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Pyrrole Alkaloids with Potential Cancer Chemopreventive Activity Isolated from a Goji Berry-Contaminated Commercial Sample of African Mango

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Supporting Information

ABSTRACT: Bioassay-guided fractionation of a commercial sample of African mango (*Irvingia gabonensis*) that was later shown to be contaminated with goji berry (*Lycium* sp.) led to the isolation of a new pyrrole alkaloid, methyl 2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]propanoate, **1**, along with seven known compounds, **2**−**8**. The structures of the isolated compounds were established by analysis of their spectroscopic data. The new compound **1**g showed hydroxyl radical-scavenging activity with an ED_{50} value of 16.7 μM, whereas 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanoic acid (**2**) was active in both the hydroxyl radical-scavenging (ED_{50} 11.9 μM) and quinone reductase-induction [CD (concentration required to double QR activity) 2.4 μM)] assays used. The isolated compounds were shown to be absent in a taxonomically authenticated African mango sample but present in three separate authentic samples of goji berry (*Lycium barbarum*) using LC-MS and ^{1}H NMR fingerprinting analysis, including one sample that previously showed inhibitory activity in vivo in a rat esophageal cancer model induced with *N*-nitrosomethylbenzylamine. Additionally, microscopic features characteristic of goji berry were observed in the commercial African mango sample.

KEYWORDS: *Lycium barbarum, goji berry, Solanaceae, pyrrole alkaloids, hydroxyl radical-scavenging activity, quinone reductase-inducing activity, Irvingia gabonensis, African mango, LC-MS, ^{1}H NMR fingerprinting, microscopic comparison*

INTRODUCTION

Irvingia gabonensis (Aubry-Lecomte ex O'Rorke) Baill. (Irvingiaceae) has become known as African mango and is unrelated botanically to the common, or Indian, mango [*Mangifera indica* (Anacardiaceae)].[1] The fruits of African mango are edible, and their use in traditional medicine has been documented for the treatment of gastrointestinal or hepatic disorders, diarrhea, infections, and as a purgative.[2,3] Recently, African mango seeds have become available on the U.S. market as a dietary supplement and have shown high in vitro antioxidant capacity,[4] significant effects on body weight loss,[5,6] blood lipid decreases,[5] and a lowering of plasma glucose[7] in experimental animal or human subject studies. Phytochemical investigations of the stem bark of *I. gabonensis* have led to the isolation of antioxidant and hepatoprotective triterpenes and phenols.[2] Nevertheless, very limited studies on the chemical constituents of the seeds have been reported. In 2011, Atawodi and co-workers[8] published the first report on the phenolic compound profile of *I. gabonensis* seeds, in which methyl gallate, ellagic acid, and other ellagic acid derivatives were detected by LC-UV-MS in the methanol extract of the seeds, which also demonstrated strong in vitro antioxidant activity. A recent study of dietary supplements wholly or partially containing African mango seed extracts using UHPLC-HRMS found evidence of contamination, adulteration, and/or mislabeling among multiple commercial samples when compared to authentic samples.[9] It is noteworthy that no alkaloids have been previously isolated or detected spectroscopically from African mango, according to the accessible and surveyed literature.

Scavenging reactive oxygen species by antioxidants and enhancing carcinogen detoxification via induction of phase II enzymes such as quinone reductase (QR) are two important cancer chemopreventive strategies.[10,11] In a continuing search for natural inhibitors of carcinogenesis, the chloroform-soluble extract of a commercially sourced African mango seed extract showed in vitro hydroxyl radical-scavenging and QR-inducing activities, and thus was selected for further study. Bioactivity-guided fractionation of this sample, using both hydroxyl radical-scavenging and QR-inducing assays, led to the isolation of a new pyrrole alkaloid, **1**, along with seven known compounds, **2**−**8**. Compounds **1**−**4** represent structural analogues of substituted pyrrole alkaloids reported recently from *Lycium chinense* Mill. (Solanaceae).[12,13]

The fruits of *L. chinense* and of the closely related species *Lycium barbarum* L. are commonly known as goji berry or wolfberry and are used almost interchangeably.[14,15] Goji has a long history of usage in Asian countries as a culinary ingredient, traditional tonic medicine, and functional food for its perceived

