide compound. Comparing the chemical shifts of C-4' ( $\delta_C$  30.4), C-5' ( $\delta_C$  41.0), and C-6a' ( $\delta_C$  140.4) in ring B of **1** with those of C-4 ( $\delta_C$  30.8), C-5 ( $\delta_C$  41.2), and C-6a ( $\delta_C$  140.8) at the same location in bidebiline A [4] indicated that the *N*-oxide was formed in ring B, not in ring B'. Thus, the structure of **1** was deduced as shown in Figure 1. In aporphine group, some compounds were characteristic of two carbonyl groups at C-4 and C-5 of ring B; however, a carbonyl group at C-4 with a double bond at C-5 and C-6

as in **1** had never been reported, and this kind of structure was rare in all synthetic and natural isoquinoline alkaloids.

Polynemoraline B (**2**) was obtained as yellowish amorphous powder from acetone. The positive Dragendorff reaction showed that **2** was an alkaloid and the positive phenylhydrazone reaction indicated that **2** contained a carbonyl group. The molecular formula was determined as  $C_{20}H_{11}N_2O_3$  on the basis of HR-EI-MS at  $m/z$  313.0734 [ $M$ ]<sup>+</sup>, consistent with 16 degrees of unsaturation. The existence of an aldehyde group was proved by EI-MS ions at  $m/z$  313 ( $M^+$ , 100), 284 ( $M-29$ , 10), and NMR spectral data. The IR spectrum showed the absorption bands of conjugated carbonyl and benzene

groups at 1658, 1645  $\text{cm}^{-1}$ . The UV absorption maxima at 208, 226, 264, 273, 295, 302, 403, and 426 nm showed characteristics of an aporphine skeleton.

In the  $^1\text{H}$ NMR spectrum of **2**, a set of 1,2-disubstituted benzene proton signals at  $\delta_{\text{H}}$  8.93 (1H, d,  $J = 8.0$  Hz, H-11), 7.64 (1H, t,  $J = 8.0$  Hz, H-10), 7.35 (1H, t,  $J = 8.0$  Hz, H-9), 8.15 (1H, d,  $J = 8.0$  Hz, H-8) was assigned to ring D. Two doublets ( $J = 7.0$  Hz) at low field were typical signals of H-4 ( $\delta_{\text{H}}$  7.17, d) and H-5 ( $\delta_{\text{H}}$  9.15, d) belonging to the pyridine moiety in oxoaporphine. The singlet at  $\delta_{\text{H}}$  6.35 (2H) represented methylenedioxy group in ring A, while another singlet at  $\delta_{\text{H}}$  7.35 might be H-3 in ring A. The proton of aldehyde group was resonated at  $\delta_{\text{H}}$  9.98 (1H, s), and one singlet of an olefinic proton at  $\delta_{\text{H}}$  7.90 (1H, s) might be deshielded by this aldehyde group. Among the 20 signals shown in the  $^{13}\text{C}$ NMR spectrum (Table 2), the signal at  $\delta_{\text{C}}$  179.5 represented the aldehyde carbon, and those at  $\delta_{\text{C}}$  148.7, 142.9 were assigned to the 1,2-dioxo-substituted benzene carbons, respectively.

Fourteen degrees of unsaturation of the aporphine molecule were calculated from the rings A, B, C, D and the aldehyde group. Based on the molecular formula, the other two degrees of unsaturation of **2** should be formed by a ring containing a double bond. The HMQC and HMBC experiments were carried out to confirm the structure of **2** and give a full assignment of  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts. The HMBC correlations between H-14 and C-13, H-12 and C-6a, 7, 13, 14 indicated that ring E was a five membered ring, and the aldehyde group was attached to C-13. Thus, the structure of **2** was elucidated as shown in Figure 2. It is a new compound named polynemoraline B. Polynemoraline C (**3**) was obtained as colorless cubic crystals from methanol. The positive Dragendorff reaction showed that **3** was an alkaloid. The molecular formula was determined to be  $\text{C}_{15}\text{H}_{13}\text{NO}_4$  from the HR-EI-MS at  $m/z$  271.0843  $[\text{M}]^+$  and EI-MS showed ion peaks at  $m/z$  271 ( $\text{M}^+$ , 100), 256 (M-15, 35) indicating that the compound should contain a methyl group. The IR spectrum showed absorption bands of conjugated carbonyl

Table 2.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, and HMBC spectral data for polynemoraline B (**2**)\*.

