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ALLELOCHEMICALS FROM *Pilocarpus goudotianus* LEAVES[1]

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Abstract—The effect on germination, shoot, and root growth by bergapten, xanthotoxin, imperatorin, xanthyletin, xanthoxyletin, luvangetin, donatin and alloxanthoxyletol from *Pilocarpus goudotianus* leaves, on *Lactuca sativa* var. nigra seedlings has been evaluated. A structure-activity correlation is discussed based on the bioassay results. Furanocoumarins appear to be the most active compounds in comparison with pyrano- and simple coumarins. The presence of an oxygenated function at C-8 decreases the germination effect in furano- and pyranocoumarins, while C-5 substituents do not cause significant changes on the activity.

Key Words—*Pilocarpus goudotianus*; Rutaceae; coumarins; furanocoumarins; pyranocoumarins; allelopathy; *Lactuca sativa.*

INTRODUCTION

Pilocarpus goudotianus is a shrub growing in Colombia and Venezuela, a member of only 20 species that comprises the genus *Pilocarpus* (Rutaceae). In a previous publication (Amaro-Luis et al., 1990), we reported the isolation of new furano- and pyranocoumarins from the leaves extract of *Pilocarpus goudotianus*. Preliminary bioassays of the crude extracts showed them to be active on germination of *Lactuca sativa* seeds.

Coumarins are known to have a wide range of biological properties, such

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[1] Part 2 in the series: *Natural Product Models as Allelochemicals*. For Part 1 see: Macías *et al.* (1992).

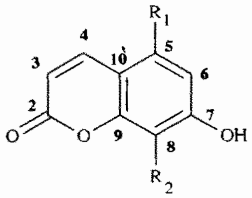
as anticoagulant (Arora and Mathur, 1963), cytostatic (Gosálvez et al., 1976; González et al., 1977a; Gawron and Glowniak, 1987), antibacterial and fungicidal (Jurd et al., 1971a,b) activities. Much attention has been paid to the photosensitizing effects on mammalian, bacterial, fungal, and viral cells caused by some furanocoumarins, effects which are attributed to photobinding of the coumarin to DNA (Kanne et al., 1982). Although various coumarins have long been known to exert stimulatory or inhibitory effects on seed germination and subsequent plant growth (Bennet and Bonner, 1953; Bose, 1958; Brown, 1981), few studies have addressed their possible allelochemical activity. The parent compound, coumarin, has been the most studied in this sense (Rodighiero, 1954). The inhibitory activity of coumarin on the germination of a number of crop and weed species such as cotton, prickly sida, redroot pigweed, etc., at a concentration of 10^{-3} M has been described (Williams and Hoagland, 1982). Simple coumarins such as umbelliferone and scopoletin were shown to inhibit Chinese cabbage root growth at 10^{-5}–10^{-7} M (Shimomura et al., 1982). Similar activities have been described for some furanocoumarins: psoralen, present in *Psoralea subacaulis* seeds, has been suggested to act as an allelopathic agent inhibiting germination and root length of competing species such as lettuce, tomato, cucumber, and radish (Baskin et al., 1967).

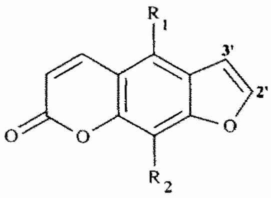
As a part of our research on bioactive natural products (coumarins, sesquiterpene lactones, lignans, phenolics, etc.) we are conducting a systematic study of their potential allelopathic activity (Macías et al., 1992). Thus, we are evaluating the regulatory effects of tested compounds on *Lactuca sativa* var. nigra (Leather and Einhellig, 1986).

