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Diterpenoids and Sesquiterpenoids from *Syzygium fluviatile*

Dingli Zhang[1], Yikao Hu[1], Li Wang[2], Shengxiong Huang[2], Yan Zhao, Mengjia Li[1], Feng Li[1] and Yong Zhao[1,*]

[1] *College of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming 650500, China*

[2] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

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Abstract: A new monocyclic diterpenoid, cassipouryl formate, (**1**) together with three known diterpenoids (**2**−**4**) and five known sesquiterpenoids (**5**−**9**) were isolated from the twigs and leaves of *Syzygium fluviatile*. The new isolate was elucidated by various spectroscopic technologies. This was the first example of the chemical constituents from this species and the first report on the diterpenoids from genus *Syzygium*.

Keywords: *Syzygium fluviatile;* Myrtaceae; diterpenoids; sesquiterpenoids..

1. Introduction

The genus *Syzygium* is one of the largest groups of the family Myrtaceae with more than 500 species distributed in the tropic and subtropical regions of the world [1]. Many of them have been used as an important crude drug for the treatment of diabetes in Southeast Asian nations [2]. Previous phytochemical research on *Syzygium* demonstrated that flavonoids, hydrolysable tannins, phenylpropanoids, chromone derivatives, phloroglucinol derivatives, and triterpenes were their major constituents [1, 3-7]. *Syzygium fluviatile* (Hemsl.) Merr. et Perry, is a shrub which distributed in Guangdong and Guangxi Province in China [8]. As far as we know, there has not report on the chemical constituents of this plant to date. In our interest in the discovery of bioactive metabolites from *Syzygium* plants, one new monocyclic diterpenoid, (**1**) together with three known diterpenoids (**2**−**4**) and five known sesquiterpenoids (**5**−**9**) (Figure 1) were isolated from the twigs and leaves of this species.

* Corresponding author: zhaooy@126.com; Phone: 86-871-65941087 Fax: 86-871-65941088

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2. Materials and Methods

2.1. Plant Material

The twigs and leaves of *Syzygium fluviatile* (Hemsl.) Merr. et Perry were collected in November 2016 from Xishuang Banna Tropical Botanical Garden, P. R. China, and were authenticated by Prof. Yu Chen at Kunming Institute of Botany, Chinese Academy of Science. Plant specimens (CEYNNU 20161102) were deposited at College of Chemistry and Chemical Engineering, Yunnan Normal University.

2.2. Extraction and Isolation

The air-dried and powdered twigs and leaves of *S. fluviatile* (5.0 kg) were extracted with 70% acetone (8 L × 3) at room temperature, then evaporated under reduced pressure to provide a residue. the residue was divided with petroleum ether (4 × 1 L), EtOAc (4 × 1 L), and n-BuOH (4 × 1 L), sequentially into three parts. The EtOAc extract (120 g) was subjected to silica gel column chromatography (CC) using a gradient system of petroleum ether/acetone (v/v, 50:0 to 0:1) to afford ten fractions (Fr A−J).

Fraction A (0.6 g) was further chromatographed over silica gel CC using a gradient elution of petroleum ether/ethyl acetate (v/v, 20:1−5:1) to afford three subfractions Fr A1−A4, then fraction A2 (70 mg) was isolated by Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1), followed by semipreparative HPLC (MeOH/H_2O 95:5) to give compounds **4** (1.5 mg, t_R = 16 min) and **7** (2.5 mg, t_R = 22 min). The Sephadex LH-20 column ($CHCl_3$/MeOH, 3:2) chromatography for fraction D (2.1 g), followed by silica gel CC with petroleum ether/ethyl acetate (v/v, 20:1) give four subfractions Fr D1−D4. The purification of fraction D3 (0.5 g) with Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) and semipreparative HPLC (MeOH/H_2O 93:7) to give compounds **1** (1.0 mg, t_R = 15 min) and **2** (1.5 mg, t_R = 12 min). Fraction E (4.0 g) was decolorized by MCI gel (MeOH/H_2O 95:5), then treated with silica gel CC using petroleum ether/ethyl acetate (v/v, 5:1) to provide three subfractions Fr E1−E3. Fraction E2 (0.7 g) afforded compounds **6** (1.6 mg, t_R = 11 min), **8** (1.5 mg, t_R = 15 min), and **9** (1.2 mg, t_R = 17 min) by Sephadex LH-20 chromatography ($CHCl_3$/MeOH, 3:2) and semipreparative HPLC (MeOH/H_2O 92:8). Fraction I (2.9 g) was decolorized in the same way, followed by chromatograhy over silica gel CC with petroleum ether/ethyl acetate (v/v, 10:1) to yield five subfractions Fr I1−I5. Sephadex LH-20 column ($CHCl_3$/MeOH, 3:2) separation of fraction I4 (0.3 g) along with the semipreparative HPLC (MeOH/H_2O 90:10) led to compounds **3** (1.5 mg, t_R = 20 min) and **5** (1.0 mg, t_R = 15 min).

