JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Constituents of *Asparagus officinalis* Evaluated for Inhibitory Activity against Cyclooxygenase-2

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As part of a project directed toward the discovery of new cancer chemopreventive agents from plants, two new natural products, asparagusic acid anti-*S*-oxide methyl ester (**1**) and asparagusic acid syn-*S*-oxide methyl ester (**2**), a new acetylenic compound, 2-hydroxyasparenyn {3′,4′-*trans*-2-hydroxy-1-methoxy-4-[5-(4-methoxyphenoxy)-3-penten-1-ynyl]-benzene} (**3**), as well as eleven known compounds, asparenyn (**4**), asparenyol (**5**), (±)-1-monopalmitin (**6**), ferulic acid (**7**), 1,3-*O*-di-*p*-coumaroylglycerol (**8**), 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (**9**), blumenol C, (±)-epipinoresinol, linoleic acid, 1,3-*O*-diferuloylglycerol, and 1,2-*O*-diferuloylglycerol, were isolated from an ethyl acetate-soluble fraction of the methanol extract of the aerial parts of *Asparagus officinalis* (Asparagus), using a bioassay based on the inhibition of cyclooxygenase-2 to monitor chromatographic fractionation. The structures of compounds **1**-**3** were elucidated by 1D- and 2D-NMR experiments (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC and NOESY). All the isolates were evaluated for their inhibitory effects against both cyclooxygenase-1 and -2, with the most active compound being linoleic acid.

KEYWORDS: ***Asparagus officinalis* L.; Liliaceae; asparagusic acid anti- and syn-*S*-oxide methyl esters; 2-hydroxyasparenyn; cyclooxygenase-2; cancer chemoprevention**

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely known to be inhibitors of cyclooxygenase (COX) enzymes, which are involved in arachidonic acid metabolism and the production of eicosanoids (*1*). Evidence has now accumulated from epidemiological studies, and investigations with human beings and animals, that NSAIDs hinder the development of colon cancer (*1*). Several epidemiological studies have shown that a substantial decrease in risk of death from colorectal cancer is associated with the use of aspirin and other NSAIDs, and have demonstrated that NSAIDs have cancer-preventive and tumor-regressive effects in the human colon (*2*−*4*). There are two forms of the cyclooxygenase enzyme, COX-1 and COX-2, and most NSAIDs inhibit both. COX-1 is expressed constitutively and is required for physiological processes such as gastrointestinal mucosa maintenance and platelet aggregation. However, COX-2 is inducible in cells such as endothelial cells, macrophages, and intestinal epithelial cells and is responsible for the biosynthesis of prostaglandins under acute inflammatory conditions (*5*−*7*). Thus, regulation of the COX pathway provides a rational approach for the discovery of cancer chemopreventive agents (*8*−*10*), as well as agents useful for the treatment of inflammatory diseases.

Asparagus (*Asparagus officinalis* L.; Liliaceae) is a popular vegetable in Western and Oriental countries alike, and several reports have been published on its secondary metabolite constituents, as well as on the estimation of its nutrient factors and on biotechnological aspects. Previous phytochemical investigations on this plant have resulted in the isolation of various compounds, including saponins (*11, 12*), saccharides (*13*−*15*), acetylenic compounds (*16*), and sulfur-containing compounds (*17*−*20*). In our ongoing project directed toward the discovery of novel naturally occurring cancer chemopreventive agents from plants (*21, 22*), the aerial parts of *Asparagus officinalis* were chosen for more detailed investigation, since the EtOAc-soluble fraction of a MeOH-soluble extract was shown to exhibit significant biological activity in a preliminary in vitro screening against COX-2. In the present study, bioassay-guided fractionation of the EtOAc-soluble fraction, by use of this in vitro COX-2 inhibition assay, led to the purification of two new natural product sulfur-containing compounds (**1** and **2**) and a new acetylenic compound (**3**), along with 11 known compounds. All isolates were evaluated for their potential to modulate the activity of both COX-1 and -2. The structural elucidation of **1**−**3** and the biological evaluation of the isolates are described herein.

