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SHORT COMMUNICATION

Polysubstituted Isochroman Derivatives with Plant Growth Regulating Properties on Wheat (*Triticum aestivum* L. cv Klein Escorpion)

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ABSTRACT

The synthesis of isochroman derivatives **4**–**9** from α-hydroxylactone **3** is reported. These heterocycles, carrying different substituents on C-3, C-4, and C-8, exhibited different degrees of inhibition of the vegetative growth of wheat (*Triticum aestivum* cv Klein Escorpion) plants, whereas plant developmental patterns such as their protein profile, carotenes/chlorophylls ratio, and weight/length relationship were not significantly affected. Microscopic observation of transverse sections of shoots and roots showed morphological changes in the treated plants, consistent with delayed development. The results suggest that among these isochromans the C-3 carbonyl moiety of the lactone and the C-4 free hydroxyl group are important but not essential for activity, and that a short side chain appended to C-3 is tolerated. However, cleavage of the C-8 methyl ether group to the related free phenol causes a drastic reduction in the growth inhibitory activity.

Key words: Chemical synthesis; Isochroman derivatives; Plant growth regulation; Wheat (*Triticum aestivum* L. cv Klein Escorpion); Structural requirements.

INTRODUCTION

A number of oxygen-bearing heterocycles cause interesting effects on plant growth (Bianchi and

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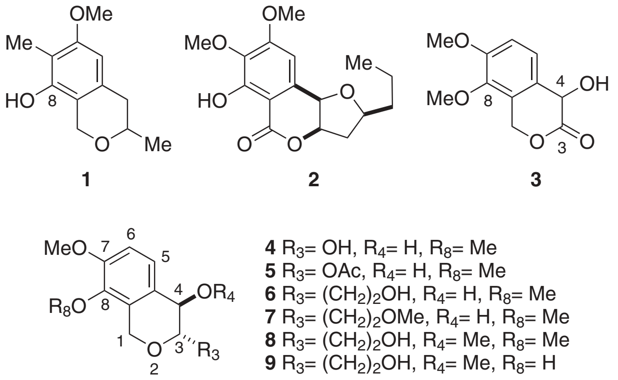
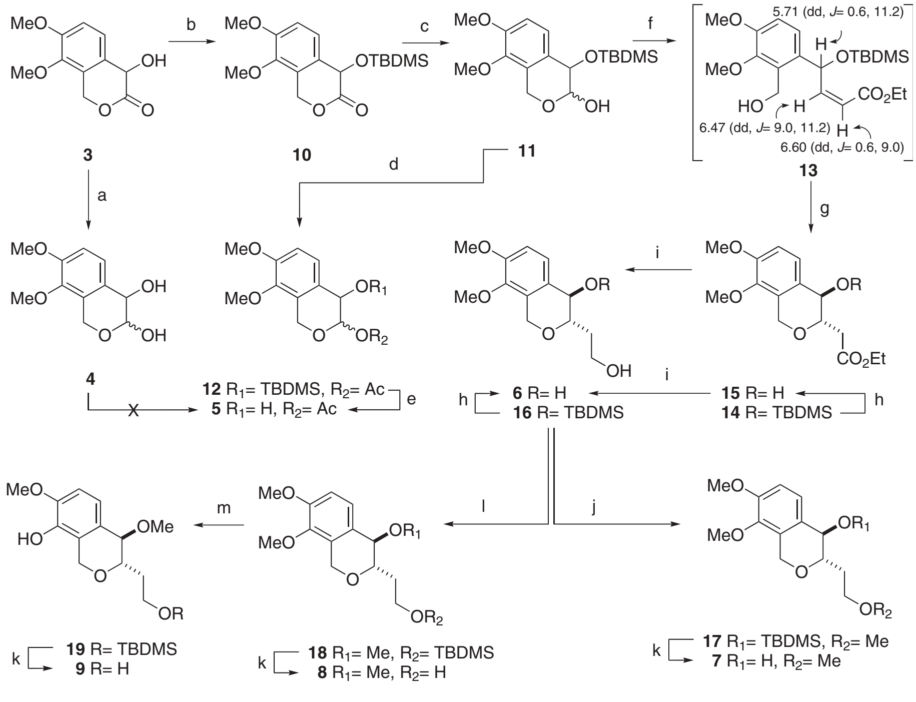


