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New Peanut (*Arachis hypogaea*) Phytoalexin with Prenylated Benzenoid and But-2-enolide Moieties

VICTOR S. SOBOLEV,*,† STEPHEN T. DEYRUP,‡ AND JAMES B. GLOER‡

National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 509, Dawson, Georgia 39842, and Department of Chemistry, 305 Chemistry Building, The University of Iowa, Iowa City, Iowa 52242

A new pigmented, optically active, low molecular weight metabolite has been isolated from peanut (*Arachis hypogaea*) kernels challenged by four species of *Aspergillus*. The structure of the new compound, termed SB-1, was elucidated by analysis of ¹H NMR, ¹³C NMR, and mass spectrometric data. The SB-1 molecule bears prenylated benzenoid and but-2-enolide moieties and represents an unusual class of compounds. The closest known analogue to SB-1 was isolated from heartwood of *Pericopsis elata*. Both *A. hypogaea* and *P. elata* belong to the family Leguminosae. The new metabolite was accumulated in different peanut genotypes challenged by five *Aspergillus* species and may be an important representative of a new class of peanut phytoalexins. SB-1 production often exceeds production of major known stilbenes.

KEYWORDS: Peanuts; *Arachis hypogaea*; groundnuts; phytoalexin; but-2-enolide; stilbene; structure elucidation; NMR; HPLC-MS

INTRODUCTION

Phytoalexins are low molecular weight, biologically active metabolites produced by plants as defensive reactions to various exogenous stimuli, particularly fungal invasion (*1*). *Aspergillus flavus* and *Aspergillus parasiticus* commonly invade peanut seeds and often produce the carcinogenic aflatoxins. Peanuts are most susceptible to invasion by these species under drought stress (*2*); when water activity exceeds 0.95, peanuts demonstrate some resistance to fungal invasion (*3*). This resistance, particularly in immature kernels, has been attributed to five stilbene phytoalexins (**Figure 1**; *4*−*9*). Knowledge of the role of stilbene phytoalexins in peanuts is limited and cannot explain why the antifungal activity of individual stilbene phytoalexins (*7, 8, 10*) does not fully correlate with peanut resistance to fungal infection. This suggests that other, as yet unidentified, compounds may be involved in peanut resistance to fungal invasion. The purpose of this study was to isolate and characterize a new substance that may serve as a peanut phytoalexin, together with known stilbenoids.

EXPERIMENTAL PROCEDURES

Reagents, Materials, and Basic Apparatus. HPLC-grade solvents used in the sample extraction, preparation of mobile phases, separations on silica gel, methylation, and isomerization were obtained form Fisher (Suwanee, GA). Celite 545 was purchased from J. T. Baker (Phillipsburg, NJ). Boron trichloride (BCl₃) in MeOH (10%, w/v) and anhydrous
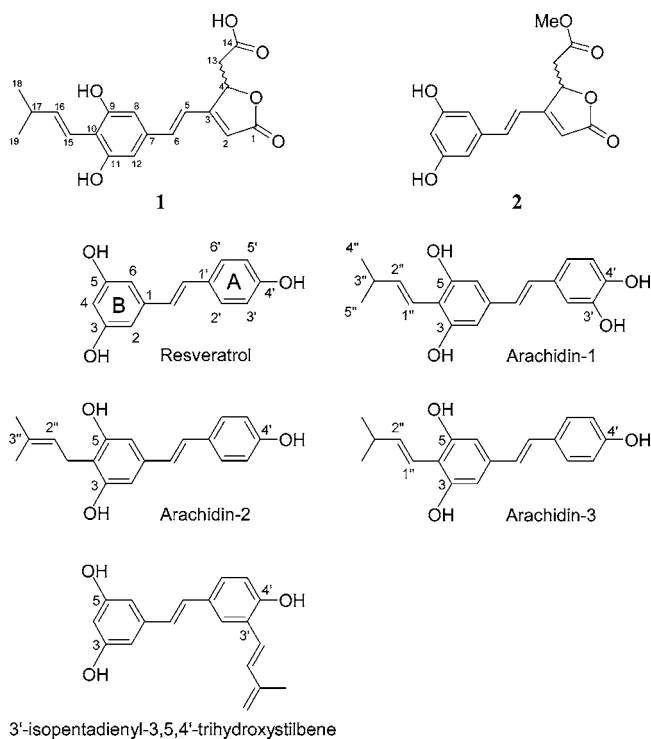

Figure 1. Structures of known peanut stilbene phytoalexins.

