Chemical Constituents from the Fermented Mycelia of the Medicinal Fungus *Xylaria nigripes*

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Nineteen compounds mainly including pyrrole-containing alkaloids and phytosterols were isolated from the EtOH extract of the fermented mycelia of *Xylaria nigripes*, a precious medicinal fungus known as Wuling Shen in Chinese. On the basis of spectroscopic and chemical means, the structures of the newly occurring compounds were determined to be (4S)-3,4-dihydro-4-(4-hydroxybiphenyl)-3-oxo-1H-pyrrolo[2,1-c][1,4]oxazine-6-carbaldehyde (1), methyl (2S)-2-[2-formyl-5-(hydroxymethyl)-1H-pyrrolo[1-yl]-3-(4-hydroxyphenyl)propanoate (2), and 3-[4-[(2R)-(2,3-dihydroxy-3-methylbutoxy)phenyl]-7-hydroxy-4H-chromen-4-one (3), respectively. The absolute configurations of 1 and 2 were deduced by the observed Cotton effects in their circular dichroism (CD) spectra, whereas that of the 1,2-diol moiety in 3 was determined using the Snatzke’s method. Their biological activities such as neuroprotective, anti-neuroinflammatory, and cytotoxic properties were also reported.

**Introduction.** – *Xylaria nigripes* (KOLTZ.) SACC. (family Xylariaceae), under the folklore name of Wuling Shen in Chinese, is a precious medicinal fungus used in traditional Chinese medicine (TCM) as a nerve tonic for insomnia and depression [1]. The wild fungus is hard to acquire since it is usually growing around the abandoned termite (*Odontotermes formosanus*) nests [2]. Nevertheless, the mycelia of *X. nigripes* can be now largely manufactured through fermentation. An on-shelf drug, under the trade name of Wuling Capsule, is a single herbal formula made from the commercially available fermented product (Wuling Powder) [3]. Wuling Capsule was approved in 1999 by the China Food and Drug Administration (CFDA) as a First Grade New TCM, and has been used in clinic for more than 15 years, exerting significant benefits for patients with insomnia, anxiety, and depression [4]. However, studies on the secondary metabolites and their biological properties of this fungus remain limited. Chemically, only a few ubiquitous constituents, including sterols [5][6], chromones [6], isoflavones [7], isocoumarins [7][8], and cerebrosides [5], were previously reported from this fungus. In our preceding work on the EtOH extract of the fermented mycelia of *X. nigripes*, four rare spirotetal pyrrole-derived alkaloids (i.e., xylapyrrosides) have been isolated as the minor components [9]. Further investigation on this extract resulted in the isolation of three new (1–3) and sixteen known (4–19) naturally occurring compounds. Reported herein are their isolation and structure elucidation, along with their neuroprotective, anti-neuroinflammatory, and cytotoxic activities.

**Results and Discussion.** – From a 75% EtOH extract of the commercially fermented mycelia of *X. nigripes*, a number of natural products were isolated, including nine pyrrole-containing alkaloids (1, 2, and 4–10) and six steroids (14–19; Fig. 1). Comparing their spectroscopic data and physicochemical properties with those reported in literature, the known compounds were identified to be (4S)-4-benzyl-3,4-dihydro-3-oxo-1H-pyrrolo[2,1-c][1,4]oxazine-6-carbaldehyde (4) [10][11], (4S)-3,4-dihydro-3-oxo-4-(propan-2-yl)-1H-pyrrolo[2,1-c][1,4]oxazine-6-carbaldehyde (5) [12], (4S)-3,4-dihydro-4-(2-methylpropyl)-3-oxo-1H-pyrrolo[2,1-c][1,4]oxazine-6-carbaldehyde (6) [12], 4-[2-formyl-5-(hydroxymethyl)-1H-pyrrolo-1-yl]butanoic acid (7) [13], methyl 4-[2-formyl-5-(methoxymethyl)-1H-pyrrolo-1-yl]butanoate (8) [14], 4-[2-formyl-5-(methoxymethyl)-1H-pyrrolo-1-yl]butanoic acid (9) [14], 5-(ethoxymethyl)-1H-pyrrolo-2-carbaldehyde (10) [14], 5-(hydroxymethyl)furan-2-carbaldehyde (11) [15], curdione (12) [16], genistein (13) [17], (3β,22E)-ergosta-7,9(11,22)-trien-3-ol (14; ergosterol D) [18], (3β,5α,8α,22E)-ergosta-6,22-diene-3,5,8-triol (15) [19], (3β,5α,6β,22E)-ergosta-7,22-diene-3,5,6-triol (16; cerasterol) [20], (3β,5α,6β,22E)-6-methoxyergosta-7,22-diene-3,5-diol (17) [21], (3β,5α,6β)-stigmastane-3,5,6-triol (18) [21], and (3β)-stigma-5-en-3-yl 6-O-[(9Z,12Z)-octadeca-9,12-dienoyl]-β-D-

