JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 875–881 875
DOI:10.1021/jf903410e

New Dimeric Stilbenoids from Fungal-Challenged Peanut (*Arachis hypogaea*) Seeds

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The peanut plant can resist fungal attacks by producing stilbene-derived phytoalexins. Once understood, such a natural phytoalexin-based mechanism of peanut resistance could be potentially manipulated to obtain fungal-resistant peanut breeding lines. Several simple stilbenoid phytoalexins from peanuts have been reported. However, more complex stilbenoid derivatives such as those that have been reported from other sources and considered important factors in plant defense have not been found in peanuts. The purpose of this research was to isolate and characterize further new oligomeric peanut stilbenoids that may act as phytoalexins. Two new prenylated stilbene dimers named arahypin-6 (**3**) and arahypin-7 (**4**) have been isolated from wounded peanut seeds challenged by an *Aspergillus caelatus* strain. The structures of these new putative phytoalexins were determined by analysis of NMR, MS, and UV spectroscopic data. Together with other known peanut stilbenoids that were also produced in the challenged seeds, these new compounds may play a defensive role against invasive fungi.

KEYWORDS: Peanuts; *Arachis hypogaea*; arahypin; groundnuts; stilbenes; stilbene dimer; stilbene oligomer; stilbenoid dimer; dimeric stilbenoid; dehydrodimer; prenylated stilbenes; stilbenoids; structure elucidation; NMR; HPLC-MS

INTRODUCTION

Peanuts (*Arachis hypogaea*) are known to produce stilbene-derived phytoalexins in response to fungal infection (*1*). However, despite considerable progress in peanut research, relatively little is known about other aspects of peanut phytochemistry. Detailed knowledge of peanut plant chemistry, particularly knowledge regarding phytoalexin production, may help to reveal mechanisms of peanut disease and/or pest resistance. It is conceivable that such mechanisms could be manipulated to improve early natural field resistance of the plant to pests. In addition, the potential medical importance or health benefits of stilbenoids from peanuts and other plants has been acknowledged by several researchers (*2*−*4*).

Several simple stilbenoid phytoalexins from peanuts have been reported (*5*, *6*). However, more complex stilbenoid derivatives such as those that have been reported from other sources (*7*−*13*) and considered important factors in plant defense have not previously been found in peanuts. Dimers and other stilbene oligomers possess biological activity (*12*, *14*−*20*). The potential therapeutic value of stilbenoid dimers has promoted research activity on the occurrence of this class of compounds in various plants around the world (*7*−*20*).

Our previous investigations of the ability of peanut plants to produce stilbenoid phytoalexins revealed the production of several new simple stilbenoids (*5*, *6*). Together with these metabolites, several higher-molecular-weight compounds with spectroscopic properties characteristic of stilbenoids (*21*) were detected in challenged peanuts and were suggested to represent stilbene oligomers. However, difficulties with their separation and purification due to small quantities present and similar chromatographic behavior among analogues has hindered their isolation and structure elucidation to date. The purpose of the research described here was to isolate and characterize some of these stilbenoids, as they may represent a new class of peanut phytoalexins.

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EXPERIMENTAL PROCEDURES

Reagents, Materials, and Basic Apparatus. HPLC-grade solvents used in the preparation of mobile phases and separations on silica gel were obtained from Fisher (Suwanee, GA). HPLC-grade H_2O was prepared with a ZD20 four-bowl Milli-Q water system (Millipore). ACS grade methanol used for seed extraction was purchased from VWR (Suwanee, GA). Silica gel for column chromatography (Silica gel 60, 0.063−0.200 mm) was purchased from EM Science (Gibbstown, NJ).

Reference Compounds. *trans*-Resveratrol (approximately 99%) was purchased from Sigma. Pure individual stilbenes *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, and *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene were obtained as described (*22*) except that preparative HPLC was used as a final purification step rather than preparative TLC. HPLC separation was achieved by using isocratic mobile phase 3 (*6*). SB-1, chiricanine A, arahypin-1, arahypin-2, arahypin-3, arahypin-4, and arahypin-5 were obtained as described (*5*, *6*).

Fungal Culture. *A. caelatus* NRRL 25528 (ex type) was used to elicit phytoalexin production in peanuts. The strain isolated from soil collected June 8, 1992 in a peanut field, Terrel Co., GA, has been deposited with the National Fungus Collections, U.S. Department of Agriculture, Beltsville, MD (BPI 737601). Living cultures of NRRL 25528 have been deposited in the ARS Culture Collection, Peoria, IL.

Published on Web 12/18/2009

Plant Material and Processing. The 31-1314 peanut runner breeding line, 2007 harvest, from the National Peanut Research Laboratory (Dawson, GA) was used. Peanut seeds were allowed to imbibe distilled water for 17 h at room temperature, and then they were chopped with a sharp hand cutter into 3−7 mm pieces, washed with distilled water, blotted with a paper towel, air-dried to the condition when sliced peanuts did not leave water spots on filter paper, and placed on stainless steel trays so that the thickness of the layer did not exceed 10 mm. The trays were evenly sprayed with the fungal spore suspension (5×10^6/mL) so that 1 mL of the suspension coved 500 cm^2 of the tray surface. Then the trays were placed into autoclave bags and incubated at 30 °C for 120 h. Every morning the bags were open under sterile conditions and fresh air was allowed into the bags. Then the bags were folded several times along the open side to prevent moisture loss and spore dispersion, and then placed into the incubator.

