Reactions of *p*-Coumaric Acid with Nitrite: Product Isolation and Mechanism Studies

Janelle L. Torres y Torres and John P. N. Rosazza*

Division of Medicinal and Natural Products Chemistry and Center for Biocatalysis and Bioprocessing, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242

p-Coumaric acid (**1**) is an abundant plant phenolic acid, a dietary chemoprotectant, and an antioxidant. The chemoprotective properties of **1** were demonstrated in vitro by its reaction with $NaNO_2$ in H_2O over a range of pH values. The reaction pathway of **1** with nitrite is dependent on pH. 4-Hydroxybenzaldehyde (**3**, 16%), 1′,4-dihydroxybenzeneacetaldehyde oxime (**5**, 59%), and 4-hydroxy-1′-oxo-benzeneacetaldehyde aldoxime (**7**, 26%) and 7-hydroxy-1,2(4*H*)-benzoxazin-4-one (**11**, 6%) were each formed at pH 2, whereas 4-(2-oxido-1,2,5-oxadiazol-3-yl)phenol (**13**) was formed at pH 3 (6%) and pH 7 and 10 (both 1%). Products were isolated and characterized by NMR and MS spectral analyses. Formation of benzoxazinone (**11**) requires the 4-phenolic functional group and the conjugated propenoic acid side chain of *p*-coumaric acid. The mechanism for nitrosation at pH 2 was examined by reacting **1** in $H_2{}^{18}O/NaNO_2$.

Keywords: *p-Coumaric acid; reaction with nitrite; nitrosation mechanism; 7-hydroxy-1,2(4H)-benzoxazin-4-one, 4-(2-oxido-1,2,5-oxadiazol-3-yl)phenol, oxime*

INTRODUCTION

p-Coumaric acid, 3-(4-hydroxyphenyl)-2-propenoic acid (**1**), is a ubiquitous plant phenolic acid that is typically esterified to arabinoxylan residues of hemicellulose or to lignin in graminaceous plants, including maize (*1*), oats (*2*, *3*), and wheat (*4*). It is also found as ester conjugates and as the free acid in fruits and vegetables such as apples (*5*), grapefruit, and oranges (*6*), and tomatoes, potatoes, and spinach (*7*). Experiments conducted in our laboratory have shown that **1** composes approximately 4% of the dry weight of maize plant parts, including stalks, roots, and cobs. In 1996, the state of Iowa produced 1.7 billion bushels (43.6 billion kilograms) of corn (*8*). Thus, **1** is an abundant, valuable, renewable aromatic compound with great potential as an antioxidant in food and nutrition industries.

The American Cancer Society (1999) estimates that exposure to chemical carcinogens present in the environment is responsible for over 50% of human cancers (*9*). Dietary nitrites can react with secondary amines to form carcinogenic *N*-nitroso compounds that are generally classified among reactive nitrogen species (RNSs). Antioxidants such as *p*-coumaric acid (**1**) and other hydroxycinnamic acids function as chemoprotective agents by reacting with RNSs such as nitrite or peroxynitrite to inhibit *N*-nitrosamine formation (*10*). For example, in vitro reaction of caffeic and ferulic acids with nitrite in human gastric fluid was found to inhibit nitrosation of dimethylamine and aminopyrine (*11*). Among hydroxycinnates tested, caffeic and ferulic acids were better inhibitors of peroxynitrite-mediated tyrosine nitration than *p*-coumaric acid at low pH (*12*). In vivo, caffeic and ferulic acids inhibited the formation of *N*-nitrosodimethylamine in the serum of rats treated with aminopyrine and $NaNO_2$ (*11*). Similarly, **1** inhibited morpholine nitrosation (*13*), and it reacted with peroxynitrite to reduce 3-nitrotyrosine formation in vitro (*14*, *15*). Although LC-MS results suggested the formation of nitrated *p*-coumaric acid (*14*), none of the products of these reactions were isolated or characterized.

Previous studies concerned with the nitrosation of **1** were all conducted in organic/aqueous mixtures and not over a range of reaction pH. The two major products of the reaction of **1** with nitrite at 0 °C in acetone/H_2O (1:2, v/v) were identified as 4-hydroxy-1′-oxo-benzeneacetaldehyde aldoxime (**7**) and 4-(2-oxido-1,2,5-oxadiazol-3-yl)phenol (**13**) in 50 and 43% yields, respectively (*16*). Products of the reaction of **1** with nitrite in EtOH/H_2O (1:1, v/v) at room temperature were characterized as 4-hydroxybenzaldehyde (**3**, 13%), 4-(2-oxido-1,2,5-oxadiazol-4-yl)phenol (3%), 4-hydroxy-1′-(hydroxyimino)-benzeneacetaldehyde (2%), and an unidentified product of molecular mass 163 ($C_8H_5NO_3$) (*17*).

This study of the reaction of **1** with nitrite in H_2O was conducted over a range of pH values to gain an understanding of the structural features of the phenolic acid responsible for antioxidant/chemoprotectant activities and to characterize the products of nitrosation under different conditions commonly found in human gastric fluid and in other tissues. We describe the isolation and characterization of products formed by the reaction of **1** with nitrite at pH 2, 3, 7, and 10. An investigation of the mechanism of nitrosation at pH 2 was conducted in reactions using $H_2{}^{18}O$ by determining the incorporations of ^{18}O into the reaction products.

MATERIALS AND METHODS

General Experimental Procedures. *p*-Coumaric acid (**1**), 4-hydroxybenzaldehyde (**3**), 4-hydroxybenzenepropanoic acid (**14**), and $H_2{}^{18}O$ (10 at. % ^{18}O) were purchased from Aldrich (Milwaukee, WI). $Na^{15}NO_2$ (>98 at. % ^{15}N) was obtained from Cambridge Isotope Laboratories (Andover, MA). Sephadex LH-

* Corresponding author. Tel.: (319) 335-4902. Fax: (319) 335-4901. E-mail: john-rosazza@uiowa.edu.

10.1021/jf001127a CCC: $20.00
Published on Web 01/26/2001

Table 1. NMR Spectral Data for (*E*)- and (*Z*)-1′,4-Dihydroxybenzeneacetaldehyde Oxime (*E*- and *Z*-5)

	E-**5**		HMBC	*Z*-**5**	
position	^{1}H (δ, J in Hz)	^{13}C (δ)	correlations (C#)	^{1}H (δ, J in Hz)	^{13}C (δ)
CH═NOH	7.37 (d, 7.2)	153.0	1′	6.79 (d, 6.2)	154.0
1	—	133.2	—	—	133.2
2	7.19 (d, 8.2)	128.6	1′, 2, 3, 5, 6	7.24 (d, 8.3)	128.8
3	6.77 (d, 8.7)	116.3	1, 3, 4, 5	6.75 (d, 8.7)	116.2
4	—	158.3	—	—	157.5
5	6.77 (d, 8.7)	116.3	1, 3, 4, 5	6.75 (d, 8.7)	116.2
6	7.19 (d, 8.2)	128.6	1′, 2, 3, 5, 6	7.24 (d, 8.3)	128.8
1′	5.12 (d, 7.2)	72.3	*C*H═NOH, 1, 2, 6	5.84 (d, 6.2)	66.8

20 was obtained from Amersham Pharmacia Biotech (Piscataway, NJ). Bakerbond octadecyl (C18) 40-μm prep LC packing and Baker silica gel 40-μm flash chromatography packing were obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Sodium nitrite was purchased from Mallinckrodt Specialty Chemicals (Paris, KY).

