Macharistol, a New Cytotoxic Cinnamylphenol from the Stems of *Machaerium aristulatum*

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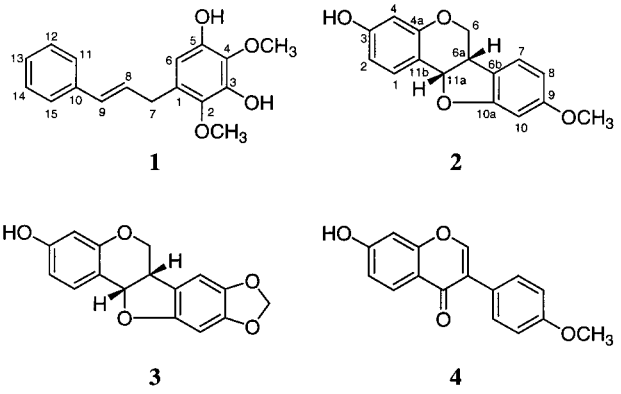
A new cinnamylphenol, macharistol (**1**), along with a known pterocarpan, (+)-medicarpin (**2**), were isolated as cytotoxic constituents from the stems of *Machaerium aristulatum*. In addition, a known pterocarpan, (+)-maackiain (**3**), and a known isoflavone, formononetin (**4**), were identified as inactive constituents. Compound **1** was evaluated in the in vivo hollow fiber assay with KB, Col-2, and hTERT-RPE1 cells and found to be inactive at the highest dose (25 mg/kg body weight) tested.

Machaerium aristulatum (Spruce ex Benth.) Ducke (Leguminosae) is a tropical plant and grows widely as a vine in the Amazon basin.[1] Plants in the genus *Machaerium* have been used by indigenous populations in South America to treat diarrhea,[2] menstruation cramps,[3] coughs,[4] and aphthous ulcers of the mouth.[4] Flavonoids, including pterocarpans,[5−9] cinnamylphenols,[6,8,10] quinoids,[8] and triterpenoids,[11] have been found as the major compound classes in some *Machaerium* species. As a part of an ongoing collaborative search for novel antineoplastic agents of plant origin, *M. aristulatum* was chosen for phytochemical investigation since a chloroform extract exhibited significant cytotoxic activity during preliminary screening. Bioassay-guided fractionation resulted in the isolation of two cytotoxic compounds, including a new cinnamylphenol, macharistol (**1**), and a known pterocarpan, (+)-medicarpin (**2**).[8,12] In addition, a second known pterocarpan, (+)-maackiain (**3**),[13] and a known isoflavone, formononetin (**4**),[12] were isolated. Formononetin (**4**) has been previously isolated from *M. aristulatum* as an antigiardial constituent along with another isoflavone, calycosin.[14] (+)-Medicarpin (**2**) has been reported as a constituent of other *Machaerium* species such as *M. vestitum*[7] and *M. nictitans*.[8]

Compound **1** showed a molecular ion peak at *m*/*z* 286.1206 in the HREIMS, corresponding to the elemental formula $C_{17}H_{18}O_4$. In the ^{1}H and ^{13}C NMR spectra, signals at δ_H 7.38/δ_C 126.1, 7.32/128.5, and 7.23/127.0 and δ_C 137.6 indicated the presence of a monosubstituted phenyl ring. Signals for a second benzene ring were observed at δ_H 6.59/δ_C 111.0, δ_C 124.8, 134.9, 144.0, 140.1, and 140.2, which included four oxygenated carbons. Two singlets at δ_H 3.83 (3H) and 3.99 (3H), which correlated with ^{13}C NMR signals at δ_C 61.0 and 60.9, respectively, in the ^{1}H−^{13}C HMQC NMR spectrum of **1**, were attributable to two methoxyl groups attached to the second benzene ring. Two hydroxyl groups of this ring appeared at δ_H 8.75 (OH-5) and 8.40 (OH-3) when the ^{1}H NMR spectrum of **1** was obtained in DMSO-d_6. One aromatic proton, attached to the second benzene ring, appeared at δ_H 6.59. Resonances for an allyl functionality appeared at δ_H 6.46/δ_C 130.9, 6.34/129.2, and 3.49/32.7. The large coupling constant (J = 16 Hz) of the olefinic protons indicated that they exhibited a *trans* arrangement. Further evidence was provided by the ROE correlation between H-7 and H-9, as shown in Table 1. One of these olefinic protons at δ_H 6.34 showed additional coupling with a ^{1}H NMR signal for the methylene protons at δ_H 3.49 (J = 7 Hz), indicating their direct connectivity. To determine the positions of the substituents of the second benzene ring, a ^{1}H−^{13}C HMBC experiment was conducted (Table 1). It was found that the olefinic group was attached to the monosubstituted phenyl group, as evidenced by the three-bond connectivity of H-9/C-11(15), whereas the methylene group at C-7 was attached to the second benzene ring from the three-bond connectivity observed for H-7/C-6. To confirm unambiguously the positions of two hydroxyl and two methoxyl groups on the second benzene ring, a HMBC experiment was carried out in DMSO-d_6. The hydroxyl group at δ_H 8.40 showed two- and three-bond connectivities with C-3 and C-4, respectively. The second



