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NOVEL BENZOFURANOID NORLIGNANS FROM THE AERIAL PARTS OF *ASPARAGUS COCHINCHINENSIS* AND THEIR BIOLOGICAL ACTIVITY

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Abstract – Three new benzofuranoid norlignans asparlignan A (**1**), B (**2**), and C (**3**) were isolated from the aerial parts of *Asparagus cochinchinensis*, in addition to previously known metabolites (**4**-**6**). The structures of these compounds were elucidated using a combination of spectroscopic analyses, including UV, IR, HRESIMS, 1D, and 2D NMR. Further, all compounds were evaluated for their anti-inflammatory activity and capability to inhibit nitric oxide (NO) production by RAW 264.7 macrophages and anticancer activity against three tumor cells.

The genus of *Asparagus* has been used as vegetables and medicines due to its soothing flavor and health benefit properties.[1] *Asparagus cochinchinensis* is an important traditional Chinese herbal plant, and was employed for treating cutaneous inflammation, cardiovascular, bacterial infection, diabetes, constipation, and throat pain.[2,3] Phytochemical studies have demonstrated that it contains phenolic, steroidal glycosides, alkaloids, and polysaccharides compounds.[4-6] However, there remains a deficiency of bioactive compounds from the aerial parts of *A. cochinchinensis*. Given this, a chemical investigation of the aerial parts of *A. cochinchinensis* led to the isolation of three novel norlignans (**1-3**), two known lignan compounds lariciresinol (**4**),[7] 5,5'-dimethoxy-7-oxolariciresinol (**5**),[8] and one known neolignan compound (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol (**6**)[9] in our study (**Figure 1**). Here, we report the isolation,

structurally elucidation, and evaluation of anti-inflammatory and anticancer activity of these compounds.

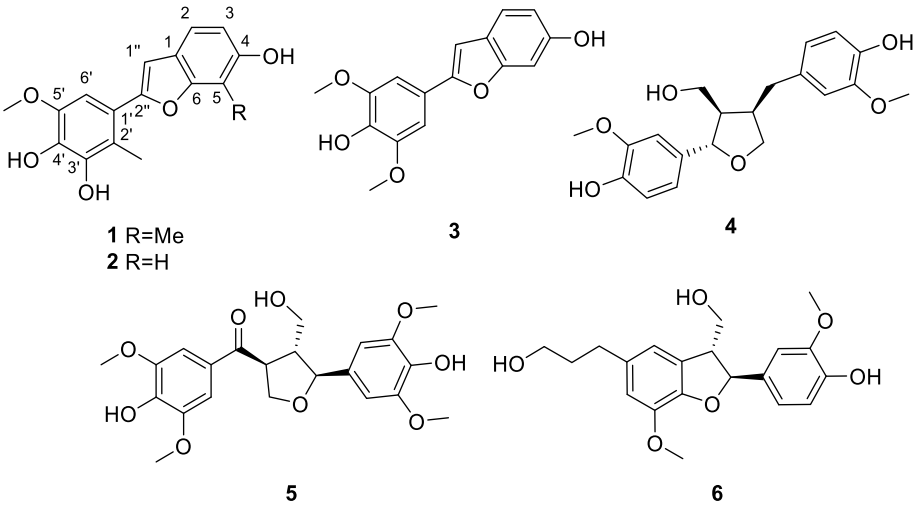

Figure 1. Compounds **1**-**6** isolated from the aerial parts *Asparagus cochinchinensis*

Asparlignan A (**1**) was obtained in the form of brown black powder and possesses the molecular formula $C_{17}H_{16}O_5$, as deduced from (-)-HRESIMS, which suggest a molecular structure with ten indices of hydrogen deficiency. ^{1}H NMR data of **1** showed characteristic signals such as a pair of aromatic protons at δ_H 7.15 (1H, d, J = 8.2 Hz, H-2) and 6.71 (1H, d, J = 8.2 Hz, H-3), two methyl groups at δ_H 2.32 (s, 2′-Me) and 2.36 (5-Me), one methoxy singlet at δ_H 3.86 (s, 5′-OMe) an olefinic hydrogen signal at δ_H 6.63 (s, H-1″), as well as, an aromatic signal at δ_H 6.87 (s, H-6′). Integrated analysis of spectroscopic data of ^{13}C NMR, DEPT, and HSQC displayed 17 carbon resonances, including 14 aromatic/olefinic carbons, and 3 aliphatic carbons. The NMR data of **1** (**Table 1**) were similar to a benzofuranoid norlignan.[10] Furthermore, analysis of 2D NMR spectra, the HMBC correlations from H-1″ to C-2/C-6 and 5-Me to C-6 suggested that one methyl was attached to the benzofuranoid at C-5 position. Meanwhile, HMBC correlation of H-2/5-Me to C-4 (δ_C 153.6) indicated one hydroxy group substitution at C-4. The two remaining hydroxy groups were located at C-4′ and C-3′, respectively, confirmed by HMBC correlations from H-6′/2′-Me to C-4′/3′. The ROESY correlations were consistent with the above speculation (**Figure 3**). **Figure 1** depicts the planar structure of asparlignan A (**1**).

