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Communication

Tsaokols A and B, unusual flavanol-monoterpenoid hybrids as α-glucosidase inhibitors from *Amomum tsao-ko*

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ABSTRACT

Tsaokols A (**1**) and B (**2**), two complicated flavanol-monoterpenoid hybrids, were isolated from the dried fruits of *Amomum tsao-ko* under the guidance of LCMS and bioassay. Their structures were determined by extensive spectroscopic analyses and electronic circular dichroism (ECD) calculations. Compounds **1** and **2** shared a flavanol backbone fused with 5/7 and 5/6 bicyclic monoterpenoid scaffolds, which were biogenetically condensed by Michael addition and acetalization. Compounds **1** and **2** exhibited significant α-glucosidase inhibitory activity with IC_{50} values of 18.8 and 38.6 μmol/L (acarbose, IC_{50} = 213 μmol/L). Docking study supported the strong interactions of **1** and **2** bonding with enzyme by mainly hydrophobic and hydrogen-bond effects. Compounds **1** and **2** could be fast distinguished by the diagnostic ions at *m/z* 289 and 313 in negative MS^2 experiments.

Amomum tsao-ko Crevost et Lemaire (Zingiberaceae) is a perennial herb mainly distributed in Southwest China and Southeast Asian countries [1,2]. Its dried fruit as one of the most ancient spices is well-known all over the world [3], which has long been used for treating stomach disorders, throat infections, and liver abscess in China [4,5]. Previous investigations on *A. tsao-ko* indicated the presence of diarylheptanoids, flavonoids, monoterpenoids, and essential oils [6–8], many of which showed antimicrobial, antiinflammatory, antioxidant, antiproliferative, and neuroprotective activities [9,10]. As a continuous search for antidiabetic candidates from natural resources [11], this LCMS and bioassay driven investigation on the dried fruits of *A. tsao-ko* afforded two intriguing flavanol-monoterpenoid hybrids, tsaokols A (**1**) and B (**2**). Herein, we report their isolation, structural elucidation, plausible biosynthetic pathway, α-glucosidase inhibitory activity, and MS/MS fragmentation.

Under the guidance of LCMS analysis and bioassay profiling, the Fr. A-5-2 of *A. tsao-ko* was detected with two interesting peaks that showed the same molecular formula of $C_{25}H_{26}O_7$ and obvious α-glucosidase inhibitory activity (Fig. S1 in Supporting information). As a result, the two peaks were purified by repeated column chromatography and semi-preparative HPLC on C_{18} column using MeCN-H_2O and MeOH-H_2O eluent to yield compounds **1** and **2** (Fig. 1).

Tsaokol A (**1**) was obtained as yellowish powder with a molecular formula of $C_{25}H_{26}O_7$ which was deduced by the positive HRESIMS at *m/z* 439.1752 ($[M + H]^+$, calcd. 439.1751), corresponding to 13 degrees of unsaturation. The IR spectrum exhibited absorption bands for hydroxyl groups (3432 cm^{-1}) and aromatic rings (1621, 1527, and 1447 cm^{-1}). All the 25 carbons were well resolved in the ^{13}C NMR (DEPT) spectrum, involving four methylenes, 13 methines, and eight quaternary carbons. In the ^1H NMR data (Table 1), an ABX spin system at δ_H 6.89 (1H, d, *J* = 1.6 Hz, H-2'), 6.77 (1H, d, *J* = 8.0 Hz, H-5'), and 6.77 (1H, dd, *J* = 8.0, 1.6 Hz, H-6'), an aromatic singlet at δ_H 5.92 (1H, s, H-6), and two oxygenated methines at δ_H 4.02 (1H, d, *J* = 8.0 Hz, H-2) and 3.95 (1H, td, *J* = 8.0, 6.0 Hz, H-3) were indicative for a catechin-like unit, when taking the typical carbons at δ_C 82.8 (C-2) and 68.8 (C-3) into consideration [12]. This deduction were well confirmed by the correlations of H-2/H-3/H-4 and H-5'/H-6' in the ^1H-^1H COSY spectrum, as well as the HMBC correlations of H-2 with C-9/C-1'/C-2'/C-6', of H-4 with C-5/C-9, and of H-6 with C-8/C-10 (Fig. 2).

