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Pondaplin: A Novel Cyclic Prenylated Phenylpropanoid from *Annona glabra*

Xiao-Xi Liu[†], Elsa Pilarinou,[♦] and Jerry L. McLaughlin[†]*

[†] *Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, USA;* [♦] *Walker Cancer Research Institute, 1634A Metropolitan Blvd., Tallahassee, Florida 32308, USA*

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Abstract: A novel cyclic prenylated phenylpropanoid, pondaplin was isolated from the ethanolic extracts of the leaves of *Annona glabra*, by directing the fractionation with the brine shrimp lethality test (BST). Pondaplin showed selective cytotoxicities at moderate potencies among six human solid tumor cell lines.

Keywords: natural products, antitumor compounds

Annona glabra L. (Annonaceae), commonly known as pond apple, is a tropical tree distributed mainly in the Americas and in southeast Asia. It is used in traditional medicine as an insecticide and a parasiticide. [1, 2] Several bioactive Annonaceous acetogenins have been previously isolated from this species. [1, 3-5] As part of our continuing efforts to find new and structurally diverse bioactive leads, a novel cyclic prenylated phenylpropanoid, pondaplin (**1**), was isolated from the bioactive ethanolic extracts of the leaves, obtained from trees native to Florida, using bioactivity-directed fractionation with the brine shrimp lethality test (BST).[6, 7] The structure of **1** (Figure 1) was identified as a cyclic prenylated *p*-coumarate by NMR spectroscopic techniques (Table 1, Figure 2). The new compound demonstrated moderate cytotoxicities among six human solid tumor cell lines with selectivities for the breast (MCF-7) and prostate (PC-3) cancer cell lines (Table 2).

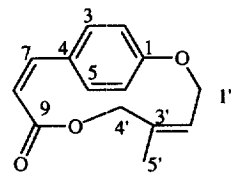

Figure 1. Structure of Pondaplin (**1**).

Compound **1** was isolated as colorless crystals, m. p. 194-195 °C. Its molecular weight was suggested by the peak at *m/z* 231 $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 231.1018 for the $[MH]^+$ ion (calcd. 231.1021) corresponding to the molecular formula, $C_{14}H_{14}O_3$.

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Compound **1** showed a UV λ_{max} at 245 nm (log ε =3.05) with a shoulder at 295 nm (log ε = 3.03) and aromatic (3040, 1603, and 1513 cm^{-1}) and carbonyl (1709 cm^{-1}) absorption peaks in its IR spectrum. The presence of a *para*-substituted aromatic system was suggested by δ_H 7.58 (dd, *J*=9.0 Hz, 2.0 Hz, H-3/5) and δ_H 6.80 (dd, *J*=8.5 Hz, 2.0 Hz, H-2/6) in the NMR (Table 1). This was confirmed by the ^{13}C chemical shifts at δ_C 157.99 (C-1), 114.94 (C-2/6), 132.97 (C-3/5), and 126.46 (C-4). The linkage of the double bond (C-7/8) to the aromatic system was indicated by single-relay COSY data. The linkage was confirmed because the olefinic doublets at δ_H 6.87 (H-7) and δ_H 5.82 (H-8) showed HMBC correlations to aromatic carbons at δ_C 132.97 (C-3/5) and to δ_C 126.46 (C-4), respectively (Figure 2).

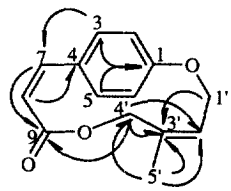

Figure 2. Selected HMBC correlations in **1**

The presence of an ester linkage in **1** was indicated by the carbonyl carbon signal at δ_C 166.59 together with IR absorption at 1709 cm^{-1}. The HMBC correlations between the signal of H-7 (δ_H 6.87) and the carbonyl group (δ_C 166.59) suggested that the carbonyl group must be located at C-9.

Table 1. ^{13}C NMR and ^1H NMR (δ, *J* in Hz) of **1**.