Uninoculated peanut material, as a control, was prepared as follows: whole kernels from the same batch of seeds that were used in the above experiments were surface-sterilized by shaking for 2 min with 2% NaOCl, rinsed three times with sterile distilled water, blotted with a sterile paper towel, and aseptically sliced into 2−3 mm pieces. Control peanut material was placed into Petri dishes in triplicate and incubated in an autoclave bag at 30 °C for 96 h. A portion of untreated sliced peanuts, as a second control, was kept frozen at −28 °C until the analysis.

HPLC-DAD-MS Analyses. Separations of seed extracts and purified stilbenoids were performed using a tandem HPLC-MS Surveyor system equipped with MS Pump Plus, Autosampler Plus, a PDA Plus Detector (Thermo Electron Corporation, San Jose, CA), and a 50 mm × 4.6 mm i.d., 2.5 μm XTerra MS C18 analytical column (Waters). H_2O (A), MeOH (B), and 2% HCOOH in H_2O (C) were used in the following gradient: initial conditions, 68% A/30% B/2% C, increase linearly to 0% A/98% B/2% C in 12 min, hold isocratic for 5 min, decrease to initial conditions in 0.01 min (mobile phase 1). The flow rate was 0.8 mL/min. The column was maintained at 40 °C.

Preparative HPLC Separations. Preparative HPLC separations were performed using a 100 mm × 19 mm i.d., 5 μm XTerra Prep RP_{18} OBD preparative column (Waters). The column temperature was 40 °C. The following isocratic mobile phases were used: mobile phase 2, 75% MeOH, 3% of 2% HCOOH in H_2O, and 22% of H_2O; mobile phase 3, 60% CH_3CN, 3% of 2% HCOOH in H_2O, and 37% of H_2O; mobile phase 4, 70% MeOH, 3% of 2% HCOOH in H_2O. The flow rate was 8.0 mL/min.

Sample Extraction and Purification. For preparative isolation of the stilbenoid dimers, 1.1 kg of inoculated and incubated peanut seeds was extracted with 5.4 L of MeOH (300 mL for each portion of 60 g of seeds) in a high-speed blender for 1 min. The combined mixture was filtered through a filter paper in a Büchner-type funnel under reduced pressure. The solid residue was resuspended in 1.4 L of MeOH, and the extraction procedure was repeated twice as described above. The combined extract solutions were filtered through a filter paper and defatted thrice each time with 0.5 L of *n*-hexane. The MeOH layer was evaporated to dryness. The residue was redissolved in $CHCl_3$ and applied to a chromatographic column (34 mm i.d.) packed with silica gel (suspended in $CHCl_3$) to the height of 330 mm (column 1). The column was subsequently eluted with 0.25 L of $CHCl_3$ (1 fraction), 1.3 L of EtOAc (3 fractions), 1.1 L of acetone (1 fraction), and 1.0 L of MeOH (1 fraction). Fractions eluted with EtOAc and containing stilbenoid dimers **3** and **4** (**Figure 1**) were separately combined, evaporated to dryness with a rotary evaporator, and subjected to further purification on a silica gel column similar to the above (packed to the height of 360 mm; column 2). The column was subsequently eluted with 0.25 L of $CHCl_3$, 1.4 L of $CHCl_3$-EtOAc (1:1), 1.2 L of EtOAc, 0.75 L of acetone, and 0.3 L of MeOH. One of the fractions eluted from column 2 with $CHCl_3$-EtOAc (1:1) and containing compound **3** was subjected to further purification on a silica gel column similar to the above (packed to the height of 360 mm; column 3). Column 3 was eluted portion wise (350 mL each) with hexane-acetone (7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, pure acetone, respectively). Combined fractions eluted from column 3 with hexane-acetone 4:1 through 2:1 and containing compound **3** were evaporated to dryness on a rotary evaporator, redissolved in MeOH, filtered, and subjected to a final purification on a preparative HPLC column using isocratic mobile phase 2. Combined fractions eluted from column 2 with acetone and MeOH and containing compound **4** were evaporated to dryness on a rotary evaporator, redissolved in MeOH, filtered, and subjected to a final purification on a preparative HPLC column using isocratic mobile phase 3. Fractions obtained from HPLC and containing chromatographically pure compounds **3** and **4** were separately evaporated with a rotary evaporator to a point where almost all of the organic solvent was removed. The remaining aqueous mixtures containing the target compounds were separately extracted four times with EtOAc (H_2O-EtOAc ratio 3:2, v/v). The combined EtOAc layers were evaporated nearly to dryness with a rotary evaporator. The residue was transferred into a 16 mL vial with MeOH and evaporated to dryness with a stream of N_2. To further reduce the surfaces of the solid target compounds **3** and **4** and to simplify their handling, the residues were redissolved in acetone, filtered, transferred into 4 mL vials, and evaporated to dryness with a stream of N_2. The vials were placed into a lyophilizer for 2 h at room temperature to remove traces of the solvents.