Apart from the flavanol unit deduced above, ten remnant carbons were assigned as a pair of *cis*-double bond [δ_H 5.72 (1H, dt,

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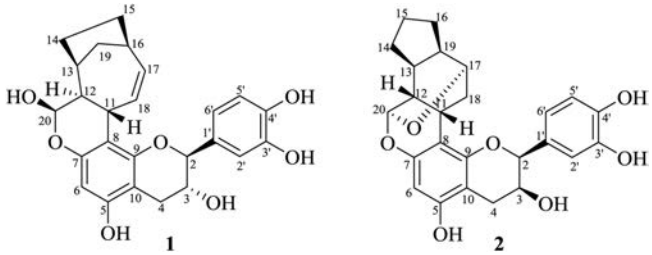


Fig. 1. Structures of tsaokols A (**1**) and B (**2**).

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** and **2** in CD_3OD.

No.	**1**		**2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	82.8 d	4.02 d (8.0)	79.9 d	4.87 br s
3	68.8 d	3.95 td (8.0, 6.0)	67.3 d	4.22 m
4	29.4 t	2.92 dd (16.2, 6.0)	29.7 t	2.90 dd (16.8, 4.2)
		2.54 dd (16.2, 6.0)		2.78 dd (16.8, 3.0)
5	154.7 s	–	156.4 s	–
6	98.1 d	5.92 s	96.3 d	5.96 s
7	151.3 s	–	150.1 s	–
8	106.4 s	–	110.6 s	–
9	155.4 s	–	152.7 s	–
10	103.2 s	–	101.4 s	–
11	31.5 d	3.67 ddd (9.6, 4.8, 1.8)	19.0 d	3.40 m
12	44.4 d	1.97 ddd (9.6, 7.8, 1.8)	32.8 d	1.78 m
13	44.8 d	1.29 m	39.6 d	2.36 m
14	23.6 t	1.75 m	30.4 t	1.72 m
		1.75 m		1.38 m
15	30.4 t	1.82 m	28.6 t	1.61 m
		1.08		1.37 m
16	46.0 d	1.86 m	29.3 t	1.90 m
				1.84 m
17	130.3 d	5.72 dt (10.2, 1.8)	69.4 d	3.63 dd (5.4, 4.2)
18	130.7 d	6.36 ddd (10.2, 3.6, 3.0)	31.4 t	2.29 dd (14.4, 11.4)
				1.40 m
19	28.7 t	1.91 m	41.6 d	2.39 m
		1.27 m		
20	94.5 d	5.34 d (1.8)	97.9 d	5.50 t (1.8)
1'	132.4 s	–	132.6 s	–
2'	115.4 d	6.89 d (1.6)	115.3 d	6.98 d (1.8)
3'	146.4 s	–	146.2 s	–
4'	146.4 s	–	146.0 s	–
5'	116.2 d	6.77 d (8.0)	116.1 s	6.77 d (8.4)
6'	120.2 d	6.77 dd (8.0, 1.6)	119.3 s	6.81 dd (8.4, 1.8)

J = 10.2, 1.8 Hz, H-17) and 6.36 (1H, ddd, J = 10.2, 3.6, 3.0 Hz, H-18); δ_C 130.3 (C-17) and 130.7 (C-18)], five sp^3 methines including an oxygenated one (δ_H 5.34, δ_C 94.5), and three methylenes. A pure-carbon bicyclo[4.2.1]non-2-ene scaffold was established by detailed inspection of ^{1}H-^{1}H COSY data: the correlations of H-11/H-12/H-13/H_2-19/H-16/H-17/H-18/H-11, H-13/H_2-14/H_2-15/H-16, and H-12/H-20. Meanwhile, the key HMBC correlations (Fig. 3) of H-20 with C-11/C-13, H_2-19 with C-12/C-14/C-15/C-17, and H-17 with C-11/C-15/C-19 facilitated the above deduction. The flavanol and monoterpenoid parts fused through C-8–C-11 and C-7–*O*–C-20 bonds were determined by the key HMBC correlations of H-11 with C-7/C-9, H-12/H-18 with C-8, and H-20 with C-7. Thus, the planar structure of compound **1** was elucidated as a flavanol-monoterpenoid hybrid as shown in Fig. 2.

The relative configuration of **1** was characterized by analyzing the coupling constants and ROESY correlations (Fig. 2). The large coupling constants of H-2/H-3 (J = 8.0 Hz) and of H-11/H-12 (J = 9.6 Hz) verified their *trans*-axis-orientation. In the ROESY experiment, key cross-peaks from H-11 to H-15a/H-2'(H-6'), from