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10.1021/jf0305229 CCC: $27.50
Published on Web 03/19/2004

MATERIALS AND METHODS

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 and DRX-500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. Regular and high-resolution mass spectrometry (MS and HRMS) were recorded on a Finnigan MAT 95 sector-field mass spectrometer, and regular and high-resolution electrospray ionization mass spectrometry (ESIMS and HRESIMS) on a Hewlett-Packard 5989B mass spectrometer with a 5998A electrospray interface. Both analytical and preparative thin-layer chromatographic (TLC) analyses were performed on precoated 250 μm thickness Merck Si 60 F_{254} glass plates. Silica gel (Merck 60A, 70−230 or 200−400 mesh ASTM) and Sorbisil C_{18} reversed-phase silica gel (Sigma, St. Louis, MO) were used for column chromatography. Preparative TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ) and distilled before use.

Plant Material. The aerial parts of *Asparagus officinalis* L. were purchased at Strube Co., South Water Market, Chicago, IL, in June 2002. A voucher specimen (PA-3268) has been deposited at the Pharmacognosy Field Station, University of Illinois, Downers Grove, IL.

Assay for Inhibition of COX Activity. Inhibition assays against both COX-1 and -2 were performed as described previously by measuring prostaglandin E_2 (PGE_2) production (*10*, *23*, *24*). Reaction mixtures were prepared in 100 mM Tris-HCl buffer, pH 8.0, containing 1 μM heme, 500 μM phenol, 300 μM epinephrine, sufficient amounts of COX-1 or -2 to generate 150 ng of PGE_2/mL, and various concentrations of test samples. The reaction was initiated by the addition of arachidonic acid (final concentration 10 μM) and incubated for 10 min at room temperature (final volume 200 μL). Then, the reaction was terminated by adding 20 μL of the reaction mixture to 180 μL of 27.8 μM indomethacin, and PGE_2 was quantitated by an enzyme-linked immunosorbent assay (ELISA) method. Samples were diluted to the desired concentration with 100 mM potassium phosphate buffer (pH 7.4) containing 2.34% NaCl, 0.1% bovine serum albumin, 0.01% sodium azide, and 0.9 mM Na_4EDTA. Following transfer to a 96-well plate (Nunc-Immuno Plate Maxisorp, Fisher) coated with a goat anti-mouse IgG (Jackson Immuno Research Laboratories, West Grove, PA), the tracer (PGE_2-acetylcholinesterase; Cayman Chemical, Ann Arbor, MI) and primary antibody (mouse antiPGE_2; Monsanto, St. Louis, MO) were added. Plates were then incubated at room temperature overnight, reaction mixtures were removed, and wells were washed with a solution of 10 mM potassium phosphate buffer (pH 7.4) containing 0.01% sodium azide and 0.05% Tween 20. Ellman's reagent (200 μL) was added to each well, and the plate was incubated at 37 °C for 3−5 h, until the control wells yielded OD = 0.5−1.0 at 412 nm. A standard curve with PGE_2 (Cayman Chemical) was generated from the same plate, which was used to quantify the PGE_2 levels produced in the presence of test samples. Results were expressed as a percentage relative to a control (solvent-treated samples). All determinations were performed in duplicate, and values generally agreed within 10%. Dose-response curves were generated for the calculation of IC_{50} values.

Extraction and Isolation. The dried and milled plant material (3 kg) was extracted with 5 L of MeOH three times by maceration. The extracts were combined and concentrated in vacuo at 40 °C. The concentrated extract was suspended in 90% MeOH and then partitioned with 1.5 L of petroleum ether three times to afford a petroleum ether-soluble syrup on drying. Next, the aqueous methanol extract was concentrated and suspended in H_2O (1.5 L) and partitioned again with 1.5 L of EtOAc three times to give an EtOAc-soluble extract and an aqueous residue. The EtOAc-soluble extract (11.3 g) was chromatographed over silica gel as stationary phase with a petroleum ether−EtOAc gradient (from 3:1 to 0:1 v/v; final stage, MeOH 100%) as mobile phase to afford 12 pooled fractions (F004−F015). Of these, fractions F004, F005, F009, and F010 showed the most potent COX-2 inhibitory activity (94%, 78%, 79%, and 64% inhibition at 10 μg/mL, respectively). Fractions F004 and F005 [eluted with petroleum ether−EtOAc (3:1 v/v), 350 mg] were combined and then chromatographed over silica gel (petroleum ether−EtOAc gradient from 9:1 to 7:3 v/v), resulting in 12 subfractions (F016−F027). Asparenyn (**4**, 1.4 mg, 0.000047%) was isolated from fraction F018 by preparative TLC (10% EtOAc/petroleum ether as developing solvent; R_f = 0.68). Additional chromatographic separation of fraction F025 was carried out by preparative TLC (4% acetone/$CHCl_3$ as developing solvent) to afford the new compound **3** (1.3 mg, 0.000043%, R_f = 0.62) and asparenyol (**5**, 15.6 mg, 0.00052%, R_f = 0.47). Linoleic acid (4.8 mg, 0.00016%; R_f = 0.65) was purified from fraction F026 by preparative TLC with EtOAc−ether−acetic acid (8:7:1) as the developing solvent.