Structure block. Structures of compounds **1**–**9**.

others 2004a). Among them are the isochromans. Watanabe and Taniguchi (1994) studied the growth regulating activity of C-1 and C-4 substituted isochroman-3-ones on rice seedlings, finding that the 4-substituted heterocycles exhibited interesting activity, whereas most of their analogs functionalized on C-1 ranked among the least active compounds. More recently, Tobler and others (1998) disclosed that certain 4-substituted isochroman-3-ones reduce damage caused to wheat by clodinafop, potentially allowing the selective control of weeds in useful plant cultivations. In addition, Cutler and others (1989, 1997, 1998) reported that polysubstituted isochroman **1** and some of its derivatives inhibited growth in the etiolated wheat coleoptile assay. Finally, Kakimoto and others (2004) found that isochromans related to the natural isocoumarin-type herbicide monocerin (**2**), were active against paddy weeds (structure block).

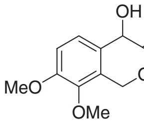
We have recently reported (Bianchi and others 2004a) the synthesis of α-hydroxylactone **3** and its plant growth regulating properties in a tobacco (*Nicotiana tabacum* cv Petit havanna) model. The observed potency of **3** indicated that the presence of a C-1 substituent on the isochroman moiety is not a requirement to elicit activity.

To identify other structural features of the isochroman derivative **3** required for activity, we report the synthesis of polysubstituted isochromans **4**–**9**, and the results of their growth regulating properties on wheat (*Triticum aestivum* L. cv Klein



Scheme. *Reagents and conditions:*(**a**) diisobutyl aluminum hydride (DIBAL-H), Toluene, −78°C, 1 h (70%); (**b**) : *tert*-butyl dimethylsilyl group (TBDMS) Cl, imidazole, : *N,N*-dimethylformamide (DMF), *N,N*-dimethyl aminopyridine (DMAP), Room temperature (RT), 4 h (87%); (**c**) DIBAL-H, toluene, −78°C, 10 min (93%); (d) acetic anhydride (Ac_2O, Ppyridine, overnight, −20°C (86%); (e) tetrabutylammonium fluoride (TBAF), tetrahydrofuran (THF), RT, 4 h (95%); (f) $Ph_3P = CHCO_2Et$, acetonitrile, reflux, 40 min; (g) potassium *tert*-butoxide (K^tBuO) (0.1 equiv.), RT, 2 min (76%); (h) TBAF, THF, RT, 30 min (**14** → **15**, 85%; **16** → **6**, 88%); (i) $LiAlH_4$, THF, 0°C, 10 min (**14** → **16**, 96%; **15** → **6**, 85%); (j) Ag_2O, MeI, acetonitrile, reflux, overnight (91%); (k) TBAF, THF, RT, overnight (**17** → **7**, 88%; **18** → **8**, 87%; **19** → **9**, 96%); (**l**) NaH, tetrabutylammonium iodide (TBAI), MeI, DMF, RT, 45 min (85%); m) 1. C_3H_7SH, NaH, DMF, 90°C, 3.5 h; 2. AcOH (77%).