One-dimensional ^{1}H, ^{13}C, and ^{15}N NMR spectra were recorded with a Bruker WM-360 MHz, a Bruker DRX-400 MHz, or a Bruker AMX-600 MHz instrument (Karlsruhe, Germany). Samples were analyzed by heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple quantum correlation (HMQC) experiments with the Bruker AMX-600 spectrometer. Samples analyzed by NMR spectroscopy were dissolved in Me_2CO-d_6, CD_3OD, DMSO-d_6, or $CDCl_3$. Chemical shifts are recorded in δ values (ppm) downfield from tetramethylsilane (^{1}H and ^{13}C NMR) or with reference to a saturated solution of NH_4NO_3 in 5% HNO_3 and 10% D_2O at 375.6 ppm, relative to NH_3 (liquid, 298 K) at 0.0 ppm (^{15}N NMR) (*18*). Coupling constants (J values) are recorded in Hertz. Electron impact mass spectra (EIMS) were obtained with either a Trio-1 MS (VG Analytical, Manchester, England) or a Voyager MS (ThermoQuest, Manchester, England) instrument. Optical rotations were measured with a JASCO P-1020 polarimeter (Kyoto, Japan), and UV spectra were obtained with a Shimadzu UV-2101PC scanning spectrophotometer. Melting points were obtained with a Mel-Temp apparatus.

Compounds were separated by TLC on either Merck silica gel GF_{254} prepared on glass plates with a Quickfit Industries (London, England) spreader (0.25 mm thick, activated at 120 °C for 20 min) or aluminum-foil-backed (Alltech) Kieselgel 60 F_{254} plates. Plates were developed by one of the following systems (prepared in volumetric ratio): (A) CH_2Cl_2/MeOH/HCOOH (95:5:0.5), (B) CH_2Cl_2/MeOH (94:6), (C) C_6H_{14}/EtOAc/HCOOH (60:40:0.5), (D) CH_2Cl_2/MeOH (95:5), or (E) CH_2Cl_2/MeOH/HCOOH (94:6:0.5). Visualization was at 254 and 366 nm by spraying with Pauly's reagent and heating. HPLC experiments were conducted with a Shimadzu LC-6A dual pumping system connected to a Shimadzu SPD-6AV UV/vis detector and a Shimadzu SCL-6B system controller (Kyoto, Japan) by pumping a mobile phase (1 mL/min) of MeOH/2% HCOOH 35:65 (v/v) through a Versapack C18 column (10μ, 250 mm × 4.6 mm, Alltech, Deerfield, IL). Chromatograms were recorded and analyzed by the Shimadzu Class VP program. Samples from reactions in progress were either injected directly from the reaction mixture or diluted with Optima grade MeOH (Fisher Chemicals, Fair Lawn, NJ). HPLC ESIMS experiments were conducted with a Hewlett-Packard Series 1100 LC/MSD instrument; samples were separated prior to ionization by the Versapack column. Chromatograms and electrospray spectra were recorded and processed by Hewlett-Packard LC/MSD Chem Station, version A.06.01, software, and the peak areas were calculated by the single ion monitoring (SIM) method.

Reaction of 1 with Nitrite at pH 2. A sample of 1.7 g (0.01 mol) of *p*-coumaric acid (**1**) dissolved in 25 mL of MeOH was added over 5 min to 500 mL of pH 2 distilled H_2O containing 5.5 g (0.08 mol) of $NaNO_2$. The reaction was stirred at room temperature for 150 min, during which time the solution turned bright yellow to pumpkin-orange. After 90, 120, and 150 min, TLC (system A) indicated that all of **1** was consumed and that a complex mixture of four major products was observed between R_f 0.50 and 0.20. After 150 min, the pumpkin-orange-colored reaction mixture was saturated with NaCl and extracted with 3 × 250 mL EtOAc. The EtOAc extracts were dried over Na_2SO_4 and concentrated to afford 1.8 g of a brick-red oil, which was stored under argon at −20 °C in a foil-wrapped flask.

The brick-red oil was separated over 190 g of flash column silica gel (40 μm, 41 × 4.1 cm, 100% CH_2Cl_2 to 9:1 CH_2Cl_2/MeOH) and monitored by TLC (system B), giving two fractions. The first fraction (884 mg) was a dark yellow mixture (R_f 0.50−0.14). The second (404 mg) was a dark orange oil (R_f 0.17−0.0). The first fraction was again purified by chromatography over flash column silica gel (90 g, 30.5 × 3.8 cm, C_6H_{14}/EtOAc/HCOOH 80:20:0.5) to give 196 mg of a light yellow powder (TLC system C, R_f 0.71, yellow), which was identified as 4-hydroxybenzaldehyde (**3**) by comparing its melting point and spectral and chromatographic properties with authentic 4-hydroxybenzaldehyde. A second fraction was a mixture containing **3** and a fluorescent component (R_f 0.61, dark orange). This fraction (51 mg) was subjected to further chromatography over C18 reversed-phase 40-μm silica gel (19.5 × 1.3 cm, 80:20−65:35 H_2O/MeOH) to afford 20 mg of 4-hydroxybenzaldehyde [**3**, 216 mg (0.16 mmol) total, 17%] and 8 mg of a yellow solid that was characterized as 7-hydroxy-1,2(4*H*)-benzoxazin-4-one (**11**, 0.05 mmol, 1%).

7-Hydroxy-1,2(4*H*)-benzoxazin-4-one (11). Yellow solid; UV (MeOH) λ_{max} (log ϵ) 254 (3.94), 308 (3.77), 318 (3.77) nm; ^{1}H NMR (Me_2CO-d_6, 600 MHz) δ 8.20 (1H, s, H-3), 7.92 (1H, d, J = 9.0 Hz, H-5), 7.02 (1H, dd, J = 2.2, 8.8 Hz, H-6), 6.88 (1H, d, J = 2.5 Hz, H-8); ^{13}C NMR (Me_2CO-d_6, 150 MHz) δ 166.8 (C-4), 165.5 (C-7), 164.0 (C-8a), 151.7 (C-3), 127.3 (C-5), 117.1 (C-6), 114.2 (C-4a), 100.4 (C-8); EIMS (70 eV) m/z 163 $[M]^+$ (100), 136 (82), 121 (26), 108 (83), 95 (21), 80 (44), 69 (24), 63 (13), 52 (39); HREIMS m/z 163.0277 (calcd for $C_8H_5NO_3$, 163.0269).

Reaction of 1 with Nitrite at pH 3. *p*-Coumaric acid (**1**, 33 mg, 0.2 mmol) dissolved in 200 μL of MeOH was added to 5 mL of a pH 3 distilled water solution containing $NaNO_2$ (55 mg, 0.8 mmol). The reaction mixture was immediately applied to a C18 reversed-phase 40-μm silica gel column (14.0 × 1.2 cm) and was eluted with H_2O/MeOH 9:1−7.5:2.5 while the fractions were monitored by HPLC (10-μL injections) and TLC (system A). One fraction was combined and concentrated to 8 mg of a clear glass, which was characterized as a 3:1 *E*/*Z* mixture of 1′,4-dihydroxybenzeneacetaldehyde oxime (**5**, R_v 4.1 mL, R_f 0.22, yellow, 0.04 mmol, 20%), and the second fraction (3 mg) was concentrated to afford a bright yellow powder, which was characterized as 4-hydroxy-1′-oxo-benzeneacetaldehyde aldoxime (**7**, R_v 9.9 mL, yellow, R_f 0.28, 0.02 mmol, 10%).

