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Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2275–2277

Two new bacterial DNA primase inhibitors from the plant *Polygonum cuspidatum*

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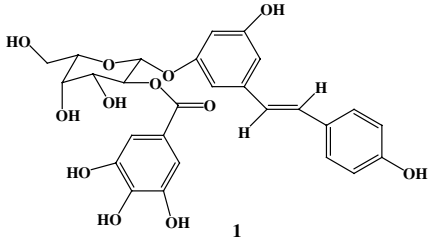
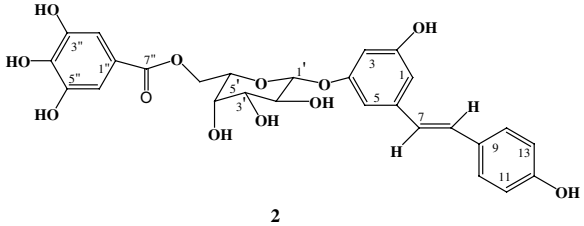
Received 13 November 2003; accepted 2 February 2004

Abstract—The 70% aqueous methanolic extract of the Peruvian plant *Polygonum cuspidatum* sp. was found to contain two novel phenolic saccharides **1** and **2**, which were identified as inhibitors of the bacterial DNA primase enzyme. Structures of these two compounds were established based on high resolution NMR studies. Compound **1** and **2** inhibited the primase enzyme with an IC_{50} of 4 and 5 μM, respectively.

Bacterial infections continue to be a significant cause of morbidity and mortality, particularly within immunocompromised patient populations. A portion of these deaths result from antibiotic resistant pathogens, for which there are very few new classes of antibiotics available for alternative therapy. Target-based drug discovery methods are being utilized to identify novel molecules that may provide alternative antimicrobial therapeutics. Bacterial DNA primase is a DNA-dependent RNA-polymerase that is required for bacterial chromosome replication.[1,2] Genetic validation studies indicate that inhibition of bacterial primase causes a rapid bactericidal response. Interaction of primase, encoded by dnaG, with the single stranded DNA-template is mediated by contacts with the replicative DNA helicase encoded by DnaB.[3–5] In the cell, the helicase progresses along the duplex DNA mediating unwinding to a single stranded DNA template. The DNA primase associates with the helicase and intermittently initiates synthesis of ssRNA primers that are required for de novo DNA synthesis. While replication of the leading-strand DNA requires only one RNA primer, replication of the lagging-strand requires >2000 primer sites. Interruption of this process would clearly cause a catastrophic event in chromosome replication. A high-throughput screen using scintillation-proximity has been devised for the *E. coli* DNA primase. The assay utilizes a biotinylated ssDNA template that contains an optimized interaction site for DnaB binding followed by an oligdT or oligodC sequence to allow measurement of the incorporation of ^{3}H-labeled ribonucleotides.



1



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As part of our continuing investigation of natural products as leads for inhibiting bacterial infection, we screened several semi-purified fractions of aqueous methanolic extracts of many plants. One of these fractions,

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doi:10.1016/j.bmcl.2004.02.006

which was derived from a plant identified as *Polygonum cuspidatum* Sieb. et Zucc. sp., was active in the primase enzyme inhibition assay, Bioassay-guided fractionation of this extract led to the isolation of two phenolic monosaccharides **1** and **2**.

The detanninized aqueous methanolic extract (1.4 g) of the plant *P. cuspidatum* sp. was prepared as a plug on 15 mL of CHP-20P (Mitsubishi Corp.) and loaded on a column (2×20 cm) with the same stationary phase, equilibrated with water and chromatographed using a water and methanol gradient system. Twenty-four enriched fractions were collected and screened in the primase assay. The fourteenth fraction (92.3 mg) showed inhibition (activity) in the primase assay. Forty-five milligrams of this fraction was used for the purification of the active compounds on a Phenomenex Luna C-18 silica column (10×250 mm), eluting with a mixture of acetonitrile and 0.05% aqueous trifluoroacetic acid (20:80 v/v). Acetonitrile was removed from the active fraction and the aqueous solution was freeze-dried to yield 2.3 and 0.6 mg of **1** and **2**, respectively.

