Food Chemistry 132 (2012) 921–924

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Heptadecanols from the leaves of *Persea americana* var. *americana*

Tzong-Huei Lee [a], Yow-Fu Tsai [b], Tzu-Tien Huang [a], Pi-Yu Chen [c], Wen-Li Liang [c,*], Ching-Kuo Lee [c,*]

[a] *Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei 110, Taiwan*
[b] *Department of Chemistry, Chung Yuan Christian University, Chung-Li 320, Taiwan*
[c] *School of Pharmacy, Taipei Medical University, Taipei 110, Taiwan*

ARTICLE INFO

Article history:
Received 26 March 2011
Received in revised form 18 October 2011
Accepted 14 November 2011
Available online 20 November 2011

Keywords:
Persea americana
Heptadecanols
Interconversion
Leaves

ABSTRACT

From the leaves of *Persea americana* var. *americana*, eleven heptadecanol derivatives were identified. Their structures were elucidated on the basis of spectroscopic analyses. The absolute configurations were determined by chemical reaction, NOESY experiment and further comparison of the optical rotation value with the literature value. Additionally, the ratios of the contents of six heptadecanol derivatives in leaves, immature fruits, mature fruits and seeds of *P. americana* were estimated by LC–MS in multiple reaction monitoring (MRM) mode.

1. Introduction

The avocado (*Persea americana* var. *americana*), a fruit tree, is native to tropical America, and is widely cultivated in central and southern Taiwan. Its fruits have long been used as a healthy food for cholesterol-lowering, blood vessel-strengthening, hormone-regulating, anti-aging and constipation-preventing (Chiu & Chang, 1998), while its leaves were reported to exhibit anti-fungal (Prusky, Keen, Sims, & Midland, 1982; Prusky et al., 1991), anti-inflammatory (Adeyemi, Okpo, & Ogunti, 2002) and anti-oxidant activities (Kim et al., 2000). Although several chemical entities have been identified from the fruits of the genus *Persea* (Adikaram, Ewing, Karunaratne, & Wijeratne, 1992; Chenga et al., 2002; Domergue, Helms, Prusky, & Browse, 2000; Gross, Gabai, Lifshitz, & Sklarz, 1974; Hashimura, Ueda, Kawabata, & Kasai, 2001; Jacques & Haslsm, 1974; Karikome, Mimaki, & Sashida, 1991; Kawagishi et al., 2001; Komae & Hayashi, 1972; Oberlies, Rogers, Martin, & Mclaughlin, 1998; Tomita, Lu, & Lan, 1965), the constituents of the other parts of these plants have been rarely studied, so far. In our preliminary pharmacological evaluation, the acetone extracts of the leaves of *P. americana* exhibited significant vasodilating activities at concentrations higher than 150 μg/ml (Huang, 2004). Based on this finding, we set out to investigate the chemical constituents of the leaves of *P. americana*; this has led to the isolation and identification of four novel chemical entities **1–4** together with seven known analogues **5–11** (Fig. 1). This paper deals with the isolation and structural elucidation of the previously unreported compounds.

2. Materials and methods

2.1. General procedures

Optical rotations were measured in a JASCO P-1020 polarimeter. ^{1}H and ^{13}C NMR were acquired with a Bruker DRX-500 SB spectrometer. Low resolution and high resolution mass spectra were obtained, using a Finnigan TSQ-46C EI/MS and JEOL SX-102A HRFAB/MS, respectively. IR spectra were recorded on a Thermo Mattson IR-300 spectrometer.

2.2. Chemicals and reagents

HPLC-grade solvents, *n*-hexane, ethyl acetate, acetone, and two reagents, 2,2-dimethoxypropane and *p*-toluenesulfonic acid monohydrate, were purchased from Merck. Open column chromatography was performed on silica gel (70–230 mesh, Merck). TLC was carried out on silica gel 60 F_{254} plates (Merck), using mixtures of *n*-hexane–ethyl acetate for development and spots were detected by spraying with vanillin-sulfuric acid, followed by heating.

2.3. Plant materials

Fresh leaves of *P. americana* were collected at Madou Township, Tainan County in September 14, 2002, and were identified by one of us (CKL). Voucher specimens (No. 20020914) have been deposited at the Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan.