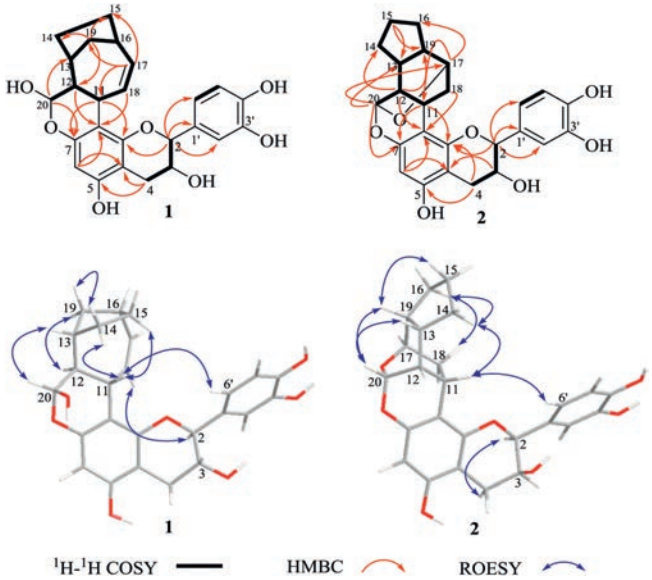


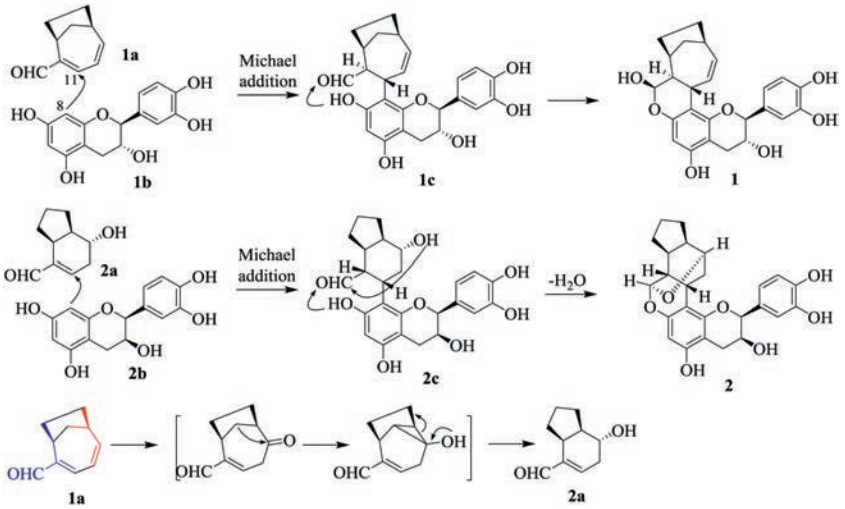
Fig. 2. Key ^{1}H-^{1}H COSY, HMBC and ROESY correlations of **1** and **2**.

H-12 to H-19b, from H-13 to H-19b and H-19a, from H-16 to H-19b and H-19a, from H-20 to H-13, and from H-2 to H-18 suggested the β-direction of H-11 and OH-20, and α-direction of H-2, H-12, H-13, H-16, H-20, and CH_2-19. The same orientation of H-12 and H-20 was consistent with their small coupling constant ($J_{H\text{-}12/H\text{-}20}$ = 1.8 Hz). With the anti-form of H-2 and H-3 deduced above, the β-configuration of H-3 was thus decided.

The absolute configuration of **1** was resolved by ECD calculations (Fig. S2 in Supporting information). The calculated ECD spectrum of 2*S*,3*R*,11*S*,12*S*,13*S*,16*R*,20*R*-form matched well with the experimental one, by which compound **1** was determined and named as tsaokol A.

Tsaokol B (**2**) had the same molecular formula of $C_{25}H_{26}O_7$ with **1**, which was deduced by its HRESIMS at m/z 439.1743 ($[M + H]^+$, calcd. 439.1751). Detailed analyses of its ^{1}H and ^{13}C NMR data revealed that compound **2** shared a similar flavanol backbone with **1** but distinct in the C_{10} monoterpenoid part (Table 1). Different from **1**, an *epi*-catechin-like unit was deduced by the small coupling constant of $J_{H\text{-}2/H\text{-}3}$ and the chemical shifts of C-2 (δ_C 79.9) and C-3 (δ_C 67.3) [12]. Besides the flavanol part, the remaining ten carbons were ascribed as six sp^3 methines (including two oxygenated ones) and four sp^3 methylenes.

