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Two Polyoxygenated Bipyrrole Alkaloids from *Speranskia tuberculate*

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Abstract: Three polyoxygenated bipyrrole alkaloids were isolated from the aerial of *Speranskia tuberculata*, including two new compounds, speranberculatines B (**1**) and C (**2**), along with a known compound, speranskatin A (**3**). Their structures were identified via NMR-spectroscopic and MS analyses. None of them showed activity in inhibiting the production of NO in LPS-induced RAW264.7 cells.

Keywords: *Speranskia tuberculata*; bipyrrole alkaloid; structure elucidation.

1. Plant Source

The aerial herbs of *Speranski*a *tuberculata* (Bunge) Bail were collected in Xinxiang County of Henan Province of China, in September 2021, authenticated by Prof. Shou-Jin Liu (Anhui University of Chinese Medicine). A voucher specimen (ACM 2021100801) was preserved in the specimen center of Anhui University of Chinese Medicine.

2. Previous Studies

The genus of *Speranskia Baill* is a small member of Euphorbiaceae family, comprising of three perennial herbs, which used as folk medicine for pain-relieving, such as rheumatic arthritis, constracture, sores, swelling and so on [1-2]. *S. tuberculata* was distributed to the Northern of China on higher altitudes of 800-1900 m hillside, grasslands or thickets. Previous investigation of chemical ingredient indicated that Pyridine-2,6 (1H, 3H) diketone alkaloids, flavonoids, sterols and pyrimidines were isolated from in this plant [3-7]. Pharmacological activities showed that the extracts of *S.*

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tuberculata had anti-inflammatory, analgesic, antioxidant and antiplatelet agglutination activities. Speranskatin A (**3**) is one of the effective components for promoting blood circulation and removing stasis [8].

3. Present Study

The air-dried and powdered herbs of *S. tuberculata* (14.5 kg) were extracted with 80 % ethanol (110 L × 3) for 2 h. And the extract was concentrated in vacuum to give a concentrated solution (10 L). The organic extracts of different polarity were given after being partitioned with petroleum ether (yield 236.5g), ethyl acetate (yield 101.2 g) and *n*-butanol (yield 340.0 g) in turn, three times, respectively. The EtOAc part (cac. 98.0 g) was first subjected to Sephadex LH-20 chromatography column (CC) and eluted with CH_2Cl_2-MeOH (1:1) to yield seven fractions (Fr.1–Fr.7). Fr. 3 (41.18 g) was further separated on MCI gel CHP 20P with MeOH-H_2O (20:80→0:100) to yield Fr.3.1–Fr.3.4; Fr.3.1 was separated by MPLC (40% MeOH) and got Fr.3.1.2 (8.0 g), then further separated and purified by silica gel ($CHCl_3$-MeOH 9:1→85:15), Sephadex LH-20 (MeOH), and semi-preparative HPLC (acetonitrile-H_2O 10: 90) to obtain compounds **1** (5.35 mg) and **2** (5.46 mg). Fr. 5 (12.83 g) was performed on Sephadex LH-20 (CH_2Cl_2-MeOH = 1:1)), MPLC (ODS-C_{18}, 25% MeOH) and semi-preparative HPLC (MeOH-H_2O 15: 85) to obtain compound **3** (55.06 mg).

Biological Activity Test: According to an established method [9], the extraction and all isolates were evaluated for their anti-informatory effect. RAW264.7 cell lines were cultured at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Gibco Inc., NY, USA) supplemented with 10% fetal bovine serum (FBS, Gibco Inc., NY, USA). Dexamethasone (DXMS) was used as positive medicine. 1.0 μg/mL lipopolysaccharide (LPS, Sigma-Aldrich Chemical Co, USA) used as the inducer. After incubation with required concentrations of substance for 24 h, the content of NO was determined at Griess method, IC_{50} value was calculated on IBM SPSS, version 23.0.

Speranskatin B (**1**): pale yellow amorphous powder; $[\alpha]^{30}{}_D$ 2.14 (*c* 0.14, MeOH); UV λ_{max} (MeOH) nm (log ε): 203.5 (4.05), 221.0 (4.06), 325.5 (3.08) nm; IR ν_{max} (KBr) cm^{-1}: 3570, 3257, 2955, 2921, 1779, 1708, 1626, 1454, 1372, 1309, 1075 cm^{-1}; (-)HREIMS *m/z*: 267.0625 $[M-H]^-$ (calc. for $C_{11}H_{11}N_2O_6$, 267.0623).

Speranskatin C (**2**): pale yellow amorphous powder; $[\alpha]^{30}{}_D$ 1.50 (*c* 0.20, MeOH); UV λ_{max} (MeOH) nm (log ε): 203.50 (3.83), 230.5 (3.85), 337.5 (2.64) nm; (-)HREIMS *m/z*: 267.0624 $[M-H]^-$ (calc. for $C_{11}H_{11}N_2O_6$, 267.0623).

