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Exotines A and B, Two Heterodimers of Isopentenyl-Substituted Indole and Coumarin Derivatives from *Murraya exotica*

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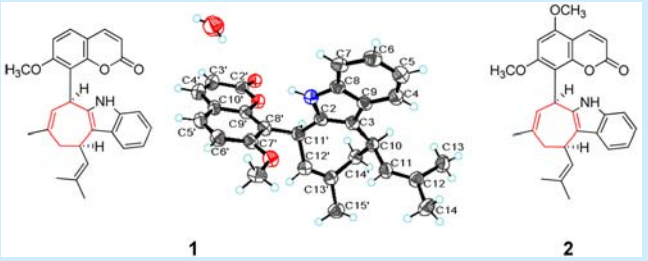
Supporting Information

ABSTRACT: Exotines A and B (**1** and **2**), two heterodimers of isopentenyl-substituted indole and coumarin derivatives linked through a new fused heptacyclic ring system, were isolated from the roots of *Murraya exotica*. Their structures were established by comprehensive NMR and MS spectroscopic data analysis, and the absolute configurations were determined by single-crystal X-ray crystallographic analysis and ECD calculations. Compounds **1** and **2** showed inhibition of nitric oxide production in lipopolysaccharide-induced BV-2 microglial cells with IC_{50} values of 9.2 and 39.9 μM, respectively.



Murraya exotica, a species of the genus *Murraya* (family Rutaceae), is widely distributed in India, Southeast Asia, and Southern China.[1,2] Coumarins have been reported to be the main bioactive constituents[3−5] in *M. exotica*, along with several indole and carbazole alkaloids.[6,7] The leaves and roots of the *Murraya* plants have been used as folk medicines for the treatment of analgesia, anesthesia, abdominal pain, eczema, rheumatism, etc. Pharmacological studies showed the antioxidant, antimicrobial, antitumor, antifungal, and anti-inflammatory activities for the crude extracts and compounds from *M. exotica*.[8−11] Our previous phytochemical investigations of the *Murraya* plants led to the isolation of numerous coumarins and flavonoids from *M. alata* and *M. paniculata*.[12,13] As a continuation of a search for bioactive metabolites from *Murraya* species, the 95% aqueous EtOH extract of the roots of *M. exotica* was investigated and two novel heterodimers of isopentenyl-substituted indole and coumarin derivatives linked through a new fused heptacyclic ring system, exotines A and B (**1** and **2**) (Figure 1), were obtained. Herein, the isolation and structural characterization of the two novel compounds and their inhibitory effects on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in BV-2 microglial cells are reported.

Exotine A (**1**) was obtained as colorless needle crystals, mp 249−250 °C, $[\alpha]_D^{25}$ +6 (*c* 0.1, MeOH). The molecular formula of **1** was established as $C_{28}H_{27}NO_3$ by the positive-ion HRESIMS (*m*/*z* 426.2060 $[M + H]^+$, calcd 426.2069) and ^{13}C NMR data, indicating 16 indices of hydrogen deficiency. The IR spectrum showed absorption bands for NH (3360 cm^{-1}) and carbonyl (1705 cm^{-1}) functionalities,[14] and the UV spectrum showed characteristic absorption for a coumarin moiety (λ_{max} 321 nm).[16]

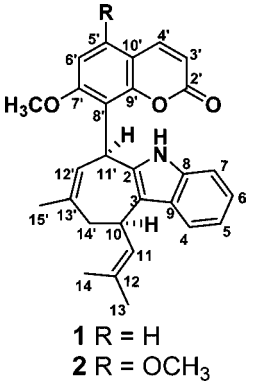


Figure 1. Exotines A (**1**) and B (**2**) from *M. exotica*.

The ^1H NMR data (Table 1) indicated the presence of an 8-substituted-7-methoxycoumarin moiety at δ_H 7.69 (d, *J* = 9.5 Hz), 6.28 (d, *J* = 9.5 Hz), 7.49 (d, *J* = 8.7 Hz), 6.95 (d, *J* = 8.7 Hz), and 3.76 (s, 3H).[16] A set of resonances for the 2,3-disubstituted indole unit in the low field region of the ^1H NMR spectrum were deduced from the $^1H-^1H$ COSY correlations and ^{13}C NMR data (Table 1).[16] Besides, the ^{13}C NMR data also revealed the presence of two olefinic groups at δ_C 128.9, 129.0, 126.6, and 137.4. These functionalities accounted for 15

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data of Exotines A (1) and B (2) in $CDCl_3$ (δ in ppm, J in Hz)

