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# Chemical Constituents of the Fruit of *Persea Americana.*

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**ABSTRACT**

Chemical investigation of the freeze-dried ripe fruit of *Persea Americana* afforded a mixture of 1,2,4-trihydroxyheptadec-16-ene (**1**), 1,2,4-trihydroxyoctadec-17-yne (**2**), and 1,2,4-trihydroxynonadecane (**3**) in a 5:2.5:1 ratio and triglyceride (**4**). The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy. The structures of **2** and **3** were confirmed by ESI-MS.

**Keywords**: *Persea americana,* Lauraceae, avocado, 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyoctadec-17-yne, 1,2,4-trihydroxynonadecane

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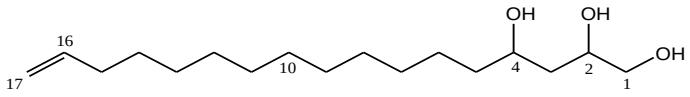
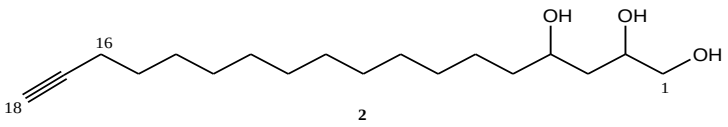
## INTRODUCTION

*Persea americana* Mill. also known as avocado is widely used for the treatment of various ailments, such as monorrhagia, hypertension, stomach ache, bronchitis, diarrhea, and diabetes. A review on the phytochemical and pharmacological properties of *P. americana* has been presented [1]. The chemical constituents of the different parts of *P. americana* include alkanols or aliphatic acetogenins, terpenoid glycosides, furan ring-containing derivatives, flavonoids, and a coumarin [1]. The pharmacological activities include vasorelactant, analgesic and anti-inflammatory, hypotensive, antiviral, antiulcer, wound healing, antihepatotoxic, antioxidant, hypoglycemic, and anti-obesity [1].

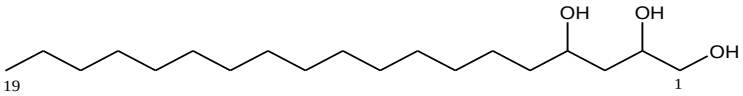
We report herein the isolation of 1,2,4-trihydroxyheptadec-16-ene (**1**), 1,2,4-trihydroxyoctadec-17-yne (**2**), and 1,2,4-trihydroxynonadecane (**3**) in a 5:2.5:1 ratio and triglyceride (**4**) (Fig. 1) from the fruits of *P. americana*.



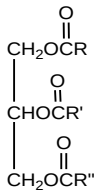

**1**




**2**




**3**




**4** R, R', R" = long chain fatty acids

## MATERIALS AND METHODS

### General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for $^{1}H$ NMR and 150 MHz for $^{13}C$ NMR spectra. The ESIMS spectrum was run on a Finnigan LCQ mass spectrometer. Column chromatography was performed with silica gel 60 (70-230 mesh). 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.

### Sample Collection

The fruit sample was bought from Project 3, Quezon City, Philippines in July 2013. It was identified as of *Persea americana* Mill. at the Bureau of Plant Industry, Manila.

### General Isolation Procedure

A glass column 18 inches in height and 1.0 inches internal diameter was packed with silica gel. The crude extract from the twigs were fractionated by silica gel chromatography using increasing proportions of

acetone in dichloromethane (10% increment) as eluents. Fifty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values 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. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

**Isolation**

The freeze-dried *P. americana* fruit (200 g) was soaked in $CH_2Cl_2$ for three days and then filtered. The fitrate was concentrated under vacuum to afford a crude extract (25 g) which was chromatographed using increasing proportions of acetone in $CH_2Cl_2$ at 10% increment. The 10% acetone in $CH_2Cl_2$ fraction was rechromatographed (3 ×) in 5% EtOAc in petroleum ether to afford **4** (35 mg). The 30% to 40% acetone in $CH_2Cl_2$ fractions were combined and rechromatographed (5 ×) in 10% EtOAc in petroleum ether to afford a mixture of **1**-**3** (150 mg) after washing with petroleum ether.

