Inhibitory Activities of Angiopteroside for HIV-1 Reverse Transcriptase and Lung Cancer Cell-line

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The structure of angiopteroside, which was isolated from rhizome of *Angiopteris evecta* Hoffm., was confirmed using X-ray crystallography. Angiopteroside showed significant activity for inhibition of HIV-1 Reverse Transcriptase (IC_{50} at 0.91 μM) as compared to the IC_{50} for ddI, a positive control, of 0.87 μM. Additionally, cytoxicity against lung cancer cell-line (Chaco), in % survival form, was also very high; 55% for angiopteroside and 63% for doxorubicin (as a positive control).

Key words: angiopteroside, HIV-1 reverse transcriptase inhibitor and anti-tumor.

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การออกฤทธิ์ยับยั้งของแองจิโอเทอโรไซด์ที่มีต่อเอนไซม์รีเวอส์ทรานสคริปเทสของเชื้อ HIV-1 และเซลล์มะเร็งปอด

สมจินตนา ทวีพานิช นันทิตา คำทอง นฤมล สวัสดิพฤกษา และโสภณ เริงสำราญ (2548) *วารสารวิจัยวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย* 30(2)

โครงสร้างของ แองจิโอเทอโรไซด์ ที่แยกได้จากหัวใต้ดินของว่านกีบแรด *Angiopteris evecta* Hoffm. สามารถยืนยันได้โดยเทคนิคเอกซ์เรย์ คริสตัลโลกราฟี แองจิโอเทอโรไซด์แสดงฤทธิ์ที่สำคัญในการยับยั้งเอนไซม์รีเวอส์ทรานสคริปเทสของเชื้อ HIV-1 (มีค่า IC50 เท่ากับ 0.91 ไมโครโมลาร์) ขณะที่ค่า IC50 ของ ddI (positive control) มีค่าเท่ากับ 0.87 ไมโครโมลาร์ ยิ่งไปกว่านั้นฤทธิ์ในการฆ่าเซลล์มะเร็งปอด (Chaco) ยังมีค่าการรอดอยู่ที่ 55 % สำหรับแองจิโอเทอโรไซด์ และ 63% สำหรับ ดอกโซรูบิซิน (positive control)

คำสำคัญ แองจิโอเทอโรไซด์ ตัวยับยั้งเอนไซม์รีเวอส์ทรานสคริปเทสของเชื้อ HIV-1
สารยับยั้งเซลล์มะเร็ง

INTRODUCTION

Angiopteris evecta Hoffm. (Marattiaceae) is widely distributed in Thailand. It has been used as a traditional medicine as a diuretic, antipyretic, tonic, analgesic and antidiarrheal. We examined the chemical constituents of *A. evecta* and isolated angiopteroside. Angiopteroside was found through the isolation of the dried rhizome of *Angiopteris evecta* Its structure, orthorhombic form, has been confirmed by Hseu[(1)] using single crystal X-ray diffraction. Its biological activities against HIV-1 Reverse Transcriptase and several cancer cell-lines were investigated.

MATERIALS AND METHODS

Instrumentation

The Nuclear Magnetic Resonance analysis was performed on a JEOL JNM-A500 Spectrometer. X-ray diffractometer readings were obtained on a BRUKER SMART CCD diffractometer at the Department of Physics, Faculty of Science and Technology, Thammasart University. The UV-visible spectrophotometer was a Tecan Group Ltd. Sunrise model.

Table 1. NMR spectra data of angiopteroside[a].

Position	δ_C	δ_H
1	165.9 s	-
2	123.6 d	6.12 (d, *J*=9.8 Hz)
3	145.3 d	7.16 (dd, *J*=4.9 and 9.8 Hz)
4	68.7 d	4.47 (dd, *J*=3.7 and 4.9 Hz)
5	78.3 d	4.70 (ddd, *J*=3.7 and 6.4 Hz)
6	16.2 d	1.45 (d, *J*=6.4 Hz)
1′	102.5 s	4.41 (d, *J*=7.6 Hz)
2′	74.9 s	3.17 (dd, *J*=7.6 and 8.5 Hz)
3′	78.0 d	3.33 (t, *J*=8.5 Hz)
4′	71.7 d	3.25 (t, *J*=8.5 Hz)
5′	78.2 d	3.29 (obsecured by DMSO-d_6)
6′	62.9 t	3.65 (dd, *J*=5.8 and 11.6 Hz) 3.88 (d, *J*=11.6 Hz)

[a] ^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz, DMSO-d_6: data in ppm.