1 **2** **3** **4**

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Table 1. NMR Data of Macharistol (**1**) (125 and 500 MHz in $CDCl_3$ or DMSO-d_6)

position	δ_C[a]	δ_C[b]	δ_H, mult. (J in Hz)[a]	δ_H, mult. (J in Hz)[b]	HMBC (H→C)	ROESY[a]
1	124.8 s	121.9 s				
2	144.0 s	143.5 s				
3	140.1 s	142.1 s				
4	140.2 s	141.7 s				
5	134.9 s	137.2 s				
6	111.0 d	110.4 d	6.59 s	6.34 s	C-1[c], C-2[c], C-4[a], C-5[c], C-7[a]	H_2−7
7	32.7 t		3.49 d (7)	3.33 d (7)	C-1[c], C-2[b], C-6[c], C-8[c]	H-6, H-9
8	129.2 d	129.6 d	6.34 dt (16, 7)	6.33 dt (16, 7)	C-1[c], C-7[c], C-9[c], C-10[c]	H-11(15)
9	130.9 d	130.0 d	6.46 d (16)	6.40 d (16)	C-7[a], C-8[c], C-10[c], C-11(15)[c]	H-11(15)
10	137.6 s	137.2 s				
11 (15)	126.1 d	125.8 d	7.38 d (7)	7.38 d (7)	C-9[b], C-13[c], C-15(11)[b]	H-8, H-9
12 (14)	128.5 d	128.5 d	7.32 t (7)	7.28 t (7)	C-10[c], C-14(12)[c]	
13	127.0 d	126.9 d	7.23 t (7)	7.18 t (7)	C-11(15)[c]	
OCH_3-2	61.0 q		3.83 s		C-2[c]	
OCH_3-4	60.9 q		3.99 s		C-4[c]	
OH-3				8.40 bs	C-3[b], C-4[b]	
OH-5				8.75 bs	C-4[b], C-5[b], C-6[b]	

[a] In $CDCl_3$. [b] In DMSO-d_6. [c] Run in both $CDCl_3$ and DMSO-d_6.

hydroxyl group at δ_H 8.75 exhibited cross-peaks with C-5 (two-bond), C-4 (three-bond), and C-6 (three-bond). Therefore, these two hydroxyls were assigned to C-3 and C-5, respectively. The methoxyl group at δ_H 3.83 and the aromatic proton at δ_H 6.59 were connected at C-2 and C-6, respectively, since the methylene protons at C-7 displayed cross-peaks with carbons at C-1 (two-bond), C-2 (three-bond), and C-6 (three-bond). Therefore, structure **1** was assigned to a new cinnamylphenol compound, macharistol [(*E*)-1-cinnamyl-2,4-dimethoxy-3,5-dihydroxybenzene].