* Corresponding authors. Tel.: +886 2 27361661x6150; fax: +886 2 23772265.
E-mail address: cklee@tmu.edu.tw (C.-K. Lee).

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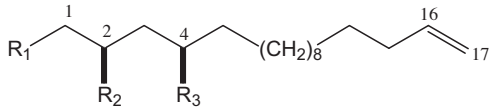
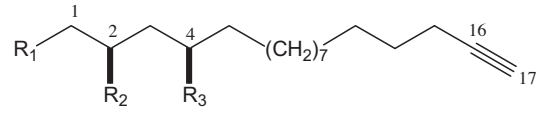
doi:10.1016/j.foodchem.2011.11.067



	R_1	R_2	R_3
1	OAc	OAc	OH
2	OH	OAc	OAc
5	OAc	OH	OAc
7	OAc	OH	OH
8	OH	OH	OAc
9	OH	OH	OH



	R_1	R_2	R_3
3	OAc	OAc	OH
4	OH	OAc	OAc
6	OAc	OH	OAc
10	OAc	OH	OH
11	OH	OH	OH

Fig. 1. Structures of compounds **1**–**11** isolated from the leaves of *Persea americana*.

2.4. Extraction and isolation

Fresh leaves (20.4 kg) of *P. americana* were extracted three times with 100 l of acetone, which was filtered and evaporated to give a black residue (1096 g). This residue was then suspended in H_2O (3.0 l) and partitioned with equal volume of *n*-hexane, and the *n*-hexane layer was evaporated to dryness under vacuum (345 g). Subsequently, the dried *n*-hexane layer was mixed with 396 g of silica gel, and was loaded onto a conditioned open column packed with 2458 g of silica gel and eluted, in a step-wise gradient, by mixtures of *n*-hexane, ethyl acetate and acetone. Five hundred millilitre was collected for each fraction and each was analysed by TLC. Then, each fraction was concentrated and re-dissolved in a minimum volume of *n*-hexane–ethyl acetate mixture and used in the subsequent HPLC system. Fr. #8 eluted by *n*-hexane–ethyl acetate (1:1), was purified by a semi-preparative HPLC (Phenomenex Luna silica 5 μm, 10 × 250 mm), using *n*-hexane–ethyl acetate (4:1) as eluent at a flow rate of 3 ml/min to afford **1** (98 mg, t_R = 8.1 min), **2** (375 mg, t_R = 9.3 min), **3** (69 mg, t_R = 10.6 min), **4** (74 mg, t_R = 11.9 min), **5** (64 mg, t_R = 15.1 min) and **6** (126 mg, t_R = 12.8 min). The same fraction was purified by the same column using *n*-hexane–ethyl acetate–acetone (7:2:1) as eluent at a flow rate of 3 ml/min to obtain **7** (154 mg, t_R = 16.2 min), **8** (94 mg, t_R = 20.1 min) and **9** (56 mg, t_R = 34.7 min). Fr. #21, eluted by acetone, was purified by the same column, using *n*-hexane–acetone (5:1) as eluent at a flow rate of 3 ml/min to yield **10** (134 mg, t_R = 10.4 min) and **11** (153 mg, t_R = 13.5 min).

1,2*R*-Diacetoxy-4*R*-hydroxy-*n*-heptadeca-16-ene (**1**): colourless oil; $[\alpha]_D^{22}$ +1.1 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3399, 1738; positive FABMS *m*/*z* 371 $[M+H]^+$; HRFABMS *m*/*z* 371.2798 $[M+H]^+$ (calcd for $C_{21}H_{39}O_5$, 371.2798); for ^1H and ^{13}C NMR data see Tables 1 and 2.

2*R*,4*R*-Diacetoxy-1-hydroxy-*n*-heptadeca-16-ene (**2**): colourless oil; $[\alpha]_D^{22}$ −0.81 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3392, 1731; FABMS *m*/*z* 371 $[M+H]^+$; HRFABMS *m*/*z* 371.2794 $[M+H]^+$ (calcd for $C_{21}H_{39}O_5$, 371.2798); for ^1H and ^{13}C NMR data see Tables 1 and 2.