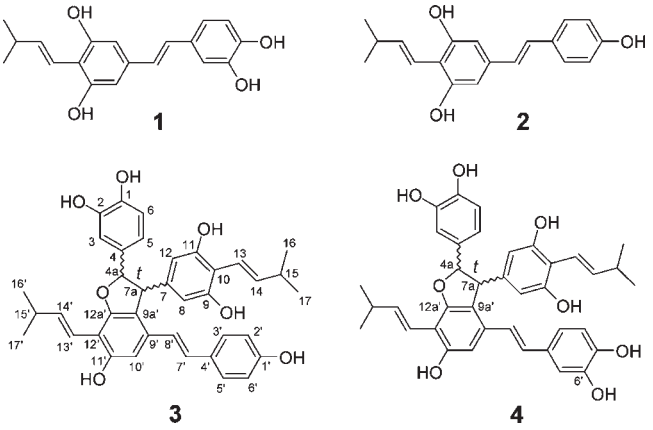

Figure 1. Structures of *trans*-arachidin-1 (**1**), *trans*-arachidin-3 (**2**), arahypin-6 (**3**), and arahypin-7 (**4**).

Oxidative Coupling Reactions. In order to obtain synthetic arachidin-1−arachidin-3 dehydrodimer (**3**), a solution of 54.8 mg (0.203 mmol, 5 equiv) of $FeCl_3 \cdot 6H_2O$ in 2 mL of H_2O was added to a solution of 12.7 mg of arachidin-1 (**1**) (0.041 mmol) and 12.0 mg of arachidin-3 (**2**) (0.041 mmol) in 5 mL of EtOAc, and the mixture was vigorously stirred with a magnetic stirrer for 2.5 h at room temperature without light. The progress of the reaction was monitored by HPLC-DAD-MS analysis of an aliquot of the EtOAc layer. After completion, the reaction mixture was separated and the EtOAc layer was dried by passing through a Pasteur pipet packed with Na_2SO_4. The EtOAc solution was evaporated to dryness under a stream of N_2, redissolved in EtOAc, and subjected to separation on a chromatographic column (22 mm i.d.) packed with silica gel (suspended in $CHCl_3$) to the height of 300 mm (column 4). The column was subsequently eluted with 1.3 L of a mobile phase with a gradient of acetone in hexane ranging from 15% to 100% of acetone in the mixture. Fractions collected from the column were analyzed by HPLC. Fractions containing stilbenoid dimer **3** (**Figure 1**) were combined, evaporated to dryness with a rotary evaporator, redissolved in MeOH, and subjected to a final preparative HPLC purification in mobile phase 4. Combined fractions obtained from HPLC and containing chromatographically pure compound **3** were evaporated with a rotary evaporator to a point where almost all MeOH was removed. The remaining aqueous mixture containing **3** was extracted four times with EtOAc (H_2O-EtOAc ratio 3:2, v/v). The combined EtOAc layers were evaporated nearly to dryness with a rotary evaporator. The residue was transferred into an appropriate vial with MeOH, filtered, and evaporated to dryness with a stream of N_2. The vial was placed into a lyophilizer for 2 h at room temperature to remove traces of the solvents.

The synthesis and isolation of arachidin-1−arachidin-1 dimer (**4**) was performed using a similar procedure. To a solution of 40.0 mg of arachidin-1 (**1**) (0.128 mmol) in 7 mL of EtOAc, a solution of 173.0 mg (0.64 mmol, 5 equiv) of $FeCl_3 \cdot 6H_2O$ in 2.8 mL of H_2O was added and the mixture was vigorously stirred with a magnetic stirrer for 1 h at room temperature without light. The reaction mixture was processed as described above. Preparative HPLC purification of **4** was performed using mobile phase 4.

Spectroscopic Measurements. ^1H and ^{13}C NMR data were acquired with a Bruker DRX-400 spectrometer. HMBC and HMQC experiments

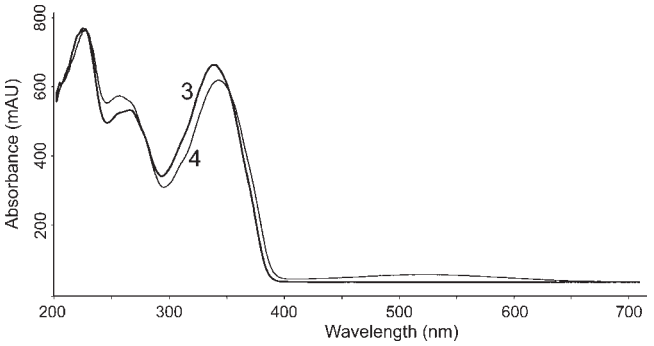

Figure 2. UV spectra of new dimeric stilbenoids in mobile phase 1: **3**, arahypin-6 (**3**); **4**, arahypin-7 (**4**).