Na₂SO₄ were purchased from Aldrich (Milwaukee, WI). Silica gel for thin-layer chromatography and for column chromatography was purchased from EM Science (Gibbstown, NJ). A 2% NaOCl solution was prepared by dilution of household bleach (Clorox). A high-speed

* Author to whom correspondence should be addressed [telephone (229) 995-7446; fax (229) 995-7416; e-mail vsobolev@nprl.usda.gov].
† National Peanut Laboratory.
‡ University of Iowa.

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blender (13000 rpm) with a 1 L glass jar (General Electric) and a model UVSL-58 multiband 254/366 nm hand-held UV lamp (Ultra-Violet Products, San Gabriel, CA) were used in the research. A 2-L, 125 mm i.d. Kimax Büchner-type filtering funnel with a 10−15-μm fritted disk was used as a column for preparative chromatography (Fisher).

Reference Compounds. *trans*-Resveratrol (∼99%) was purchased from Sigma. Pure individual peanut stilbenes, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, and *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene, were obtained as described in ref *11*, with the exception of using preparative HPLC instead of preparative TLC. HPLC separation was achieved by using both gradient systems below. The identities of the reference compounds as well as stilbenes extracted from challenged peanuts were confirmed by ESI-MS/MS (MS^2) and UV spectroscopy. The values determined in this research are given in parentheses as [M + H]$^+$ values followed by UV absorption maxima: *trans*-resveratrol (*m/z* 229; 305 and 317 nm); *trans*-arachidin-1 (*m/z* 313; 339 nm); *trans*-arachidin-2 (*m/z* 297; 308 and 322 nm); *trans*-arachidin-3 (*m/z* 297; 334 nm); *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene (*m/z* 295; 296 nm). The above results were in agreement with published data (*4−9*, *11*).

Fungal Cultures. *A. flavus* NPL TX15-2 (aflatoxin producer, cyclopizonic acid nonproducer), *A. flavus* NPL GA4-4 (aflatoxin nonproducer, cyclopiazonic acid producer), *A. parasiticus* NPL 32 (aflatoxin producer), *Aspergillus niger* NRRL 326 (ex type), and *Aspergillus caelatus* NRRL 25528 (ex type) were used to elicit phytoalexin production in peanuts.

Plant Material and Processing. For phytoalexin production studies, mature, sound peanut pods of the cultivar Georgia Green were surface-sterilized with 2% NaOCl (*12*). The seeds were aseptically removed from the hulls. About one-third of each kernel from the side opposite the embryo was removed with a microtome knife and discarded. A suspension of fungal spores (10^6/mL) from one of the above *Aspergillus* cultures was sprayed over the sliced sides of the kernels. The kernels were then incubated without light at 30 °C for 96 h. Phytoalexin production was determined in individual 0.5-mm-thick slices from the outer wound site after 0, 24, 48, 72, and 96 h of incubation according to the following method. Individual slices were ground in an agate mortar with an equal amount (w/w) of Celite 545. The mixture was extracted with 1 mL of MeOH for 30 min with periodic shaking every 5 min. The extract was filtered through a glass fiber filter. From 5 to 30 μL of the filtrate was analyzed by HPLC.

The following peanut market types were used to confirm their ability to produce compound **1**: Virginia (cultivar NCV11); Valencia (cultivar GT-101); Spanish (cultivar AT9899); and Runners (cultivars Georgia Green, Virugard, Anordon, Sanoleic 97R, AP-3, and GAO3L). Peanut kernels were sliced into ∼2-mm pieces, inoculated with *A. parasiticus* NPL 32, incubated at 30 °C for 72 h, and extracted as described above.