1,2*R*-Diacetoxy-4*R*-hydroxy-*n*-heptadeca-16-yne (**3**): colourless oil; $[\alpha]_D^{22}$ +5.7 (*c* 1.5, $CHCl_3$); IR (neat) ν_{max} 3396, 2100, 1735; FABMS *m*/*z* 369 $[M+H]^+$; HRFABMS *m*/*z* 369.2638 $[M+H]^+$ (calcd for $C_{21}H_{37}O_5$, 369.2641); for ^1H and ^{13}C NMR data see Tables 1 and 2.

2*R*,4*R*-Diacetoxy-1-hydroxy-*n*-heptadeca-16-yne (**4**): colourless oil; $[\alpha]_D^{22}$ +4.0 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3466, 2116, 1736; FABMS *m*/*z* 369 $[M+H]^+$; HRFABMS *m*/*z* 369.2639 $[M+H]^+$ (calcd for $C_{21}H_{37}O_5$, 369.2641); for ^1H and ^{13}C NMR data see Tables 1 and 2.

2.5. Acetonide derivative of **1**

To a solution of compound **1** (6.0 mg), in anhydrous CH_3CN, was added CH_3ONa (12 mg) in one portion at room temperature. After completion of the deacetylation, the resulting triol compound **9** and 2,2-dimethoxypropane were then dissolved in anhydrous CH_2Cl_2 and treated with an amount of *p*-toluenesulfonic acid monohydrate, as catalyst, as described in the literature Kashman, Néeman, and Lifshitz (1970). After starting material was consumed, monitored by TLC, the reaction mixture was neutralised with Et_3N and then the solvent was removed to afford a 2,4-*O*-isopropylidene derivative **12** (3.6 mg).

2.6. Chemical quantification by a MRM mode of LC–MS–MS

Fresh leaves, immature fruits, mature fruits and seeds (each 25 g) were extracted ultrasonically with acetone (3 × 250 ml). The extracts were pooled and filtered through a 0.22 μm nylon membrane before injection for LC–MS–MS analyses. LC was performed with an HPLC (Agilent 1100), using a 4.6 × 250 mm, 5 μm particle, Biosil Aqu-ODS-W column (Biotic Chemical Co., Ltd., Taiwan) at a flow rate of 0.8 ml/min. The mobile phase was started at 100% H_2O, then changed to 100% MeOH in 30 min, and maintained at 100% MeOH for 20 min. Mass spectrometry was performed with a 4000Q TRAP (Applied Biosystem) equipped with an ESI probe and a tandem quadrupole analyser. Quantification was performed in MRM mode, and the ion transitions are listed in Table 3.

Table 1
^{13}C NMR (125 MHz) spectroscopic data for compounds **1**–**4** (in $CDCl_3$, δ in ppm).

No.	**1**[a]	**2**[a]	**3**[a]	**4**[a]
1	65.0 t	64.2 t	64.9 t	64.0 t
2	70.1 d	71.1 d	70.1 d	71.2 d
3	38.1 t	34.8 t	38.0 t	34.8 t
4	69.1 d	72.4 d	68.9 d	72.0 d
5	37.8 t	34.3 t	34.3 t	34.2 t
6	25.4 t	25.1 t	25.2 t	25.1 t
7	29.7 t	31.6 t	29.6 t	29.4 t
8	29.6 t	29.7 t	29.5 t	29.3 t
9	29.6 t	29.6 t	29.5 t	29.2 t
10	29.5 t	29.5 t	29.4 t	29.2 t
11	29.5 t	29.4 t	29.4 t	28.9 t
12	29.4 t	29.3 t	29.0 t	28.7 t
13	29.1 t	29.1 t	28.7 t	28.6 t
14	28.9 t	28.9 t	28.4 t	28.3 t
15	33.8 t	33.8 t	18.3 t	18.4 t
16	139.3 d	139.3 d	68.0 s	67.9 s
17	114.1 t	114.1 t	84.8 d	84.8 d
CH_3COO-1	170.6 s		171.0 s	
CH_3COO-1	21.2 q		21.3 q	
CH_3COO-2	170.8 s	179.8 s	171.1 s	170.8 s
CH_3COO-2	20.8 q	22.6 q	20.8 q	21.1 q
CH_3COO-4		170.9 s		170.9 s
CH_3COO-4		21.2 q		21.2 q

[a] Multiplicities were obtained from DEPT experiments.

