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A NEW TRIHYDROXY FATTY ACID ESTER FROM THE ROOT OF *Isatis indigotica*

Dong-Dong Sun,[1*] Wei-Wei Dong,[2] Xiang Li,[1] Han-Qing Zhang,[3] and Xian-Feng Huang[4]

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From the EtOAC extract of the root of Isatis indigotica*, a new trihydroxylated monounsaturated fatty acid ester **1**, together with two known fatty acids **2–3** that were separated for the first time, has been isolated. The structure of this new linoleic acid-derived metabolite was established as methyl-8,11,12-trihydroxy-9Z-octadecenoic acid on the basis of spectroscopic data.*

Keywords*: Isatis indigotica*, fatty acid ester.

Isatis indigotica Fort. has been used in traditional Chinese medicine to treat headache, rheumatism, and virosis [1]. Two sphingolipids have been found in the 95% EtOH extract of the root of *I. indigotica* in our previous study [2]. In continuation of our investigation on the chemical constituents of *Isatis indigotica*, a new fatty acid ester was isolated from the root of this plant, along with the known compounds 8,11,12-trihydroxy-9*Z*-octadecenoic acid (**2**) and 11,12-dihydroxy-7,9-octadecadienoic acid (**3**). The present report deals with the isolation and structural elucidation of these compounds.

Purification of the EtOAc fraction of the root of *I. indigotica* afforded two pure known ceramides **2**, **3** and the new ester **1**.

The molecular formula of **1** was determined to be $C_{19}H_{36}O_5$ by HR-ESI-MS. The IR spectra of **1** revealed the absorption bands of hydroxyls at 3380 and 1015 cm^{-1} and a carbonyl at 1740 cm^{-1}. Also, the bands appearing at 2850, 1450, 1420, and 720 cm^{-1} revealed its paraffinic nature [3]. Compound **1** was considered to be a straight-chain compound due to a terminal methyl group at δ 14.1 ppm [4] in its ^{13}C NMR spectrum. The ^{1}H NMR spectrum of **1** showed the presence of one terminal methyl at δ 0.88 (3H, t) and methylenes at δ 1.30 (br.m). The ^{13}C NMR (distortionless enhancement by polarization transfer) spectrum of **1** further furnished a quaternary carbon, 5 methines, 11 methylenes, 1 terminal methyl, and 1 methoxyl (Table 1), in which one carbonyl carbon ($COOCH_3$) at 174.5 was revealed. These data revealed that **1** was an unbranched fatty acid ester.

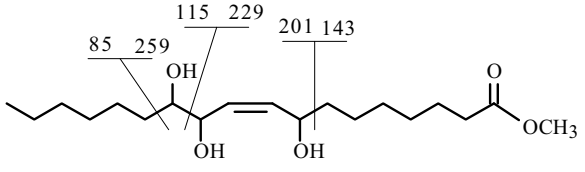

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Fig. 1. ESI-MS fragment analysis of compound **1**.

1) Nanjing University of Chinese Medicine, 210046, Jiangsu, P. R. China, fax: +86 25 85811006, e-mail: sun_21373@163.com; 2) Nanjing University of Technology, 210009, Jiangsu, P. R. China; 3) Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, 210014, Jiangsu, P. R. China; 4) Nanjing University, 210093, Jiangsu, P. R. China.
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TABLE 1. ^{1}H and ^{13}C NMR of **1** (300 and 75 MHz, DMSO-d_6, δ, ppm J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1 (C=O)		174.5	10 (CH)	5.65 (1H, dd, J = 11.2, 5.4)	135.4
2 (CH_2)	2.25 (1H, t, J = 7.5)	35.1	11 (CH)	3.98 (1H, dd, J = 6.3, 5.4)	75.9
3–6;14–17(CH_2)	1.30 (16H, m)	24–32	12 (CH)	3.51 (1H, dd, J = 6.3, 5.3)	74.8
7 (CH_2)	1.58 (2H, m)	38.5	13 (CH_2)	1.55 (2H, m)	36.9
8 (CH)	4.05 (1H, dd, J = 6.2, 5.7)	72.5	18 (CH_3)	0.88 (3H, t, J = 6.9)	14.1
9 (CH)	5.58 (1H, dd, J = 11.2, 5.7)	132.1	OCH_3	3.65 (3H, s)	51.8

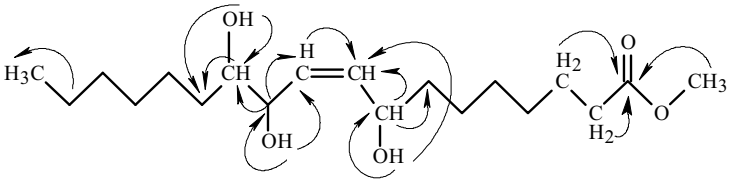

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Fig. 2. Selected HMBC correlations of **1**.

