Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(10):4558-4561

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Fatty acid derivatives from seeds of *Annona squamosa* Linn.

Chandrababu Naidu R[1], Niranjana Kumar A[1], Komuraiah B[1], Satya Srinivas KVN[1], Kotesh Kumar J[1*], Ramakrishna KVS[2], Laxman Nayak V[2] and Sistla Ramakrishna[2]

[1]Natural Products Chemistry, CIMAP-Research Centre (CSIR), Boduppal, Hyderabad-500039, Andhra Pradesh, India
[2]Indian Institute of Chemical Technology (CSIR), Uppal Road, Hyderabad-500607, Andhra Pradesh, India

---

ABSTRACT

*Fatty acid derivatives, (Z)-2-hydroxy-3-(octadec-9-enoyloxy) propanoic acid (**1**) and Hexadecanoic acid-2,3-dihydroxy propyl ester (**2**) were isolated from the seeds of Annona squamosa. Compound **1** is novel and isolated for the first time from a plant source and **2** is reported for the first time from A. squamosa. Compounds **1** & **2** were evaluated for cytotoxic activity in vitro against human cancer cell lines (A549, ACHN, HELA, PC-3, B-16, HT-29, and MCF-7). Compound **1** showed potential cytotoxicity against HELA (IC_{50} 5.45µg/ml) and MCF-7 (IC_{50} 4.66µg/ml) cell lines where as **2** showed potential cytotoxicity against HELA (IC_{50} 9.16µg/ml) cell lines.*

Key words: *Annona squamosa*, Fatty acid derivatives, cytotoxic activity, Cancer cell lines

---

INTRODUCTION

Annonaceae is a large family of tropical and subtropical trees and shrubs, comprising 130 genera and about 2300 species [1-2]. Many natural products, including alkaloids [3-6], *ent*-kauranes [7-8], flavonoids [9-10], annonaceous acetogenins [2] and cyclopeptides [11], have been isolated from annonaceous plants. These products showed cytotoxicity [2,12], antimalarial [2,3], pesticidal [2,13], antiplatelet [9], anti-inflammatory [7-8], and vasorelaxant activities [14]. *Annona squamosa*, commonly known as the custard apple, is a well-known edible fruit and native of West Indies. The plant has been naturalized throughout India in plains as well as on hills [14]. Its seeds exhibit insecticidal and abortifacient properties [15]. *A. squamosa* consists of a variety of compounds *i.e.*, fatty acids [16], acetogenins [17] amino acids [18], kaurenes [19], steroids [20] *etc*. In the present work, we report the isolation and identification of a novel fatty acid derivative **1** and a rare compound **2** from the seeds of *A. squamosa*. Compound **2** [21,22] was isolated for the first time from *A. squamosa* seeds. Both structures were confirmed by spectroscopy.

EXPERIMENTAL SECTION

2.1. Plant material

The seeds of *A. squamosa* were collected from Ranga Reddy District of Andhra Pradesh, India during October, 2011 and were identified by Prof. V.S. Raju of Kakatiya University, Warangal, Andhra Pradesh, India. A voucher specimen (CRC-AS-002) was deposited in the CIMAP-Research Center, Hyderabad.

2.2. Extraction and Isolation

Fresh seeds of *A. squamosa* (250g) were pulverized and macerated in hexane solvent at room temperature for 48h. Later, the solvent was filtered and evaporated at reduced pressures to obtain pale yellow crude (20g). The crude extract was directly subjected to column chromatography over silica gel (100-200 mesh) and eluted successively with solvents hexane and mixtures of ethylacetate (EtOAc) in hexane. Later fractions collected in the hexane yielded compound **1** (6g, TLC 95:5 Hexane:EtOAc, rf:0.47). Fractions collected using hexane:EtOAc (80:20) have afforded compound **2** (90mg, TLC 1:1 Hexane:EtOAc, Rf 0.46).