Table 1. Effect of Compounds 3 – 9 on the Vegetative Growth of Wheat[a]



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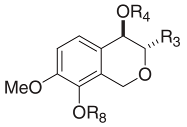

4 R_3= OH, R_4= H, R_8= Me
5 R_3= OAc, R_4= H, R_8= Me
6 R_3= $(CH_2)_2OH$, R_4= H, R_8= Me
7 R_3= $(CH_2)_2OMe$, R_4= H, R_8= Me
8 R_3= $(CH_2)_2OH$, R_4= Me, R_8= Me
9 R_3= $(CH_2)_2OH$, R_4= Me, R_8= H

Compd. N°[b] Conc. (μg/mL)	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0 (Control)	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
0.1	XXXXXXX	ND	XXXXXXX	XXXXX	ND	XXXXXXX	XXXXXXX
1	XXXXXX	ND	XXXXXXX	XXXXX	XXXXXXX	XXXXXX	XXXXXXX
10	XXXXXX	ND	XXXXXXX	XXXXX	XXXXX	XXXXXX	XXXXXXX
100	XXX	XXXXXXX	XXXXX	XXXXX	XXXX	XXXXX	XXXXXX
1000	X	XXXXX	XX	X	X	XX	XXXXX
AlogP[c]	0.62	0.25	0.75	0.80	1.40	1.44	0.74

[a] *The seeds were individually placed in test tubes containing 1.0 mL of MS-agar medium and the indicated concentrations of compounds* **3** *–* **9***, in an environment-controlled growth chamber at 25°C, employing a 16h/8h photoperiod. Controls (set C1) with no added isochromans were simultaneously prepared for each concentration level. Plant length (cm) was recorded on the 10th day.*

[b] *Experiments were run in duplicate. ND: Not determined; X: < 1 cm; XX: 1–3 cm; XXX: 3–5 cm; XXXX: 5–8 cm; XXXXX: 8–11 cm; XXXXXX: 11–14 cm and XXXXXXX: > 14 cm.*

[c] *Calculated with the aid of ALOGPs 2.1 from the Virtual Computational Chemistry Laboratory, available at http://www.vcclab.org.*

Escorpion). The set of synthetic compounds was designed to evaluate the influence of modifications at the level of C-3, the effect of a free C-4 hydroxyl group, and the importance of the C-8 methyl ether (scheme). This study could be useful for developing more effective plant growth regulators based on this heterocyclic motif.

MATERIALS AND METHODS

Biological Evaluation

Effect of compounds ***3–9*** *on the vegetative growth of wheat plants* – Seeds of *Triticum aestivum* cv Klein Escorpion were sterilized by successive 15 min immersions in ethanol and in sodium hypochlorite (1 mL of a 1.6% solution) containing Tween 80 (0.05%), followed by four rinses with sterilized distilled water. All subsequent manipulations were carried out under a horizontal laminar flow. Seeds were individually placed in test tubes containing 1.0 ml of MS-agar medium (Murashige and Skoog 1962) added with 0.1–1000 μg/ml of compounds **3–9**, and incubated in a growth chamber at 25°C, with a 16 h/8 h photoperiod.

Two sets of controls (C1 and C2) with no added isochromans were concomitantly prepared. Plant length and fresh weight were recorded on the 10th day for the treated plants and for the set of controls C1 (Table 1). The same growth parameters were recorded during days 5–10 for the second set of controls (C2, data plotted in Figure 1a).

Protein profiles of soluble and membrane-associated proteins were obtained according to Palatnik and others (1997) and pigment levels (carotenes and chlorophylls) were determined in ethanolic extracts after Lichtenthaler (1987). Statistical analysis was performed with Statgraphics Plus v. 5.1.

Observation of roots and shoots by optical microscopy – Radicles and shoots of control and treated plants were fixed in a formaldehyde-acetic acid (AcOH)-50% ethanol (1:1:18, v/v) solution, dehydrated through a graded *n*-butanol series (50%, 70%, 85%, 95%, 100%) and embedded in paraffin wax. Transverse sections (6–8 μm thick) were then cut, stained with cresyl violet, and mounted in Canada balsam (Ruzin 1999). Shoots were transversely cut at a level just above the coleoptile. The photomicrographs were taken with a Zeiss Axiolab microscope fitted with an MC 80 camera.