3. Results and Discussion

Compound **1**, a colorless gum (CH_3OH), of which molecular formula was determined as $C_{21}H_{38}O_2$ by positive HR-EI-MS (*m*/*z* 322.2878 $[M]^+$, calc. 322.2872), requiring 3 degrees of unsaturation. Strong UV absorption at 197 nm implied it had isolated chromophores. IR spectrum disclosed the existence of ester carbonyl (1731 cm^{-1}) and olefinic bond (1639 cm^{-1}). One olefinic methine at δ_H 5.36 (1H, td, *J* = 7.2, 0.8 Hz), one oxymethylene at δ_H 4.68 (2H, d, *J* = 7.1 Hz), one formyl group at δ_H 8.07 (1H, s), two tertiary methyl at δ_H 0.85 (3H, d, *J* = 6.6 Hz) and δ_H 0.84 (3H, d, *J* = 6.6 Hz), three quaternary methyls (δ_H 0.86, s; 0.87, s; and 1.71, s) including one bearing sp^2 one were observed in the ^1H NMR. The ^{13}C NMR spectra demonstrated 21 carbon signals covering a formyl carbon (δ_C 161.1), two olefinic ones (δ_C 143.8, and 117.3), five methyls (δ_C 22.7, 22.6, 19.8, 19.7, and 16.4), nine methylenes (δ_C 24.8−60.8) including one oxidized, three sp^3 methines (δ_C 27.9, 32.7 and 32.8), and one sp^3 quaternary carbon. Considering of 3 degrees of unsaturation these evidences unambiguously disclosed that it was a formate of monocyclic diterpenoid. All the NMR data of **1** were closely similar to those of the known compound **2**, cassipourol [9], except for the only difference of a formyloxy at C-15 in **1** instead of a hydroxyl in cassipourol. This hypothesis was

supported by the HMBC cross peaks (as Figure 2) between H-1' (δ_H 8.07) and C-15 (δ_C 60.8), and between H-15 (δ_H 4.68) and C-13 (δ_C143.8), C-14 (δ_C 117.3) and C-1' (δ_C 161.1).

