Mappain, a New Cytotoxic Prenylated Stilbene from *Macaranga mappa*

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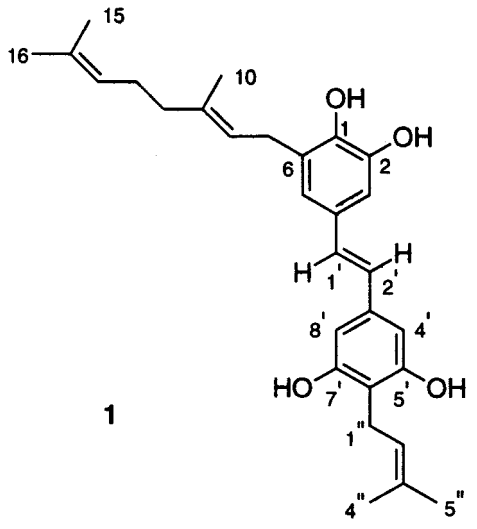
Received May 26, 2000

A new prenylated stilbene, mappain (**1**), was isolated from leaves of *Macaranga mappa* by bioassay-guided fractionation. The structure was established by application of spectroscopic methods. Mappain is cytotoxic but it appears to be a poor substrate for P-glycoprotein-mediated transport because it is equally potent and effective against the drug-sensitive SK-OV-3 and drug-resistant SKVLB-1 ovarian cancer cell lines, exhibiting an IC_{50} value of 1.3 μM in both cases.

In the course of an investigation to identify new anticancer lead compounds from Asian botanical sources, we collected *Macaranga mappa* (L.) Muell. Arg. (Euphorbiaceae) (from the Lyon Arboretum of the University of Hawaii. The lipophilic crude extract of leaves of this species displayed significant cytotoxicity against both drug-resistant (SKVLB-1) and drug-sensitive (SK-OV-3) ovarian cancer cell lines (IC_{50} of 3.5 μg/mL). A literature review indicated that the genus *Macaranga* with its 300 species[1] has not been investigated phytochemically in great detail. A prenylated flavonol[2] and a hexahydroxanthene derivative, vedelianin,[3] were isolated from *M. vedeliana* from New Caledonia. Prenylated flavanones[4] have been reported from *M. pleiostemona*, and chromenoflavones have been isolated form *M. indica*.[5] Consequently, the *M. mappa* extract was selected for bioassay-directed fractionation, which yielded mappain (**1**) as the active constituent. While this work was in progress, two publications from the Laboratory of Drug Discovery Research and Development of the NCI appeared describing the isolation and structure elucidation of the related schweinfurthins[6] and of a prenylated flavone[7] from *M. schweinfurthii*. A total synthesis of schweinfurthin C has been reported recently.[8]



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Freeze-dried leaf material was extracted with CH_2Cl_2−*i*-PrOH, and after evaporation the extract was subjected to a modified Kupchan partition scheme. The $CHCl_3$-soluble fraction was cytotoxic and was subjected to flash chromatography on Si gel. Final purification was achieved by gel filtration over LH-20 followed by flash chromatography on Si gel. Mappain (**1**) was isolated as a beige powder in 0.026% yield of the fresh weight.

The HREIMS of **1** showed a molecular ion of *m*/*z* 448 (found 448.2596), which matched a molecular formula of $C_{29}H_{36}O_4$(Δ 1.8 mmu). The ^{13}C NMR spectrum of **1** displayed 27 signals comprising five methyl, four methylene, eight methine, and 12 quaternary carbon atoms based on analysis of the HMQC spectrum. The difference between the molecular formula deduced by mass spectrometry and the number of carbon atoms visible in the ^{13}C NMR spectrum suggested the presence of an element of symmetry in one part of the structure of **1**. This was confirmed by the analysis of the ^1H NMR spectrum in $CDCl_3$. Thus, the presence of one two-proton singlet at 6.53 ppm and one two-proton singlet for exchangeable hydrogen atoms at 5.13 ppm suggested that this element of symmetry was present in an aromatic ring in the form of a 1.3 bisphenol (ring B). Further analysis of the downfield portion of the ^1H NMR spectrum indicated two additional aromatic signals in the form of a pair of one-proton doublets (J = 1.8 Hz) at 6.78 and 6.93 ppm, respectively, suggesting the meta-orientation of two protons on an aromatic ring, as well as a pair of one-proton doublets (J = 16.1 Hz) at 6.74/6.83 ppm. This latter system was indicative of a trans double bond linking two aromatic rings and suggested that **1** was a substituted stilbene, which was corroborated by the UV/vis spectrum [λ_{max} 330 (log ϵ 3.55) nm, MeOH]. Two of the remaining three substituents on ring A of this stilbene were identified in the form of two exchangeable protons resonating at 5.38 and 5.48 ppm, respectively, suggesting the presence of two phenolic groups. This analysis left one position on each of the two aromatic rings of the stilbene system for the attachment of the remaining structural elements, which were composed of 15 carbon atoms and 26 hydrogen atoms.

