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Isolation and Structure Elucidation of 9-Hydroxy-10*E*, 12*Z*-octadecadienoic Acid from *Dioscorea luzonensis*

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ABSTRACT

Chemical investigation of the dichloromethane extract of *Dioscorea luzonensis* Schauer. led to the isolation of 9-hydroxy-10*E*,12*Z*-octadecadienoic acid or α-dimorphecolic acid (**1**) from the skin of the tuber. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry.

Keywords: Dioscorea luzonensis, Dioscoreaceae, 9-hydroxy-10*E*,12*Z*-octadecadienoic acid, α-dimorphecolic acid

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INTRODUCTION

Dioscorea luzonensis, also known as wild yam or camangeg, is a plant endemic to The Philippines. It is a wild root crop that grows naturally in Ilocos province of the Philippines. The tubers of this plant are usually harvested during the early part of August and these are used as a vegetable and in making delicacies such as haleya, a type of jam or vegetable puree [1]. The tuber has a unique brown color with fine roots on its surface and an elongated and irregular morphology. The tuber can also be eaten after 20 to 30 minutes boiling. The skin of the tuber is usually considered to be inedible and it is removed when it is used as a food supplement. The inner portion of the tuber is the edible portion with uniform white color.

Dioscorea luzonensis has no reported biological activities. We earlier reported the isolation of long chain alkyl *trans*-ferulates, β-sitosterol, and fatty acids from the skin of the tuber; and ursolic acid and fatty acids from the inner portion of the tuber of *D. luzonensis* [2]. We report herein the isolation of 9-hydroxy-10*E*,12*Z*-octadecadienoic acid or dimorphecolic acid (**1**) from the skin of *D. luzonensis* tuber. The structure of **1** is presented in Fig.1. To the best of our knowledge this is the first report on the isolation of **1** from *D. luzonensis.*

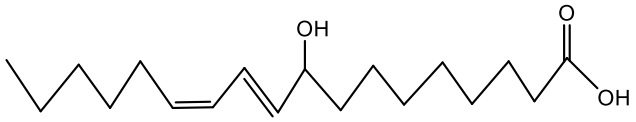

Fig. 1. Chemical structure of 9-hydroxy-10E,12Z-octadecadienoic acid or dimorphecolic acid (1) from *D. luzonensis.*

MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 400 in $CDCl_3$ at 400 MHz. Column chromatography was performed with silica gel 60 (70-230 mesh). The ESIMS was recorded on Agilent Technologies 6120 Quadrapole LC/MS. Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f value were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Plant Material

The tubers were bought from Batac Public Market in Batac, Ilocos Norte, Philippines in November 2014. The sample was authenticated by Flordeliz Rapacon Estira of Mariano Marcos State University, Batac, Ilocos Norte, Philippines.

Isolation

The freeze-dried skin of the tuber (66.27 g) of *D. luzonensis* was cut into small pieces, ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated from the filtrate under

vacuum to afford a crude extract (0.6 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increments by volume as eluents. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 20% EtOAc in petroleum ether to afford **1** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *D. luzonensis* yielded compound **1**. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy. The ^{1}H NMR spectrum of **1** indicated resonances for olefinic protons at δ 6.47 (dd, *J* = 11.0, 15.2 Hz), 5.95 (t, *J* = 11 Hz), 5.64 (dd, *J* = 6.9, 15.2 Hz), and 5.43 (dt, *J* = 7.4, 11.0); a hydroxymethine proton at δ 4.15 (bq, 6.7); α-methylene protons at δ 2.32 (t, *J* = 7.5 Hz), allylic methylene protons at δ 2.15 and methylene protons at δ 1.23–1.61; and virtually coupled methyl protons at δ 0.87. These resonances indicated a fatty acid with two double bonds and a hydroxyl group. The deshielded nature of the olefinic protons and the hydroxymethine proton suggested that the double bonds are conjugated and adjacent to the hydroxyl group. The conjugated system is supported by the COSY correlations of the hydroxymethine proton at δ 4.15 and the olefinic proton at δ 5.64, which was in turn coupled to the proton at δ 6.47 by 15.2 Hz. The latter proton was coupled to the proton at δ 5.95, which was in turn coupled to the proton at δ 5.43. The large coupling constant of 15.2 Hz between the olefinic protons at δ 5.64 and 6.47 indicated *trans* coupling, while the smaller coupling constant of 11 Hz between the olefinic protons at δ 5.95 and 5.43 suggested *cis coupling.*

The ^{13}C NMR spectrum indicated resonances for a carboxylic acid at δ 176.9; olefinic carbons at δ 125.9. 127.6, 135.1 and 135.7; a hydroxymethine carbon at δ 72.9; methylene carbons at δ 37.2, 33.5, 31.4, 29.7, 29.3, 29.1, 28.9, 27.7, 25.3, 24.7, and 22.5, and a methyl carbon at 14.0. These eighteen carbon resonances with two double bonds, a hydroxymethine and a carboxylic acid indicated a molecular formula of $C_{18}H_{32}O_3$ corresponding to a molecular weight of 296.3. This was confirmed by an ESIMS which gave a peak for *m/z* $[M–H]^-$ at 295.3.

Protons attached to carbons were verified by HSQC and the structure of **1** was supported by HMBC 2D NMR data. The carboxylic acid was long-range correlated to the α-methylene protons at δ 2.32 and the β-methylene protons at δ 1.60. Long-range correlations were also observed between the methyl protons at δ 0.87 and the carbons at δ 31.4 and 22.5. Importantly, the allylic methylene protons at δ 2.15 are also correlated with methylene group at 31.4, as well as another methylene group at δ 29.1. This establishes a five sp^3 carbon chain from the methyl group terminus to the allylic methylene group. Furthermore, the allylic methylene protons at δ 2.15 are long-range correlated to the olefinic carbons at δ 133.1 and 127.6. Long-range correlations were also observed between the olefinic proton at δ 5.65 and the carbon at δ 127.6; and the oxymethine proton at δ 4.15 and the carbon at δ 125.9.

Literature search revealed that **1** is 9-hydroxy-10*E*,12*Z*-octadecadienoic acid or dimorphecolic acid (**1**) as evidenced by similar ^{1}H NMR data [3]. This conjugated linoleic acid derivative showed inhibitory activity against fat accumulation [3]. Another study reported that **1** inhibited the growth of *Bacillus subtilis* SBUG 14, *Micrococcus flavus* SBUG 16 and *Staphylococcus aureus* SBUG 11 and ATCC 25923 [4]. Furthermore, **1** was found to be cytotoxic against P388 mouse leukemia cells, slightly inhibited the growth of a Balb/c mouse 3T3 fibroblast cells and strongly inhibited simian virus 40-transformed 3T3 cells [5]. In another study, **1** was found to be cytotoxic against human myeloma RPMI 8226 (ATCC CCL-165), chronic myelogenous leukemia K-562 (ATCC CCL-243), human hepatocellular carcinoma HepG2 (ATCC HB-8065) and adenocarcinoma MCF-7 (HTB-22) with IC_{50} values of 21.2±1.8, 10±2, 59±6, and 56±4 μM±S.D., respectively [6].

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