(*E*)- and (*Z*)-1′,4-Dihydroxybenzeneacetaldehyde Oxime [(*E*)- and (*Z*)-5]. Clear glass; $[\alpha]^{25}_D$ 0.0° (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR in Table 1; EIMS (70 eV) m/z 167 $[M]^+$ (11), 123 (72), 121 (100), 95 (28), 65 (23); HREIMS m/z 167.0572 (calcd for $C_8H_9NO_3$, 167.0582).

4-Hydroxy-1′-oxo-benzeneacetaldehyde Aldoxime (7). Bright yellow powder; UV (MeOH) λ_{max} (log ϵ) 230 (3.80), 305 (3.73) nm; ^{1}H NMR signals in Me_2CO-d_6 and CD_3OD and ^{13}C NMR in Me_2CO-d_6 were nearly identical to the reported values (*16*, *19*); EIMS (70 eV) m/z 165 $[M]^+$ (20), 147 (11), 137 (6), 121 (100), 93 (26), 65 (31); HREIMS m/z 165.0428 (calcd for $C_8H_7NO_3$, 165.0426).

Table 2. Yields (HPLC) of Products Obtained from Reactions of *p*-Coumaric Acid (1) with Nitrite in H_2O over a Range of pH Values

	pH			
compound	2	3	7[a]	10[a]
3	16 ± 5%	−	−	−
5	59 ± 7%	25 ± 7%	−	−
7	26 ± 3%	67 ± 5%	23 ± 3%	17 ± 3%
11	6 ± 2%	6 ± 1%	−	−
13	−	6 ± 1%	1.4 ± 0.8%	1.4 ± 0.4%

[a] Results from duplicate analyses.

HPLC Analysis of Products Formed at pH 2. The reaction was carried out as described above on a 50-mL scale at pH 2 (8:1 molar ratio of $NaNO_2$/**1**). The reaction was monitored by HPLC. After 1, 25, 55, 80, 105, and 130 min, 1-mL volumes of the reaction mixture were sampled and diluted with 2 mL of MeOH, and 5-μL samples were analyzed. The peaks were identified by comparison with retention volumes of the isolated standards. The product yields in solution were quantitated (from three separate experiments) from established standard curves, and yields of each compound in solution after 130 min are given in Table 2.

Reaction of 4-Hydroxybenzenepropanoic Acid (14) with Nitrite. A sample of 32 mg of **14** dissolved in 300 μL of MeOH was added to 5 mL of pH 2 distilled H_2O containing 57 mg (0.8 mmol) of $NaNO_2$. After 30 and 60 min, 200-μL volumes of the reaction mixture were sampled and diluted with 400 μL of MeOH, and 5-μL samples were analyzed by HPLC (280 nm), indicating that **15** (R_v 23.0 mL) was present in about 10% yield. After 60 min, ammonium sulfamate ($NH_4SO_3NH_2$, 47 mg, 0.4 mmol) was added to the dark orange-red-colored reaction (*20*), and it was extracted with 3 × 2.5 mL EtOAc. EtOAc extracts were dried over Na_2SO_4 and evaporated to yield 28 mg of a red mixture. The mixture was separated over 40-μm C18 reversed-phase silica gel (12.3 × 1.2 cm, H_2O/MeOH 9:1−6:4). One fraction contained 23 mg of unreacted **14** (TLC, system A, R_f 0.35, yellow), whereas a second gave 2 mg of a yellow solid that was characterized as **15** (R_f 0.63, yellow, 9 μmol, 5%).

4-Hydroxy-3-nitrobenzenepropanoic Acid (15). Yellow solid; UV (MeOH) λ_{max} (log ϵ) 215 (4.14), 275 (3.69), 358 (3.37) nm; ^{1}H and ^{13}C NMR data were nearly identical to those reported (*21*, *22*); EIMS (70 eV) *m*/*z* 211 $[M]^+$ (13), 193 (21), 175 (17), 152 (100), 151 (36), 147 (36), 106 (15), 72 (60), 55 (17); HREIMS *m*/*z* 211.0482 (calcd for $C_9H_9NO_5$, 211.0481).

Preparation of 3-(4-Hydroxyphenyl)-2-propenoic Acid Methyl Ester (16) (*23*). H_2SO_4 (1 mL) was added to anhydrous **1** (1.0 g, 6 mmol) dissolved in 23 mL of anhydrous MeOH and refluxed under N_2 for 2 h at 45 °C. The reaction was monitored by TLC (system E, R_f 0.57, dark orange), which indicated that 3-(4-hydroxyphenyl)-2-propenoic acid methyl ester (**16**) was produced in about 80% yield after 3.5 h. The reaction mixture (30 mL) was poured over 24 g of crushed ice and stirred until the ice dissolved and the solution became milky white. The pH was adjusted to 8.0 with 78 mL of 1 M $NaHCO_3$, and the solution was extracted with 3 × 130 mL CH_2Cl_2. Pooled organic extracts were dried over Na_2SO_4 and concentrated to afford 920 mg (5.2 mmol) **16** in 86% yield. Recrystallization from MeOH/H_2O provided 838 mg (4.7 mmol) white needles (78% overall yield) of **16** that gave a melting point of 134.5−136 °C [lit. 137 °C (*24*)], NMR spectral properties corresponding to previous reports (*24*, *25*), and HREIMS *m*/*z* 178.0630 (calcd for $C_{10}H_{10}O_3$, 178.0630).

Reaction of 3-(4-Hydroxyphenyl)-2-propenoic Acid Methyl Ester (16) with Nitrite. Reaction of 354 mg (2.0 mmol) of **16** with 553 mg (8.0 mmol) of $NaNO_2$ at pH 2 as before, but in a 2:1 (v/v) mixture of distilled water/acetone, afforded after extraction and chromatography 104 mg of 3-(4-hydroxy-3-nitrophenyl)-2-propenoic acid methyl ester (**17**, R_f 0.86, TLC system E, yellow, 0.47 mmol, 23%). The bright yellow powder gave a melting point of 146.5−148.0 °C [corrected, lit. 142−144 °C, (*26*)]; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 10.77 (1H, s, O*H*), 8.30 (1H, d, J = 2.1 Hz, H-2′), 7.81 (1H, dd, J = 2.1, 8.9 Hz, H-6′), 7.67 (1H, d, J = 16.0 Hz, H-3), 7.25 (1H, d, J = 8.9 Hz, H-5′), 6.46 (1H, d, J = 16.0 Hz, H-2), 3.86 (3H, s, COOCH_3); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.18 (1H, d, J = 2.4 Hz, H-2′), 7.75 (1H, dd, J = 2.4, 8.6 Hz, H-6′), 7.63 (1H, d, J = 15.7 Hz, H-3), 7.02 (1H, d, J = 8.7 Hz, H-5′), 6.40 (1H, d, J = 15.8 Hz, H-2), 3.77 (3H, s, COOCH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 166.7 (*C*OOCH$_3$), 156.0 (C-4′), 141.7 (C-3), 136.0 (C-6′), 133.6 (C-3′), 127.0 (C-1′), 124.9 (C-2′), 120.8 (C-5′), 118.8 (C-2), 51.7 (COO*C*H$_3$); EIMS (70 eV) *m*/*z* 223 $[M]^+$ (81), 192 (100), 146 (22), 118 (16), 89 (31); HREIMS *m*/*z* 223.0498 (calcd for $C_{10}H_9NO_5$, 223.0481).