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glucopyranoside (19) [22]. Interestingly, all the alkaloids (1, 2 and 4–10, Fig. 1) possess a common 2-formyl-5-(hydroxymethyl)pyrrole skeleton, which could be also found in xylapyrrosides A and B, and other related spiroalkaloids produced by this fungus [9]. To our knowledge, this class of 2-formyl-5-(hydroxymethyl)-pyrrole-containing alkaloids has never been reported from any mushrooms as the secondary metabolites until the present study. In addition, the above known compounds (except 13 and 16) were identified for the first time from X. nigripes.

Compound 1 was identified as a colorless gum and its molecular formula was determined to be C14H15NO6 based on a pseudo-molecular ion [M + H]+ at m/z 272.0917 (calc. 272.0928) in the HR-ESI-MS (positive-ion mode). The IR absorption bands suggested the presence of a lactone (1747 cm⁻¹) and an α,β-unsaturated CO (1647 cm⁻¹) group. An absorption band at 291 nm in the UV spectrum of 1 was indicative of a pyrrole-2-aldehyde moiety [23], and this was confirmed by the presence of a formyl H-atom at δ(H) 9.59 (s, H–C(7)) and two mutually-coupling H-atoms at δ(H) 7.08 (d, J = 4.0, H–C(3)) and 6.00 (d, J = 4.0, H–C(4)) for a typical 2,5-disubstituted pyrrole ring [9][23] in the 1H-NMR spectrum (Table). The 1H-NMR spectrum of 1 also displayed signals for a para-substituted benzene ring (δ(H) 6.68, 6.62 (each 2 H, br. d, J = 8.8)), an isolated –CHCH₂– group (δ(H) 5.99 (dd, J = 4.8, 3.2, H–C(2))), 3.46 (dd, J = 14.4, 3.2, H₅–C(3)), 3.35 (dd, J = 14.4, 4.8, H₆–C(3)), and an O–CH₂ group with an AB system (δ(H) 4.89 and 3.65 (AB, J = 14.8, CH₂(6)); Table). The 13C-NMR data of 1 (Table) exhibited 15 signals including two CO groups (δ(C) 179.2, 167.9), ten olefinic C-atoms (δ(C) 105.7–155.9) due to two aromatic rings, one sp³ CH group (δ(C) 59.3) and two sp² CH₂ groups (δ(C) 63.7, 39.5). The above NMR data of 1 together with its ESI-MS data were all identical to those of a synthetic compound, 2-[5-(hydroxymethyl)-2-formyl-1H-pyrrol-1-yl]-3-(4-hydroxyphenyl)propionic acid lactone, which was synthesized by a roasting reaction between l-tyrosine and D-glucose [11], indicating a (S)-configuration for compound 1. This was in agreement with the observation of two positive Cotton effects around 208 and 285 nm in the circular dichroism (CD) spectrum of 1, which matched well with those of compounds 4–6 [24]. Thus, the absolute configuration of 1 was unambiguously determined as (S) and it was reported herein as a new naturally occurring pyrrole lactone. Indeed, the known congeners 4–6 have also been reported to be generated by reactions of D-glucose with a corresponding l-amino acid: l-phenylalanine for 4, l-valine for compound 5, and l-leucine for 6 [11][23][25]. In these reactions, the stereogenic center at the l-amino acid moieties (i.e., C(2) in compounds 1 and 4–6) are conserved. However, no data on absolute configuration, such as specific rotations and CD spectra, could be found in any literature. Until recently, their absolute configurations have been well-defined by the lactone sector rule, and by comparison of their experimental CD spectra with those predicted by time-dependent DFT (TDDFT) calculations [24].