	^{13}C NMR (125 MHz)	^1H (500 MHz) (*J* in Hz)
1	157.99	-
2	114.94	6.80 (dd, 9.0, 2.0)
3	132.97	7.58 (dd, 8.5, 2.0)
4	126.46	-
5	132.97	7.58 (d, 9.0)
6	114.94	6.80 (d, 9.0)
7	144.21	6.87 (d, 12.0)
8	116.06	5.82 (d, 12.5)
9	166.59	-
1'	58.53	4.17 (d, 7.0)
2'	126.97	5.62 (td, 6.5, 1.5)
3'	132.19	-
4'	68.77	4.54 (s)
5'	13.83	1.67 (s)

The remaining four resonances at δ_H 5.62 (1H, td, *J*=6.5 Hz, 1.5, H-2'), 4.54 (2H, s, H-4'), 4.17 (2H, d, J=7.0 Hz, H-1'), and 1.67 (3H, s, H-5') and five peaks at δ_C 132.19 (C-3'), 126.97 (C-2'), 68.77 (C-4'), 58.53 (C-1'), and 13.83 (C-5') in the NMR spectra (Table 1) were characteristic spectral features for the oxygen-substituted prenyl group, which was

confirmed by HMBC correlations among H-1'/C-3', H-4'/C-2'. H-4'/C-3', H-5'/C-2', H-5'/C-3', and H-5'/H-4' (Figure 2).

The linkage between the ester group and the prenyl group was established via HMBC correlation between the methylene group (H-4') and the carbonyl carbon (C-9). The presence of an additional downfield-shifted methylene carbon signal at δ_C 58.53 (C-1') could only be accounted for by its connection to an oxygen atom. This was also revealed by the ^1H shifts of H-2/6 (δ_H 6.80) and the ^{13}C NMR shift of C-1 (δ_C 157.99) in the aromatic system.

The coupling constant of the pair of olefinic protons at δ_H 6.87 (H-7, br d, J =12.0 Hz) and 5.82 (H-8, br d, J=12.5 Hz) indicated the (*Z*)-configuration for the double bond at C-7/8. The stereochemistry of the other double bond (C-2'/3') was suggested by NOESY data. In the NOESY spectrum, the triple doublet signal of olefinic protons at δ_H 5.62 showed a cross peak to the methyl group (δ_H 1.67, s, 3H, H-5'), which indicated the (*Z*)-configuration.

Many of phenylpropanoids exhibit diverse biological activities of which the most noteworthy are antimicrobial, anticancer, and hypotensive properties. [8-10] Moreover, phenylpropanoid derivatives are already known to inhibit some enzymes such as cAMP phosphodiesterase and prostaglandin synthetase. [11, 12] The biological activities of **1** against six solid tumor cell lines are summarized in Table 2. The compound was moderately and selectively active across the six human tumor cell lines in our seven-day MTT human solid tumor cytotoxicity tests. [13] Since this is a small molecule and total synthesis should not be difficult, structural modifications with possible enhancement of bioactivity seems reasonable.

Table 2. Bioactivity of **1**.

Compound		**1**	adriamycin[g]
BST[1] ED_{50} (μg/ml)		3.9×10^1	-
Human	A-549[a]	1.1×10^1	1.5×10^{-3}
Tumor	MCF-7[b]	2.3	1.1×10^{-1}
Cell	HT-29[c]	5.0	2.6×10^{-2}
Lines	A-498[d]	2.6×10^1	3.0×10^{-3}
ED_{50}	PC-3[e]	3.6	1.9×10^{-2}
(μg/ml)	PACA-2[f]	1.9×10^1	1.6×10^{-3}

[1]Brine shrimp lethality test; [6] [a] Human lung carcinoma; [b] Human breast carcinoma; [c] Human colon adenocarcinoma; [d] Human kidney carcinoma; [e] Human prostate adenocarcinoma; [f] Human pancreatic carcinoma; [g] positive control standard.

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13. *In vitro* cytotoxicities, against human tumor cell lines, were carried out at the Purdue Cancer Center, Cell Culture Laboratory, using standard 7-day MTT assays for A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma). A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma) and PACA-2 (human pancreatic carcinoma). Adriamycin is always used as a positive antitumor control in the same runs; ED_{50} values of ≤ 4 μg/ml are considered significantly active.