An octahydroindene unit was constructed by the ^{1}H-^{1}H COSY correlations of H-13/H-14/H-15/H-16/H-19/H-13, and H-11/H-12/H-13/H-19/H-17/H-18/H-11, as well as the key HMBC correlations of H-16 with C-13/C-14/C-17, and H-14 with C-12/C-16/C-19. A dioxygenated methine (C-20) directly linking with C-12 was confirmed by H-20 (δ_H 5.50) that showed ^{1}H-^{1}H COSY correlation with H-12, and HMBC correlations with C-11 and C-13. Similar with **1**, the HMBC correlations of H-11 with C-7 and C-9, and of H-20 with C-7 in **2** connected the C_{10} monoterpenoid and flavanol units by the C-8–C-11 and C-7–*O*–C-20 linkages. The residual one unsaturation degree required an additional ring in the structure of **2**, and thus established the ether bond between C-17 and C-20, which was confirmed by the HMBC correlations from H-20 to C-17 and from H-17 to C-20. In the ROESY spectrum, the correlations of H-11 with H-12, H-14b and H-2'(6'), of H-18a with H-16b, and of H-20 with H-13 and H-19, assigned the β-orientation of H-11 and H-12, the α-orientation of H-2, H-3, H-13, H-19, and the ether linkage between C-17 and C-20. By comparing the calculated and experimental ECD spectra, the absolute configuration of **2** was clarified as 2*S*,3*S*,11*S*,12*R*,13*S*,17*R*,19*R*,20*S*.



Scheme 1. Hypothetical biosynthetic pathways of **1** and **2**.

Structurally, tsaokols A (**1**) and B (**2**) are two unusual flavanol-monoterpenoid hybrids fused with pure-carbon bicyclo[4.2.1]non-2-ene and octahydroindene, respectively. Plausibly biosynthetic pathways for **1** and **2** were proposed in Scheme 1. The flavanol parts (**1b** and **2b**) were widely present in *A. tsao-ko*, which were condensed with different C_{10} parts (**1a** or **2a**) by consecutive Michael addition and acetalization to form compounds **1** and **2**. Presently, several bicyclononane aldehydes with the skeleton of octahydroindene had been isolated from this species [8], whereas their biosynthetic origin was never discussed. By inspection of the features of **1a** and **2a**, a key intermediate with three-membered ring was suggested for their transformation. Although the bicyclo[4.2.1]non-2-ene can be well interpreted from the precursor of geranyl pyrophosphate (GPP), monoterpenoids with such a skeleton has not been reported.

Tsaokols A (**1**) and B (**2**) were investigated *in vitro* for their α-glucosidase inhibitory activity, both of which displayed obvious inhibition with IC_{50} values of 18.8 and 38.6 μmol/L, respectively, much more potent than the positive control, acarbose (IC_{50} = 213 μmol/L). For better understanding the binding modes of **1** and **2** with α-glucosidase, *in silico* docking study with 3TOP was further performed.

The docking poses of compounds **1** and **2** were shown in Fig. S3 (in Supporting information). The prominent interactions of **1** with enzyme were hydrophobic effects with Phe1559 and Phe1560, hydrogen bonds with Asp1157, Asp1526, Lys1460, Arg1510, Thr1586, and Tyr1251, pi-stacking with Phe1560, and pi-cation with Lys1460. As a comparison, compound **2** bonded to enzyme mainly by hydrophobic effects with Trp1369 and Phe1560, hydrogen bonds with Asp1526, Thr1586, Lys1460, and Trp1369, and pi-stacking with Phe1560, Trp1369, and Trp1355.

Tsaokols A (**1**) and B (**2**) were further investigated for their MS/MS fragmentation in both positive and negative modes by ESI ion source (Scheme S1 in Supporting information). In the first stage MS, they both showed the $[M+H]^+$ ion at *m/z* 439 and $[M–H]^-$ ion at *m/z* 437, respectively. When the $[M+H]^+$ ion was selected for MS/MS experiment, compounds **1** and **2** produced quite similar fragmentation ions at *m/z* 421, 287, and 269, which could be well explained by the neutral loss of H_2O, $A^{1,3}$ *retro*-Diels-Alder (RDA) cleavage, and further loss of H_2O. Interestingly, their MS/MS fragmentations in negative mode were obviously different. For compound **1**, the MS^2 ion at *m/z* 289 was due to the departure of the C_{10} monoterpenoid part. As a comparison, the characteristic $A^{1,2}$ cleavage was occurred in the negative MS^2 experiment of **2** yielding the ion at *m/z* 313. Thus, compounds **1** and **2** could be well differentiated by their respectively diagnostic ions of 437-289 and 437-313 in negative MS mode.

In conclusion, two unusual flavanol-monoterpenoid hybrids, tsaokols A (**1**) and B (**2**), with intriguing α-glucosidase inhibitory activity were isolated from *A. tsao-ko* under the guidance of LCMS and bioassay. Compounds **1** and **2** are a pair of isomers showing quite similar fragmentation pathways in positive MS/MS experiments, whereas they can be fast distinguished by the diagnostic MS^2 ions at *m/z* 289 and 313 in negative mode. Compounds **1** and **2** are two intriguing antidiabetic candidates needing further investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2020.08.050.

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