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Cytotoxic triterpenes from *Antrodia camphorata* and their mode of action in HT-29 human colon cancer cells

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ABSTRACT

Five lanostane (**2, 3, 4**, **6** and **8**) and three ergostane-type (**1**, **5** and **7**) triterpenes isolated from the fruiting bodies of *Antrodia camphorata* were evaluated for their *in vitro* cytotoxic data against various cancer cell types. The three zhankuic acids, **1**, **5** and **7** displayed the most potent cytotoxic effect with an IC_{50} value of 22.3–75.0 μM. The compound **3** was selectively cytotoxic in three colon cancer cell lines (HT-29, HCT-116 and SW-480) and a breast cancer model (MDA-MB-231), whereas **8** only showed its cytotoxicity against MDA-MB-231. None of these isolates was toxic to mammary epithelial (MCF10A) and primary foreskin fibroblast (HS68) cells, two human normal cell lines. The compounds **1**, **5** and **7** were also demonstrated to induce apoptosis in HT-29 and SW-480 cells, as confirmed by sub-G1 cell cycle arrest. In HT-29 cells, the expression of apoptosis-associated proteins poly-(ADP-ribose) polymerase cleavage, Bcl-2 and procaspase-3 were suppressed by compounds **1**, **5** and **7**. A mixture containing 4 μM each of compounds **1**, **5** and **7** also showed a synergistic cytotoxic effect in HT-29 cells.

1. Introduction

Cancer is a major cause of death worldwide and causes serious problems in human life, including mental and physical agony and economic strain. Therefore, many kinds of cancer therapies, including various anticancer agents, have been developed. However, they also have several problems such as serious side effects and drug resistance [1]. To resolve these difficulties, development of cancer chemopreventive agents and improvement of cancer treatment are very important. Accordingly, screening of natural products as potential anticancer agents, in the form of functional foods or nutraceuticals has become an important undertaking [2]. The rising interest in the pharmacological properties of natural triterpenoids [3] led us investigate *in vitro* cytotoxicities of five lanostane (**2, 3, 4**, **6** and **8**) and three ergostane-type (**1**, **5** and **7**) triterpenes isolated from *Antrodia camphorata* (Polyporaceae).

A. camphorata is a parasitic fungus grown on the hardwood of *Cinnamomum kanehirai* Hay (Lauraceae), has been widely used as a Chinese remedy for food and drug intoxication, diarrhea, abdominal pain, hypertension and cancer [4]. Crude extracts from the fruiting bodies and mycelium of *A. camphorata* show potent anticancer activities in human leukemia HL-60, breast cancer MDA-MB-231 (estrogen-nonresponsive) and MCF-7 (estrogen-responsive)

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cells, but not in healthy breast cells (HBL100) and umbilical vein endothelial cells [5–7]. The extracts from fruiting bodies show cytotoxic effect in bladder cancer cells and arrest the cell cycle in the G2/M phase [8]. The ethanol extract from mycelium inhibits the proliferation of human lung cancer cell A-549, but not the normal fetal lung fibroblast MRC-5 cell [9]. The methanol extract of mycelium inhibits cell viability and induces apoptosis in Hep G2 via G0/G1 cell cycle arrest followed by the activation of the caspase-3 and -8 cascades [10]. The ethyl acetate extract (EAC) inhibits cell growth in two liver cancer cells, Hep G2 and PLC/PRF/5 through the regulation of Bcl-2 family protein expression [11]. *A. camphorata* treatment could be effective in inhibiting breast cancer cell MDA-MB-231 proliferation and inducing apoptosis *in vitro* and *in vivo* [12]. Interestingly, the authors also show the nontoxicity of *A. camphorata* with a daily oral administration of 500 mg/kg for 28 days in rats, which increase its potential for application in food and drug products [13]. Hence, it is of significant interest to isolate and identify such exact compounds which are responsible for the anticancer activity of this fungus.

