# Bioactive Constituents from the Twigs of Asimina parviflora

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*J. Nat. Prod.*, **1992**, 55 (10), 1462-1467• DOI: 10.1021/np50088a012 • Publication Date (Web): 01 July 2004

*Journal of Natural Products*
*Vol. 55, No. 10, pp. 1462-1467 October 1992*

# BIOACTIVE CONSTITUENTS FROM THE TWIGS OF *ASIMINA PARVIFLORA*

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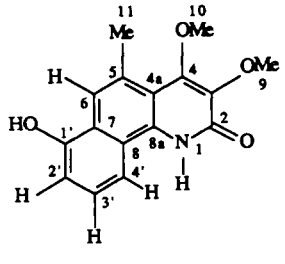
ABSTRACT.—The EtOH extract of *Asimina parviflora* (Annonaceae), when subjected to activity-directed fractionation using lethality to brine shrimp, led to the isolation and identification of five bioactive compounds: asimicilone [**1**], which is a new 2-quinolone alkaloid, 6-cis-docosenamide [**2**], which is a new amide of a long hydrocarbon chain fatty acid, and three known compounds, asimicin, (+)-syringaresinol, and β-sitosterol-β-D-glucopyranoside. The structure determination of the new alkaloid was performed by extensive nmr analyses, including HMQC and HMBC. Selective cytotoxic activities of these compounds in three human solid tumor cell lines are also reported.

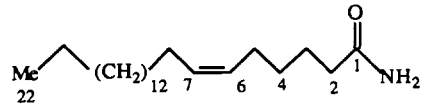
*Asimina parviflora* (Michx.) Dunal., dwarf paw paw, is a small shrub native to the southeastern United States (1). In our search for new potential anticancer and pesticidal constituents from the Annonaceae, we investigated the twigs for bioactive compounds, using the brine shrimp toxicity (BST) bioassay (2,3) to direct the fractionation. No previous phytochemical work has been reported with this species. Five bioactive compounds, including two new compounds, were isolated and identified. In this paper we report the spectral analysis and characterization of the two new compounds and cytotoxic activities of all the compounds in three human solid tumor cell lines.

## RESULTS AND DISCUSSION

Dried and pulverized twigs of *A. parviflora* were successively extracted and partitioned according to a standard scheme (see Experimental). The most bioactive fraction, the 90% MeOH partition residue [BST $LC_{50}$ 0.43 (0.25–0.73) μg/ml], was subjected to repeated cc and subsequent centrifugal radial chromatography (Chromatotron) separations, directed by the BST bioassay at each step, to yield the five bioactive compounds. Compound **1** is a new 2-quinolone alkaloid, whereas compound **2** is a new amide of a long hydrocarbon chain fatty acid. The third compound, asimicin, is a very potent acetogenin previously reported from the paw paw, *Asimina triloba* (L.) Dunal. (4), and the fourth and fifth compounds are the known (+)-syringaresinol (a lignan) and β-sitosterol-β-D-glucopyranoside. The structures of all these compounds were identified by spectral analysis.




**1**




**2**

The hrms of compound **1** showed the molecular ion at *m/z* 285.1004 corresponding to the molecular formula $C_{16}H_{15}NO_4$ (calcd 285.1001). The ir absorptions at 3353 and 3203 $cm^{-1}$, together with those at 1656 and 1649 $cm^{-1}$, indicated the presence of an amide group. The uv maxima at 282, 293, 331, and 348 nm suggested the presence of an extended aromatic system. The $^1$H-nmr spectrum of **1** showed the presence of one aromatic Me group (δ 2.77), two aromatic MeO groups (δ 3.93 and 3.99), one isolated aromatic proton (δ 6.46), three mutually coupled aromatic protons (δ 6.97, 7.34, and 7.67), and two one-proton singlets further downfield at δ 8.89 and 9.45, for NH and OH protons. The $^{13}$C-nmr spectrum also indicated the presence of one Me and two MeO (aromatic) carbons (δ 23.33, 63.20, and 63.81, respectively), three oxygen-bearing aromatic carbons (δ 148.81, 152.38, and 152.44 ppm), a carbonyl carbon (δ 161.89 ppm), and nine other aromatic carbons. These uv, ir, and $^1$H-nmr data suggested that the structure of this compound is very similar to that of geovanine, an azanthracene alkaloid (5), except for an OH group instead of a MeO group at one position. To confirm the structure, 2D nmr experiments, HMQC (heteronuclear multiple quantum correlation), and HMBC (heteronuclear multiple bond correlation), were performed. The results proved that **1** is a new 2-quinolone and not an azanthracene alkaloid.

