JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 10281−10287 **10281**

Biosynthesis Enhancement and Antioxidant and Anti-inflammatory Activities of Peanut (*Arachis hypogaea* L.) Arachidin-1, Arachidin-3, and Isopentadienylresveratrol

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Peanut is a potent plant to be induced to synthesize bioactive stilbenoids. Bioactivities of those stilbenoids except resveratrol have been meagerly investigated. When peanut kernels (Tainan 14, a Spanish cultivar) were imbibed, incubated 3 days for germination, sliced, incubated with artificial aeration, periodically sampled, lyophilized, extracted with methanol, and subjected to reverse-phase HPLC analysis, four major fractionations were detected and identified as *trans*-resveratrol (Res), *trans*-arachidin-1 (Ara-1), *trans*-arachidin-3 (Ara-3), and *trans*-isopentadienylresveratrol (IPD). During incubation of the peanut slices, contents of Res, Ara-1, and Ara-3 increased tremendously from initially trace or not detectable amounts up to 147.3, 495.7, and 2414.8 μg/g, corresponding to 20, 16, and 24 h of incubation, while IPD contents continued to increase up to 28 h (4474.4 μg/g). When the four stilbenoids and butylated hydroxytoluene (BHT) were subjected to antioxidant characterization by various measures, all have exhibited varied potencies of antioxidant activity. In particular, retardation of absorbance increase at 234 nm as formation of the conjugated diene hydroperoxides in a real pork oil system stored at 60 °C, supplement of Ara-1 at 100 μM has shown equivalent or even greater activity than did BHT. When the media were supplemented with Res, Ara-1, Ara-3, and IPD at 15 μM for cultivation of mouse macrophage RAW 264.7 cells activated by lipopolysaccharide (LPS), the LPS-induced extracellular production of prostaglandin E2 (PGE_2) and nitric oxide (NO) was significantly inhibited by Ara-1 ($p < 0.001$), Res ($p < 0.001$), Ara-3 ($p < 0.01$), and IPD ($p < 0.01$). It is noteworthy and of merit that all test stilbenoids have exhibited potent antioxidant and anti-inflammatory activities and varied as affected by number of hydroxyl groups and isopentenyl or isopentadienyl moiety.

KEYWORDS: ***Arachis hypogaea* L.; peanut; groundnut; resveratrol; stilbenoids; arachidin; antioxidant; anti-inflammation**

INTRODUCTION

Resveratrol (*trans*-3,5,4′-trihydroxystilbene) (**Figure 1**) is a phytochemical bearing potent bioactivities including antioxidation, chemoprevention of cardiovascular diseases and cancers, and even extension of lifespan (*1*−*9*). Resveratrol, as a phytoalexin, has been demonstrated mainly in grapes and peanuts (*10*) biosynthesized by induction as a secondary metabolite against fungal infection or under stress. It has been detected in infected peanut hypocotyls, kernels, roots, leaves, pods, processed foods, tissue-cultured callus, sprouts, and root mucilage (*11*−*21*), indicating that peanut is a potent bioresource of resveratrol. Since resveratrol was first detected in the infected hypocotyls of African peanuts by Ingham (*11*), *trans*-4-(3-methyl-1-butenyl)-3,5,4′-trihydroxystilbene (arachidin-3) (**Figure 1**) has been detected in germinating peanut seeds challenged with native microflora (*22*). Arachidin-1 [*trans*-4-(3-methyl-1-butenyl)-3,5,3′,4′-tetrahydroxystilbene] was detected by Aguamah et al. (*23*) and Wotton and Strange (*24*) and isopentadienylresveratrol (IPD) [*trans*-3′-(3-methyl-1,3-butadienyl)-3,5,4′-trihydroxystilbene] was isolated later and identified by Cooksey et al. (*25*), all from the sliced imbibed peanut kernels and subjected to with or without fungal challenge. A new peanut phytoalexin SB-1 detected in the peanut kernels challenged with *Aspergillus* species and seven prenylated stilbenes detected in the peanut root mucilage have been reported recently by Sobolev et al. (*21*, *26*). The biomedicinal activities other than antifungal activity of those stilbenoids (except resveratrol) have been meagerly investigated. As most of those phytoalexins are not

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10.1021/jf0620766 CCC: $33.50
Published on Web 11/22/2006

