Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 16 (2006) 581–583

# Angelmarin, a novel anti-cancer agent able to eliminate the tolerance of cancer cells to nutrient starvation

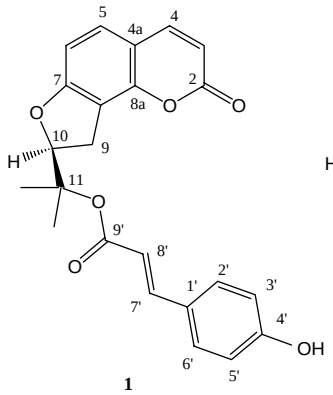
Suresh Awale,[a] Eduardo M. N. Nakashima,[a] Surya K. Kalauni,[a] Yasuhiro Tezuka,[a] Yukiko Kurashima,[b] Jie Lu,[b] Hiroyasu Esumi[b] and Shigetoshi Kadota[a,*]

[a]*Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan*
[b]*Investigative Treatment Division, National Cancer Center Research Institute East, Chiba 277-8577, Japan*

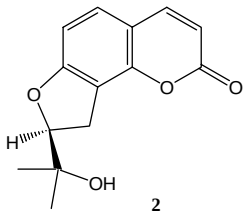
Received 7 September 2005; revised 28 September 2005; accepted 15 October 2005
Available online 8 November 2005

**Abstract**—The $CH_2Cl_2$-soluble extract of *Angelica pubescens* was found to kill PANC-1 cancer cells preferentially under nutrition starvation at a concentration of 50 μg/ml, with virtually no cytotoxicity under nutrient-rich conditions. Further bioassay-guided fractionation and isolation led to the isolation of a novel compound named angelmarin as the primary compound responsible for the preferential cytotoxicity; the compound exhibited 100% preferential cytotoxicity against PANC-1 cells at a concentration of 0.01 μg/ml.


The physiology of solid tumors differs from that of normal tissues in a number of important aspects. Compared to normal cells, tumor cells proliferate extremely rapidly, and the demand for essential nutrients as well as oxygen always exceeds the supply, due to the unregulated growth caused by genetic and epigenetic alterations of the cells and the insufficient and inappropriate vascular supply.[1–3] However, cancer cells show an inherent ability to tolerate extreme conditions, such as that characterized by low nutrient and oxygen supply, by modulating their energy metabolism.[4] Previously, we reported that certain pancreatic cancer cell lines, such as PANC-1, AsPC-1, BxPC-1, and KP-3, exhibit marked tolerance and survive for prolonged periods of time even under extremely nutrient-starved conditions.[4] Thus, we hypothesized that tolerance to nutrient starvation might be a part of the biological response to insufficient blood supply, and the development of drugs aimed at countering this resistance of the cells to nutrient deprivation may serve as a novel biochemical approach to cancer therapy.[4–6] In this regard, we developed a novel screening methodology based on this anti-austerity strategy[6] and screened 500 medicinal plant extracts used in Japanese Kampo medicine. We found from this screening that a $CH_2Cl_2$-soluble extract of *Angelica pubescens* exhibited preferential cytotoxicity against nutrient-deprived cancer cells at the concentration of 50 μg/ml. We thus carried out further bioassay-guided fractionation and isolation, which led to the isolation of a new compound named angelmarin (**1**) as the primary compound responsible for exhibiting preferential cytotoxicity at a concentration of 0.01 μg/ml. In this paper, we report the structure elucidation of angelmarin (**1**) together with its potent in vitro preferential cytotoxic activity.