Compound 2, purified as a colorless gum, has a molecular formula of C₁₇H₁₄NO₄ as deduced by HR-ESI-MS (m/z 326.0985 ([M + Na]+)). The 1H- and 13C-NMR spectroscopic data (Table), as well as the UV spectrum, were quite similar to those of 1, indicating 2 was also a conjugate of 2-formyl-5-(hydroxymethyl)pyrrole and tyrosine. The most distinct difference between these two compounds was that 2 possesses an additional MeO group (δ(H) 3.77 (s), δ(C) 52.7). Moreover, from the ESI-MS data, nine degrees of unsaturation were required for 2, one
Table. \(^1\)H- (400 MHz, J values in Hz) and \(^13\)C- (100 MHz) NMR Data for 1–3

<table>
<thead>
<tr>
<th>Position</th>
<th>1(^a))</th>
<th>2(^b))</th>
<th>Position</th>
<th>3(^c))</th>
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<tr>
<td></td>
<td>δ(H)</td>
<td>δ(C)</td>
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<td>δ(H)</td>
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<tr>
<td>1</td>
<td>167.9</td>
<td>169.7</td>
<td>2</td>
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<td>2</td>
<td>5.99 (dd, J = 4.8, 3.2)</td>
<td>4.84 (br. s)</td>
<td>3</td>
<td>3.65 (dd, J = 14.4, 3.2)</td>
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<td></td>
<td>3.35 (dd, J = 14.4, 4.8)</td>
<td>3.25 (dd, J = 14.4, 10.4)</td>
<td>4</td>
<td>7.98 (d, J = 8.8)</td>
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<tr>
<td>4</td>
<td>132.4</td>
<td>132.3</td>
<td>5</td>
<td>6.95 (br. d, J = 8.8)</td>
</tr>
<tr>
<td>5</td>
<td>6.68 (br. d, J = 8.8)</td>
<td>6.66 (br. d, J = 8.4)</td>
<td>6</td>
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<td>6</td>
<td>6.62 (br. d, J = 8.8)</td>
<td>6.61 (br. d, J = 8.4)</td>
<td>7</td>
<td>154.5</td>
</tr>
<tr>
<td>7</td>
<td>6.62 (br. d, J = 8.8)</td>
<td>6.61 (br. d, J = 8.4)</td>
<td>8</td>
<td>115.4</td>
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<tr>
<td>9</td>
<td>6.62 (br. d, J = 8.8)</td>
<td>6.61 (br. d, J = 8.4)</td>
<td>10</td>
<td>115.4</td>
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<tr>
<td>10</td>
<td>3.77 (s)</td>
<td>52.8</td>
<td>2'</td>
<td>7.50 (br. d, J = 8.4)</td>
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<tr>
<td>2'</td>
<td>130.2</td>
<td>133.4</td>
<td>3'</td>
<td>7.00 (d, J = 3.2)</td>
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<tr>
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<td>7.00 (d, J = 3.2)</td>
<td>4'</td>
<td>4.27 (br. d, J = 9.6)</td>
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<tr>
<td>4'</td>
<td>6.00 (d, J = 4.0)</td>
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<td>6'</td>
<td>3.56 (d, J = 7.8)</td>
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<td>4.21 (d, J = 12.4)</td>
<td>7'</td>
<td>1.16 (s)</td>
</tr>
<tr>
<td>7'</td>
<td>3.65 (AB, J = 14.8)</td>
<td>3.84 (d, overlapped)</td>
<td></td>
<td>1.10 (s)</td>
</tr>
</tbody>
</table>

