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Anti-inflammatory activity of coumarins from *Ligusticum lucidum* Mill. subsp. *cuneifolium* (Guss.) Tammaro (Apiaceae)

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Abstract

Four coumarin derivatives [selidinin 1, (+)-praeruptorin A 2, visnadin 3 and (R)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin 4] were isolated from the aerial parts of *Ligusticum lucidum* Mill. subsp. *cuneifolium* (Guss.) Tammaro (Apiaceae). This is the first report on identification of these compounds in *Ligusticum* genus. Their topical anti-inflammatory activity was evaluated as inhibition of the Croton oil-induced ear dermatitis in mice. Each compound induced a significant oedema reduction and compound 4 exerted an effect similar to that of the reference drug indomethacin.

Keywords

Ligusticum lucidum, coumarins, anti-inflammatory activity.

Introduction

The Apiaceae family includes many plants distributed all over the world and a great number of these species are used as traditional ethnomedical remedies or food. Previous studies on coumarin occurrence in the family of Apiaceae, one of the richest source of these compounds, showed a profile comprising a wide range of phenylpropanoids, in particular substituted coumarins, fused furano- and pyranocoumarins (Wierzchowska-Renke 1974) and finally prenyloxycoumarins (Curini 2006). Nevertheless, little information about the chemical constituents within the genus *Ligusticum* is available. In fact, a previous phytochemical investigation on the whole plant of *Ligusticum lucidum* Mill. reported only its fatty acid composition (Kleiman 1982). To the best of our knowledge, no other phytochemical studies have been carried out on this plant to date and in particular no

investigations were done on the subsp. *cuneifolium*.

Materials and methods

Ligusticum lucidum Mill. subsp. *cuneifolium* (Guss.) Tammaro (Apiaceae) is a perennial herb living on rocky calcareous gravels of the Majella Mountains (Abruzzo, Italy), at altitudes ranging from 1000 to 1300 m. It's an endemic entity and to date there is no record about its use in local ethnomedical traditions.

Aerial parts of *L. lucidum* subsp. *cuneifolium* were collected at flowering stage in its native "locus classicus" (Tammaro 1989), at the end of July 2005. Identification of the plant was carried out by Prof. Fernando Tammaro from the Botanical Section of the Faculty of Biotechnology of University of L'Aquila, Italy. A voucher specimen (No. 2871/1) was deposited in the Herbarium (AQUI) of University of L'Aquila, Italy.

The structure of compounds **1-4** was established by spectroscopic and spectrometry methods, particularly NMR and GC-MS techniques, and confirmed by comparison with the literature data (Goswani 2006, Liu 2004, Ikeshiro 1992, Gray 1981).

The topical anti-inflammatory activity of these natural compounds was evaluated using the Croton oil-induced ear oedema test in mice as experimental model of inflammation, in comparison to that of the non steroidal anti-inflammatory drug (NSAID) indomethacin (Tubaro 1685).

Male CD-1 mice (28–32 g; Harlan Italy, Udine, Italy) were anaesthetised with ketamine hydrochloride (145 mg/kg, intraperitoneally; Virbac, Milan, Italy). Inflammation was induced on the right ear (surface: about 1 cm²) applying 80 µg of Croton oil (Sigma Chemical Co., St. Louis, USA) dissolved in acetone. Control animals received only the

irritant, whereas the others received both the irritant and the compounds under study, dissolved in acetone. After 6 hours, mice were killed to excise a plug (6 mm Ø) from both the treated and untreated ears. Oedema was quantified as weight difference between the two plugs. The anti-inflammatory activity was expressed as percent of oedema reduction in mice treated with the compounds under test with regard to control mice. Oedema values, expressed as mean $\pm$ standard error of the mean, were analyzed by one-way analysis of variance followed by Dunnett's test for multiple comparison of unpaired data. Significance was considered for probability levels lower than 0.05. Experiments complied with the Italian D.L. n. 116 of January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986.

Results and discussion

We report herein the isolation, structural characterization and anti-inflammatory activity of four coumarins from aerial parts of *L. lucidum* subsp. *cuneifolium*

Compounds **1-4** (Figure 1) were isolated for the first time from the genus *Ligusticum*. Among them selinidin **1** is known to occur in other species of *Ligusticum* (Gupta 1975). Selidinin **1**, (+)-Praeruptorin A **2** and visnadin **3** are common in other genera belonging to the family of Apiaceae, such *Ammi* (Duarte 1997), *Angelica* (Akihisa 2003), *Peucedanum* (Chen 1996) and *Selinum* (Seshadri 1967). On the contrary, (R)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin **4** has been previously isolated only from three species belonging to genus *Coleonema* (Rutaceae), namely *C. album* (Gray 1981), *C. aspalathoides* and *C. calycinum* (Gray 1986), being identified in the genus *Ligusticum* for the first time.

Results obtained for topical anti-inflammatory activity tests revealed that each coumarin reduced the oedematous response to a certain extent at the dose of 0.3 $\mu\text{mol}/\text{cm}^2$ (Table 1).