***1,2,4-Trihydroxyheptadec-16-ene*** (**1**): colorless powdery solid. $^1$H NMR (600 MHz, $CDCl_3$): δ 3.49 (dd, 11, 6.5, H-1), 3.65 (dd, 11, 3.6, H-1'), 3.94 (m, H-2), 1.55 (m, H-3), 3.91 (H-4, m), 1.48 (H-5, m), 1.23-1.44 (m, H-6-H-14), 2.02 (m, H-15), 5.79 (dd, 16.8, 10.2, H-16), 4.90 (dd, 10.2, 2.4, H-17 *cis*), 4.95 (dd, 16.8, 2.4, H-17 *trans*); $^{13}$C NMR (150 MHz, $CDCl_3$): δ 66.76 (C-1), 72.67 (C-2), 38.99 (C-3), 72.33 (C-4), 38.22 (C-5), 29.58, 29.48, 29.13, 28.92, 25.35 (C-6-C-14), 33.79 (C-15), 139.23 (C-16), 114.06 (C-17).

***1,2,4-Trihydroxyoctadec-17-yne*** (**2**): colorless powdery solid. $^1$H NMR (600 MHz, $CDCl_3$): δ 3.49 (dd, 11, 6.5, H-1), 3.65 (dd, 11, 3.6, H-1'), 3.94 (H-2, m), 1.57 (m, H-3), 3.91 (H-4, m), 1.42 (H-5, m), 1.26-1.43 (m, H-6-H-14), 2.15 (td, 6.6, 3.0, H-16), 1.92 (t, 3.0, H-17); $^{13}$C NMR (150 MHz, $CDCl_3$): δ 66.76 (C-1), 72.67 (C-2), 38.90 (C-3), 72.33 (C-4), 38.22 (C-5), 29.55, 29.48, 29.08, 28.47, 25.35 (C-6-C-15), 18.37 (C-16), 84.79 (C-17), 68.02 (C-18). ESI-MS *m/z* = 321.14 $[M+Na]^+$ ($C_{18}H_{34}O_3Na$).

***1,2,4-Trihydroxynonadecane*** (**3**): colorless powdery solid. $^1$H NMR (600 MHz, $CDCl_3$): δ 3.49 (dd, 11.4, 6.6, H-1), 3.65 (dd, 11.4, 3.0, H-1'), 3.94 (m, H-2), 1.55 (m, H-3), 3.94 (H-4, m), 1.49 (H-5, m), 1.23-1.44 (m, H-6-H-18), 0.88 (t, 7.2, H-19); $^{13}$C NMR (150 MHz, $CDCl_3$): δ 66.76 (C-1), 72.67 (C-2), 38.90 (C-3), 72.33 (C-4), 38.22 (C-5), 31.90, 29.67, 29.58, 29.34, 28.92, 25.35 (C-6-C-18), 14.10 (C-19). ESI-MS *m/z* = 339.09 $[M+Na]^+$ ($C_{19}H_{40}O_3Na$).

***Triglyceride*** (**4**): Triglyceride: $^1$H NMR (600 MHz, $CDCl_3$): δ 4.27 (dd, 4.2, 12.0), 4.12 (dd, 6.0, 12.0, glyceryl $CH_2O$), 5.24 (glyceryl CHO), 2.29 (α-$CH_2$), 5.32 (olefinic H), 2.75 (double allylic $CH_2$), 2.00 (allylic, $CH_2$), 1.58 (β-$CH_2$), 1.23-1.34 ($CH_2$), 0.87 (t, 6.6, $CH_3$); $^{13}$C NMR (150 MHz, $CDCl_3$): δ 62.09 (glyceryl $CH_2$), 68.87 (glyceryl CH), 173.26 (C=O α), 172.85 (C=O β), 34.05 (C-2α), 34.19 (C-2β), 24.84 (C-3α), 24.86 (C-3β), 29.08 (C-4α), 29.04 (C-4β), 29.19 (C-5α), 29.27 (C-5β), 29.12 (C-6α), 29.17 (C-6β), 29.62 (C-7α), 29.66 (C-7β), 29.19 (both C-8), 130.01 (C-9α), 129.98 (C-9β), 128.07 (both C-10), 25.62 (both C-11), 127.88 (both C-12), 130.23 (both C-13), 27.19 (both C-14), 29.36 (both C-15), 31.52 (both C-16), 22.57 (both C-17), 14.07, 14.11 (both C-18).