\(^a\) Recorded in CDCl\(_3\). \(^b\) Recorded in (D\(_2\))MSO.

less than that of compound 1. Considering that two CO groups, a pyrrole, and a benzene ring accounted for the nine degrees of unsaturation, compound 2 was hence assumed to be a lactone ring-opened product of 1, with the COOH group esterified (C(1): δ(C) 169.7). This was further confirmed by 1H-\(^1\)H-COSY and HMBC experiments (see Supporting Information\(^1\)). Especially, HMB correlations from the MeO group to C(1), from CH\(_2\)(3) (δ(H) 3.65, 3.25) to C(1), C(2) (δ(C) 61.3) and C(5)/C(9) (δ(C) 130.3), and from H–C(5)/H–C(9) (δ(H) 6.66) to C(3) (δ(C) 37.1) were observed. It is worth mentioning that the H-atom at C(2) in 2 resonated as a broad singlet instead of a doublet of doublets in compound 1. This phenomenon is probably due to the steric hindrance of the Ph ring, which has also been observed for two similar compounds, methyl 2-[2-formyl-5-(methoxymethyl)-1H-pyrrol-1-yl]-3-(4-hydroxyphenyl)propanoate [26] and methyl 2-[2-formyl-5-(methoxymethyl)-1H-pyrrol-1-yl]-3-phenylpropanoate [27].

Similar Cotton effects observed for 2 (Δε\(_{255}\) + 8.00, Δε\(_{295}\) + 7.64) and 1 (Δε\(_{285}\) + 3.42, Δε\(_{295}\) + 3.94) in their CD spectra indicated that they shared the same (2S) absolute configuration. Accordingly, the structure of 2 was characterized as methyl (2S)-2-[2-formyl-5-(hydroxymethyl)-1H-pyrrol-1-yl]-3-(4-hydroxyphenyl)propanoate.

However, in contrast to the negative optical rotations observed for those amino acid-derived pyrrole alkaloids (4: [α]\(^20\) D = +46.8, 5: [α]\(^20\) D = −88, 6: [α]\(^20\) D = −32, in acetone) recently reported by Zhu et al. [24], positive values were obtained for compounds 1, 2, and 4–6 (1: [α]\(^20\) D = +52.0, 2: [α]\(^20\) D = +36.7, 4: [α]\(^20\) D = +72.3, 5: [α]\(^20\) D = +46.8, 6: [α]\(^20\) D = +55.4, in acetone). Similar values were also acquired, when CH\(_2\)Cl\(_2\) was used as the solvent (see Exper. Part). Actually, such conflicts could be always found for those synthetic analogues [28]. This could be explained by a significant conformational rearrangement under different measurement conditions that results in a distinct change of optical activity, which has been fully discussed for α-amino acids; e.g., see [29].

Compound 3 was isolated as white amorphous powder, which was assigned the molecular formula C\(_{22}\)H\(_{32}\)O\(_8\)Na by the HR-ESI-MS (m/z 379.1144 ([\(M + Na\]^+)). The IR spectrum exhibited absorption bands at 3554 and 1624 cm\(^{-1}\) attributed to OH and conjugated CO functionalities, respectively. The UV absorption maximum at 249 nm was typical of an isoflavone [30], which was supported by the presence of characteristic NMR resonances at δ(H) 8.34 (s, H–C(2)) and δ(C) 153.6 (C(2)) [31]. Also, the aromatic region of the 1H-NMR spectrum (Table) of 3 displayed signals of a typical ABX spin system (δ(H) 7.98 (d, J = 8.8, H–C(5)), 6.95 (br. d, J = 8.8, H–C(6)), 6.88 (br. s, H–C(8))) assignable to the three aromatic H-atoms on ring C, and signals of a para-substituted ring B with an AA′BB′ spin system (δ(H) 7.50 (br. d, J = 8.4, H–C(2')) and H–C(6')), 7.00 (br. d, J = 8.4, H–C(3') and H–C(5'))). The above data were superimposable on those of daidzein [17], indicating that compound 3 was a daidzein derivative.