	1		2	
no.	δ_H	δ_C	δ_H	δ_C
2		132.5		133.1
3		114.8		114.5
4	7.36 m	118.1	7.35 m	118.0
5	7.00 m	118.8	6.98 m	118.7
6	7.00 m	120.6	6.99 m	120.5
7	7.04 m	109.9	7.03 m	109.8
8		133.7		133.6
9		129.6		129.7
10	4.12 m	33.0	4.11 m	33.0
11	5.47 d (9.8)	128.9	5.44 d (9.6)	129.0
12		129.0		128.9
13	1.74 s	25.8	1.74 s	25.8
14	1.96 s	18.1	1.95 s	18.1
2′		160.5		160.9
3′	6.28 d (9.5)	113.6	6.19 d (9.7)	111.3
4′	7.69 d (9.5)	143.6	8.06 d (9.7)	138.7
5′	7.49 d (8.7)	128.2		156.5
6′	6.95 d (8.7)	108.4	6.41 s	91.1
7′		161.2		162.0
8′		118.5		110.6
9′		153.1		153.9
10′		113.2		103.8
11′	6.01 s	32.8	5.89 s	32.4
12′	5.51 dd (1.6, 3.2)	126.6	5.51 dd (1.7, 3.2)	127.4
13′		137.4		137.1
14′α	3.16 d (13.1)	37.7	3.15 d (13.1)	37.7
14′β	2.25 dd (4.9, 13.1)		2.23 dd (4.9, 13.1)	
15′	1.83 s	27.3	1.82 s	27.3
5′-OCH_3			4.00 s	56.0
7′-OCH_3	3.76 s	56.3	3.75 s	56.3
*N*H	7.01 br s		7.10 br s	

indices of hydrogen deficiency, requiring the presence of an additional ring in **1**.

The HMBC correlations from H_3-13/H_3-14 to C-11/C-12, from H_3-15′ to C-12′/C-13′/C-14′, and from H_2-14′ to C-3/C-12′/C-13′, as well as the ^{1}H−^{1}H COSY correlations of H-11/H-10 and H-11′/H-12′ indicated the presence of two isopentenyl groups in **1**. These two isopentenyl groups were linked at C-3 of the indole moiety and C-8′ of the coumarin unit, respectively, on the basis of HMBC correlations from H-10 to C-2/C-3/C-9 and from H-11′ to C-7′/C-8′/C-9′. Moreover, the HMBC correlations from H_2-14′ to C-3, from H-10 to C-13′/C-14′, and from H-11′ to C-2/C-3 as well as the ^{1}H−^{1}H COSY correlation of H-10/H_2-14′ suggested the connections of C-10/C-14′ and C-2/C-11′. Thus, a heptacyclic ring was formed between the isopentenyl-substituted indole and coumarin moieties (Figure 2), and the planar structure of exotine A (**1**) was defined as a heterodimer as shown in Figure 2.

In the NOESY spectrum, the NOEs of H-14′α/H-11′, H-14′α/H-10, H-14′β/H_3-15′, and H-12′/H_3-15′ indicated a preferred boat conformation of the heptacycle, and the α-configurations of H-10 and H-11′ (Figure 2). The absolute configuration of **1** was defined as 11′*S*,10*R* by single-crystal X-ray diffraction analysis (Figure 3) using an anomalous scattering of Cu Kα radiation with a Flack parameter of 0.0(5).[17]

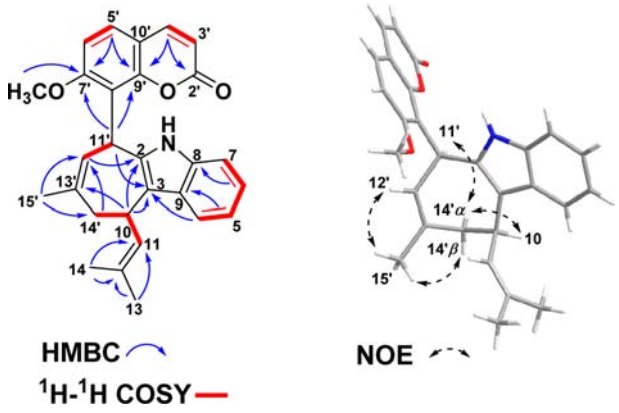

Figure 2. Key HMBC, ^{1}H−^{1}H COSY, and NOE correlations of **1**.

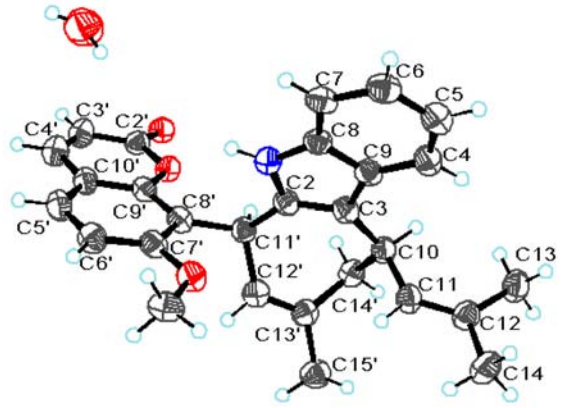

Figure 3. ORTEP drawing of **1** by Cu Kα radiation.

Moreover, we calculated the ECD spectrum of **1** using the TDDFT method at the B3LYP/6-311G(d) level to further confirm the X-ray result. The calculated ECD spectrum of (11′*S*,10*R*)-**1** agreed well with the experimental one (Figure 4).

