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Bicyclononane aldehydes and antiproliferative constituents from *Amomum tsao-ko*

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Abstract

A racemic mixture of a new bicyclononane aldehyde, (1RS,5SR,6RS)-5-hydroxybicyclo[4.3.0]non-2-ene-2-carbaldehyde (**1**) was isolated from the fruits of *Amomum tsao-ko*, together with twelve known compounds (**2-13**). The structure of **1** was determined on the basis of extensive spectroscopic analysis, including 1 D and 2 D NMR data. The antiproliferative activity of compounds **1-13** was assessed in the murine neuroblastoma cell line, N2a.

Key words

Amomum tsao-ko, Zingiberaceae, bicyclic aldehydes, antiproliferative activity

Amomum tsao-ko Crevost et Lemaire (Zingiberaceae) has been used for centuries as food, spice and perfume in China, Japan and Korea. Its fruits also have found applications in traditional Chinese medicine (TCM) for the treatment of stomach illness, digestive disorders and throat infections [1]. In previous phytochemical investigations of the species, bicyclononane aldehydes, tsaokoin, isotsaokoin [2], [3], [4], [5], 6-hydroxyindan-4-carbaldehyde [2], trans-2,3,3a,7a-tetrahydro-1H-indene-4 -carbaldehyde, *cis*-2,3,3a,7a-tetrahydro-1H-4-carbaldehyde, 4-indanecarbaldehyde and 5-indanecarbaldehyde [5], diarylheptanoids such as curcumin, tsaokoarylone, 1,7-bis(4-hydroxyphenyl)hepta-4*E*,6*E*-dien-3-one, (+)-hannokinol, meso-hannokinol and 6-(4-hydroxyphenyl)-4-hydroxyhexan-2-one [6], and catechin [7] have been reported.

The identification of a bicyclic aldehyde as active compound in a yeast-based screening for phosphoinositide-3 kinase (PI3K) (unpublished results) led us to extend our search to other known sources for aldehydes, such as *Amomum tsao-ko*. We here report on the isolation of a new bicyclononane aldehyde, (1RS,5SR,6RS)-5-hydroxybicyclo[4.3.0]non-2-ene-2-carbaldehyde (**1**) and twelve known compounds **2-13** from the fruits of this species, and on their antiproliferative activity in the murine neuroblastoma cell line, N2a .

Compound **1** (Fig. 1) was obtained as pale brown oil. A molecular formula of $C_{10}H_{16}O_2$, identical with that of tsaokoin (**3**), was established on the basis of the HRESIMS ($[M+Na]^+$, *m/z* 189.0902). UV absorption maxima at 222 and 232 nm indicated the presence of an *α,β*-unsaturated aldehyde [3]. The ^{1}H and ^{13}C-NMR spectral data of **1** (Table 1) and tsaokoin (**3**) were only slightly different and suggested that **1** was an

isomer of **3**. Combined analysis of the ^{13}C NMR spectrum (one aldehyde carbonyl, five methine, and four methylene carbons), the ^{1}H-^{1}H COSY spectrum (cross-peaks: H-1/H-6 and H-9, H-3/H_2-4, H-5/H_2-4 and H-6, H_2-7/H-6 and H-8, and H_2-9/H-8 and H-1), and key HMBC correlations (H-1/C-9 and C-10, H-3/C-1, C-5 and C-10, H_2-4/C-2 and C-6, H-5/C-1, C-3 and C-7, H-6/C-2, C-8 and C-9, and H-8/C-1 and C-6) established the planar structure of **1** as 5-hydroxybicyclo[4.3.0]non-2-ene-2-carboxaldehyde. The relative stereochemistry of **1** was established by the analysis of ^{1}H coupling constants and NOESY spectra. NOESY difference experiments confirmed spatial proximity of H-1 and H-9α, and enhancement of H-4β, H-5, H-7β and H-9β upon presaturation of H-6. Thus, the two rings were *trans*-fused, and H-1 and H-6 located on the opposite faces of the molecule (Fig. 2). The splitting pattern and small coupling constants for H-5 [δ_H = 4.15 (1H, d, J = 4.4 Hz)] indicated that the oxygenated proton was pseudoequatorial (β-oriented) and the hydroxyl group at C-5, therefore, in α-position. Also, a large vicinal coupling constant of 9.8 Hz between H-6 and H-7α suggested that H-6 was pseudoaxial (β-oriented) in the six-membered ring. Since the compound showed no optical rotation, compound **1** was concluded to be racemic. Thus, the structure of **1** was established as (1RS,5SR,6RS)-5-hydroxybicyclo[4.3.0]non-2-ene-2-carbaldehyde. Related aldehydes, such as tsaokoin and isotsaokoin, have also been reported as optically inactive compounds [4].