As part of a study program to evaluate the therapeutic properties of *A. camphorata*, this paper has demonstrated the *in vitro* cytotoxic effects of eight triterpenoids (Fig. 1) against various cancer types: colon, liver, breast and lung cancer cell lines. Although the *in vitro* cytotoxic activity of zhankuic acids A (**5**) and C (**7**) in P-388 murine leukemia cells had been reported [14], however no information about their mechanism of action. In this study, among eight selected compounds, three ergostane-types were capable of blocking cell cycle progression at the sub-G1 phase and inducing apoptosis through the cleavage of the downstream poly(ADP-ribose) polymerase, pro-caspase-3 and Bcl-2. In addition, the combination of ergostane-triterpenes exhibited a potential synergistic cytotoxic effect in HT-29 cells.

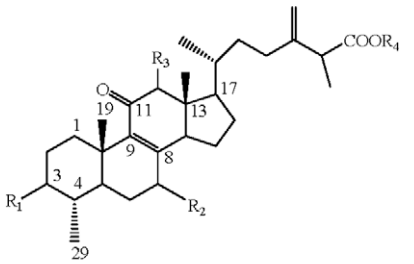
2. Materials and methods

2.1. Materials

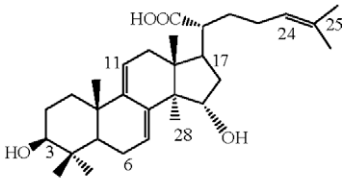
All the materials were obtained commercially and used without further purification. NMR spectra were measured on a Varian Unity Inova-600 VXR-300/51 spectrometer with TMS as an internal standard. Silica gel for column chromatography (CC) (0.063–0.200 mm), was a product of Merck Company. TLC was performed on Merck TLC plates (0.23 mm thickness), with compounds visualized by spraying with 8% (v/v) H_2SO_4 in ethanol and then heating on a hot plate. The wild fruiting bodies of *A. camphorata* were collected from the Yuli, Hualien County, Taiwan, in December 2006. A voucher specimen (YMT 6002) was deposited in the Herbarium of the Institute of Biochemical Sciences and Technology, Chaoyang University of Technology, Taiwan, ROC.

2.2. Extraction and isolation

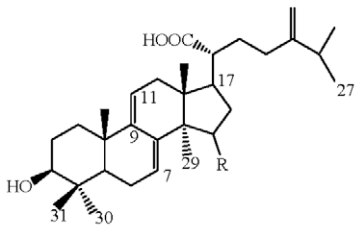
The compounds **1**–**8** were separated from the fruiting bodies of *A. camphorata*, according to the extraction and isolation procedures described by Male et al. [15]. Briefly, air-dried powder of *A. camphorata* was extracted with $CHCl_3$ using a soxhlet extractor. After solvent evaporation, the residue subjected to silica gel column chromatography was eluted with increasing polarity using mixtures of *n*-hexane/EtOAc. Following the TLC analysis, eluates of



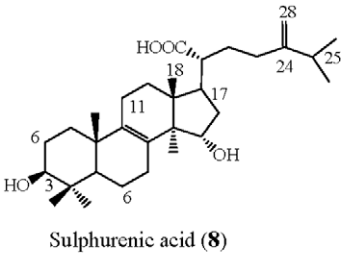
Methyl antcinate B (**1**): R_1=R_2=(=O), R_3=H, R_4=CH_3
Zhankuic acid A (**5**): R_1=R_2=(=O), R_3=R_4=H
Zhankuic acid C (**7**): R_1=R_3=(α-OH), R_2=(=O), R_4=H



3β, 15α-dihydroxy lanosta-7,9 (11), 24-triene-21-oic acid (**4**)



Dehydroeburicoic acid (**2**): R=H
15α-Acetyl-dehydrosulphurenic acid (**3**): R= (α-OAc)
Dehydrosulphurenic acid (**6**): R=(α-OH)



Sulphurenic acid (**8**)

Fig. 1. Chemical structures of the eight isolates from *Antrodia camphorata*.