HPLC-DAD-MS Analyses. Analyses were performed using an HPLC system equipped with an LC-10ATvp pump (Shimadzu), an SPD-M10Avp diode array detector covering the 200−600 nm range with Shimadzu Client/Server software, version 7.3, and a model 717 plus autosampler (Waters). The separation was performed on a 150 mm × 4.6 mm i.d., 3.5 μm XTerra RP18 analytical column (Waters). H$_2$O (A), MeOH (B), and 1% HCOOH in H$_2$O (C) were combined in the following gradient: initial conditions, 45% A/50% B/5% C, held for 0.5 min, increased linearly to 0% A/95% B/5% C in 12 min, held isocratic for 3 min, decreased to initial conditions in 0.01 min. The flow rate was 1.2 mL/min. The column was maintained at 35 °C in a model 105 column heater (Timberline Instruments, Boulder, CO). The eluate from the diode array detector was split with a T-unit (Upchurch Scientific, Oak Harbor, WA) for optimal MS performance. The flow rate through the ESI probe was set at 0.35 mL/min. MS analyses were performed using a Finnigan LCQ Advantage MAX ion trap mass spectrometer equipped with an ESI interface and operated with Xcalibur version 1.4 software (Thermo Electron Corp., San Jose, CA). The data were acquired in the full-scan mode (MS) from *m/z* 100 to 2000. The most intense response for the [M + H]$^+$ ion of **1**, *m/z* 345, was obtained with a heated capillary temperature of 250 °C, a sheath gas flow of 45 units, a capillary voltage of 6 V, and a source voltage of 4.5 kV. In MS^2 analyses, the [M + H]$^+$ and [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, 30 or 35%, 0.24, and 30 ms, respectively. Concentrations of *trans*-resveratrol, *trans*-arachidin-1, *trans*-arachidin-2, *trans*-arachidin-3, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene, and **1** were determined by reference to peak areas of corresponding pure standards at 317, 339, 322, 334, 296, and 364 nm, respectively.

Isolation of 1. Georgia Green peanut kernels (1.3 kg) were chopped in a food processor to obtain 3−5 mm size particles. Peanut particles were washed several times with distilled water to remove small particles and peanut juice and were then blotted with a paper towel to remove excess water and prevent bacterial growth during incubation. Approximate 1-cm layers of peanut particles were placed in trays and inoculated by spraying with a spore suspension (10^6) of *A. flavus* NPL TX15-2. After incubation for 72 h without light at a relative humidity close to 100%, the kernels were extracted twice with MeOH (4 L + 2 L) in a high-speed blender. Combined extracts were filtered through filter paper and evaporated nearly to dryness with 150 g of silica gel for column chromatography with a rotary evaporator. To remove excess water from the silica gel, an appropriate amount of CH$_3$CN was added near the end of evaporation, and the mixture was evaporated to dryness. The silica gel appeared as a free-flowing powder.

For isolation of the target compound **1**, a 2-L filtering funnel packed with 350 g of silica gel for TLC (suspended in *n*-hexane) was used as a column for preparative chromatography. Dry silica gel with the peanut extract adsorbed was resuspended in *n*-hexane and applied to the column. The silica gel was allowed to settle and was covered with 2−2.5 cm of glass wool. The column was then sequentially eluted under low vacuum with 1 L of *n*-hexane, 2 L of CHCl$_3$, 2 L of EtOAc, 1.5 L of acetone, and 3.5 L of MeOH. Separation of fluorescent compounds was monitored with a hand-held UV lamp. Eluates from the column were analyzed by HPLC. The target compound (82% purity, area percent at 364 nm) was eluted with the first portion of MeOH (1 L) and subjected to further purification. It was evaporated to dryness with 80 g of silica gel for column chromatography with a rotary evaporator and applied to a 2-L funnel with 400 g of silica gel for TLC as described above. The column was sequentially eluted under low vacuum with a CH$_2$Cl$_2$/MeOH mixture as follows: 3.3 L (10:1, v/v, respectively), 1.2 L (10:2), 1.3 L (10:3), 1.4 L (10:4), 1.5 L (10:5), and 1.6 L (10:6). The target compound (91% purity, area percent at 364 nm) was eluted in the 10:4 fraction. The eluate was evaporated to dryness with a rotary evaporator, redissolved in MeOH, filtered, and subjected to final purification on a 100 × 19 mm i.d., 5 μm XTerra Prep RP$_{18}$ OBD preparative HPLC column (Waters). H$_2$O (A), MeOH (B), and 1% HCOOH in H$_2$O (C) were combined in the following gradient: initial conditions, 65% A/30% B/5% C, held for 1 min, increased linearly to 0% A/95% B/5% C in 10 min, held isocratic for 4 min, decreased to initial conditions in 0.01 min. The flow rate was 9.99 mL/min. Combined eluates containing **1** were diluted with distilled water and extracted three times with EtOAc. Combined EtOAc layers were evaporated nearly to dryness with a rotary evaporator. The compound was crystallized as a yellow solid from an H$_2$O/MeOH/CH$_3$CN mixture. The crystals were then filtered, washed with CH$_3$CN, and dried for 48 h in a desiccator with silica gel. SB-1 (39 mg) showed 99.8% purity (area percent at 364 nm).

