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A novel isocoumarin with anti-influenza virus activity from *Strobilanthes cusia*

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ABSTRACT

Strobilanthes A (1), a novel isocoumarin with an unusual tetrahydro-4 H-pyran-4-one moiety fused isocoumarin core skeleton, together with a known compound (2) was isolated from *Strobilanthes cusia*. Its chemical structures were elucidated by 2D NMR spectroscopy, mass spectrometry and single-crystal X-ray diffraction analysis. The biosynthetic pathway of 1 could be supposed to be originally derived from 3-methylisocoumarin, a product of AA-MA pathway. Both of two compounds displayed anti-influenza virus activity *in vitro*.

1. Introduction

Strobilanthes cusia (Nees) Kuntze, a species of Acanthaceae family, is a well known folk medicine in Myanmar, India, Thailand, and southern part of China [1,2]. As a Traditional Chinese Medicine (TCM), the root of *S. cusia* called "Nan-Ban-Lan-Gen", has been used to treat influenza, epidemic cerebrospinal meningitis, encephalitis B, viral pneumonia, and mumps for a long time [3]. Our previous study reported three new indole alkaloid glycosides, strobilanthosides A–C, and five phenylethanoidglycosides isolated from the aerial parts of *S. cusia* [4]. Continuing studies on bioactive compounds of *S. cusia* resulted in isolation of a novel skeleton isocoumarin strobilanthes A (1) and a known compound 2(3H)-Benzoxazolinone (2) [5] with activity against influenza virus from the root of *S. cusia* cultivated in Guizhou province, China.

2. Experimental

2.1. General experimental procedures

Optical rotation values were determined on a Jasco P-1020 polarimeter. IR spectra were determined on a Bruker Tensor-27 infrared spectrophotometer with KBr disks. UV spectra were recorded on a Shimadzu double-beam 210A spectrophotometer (Shimadzu, Kyoto, Japan). 1D and 2D NMR spectra were performed on Bruker AM-400, DRX-500, and Avance III-600 spectrometers (Bruker Bio-Spin GmbH, Rheinstetten, Germany) with TMS as the internal standard. ESIMS and HREIMS analyses were carried out on a API Qstar-Pulsar-1 mass spectrometer (Applied Biosystems/MDS Sciex, Ontario, Canada) and Waters AutoSpec Premier P776 (Waters, Milford, MA, USA), respectively. The single-crystal X-ray diffraction experiment (Cu Kα radiation) was performed on a Bruker APEX DUO instrument. Column chromatography was performed using silica gel 80–100; 300–400 mesh; GF-254 (SiO2; Qingdao Meigao Chemical Co.), C_{18} reversed-phase silica gel (SiO2, 40–75 μm; Fuji Silysia Chemical Ltd.), and Sephadex LH-20 gel (GE Healthcare Bio-Sciences AB). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Co. Ltd., Qingdao, China).

2.2. Material

The roots of *S. cusia* were collected during September 2013 from Qiannan Buyi and Miao Autonomous Prefecture, Guizhou Province, China. The plant was identified by one of the authors (W. G.). A voucher specimen (DL-006) was deposited in the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powdered roots of *S. cusia* (70 kg) were extracted three times with MeOH–H_2O (375 × 3, 95:5, *v*/*v*) under conditions of

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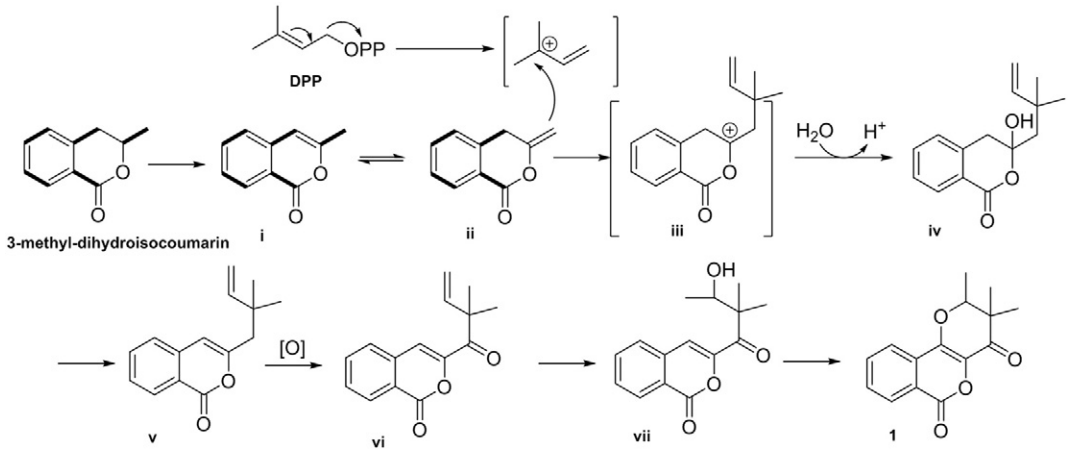

