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Tricuspone, a Rearranged Diterpenoid from *Salvia tricuspis*

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The genus of *Salvia* is the largest one of the Lamiaceae with *ca*. 1000 species that extensively distribute throughout the world.[1] Several species, such as *S. miltiorrhiza*, *S. yunnanensis*, *S. przewalskii*, *S. przewalskii* var. *mandarinorum* and *S. plebeia*, have long been used as traditional Chinese medicines (TCM) for the therapy of hypertension, coronary heart disease, dysmenorrhea and inflammation *etc*..[2] Phytochemical researches of many *Salvia* species have been conducted, and several kinds of constituents, including sesquiterpenoids,[3,4] triterpenoids,[5] sterols,[6] flavones and flavone glycosides,[7] have been isolated from some species. However, the most characteristic secondary metabolites of this genus are clerodane and abietane diterpenoids.[1,8-10] *S. tricuspis* Franch. is an annual or biennial plant which only grows in central China. To the best of our knowledge, there has been no report of medical applications of this plant and no report of its chemical investigation up to now. As part of the ongoing project to screen natural terpenoids for potential antitumor and antibacterial agents, we investigated the secondary metabolites of *S. tricuspis*, and a novel rearranged abietane diterpenoid with 6/6/5 ring system, named as tricuspone (**1**) (Fig. 1), was obtained. This paper deals with the isolation and structural elucidation of this compound, and antibacterial and cytotoxic activities of tricuspone (**1**) are also evaluated.

Tricuspone (**1**) was isolated as colorless crystal. Its HRESIMS spectrum showed the molecular ion peak at *m/z* 363.2168 ([M+H]$^+$; calcd. for $C_{21}H_{31}O_5$: 363.2166), corresponding to the molecular formula of $C_{21}H_{30}O_5$. The IR spectrum indicated the presence of hydroxyl group (3262 cm^{-1}), double bond (1661 cm^{-1}), ester carbonyl (1744 cm^{-1}) and ketone carbonyl moiety (1729 cm^{-1}). In the ^{1}H NMR spectrum (Table 1), the signals of two secondary methyl at δ_H 1.18 (3H, d, J = 6.6 Hz, H-16) and 0.95 (3H, d, J = 6.9 Hz, H-17), coupled to a methine group at δ_H 1.91 (1H, m, H-15), suggested the existence of an isopropyl group. The ^{1}H NMR spectrum also showed three tertiary methyl group at δ_H 1.43 (3H, s, H-20), 0.96 (3H, s, H-18) and 0.90 (3H, s, H-19). The broad singlet centered at δ_H 4.02 (1H, brs) was assigned to a hydroxyl group with the aid of HMQC spectrum. The ^{1}H NMR signal at δ_H 3.82 (3H, s) and the corresponding ^{13}C NMR signal at δ_C 54.0 indicated the presence of a methoxyl group. Apart from the signal of the methoxyl group, there are 20 carbon signals left in the ^{13}C NMR and DEPT spectra (Table 1), including the signals of two α,β-unsaturated ketone carbons at δ_C 196.5 (C-14) and 194.7 (C-7), an ester carbonyl at δ_C 173.6 (C-12), a tetrasubstituted double bond at δ_C 189.0 (C-9) and 134.2 (C-

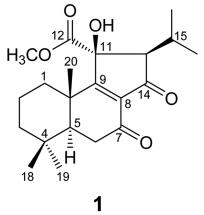

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Figure 1. The structure of tricuspone (**1**).

Table 1. ^{1}H-, ^{13}C-NMR and HMBC data of tricuspone (**1**) ($CDCl_3$, δ ppm, TMS)[a,b]

No.	δ_H mult (J in Hz)	δ_C	HMBC
1α	1.01 ddd (12.8, 11.4, 3.4)	33.3 t	2, 3
1β	2.24 brd (12.8)		3
2α	1.55 m	18.1 t	10
2β	1.76 brdddd (13.7, 13.7, 3.4, 3.2)		1
3α	1.20 m (overlapped)	41.2 s	1
3β	1.51 brd (13.5)		1
4	-	33.7 s	-
5	1.70 dd (13.7, 3.9)	51.7 d	3, 5, 7, 10
6α	2.58 dd (17.9, 3.9)	36.6 t	5, 7, 8, 10
6β	2.50 ddd (17.9, 13.7)		5, 7, 8, 10
7	-	194.7 s	-
8	-	134.2 s	-
9	-	189.0 s	-
10	-	41.1 s	-
11	-	83.4 s	-
12	-	173.6 s	-
13	2.65 d (8.5)	68.1 d	11, 12, 14, 15, 16, 17
14	-	196.5 s	-
15	1.91, m	26.1 d	11, 13, 14, 16, 17
16	1.18 d (6.6)	21.0 q	13, 15, 17
17	0.95 d (6.9)	21.5 q	13, 15, 16
18	0.96 s	21.1 q	3, 4, 5, 19
19	0.90 s	32.8 q	3, 4, 5, 18
20	1.43 s	18.8 q	1, 5, 9, 10
CH_3O	3.82 s	54.0 q	11
11-OH	4.02 brs	-	9, 11, 12, 13

[a]Measured at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. [b]Assigned by ^{1}H-^{1}H COSY, HMQC and HMBC spectra.