Fraction F009 [eluted with petroleum ether−EtOAc (1:1 v/v), 350 mg] was chromatographed over silica gel ($CHCl_3$−acetone gradient from 19:1 to 9:1 v/v), resulting in eight subfractions (F028−F035). Compounds **1** (2.0 mg, 0.000067%) and **2** (0.5 mg, 0.000017%) were obtained from F029 by preparative TLC with petroleum ether−acetone (3:2 v/v) as the developing solvent (R_f = 0.87 and 0.78, respectively). Purification of fraction F032 was carried out by preparative TLC with *n*-hexanes−EtOAc (4.5:5.5 v/v, repeated twice) as the developing solvent to give blumenol C (1.3 mg, 0.000043%; R_f = 0.65). (±)-1-Monopalmitin (**6**, 15.2 mg, 0.00051%) was purified by recrystallization (from petroleum ether/EtOAc) from fraction F034.

The final active fraction, F010 [eluted with petroleum ether−EtOAc (2:3 v/v), 1130 mg], was purified over a silica gel column with $CHCl_3$−acetone (19:1 → 0:1 v/v) used as the solvent system, yielding in turn the known compounds (±)-epipinoresinol (7.2 mg, 0.00024%), ferulic acid (**7**, 12.1 mg, 0.0004%), 1,3-*O*-di-*p*-coumaroylglycerol (**8**, 19.2 mg, 0.00064%), 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (**9**, 81.5 mg, 0.0027%), 1,3-*O*-diferuloylglycerol (36.2 mg, 0.0012%), and 1,2-*O*-diferuloylglycerol (6.2 mg, 0.00021%).

Table 1. NMR Data for Compounds **1** and **2** (in $CDCl_3$)[a]

	1		**2**	
position	δ_C	δ_H multiplicity (*J*, Hz)	δ_C	δ_H multiplicity (*J*, Hz)
2	38.9 t	3.88 dd (10.4, 7.1) 3.62 t (10.3)	38.6 t	4.34 dd (11.1, 4.9) 3.54 dd (11.1, 4.9)
3	48.3 d	4.20 m	49.6 d	3.82 m
4	64.6 t	3.70 dd (12.6, 4.6) 3.34 t (12.3)	65.6 t	4.06 dd (13.0, 4.1) 3.46 dd (13.0, 7.4)
*C*OOMe	171.0 s		170.2 s	
COO*Me*	53.0 q	3.80 s	52.9 q	3.77 s

[a] Experiments were run at 500 MHz for ^1H and 125 MHz for ^{13}C. Assignments are based on DEPT, COSY, HMQC, and HMBC experiments.

Asparagusic acid anti-*S*-oxide methyl ester (1) was obtained as a colorless solid: $[\alpha]_D^{20}$ −0.2° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 237 (3.57), 252 (3.56) nm; IR ν_{max} (NaCl) 2926, 2851, 1734, 1436, 1337, 1243, 1215, 1168, 1073 cm^{-1}; ESIMS *m*/*z* 181 ($[M + H]^+$); HRESIMS *m*/*z* 202.9819 ($[M + Na]^+$, calcd 202.9813 for $C_5H_8O_3NaS_2$); ^1H NMR and ^{13}C NMR data, see **Table 1**.