The singlet at δ 6.46 showed a direct correlation to the carbon at δ 123.29 (C-6). This proton, H-6, showed three-bond correlations to C-1′ (δ 152.44), C-11 (δ 23.33, Me carbon), and C-4a (δ 114.33) as well as a four-bond correlation to C-2′ (δ 114.65). The most deshielded aromatic proton resonated at δ 7.67 as a doublet and indicated a direct correlation to C-2′ (δ 114.65) and three-bond correlations to C-7 (δ 118.45) and C-4′ (δ 111.91). This proton also showed a two-bond correlation with C-1′. The doublet of doublets at δ 7.34 (H-3′) showed a direct correlation to C-3′ (δ 126.02) and three-bond correlations to C-8 (δ 125.82) and C-1′, as well as a four-bond correlation to C-8a (δ 127.35). The doublet at δ 6.97 (H-4′) showed a direct correlation to C-4′ and three-bond correlations to C-7 and C-2′. The NH proton (δ 8.89) showed a three-bond correlation to C-3 (δ 152.38 ppm) as well as a four-bond correlation to C-4′. The positions of the MeO groups were also easily demonstrated from these spectra. The protons of the MeO group resonating at δ 3.94 (C-9, δ 63.20) showed a three-bond correlation to C-3. The protons of the other MeO group, which resonated at δ 3.99 (C-10, δ 63.81), showed a three-bond correlation to C-4 (δ 148.81) and a five-bond correlation to C-5 (δ 135.41). The Me protons showed a four-bond correlation to C-4. These HMBC and HMQC correlations are summarized in Figure 1 and Table 1 and confirmed the structure of **1** as 7,8-(1′-hydroxybenzo)-3,4-dimethoxy-5-methyl-2-quinolone, a novel quinolone alkaloid which we have named asimicilone.

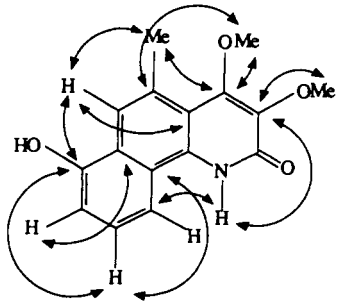



FIGURE 1. Proton-carbon multiple-bond correlations established from HMBC spectra of asimicilone [**1**].

TABLE 1. $^1$H- and $^{13}$C-nmr Chemical Shift Assignments and Observed Proton-Carbon Multiple Bond Correlations[a] (via HMQC and HMBC) for Asimicilone [**1**].

| Position | δ $^1$H[b] $CDCl_3$ | δ $^{13}$C $CDCl_3$ | Three-bond Correlations | Four-bond Correlations |
|---|---|---|---|---|
| 1 | 8.89 (br s) | | | |
| 2 | | 161.89 | | |
| 3 | | 152.38 | H-1, OMe-9 | |
| 4 | | 148.81 | OMe-10 | Me-11 |
| 4a | | 114.33 | H-6 | |
| 5 | | 135.41[c] | | |
| 6 | 6.46 (s) | 123.29 | Me-11 | |
| 7 | | 118.45 | H-2′, H-4′ | |
| 8 | | 125.82 | H-3′ | |
| 8a | | 127.35 | | H-3′ |
| 1′ | | 152.44[d] | H-3′ | H-4′ |
| 2′ | 7.67 (d, $J$ = 8.4)[e] | 114.65 | H-4′ | H-6 |
| 3′ | 7.34 (dd, $J$ = 7.5) | 126.02 | | |
| 4′ | 6.97 (d, $J$ = 7.47) | 111.91 | H-2′ | H-1 |
| 9-OMe | 3.94 (s) | 63.20 | | |
| 10-OMe | 3.99 (s) | 63.81 | | |
| 11-Me | 2.77 (s) | 23.33 | H-6 | |
| OH | 9.45 (s) | | | |

[a]Detected at three different $J$ values (5, 10 and 15 Hz).
[b]Determined by $^1$H and HMQC spectra.
[c]Five-bond correlation was observed with OMe-10.
[d]Two-bond correlation was observed with H-2′.
[e]$J$ values are in Hz.