Methylation of 1. The methyl ester of **1** was obtained by treatment of 2 mg of **1** with 1.5 mL of BCl$_3$ in MeOH (10% w/v) (Alltech, Deerfield, IL). The reaction mixture was heated for 3 min at 40 °C. Equal amounts of water and toluene (10 mL) were added to stop the reaction and to extract the methyl ester derivative. The toluene layer was dried over Na$_2$SO$_4$, filtered, evaporated to dryness with a stream of N$_2$, redissolved in MeOH, and subjected to preparative separation by HPLC. H$_2$O (A), MeOH (B), 1% HCOOH in H$_2$O (C), and CH$_3$CN (D) were combined in the following gradient: initial conditions, 65% A/15% B/5% C/15% D, increased linearly to 5% A/50% B/5% C/40% D in 15 min, decreased to initial conditions in 0.01 min. The flow rate was 9.99 mL/min. Combined eluates containing the methyl ester of **1** were diluted with distilled water and extracted three times with EtOAc. Combined EtOAc layers were evaporated nearly to dryness with a rotary evaporator, and a ∼20-fold volume of CH$_3$CN was added to the flask.
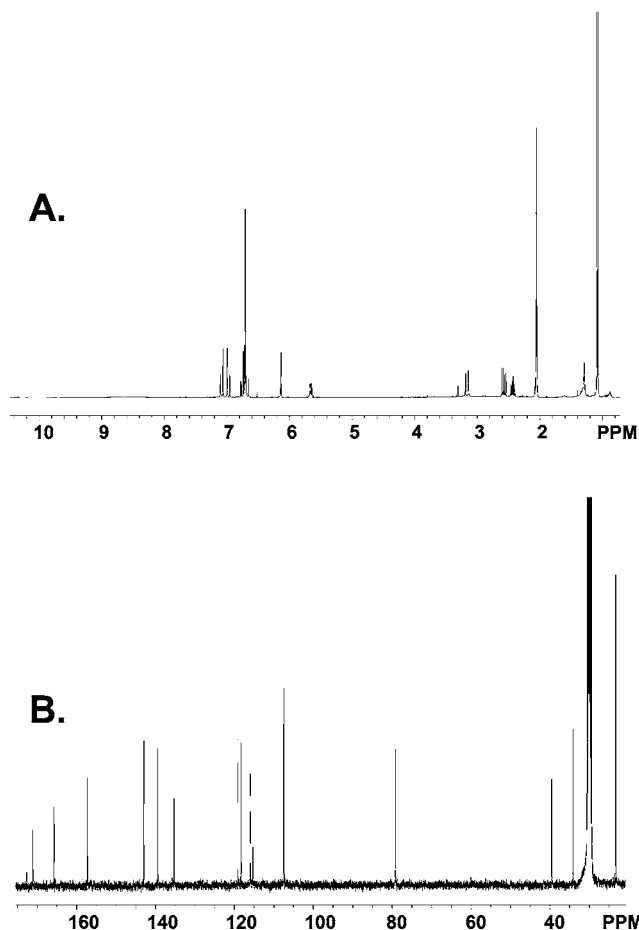
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Figure 2. (**A**) ^{1}H NMR spectrum of **1** in acetone-d_6 at 400 MHz; (**B**) ^{13}C NMR spectrum of **1** in acetone-d_6 at 100 MHz.

