SHORT REPORT

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Fissistigmol A: A New Polyacetylene alcohol from *Fissistigma Minuticalyx*

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Abstract: A new polyacetylene alcohol, Fissistigmol A (**1**), along with four known polyacetylene alcohol compounds (**2-5**) were isolated from the aerial part of *Fissistigma minuticalyx* (McGr. et W. W. Sm.). Their structures were elucidated based on the spectroscopic evidence, mainly including NMR and induced ECD, and HRESIMS data. In addition, bioactivity assay showed that **1-5** showed notable nitric oxide (NO) inhibitory effects (IC_{50} < 10 μM) on the model of the lipopolysaccharide (LPS)-activated RAW 264.7 macrophages.

Keywords: Polyacetylene alcohol; Annonaceae; *Fissistigma minuticalyx;* nitric oxide (NO) inhibitory activity.

1. Plant Source

Fissistigma minuticalyx (McGr. et W. W. Sm.) is a plant of the genus *Fissistigma* of the Annonaceae family. It has been commonly used as medicinal materials for a long period in the treatment of rheumatoid arthritis in Chinese folk medicine.

To investigate the phytochemistry of *F. Minuticalyx*, we collected the branches and leaves of *F. Minuticalyx* from Pu'er City in Yunnan Province (in Southwest of China) in April 2017. The sample of *F. Minuticalyx* were identified by Dr. Cheng Xiang (Medicinal botanist), furthermore, a voucher specimen (No. *Fissistigma*-2017-04) has been deposited at the Faculty of Life Science and Technology, Kunming University of Science and Technology.

2. Previous Studies

Phytochemisry researches of *Fissistigma* genus indicated main components of these plants included alkaloids[1], flavonoids [2], and terpenoids [3]. Some of these chemical substances could exhibited anti-inflammatory and antioxidant activities [4,5].

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3. Present Study

General Experimental Procedures: The column chromatography were carried out by a series of column chromatography, silica gel column (100-200 and 200-300 mesh, Qingdao Ocean Chemical Co.,Ltd.), ODS-C18 column (40-63 μm, Fuji, Japan), MCI gel (CHP 20P, 75–150 mm, Mitsubishi Chemical Corporation, Tokyo, Japan) and Sephadex LH-20 (20-100 μm ,GE Healthcare, Bio-Science AB, Uppsala). HPLC and semi-HPLC for analysis and purification were performed via Agilent 1200 series liquid chromatograph (Agilent, USA) and LC3000 liquid chromatograph (Beijing Tong Heng Innovation Technology Co., Ltd. China). The 1D and 2D NMR spectra were measured on Bruker HD 600 MHz NMR spectrometer (Bruker, Germany). HRESIMS was conducted on Agilent Q-TOF-MS (6530B). Optical rotations were recorded in methanol on a Jasco P-1020 polarimeter. CD spectra were obtained on an APP Chirascan spectropolarimeter. UV spectra were performed on a Shimadzu UV2401A spectrometer.

Extraction and Isolation: The dried branches and leaves of *F. minuticalyx* (10.0 kg) were extracted by heating reflow with 95 % EtOH to afford 920.0 g of crude extract, which was suspended in water and partitioned with petroleum ether, and ethyl acetate to yield petroleum ether (96.3 g), ethyl acetate (196.6 g), and aqueous fractions. The petroleum ether fraction was subjected to silica gel column chromatography and eluted with a gradient of petroleum ether-ethyl acetate (1: 0, 5: 1, 3: 1, 1: 1, 1: 3, 1: 5, 0: 1, v/v) and methanol. All fractions were collected and monitored by TLC. Depending upon the TLC similar fraction were combined and concentrated to get Fr.P1 to Fr.P8 fractions in which Fr.P7 (3.1 g) was separated by reversed-phase ODS (MeOH-H_2O, 10%-100%) and semi-preparative HPLC (MeOH-H_2O 42%) to obtain compound **2** (4.2 mg, t_R = 15.5 min) and compound **3** (2.1 mg, t_R = 16.8 min); Fr.P8 (2.4 g) was chromatographed over an ODS column using a gradient of MeOH-H_2O (10%-100%) and semi-preparative HPLC (MeOH- H_2O 35%) to obtain compound **5** (2.6 mg, t_R = 16.4 min). The ethyl acetate fraction was chromatographed on a silica gel column and eluted with a gradient of petroleum ether-ethyl acetate (v/v 1:0, 5:1, 3:1, 1:1, 1:3, 1:5, 0:1) to give a total of 6 fractions Fr.E1 to Fr.E6. Fr.E5 (6.9 g) was separated by normal phase silica gel (petroleum ether-ethyl acetate, gradient elution-volume ratio 50%-100%), MCI (MeOH-H_2O, gradient elution-volume ratio 10%-100%), Sephadex LH-20 (MeOH) and semi-preparative HPLC (MeOH-H_2O 40%) to obtain compound **1** (4.9 mg, t_R = 11.4 min). Fr.E6 (9.1 g) was separated by silica gel column gradient elution (petroleum ether-ethyl acetate, 30%-100% v/v), ODS column gradient elution (MeOH-H_2O, 30%-100% v/v), Sephadex LH-20 (MeOH) and semi-preparative HPLC (65% MeOH- H_2O) to afford compound **4** (1.4 mg, t_R =13.7 min).