Analysis of the remaining signals in the ^1H NMR spectrum of **1** indicated the presence of five methyl singlets between 1.60 and 1.83 ppm, a four-proton multiplet at 2.10 ppm, two closely spaced two-proton doublets (J = 7.1 Hz) between 3.38 and 3.42 ppm, as well as three olefinic methine multiplets between 5.07 and 5.34 ppm. These resonances and the remaining 15 carbon resonances were assigned to a prenyl and a geranyl group, respectively,

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10.1021/np000265r CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 11/17/2000

mainly by straightforward analysis of HMBC and COSY spectra.

Placement of the substituents on the aromatic rings of **1** was accomplished by analysis of the HMBC spectrum. Thus, the two-proton singlet at 6.53 ppm for the aromatic protons on ring B showed HMBC correlations to both of the carbon atoms of the styrene double bond. This supported the placement of these protons at C-4′/C-8′ rather than at C-5′/C-7′, as would be expected also from biogenetic considerations. The resonance at 112.9 ppm is assigned to the nonprotonated carbon atom C-6′, based on correlations to the two-proton singlet at 6.53 ppm (H-4′, H-8′) as well as to the two-proton singlet at 5.13 ppm for the two exchangeable phenolic protons (OH-5′, OH-7′). An additional correlation of the resonance due to C-6′ to the two-proton doublet at 3.42 ppm (H-1″) identified C-6′ as the site of attachment of the prenyl group.

The placement of the two aromatic protons of ring A in the ortho position relative to the styrene double bond was supported by HMBC correlations from the proton resonance at 6.83 ppm due to the proximal styryl proton to both C-3 and C-5. Placement of the phenolic substituents at C-1 and C-2 was based on HMBC correlations of H-3 to C-1 and C-2, whereas H-5 correlated only to C-1. Attachment of the geranyl group via the two-proton doublet at 3.38 ppm to ring A was based on HMBC correlations of the carbon atoms C-6 (127.4 ppm), C-5, and C-1 to the proton signal at 3.38 ppm.

Mappain (**1**) may thus be regarded as the biogenetic precursor of vedelianin[3] and a lower homologue of schweinfurthin C,[6] carrying a prenyl group in place of a geranyl group in ring B. It is interesting to note that, although **1** and schweinfurthin C are structurally related, they appear to differ substantially in their biological effects. Mappain is significantly cytotoxic (Table 2), whereas schweinfurthin C is reported to be inactive. The biological differences are maintained in regard to schweinfurthins A and B. These latter two compounds were shown to be particularly effective against cancer cells of CNS origin and were ineffective against ovarian cells,[6] whereas **1** was effective against both an ovarian (SK-OV-3) and a breast cancer (MDA-MB-435) cell line, with IC_{50} values in the low micromolar range (Table 2). We have shown previously that these cell lines are sensitive to other cytotoxic agents.[9,10]

Moreover, unlike many other cytotoxins, **1** appears to be a poor substrate for P-glycoprotein-mediated transport because the parental cell line SK-OV-3 and the multidrug-resistant SKVLB-1 cell line are equally sensitive to mappain (**1**) (Table 2). The SKVLB-1 cell line is multidrug-resistant due to the overexpression of P-glycoprotein.[11] It was derived from the SK-OV-3 cell line by selection with vinblastine and, in our hands, shows a resistance factor of 6400 for vinblastine and of 690 for colchicine.[10] The resistance factor is defined as the IC_{50} value for the drug-resistant line divided by the IC_{50} value for the drug-sensitive parental cell line. Using these two cells lines, we have determined resistance factors for a number of natural products. We obtained values ranging from 1 for **1** and isolaulimalide,[9] indicative of no resistance, to greater than 58 000 for Taxol, suggesting that the latter compound is a substrate for extracellular transport by P-glycoprotein.[9]

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** in $CDCl_3$

position	δ_H (J in Hz)	δ_C[a]	long-range correlations[b]
1		141.9	OH-1, H-3, H-5, H-7
2		144.4	OH-2, H-3
3	6.94 d, 1.8	110.8	H-5, H-1′
4		137.6	H-5, H-1′
5	6.78 d, 1.8	120.6	H-7, H-1′
6		127.4	H-7
7	3.38 d, 7.1	30.0	H-5
8	5.34 m	121.5	H-7
9		139.1	H-10, H-11
10	1.80 s	16.2	
11	2.10 m	39.6	H-10
12	2.10 m	26.3	H-11
13	5.07 m	123.7	H-12, H-15, H-16
14		132.2	H-12, H-15, H-16
15	1.60 s	17.7	H-16
16	1.70 s	25.7	H-15
1′	6.85 d, 16.1	128.5	H-3, H-5
2′	6.74 d, 16.1	126.2	H-4′
3′		130.0	H-2′
4′	6.53 s	106.3	H-1′, H-2′
5′		155.0	OH-5′, H-1″
6′		112.9	H-4′/8′, OH-5′,-7′, H-1″
7′		155.0	OH-7′, H-1″
8′	6.53 s	106.3	H-1′, H-2″
1″	3.42 d, 7.1	22.5	
2″	5.28 m	121.4	H-1″
3″		135.5	H-1″, H-4″, H-5″
4″	1.83 s	17.9	H-5″
5″	1.76 s	25.8	H-4″
OH-1	5.48 s		
OH-2	5.38 s		
OH-5′	5.13 s		
OH-7′	5.13 s		

[a] From HMQC spectrum. [b] From HMBC spectrum.