Compounds **2**-**13** were identified as 6-hydroxyindan-4-carbaldehyde (**2**) [2], tsaokoin (**3**) [3], [4], (2*E*,6*E*)-8-hydroxy-2,6-dimethyl-2,6-octadienal (**4**) [8], tsaokoarylone (**5**) [6], (2*E*,8*E*)-10-hydroxy-decadienal (**6**) [8], 4-hydroxy-benzaldehyde (**7**) [9], 4-methoxy-3-hydroxy-benzaldehyde (**8**) [9],

(2*E*,6*E*)-8-hydroxy-2,6-dimethyl-2,6-octadienal acetate (**9**) [8], (*2E*)-decenol (**10**) [10], geraniol (**11**) [11], (2*E*)-decenal (**12**) [12] and geraniol acetate (**13**) [11] by comparison of spectroscopic data with published values (UV, NMR and MS). To our knowledge, bicyclo[4.3.0]nonane-2-aldehydes have been found only in this plant species [2], [4], [5]. Interestingly, isotsaokoin previously reported to co-occur with tsakoin has not been detected in the course of the current investigation. The linear aldehyde 10-hydroxy-decadienal (**6**) was obtained for the first time from a natural source.

The antiproliferative activity of compounds **1-13** was assessed in the murine neuroblastoma cell line, N2a, by means of the MTT assay. Compounds **5** and **6** showed antiproliferative activity with IC_{50} values between 46 and 52 µM. The other compounds were inactive at concentrations up to 200 µM. Besides diarylheptanoid **5**, only linear aldehyde **6** showed antiproliferative activity, whereas cyclic, branched and unsaturated aldehydes **1-4**, **7-9**, and **12** were inactive. Antiproliferative activity thus seems not conferred solely by the aldehyde moiety.

Materials and Methods

Optical rotations were measured on a M341 polarimeter (Perkin Elmer, USA). EIMS data were obtained on a GCMS QP 5000 (Shimadzu, Japan) at an ionizing voltage of 70 eV. HR-ESIMS were recorded on a microTOF ESI-MS system (Bruker Daltonics, Germany) connected to an 1100 series HPLC (Agilent; Waldbronn, Germany). The NMR spectra were recorded in $CDCl_3$ or CD_3OD on an AVANCE III 500 MHz spectrometer equipped with a 1 mm TXI Microprobe (Bruker BioSpin, Fällanden, Switzerland). Flash chromatography was carried out on a Biotage Silica gel 60 cartridge

using a Bottomless Solvent Reservoir (Analogix; Burlington, WI, USA).

Semi-preparative HPLC was carried out on an Agilent 1100 system consisting of a quaternary pump, degasser and PDA coupled to a liquid handler 215 (Gilson; Mettmenstetten, Switzerland) as injector. Separations were performed on a Waters Sunfire^{TM} C18 column (5 µm, 10 x 150 mm) (Waters, Ireland). Silica gel 60 F_{254} precoated Al sheets (0.25 mm) and silica gel 60 GF_{254} plates (1.00 mm) (both Merck; Darmstadt, Germany) were used for TLC and preparative TLC, respectively.