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benefits in antiaging, as well as enhancements in vision and liver and kidney functions.[16,17] In recent years, goji has become increasingly popular in Europe and North America as a "superfruit" and botanical dietary supplement. The in vivo inhibitory activity of powdered *L. barbarum* (wolfberry) fruits when evaluated in an *N*-nitrosomethylbenzylamine-induced esophageal cancer model was reported recently in rats.[18] Other recent studies have indicated that goji berry extracts and *L. barbarum* polysaccharides possess a range of biological effects, including antiaging, antioxidant, antitumor, immunomodulatory, and cytoprotective activities.[17,19−21] The published chemical constituents of goji have been thoroughly reviewed, with the occurrence of pyrrole alkaloids well established.[16]

Due to the unexpected presence of the pyrrole alkaloids isolated, **1**−**4**, and their structural similarity to constituents found in goji berries,[12,13] as well as the recent report of contamination in commercial African mango samples,[9] the identity of the source material investigated came into question. The present study includes the bioactivity-guided isolation, identification, and biological evaluation of compounds **1**−**8** in addition to comparison of the commercial product with authentic African mango seeds and *L. barbarum* samples by chemotaxonomy using LC-MS and ^{1}H NMR fingerprinting as well as microscopic analysis.

MATERIALS AND METHODS

Instrumentation for Compound Isolation and Characterization. Optical rotations were measured using a PerkinElmer 343 automatic polarimeter (PerkinElmer, Waltham, MA, USA). UV spectra were collected on a Hitachi U-2910 spectrophotometer (Hitachi, Tokyo, Japan). IR spectra were obtained with a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA). NMR spectroscopic data were recorded at room temperature on a Bruker Avance DRX-400 MHz spectrometer (Bruker, Billerica, MA, USA) using standard Bruker pulse sequences. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained on a Micromass Q-Tof II (Micromass, Wythenshawe, UK) mass spectrometer operated in the positive-ion mode, with sodium iodide being used for mass calibration. Column chromatography was performed with Sephadex LH-20 (Supelco, Bellefonte, PA, USA) and 65 × 250 or 230 × 400 mesh silica gel (Sorbent Technologies, Atlanta, GA, USA). Analytical thin-layer chromatography (TLC) was conducted on precoated 250 μm thickness Partisil Si gel 60F_{254} glass plates. A 150 mm × 19 mm i.d., 5 μm, XBridge PrepC_{18} column with a 10 mm × 19 mm i.d. guard column of the same material (Waters, Milford, MA, USA) was used for semipreparative HPLC, along with a Hitachi system composed of an L-2130 prep pump, an L-2200 autosampler, and an L-2450 diode array detector (Hitachi, Tokyo, Japan).

Chemicals. All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ, USA). 2′,7′-Dichlorodihydrofluorescein diacetate (H_2DCF-DA), esterase, ferrous sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), quercetin, dimethyl sulfoxide (DMSO), digitonin, EDTA, Trizma base, Tween 20, flavin adenine dinucleotide phosphate (FAD), glucose-6-phosphate (G-6-P), nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate dehydrogenase (G-6-P-D), menadione, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bovine serum albumin (BSA), L-sulforaphane, and deuterated NMR solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture media and supplements were obtained from Life Technologies, Inc. (Grand Island, NY, USA). Gallic acid, methyl gallate, and ellagic acid that served as standards for HPLC-PDA analysis were authentic samples isolated in previous studies and stored in a compound library at The Ohio State University.

Plant Material. The commercial sample labeled as powdered seeds of African mango (*I. gabonensis*), used for isolation, was obtained by Nature's Sunshine Products, Inc., from a manufacturer in the People's Republic of China. A representative sample (code OSUADK-CCP0024) was deposited in the Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University. An authentic sample of African mango seed powder, collected in Nigeria and imported by Nutralliance (Yorba Linda, CA, USA), was purchased by Nature's Sunshine Products, Inc. (code OSUADK-CCP0025; same repository). Three samples of authentic goji berries were obtained, two from the Ningxia province of People's Republic of China, by Nature's Sunshine, Inc., and another directly from a drug store in Chengdu, Sichuan, People's Republic of China (codes OSUADK-CCP0011, OSUADK-CCP0015, and OSUADK-CCP0026, respectively; same repository). The seeds of the last-mentioned product were separated manually from the fruit pulp. These five samples (OSUADK-CCP0024, OSUADK-CCP0026, OSUADK-CCP0015, OSUADK-CCP0011, and OSUADK-CCP0025) are referred to as products A−E, respectively, henceforth in this paper.