Table 3. Mass Spectral Data for 11 Isolated from Reactions of *p*-Coumaric Acid (1) with Nitrite in H_2O and $H_2{}^{18}O$

	isolated standard **11**		**11** from 10% $H_2{}^{18}O$ reaction	
ion (*m*/*z*)	ion relative intensity (%)	fragment ion	ion relative intensity (%)	fragment ion
168	0.03	$[M + H + 4]^+$	0.91	$[M + H + 4]^+$
166	0.84	$[M + H + 2]^+$	20.84	$[M + H + 2]^+$
165	9.07	$[M + H + 1]^+$	11.01	$[M + H + 1]^+$
164	100	$[M + H]^+$	100	$[M + H]^+$

Reaction of *p*-Coumaric Acid (1) with $NaNO_2$ at pH 2 in $H_2{}^{18}O$. A sample of 5 mg (0.02 mmol) of *p*-coumaric acid (**1**) dissolved in 30 μL of MeOH and 22 μL of 6 N HCl were added to 11 mg (0.16 mmol) of $NaNO_2$ dissolved in 1.0 g of $H_2{}^{18}O$ (10 at. % ^{18}O). The reaction mixture was stirred for 60 min until it became dark yellow. Samples of 5 μL were withdrawn from the reaction after 0 and 60 min and directly injected without workup for HPLC analysis. After 60 min, the pH was adjusted to 8.0 with 300 μL of 1 M $NaHCO_3$ and extracted with 3 × 0.5 mL EtOAc. Pooled EtOAc extracts were evaporated to yield 4 mg of a bright orange oil, which was redissolved in 200 μL of Optima MeOH, and 12 × 15 μL samples were injected for HPLC purification. Peaks eluting at R_v 16 mL were collected, pooled, and extracted with three half-volumes of EtOAc. The EtOAc layers were combined and evaporated to yield 55 μg of **11** (0.3 μmol, 2% yield).

Analysis of ^{18}O-Labeled 11 by HPLC ESIMS. The unlabeled standard **11** and the reaction product were each dissolved to concentrations of 1 mg/mL in Optima MeOH. Samples of 5 μL were resolved over a Versapack C18, 10μ column connected to a Hewlett-Packard Series 1100 LC/MSD instrument and ionized under API-ES conditions. The percentage incorporation of ^{18}O was determined by comparing peak areas for ions of the unlabeled standard and the compound isolated from the reaction in $H_2{}^{18}O$, 10 atom % ^{18}O (Table 3).

Reaction of 1 with Nitrite at pH 7 and 10. The same method was used for reaction of *p*-coumaric acid (**1**) with nitrite at both pH 7 and pH 10. $NaNO_2$ (552 mg, 8.0 mmol) was dissolved in 50 mL of distilled H_2O, pH 7.0. *p*-Coumaric acid (**1**, 165 mg, 1.0 mmol) dissolved in 2 mL of MeOH was added to the $NaNO_2$ solution. The color became bright yellow within seconds of the addition of **1**. A 1-mL sample of the reaction mixture was diluted with 2 mL of MeOH, and injection of 5 μL of the MeOH solution for HPLC analysis indicated that **13** (R_v 21.3 mL) was gradually produced over 150 min to a maximum of 1% yield. TLC analysis (system D) showed five spots after 30 min and through 150 min (R_f 0.50−0.10), with about 70% of **1** remaining unreacted. After 150 min, the reaction mixture was extracted with 3 × 25 mL EtOAc. Pooled EtOAc extracts were dried over Na_2SO_4 and concentrated to yield 38 mg of a yellow-orange oil. The oil was separated over 7 g of 40-μm silica gel (19.0 × 1.1 cm, CH_2Cl_2/MeOH 98:2−96:4), giving three fractions (TLC system D). Evaporation of the first fraction afforded a bright yellow powder (1.5 mg, 8 μmol), which was characterized as **13** (1%, R_f 0.49, yellow), the second gave 3 mg (16 μmol) of **7** (2%, R_f 0.24, yellow), and the third fraction contained 13 mg of unreacted **1** (R_f 0.40, orange). The yields (HPLC) of the products in reactions at pH 7 and pH 10 were very similar: **13** (1%), **7** (23% at pH 7, 17% at pH 10) (Table 2).

4-(2-Oxido-1,2,5-oxadiazol-3-yl)phenol (13). Bright yellow powder; ^{1}H NMR [Me_2CO-d_6, DMSO-d_6, $CDCl_3$/Me_2CO-d_6 9.5:0.5 (v/v)] and ^{13}C NMR (Me_2CO-d_6) signals were nearly identical to those reported (*16*, *17*); EIMS (70 eV) *m*/*z* 178 $[M]^+$ (31), 148 (3), 118 (100), 89 (10); HREIMS *m*/*z* 178.0380 (calcd for $C_8H_6N_2O_3$, 178.0378).

Preparation and Characterization of ^{15}N-13. The same procedure used for the preparation of unlabeled **13** was followed. $Na^{15}NO_2$ (>98 at. % ^{15}N, 280 mg, 4.0 mmol) was dissolved in 25 mL of H_2O, and the pH was adjusted to 10.0 with 54 μL of 0.25 N NaOH. *p*-Coumaric acid (**1**, 83 mg, 0.50 mmol) dissolved in 1 mL of MeOH was added to the $Na^{15}NO_2$ solution. Samples (5 μL) diluted with MeOH were analyzed by HPLC as before. HPLC results indicated that **13** and **7** were produced within 25 min (1 and 13%, respectively) and that the reaction was complete after 90 min (Table 2). The bright yellow reaction mixture was then extracted with 3 × 12 mL EtOAc. Extracts were pooled, dried over Na_2SO_4, and concentrated to 15 mg of yellow residue, which was separated over 3 g of 40-μm flash silica gel (1.1 × 8.6 cm, CH_2Cl_2/MeOH 98:2). Fractions 4−10 (TLC system D) were pooled and evaporated to yield 1 mg (6 μmol, 1%) of the yellow powder ^{15}N-**13**.

^{15}N-4-(2-Oxido-1,2,5-oxadiazol-3-yl)phenol (^{15}N-13). Yellow powder; ^{1}H NMR (Me_2CO-d_6, 360 MHz) δ 9.12 (1H, dd, J = 3.9, 12.5 Hz, H-4′), 7.94 (2H, d, J = 8.6 Hz, H-3, H-5), 7.01 (2H, d, J = 9.1 Hz, H-2, H-6); ^{13}C NMR (Me_2CO-d_6, 90 MHz) δ 160.4 (C-1), 145.8 (C-4′, J = 2.5, 5.8 Hz), 128.5 (C-3, C-5), 116.9 (C-2, C-6), 114.6 (C-3′, C-4); ^{15}N NMR (36.5 MHz) δ 379.3 (N-5′), 351.1 (N-2′); EIMS (70 eV) *m*/*z* 180 $[M]^+$ (19), 149 (11), 118 (100), 89 (27), 63 (13); HREIMS *m*/*z* 180.0319 (calcd for $C_8H_6{}^{15}N_2O_3$, 180.0319).