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC
1	142.9		
1a	113.6		
1b	116.9		
2	148.7		
3	102.8	7.35 (1H, s)	C-1, 1b, 2, 4
3a	124.1		
4	113.6	7.17 (1H, d, $J = 7.0$ Hz)	C-1b, 3, 5
5	124.6	9.15 (1H, d, $J = 7.0$ Hz)	C-3a, 4, 6a
6a	133.5		
7	114.0		
7a	126.6		
8	123.2	8.15 (1H, d, $J = 8.0$ Hz)	C-7, 7a, 9
9	125.4	7.35 (1H, t, $J = 8.0$ Hz)	C-7a, 8
10	128.0	7.64 (1H, t, $J = 8.0$ Hz)	C-11, 11a
11	128.6	8.93 (1H, d, $J = 8.0$ Hz)	C-1a, 10, 11a
11a	129.2		
12	116.8	7.90 (1H, s)	C-6a, 7, 13, 14
13	127.0		
14	179.5	9.98 (1H, s)	C-13
1-OCH <sub>2</sub> O-	101.7	6.35 (2H, s)	C-1, 2

\*Measured in  $\text{CDCl}_3$  at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$  NMR, respectively, with assignments confirmed by HMQC and HMBC spectra.

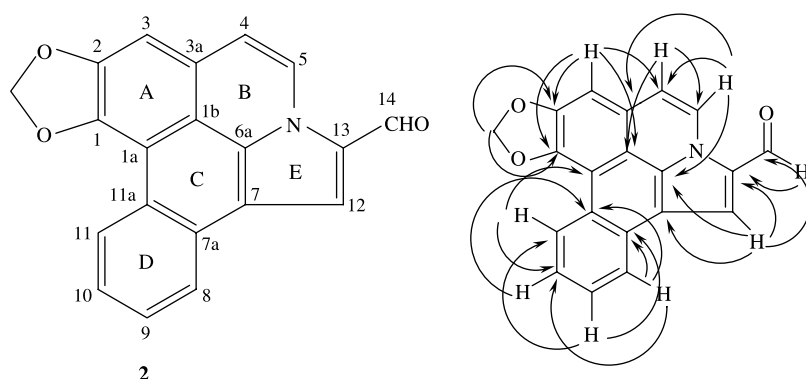


Figure 2. Structure and key HMBC correlations of **2**.

group at  $1718\text{ cm}^{-1}$  and aryl group at  $1618$ ,  $1574$ , and  $1510\text{ cm}^{-1}$ . The UV spectrum of **3** exhibited the presence of a condensed aromatic chromophore absorptions at  $206$ ,  $214$ ,  $237$ ,  $278$ , and  $322\text{ nm}$ .

$^1\text{H-NMR}$  spectrum of **3** exhibited four aryl protons at  $\delta_{\text{H}}$   $8.75$  (1H, d,  $J = 4.8\text{ Hz}$ ),  $7.23$  (1H, d,  $J = 4.8\text{ Hz}$ ),  $7.99$  (1H, s),  $6.85$  (1H, s); among them, the protons at  $\delta_{\text{H}}$   $8.75$  and  $7.23$  were two characteristic protons at ( $\alpha$  and  $\beta$ ) positions of pyridine moiety, the protons at  $\delta_{\text{H}}$   $4.03$  (3H, s),  $3.97$  (3H, s) were two methoxy groups of the condensed-nuclei aromatics. The other signal at  $\delta_{\text{H}}$   $2.87$  (3H, s) might be the methyl group at C-4 position of pyridine.