RESULTS AND DISCUSSION

Synthesis

Synthesis of the proposed polysubstituted isochromans was favored by an efficient access to the starting α-hydroxylactone **3** (Bianchi and Kaufman

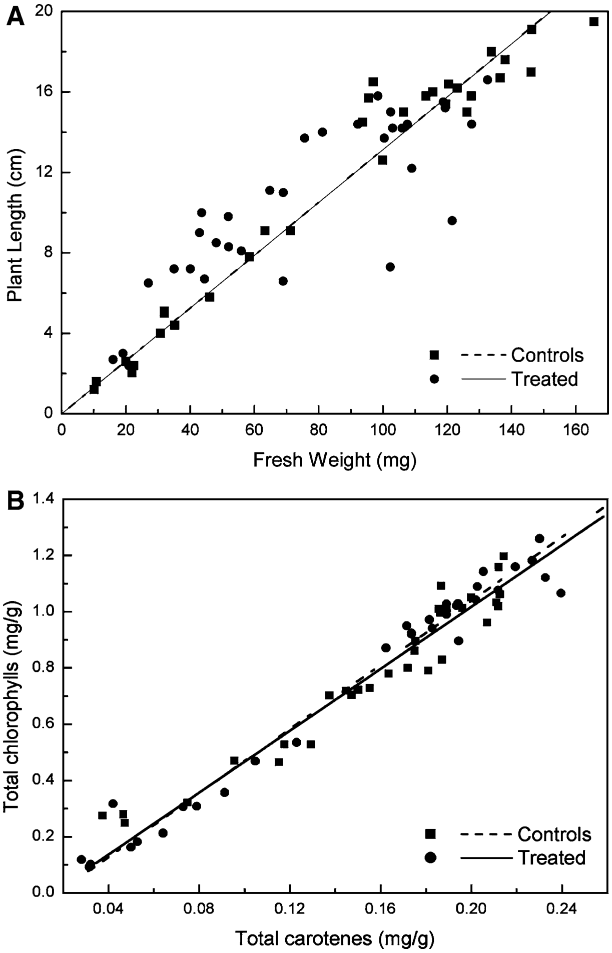

Figure 1. (**a**) Plot of length vs. weight of control (set C2, days 5–10) and treated plants (day 10). The equations of the straight lines fitting the data are: *Plant Length* (cm) = 0.00 (± 0.02) + 0.133 (± 0.006)* *Fresh Weight* (mg), $r = 0.842$, N = 32, $p < 0.05$ for the treated plants and *Plant Length* (cm) = 0.00 (± 0.01) + 0.131 (± 0.002)* *Fresh Weight* (mg), $r = 0.979$, $n = 32$, $p < 0.05$, for the controls. No statistically significant difference was found among their slopes and intercepts at the $p < 0.05$ level. (**b**) Plot of the concentration of carotenes (in mg per gram of fresh weight) versus the amount of chlorophyll (mg/g fresh weight), in control and treated plants. The equations of the straight lines fitting the data are as follows: *Total chlorophylls* (mg/g) = −0.08 (± 0.03) + 5.54 (± 0.17) * *Total carotenes* (mg/g), $n = 32$, $r = 0.986$, $p < 0.05$, for the treated plants and *Total chlorophylls* (mg/g) = −0.03 (± 0.04) + 5.14 (± 0.25) * *Total carotenes* (mg/g), $n = 32$, $r = 0.966$, $p < 0.05$, for the controls. The analysis of variance (ANOVA) test indicated that no statistically significant differences were found between the two lines at the $p < 0.05$ level.

2004a); complete experimental details are given in the Appendix (see http://www.springerlink.com). α-Hydroxylactols **4** were conveniently obtained (Scheme) in 70% yield as a 1.3:1 inseparable mixture of diastereomers, by partial reduction of **3** with diisobutyl aluminum hydride (DIBAL-H) in toluene at −78°C. Because selective acetylation of **4** to provide direct access to **5** proved unfeasible, compound **3** was first protected in 87% yield as the *tert*-butyl dimethylsilyl group (TBDMS) ether derivative **10**. Next, the one-pot partial reduction of **10** with DIBAL-H, followed by acetylation of the resulting inseparable mixture (1.3:1) of lactols **11**, furnished acetates **12** (4:1, inseparable mixture) in 80% overall yield.