The hrms of compound **2** showed the molecular ion at $m/z$ 337.3339 corresponding to the molecular formula $C_{22}H_{43}NO$ (calcd 337.3345). The ir spectrum of this compound showed absorptions at 3359 $cm^{-1}$, together with those at 1653 and 1632 $cm^{-1}$, indicating the presence of an amide. The 1D and 2D $^1$H-nmr showed a two-proton multiplet at δ 5.34 which was assignable to two olefinic protons at the C-6 and C-7 positions. This signal was coupled to a four-proton multiplet at δ 2.01, which corresponded to the protons adjacent to the olefinic protons at C-5 and C-8. The two-proton triplet at δ 2.22 ($J$ = 7.63 Hz) was indicative of H-2, adjacent to the carbonyl group. Furthermore, this H-2 signal was coupled to a two-proton multiplet at δ 1.6 that was assignable to H-3. This multiplet at δ 1.6 was also coupled to a multiplet at δ 1.4 which was assigned to H-5. The multiplet at δ 2.01 was also coupled to a broad singlet at δ 1.26, probably due to the coupling between H-8 and H-9, and this broad singlet at δ 1.26 was also coupled to the triplet at δ 0.87, due to the coupling between H-21 and H-22. All of these couplings could be clearly seen in the off-diagonal element of the 2D COSY spectrum of this compound. These data suggested that **2** is simply an amide of a 22-carbon straight chain fatty acid with a double bond at the 6 position. This compound, named 6-cis-docosenamide, is also a new natural compound.

The third compound, asimicin, was easily identified by comparisons of tlc and spectral data (4,6) of the standard compound (isolated from *A. triloba*) available in our laboratory. The lignan, (+)-syringaresinol, was identified by comparing the spectral data with previously reported data (8,9). The ubiquitous β-sitosterol-β-D-glucopyranoside was identified by its solubility, $R_f$ value comparisons with a standard sample, and ms data.

Biological activities of the five isolated compounds are reported in Table 2. Among

TABLE 2. Bioactivities[a] of the Isolated Compounds.

| Compound | BST $LC_{50}$ (μg/ml) | A-549 $LC_{50}$ (μg/ml) | MCF-7 $LC_{50}$ (μg/ml) | HT-29 $LC_{50}$ (μg/ml) |
|---|---|---|---|---|
| Asimicilone [1] | 2.15 (0.2–4.3)[b] | 2.13 | 7.22 | 3.25 |
| 6-Docosenamide [2] | 2.45 (0.6–5.2) | 3.16 | 2.91 | $<10^{-2}$ |
| Asimicin[c] | 0.025 (0.015–0.042) | $8.43 \times 10^{-4}$ | $8.52 \times 10^{-1}$ | $<10^{-15}$ |
| (+)-Syringaresinol | 0.65 (0.2–1.6) | 13.92 | 30.31 | $2.27 \times 10^{-1}$ |
| β-Sitosterol glucoside | 0.14 (0.02–2.1) | 4.73 | 44.39 | $4.44 \times 10^{-1}$ |
| Adriamycin | 0.088 (0.01–0.6)[d] | $5.04 \times 10^{-4}$ | $2.31 \times 10^{-1}$ | $4.45 \times 10^{-3}$ |

[a]BST = brine shrimp toxicity bioassay; A-549 lung carcinoma cell line; MCF-7 breast carcinoma cell line; HT-29 colon cancer cell line.

[b]Numbers in parentheses show 95% confidence intervals.

[c]Data from Zhao *et al.* (6).

[d]Data from Anderson *et al.* (13).

these, asimicin is by far the most bioactive compound isolated from *A. parviflora* to date in the BST assay as well as in the three human solid tumor cell lines (colon cancer HT-29, lung carcinoma A-549, and breast carcinoma MCF-7) (9–11). The presence of asimicin in *A. parviflora* suggests that this species could, like *A. triloba*, serve as a source for the pesticidal Annonaceous acetogenins should their commercial development become feasible (12). Asimicilone [**1**] showed borderline, but significant, cytotoxicities, and 6-cis-docosenamide [**2**] showed significant cytotoxicities with relative potency against the HT-29 (colon) cell line.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were measured in capillaries on a Mel-temp apparatus and were uncorrected. Uv spectra were obtained on a Beckman DU-7 spectrophotometer. Ir spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer. Low resolution cims and eims data were collected on a Finnigan 4000 mass spectrometer. Exact masses for hrms measurements were obtained on a Kratos MS50 spectrometer through peak matching. $^1$H- and $^{13}$C-nmr spectra were recorded on a Varian VXR-500S ($^1$H at 500 MHz and $^{13}$C at 125.75 MHz) spectrometer. COSY, HMQC, and HMBC 2D spectra were obtained on the Varian VXR-500S using the Varian pulse sequences.