Asparagusic acid syn-*S*-oxide methyl ester (2) was obtained as a colorless solid: $[\alpha]_D^{20}$ 0.3° (*c* 0.05, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 236 (3.60), 257 (3.54) nm; IR ν_{max} (NaCl) 2924, 2853, 1738, 1455, 1372, 1247, 1201, 1168, 1025 cm^{-1}; ESIMS *m*/*z* 181 ($[M + H]^+$); HRESIMS *m*/*z* 202.9820 ($[M + Na]^+$, calcd 202.9813 for $C_5H_8O_3NaS_2$); ^1H NMR and ^{13}C NMR, see **Table 1**.

2-Hydroxyasparenyn (3) was obtained as a yellowish powder: mp 132−134 °C; UV (EtOH) λ_{max} (log ϵ) 208 (4.47), 285 (4.29), 301 (4.16) nm; IR ν_{max} (NaCl) 3450, 2924, 2847, 2198, 1508, 1456, 1230, 1032, 1019, 824 cm^{-1}; EIMS *m*/*z* (rel int) 310 ($[M]^+$, 3), 187 (100), 172 (5), 115 (7); HREIMS *m*/*z* 310.1225 (M^+, $C_{19}H_{18}O_4$, calcd 310.1205); ^1H NMR and ^{13}C NMR, see **Table 2**.

Asparenyn (4) was obtained as a yellowish powder: mp 123−125 °C; EIMS *m*/*z* (rel int.) 294 ($[M]^+$, 2), 171 (100), 128 (11); ^1H and ^{13}C NMR data were in agreement with the reported literature values (*16*).

Table 2. NMR Data for Compounds **3**–**5** (in $CDCl_3$)[a]

position	**3** δ_C	**3** δ_H multiplicity (*J*, Hz)	**4** δ_C	**5** δ_C
1	147.0 s		159.6	155.8
2	147.3 s		113.9	115.5
3	117.5 d	7.00 d (1.8)	133.0	133.2
4	125.6 s		115.2	115.5
5	124.3 d	6.96 dd (8.3, 1.8)	133.0	133.2
6	110.5 d	6.77 d (8.3)	113.9	115.4
1′	90.6 s		90.6	90.5
2′	85.7 s		85.9	85.8
3′	112.5 d	6.01 br dt (15.9)	112.5	112.6
4′	137.3 d	6.31 dt (15.9, 5.3)	137.1	137.1
5′	68.5 t	4.57 dd (5.3, 1.6)	68.5	68.6
1″	152.5 s		152.5	152.5
2″/6″	115.9 d	6.82–6.87 m	115.8	115.9
3″/5″	114.7 d	6.82–6.87 m	114.6	114.7
4″	154.1 s		154.1	154.1
OMe-1	56.0 q	3.90 s	55.3	
OMe-4″	55.8 q	3.77 s	55.7	55.8
OH-2		5.57 br s		

[a] Experiments were run at 500 MHz for ^1H and 125 MHz for ^{13}C. Assignments are based on DEPT, COSY, HMQC, HMBC, and NOESY experiments.

Asparenyol (5) was obtained as a yellowish powder: mp 138−140 °C; EIMS *m*/*z* (rel int.) 280 (M^+, 5), 157 (100), 131 (5), 128 (9); ^1H and ^{13}C NMR data were in agreement with the reported literature values (*16*).

(±)-1-Monopalmitin (6) was obtained as a white powder: mp 67−69 °C; $[\alpha]_D^{20}$ −0.1° (*c* 1.0, $CHCl_3$); LRESIMS *m*/*z* 331 ($[M + H]^+$); ^1H and ^{13}C NMR data were in agreement with the reported literature values (*25*).

Ferulic acid (7) was obtained as yellowish needles: mp 167−169 °C; EIMS *m*/*z* (rel int.) 194 ($[M]^+$, 100), 179 (15), 133 (14), 77 (7); ^1H and ^{13}C NMR data were in agreement with the reported literature values (*26*).

1,3-*O*-Di-*p*-coumaroylglycerol (8) was obtained as a white powder: mp 193−195 °C; $[\alpha]_D^{20}$ 0.1° (*c* 1.0, MeOH); EIMS *m*/*z* (rel int.) 384 ($[M]^+$, 3), 164 (6), 147 (100), 119 (8), 91 (5); ^1H and ^{13}C NMR data were in agreement with the reported literature values (*27*).