In this paper we report the effect on *L. sativa* seed germination and root and shoot length of 10 different coumarins in aqueous solutions at concentrations of 10^{-4}–10^{-9} M. Simple coumarins 5,7-dihydroxycoumarin (**1**) and 7,8-dihydroxycoumarin (**2**); furanocoumarins bergapten (**3**), xanthotoxin (**4**) and imperatorin (**5**); and the pyranocoumarins xanthyletin (**6**), xanthoxyletin (**7**), luvangetin (**8**), donatin (**9**), and alloxanthoxyletol (**10**) were tested (Figure 1).

METHODS AND MATERIALS

Tested Coumarins. 5,7-Dihydroxycoumarin (**1**) [mp 280–282°C, AcOEt, (Kaufman and Kelly, 1965) mp 280°C, water] was prepared from phloroglucinol and ethylpropiolate as described by Kaufman and Kelly (1965). 7,8-Dihydroxycoumarin (**2**) [mp 249–251°C, MeOH, (Shimomura et al., 1980) mp 253°C, EtOH] was obtained by Pechmann condensation of pyrogallol with malic acid (Sethna and Phadke, 1953). Bergapten (**3**) [mp 187–189°C, petrol/AcOEt, (González et al., 1977b) mp 188–189°C, EtOH], xanthotoxin (**4**) [mp 144–146°C, petrol/AcOEt, (González et al., 1977b) mp 145–146°C, benzene/





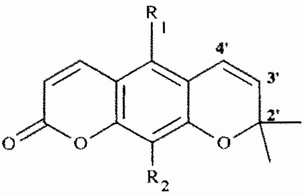
1 $R_1 = OH$ $R_2 = H$

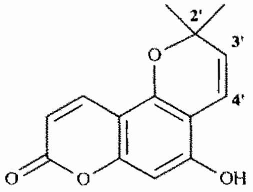
2 $R_1 = H$ $R_2 = OH$

3 $R_1 = OMe$ $R_2 = H$

4 $R_1 = H$ $R_2 = OMe$

5 $R_1 = H$ $R_2 = OCH_2CH{=}C(CH_3)_2$





6 $R_1 = R_2 = H$

7 $R_1 = OMe$ $R_2 = H$

8 $R_1 = H$ $R_2 = OMe$

9 $R_1 = H$ $R_2 = OCH_2CH{=}C(CH_3)_2$ =

10

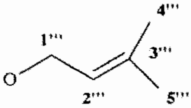


FIG. 1. Natural and synthetic coumarins tested for allelopathic activity.

AcOEt], imperatorin (**5**) [mp 98–100°C, petrol/AcOEt, (González et al., 1982) mp 102–103°C, benzene/AcOEt], xanthyletin (**6**) [mp 127–128°C, petrol/AcOEt, (González et al., 1977b) mp 128–130°C, petrol/AcOEt], xanthoxyletin (**7**) [mp 129–131°C, petrol/AcOEt, (Lassak and Pinhey, 1969) mp 131–132°C, AcOEt], luvangetin (**8**) [mp 106–107°C, petrol/AcOEt, (Agulló-Martínez et al., 1967) mp 106–107°C, benzene], donatin (**9**) (oil) and alloxanthoxyletol (**10**) (mp 217–219°C, dec. AcOEt) were isolated from *Pilocarpus goudotianus* leaves (Amaro-Luis et al., 1990).

Weight percentages (weight of isolated coumarins/weight of dry plant × 100) of coumarins isolated from *Pilocarpus goudotianus* leaves (Amaro-Luis et al., 1990) were the following: bergaptene (**3**), 0.015%; xanthotoxin (**4**), 0.02%;

imperatorin **(5)**, 1.8 × 10^{-3}%; xanthyletin **(6)**, 0.12% xanthoxyletin **(7)**, 1.4 × 10^{-3}%; luvangetin **(8)**, 6.1 × 10^{-3}%; donatin **(9)**, 5.9 × 10^{-3}%; alloxanthoxiletol **(10)**, 4.1 × 10^{-4}%.