were performed on a Bruker AMX-600 instrument. Chemical shift values were referenced to the solvent signals for $CDCl_3$ (δ_H 7.24/δ_C 77.0) or acetone-d_6 (δ_H 2.04/δ_C 29.8). Optical rotation measurements were made with a Rudolph Research Autopol III polarimeter, and CD data were acquired using an Olis DSM 17 CD spectrophotometer. HR ESI-MS and HR EI-MS data were recorded on a Micromass Autospec instrument. ESI-MS/MS^2 data were obtained on a Finnigan LCQ Advantage MAX ion trap mass spectrometer equipped with APCI and ESI interfaces and operated with Xcalibur version 1.4 software (Thermo Electron Corporation, San Jose, CA). All data, when using the APCI interface, were acquired in the full-scan positive polarity mode from m/z 100 to 2000. Capillary temperature was 170 °C, APCI vaporizer temperature 240 °C, sheath gas flow 60 units, auxiliary/sweep gas flow 5 units, source voltage 5 kV, and source current 6 μA. Data, when using the ESI interface, were acquired in the full-scan positive polarity mode from m/z 100 to 2000. Capillary temperature was 270 °C, capillary voltage 5 V, sheath gas flow 60 units, auxiliary/sweep gas flow 20 units, and source voltage 4.5 kV. In MS^2 analyses, the $[M + H]^+$ ions observed for each chromatographic peak in full-scan analyses were isolated and subjected to source collision-induced dissociation (CID) using He buffer gas. In all CID analyses, the isolation width, relative fragmentation energy, relative activation Q, and activation time were m/z = 2.8, 35−40%, 0.25, and 30 ms, respectively. The results of MS^2 experiments are represented throughout the text as follows: m/z aaa@bb, where aaa is parent ion and bb is normalized collision energy (%).

Arahypin-6 (*3*). Yellow-brownish glass; UV (CH_3OH) λ_{max} (log ε) 224 (4.71), 272 (4.41), 339 (4.43); UV (mobile phase 1), see **Figure 2**; ^1H, ^{13}C, and HMBC NMR data, see **Table 1**; ESI-MS, m/z 607 ($[M + H]^+$; rel int 100); ESI-MS^2, m/z 607@35, see **Figure 3A**; HR EI-MS, m/z 606.2615 $[M]^+$; calcd for $C_{38}H_{38}O_7$, 606.2616.

Arahypin-7 (*4*). Yellow-brownish glass; UV (CH_3OH) λ_{max} (log ε) 224 (4.71), 270 (4.47), 310 sh (4.17), 347 (4.42); UV (mobile phase 1), see **Figure 2**; ^1H NMR (acetone-d_6, 400 MHz) δ 6.27 (s, H_2-8/12), 5.43 (d, J = 4.3 Hz, H-4a), 4.37 (d, J = 4.3 Hz, H-7a), 2.46 (m, H-15′), 2.39 (m, H-15), 1.09 (d, J = 6.7 Hz, H_3-17), 1.08 (d, J = 6.7 Hz, H_3-16), 1.047 (d, J = 6.7 Hz, H_3-16′), 1.046 (d, J = 6.7 Hz, H_3-17′). The remaining signals in the ^1H NMR spectrum were severely overlapped in the region from δ 6.61 to 6.84 and could not be distinguished for accurate assignments. However, integration of this region indicated that the appropriate number of protons is present. ESI-MS, m/z 623 ($[M + H]^+$; rel int 100). ESI-MS^2, m/z 623@40, see **Figure 3B**; HR ESI-MS m/z 623.2641 $[M + H]^+$; calcd for $C_{38}H_{38}O_8$ + H, 623.2644.

RESULTS AND DISCUSSION

Structure Elucidation. Challenged peanuts have been shown to produce simple, monomeric stilbene derivatives (*5*, *6*). Molecular masses of these known simple stilbenoids do not exceed 344 Da (*5*). In our previous experiments, several stilbenoid-like compounds with molecular masses over 450 Da were also detected. All of those compounds were eluted from an analytical reversed-phase column after elution of all major known simple stilbenoids. Those higher-molecular-weight compounds formed a "serrated" area in the chromatogram shown in **Figure 4**. These compounds, represented by corresponding peaks in this area were suggested to be stilbenoid derivatives based on their characteristic absorption (*21*) in the 340 nm region as well as their mass spectra. The UV spectrum of **3** (**Figure 2**) displayed absorption peaks indicating the presence of a conjugated system in the molecule. Both ESI and APCI mass spectra of **3** (**Figure 3A**, only ESI spectra are shown in the figure) revealed a molecular weight of 606 Da and a characteristic loss of 56 Da (C_4H_8), a loss derived from the prenylated chain of several stilbenes (*5*, *6*). The structure of **3** was deduced from UV, MS, and NMR data.