## RESULTS AND DISCUSSION

Silica gel chromatography of the freeze-dried ripe fruit of *Persea americana* afforded 1,2,4-trihydroxyheptadec-16-ene (**1**), 1,2,4-trihydroxyoctatadec-17-yne (**2**), 1,2,4-trihydroxynona decane (**3**) and triglyceride (**4**). Compounds **1**, **2** and **3** were obtained as a mixture in 5:2.5:1 ratio, respectively based on integrals of the resonances at δ 5.79 for the olefinic proton (H-16) of **1**, δ 2.15 for the methylene protons ($H_2$-15) of **2** and δ 0.88 for the methyl protons ($H_3$-19) of **3**. The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy. The structures of **2** and **3** were confirmed by ESI-MS. Compound **2** gave a pseudomolecular ion *m/z* = 321.14 $[M+Na]^+$ corresponding to a molecular formula of $C_{18}H_{34}O_3Na$, while **3** gave a pseudomolecular ion *m/z* = 339.09 $[M+Na]^+$ corresponding to a molecular formula of $C_{19}H_{40}O_3Na$. Compounds **1**, **2** and **3** gave similar $^1$H and $^{13}$C NMR resonances to those reported in the literature for 1,2,4-trihydroxyheptadec-16-ene, 1,2,4-trihydroxyheptadec-16-yne, and 1,2,4-trihydroxynonadecane, respectively [2]. The only difference between 1,2,4-trihydroxyoctatadec-17-yne **(2**) and 1,2,4-trihydroxyheptadec-16-yne (**2'**) is the number of carbon atoms. Whereas the isolated compound (**2**) contained eighteen carbons, the compound (**2'**) reported in the literature had seventeen carbons. The structure of **4** was confirmed by comparison of its $^1$H and $^{13}$C NMR data with those reported in the literature for triglyceride [3].

Although bioassays were not conducted on **1**-**3**, literature search revealed that **1**, **2'** and **3** exhibit diverse biological activities. Compounds **1**, **2'** and **3** obtained from the unripe fruit of *P. americana* exhibited cytotoxicity against six human tumor cell lines: lung carcinoma (A-549), mammary adenocarcinoma (MCF-7), colon adenocarcinoma (HT-29), kidney carcinoma (A-498), pancreatic carcinoma (PaCa-2), and prostate adenocarcinoma (PC-3) in culture and showed selectivity for PC-3 cells with **3** being nearly as potent as the positive control [2]. Compound **3** was a more effective insecticide than rotenone and the positive control when tested against yellow fever mosquito larva [2]. Compounds **1** and **3** exhibited antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}R_v$ with MIC values of 35.7 and 24.9 µg/ml, respectively [4]. Another study reported that **1** inhibited inflammation using the carrageenan-induced edema method, by about 66% at a dosage of 0.2 g/kg body weight and reduced platelet aggregation induced by $CaCl_2$ [5]. Compounds **1** and **2'** exhibited trypanocidal against epimastigotes of *T. cruzi* with $IC_{50}$ values of 140 and 123 µM, respectively and cytotoxicity against HeLa cells with $IC_{50}$ values of 192 and 222 µM, respectively [6]. Compounds **1** and **2'** exhibited antibacterial [7, 8] and antifungal [9] properties.

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