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A structure and an absolute configuration of (+)-alternamin, a new coumarin from *Murraya alternans* having antidote activity against snake venom

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Abstract—(+)-Alternamin (**1**), a new dihydrofuranocoumarin, was isolated from the aerial parts of *Murraya alternans* (Kurz) Swingle. The analysis 2-D NMR correlation of (+)-**1** led to either of linear dihydrofuranocoumarin (**2A**, **2C**) or angular one (**2B**). An IR and a vibrational circular dichroism (VCD) studies were conducted to distinguish the structure and to assign the absolute configuration. By comparison of the observed spectra with the calculated spectra for (*S*)-**2A**, (*S*)-**2B**, and (*R*)-**2C**, the molecular structure of (+)-**1** was determined to be (*S*)-(+)-5,8-dimethoxymarmesin. The compound exhibited antidote activity against snake venom from *Trimeresurus flavoviridis*, affording experimental support for the pharmacological use of *M. alternans*.

Murraya alternans (Kurz) Swingle (Myanmar name: Naganaing), belonging to the Rutaceae family, is a small tree inhabiting the deltaic areas of southern Myanmar, including the Hinthada, Pathein, and Bago areas. It is thought that the fresh juice of the Naganaing leaves can neutralize the toxic action of snake venoms. According to Myanmar tradition, the Naganaing leaves taste bitter to a normal person, while a person with snake venom in his body does not taste the bitterness of the leaves. A variety of compounds have been isolated from *Murraya* species,[1] however, to the best of our knowledge, none of them is known to have antidote activity to snake venom.

In searching for an active compound, we have isolated a new coumarin, (+)-alternamin (**1**), from *M. alternans*. However, the lack of an aromatic proton in **1** hampered the discrimination among **2A**, **2B**, and **2C** (Fig. 1) by 2-D NMR correlation. Moreover, since both enantiomers, linear[2] and angular,[3] have been reported for α-hydroxyisopropyldihydrofuranocoumarin, the absolute configuration of (+)-**1** is particularly of interest. In this study, we conducted IR and vibrational circular dichroism (VCD) to elucidate the structure and absolute configuration. VCD measures the difference in absorption for left versus right circularly polarized infrared radiation. This technique has been utilized as a reliable and convenient tool to determine the absolute configuration and the conformation of chiral compounds.[4] VCD is sensitive not only to the molecular chirality but also to the structure, however, its use in differentiating structural isomers has been limited to only one recent report that studied a synthetic compound with known absolute stereochemistry.[5] Here, we report the isolation of (+)-**1** and its structural and absolute configuration elucidation with the aid of VCD as well as IR. Finally, (+)-**1** is shown to be the first active antidote compound against snake venom isolated from *M. alternans*.

The whole plant of *M. alternans* was air dried and powdered. The plant (25 g) was extracted with ethanol,

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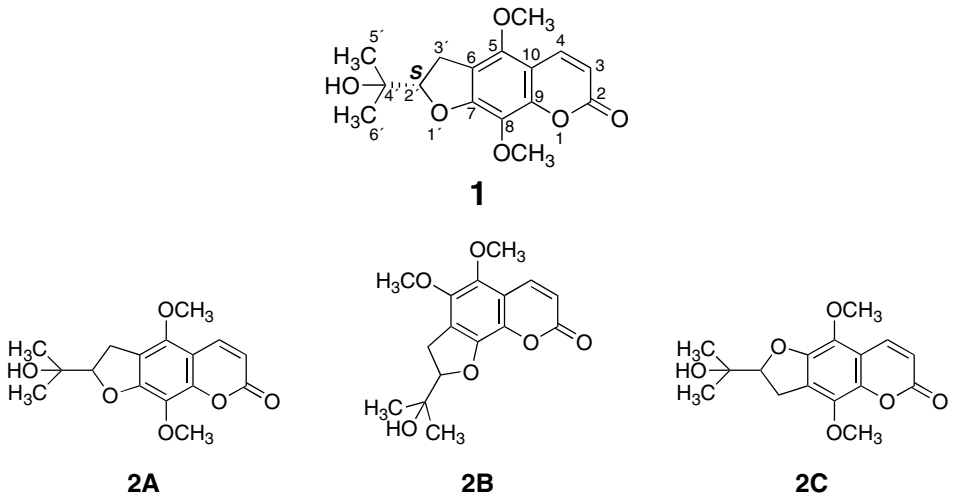

Figure 1. Possible structures for **1**. Compound **2A**, **2B**, and **2C** are classified as linear, angular, and linear dihydrofuranocoumarin, respectively.

and the extract (1.6 g) was fractionated by silica-gel column chromatography to afford five fractions (MA-A to MA-E). Among these fractions, MA-B one, which was eluated by hexane–ethyl acetate (1:1–3:7), exhibited complete inhibition of hemorrhage caused by snake venom of *Trimeresurus flavoviridis* at a dose of 0.25 mg/mL. The ethanol extract also inhibited venom-induced hemorrhage at the same concentration.[6a] The antidote active fraction (113 mg) was further separated by repeated silica-gel column chromatography to afford 26 mg of (+)-alternamin (**1**).

