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Compounds with DNA cleaving activity from *Kadsura ananosma*

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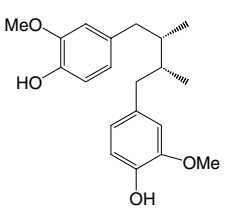
Two DNA cleavage agents, meso-dihydroguaiaretic acid (**1**) and isobavachalcone (**2**) together with the known α-ylangene, β-sitosterol, daucosterol, pentacosane, hexacosanic acid and cerotic acid 1-monoglyceride were isolated from the stem barks of *Kadsura ananosma* Kerr for the first time. Compounds **1** and **2** showed relaxation of supercoiled DNA to nicked DNA. **1** represented a new structural type of DNA cleavage agent, while **2** was reported to show DNA strand-scission activity for the first time. **1** also showed significant cytotoxic effect on Hela and Leukemia cells *in vitro*.

DNA strand breakage process is involved in various biological stages such as inflammation, mutagenesis, carcinogenesis, or aging (Mibu et al. 2003). As a consequence of the clinical utility of DNA cleavage agents such as bleomycin, considerable effort has been made to identify and characterize naturally occurring molecules capable of mediating DNA strand scission, as such species may serve as lead structures for the development of novel anti-tumor drugs (Ma et al. 2004; Seo et al. 2003). *Kadsura ananosma* Kerr (Schisandraceae) is a plant indigenous to Yunnan, China (Yunnan Provincinal Crude Drugs Company, 1993). Previously, a lignan, four sesquiterpenoids and three triterpenoid acids showing cytotoxicity against CCRF-CEM leukemia cells and HeLa cells were isolated from the plant (Chen et al. 2004, 2001; Zou et al. 1993). In the course of our search for plant derived DNA cleavage agents, we isolated two DNA strand-nicking principles from the plant stem barks of *K. ananosma* and the results are reported herein.

By repeated column chromatography of a CH_2Cl_2 extract of the stem bark eight compounds were obtained. By spectroscopic analysis (NMR and MS) and literature comparison, their structures were elucidated as meso-dihydroguaiaretic acid (**1**), isobavachalcone (**2**), α-ylangene (**3**), β-sitosterol (**4**), daucosterol (**5**), pentacosane (**6**), hexacosanic acid (**7**) and cerotic acid 1-monoglyceride (**8**) (Chen et al. 1998; Yu et al, 2005; Hunter et al. 1964). All of the compounds were isolated from the plant for the first time.

In view of the structural similarity of **1** and **2** with the natural DNA cleavage agents resveratrol (Fukuhara et al. 1998) and hamilcone (Huang et al. 1996), compounds **1** and **2** were tested for their ability to relax PBR 322 plasmid DNA, a supercoiled, covalently closed, circular DNA in the presence of Cu^{2+}, using a cell free DNA cleavage assay (Deng et al. 2000). Compound **1** showed 57% relaxation of supercoiled DNA (form I) to nicked DNA (form II) at 5 μg/ml in the presence of 20 μM Cu^{2+}, while **2** was found to relax 49% of form I DNA to form II DNA at 10 μg/ml in the presence of 20 μM Cu^{2+}. Compound **1** represented a new structural type of DNA cleavage agent, while **2** was reported to show DNA strand-scission activity for the first time. In the cytotoxicity assays, **1** exhibited cytotoxic activities on Hela and leukemia cells, with IC_{50} values of 3.6 and 36 μg/ml, respectively. It proved to be more cytotoxic to Hela cells than to leukemia cells.



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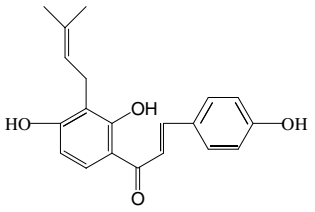

2

Experimental

MS were determined on an API Qstar Pulsa LC/TOF mass spectrometer. NMR spectra were measured on a Bruker DRX-500 spectrometer with TMS as int. standard. Optical rotation was run on a Horiba-SEPA-300 polarimeter. Silica gel (200–300 mesh) was used for column chromatography and silica gel GF_{254} for TLC (Qingdao Marine Chemical Co., China). Solvents were of the industrial purity and distilled prior to use. The stem bark of *K. ananosma* was collected from Men-Na County of Yunnan Province, China in February 1998, and identified by Mr. Hong Wang, a botanist of Xi-Shuang-Bang-Na Botanical Garden, Chinese Academy of Sciences, where a voucher specimen (No.9802015) is deposited.

The dried powered stem bark of *K. ananosma* (6.0 kg) was extracted with CH_2Cl_2 exhaustively. The CH_2Cl_2 extract was concentrated *in vacuo* to give a residue (500 g). From this, 83 g were subjected to silica gel column chromatography eluting with 400 ml portions of a solvent gradient system with increasing polarity from petroleum ether to ethyl acetate to offer eight fractions (A–I). Fractions B, F and G were subjected to repeated column chromatography to yield **1** (260 mg), **2** (20 mg), **3** (9 mg), **4** (12 mg), **5** (20 mg), **6** (50 mg), **7** (300 mg) and **8** (50 mg).

