Arch Pharm Res Vol 26, No 3, 207-209, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

A New (*E*)-4-Hydroxy-dodec-2-enedioic Acid from the Stem Bark of *Albizzia julibrissin*

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(Received December 31, 2002)

A new unsaturated hydroxy acid was isolated from the stem bark extract of *Albizzia julibrissin* through repeated silica gel and Sephadex LH-20 column chromatography. The chemical structure of the new acid was determined as (*E*)-4-hydroxy-dodec-2-enedioic acid on the basis of several spectral data including 2D-NMR. The stereochemical feature of the double bond was determined to be *E* on the basis of the coupling pattern of related proton signals in the ^{1}H-NMR and COSY experiments.

Key words: *Albizzia julibrissin,* Leguminosae, Unsaturated hydroxy acid, (*E*)-4-Hydroxy-dodec-2-enedioic acid

INTRODUCTION

Albizzia julibrissin Durazz (Leguminosae) is a small and spreading tree with smooth, gray-brown bark and doubly pinnate leaves. Clusters of pink flower heads are borne in summer. It grows abundantly in Korea and the dried stem bark of this plant has been used in China, Japan and Korea for the treatment of insomnia, diuresis, sthenia, ascariasis and contusion (Kim, 1996; Ikeda *et al.*, 1997; Kang *et al.*, 2000). Previously, we reported the isolation of 3',4',7-trihydroxyflavone, α-spinasterol glucoside (Chamsuksai *et al.*, 1981) and triterpenes (Kang and Woo, 1983; Woo and Kang, 1984). In the course of our continuing work, a new unsaturated hydroxy acid was isolated. This paper describes the isolation and structure determination of the new acid. The position of the double bond was determined by analysis of ^{1}H-^{1}H COSY and HMBC NMR data.

MATERIALS AND METHODS

General experimental procedures

Optical rotation was determined in MeOH on a Perkin-Elmer 341MC polarimeter. UV and IR spectra were recorded on a Varian Carry UV-visible spectrophotometer and a Perkin Elmer FT-IR spectrometer, respectively. HR-FAB mass data were recorded on a JEOL JMS-HX110/110A spectrometer. EI mass data were recorded on a JEOL JMS-700 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured by JEOL JNM-ECP 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometer. Chemical shifts were referenced to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). DEPT, COSY, HMQC, and HMBC spectra were recorded on a JEOL JNM-ECP 400 using pulsed field gradients. Column chromatography was carried out using silica gel (Merck, 70-230 mesh) and Sephadex LH-20 (Sigma, 25-100 μ). TLC was performed on the precoated Merck Kieselgel 60 F_{254} plate (0.25 mm), and 50 % H_2SO_4 was used as a spray reagent.

Plant material

The stem bark of *A. julibrissin* was purchased from a commercial supplier in 2001 and authenticated by Prof. J. S. Choi of the Faculty of Food Science and Biotechnology, Pukyong National University. A voucher specimen has been deposited in the laboratory of Prof. J. S. Choi.

Extraction and isolation

The stem bark (18.2 kg) of *A. julibrissin* was refluxed with MeOH for 3 h (18 L × 3). The total filtrate was concentrated to dryness *in vacuo* at 40 °C to render the MeOH extract (2.97 kg), and this extract was suspended in distilled water and partitioned with CH_2Cl_2 (931.8 g), EtOAc (86.2 g), *n*-

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BuOH (649.5 g), and H_2O (1181.8 g) in sequence. The EtOAc (86.2 g) fraction was chromatographed on a Si gel column using CH_2Cl_2-MeOH (gradient) to yield 29 subfractions. The fraction 19 (14.69 g) was further chromatographed on a Sephadex LH-20 column with MeOH to give compound **1** (30 mg).

(*E*)-4-Hydroxy-dodec-2-enedioic acid (1)

Amorphous powder; $[\alpha]_D^{20}$ - 0.21° (MeOH, c 0.01%); UV λ_{max} (MeOH) : 259 nm (log ε 2.86); IR ν_{max} (KBr) 3537, 2932, 2843, 2358, 1701, 1690, 1466, 1425, 1406, 1280, 1265, 1067, 977 (cm^{-1}); HR-FABMS : $[M + H]^+$ found *m/z* 245.1389 (Δ – 0.2 mmu); ^{1}H-NMR (400 MHz, CD_3OD, δ_H): see Table I; ^{13}C-NMR (100 MHz, CD_3OD, δ_C) : see Table II.

(*E*)-4-Hydroxy-dodec-2-enedioic acid dimethyl ester (1a)

Compound **1** (15 mg) was methylated with diazomethane at room temperature for 24 h to yield compound **1a** (6 mg). EIMS : $[M]^+$ *m/z* 272 (7.61%); ^{1}H-NMR (400 MHz, CD_3OD, δ_H) : see Table I; ^{13}C-NMR (100 MHz, CD_3OD, δ_C) : see Table II.