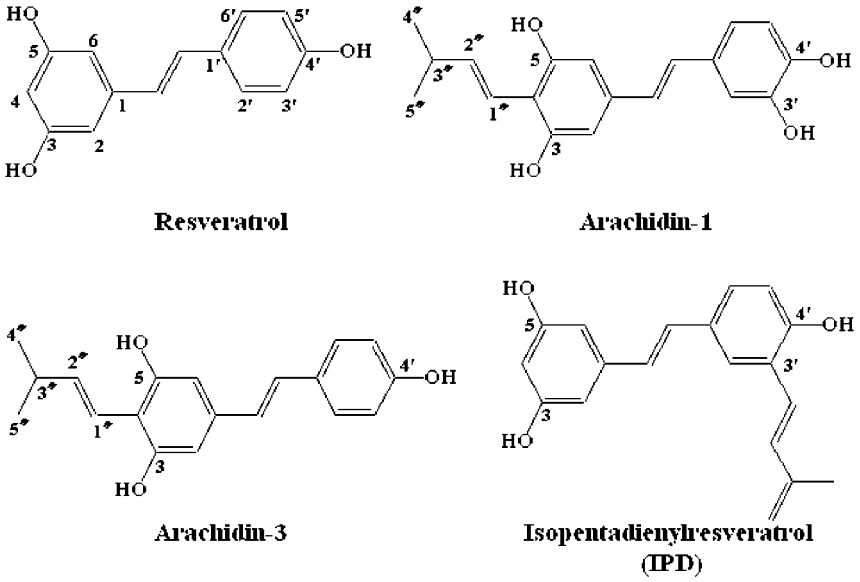

Figure 1. Chemical structures of resveratrol, *trans*-3,5,4′-trihydroxystilbene; arachidin-1, *trans*-4-(3-methyl-1-butenyl)-3,5,3′,4′-tetrahydroxystilbene; arachidin-3, *trans*-4-(3-methyl-1-butenyl)-3,5,4′-trihydroxystilbene; and isopentadienylresveratrol (IPD), *trans*-3′-(3-methyl-1,3-butadienyl)-3,5,4′-trihydroxystilbene.

commercially available, to prepare substantial amounts in the laboratory is a prerequisite to be able to conduct relevant experiments.

Cyclooxygenase-2 (COX-2) is an inducible enzyme expressed in activated macrophages, fibroblasts, and several other cells in production of prostaglandin E2 (PGE_2), as one of the major inflammatory metabolites. In vitro, COX-2 expression was induced in response to stimuli such as lipopolysaccharide (LPS) and growth factors (*27*, *28*). Besides COX-2, inducible nitric oxide synthase (iNOS) is another enzyme contributing to inflammation by production of nitric oxide (NO). Normally, similar to COX-2, iNOS is not present in the resting cells but induced by various stimuli, including LPS and tumor necrosis factor α (TNF-α) (*29*). NO being reactive to activate COX-2 to synthesize PGE_2 and eventually enhance inflammation has been reported (*30*). Various natural polyphenols including resveratrol are known to have anti-inflammatory activity (*4*). On the basis of the fact that arachidin-1, arachidin-3, and isopentadienylresveratrol (IPD) are peanut original and are structurally similar to resveratrol, it is worthwhile to investigate their effects on suppression of PGE_2 and NO production.

Biosynthesis of resveratrol and some other stilbenoids enhanced by imbibation of peanut kernels and subjected to biotic or abiotic inductions, such as germination, artificial microbial inoculation, wounding, UV irradiation, or ultrasonic treatment, has been extensively investigated (*19*, *20*, *23*, *31*, *32*). From the preliminary experiments extensively conducted in our laboratory, it was noticed that incubation of the imbibed kernels for germination prior to slicing and further incubation of the slices with artificial aeration was critically important in enhancement of simultaneous biosynthesis of resveratrol, arachidin-1, arachidin-3, and IPD (**Figure 1**). In this study, a custom-made glass apparatus (**Figure 2**) has been applied for aerobic incubation with artificial aeration of the peanut slices prepared from 3-day germinated kernels to enhance biosynthesis of the stilbenoids. The stilbenoids were further isolated, identified, and subjected to antioxidant characterization by various measures including determinations of antioxidative potency (AOP), reducing power, free radical (1,1-diphenyl-2-picryl-hydrazyl, DPPH) scavenging activity, and retardation of absorbance increase at 234 nm as formation of the conjugated diene hydroperoxides

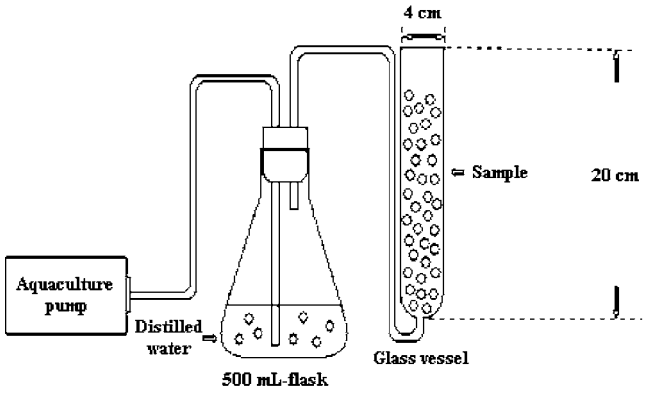

Figure 2. Illustrative drawing of a custom-made glass apparatus used for biosynthesis enhancement of peanut stilbenoids affiliated with artificial aeration.

(CDHP) in a real pork oil system. Investigations of their involvement in suppression of PGE_2 and NO production, as indicators of anti-inflammatory potency, by a cell model of mouse macrophage of RAW 264.7 were extended.