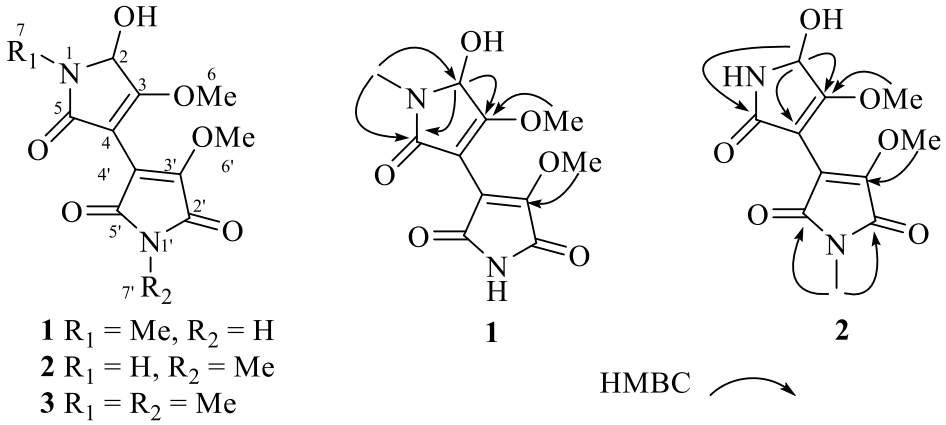

Figure 1. Structure of compounds **1**-**3** and key HMBC correlations of **1** and **2**

Compound **1** was obtained as a pale yellow amorphous power, its molecular formula was deduced to be $C_{11}H_{11}N_2O_6$ on a pseudo-molecular ion at *m/z* 267.0625[M-H]$^-$ in HRESIMS. The ^{1}HNMR spectrum (Table 1) of **1** exhibited three methyl group singlet signals at δ_H 4.05, 4.03 and 3.04, and an oxymethine proton signal at 5.30 (1H, s); The ^{13}C NMR spectrum revealed 11 carbon resonances, including three lactam carbonyl signals, two sets of double bond carbon signals, an oxygenated methine, and three methyl carbons. Comparison of the above NMR spectral data of **1** with those of **3** (Shi et al, 2000), they were similar, except a group *N*-CH_3 signal (δ_H 3.00, δ_C 24.0) omitted in **1**. The HMBC spectra (Figure 1) showed correlations of 3.04 (3H, s, *N*-CH_3) to C-2, C-5, indicated it was attached to position-N-1. Moreover, the HMBC correlations from H-2 to C-2, C-5, H_3-6 to C-3, and H_3-6′ to C-3′ were observed. Thus, the structure of **1** was identified as (+)-2-hydroxy-3,3′-dimethoxy-1-methyl-4,4′-bipyrrole-5,2′,5′(*2H*)-trione, named as speranberculatine B.

Compound **2** was determined to have a molecular formula of $C_{11}H_{11}N_2O_6$ with the molecular ion at *m/z* 267.0623[M-H]$^-$ in the negative HRESIMS spectrum. Compared with **1**, the NMR spectroscopic properties were similar (Table 1) except those of *N*-CH_3. The HMBC correlations (Figure 1) from the signal of protons δ_H 2.96 (3H, s, *N*-CH_3) to C-2′ and C-5′, which confirmed the methyl was connected to *N*-1′ position. Therefore, compound **2** was elucidated as (+)-2-hydroxy-3,3′-dimethoxy-1′-methyl-4,4′-bipyrrole-5,2′,5′(*2H*)-trione, named as speranberculatine C.

Table 1. The ^{1}H- and ^{13}C-NMR data of compounds **1-3** (CD_3OD, 600/125 MHz)

No.	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.30 (1H, s)	82.4	5.51 (1H, s)	78.4	5.22 (1H, s)	82.3
3	-	173.2	-	174.6	-	172.8
4	-	96.6	-	96.4	-	96.5
5	-	171.9	-	174.5	-	171.8
6	4.03 (3H, s)	59.5	4.02 (3H, s)	59.8	3.99 (3H, s)	59.5
7	3.04 (3H, s)	26.1		-	2.94 (3H, s)	26.2
2'	-	172.8		172.6		172.5
3'	-	157.8	-	157.8	-	157.8
4'	-	102.8	-	101.9	-	102.1
5'	-	167.8	-	167.0	-	167.0
6'	4.05 (3H, s)	59.7	4.07 (1H, s)	59.5	4.08 (3H, s)	59.8
7'			2.96 (3H, s)	24.0	3.00 (3H, s)	24.0

The anti-inflammatory of ethanol extraction, Pet-soluble fraction, EtOAc-soluble fraction and *n*-BuOH fraction of was evaluated *in vitro* against RAW264.7 cell lines. As a result, the EtOAc-soluble fraction exhibited inhibitions on NO production with the IC_{50} value 46.52 μg/ml. Whereas, speranskatins A-C showed no anti-inflammatory activity.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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