

Phytochemical Analysis of *Phillyrea latifolia* L., a New Source of Oleuropeoside

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Phenylpropanoid Glycosides, *Phillyrea latifolia*, Secoiridoid Glycosides

As a part of our studies on the biologically active substances from Spanish plants, we have undertaken an investigation of the chemical constituents of a typical mediterranean species, *Phillyrea latifolia* L. (Oleaceae). Two secoiridoid glycosides, three phenylpropanoid glycosides, one lignane and two triterpenic acids were isolated from the leaves of this species and identified. The phytochemical analysis of the aerial parts of *P. latifolia* revealed that it is a rich source of oleuropeoside.

Oleaceae plants have been used for medicinal purposes and spices for many centuries. A number of biologically active substances have been isolated from plants of the Oleaceae. Secoiridoid glycosides are the major secondary metabolites in Oleaceae. Several plants of this family, e.g. *Olea europaea*, have been studied from a pharmacological point of view. Oleuropeoside is the major iridoid in olive leaves. Clinical data on the beneficial effects of olive leaves in the treatment of hypertensive disease has been available since the 1950s (Hansel *et al.*, 1996). This compound increased coronary blood flow and showed antiarrhythmic and spasmolytic effects (Ghisalberti, 1998). It showed a hypoglycemic effect and increased tolerance of orally administered glucose (Trovato *et al.*, 1993). In fact, olive leaves are used in folk medicine as an antidiabetic drug (González *et al.*, 1992). Oleuropeoside has also shown antimicrobial and has been shown to be a potent antioxidant endowed with antiinflammatory properties (Visioli *et al.*, 1998a), it is a potent scavenger of superoxide radicals and inhibitors of neutrophil respiratory burst (Visioli *et al.*, 1998b).

A previous study of the constituents of the leaves from *Phillyrea latifolia* revealed that it is a rich source of oleuropeoside. Because of the reported pharmacological activities of this compound, we have undertaken an investigation of this species. *Phillyrea latifolia* is found in Spain,

in the Mediterranean Europe and in the north of Africa. *P. latifolia* leaves were well known in the Mediterranean historical medicine for their oropharyngeal antiinflammatory effects. At the present time the use of this species is as diuretic (Ballero and Fresu, 1993), as antipyretic (Bellakhdar, 1997) and as antispasmodic against stomach aches (Merzouki *et al.*, 1997). Aerial parts of *P. latifolia* showed antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Husain and Tobji, 1997).

No previous phytochemical study on *Phillyrea latifolia* leaves has been reported till now. This paper led to isolation of ursolic acid (**1**), oleanolic acid (**2**), phillyrin (**3**), ligustroside (**4**), oleuropeoside (**5**), salidroside (**6**), coniferin (**7**) and syringin (**8**) from the leaves of *Phillyrea latifolia*.

Materials and Methods

Plant material was collected in Jaén (Spain) (March, 1997) and identified by Pr. Carmen Bartolomé Esteban, Department of Vegetal Biology, University of Alcalá, Madrid, Spain. A voucher specimen (CR 97) is kept in the University of Alcalá.

Leaves of *Phillyrea latifolia* (800 g) were extracted with acetone at room temperature. The extract (46 g) was chromatographed on Sephadex LH-20 and eluted by MeOH to afford four frac-



tions (F₁-F₄). Fraction F₂ (24 g) was submitted to CC on silica gel (G-60, 26–40 μ , Merck) using a linear gradient of CHCl₃/MeOH (97/3; 95/5; 93/7; 90/10; 85/15 v/v) to afford ten main groups of fractions (A₁-A₁₀). Fractions A₂ and A₃ yielded ursolic acid (83 mg, **1**) and oleanolic acid (136.4 mg, **2**), respectively. Fraction A₆ (557.4 mg) was purified by flash chromatography on silica gel and eluted successively by CHCl₃, CHCl₃/MeOH (98/2) and MeOH affording phillyrin (16 mg, **3**) and ligustroside (23 mg, **4**). Fraction A₈ (1.9 g) on further flash chromatography on silicagel [CHCl₃, CHCl₃/MeOH (98/2) and MeOH] gave oleuropeoside (404 mg, **5**). Fraction A₉ (2.8 g) was submitted to flash chromatography on silicagel using as eluents CHCl₃/MeOH (95/5) and (90/10) to obtained oleuropeoside (233 mg, **5**), salidroside (65.3 mg, **6**), coniferin (42 mg, **7**) and syringin (30.4 mg, **8**).

Results and Discussion

In our study of the phytochemistry of this species we have isolated compounds **1-8** from the acetonic extract. Structures of isolated compounds are shown in Figure 1.