Table 1. NMR Spectroscopic Data for **1**

position	δ_H (multiplicity, J_{HH})	$\delta_C{}^b$	HMBC (H# → C#)
1		172.6	
2	6.14 (d, 1.2)	115.9	1, 3, 4, 5, 6^c, 13^c
3		165.7	
4	5.68 (m)	79.0	1, 2, 3, 13, 14
5	6.98 (d, 17)	118.3	2, 3, 4, 6, 7, 8/12^c
6	7.08 (d, 17)	139.4	3, 4^c, 5, 7, 8/12
7		135.2	
8/12	6.70 (s)	107.4	6, 8/12, 9/11, 10
9/11	8.43(s); OH	157.2	
10		115.3	
13	3.19 (dd, 17, 3.0)	39.4	3, 4, 14
	2.58 (dd, 17, 9.1)		3, 4, 14
14	10.95 (br s); OH	171.0	
15	6.67 (d, 16)	119.0	9/11, 10, 16, 17, 18/19^c
16	6.74 (dd, 16, 6.6)	142.9	9/11^c, 10, 15, 17, 18/19
17	2.42 (octet, 6.6)	34.1	15, 16, 18/19
18/19	1.08 (d, 6.6)	23.2	16, 17, 18/19

^{a} ^{1}H NMR data were recorded at 400 MHz; ^{13}C NMR data were recorded at 100 MHz; both in acetone-d_6. ^{b} Protonated carbon signal assignments were supported by HMQC data. ^{c} These four-bond correlations were weak, but observable.

H)]$^-$; 14], 389 [(M + HCOOH − H)$^-$; 100], 687 [(2M − H)$^-$; 25]; positive ESI-MS, *m/z* 345 [(M + H)$^+$; 100], 362 (13); positive ESI-MS^2, *m/z* 345@30, 243 (41), 253 (13), 271 (100), 281 (27), 285 (47), 299 (25), 309 (24), 327 (34).

Methyl ester of **1**: yellow solid; mp 155−158 °C; UV (CH$_3$OH) λ_{max} (log ϵ) 223 (4.46), 263 (4.06), 363 (4.44); ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.53 (s, OH-9/11) 7.07 (d, *J* = 16 Hz, H-6), 6.96 (d, *J* = 16 Hz, H-5), 6.75 (dd, *J* = 16, 6.5 Hz, H-16), 6.69 (s, H-8/12), 6.66 (d, *J* = 16 Hz, H-15), 6.14 (d, *J* = 1.2 Hz, H-2), 5.67 (m, H-4), 3.69 (s, OCH$_3$-14), 3.18 (dd, *J* = 17, 3.3 Hz, H-13a), 2.60 (dd, *J* = 17, 8.9 Hz, H-13b), 2.43 (octet, *J* = 6.5 Hz, H-17), 1.08 (d, *J* = 6.5 Hz, H$_3$-18/19); negative ESI-MS, *m/z* (relative abundance, %) 357 [(M − H)$^-$; 8], 715 [(2M − H)$^-$; 63], 1072 [(3M − 2H)$^-$; 100]; negative ESI-MS^2, *m/z* 357@30, 281 (54), 283 (30), 313 (59), 357 (100).

Cis-isomer of **1**: yellow glass; UV (CH$_3$OH) λ_{max} (log ϵ) 224 (4.39), 262 (4.16), 352 (4.04); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.60 (br s, OH-9/11), 8.14 (s, OH-14), 6.83 (d, *J* = 12 Hz, H-6), 6.73 (dd, *J* = 16, 6.5 Hz, H-16), 6.65 (d, *J* = 16 Hz, H-15), 6.52 (s, H-8/12), 6.25 (br d, *J* = 12 Hz, H5), 6.02 (br s, H-2), 5.54 (m, H-4), 2.88 (dd, *J* = 16, 3.3 Hz, H-13a), 2.48 (dd, *J* = 16, 9.0 Hz, H-13b), 2.42 (octet, *J* = 6.5 Hz, H-17), 1.07 (d, *J* = 6.5, H$_3$-18/19); ^{13}C NMR (acetone-d_6, 100 MHz) δ 172.6, 170.8, 165.4, 157.2 (2C), 142.5, 139.7, 135.4, 119.5, 119.0, 118.4, 114.0, 108.3 (2C), 80.5, 39.9, 34.0, 23.2 (2C); negative ESI-MS, *m/z* (relative abundance, %) 343 [(M − H)$^-$; 96], 389 [M + HCOOH − H]$^-$; 100], 687 [(2M − H)$^-$; 53]; positive ESI-MS, *m/z* 345 [(M + H)$^+$; 100], 362 (41), 706 (16), 711 [2M + Na]$^+$; 16]; positive ESI-MS^2, *m/z* 345@30, 271 (100), 281 (36), 285 (46), 299 (33), 309 (11), 327 (98).