The remaining signals shown in the 1H-NMR spectrum (Table) included an O–CH\(_2\) group (δ(H) 4.27 (br. d, J = 9.6, H–C(1'))), 3.84 (dd, J = 9.6, 7.8, H–C(1'))), an O-bearing CH group (δ(H) 3.56 (br. d, J = 7.8, H–C(2'))), and two Me singlets (δ(H) 1.16 (s, Me(4')), 1.10 (s, Me(5'))). These signals could be easily assigned to a 1,2,3-trihydroxyalted...
isopentyl unit. Correspondingly, three O-bearing C-atoms at δ(C) 70.3 (C(1′′)), 76.2 (C(2′′)), and 71.2 (qC, C(3′′)), together with two Me groups at δ(C) 27.8 (Me, C(4′′)) and 24.7 (Me, C(5′′)), were observed in the high field region of the 13C-NMR spectrum (Table) of 3. Furthermore, the linkage position between the isoflavone skeleton and the isopentyl chain was determined by the observation of a clear 1J correlation from H–C(4′) to C(4′) (δ(C) 159.1) in the HMBC spectrum of 3 (Fig. 2). Additionally, HMB correlations from H–C(5) to C(4′) (δ(C) 175.1) and from H–C(6) to C(10) (δ(C) 117.1) confirmed that the OH group at the C-ring should be located at C(7), but not C(6).

To determine the absolute configuration of 2′′,3′′-diol moiety in compound 3, an induced circular dichroism (ICD) spectrum which involved the in situ complexation of a 1,2-diol with dimolybdenum tetracetate [Mo4(AcO)6] in DMSO solution (Snatzke’s method) was applied [32]. According to the empirical rule proposed by Snatzke and Flerek [32], the sign of the Cotton effect at around 310 nm in the ICD spectrum originates from the chirality of the vic-diol expressed by the O–C–C–O torsion angle in the favored conformation. As shown in Fig. 3, the metal complex of compound 3 in DMSO gave a significant ICD spectrum, in which the negative Cotton effect observed at 306 nm permitted the assignment of the (R)-configuration at C(2′′). Therefore, the structure of 3 was established as (2′′R)-4′-(2,3-dihydroxy-4-methylbutoxy)daidzein. But, it should be noted that the fungus (X. nigripes) itself seems not to produce the substituted daidzein but might transform the ingredients (daidzein and genistein (13)) of the soy meal medium into the compound 3 [33].

Considering the traditional application of X. nigripes and Wuling Capsule in treating central nervous system (CNS) associated diseases, e.g., anxiety disorders and depression, the compounds isolated in suitable amounts (1–4, 8, 9, 11, 12, and 14–18), were evaluated for their therapeutic potential against CNS-related disorders. Several well-established cell-based models have been utilized, including neuroprotection [34][35], anti-neuroinflammation [35], and acetylcholine esterase (AChE) inhibition [34]. As shown in Fig. 4, (3β,5α,6β,22E)-6-methoxyergosta-7,22-diene-3,5-diol (17), at the concentration of 10 μM, exhibited a significant neuroprotective effect by attenuating β-amyloid(25-35) (Aβ25,35)-induced cell damage in human SH-SY5Y neuroblastoma cells [34] (20.9% of increase in cell viability, Fig. 4). Meanwhile, in the anti-neuroinflammatory test, the above mentioned isolates were evaluated for their inhibitory effects against the lipopolysaccharide (LPS)-induced nitric oxide (NO) production in BV-2 microglial cells [35]. As a result, only (3β,5α,8α,22E)-ergosta-6,22-diene-3,5,8-triol (15) could significantly decrease NO production with an IC50 value of 27.6 μM, which was comparable to that of the positive control, Nω-monomethyl-l-arginine (l-NMMA, IC50 = 14.4 μM). Additionally, none of the tested compounds showed significant inhibitory effects against AChE [34] (inhibition rate < 50% at 40 μM). Taken together, the above findings provide new clues on the mechanism of the therapeutic effect of Wuling Capsule on improving CNS associated diseases. Undoubtedly, other related bioactivities of the pyrrole-containing alkaloids (1, 2, 4–10) need to be evaluated in a further study.

