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New arahypins isolated from fungal-challenged peanut seeds and their glucose uptake-stimulatory activity in 3T3-L1 adipocytes

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ABSTRACT

Two new stilbene dimers (arahypin-8 and arahypin-9) and one monomeric stilbene derivative arahypin-10 were isolated for the first time from wounded peanut seeds challenged by a *Rhizopus oligoporus* strain, a starter culture for soybean fermentation in Southeast Asia. The structures of the three new compounds were elucidated on the basis of HRESIMS, UV, 1D and 2D NMR spectroscopy and the plausible mechanism of their formation was proposed. In addition, these compounds showed insulin sensitizing effect by enhancing insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes.

1. Introduction

Fungi-stressed stilbenoid phytoalexins from peanuts are of considerable interest due to their potent bioactivities as antioxidant, anticancer, and anti-inflammatory agents (Chang et al., 2006; Lopes et al., 2011). A number of stilbenoids have been isolated by different research groups from peanut seeds treated with various stress methods as elicitors (Cooksey et al., 1988; Sobolev et al., 1995, 2009, 2010; Wu et al., 2011). In our previous study (Wu et al., 2011), germinating peanuts stressed by *Rhizopus oligoporus*, a popular food fermentation fungi in Southeast Asia, were found to produce a variety of stilbene phytoalexins and enhance polyphenolic antioxidants. In this study, two novel stilbene dimers and a new stilbene monomer were isolated from wounded peanuts stressed by *R. oligoporus* via a different approach. The anti-diabetic effect of the three compounds was also examined through evaluating the insulin sensitizing activity in a cell-based glucose uptake assay.

2. Results and discussion

2.1. Structure elucidation of new arahypins

Upon analysis of HRESIMS, compound **3** ($C_{19}H_{18}O_3$) and compound **2** ($C_{38}H_{38}O_7$) have the same molecular formulae as arahypin-5 and arahypin-6 isolated from peanut before (Sobolev et al., 2009, 2010). However, the UV spectra indicated that **3** (λ_{max} 230, 275, and 323 nm) and **2** (λ_{max} 227 and 320 nm) are isomers of arahypin-5 (λ_{max} 217 and 339 nm) and arahypin-6 (λ_{max} 224, 272, and 339 nm). Compound **1** ($C_{38}H_{36}O_6$) and compound **2** share a similar UV spectrum, which suggests resemblance of their structural patterns. The three new compounds cannot be found in either nonviable or unstressed viable control samples, indicating that they are products of stressed peanuts in response to the fungi action.

The proton NMR spectra of compound **1** (arahypin-8) showed that there were only three methyl groups in the compound. To date, all of the reported dimeric stilbenoids have four methyl groups. The HMBC correlations from H-18′ (δ_H 1.83) and H-19′ (δ_H 1.82) to C-16′ (δ_C 129.1) and C-17′ (δ_C 134.6) indicate the presence of two prenyl derived methyl groups. The methyl group at δ_H 1.53 supports that the carbon next to this methyl group is connected with an oxygen. There are two protons at δ_H 6.55 and two protons at δ_H 6.56; four carbons at δ_C 106.3 and four carbons at δ_C 160.3. These data revealed two 3,5-dihydroxyphenyl groups in the molecule. The entire structure of **1** is established by assigning signals from ^1H, ^{13}C, COSY, HMQC and HMBC experiments. The NMR data is summarized in Table 1.

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Table 1
NMR data of arahypin-8 (**1**), arahypin-9 (**2**) and arahypin-10 (**3**).