Table 2. Cytotoxicity of **1** in Drug-Sensitive and Drug-Resistant Cell Lines

cell lines	IC_{50} value (μM) ± SD[a]
human ovarian cancer SK-OV-3[b]	1.3 ± 0.1 (n = 4)
human ovarian cancer SKVLB[c]	1.3 ± 0.2 (n = 4)
human breast cancer MDA-MB-435[b]	1.0 ± 0.1 (n = 4)

[a] n = number of replicates. Four complete experiments were run, and the IC_{50} values calculated for each. Within each experiment either triplicate or quadruplicate replicates were run for each data point. [b] Drug-sensitive cell line. [c] Drug-resistant cell line.

Experimental Section

General Experimental Procedures. Optical rotation data were obtained on a JASCO DIP-370 instrument. UV/vis spectra were measured on a Hewlett-Packard model 8453 diode-array spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR instrument. NMR spectra were recorded on a Varian INOVA WB 400 instrument with a 5-mm Z-gradient probe. MS was performed on a ZAB E instrument in the EI mode. TLC was carried out on Merck Si gel precoated glass plates (0.2 mm thickness), and visualization was performed with phosphomolybdic acid spray or iodine vapor.

Plant Material. *M. mappa* was collected at the Lyon Arboretum, University of Hawaii, Oahu, HI. The leaf material was frozen within 3 h of collection. Voucher specimens (accession number Mooberry 178) were deposited in the Department of Botany herbarium and authenticated by Dr. Will McClatchey.

Extraction and Isolation. Crushed, freeze-dried leaf material (100 g from 350 g fresh wt) was extracted twice with 1.2 L CH_2Cl_2−*i*-PrOH (7:3) each for 24 h at room temperature. The solids were filtered off, and the filtrate was concentrated to dryness in vacuo below 40 °C. The resulting solid (7 g) was dissolved in 250 mL of MeOH−H_2O (9:1) and extracted with 3 × 250 mL of hexanes. The aqueous MeOH layer was diluted by addition of H_2O (100 mL) and extracted with 3 × 350 mL of $CHCl_3$. The combined $CHCl_3$ extracts were evaporated to dryness (3 g) and dissolved in CH_2Cl_2−hexanes (1:1). This

solution was applied to a Si gel column equilibrated in CH_2Cl_2−hexanes (1:1). The column was washed with 3 bed volumes each of CH_2Cl_2−hexanes (1:1), pure CH_2Cl_2, CH_2Cl_2−EtOAc (1:1), and EtOAc. The CH_2Cl_2−EtOAc (1:1) fraction contained all of the biological activity. The fraction was evaporated to dryness (1.8 g) and chromatographed over Si gel with EtOAc−hexanes (1:2). The active fractions were combined, evaporated to dryness (0.55 g), and dissolved in MeOH. The MeOH solution was applied to a column of Sephadex LH-20 (15 × 950 mm) equilibrated in MeOH and eluted with MeOH. Active fractions were evaporated to dryness (0.2 g) and purified by flash chromatography with methyl−*tert*-butyl ether−hexanes (1:1) to yield **1** (90 mg) as a beige powder.

Mappain (1): beige powder; $[\alpha]^{20}_D$ 0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 330 (log ϵ 3.55) nm; IR (NaCl) ν_{max} 3415, 2970, 2925, 1620, 1500, 1440, 1300, 1160, 1045, 960, 850 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 449 (14), 448 $[M]^+$(49), 363 (20), 268 (18), 207 (34), 123 (38), 91 (50), 69 (100); HREIMS *m*/*z* 448.2596 (calcd for $C_{29}H_{36}O_4$, 448.2578).

Cell Culture and Bioassay. The SK-OV-3 ovarian cancer cell line (HTB-77) was obtained from American Type Culture Collection (Manassas, VA) and the drug-resistant SKVLB-1 ovarian cancer subline, which was derived from SK-OV-3 cells by selection with vinblastine, was kindly provided by Dr. V. Ling (Vancouver, BC). The MDA-MB-435 cell line was obtained from Dr. M. M. Hijazi (Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC) Cytotoxicity was determined using the sulforhodamine B assay.[12]

Acknowledgment. Support of this work by the Department of Defense Breast Cancer Initiative (DAMD17-97-7212) is gratefully acknowledged. We thank Ms. Jennifer Hewett and Mr. Bob Hirano of the Lyon Arboretum for the plant collections; Dr. Will McClatchey of the Department of Botany, University of Hawaii, for taxonomy; and Ms. Georgia Tien for conducting the cytotoxicity assays.

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NP000265R