**1**




**2**

Angelmarin (**1**)[7] was isolated as a colorless amorphous solid and showed $[\alpha]_D^{25}$ +218.7° (*c* 0.025, $CHCl_3$). Its molecular formula was determined by HR-FABMS to

*Keywords*: PANC-1; Anti-austerity; Screening strategy; Angelmarin; *Angelica pubescens*; Anti-cancer agent.
* Corresponding authors. Tel.: +81 76 434 7625; fax: +81 76 434 5059; e-mail: kadota@ms.toyama-mpu.ac.jp

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.046

be $C_{23}H_{20}O_6$ [*m*/*z* 393.1295 $(M+H)^+$]. The IR spectrum of **1** showed the absorptions due to hydroxyl (3280 $cm^{-1}$), ester carbonyl (1705 $cm^{-1}$), and phenyl (1610, 1460 $cm^{-1}$) groups. The $^1H$ NMR spectrum of **1** revealed signals due to two tertiary methyls ($\delta_H$ 1.61, 1.65), an oxygen-substituted methine ($\delta_H$ 5.21), an aliphatic methylene ($\delta_H$ 3.38), two sets of ortho-coupled aromatic protons ($\delta_H$ 6.24, 7.68; 6.79, 7.31), two *trans* olefinic protons ($\delta_H$ 6.14, 7.35), together with two pairs of *p*-substituted phenyl protons ($\delta_H$ 6.83, 7.28). The $^{13}C$ NMR spectrum showed the signals of 21 carbons including those of two ester carbonyls and four $sp^2$ and two oxygenated $sp^3$ carbons, one aliphatic carbon, and two phenyl groups. These data resembled those of columbianetin[8] (**2**) isolated from the same extract, with the exception of the presence of additional signals ascribable to a *p*-hydroxycinnamoyl group. The COSY and HMQC spectra revealed the partial connectivities (bold line) between $C_3$–$C_4$, $C_5$–$C_6$, $C_9$–$C_{10}$, $C_{7'}$–$C_{8'}$ and $C_{2'(6')}$–$C_{3'(5')}$. These partial structures were connected based on the long-range correlations observed in the HMBC spectrum (Fig. 1a), leading to a planar structure of *p*-hydroxycinnamoyl ester of **2**. Furthermore, saponification of angelmarin (**1**)[8] afforded **2** having the optical rotation value ($[\alpha]_D^{25}$ +198°) identical to the reported value ($[\alpha]_D^{22}$ +209°),[9] suggesting the absolute stereochemistry of **1** to be 10*S*. In addition, the CD spectrum of **1** showed a positive Cotton effect ($[\theta]_{338}$ +34314, $[\theta]_{298}$ −16932) due to an exciton chirality (Fig. 1b),[10,11] suggesting the spatial orientation of two chromophores to be in a clockwise fashion (Fig. 1b).[9,10] Thus, the structure of **1** was concluded as 11-*O*-(*p*-hydroxycinnamoyl)columbianetin.

Angelmarin (**1**) was tested for its preferential cytotoxicity using PANC-1 cancer cell line.[12] As shown in Figure 2, PANC-1 is highly resistant to even extreme nutrient deprivation and can survive under this condition for more than 48 h.[5] In this study, this tolerance to nutrient deprivation was remarkably eliminated by angelmarin (**1**) in a concentration- as well as time-dependent manner. Cells exposed to angelmarin (**1**) at a concentration of 0.01 μg/ml showed 100% cell death within 24 h of starvation, and this sensitivity to starvation was more pronounced when angelmarin (**1**) was added at a concentration of 10 μg/ml, at which 100% cell death was observed within 6 h. On the other hand, preferential cytotoxicity test for columbianetin (**2**) and *p*-hydroxycinnamic acid indicated that they are inactive in both nutrient-rich and nutrient-deprived condition at a concentration of 100 μg/ml. This suggests the requirement of unique structural feature of angelmarin (**1**) to expose preferential cytotoxic activity.

Pancreatic cancer is one of the most serious forms of cancer associated with the lowest 5-year survival rates known for cancers,[13,14] and continues to be one of the major health problems that remain unresolved at the start of the 21st century. Currently, surgery is the only treatment modality that offers any prospect of potential cure.[14,15] It shows resistance to almost all known chemotherapeutic agents such as 5-fluorouracil, taxol, doxorubicin, cisplatin, and camptothecin, and exhibits only minimal activity as a single agent in pancreatic cancer.[14] Moreover, these agents were found to be virtually inactive under nutrient-deprived conditions, while they exhibit only weak toxicity under normoxic conditions.[6] This indicates a different mechanism that may underlie the anti-tumor activity of angelmarin (**1**) against tumor cells growing in a low-nutrient and low-oxygen environment. Further studies on its mechanism of action and in vivo anti-tumor activity are underway and will be reported as a full paper.