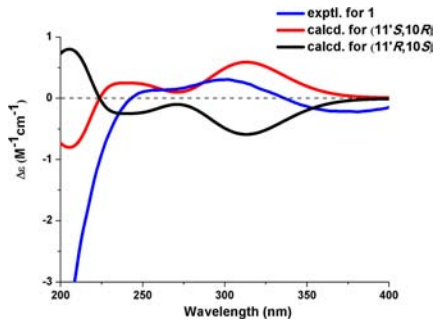

Figure 4. Comparison of the experimental ECD spectrum of **1** in MeOH (blue) with calculated ECD spectra for (11′*S*,10*R*)-**1** (red) and (11′*R*,10*S*)-**1** (black).

However, the atropisomerism between the planes of coumarin and indole moieties was found to also have a profound effect on the calculated ECD spectra. For example, it is hard to differentiate the calculated ECD spectra between (11′*S*,10*R*)-1*R* and (11′*R*,10*S*)-1*R* (Figure 5), suggesting that the relative spatial position of coumarin and indole moieties is also the necessary factor to be considered in the assignment of the absolute configuration of **1**. Finally, the absolute configuration

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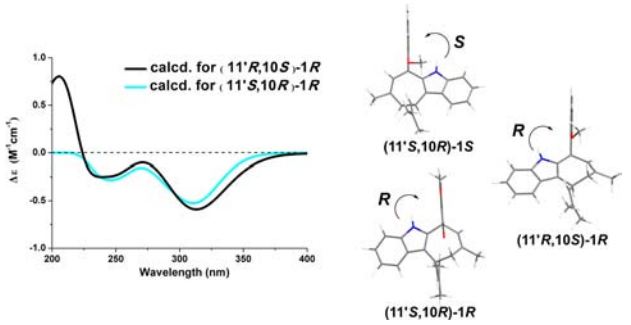


Figure 5. Comparison of the experimental ECD spectrum of (11′*R*,10*S*)-**1***R* in MeOH (black) with the calculated ECD spectrum of (11′*S*,10*R*)-**1***R* (blue).

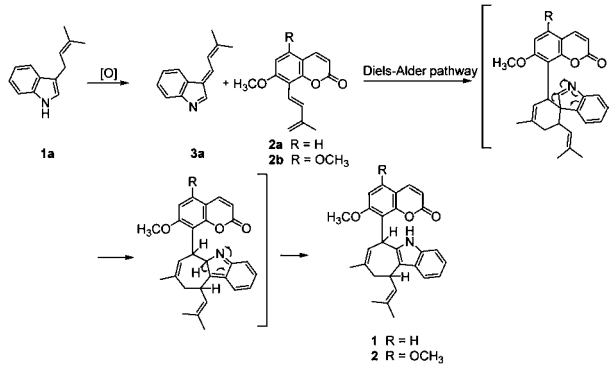
of exotine A (**1**) was deduced as (11′*S*,10*R*)-*S* based on the X-ray diffraction analysis and ECD spectrum.

Exotine B (**2**) was obtained as colorless needles, mp 250−251 °C, with $[\alpha]_D^{25}$ +14 (*c* 0.1, MeOH). Its molecular formula was determined as $C_{29}H_{29}NO_4$ on the basis of a protonated ion at *m*/*z* 456.2176 $[M + H]^+$ (calcd for $C_{29}H_{30}NO_4$, 456.2175) in the positive-ion HRESIMS and ^{13}C NMR data. The similar UV, IR, and NMR data to those of **1** indicated **2** shares the same structural scaffold with **1**. The only difference was the presence of an additional methoxy group [δ_H 4.00 (3H, s), δ_C 56.0] in **2**, which was located at C-5′ based on the HMBC correlation from $-OCH_3$ to C-5′. The similar NOESY correlations and ECD spectra between **1** and **2** suggested the (11′*S*,10*R*)-*S* configuration of **2**.

There were numerous articles about the isolation and synthesis of tetrahydrocyclohepta[*b*]indole derivatives,[18−20] and their various pharmacological properties such as the inhibition of deacetylase SIRT121 and antitubercular activity.[22] Yet, such unusual heterodimers of isopentenyl-substitued indole and coumarin derivatives, linked through a new fused heptacyclic ring skeleton, have never been reported. A plausible biogenetic pathway to exotines A (**1**) and B (**2**) was proposed via a hetero-Diels−Alder cycloaddition as illustrated in Scheme 1, using 3-prenylindole and *trans*-dehydroosthol derivatives, which had been isolated from *Murraya* species,[23−25] as the precursors.

To evaluate the biological properties of the isolates with such a new chemical skeleton, exotines A (**1**) and B (**2**) were tested on LPS-induced NO production in BV-2 microglial cells. As a result, exotine A (**1**) showed inhibition of NO production with an IC_{50} value of 9.2 μM, comparable to that of the positive control, quercetin (17.4 μM). As for exotine B (**2**) with one more methoxy group, the activity decreased (39.9 μM).

Scheme 1. A Plausible Biogenetic Pathway to 1 and 2



■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02230.

Experimental section (general experimental procedures, plant material, extraction and isolation, X-ray crystallographic analysis, and bioassay) and full spectroscopic data (NMR, MS, and IR) for exotines A (**1**) and B (**2**) were provided (PDF)

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Notes

The authors declare no competing financial interest.

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