The ¹H and ¹³C NMR data of all compounds were assigned using a variety of 2D-NMR experiments including ¹H-¹H-COSY, HMQC, HMBC and NOESY experiments. Identification and assignment of the isolated compounds were performed by comparison of their spectroscopic data with those of authentic samples (oleanolic and ursolic acids, Extrasynthese, France) and/or previously reported data (Damtoft *et al.*, 1992; Inouye and Nishioka, 1972; Maillard *et al.*, 1992; Chiba *et al.*, 1980).

¹H NMR and ¹³C NMR spectra (in CD₃OD, TMS as int. standard; chemical shifts in δ ppm) were obtained using Bruker WM spectrometer [300 MHz (¹H NMR) and 75 MHz (¹³C NMR)].

The results obtained from analysis of isolated compounds were:

Phillyrin (3): ¹³C NMR: 51.2 (C-1), 83.3 (C-2), 72.0 (C-4), 55.8 (C-5), 89.0 (C-6), 70.7 (C-8), 132.8 (C-1'), 110.8 (C-2'), 147.8 (C-3'), 150.3 (C-4'), 112.8 (C-5'), 119.2 (C-6'), 137.5 (C-1''), 111.5 (C-2''), 149.5 (C-3''), 150.9 (C-4''), 118.0 (C-5''), 119.8 (C-6''), 56.7 (3''-OCH₃), 56.5 (4''-OCH₃), 56.4 (3'-OCH₃), 102.8 (C-1'''), 74.9 (C-2'''), 78.2 (C-3'''), 71.3 (C-4'''), 77.8 (C-5'''), 62.4 (C-6''').

Ligustroside (4): ¹³C NMR: 95.13 (C-1), 155.14 (C-3), 109.32 (C-4), 31.82 (C-5), 41.24 (C-6), 173.18 (C-7), 124.91 (C-8), 130.49 (C-9), 13.58 (C-10), 168.64 (C-11), 51.90 (C-12), 66.90 (C-1'), 35.19 (C-2'), 130.0 (C-3'), 130.99 (C-4'), 116.27 (C-7'), 130.99 (C-8'), 100.80 (C-1''), 74.77 (C-2''), 78.45 (C-3''), 71.48 (C-4''), 77.95 (C-5''), 62.78 (C-6'').

Oleuropeoside (5): ¹H NMR: 5.90 (H-1), 7.5 (H-3), 3.95 (H-5), 2.43 and 2.69 (H-6_a and H-6_b), 6.06 (H-8), 1.65 (H-10), 3.70 (H-12), 4.19 and 4.09 (H-1'^a and H-1'^b), 2.75 (H-2'), 6.65 (H-4'), 6.67 (H-7'), 6.53 (H-8'), 4.79 (H-1''), 3.35 (H-2''), 3.40 (H-3''), 3.35 (H-4''), 3.35 (H-5''), 3.87 and 3.66 (H-6'^a and H-6'^b); ¹³C NMR: 95.3 (C-1), 155.1 (C-3), 109.4 (C-4), 31.8 (C-5), 41.3 (C-6), 173.2 (C-7), 124.9 (C-8), 130.8 (C-9), 13.5 (C-10), 168.7 (C-11), 51.9 (C-12), 66.8 (C-1'), 35.4 (C-2'), 130.8 (C-3'), 116.5 (C-4'), 146.2 (C-5'), 144.9 (C-6'), 117.1 (C-7'), 121.3 (C-8'), 101.0 (C-1''), 74.8 (C-2''), 78.0 (C-3''), 71.5 (C-4''), 78.4 (C-5''), 62.8 (C-6'').

Salidroside (6): ¹H NMR: 4.00 and 3.68 (H-1^a and H-1^b), 2.81 (H-2), 7.04 (H-4), 6.67 (H-5), 6.67 (H-7), 7.04 (H-8), 4.26 (H-1'), 3.15 (H-2'), 3.32 (H-3'), 3.26 (H-4'), 3.23 (H-5'), 3.83 and 3.64 (H-6'^a and H-6'^b); ¹³C NMR: 72.1 (C-1), 36.4 (C-2), 130.7 (C-3), 130.9 (C-4), 116.1 (C-5), 156.5 (C-6), 116.1 (C-7), 130.9 (C-8), 104.4 (-1'), 75.1 (C-2'), 78.1 (C-3'), 71.7 (C-4'), 78.0 (C-5'), 62.8 (C-6').

Coniferin (7): ¹H NMR: 4.21 (H-1), 6.32 (H-2), 6.54 (H-3), 6.74 (H-5), 6.74 (H-9), 4.86 (H-1'), 3.45 (H-2'), 3.40 (H-3'), 3.40 (H-4'), 3.20 (H-5'), 3.77 and 3.66 (H-6^a and H-6^b); ¹³C NMR: 63.5 (C-1), 130.1 (C-2), 131.3 (C-3), 135.0 (C-4), 105.6 (C-5), 154.4 (C-6), 135.3 (C-7), 154.4 (C-8), 105.6 (C-5), 57.1 (-OCH₃), 105.4 (C-1'), 75.8 (C-2'), 77.9 (C-3'), 71.4 (C-4'), 78.4 (C-5'), 62.7 (C-6').