MATERIALS AND METHODS

Peanut Cultivar, Germination, Pretreatment and Incubation. Sound, graded and mature peanut kernels of Tainan 14 (*Arachis hypogaea* L., a Spanish cultivar) were imbibed by soaking with tap water for 16 h at the ambient temperature (23−25 °C), drained, and subjected to incubation on a punctured plastic tray at the ambient temperature for germination. During germination, the kernels were daily washed with tap water to lessen microbial growth. Then, the germinated kernels were sliced manually (ca. 0.4 mm thickness) with a razor blade and incubated in a custom-made glass apparatus (**Figure 2**). After deposition of ca. 50 g of peanut slices into the glass vessel, the vessel was wrapped with aluminum foil to protect from light exposure and artificially aerated with humidified air by an aquaculture pump (ca. 1.5−2.0 L/min flow rate as measured with a flow meter) for incubation at 23−25 °C. During incubation, samples (ca. 1.0 g aliquots) were taken every 4 h up to 28 h. Two aliquots were sampled after the slices in the vessel were poured out and thoroughly mixed. Duplicate experiments were conducted. The samples were then lyophilized and pulverized into powder and stored at −25 °C for further analyses.

Extraction and HPLC Analysis of the Peanut Stilbenoids. For methanol extraction, the procedure reported by Chen et al. (*15*) was

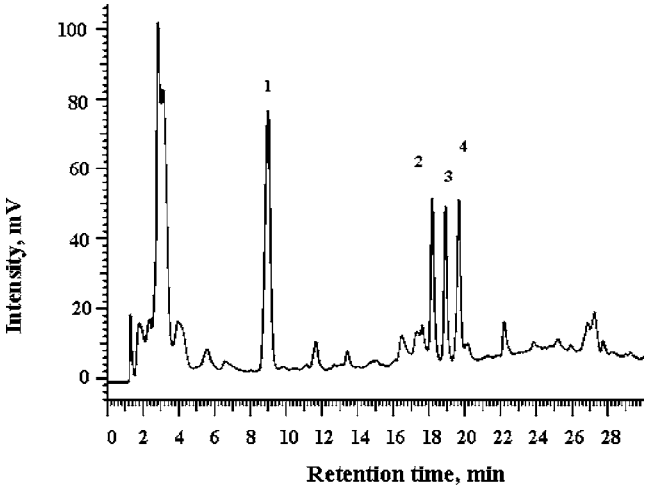

Figure 3. HPLC chromatogram of the peanut stilbenoids extracted from peanut slices subjected to aerobic incubation for 8 h: peak 1, *trans*-resveratrol; peak 2, *trans*-arachidin-1; peak 3, *trans*-arachidin-3; and peak 4, *trans*-isopentadienylresveratrol (IPD).

followed. Then the extracts were membrane-filtered (0.45 μm) and subjected to HPLC analysis (L-7100 pump, L-7420 UV detector, and L-7455 diode array detector, Hitachi Co., Ltd., Tokyo, Japan) with a RP-18 column (Hypersil ODS, 250 × 4.6 mm, 5 μM, Thermal Hypersil Ltd., Cheshire, U.K.). The mobile phase contains A (100% methanol) and B (water). The analysis was initiated with 50% A and 50% B, programmed to 100% A and 0% B in 22 min, held for an additional 3 min, programmed to 50% A and 50% B in 2 min, and held for an additional 3 min. The flow rate and monitoring wavelength were 1.0 mL/min and 254 nm, respectively. Authentic resveratrol (Sigma Co., St. Louis, MO) was run under identical conditions as a reference for identification and quantification of resveratrol. For quantifying other HPLC-separated fractions (peaks 2, 3, and 4 shown in **Figure 3**), their concentrations were respectively determined after each molecular structure was identified by NMR analyses and applied to construct a reference relationship between the injected concentrations and their corresponding HPLC peak areas.

Isolation and Identification. For isolating fractions of peaks 2, 3, and 4 shown in **Figure 3**, a semipreparative column (Hyperprep HS C18, 250 × 10 mm, 8 μm, Thermal Hypersil Ltd., Cheshire, U.K.) was equipped and run at 3.0 mL/min flow rate and 0.5 mL injection volume. The collected fractions were subjected to vacuum drying by a freeze-drier (Lyvotac GT2, Finn-Aqua, Heraus, Germany) and stored in brown vials at −25 °C for later use. Samples except peak 1 (resveratrol) after dissolving in CD_3OD were subjected to ^1H and ^{13}C NMR spectroscopic analyses for structural elucidation. Nuclear magnetic resonance spectra were recorded on Bruker AMX-400 and Avance 500 Fourier transform (FT-) NMR spectrometers. All chemical shifts were reported in parts per million (ppm) from tetramethylsilane as an internal standard.