3. Results and discussion

An acetone extract of the leaves of *P. americana* was partitioned in a preliminary manner to give an *n*-hexane-soluble layer. Gravity column separation of this layer, over silica gel, followed by HPLC

Table 2
^{1}H NMR (500 MHz) spectroscopic data for compounds **1**–**4** (in $CDCl_3$, δ in ppm, J in Hz).

No.	1	2	3	4
1	4.09 (dd, 12.0, 6.4)	3.64 (dd, 12.1, 5.5)	4.08 (dd, 12.1, 6.5)	3.64 (dd, 12.1, 5.7)
	4.26 (dd, 12.0, 3.1)	3.72 (dd, 12.1, 3.5)	4.26 (dd, 12.1, 3.1)	3.73 (dd, 12.1, 3.4)
2	5.21 (m)	4.92 (m)	5.21 (m)	4.92 (m)
3	1.72 (t, 6.5)	1.87 (t, 6.4)	1.71 (t, 6.6)	1.87 (t, 6.4)
4	3.67 (m)	4.94 (m)	3.67 (m)	4.94 (m)
5	1.42 (m)	1.53 (m)	1.41 (m)	1.52 (m)
6	1.23 (m)	1.24 (m)	1.23 (m)	1.23 (m)
7	1.23 (m)	1.24 (m)	1.23 (m)	1.23 (m)
8	1.23 (m)	1.24 (m)	1.23 (m)	1.23 (m)
9	1.23 (m)	1.24 (m)	1.23 (m)	1.23 (m)
10	1.23 (m)	1.24 (m)	1.23 (m)	1.23 (m)
11	1.23 (m)	1.24 (m)	1.23 (m)	1.23 (m)
12	1.23 (m)	1.24 (m)	1.23 (m)	1.23 (m)
13	1.23 (m)	1.24 (m)	1.35 (m)	1.36 (m)
14	1.34 (m)	1.35 (m)	1.48 (m)	1.51 (m)
15	2.01 (m)	2.01 (m)	2.14 (td, 7.1, 2.7)	2.16 (td, 6.9, 2.5)
16	5.78 (ddt, 17.0, 10.1, 6.6)	5.79 (m)		
17	4.96 (dd, 17.0, 1.2)	4.91 (m)	1.90 (t, 2.7)	1.91 (t, 2.5)
	4.89 (dd, 10.1, 1.2)	4.97 (m)		
CH_3COO-1	2.03 (s)		2.03 (s)	
CH_3COO-2	2.03 (s)	2.03 (s)	2.03 (s)	2.03 (s)
CH_3COO-4		2.06 (s)		2.06 (s)

Table 3
The ratios of the contents of **1**–**3** and **4**–**6** in leaves, immature fruits, mature fruits and seeds of *P. americana*.

Parts of *P. americana*	**1**–**3**[a,c]	**4**–**6**[b,c]
Leaves	65.6	6.3
Immature fruits	93.8	8.8
Mature fruits	50.0	1.0
Seeds	46.9	4.1

[a] The ratios were estimated from the peak height at R_t 29.6 min in the LC–MS chromatogram. The ion transitions for **1**–**3** are 371→311 (de-acetyl) and 371→251 (de-diacetyl).
[b] The ratios were estimated from the peak height at R_t 28.2 min in the LC–MS chromatogram. The ion transitions for **4**–**6** are 369→309 (de-acetyl) and 369→247 (de-diacetyl).
[c] All the ratios of the contents were calculated from the peak height of compounds in each material over the peak height of **4**–**6** in mature fruits.