Extraction and Isolation. The commercial sample labeled as the powdered seeds of African mango (product A, 5 kg) was extracted by maceration with MeOH (3 × 10 L) at room temperature, for 3 days each. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanol extract (ca. 1000 g) was suspended in H_2O (1 L) and then partitioned in turn with hexanes (3 × 1 L), $CHCl_3$ (3 × 1 L), EtOAc (3 × 1 L), and *n*-BuOH (3 × 1 L) to afford dried hexanes (40.1 g), $CHCl_3$ (5.0 g), EtOAc (6.5 g), *n*-BuOH (166.0 g), and H_2O-soluble (ca. 800 g) extracts. The $CHCl_3$-soluble extract showed activities in both hydroxyl radical-scavenging (ED_{50} 6.3 μg/mL) and QR induction (CD 12.4 μg/mL) assays and hence was selected for further fractionation. The $CHCl_3$-soluble extract was subjected initially to chromatography over a silica gel column, eluted with $CHCl_3$/MeOH (50:1 to pure MeOH, stepwise), to afford 12 fractions (fractions F01−F12). Fractions F01−F03 showed activity in either the hydroxyl radical-scavenging assay or the QR induction assay and were chosen for further purification.

Fraction F01 (ca. 890 mg) was chromatographed over an open C_{18} reversed-phase column, eluted with MeOH/H_2O (5:95 to pure MeOH, stepwise) to afford eight subfractions. Subfraction F0102 (ca. 400 mg, eluted by MeOH/H_2O 20:80) was subjected to passage over a Sephadex LH-20 column with MeOH/H_2O (20:80 to pure MeOH, stepwise) as the eluting solvent system to yield compound **8** (eluted by MeOH/H_2O 50:50; 3.0 mg) and a further subfraction F010211 (ca. 65 mg). Subfraction F010211 was separated by HPLC using a 150 mm × 19 mm i.d., 5 μm, XBridge PrepC_{18} column with a 10 mm × 19 mm i.d. guard column of the same material (Waters) and with CH_3CN/H_2O by isocratic elution (3:97; 8 mL/min) to afford compounds **5** (t_R 68.6 min; 5.7 mg) and **6** (t_R 55.0 min; 0.8 mg) and a further subfraction, F01021103 (ca. 40 mg, eluted with pure CH_3CN). F01021103 was purified further by HPLC on the same column with CH_3CN/H_2O (0.025% NH_4OH) isocratic elution (3:97; 8 mL/min) to obtain compound **2** (t_R 12.3 min; 4.5 mg).

Fractions F02 (ca. 180 mg) and F03 (ca. 120 mg) were subjected separately to Sephadex LH-20 column chromatography, eluted by MeOH/H_2O (50:50, 75:25, and pure MeOH) to remove pigments and afforded several subfractions. Subfraction F0203 (ca. 100 mg) was purified further by HPLC on the same XBridge PrepC_{18} column as above, by a gradient elution (A, CH_3CN; B, H_2O, 3−15% A over 70 min, 15−100% A from 70 to 85 min; 8 mL/min), to render the purification of compounds **1** (t_R 49.5 min; 4.8 mg), **3** (t_R 46.7 min; 1.3 mg), and **7** (t_R 16.5 min; 20.0 mg). Subfraction F0303 (ca. 80 mg) was also purified by HPLC on the same XBridge PrepC_{18} column, eluted by a CH_3CN/H_2O gradient (A, CH_3CN; B, H_2O, 3−15% A over 70 min, 15−100% A from 70 to 85 min; 8 mL/min), to yield compound **4** (t_R 22.7 min; 6.8 mg).

Methyl 2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]-propanoate, 1, was isolated (ca. 0.0001% of dry weight) as a colorless resin: $[\alpha]^{20}_D$ +28.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 291 (4.09) nm; IR (film) ν_{max} 3424, 3120, 2946, 2850, 2803, 2727, 1749, 1655 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 9.31 (1H, s, CHO), 7.07 (1H, d, J = 4.0 Hz, H-3), 6.28 (1H, d, J = 4.0 Hz, H-4), 5.43 (1H, q, J = 7.0 Hz, H-1′), 4.63 (2H, ABq, $\Delta\nu$ = 8.1 Hz, J = 13.9 Hz, H-6), 3.67 (3H, s, OCH_3), 1.67 (3H, d, J = 7.0 Hz, CH_3); ^{13}C NMR (100 MHz,

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CD_3OD) δ 180.5 (CHO), 172.5 (COO), 144.6 (C-5), 133.4 (C-2), 127.4 (C-3), 111.4 (C-4), 56.6 (C-6), 55.8 (C-1′), 52.8 (OCH_3), 18.0 (CH_3); HRESIMS m/z 234.0733 $[M + Na]^+$ (calcd for $C_{10}H_{13}NO_4Na$, 234.0742).

