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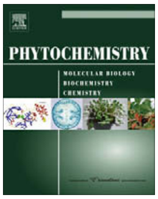

Secoiridoid glucosides and unusual recyclized secoiridoid aglycones from *Ligustrum vulgare*

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ABSTRACT

Phytochemical investigation of the dried leaves and twigs of *Ligustrum vulgare* has led to the isolation of the secoiridoid glucosides, (2″*R*)- and (2″*S*)-10-hydroxy-2″-methoxyoleuropeins (**1** and **2**), and the secoiridoid aglycones, ligustrohemiacetals A (**3**) and B (**4**). Their structures were elucidated by spectroscopic and chemical means. Enzymatic hydrolysis of 10-hydroxyoleuropein to the analog of ligustrohemiacetals A and B led to the structural revision of jasmolactones.

1. Introduction

Ligustrum vulgare L. (Oleaceae) is a shrub that is found in Europe and Northwestern Asia. Earlier investigation of the plant species resulted in identification of the secoiridoid glucosides oleuropein, ligstroside, and nuezhenide in fruits (Willems, 1988) and oleuropein, ligstroside, ligustalosides A and B, and flavonoid glycosides in leaves (Romani et al., 2000). Isolation of oleuropein, ligstroside, oleuropein aglycone, ligstroside aglycone, luteolin-7-*O*-glucoside, apigenin-7-*O*-rutinoside, *p*-hydroxyphenethyl alcohol, and 3,4-dihydroxyphenethyl alcohol from the leaves has also been reported (Kiss et al., 2008). In the course of our chemical studies on the glycosides of oleaceous plants (Takenaka et al., 2002), we have reinvestigated the constituents of the leaves and twigs of *L. vulgare* and isolated two secoiridoid glucosides, **1** and **2**, as well as the unusual secoiridoid aglycones **3** and **4**. This paper deals with the structural determination of these novel compounds.

2. Results and discussion

The $CHCl_3$ and *n*-BuOH soluble fractions of the methanolic extract of the dried leaves and twigs of *L. vulgare* were separated by a combination of column chromatography, prep. TLC, and prep. HPLC, affording two secoiridoid glucosides, (2″*R*)-10-hydroxy-2″-methoxyoleuropein (**1**) and (2″*S*)-10-hydroxy-2″-methoxyoleuropein (**2**), and two new secoiridoids, named ligustrohemiacetals A (**3**) and B (**4**), together with nine known compounds, ligstroside, kaempferol 3,7-*O*-bis-α-L-rhamnopyranoside (Tanaka et al., 1981; Kitajima et al., 1989), *p*-hydroxyphenethyl alcohol, 3,4-dihydroxyphenethyl alcohol, 1-*O*-*trans*-cinnamic acid β-D-glucopyranoside (Latza et al., 1996), secologanoside (Calis and Sticher, 1984), 10-hydroxyoleoside dimethyl ester (**8**) (Trujillo et al., 1996), 10-hydroxyoleuropein (**6**) (Inoue et al., 1982), and 10-hydroxyligstroside (**7**) (Shen et al., 1990). The latter five known compounds were isolated for the first time from this species. (See Fig. 1).

Compound **1** was isolated as a colorless amorphous powder, $[\alpha]_D$ − 141. The HR-SIMS of **1** exhibited a pseudomolecular ion $[M-H]^-$ at *m*/*z* 585.1799, indicating a molecular formula of $C_{26}H_{34}O_{15}$. It showed an UV maxima at 232 and 281 nm and IR bands at 3368 (OH), 1734 (ester), 1701, and 1630 (α,β-unsaturated ester) cm^{-1}. Its ^1H NMR spectra (Table 1) displayed signals due to a 10-hydroxyoleoside 11-methyl ester (**5**) moiety [H-3 at δ 7.53, H-8 at δ 6.15 (ddd, *J* = 8.0, 6.0, 1.0 Hz), H-1 at δ 5.97 (br s), H-1’ at δ 4.82 (d, *J* = 8.0 Hz), H_2-10 at δ 4.18 (ddd, *J* = 13.0, 6.0, 1.0 Hz) and 4.30 (dd, *J* = 13.0, 8.0, 1.0 Hz), and a carbomethoxyl group at δ 3.71 (s)] as well as an aromatic ABX spin system at δ 6.65–6.76, indicating that **1** was structurally similar to 10-hydroxyoleuropein (**6**). However, there were marked differences in their spectra with a methoxyl signal at δ 3.22 (3H, s) and an oxygenated methine proton at δ 4.29 (dd, *J* = 8.0, 4.5 Hz) in **1** instead of the methylene