The acetates were selectively desilylated with tetrabutylammonium fluoride (TBAF), giving 90% of **5**, as a 4:1 inseparable mixture of diastereomers. Differences in the diastereomeric proportions between **12** and **11** may be assigned to preferential acetylation of one of the diastereomers, followed by equilibration of the remaining mixture of lactols.

To install the proposed C-3 side chain, a one-pot Wittig-oxa-Michael strategy was employed, (Bianchi and others 2004b). To that end, lactol **11**, obtained in 93% yield from **10**, was submitted to reaction with ethoxycarbonyl methylene triphenylphosphorane, and the α,β-unsaturated ester resulting from the Wittig reaction of the aldehydic form of the lactol was cyclized *in situ* with a catalytic amount of potassium *tert*-butoxide, giving a single diastereomer (**14**) in 76% overall yield.

In agreement with literature precedents, isolation of the *trans* diastereomer **14** was considered as being the result of a Michael-type attack of the alkoxide to a *Z*-olefinic intermediate with the structure of **13**, through a chair-like transition state (Ramírez and others 1997). Support for this hypothesis was gained after ^{1}H NMR analysis of an analytical sample of intermediate **13**, which revealed a coupling constant of 9.0 Hz between the two olefinic protons.

$LiAlH_4$ reduction of **14** gave 96% of alcohol **16**, which upon desilylation furnished diol **6** in 88% yield. Diol **6** could also be accessed from **14**, by inverting the sequence of transformations; thus, desilylation of **14** furnished 85% of ester **15** (Bianchi and others 2004b), which in turn provided diol **6** in 85% yield upon $LiAlH_4$ reduction.

The silyl ether **16** proved to be an excellent starting material for obtaining monomethyl ethers **7** and **8**, as well as phenol **9**. On the one hand, silver(I) oxide-mediated etherification (Finch and others 1975) of **16** furnished 91% of methyl ether **17**, which gave 88% yield of product **7** after desilylation. On the other side, reaction of **16** with sodium hydride effected the relatively rare high order 1,6-migration of the silyl moiety from the benzylic oxygen to the less congested primary carbinol (Morton and Neilson 1982). Subsequent addition of methyl iodide afforded benzylic methyl ether **18** in 85% yield and, in turn, compound **18** furnished 87% of the desired primary alcohol **8** after desilylation. Finally, chemoselective cleavage of the *ortho*-disubstituted methyl ether moiety of **8** with sodium propylmercaptide in dimethylformamide (Kaufman 1996) provided 77% of **19**, which was transformed into the required phenol **9** by a TBAF desilylation.

Growth Regulating Activity

Seeds were individually grown in Murashige-Skoog agar medium containing 0.1–1000 μg/ml of the test compounds. These concentrations did not inhibit the germination process and, compared to the controls, they did not delay germination. The plant growth of treated and control (C1) specimens was determined as plant length on the day 10, when plant fresh weights were also recorded. The results, shown in the Table 1, indicated that plants treated with 1000 μg/ml of compounds **3**, **6**, and **7**, all of them bearing a free C-4 alcoholic function, exhibited very poor vegetative growth. However, at the tested concentrations the compounds were not acting as herbicides. At a concentration of 100 μg/ml, compounds **3** and **5**–**8** were those exhibiting the highest inhibitory activity. In addition, compounds **6** and **7** still retained good activity, showing a reduction of plant height between 30% and 48%; however, lactol **4** and phenol **9** were the less active compounds.