Compound **1** showed a molecular ion at m/z 543 $(M+H)^+$ and a sodiated ion m/z 565 $(M+Na)^+$ in the FAB mass spectrum (FABMS) suggesting a molecular weight of 542 Da. The molecular formula of **1** was established as $C_{27}H_{26}O_{12}$ by HRMS (high resolution mass spectrometry).[6] The molecular formula revealed 15 unsaturations in the molecule. The UV spectrum (MeOH) of **1** showed absorption maxima at 214, 304 and 318 nm and the IR spectrum (neat) showed peaks at 1684, 1603, 1514, 1462, 1207 and 1032 cm^{-1}, suggesting the presence of an ester functionality. ^1H and ^{13}C NMR chemical shifts of **1** and **2** are listed in Table 1. The ^1H NMR indicated the presence of several aromatic protons and the presence of one sugar. D_2O exchange revealed five phenolic –OH's and at least two hydroxyl groups. The ^{13}C NMR also showed 27 carbon signals in agreement with the number of carbons revealed by HRMS. APT ^{13}C NMR identified them as one >C═O, twenty olefinic (eleven ═CH─, nine ═C<), one anomeric methine (O─CH─O), four >CH─O, one O─CH_2. This information suggested that the compound probably contains three aromatic rings, a double bond and one sugar. When a carbonyl functionality was included, it accounted for all the carbons and degree of unsaturations. The phenolic nature, high oxygen content and absence of nitrogen suggested the compound must be a typical lignan. Comparison of the ^{13}C NMR chemical shifts of aromatic rings suggested the compound contain a gallic acid residue.[7] Using contemporary 2D NMR techniques (COSY, HMQC, HMQC–TOCSY and HMBC) the structure was established as shown in **1**. The attachment of aromatic residues to the sugar was established by HMBC correlation studies.

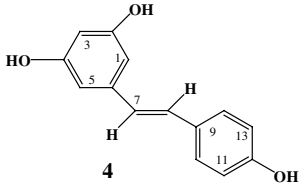
This structure was further confirmed by degradation. The compound was hydrolyzed with 6 N HCl overnight in a sealed tube under nitrogen heating at 100 °C. Then

Table 1. ^1H and ^{13}C NMR chemical shifts for **1** and **2**

C#	1	1	2	2	4	
1	6.54 (t, $J = 2$ Hz)	107.7	6.57 (t, $J = 2$ Hz)	106.8	6.37 (d, $J = 2.2$ Hz)	104.3
2	9.42 (–OH)	158.4	9.44 (–OH)	158.4	9.17 (–OH)	158.5
3	6.18 (t, $J = 2$ Hz)	102.8	6.34 (t, $J = 2$ Hz)	102.7	6.10 (t, $J = 2.2$ Hz)	101.7
4		158.4		158.7		158.5
5	6.62 (t, $J = 2$ Hz)	104.7	6.61 (t, $J = 2$ Hz)	105.1	6.37 (d, $J = 2.2$ Hz)	104.3
6		139.5		139.4		139.2
7	6.80 (d, $J = 16.2$ Hz)	124.9	6.81 (d, $J = 16.4$ Hz)	125.2	6.79 (d, $J = 16.3$ Hz)	125.6
8	6.95 (d, $J = 16.2$ Hz)	128.7	6.97 (d, $J = 16.4$ Hz)	128.6	6.91 (d, $J = 16.3$ Hz)	127.8
9		127.9		127.9		128.0
10	7.36 (d, $J = 8.5$ Hz)	127.9	7.34 (d, $J = 8.5$ Hz)	127.9	7.38 (d, $J = 8.5$ Hz)	127.8
11	6.73 (d, $J = 8.5$ Hz)	115.5	6.74 (d, $J = 8.5$ Hz)	115.5	6.74 (d, $J = 8.5$ Hz)	115.5
12	9.55 (–OH)	157.3	9.56 (–OH)	157.3	9.53 (–OH)	157.2
13	6.73 (d, $J = 8.5$ Hz)	115.5	6.74 (d, $J = 8.5$ Hz)	115.5	6.74 (d, $J = 8.5$ Hz)	115.5
14	7.36 (d, $J = 8.5$ Hz)	127.9	7.34 (d, $J = 8.5$ Hz)	127.9	7.38 (d, $J = 8.5$ Hz)	127.8
1′	5.12 (d, $J = 8$ Hz)	98.7	4.89 (d, $J = 7.7$ Hz)	100.6		
2′	4.93 (dd, $J = 8$, 8.5 Hz)	73.6	3.26 (dd, $J = 7.7$, 9 Hz)	73.3		
3′	3.58 (m)	74.2	3.35 (m)	76.3		
	5.36 (–OH, d, $J = 5.5$ Hz)					
4′	3.30 (m),	70.0	3.40 (m)	69.2		
	5.26 (–OH, d, $J = 5.5$ Hz)					
5′	3.48 (m)	77.3	3.67 (ddd, $J = 2$, 3.7, 9.5 Hz)	73.6		
6′	3.54 (m), 3.76 (m),	60.5	4.32 (dd. $J = 3.7$, 12.3 Hz)	62.9		
	4.73 (–OH, t, $J = 5.5$ Hz)		4.39 (dd, $J = 2$, 12.3 Hz)			
1″		119.5		119.3		
2″	6.97 (s)	108.7	6.96 (s)	108.6		
3″	9.22 (–OH)	145.5	9.26 (–OH)	145.5		
4″	8.93 (–OH)	138.4	8.91 (–OH)	138.5		
5″	9.22 (–OH)	145.5	9.26 (–OH)	145.5		
6″	6.97 (s)	108.7	6.96 (s)	108.6		
7″		165.0		165.8		