Scheme 1. The proposed biosynthetic pathway of 1.

reflux for 4 h. The solvent was evaporated under reduced pressure to yield a residue, which was suspended in H_2O and extracted successively with petroleum ether and EtOAc. The EtOAc-soluble fraction (592 g) was subjected to silica gel column chromatography (80–100 mesh) with a gradient elution of $CHCl_3$–MeOH [1:0, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1 (*v*/*v*)] to afford seven fractions. Fraction 4 was purified by C_{18} reversed-phase column followed by Sephadex LH-20 column and silica gel column chromatography to give compound 1 (40 mg). Fraction 3 was loaded onto a C_{18} reversed-phase column and eluted with a MeOH–H_2O step gradient (50% MeOH → 90% MeOH) to give subfractions 2a–2e. Subfraction 2b was fractionated on a Sephadex LH-20 column (MeOH) to obtain compound 2 (2.43 g).

2.3.1. Strobilanthes A (1)

$C_{15}H_{14}O_4$, colorless needle crystal; $[\alpha]_D^{18} = +79$ (c = 0.02, MeOH); IR (KBr) ν_{max} 3439, 1736, 1678, 1627, 1414, 1251, 1106 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESIMS (positive) *m*/*z* 281 $[M + Na]^+$; HRESIMS *m*/*z* 281.0792 $[M + Na]^+$ (calcd for $C_{15}H_{14}NaO_4$, 281.0790).

2.3.2. 2(3H)-Benzoxazolinone (2)

Pale reddish crystals (EtOH), mp 139° (ref. [5] 137–138°). UV λ_{max}^{MeOH}nm: 274. ^1H NMR (400 MHz, CD_3OD) δ: 10.08 (1H, *br* s, NH), 7.21–7.09 (4 H, m, H-4, H-5, H-6, H-7). ^{13}C NMR (100 MHz, CD_3OD) δ: 156.3 (C-2), 143.8 (C-7a), 129.5 (C-3a), 124.1 (C-7), 122.6 (C-4), 110.2 (C-6), 110.0 (C-5).

Table 1
^1H and ^{13}C NMR spectroscopic data for strobilanthes A (1) recorded at 400 MHz and 100 MHz in $CDCl_3$.

position	1	
	δ_C, type	δ_H, (*J* in Hz)
1	159.1, qC	
3	113.5, qC	
4	147.9, qC	
5	130.3, CH	8.36, d, (8.0)
6	134.7, CH	7.88, td (8.0, 1.3)
7	132.0, CH	7.76, td (8.0, 1.3)
8	123.0, CH	8.00, d (8.0)
9	131.0, qC	
10	130.7, qC	
11	189.3, qC	
12	44.9, qC	
13	83.0, CH	4.47, q (6.7)
14	17.2, CH_3	1.23, s
15	14.5, CH_3	1.15, s
16	19.7, CH_3	1.54, d (6.6)

2.4. X-ray diffraction study on Strobilanthes A (1)

Crystal data for cu_hgw12a_0m: $C_{15}H_{14}O_4$, $M = 258.26$, orthorhombic, $a = 7.1841(5)$ Å, $b = 17.2426(11)$ Å, $c = 20.3430(13)$ Å, $\alpha = 90.00°$, $\beta = 90.00°$, $\gamma = 90.00°$, $V = 2519.9(3)$ Å^3, $T = 100(2)$ K, space group *P*212121, $Z = 8$, μ(CuKα) = 0.817 mm^{-1}, 20,732 reflections measured, 4522 independent reflections ($R_{int} = 0.0598$). The final R_1 values were 0.0530 ($I > 2\sigma(I)$). The final *wR* (F^2) values were 0.1447 ($I > 2\sigma(I)$). The final R_1 values were 0.0536 (all data). The final *wR* (F^2) values were 0.1459 (all data). The goodness of fit on F^2 was 1.051. Flack parameter = 0.00(18). The Hooft parameter is 0.06(6) for 1863 Bijvoet pairs.

