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ISOLATION AND STRUCTURE ELUCIDATION OF ALKALOIDS FROM *PINELLIA TERNATA*

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Abstract – A new compound, 3-(6,7-dimethoxyisoquinolin-1-yl)-4,7-dimethoxy-3-methylisobenzofuran-1(3*H*)-one (**1**) named alkterlactone was isolated from *Pinellia ternata*, together with four known compounds *N*-*trans*-feruloyloctopamine (**2**), 2'-*O*-methyladenosine (**3**), 5'-*S*-methyl-5'-thioadenosine (**4**) and 2'-deoxy-thymidine (**5**). Compounds **2–5** were isolated for the first time from *Pinellia ternata*. The structures of these compounds were elucidated and characterized on the basis of 1D NMR, 2D NMR and MS data.

Pinellia ternata (Thunb.) Berit. called *banxia* in China is a classic traditional Chinese medicine widely distributed in Sichuan, Guizhou and Anhui Provinces of China. Its rhizome is used in clinic for antiemetic, antitussive, sedative and anti-inflammatory purposes.[1,2] However, crude *banxia* may cause side effects such as tongue numbing, tongue swelling, salivation, slurred speech and hoarseness. Unfortunately, the toxic ingredients have not yet been identified unambiguously. So processed products of *banxia* are always used in clinic, especially *Rhizoma Pinelliae Preparata* (*qingbanxia*) and *Rhizoma Pinelliae Preparatum* (*fabanxia*), both of which are recorded in Chinese Pharmacopoeia (2010 Edition). *Qingbanxia* is the product of raw *banxia* processed with alum and *fabanxia* is the product of raw *banxia* processed with lime solution and *Glycyrrhiza uralensis*.

Previously, phytochemicals from this plant that have been characterized include alkaloids,[3,4] volatile oils,[5] polysaccharides,[6] amino acids,[7] triterpenes, sterols, fatty acids,[8] cerebrosides,[9] and proteins.[10] Here, We report the isolation and characterization of a new alkaloid, 3-(6,7-dimethoxyisoquinolin-1-yl)-4,7-dimethoxy-3-methylisobenzofuran-1(3*H*)-one (**1**) and four known alkaloids (**Figure 1**)

N-trans-feruloyloctopamine (**2**), 22-*O*-methyladenosine (**3**), 5'-*S*-methyl-5'-thioadenosine (**4**) and 2'-deoxythymidine (**5**), which were isolated for the first time from *banxia*.

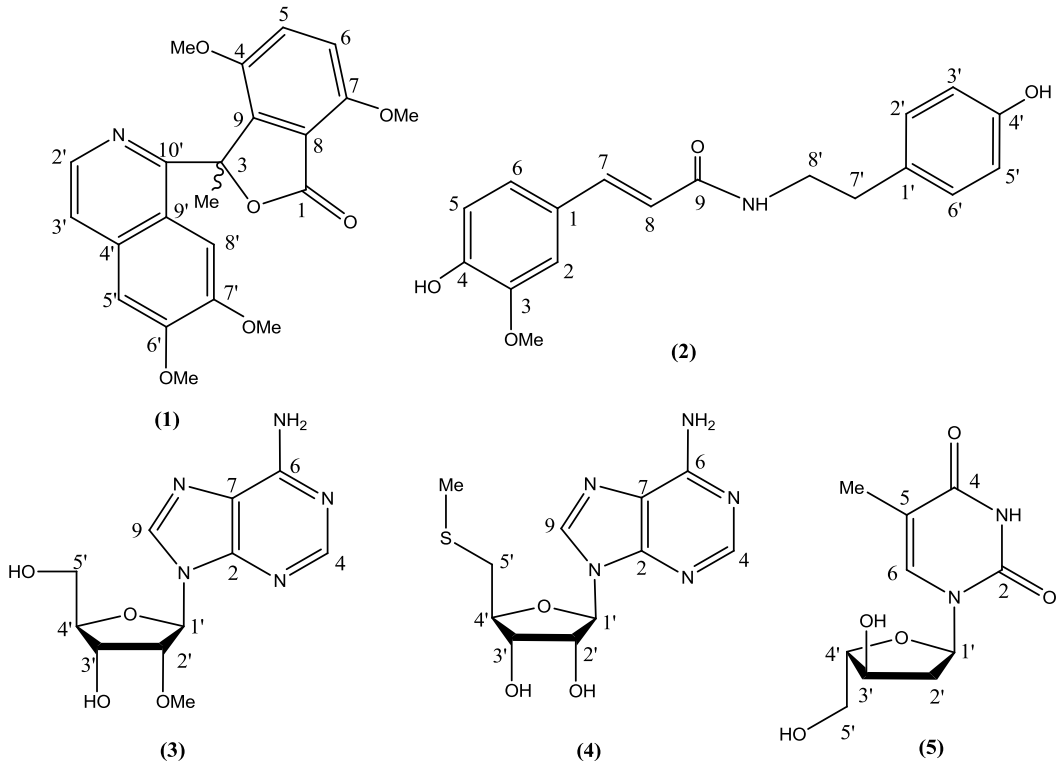


Figure 1. Structures of compounds **1-5**.