For peak 2, the NMR signals are summarized as follows: ^1H NMR (CD_3OD, 400 MHz) δ 1.09 (6H, d, J = 6.9 Hz, H-4″ and -5″), 2.40 (1H, m, H-3″), 6.46 (2H, s, H-2′ and -6′), 6.59 (2H, m, H-1″ and -2″), 6.68 (1H, d, J = 16.2 Hz, H-α), 6.73 (1H, d, J = 8.2 Hz, H-5′), 6.82 (1H, dd, J = 8.2, 1.7 Hz, H-6′), 6.86 (1H, d, J = 16.2 Hz, H-β), 6.96 (1H, d, J = 1.7 Hz, H-2′); ^{13}C NMR (CD_3OD, 100 MHz) δ 23.4 (C-4″ and -5″), 34.4 (C-3″), 105.9 (C-2 and -6), 113.0 (C-4), 113.8 (C-2′), 116.4 (C-5′), 119.4 (C-1″), 120.1 (C-6′), 126.8 (C-α), 129.1 (C-β), 131.2 (C-1′), 138.0 (C-1), 141.5 (C-2″), 146.5 (C-3′ and -4′), 157.4 (C-3 and -5).

For peak 3, the NMR signals are summarized as follows: ^1H NMR (CD_3OD, 500 MHz) δ 1.09 (6H, d, J = 6.7 Hz, H-4″ and -5″), 2.40 (1H, m, H-3″), 6.47 (2H, s, H-2′ and -6′), 6.59 (2H, m, H-1″ and -2″), 6.74 (1H, d, J = 16.3 Hz, H-α), 6.76 (2H, d, J = 8.0 Hz, H-3′ and -5′), 6.92 (1H, d, J = 16.3 Hz, H-β), 7.34 (2H, d, J = 8.0 Hz, H-2′ and -6′); ^{13}C NMR (CD_3OD, 100 MHz) at δ 23.4 (C-4″ and 5″), 34.4 (C-3″), 105.9 (C-2 and -6), 113.0 (C-4), 116.5 (C-3′ and -5′), 119.5 (C-1″), 126.8 (C-α), 128.7 (C-2′ and-6′), 128.8 (C-1′), 130.6 (C-β), 138.0 (C-1), 141.6 (C-2″), 157.4 (C-3 and -5), 158.3 (C-4′).

For peak 4, the NMR signals are summarized as follows: ^1H NMR (CD_3OD, 500 MHz) at δ1.99 (3H, s, H-5″), 5.04 (1H, s, H-4″a), 5.12 (1H, s, H-4″b), 6.17 (1H, t, J = 2.2 Hz, H-4), 6.47 (2H, d, J = 2.2 Hz, H-2 and -6), 6.78 (1H, d, J = 8.0 Hz, H-5′), 6.84 (1H, d, J = 16.5 Hz, H-α), 6.88 (1H, d, J = 16.5 Hz, H-1″), 6.98 (1H, d, J = 16.5 Hz, H-α), 6.99 (1H, d, J = 16.5 Hz, H-2″), 7.26 (1H, dd, J = 8.0, 20 Hz, H-6′), 7.58 (1H, d, J = 2.0 Hz, H-2′); ^{13}C NMR (CD_3OD, 125 MHz) at δ 18.8 (C-5″), 102.7 (C-4), 105.8 (C-2 and-6), 116.9 (C-4″ and -5′), 124.8 (C-1″), 125.7 (C-2′), 125.9 (C-1′), 127.3 (C-α), 127.5 (C-6′), 129.4 (C-β), 130.6 (C-3′), 132.5 (C-2″), 141.3 (C-1), 144.1 (C-3″), 156.0 (C-4′), 159.7 (C-3 and -5).

Determination of Antioxidative Potency, Reducing Power, and Diphenylpicrylhydrazyl Scavenging Activity. The procedure reported by Hsu et al. (*33*) for antioxidative potency (AOP) determination was followed. For comparison on a molecular basis, 100 μM resveratrol, arachidin-1, arachidin-3, IPD, and butylated hydroxytoluene (BHT), all in methanol, were prepared. For determination of reducing power, the procedure reported by Yen and Chen (*34*) was followed. Briefly, to a series of glass test tubes containing 0.5 mL of the antioxidants were added 0.5 mL of 0.2 M sodium phosphate buffer, pH 6.6, and 0.5 mL of 1.0% $K_3Fe(CN)_6$ aqueous solution, and the mixtures were incubated at 50 °C in a water bath for 20 min. After rapid cooling of the samples in an ice bath, 0.5 mL of 10% trichloroacetic acid was added to each one, and they were centrifuged at 8000*g* for 10 min. One milliliter of the supernatant was mixed with 1.0 mL of deionized water and 0.2 mL of 1.0% $FeCl_3$ aqueous solution, and the mixture was incubated at ambient temperature (25−26 °C) for 10 min, followed by absorbance determination at 700 nm.

For determination of DPPH scavenging activity, the method of Shimada et al. (*35*) was followed. For each determination, 0.25 mL of 2.0 mM DPPH solution (in methanol) was mixed with 2.5 mL of 400 μM resveratrol, arachidin-1, arachidin-3, IPD, or BHT, each in methanol, and the mixture was incubated in darkness at 25 °C for 30 min. The absorbance of the solution after incubation was determined at 517 nm. Methanol was run concurrently as a blank and applied for determination of DPPH scavenging activity according to the reported equation.