1-*O*-Feruloyl-3-*O*-*p*-coumaroylglycerol (9) was obtained as a pale yellow oil: $[\alpha]_D^{20}$ −0.2° (*c* 1.0, MeOH); EIMS *m*/*z* (rel int.) 414 ($[M]^+$, 17), 268 (4), 194 (11), 177 (48), 164 (7), 147 (100), 119 (9), 91 (7); ^1H and ^{13}C NMR data were in agreement with the reported literature values (*28*).

Linoleic acid was obtained as a colorless oil: LREIMS *m*/*z* (rel int.) 280 ($[M]^+$, 45), 182 (9), 136 (15), 123 (16), 96 (51), 82 (82), 67 (100); ^1H NMR data were in agreement with the reported literature values (*29*).

RESULTS AND DISCUSSION

Purification of the ethyl acetate-soluble fraction of the methanol extract of the aerial parts of *Asparagus officinalis* L., via a bioassay based on the inhibition of COX-2 to monitor chromatographic fractionation, led to the isolation of two new natural sulfur-containing compounds (see **Figure 1**), asparagusic acid anti-*S*-oxide methyl ester (**1**) and asparagusic acid syn-*S*-oxide methyl ester (**2**), and a new acetylenic compound, 2-hydroxyasparenyn {2-hydroxy-1-methoxy-4-[5-(4-methoxyphenoxy)-3-penten-1-ynyl]-benzene} (**3**), as well as 11 known compounds, asparenyn (**4**) (*16*), asparenyol (**5**) (*16*), (±)-1-monopalmitin (**6**) (*25*), ferulic acid (**7**) (*26*), 1,3-*O*-di-*p*-coumaroylglycerol (**8**) (*27*), 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (**9**) (*28*), blumenol C (*30*), (±)-epipinoresinol (*31*), linoleic acid (*29*), 1,3-*O*-diferuloylglycerol (*28*), and 1,2-*O*-diferuloylglycerol (*32*). The structures of the known compounds were identified by physical (mp, $[\alpha]_D$) and spectroscopic data (^1H NMR, ^{13}C NMR, 2D NMR, and MS) measurement and by comparison with published values. Compounds **6**, **8**, **9**, (±)-epipinoresinol, 1,3-*O*-diferuloylglycerol, and 1,2-*O*-diferuloylglycerol have not been reported from any species in the genus *Asparagus* previously.

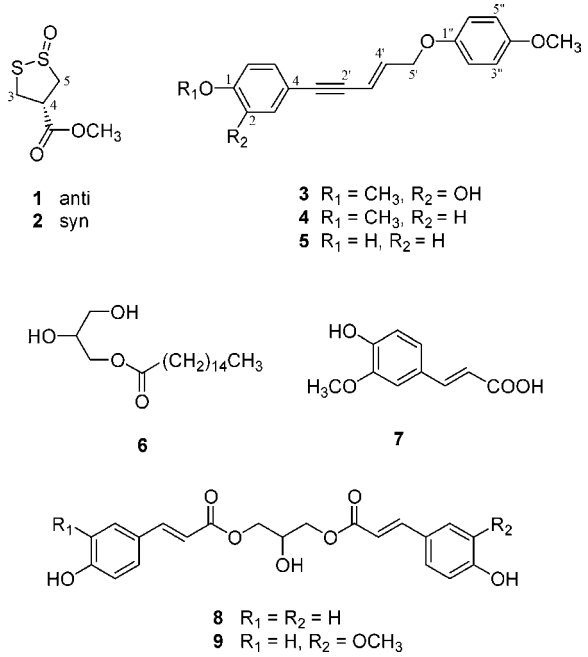

Figure 1. Structures of compounds **1**–**9** isolated from *Asparagus officinalis*.