Lettuce Seed Germination Bioassay. Seeds of lettuce, *Lactuca sativa* var. nigra, 1989 crop, were obtained from Estación Experimental Rancho de La Merced, Dirección General de Investigación y Extensión Agrarias, Junta de Andalucía, Jerez, Spain. All undersized and damaged seeds were discarded and the assay seeds were preselected for uniformity of size.

Bioassays were performed by germinating 25 lettuce seeds for five days (three for germination and two for root and shoot growth) in the dark at 25°C in 9-cm plastic Petri dishes containing a 10-cm sheet of Whatman No. 1 filter paper, and 7 ml of test or control solution. Test solutions, 10^{-4} M, were prepared using DMSO (1% v/v) as the initial solubilizing agent. Test solutions, 10^{-5}–10^{-9}M were obtained by diluting the previous solution. Studies with parallel deionized water controls and with the same DMSO concentration were also conducted. Four replicates of each treatment and of parallel controls were carried out. The number of seeds per replicate and the time and temperature of germination were chosen according to a number of preliminary experiments, varying the number of seeds, volume of test solution per dish, and the incubation period.

All the pH values were controlled before the bioassay and were between 6.5 and 7.0.

Statistical Treatment. The germination and root and shoot length values were tested by Student's *t* test; the differences between the experiment and the control were significant at value of $P = 0.01$ (Table 1).

RESULTS AND DISCUSSION

It has been reported that the presence of an alkylating α,β-unsaturated carbonyl function is related with its potential allelopathic activity (Fischer, 1986). Macías et al. (1992) reported that the presence of an α-methylene-γ-lactone group in sesquiterpene lactones is more related with the effects on seedling than on shoot and root lengths, where the effect of the conformational changes are crucial.

In this study we are evaluating some coumarins as a class of compounds with planar structures that contain an α,β-unsaturated δ-lactone moiety integrated in an aromatic system. We also include synthetic simple coumarins to compare them with the natural products and to establish a structure–activity correlation.

Table 1 and Figure 2 show that only at the highest concentration (10^{-4} M) do these compounds show a significant activity on germination and shoot and

TABLE 1. STATISTICAL RESULTS OF EFFECT OF SYNTHETIC COUMARINS **1** AND **2** AND NATURAL COUMARINS **3–10** ISOLATED FROM *Pilocarpus goudotianus* ON *L. sativa* VAR. NIGRA[a]

	Germination (%)						Radicle length (%)						Shoot length (%)					
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M
Simple coumarins																		
5,7-Dihydroxycoumarin (**1**)	−24	+1	+8	+22	−2	+2	−5[b]	+15	+6[b]	+8[c]	+17	+13	0	+5[b]	+7[b]	+11[c]	+13	+1[b]
7,8-Dihydroxycoumarin (**2**)	−38	−1	+2	+13	−2	+8	−4[b]	+10[c]	+7[b]	+12	+20	+8[c]	−8[b]	+ 3[b]	+2[b]	+2[b]	+18	−13
Furanocoumarins																		
Bergaptene (**3**)	−87	−1	+3	−5	−6	0	−16[c]	+23	+3[b]	+17	+14	+10[c]	−32[c]	+16	−1[b]	+13	+3[b]	−11[c]
Xanthotoxin (**4**)	−82	+3	+1	+10	−5	+3	−39	+3[b]	+1[b]	+4[b]	+22	+12	−61	−4[b]	−4[b]	+15	+7[b]	+3[b]
Imperatorin (**5**)	−47	+5	−6	+9	−10	+9	+25	+21	+9[b]	+14	+20	+7[b]	−22[c]	+8[b]	+8[b]	+10	+8[b]	−9
Pyranocoumarins																		
Xanthyletin (**6**)	−78	−5	−2	+16	−3	−1	−5[b]	+3[b]	−1[b]	+2[b]	+20	+17	−29[c]	−6[b]	−5[b]	+3[b]	+15	+10
Xanthoxyletin (**7**)	−67	+1	−16	+14	−5	+7	+14[c]	+17	+4[b]	0	+11	+1[b]	−22[c]	−8[b]	−3[b]	−4[b]	−6[b]	−20
Luvangetin (**8**)	+16	−6	−4	+8	−12	−1	−56	+15	+10[c]	+10[c]	+5[b]	+22	−43	+15	+10[c]	+5[c]	+5[b]	+10[c]
Donatine (**9**)	−20	−8	−1	+10	−9	+1	+3[b]	+16	+1[b]	+4[b]	+13	+2[b]	−38	+7[b]	+8[b]	+13	+7[b]	−10[c]
Angular pyranocoumarins																		
Alloxanthoxiletol (**10**)	+25	+1	0	+20	−5	−17	+12[c]	+23	+6[b]	+9	+18	−17	+14[c]	+12	+11[c]	0	+20	+8[b]