	Arahypin-8[a]			Arahypin-9[a]			Arahypin-10[b]		
	δ_H (*J* in Hz)	δ_C	HMBC correlations	δ_H (*J* in Hz)	δ_C	HMBC correlations	δ_H (*J* in Hz)	δ_C	HMBC correlations
1		141.5			141.5			140.4	
2	6.55, d (2.0)	106.3	C-3, C-4, C-6, C-7	6.50, d (1.7)	106.3	C-3, C-4, C-6, C-7	6.53, bs	105.9	C-4, C-7
3		160.3			160.2			157.0	
4	6.27, t (2.0)	103.3	C-2, C-3, C-5, C-6	6.24, brs	103.3	C-2, C-3, C-5, C-6	6.25, bs	102.0	
5		160.3			160.2			157.0	
6	6.55, d (2.0)	106.3	C-2, C-4, C-5, C-7	6.50, d (1.7)	106.3	C-2, C-4, C-5, C-7	6.53, bs	105.9	C-4, C-7
7	6.97, d (16.6)	127.9	C-1, C-2, C-6, C-8, C-9	6.87, d (16.3)	127.8	C-1, C-2, C-6,C-8, C-9	6.79, d (16.0)	125.8	C-2, C-6, C-9
8	7.07, d (16.6)	129.9	C-1, C-7, C-9, C-10	6.97, d (16.3)	129.7	C-1, C-7, C-9, C-10	6.93, d (16.0)	129.1	C-1, C-10, C-14
9		131.0			131.5			129.8	
10	7.72, d (2.1)	126.7	C-8, C-11, C-12, C-15	7.55, brs	126.7	C-8, C-9, C-12, C-15	7.10, bs	124.4	C-8, C-16
11		125.8			125.5			121.3	
12		156.2			155.9			152.9	
13	6.90, d (8.4)	117.6	C-9, C-11, C-12, C-14	6.85, d (8.4)	117.5	C-9, C-11, C-12, C-14	6.77, d (8.4)	116.6	C-11, C-12
14	7.31, dd (8.4, 2.1)	128.3		7.28, brd (8.4)	127.9		7.22, bd (8.4)	127.6	
15	7.05, d (16.3)	123.8	C-10, C-12, C-16, C-17	6.90, d (16.4)	125.1	C-10, C-12, C-17	5.63, d (9.8)	131.1	C-16, C-17
16	6.62, d (16.3)	136.9	C-11, C-15, C-18, C-19	6.47, d (16.4)	135.3	C-11, C-17, C-18, C-19	6.33, d (9.8)	122.2	C-10, C-11, C-12
17		78.1			78.4			76.6	
18	a. 2.09, m	40.8	C-16, C-17, C-19, C-11′, C-15′, C-16′	a. 2.55, dd (14.0, 7.5)	41.3	C-16, C-17, C-19, C-11′, C-15′, C-16′	1.44, s	28.1	C-16, C-17, C-18/19
	b. 1.78, m			b. 1.79, m					
19	1.53, s	25.0	C-16, C-17, C-18	1.54, s	30.1	C-16, C-17, C-18			
1′		141.5			138.2				
2′	6.56, d, 2.0	106.3	C-3′, C-4′, C-6′, C-7′	6.53, brs	107.1	C-3′, C-4′, C-6′, C-7′			
3′		160.3			157.8				
4′	6.27, t, 2.0	103.3	C-2′, C-3′, C-5′, C-6′	6.83, m	116.9	C-2′, C-6′			
5′		160.3			157.4				
6′	6.56, d, 2.0	106.3	C-2′, C-4′, C-5′, C-7′	6.60, brs	108.5	C-2′, C-4′, C-5′, C-7′			
7′	6.85, d, 17.4	127.8	C-1′, C-2′, C-6′, C-8′, C-9′	6.80, d, 16.0	127.4	C-1′, C-2′, C-6′, C-9′			
8′	7.02, d, 17.4	129.7	C-1′, C-7′, C-9′, C-10′	6.91, d, 16.0	129.4	C-1′, C-9′, C-10′, C-14′			
9′		131.1			130.8				
10′	7.22, d (2.1)	129.3	C-8′, C-11′, C-12′, C-15′	7.05, brs	114.5	C-8′, C-12′, C-14′			
11′		126.3			115.8				
12′		154.2			146.8				
13′	6.85, d (8.5)	119.1	C-9′, C-11′, C-12′, C-14′		146.7				
14′	7.39, dd (8.5, 2.1)	127.9		6.89, m	120.5	C-8′, C-10′, C-12′			
15′	3.90, m	33.3	C-18, C-11′, C-12′, C-14′, C-17′	3.07, m	29.1				
16′	5.20, d, (6.8)	129.1		2.35, m 1.23, m 1.23, m	45.1	C-18, C-11′, C-15′, C-17′, C-18′, C-19′			
17′		134.6		1.90, m	27.1				
18′	1.83, s	18.8	C-16′, C-17′, C-19′	0.93, d (6.5)	22.3	C-16′, C-17′, C-19′			
19′	1.82, s	26.7	C-16′, C-17′, C-18′	0.87, d (6.5)	25.3	C-16′, C-17′, C-18′			