The dried fruits of *A. tsao-ko* were purchased from Yong Quan GmbH (Hagen, Germany) in October 2007. A voucher specimen (P 398) is deposited at the Institute of Pharmaceutical Biology, University of Basel, Switzerland. The fruits (1.0 kg) were milled and extracted at room temperature exhaustively with EtOAc (5 L) for 48 h. After evaporation of the solvent, a viscous extract (34 g) was obtained. A portion (30 g) of the EtOAc extract was separated on a silica gel (40-63 µm) column (8 x 80 cm) eluted with a gradient of petroleum ether/EtOAc [7 : 1 (Fraction 1, 0.9 L, 4.2 g) → 6 : 1 (Fraction 2, 0.8 L, 2.5 g) → 5 : 1 (Fraction 3, 0.9 L, 2.1 g) → 4 : 1 (Fraction 4, 1.5 L, 3.7 g) → 3 : 1 (Fraction 5, 0.9 L, 1.2 g) → 2 : 1 (Fraction 6, 0.8 L, 3.4 g) → 1 : 1 (Fraction 7, 0.8 L, 3.7 g) → 0 : 1 (Fraction 8, 0.6 L, 3.8 g)] to yield 8 fractions. Fraction 7 (3.2 g) was chromatographed on a Biotage Silica gel 60 cartridge (150 x 40 mm) with petroleum ether/EtOAc (3 : 2) to afford subfractions 7.1 to 7.5. Subfraction 7.3 (0.6-0.9 L, 210 mg) was submitted to semi-preparative HPLC (H_2O : CH_3CN 95% : 5 → 0% : 100%, 25 min, 5 mL/min) to give **1** (0.9 mg, 0.00012%, t_R 12.5 min), **3** (51 mg, t_R 10.6 min) and **4** (2.7 mg, t_R 13.7 min). Subfraction 7.5 (1.1-1.2 L, 170 mg)was also separated by semi-preparative HPLC (H_2O : CH_3CN 95% : 5 → 0% : 100%, 25 min, 5 ml/min) to give **5** (2.5 mg, t_R 9.2 min) and **6** (1.9 mg, t_R 15.9 min). Fraction 6 (3.0 g) was separated by a Biotage Silica gel 60 cartridge (150 x 40 mm, petroleum ether/EtOAc 2 : 1) to

afford subfractions 6.1-6.4. Subfraction 6.3 (600-900 mL, 121 mg) was submitted to semi-preparative HPLC (H_2O : CH_3CN 95% : 5 → 0% : 100%, 25 min, 5 mL/min) to give **2** (2.1 mg, t_R 11.2 min), **7** (2.1 mg, t_R 7.2 min) and **8** (2.4 mg, t_R 8.9 min). Fraction 5 (500 mg) was purified by a Sephadex LH-20 column ($CHCl_3$/MeOH, 1 : 3, v/v, 0.5 L) to afford **9** (3.9 mg). Fraction 4 (1.5 g) was purified by a Biotage Silica gel 60 cartridge (150 x 40 mm, petroleum ether/EtOAc 2 : 1) and preparative TLC (Hexane/EtOAc 4 : 1) to afford **10** (17 mg) and **11** (8.7 mg). Purification of fraction 2 (1.3 g) by Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1, v/v, 1.5 L) and preparative TLC (Hexane/EtOAc 10 : 1) led to **12** (67 mg) and **13** (15 mg), respectively.

(1RS,5SR,6RS)-5-hydroxybicyclo[4.3.0]non-2-ene-2-carbaldehyde (**1**): Pale brown oil; $[\alpha]_D^{25}$: 0 (c, 0.6, $CHCl_3$); UV (MeOH) λ_{max} (nm): 222, 232; ^{1}H-and ^{13}C-NMR: see Table 1; HR-ESIMS: *m/z* = 189.0902 $[M+Na]^+$ (calcd. for $C_{10}H_{14}O_2Na$: 189.0891); EIMS: *m/z* (rel. int.) = 166 $[M]^+$ (100), 165 (13), 148 (24), 137 (27), 130 (21), 119 (61), 109 (59), 105 (55).

N2a (murine neuroblastoma) was purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). Cells were maintained in DMEM (Sigma-Aldrich, Buchs, Switzerland), supplemented with 10% heat-inactivated fetal calf serum (FCS, Amimed; Basel, Switzerland), 100 U/mL Penicillin/Streptomycin (Invitrogen; Basel, Switzerland), and 2 mM L-glutamine (Invitrogen; Basel, Switzerland) in a humidified atmosphere of 5% CO_2 in air at 37°. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide salt, Sigma-Aldrich; Buchs, Switzerland) assay was performed as previously described [13]. Briefly, 1×10^5 cells (50 µL) were placed in each well of a flat 96-well plate. After 24 h incubation, the

medium containing different concentrations of compounds **1-13** was added, and 0.1% DMSO was used as solvent control. After 24 h, 30 μL MTT (5 mg/mL) were added into each well and incubated for 3 h. MTT solution was discarded and 100 μL DMSO were added to dissolve the formazan crystals. The absorbance in control and drug treated wells was measured in an automated microplate reader (Bio-Rad 550) at 570/630 nm. Colchicine (Sigma-Aldrich, Buchs, Switzerland) was used as the positive control with approx. 98% purity (HPLC). The HPLC analyses of compounds **1-13** are shown as supporting information.