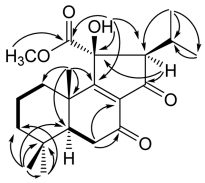

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Figure 2. The key HMBC and ^{1}H-^{1}H COSY correlations of tricuspone (**1**).

8), and an oxygenated quarternary carbon at δ_C 83.4 (C-11). These data, especially the presence of an isopropyl group, supported an abietane skelecton.[11] All signals of protons and carbons in tricuspone (**1**) were assigned by 2D NMR spectra (Table 1). The ^{1}H-^{1}H COSY spectrum showed the cross peaks through δ_H 1.01 and 2.24 (H-1) to δ_H 1.20 and 1.51 (H-3), and δ_H 1.70 (H-5) to δ_H 2.50 and 2.58 (H-6) (Fig. 2). The HMBC correlations of methyl protons signals at δ_H 0.96 (s, H-18) and 0.90 (s, H-19) with carbons at δ_C 41.2 (C-3), δ_C 33.7 (C-4) and δ_C 51.7 (C-5), methyl protons signal at δ_H 1.43 (s, H-20) with carbons at δ_C 33.3 (C-1), δ_C 51.7 (C-5), δ_C 189.0 (C-9) and δ_C 41.1 (C-10), and methylene protons signals at δ_H 2.50 and 2.58 (H-6) with carbons at δ_C 194.7 (C-7) and δ_C 134.2 (C-8) established the A and B rings of abietane diterpene with a double bond between C-8 and C-9 as well as a ketone group at C-7 (Fig. 2). However, the residual carbons could not construct the C ring of a normal abietane diterpenoid. In the HMBC spectrum, the proton of hydroxyl group correlated to the carbon at δ_C 189.0 (C-9), δ_C 83.4 (C-11), δ_C 173.6 (C-12), δ_C 68.1 (C-13) synchronously, which indicated that C ring of the abietane decreased from a six- to a five-numbered ring, and C-12 extruded out and formed a methyl ester. This conclusion was further confirmed by the HMBC correlations of methine signal at δ_H 2.65 (H-13) with the carbons at δ_C 83.4 (C-11) and δ_C 173.6 (C-12), and the methoxy protons signals at δ_H 3.82 (CH_3O) with the carbonyl carbon at δ_C 173.6 (C-12). The ketone group at C-14 was established by the HMBC correlations of methine signals at δ_H 2.65 (H-13) and δ_H 1.91 (H-15) with carbon at δ_C 196.5 (C-14). Fortunately, the single crystal of tricuspone (**1**) was obtained by recrystallizing in acetone, and the structure including the absolute configuration of tricuspone (**1**) was further established by the single crystal X-ray diffraction analysis with graphite-monochromated CuKα_{ave} radiation (Fig. 3). The absolute configurations of C-5, C-10, C-11 and C-13 were assigned as 5*S*, 10*S*, 11*S* and 13*R* respectively. Accordingly, tricuspone (**1**) should derive from a normal abietane diterpenpid. Abietane diterpenoid is a relatively abundant class of natural products. A normal abietane diterpenoid possesses 6/6/6 ring system. Through the break of C-C brond, migration of methyl group or formation of new C-C brond, normal abietane could transform into irregular abietane diterpenes, such as secoabietanes and secofriedoabietanes, abeoabietanes, furanoabietanes, nor- and homoabietanes, and abietane

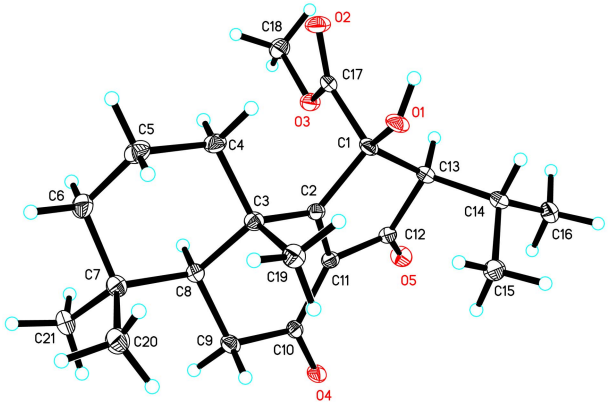

Figure 3. X-ray single-crystal structure for tricuspone (**1**).