Retardation of Absorbance Increase at 234 nm of Pork Oils as an Indicator of Formation of Conjugated Diene Hydroperoxides (CDHP). The procedure reported by Chiou et al. (*36*) was followed. Briefly, fresh pork fat was diced and cooked at 125 °C with agitation every 30 min for 2 h. After cooling to ca. 50 °C, the free oils at the top layer were withdrawn and centrifuged (8000*g*) for 1 min. Aliquots (1 mL) of the supernatant oil were deposited in a series of 10 mL brown vials and respectively supplemented with 100 μL of resveratrol, arachidin-1, arachidin-3, IPD, and BHT solutions (in methanol) to reach 100 μM as the final supplemented concentration. The vials were stored at 60 °C for 9 days. From each vial, 2.5 μL of oil was sampled after shaking to mix and combined with 2.5 mL of isooctane and subjected to absorbance determination at 234 nm as a quantitative measure of CDHP formation.

Cultivation of Mouse Macrophage RAW 264.7 Cells and Cell Viability Determination. RAW 264.7 cells were cultured with Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a 75 mm^2 tissue culture flask. The flasks were incubated at 37 °C with 95% air humidity and 5% CO_2 atmosphere.

Cell viability was evaluated by direct microscopic cell counting. Aliquots of 5 × 10^5 RAW 264.7 cells were cultured 1 day in a series of 6-well plates. After respective introduction of resveratrol, arachidin-1, arachidin-3, and IPD at various concentrations, namely, 0, 7.5, 15, and 30 μM, the plates were further cultivated for 24 h and cell numbers were counted under a microscope. As compared and evaluated accordingly, 15 μM resveratrol, arachidin-1, arachidin-3, and IPD did not result in obvious cytotoxicity (relative viabilities were higher than 80%). Thus, 15 μM was chosen and applied for the following assays.

Prostaglandin E2 Determination. Aliquots of 5 × 10^5 RAW 264.7 cells were cultured overnight in a series of 24-well plates. Immediately after introduction of 100 ng/mL LPS for activation, the cells were

Table 1. Changes of *trans*-Resveratrol, *trans*-Arachidin-1, *trans*-Arachidin-3, and *trans*-Isopentadienylresveratrol Contents during Aerobic Incubation of the Slices Prepared from 3-Day Germinated Peanut Kernels

incubation time, h	contents of peanut stilbenoids, μg/g[a]			
	Res	Ara-1	Ara-3	IPD
0	trace	nd	nd	nd
4	trace	nd	nd	nd
8	66.8 ± 2.3 c	120.9 ± 8.2 d	827.4 ± 67.0 c	938.8 ± 96.7 e
12	116.1 ± 3.2 b	145.4 ± 2.2 cd	1795.4 ± 15.0 b	1826.5 ± 80.5 d
16	130.2 ± 4.3 ab	495.7 ± 39.7 a	2217.8 ± 15.0 a	2871.3 ± 107.2 c
20	147.3 ± 14.0 a	244.9 ± 7.5 b	2412.4 ± 164.7 a	3856.9 ± 361.3 b
24	142.4 ± 4.9 a	179.1 ± 15.4 c	2414.8 ± 192.2 a	4171.2 ± 503.5 ab
28	102.4 ± 8.1 b	83.5 ± 14.9 d	1808.8 ± 111.5 b	4474.4 ± 167.6 a

[a] Each value represents mean ± SD ($n = 3$) of the freeze-dried weight. nd, not detectable. Values in each column with different letters are significantly different ($p < 0.05$).

respectively treated with 15 μM of resveratrol, arachidin-1, arachidin-3, and IPD and further incubated for 23 h. The extracellular medium containing the synthesized PGE_2 was determined by a PGE_2 enzyme immunoassay kit (Cayman, Ann Arbor, MI). PGE_2 production was quantified by absorbance determination at 420 nm.

Nitric Oxide Determination. Cells were cultivated, LPS-activated, and treated with 15 μM resveratrol, arachidin-1, arachidin-3, and IPD, following the above procedure done for PGE_2 determination. The extracellular medium containing NO_2^- was determined by Griess reagent system (Promega, Madison, WI). Briefly, each collected supernatant was incubated with an equal volume of Griess reagent and incubated at room temperature for 10 min, and NO production was determined by measurement of absorbance at 550 nm. $NaNO_2$ was used to generate a standard curve for estimation of NO concentrations.

