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# Asterogynins: Secondary Metabolites from a Costa Rican Endophytic Fungus

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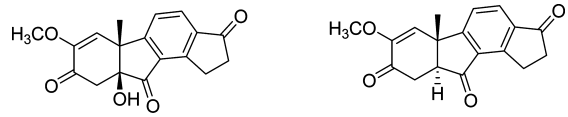
## ABSTRACT




**1** **2**

**An endophytic fungus isolated from the small palm *Asterogyne martiana* produced two unusual steroid-like metabolites, asterogynin A (1) and asterogynin B (2), along with the known compounds viridiol (3) and viridin (4). Asterogynins A and B were characterized by NMR and MS spectroscopic analysis.**

Fungi have made noteworthy contributions to our store of naturally occurring small molecules as they have contributed more than a quarter by most reckonings, and these contributions have come from only a tiny fraction of the world's estimated 1.5 million fungal species. Only ∼5% of the fungal species have been scientifically studied, and a minority of these have been studied chemically.[1−3] The endophytic fungi that live inside of vascular plants constitute one of the richest sources of poorly examined fungi. As part of a longstanding collaborative research project with INBio (National Biodiversity Institute), we have begun characterizing some of the chemical diversity of Costa Rican endophytes. Costa Rica's location on the slender land bridge between North and South American organisms makes it a natural mixing bowl for the organisms of both continents. As a result, the country's many different ecological niches contain over 9000 species of vascular plants.

In one recent project, extracts from Costa Rican endophytes were screened for their ability to bind *Pf*Hsp86, an essential protein-folding chaperone from *Plasmodium falciparum*, the parasite responsible for the most deadly form of human malaria. *Plasmodium falciparum* encodes three full-length Hsp90 genes for the proteins *Pf*Hsp86, *Pf*GRP94, and *Pf*TRAP1. *Pf*Hsp86 has 59% amino acid identity with human Hsp90α2, and its highly conserved ATP-binding Bergerat fold has 75% identity. *Pf*Hsp86 could be a drug target for malaria as the *Plasmodium* parasites transition between cold-blooded mosquito vectors and warm-blooded and often febrile human hosts, a transition that should create a substantial requirement for assisted protein folding.[4] Some known human Hsp90 inhibitors, like geldanamycin, inhibit parasite growth through *Pf*Hsp86 inhibition.[4]

The dichloromethane extract of CR1488E, which was isolated from the host plant *Asterogyne martiana* (Arecaceae) and whose closest relative based on DNA sequencing is *Chalara alabamensis*, was active with an $EC_{50}$ value of ∼24 μg/mL. Here we report the isolation and structure elucidation of asterogynins A (**1**) and B (**2**) from CR1488E.

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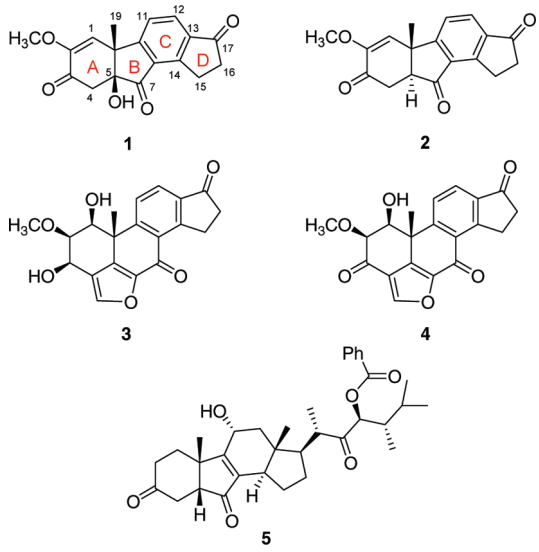
The dichloromethane extract of CR1488E was separated by C-18 prep-HPLC to yield compounds **1**, **2**, **3**, and **4**. Compounds **3** and **4** were identified as the known natural products viridiol[5] and viridin,[6] respectively. The viridin class of steroidal furans contains potent antifungal agents and covalent inhibitors of phosphatidylinositol 3-kinase (PI3 kinase) and polo-like kinase.[7] As viridin-like compounds react with the ATP-binding site of kinases, it is likely that they could also bind the ATP pocket of chaperone and other proteins.