The  $^{13}\text{C-NMR}$  (Table 3) spectrum showed 15 carbon signals including one ester carbonyl group at  $\delta_{\text{C}}$   $160.7$ , two methoxy groups at  $\delta_{\text{C}}$   $56.4$ ,  $56.3$ , and one methyl group at  $\delta_{\text{C}}$   $23.0$ . The remaining 11 carbon resonances were aromatic carbon signals at  $\delta_{\text{C}}$   $153.7$ ,  $153.6$ ,  $153.0$ ,  $152.8$ ,  $147.8$ ,  $146.7$ ,  $125.7$ ,  $115.4$ ,  $110.5$ ,  $105.4$ ,  $99.7$ , among which the former six signals would be carbons connected with oxygen or nitrogen atoms.

The structure of **3** (Figure 3) was confirmed as 8,9-dimethoxy-4-methyl-5H-chromeno[4,3-b]-pyridine-5-one by X-ray crystallographic analysis (Figure 4). The crystal belonged to space  $P_1$ , the unit cells parameter were

Table 3.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, and HMBC spectral data for polynemoraline C (**3**)\*.

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC
1a	152.8		
2	153.6	$8.75$ (1H, d, $J = 4.8\text{ Hz}$ )	C-3, 4
3	125.7	$7.23$ (1H, d, $J = 4.8\text{ Hz}$ )	C-4, 4- $\text{CH}_3$
4	153.7		
4a	115.4		
5	160.7		
6a	147.8		
7	99.7	$6.85$ (1H, s)	C-6a, 8, 9, 10a
8	153.0		
9	146.7		
10	105.4	$7.99$ (1H, s)	C-8, 9, 10a
10a	110.5		
4- $\text{CH}_3$	23.0	$2.87$ (3H, s)	C-3, 4, 4a, 5
8- $\text{OCH}_3$	56.3	$3.97$ (3H, s)	C-8
9- $\text{OCH}_3$	56.4	$4.03$ (3H, s)	C-9

\*Measured in  $\text{CDCl}_3$  at  $500\text{ MHz}$  for  $^1\text{H}$  and  $125\text{ MHz}$  for  $^{13}\text{C}$  NMR, respectively, with assignments confirmed by HMQC and HMBC spectra.

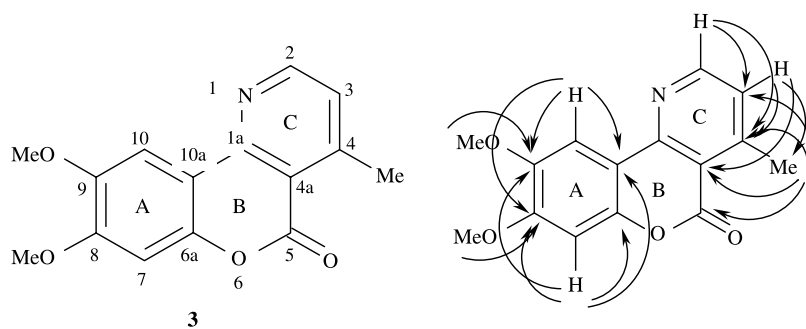


Figure 3. Structure and key HMBC correlations of **3**.

as follows:  $a = 6.971(1)$ ,  $b = 8.348(1)$ ,  $c = 11.535(1)\text{\AA}$ ,  $\alpha = 108.07(1)$ ,  $\beta = 91.54(1)$ ,  $\gamma = 100.02(1)^\circ$ ,  $V = 626.08(14)\text{\AA}^3$ ,  $Z = 2$ . Data collection was performed on a MAC DIP-2030K diffractometer with graphite monochromated Mo  $K\alpha$ . Total of 1900 unique reflections were collected. The structure was resolved by direct methods (Shelxs 97), and  $R$  and  $R_w$  values were 0.0831 and 0.2328 and 1684 for observed reflections ( $|F|^2 \geq 2\sigma|F|^2$ ).