Statistics. At least three replicates for each treatment were conducted. Means of the determinations with standard deviation are expressed. Analysis of variance ($p = 0.05$) among the test antioxidants and production of the stilbenoids during incubation of the peanut slices were analyzed by SAS (strategy analysis system). Differences of data between LPS-treated control and each treatment with various peanut stilbenoids were analyzed by Student's *t*-test. Statistical probability was expressed as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

RESULTS AND DISCUSSION

When peanut kernels were imbibed, germinated, sliced, incubated with artificial aeration, extracted with methanol, and subjected to HPLC analysis, four major well-resolved peaks were observed (**Figure 3**). Peak 1 was detected as *trans*-resveratrol; the other fractions (peaks 2, 3, and 4) were fractionated by semipreparative HPLC, vacuum-dried, dissolved in CD_3OD, and subjected to ^1H and ^{13}C NMR analyses for structural elucidation. According to their NMR data (presented under Methods and Materials), peak 2 was identified as *trans*-4-(3-methyl-1-butenyl)-3,5,3′,4′-tetrahydroxystilbene (*trans*-arachidin-1, Ara-1) on the basis of the achieved NMR spectra. Similarly, peaks 3 and 4 were identified as *trans*-4-(3-methyl-1-butenyl)-3,5,4′- trihydroxystilbene (*trans*-arachidin-3, Ara-3) and *trans*-3′-(3-methyl-1,3-butadienyl)-3,5,4′-trihydroxystilbene (*trans*-isopentadienylresveratrol, IPD). After structural identification of the stilbenoids, molecular comparisons of biological activities could be made in terms of the known molecular weights.

When the peanut slices were incubated under specific conditions for 28 h, resveratrol, arachidin-1, and arachidin-3 contents increased tremendously from initially trace or not detected amounts to 147.3, 495.7, and 2414.8 μg/g corresponding to 20, 16, and 24 h of incubation (**Table 1**). Upon further incubation of the slices, resveratrol, arachidin-1, and arachidin-3 contents decreased slightly while IPD contents continued to increase with time. After 28 h of incubation, the average IPD content was 4474.4 μg/g. Obviously, those stilbenoid metabolites were biosynthesized interactively and correlatively during incubation under the specified condition. In particular, it is of merit to achieve a simultaneous biosynthesis of considerable amounts of resveratrol, arachidin-1, arachidin-3, and IPD in peanut kernels subjected to imbibation, germination, slicing, and incubation with artificial aeration for 16−24 h. Since molecular extinction coefficients are different among the test molecules monitored at 254 nm, the peak sizes shown in **Figure 3** did not directly reflect their real quantities as shown in **Table 1**.

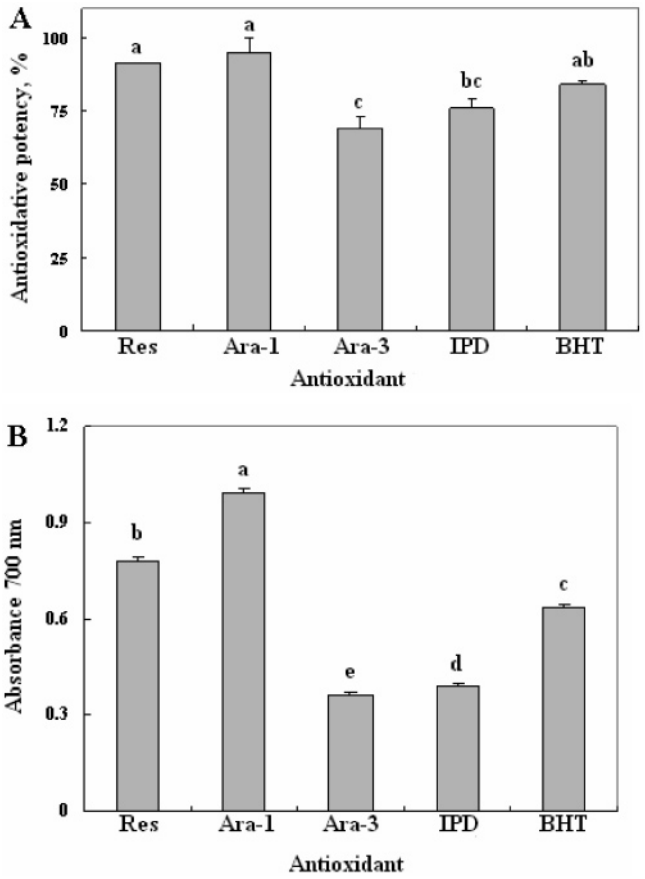

Figure 4. (**A**) Antioxidative potencies and (**B**) reducing powers of *trans*-resveratrol (Res), *trans*-arachidin-1 (Ara-1), *trans*-arachidin-3 (Ara-3), *trans*-isopentadienylresveratrol (IPD), and butylated hydroxytoluene (BHT) at 100 μM. Means of determinations ± SD ($n = 3$) shown by bars with different letters are significantly different ($p < 0.05$).