Evaluation of Hydroxyl Radical-Scavenging Activity. Hydroxyl radical-scavenging activity was tested according to the method previously reported.[22,23]

Evaluation of Quinone Reductase-Inducing Activity. The in vitro QR-inducing activity of the extracts, fractions, and pure isolates was assayed according to a previously published method.[24,25]

Microscopic Examination Procedure. The clean samples of products B−D were pulverized and passed through a no. 4 sieve to obtain fine granules. Products A and E were obtained as powders and thus passed through the sieve directly. The powdered materials of products A−E were each placed on a slide and stirred with a fine-pointed needle to distribute the powders evenly, followed by the application of 2 drops of clearing agent, Visikol (Phytosys LLC, New Brunswick, NJ, USA), and gentle heat until air bubbles moved to the edge of the slide. Two drops of diluted glycerin and a coverslip were added to the slide, followed by immediate observation under a Nikon Eclipse TE300 optical microscope (Nikon, Tokyo, Japan), along with an A01F881036 camera module (Roper Scientific, Trenton, NJ, USA) for digital imaging of the microscopic structures observed.

Sample Preparation and HPLC-PDA, LC-IT-MS, and ^{1}H NMR Fingerprinting Analysis. Each sample (3 g) was extracted with 10 mL of methanol by sonication at room temperature for 30 min. The methanol extracts were dried in vacuo (40 °C), followed by another ultrasonic extraction with 10 mL of chloroform at room temperature for 30 min, to obtain a chloroform-soluble extract of each sample, which was then dried in vacuo at 40 °C and stored at −20 °C before analysis. All analyses were completed within 24 h of extraction.

The HPLC-PDA analysis was performed using a 150 mm × 4.6 mm i.d., 5 μm, XBridge C_{18} analytical column (Waters), along with a Hitachi system composed of an L-2130 pump and an L-2450 diode array detector (Hitachi). The mobile phase consisted of 0.05% trifluoroacetic acid in water (A) and acetonitrile (B) using a gradient program of 3−20% B from 0 to 60 min and 20−100% B from 60 to 70 min. The mobile phase flow rate and the injection volume were 1 mL/min and 10 μL, respectively. The dried chloroform-soluble extract of each sample was dissolved in 1 mL of HPLC grade methanol, and filtered through a Fisher Scientific 13 mm syringe filter (0.2 μm) prior to injection. After comparison of the chromatograms of the chloroform-soluble extract solution recorded at wavelengths within 200−550 nm, it was found that 254 nm could best represent the profile of the analytes.

The LC-IT-MS analysis employed the same separation conditions and Xbridge C_{18} analytical column as used for the HPLC-PDA analysis mentioned above, on a Waters Alliance 2690 separation module. The injection volume was 10 μL. The mobile phase flow rate was maintained at 1 mL/min and was split approximately 50:1 postcolumn using a microsplitter valve (Upchurch Scientific, Oak Harbor, WA, USA) for the introduction to the ESI source. The electrospray ionization ion trap mass spectrometry (ESI-IT-MS) was performed on a Bruker dual funnel amaZon ETD ion trap mass spectrometer (Bremen, Germany) equipped with an orthogonal electrospray source operated in positive-ion mode. Sodium iodide was used for mass calibration for a calibration range of m/z 100−1000. Optimal ESI conditions were as follows: capillary voltage, 4500 V; source temperature, 250 °C; ESI drying gas (nitrogen), 4.0 L/min; and nebulizer, 10 psi. The ion trap was set to UltraScan mode with a target mass of m/z 500 pass ions from m/z 100 to 1000.

The ^{1}H NMR spectroscopic fingerprinting was conducted using the previously described chloroform-soluble extracts of products A−E. The samples were dissolved in 600 μL of $CDCl_3$, and spectra were measured at 300 K using a Bruker Avance-III HD400 spectrometer.