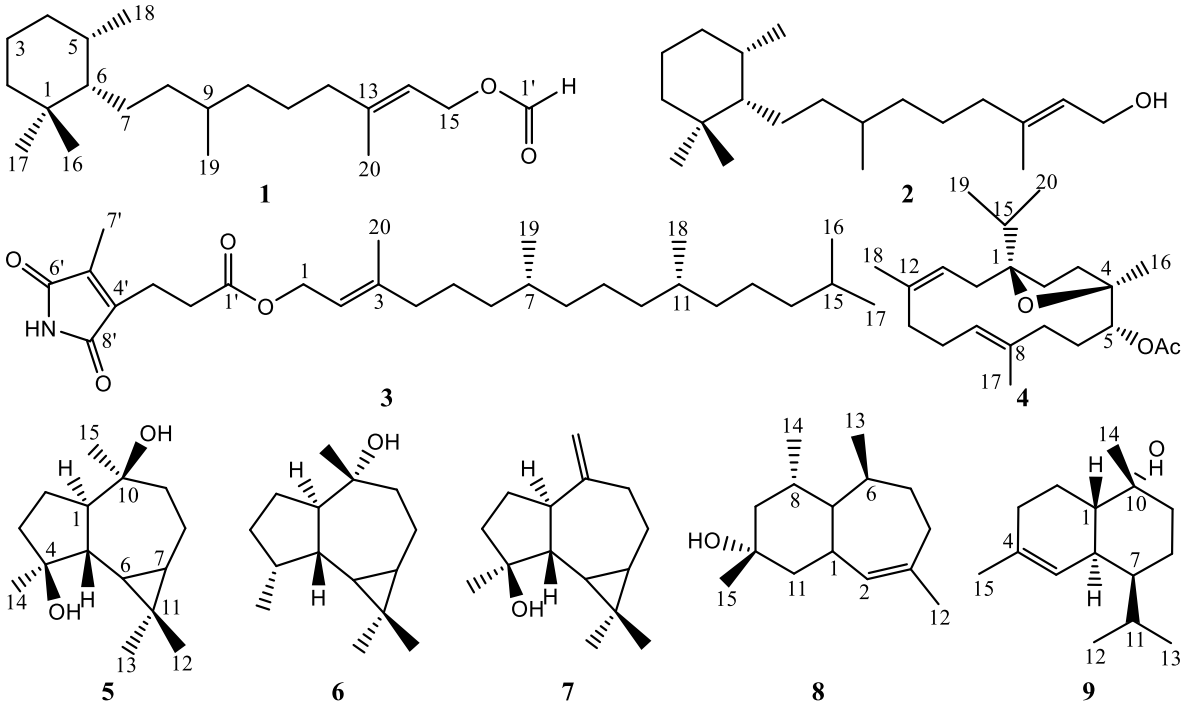

Figure 1. The chemical structures of compounds **1**–**9**

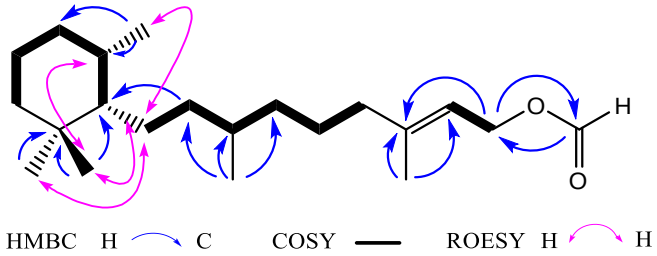

Figure 2. Key 2D NMR correlations of **1**

Further HMBC correlations combing with the ^{1}H-^{1}H COSY correlations (Figure 2) of H-2/H-3/H-4/H-5/H-6, of H-7/H-8/H-9/H-10/H-11/H-12 and of H-14/H-15 confirmed the above deduction. The stereochemistry of compound **1** was presumed to be as shown (as Figure 2) on the basis of the ROESY cross peaks from H-7 (δ_H 1.34) to H-17 (δ_H 0.86) and H-18 (δ_H 0.84) and from H-6 (δ_H 1.07) and H-5 (δ_H 1.54) to H-16 (δ_H 0.87). Additionally, their almost identical chemical shifts of C-5−C-7 and C-18 also supported this deduction. Detailed examination of the ^{13}C NMR data of C-12−C-15 and C-20 suggested that compound **1** had the same *E*-configuration of the double bond at C-13 as cassipourol [9], which was confirmed by the ROESY connections of CH_3-20 (δ_H 1.71) and H-15 (δ_H 4.68), and of H-12 (δ_H 2.01) and H-14 (δ_H 5.36). Consequently, the structure of **1** was established as cassipouryl formate. The biosynthetic pathway of diterpenoids **1**–**4** was discussed as the reference [10] (Figure 3).

Table 1. ^{1}H NMR and ^{13}C NMR data for compounds**1**−**4** (δ in ppm)[a].