The compounds **1-5** were isolated using silica gel, Sephadex LH-20 gel column chromatography and preparative liquid chromatography from a 95% EtOH extract of *banxia*. The structures of compounds were characterized by examination of their HR ESI-MS, NMR (1D and 2D) data and comparison with literature reports.

Compound **1** was obtained as a colorless solid; $[\alpha]_D^{25}$ -0.7° (c 0.003, CH_3OH). UV (CH_3OH) λ_{max} (nm) (log ε): 239, 315, 326 (4.4, 3.5, 3.5). Its molecular formula was assigned as $C_{22}H_{21}NO_6$, suggesting thirteen degrees of unsaturation, on the basis of the $[M+H]^+$ ion peak at *m/z* 396.1424 (calcd. for $C_{22}H_{21}NO_6$, 396.1447) in the HR-ESI-MS. ^{1}H-NMR (CD_3OD, 500 MHz) showed six downfield proton signals, including an AB-pattern for two heteroaromatic protons at δ 8.23 (1H, d, 5.5 Hz) and 7.56 (1H, d, 5.5 Hz), and another AB-pattern for two aromatic protons at δ 7.37 (1H, d, 8.0 Hz), δ 7.27 (1H, d, 8.5 Hz), and two aromatic protons at δ 7.46 (1H, s), δ 7.21 (1H, s). Fifteen highfield proton signals, including five singlet protons at δ 3.99 (3H, s, OCH_3), δ 3.90 (3H, s, OCH_3), δ 3.86 (3H, s, OCH_3), δ 3.78 (3H, s, OCH_3), δ 2.09 (3H, s, CH_3) were also observed. The ^{13}C-NMR data (**Table 1**), DEPT and HSQC spectra of compound **1** allowed the assignment of 22 carbon signals to six tertiary, 11 quaternary carbons, four methoxy and one methyl.

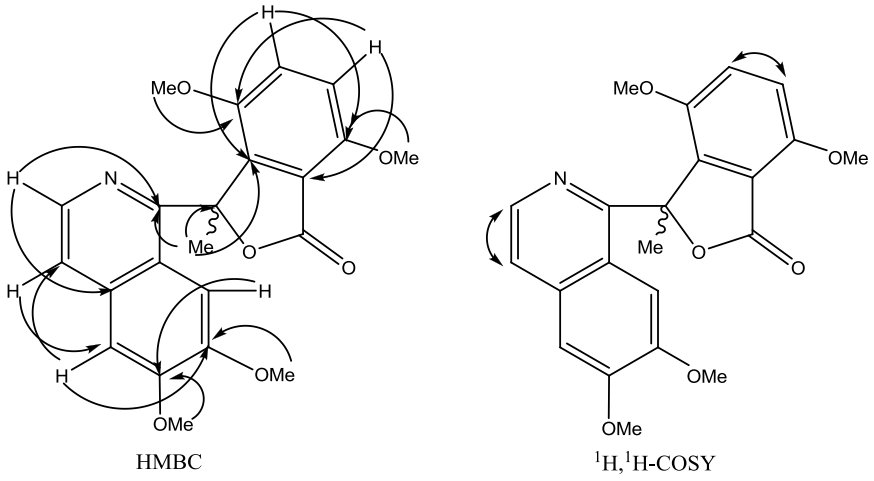
Table 1. NMR data of compound **1** (CD_3OD, 500 MHz, 100 MHz)

Position	δ_C	δ_H
1	169.9	
3	91.4	
4	154.4	
5	121.7	7.37 (1H, d, 8.0 Hz)
6	121.1	7.27 (1H, d, 8.5 Hz)
7	149.2	
8	118.6	
9	148.9	
2′	140.8	8.23 (1H, d, 5.5 Hz)
3′	122.3	7.56 (1H, d, 5.5 Hz)
4′	136.7	
5′	107.2	7.21 (1H, s)
6′	151.6	
7′	154.2	
8′	105.6	7.46 (1H, s)
9′	123.2	
10′	155.7	
4- OMe	57.6	3.86 (3H, s)
7- OMe	62.8	3.99 (3H, s)
6′-OMe	56.5	3.78 (3H, s)
7′-OMe	56.7	3.90 (3H, s)
3-Me	29.7	2.09 (3H, s)

The carbon skeleton of compound **1** is as same as the carbon skeleton of 9-methyldecumbenine C which has been reported in literature.[11] The ^{1}H-NMR data was similar to that of 1-[1'-(6',7'-dimethoxyphtha1ide)]-6,7-dimethoxyisoquinoline synthesized by V. Smul.[12] The major difference in compound **1** was the two methoxy groups at C-4 and C-7 in contrast to methoxy groups at C-6' and C-7' in that compound. What's more, H-3 was replaced by methyl in compound **1**. These differences were confirmed by HMBC spectrum and ^{1}H, ^{1}H-COSY spectrum (**Figure 2**).