RESULTS AND DISCUSSION

Isolation, Structure Elucidation, and Biological Evaluation of the Constituents. The $CHCl_3$-soluble extract of product A (a commercial sample labeled as powdered seeds of African mango) was found to be the most potent among the hexanes-, $CHCl_3$-, EtOAc-, *n*-BuOH-, and H_2O-soluble extracts in the in vitro hydroxyl radical-scavenging (ED_{50}, concentration scavenging hydroxyl radical by 50%, 6.3 μg/mL) and QR induction (CD, concentration required to double QR activity, 12.4 μg/mL) assays. Therefore, it was selected for fractionation. Fractions F01−F03 of the $CHCl_3$-soluble extract showed hydroxyl radical-scavenging activity, with ED_{50} values of 3.3, 5.6, and 6.8 μg/mL, respectively. In addition, fractions F01 and F02 exhibited QR-inducing activity, with CD values of 6.9 and 10.2 μg/mL, respectively. Accordingly, fractions F01−F03 were used for further detailed purification. In this way, bioactivity-guided fractionation of product A led to the isolation of a new pyrrole alkaloid, **1**, and seven known compounds, **2**−**8**, as shown in Figure 1.

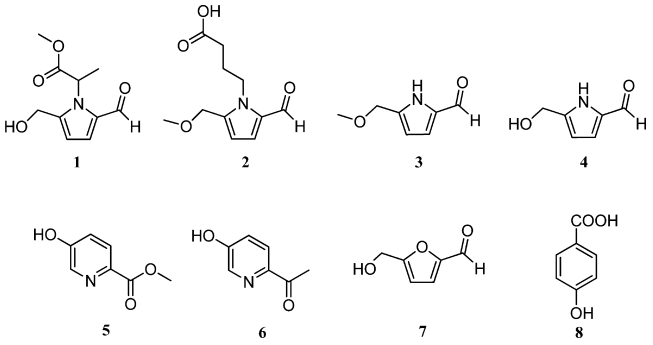

Figure 1. Structures of compounds isolated from a goji berry-contaminated commercial sample labeled African mango (*Irvingia gabonensis*).

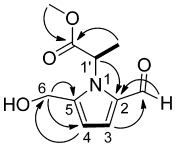

Figure 2. ^{1}H−^{1}H COSY (bold lines) and key HMBC (arrows) correlations of the new compound **1**.

Table 1. Hydroxyl Radical-Scavenging and Quinone Reductase-Inducing Activities of the Isolated Active Compounds 1, 2, and 6

	hydroxyl radical scavenging	QR induction		
compound[a]	ED_{50}[b] (μM)	CD[c] (μM)	IC_{50}[d] (μM)	CI[e]
1	16.7	43.1	>100	>2.3
2	11.9	2.4	>100	>42.6
6	>20	48.5	>100	>2.1
quercetin[f]	1.3			
L-sulforaphane[g]		0.77	16.7	21.7

[a]Compounds **3**−**5**, **7**, and **8** did not show hydroxyl radical-scavenging or QR-inducing activity at the concentrations tested. [b]ED_{50}, concentration scavenging hydroxyl radical by 50%. Compounds with ED_{50} values of <20 μM are considered active. [c]CD, concentration required to double QR activity. Compounds with CD values of <20, 20−100, and >100 μM are considered significantly active, weakly active, and inactive, respectively. [d]IC_{50}, concentration inhibiting cell growth by 50%. [e]CI, chemoprevention index (= IC_{50}/CD). [f]Positive control for hydroxyl radical-scavenging assay. [g]Positive control for QR induction assay.

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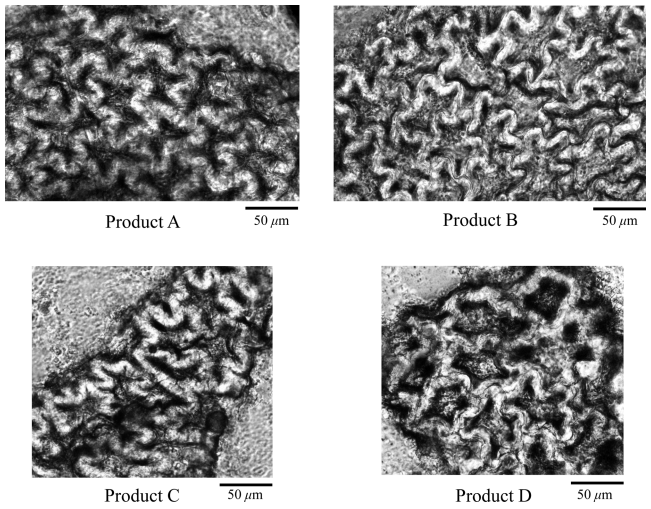

Figure 3. Microscopic structures of testa sclereids (stone cells) observed in products A–D.