On the basis of X-ray crystallographic analysis, and HMQC and HMBC experiments, the  $^{13}\text{C}$  and  $^1\text{H}$  signals were assigned

as shown in Table 3. Thus, **3** was determined as a novel coumarin-type alkaloid named polynemoriline C.

Polynemoriline D (**4**) was obtained as white needles from acetone. The positive Dragendorff reactions showed that **4** was an alkaloid. And the positive  $\text{FeCl}_3\text{-K}_3\text{Fe}(\text{CN})_6$  reaction suggested the presence of phenolic hydroxyl group. The molecular formula was established as  $\text{C}_{16}\text{H}_{13}\text{NO}_3$  by HR-EI-MS at  $m/z$  267.0915  $[\text{M}]^+$ . The IR spectrum showed absorption bands of hydroxy groups at  $3002\text{ cm}^{-1}$  and benzene moiety at  $1624$ ,

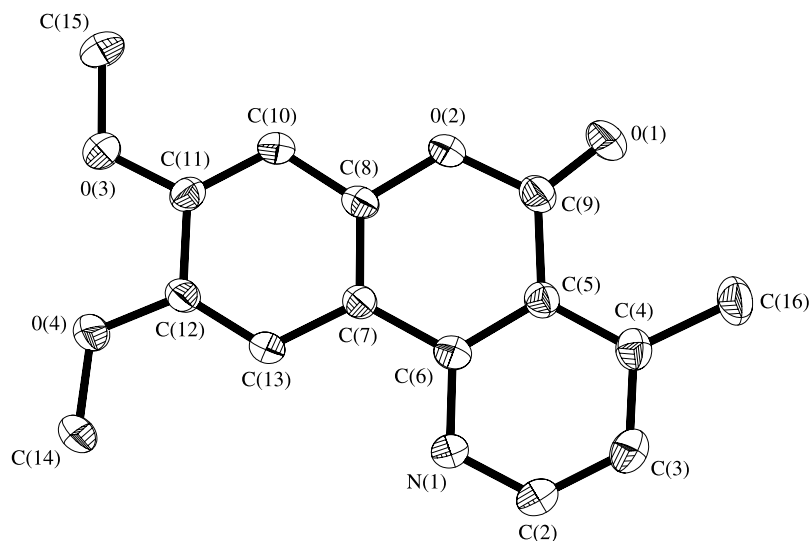


Figure 4. The X-ray crystallography structure of **3**.



1581, and 1529  $\text{cm}^{-1}$ . The UV spectrum of **4** exhibited absorption maxima at 202, 236, 282 nm, characteristic of a condensed aromatic chromophore in the basic structure.

$^1\text{H}$ -NMR spectrum of **4** exhibited one singlet and two doublets at  $\delta_{\text{H}}$  8.37 (1H, s), 8.23 (1H, d,  $J = 5.4$  Hz), 7.57 (1H, d,  $J = 5.4$  Hz) characteristic for protons of pyridine moiety. Four aryl proton signals at  $\delta_{\text{H}}$  7.29 (1H, dt,  $J = 1.8, 7.5$  Hz), 7.11 (1H, dd,  $J = 1.8, 7.5$  Hz), 7.00 (1H, dd,  $J = 1.8, 7.5$  Hz), 6.93 (1H, dt,  $J = 1.8, 7.5$  Hz) were due to the 1,2-disubstituted benzene. Two broad downfield singlets at  $\delta_{\text{H}}$  10.45 (1H, brs), 9.39 (1H, brs) suggested **4** as a phenol compound. The remaining signals were presenting an aryl proton at  $\delta_{\text{H}}$  7.22 (1H, s) and methoxyl protons at  $\delta_{\text{H}}$  3.62 (3H, s).

