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ACYLATED TRITERPENOIDS FROM *LIGUSTRUM OVALIFOLIUM**

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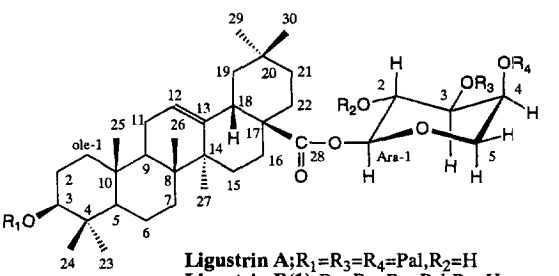
Key Word Index—*Ligustrum ovalifolium*; Oleaceae; flower; acylated triterpenoid; Ligustrin.

Abstract—Two new acylated triterpenoids, 2,4-di-*O*-palmitoyl-1-*O*-(3-*O*-palmitoyloleanoyl)-α-L-arabinopyranose and 1-*O*-(3-*O*-palmitoyloleanoyl)-α-L-arabinopyranose, were isolated from the flower of *Ligustrum ovalifolium*. Their structures were established on the basis of chemical and spectral data. © 1997 Elsevier Science Ltd

INTRODUCTION

We have reported the isolation of two new acylated compounds, named ligustrin A [3,4-di-*O*-palmitoyl-1-*O*- (3-*O*-palmitoyloleanoyl)-α-L-arabinopyranose][1] and ligustrol A [*p*-hydroxyphenetyl palmitate][2], from the flower of *Ligustrum ovalifolium* HASSK [3–5]. Further investigation of this plant led to the isolation of two new acylated triterpenoids, which we have named ligustrins B and C, respectively. This paper deals with the structural elucidation of these compounds.



Ligustrin A; $R_1=R_3=R_4=$Pal,$R_2=$H
Ligustrin B(1); $R_1=R_2=R_4=$Pal,$R_3=$H
Ligustrin C(2); $R_1=$Pal,$R_2=R_3=R_4=$H
Pal=$CO(CH_2)_{14}CH_3$

RESULTS AND DISCUSSION

The isolation procedure is described in detail in the experimental section. Compound **1**, named ligustrin B, was obtained as colorless oil, $[\alpha]_D$ +32.1° ($CHCl_3$). The IR spectrum of **1** indicated the presence of hydroxy (3441 cm^{-1}) and ester (1724 cm^{-1}) groups. The FAB-mass spectrum gave a $[M+H]^+$ ion at *m/z* 1303 and a molecular plus sodium ion at *m/z* 1325 $[M+Na]^+$, which were similar to those of ligustrin A. Furthermore, hydrolysis of **1** with 5% NaOH–MeOH yielded oleanolic acid, palmitic acid and arabinose (oleanolic acid was identified by TLC and palmitic acid and arabinose were identified by GC after derivatizations, respectively). The ^1H and ^{13}C NMR chemical shifts, except for the signals owing to the arabinosyl moieties were coincident with those of **1** and ligustrin A. In the ^1H NMR spectrum, the 3-H_{ara} signal (δ 3.83) of **1** was shifted upfield by 1.19 ppm compared with that of ligustrin A. These findings suggested that three palmitoyl groups are located at C_{ole}-3, C_{ara}-2 and C_{ara}-4, respectively. This deduction was supported by ^1H-^1H COSY and HMBC spectrum. The placement of the two of three palmitoyl groups on C_{ole}-3 and C_{ara}-4 were deduced from the HMBC spectrum, that is, cross peaks were observed between 3-H_{ole} and δ 173.8 and between 4-H_{ara} and δ 173.7. The carbon resonances at δ 173.7 and 173.8 showed HMBC correlations with the methylene protons at δ 2.29 and 2.41 which are assigned to α-methylene moieties of palmitoyl groups, respectively. The remaining a palmitoyl group on C_{ara}-2 was deduced from ^1H-^1H COSY, a cross peak was observed between 2-H_{ara} and α-methylene protons (δ 2.41) of the palmitoyl group. This long-range coupling (through five bonds) is explained by a co-planar zig-zag configuration (*W*-type) of the internuclear bonds. The structure of **1** is therefore 2,4-di-*O*-palmitoyl-1-*O*-(3-*O*-palmitoyloleanoyl)-α-L-arabinopyranose.

Compound **2**, named ligustrin C, was obtained as colorless oil, $[\alpha]_D$ +37.5° ($CHCl_3$). The FAB-mass

* Part 18 in the series, 'Studies on the Constituents of *Ligustrum* Species. XVII'. For part 17 see ref. [2].