RESULTS AND DISCUSSION

Structure Elucidation. The molecular formula for **1** was determined to be C$_{19}$H$_{20}$O$_6$ with 10 units of unsaturations on the basis of HRESIMS data. Analysis of ^{1}H, ^{13}C, and DEPT NMR data and homonuclear decoupling results revealed the presence of an isopropyl group linked to a *trans*-olefin, a 1,2,3,5-tetrasubstituted benzene ring with *C*2 symmetry, a second, isolated *trans*-olefin moiety, an OCHCH$_2$ unit, a trisubstituted olefin, and two ester or acid carbonyls. ^{13}C NMR shifts indicated that the benzene ring was 1,3-dioxygenated. The trisubstituted olefin proton signal (H-2) showed allylic coupling (1.2 Hz) to the oxygenated methine signal (H-4) of the OCHCH$_2$ unit, leading to construction of a substructure corresponding to the C-2/C-3/C-4/C-13 portion of **1**.

These units were interconnected by analysis of HMBC data. Correlations of H-2 with carbonyl carbon C-1, nonprotonated
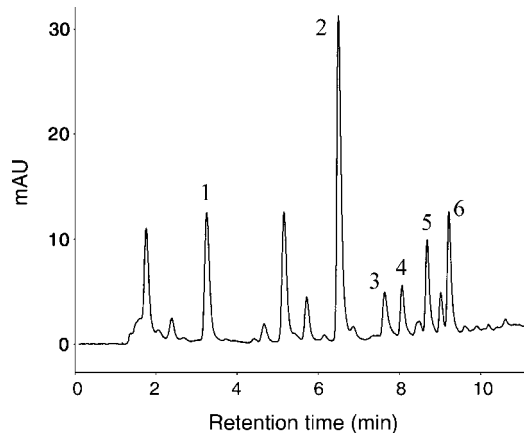
The mixture was then evaporated to dryness. The methyl derivative was obtained as a yellow solid (0.3 mg) by crystallization from H$_2$O/MeOH/CH$_3$CN mixture. The derivative was 98% pure (area percent at 364 nm).

Trans−Cis Isomerization of 1. The *cis*-isomer of **1** was obtained by irradiating **1** with UV light at 365 nm. A solution of 25 mg of **1** in 60 mL of MeOH was placed into four 15-mL borosilicate vials equipped with lids with Teflon septa. The vials were placed in a horizontal position on a sheet of aluminum foil inside a UV viewing cabinet equipped with 4 × 15 W fluorescent lamps and were irradiated from a distance of 15 cm for 5 h. The combined irradiated solutions were then evaporated to dryness on a rotary evaporator, redissolved in MeOH, filtered, and separated by preparative HPLC. The isolation conditions for the *cis*-isomer were the same as for the methyl ester of **1**. The *cis*-isomer was obtained as a yellow glass (4.2 mg) with 98.2% purity (area percent at 262 nm).

General Experimental Procedures for Characterization. The optical rotation of **1** was measured with a Rudolph Research Autopol III automatic polarimeter. UV measurements were performed with a Varian Cary 100 Bio UV−vis spectrophotometer. ^{1}H, ^{13}C NMR, and homonuclear decoupling experiments were performed on a Bruker DRX-400 or a Bruker AC-300. HMBC and HMQC experiments were performed on a Bruker AMX-600 spectrometer. Chemical shift values were referenced to the solvent signals for acetone-d_6 (δ_H 2.05/δ_C 29.92). HRESIMS 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.

1: yellow solid; mp 196−199 °C; [α]$^{25}_D$ +37° (*c* 0.31, CH$_3$OH); UV (CH$_3$OH) λ_{max} (log ϵ) 223 (4.36), 262 (3.99), 364 (4.33); ^{1}H NMR and ^{13}C NMR spectra are shown in **Figure 2**, panels **A** and **B**, respectively; ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC data are given in **Table 1**; HRESIMS, *m/z* 345.1339 [M + H]$^+$, calcd for C$_{19}$H$_{21}$O$_6$, 345.1337; negative ESI-MS, *m/z* (relative abundance, %) 343 [(M −

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Figure 3. HPLC (at 317 nm) of MeOH extract of Georgia Green peanut kernels after incubation with *A. flavus* TX15-2 for 48 h. Peaks: 1, *trans*-resveratrol; 2, SB-1; 3, *trans*-arachidin-1; 4, *trans*-arachidin-2; 5, *trans*-arachidin-3; 6, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene.