$^{13}\text{C}$ -NMR and HMQC spectra of **4** (Table 4) exhibited 15 signals including a methoxyl group at  $\delta_{\text{C}}$  59.9 and 14 aryl carbons. Among them, five signals at  $\delta_{\text{C}}$  155.2, 154.0, 149.2, 147.1, 141.6 might be carbons attached to O or N. The HMQC and HMBC experiments confirmed the skeleton and

substituted pattern in the molecule of **4** and provided the  $^1\text{H}$  and  $^{13}\text{C}$  NMR full assignments as shown in Table 4.

The connection of rings B and C between C-8 and C-1' could not be settled down from the HMBC experiment since the overlapping of cross-peaks between H-6' at  $\delta_{\text{H}}$  7.11 and two carbons at  $\delta_{\text{C}}$  129.2 (C-5') and 129.1 (C-8). In NOE spectrum, the signal at  $\delta_{\text{H}}$  7.11 (H-6') was enhanced by irradiating the proton at  $\delta_{\text{H}}$  3.62 (7-OCH<sub>3</sub>), while the signals at  $\delta_{\text{H}}$  3.62 and  $\delta_{\text{H}}$  8.37 (H-1) were enhanced by irradiating the proton at  $\delta_{\text{H}}$  7.11 (H-6'), thus the C-8–C-1' connection between rings B and C was confirmed. The observation of NOE between H-5 ( $\delta_{\text{H}}$  7.22) and H-4 ( $\delta_{\text{H}}$  7.57), but not between H-5 and OCH<sub>3</sub> ( $\delta_{\text{H}}$  3.62), suggested that the methoxyl group should be located at C-7, not at C-6. In addition, enhancement of signals at  $\delta_{\text{H}}$  6.93 (H-4') and 9.39 (2'-OH) by irradiation of the proton at  $\delta_{\text{H}}$  7.00 (H-3') confirmed the positions of 2'-OH. Finally, the structure of **4** was elucidated as shown in Figure 5. Generally in the reported isoquinoline alkaloid, the aryl group was mostly located at C-1 of ring A, not at C-8 as in **4**.

Table 4.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, and HMBC spectral data for polynemoraline D (**4**)\*.

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC
1	149.2	8.37 (1H, s)	C-1a, 3, 4a, 8
1a	122.8		
3	141.6	8.23 (1H, d, $J = 5.4$ Hz)	C-1, 4
4	118.6	7.57 (1H, d, $J = 5.4$ Hz)	C-1a, 3, 4a, 5
4a	133.9		
5	108.2	7.22 (1H, s)	C-1a, 4, 4a, 6, 7
6	154.0		
7	147.1		
8	129.1		
1'	121.1		
2'	155.2		
3'	115.5	7.00 (1H, dd, $J = 1.8, 7.5$ Hz)	C-1', 2', 4', 5'
4'	118.8	6.93 (1H, dt, $J = 1.8, 7.5$ Hz)	C-2', 3', 6'
5'	129.2	7.29 (1H, dt, $J = 1.8, 7.5$ Hz)	C-2', 6'
6'	131.6	7.11 (1H, dd, $J = 1.8, 7.5$ Hz)	C-1', 2', 4', 5'
6-OH		10.45 (1H, brs)	
7-OCH <sub>3</sub>	59.9	3.62 (3H, s)	C-7
2'-OH		9.39 (1H, brs)	C-1', 2'

\*Measured in DMSO-*d*<sub>6</sub> at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$  NMR, respectively, with assignments confirmed by HMQC, HMBC, and NOE spectra.