2.3. Cytotoxicity by MTT Assay

A549 (Lung cancer), ACHN (renal cancer cell), HELA (cervical cancer), B-16 (mouse melanoma), HT-29 (colon cancer), prostate cancer (PC-3), and MCF-7 (Breast cancer) cell lines were obtained from National center for Cell science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagle Medium), MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Trypsin, EDTA were purchased from Sigma Chemicals Co. (st.Louis, MO), Fetal bovine serum were purchased from Arrow labs, 96 well flat bottom tissue culture plates were purchased from Tarson. A549 (Lung cancer), ACHN (renal cancer cell), HELA (cervical cancer), B-16 (mouse melanoma), HT-29 (colon cancer), prostate cancer (PC-3), and MCF-7 (Breast cancer) cell lines grown as adherent in DMEM media. Supplemented with 10% fetal bovine serum, 100 µg/ml penicillin, 200 µg/ml streptomycin, 2 mM L-glutamine, and culture was maintained in a humidified atmosphere with 5%CO2. Stock solution of 10mg/ml stock solution in DMSO, from the above stock various dilutions was made with sterile water to get required concentration.

2.3.1. Cytotoxicity evaluation

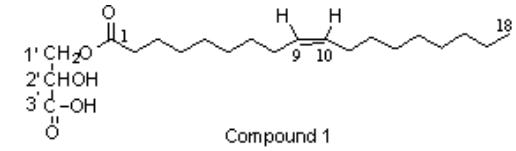
Toxicity of test compound in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product. $1x10^4$ Cells (counted by Trypan blue exclusion dye method) in 96- well plates were incubated with compounds with series of concentrations tested for 48 hrs at 37^0C in DMEM/MEM with 10% FBS medium. Then the above media was replaced with 90µl of fresh serum free media and 10 µl of MTT reagent (5mg/ml) and plates were incubated at 37^0C for 4h, there after the above media was replaced with 200µl of DMSO and incubated at 37^0C for 10min.The absorbance at 570nm was measured on a spectrophotometer (spectra max, Molecular devices) IC–50 values were determined from plot: % inhibition (from control) versus concentration.

RESULTS AND DISCUSSION

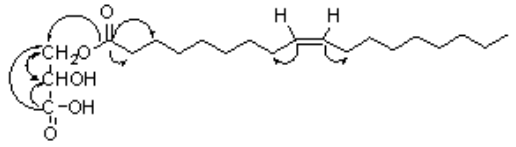
Compound **1**, $[\alpha]^{25}_D$ -15.7499 (c=0.25, chloroform), ESI-MS m/z 371 (M^+), was isolated as a colorless oil from n-hexane. Its molecular formula was established as $C_{21}H_{38}O_5$ by spectroscopy coupled with mass data. It showed IR absorptions (ν_{max}) at 3466, 3007, 2918, 1739, 1647, 1464 and 1118 cm^{-1} revealing the presence of OH, CH stretch of alkene, aliphatic CH stretch, carbonyl of ester, carbonyl of acid, C=C stretch and C-O stretch of ester functionalities in the molecule. 300 MHz ^{1}H-NMR spectrum of **1**, measured in $CDCl_3$ (Table 1) displayed one terminal methyl signal at δ 0.85 ppm (H18), two alkene protons at δ5.35 ppm (H9&10; J=5.41) in *cis*- geometry, one CH resonance (attached to OH) at δ5.28 ppm (H2′), one CH_2 triplet (attached to OH) at δ4.29 ppm (H3′), two CH_2 multiplets (adjacent to unsaturation) at δ 2.01 ppm (H8&11), one CH_2 multiplet (adjacent to carbonyl) at δ2.31 ppm (H2), one CH2 triplet at δ1.63 ppm (H3), ten CH_2 multiplets, in a bunch suggesting that they were present in closely matching environment, at δ1.28 ppm (H4-7 & 12-17). The ^{13}C NMR (Table 1) of **1** coupled with DEPT spectrum displayed the coexistence of fifteen methylene groups, three methine groups, two quaternary carbons as carbonyl groups and one methyl group. In ^{13}C-NMR, two quaternary carbons resonated at δ173.71 and 173.26 ppm was attributed to ester linked carbonyl (C1) and acid carbonyl (C1′) moieties respectively. Presence of two signals at δ130.40 & 130.09 ppm were attributed to alkene carbons (C9&10) suggesting the presence of single unsaturation. Two carbon signals appeared at δ 69.27 (C2′) and δ 62.49 ppm (C3′) suggested that these were attached to Oxygen functionality. Fourteen CH_2 signals resonated between δ23.08 and 34.58 ppm suggested that all of them were in a chain with minor difference of environment. Terminal CH_3 signal was resonated at δ 14.50 ppm (C-16). Interpretation of 2D NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and HMBC) with 1D NMR support revealed **1** has a oleic acid (C_{18}) unit, in which a double bond is present at C9&C10 position, and it is esterified to a three carbon alcohol, glyceric acid. The two signals at δ4.29 and 5.28 ppm were confirmed as H3′ & H2′ of the glyceric acid moiety due to the correlations observed in COSY & HMBC experiment. In HMBC (Fig 1), the long range correlations observed between C1 and H3′, H2, H3 were crucial and hence, confirmed the attachment suggested for