Compounds **1** and **2** showed moderate cytotoxic activity in the KB cell line with EC_{50} values of 1.7 and 2.4 μg/mL, respectively, whereas (+)-maackiain (**3**) and formononetin (**4**) were inactive. In addition, moderate activity was observed for compound **1** in the Col-2, LNCaP, hTERT-RPE1, and HUVEC cell lines, with EC_{50} values of 3.0, 2.4, 1.3, and 3.0 μg/mL, respectively. Additional studies were performed with compound **1** in which the KB, Col-2, and hTERT-RPE1 cell lines were evaluated with the in vivo murine hollow fiber test. At the highest dose (25 mg/kg body weight) tested, compound **1** showed 23% and 0% inhibition of growth of KB cells implanted at the intraperitoneal (i.p.) and subcutaneous (s.c.) compartments of mice, respectively. At this dose, inhibition of growth of Col-2 cells (35%) was also observed both at the i.p. and s.c. sites. The growth of hTERT-RPE1 was inhibited by 31% and 45% at the i.p. and s.c. sites, respectively. No significant weight loss was observed in test mice in all cases.

These preliminary studies were limited by the quantity of material currently available from the natural source. However, some of the data (e.g., the response observed with Col-2 cells implanted at the s.c. site) provide encouragement for further work. It would be reasonable to evaluate the test compound with additional cells in culture, such as the 60-cell line panel of the NCI, followed by more complete dose−response studies with in vivo models, based on cell culture data. To facilitate work of this nature, it should be possible to obtain a sufficient quantity of material from the natural source.

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Rudolph Research Autopol III automatic polarimeter (Flanders, NJ) at 25 °C. UV and IR spectra were recorded on a Varian Cary 3G UV−vis spectrophotometer and a Nicolet Avatar 360 FT-IR spectrometer, respectively. ^{1}H, ^{13}C, COSY, ROESY, HMQC, and HMBC NMR experiments were performed on a Bruker AMX 500 instrument. TMS was used as internal standard. EIMS were recorded on a HP 5989A instrument, and HREIMS were obtained using a VGZAB-E magnetic sector instrument. Flash column chromatography was carried out on Si gel 60 (70−230 mesh, Merck, Darmstadt, Germany) under nitrogen. Column chromatography was monitored by TLC (Si gel 60 F_{254} plates, 0.25 mm thickness) with visualization under UV light (254 and 365 nm) and 1% sulfuric acid in EtOH. Preparative HPLC was carried out on a Waters 3000 system controller attached to a MetaChem Inertsil ODS 3 (250 × 25 mm i.d., 3 μm) column and a MetaChem Inertsil ODS 3 (50 × 10 mm i.d., 8 μm) guard column. The peaks were detected at 254 nm using a Waters 486 turnable absorbance detector and recorded at a Waters 740 data module integrator. The flow rate was 8 mL/min.

Plant Material. The stems of *Machaerium aristulatum* were collected in November 1997, at the District of Yarinacocha, Province of Coronel Portillo, Department of Ucayali, Peru. A voucher specimen (A3742) has been deposited in the Field Museum of Natural History, Chicago, IL, under the accession number 2193120.

Extraction and Isolation. The dried stems of *M. aristulatum* (800 g) were ground and extracted with MeOH (2 × 2 L) for 24 h by percolation. The volume of the MeOH extract was reduced and H_2O was added to make a MeOH−H_2O (9:1) solution. This aqueous MeOH extract was partitioned with hexane (300 mL), and the aqueous MeOH partition (300 mL) was extracted with $CHCl_3$ (2 × 300 mL). The $CHCl_3$ extract was washed with a 1% saline solution, then evaporated, affording 5.3 g of a residue, which showed significant cytotoxic activity against the KB cell line.[15] Then the $CHCl_3$ residue was mixed with Celite (15 g) and subjected to Si gel column chromatography (200 g). Elution was conducted with solvents of increasing polarity [CH_2Cl_2−MeOH (9.5:0.5 → 9.0:1.0), $CHCl_3$−MeOH (9.0:1.0 → 10:1)], affording seven fractions (I−VII). Fraction III (1.1 g), eluted with CH_2Cl_2−MeOH (99:1), was subjected to Si gel column chromatography using hexane−EtOAc (9:1 → 1:1) mixtures for elution, giving 20 fractions (III-1 to III-20). Fraction III-3 (165 mg), eluted with hexane−EtOAc (17:3), was then separated by reversed-phase preparative HPLC with CH_3CN−H_2O (65:35) as mobile phase, to yield pure (+)-medicarpin (**2**, 40 mg) (t_R 10.5 min) and (+)-maackiain (**3**, 26 mg) (t_R 9.7 min). Fraction III-7 (109 mg), eluted with hexane−EtOAc (7:3), was further purified by reversed-phase preparative HPLC using CH_3CN−H_2O (7:3) as mobile phase, to afford macharistol (**1**, 70 mg) (t_R 12.4 min). Fraction IV (593 mg) was subjected to Si gel column chromatography using hexane−acetone (9:1 → 2:3) and afforded 17 fractions (IV-1 to IV-17). Formononetin (**4**, 8 mg) was isolated by trituration with MeOH of fraction IV-8 [eluted with hexane−acetone (7:3)].