When resveratrol, arachidin-1, arachidin-3, IPD, and butylated hydroxytoluene (BHT) were subjected to determinations of antioxidative potency (AOP) and reducing power at 100 μM, all test compounds showed potent antioxidant activity (**Figure 4**). In comparison, AOPs of resveratrol, arachidin-1, and BHT did not differ significantly from one another. The highest reducing power was obtained by arachidin-1, followed in order by resveratrol, BHT, IPD, and arachidin-3. When BHT, resveratrol, arachidin-1, arachidin-3, and IPD at 400 μM were subjected to determinations of DPPH scavenging activity (**Figure 5**), resveratrol showed equivalently potent activity as BHT, and both had significantly higher activity than arachidin-1, arachidin-3, and IPD. In a real pork oil system subjected to storage at 60 °C in retardation of absorbance increase at 234 nm as formation of CDHP, all supplemented antioxidants at 100 μM showed inhibitory effects against CDHP formation

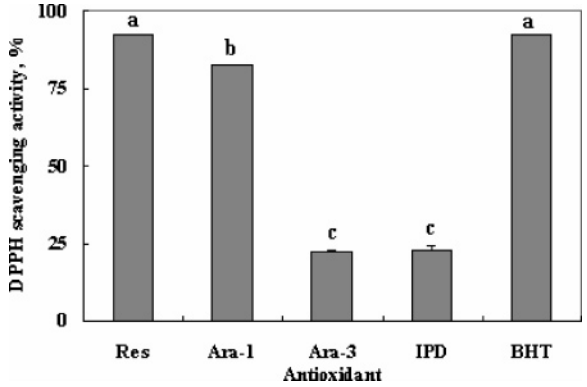

Figure 5. Diphenylpicrylhydrazyl (DPPH) scavenging activities of *trans*-resveratrol (Res), *trans*-arachidin-1 (Ara-1), *trans*-arachidin-3 (Ara-3), *trans*-isopentadienylresveratrol (IPD), and butylated hydroxytoluene (BHT) at 400 μM. Means of determinations ± SD ($n = 3$) shown by bars with different letters are significantly different ($p < 0.05$).

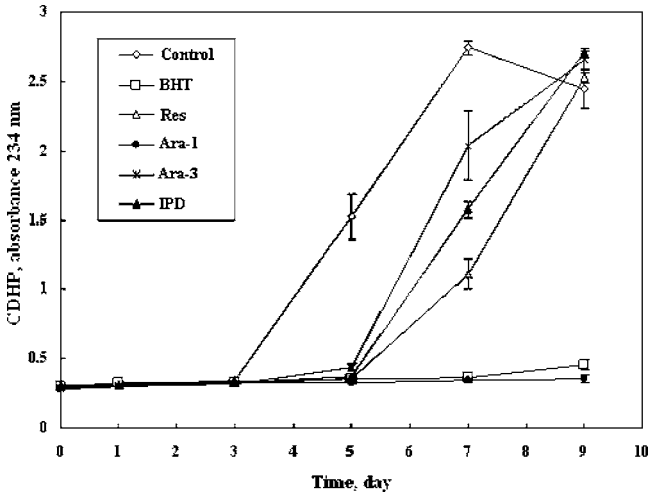

Figure 6. Retardation of absorbance increase at 234 nm as formation of conjugated diene hydroperoxides (CDHP) during storage at 60 °C of pork oils supplemented with 100 μM *trans*-resveratrol (Res), *trans*-arachidin-1 (Ara-1), *trans*-arachidin-3 (Ara-3), *trans*-isopentadienylresveratrol (IPD), and butylated hydroxytoluene (BHT). Means of determinations ± SD ($n = 3$).

(**Figure 6**). The lag times for absorbance increase at 234 nm have been extended by addition of the test stilbenoids. It is noteworthy to point out the finding that arachidin-1 showed even greater activity than BHT in retardation of CDHP formation.

From the structural nature of the test peanut stilbenoids (**Figure 1**), the stilbenoids mainly differ in number of hydroxyl groups and isopentenyl or isopentadienyl moiety. When a comparison was made between resveratrol and arachidin-3, the latter exhibited lower antioxidant activity, mainly due to bearing an isopentenyl moiety in addition to the resveratrol molecule. When a similar comparison was made between arachidin-1 and arachidin-3, the former exhibited greater antioxidant activity, mainly due to bearing an extra hydroxyl group. When the comparison was made between arachidin-3 and IPD, the difference in antioxidant activity was limited, indicating that antioxidant activity as affected by the position and structure of the isopentenyl or isopentadienyl moiety covalently linked to the resveratrol molecule was minor. Among resveratrol and its derivatives, in conjunction with the *trans*-olefin structure of the parent stilbene skeleton, the hydroxyl groups were the most important determinants of bioactivity (*7*). The 4′-hydroxyl group of resveratrol was reported to be more reactive than the 3- and 5-hydroxyl groups because of resonance effects. However, the hydroxyl group in the 4′ position of resveratrol is not the sole determinant for antioxidant activity. In addition to the 4′-hydroxyl group, antifungal activities of those compounds are also affected by their molecular lipophilicity (*37*).