[a] Values are expressed as percentages from the control and are significantly different with $P < 0.01$ for Student's *t* test.

[b] Values significantly different with $0.05 > P$.

[c] Values significantly different with $0.01 < P < 0.05$

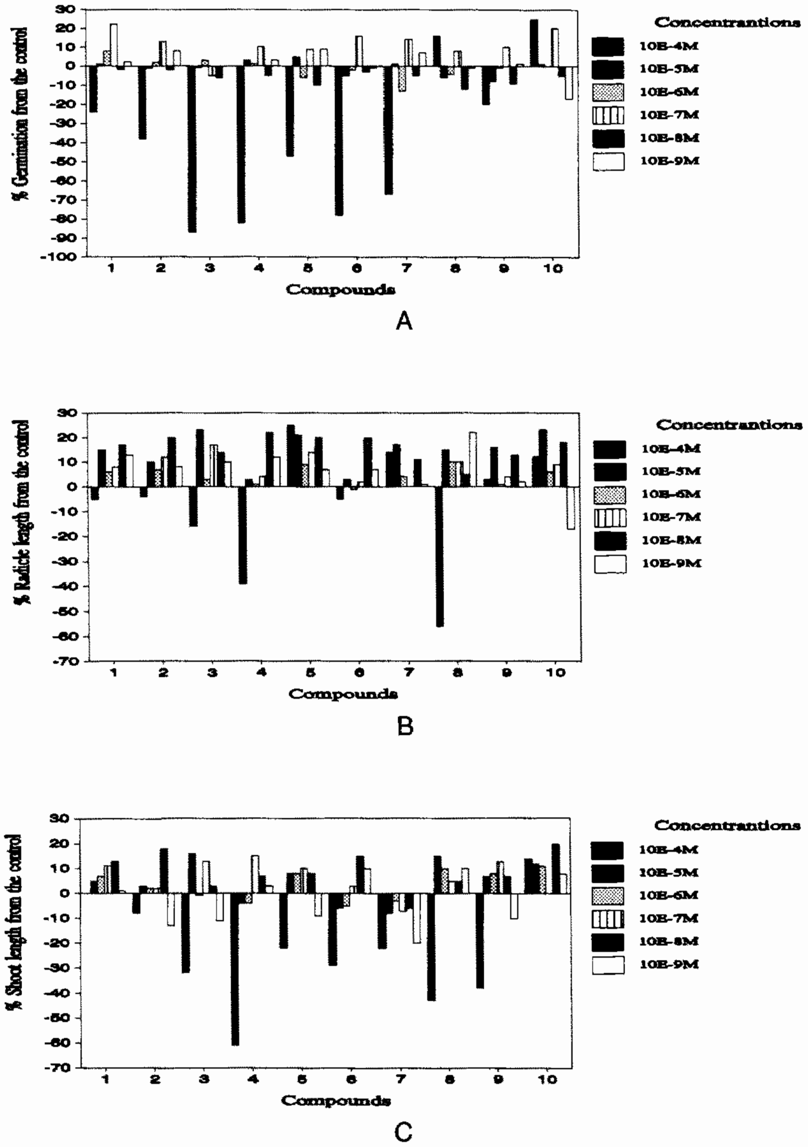


FIG. 2. Effects of coumarins 1–10 on the germination and growth of *L. sativa*. (A) Effect of coumarins 1–10 on the germination of *L. sativa*. (B) Effect of coumarins 1–10 on the radical length of *L. sativa*. (C) Effect of coumarins 1–10 on the shoot length of *L. sativa*.

similar phenolic compounds (Rodighiero, 1954; Williams and Hoagland, 1982; Inderjit and Dakshini, 1991).