These compounds have been isolated for the first time from *Phillyrea latifolia*.

The *Phillyrea* genus comprises three species: *P. media*, *P. angustifolia* and *P. latifolia*. Among these only *P. media* has furnished oleuropeoside (Popov *et al.*, 1975).

Considering the bioactivity of oleuropeoside, and olive leaves, it is important to take into account the fact that *Phillyrea latifolia* also possesses a high amount of oleuropeoside. The presence of this compound, increases the possible pharmacological value of this species, because we now report that it is a potential source of oleuropeoside.

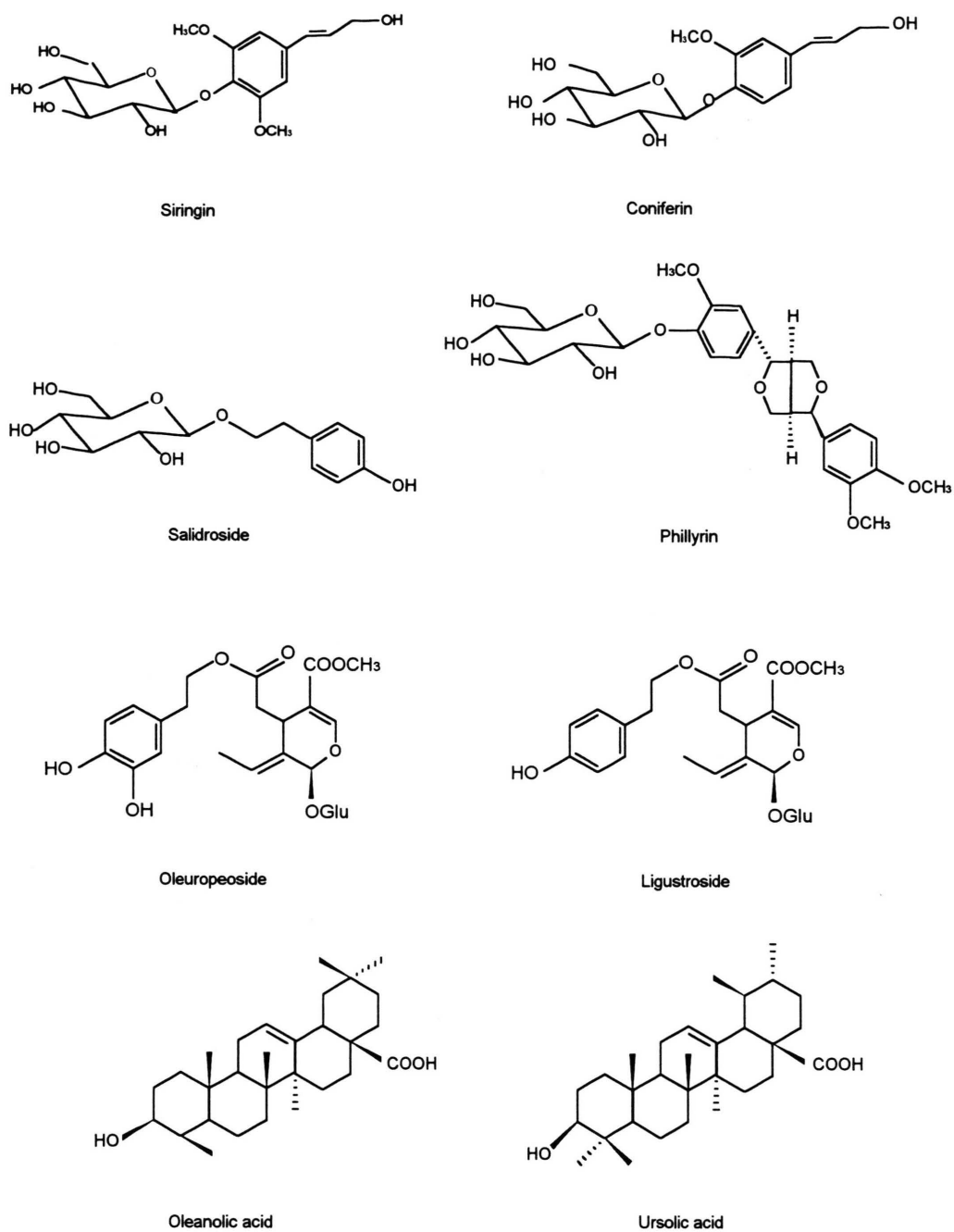


Fig. 1. Structures of isolated compounds.

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