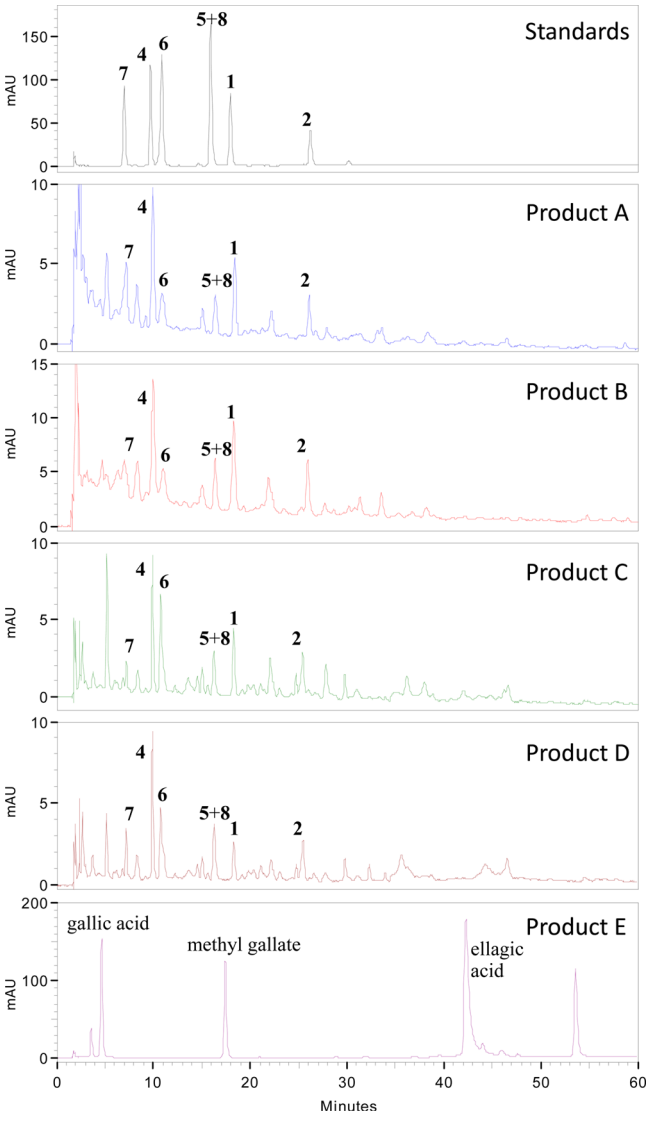

Figure 4. HPLC chromatograms (254 nm) of products A–E and standard compounds isolated from product A.

The new compound, **1**, was isolated as a colorless resin. The molecular formula was determined as $C_{10}H_{13}NO_4$ on the basis of the sodiated molecular ion peak in the HRESIMS. The IR spectrum showed absorptions of hydroxy (3424 cm^{-1}), ester carbonyl (1749 cm^{-1}), and conjugated aldehyde (2803, 2727, and 1655 cm^{-1}) groups. The UV spectrum of **1** exhibited a maximum absorption at 291 nm, which is characteristic of pyrrole 2-carbaldehyde.[13] The proton signal at δ_H 9.31 and the carbon signal at δ_C 180.5 correlated in the HSQC spectrum and indicated the presence of an aldehyde group. Two proton signals at δ_H 4.63 (2H, ABq, $\Delta\nu$ = 8.1 Hz, J = 13.9 Hz, H-6) resulted from a magnetically inequivalent oxygenated methylene group with geminal coupling. The HMBC correlation (Figure 2) of the methoxy group (δ_H 3.67, 3H, s, OCH_3) to the carbonyl at δ_C 172.5 revealed that this methoxy group forms an ester with this carbonyl. The quartet of H-1′ (δ_H 5.43, J = 7.0 Hz) and doublet of the methyl group (δ_H 1.67, J = 7.0 Hz), as well as their correlation in the COSY spectrum, clearly indicated the connection of the methyl group (δ_C 18.1) to C-1′ (δ_C 55.8). Furthermore, HMBC correlation of this methyl group (δ_H 1.67) to the ester carbonyl C-2′ (δ_C 172.5) suggested the connection of C-1′ (δ_C 55.8) to C-2′. The chemical shifts and coupling constants of the two protons at δ_H 7.07 (1H, d, J = 4.0 Hz, H-3) and δ_H 6.28 (1H, d, J = 4.0 Hz, H-4) implied the presence of a pyrrole ring. Moreover, the substitution pattern of the pyrrole ring should be 2,5- because a 2,3- or a 2,4-disubstituted pyrrole would have coupling constants of 1.3–2.9 or 2.3–3.2 Hz,[13] respectively. Also, the two carbons of the pyrrole ring at the lower field region (δ_C 144.6 and 133.4) were not present in the ^{13}C DEPT 135 spectrum, suggesting that C-2 and C-5 of this ring system are substituted (quaternary carbons). This was confirmed by the HMBC correlation of H-6 (δ_H 4.63) to C-4 (δ_C 111.4) and C-5 (δ_C 144.6), H-4 (δ_H 6.28) to C-6 (δ_C 56.6), H-3 (δ_H 7.07) to the aldehyde carbon (δ_C 180.5), and the aldehyde proton (δ_H 9.31) to C-2 (δ_C 133.4). In addition, the connection between C-1′ and N-1 was clearly indicated by the HMBC correlation of H-1′ (δ_H 5.43) to C-2 (δ_C 133.4). Therefore, the structure of **1** was established unambiguously as methyl 2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]propanoate.

