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A New Polyphenolic Compound from *Rubus aleaefolius* and Its Inhibitory Activity on Mammalian Cell Cycle at G_0/G_1 Phase

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Abstract: Rubuphenol (**1**), a new polyphenolic compound, was isolated together with the known ellagic acid (**2**) as new cell cycle inhibitors from *Rubus aleaefolius* Poir. through a bioassay-guided separation procedure and the structure of **1** was elucidated by spectroscopic method. Compounds **1** and **2** inhibited the cell cycle of tsFT210 cells at the G_0/G_1 phase respectively with the MIC values of 14.6 μM and 10.3 μM.

Keywords: Rubuphenol, ellagic acid, polyphenol, *Rubus aleaefolius*, cell cycle inhibitor

Rubus aleaefolius Poir. (Rosaceae), a traditional Chinese medicine "Cu Ye Xuan Gou Zi"[1], is also used as a folk medicine to cure certain cancers in partial area of China. However the chemical constituents of the plant have so far not been reported. In the course of our screening for new anticancer agents from natural resources using mammalian tsFT210 cells[2,3], we found that the alcoholic extract of *R. aleaefolius* significantly inhibited the cell cycle of tsFT210 cells at the G_0/G_1 phase and we have now isolated a new polyphenolic compound named rubuphenol **1** together with the known ellagic acid **2** as new cell cycle inhibitors from *R. aleaefolius* through a bioassay-guided separation procedure.

The roots (3 kg) of *R. aleaefolius* were extracted at room temperature with 3 L of 60% aqueous alcohol (3 times) to give an alcoholic extract (435 g). The alcoholic extract showed a strong G_0/G_1 inhibitory activity on the cell cycle of tsFT210 cells and thus the following separation procedure was monitored by the same activity. The whole extract was suspended in water (3 L) and extracted successively with the same volume of chloroform, ethyl acetate and butanol to afford an active ethyl acetate extract (78 g) from which **1** (10 mg) and **2** (132 mg) were isolated through repeated solvent-extraction and column chromatography on Sephadex LH-20 and ODS.

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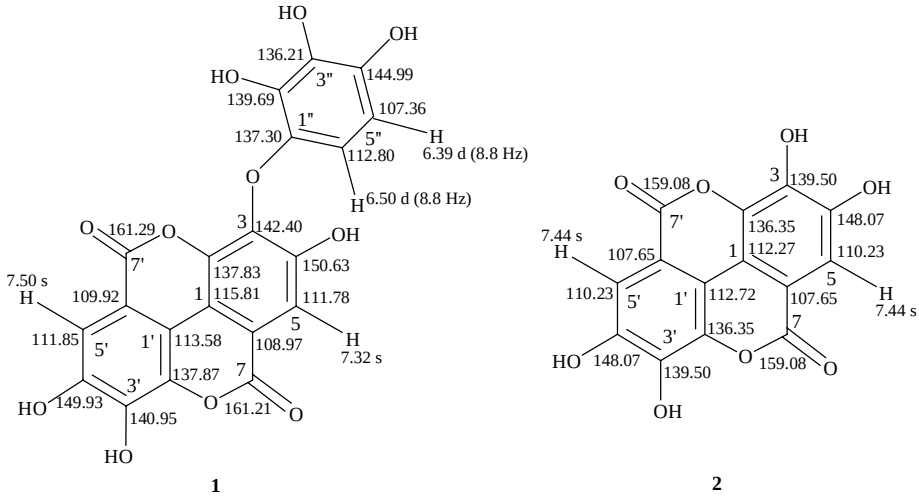
Figure 1 Structures and NMR Data (**1** in CD_3OD, **2** in DMSO-d_6)



Rubuphenol **1** was obtained from MeOH solution as amorphous powders and decomposed at above 275°C. It showed no optical activity and gave a blue color with the ferric chloride reagent, revealing that **1** is a phenolic compound. It gave *quasi*-molecular ion peaks at *m/z* 427 $[M+H]^+$ and 425 $[M-H]^-$ respectively in the positive and negative ESI-MS measurements and its molecular formula $C_{20}H_{10}O_{11}$ was determined by negative HR-SIMS-MS (measured 425.0151, *calcd.* 425.0150). The UV spectrum of **1** in MeOH solution showed a characteristic absorption curve with maximum absorptions at 215 (log ε 4.99), 250sh, 258 (4.64), 272 (4.62) and 351 nm (4.13) and in the IR spectrum, **1** showed absorption bands at 3518, 3396 and 3262 cm^{-1} (free and hydrogen-bonded OH groups), 1737 and 1726 cm^{-1} (ester carbonyl groups), 1620, 1587 and 1494 cm^{-1} (aromatic rings), 1348 cm^{-1}, and 1243 cm^{-1} (=C-O groups).

The ^1H and ^{13}C NMR (600 MHz and 150 MHz) data of **1** in methanol-d_4 solution (**Figure 1**) indicated the presence of two isolated methine (δ_H 7.32 s, H-5, δ_C 111.78 d, C-5 and δ_H 7.50 s, H-5', δ_C111.85 d, C-5') and two neighboring methine (δ_H 6.39 d, *J*=8.8 Hz, H-6", δ_C 107.36 d, C-6" and δ_H 6.50 d, *J*=8.8 Hz, H-5", δ_C 112.80 d, C-5") groups together with two conjugated ester carbonyl, ten oxygenated quaternary and four quaternary carbons (**Figure 1**) in **1**. Six proton signals have not been observed in the ^1H NMR spectrum in methanol-d_4 solution, revealing the presence of six free hydroxyl groups in **1**. These ^1H and ^{13}C NMR data suggested that **1** is an ellagic acid derivative with an *O*-phenyl moiety bearing adjacent trihydroxy groups[4].

