A New Dimeric Stilbene with Tyrosinase Inhibitiory Activity From *Artocarpus gomezianus*

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A new dimeric stilbene, namely, artogomezianol (**1**), and the known compound andalasin A (**2**) were isolated from the roots of *Artocarpus gomezianus*. Both **1** and **2** showed moderate tyrosinase inhibitory activity with IC_{50} values of 68 and 39 μM, respectively.

Stilbenoids are bibenzyl compounds produced via the mixed phenylpropanoid/polyketide biosynthetic pathway.[1] They are widely distributed in the plant kingdom, being reported from bryophytes and pteridophytes through gymnosperms and angiosperms, and many of these compounds have demonstrated a number of interesting biological activities, such as antibacterial, antifungal, estrogenic, and cancer-chemopreventive actions.[2] In the Moraceae, several members of the genus *Artocarpus*, including *A. incisus*, *A. chaplasha*, and *A. lakoocha*, have been known to produce stilbenoids of various structural types.[3−7] Our recent study on the roots of *A. gomezianus* Wall ex Tre'c. revealed the presence of a stilbene and a number of flavones, some of which displayed potent tyrosinase inhibitory activity.[7,8] Tyrosinase (EC 1.14.18.1) is a copper-containing monooxygenase enzyme widely found in nature, including fungi, higher plants, and animals.[9,10] It is one of the key enzymes involved in the molting process of insects, and identification and investigation of inhibitors of this enzyme may provide important clues for developing new insect control agents. In plants, the enzyme is responsible for the browning of some fruits and vegetables; therefore its inhibitors may have potential uses as food preservatives. In man, potent tyrosinase inhibitors, such as kojic acid and related compounds, have been used as whitening agents in cosmetic products, due to their ability to suppress dermal melanin production. As a continuation of our chemical studies of Thai medicinal plants,[11] we report herein the isolation of a new dimeric stilbene and a known compound with tyrosinase inhibitory activity, namely, artogomezianol (**1**) and andalasin (**2**), from the MeOH fraction of the root extract of *A. gomezianus*.

A molecular formula of $C_{28}H_{24}O_8$ was deduced for compound **1** from its $[M - H]^+$ ion at *m/z* 487.1431 (calcd for $C_{28}H_{23}O_8$ 487.1393) in the HRFABMS. The UV absorptions at 286 and 338 nm were characteristics of a stilbene skeleton.[2] The IR spectrum showed the presence of aromatic and olefinic structures (1605 and 1511 cm^{-1}) with hydroxy groups (3750−3273 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) displayed 13 aromatic and olefinic protons at δ 7.20−5.90, together with three aliphatic protons at δ 4.70 (1H) and 2.95 (2H), reminiscent of a dimeric structure comprising a stilbene unit and a dihydrostilbene moiety linked by a C−C (sp^2−sp^3) bond.[12] In the ^1H NMR spectrum of **1**, the stilbene monomeric part displayed proton signals similar to those of oxyresveratrol (**3**; 2,4,3′,5′-tetrahydroxystilbene),[7] except for the absence of the H-5 resonance, with two *trans*-olefinic proton signals at δ 7.08 (1H, d, J = 16.2 Hz, H_α) and 6.63 (1H, d, J = 16.2 Hz, H_β) and three aromatic proton resonances at δ 6.31 (2H, d, J = 2.1 Hz, H-2′ and H-6′) and 6.05 (1H, dd, J = 2.1, 2.1 Hz, H-4′) (Table 1). In support of this, C-5 of **1** was shown in the ^{13}C NMR and DEPT spectra to be a quaternary sp^2 carbon at δ 122.5, in contrast with that of **3**, which appeared as a methine sp^2 carbon at δ 107.4 (Table 1). These observations suggested that this stilbene monomer was derived from **3**. The argument was further corroborated by the upfield shifts observed in structure **1** for the carbons in the *ortho* (2.8 and 0.9 ppm for C-4 and C-6, respectively) and *para* positions (2.4 ppm for C-2) in relation to C-5, as compared with their counterparts in **3**. The $^1H-^1H$ COSY spectrum of **1** revealed the following signals for the dihydrostilbene substituent: six aromatic protons at δ 6.85 (1H, d, J = 8.5 Hz, H-6″), 6.21 (1H, d, J = 2.4 Hz, H-3″), 6.10 (1H, dd, J = 8.5, 2.4 Hz, H-5″), 6.09 (2H, d, J = 2.1 Hz, H-2‴ and H-6‴), 5.90 (1H, dd, J = 2.1, 2.1 Hz, H-4‴); one methine proton at δ 4.70 (1H, dd, J = 7.9, 7.9 Hz, H-α′); and two methylene protons at δ 2.95 (2H, d, J = 7.9 Hz, H_2-β′). The ^{13}C NMR, DEPT, and HMQC spectra displayed the C-α′ and C-β′ resonances at δ 36.7 and 40.0, respectively. From these spectral data, it could be inferred that the second monomer was a dihydro derivative of **3**, i.e., dihydrooxyresveratrol, in which one of its aliphatic carbons (C-α′) was connected to C-5 of the first monomer. Confirmation of the proposed structure of **1** was obtained from the long-range C−H couplings observed in the HMBC spectrum (Figure 1). The C-6 carbon of **1** showed three-bond correla-