The results indicate that partial reduction of the carbonyl of **3** rendered the essentially inactive lactols **4**, suggesting the relative importance of the lactone moiety for the growth inhibition effect. The activity was partially restored when **4** was transformed into the related acetates **5**; this confirmed that the lactonic carbonyl is important but not essential for the activity, and it evidenced some steric tolerance at the C-3 level. The revelation that **6** and **7** were almost as active as **3** even at low concentrations (0.1–10 μg/ml) reinforced these observations, demonstrating that a short chain appended to C-3 is well tolerated, being of little effect on the growth inhibitory activity.

An empirical and general rule indicates that among natural plant growth inhibitors, free hydroxyl groups on the heterocyclic motif convey good levels of activity (Cutler and others 1979, 1980). A similar situation takes place among the pyranonaphthoquinone antibiotics, where the presence of a benzylic oxygen-bearing ring has been linked to their biological activity (Omura and others 1980). However, comparative analysis of the performances of diol **6** and the related C-4 methyl ether **8** indicated that a C-4 alcoholic group seems to be beneficial but not essential for the observed activity, whereas comparison of **8** and the related phenol **9** revealed that the presence of a C-8 methyl ether could be key for the potency of the growth retarding effect in this series of compounds. Interestingly, Cutler reported that some synthetic C-8 alkyl ether derivatives of **1** are more powerful growth inhibitors in the etiolated wheat coleoptile assay than their parent compound (**1**), which bears a free phenol as substituent on C-8 (Cutler and others 1997, 1998).

To further assess that this series of isochromans act as growth retarding agents, the lengths of plants treated over 10 days with **3**–**9** were plotted against their corresponding fresh weight (both measured on the day10). The slopes and intercepts of the straight lines (Figure 1a) were statistically compared by means of an ANOVA test with the parameters provided by the plot of the same variables recorded for a set of control plants from day 5 to day 10 (set C2).

Interestingly, no statistically significant differences were observed between the two straight lines at the $p < 0.05$ level; in addition, straight lines corresponding to individual compounds (**3**, **5**–**9**) were also not statistically different from that corresponding to the control ($p < 0.05$). This indicated that treated plants observed on the 10th day exhibited length and weight parameters not statistically different from those displayed by 5–10-day-old control plants, thus confirming the proposed growth retarding effect.

When the AlogP values of the isochromans were correlated with the resulting lengths of the treated plants at the 10th day (Table 1), a clear trend was observed, where the most active compounds were the most lipophilic members of the set. AlogP is the logarithm of the octanol-water distribution coefficient, a global molecular property that is a measure of lipophilicity (Viswanadhan and others 1989). However, the small size of the set of synthetic isochromans made difficult the establishment of a statistically significant relationship between growth regulating activity and lipophilicity.

The concentrations of carotenes and total chlorophylls were also determined on day 10 in the groups of controls (C1) and treated plants, and straight lines were obtained when the levels of carotenes were plotted against the concentrations of the total chlorophylls in the two groups (Figure 1b). The two regression lines were not statistically different at $p < 0.05$, revealing that the biosyntheses of chlorophylls and carotenes remained statistically unaffected by the treatment.