In the HMBC spectrum, the observation of diagnostic correlations from methyl protons to C-9, C-10' and C-3, allowed the methyl group to be attached to C-3. Four methoxy groups (methoxy protons at δ_H = 3.78, 3.90, 3.86, 3.99) were assigned to C-6', C-7', C-4 and C-7, respectively, based on the correlations of H (δ 3.78, 3H, s) with C-6', H (δ 3.90, 3H, s) with C-7', H (δ 3.86, 3H, s) with C-4 and H (δ 3.99, 3H, s) with C-7. The attribution of the carbons and hydrogen were further established by the HMBC spectrum. These data pooled together would suggest compound **1** to be 3-(6,7-dimethoxyisoquinolin-1-yl)-4,7-dimethoxy-3-methylisobenzofuran-1(3*H*)-one.

Figure 2. Key HMBC and ^{1}H, ^{1}H-COSY correlations of compound **1**.



C-3 is a chiral carbon. Since the specific rotation values of compound **1** was -0.7° (*c* 0.003, CH_3OH), which is close to zero and CD spectrum indicated no obvious cotton effects. We deduced the compound **1** to be racemates. These data established the structure of compound **1** as 3-(6,7-dimethoxyisoquinolin-1-yl)-4,7-dimethoxy-3-methylisobenzofuran-1(3*H*)-one. The novel natural product is tentatively named alkterlactone. Further work on the biological activities of compounds **1-5** and structural determination of other interesting alkaloids are in progress.

EXPERIMENTAL

General. Agilent 6320 Ion TRAP LC/MS and Waters Xevo^{TM} UPLC-QTof were employed for MS analysis. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker Avance DRX 500 instrument or Varian Unity VNS 600 using DMSO-d_6 or CD_3OD as solvent, with TMS as internal standard. The specific rotation was measured on AUTOPOL IV Automatic Polarimeter (Rudolph, Hackettstown, NJ, USA). UV spectra were recorded on an Agilent 8453 UV/Vis Spectrophotometer (Agilent, Santa Clara, CA, USA). CD spectra were taken on a JASCO J-815 Spectropolarimeter (JASCO, Tokyo, Japan) using a 0.1 cm standard cell and spectrophotometric-grade MeOH. IR spectra were taken on a Nicolet 5700 FTIR Spectrometer (Thermo, Waltham, MA, USA). Compounds were purified by Dalian Elite P230p preparative high performance liquid chromatography [(Dalian, Liaoning Province, China), Phenomenex Luna C_{18} columns (250 mm × 4.6 mm and 250 mm × 21.2 mm i.d.)]. Sephadex LH-20 was purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden). AB-8 resin (20–60 mesh) was acquired from Nankai University (Tianjin, China). Silica gel (160–200 mesh, 200–300 mesh) for column chromatography was purchased from Qingdao Marine Chemical Plant (Qingdao, Shandong Province, China). All other chemicals were of analytical reagent grade and used without any further purification.

Plant Material. Crude roots of *banxia* were collected from Hezhang County, Guizhou Province, China, in June 2011. The species was identified by Professor Zhang. J. (National Institutes for Food and Drug Control, NIFDC for short). The voucher specimens were deposited at the herbarium of NIFDC. The roots were air-dried and ground to a powder using a grinding mill (Tianjin, China).