Table 1. NMR Spectroscopic Data for Arahypin-6 (**3**) in $CDCl_3$[a]

position	δ_H (multiplicity, J_{HH})	δ_C[b]	HMBC (H# → C#)
1	5.12 s, OH	143.8	1[c]
2	5.13 s, OH	143.8	2[c]
3	6.83 d (2.0)	112.5	1, 5
4		135.2	
4a	5.45 d (5.3)	92.7	3, 5, 7, 12a′
5	6.76 dd (2.0 8.3)	118.2	1, 2 wk, 3
6	6.82 d (8.3)	115.5	2, 4
7		144.5	
7a	4.36 d (5.3)	56.6	4, 4a, 7, 8/12, 9a′, 12a′
8, 12	6.30 s	107.0	7, 8/12, 9/11, 10
9, 11	5.08 s, OH	154.2	8/12, 9/11, 10[c]
10		111.0	
13	6.24 dd (1.2, 16.6)	117.2	9/11, 15
14	6.10 dd (6.7, 16.6)	146.0	10, 13, 15, 16/17
15	2.54 m	32.3	
16	1.102 d (6.8)	22.7	14, 15, 16/17
17	1.106 d (6.8)	22.7	14, 15, 16/17
1′	4.71 s, OH	155.5	
2′, 6′	6.70 d (8.7)	115.5	1′, 2′/6′, 4′
3′, 5′	7.09 d (8.7)	127.8	1′, 3′/5′, 7′
4′		130.3	
7′	6.79 d (16.2)	129.0	4′, 3′/5′, 9′
8′	6.510 d (16.2)	122.8	4′, 7′, 9a′, 10′
9′		133.5	
9a′		119.2	
10′	6.66 s	104.0	8′, 9a′, 11′, 12′
11′	5.14 s, OH	154.0	
12′		108.0	
12a′		159.0	
13′	6.512 m[d]	117.2	11′, 12a′, 14′, 15′
14′	6.512 m[d]	142.7	12′, 15′, 16′/17′
15′	2.54 m	32.7	
16′, 17′	1.11 d (6.8)	22.4	14′, 15′, 16′/17′

[a] ^1H NMR data were recorded at 400 MHz; ^{13}C NMR data were recorded at 150 MHz. [b] Carbon assignments were made using HMQC and HMBC data. [c] These entries indicate correlations from the corresponding phenolic OH signal. [d] These signals are coincident in the spectrum, resulting in an apparent doublet.

Compound **3** was assigned the molecular formula $C_{38}H_{38}O_7$ (unsaturation index = 20) by analysis of NMR and HRMS data. NMR data (**Table 1**) revealed the presence of two isolated *trans*-3-methyl-1-butenyl groups, an isolated *trans*-olefin unit, a monooxygenated *para*-disubstituted benzene ring, a *meta*-dioxygenated 1,2,3,5-tetrasubstituted benzene ring with C2 symmetry, a *meta*-dioxygenated pentasubstituted benzene ring, and an *ortho*-dioxygenated 1,2,4-trisubstituted benzene ring. ^1H NMR signals corresponding to six phenolic OH groups were also observed as well as resonances corresponding to an isolated OCH−CH system. The remaining oxygen atom must be present as an ether group involving the oxygen atom of this unit and one of the seven oxygenated aromatic carbons.

HMQC and HMBC NMR data (**Table 1**) were used to establish the connectivity of these units. One of the *para*-disubstituted ring ^1H NMR signals (H-3′/H-5′) correlated to C-7′ of the isolated olefin unit in the HMBC spectrum, linking the *para*-disubstituted