RESULTS AND DISCUSSION

The reaction of *p*-coumaric acid (**1**) with nitrite was carried out under a variety of conditions likely to be found in living systems. Although some products formed by the reaction of **1** with nitrite in different H_2O/organic solvent mixtures have been identified, its reaction in H_2O at room temperature has not been described. Furthermore, little previous work has been reported on the nitrosation of dietary phenolic compounds at other than acidic pH, and to our knowledge, the reaction of *p*-coumaric acid with nitrite at basic pH is unknown.

We previously studied the nitrosation of ferulic acid, with isolation and spectral identification of the products obtained at pH 2 (*27*). A novel benzoxazinone, vanillin, and 2-methoxy-4,6-dinitrophenol were characterized as products of the reaction of ferulic acid with nitrite. However, in this study, the range of products obtained by nitrosation of *p*-coumaric acid was much greater than observed with ferulic acid. Different reaction conditions afforded complex mixtures of *p*-coumaric acid products by TLC and HPLC, and the identities of the major reaction products were established by their isolation and spectral characterization.

Two products were obtained from reactions of **1** with nitrite at room temperature in H_2O at pH 2. 4-Hydroxybenzaldehyde (**3**), was isolated as a light yellow powder and identified by comparison of its chromatographic (TLC system A, R_f 0.44, yellow), mass, and NMR spectral characteristics with those of authentic 4-hydroxybenzaldehyde.

A new compound, 7-hydroxy-1,2(4*H*)-benzoxazin-4-one (**11**), was also characterized. HREIMS gave an empirical formula of $C_8H_5NO_3$, indicating the incorporation of one nitrogen atom into the structure of **1** and the loss of one carbon atom. ^{1}H NMR indicated four protons: three were part of an A, B, X system, for which the ^{1}H signal at δ 7.02 (dd) was *o*-coupled to that at δ 7.92 (d, J = 9.0 Hz) and *m*-coupled to another at δ 6.88 (d, J = 2.5 Hz). Another proton (δ 8.20, s) gave a δ value (8.18) similar to that for H-3 of a related, ferulic acid-derived product (*27*). The ^{13}C NMR spectrum showed signals for eight carbons of an extended π system, one of which was a carbonyl carbon (δ 166.8). Two others were also oxygen-bearing carbons (δ 164.0 and 165.5), and four more comprised the aromatic ring (δ 100.4, 117.1, 127.3, and 114.2). Signals for C-3 (δ 151.7) and C-4 (δ 166.8) were nearly identical to those for the related 6-methoxy derivative (*27*). HMBC and HMQC spectral analyses confirmed the following connectivities of the protons and carbons for **11**: H-3 (C-4 and C-4a), H-5 (C-4, C-7, and C-8a), H-6 (C-4a, C-7, and C-8), and H-8 (C-4a, C-6, C-7, and C-8a). The correlation of H-6 with C-7 and the lack of correlation with C-8a indicated that the ^{13}C signal at δ 165.5 corresponded to C-7 and that at δ 164.0 to C-8a. Thus, the structure of **11** was confirmed as the new compound, 7-hydroxy-1,2(4*H*)-benzoxazin-4-one.

Two major products were isolated from the reaction of **1** with nitrite at pH 3 by immediately separating the reaction mixture over a C-18 40-μm reversed-phase flash chromatography column. HPLC (10-μL injections) and TLC (system A) were used to identify chromatographically similar fractions that were pooled and evaporated to give 8 mg (20% yield) of **5** as a clear glass (R_v 4.1 mL, R_f 0.22, yellow) and 3 mg (10% yield) of **7** as a bright yellow powder (R_v 9.9 mL, R_f 0.28, yellow).

HREIMS of **5** gave $C_8H_9NO_3$, indicating the addition of one N atom and the loss of one C atom of **1**. EIMS gave $[M]^+$ at *m*/*z* 167 and fragmentations at *m*/*z* 123 ($[M-CH_2NO]^+$) and *m*/*z* 121 ($[M-CH_4NO]^+$, base peak). Comparison of the 600-MHz ^{1}H NMR spectrum in CD_3OD (Table 1) with those for several oxime structures indicated that the isolated product was a 3:1 mixture of 1′,4-dihydroxybenzeneacetaldehyde oxime isomers, (*E*)-**5** and (*Z*)-**5** (*28*−*31*), which were not separated. ^{1}H NMR signals for *E*-**5** were shifted downfield by exactly 0.46 ppm versus those reported for 1′,4-dihydroxybenzeneacetaldehyde oxime (*31*). For *E*-**5**, signals were evident for a *p*-disubstituted aromatic ring derived from **1** and two coupled side-chain protons (δ 5.12 and δ 7.37, J = 7.2 Hz). ^{13}C NMR results (Table 1) for *E*-**5** confirmed the presence of six aromatic carbons and two connected methines. For *Z*-**5**, ^{1}H NMR results (Table 1) indicated a *p*-substituted aromatic ring from **1** and two coupled side-chain signals (δ 5.84 and 6.79, J = 6.2 Hz). The ^{13}C NMR spectrum (Table 1) for *Z*-**5** was similar to that for *E*-**5**. HMBC (Table 1) and HMQC analyses permitted a clear differentiation of the two isomers by indicating the position of the oxime functionality of each isomer and permitting the assignment of the signals at δ 153.0 and 158.3 to the (*E*) isomer and those at δ 154.0 and 157.5 to the (*Z*) isomer. Because the specific rotation of **5** ($[\alpha]^{25}_D$) was 0.0°, the (*Z*) and (*E*) isomers each must exist as racemates of the (*R*) and (*S*) enantiomers.

The structure of the bright yellow powder was identified as that of 4-hydroxy-1′-oxo-benzeneacetaldehyde aldoxime (**7**) by NMR and MS spectral analyses and by comparison with the literature (*16*, *19*). HREIMS of **7** gave $C_8H_7NO_3$, indicating the loss of one carbon atom and the addition of one nitrogen atom to **1**. ^{1}H and ^{13}C NMR spectroscopy in acetone-d_6 indicated the presence of a *p*-disubstituted aromatic derived from **1**. Signals at δ 187.8 and 150.0 correspond to carbonyl and oxime carbons. HMBC indicated that the C*H*═NOH methine proton was correlated only to the carbonyl carbon,