[a] Recorded in acetone-d_6.
[b] Recorded in CDCl3.

The HMBC correlations from H-2 (δ_H 6.55) to C-7 (δ_C 127.9), H-8 (δ_H 7.07) to C-1 (δ_C 141.5) combining that coupling constants between H-7 and H-8 is 16.6 Hz, suggest that a *trans* double bond is attached to C-1 of the 3,5-dihydroxyphenyl group. Coupling constant between H-15 (δ_H 7.05) and H-16 (δ_H 6.62) is 16.3 Hz, which supports a *trans* double bond. HMBC correlations from H-15 to C-17 (δ_C 78.1), H-16 to C-18 (40.8) and H-19 (δ_H 1.53) to C-17 and C-18 revealed that C-18, which is derived from a methyl group of one of the constituent stilbene monomers, is a methylene unit that links the dimer. The HMBC correlations from H-18 (δ_H 2.09 & 1.78) to C-11′ (δ_C 126.3), C-15′ (δ_C 33.3) and C-16′ (δ_C 129.1) establish connections between the two monomers to give the isolated product (Fig. 1). In addition, the relative stereochemistry of compound **1** was measured by NOESY experiment. H-15′ showed correlation with H-19, which indicated that both of the protons locate at the same side of the six-member ring. The relative stereochemistry of compound **1** was thus established.

The proton NMR spectra of compound **2** (arahypin-9) showed that there were only three methyl groups in the compound. The two methyl resonance doublets at δ_H 0.93 and 0.87 suggest that both groups terminate an aliphatic chain. This is a clear difference between compounds **1** and **2**. The methyl group at δ_H 1.54, two protons at δ_H 6.50, two carbons at δ_C 106.3 and two carbons at δ_C 160.2 revealed that the structure of monomer, composed of C-1–C-19, is not changed. However, it is notable that H-2′ and H-6′ are not equivalent in compound **2**, indicating that 3′-OH or 5′-OH may form ether or ester bond with other carbons. Carbon signals at δ_C 146.7 and 146.8 support a 1,2-dihydroxyphenyl structure originating from the constituent monomer 3′-prenyl-3,5,4′,5′-tetrahydroxystilbene. The entire structure of compound **2** is established by assigning signals from ^{1}H, ^{13}C, COSY, HMQC and HMBC experiments. The HMBC correlations from H-18 (δ_H 2.55 and 1.79) to C-11′ (δ_C 115.8), C-15′ (δ_C 29.1) and C-16′ (δ_C 45.1) combining the HMBC correlations from H-10′ to C-8′ (δ_C 129.4), C-12′ (δ_C 146.8) and C-14′ (δ_C 120.5) establish connections between the two monomers (Fig. 1). The NMR data is summarized in Table 1.

The UV spectrum of compound **3** (arahypin-10) is similar to that of its dimethyl ether analog, lonchocarpene, previously isolated from the roots of *Lonchocarpus nicou* (Kaouadji et al., 1986). The structure of compound **3** was established by analysis of its 1D and 2D NMR data summarized in Table 1. HMBC correlations of compound **3** are shown in Fig. 1.

Arahypin-10 may be formed from cyclization reaction of its diene precursor trans-3′-isopentadienyl-3,5,4′-trihydroxystilbene