Figure 4. Production of phytoalexins by Georgia Green peanut kernels challenged by *A. flavus* NPL GA4-4 (IPD, *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene).

Figure 5. Proposed sequence of SB-1 (**d**) formation from *trans*-arachidin-1 (**a**).

olefinic carbon C-3, and C-5 of the isolated *trans*-olefin unit, along with correlations of H-4 and H_2-13 to the second carbonyl (C-14), allowed connection of C-1 to C-2, C-3 to C-5, and C-13 to C-14. A key correlation from H-4 to C-1 together with the downfield shift of H-4 enabled assignment of the five-membered lactone ring. H-6 correlated to C-7 and C-8/C-12 of the benzene ring, enabling connection of C-6 to C-7. Correlations from H-15 to C-10 and C-9/C-11 connected the C15−C19 prenyl unit to C-10 of the benzene ring, thus completing the assignment of the carbon skeleton of **1**.

The presence of three exchangeable protons reflected in the molecular formula and the DEPT results indicated that the aromatic ring must have two (degenerate) phenolic OH groups and that C-14 must be part of a free carboxylic acid. The presence of the carboxylic acid functionality was confirmed by the formation of a methyl ester upon treatment with BCl_3/MeOH. The ^{1}H NMR spectrum of the product showed one new methoxy signal, whereas the mass spectrum indicated an increase of 14 Da. The ^{1}H NMR data clearly indicated that the new methyl group was added as a methyl ester rather than as a methyl ether involving one of the phenolic OH groups because the symmetry of the aromatic system was retained.

SB-1 (**1**) possessed optical activity; however, the absolute configuration at the C4 chiral center was not elucidated.

Like all peanut stilbene phytoalexins (*6, 11*), **1** exists naturally as the *trans*-isomer but can be converted into the corresponding *cis*-isomer by exposure to UV−vis light radiation. In our experiments, the reaction did not go to completion; the ratio of *cis*- to *trans*-isomer was about 1:4. UV irradiation of **1** led to isomerization to form the corresponding C5−C6 *cis*-isomer, which was separated by HPLC and identified by NMR analysis. The only significant difference in the ^{1}H NMR spectrum was the expected reduction in the J_{H5-H6} value (from 17 to 12 Hz).