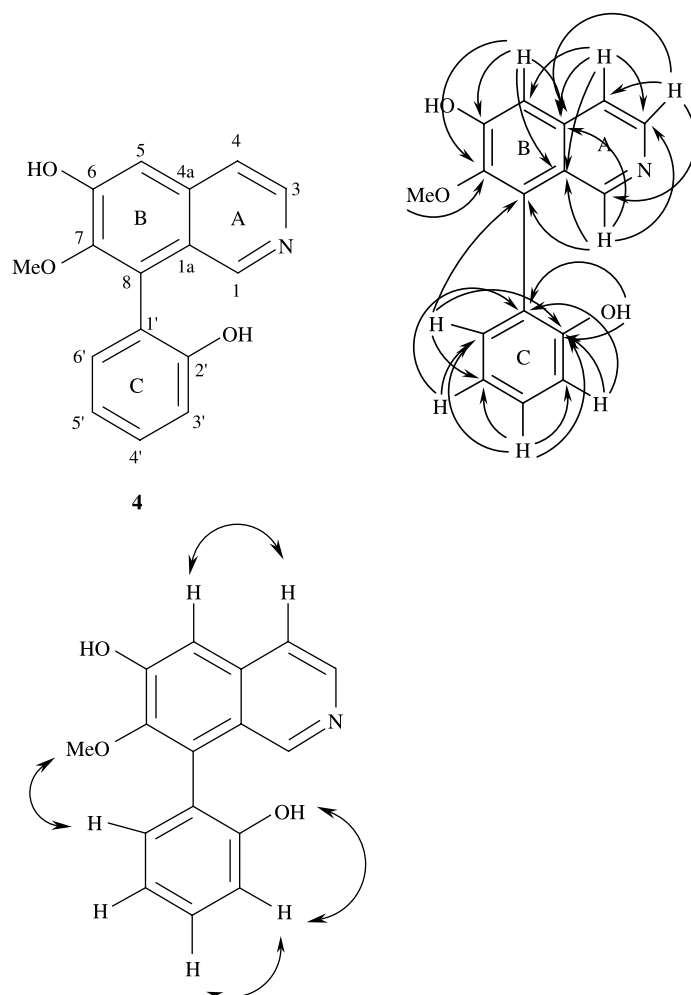


Figure 5. Structure, key HMBC, and NOE correlations of **4**.

### 3. Experimental

#### 3.1 General experimental procedures

Melting points were determined on a XT4 100 × micro melting point apparatus and are uncorrected. Optical rotations were obtained on a PE Model 343 polarimeter. IR spectra were recorded on a Nicolet IMPACT-400 spectrophotometer as KBr disks. UV spectra were recorded on a Hitachi UV-240 and Shimadzu UV-241 spectrophotometer. Mass spectra were obtained on the VGZAB-2F(EI-MS), ZAB-spec(FAB-MS), LC/MSD Trap-SL(ESI-MS) instruments.  $^1\text{H-NMR}$  (500 MHz) and  $^{13}\text{C-NMR}$  (125 MHz) spectra were recorded with

Varian Inova-500 spectrophotometer. The single crystal X-ray diffraction analysis was carried out on an R3m/E four circle diffractometer.

#### 3.2 Plant material

The branches and leaves of *P. nemoralis* A DC. (15 kg) were collected from Hainan province in March, 2004, and identified by Professor Wanzhi Song. A voucher specimen (ID-3764) is deposited in the Herbarium of the Institute of Materia Medica, the Chinese Academy of Medical Sciences, and the Peking Union Medical College.

