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A Novel Cyclooxygenase-Inhibitory Stilbenolignan from the Seeds of *Aiphanes aculeata*

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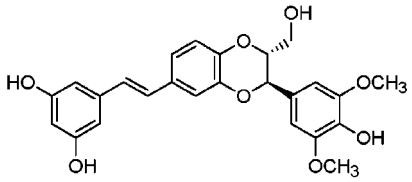
ABSTRACT



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Aiphanol (1), a novel stilbenolignan, along with isorhapontigenin (2), piceatannol (3), and luteolin, were isolated by bioassay-guided fractionation from the seeds of *Aiphanes aculeata* Willd. (Arecaceae). The structure of compound 1 was elucidated by spectroscopic methods. Compound 1 is based on an unprecedented stilbenolignan skeleton in which a stilbene moiety is linked with a phenylpropane unit through a dioxane bridge. Compounds 1 and 2 exhibited significant inhibitory activities against cyclooxygenases-1 and -2.

Stilbenoids have been found in a number of plant species and are of interest from a pharmacological point of view.[1−3] Recently, Pezzuto and colleagues established the cancer chemopreventive potential of *trans*-resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) in various assays reflective of the three major stages of carcinogenesis.[4,5] In our search for naturally occurring cancer chemopreventive agents, the seeds of *Aiphanes aculeata* Willd. (Arecaceae),[6] collected in Peru, were investigated. No previous biological and phytochemical investigations on this plant have been reported. The bioassay-guided chromatographic separation of an EtOAc-soluble extract of *A. aculeata* using the in vitro cyclooxygenase-1 (COX-1) inhibitory assay resulted in the isolation of a novel stilbenolignan, aiphanol (**1**), as well as the known stilbenes, isorhapontigenin (**2**)[7] and piceatannol (**3**),[8] and the flavone, luteolin.[9] Compound **1** represents a novel carbon skeleton having a stilbene−phenylpropane unit with a dioxane moiety. This communication deals with the isolation and structural characterization of **1** and the biological evaluation of the four compounds isolated.

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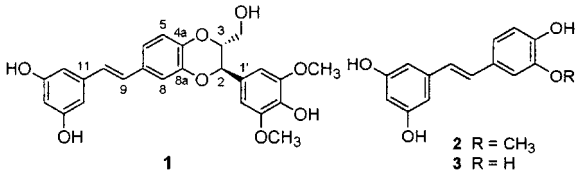
The dried seeds of *A. aculeata*[10] (5.8 kg) were ground and extracted with MeOH by maceration. After filtration and concentration, the resultant extract was partitioned with hexane and EtOAc, respectively, to afford hexane-soluble (48.0 g) and EtOAc-soluble (51.0 g) residues. Bioassay-guided fractionation of the EtOAc-soluble residue using the cyclooxygenase-1 (COX-1) inhibitory assay, applying successive Si gel and Sephadex LH-20 column chromatography and HPLC steps, resulted in the isolation of aiphanol[11] (**1**, 6.0 mg, 0.00008% w/w), along with three known constituents, isorhapontigenin[7] (**2**, 8.0 mg, 0.00014% w/w), piceatannol[8] (**3**, 250 mg, 0.0043% w/w), and luteolin[9] (9.0 mg, 0.00015% w/w).



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Compound **1** was obtained as an amorphous brown powder and was shown to possess a molecular formula of $C_{25}H_{24}O_8$ by HRMS. The ^{1}H NMR spectrum of **1** showed protons of an AMX system at δ_H 6.90 (1H, d, J = 8.3 Hz, H-5), δ_H 7.08 (1H, dd, J = 1.9 and 8.4 Hz, H-6), and δ_H 7.13 (1H, d, J = 1.9 Hz, H-8), protons of an AX_2 system at δ_H 6.56 (2H, d, J = 2.0 Hz, H-12) and δ_H 6.28 (1H, brt, J = 1.9 Hz, H-14), and signals of a trans double bond at δ_H 6.94 (1H, d, J = 16.4 Hz, H-9) and δ_H 7.02 (1H, d, J = 16.3 Hz, H-10). These signals were suggestive of the presence of a stilbene moiety,[7,8] which was substantiated by the HMQC NMR experiment. Additionally, signals at δ_H 4.97 (1H, d, J = 8.1 Hz, H-2), δ_H 4.14 (1H, multiplet, H-3), δ_H 3.53 (1H, dd, J = 4.1, 12.3 Hz, CH_2OH), δ_H 3.74 (1H, dd, J = 2.3, 12.4 Hz, CH_2OH), δ_H 6.84 (2H, singlet, H-2′), and δ_H 3.86 (3H, singlet, OCH_3) were observed. Careful analysis of the COSY and HMBC NMR data indicated that compound **1** also has a phenylpropane unit.[12] The deshielded doublet at δ_H 4.97 (H-2), typical of a benzylic methine substituted by an oxygen, and the multiplet at δ_H 4.14 (H-3), which were coupled to each other, implying the existence of a 1,4-dioxane ring between a stilbene moiety and a phenyl ring.[12−16] On the basis of this observation, it is proposed that compound **1** is a stilbene-phenylpropane with a 1,4-dioxane ring. The linkage of stilbene and phenylpropane units through a 1,4-dioxane bridge was deduced by HMBC NMR experiments (Figure 1). Thus, after optimizing the J value [$^{2,3}J$(C,H)] for a long-range correlation to 4 Hz, the HMBC cross-peaks for H-2/C-8a and H-3/C-4a were observed. Also, a long-range correlation between the methoxyl signal and C-3′ indicated the position of this methoxyl group as C-3′. The relative trans stereochemistry of the dioxane moiety was confirmed by J value comparison and from the NOESY NMR experiment (Figure 1). Thus, the coupling constant (J = 8.1 Hz) between H-2 and H-3 and a NOE correlation between H-3 and H-2′ clearly indicated a trans configuration of the chiral centers of the dioxane ring.[12−16] Therefore, the structure of this novel stilbenolignan, aiphanol (**1**), was elucidated as 5-[2-[3-(hydroxy-3,5-dimethoxyphenyl)-2-hydroxymethyl-2,3-dihydrobenzo[1,4]dioxin-6-yl]vinyl]benzene-1,3-diol. There are several reports of natural compounds which have a dioxane moiety.[12−16] To the best of our knowledge, aiphanol (**1**) represents the first example of a stilbenolignan linked through a dioxane bridge.