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spectrum gave a molecular plus hydrogen ion at *m/z* 827 $[M+H]^+$ and a molecular plus sodium ion at *m/z* 849 $[M+Na]^+$. The ^1H NMR spectrum exhibited signals belonging to a oleanoyl, an arabinosyl and a palmitoyl moieties. The ^1H and ^{13}C NMR chemical shifts of oleanoyl moiety were coincident with those of **2** and ligustrin A. In the ^1H NMR spectrum, 3-H_{ara} (δ 3.68) and 4-H_{ara} (δ 3.99) signals of **2** were shifted upfield by 1.34 and 1.29 ppm, respectively, compared with those of ligustrin A. These findings suggested that a palmitoyl group is located at C_{ole}-3. This deduction was supported by HMBC correlation from 3-H_{ole} to δ 173.8. The structure of **2** is therefore 1-*O*-(3-*O*-palmitoyloleanoyl)-α-L-arabinopyranose.

EXPERIMENTAL

General. ^1H and ^{13}C NNR: 270 and 67.8 MHz, respectively, TMS as int. standard. FAB-MS: Jeol JMS-DX 303 mass spectrometer (matrix; TEA:NBA = 1:1). CC: Kieselgel 60 (Merck; 230–400 mesh). Prep. HPLC: Tosoh HPLC system using TSK gel OH-120 column (Tosoh Co., 7.8 mm i.d. × 30 cm) with UV detector. A flow rate of 1.5 ml min^{-1} of hexane–EtOH (50:1) was maintained with selective detection at 202 nm. GC was carried out on a Shimadzu GC-7A equipped with FID [column; 3% silicone SE-52 on Chromosorb W (AW) (60–80 mesh), 3 mm i.d. × 2 m]. TLC was performed on Merck precoated Kieselgel $60F_{254}$, and spots were detected by spraying 50% H_2SO_4, followed by heating.

Plant material. The flowers of *Ligustum ovalifolium* HASSK were collected at the flowering stage, and identified by Prof. Shuji Hisamichi (Department of Pharmacognosy of our college) from Sendai, Miyagi, Japan, May 1986. A voucher specimen is deposited in the laboratory of M. Kikuchi.

Extraction and isolation. Fresh flowers (4.6 kg) were extracted with Et_2O at room temp., and concd to give an extract (69.0 g). The extract (5.0 g) was chromatographed on a silica gel column using hexane–Me_2CO (4:1) and the elute was sepd into 11 frs (frs 1–11). Fr. 5 was subjected to prep. HPLC to give **1** (5 mg). Fr. 7 was rechromatographed on a silica gel column using hexane–Me_2CO (5:1) to give **2** (60 mg).

Ligustrin A. ^1H NMR (270 MHz, $CDCl_3$): δ 5.54 (1H, *d*, *J* = 7.3 Hz, 1-H_{ara}), 5.33 (1H, *m*, 12-H_{ole}), 5.28 (1H, *m*, 4-H_{ara}), 5.02 (1H, *dd*, *J* = 9.3, 3.5 Hz, 3-H_{ara}), 4.49 (1H, *t*, *J* = 7.7 Hz, 3-H_{ole}), 3.99 (1H, *dd*, *J* = 12.3, 3.1 Hz, 5-H_{ara}), 3.96 (1H, *m*, 2-H_{ara}), 3.73 (1H, *br d*, *J* = 12.3 Hz, 5-H_{ara}), 2.88 (1H, *m*, 18-H_{ole}), 2.37, 2.31, 2.29 (each 2H, *t*, *J* = 7.6 Hz, —$COC\underline{H}_2CH_2$—). ^{13}C NMR (67.8 MHz, $CDCl_3$): oleanoyl moiety; δ 38.2 (C-1), 25.2 (C-2), 80.5 (C-3), 37.7 (C-4), 55.3 (C-5), 18.2 (C-6), 32.8 (C-7), 39.3 (C-8), 47.5 (C-9), 36.9 (C-10), 22.7 (C-11), 122.8 (C-12), 143.1 (C-13), 41.8 (C-14), 27.8 (C-15), 23.4 (C-16), 46.8 (C-17), 41.2 (C-18), 45.8 (C-19), 30.6 (C-29), 33.8 (C-21), 32.1 (C-22), 28.0 (C-23), 16.7 (C-24), 15.4 (C-25), 17.0 (C-26), 25.7 (C-27), 175.9 (C-28), 33.0 (C-29), 23.6 (C-30). arabinose moiety; δ 94.2 (C-1), 68.8 (C-2), 72.9 (C-3), 67.4 (C-4), 64.5 (C-5). palmitoyl moiety; δ 173.7, 173.3, 172.9 (C=O), 34.9, 34.2, 34.1, 31.9, 29.7, 29.67, 29.6, 29.5, 29.46, 29.4, 29.3, 29.29, 29.2, 29.17, 29.1, 25.0, 24.7, 23.0 (CH_2), 14.1 (CH_3).