### 3.3 Extraction and isolation

The dried plant materials (15 kg) were powdered and refluxed with 95% EtOH thrice under reflux to give 1014 g of extract, which was suspended in water and partitioned successively with petroleum ether (227 g), CHCl<sub>3</sub>, EtOAc, and *n*-BuOH (202 g). On the basis of TLC analysis, the CHCl<sub>3</sub> and EtOAc fractions were combined (198 g) and subjected to column chromatography over silica gel eluted with a CHCl<sub>3</sub>-CH<sub>3</sub>OH mixture with increasing polarity. Combination of similar fractions on the basis of TLC analysis afforded 14 fractions. The pure compounds were obtained either by direct crystallization or after further purification on column chromatography. Fraction 2 (18.3 g) was subjected to repeated silica gel column chromatography using a CHCl<sub>3</sub>-CH<sub>3</sub>OH mixture of increasing polarity (100:1-50:1) and Sephadex LH-20 column chromatography eluted with CHCl<sub>3</sub>-petroleum ether-CH<sub>3</sub>OH (5:5:1), which resulted in the isolation of the new compound **1**. Fraction 3 (2.3 g) was subjected to repeated silica gel column chromatography using a CHCl<sub>3</sub>-CH<sub>3</sub>OH mixture of increasing polarity (50:1-30:1) and Sephadex LH-20 column chromatography eluted with CHCl<sub>3</sub>-petroleum ether-CH<sub>3</sub>OH (5:5:1) to give two new compounds **2** and **3**. Fraction 7 (10.5 g) was subjected to repeated silica gel column chromatography using a CHCl<sub>3</sub>-CH<sub>3</sub>OH mixture (20:1) and Sephadex LH-20 column chromatography eluted with CH<sub>3</sub>OH affording the new compound **4**.

#### 3.3.1 Polynemoraline A (1)

Yellowish amorphous powder from acetone. mp > 260 (decomposed).  $[\alpha]_D^{25} = 0$  (*c* 0.25, CHCl<sub>3</sub>). Dragendorff reaction positive. UV  $\lambda_{\max}$  (MeOH): 219, 243, 261, 304, 317, 337, 396, 440 nm. IR (KBr)  $\nu_{\max}$ : 3342, 2992, 2846, 1697, 1655, 1587 cm<sup>-1</sup>. <sup>1</sup>H and <sup>13</sup>C NMR spectral data see Table 1. EI-MS *m/z* (%): 552 (M<sup>+</sup>, 100), 536 (M-16, 35). HR-EI-MS *m/z*

552.1295 [M]<sup>+</sup> (calcd for C<sub>34</sub>H<sub>20</sub>N<sub>2</sub>O<sub>6</sub>, 552.1321).

#### 3.3.2 Polynemoraline B (2)

Yellowish amorphous powder (acetone). mp > 210 (decomposed). Dragendorff and phenylhydrazone reactions positive. UV  $\lambda_{\max}$  (MeOH): 208, 226, 264, 273, 295, 302, 403, 426 nm. IR (KBr)  $\nu_{\max}$ : 1658, 1645 cm<sup>-1</sup>. <sup>1</sup>H and <sup>13</sup>C NMR spectral data see Table 2. HR-EI-MS *m/z* 313.0734 [M]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>11</sub>NO<sub>3</sub>, 313.0739). EI-MS *m/z* (%): 313 (M<sup>+</sup>, 100), 284 (M-29, 10).

#### 3.3.3 Polynemoraline C (3)

Colorless cubic crystals from methane, mp 162-163. UV  $\lambda_{\max}$  (MeOH): 206, 214, 237, 278, 322 nm. IR (KBr)  $\nu_{\max}$ : 1718, 1618, 1574, 1510 cm<sup>-1</sup>. <sup>1</sup>H and <sup>13</sup>C NMR spectral data see Table 3. HR-EI-MS *m/z* 271.0843 [M]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>13</sub>NO<sub>4</sub>, 271.0840). EI-MS *m/z* (%): 271 (M<sup>+</sup>, 100), 256 (M-15, 35).

#### 3.3.4 Polynemoraline D (4)

White needles from acetone, mp 167-168. Dragendorff and FeCl<sub>3</sub>-K<sub>3</sub>Fe(CN)<sub>6</sub> reactions positive. UV  $\lambda_{\max}$  (MeOH): 202, 236, 282 nm. IR (KBr)  $\nu_{\max}$ : 3002, 1624, 1581, 1529 cm<sup>-1</sup>. <sup>1</sup>H and <sup>13</sup>C NMR spectral data see Table 4. HR-EI-MS *m/z* 267.0915 [M]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>13</sub>NO<sub>3</sub>, 267.0895).

## References

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