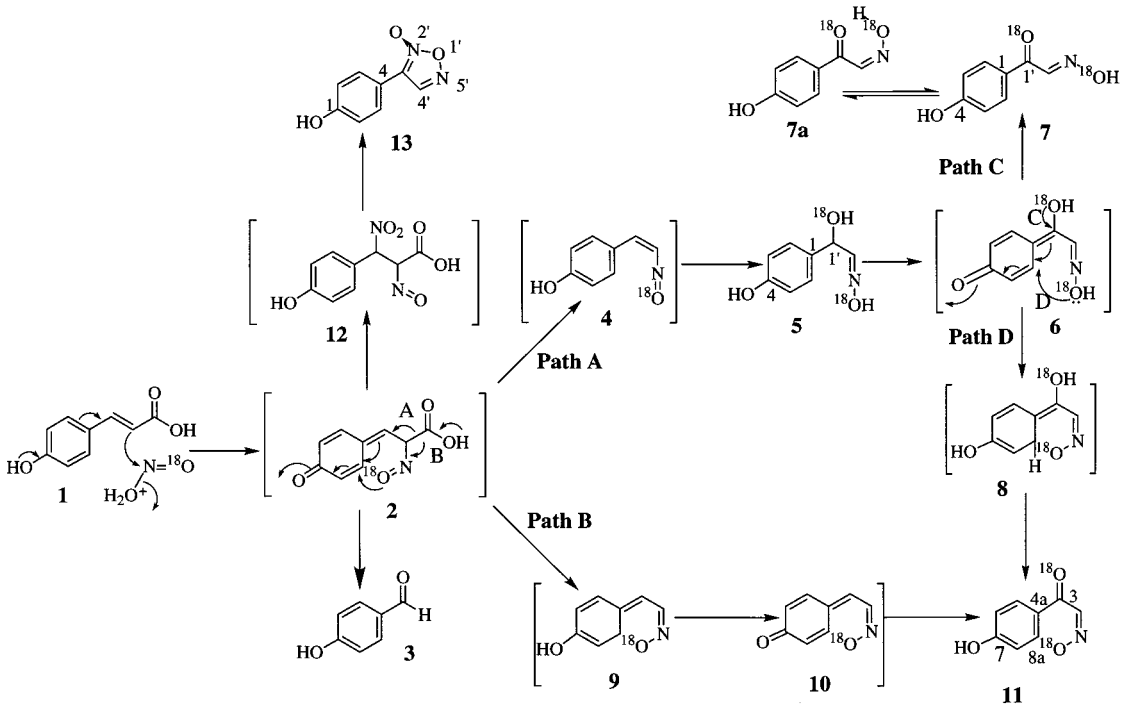

Figure 1. Products obtained by the reaction of *p*-coumaric acid (**1**) with nitrite under different conditions.

whereas aromatic protons H-2 and H-6 were correlated with the aromatic carbons and C-1′. The aldoxime **7** was previously isolated by reaction of **1** with $NaNO_2$ in acetone/water (*16*).

Aldoxime **7** can occur in either the (*E*) (**7**) or the (*Z*) (**7a**) conformation. ^{1}H NMR spectroscopy and X-ray crystallography confirmed that *Penicillium olsonii* yielded the (*E*) isomer **7** (*19*). By ^{1}H NMR spectroscopy, the C*H*═NOH methine proton of the (*Z*) isomer (**7a**) gives a signal at δ 7.77 in Me_2CO-d_6 (*16*), whereas **7** resonates at δ 8.06 in CD_3OD (*19*). The aldoxime isolated in this work gives singlet signals for C*H*═NOH at δ 7.93 in Me_2CO-d_6 and at δ 8.05 in CD_3OD, thus confirming the structure of the aldoxime as *E*-**7**. Although, in principle, **7** could tautomerize to **7a** stabilized by hydrogen bonding (*16*), our NMR results indicate the presence of the single isomer **7**.

HPLC analysis (Figure 2) of the reaction of **1** with nitrite at pH 2 in H_2O versus time indicates that *p*-coumaric acid (**1**, 3.1 mg/mL) was rapidly consumed within 25 min to give **3** (0.2 mg/mL), **11** (0.2 mg/mL), **5** (2.0 mg/mL), and **7** (0.8 mg/mL). The major peak eluting at R_v 7.0 mL was attributed to nitrite. During the reaction, products are likely formed immediately, and their relative concentrations remain essentially unchanged for 130 min. HPLC analysis (Table 2) also indicates that **5** and **7**, isolated from the reaction at pH 3, were actually produced at pH 2 in good yield.

Nitrosation at the *p* or *o* positions of phenols under acidic conditions is a well-known reaction, in which the nitrosating species is considered to be $H_2NO_2^+$. *p*-Coumaric acid (**1**) has a conjugated side-chain double bond that can also participate in the nitrosation reaction. The products isolated when **1** and nitrite were reacted at pH 2 indicate that the side chain was the primary reactive site. This suggests that tautomerization of **1** to a quinoid intermediate preceded nitrosation to give **2** (Figure 1). With 4-hydroxybenzenepropanoic acid (**14**), nitrosation on the aromatic ring and oxidation gave **15** by HREIMS and ^{1}H and ^{13}C NMR spectroscopy

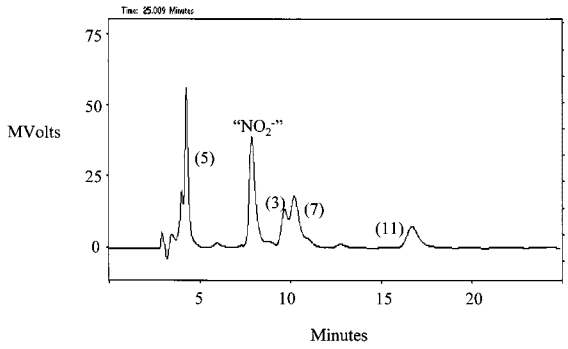

Figure 2. HPLC profile of the products obtained by reaction of *p*-coumaric acid (**1**) with nitrite at pH 2.

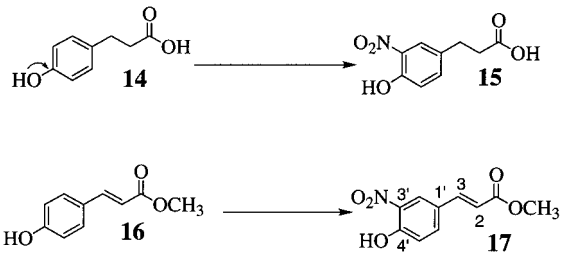

Figure 3. Products obtained by the reactions of 4-hydroxybenzenepropanoic acid (**14**) and *p*-coumaric acid methyl ester (**16**) with $NaNO_2$ at pH 2.

(*21, 22*) (Figure 3). Yields of **15** were consistently low by HPLC analysis, indicating that reaction of nitrite with the vinyl side chain of *p*-coumaric acid (**1**) is preferred.

Reaction of **16** in acidic nitrite aqueous/acetone solutions gave 3-(4-hydroxy-3-nitrophenyl)-2-propenoic acid methyl ester (**17**) in 25% yield and only traces of other unidentified reaction products. This reaction was performed in acetone and H_2O because the product was formed in extremely low yield in H_2O. The structure of **17** was confirmed by melting point (*26*), MS and NMR spectral analyses, and comparisons with spectra for **15**

and **16** and published ^{13}C NMR data for **16** (*25*). HREIMS gave $C_{10}H_9NO_5$, and ^1H NMR in both $CDCl_3$ and CD_3OD and ^{13}C NMR spectra gave signals similar to those for **16** for the side chain and to those for **15** for the NO_2-substituted aromatic ring. Zioudrou et al. (*32*) also obtained **17** by reaction of **16** in acidic nitrite, using aqueous/dioxane mixtures. Results obtained by nitration of **14** and **16** underline the importance of the free carboxyl group and side-chain unsaturation in reactions of *p*-coumaric acid (**1**) with nitrite under acidic conditions.