(+)-Alternamin (**1**), $[\alpha]_D$ +31.5 (*c* 0.25, $CHCl_3$) was found to have the molecular formula $C_{16}H_{18}O_6$ determined by high-resolution FABMS (*m/z* 306.1046, Δ −0.7 mmu). The ^1H and ^{13}C NMR spectra along with DEPT spectra in chloroform-*d* of **1** demonstrated the presence of two methyl groups, two methoxyl groups, one methylene carbon, one methine carbon, one oxygen attached quaternary carbon (δ_C 91.3), a pair of disubstituted double bonds (δ_C 110.9, δ_H 6.13 and δ_C 139.2, δ_H 7.89) and seven sp^2 carbons, including one carbonyl group.[7] HMQC and HMBC (Fig. 2) spectra proposed three partial structures for (+)-alternamin (**1**), a dihydrobenzofuran moiety due to an intramolecular cyclization of the isoprenyl side chain, a methoxybenzene, and a coumarin moiety. The benzene ring in each partial structure is common, so the proposed partial structures shown in Figure 2 suggested the possible structures with different positions of the dihydrofuran ring (**2A**, **2B**, and **2C** in Fig. 1). This proposal was plausible since furanocoumarins are known to biosynthetically occur both as a linear type and as an angular type.[2b,3a] Observed NOE signals between 5-OCH_3 (δ 3.95) and H-4 (δ 7.89) and between 5-OCH_3 and H-3′ (δ 3.39, 3.34) suggested that the reliable structure could be **2A**.[8]

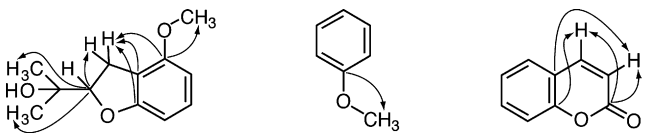

Figure 2. HMBC correlations and partial structures of **1**.

To confirm the structure and the absolute configuration of (+)-**1**, a nonempirical analysis of IR and VCD using the density functional theory (DFT) calculation method was performed for **2A**, **2B**, and **2C**. Prior to the calculation of IR and VCD, conformational analyses were carried out to define the stable conformations and their relative energies, arbitrarily starting from (*S*)-**2A**, (*S*)-**2B**, and (*R*)-**2C** (the α-hydroxyisopropyl group directed back in all structures). First, conformational analyses using the CONFLEX program with the MMFF94S force fields were performed.[9] 9, 8, and 4 conformers were selected from 37 (**2A**), 59 (**2B**), and 13 (**2C**) nonredundant ones, whose sum of the Boltzmann weighted populations were over 95%. The geometries of the selected conformers were optimized with DFT calculations at the B3PW91/6-311++G(d,p) level of theory. The IR and VCD spectra were calculated for the optimized geometries, based on the harmonic vibrational analyses at the B3PW91/6-311++G(d,p) level. The sets of frequencies and intensity were convoluted with Lorentz-like profile functions. The final spectra were obtained as a weighted average of them based on their Boltzmann weighted populations with 6, 6, and 4 conformers for (*S*)-**2A**, (*S*)-**2B**, and (*R*)-**2C**, respectively.[10] The frequencies of the spectra were scaled with a factor 0.97. Calculated IR and VCD spectra of (*S*)-**2A**, (*S*)-**2B**, and (*R*)-**2C** were considerably different, and the difference is even more obvious in the VCD spectra (Fig. 3).

The experimental IR and VCD spectra of (+)-**1** were measured in $CDCl_3$ and then compared with the theoretical spectra of (*S*)-**2A**, (*S*)-**2B**, and (*R*)-**2C** (Fig. 3). The observed IR and VCD spectra showed good agreement with the calculated ones for (*S*)-**2A**, but exhibited an apparent discrepancy with those for (*S*)-**2B** and (*R*)-**2C**. The correspondence of the signs of overall VCD peaks also confirmed the absolute configuration of (+)-alternamin (**1**) as (*S*). This result straightforwardly led us to ascertain the structure of naturally occurring (+)-alternamin (**1**) as (*S*)-**2A**. The potential for the simultaneous determination of the structure and the absolute configuration revealed in this work will encourage further VCD application in structural