On the basis of the spectroscopic data measurements and the comparison of the data obtained with published values, the structures of the known compounds **2**–**8** were identified as 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanoic acid, **2**,[13] 5-(methoxymethyl)-1*H*-pyrrole-2-carbaldehyde, **3**,[26] 5-(hydroxymethyl)-1*H*-pyrrole-2-carbaldehyde, **4**,[27,28] methyl-5-hydroxy-2-pyridinecarboxylate, **5**,[29] 5-hydroxy-2-pyridyl methyl ketone, **6**,[30] 5-hydroxymethyl-2-furancarbaldehyde, **7**,[31] and 4-hydroxybenzoic acid, **8**.[32]

In the present study, all of the isolates, **1**–**8**, were evaluated in vitro using the hydroxyl radical-scavenging and QR induction assays. The new compound, **1**, and the known pyrrole alkaloid, **2**, showed activities in both assays, as shown in Table 1. When the QR-inducing activities were compared among the structurally related pyrrole alkaloids, **1**–**4**, it was observed that **2**, containing a butanoic acid side chain at position N-1, exhibited the greatest potency (CD 2.4 μM); **1**, with a methyl propanoate substitution attached to N-1, showed much less potency (CD 43.1 μM), whereas **3** and **4**, each bearing no substituent to N-1, displayed no discernible activity (both CD >100 μM), suggesting that the length of the side chain at N-1 may significantly affect the QR-inducing activity of this type of compound.

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Table 2. LC-MS Data of Constituents Identified in a Representative Authentic Goji Berry Sample (Product C)

t_R (min)	UV λ_{max} (nm)	molecular formula	$[M + H]^+$	$[M + Na]^+$	identification
7.0	230, 284	$C_6H_6O_3$	127.1	149.1	compound **7**
9.7	254, 299	$C_6H_7NO_2$	126.1	148.1	compound **4**
10.9	217, 261, 294	$C_7H_7NO_2$	138.1	160.0	compound **6**
15.9	254, 285	$C_7H_6O_3$, $C_7H_7NO_3$	139.1, 154.1	161.1, 176.0	compounds **5** and **8**
18.0	258, 296	$C_{10}H_{13}NO_4$	212.1	234.1	compound **1**
26.2	258, 296	$C_{11}H_{15}NO_4$	226.1	248.1	compound **2**

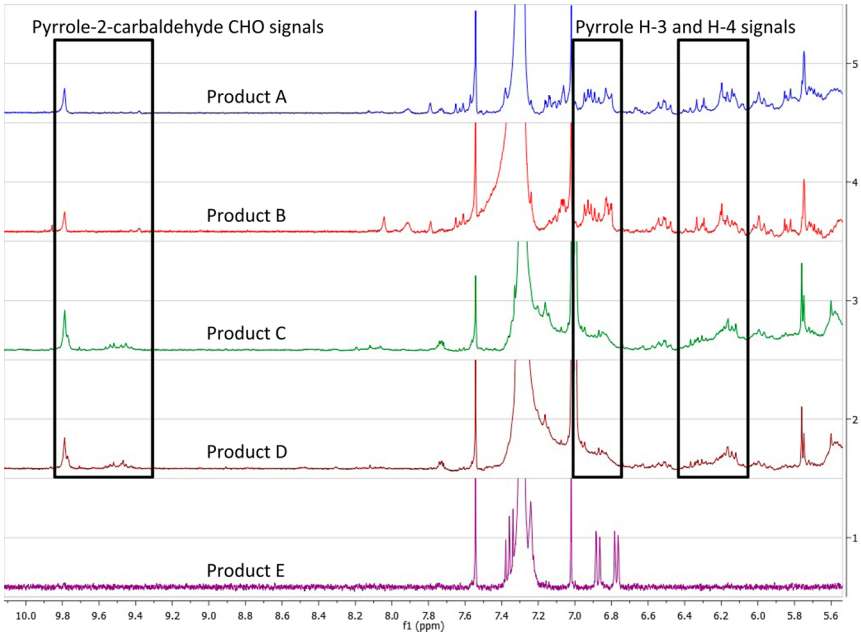

Figure 5. ^{1}H NMR fingerprinting (δ_H 5.5−10.1 ppm) of products A−E ($CDCl_3$, 400 MHz).