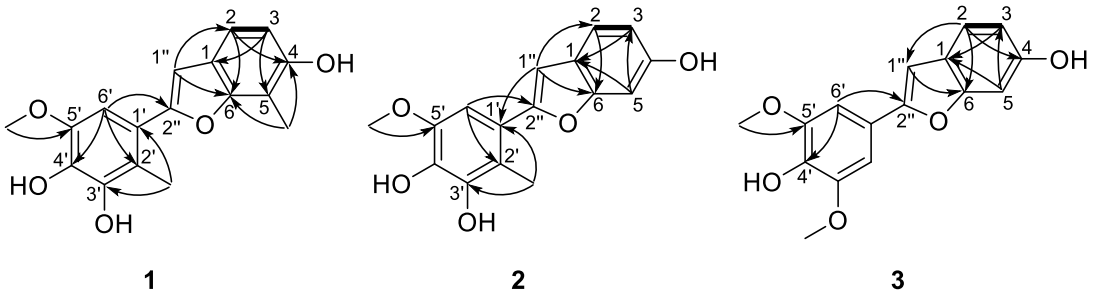

Figure 2. The key ^{1}H-^{1}H COSY (bold) and HMBC (arrows) correlations of compounds **1**-**3**

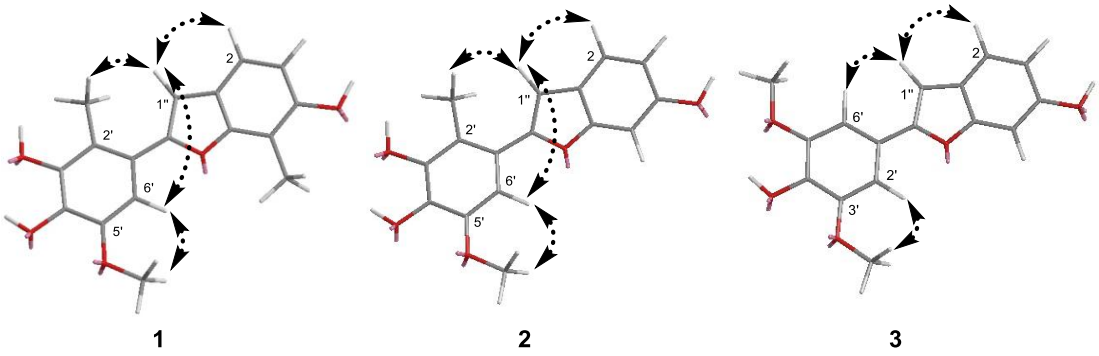

Figure 3. Key ROESY correlations of compounds **1**-**3**

Aspalignan B (**2**) was isolated in the form of amorphous powder and its molecular formula $C_{16}H_{14}O_5$ was assigned from HRESIMS data. The NMR spectra suggested that it was an analogue of compound **1**. Compared with those of **1**, 1D NMR spectra of **2** displayed an additional aromatic signal and lost one methyl signal. This difference were supported by an ABX spin-like system [δ_H 7.33 (1H, d, J = 8.2 Hz), 6.71 (1H, d, J = 8.2 Hz), and 6.89 (1H, br.s)] and key HMBC correlations from H-5 (δ_H 6.89, br.s) to C-1/3 (**Figure 1**). Therefore, compound **2** was characterized as shown and named aspalignan B.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of **1**-**2** in CD_3OD (δ in ppm, J in Hz)

Position	**1**		**2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	-	122.8, C	-	123.3, C
2	7.15 (d, 8.2)	118.3, CH	7.33 (d, 8.2)	121.7, CH
3	6.71 (d, 8.2)	112.5, CH	6.71 (d, 8.2)	112.8, CH
4	-	153.6, C	-	156.3, C
5	-	108.2, C	6.89 (br.s)	98.3, CH
6	-	155.6, C	-	156.6, C
1″	6.63 (s)	105.0, CH	6.66 (s)	104.7, CH
2″	-	156.4, C	-	156.3, C
1′	-	123.0, C	-	122.7, C
2′	-	117.3, C	-	117.3, C
3′	-	145.1, C	-	145.1, C
4′	-	135.4, C	-	135.4, C
5′	-	147.1, C	-	147.1, C
6′	6.87 (s)	104.0, CH	6.87 (s)	103.9, CH