Extraction and isolation. The powder (30 kg) was extracted three times with hot 95% EtOH (12 L), for 2 h each time. The extract was concentrated to afford a syrup (0.8 kg), which was dissolved in 95% EtOH (1 L). The solution was chromatographed over a AB-8 resin (20–60 mesh) column (100 × 12 cm i.d.) eluting with a gradient of EtOH-H_2O (0:100, 30:70, 60:40, 90:10) to give four fractions. The 90% EtOH eluent and 60% EtOH eluent were combined and were concentrated under vacuum to obtain the extract (132 g). The extract was chromatographed over a silica gel (160–200 mesh) column (100 × 12 cm i.d.) with petroleum ether first and then CH_2Cl_2/MeOH (100% CH_2Cl_2, CH_2Cl_2:MeOH = 100:1, CH_2Cl_2:MeOH = 80:1, … 100% MeOH) to afford 10 fractions (M01–M10). Fraction M02 (19.0 g) was chromatographed over a silica gel (200–300 mesh) column (100 × 6.0 cm i.d.) with CH_2Cl_2/MeOH (100% CH_2Cl_2 to 100% MeOH) to afford 105 fractions. Fractions 28-46 (1.2 g) were chromatographed over a silica gel (200–300 mesh) column (45 × 2.5 cm i.d.) with CH_2Cl_2/MeOH (50:1 to 10:1) to afford 11 fractions. Fractions 4-8 (0.54 g) were subjected to Sephadex LH-20 column chromatography (100 × 2.5 cm i.d.) with MeOH to afford 25 fractions. Fractions 7-10 (260 mg) were subjected to Sephadex LH-20 column chromatography (100 × 2.0 cm i.d.) with MeOH to afford 28 fractions. The fraction 12 was purified by preparative HPLC to yield compound **1** (3.3 mg) using MeOH-H_2O (70:30) as mobile phase at a flow rate of 10 mL/min. Fraction M04 (0.53 g) was subjected to Sephadex LH-20 column chromatography (100 × 2.5 cm i.d.) with MeOH to afford 26 fractions. The fractions 14-15 were purified by preparative HPLC to yield compound **2** (6.7 mg) using MeOH-H_2O (55:45) as mobile phase at a flow rate of 10 mL/min. The 30% EtOH eluent was concentrated under vacuum to obtain the extract (178 g). The extract was chromatographed over a silica gel (160–200 mesh) column (100 × 12 cm i.d.) with CH_2Cl_2/MeOH (100% CH_2Cl_2, CH_2Cl_2:MeOH = 100:1, CH_2Cl_2:MeOH = 50:1, … 100% MeOH) to afford 10 fractions (N01-N10). Fraction N04 (5.6 g) was chromatographed over a silica gel (200–300 mesh) column (45 × 4.0 cm i.d.) with CH_2Cl_2/MeOH (100% $CHCl_2$ to 100% MeOH) to afford 33 fractions. Fractions 19-23 (820 mg) were subjected to Sephadex LH-20 column chromatography (100 × 2.5 cm i.d.) with MeOH to afford 22 fractions. Fractions 21-22 were compound **4** (13.9 mg). Fractions 18-20 were purified by preparative HPLC to yield compound **3** (4.0 mg) using MeOH-H_2O (25:75) as mobile phase at a flow rate of 10 mL/min. Fractions 12-14 were purified by preparative HPLC to yield compound **5** (4.0 mg) using MeOH-H_2O (10:90) as mobile phase at a flow rate of 10 mL/min.

(±)-Alkterlactone (**1**): Colorless solid. $[\alpha]_D^{25}$ −0.7° (*c* 0.003, CH_3OH). UV (CH_3OH) λ_{max} (nm) (log ε):

239, 315, 326 (4.4, 3.5, 3.5). HR-ESI-MS: *m/z* 396.1424 $[M+H]^+$ (calcd. for $C_{22}H_{21}NO_6$, 396.1447). The ^{1}H- and ^{13}C-NMR spectral data are listed in **Table 1**.

***N-trans*-Feruloyloctopamine** (**2**): Colorless oil. ESI-MS: *m/z* 314 $[M+H]^+$. $C_{18}H_{19}NO_4$. The ^{1}H- and ^{13}C-NMR spectral data are consistent with published data.[13,14]

2′-*O*-Methyladenosine (**3**): Colorless solid. ESI-MS: *m/z* 282 $[M+H]^+$. $C_{11}H_{15}N_5O_4$. The ^{1}H- and ^{13}C-NMR spectral data are consistent with published data.[15]

5'-*S*-Methyl-5'-thioadenosine (**4**): Colorless oil. ESI-MS: *m/z* 298 $[M+H]^+$. $C_{11}H_{15}N_5O_3S$. ^{13}C-NMR (DMSO, 100 MHz): δ 156.1 (C-6), 152.7 (C-2), 149.6 (C-4), 139.9 (C-9), 119.2 (C-7), 87.4 (C-1′), 83.8 (C-4′), 72.7 (C-2′), 72.7 (C-3′), 36.1 (C-5′), 15.6 (-CH_3). The ^{1}H-NMR spectral data are consistent with the published data.[16,17] The ^{13}C-NMR spectral data has not been reported previously.

2'-Deoxy-thymidine (**5**): Colorless solid. ESI-MS: *m/z* 243 $[M+H]^+$. $C_{10}H_{15}N_2O_5$. The ^{1}H- and ^{13}C-NMR spectral data are consistent with published data.[18]

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