Compound **1** was obtained as colorless crystals and gave a molecular ion $[M + Na]^+$ at *m*/*z* 202.9819 by HRESIMS, consistent with an elemental formula of $C_5H_8O_3NaS_2$. It exhibited an IR maximum at 1734 cm^{-1}, suggesting the presence of a carbonyl group. The ^1H NMR spectrum (**Table 1**) showed signals for both an ABX [δ 4.20 (1H, m, H-4), δ 3.88 (1H, dd, J_{AB} = 10.4 Hz, J_{AX} = 7.1 Hz, H-3a), and δ 3.62 (1H, t, J_{AB} = 10.3 Hz, H-3b)] and an A′B′X pattern [δ 3.70 (1H, dd, $J_{A'B'}$ = 12.6 Hz, $J_{A'X}$ = 4.6 Hz, H-5a), δ 3.34 (1H, t, $J_{A'B'}$ = 12.3 Hz, H-5b)]. One additional singlet proton signal at δ 3.80 (3H, OCH_3) was also observed in the ^1H NMR spectrum. The ^{13}C NMR spectrum and a DEPT experiment (**Table 1**) on **1** showed the presence of signals for two methylene groups (δ 64.6 and δ 38.9) bearing sulfur atoms, as well as one methine group (δ 48.3), one methoxyl group (δ 53.0), and one quaternary carbonyl carbon (δ 171.0). All of these data were in accordance with the assignment of **1** as a sulfur-containing compound, asparagusic acid *S*-oxide methyl ester, and its structure was supported by 2D NMR experiments [correlation (COSY), nuclear Overhauser effect (NOESY), heteronuclear multiple quantum coherence (HMQC), and HMBC spectroscopy]. Compound **1**, previously reported as a synthetic compound, exhibited ^1H NMR data similar to literature values (*18*). The relative stereochemistry of the *S*-oxide in **1** with respect to the $COOCH_3$ group was assigned as anti on the basis of chemical shift comparison with synthetic **1**. Thus, compound **1** was assigned as the new naturally occurring sulfur-containing compound asparagusic acid anti-*S*-oxide methyl ester (*trans*-1,2-dithiolane-4-carboxylic acid-1-oxide methyl ester).

Compound **2** was obtained as colorless crystals and gave a molecular ion $[M + Na]^+$ at *m*/*z* 202.9820 by HRESIMS, consistent with an elemental formula of $C_5H_8O_3NaS_2$. It also exhibited an IR maximum at 1738 cm^{-1}, suggesting the presence of a carbonyl group. Comparison of the ^1H and ^{13}C NMR spectral data (**Table 1**) obtained for **2** with those of **1** indicated that **2** is a stereoisomer of **1**. Compound **2**, previously also reported as a synthetic compound, exhibited ^1H NMR data

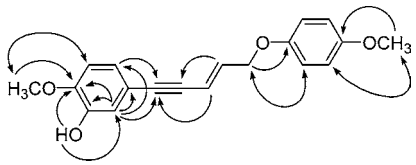

Figure 2. Selected correlations observed in the HMBC (→) and NOESY (↔) spectra of **3**.

similar to literature values (*18*). The relative stereochemistry of the *S*-oxide in **2** with respect to the $COOCH_3$ group was assigned as syn on the basis of comparison of its chemical shift with that of synthetic **2**. Thus, compound **2** was also assigned as the new naturally occurring sulfur-containing compound asparagusic acid syn-*S*-oxide methyl ester (*cis*-1,2-dithiolane-4-carboxylic acid-1-oxide methyl ester).

Compound **3** was obtained as a yellowish powder and gave a protonated molecular ion $[M]^+$ at *m*/*z* 310.1225 by HREIMS, consistent with an elemental formula of $C_{19}H_{18}O_4$. It exhibited an IR absorption at 2198 cm^{-1}, suggesting the presence of an acetylenic group (*16*). Assignments of the resonances of all of the hydrogen and carbon atoms in the molecule (**Table 2**) were made by application of one- and two-dimensional (1D and 2D) homo- and heteronuclear NMR experiments (^1H NMR, ^{13}C NMR, DEPT, COSY, NOESY, HMQC, and HMBC). Thus, the ^1H NMR spectrum of **3** showed resonances for a 1,3,4-trisubstituted aromatic ring, indicated by a typical ABX system at δ 6.77 (1H, d, $J = 8.3$ Hz, H-6), δ 6.96 (1H, dd, $J = 8.3$ and 1.8 Hz, H-5), and δ 7.00 (1H, d, $J = 1.8$ Hz, H-3), and for a *para*-disubstituted aromatic ring at δ 6.82−6.87 (4H, m, H-2″, 3″, 5″, and 6″). Resonances for an AMX_2 system at δ 4.57 (2H, dd, $J = 5.3$ and 1.6 Hz, H-5′), δ 6.01 (1H, br dt, $J = 15.9$ Hz, H-3′), and δ 6.31 (1H, dt, $J = 15.9$ and 5.3 Hz, H-4′) were also observed, suggesting the presence of a *trans*-propenylene group attached to an oxygen atom by its methylene group (*16*). The residual signals in the ^1H NMR spectrum were three singlets at δ 3.77 (3H, OMe-4″), δ 3.90 (3H, OMe-1), and δ 5.57 (1H, OH-2). The ^{13}C NMR and DEPT spectra of **3** (**Table 1**) showed seven quaternary carbons including two acetylenic carbons (δ 85.7 and 90.6) as well as nine tertiary carbons, one secondary carbon (δ 68.5), and two methoxy groups (δ 55.8 and 56.0).