*Fissistigmol A (**1**):* Brown oil; $[\alpha]_D^{25}$ +15.06 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.44), 253 (3.94), 268 (4.44), 284 (3.60) nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 1.74 (2H, m, H-2), 1.82 (3H, dd, *J* = 6.9, 1.8 Hz, H-14), 2.05 (3H , s, H-2'), 2.16 (2H, q, *J* = 7.2 Hz, H-3), 4.08 (2H, t, *J* = 6.5 Hz, H-1), 4.14 (1H, td, *J* = 6.5, 1.1 Hz, H-6), 4.30 (1H, m, H-7), 5.53 (1H, brd, J = 15.8 Hz, H-12), 5.57 (1H, ddt, *J* = 15.5, 6.7, 1.3 Hz, H-5), 5.85 (1H, dtd, *J* = 15.5, 6.8, 1.1 Hz, H-4), 6.35 (1H, dq, *J* = 15.8, 6.9 Hz, H-13); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 19.1 (CH_3, C-14), 21.2 (CH_3, C-2'), 28.0 (CH_2, C-2), 28.8 (CH_2, C-3), 63.8 (CH_2, C-1), 66.9 (CH, C-7), 71.5 (C, C-10), 71.6 (C, C-9), 75.5 (CH, C-6), 78.2 (C, C-11), 79.0 (C, C-8), 109.5 (CH, C-12), 127.9 (CH, C-5), 134.5 (CH, C-4), 144.8 (CH, C-13), 171.5 (C, C-1'); HRESIMS *m/z* 299.1258 $[M + Na]^+$ (calcd. for $C_{16}H_{20}NaO_4$, 299.1254).

A new polyacetylene alcohol

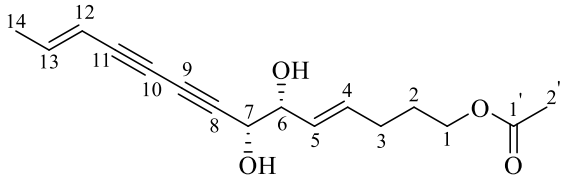

Figure 1. Structure of Fissistigmol A (**1**)