Ligustrin B (**1**). Colorless oil, $[\alpha]_D$ +32.1° ($CHCl_3$, *c* 0.3). FAB-MS *m/z*: 1303 $[M+H]^+$, 1325 $[M+Na]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3441, 2928, 2856, 1724, 1600. ^1H NMR ($CDCl_3$): δ 5.45 (1H, *d*, *J* = 6.9 Hz, 1-H_{ara}), 5.33 (1H, *br s*, 12-H_{ole}), 5.14 (1H, *br s*, 4-H_{ara}), 4.49 (1H, *t*, *J* = 7.7 Hz, 3-H_{ole}), 4.03 (1H, *dd*, *J* = 13.3, 2.6 Hz, 5-H_{ara}), 3.83 (1H, *m*, 3-H_{ara}), 3.81 (1H, *m*, 2-H_{ara}), 3.69 (1H, *dd*, *J* = 13.3, 1.3 Hz, 5-H_{ara}). 2.88 (1H, *m*, 18-H_{ole}), 2.41 (4H, *t*, *J* = 7.5 Hz, —$COC\underline{H}_2CH_2$—), 2.29 (2H, *t*, *J* = 7.5 Hz, —$COC\underline{H}_2CH_2$—). ^{13}C NMR ($CDCl_3$): oleanoyl moiety; δ 38.2 (C-1), 25.2 (C-2), 80.5 (C-3), 37.7 (C-4), 55.3 (C-5), 18.2 (C-6), 32.8 (C-7), 39.3 (C-8), 47.5 (C-9), 36.9 (C-10), 22.7 (C-11), 122.7 (C-12), 143.1 (C-13), 41.8 (C-14), 27.8 (C-15), 23.4 (C-16), 46.9 (C-17), 41.2 (C-18), 45.8 (C-19), 30.6 (C-20), 33.8 (C-21), 32.1 (C-22), 28.1 (C-23), 16.7 (C-24), 15.4 (C-25), 17.0 (C-26), 25.7 (C-27), 176.1 (C-28), 33.0 (C-29), 23.5 (C-30). arabinose moiety; δ 94.2 (C-1), 71.0 (C-2), 72.1 (C-3), 69.7 (C-4), 64.6 (C-5). palmitoyl moiety; δ 173.8, 173.7 (C=O), 34.9, 34.3, 31.9, 29.7, 29.66, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.2, 24.9, 23.6, 23.0 (CH_2), 14.1 (CH_3). A soln of **1** (3 mg) in $CHCl_3$ (1 ml) in 5% NaOH–MeOH (1 ml) was heated under reflux for 30 min. The reaction mixt. was neutralized with 5% HCl and extracted with $CHCl_3$. The $CHCl_3$ layer (1.2 mg) was subjected to TLC and GC analyses to identifies oleanolic acid [TLC; R_f 0.33, hexane–Me_2CO (3:1)] and methyl ester derivative of palmitic acid (GC; R_t 9.6 min, column temp: 190° inj temp: 220°, carrier gas: N_2 20 ml min^{-1}). The H_2O layer (0.2 mg) was subjected to GC analysis to identify the TMS derivative of methyl arabinose (R_t 3.5 min, column temp: 180°, inj temp: 210°, carrier gas: N_2 20 ml min^{-1}).

Ligustrin C (**2**). Colorless oil $[\alpha]_D$ +37.5° ($CHCl_3$, *c* 2.4). FAB-MS *m/z*: 827 $[M+H]^+$, 849 $[M+Na]^+$. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3424, 2928, 2856, 1719, 1615. ^1H NMR ($CDCl_3$): δ 5.43 (1H, *d*, *J* = 6.9 Hz, 1-H_{ara}), 5.31 (1H, *br s*, 12-H_{ole}), 4.49 (1H, *t*, *J* = 7.9 Hz, 3-H_{ole}), 3.99 (2H, *m*, 4-H_{ara} and 5-H_{ara}), 3.83 (1H, *dd*, *J* = 7.8, 6.9 Hz, 2-H_{ara}), 3.68 (2H, *m*, 3-H_{ara} and 5-H_{ara}), 2.87 (1H, *m*, 18-H_{ole}), 2.29 (2H, *t*, *J* = 7.5 Hz, —$COC\underline{H}_2CH_2$—). ^{13}C NMR ($CDCl_3$): oleanoyl moiety; δ 38.1 (C-1), 25.1 (C-2), 80.5 (C-3), 37.7 (C-4), 55.2 (C-5), 18.2 (C-6), 32.7 (C-7), 39.3 (C-8), 47.5 (C-9), 36.9 (C-10), 22.6 (C-11), 122.7 (C-12), 143.1 (C-13), 41.7 (C-14), 27.7 (C-15), 23.4 (C-16), 46.8 (C-17), 41.2 (C-18), 45.8 (C-19), 30.6 (C-20), 33.8 (C-21), 32.0 (C-22), 28.0 (C-23), 16.7 (C-24), 15.4 (C-25), 16.9 (C-26), 25.6 (C-27), 176.2 (C-28), 33.0 (C-29), 23.5 (C-30). arabinose moiety; δ 94.3 (C-1), 70.6 (C-2), 73.2 (C-3), 67.8 (C-4), 66.2 (C-5). palmitoyl moiety; δ 173.8 (C=O), 34.8, 31.9, 29.6, 29.58, 29.5, 29.4, 29.3, 29.2, 29.1, 24.7, 23.5, 22.9 (CH_2), 14.1 (CH_3).

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