5-Me	2.36 (s)	8.7, Me	-	-
2′- Me	2.32 (s)	13.4, Me	2.30 (s)	13.4, Me
5′-OMe	3.86 (s)	56.5, Me	3.86 (s)	56.5, Me

The molecular formula of aspalignan C (**3**) was determined by HRESIMS to be $C_{16}H_{14}O_5$. In ^{1}H NMR (**Table 2**) spectrum of **3** indicated the presence of a symmetrical structure [δ_H 7.09 (2H, s, H-2′/6′), 3.92 (6H, s, -OMe)]. The NMR data of **3** was furthermore found to be similar to **1** and **2**, and the obvious difference was the absence of methyl group signal in **3**. Meanwhile, HMBC correlations from H-2 to C-4 (δ_C 156.3) supported a hydroxy group substitution at C-4. The symmetrical aromatic ring suggested it to be connected at C-2″ by the key HMBC plots from H-2′/6′ to C-2″. Consequently, the structure of compound **3** was elucidated as a new benzofuranoid norlignan and named aspalignan C (**3**).

Table 2. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data of **3** in CD_3OD (δ in ppm, J in Hz)

Position	δ_H (J in Hz)	δ_C
1	-	123.4, C
2	7.32 (d, 8.2)	121.6, CH
3	6.72 (dd, 8.2, 2.0)	113.0, CH
4	-	156.3, C
5	6.90 (br.s)	98.4, CH
6	-	157.1, C
1″	6.92 (s)	100.7, CH
2″	-	156.5, C
1′	-	123.3, C
2′/6′	7.09 (s)	102.9, CH
3′/5′	-	149.5, C
4′	-	137.1, C
3′/5′-OMe	3.92 (s)	56.8, Me

Here, we assessed the ability of isolated compounds **1-6** to inhibit NO production in LPS-stimulated RAW 264.7 cells. No obvious cytotoxicities were observed for the cells when treated with compounds **1**, **2**, and **4-6** at the test concentrations, whereas compound **3** exhibited cytotoxicity on RAW 264.7 (cell viability <85% at 25 μM). The results (**Table 3**) revealed that compounds **1** and **2** displayed moderate NO inhibitory activities, with IC_{50} values of 21.1 and 28.6 μM, respectively (the positive control

L-NG-monomethylarginine hydrochloride with IC_{50} = 12.2 μM).

Simultaneously, the cytotoxicities of these compounds against three cancer cell lines (HL-60, A-549, and MCF-7) were assessed by the MTT method. The results revealed that all compounds had no cytotoxicities (IC_{50} > 40 μM) against three cancer cell lines (the positive control cisplatin with IC_{50} = 1.6, 8.4, 20.3 μM, respectively).

Table 3. IC_{50} Values of compounds **1–6** inhibiting NO production in RAW 246.7 cells

compound	IC_{50} (μM)[a]
1	21.1 ± 1.5
2	28.6 ± 0.8
3	-[b]
4	>50
5	>50
6	41.2 ± 0.3
L-NMMA	12.2 ± 1.1

[a]IC_{50} Values were expressed as mean ± SD (n = 3). [b]The samples showed cytotoxic effects to cells at 25 μM (less than 15% cell survival).

In summary, three previously undescribed benzofuranoid norlignans (**1-3**) and three known ones (**4-6**) were isolated from the aerial parts of *Asparagus cochinchinensis*. Their structures were identified via various spectroscopic methods. All isolates were evaluated for their inhibitory effects on NO production in LPS-induced RAW 264.7 cells, showing potential inhibitory activity with IC_{50} values of 21.1-41.2 μM. Unfortunately, no compounds displayed cytotoxicities against three human cancer cell lines with IC_{50} > 40 μM. This study not only enriched the diversity of components from *Asparagus cochinchinensis* but also deserved further research as anti-inflammatory agents.