In addition, steroids 14, 17, and 18 exhibited remarkable cytotoxic effects against human U2OS lung osteosarcoma cells, with IC50 values of 0.93, 6.0, and 13.2 μM, respectively. Compound 17 also showed cytotoxicity against A549 human lung carcinoma cell line (IC50 = 11.9 μM). Staurosporine was used as a positive control, with IC50 values of 0.01 and 0.003 μM, respectively.

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Experimental Part

General. SiO₂-precoated plates (GF254, 0.25 mm, Kang-Bi-Nuo Silysta Chemical Ltd., Yantai, P. R. China) were used for TLC. Spots were visualized using UV light (254 and/or 366 nm) and by spraying with 5% (v/v) H₂SO₄/MeOH or 15% CeSO₄/MeOH followed by heating to 120 °C. Column chromatography (CC) was performed using SiO₂ (200 – 300 mesh, Ji-Yi-Da Silysta Chemical Ltd., Qingdao, P. R. China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and ODS (50, YMC Co. Ltd., Kyoto, Japan). Semi-prep. HPLC: Waters e2695 system coupled to a Waters 2998 Photodiode Array Detector (PAD) and a Waters 2424 Evaporative Light-scattering Detector (ELSD); a Sunfire ODS column (5 μm, 250 mm × 10 mm) was utilized. Optical rotations: JASCO P-1020 polarimeter. UV and IR spectra: Shimadzu UV-2550 and Nicolet AVATAR 360 FT-IR spectrophotometer; resp. CD Spectra: Jasco 810 spectrometer. NMR Spectra: Varian Mercury Plus 400 or Bruker Advance II 400; spectrometer; chemical shifts were given in δ [ppm] with tetramethylsilane (TMS) as an internal standard. ESI-MS: Agilent 1100 Series mass spectrometer, and high-resolution mass spectra (HR-ESI-MS) were recorded on a Bruker Daltonics microTOF-QII mass spectrometer.

Fungi Material. The commercially fermented mycelia of X. nigripes, also called Wuling Powder, were produced by Zhejiang Jolly Pharmaceutical Company (Deqing County in Zhejiang Province, P. R. China). In September 2008, the crude samples were dried on site and then shipped to the laboratory, where they were frozen upon arrival.