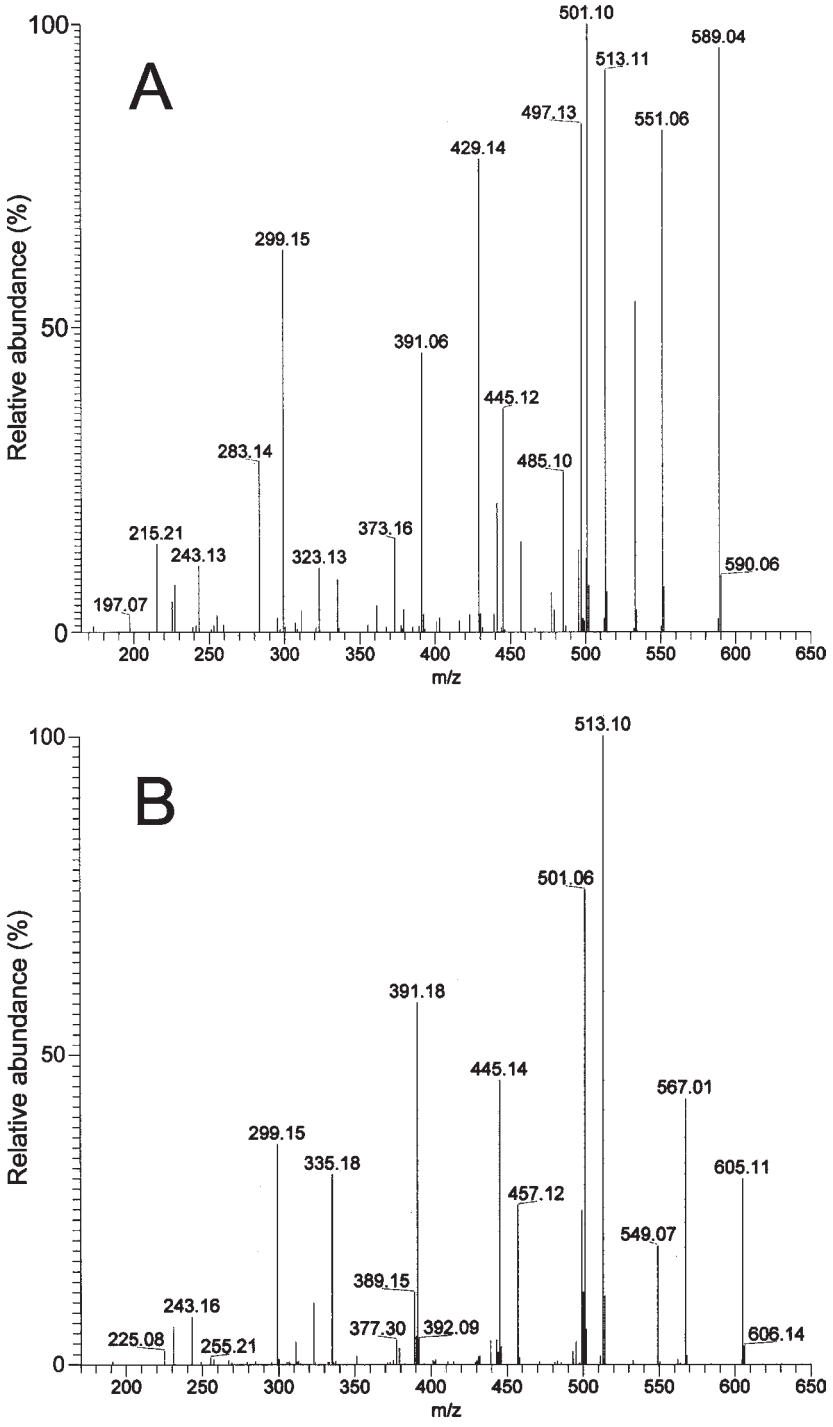

Figure 3. ESI-MS^2 data: (**A**) arahypin-6 (**3**) and (**B**) arahypin-7 (**4**).

benzene ring to the isolated double bond. H-8′ of the same olefin unit showed correlations to C-9a′ and C-10′ of the pentasubstituted benzene ring, while H-7′ showed a correlation to C-9. These data required connection of C-8′ to C-9′. The oxygenated carbons of the pentasubstituted ring (C-12a′ and C-11′) were correlated with an olefinic proton signal of one of the 3-methyl-1-butenyl side-chains (H-13′), thereby locating this side-chain at C-12′. The other 3-methyl-1-butenyl side-chain showed a correlation from one of its olefin protons (H-13) to the oxygenated carbon signals of the symmetrical 1,2,3,5-tetrasubstituted benzene ring (C-9/C-11), thereby locating the second side-chain at C-10. The sp^3 methine H-7a showed correlations to C-8/C-12 of this same symmetrical benzene ring, requiring its attachment to C-7. H-7a also showed correlations to C-9a′ and C-12a′, linking C-7a to the pentasubstituted benzene ring at C-9a′. The remaining 1,2,4-trisubstituted benzene ring was connected to the oxygenated carbon (C-4a) of the OCH−CH system on the basis of correlations of H-4a to C-3 and C-5. The presence of an ether unit linking C-4a and C-12a′ was established on the basis of a HMBC correlation of H-4a with C-12a′. This requires all of the other oxygen atoms to be present as OH groups, thereby enabling completion of the assignment of the structure as shown in **3**. Only four of the phenolic OH signals showed HMBC correlations to nearby carbons, but all observed correlations were consistent with the proposed structure. Compound **3** appears to be composed of one unit each of arachidin-1 and arachidin-3. The relative