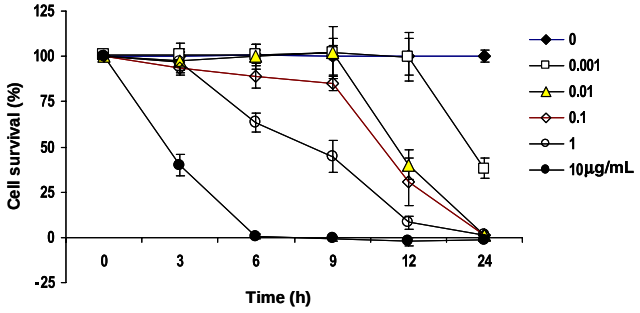



**Figure 2.** Effect of angelmarin (**1**) on survival of PANC-1 cells under nutrient-deprived conditions.

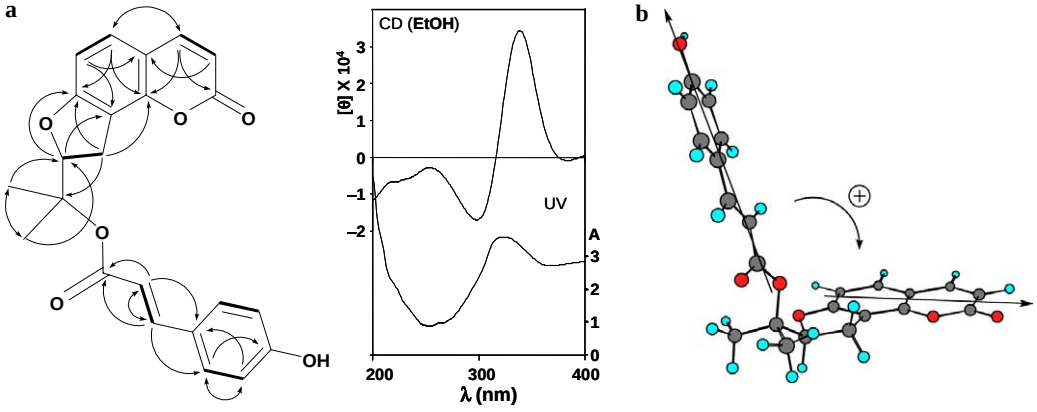



**Figure 1.** (a) $^1H$–$^1H$ COSY (bold line) and key HMBC correlations (arrows) observed for **1** and (b) CD and UV spectra of **1**.

## Acknowledgments

This work was supported in part by a grant from the Ministry of Health and Welfare for the Second-Term Comprehensive 10-year Strategy for Cancer Control and Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare and the Ministry of Education, Science, Sports and Culture.