Production of 1 by Peanut Kernels. A typical HPLC chromatogram of the MeOH extract of a fungus-challenged peanut kernel is given in **Figure 3**. In most of the experiments, SB-1 was the major metabolite between 48 and 72 h of incubation at 30 °C. In all of the experiments phytoalexins were not detected in control peanuts or in peanuts immediately after inoculation with fungal spores. **Figure 4** (one experiment) demonstrates production of phytoalexins by Georgia Green peanut kernels challenged by *A. flavus* NPL GA4-4. Accumulation of **1** increased from 48 to 72 h and exceeded accumulation of other phytoalexins, reaching 4.51 mg/g. At the same time, a concomitant decrease in resveratrol concentration from its
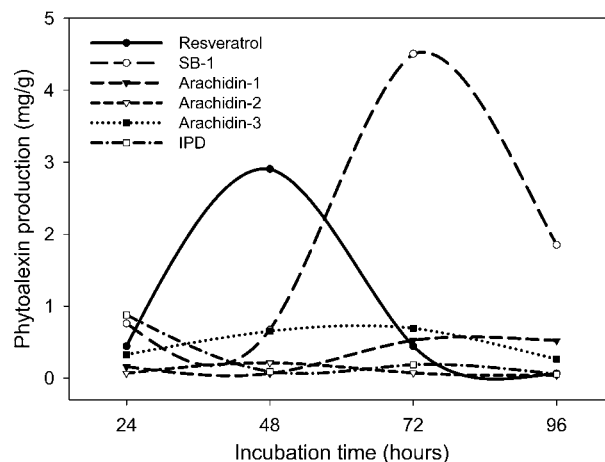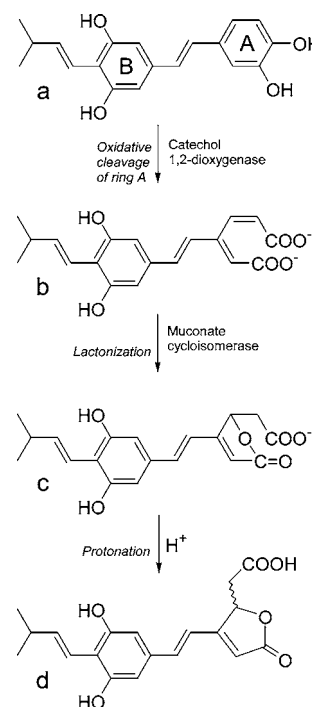
highest level (2.91 mg/g) at 48 h to a lower level (0.44 mg/g) at 72 h was observed. Accumulation of the arachidins and *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene varied during incubation; however, their concentrations never approached the high concentrations of resveratrol and **1**. A similar pattern of phytoalexin production was observed when Georgia Green kernels were inoculated with other *Aspergillus* strains. Different peanut cultivars also produced the same set of phytoalexins, including **1**, after incubation with *A. flavus* TX15-2. The highest levels of resveratrol and **1** were present in infected kernels at different times. Resveratrol is the first stilbene that is produced in significant quantities by peanuts under biotic stress (*11*). The structure of this simplest of all known peanut stilbenes can serve as a building block for more hydrophobic stilbenes (**Figure 1**) with higher biological activities. Isoprenylation increased the fungitoxicity of the corresponding nonprenylated stilbenes that had higher polarity and lower activity (*6*). Similar effects were observed for isoflavonoid phytoalexins (*13*).

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Production of **1** in different peanut genotypes was elicited by different soil fungi, including toxigenic and nontoxigenic *A. flavus* and *A. parasiticus*, as well as *A. caelatus* and *A. niger*. This reconfirms the fact that the peanut plant has a basic, genetically preprogrammed response to fungal invasion (*4−8, 11*). Such a response also suggests that **1** may serve as a phytoalexin.

Origin of 1. The origin of the new metabolite is unknown; however, the number of carbon atoms corresponds to that of stilbenes, suggesting that **1** may derive from the coexisting stilbene, arachidin-1. The *o*-dihydroxybenzene moiety in arachidin-1 (**Figure 5a**) might be oxidatively cleaved to produce a *cis,cis*-muconic acid derivative (**b**). Lactonization of the muconate (**b**) would afford the muconolactone (**c**), which, followed by protonation, would give **1** (**d**). Because **1** is optically active, enzymes are likely involved in its biosynthesis from the suspected stilbene precursor. Although this proposed sequence is speculative, the above steps seem to be reasonable on the basis of analogous sequences described in the literature (*14*). The new metabolite is suggested to be an important representative of a new class of peanut phytoalexins because its production (**Figure 4**) often exceeds the production of known major stilbenes (**Figure 1**). It is also possible that **1** is accumulated as the product of fungal enzymatic detoxification of arachidin-1 or other related stilbenes. However, because its structure is closely related to but-2-en-4-olide **2** synthesized by the legume *Pericopsis elata* (*15*), **1** is likely an unmodified peanut metabolite. The biological activity of the new compound is the subject of future investigation.

ABBREVIATIONS USED

DEPT, distortionless enhancement by polarization transfer; ESI, electrospray ionization; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum correlation; HPLC, high-performance liquid chromatography; HRESIMS, high-resolution electrospray ionization mass spectrometry.

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Supporting Information Available: ^{1}H NMR spectrum of methyl ester of **1** in acetone-d_6 at 300 MHz; ^{1}H NMR spectrum of the *cis*-isomer of **1** in acetone-d_6 at 400 MHz; ^{13}C NMR spectrum of *cis*-isomer of **1** in acetone-d_6 at 100 MHz; positive ESI-MS[2] spectrum of **1**, *m/z* 345@35; negative ESI-MS[2] spectrum of **1**, *m/z* 343@30 (infusion in MeOH−IPA). This information is available free of charge via the Internet at http://pubs.acs.org.

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