PLANT MATERIAL.—Twigs of *A. parviflora* were collected and identified by one of us (DRE) near Tifton, Georgia, during August 1990. A voucher specimen is preserved in the Herbarium of the Department of Medicinal Chemistry and Pharmacognosy, Purdue University.

BIOASSAYS.—The extracts, fractions, and pure compounds were routinely tested for lethality to brine shrimp larvae (2,3). In vitro cytotoxicity tests (7 days) to determine $ED_{50}$ values against human tumor cell lines, were carried out at the Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma) (9), MCF-7 (human breast carcinoma) (10), and HT-29 (human colon carcinoma) (11) with adriamycin as a positive control; $ED_{50}$ values $< 4$ μg/ml for pure compounds are considered significant.

EXTRACTION AND ISOLATION.—The twigs (<1 cm diameter) were dried in an oven (<40°) and pulverized; a portion (3.5 kg) was exhaustively extracted by percolation with 95% EtOH. The EtOH was evaporated under vacuum to yield 350 g of residue. This residue was partitioned between $H_2O$ and $CH_2Cl_2$ (1:1), and the solvents of these two fractions were removed under vacuum to obtain the $H_2O$-soluble fraction (230 g) and the $CH_2Cl_2$-soluble fraction (100 g). The $CH_2Cl_2$ residue was further partitioned between hexane and 90% aqueous MeOH to yield the residue of the 90%-aqueous-MeOH-soluble fraction (57 g) and the hexane-soluble fraction (38 g). The bioactivities were concentrated into the 90% MeOH residue (BST $LC_{50}$ 0.4262 μg/ml). This residue (57 g) was adsorbed to 30 g of Celite (uncalcined, Johns-Manville) and subjected to vacuum liquid column chromatography over Si gel (230–400 mesh) using 400-ml portions of each of following solvent mixtures: hexane-$CH_2Cl_2$ (3:1 and 1:1), $CH_2Cl_2$, $CH_2Cl_2$ and MeOH (1%, 2%, 5%, 10%, 25%, 50%), and MeOH. Fractions of about 400 ml were collected. Fraction 5 (BST $LC_{50}$ 4.02 μg/ml) was repeatedly chromatographed over Si gel (230–400 mesh) columns and Si gel

Chromatotron plates using $CH_2Cl_2$-MeOH (9:1 and 8:2) to isolate compound **1** as yellow needles (in MeOH, 8 mg) and (+)-syringaresinol, as colorless needles (in EtOH, 15 mg). Repeated chromatography of fraction 3 (BST $LC_{50}$ 0.377 μg/ml) in a similar manner gave asimicin (8 mg), and β-sitosterol-β-D-glucopyranoside (22 mg) as white powders, while fraction 8 (BST $LC_{50}$ 7.6 μg/ml) gave compound **2** as a whitish wax (10 mg).

*Asimicilone* [**1**].—Compound **1** started to decompose at 151°, melted at 191°; uv (EtOH) λ max (log ε) 242 (4.7), 282 (4.79), 293 (4.79), 331 (4.190), 348 (4.11) nm; ir (KBr) ν max 3353, 3203 (N-H), 1656, 1649 (carbonyl), 1454, 1364 $cm^{-1}$; cims (isobutane) *m/z* (%) $[MH]^+$ 286 (100); eims *m/z* (%) $[M]^+$ 285 (34), $[M-Me]^+$ 270 (100), $[M-Me-NH]^+$ 255 (17), $[M-Me-NH-CO]^+$ 227 (16), 199 (5), 170 (4), 115 (8), 63 (12), 51 (13); hreims *m/z* 285.1004 for $C_{16}H_{15}NO_4$ (calcd 285.1001); $^1H$ nmr (500 MHz, $CDCl_3$), $^{13}C$ nmr (125 MHz, $CDCl_3$), HMQC, and HMBC see Table 1 and Figure 1.