From the HMBC spectrum (**Figure 2**), it was inferred that compound **3** has an acetylenic group and methylene group linked to the benzylic group and benzoyl group, respectively. All of the positions of the methoxy and hydroxyl groups were determined unambiguously by HMBC and NOESY NMR experiments (**Figure 2**). Comparison of the above data with both the literature (*16*) and the present work (**Table 2**) indicated that the structure of **3** is closely related to those of the acetylenic compounds, asparenyn (**4**) and asparenyol (**5**), which were also isolated in the present investigation, except for the 1,3,4-trisubstituted aromatic ring part. Therefore, the structure of this new acetylenic compound, 2-hydroxyasparenyn (**3**), was elucidated as 3′,4′-*trans*-2-hydroxy-1-methoxy-4-[5-(4-methoxyphenoxy)-3-penten-1-ynyl]-benzene.

All the isolates obtained were evaluated for their COX-1 and -2 inhibitory activity (**Table 3**). Compounds **1**−**5** were not active. (±)-1-Hexadecanoylglycerol (**6**) showed very weak activity against COX-1 and moderate activity against COX-2. Ferulic acid (**7**), 1,3-*O*-di-*p*-coumaroylglycerol (**8**), and 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (**9**) exhibited weak activity against COX-1, while linoleic acid showed significant activity against COX-2 and moderate activity against COX-1. The inhibitory effects against both COX-1 and -2 of some naturally occurring and modified fatty acids have been reported recently (*29, 33, 34*). Thus, the present in vitro test data on linoleic acid are consistent with recent results on its inhibitory activity against COX (*29, 33, 34*). Since the present phytochemical study on the aerial parts of *A. officinalis* was carried out by activity-guided chromatography by use of COX-2 to monitor fractionation, it may be expected that the compounds included in the body of **Table 3** are responsible for the COX-2 inhibitory activity originally present in the EtOAc-soluble crude extract.

Table 3. Inhibitory Activities of Compounds from *A. officinalis* against Cyclooxygenase-1 and -2[a]

	COX-1		COX-2	
compound	% inhib at 100 μg/mL	IC_{50} (μg/mL)	% inhib at 100 μg/mL	IC_{50} (μg/mL)
6	50	ND[b]	67	45.4
7	55	33.7	1	ND
8	62	ND	30	ND
9	51	ND	0	ND
linoleic acid	100	14.6	100	0.53
trans-resveratrol[c]	100	0.25	100	0.30

[a] The new natural products **1** and **2**, the new compound **3**, and the known compounds **4**, **5**, blumenol C, (±)-epipinoresinol, 1,3-*O*-diferuloylglycerol, and 1,2-*O*-diferuloylglycerol were inactive (IC_{50} >100 μg/mL) in the COX-1 and COX-2 assays. [b] Not determined. [c] *trans*-Resveratrol was used as a positive control.

ACKNOWLEDGMENT

We thank Dr. K. Fagerquist, Mass Spectrometry Facility, Department of Chemistry, University of Minnesota, Minneapolis, MN, and Dr. J. A. (Art) Anderson, Research Resources Center (RRC), University of Illinois at Chicago (UIC), for the mass spectral data. We are grateful to the RRC, UIC, for providing spectroscopic equipment and to Dr. R. A. Kleps of the RRC, UIC, for facilitating the running of the 500 MHz NMR instrument.

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Received for review July 22, 2003. Revised manuscript received January 23, 2004. Accepted January 25, 2004. This research was supported by program project P01 CA48112, funded by the National Cancer Institute, NIH, Bethesda, MD.

JF0305229