Table 2. Cytotoxic activity against tumor cell lines of angiopteroside (% Survival).

Compound[1] (10 μg/ml)	**% survival**					
	HS 27 (fibroblast)	**Kato-3 (gastric)**	**BT 474 (breast)**	**Chago (lung)**	**SW 620 (colon)**	**HEP-G2 (hepatoma)**
angiopteroside	78	112	113	**55**	91	105
doxorubicin[2]	35	54	28	63	20	17

Note [1]dissolved in ethanol.

[2]doxorubicin: Doxorubicin hydrochloride was used as positive control.

Methods

The plant samples of *Angiopteris evecta* Hoffm. used in this study were collected from Kanchanaburi Province, Thailand in June 2000. The methanol extract (126 g) of ground *A. evecta* rhizome (2.7 kg, wet wt.) was reextracted with hexane and EtOAc to give hexane extract (2.0 g) and EtOAc extract (12.5 g), respectively. The EtOAc extract (12.5 g) was fractioned by silica gel column chromatography and eluted with hexane-EtOAc gradient in a stepwise fashion. Angiopteroside was obtained from the elution of silica gel column chromatography with 100% ethyl acetate and was purified by re-crystallization with methanol to obtain a white solid crystal. The *in vitro* HIV-1 Reverse Transcriptase inhibitory activity of angiopteroside was determined by the Boehringer Mannheim ELISA kit.[2]

Bioassay of angiopteroside's cytotoxic activity against 6 tumor cell lines; HS 27 (fibroblast), Kato-3 (gastric), BT 474 (breast), Chago (lung), SW 620 (colon) and HEP-G2 (hepatoma), cultured *in vitro* was performed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method. In principle, the viable cell number/well is directly proportional to the production of formazan, which following solubilization, can be measured spectrophotometrically.[3]

RESULTS AND DISCUSSION

The structure of angiopteroside was established by 1D and 2D NMR as shown in Table 1, and its relative configuration was also confirmed by single crystal X-ray diffraction. In addition, the X-ray analysis revealed that this compound is a monoclinic polymorph of angiopteroside monohydrate. This differed from the previous polymorph reported by Hseu[1] (see Figure 1). We used 2′,3′-dideoxyinosine (ddI) as a control substance. Angiopteroside and ddI exhibited HIV-1 Reverse Transcriptase inhibitory activity with IC_{50} at 0.91 μM and 0.87 μM, respectively. From the results, angiopteroside possesses an equivalent inhibitory activity against HIV-1 RT when compared with ddI. Some parts of the structure of angiopteroside is similar to 3′-azido-3′-deoxythymidine (AZT) and 2′, 3′-dideoxyinosine (ddI) (see Figure 2). Since, AZT and ddI are nucleoside reverse transcriptase inhibitors then similarly angiopteroside may act as a terminator of the nascent DNA chain by blocking the viral reverse transcription process upon intercellular phosphorylation. It might become a competitive inhibitor with respect to the deoxynucleoside triphosphate substrate.[4,5] This possibility needs further investigation.

Additionally angiopteroside was tested against several tumor cell-lines. The results in Table 2 suggested that angiopteroside showed anti-tumor activity for lung cancer. The cytotoxicity of angiopteroside appears very specific since it was only active against the lung cell line and was unaffective against other cell-lines including fibroblast. Consequently, the biological activity of angiopteroside for inhibition of HIV-1 RT and lung cancer shows potential as a treatment source and angiopteroside may be a lead compound for further study and drug development.