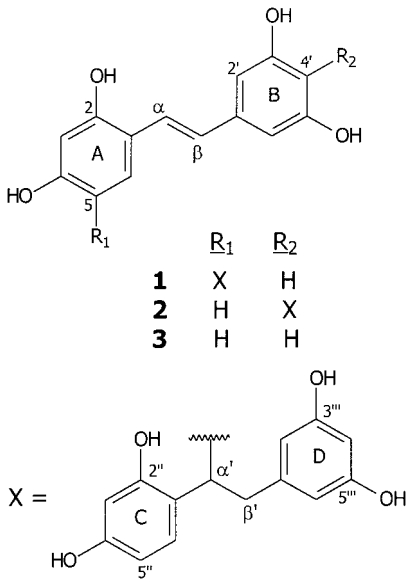

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Table 1. NMR Data of **1** as Compared with **3** in DMSO-d_6[a]

	artogomezianol (**1**)		oxyresveratrol (**3**)[b]	
position	^{1}H (mult., *J* in Hz)	^{13}C (mult. by DEPT))	^{1}H (mult., *J* in Hz)	^{13}C (mult. by DEPT)
1		114.3 (s)		115.4 (s)
2		153.7 (s)		156.1 (s)
3	6.30 (s)	102.6 (d)	6.33 (d, 2.4)	102.7 (d)
4		155.4 (s)		158.2 (s)
5		122.5 (s)	6.25 (dd, 8.4, 2.4)	107.4 (d)
6	7.20 (s)	126.4 (d)	7.34 (d, 8.4)	127.3 (d)
α	7.08 (d, 16.2)	124.0 (d)	7.15 (d, 16.5)	123.3 (d)
β	6.63 (d, 16.2)	124.2 (d)	6.77 (d, 16.5)	124.7 (d)
1′		140.2 (s)		140.1 (s)
2′	6.31 (d, 2.1)	103.9 (d)	6.35 (d, 1.8)	104.2 (d)
3′		157.8 (s)		158.5 (s)
4′	6.05 (dd, 2.1, 2.1)	101.3 (d)	6.08 (br s)	101.5 (d)
5′		157.8 (s)		158.5 (s)
6′	6.31 (d, 2.1)	103.9 (d)	6.35 (d, 1.8)	104.2 (d)
1″		121.6 (s)		
2″		155.6(s)[c]		
3″	6.21 (d, 2.4)	102.5 (d)		
4″		155.9(s)[c]		
5″	6.10 (dd, 8.5, 2.4)	105.6 (d)		
6″	6.85 (d, 8.5)	128.8 (d)		
α′	4.70 (dd, 7.9, 7.9)	36.7 (d)		
β′	2.95 (d, 7.9)	40.0 (t)		
1‴		143.8 (s)		
2‴	6.09 (d, 2.1)	107.0 (d)		
3‴		158.5 (s)		
4‴	5.90 (dd, 2.1, 2.1)	99.9 (d)		
5‴		158.5 (s)		
6‴	6.09 (d, 2.1)	107.0 (d)		

[a] ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR with solvent signal as reference. [b] Data obtained in this study. [c] Interchangeable assignments.

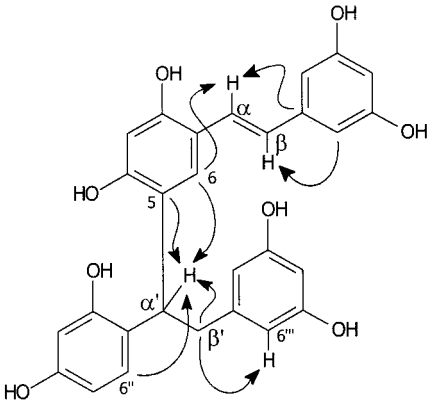

Figure 1. Selected HMBC correlations for **1**.

tions with H-α and H-α′, confirming the involvement of C-5 and C-α′ of the first and the second units in the interstilbenoid C−C linkgage. The other important three-bond couplings were found between H-α′ and C-6″, and H-6‴ and C-*β*′. In addition, two-bond couplings between H-α′ and C-5, and H-α′ and C-*β*′, were displayed. Compound **1** was named artogomezianol. The other oxyresveratrol-derived dimeric stilbene (**2**) was also identified from this plant as an unknown structure; however, prior to submission of this paper, **2** has been reported as a new stilbene dimer named andalasin A from *Morus macroura*.[13]

As mentioned earlier, stilbenes and their oligomers have wide distribution in the plant kingdom.[2] With regard to the dimers, a large number have been reported from the bryophytes. In gymnosperms, stilbene dimers have been found in the genera *Gnetum* and *Welwitschia*. In angiosperms, the Dipterocarpaceae are known to produce a number of stilbene oligomers; however, no monomers have been isolated. As for the family Moraceae, the occurrence of stilbene oligomers is indeed rare. Apart from **1** and **2**, the only known stilbene oligomer in this family is excelsaoctaphenol isolated from the wood of *Chlorophora excelsa*.[14] Its structure consists of two momeric oxyresveratrol units linked by a dimethyldecadiene linkage. Compounds **1** and **2**, however, appear to be formed by the coupling of the oxyresveratrol and dihydro-oxyresveratrol monomers via direct C−C bond formation. A rather closely structurally related example is the stilbene dimer gnetifolin D from *Gnetum parvifolium*.[12]