*6-cis-Docosenamide* [**2**].—Mp 42–44°; ir (film) ν max 3359 (N-H), 1653, 1632 (carbonyl), 1461 $cm^{-1}$; cims (isobutane) *m/z* (%) $[MH]^+$ 338 (100); eims *m/z* (%) $[M]^+$ 337 (3.8), $[M-NH_3]^+$ 320 (2.1), 294 (1), 178 (2), 151 (6), 126 (4.6), 100 (4), 72 (100); hreims *m/z* 337.3339 for $C_{22}H_{43}NO$ (calcd 337.3345); $^1H$ nmr (500 MHz, $CDCl_3$) 0.87 (3H, t, H-22); 1.26 (28H, br s, H-9–H-21), 1.4 (2H, m, H-4), 1.6 (2H, m, H-3), 2.01 (4H, m, H-5, -8), 2.22 (2H, t, $J$ = 7.63 Hz, H-2), 5.34 (2H, m, H-6, -7); $^1H$-$^1H$ COSY (500 MHz, $CDCl_3$).

*Asimicin*.—Mp 67–68° [lit. (4) 68–69°]; ir (film) ν max 3416 (OH), 1753 (carbonyl) $cm^{-1}$; cims (isobutane) *m/z* (%) $[MH]^+$ 623 (16), $[MH-H_2O]^+$ 605 (73), $[MH-CO]^+$ 595 (100), $[MH-2\times H_2O]^+$ 587 (43), $[MH-CO-H_2O]^+$ 577 (45), $[MH-3\times H_2O]^+$ 569 (18), $[MH-CO-2\times H_2O]^+$ 559 (25), $[MH-3\times H_2O-CO]^+$ 541 (11), 311 (37), 293 (7), 241 (5), 171 (4), 141 (6); $^1H$ nmr (500 MHz, $CDCl_3$, in δ) 0.87 (3H, t, $J$ = 7.1 Hz, H-34), 1.26 (16H, br s, H-6–H-13, H-26–H-33), 1.38–1.48 (3H, m, H-5, -14, -25), 1.42 (1H, d, $J$ = 6.8 Hz, H-37), 1.6–2.02 (4H, m, H-17, -18, -21, -22), 2.39 (1H, dddd, $J$ = 15, 8.2, 1.2 Hz, $H_b$-3), 2.54 (1H, dddd, $J$ = 15, 3.5, 1.2 Hz, $H_a$-3), 3.38 (2H, m, H-15, -24), 3.84 (4H, m, H-4, -19, -20, -23), 5.06 (1H, qddd, $J$ = 6.8, 1.2 Hz, H-36), 7.18 (1H, ddd, $J$ = 1.4 Hz, H-35).

*(+)-Syringaresinol*.—Mp 172–174° [lit. (7) 173–175°]; ir (KBr) ν max 3415 (OH), 1608, 1516, 1461, 1330, 1216, 1113, 732 $cm^{-1}$; cims (isobutane) *m/z* (%) $[M]^+$ 418 (41), $[M-MeO]^+$ 387 (2), 235 (5), 210 (13), 193 (25), 181 (100), 167 (90); $^1H$ nmr (500 MHz, $CDCl_3$), 3.08 (2H, ddd, $J$ = 6.8, 4.4, 3.6 Hz, H-1, -5), 3.89 (12H, s, 4 × MeO), 3.90 (2H, dd, $J$ = 9.1, 3.6 Hz, $H_a$-4, -8), 4.26 (2H, dd, $J$ = 9.1, 6.8 Hz, $H_b$-4, -8), 4.72 (2H, d, $J$ = 4.3 Hz, H-2, -6), 5.49 (2H, br s, 2 × OH), 6.56 (4H, s, H-2′, -6′).

β-*Sitosterol*-β-D-*glucopyranoside*.—Mp 285° (dec); co-tlc with standard sample fabms (DTT/DTE) *m/z* $[MH-Glu]^+$ 397.

ACKNOWLEDGMENTS

This research was supported by grant no. CA30909 from the National Cancer Institute, National Institute of Health. We thank the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the cytotoxicity testing.

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*Received 3 April 1992*