Extraction and Isolation. A 20 kg aliquot was extracted with 75% EtOH (3 × 201) at r.t. to afford a brown residue (1.3 kg), which was suspended in H₂O (2.0 l) and then successively extracted with petroleum ether (PE, 3 × 1.5 l), AcOEt (3 × 1.5 l), and BuOH (3 × 1.5 l). The AcOEt-soluble fraction (281.6 g) was subjected to CC over SiO₂ (9.0 mg) was chromatographed over a SiO₂ column with CH₂Cl₂/MeOH (50:1 → 10:1, v/v), and six subfractions (Fr. 10a – 10f) were collected. Compounds 8 (8.2 mg) and 9 (30.0 mg) were isolated from Fr. 10b (2.5 g) by SiO₂ CC with CH₂Cl₂/MeOH (10:1 → 2:1, v/v), and each was finally refined by GPC over Sephadex LH-20 (MeOH). Fr. 10c (2.1 g) was subjected to Sephadex LH-20 (MeOH), and C18 HPLC (MeOH/H₂O 20:80, v/v; flow rate, 3.0 ml/min) to yield compound 1 (18.5 mg, t₁R = 5.3 min). Compound 13 (50.8 mg) was crystallized from Fr. 10d, and compound 7 (10.0 mg) was purified from Fr. 10f (1.5 g) by size-exclusion chromatography over Sephadex LH-20 (MeOH). Fr. 11e (3.95 g) was fractionated by SiO₂ CC (CH₂Cl₂/MeOH 50:1 → 30:1, v/v), and six subfractions (Fr. 11a – 11f) were obtained. Fr. 11a (174.0 mg) was chromatographed over SiO₂ with PE/AcOEt (20:1 → 2:1, v/v) and then Sephadex LH-20 (CH₂Cl₂/MeOH 2:1, v/v) to yield compounds 4 (1.0 mg) and 6 (4.0 mg). Compound 3 (24.3 mg) was isolated from Fr. 11e (350 mg) by GPC on Sephadex LH-20 (MeOH) followed by flash CC over SiO₂ (CH₂Cl₂/MeOH 25:1, v/v). Purification of the Fr. 11b – 11d led to the isolation of four spirocyclic pyrrole alkaloids as described previously [9]. Fr. 12 (11.6 g) was loaded on a SiO₂ column with CH₂Cl₂/MeOH gradient (50:1 → 10:1, v/v) to yield five subfractions (Fr. 12a – 12e). Subsequent separation of Fr. 12b (102.5 mg) by SiO₂ CC (PE/AcOEt 10:1 → 2:1, v/v) led to the isolation of compound 17 (9.1 mg). Compound 16 (78.3 mg) was crystallized from Fr. 12c (350.0 mg) by GPC on Sephadex LH-20 (MeOH), and compound 18 (6.5 mg) was obtained from Fr. 12d (0.8 g) by ODS CC (from MeOH/H₂O 9:1 to MeOH neat, v/v) and further purification by GPC on Sephadex LH-20 with MeOH. (4S)-3,4-Dihydro-4-(4-hydroxybenzyl)-3-oxo-1H-pyrrole-2,1-[C]l/4 oxazine-6-carbaldehyde (1). Colorless gum. [α]₂₅ = +52.0 (c = 0.1, acetone); [α]₂₅ = +36.0 (c = 0.1, CH₂Cl₂). UV (MeOH): 291 (2.95), CD (c = 1.84 × 10⁻³ m, MeOH); 285 (+ 3.94), 227 (− 1.00), 208 (− 3.42). IR: 3419, 1747, 1647, 1516, 1544, 1237, 1043. ¹H- and ¹³C-NMR (D₂O): see [11]; ¹H- and ¹³C-NMR (CDCl₃); see Table 1. ESI-MS: 272 ([M + H]+). HR-ESI-MS: 272.0917 ([M + H]+), C₂₀H₂₈N₂O₂ calc. 272.0928.

Methyl (2S)-2-[2-Formyl-5-(hydroxymethyl)-1H-pyrrole-1-yl]-3-(4-hydroxyphenyl)propionate (2). Colorless gum. [α]₂₅ = +36.7 (c = 0.05, acetone); [α]₂₅ = +25.0 (c = 0.1, CH₂Cl₂). UV (MeOH): 291 (2.44), CD (c = 1.65 × 10⁻³ m, MeOH): 286 (+ 7.64), 242 (− 0.88), 207 (± 8.00). IR: 3463, 1735, 1645, 1514, 1494, 1243. ¹H- and ¹³C-NMR: see Table 1.
in a humidified environment with 5% CO₂. The anti-inflammatory activity was tested against A549-induced injury in SH-SYSY neuroblastoma cells using the protocol described earlier [34] [35].

Cytotoxicity Assay: The U2OS human osteosarcoma and A549 human lung carcinoma cell lines were purchased from the cell bank of Shanghai Institute of Cell Biology (Shanghai, P.R. China). The U2OS cells were maintained in Dulbecco's modified Eagle's medium containing 1800 mg/l NaHCO₃, supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in a humidified atmosphere with 5% CO₂. The cell viability was determined by MTT assay as described earlier [36]. Staurosporine (Sigma–Aldrich, catalog No. S6942-200UL) was used as a positive control.


REFERENCES


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