purification, afforded four previously unreported heptadecanols (**1**–**4**), along with seven known compounds. The known compounds were identified as 1-acetoxy-2*R*,4*R*-dihydroxy-*n*-heptadec-16-ene (**7**) (Domergue et al., 2000), 4-acetoxy-1*R*,2*R*-dihydroxy-*n*-heptadec-16-ene (**8**) (Domergue et al., 2000), 1,2*R*,4*R*-trihydroxy-*n*-heptadec-16-ene (**9**) (Oberlies et al., 1998), 1-acetoxy-2*R*,4*R*-dihydroxy-*n*-heptadec-16-yne (**10**) (Domergue et al., 2000), and 1,2*R*,4*R*-trihydroxy-*n*-heptadec-16-yne (**11**) (Oberlies et al., 1998). Although 1,4*R*-diacetoxy-2*R*-hydroxy-*n*-heptadeca-16-ene (**5**) and 1,4*R*-diacetoxy-2*R*-hydroxy-*n*-heptadec-16-yne (**6**) were well-known compounds, only registered CAS numbers 1083200-70-7 and 1174388-00-1, respectively, were shown in the SciFinder database (Chemical Abstracts Service, USA).

Compound **1** was obtained as a colourless oil and its IR absorptions at 3399 and 1738 cm^{-1} indicated the presence of a hydroxy and an acetoxy carbonyl functionality, respectively. Twenty-one carbon resonances, attributed to two quaternary carbons (CH_3COO-1 and CH_3COO-2), one oxymethylene (C-1), two carbinoyl methines (C-2 and C-4), one olefinic methine (C-16), one exomethylene (C-17) and twelve methylenes (C-3, C-5 and C-6–C-15), and two methyls (CH_3COO-1 and CH_3COO-2) were observed in the ^{13}C NMR spectrum coupled with the DEPT spectrum of **1** (Table 1). On account of the molecular formula, $C_{21}H_{38}O_5$, as assigned by HREIMS, the double bond equivalence (DBE) of **1** was determined to be three, consistent with two acetoxy carbonyl groups and one olefinic functionality, which were further supported by two carbonyl signals at δ_C 170.6 (CH_3COO-1) and 170.8 (CH_3COO-2) and one pair of olefinic carbons at δ_C 139.3 (C-16)/114.1 (C-17) in the ^{13}C NMR spectrum of **1**. The above assignments were characteristic for a linear aliphatic skeleton bearing one hydroxy, two acetoxys and one double bond. The connectivity of **1** was further deduced by its ^{1}H NMR (Table 2) and selected COSY [δ_H 4.09, 4.26 (H_2-1)/δ_H 5.21 (H-2); δ_H 5.21 (H-2)/δ_H 1.72 (H_2-3); δ_H 1.72 (H_2-3)/δ_H 3.67 (H-4); δ_H 5.78 (H-16)/δ_H 4.96, 4.89 (H_2-17)] and key HMBC (H_2-1/CH_3COO-1 and H-2/CH_3COO-2) correlations (Fig. 2). The relative configurations of carbinoyl H-2 and H-4 of **1** were deduced to be both axial-oriented (both R^* or S^*) by the $J_{H\text{-}2/H\text{-}3ax}$ (12.0 Hz), $J_{H\text{-}4/H\text{-}3ax}$ (12.0 Hz) and NOESY assignments (Fig. 3) of its acetonide derivative, **12**.

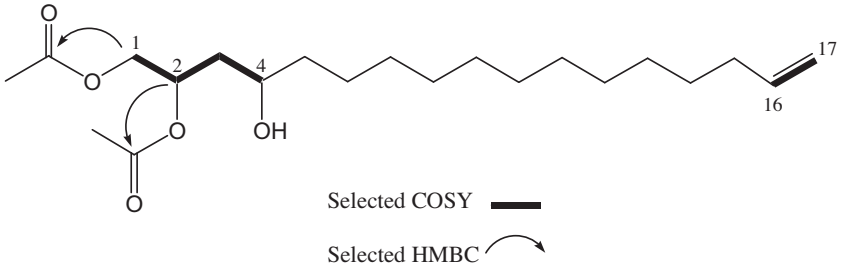
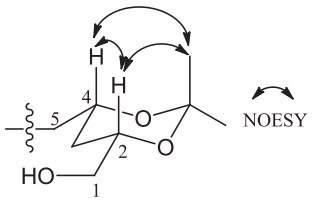


Fig. 2. Selected COSY and HMBC of **1**.