compound **1**. All the 1D and 2D NMR data along with IR and mass experimental data showed that the compound **1** to be (Z)-(-)-2-hydroxy-3-(oleoyloxy) propanoic acid (Fig 1).

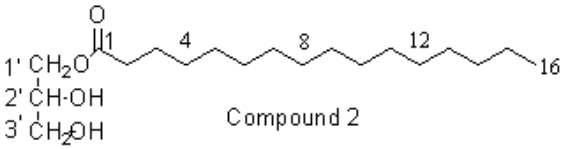
Figure-1: Structures of (Z)-2-hydroxy-3-(oleoyloxy) propanoic acid 1, Hexadecanoic acid-2, 3-dihydroxy propyl ester 2



Compound 1



Important HMBC Correlations for Compound 1



Compound 2

Table1: 300 MHz NMR data for Compound 1 taken in CDCl3

Carbon Position	δH values	δC values	Multiplicity (DEPT)
1	--	173.71	-C-
2	2.31 (m)	34.42	CH2
3	1.63 (t)	25.25	CH2
4	1.28(m)	29.51	CH2
5	1.28(m)	29.72	CH2
6	1.28(m)	29.92	CH2
7	1.28(m)	30.16	CH2
8	2.01 (m)	27.61	CH2
9	5.35 (t)	130.40	CH
10	5.35 (t)	130.09	CH
11	2.01 (m)	27.56	CH2
12	1.28(m)	30.09	CH2
13	1.28(m)	29.87	CH2
14	1.28(m)	29.67	CH2
15	1.28(m)	29.57	CH2
16	1.28(m)	32.30	CH2
17	1.28(m)	23.08	CH2
18	0.85 (t)	14.50	CH3
1′	--	173.26	-C-
2′	5.28 (m)	69.27	CH
3′	4.29 (t)	62.49	CH2

Compound **2**, ESI-MS (m/z 331 (M^+), white amorphous powder, $C_{19}H_{38}O_4$, UV (λmax, MeOH) 201 nm, IR (ν_{max}) at 3308, 3254, 2956, 1730, and 1180 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.21(md), 3.91(md), 3.66 (mdd), 2.36 (m), 2.34 (m), 2.31 (m), 1.61 (t), 1.24(m), 0.87 (t); ^{13}C NMR (300 MHz, $CDCl_3$): δ 174.78 (C-1), 34.56 (C-2), 25.31(C-3), 29.53(C-4), 30.08 (C-5), 34.56 (C-6), 32.32 (C-7), 32.32 (C-8), 32.32 (C-9), 29.85(C-10), 29.75 (C-11), 29.64 (C-12), 30.00 (C-13), 34.56 (C-14), 23.08 (C-15), 14.51 (C-16), 70.67 (C-2′), 65.55 (C-1′), 63.75 (C-3′).

3.1. Cytotoxic activities

The fatty acid derivatives **1** & **2** were tested for their cytotoxic activity against different cancer cell lines, A549 (Lung cancer), ACHN (renal cancer cell), HELA (cervical cancer), B-16 (mouse melanoma), HT-29 (colon cancer), prostate cancer (PC-3), and MCF-7 (Breast cancer) as determined by MTT assay (Table 2). Compound **1** showed potential cytotoxicity against HELA (IC_{50} 5.45µg/ml) and MCF-7 (IC_{50} 4.66µg/ml) human cancer cell lines where as **2** showed potential cytotoxicity against HELA (IC_{50} 9.16µg/ml).