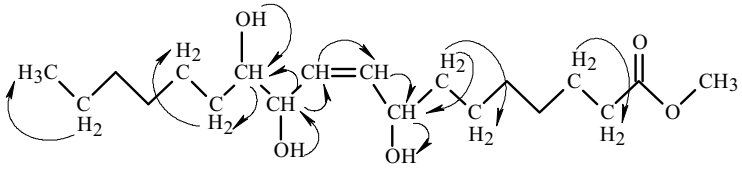

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Fig. 3. Selected ^{1}H–^{1}H COSY correlations of **1**.

The ^{13}C NMR spectral signals at δ 72.5 (CH), 75.9 (CH), and 74.8 (CH) and the ^{1}H NMR resonances at δ 4.05 (1H, dd, J = 6.2, 5.7), 3.98 (1H, dd, J = 6.3, 5.4), and 3.51(1H, dd, J = 6.3, 5.3) infer the existence of three hydroxyl groups in the molecule. Furthermore, the ^{1}H NMR signals at 5.58 (1H, dd, J = 11.2, 5.7) and 5.65 (1H, dd, J = 11.2, 5.4) and ^{13}C NMR signals at δ 132.1 (CH) and 135.4 (CH) indicated the presence of a double bond in **1**. The location and configuration of the double bond were determined as follows. The corresponding fragment ions at *m/z* 85, 115, and 229 (Fig. 1) due to the formation of $[C_6H_{13}]^+$, $[CH_3(CH_2)_5CH(OH)]^+$, and $[CH(OH)CH{=}CHCH(OH)(CH_2)_6COOCH_3]^+$ species arising from cleavages between C-12 and C-13 and between C-11 and C-12 supported the location of the olefinic linkage between C-9 and C-10 in the molecule. The *cis* stereochemistry of the double bond was deduced from the large vicinal coupling constant ($J_{9,10}$ = 11.2) displayed between H-9 and H-10 [5]. Analysis of the 2D NMR spectra HMBC (Fig. 2) and ^{1}H–^{1}H COSY (Fig. 3) led to the assignment of proton and carbon signals for **1**, which strongly confirmed that **1** contained the partial structures -CH_2-CH(OH)-CH(OH)-CH=CH-CH(OH)-CH_2- (C-7 to C-13) and -CH_2-CO-O-CH_3. Consequently, the preceding evidence led to the establishment of the structure of **1** as methyl-8,11,12-trihydroxy-9*Z*-octadecenoic acid.

EXPERIMENTAL

General. Melting points: Gallenkamp apparatus. Optical rotations: Jasco P-1020. IR: Hitachi 260–30. NMR: Bruker AMX or Varian UNITY INOVA 300 NMR in DMSO-d_6. ESI-MS^n: API 3000. HR-ESI-MS: PE LC/MS spectrometer.

Column chromatography (CC) was performed over silica gel (100–300 mesh; Qingdao Marine Chemical Ltd., Qingdao, People′s Republic of China). Thin-layer chromatographic analysis was carried out in plates precoated with silica gel F_{254} (Qingdao Marine Chemical Ltd.), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. All solvents were distilled before use.

Plant Material. *I. indigotica* root (Brassicaceae) were purchased from a traditional Chinese medicine company (Anguo Co.) of Hebei Province in 2007 and identified by Prof. Jian-Wei Chen (School of Pharmacy, Nanjing University of Chinese Medicine, Jiangsu, China). The samples were authenticated and deposited in the Herbarium of Nanjing University of Chinese Medicine, Nanjing, P. R. China (SDD-BLG-003).

Extraction and Isolation. The root (50 kg) was extracted with 95% EtOH at room temperature. The extract (2100 g) was suspended in water and partitioned to provide EtOAc (500 g) and *n*-BuOH (220 g) fractions. The EtOAc extract (500 g) was subjected to Si-gel CC (200–300 mesh) and eluted with mixtures of $CHCl_3$–MeOH (200:1–1:1) to provide ten fractions (P1–P10). The P5 fraction (20 g) was subjected to silica gel (200–300 mesh) column chromatography using $CHCl_3$–Me_2CO (100:1–1:1) as eluent to afford six other fractions (P11–P16). The P13 fraction (5 g) was further subjected to Sephadex LH-20 CC ($CHCl_3$–MeOH 9:2) and purified using a Lobar A RP-18 column (82% MeOH) to afford **1** (20 mg), **2** (30 mg), and **3** (150 mg).

The known compounds **2–3** were identified by comparison of their ^1H and ^{13}C NMR spectral data with those in the literature [6].

Methyl-8,11,12-trihydroxy-9*Z*-octadecenoic Acid (1). White amorphous powder: mp 82–83°C; $[\alpha]_D^{20}$ +9.6° (*c* 0.007, $CHCl_3$). IR (KBr, cm^{-1}): 3500–3100, 2850, 1740, 1015, and 720. ESI-MS *m/z*: 880 $[M + Na]^+$ 367, 336, 308, 282, 252, 224, 166, 138, 108, 82, 54; HR-ESI-MS *m/z* $[M + Na]^+$ 367.2463 (calcd for $C_{19}H_{36}O_5Na$, 367.2460).

^1H and ^{13}C NMR data, see Table 1.

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