12

Fig. 3. Selected NOESY of **12**.

The absolute configurations of C-2 and C-4 were further confirmed to be both *R* based on comparison of the optical rotation value $[\alpha]_D^{22}$ −8.3 (*c* 1.0, $CHCl_3$) of **9** with the configurational isomers ($[\alpha]_D$ −6.4 for 2*R*,4*R*-heptadecanol; $[\alpha]_D$ +6.0 for 2*S*,4*S*-heptadecanol) in the literature Sugriyama, Sato, and Yamashita (1982). Accordingly, **1** was characterised as shown, and was named as 1,2*R*-diacetoxy-4*R*-hydroxy-*n*-heptadeca-16-ene.

All the physical data of **2** closely resemble those of **1,** indicating that **2** was the isomer of **1**. The major differences involved were only the locations of the acetoxy functionalities, as evidenced from the HMBC spectrum of **2**. Cross peaks of H-2/CH_3COO-2 and H-4/CH_3COO-4 in the HMBC spectrum of **2,** corroborating the acetoxy moieties, were located at C-2 and C-4 of **2**. Thus, the structure of **2** was determined to be as shown, and was named as 2*R*,4*R*-diacetoxy-1-hydroxy-*n*-heptadeca-16-ene.

Compounds **3** and **4** also possessed skeletons identical to those of **1** and **2**, respectively, except that their H-16 was absent and H-17 up-field shifted to around δ_H 1.90 in the ^1H NMR spectra of **3** and **4**. This difference unambiguously indicated that a Δ^{16}, in both **1** and **2,** was replaced by an acetylenic moiety in **3** and **4**, which was also reflected in their IR absorptions of 2100 and 2116 cm^{-1}, respectively. Conclusively, **3** and **4** were identified as shown, and were named as 1,2*R*-diacetoxy-4*R*-hydroxy-*n*-heptadeca-16-yne and 2*R*,4*R*-diacetoxy-1-hydroxy-*n*-heptadeca-16-yne, respectively.

Aliphatic alcohols with odd carbons are rarely found in higher plants. To our knowledge, heptadecane with 1,2,4-trihydroxy groups, such as **1**–**11** have been found only in *Persea* spp. so far. It was found that the pure isolates **1**–**3** and **4**–**6** were prone to interconvert into respective mixtures of **1**–**3** and **4**–**6** when they were dissolved in $CHCl_3$ with slight acidity. The interconversion to their respective mixtures was speculated to be caused by the migration of the acetyl moieties.

The ratios of the contents of heptadecanols with a terminal double bond, including **1**–**3**, and heptadecanols with a terminal triple bond, including **4–6** in leaves, immature fruits, mature fruits and seeds of *P. americana* were estimated by a LC system coupled with tandem MS in multiple reaction monitoring (MRM) mode (Table 3). It was found that the contents of **1**–**3** were at least tenfold those of **4**–**6** in four parts of *P. americana*. The total contents of **1**–**6** in immature fruits were estimated to be higher than those in the other three parts of *P. americana*.

Heptadecanols isolated from avocado have never been reported to exhibit cytotoxic (Oberlies et al., 1998), insecticidal (Oberlies et al., 1998), anti-fungal (Adikaram et al., 1992; Domergue et al., 2000), liver injury-suppressing (Kawagishi et al., 2001) or acetyl-CoA carboxylase inhibitory (Hashimura et al., 2001) activities. In this study, all the pure isolates **1**–**11** were evaluated for their biological activities, including vasodilating, DPPH-scavenging, anti-xanthine oxidase and anti-tyrosinase. However, they exhibited no significant activity, even at concentrations higher than 100 μM.

Acknowledgments

We are grateful to Ms. Shwu-Huey Wang, Ms. Shou-Ling Huang and Ms. Shu-Yun Sun of the Instrumentation Center of Taipei Medical University and the Instrumentation Center of the College of Science, National Taiwan University, respectively, for the NMR and MS data acquisition. We also wish to thank the National Science Council and Center of Excellence for Clinical Trial and Research in Neuroscience, WanFang Hospital for their financial support.

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