Supporting information

^{1}H-NMR, ^{13}C-NMR, COSY, HSQC and HMBC spectra of **1,** ^{1}H-NMR and ^{13}C-NMR spectra of **3**, and HPLC chromatograms of compounds **1-13** are available as Supporting Information.

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References

1 Nanjing University for Chinese Medicine. Dictionary of Traditional Chinese Medicine. Shanghai: Shanghai Science and Technique Publishing House; 2006: 2124.

2 *Lee KY*, *Kim SH*, *Sung SH*, *Kim YC*. Inhibitory constituents of lipopolysaccharide-induced nitric oxide production in BV2 microglia isolated from *Amomum tsao-ko*. Planta Medica 2008; 74: 867-869.

3 *Song QS, Teng RW, Li*u *XK, Yang CR*. Tsaokoin, a new bicyclic nonane from *Amomum tsao-ko*. Chin Chem Lett 2001; 12: 227-30.

4 *Moon SS, Lee JY, Cho SC*. Isotsaokoin, an antifungal agent from *Amomum tsao-ko*. J Nat Prod 2004; 67: 889-91.

5 *Starkenmann C, Mayenzet F, Brauchli R, Wunsche L, Vial C*. Structure elucidation of a pungent compound in black cardamon: *Amomum tsao-ko* Crevost et Lemarie (Zingiberaceae). J Agric Food Chem 2007; 55: 10902-7.

6 *Moon SS, Cho SC, Lee JY*. Tsaokoarylone, a cytotoxic diaryheptanoid from *Amomum tsao-ko* fruits. Bull Korean Chem Soc 2005; 26: 447-50.

7 *Martin TS, Kikuzaki H, Hisamoto M, Nakatani N*. Constituents of *Amomum tsao-ko* and their radical scavenging and antioxidant activities. J Am Oil Chem Soc 2000; 77: 667-73.

8 *Dawson GW, Pickett JA, Smiley DWM*. The aphid sex pheromone cyclopentanoids: synthesis in the elucidation of structure and biosynthetic pathways. Bioorg Med Chem 1996; 4: 351-61.

9 *Jia R, Chen ZX, Corvini PFX, Kappler A, Brune A, Haider K et al*. Synthesis of [^{13}C]- and [^{14}C]-labeled phenolic humus and lignin monomers. Chemosphere 2005; 60: 1169-81.

10 *Mayer SF, Steinreiber A, Orru RVA, Faber K*. Chemoenzymatic asymmetric total syntheses of antitumor agents (3*R*,9*R*,10*R*)- and (3*S*,9*R*,10*R*)-panaxytriol and (*R*)- and (*S*)-falcarinol from *Panax ginseng* using an enantioconvergent enzyme-triggered cascade reaction. J Org Chem 2002; 67: 9115-21.

11 *Mino T, Hasegawa T, Shirae Y, Sakamoto M, Fujita T*. N,O-ligand accelerated zinc-catalyzed transesterification of alcohols with vinyl esters. J Organomet Chem 2007; 692: 4389-96.

12 *Takehiko Y, Masatoshi M, Masato A, Naoya I, Toshiyuki H, Takaaki N et al*. Crucial structural factors and mode of action of polyene amides as inhibitors for mitochondrial NADH-ubiquinone oxidoreductase (Complex I). Biochem 2007; 46: 10365-72.

13 *Cory AH, Owen TC, Barltrop JA, Cory JG*. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assay in culture. Cancer Commun 1991; 3: 207-12.

Figure Legends

Table 1: ^{1}H- and ^{13}C-NMR data of **1** and **3** (500 MHz for ^{1}H, 125 MHz for ^{13}C).

Table 2: Antiproliferative activity of compounds **1-13** in the N2a cell line (IC_{50}, μM)

Fig. 1: Structures of compound **1-13**

Fig. 2 Energy minimized conformation (MM2) of **1** with key NOESY contacts

Table 1 ^{1}H- and ^{13}C-NMR data of **1** and **3** (500 MHz for ^{1}H, 125 MHz for ^{13}C).