Compound **1,** Fissistigmol A, obtained as brown oil, possessed a molecular formula of $C_{16}H_{20}O_4$ by the positive HR-ESI-MS (*m/z* 299.1258 $[M + Na]^+$, calcd. 299.1254), requiring seven indices of hydrogen deficiency. By comparing datas reported in the literature[6] and the ^1H NMR spectrum showed two pairs of trans olefinic protons at δ_H 6.35 (1H, dq, *J* = 15.8 6.9 Hz, H-13), 5.53 (1H, brd, *J* = 15.8 Hz, H-12); 5.85 (1H, dtd, *J* = 15.5 6.8, 1.1 Hz, H-4) and 5.57 (1H, ddt, *J* = 15.5, 6.7, 1.3 Hz, H-5), two mutually coupling oxygenated ethane protons at δ_H 4.30 (1H, d, *J* = 6.4 Hz, H-7) and 4.14 (1H, br dd, *J* = 6.7, 6.4 Hz, H-6), three methylene signals at δ_H 1.74 (2H, m, H-2); δ_H 2.16 (2H, q, *J* = 7.2 Hz, H-3) and δ_H 4.08 (2H, t, *J* = 6.5 Hz, H-1), as well as two methyl signals at δ_H 2.05 (3H, s, H-2') and 1.82 (3H, dd, *J* = 6.9, 1.8 Hz, H-14). The ^{13}C NMR spectrum showed a total of 16 carbon signals, including four olefinic carbons, four alkyne carbons, three oxygenated sp^3 carbons, as well as characteristic acetyl carbons at δ_C 171.3 and 21.0. The above NMR features were very similar to those of lobetyol, a linear polyacetylene alcohol [11]. The ^1H-^1H-COSY correlations (Figure 2) established the connections of H-14/H-13/H-12 and H-7/H-6/H-5/H-4/H-3/H-2/H-1. In view of obvious downfield shift of H-1, the acylation position was deduced at C-1. The HMBC correlation (Figure 2) from H-1 to the acetyl carbonyl carbon [171.3 (C-1′)] further confirmed the acetylation at C-1.

Summarizing the relevant NMR data of the threo or erythrodiol structure with similar partial structures in the literature [7,8,9], it is found that the coupling constant law is: the coupling constant of threo vicinal diol is 5.5-7.0 Hz, while the coupling constant of erythro diol is 3.0-4.0 Hz. Based on the coupling constant between H-7 and H-6 (*J* = 6.5 Hz), it can be inferred that the vicinal diol of compound **1** is in the threo configuration. The absolute configuration of the acyclic chiral neighboring diols in **1** was determined by $Mo_2(OAc)_4$-induced ECD method [10,11]. According to Snatzke's rule, a negative cotton effect at 322 nm shown in the ICD spectrum of compound **1** indicated the configuration of diol in **1** should be 6*R*,7*R*. In addition, the specific optical rotation calculation via DFT computational methods (B3LYP/6-311+g(d,p)//B3LYP/6-31g(d), see Supplementary Materials) provided additional evidence for the absolute configuration of this chiral compound. Therefore, the structure of **1** was established as (4*E*,6*R*,7*R*,12*E*)-6,7-dihydroxytetradeca-4,12-dien-8,10-diyne-1-yl acetate, and named Fissistigmol A, as illustrated in Figure **1**.

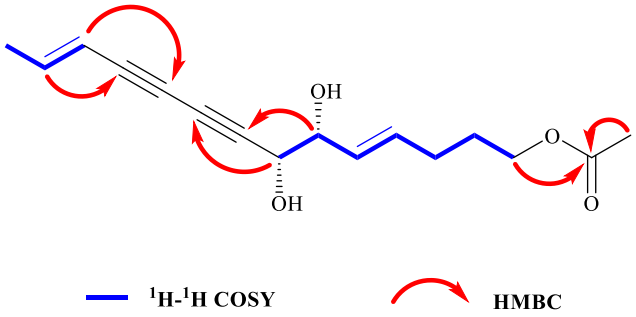

Figure 2. Important ^1H-^1H-COSY and HMBC correlations for Fissistigmol A (**1**)

The known compounds were identified as lobetyol (**2**) [12], isolobetyol (**3**) [13], pilosulyne A (**4**) [14], lobetyolin (**5**) [15] by comparing their NMR and MS data with those reported in the literature. All of them were found in this plant for the first time.

All the isolates were tested for their inhibitory effects on NO production in LPS-activated RAW 264.7 macrophages with dexamethasone (DMX) as the positive control. As shown in Table 1, all compounds showed significant activities against RAW 264.7 cells with IC_{50} values less than 10 μM.

Table 1. Effects of Compounds on the NO production in LPS-activated RAW 264.7 cells [a]

Compounds	IC_{50} ± SD (μM)
DXM [b]	0.55 ± 0.35
1	8.50 ± 0.66
2	3.04 ± 0.98
3	2.45 ± 0.57
4	6.61 ± 0.55
5	2.78 ± 0.42

[a] Results were expressed as IC50 values in μM (n = 3).
[b] Positive control.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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