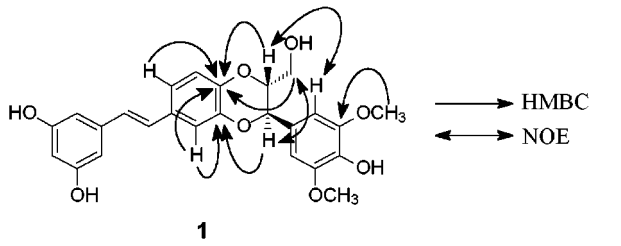

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Figure 1. Selected HMBC and NOE correlations of **1**.

All of the isolates obtained were evaluated for their potential to inhibit cyclooxygenase-1 and -2 (COX-1 and COX-2). Assays were performed according to established protocols.[2,17] Aiphanol (**1**) and isorhapontigenin (**2**) demonstrated IC_{50} values of 1.9 and 1.5 μM, respectively, when evaluated with COX-1, and 9.9 and 6.2 μM, respectively, when evaluated with COX-2. Piceatannol (**3**), the demethyl derivative of **2**, and luteolin were inactive (IC_{50} values >100 μg/mL) in both the COX-1 and COX-2 inhibition assays.

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(10) The seeds of *A. aculeata* were collected in Peru in July 1999 by J. Schunke Vigo, J. G. Graham, and F. Cabieses and dried. A voucher specimen has been deposited at the Field Museum of Natural History, Chicago, IL (accession no. 2222531).

(11) Aiphanol (**1**): brown powder; $[\alpha]^{20}_D$ −21.8° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 233 (5.37), 322 (5.32) nm; CD (MeOH) nm $\Delta\epsilon_{204}$ +16.9, $\Delta\epsilon_{208}$ −13.1, $\Delta\epsilon_{358}$ −7.8, $\Delta\epsilon_{379}$ +7.4; ^{1}H NMR (Me_2CO-d_6, 500 MHz) δ 3.53 (1H, dd, J = 4.1, 12.3 Hz, CH_2OH), 3.74 (1H, dd, J = 2.3, 12.4 Hz, CH_2OH), 3.86 (3H, s, OCH_3), 4.14 (1H, m, H-3), 4.97 (1H, d, J = 8.1 Hz, H-2), 6.28 (1H, brt, J = 1.9 Hz, H-14), 6.56 (2H, d, J = 2.0 Hz, H-12), 6.84 (2H, s, H-2′), 6.90 (1H, d, J = 8.3 Hz, H-5), 6.94 (1H, d, J = 16.4 Hz, H-9), 7.02 (1H, d, J = 16.3 Hz, H-10), 7.08 (1H, dd, J = 1.9, 8.4 Hz, H-6), 7.13 (1H, d, J = 1.9 Hz, H-8); ^{13}C NMR (Me_2CO-d_6, 125 MHz) δ 56.7 (OCH_3), 61.9 (CH_2OH), 77.5 (C-2), 79.7 (C-3), 102.8 (C-14), 105.8 (C-12), 106.1 (C-2′), 115.4 (C-8), 117.8 (C-5), 120.9 (C-6), 128.07 (C-9), 128.10 (C-1′), 128.7 (C-10), 131.8 (C-7), 137.3 (C-4′), 140.6 (C-11), 144.5 (C-4a), 145.1 (C-8a), 148.8 (C-3′), 159.6 (C-13); FABMS m/z 452 $[M^{\bullet}]^+$, 307 (60), 289 (35), 154 (90), 137 (100), 107 (65); HRFABMS m/z $[M^{\bullet}]^+$ 452.1462 (calcd for $C_{25}H_{24}O_8$ 452.1471); HRAPCITOFMS m/z $[M + H]^+$ 453.1546 (calcd for $C_{25}H_{25}O_8$ 453.1549).

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