	1			**1**	
No	δ_H	δ_C	**No**	δ_H	δ_C
1		36.6	12	2.01 m, 1.14 m	39.8
2	1.14 m	39.4	13		143.8
3	1.38 m	24.8	14	5.36 td (7.2,	117.3
4	1.26 m	37.3	15	4.68 d (7.2)	60.8
5	1.54 m	27.9	16	0.87 s	22.7
6	1.07 m	32.8	17	0.86 s	22.6
7	1.20 m, 1.34	24.5	18	0.84 d (6.6)	19.7
8	1.38 m	37.3	19	0.85 d (6.6)	19.8
9	1.38 m	32.7	20	1.71 s	16.4
10	1.26	37.4	1'	8.07 s	161.1
11	1.38 m, 1.45	25.0			

[a] ^{1}H NMR and ^{13}C NMR Data for **1** in $CDCl_3$ at 600 MHz and 150 MHz, respectively.

The known compounds were assigned as cassipourol (**2**) [9], 4',5'-dehydrodiodictyonema (**3**) [11], acetyl incensole (**4**) [12], aromadendrane-4β,10β-diol (**5**) [13], (-)-globulol (**6**) [14], spathulenol (**7**) [15], 3β,6β,8α,10β-tetramethylwiddrane-2(3)-en-10α-ol (**8**) [16], and α-cadinol (**9**) [17] according to their NMR and MS data as well as the comparison with the reported values.

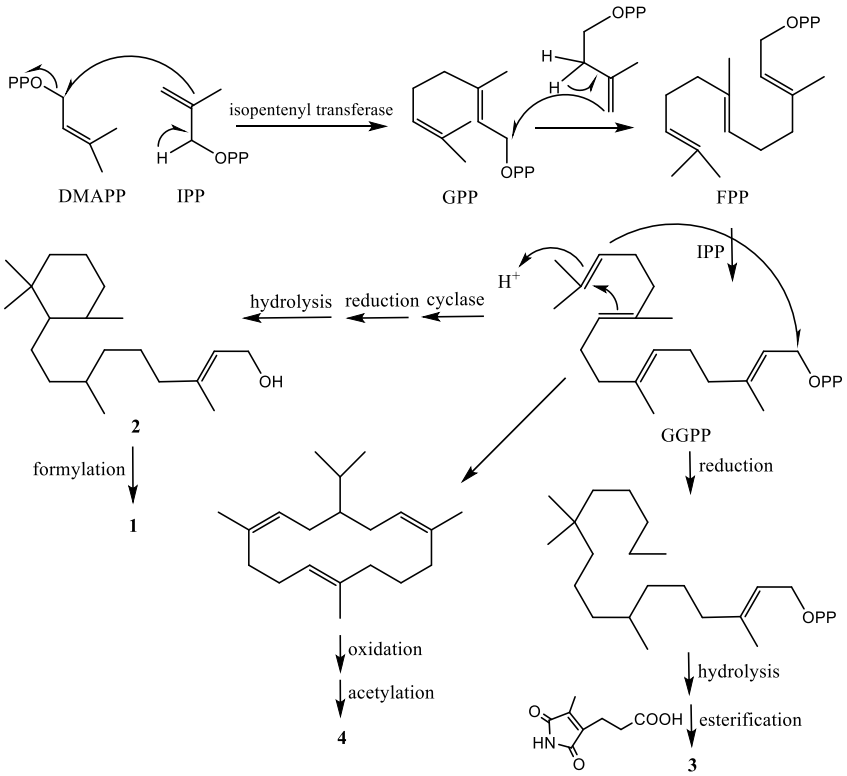

Figure 3. Biosynthetic pathway for compounds **1**−**4**.

In summary, one new monocyclic diterpenoid, together with three known diterpenoids and five sesquiterpenoids were isolated from the twigs and leaves of *S. fluviatile*. This was the first examples for the chemical constituents of *S. fluviatile* and the first report on the diterpenoids of genus *Syzygium*.

This discovery provided new evidences not only for the chemical diversity of the genus *Syzygium*, but also for the chemotaxonomic makers of *S. fluviatile.*

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Supporting Information

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ORCID

Dingli Zhang: 0000-0002-9073-6761
Yikao Hu: 0000-0002-3616-8556
Li Wang: 0000-0002-7004-4725
Shengxiong Huang: 0000-0002-3616-8556
Yan Zhao: 0000-0002-1638-7751
Mengjia Li: 0000-0002-7454-3767
Feng Li: 0000-0002-3943-5058
Yong Zhao: 0000-0002-3996-2480

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