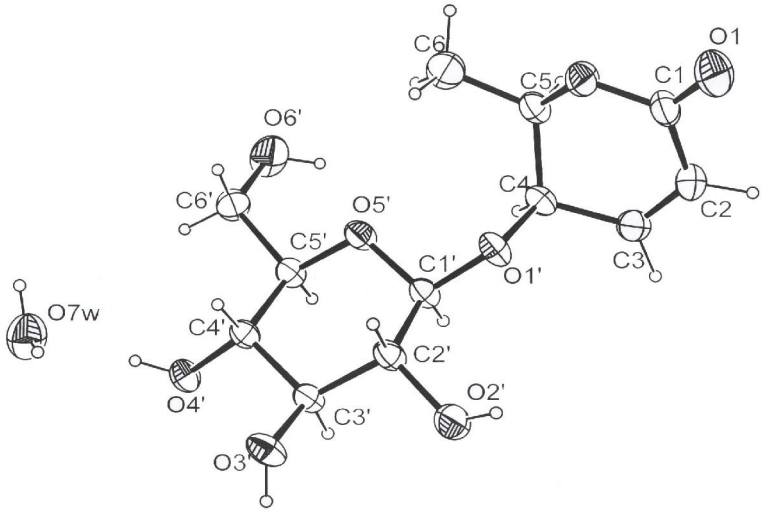

Figure 1. The ORTEP drawing of angiopteroside.

(Crystal data of angiopteroside: $C_{12}H_{18}O_8 \cdot H_2O$, M = 290.2, monoclinic, space group $P2_1$, a = 7.8345(1), b = 6.9233(1), c = 12.9322(1) Å, V = 701.18(2) Å^3, Z = 2, D_{cal} = 1.460 g cm^{-3}, μ = 0.126 mm^{-1}, (Cu-K$_\alpha$, λ = 0.71073 Å), T = 293(2) K, Bruker SMART CCD area detector diffractometer, Θ_{max} 30.46^o, 3533 unique reflections. Structure solution by direct method, full-matrix least squares refinement on F^2 using SHELXL 97 with all non-hydrogen atoms anisotropic and all hydrogen atoms were found from electron density maps. The final cycle converged to R_1 = 0.0299 and wR_2 = 0.0817).

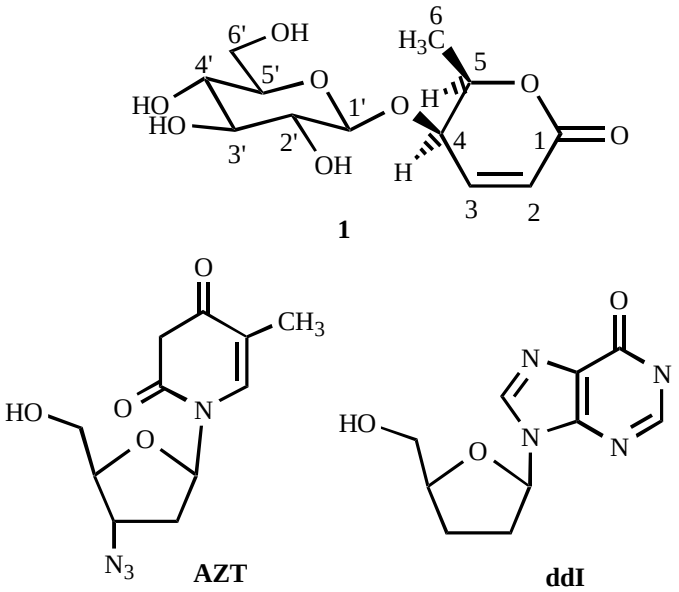

Figure 2. Structures of Angiopteroside(1), AZT and ddI.

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REFERENCES

1. Hseu, T. H. (1981) "Structure of Angiopteroside (4-O-β-D-Glucopyranosyl-L-thero-2-hexane-5-olide) Mono-hydrate, a Fern Glycoside from *Angiopteris lygodiifolia*" Ros. Acta. Cryst. **B37**, 2095 - 2098.
2. Rice, W. G., Schaeffer, C. A., Mcdougal, J. S., Orloff, S. L., Summers, M. F., South, T. L., Mendelevev, J. and Kun, E., (1993) "Novel zinc-ejecting c-nitroso compounds inhibit the infectious and expressive phases of HIV-1 life-cycle" *J. Cell. Biochem.* 53S, 22.
3. Twentyman, P. R., Fox, N. E. and Rees, J. K., (1989) "Chemosensitivity testing of fresh leukaemia cells using the MTT colorimetric assay" Br. *J. Haematol*. **71**, 19-24.
4. De Clercq, E., (2002a) "New anti-HIV agents and targets" *Med. Res. Rev.* **22**, 531-565.
5. De Clercq, E., (2002b) "Strategies in the design of antiviral drugs" *Nat. Rev. Drug. Discov.* **1**, 13-25.

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