* NMR's were run in DMSO-d_6; similar chemical shifts may be interchanged.

the contents were diluted with water, extracted with ethyl acetate and organic layer dried and finally purified on HPLC. Two compounds **3** and **4** were isolated from this degradation mixture. The mass ion, ^{1}H and ^{13}C NMR spectral data of **3** suggested it to be gallic acid. The identity **3** was further confirmed by HPLC comparison with authentic sample of gallic acid. Compound **4** showed molecular ion in FABMS with m/z 229 (M+H) suggesting molecular weight 228. ^{1}H and ^{13}C NMR chemical shifts of **4** are tabulated in Table 1. Both ^{1}H and ^{13}C NMR chemical shifts are similar to that of phenolic part of compound **1**. Based on spectral data the structure of this fragment was established as **4**. This was further verified by COSY and HMBC spectral analysis. This structure is further confirmed using authentic compound previously isolated in our laboratory.[8] Compound **4** is previously known in literature as resveratrol.[9–11] The sugar in **1** appears to be glucopyranose based on the relative stereochemistry established from NMR chemical shifts and ^{1}H–^{1}H coupling studies of the parent molecule. However, the absolute structure has not been established.



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Compound **2** showed a sodiated ion m/z 565 $(M+Na)^{+}$ in FABMS suggesting the molecular weight of 542 Da. The molecular formula of **2** was established as $C_{27}H_{26}O_{12}$ by HRMS,[6] suggesting **1** and **2** are structural isomers. Compound **2** showed UV maxima (MeOH) at 214, 304 and 318 nm. The ^{1}H and ^{13}C NMR chemical shifts of **2** are tabulated in Table 1, revealing close structural similarities between **1** and **2**. Comparison of individual ^{1}H and ^{13}C NMR chemical shifts of gallic acid, sugar and stilbene portion revealed that in compound **2** these moieties were intact. The structure **2** was established by long-range C–H coupling studies using HMBC.

No hydrolysis experiments were performed to identify the sugar separately, due to the lack of material their structures were deduced mainly from the 2D NMR experiments.

Compounds **1** and **2** were active in the primase enzyme inhibition assay with IC_{50} values of 4 and 5 μM, respectively. However, **1** was also found to have potent activity in a fluorescent intercalator dye displacement assay (FID) that detects interactions with double-stranded DNA templates.[12] The FID $IC_{50} = 0.7\,\mu M$ when using a 250 nM DNA template (41 bp in length) with 250 nM TO-PRO-1 (Molecular Probes, Eugene, OR). Therefore, the inhibition of DNA primase activity may be mediated by disrupting the interaction of the protein with the ssDNA template.

Acknowledgements

We graciously acknowledge Microsource Discovery System through their collaboration with PeruBotanica, in supplying extract of the plant *P. cuspidatum*.

References and notes

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