Macharistol (1): scarlet amorphous solid; $[\alpha]_D$ −3.6° (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 282 (3.79), 252 (4.35)

nm; IR (film) ν_{max} 3028, 2941, 2360, 2338, 1652, 1616, 1498, 1472, 1377, 1293, 1195, 1067 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 286 [M^+] (100), 271 (9), 255 (21), 223 (9), 169 (8), 165 (15), 153 (10), 141 (8), 128 (12), 115 (31), 91 (57); HREIMS *m/z* 286.1206 [M^+] (calcd for $C_{17}H_{18}O_4$, 286.1205).

(+)-Medicarpin (2): physical and ^1H NMR data were comparable to the literature values;[12] ^{13}C NMR (pyridine-d_6, 500 MHz) δ 161.7 (C-9, s), 161.5 (C-10a, s), 160.6 (C-3, s), 157.5 (C-4a, s), 132.9 (C-1, d), 125.4 (C-7, d), 120.1 (C-6b, s), 112.0 (C-11b, s), 112.0 (C-2, d), 106.6 (C-8, d), 104.3 (C-4, d), 97.2 (C-10, d), 79.3 (C-11a, d), 66.8 (C-6, t), 55.4 (OCH_3-9), 40.1 (C-6a, d).

(+)-Maackiain (3): physical and spectral data were comparable with literature values.[13]

Formononetin (4): physical and spectral data were comparable with literature values.[12]

Cell Culture Panel Bioassay. Extracts, fractions, and compounds **1**−**4** were tested in a human oral epidermoid carcinoma (KB) cell line using established protocols.[15] In addition, compound **1** was evaluated against the other human cancer cell lines comprising our cytotoxicity screening panel. Assays involving colon (Col-2), prostate (LNCaP), and lung (Lu1) cancer cell lines utilized established protocols,[16] while HUVEC (human umbilical vein endothelial cells) and hTERT-RPE1 (human telomerase reverse transcriptase−retinal pigment epithelial) cells were grown in more specialized media. HUVEC were purchased and grown in media and components supplied in the EGM-2 BulletKit (Clonetics Corporation, Walkersville, MD) with 2% fetal bovine serum (FBS), while the hTERT-RPE1 line (Clontech Laboratories, Palo Alto, CA) was maintained in DMEM/F-12 (Invitrogen Corporation, Carlsbad, CA) containing 10% FBS. The HUVEC line constitutes a test system to identify samples with potential antiangiogenic activity. The telomerase-immobilized normal human cell line, hTERT-RPE1, was adopted as a substitute for a primary cell line for determining toxicity to normal human cells. Both these cell lines were tested at an initial cell seed concentration of 5×10^4 cells/mL using standard assay procedures.[16]

In Vivo Hollow Fiber Assay. The in vivo hollow fiber test was performed using a literature procedure with some modification.[17,18] Compound **1** was tested against the KB, Col-2, and hTERT-RPE1 cell lines in both the i.p. and s.c. compartments of female athymic NCr *nu/nu* mice. Cell growth was assessed with fibers containing 7.5×10^5, 10^6, or 2.5×10^6 cells/mL of KB, Col-2, and hTERT-RPE1, respectively. Compound **1** was coprecipitated with PVP (MW 360 000, Sigma) to increase solubility[19] and then dissolved in PBS. Formulations containing 6.25, 12.5, and 25 mg/kg body weight of compound **1** were administered once daily by i.p. injection from day 3−6 after implantation of fibers. Body weights were determined daily. On day 7, mice were sacrificed by cervical dislocation, and fibers were retrieved and processed as described previously.[17,18]

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