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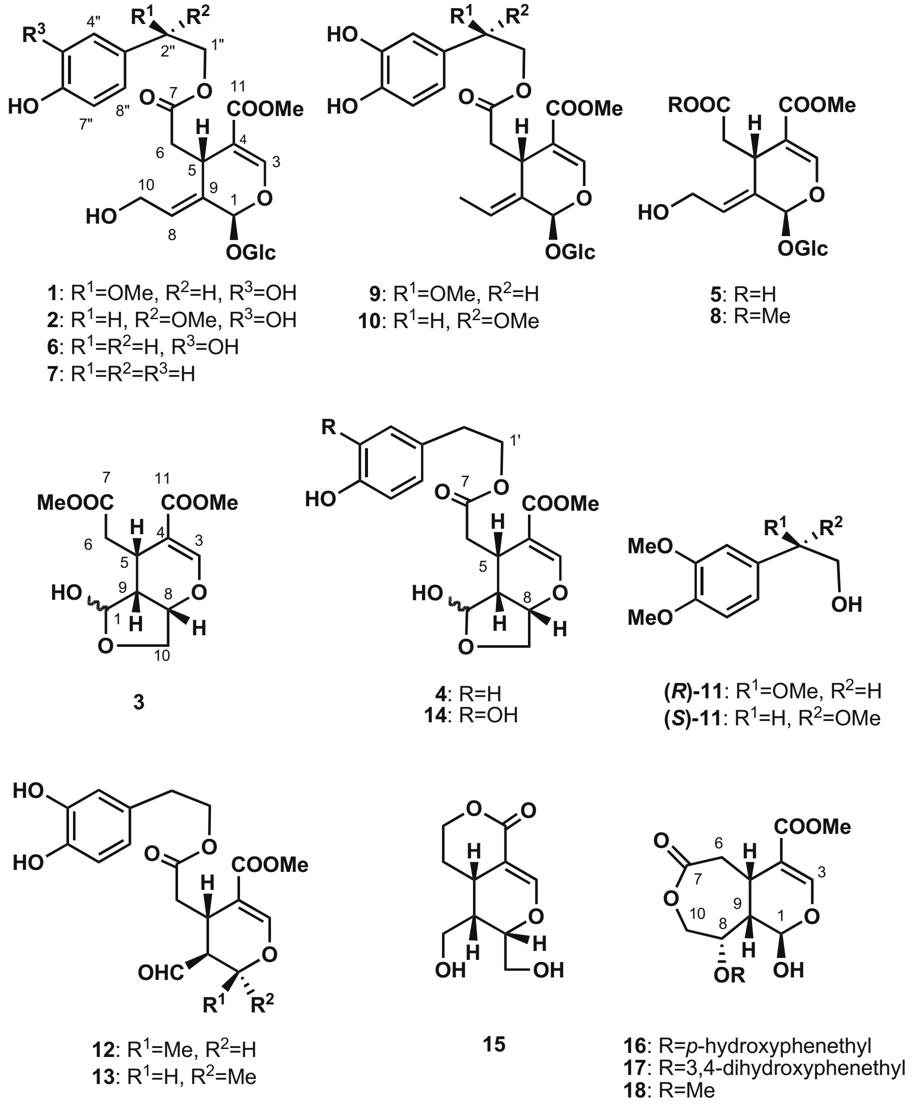

Fig. 1. Structures of compounds **1**–**18**.

protons assigned to H_2-2″ in **6**. The ^{13}C NMR signals of **1** (Table 1) were superimposable to those of **6**, except for the presence of the additional methoxyl signal and chemical shifts of C-1″ and C-2″. HMBC experiments with **1** showed significant correlations between H_2-1″ (δ_H 4.08 and 4.09) and C-7 (δ_C 172.9) and between the methoxyl group (δ_H 3.22) and C-2″ (δ_C 82.6). These findings indicated that **1** possessed a 2-(3,4-dihydroxyphenyl)-2-methoxyethanol unit instead of the 2-(3,4-dihydroxyphenyl)ethanol unit found in **6**.

Compound **2** was isomeric with **1**, and showed UV, IR, MS, and NMR spectroscopic features that were closely similar to those of **1**. The only significant difference observed between the two compounds was in the signals of H_2-1″ in the ^1H NMR spectra, suggesting **2** to be a C-2″ epimer of **1**. This was supported by comparison of their NMR spectroscopic data with those of (2″*R*)-2″-methoxyoleuropein (**9**) and (2″*S*)-2″-methoxyoleuropein (**10**), which have been isolated from *Jasminum officinale* L. var. *grandiflorum* (L.) Kobuski (Oleaceae) (Tanahashi et al., 1999).

The absolute configurations of the C-2″ of **1** and **2** were determined in the same way as for **9** and **10** (Tanahashi et al., 1999). A mixture of **1** and **2** in a ratio of 1:4 was hydrolyzed before being methylated with CH_2N_2 to give 2-methoxy-2-(3,4-dimethoxyphenyl)ethanol (**11**). Chiral HPLC analysis showed that the product was an enantiomeric mixture of (*R*)-**11** and (*S*)-**11** in a ratio of 1:4. These results led us to conclude that compounds **1** and **2** are (2″*R*)- and (2″*S*)-10-hydroxy-2″-methoxyoleuropeins, respectively.

Compound **3**, was obtained as an amorphous powder, and was determined to have the molecular formula $C_{12}H_{26}O_7$ from its HR-EIMS. Its UV and IR spectra suggested the presence of an enol-ether system conjugated to a carbonyl group (236 nm, 1705, 1630 cm^{-1}), which is typical for secoiridoid nuclei. In addition, its ^1H NMR spectrum (Table 2) showed signals for an olefinic proton at δ 7.60 (d, J = 1.5 Hz) and two methoxyl groups at δ 3.669 and 3.674 (each s), implying the presence of a dihydropyran ring structurally related to the aglycone part of 10-hydroxyoleoside dimethyl ester (**8**), a constituent of this plant material. However, no signals for an ethylidene group or a glucose moiety were recognized, but resonances for two methine protons at δ 5.19 (d, J = 7.0 Hz) and δ 4.57 (dd, J = 4.0, 2.0 Hz) and oxygenated methylene at δ 3.93 (d, J = 10.0 Hz) and δ 4.13 (brdd, J = 10.0, 2.0 Hz) were observed. These proton signals were accompanied by weak ones (Table 2), suggesting **3** to be an isomeric mixture in the ratio of 4:1. Its ^{13}C NMR spectrum (Table 3) showed, besides two methoxyl and two carbonyl carbon signals, eight carbons, four of which resonated at