Position	**1**				**3**	
	δ(H)[a]	δ(C)[a]	δ(C)[b]	HMBC (H→C)	δ(H)[a]	δ(C)[a]
1	2.25 m	34.7	34.0	9, 10	2.92 m	37.2
2		144.5	143.3			144.8
3	6.56 dd (4.0, 2.0)	148.4	148.7	1, 2, 4, 5, 10	6.63 ddd (4.6, 2.3, 1.4)	146.9
4α	2.60 ddd (20.8, 4.0, 4.0)	37.3	35.1	2, 3, 5, 6	2.38 m	31.1
β	2.47 dt (20.8, 2.0)			2, 3, 5, 6, 10	2.48 ddd (18.5, 5.3, 5.2)	
5	4.15 br d (4.4)	65.0	63.5	1, 3, 6, 7	4.00 ddd (9.2, 4.8, 4.4)	68.3
6	1.39 ddd (10.3, 9.8, 4.5)	48.1	47.1	1, 2, 7, 8, 9	2.41 m	42.8
7α	1.52 dd (11.7, 9.8)	24.2	22.7	6, 8, 9	1.73 m	25.1
β	1.42 m			5, 6, 8	1.53 m	
8	1.66 m	22.3	20.4	1, 6, 7, 9	1.51 m	24.9
9α	2.42 m	27.5	26.2	1, 6, 7	1.37 m	32.2
β	1.13 ddd (12.3, 9.7, 9.6)			1, 2, 7	2.05 m	
10	9.31 s	193.7	193.0	1, 2, 3, 6	9.35 s	194.0

[a] in $CDCl_3$, [b] in CD_3OD

Table 2 Antiproliferative activity of compounds **1-13** in the N2a cell line (IC_{50}, μM) (mean ± SD, n=3)

	N2a
1	>200
2	>200
3	>200
4	82 ± 2
5	46 ± 7
6	52 ± 2
7	>200
8	>200
9	157 ± 18
10	>200
11	>200
12	>200
13	>200
Colchicine[a]	2.3 ± 1.6

[a] positive control

Fig. 1

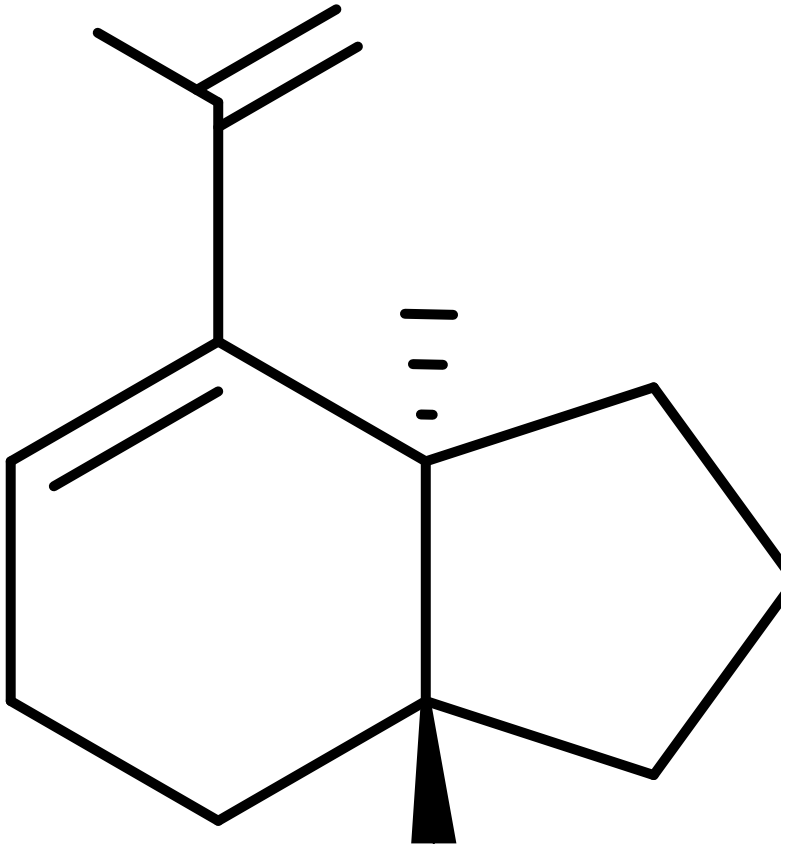

Fig. 2