In particular, the most active compound, (R)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin **4**, induced 68 % oedema reduction, exerting an effect higher than that of the same dose of the NSAID indomethacin (58 % oedema reduction). Oedema reductions provoked by the other coumarins ranged between 22% [(+)-praeruptorin A **2**] and 43 % (visnadin **3**)

Thus, the anti-inflammatory activity of these compounds appears to be modulated by the substituents on their aromatic ring. In particular, the presence of the 7-(2',3'-epoxy-3'-methylbutoxy) group (coumarin **4**) is accompanied by a high anti-inflammatory activity (68 % oedema reduction) whereas that of a saturated pyrano-fused ring (coumarins **1-3**) is associated to a lower effect (22-43 % oedema reduction). The substituents on the pyrane ring seem to slightly influence the antiphlogistic effect of these compounds as well. Indeed, a significant increase of activity can be observed in visnadin **3** (43 % oedema reduction), characterised by the presence of 3'- β -O-dihydroangeloyl and 4'- β -O-acetyl residues, with respect to selinidin **1** or (+)-praeruptorin A **2** (27 % or 22 % oedema reduction, respectively), characterized by an α -acyl configuration in the same positions [3'- α -O-angeloyl (**1** and **2**) and 4'-O-acetyl (**2**)].

In conclusion, these findings suggest (R)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin **4** as a potential lead compound of a novel class of anti-inflammatory agents. Studies to elaborate a synthetic route to obtain **4** in sufficient amounts to get further insights into its mechanism of action and to synthesize structural analogues of **4** for *in vitro* and *in vivo* studies on their anti-inflammatory properties are in progress in our laboratories.

Experimental procedures

Air-dried and finely powdered aerial parts (100 g) of *L. lucidum* subsp. *cuneifolium* were defatted with *n*-hexane (2 x 200 mL) and subsequently extracted with CH₂Cl₂ (2 x 400 mL) by exhaustive maceration to give 2.1 g of dry extract. The extract was subjected to silica gel chromatography using CH₂Cl₂ and increasing polarity mixtures of CH₂Cl₂/MeOH (99.5:0.5 to 97:3) as eluents. Two main fractions were obtained (180 mg and 130 mg, respectively) and subsequently purified by preparative TLC on 20 x 20 cm silica 60 F₂₅₄ gel-coated glass sheets with CH₂Cl₂/MeOH 99.5:0.5 and 99:1 mixtures as mobile phases respectively, yielding compounds **1** (16 mg), **2** (11 mg), **3** (8 mg) and **4** (11 mg).

Selinidin (1): white solid; mp: 97 - 98°C; IR (KBr): 1655, 1630 cm⁻¹; ¹H NMR;⁶ ¹³C NMR;⁶ Anal. Calcd. for C₁₉H₂₀O₅: C, 69.50; H, 6.14; O, 24.36. Found C, 69.47, H, 6.12; O, 24.38.

(+)-Praeruptorin A (2): white solid; mp: 152 - 154°C; IR (KBr): 1680, 1650, 1635 cm⁻¹; ¹H NMR;⁷ ¹³C NMR;⁷ Anal. Calcd. for C₂₁H₂₂O₇: C, 65.28; H, 5.74; O, 28.98. Found C, 65.27, H, 5.72; O, 28.95.

Visnadin (3): white solid; white solid; mp: 86 - 87°C; IR (KBr): 1685, 1680, 1630 cm⁻¹; ¹H NMR;⁸ ¹³C NMR;⁸ Anal. Calcd. for C₂₁H₂₄O₇: C, 64.94; H, 6.23; O, 28.83. Found C, 64.97, H, 6.22; O, 28.80.

(R)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin (4): white solid; mp: 102 - 104°C; IR (KBr): 1640 cm⁻¹; ¹H NMR;⁹ ¹³C NMR (50 MHz, CDCl₃ δ) 18.7, 24.4, 57.8, 60.7, 68.4,

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101.6, 112.2, 112.6, 113.3, 128.5, 143.1, 155.5, 160.2, 160.9; Anal. Calcd. for C₁₄H₁₄O₄: C, 68.28; H, 5.73; O, 25.99. Found C, 68.26, H, 5.71; O, 25.98.

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Table 1. Anti-inflammatory activity of coumarins **1-4**(administered dose: 0.3 $\mu\text{mol}/\text{cm}^2$).

Compound	N°. animals	Dose ($\mu\text{mol}/\text{cm}^2$)	Oedema (mg)	%
			Mean $\pm$ S.E.	Reduction
Controls	10	--	6.9 $\pm$ 0.2	--
1	10	0.3	5.1 $\pm$ 0.3*	27
2	10	0.3	5.4 $\pm$ 0.4*	22
3	10	0.3	3.9 $\pm$ 0.3*,**	43
4	10	0.3	2.2 $\pm$ 0.2*	68
Indomethacin	10	0.3	2.9 $\pm$ 0.3*	58

* $p < 0.05$ at the analysis of variance, as compared with controls;..** $p < 0.05$ at the analysis of variance, as compared with compounds **1** or **2**.

Figure 1

Coumarins isolated from *Ligusticum lucidum*: selinidin **1**, (+)-praeruptorin A **2**, visnadin **3**, and a prenyloxycoumarin, (R)-(+)-7-(2',3'-epoxy-3'-methylbutoxy)-coumarin **4**.

1 R¹ = α -O-angeloyl,

R² = -H

2 R¹ = α -O-angeloyl,

R² = - α -OAc

3 R¹ = β -O-dihydroangeloyl,

R² = - β -OAc