Pyrrole alkaloid **2** was initially isolated from goji berry (*L. chinense*).[13] Compounds **1**, **3**, and **4** are analogues of **2** and are also pyrrole alkaloids. In contrast, no alkaloid has been previously isolated or detected spectroscopically from African mango. In addition, both goji berry and African mango reportedly can be used as antiobesity medicinal plants.[33] Due to the unexpected presence of these pyrrole alkaloids and their structural similarity to and occurrence as constituents found in goji berries,[12,13] as well as the public concern and a recent report about frequent contamination in commercial African mango samples,[9] a comprehensive comparison of the commercial sample labeled as African mango seeds (product A) with authentic goji berry samples (products B−D) and African mango seeds (product E) was conducted via microscopic analysis as well as chemotaxonomy using LC-MS and ^{1}H NMR fingerprinting, as shown in the following sections.

Microscopic Examination. In the powders of products A−D, irregular polygonal testa sclereids (stone cells) were observed with thickened and curved walls as well as distinct striations in surface view (Figure 3), characteristic of the seeds of goji berry.[14] This microscopic feature was not found in the authentic sample of African mango seed powder (product E).

HPLC-PDA and LC-IT-MS Analysis. Products A−D shared very similar HPLC chromatograms, which differed significantly from that of product E (Figure 4). Compound **3** had been all consumed for bioassay and thus could not be used as a standard. However, compounds **1**, **2**, and **4**−**8** isolated from product A were present in the authentic goji berry samples (products B−D), as identified by comparison of the retention times, UV profiles, and mass spectra of the peaks with standard compounds that had been identified by NMR (Table 2). In contrast, the HPLC chromatogram of the authentic sample of African mango seeds (product E) was shown to be very different, containing gallic acid, methyl gallate, ellagic acid, and their derivatives as major constituents, as identified by the same analytical methods mentioned above. These substances have been previously detected in African mango.[8,9]

^{1}H NMR Fingerprinting. Visual inspection of the ^{1}H NMR spectra revealed that all samples tested contained triacylglycerols as major constituents. By comparison, however, products A−D each contained pyrrole alkaloids as minor constituents with characteristic signals at δ_H 6.0−6.4 ppm that were not observed in product E. The presence of signals in the region of δ_H 9.4−9.8 ppm can be attributed to the aldehyde functionality of the isolated pyrrole-2-carbaldehyde compounds and further distinguished the botanical identity of product A from product E. Additionally, the signals that were detected only in product E may be ascribed to the previously reported constituents of African mango, namely, methyl gallate, ellagic acid, or their derivatives.[8,9] The region of the NMR spectra pertinent to these comparisons is shown in Figure 5. The relative cleanness of the proton NMR spectrum of the authentic African mango seed extract suggests that ^{1}H NMR fingerprinting may be a useful tool for the future authentication and/or quality control of such samples.

Accordingly, chemotaxonomic examination by LC-IT-MS and ^{1}H NMR fingerprinting using authentic samples of goji berries and African mango suggested that there was evidence of

contamination, adulteration, and/or mislabeling of the commercial African mango sample evaluated (product A), in agreement with a concerning trend previously reported.[9] It is worth noting that goji berry from the same batch as product C was previously reported to exhibit in vivo inhibitory activity in a rat *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal cancer model, and the detection of the in vitro active compounds **1**, **2**, and **6** in product C suggests that these compounds may possess potential in vivo cancer chemopreventive activity and hence merit further investigation.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR, ^{13}C NMR, ^{13}C DEPT 135, HSQC, HMBC, ^{1}H−^{1}H COSY, HRESIMS, and UV and IR spectra for compound **1**; HPLC-PDA and LC-IT-MS data for identification of the isolated compounds in the authentic goji berry and African mango samples; ^{1}H NMR fingerprinting of the $CHCl_3$-soluble extracts of products A−E, and protocols used to evaluate hydroxyl radical-scavenging and QR-inducing activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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