The $^1$H NMR spectrum of **1**[8] in $CD_3OD$ showed two aromatic protons and one olefinic proton, one methoxy, three methylenes, and one methyl group. The $^{13}$C NMR spectrum exhibited 18 signals, including three carbonyls, six aromatic carbons, one double bond, two quaternary and three secondary carbons, one methoxy, and one methyl group. These assignments were further confirmed by the HSQC spectrum. The HRMS (positive-ion mode) had an ion peak at *m/z* 295.0969, consistent with a molecular composition of $C_{18}H_{15}O_4$ ([M − $H_2O$ + H], calcd 295.0970), a molecular formula that required 11 double-bond equivalents. Besides three carbonyls, one double bond, and an aromatic ring, there must be three more rings in the molecule. In the COSY spectrum of **1**, two cross-peaks from two coupling systems [CH═CH (aromatic: $\delta_H$ 7.78, d, $J$ = 8.0 Hz, H-11; 8.01, d, $J$ = 8.0 Hz, H-12) and $CH_2$−$CH_2$ ($\delta_H$ 3.37, m, H-15; 2.74, m, H-16)] were observed. Rings C and D were readily established from the HMBC correlations between the carbonyl at ring D and one aromatic proton and the two coupling methylenes. The $^{13}$C chemical shifts of the carbons in rings C and D ($\delta_c$ 130.6, C-8; 166.1, C-9; 131.6, C-11; 124.8, C-12; 139.0, C-13; 156.8, C-14; 25.3, C-15; 37.0, C-16; 207.9, C-17) matched those of demethoxyviridin and its analogues[9] very well, which further confirmed these two rings. The carbonyl in ring A ($\delta_c$ 192.5, C-3) had to be an α,β-unsaturated ketone ($\delta_c$ 120.7, C-1; 150.5, C-2) since its $^{13}$C chemical shift was <195 ppm, and the olefinic proton ($\delta_H$ 6.05, s, H-1) showed a strong HMBC correlation to that carbonyl carbon. In the HMBC spectrum (Figure 1), the

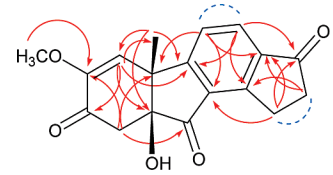


**Figure 1.** Key HMBC (arrows) and COSY (dashed curves) correlation of (**1**).

methyl group ($\delta_H$ 1.54, s, $H_3$-19) had correlations with the protonated olefinic carbon at $\delta_c$ 120.7 (C-1), which indicated that it must be at the β-position of the α,β-unsaturated ketone, one aromatic carbon ($\delta_c$ 166.1, C-9), and two quaternary carbons ($\delta_c$ 49.0, C-10; 82.5, C-5), one of which was oxygenated. Although no HMBC correlations in $CD_3OD$ between the third methylene and any carbon was observed, rings A and B were deduced to be six- and five-membered rings, respectively, with the oxygenated quaternary carbon connected to the methylene ($\delta_c$ 44.5, C-4) at ring A and carbonyl ($\delta_c$ 204.2, C-7) at ring B. To check this, both HSQC and HMBC spectra of compound **1** were collected in $C_6D_5N$, and correlations between the methylene at ring A and C-2, C-3, C-5, C-7, and C-10 were observed. In the ROESY spectrum of **1** in $C_6D_5N$, $H_3$-19 showed correlation to 5-OH (Figure 2), indicating a *cis* relationship between these two functional groups. Hence, the structure of **1** was determined as shown.

Compound **2**[10] had a molecular formula of $C_{18}H_{16}O_4$. The only difference between **1** and **2** was the substituent at C-5. In the HMBC spectrum of **2**, the methyl group had correlations to the protonated olefinic carbon, one aromatic carbon and the quaternary carbon, and the tertiary carbon, indicating a methine at the 5 position. No ROESY cross-peak between $H_3$-19 and 5-H was observed. Hence, the structure of **2** was determined as shown.