Both **1** and **2** were evalauated for tyrosinase inhibitory activity using procedures previously described.[3,7−9] Moderate activity was observed for each compound, with IC_{50} values of 68 and 39 μM, respectively (kojic acid as positive control, IC_{50} 27 μM). As expected, **2**, having two 4-substituted resorcinol moieties (rings A and C), was nearly two times as inhibitory as **1**, which possesses only one 4-substituted resorcinol structure (ring C). The relationships of 4-substituted resorcinol skeleton and tyrosinase inhibitory activity have been extensively discussed.[15]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were obtained on a Shimadzu UV-160 spectrophotometer, and IR spectra were recorded with a Perkin-Elmer FT-IR 1760X spectrophotometer. HRFAB mass spectra were obtained with a Finnigan MAT TSQ 700 spectrometer. ^{1}H (500 MHz) and ^{13}C (125 MHz), DEPT, HMQC, HSQC, and HMBC spectra were obtained with a JEOL JMN-A or a Varian Inova NMR spectrometer. Samples were recorded in DMSO-d_6 with solvent signal as internal standard.

Plant Material. The roots of *A. gomezianus* were collected from Trang Province, Thailand, in May 1998. Authentication was performed by comparison with herbarium specimens at the Royal Forest Department, Ministry of Agriculture-Cooperatives. A voucher specimen, KL-052541, is on deposit at the Department of Pharmacognosy, Chulalongkorn University.

Extraction and Isolation. Dried powdered roots of *A. gomezianus* (8.5 kg) were extracted successively with petroleum ether, ethyl acetate, and MeOH.[8] The MeOH extract (200 g) was fractionated by quick column chromatography (SiO_2; hexane−EtOAc gradient). Fractions 33−38 (12.7 g) were pooled and dried and further purified on a vacuum-liquid column (SiO_2; $CHCl_3$−MeOH gradient) to give four fractions (A−D). Fraction D (240 mg) was further separated by gel filtration (Sephadex LH20, MeOH) and preparative TLC (SiO_2; acetone−toluene, 4.5:5.5). Purification of the obtained residue on a Sephadex LH20 (MeOH) column gave **1** as a yellow solid (9 mg). Fraction C was further separated on SiO_2 (hexanes−EtOAc gradient) and Sephadex LH20 (MeOH) and then repurified on Sephadex LH20 ($CHCl_3$−MeOH gradient) to furnish **2** as a yellow solid (22 mg). Compound **2** was identified by comparison of its $[\alpha]_D$, ^1H and ^{13}C NMR, and MS data with reported values.[13]

Artogomezianol (1): yellowish amorphous solid; $[\alpha]^{28}_D$ −1.4° (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log) ϵ 286 (1.93), 338 (1.96) nm; IR (KBr) ν_{max} 3750−3273, 2925, 1605, 1511, 1166, 1057, 852 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* $[M - H]^+$ 487.1431 (calcd 487.1393 for $C_{28}H_{23}O_8$).

Evaluation of Tyrosinase Inhibitory Activity. Tyrosinase inhibitory activity was determined as previously described, using the modified dopachrome method with L-DOPA as the substrate.[3,7−9] Assays were conducted in a 96-well microtiter plate, and a BIO-RAD 450 plate reader was used to measure the absorbance at 475 nm. For each concentration of the sample solution, four wells designated as A, B, C, and D each contained a reaction mixture (180 μL) as follows: (A) 20 μL of mushroom tyrosinase in 20 mM phosphate buffer (480 units/ml, Sigma), 140 μL of 20 mM phosphate buffer (pH 6.8), and 20 μL of methanol; (B) 160 μL of 20 mM phosphate buffer (pH 6.8) and 20 μL of methanol; (C) 20 μL of mushroom tyrosinase solution (480 units/ml), 140 μL of 20 mM phosphate buffer (pH 6.8), and 20 μL of sample solution; (D) 160 μL of 20 mM phosphate buffer (pH 6.8) and 20 μL of sample solution. Each well was mixed and incubated at 25 °C for 10 min. Then, 20 μL of 0.85 mM L-DOPA (Sigma) in phosphate buffer (pH 6.8) was added. After incubation at 25 °C for 20 min, the amount of dopachrome in each reaction mixture was measured as the difference of the optical density before and after incubation. The percent inhibition of tyrosinase activity was calculated using the equation $\%\text{Inhibition} = 100[(A - B) - (C - D)]/(A - B)$, where *A* represents the difference of optical density before and after incubation without test sample; *B*, the difference of optical density before and after incubation without test sample and without enzyme; *C*, the difference of optical density before and after incubation with test sample; *D*, the difference of optical density before and after incubation with test sample, but without enzyme. Kojic acid (Sigma) was used as a positive standard.

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