Reaction of **1** with nitrite under standard conditions, but under argon, gave the same HPLC product profile as reactions exposed to air. This result suggests that oxygen atoms in isolated products were introduced either from H_2O, nitrite, or a combination of these and not from atmospheric oxygen. Thus, **1** was reacted with nitrite in $H_2{}^{18}O$ (10 atom % ^{18}O) at pH 2, and **11** was isolated by HPLC and subjected to electrospray MS analysis.

The percentage incorporation of ^{18}O was determined by comparing relative peak intensities for $[M + H + 2]^+$ and $[M + H + 4]^+$ ions of unlabeled **11** to those for **11** isolated from the $H_2{}^{18}O$ experiment (Table 3). Ions at *m*/*z* 166 ($[M + H + 2]^+$) and *m*/*z* 168 ($[M + H + 4]^+$) were increased in intensity by 20.0 and 0.88%, respectively. The 20% increase in intensity for *m*/*z* 166 indicated that labeled oxygen atoms were incorporated into two different positions in **11**. A doubly ^{18}O-labeled compound would require two sequential steps incorporating $H_2{}^{18}O$ into **1**. With 10% $H_2{}^{18}O$, the doubly labeled $[M + H + 4]^+$ product would show an increase of 1%. The increase in the intensity of the ion at *m*/*z* 168 of 0.88% indicates that two atoms of ^{18}O were incorporated into **11.** We believe that the two oxygen atoms derive from $H_2{}^{18}O$ itself and from nitrite that becomes labeled through oxygen exchange.

The results suggest a mechanism for the formation of **11** from the reaction of **1** with nitrite in H_2O under acidic conditions (Figure 1). Concerted tautomerization of **1** and attack of $H_2NO_2{}^+$ would give a nitrosated and doubly vinylogous β-keto acid **2**. Decarboxylation to 4-hydroxy-2′-nitrosostyrene (**4**) (path A) and water addition to **4** gives **5**. Oxidation of **5** to **6** and tautomerization (path C) affords **7**. Intramolecular Michael addition of the oxime oxygen atom of **6** (path D) affords **11** by aromatization and oxidation of **8**. A pathway similar to D was proposed for the formation of 7-hydroxy-6-methoxy-1,2(4*H*)-benzoxazin-4-one from ferulic acid (*27*). An alternative path B shows the concerted decarboxylation of **2** to benzoxazine **9**, which, upon further oxidation, gives **11**. Our labeling results do not permit a distinction between these two pathways.

HPLC analysis of reactions of *p*-coumaric acid (**1**) with nitrite at pH values of 1, 3, 7, and 10 shows that reaction profiles differed significantly as a function of pH (Table 2). Intractable mixtures of numerous products were observed at pH 1. As the pH of the reaction mixture increased, the reactivity of **1** decreased. Whereas **1** was completely consumed within 25 min at pH 2 and 3, 68 and 65% remained after 150 min at pH 7 and 10, respectively. Although aldoxime **7** was formed over the entire pH range, **3** was observed only at pH 2, and **11** was not observed in reactions above pH 3. At pH 3, four products were formed gradually over 120 min: **7** (67 ± 5%,), **5** (25 ± 7%), **11** (6 ± 1%), and **13** (6 ± 1%). The HPLC-measured yields of products in reactions at pH 7 and 10 were very similar: **13** (1%) and **7** (23% at pH 7 and 17% at pH 10) (Table 2). Therefore, a preparative-scale reaction was performed at pH 7 in order to isolate and characterize **13**.

HREIMS of **13** gave a formula of $C_8H_6N_2O_3$, indicating the presence of two nitrogen atoms and the absence of one carbon atom in comparison with **1**. ^1H NMR spectroscopy was performed in three different solvents [Me_2CO-d_6, CD_3OD, and $CDCl_3$/Me_2CO-d_6 9.5:0.5 (v/v)] in order to compare signals with those for **13** isolated earlier from acidic water/organic solvent mixtures (*16*, *17*). Product 13 was distinguished from the geometric oxadiazol-4-yl isomer by NMR spectroscopy: The oxadiazole ring proton of the nonphenolic analogue of 13 resonates at δ 8.55 in $CDCl_3$, whereas that of the nonphenolic analogue of the oxadiazol-4-yl isomer resonates at δ 7.26 (*33*). Plucken et al. (*16*) crystallized **13**, which was isolated from an acidic acetone/water mixture, and assigned the oxadiazole ring proton to the ^1H NMR signal at δ 8.55 in $CDCl_3$/Me_2CO-d_6 (9.5:0.5, v/v). The oxadiazole ring proton for our isolated compound gave a signal at δ 8.54 in the same solvent mixture, thus confirming that the isomer obtained by reaction of **1** with nitrite at pH 7 in H_2O was **13** and not the geometric oxadiazol-4-yl isomer.

Reaction of **1** with $Na^{15}NO_2$ (>98 at. % ^{15}N) at pH 10 in H_2O gave doubly ^{15}N-labeled **13**, which was useful in confirming the structure of **13**. HREIMS gave an empirical formula of $C_8H_6{}^{15}N_2O_3$, showing the incorporation of two ^{15}N atoms for labeled **13**. Both nitrogens of **13** were ^{15}N-labeled, as evidenced by a dd signal (J = 3.9, 12.5) at δ 9.12 due to coupling with each of its labeled nitrogens. The ^{15}N NMR spectrum showed two signals at δ 351.1 (s) and 379.3 (s), and the ^{13}C-signal at δ 145.9 was split into a dd (J = 2.5, 5.8). $^1H-{}^{15}N$ HMBC identified the positions of the nitrogen atoms by correlating the signal for the *N*-oxide nitrogen atom N-2′ (δ 351.1) with H-3, H-5, and H-4′ but coupling N-5′ with only H-4′. These assignments were supported by published ^{15}N NMR spectral data for similar structures (*34*).

The results support a mechanism for the formation of **13** at pH 7 by nitrosation of *p*-coumaric acid (**1**) in H_2O similar to that reported by Plucken et al. (*16*) (Figure 1). In this process, **1** tautomerizes and attacks the nitrosyl cation (NO^+) in concerted fashion to form **2**. Subsequent nitrite addition and rearomatization forms a pseudonitrosite (**12**) that decarboxylates and cyclizes to **13**.

These experiments demonstrate the products at a range of pH values for the reaction of *p*-coumaric acid (**1**) with nitrite in H_2O and suggest logical mechanisms for the formation of these products from **1**. Although **1** is most reactive at acidic pH, it might also serve as a nitrite scavenger at higher pH values. This work clarifies the mechanism by which *p*-coumaric acid (**1**) might act as an effective chemoprotective agent by quenching nitrosating agents in several biological compartments, including salivary and gastric fluids.

LITERATURE CITED

(1) Hartley, R. D.; Ford, C. W. Phenolic constituents of plant cell walls and wall biodegradability. In *Plant Cell Wall Polymers*; Lewis, N. G., Paice, M. G., Eds.; American Chemical Society: Washington, D.C., 1989; pp 137−145.

(2) Xing, Y.; White, P. J. Identification and function of antioxidants from oat groats and hulls. *J. Am. Oil Chem. Soc.* **1997**, *74*, 303−307.