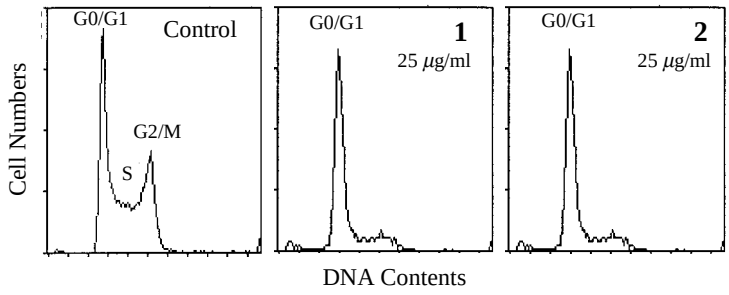
Then, analysis of the pulse field gradient (PFG) HMBC spectrum enabled us readily to confirm the ellagic acid moiety and the *O*-trihydroxyphenyl group in **1**. In the PFG-HMBC spectrum, H-5 in **1** correlated with C-1, C-3, C-4, C-6, and C-7 and showed weak but significant correlations with C-2 and C-1', while H-5' correlated with C-1', C-3', C-4', C-6' and C-7' and gave weak but significant correlation peaks with C-2' and C-1. Thus the proton and carbon signals for ellagic acid skeleton in **1** could be

fully recognized and assigned unambiguously to confirm this moiety (**Figure 1**). On the other hand, in the PFG-HMBC spectrum, H-5" in **1** correlated with C-1", C-3" and C-4" and gave a weak but significant correlation peak with C-2"，while H-6" correlated with C-1", C-2" and C-4" and further showed a weak but significant correlation peak with C-3". These data evidenced the *O*-phenyl moiety bearing adjacent trihydroxy groups in **1** and established the unambiguous assignments of all ^{1}H and ^{13}C signals for this moiety (**Figure 1**). These data are consistent with those of phenyl group in 4-*O*-(2",3",4"-trihydroxyphenyl)-ellagic acid[4,5], indicating the same substitution pattern of the phenyl group in **1**.

Then, location of the *O*-phenyl group at C-3 position could be demonstrated by the difference NOE experiments for **1** in methanol-d_4 solution, where no NOE was observed between H-5 and H-6" in **1**[6]. This provided negative evidence for the ether linkage in **1** between C-4 and C-1". Thus the structure of **1** could be concluded to be 3-*O*-(2",3",4"-trihydroxyphenyl)-ellagic acid. To our best knowledge, rubuphenol (**1**) is a new phenolic compound and is the first example of ellagic acid derivative with a single ether linkage at the C-3 position to link an *O*-multihydroxyphenyl group[4,5].

Compounds **1** and **2** inhibited the cell cycle of tsFT210 cells at the G_0/G_1 phase respectively with the MIC values of 14.6 μM and 10.3 μM. Typical flow cytometric histograms for **1** and **2** are given in **Figure 2**. The present result provides rubuphenol (**1**), a new polyphenolic compound, and the known ellagic acid **2**, as new G_0/G_1 phase inhibitors of the mammalian cell cycle, which may possess potential anticancer effect[2,3].

Figure 2 Flow Cytometric Histograms for Compounds **1** and **2**



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References and a Note

1. Jiangsu New Medical College, *The Dictionary of Traditional Chinese Medicines*, 1st ed., Shanghai Science and Technology Publisher, Shanghai, **1999**, Volume *2*, p.2259.
2. H. Osada, C. B. Cui, R. Onose, F. Hanaoka, *Bioorg. Med. Chem.*, **1997**, *5*, 193.

3. H. Wang, C. B. Cui, B. Han, Z. H. Liu, Y. X. Song, P. J. Zhou, *Chinese J. Antibiot.*, **2001**, *26*, 19.
4. S. W. Yang, B. N. Zhou, J. H. Wisse, R. Evans, H. van der Werff, J. S. Miller, D. G. I. Kingston, *J. Nat. Prod*., **1998**, *61*, 901.
5. H. H. Barakat, S. A. M. Hussein, M. S. Marzouk, I. Merfort, M. Linscheid, M. A. M. Nawwar, *Phytochem*., **1997**, *46*, 935.
6. For eschweilenol A [4-*O*-(2",3",4"-trihydroxyphenyl)-ellagic acid], the positive NOE was observed between H-5 and H-6" in the methanol-d_4 solution, evidencing location of the ether linkage at the C-4 position in eschweilenol A (*see reference 4*). While in the case of rubuphenol (**1**) in the present study, no NOE was observed between H-5 and H-6" in the difference NOE experiments under the conditions, 11 mg of samples in 0.4 mL methanol-d_4 solution, 32 scans for each experiment, and variation of the mixing times between 0.05-0.5 seconds with 0.025 seconds steps, supporting that the ether linkage in **1** should be located at the C-3 position.

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