2.5. Anti-influenza A virus (H1N1) assay

The antiviral activity against H1N1 was evaluated by the CPE inhibition assay. Confluent MDCK cell monolayers were incubated with influenza virus (A/Puerto Rico/8/34 (H1N1), PR/8) at 37 °C for 1 h and the multiplicity of infection (MOI) of PR/8 virus was about 0.1. After removing the virus dilution, cells were maintained in infecting media (RPMI 1640, 4 μg/mL of trypsin) containing different concentrations of test compounds at 37 °C. After 48 h incubation at 37 °C, the cells were fixed with 100 μL of 4% formaldehyde for 20 min at room temperature. After removal of the formaldehyde, the cells were stained with 0.1% crystal violet for 30 min. The plates were washed and dried, and the intensity of crystal violet staining for each well was measured in a microplate reader (Bio-Rad, USA) at 570 nm. The IC_{50} value was calculated as the compound concentration required inhibiting influenza virus yield at 48 h post-infection by 50%. Ribavirin was used as the positive control with an IC_{50} value of 137.3 ± 0.4 μM.

3. Results and discussion

A methanol (MeOH) extract of dried roots of *S. cusia* was suspended in water and partitioned successively with petroleum ether, EtOAc and *n*-butanol. The EtOAc-soluble part was subjected to column chromatography over silica gel, RP C18 silica gel, and Sephadex LH-20 followed by semipreparative HPLC to afford strobilanthes A (1) and 2(3H)-Benzoxazolinone (2).

Compound 1 was obtained as a colorless needle crystal (MeOH) with $[\alpha]_D^{18} = +79$ (c = 0.02, MeOH), its molecular formula, $C_{15}H_{14}O_4$, was assigned based on HREIMS (*m*/*z* 281.0792 $[M + Na]^+$, calcd for $C_{15}H_{14}O_4$ $[M + Na]^+$, 281.0790), corresponding to 9° of unsaturation. The UV absorptions at 253, 321, and 334 nm indicated the presence of aromatic moiety in 1, and the IR absorptions at 1736, 1678 cm^{-1} showed the presence of different conjugated carbonyl group in 1. The

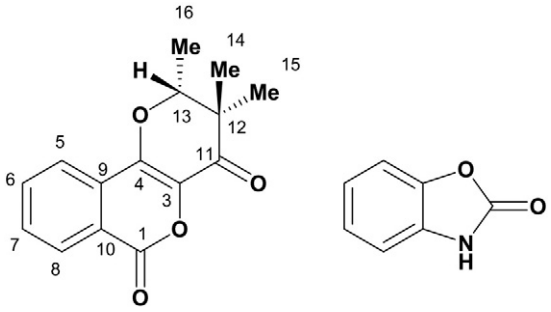

Fig. 1. Structures of 1 and 2.