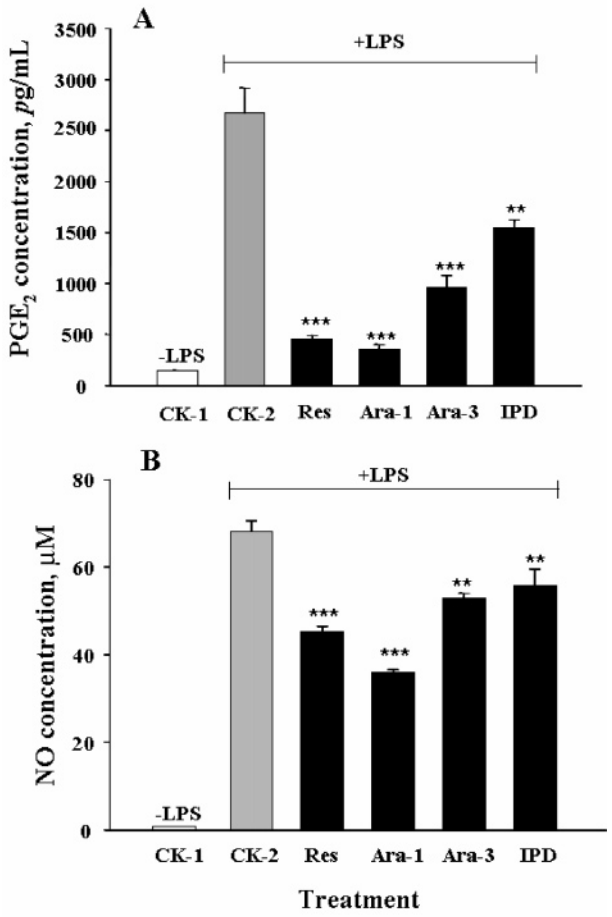

Figure 7. Inhibition of lipopoysaccharide- (LPS-) induced extracellular production of (**A**) prostaglandin E2 (PGE_2) and (**B**) nitric oxide (NO) of RAW 264.7 cells by *trans*-resveratrol (Res), *trans*-arachidin-1 (Ara-1), *trans*-arachidin-3 (Ara-3), and *trans*-isopentadienylresveratrol (IPD) at 15 μM. Means of determinations ± SD ($n = 3$) are shown. CK-1, control 1 without LPS induction; CK-2, control 2 with LPS induction. *p* Values are calculated by pair comparison to that of CK-2 and expressed as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

When RAW 264.7 cells were activated by LPS and respectively treated with resveratrol, arachidin-1, arachidin-3, and IPD at 15 μM, PGE_2 and NO production were both significantly inhibited (**Figure 7**). In comparison to control, LPS-induced PGE_2 production by the cells was significantly inhibited by arachidin-1 ($p < 0.001$), resveratrol ($p < 0.001$), arachidin-3 ($p < 0.001$), and IPD ($p < 0.01$). At the same supplemented level, LPS-induced NO production by the cells was significantly inhibited by arachidin-1 ($p < 0.001$), resveratrol ($p < 0.001$), arachidin-3 ($p < 0.01$), and IPD ($p < 0.01$). In a general inflammation reaction, arachidonic acid is released from cell membrane catalyzed by phospholipase A_2 and then converted to PGH_2 by cyclooxygenase and further metabolized to PGE_2. In addition, NO synthesized by inducible nitric oxide synthase (iNOS) has been implicated as a mediator of inflammation. Resveratrol has been observed to inhibit cyclooxygenase activity (*1*, *38*) and suppress LPS-induced NO production by macrophages (*39*, *40*). Generally, all test peanut stilbenoids have exhibited anti-inflammatory activities. This could be attributed

to the fact that each of the stilbenoids bears a 4′-hydroxyl group, as the most important determinant of bioactivity (*7*). When effects of stilbene (no hydroxyl group), rhaponticin (dihydroxy), resveratrol (trihydroxy), and piceatannol (tetrahydroxy) on TNF-induced NF-κB activation were compared, both resveratrol and piceatannol blocked TNF-induced NF-κB activation, but stilbene under these conditions was ineffective (*41*). Thus, similar to the antioxidant characterization obtained in this study, the hydroxyl groups as well as isopentenyl or isopentadienyl moiety of the test stilbenoids are critical for their bioactivity performance. As expected, all test stilbenoids showed varied potencies in suppression of LPS-induced inflammation of the RAW 264.7 cells as affected by the number and position of other hydroxyl groups, in addition to the 4′-hydroxyl group and of the covalently linked isopentenyl or isopentadienyl moiety.

In conclusion, it is of merit to simultaneously biosynthesize bioactive stilbenoids of resveratrol, arachidin-1, arachidin-3, and IPD by subjecting imbibed peanut kernels to germination, slicing, and incubation with artificial aeration in a custom-made practical apparatus for 16−24 h. All stilbenoids have exhibited potent antioxidant activity. In particular, arachidin-1 showed equivalent or even better antioxidant activity than did BHT. In addition, all test stilbenoids at 15 μM have shown effectiveness in suppression of the LPS-induced production of PGE_2 and NO by mouse macrophage RAW 264.7 cells. Since all those molecules are structurally correlated and exhibit more or less different bioactivities, further investigations on biosynthesis mechanism as well as application of these natural phytochemicals for nutraceutical and chemopreventive purposes are equally important in the future.

ACKNOWLEDGMENT

Helpful assistance in the laboratory by Show-Phon Learn is acknowledged.

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Received for review July 23, 2006. Revised manuscript received October 13, 2006. Accepted October 15, 2006. Financial support by the National Science Council (NSC 94-2313-B 415-011), Republic of China, is acknowledged.

JF0620766