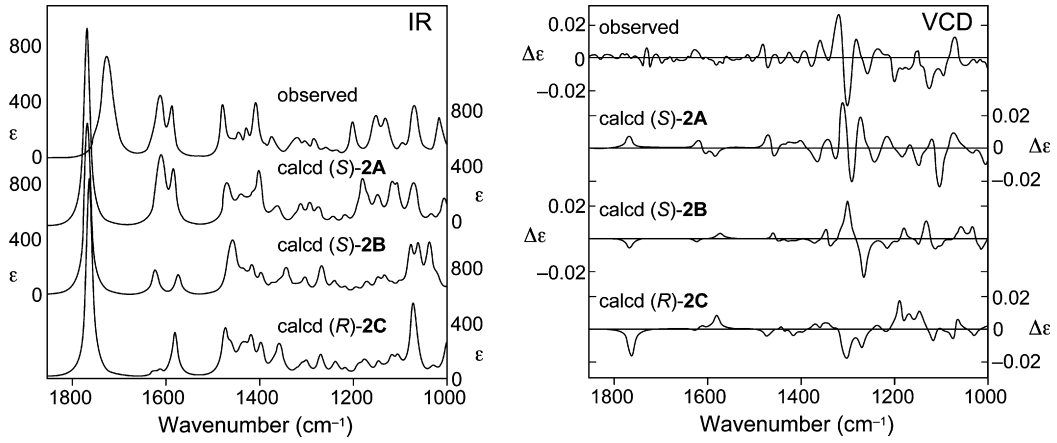

Figure 3. Comparison of IR (left frame) and VCD (right frame) spectra observed with those calculated for (*S*)-**2A**, (*S*)-**2B**, and (*R*)-**2C**. ($CDCl_3$, *c* 0.15 M, *l* 71 μm).

analysis studies. Some recent electronic circular dichroism (ECD) studies suggested that ECD also has the potential for the same purpose,[11] while totally chiroptical analysis including VCD, ECD, and optical rotation with the aid of theoretical calculation, has become a trend for determination of absolute configuration.[12] The antidote activity of (+)-alternamin (**1**) against *T. flavoviridis* venom was evaluated by the modified method reported by Bjarnason and Tu.[6b] A solution of (+)-**1** at the concentration of 0.25 mg/mL inhibited hemorrhage induced by the venom by 24% as compared to control. This evidence confirmed, for the first time, that the plant contains compounds with high antivenom activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.06.156.

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6. (a) Hemorrhagic activity was assayed as follows. White mice (4 w, male, ddY strain) were injected subcutaneously in the abdomen with 0.1 mL of test solution, and killed after 24 h. The skin was rapidly removed, and the diameter of each hemorrhagic spot was measured. A test solution was prepared by mixing a solution of snake venom of *T. flavoviridis* (0.075 mg/mL in saline, 50 μL), and a sample solution (0.50 mg/mL in 20% DMSO–saline, 50 μL) followed by 1 h incubation. In the same way, a solution without sample was injected to the control group. The inhibitory activity was evaluated by the ratio of the diameter of the hemorrhage of the test group and the control group; (b) Bjarnason, J. B.; Tu, A. T. *Biochemistry* **1978**, *17*, 3395–3404.
7. (+)-Alternamin (**1**): ^{1}H NMR ($CDCl_3$) δ 7.89 (1H, d, $J = 9.7$ Hz; H-4), 6.13 (1H, d, $J = 9.7$ Hz; H-3), 4.75 (1H, dd, $J = 9.1$, 8.4 Hz; H-2′), 3.97 (3H, s; C-8-OCH_3), 3.95 (3H, s; C-5-OCH_3), 3.39 (1H, dd, $J = 15.3$, 8.4 Hz; H-3′), 3.34 (1H, dd, $J = 15.3$, 9.1 Hz; H-3′), 1.39 (3H, s; H-5′), 1.27 (3H, s; H-6′); ^{13}C NMR ($CDCl_3$) δ 160.8 (C-2), 155.7 (C-7), 148.1 (C-9), 147.5 (C-5), 139.2 (C-4), 127.2 (C-8), 112.9 (C-6), 110.9 (C-3), 106.8 (C-10), 91.3 (C-2′), 71.5 (C-4′), 61.2 (C-8-OCH_3), 60.0 (C-5-OCH_3), 28.9 (C-3′), 25.9 (C-5′), 24.6 (C-6′); IR ($CDCl_3$) 3452, 1722, 1373, 1281, 828, 664 cm^{-1}, UV (CH_3CN, nm) λ_{max} (ε): 211 (40,700), 230 (sh, 14,700), 262 (6900), 331 (18,400); CD (CH_3CN) λ_{ext} ($\Delta\varepsilon$): 211 (+8.6), 235 (−1.3), 247 (+0.2), 266 (+0.3), 332 (−1.3).
8. Differential NOE experiments of **1** were performed as well as a NOESY one. Small difference of chemical shift between C-5-OCH_3 and C-8-OCH_3 made their complete assignments uncertain.

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10. The weights of the highest-lying conformers used for each isomer were 2–6%. Therefore, contributions to the whole calculated spectra of the energetically higher conformers could be fairly diminutive.
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