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(8) **Asterogynin A (1)**: colorless powder; $[\alpha]^{26}_D$ −5.4 (*c*, 0.11 MeOH); UV (MeOH) $\lambda_{max}$ (log *e*) 235, 312 nm; $^1$H NMR (400 MHz, pyridine-$d_5$) d 1.84 s ($H_3$-19), 2.62 m ($H_2$-16), 3.26 m ($H_2$-15), 3.26 m (H-4a), 3.46, $J$ = 16 Hz, d (H-4b), 3.57 s (−OMe), 6.12 s (H-1), 7.76 d, $J$ = 8.0 Hz (H-11), 8.12 d, $J$ = 8.0 Hz (H-12), 8.92 s (5-OH); $^1$H NMR (600 MHz, $CD_3OD$) d 1.54 s ($H_3$-19), 2.74 m ($H_2$-16 and H-4a), 2.94 m (H-4b), 3.37 m ($H_2$-15), 3.56 s (−OMe), 6.05 s (H-1), 7.78 d, $J$ = 8.0 Hz (H-11), 8.01 d, $J$ = 8.0 Hz (H-12); $^{13}$C NMR (150 MHz, $CD_3OD$) d 25.3 (C-15), 25.8 (C-19), 37.0 (C-16), 44.5 (C-4), 49.0 (C-10), 55.8 (OMe), 82.5 (C-5), 120.7 (C-1), 124.8 (C-12), 130.6 (C-8), 131.6 (C-11), 139.0 (C-13), 150.5 (C-2), 156.8 (C-14), 166.1 (C-9), 192.5 (C-3), 204.2 (C-7), 207.9 (C-17); HRMS *m/z* 295.0969 ([M − $H_2O$ + H], calcd for $C_{18}H_{15}O_4$, 295.0970).
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(10) **Asterogynin B (2)**: colorless powder; $[\alpha]^{26}_D$ +46 (*c*, 0.03 MeOH); UV (MeOH) $\lambda_{max}$ (log *e*) 235, 312 nm; $^1$H NMR (600 MHz, $CD_3OD$) d 1.84 s ($H_3$-19), 2.14 and 3.29 m ($H_2$-4), 3.31 and 3.40 m ($H_2$-15), 3.45 s (−OMe), 5.91 s (H-2), 7.80 d, $J$ = 7.8 Hz (H-11), 8.03 d, $J$ = 7.8 Hz (H-12); $^{13}$C NMR (150 MHz, $CD_3OD$) d 24.5 (C-19), 25.4 (C-15), 36.4 (C-16), 38.9 (C-4), 45.6 (C-10), 55.2 (OMe), 53.3 (C-5), 123.3 (C-1), 124.0 (C-12), 130.8 (C-8), 132.6 (C-11), 138.6 (C-13), 149.8 (C-2), 156.0 (C-14), 168.1 (C-9), 192.4 (C-3), 207.8 (C-17); HRMS *m/z* 297.1123 (calcd for $C_{18}H_{17}O_4$, 297.1127).

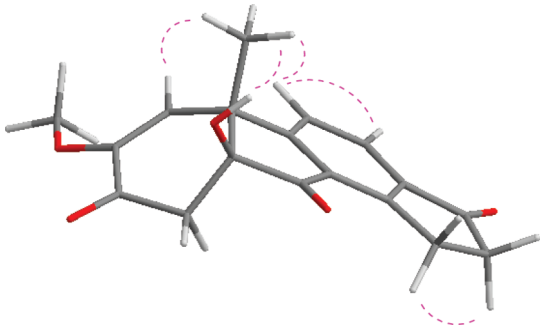

**Figure 2.** Key ROESY correlation of **1**.

Because of their overall structure and association with viridin (**4**) and viridiol (**3**), asterogynins A (**1**) and B (**2**) are likely sterol derivatives with a tetracyclic (6−5−6−5) carbocyclic ring system, which differs from the tetracyclic (6−6−6−5) of **3**, **4**, and other steroids. Only a few B-norsteroids (**5** is typical)[11] with more complex structures have been previously reported from other sources. The asterogynins A (**1**) and B (**2**) are quite different in having no remnants of the typical steroid side chain. They also lack the furan ring of **3** and **4**. Viridin (**4**) is a modified steroid,[12] and it is therefore likely that asterogynins A (**1**) and B (**2**) are also derived from the steroidal pathway. Whether they are further elaborations of viridin/viridiol through oxidative removal of the furan ring or arise from a separate pathway is not clear.

All four compounds were tested against *Pf*Hsp86, but only compound **3** was active with an $EC_{50}$ value of 5.5 ± 1.2 $\mu$g/mL. Further biological evaluations of compounds **1** and **2** are now being conducted and will be reported in due course.

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**Supporting Information Available:** Experimental procedure, and selected NMR (1D, 2D) and HRMS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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