(3) Dimberg, L. H.; Molteberg, E. L.; Solheim, R.; Frolich, W. Variation in oat groats due to variety, storage and heat treatment. I: Phenolic compounds. *J. Cereal Sci.* **1996**, *24*, 263−272.
(4) Pan, G. X.; Bolton, J. L.; Leary, G. J. Determination of ferulic and *p*-coumaric acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment. *J. Agric. Food Chem.* **1998**, *46*, 5283−5288.
(5) Plumb, G. W.; Chambers, S. J.; Lambert, N.; Bartolome, B.; Heaney, R. K.; Wanigatunga, S.; Aruoma, O. I.; Halliwell, B.; Williamson, G. Antioxidant actions of fruit, herb and spice extracts. *J. Food Lipids* **1996**, *3*, 171−188.
(6) Naim, M.; Zehavi, U.; Nagy, S.; Rouseff, R. L. Hydroxycinnamic acids as off-flavor precursors in citrus fruits and their products. In *Phenolic Compounds in Food and their Effects on Health I: Analysis, Occurrence, and Chemistry*; Ho, C.-T., Lee, C. Y., Huang, M.-T., Eds.; ACS Symposium Series 506; American Chemical Society: Washington, D.C., 1992; pp 180−191.
(7) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362−372.
(8) Pratt, B. *Agricultural Statistics, 1998*; U.S. Department of Agriculture, National Agricultural Statistics Service: Washington, D.C., 1998; p I-26.
(9) American Cancer Society. *Cancer Facts and Figures, 1999*; American Cancer Society: Atlanta, GA, 1999; pp 29−31.
(10) NAS/NRC. *The Health Effects of Nitrate, Nitrite, and N-nitroso Compounds*; Committee on Nitrite and Alternative Curing Agents, National Research Council, National Academy Press: Washington, D.C., 1981.
(11) Kuenzig, W.; Chau, J.; Norkus, E.; Holowaschenko, H.; Newmark, H.; Mergens, W.; Conney, A. H. Caffeic and ferulic acid as blockers of nitrosamine formation. *Carcinogenesis* **1984**, *5*, 309−313.
(12) Pannala, A. S.; Razaq, R.; Halliwell, B.; Singh, S.; Rice-Evans, C. A. Inhibition of peroxynitrite dependent tyrosine nitration by hydroxycinnamates: nitration or electron donation? *Free Radical Biol. Med.* **1998**, *24*, 594−606.
(13) Li, P.; Wang, H.-Z.; Wang, X.-Q.; Wu, Y.-N. The blocking effect of phenolic acid on *N*-nitrosomorpholine formation *in vitro*. *Biomed. Environ. Sci.* **1994**, *7*, 68−78.
(14) Kato, Y.; Ogino, Y.; Aoki, T.; Uchida, K.; Kawakishi, S.; Osawa, T. Phenolic antioxidants prevent peroxynitrite-derived collagen modification *in vitro*. *J. Agric. Food Chem.* **1997**, *45*, 3004−3009.
(15) Niwa, T.; Doi, U.; Kato, Y.; Osawa, T. Inhibitory mechanism of sinapinic acid against peroxynitrite-mediated tyrosine nitration of protein *in vitro*. *FEBS Lett.* **1999**, 459, 43−46.
(16) Plucken, U.; Winter, W.; Meier, H. Strukturuntersuchungen an Oxadiazolring-Systemen. *Liebigs Ann. Chem.* **1980**, 1557−1572.
(17) Kikugawa, K.; Hakamada, T.; Hasunuma, M.; Kurechi, T. Reaction of *p*-hydroxycinnamic acid derivatives with nitrite and its relevance to nitrosamine formation. *J. Agric. Food Chem.* **1983**, *31*, 780−785.
(18) Levy, G. C.; Lichter, R. L. *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*; Wiley: New York, 1979; pp 32−33.
(19) Amade, P.; Mallea, M.; Bouaicha, N. Isolation, structural identification and biological activity of two metabolites produced by *Penicillium olsonii* Bainier and Sartory. *J. Antibiot.* **1994**, *47*, 201−207.
(20) Williams, D. L. H. *Nitrosation*; Cambridge University Press: Cambridge, U. K., 1988; pp 1−8, 108−109, 156−157.
(21) *Nuclear Magnetic Resonance Spectra*; Sadtler Research Laboratories: Philadelphia, PA, 1977; Vol. 41, p 25445M.
(22) *Sadtler Standard Carbon-13 NMR Spectra*; Sadtler Research Laboratories; Philadelphia, PA, 1983; p 14679C.
(23) Harwood, L. M.; Moody, C. J. *Experimental Organic Chemistry*; Blackwell Scientific Publications: London, 1989; pp 441−443.
(24) Shimomura, H.; Sashida, Y.; Mimaki, Y.; Iida, N. Regaloside A and B acylated glycerol glucosides from *Lilium regale*. *Phytochemistry* **1988**, *27*, 451−454.
(25) Scalbert, A.; Monties, B.; Lallemand, J.-Y.; Guittet, E., Rolando, C. Ether linkage between phenolic acids and lignin fractions from wheat *Triticum aestivum* cultivar Champlein straw. *Phytochemistry* **1985**, *24*, 1359−1362.
(26) Johnson, T. B.; Kohmann, E. F. 3-Nitro-4-hydroxycinnamic acid and its methyl ether. *J. Am. Chem. Soc.* **1915**, *37*, 162−167.
(27) Rousseau, B.; Rosazza, J. P. N. Reaction of ferulic acid with nitrite: formation of 7-hydroxy-6-methoxy-1, 2(4*H*)-benzoxazin-4-one. *J. Agric. Food Chem.* **1998**, *46*, 3314−3317.
(28) Convert, O.; Pinson, J.; Armand, J. Configuration de quelques α-oximinoalcools. *C. R. Acad. Sci.* **1972**, *274*, 296−299.
(29) Karabatsos, G. J.; Taller, R. A. Structural studies by nuclear magnetic resonance-XV. Conformations and configurations of oximes. *Tetrahedron* **1968**, *24*, 3347−3360.
(30) Varma, R. S.; Kabalka, G. W. Reduction of nitroalkenes with stannous chloride in nonacidic and nonaqueous medium. Synthesis of α-substituted oximes. *Chem. Lett.* **1985**, 243−244.
(31) Shimada, M.; Conn, E. E. The enzymatic conversion of *p*-hydroxyphenylacetaldoxime to *p*-hydroxymandelonitrile. *Arch. Biochem. Biophys.* **1977**, *180*, 199−207.
(32) Zioudrou, C.; Meyer, W. L.; Fruton, J. S. Reactions of nitrous acid with *p*-hydroxycinnamic acid and its derivatives. *J. Am. Chem. Soc.* **1957**, *79*, 4114−4116.
(33) Gasco, A.; Boulton, A. J. Furoxans and benzofuroxans. In *Advances in Heterocyclic Chemistry*; Katritzky, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1981; Vol. 29, pp 251−340.
(34) Kamienski, B.; Schilf, W.; Sitkowski, J.; Stefaniak, L.; Webb, G. A. A ^{13}C and ^{15}N NMR study of some furoxans and related compounds. *J. Crystallogr. Spectrosc. Res.* **1989**, *19*, 1003−1008.

Received for review September 11, 2000. Revised manuscript received December 21, 2000. Accepted December 21, 2000. J. Torres y Torres thanks the NIH (GM08365), the Center for Biocatalysis and Bioprocessing, and the American Foundation for Pharmaceutical Education for predoctoral fellowship awards. We acknowledge financial support from the USDA through the Biotechnology Byproducts Consortium.

JF001127A