## References and notes

1. Dang, C. V.; Semenza, G. L. *Trends Biochem. Sci.* **1999**, *24*, 68.
2. Southerland, R. M. *Science* **1988**, *240*, 178.
3. Helmlinger, G.; Yuan, F.; Dellian, M.; Jain, R. K. *Nat. Med.* **1997**, *3*, 177.
4. Izuishi, K.; Kato, K.; Ogura, T.; Kinoshita, T.; Esumi, H. *Cancer Res.* **2000**, *60*, 6201.
5. Lu, J.; Kunimoto, S.; Yamazaki, Y.; Kaminishi, M.; Esumi, H. *Cancer Sci.* **2004**, *95*, 547.
6. Esumi, H.; Lu, J.; Kurashima, Y.; Hanaoka, T. *Cancer Sci.* **2004**, *95*, 685.
7. **Angelmarin** (**1**). Colorless amorphous solid. $[\alpha]_D^{25}$ +218.7° (*c* 0.025, $CHCl_3$). IR $\nu_{max}$ ($CHCl_3$) $cm^{-1}$ 3280, 1910, 1705, 1610, 1585, 1506, 1490, 1460, 1405, 1388, 1370, 1325, 1260, 1168, 1140, 1065, 980, 835. CD $\lambda_{max}$ (EtOH, 0.255 mM) nm: 338 ([$\theta$] +34314), 298 ([$\theta$] −16932). HR-FABMS 393.1295 [calcd for $C_{23}H_{21}O_6$ $(M+H)^+$ 393.1338]. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 1.61 (3H, s, $H_3$-13), 1.65 (3H, s, $H_3$-12), 3.38 (2H, ddd, *J* = 16.3, 9.5, 8.0 Hz, $H_2$-9), 5.21 (1H, dd, *J* = 9.5, 8.0 Hz, H-10), 6.14 (1H, d, *J* = 15.9, H-8′), 6.24 (1H, d, *J* = 9.5 Hz, H-4), 6.79 (1H, d, *J* = 8.3 Hz, H-6), 6.83 (2H, d, *J* = 8.5 Hz, H-3′,5′), 7.28 (2H, d, *J* = 8.5 Hz, H-2′,6′), 7.31 (1H, d, *J* = 8.3 Hz, H-5), 7.35 (1H, d, *J* = 15.9, H-7′), 7.68 (1H, d, *J* = 9.5 Hz, H-3). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 21.1 (C-13), 22.1 (C-12), 27.5 (C-9), 82.2 (C-11), 89.1 (C-10), 106.9 (C-6), 111.98 (C-3), 113.0 (C-4a), 113.6 (C-8), 114.5 (C-7′), 115.9 (C-3′,5′), 116.1 (C-8′), 126.6 (C-1′), 128.9 (C-5), 129.8 (C-2′,6′), 144.3 (C-4), 151.1 (C-8a), 158.3 (C-4′), 161.5, (C-2), 164.1 (C-7), 166.5 (C-9′).
8. Angelmarin (**1**, 30 mg) was treated with LiOH in MeOH–$H_2O$ (1 N, 2 ml) with stirring at room temperature for 28 h. The reaction mixture was then neutralized by adding 1 N HCl (2 ml) and extracted with EtOAc (2 ml × 3). The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by preparative TLC (acetone–$CHCl_3$, 2:8) to afford **2** (8 mg) with $[\alpha]_D^{25}$ +198° (*c* 0.025, $CHCl_3$).
9. Perel'son, M. E.; Nikonov, G. K.; Pek, G. Y.; Sheinker, Y. N. *Dokl. Akad. Nauk SSSR* **1964**, *159*, 154.
10. Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989.
11. Morita, H.; Dota, T.; Kobayashi, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3665.
12. PANC-1 cancer cells were seeded in 96-well plates ($1 \times 10^4$/well) and incubated in fresh DMEM at 37 °C under a 5% $CO_2$/95% air for 24 h. The cells were then washed with PBS, and the medium was changed to either DMEM or nutrient-deprived medium (absence of glucose, amino acid, and serum), followed by immediate addition of serial dilutions of the test samples. After 24-h incubation, the cells were washed again with PBS, then 100 μl of DMEM with 10% WST-8 cell counting kit solution was added to the wells, and the plate was incubated for a further 2 h. Then, the absorbance of the wells at 450 nm was measured. The viable cell number was determined using a previously prepared calibration curve (Dojindo, Kumamoto).
13. Li, D.; Xie, K.; Wolff, R.; Abbruzzese, J. L. *Lancet* **2004**, *363*, 1049.
14. Shore, S.; Vimalachandran, D.; Raraty, M. G. T.; Ghaneh, P. *Surg. Oncol.* **2004**, *13*, 201.
15. Chung, H. W.; Bang, S. M.; Park, S. S.; Chung, J. B.; Kang, J. K.; Kim, Ju. W.; Seong, J. S.; Lee, W. J.; Song, S. Y. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *60*, 1494.