^{13}C NMR and DEPT data (Table 1) revealed 15 carbon signals comprising ten sp^2 carbon atoms (4×CH and 6×qC) and five sp^3 carbon atoms (3×CH_3, 1×CH and 1×qC). Two sp^2 carbon signals (δ_C 189.3 and 159.1) indicated the existence of two carbonyl groups, and the rest eight sp^2 carbon atoms were assumed as four double bond in 1. Since these ten sp^2 carbon atoms were accounted for six out of nine degrees of unsaturation, the remaining four degrees of unsaturation were assumed for presence of a tricyclic system in 1(Fig. 1). Its ^{1}H–^{1}H COSY spectrum revealed that 1 possessed only one spin coupling system: H-5/H-6/H-7/H-8, corresponding to its ^{1}H NMR data (δ_H 8.36, d, 8.0; δ_H 7.88, td, 8.0, 1.3; δ_H 7.76, td, 8.0, 1.3; δ_H 8.00, d, 8.0), which suggested the presence of *o*-disubstituted phenyl moiety in 1 (Fig. 2). In the HMBC spectrum, cross-peaks of H-5 (1H, δ_H 8.36, d, 8.0) to C-1(δ_C 159.1) and C-6 (δ_C 134.7), and H-8 (1H, δ_H 8.00, d, 8.0) to C-4 (δ_C 147.9) and C-7 (δ_C 132.0), combined with its ^{1}HNMR data as above and its IR and UV data, indicated the presence of isocoumarin core in 1 [6]. Furthermore, the HMBC cross-peaks of H-13 (1H, δ_H 4.47, q, 6.7) to C-4 (δ_C 147.9) and C-12 (δ_C 44.9), and H-16 (3H, δ_H 1.54, d, 6.6) to C-12 (δ_C 44.9) and C-13 (δ_C 83.0), as well as H-14 (3H, δ_H 1.23, s) and H-15 (3H, δ_H 1.15, s) to C-11 (δ_C 189.3), C-12 (δ_C 44.9) and C-13 (δ_C 83.0), indicated the presence of fragment of O-CH(Me)-CMe_2-CO. Thus, planar structure of 1 was proposed as shown in Fig. 2.

To confirm the structure and absolute configuration of 1, compound was crystallized from MeOH to afford an orthorhombic crystal with the space group $P2_12_12_1$, which was analyzed by X-ray crystallography. The final refinement of the Cu Kα data resulted in a flack [7] parameter of 0.00 (18) and the Hooft [8] parameter of 0.06 (6), which allowed unambiguous assignment of the absolute configuration of C-13 of 1 as *R*-configuration (Fig. 3).

The biosynthetic pathway of 1 could be supposed to be derived from 3-methylisocoumarin, a product of AA-MA biosynthetic pathway [9]. The migration of double bonds both for intermediate I and DPP resulted in formation of intermediate IV, which was further undergone dehydrolation and oxidation to give VI. The intramolecular condensation of VII to generate strobilanthes A (1). (See Scheme 1)

The inhibitory effects on influenza A virus (H1N1) of compounds 1 and 2 were evaluated *in vitro* [10–11], which exhibited moderated inhibitory activity with IC_{50} = 29.2 ± 5.8 μM, CC_{50} = 474.0 ± 6.4 μM,

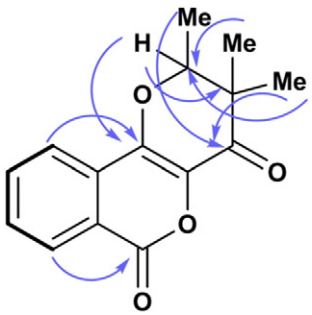

Fig. 2. ^{1}H–^{1}H COSY (bold) and key HMBC correlations of 1.

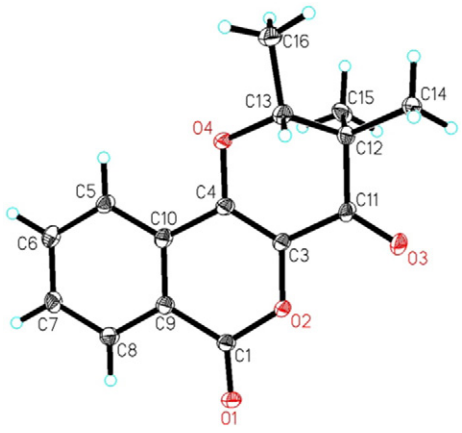

Fig. 3. Single-crystal X-ray structure of 1.

and selective index (SI) of 16.2 for 1, and IC_{50} = 46.0 ± 8.4 μM, CC_{50} = 351.2 ± 10.9 μM, and selective index (SI) of 7.6 for 2, respectively (Ribavirin as positive control, IC_{50} = 32.8 ± 4.3 μM, CC_{50} = 797.0 ± 14.6 μM, and SI = 24.3).

Conflict of interest

The authors declare no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2015.10.009.

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