The germination inhibitory effect of furanocoumarins usually is much more pronounced than those shown by pyranocoumarins and simple coumarins (Figure 2A). The activity displayed by pyranocoumarins **6** and **7** (−78% and −67%, respectively) is comparable to that shown by **3** and **4** (−87% and −82%), while **8** and **9**, with an oxygenated functionalization at C-8 have no significant effect (+16% and −20%). Therefore, the presence of a condensed furanic or pyranic ring appears to enhance the activity compared to simple coumarins. The presence of an oxygenated function at C-5 does not show significant changes on the activity, while functionalization at C-8 decreases the effect: imperatorin (**5**), with an voluminous isoprenyl side chain at C-8 presents −47% of inhibition in comparison with **3** (−87%), and luvangetin (**8**) and donatin (**9**) (+16%, −20%), which are lower in comparison with **6** (−78%).

The case of alloxanthoxiletol (**10**) is rather different from the other compounds tested, perhaps due to a different geometry of the molecule, which implies a different possibility of access to the active site.

With respect to the root and shoot length parameters (Figure 2B and C, respectively), there is no significant effect, except for furano- and pyranocoumarins **4**, **8**, and **9**, which are functionalizated at C-8. The presence of an oxygenated function at C-8 in these two families of coumarins could be related with the activity: the most active compounds are **4** and **8** for root length (−39%, −56%), and **4**, **8**, and **9** for shoot length (−61%, −43%, −38%, respectively). The lack of a significant change on the profile of activity of these compounds could be attributed to the fact that the adoption of different spatial dispositions other than a planar conformation is not possible. Therefore, the activity could be more directly related to the presence of several oxygenated side chains at C-8 than with conformational changes. This fact is in agreement with the previous observations reported for sesquiterpene lactones (Macías et al., 1992), where the conformational changes are more related with root and shoot length profiles of activity than with germination effects.

Due to the high coumarin concentration (10^{-4} M) needed for the activity, the coumarins most likely could be responsible for the allelopathic behavior in those plants where they are present as major constituents. Low coumarin plant concentrations could be related with different bioactivities (antifungal, antifeedant, etc.), including plant growth regulation. Nevertheless, in this case, it is not possible to establish a direct correlation between the presence of coumarins and the allelopathic activity shown by the plant.

The crude extract, obtained by soaking dry leaves in water (3:1, water–dry plant weight) was shown to be active on *L. sativa* bioassays.

From the isolated coumarin percentages of *P. goudotianus*, the molar concentrations of these compounds in the 1:10 aqueous extract (obtained by diluting the original aqueous extract with deionized water 1:10, 1:20, and 1:40) are: bergaptene (**3**), 2.3×10^{-5} M; xanthotoxin (**4**), 3.1×10^{-5} M; imperatorin (**5**), 2.2×10^{-6} M; xanthyletin (**6**), 1.7×10^{-4} M; xanthoxyletin (**7**), 1.8×10^{-6} M; luvangetin (**8**), 7.8×10^{-6} M; donatin (**9**), 6.3×10^{-6} M; alloxanthoxiletol (**10**), 5×10^{-7} M.

The concentrations of compounds **6**, **4**, and **3** in the 1:10 aqueous extract are in the same range as those that were active in the bioassay. Thus, in this case, coumarins, and particularly compounds **6**, **4**, and **3**, are very likely responsible for the allelopathic activity of *P. goudotianus* aqueous extract.

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