Table I. ^{1}H NMR data of **1** and **1a** (400 MHz, CD_3OD)

Position	1	1a
1		3.64 (3H , s)
2	5.96 (1H, dd, 1.5, 15.6 Hz)	6.00 (1H, dd, 1.7, 15.6 Hz)
3	6.91 (1H, dd, 5.0, 15.6 Hz)	6.93 (1H, dd, 5.0, 15.6 Hz)
4	4.21 (1H, dd, 5.0, 11.8 Hz)	4.21 (1H, dd, 5.0, 11.3 Hz)
5	1.53 (2H, m)	1.53 (2H, m)
6-9	1.34 (2H × 4, brs)	1.32 (2H × 4, brs)
10	1.59 (2H, m)	1.59 (2H, m)
11	2.27 (2H, t, 7.4 Hz)	2.30 (2H, t, 7.4 Hz)
12		3.72 (3H, s)

Multiplicities and coupling constants are in parentheses.
Assignments based on DEPT, COSY, HMQC, and HMBC experiments.

Table II. ^{13}C NMR data of **1** and **1a** (100 MHz, CD_3OD)

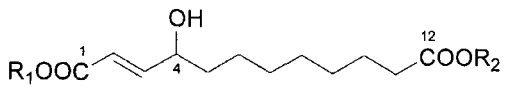
Position	1	1a
1	170.9	169.5
2	121.7	121.0
3	153.5	153.7
4	72.4	72.4
5	38.4	38.3
6	27.2	27.1
7	31.2	31.2
8	30.9	30.9
9	31.1	31.1
10	26.9	26.8
11	35.7	35.6
12	178.5	176.8
OCH_3		52.7, 52.8

Assignments based on DEPT, COSY, HMQC, and HMBC experiments.

RESULTS AND DISCUSSION

Compound **1**, obtained as amorphous powder in MeOH, showed the absorbance bands at 1690, 1701, 2358, 3537 cm^{-1} in the IR spectrum (KBr) due to α,β-unsaturated ketone, aliphatic COOH, aliphatic CH, and hydroxy group, respectively. The ^{13}C-NMR spectrum of **1** exhibited two carboxyl (δ 178.5, 170.9), two olefinic methine (δ 153.5, 121.7), one oxygenated methine (δ 72.4), and seven methylene (δ 38.4, 27.2, 31.2, 30.9, 31.1, 26.9, 35.7) carbon signals, indicating **1** to be a C_{12} diacid containing one double bond and a hydroxyl group. This result was confirmed by its molecular ion peak $[M + H]^+$ at *m/z* 245.1389 (Δ – 0.2 mmu) in the HRFAB-MS spectrum. In addition, in the ^{1}H-NMR spectrum of **1**, two olefinic methane (δ 5.96, 6.91 (each 1H)), one oxygenated methine (δ 4.21 (1H)), and seven methylene (δ 1.53 (2H, m), 1.34 (2H × 4, brs), 1.59 (2H, m), 2.27 (2H, t)) proton signals were observed. The ^{1}H-^{1}H connectivity between H-2 and H-11 was observed in the ^{1}H-^{1}H COSY of **1**, indicating the position of one double bond and one hydroxyl to be C-2 and C-4, respectively. All proton signals except those due to four methylene groups of aliphatic region in the ^{1}H-NMR were assigned unambiguously by HMBC (Table I) and COSY experiments. The assignment of carbon signals was reinforced by the HMQC experiment. The geometry of C-2 double bond was suggested to be *E*-form by the coupling constant (*J* = 15.6 Hz) between H-2 and H-3. The presence of two carboxyl groups in the molecule was confirmed by methylation of **1**. Compound **1** (15 mg) was methylated with diazomethane treatment and then purified through Sephadex LH-20 column chromatography eluting with MeOH to afford compound **1a** (6 mg). Compound **1a** was identified as (*E*)-4-hydroxy-dodec-2-enedioic acid dimethyl ester on the basis of several spectral evidences. Thus, the structure of **1** was determined to be (*E*)-4-hydroxy-dodec-2-enedioic acid (Fig. 1). Currently, we are studying the absolute configuration of C-4 in **1**.

α-Linolenic acid, the most abundant octadecatrienoic acid in nature, has unsaturation at C-9, C-12 and C-15. As the relevant monohydroxyoctadecatrienoic acids from natural source, 2-hydroxy-9,12,15-triene (2-hydroxylinolenic



1 : R_1=R_2= H
1a : R_1=R_2= CH_3

Fig. 1. Chemical structures of **1** and **1a**

acid, from the seed oil of *Thymus vulgaris*) and 18-hydroxy-9,11,13-triene (kamloenic acid, from the seeds of *Mallotus discolor*) have been reported (Bang *et al.*, 2002). To the best of our knowledge, this is the first report of the occurrence of compound **1** in nature.

ACKNOWLEDGEMENTS

The high resolution positive ion FAB-MS spectra were provided by the Korea Basic Science Institute. This study was supported by a grant from the Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6-01NA01-0002).

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