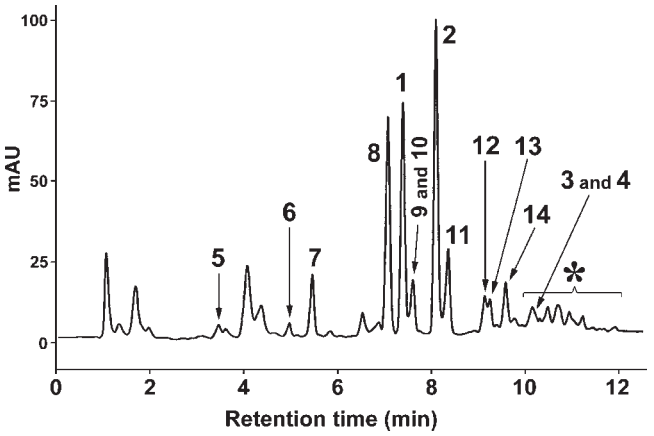

Figure 4. HPLC of peanut seed extracts after 96 h of seed incubation with *A. caelatus*. **1**, arachidin-1 (**1**); **2**, arachidin-3 (**2**); **3**, arahypin-6 (**3**); **4**, arahypin-7 (**4**); **5**, resveratrol; **6**, arahypin-2; **7**, arahypin-3; **8**, SB-1; **9**, arachidin-2; **10**, arahypin-4; **11**, 3′-isopentadienyl-3,5,4′-trihydroxystilbene; **12**, arahypin-5; **13**, chiricanine A; **14**, arahypin-1; ∗, area of retention of stilbenoid oligomers. The chromatogram was recorded at 338 nm.

configuration of **3** at C-7a and C-4a was determined on the basis of the corresponding vicinal ^{1}H NMR *J*-value of 5.3 Hz, which is indicative of a *trans* orientation in a ring system of this type (*23*). In contrast, the *J*-value for a *cis* orientation has been reported as 8.3 Hz (*24*). Compound **3** appears to be a new natural compound, and it has not been reported previously in the literature. Therefore, the common name arahypin-6 is proposed for **3**.

Arahypin-7 (**4**) has the molecular formula $C_{38}H_{38}O_8$ (unsaturation index = 20), differing from that of **3** by addition of an oxygen atom, as established by analysis of NMR and HRMS data. The MS^2 data for **4** displayed several ions of similar relative abundance that were also observed in the data for **3** (**Figure 3**), indicating close structural similarities between **3** and **4**. The NMR data for **3** and **4** were also very similar, although the aromatic and olefinic signals in the ^{1}H NMR spectrum of **4** showed much more overlap, regardless of which of several NMR solvents was employed. Even so, the data for **4** clearly lacked the *para*-disubstituted aromatic ring signals, replacing them instead with signals for a second *ortho*-dioxygenated 1,2,4-trisubstituted benzene ring. Thus, the structure of **4** was presumed to differ from that of **3** by addition of one OH group at position 6′ on the *para*-disubstituted aromatic ring of **3**. This relationship was consistent with the very similar HPLC behavior of **3** and **4** (6 s between peak apexes; compound **4** was eluted first). In the acidic mobile phase 1, arahypin-7 (**4**) demonstrated a characteristic absorbance at 522 nm (**Figure 2**), which suggested the presence of a vicinal dihydroxy substituted aromatic moiety similar to that of arachidin-1 (**1**), whose UV spectrum also showed similar absorbance at 522 nm. Although the degree of overlap in the ^{1}H NMR spectrum did not permit complete assignment of all of the signals for **4**, key signals that did resolve were fully consistent with the proposed structure, which is essentially a dimer of arachidin-1 formed in a process directly analogous to that involved in the formation of **3**. Because of the severe overlap, together with difficulties in purifying sizable samples of **4** from the complex matrix, the structure in this instance was verified by synthesis. Compound **4** also appears to be a new compound not described previously in the literature. The common name arahypin-7 is proposed for **4**.

Synthetic Approach to Dimers 3 and 4. In order to further verify the structure of **4**, and to determine whether such compounds might be formed chemically under certain conditions, efforts were undertaken to form **3** and **4** synthetically from the corresponding presumed monomer units **1** and/or **2**. Oligostilbenoids can be formed from resveratrol by oxidative phenolic coupling, and similar to the case of resveratrol **1** and **2** possess the typical resorcinol arrangement (*26*). Synthetic stilbenoid oligomers in general and stilbenoid dimers in particular have attracted interest among scientists because of their beneficial biological activity and their relatively rare occurrence in nature (*25*, *26*). Numerous publications have addressed the issue of not-so-straightforward oxidative coupling reactions of simple stilbenoids. One of the most recent publications (*26*) provides a brief but informative review on the subject as well as the results of systematic research on the coupling reaction. We attempted to synthesize compounds **3** and **4** by using the described methods (*25*, *26*) as a starting point for our experimental synthetic procedures. The solvents that worked best for the reported model reaction (*26*), particularly CH_2Cl_2, as well as other solvents in which the initial simple stilbenoids, arachidin-1 and arachidin-3, were insoluble, did not lead to formation of any stilbenoid dimers. Toluene with suspended stilbenoids and crystalline $FeCl_3 \cdot 6H_2O$ did afford the coupling reaction, albeit in modest yields. The formed dimers were detected as a suspension after 24 h of stirring at room temperature. Silver acetate did not assist the coupling reaction in tested solvents within a reasonable period of time (24 h). The most effective solvent system found for the arachidin-1−arachidin-3 and arachidin-1−arachidin-1 heterogeneous coupling reactions was a combination of EtOAc with at least a 5 equiv excess of $FeCl_3 \cdot 6H_2O$ dissolved in H_2O. To achieve the best results, it was found that the reaction needed to be worked up before substantial formation of products other than the desired dimers could take place, that is, after 1 h of stirring. The target dehydrodimers **3** and **4** were the major products of the reactions at that stage, and they could be obtained with 12−15% recovery. The conditions employed led to formation of the stilbenoid dehydrodimers that were identical in every respect to naturally occurring compounds **3** and **4** and, therefore, helped to confirm the structures of these new peanut-derived compounds.