Table2: Cytotoxicity of compound 1 and 2 (values in µg/ml)

Human cancer Cell lines	COMPOUND 1 IC_{50}	COMPOUND 2 IC_{50}	DOXORUBICIN IC_{50}
A549	36.31±0.84	29.07±1.02	1.12±0.08
ACHN	15.41±1.39	12.99±0.61	1.47±0.04
HELA	5.45±0.27	9.16±1.06	2.95±0.18
B-16	18.77±0.89	24.54±0.57	1.63±1.09
HT-29	12.98±1.97	22.93±1.38	0.99±0.27
PC-3	17.78±0.8	34.95±1.23	1.20±0.46
MCF-7	4.66±0.6	12.05±2.06	3.39±0.95

Acknowledgments

We thank Director, CIMAP-Lucknow and Dr. K.P. Sastry, Scientist-In-Charge, CRC-Hyderabad for their constant encouragement and financial support.

REFERENCES

1.

[1] BM John; G Barbara; WC Lars; DP Micheal; CB Paul; WC Mark; JA Paul, *American J. of Bot.*, **2004**, 91(4), 590–600.
[2] A Bermejo; B Figadere; MC Zafra Polo; I Barrachina; E Estornell; D Cortes, *Nat. Prod. Rep.*, **2005**, 22, 269.
[3] P Padma; RL Khosa; M Sahai, *Indian J. Nat. Prod.*, **1995**, 11, 3.
[4] F Bracher, *PZ Wiss*, **1992**, *5*, 109-117.
[5] A Cave, M Leboeuf, PG Waterman, SW Pelletier, Alkaloids: Chemical and Biological Perspectives, Wiley, New York **1987**, 5, 133.
[6] AI Da Rocha; AI Reis Luz; WA Rodrigues, *Acta Amazon*, **1981**, 11, 537.
[7] SH Yeh; FR Chang; YC Wu; YL Yang; SK Zhuo; TL Hwang, *Planta Med*., **2005**, 71, 904.
[8] YL Yang; FR Chang; TL Hwang; WT Chang; YC Wu, *Planta Med.*, **2004**, 70, 256.
[9] FR Chang; JL Wei; CM Teng; YC Wu, *J. Nat. Prod.*, **1998**, 61, 1457.
[10] TR Seetharaman, *Fitoterapia*, **1986**, 57, 198.
[11] H Morita; Y Sato; J Kobayashi, *Tetrahedron*, **1999**, 55, 7509.
[12] FQ Alali; XX Liu; JL McLaughlin, *J. Nat. Prod.*, **1999**, 62, 504.
[13] XP Fang; MJ Rieser; ZM Gu; GX Zhao; JL McLaughlin, *Phytochem. Anal.*, **1993**, 4, 27.
[14] The wealth of India; Raw Materials, A Dictionary of Indian Raw Materials and Industrial Products, Council of Scientific and Industrial Research, New Delhi, **1984**, I, 80.
[15] RN Chopra, SL Nayar, IC Chopra, (Eds.) Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, **1956**, 20.
[16] I Junya; C Warinthorn; G Wandee, *Southeast Asian J. Tro. Med. Public Health*, **2006**, 37(3), 532.
[17] PH Chuang; PW Hsieh; YL Yang; KF Hua; FR Chang; J Shiea; SH Wu; YC Wu, *J. Nat. Prod.*, **2008**, 7, 1365.
[18] SV Rao; K Ramachandran; SH Zaher, *J. Indian Chem. Soc.*, (Industrial and News Edition), **1955**, 18, 215.
[19] F Bholmann; N Rao, *Chem. Ber.*, **1973**, 106, 841.
[20] M Behari; RK Sharma, *J. Indian Chem. Soc.*, **1986**, 63, 255.
[21] Atta-Ur-Rahman; N Sultana; D Shahwar; Md Iqbal Choudhary, *Natural Product Research*, **2008**, 22(15), 1350.
[22] JL Lu; JA Duan; YP Tang; YL Ge; Y Chen. *Asian Chemistry Letters*, **2009**, 13(1&2), 27-34.