EXPERIMENTAL

General experimental procedures. The NMR data were recorded on a Bruker 500 and 600 MHz spectrometer (Bruker, USA). IR spectra were recorded with KBr disks by a Bruker vertex-70 spectrometer (Bruker, USA). The high-resolution mass spectrum was acquired via Shimadzu LC-IT-TOF (Shimadzu, Japan). MPLC separation was performed on a Büchi sepacore (Büchi Labortechnik AG, Flawil, Switzerland) with YMC gel ODS C18 column (45-60 μm, YMC Co., Ltd., Kyoto, Japan). Column chromatography (CC) was carried out using silica gel (200-300 mesh, Qingdao Marine Chemical Co. Ltd., China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Thin-layer chromatography (TLC) was undertaken on HSGF254 plates (Qingdao Marine Chemical Co. Ltd., China).

Semi-preparative HPLC was conducted on an LC-3000 semi-preparation gradient HPLC system (Chuangxintongheng, Beijing, China), equipped with a UV–vis detector and a semipreparative RP-HPLC column (Shiseido CAPCELL PAK C_{18} column, 250×20 mm, 5μm, Japan).

Plant material. The fresh aerial parts of *Asparagus cochinchinensis* were collected at Lu'an, Anhui province (China), in September 2020 and identified by associate Prof. Tao Xu.

Extraction and isolation. The fresh aerial parts of (3 kg) were extracted three times with MeOH to give a crude extract, then the extract was suspended in water and extracted with EtOAc, affording an EtOAc soluble extract (50 g). The EtOAc part was divided into five fractions (A-E) using a silica gel column (200-300 mesh) and eluted sequentially with $CHCl_2$-MeOH (100:0→0:1, v/v). Fraction B (600 mg) was subjected to Sephadex LH-20 column (MeOH) and separated by semipreparative HPLC (MeOH-H_2O, 50:50 v/v) to furnish compound **6** (8 mg, t_R=26.8 min). Fraction C (1 g) was separated using MPLC eluted with MeOH-H_2O (30:70-100:0, v/v), and followed by Sephadex LH-20 column (MeOH) and purified by pre-HPLC (MeCN-H_2O, 50:50 v/v) to obtain compounds **3** (2 mg, t_R=25.3 min) and **1** (12 mg, t_R=28.4 min). Fraction D (2 g) was chromatographed through flash chromatography over MCI gel eluted with MeOH-H_2O (30:70-0:1, v/v) and RP-18 column (MeOH-H_2O, 20:80-100:0, v/v) to yield three subfractions D1-D3. D2 (400 mg) was chromatographed on Sephadex LH-20 column (eluted with MeOH) and applied to pre-TLC (petroleum ether: acetone 2:1, v/v) to yield compound **4** (6 mg) and compound **5** (8 mg). D3 (100 mg) was also chromatographed on Sephadex LH-20 column (MeOH) and purified by semipreparative HPLC with MeCN-H_2O elution system (50:50) to afford compound **2** (10 mg, t_R=26.2 min).

Asparlignan A (1): Brown black powder; UV (MeCN) λ_{max} 200, 306 nm; IR (KBr): ν_{max} 3435, 1626, 1509, 1297, 1102 cm^{-1}; HRESIMS $[M-H]^-$ *m/z*: 299.0923 (calcd. for $C_{17}H_{15}O_5$, 299.0925); ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 1.

Asparlignan B (2): Brown black powder; UV (MeCN) λ_{max} 210, 314 nm; IR (KBr): ν_{max} 3425, 1621, 1503, 1292, 1114 cm^{-1}; HRESIMS $[M-H]^-$ *m/z*: 285.0766 (calcd. for $C_{16}H_{13}O_5$, 285.0768); ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 1.

Asparlignan C (3): Brown black powder; UV (MeCN) λ_{max} 212, 320 nm; IR (KBr): ν_{max} 3418, 1620, 1512, 1217, 1116 cm^{-1}; HRESIMS $[M-H]^-$ *m/z*: 285.0769 (calcd. for $C_{16}H_{13}O_5$, 285.0768); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) see Table 2.

Anti-inflammatory assay. The experimental procedures were followed by the literature.[11] The cells were seeded in the 96-well plates for 24 h, pretreated with the six test compounds, and co-incubated with LPS (1 μg/mL) for 24 h. NO production was analyzed through Griess reaction. Precisely, the cell culture supernatant (50 μL) and Griess reagent (50 μL) were mixed and monitored at 570 nm using a microplate reader. All experiments were performed in triplicates.

Anticancer assay. All compounds were tested for cytotoxicity against MCF-7 (human breast cancer cell line), A-549 (human lung cancer cell line), and HL-60 (human acute promyelocytic leukemia cell line) utilizing MTT method as previously reported.[12,13] Cisplatin was used as a positive control.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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