Stilbenoid Production and Suggested Role of New Stilbenoid Dimers. An *A. caelatus* strain was chosen as a phytoalexin elicitor because it demonstrated stable growth and satisfactory reproducibility of the results in our previous experiments (*6*). As expected, inoculated peanuts produced numerous stilbenoids (**Figure 4**), while no stilbenoids were detected in frozen control seeds and only traces of resveratrol and arachidin-3 (**2**) were detected in incubated control seeds. Accumulation of dimers **3** and **4** in challenged peanuts was observed in the second half of the incubation period. Among the longer-retained compounds (**Figure 4**), **3** and **4** were the major compounds along with other minor compounds that bore UV similarities to **3** and **4** and molecular masses around 600 Da. However, the UV and MS spectrometric properties of those compounds could be estimated only after preliminary cleanup procedures due to a significant number of compounds present with very close chromatographic properties. These compounds overlapped with each other and were eluted as a relatively narrow band. Among the compounds whose structures can be also suggested as dehydrodimers are those that produced *m*/*z* 641 and *m*/*z* 591 positive M + H ions. The *m*/*z* 641 pseudomolecular ion could represent an arachidin-1−arahypin-3 dehydrodimer or arachidin-1−arahypin-2 dimer. However, it is more likely that the first of these possibilities is mainly formed due to the presence of a higher concentration of the corresponding precursor, arahypin-3, compared to arahypin-2. Similarly, the *m*/*z* 591 may represent the following dimers: arachidin-3−arachdin-3, arachidin-1−chiricanine A, and arachidin-1−arahypin-1. Arachidin-3−arachdin-3 and arachidin-1−chiricanine A dimers are considered most likely. The MS

patterns of these compounds resembled those of **3** and **4**. However, the small quantities and the difficulty of isolation of these compounds have thus far precluded preparation of sufficient quantities in pure form for NMR analysis.

A few research groups have demonstrated that simple stilbenoids can be transformed into oligomers by treatment with different biochemical catalysts (*12*, *27*−*29*). However, the reactions were performed only *in vitro* with purified enzymes (*12*, *28*, *29*) or fungal culture filtrates (*27*). There is still no evidence that living microorganisms metabolize/detoxify simple stilbenoids to corresponding oligomers. At present, our experimental data do not permit a conclusion as to whether stilbenoid dimers **3** and **4** are produced by peanuts *in vivo* or whether they are perhaps formed only *in vitro* from simple peanut stilbenoids under favorable incubation conditions. However, no significant optical rotation values were observed for samples of **3** and **4** isolated from challenged peanut extracts, and the CD curves for the samples were comparable to those of solvent blanks. Thus, these samples are apparently racemic, suggesting that their formation is likely to have been nonenzymatic.

Stilbenoid dimers represent the most abundant group in the class of stilbenoid-derived oligomers and are known for their diverse biological activities, including antifungal, antinematodal, antioxidant, cancer chemopreventive, anti-inflammatory, anti-HIV, antimutagenic, cytotoxic, hepatoprotective, blood glucose reductive, and tyrosinase inhibitory effects (*10*, *12*−*16*, *18*, *19*, *30*). Based on the similarity of structures **3** and **4** with known natural oligomers, it is reasonable to expect some biological activity from **3** and **4** as well. Taking into account the importance of the knowledge of natural plant defense mechanisms as well as published high biological activity of known stilbenoid oligomers, a systematic study of biological activity on new dimeric stilbenoids is planned. The results will be reported in a timely manner elsewhere.

The present study revealed the production of two new dimeric stilbenoids **3** and **4** (**Figure 1**) by fungal-challenged peanut seeds. These new stilbenoids may possess antifungal properties, analogous to those previously reported for a natural resveratrol trimer (*30*).

ABBREVIATIONS USED

DAD, diode array detector; HPLC, high-performance liquid chromatography; MS, mass spectrometer, mass spectrometry, mass spectra; ESI, electrospray ionization; APCI, atmospheric pressure chemical ionization; HR ESI-MS, high resolution electrospray ionization mass spectrometry; EI-MS, electron impact mass spectrometry; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum correlation.

ACKNOWLEDGMENT

We express our gratitude to A. Larkin for help in the lab, B. Horn for the *A. caelatus* NRRL 25528 strain, and C. Chen for a sample of peanut seeds.

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Received for review September 29, 2009. Revised manuscript received November 27, 2009. Accepted December 1, 2009. Support for the University of Iowa group from the National Science Foundation (CHE-0718315) is gratefully appreciated.