Meso-dihydroguaiaretic acid (**1**), white amorphous powder; m.p. 85–86 °C; $[\alpha]_D^{25}$ 0 ° (c 0.283, MeOH); ^{1}H NMR ($CDCl_3$) δ 6.80 (2 H, d, J = 8.0), 6.64 (2 H, dd, J = 8.0, 1.3), 6.59 (2 H, d, J = 1.3), 3.84 (6 H, s, OMe × 2), 2.71 (2 H, dd, J = 13.5, 5.0), 2.26 (2 H, dd, J = 13.5, 9.2), 1.74 (2 H, m, CH × 2), 0.82 (6 H, d, J = 6.7 Hz, Me × 2); ^{13}C NMR ($CDCl_3$) δ 16.3, 39.1, 39.4, 56.2, 112.1, 114.6, 122.4, 134.6, 144.4, 147.2; EIMS m/z (rel. int.): 330 (M^+, 86), 165(23), 137(100), 122(39).

Isobavachalcone (**2**), yellow amorphous powder; ^{1}H NMR (CD_3COCD_3) δ 14.00 (1 H, s, OH), 9.43 (1 H, s, OH), 9.08 (1 H, s, OH), 7.98 (1 H, d, J = 8.9), 7.84 (1 H, d, J = 15.4), 7.77 (1 H, d, J = 15.1), 7.74 (2 H, d, J = 6.6), 6.93 (2 H, d, J = 6.7), 6.53 (1 H, d, J = 8.9), 5.28 (1 H, m), 3.37 (2 H, d, J = 7.3), 1.79 (2 H, d, J = 7.4), 1.65 (3 H, d, J = 8.5); ^{13}C NMR (CD_3COCD_3) δ 192.2, 164.3, 161.9, 160.1, 144.0, 130.8, 130.8, 130.6, 129.3, 126.7, 122.4, 117.6, 115.9, 115.9, 115.3, 113.5, 107.1, 25.0, 21.4, 17.0; EIMS m/z (rel. int.): 324 (M^+, 78), 281 (85), 269 (61), 203 (50), 189 (63), 176 (76), 161 (92), 149 (100), 120 (85), 107 (54), 91 (74), 77 (46), 65 (59).

The DNA strand-scission assay was performed based on a modified Hecht procedure (Deng et al. 2000; Huang et al. 1996). Compounds were dissolved in DMSO-MeOH (1 : 1); 1 μl of each sample was added to a 13-μl reaction mixture (total volume) containing 600 ng of PBR 322 DNA and 20 μM $CuCl_2$ in 10 mM tris-HCl, pH 8.0. The reactions were incubated at 37 °C for 1 h, terminated by addition of 2 μl of 0.125% bromophenol blue in 30% glycerol, and applied to a 1% agarose gel containing 0.7 μg/mL ethidium bromide. The gel was run in 89 mM Tris containing 8.9 mM boric acid and 2.0 mM Na_2-EDTA at 110 V for 1 h, then visualized by UV irradiation. Agarose gels were quantified for percent DNA cleavage utilizing 1D Image Analysis software, Windows Extra Package, Version 3.5.

Cytotoxicity assays (IC_{50} μg/mL) were carried out against two types of cancer cells including murine leukemia (ATCC: CCRF-CEM) and Hela (ATCC-17). Methodology of the *in vitro* cytotoxicity screening was con-

ducted by measuring toxicity against cancer cells using the NIH-NCI protocol (Grever et al. 1992; Alley et al. 1988). Cytotoxicity was determined by measuring cell viability.

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References

Alley MC, Scudiero DA, Monks PA et al. (1988) Feasibility of drugs screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 48: 589–601.

Chen YG, Qin G.W, Xie YY et al. (1998) Lignans from *Kadsura angustifolia*. J Asian Nat Prod Res 1: 125–131.

Chen YG, Hai LN, Liao XR et al. (2004) Ananosic acids B and C, two new 18 (13→12) abeo lanostane triterpenoid from *Kadsura anaosma*. J Nat Prod 67: 875–877.

Chen YG., Xie YY, Cheng KF et al. (2001) Compounds from *Kadsura ananosma*. Phytochemistry 58: 1277–1280.

Deng JZ, Newman DJ, Hecht SM (2000) Use of Compare analysis to discover functional analogues of bleomycin. J Nat Prod 63 : 1269–1272.

Fukuhara K, Miyata N (1998) Resveratrol as a new type of DNA-cleaving agent. Bioorg Med Chem Lett 8: 3187–3192.

Grever MR, Schepartz SA, Chabner BA (1992) The National Cancer Institute: Cancer Drug Discovery and Development Program, Seminars in Oncology 19: 622–638.

Huang L, Fullas F, Mcgivney RJ et al. (1996) New compounds with DNA strand-scission activity from the combined leaf and stems of *Uvaria hamiltoni*. J Nat Prod 59: 290–292.

Hunter GL, Brogden WB (1964) Structure of ylangene. J Org Chem 29: 982–983.

Ma J, Hecht SM (2004) Javaniside, a novel DNA cleavage agent from *Alangium javanicum* having an unusual oxindole skeleton. Chem Commun: 1190–1191.

Mibu N, Yukawa M, Kashige N et al (2003) Synthesis and DNA strand breakage activity of some 1,4-diazepines. Chem Pharm Bull 51: 27–31.

Seo EK, Lee D, Shin YG et al (2003) Bioactive prenylated flavonoids from the stem bark of *Artocarpus kemando*. Arch Pharm Res 26: 124–127

Yunnan Provincinal Crude Drugs Company (1993) Name Lists of Chinese Herbal Medicine Resources in Yunnan. Science Press, Beijing, p. 151.

Yu LL, Chen YG, Liu JC et al (2005) Chalcones from the seeds of *Psoralea corylifolia*. Polish J Chem 79: 1173–1178.

Zou C, Pu XY, Zhou J (1993) Sesquiterpenoids from *Kadsura ananosma*. Acta Botanica Yunnanica 15: 196–200.