dimmers.[12] A lot of rearranged abietane diterpenoid have been isolated from *Salvia* species.[13-15] To the best of our knowledge, tricuspone (**1**) is the first example of rearranged abietane diterpenoid with 6/6/5 ring system isolated from natural resources, which has not been reported previously. Two modified *ent*-abietane diterpenoid with 6/6/5 ring system, including a dimer and a diterpenoid-aryphloroglucinol hybrid, have ever been isolated from the roots of *Euphorbia fischeriana*.[16,17]

Tricuspone (**1**) exhibited potential antibacterial activity against *Staphylococcus aureus* with MIC values of 167 μg/mL. The MIC of control drug Cefalexin was 51 μg/mL for *S. aureus*. At the concentration of 100 μg/mL, tricuspone (**1**) almost exhibited no antibacterial activity against *Escherichia coli*. In the MTT assay, tricuspone (**1**) showed no cytotoxic activity against human oral epidermoid carcinoma (KB) and human breast cancer (MCF-7) cell lines at the concentration of 80 μM.

Experimental Section

General Procedures. Melting point was determined on Kofler melting point apparatus and uncorrected. Optical rotation was measured on a Perkin-Elmer 341 polarimeter. IR spectrum was recorded with a Bruker Vertex 70 FT-IR spectrometer. ^{1}H, ^{13}C NMR (DEPT) and 2D NMR were recorded on a Bruker AVANCE 500 spectrometer. HRESIMS spectrum was obtained on a Bruker APEX II spectrometer. Silica gel (200-300 and 300-400 mesh) used for column chromatography (CC) and silica GF_{254} for thin layer chromatography (TLC) were purchased from *Qingdao Marine Chemical Factory* in China. The purity of the samples were checked on TLC (silica gel, GF_{254} and C-18) under UV light at 254 nm or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

Plant Material. The whole plants (1.8 kg) of *S. tricuspis* were collected in Funiu Mountains, Henan Province, China in September 2009. The specimen was identified by Long-Xiao Gao from Henan Wild Ornamental Arboretum. A voucher specimen (NO. HN09003) was deposited at the herbarium in the Laboratory of Botany, Marine College, Shandong University.

Extraction and Isolation. The ground air-dried whole plant of *S. tricuspis* (1.8 kg) extracted with MeOH (7 days × 3) at room temperature. The extract was concentrated under reduced pressure and the residue (50.0 g) was suspended in water (60 ºC, 300 mL). This suspension was extracted successively with equal volumes of petroleum ether (bp 60-90 ºC) and EtOAc. The EtOAc soluble fraction (16.9 g) was chromatographed over a silica gel column (200-300 mesh, 200 g) with *n*-hexane-acetone (10:1, 8:1, 5:1, 3:1) elution to give four fractions (Fr1-Fr4). Fr3 (with *n*-hexane-acetone 5:1, 6.0 g) was chromatographed on an active carbon powder column (3.0 g), and then a silica gel column (200-300 mesh, 70 g) with petroleum ether-EtOAc (15:1, 10:1) elution to give 45 samples. Tricuspone (**1**) (53 mg) was recrystallized in acetone from 21-25 samples.

Tricuspone (1). Colorless crystal, mp 208-209 ºC; $[\alpha]_D^{20}$ = +79.5 (*c* 0.206, $CHCl_3$); UV (MeOH): λ_{max} (ε) 207 (1164), 243 (882) nm; IR (KBr): 3262, 2991, 2956, 2868, 2850, 1744, 1729, 1661, 1601, 1459, 1428, 1377, 1370, 1214, 1206, 1140 cm^{-1}; HR-ESI-MS *m/z* 363.2168 $[M+H]^+$ (calcd. for $C_{21}H_{31}O_5$: 363.2166).^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (DEPT) (125 MHz, $CDCl_3$) data: see Table 1.

X-ray Structure Determination of Tricuspone (1). $C_{21}H_{30}O_5$ (*fw* = 362.45), 133 K, Monoclinic, P2(1), a = 8.1747(2) Å, b = 11.4599 (3) Å, c = 10.9460(3) Å, β = 109.5740 (10)°, V = 966.17(4) $Å^3$, Z = 2, μ (Cu-Kα_{ave}) = 0.710 mm^{-1}, ρ_{cal} = 1.246 g cm^{-3}; crystal dimensions: 0.12 × 0.09 × 0.05 mm; 13101 reflections measured (θ = 5.74 to 64.99°), 3068 were unique (R_{int} = 0.0348). Final *R* indicates *R*1 = 0.0340, *wR*2 = 0.0764 [$I > 2\sigma(I)$] for 242 parameters. Data were collected using a Bruker Smart Apex CCD diffractometer using graphite-monochromated CuKα_{ave} radiation λ = 1.54178 Å. The structure was solved by direct methods and refined by full-matrix least-squares on F2 using Bruker SHELXS-97. The final R and R_w factors were 0.0307 and 0.0767, respectively. CCDC 846444 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Antibacterial Assay. The antibacterial activity of tricuspone (**1**) against *S. aureus* and *E. coli* was evaluated with the method in reference.[18]

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