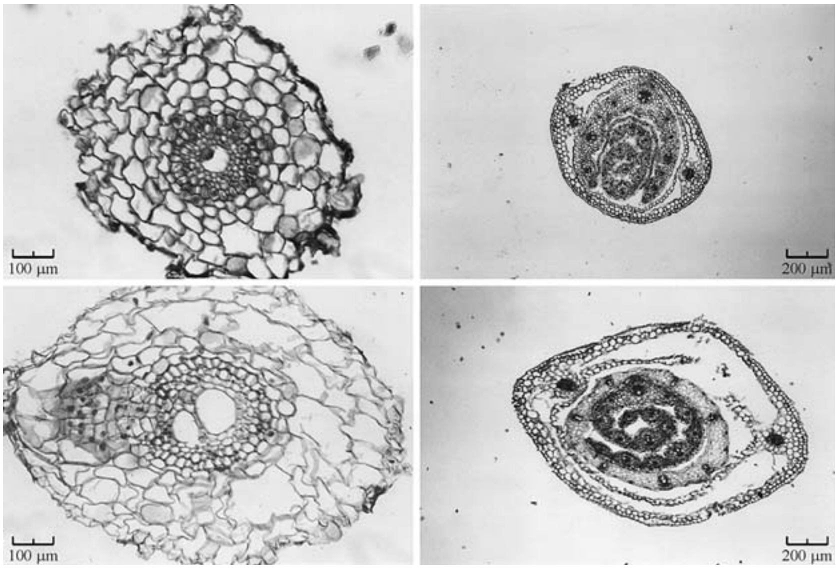

Figure 2. Optical microscopic observation of transverse sections of radicles (left) and shoots (right) of a plant treated with 10 μg/ml of compound **6** (above) and a control specimen (below), showing morphological changes produced by the isochroman derivative.

In addition, a sodium dodecyl sulfate-polyacrylaminde gel electrophoresis (SDS-PAGE) analysis of soluble and membrane-associated protein fractions provided evidence that the protein profiles of the treated and control plants were essentially equivalent in both cases (data not shown), suggesting that the tested compounds did not produce significant perturbation effects on important metabolic pathways. However, this result cannot rule out the fact that the tested compounds may be able to perturb some key metabolic pathway involving only a subset of specific proteins, with only very subtle modifications of the SDS-PAGE protein pattern. In this event, the synthetic compounds could be useful as probes for acquiring better information about the molecular processes involved in plant growth regulation.

Finally, shoots and roots of treated (10 μg/ml of compound **6**) and control plants were cut transversely, stained, and observed microscopically (Figure 2). The transvere section of the control roots showed a normal anatomy, where the protoxylem, two completely differentiated metaxylematic vessels and a differentiated phloem could be clearly observed. However, the transverse section of the treated roots revealed a scarcely developed anatomy, with a poorly lignified protoxylem, and a metaxylematic vessel in the process of differentiation. In addition, the poles of the phloem were scarcely differentiated in this specimen.

Examination of the transverse section of the coleoptile of the control plants showed the coleoptile, including a well-differentiated foliar vein belonging to an expanded leaf and a second leaf in a convoluted prefoliation stage ready to expand; however, the transverse section of the treated plants showed less development, exhibiting a coleoptile enclosing a foliar apex with two non-expanded leaves. Interestingly, silica deposition in the leaves was markedly lower in the treated specimens compared with the control plants (Sangster 1970).

Thus, the microscopic observation revealed a greater expansion and cellular differentiation in the control specimens with respect to the treated plants, which were less developed owing to a lack of expansion of their walls. The observed results are analogous to those produced by other isochromans proposed to act as auxin transport inhibitors based on the inhibition of the elongation of hypocotyles of lettuce (Watanabe and Taniguchi 1994), and as plant growth regulators as revealed by the wheat coleoptile assay (Cutler and others 1989, 1998).

In conclusion, the synthesis of isochroman derivatives **4**–**9** from the readily available α-hydroxylactone **3** was performed, and compounds **3**–**9** were tested as plant growth regulators in a whole plant wheat (*Triticum aestivum* cv Klein Escorpion) model. Compound **3** as well as isochromans **5**–**8** were the most active at the 100 μg/ml level, and compounds **6** and **7** retained good activity at the 10 μg/ml level, inhibiting the vegetative growth of the wheat seedlings. The isochromans lead to reduced plant length and lower fresh weight, without significantly affecting the plant length to weight relationship and without significantly disturbing pigment and protein profiles.

The results suggest that the carbonyl group of the lactone moiety and the free C-4 alcohol are important for improved activity, but neither of them is strictly required. It was also observed that a short side chain attached to C-3 is well tolerated; however, transformation of the C-8 methyl ether into a free phenol yields a poorly active growth inhibitor, indicative of the relevance of this structural feature. The growth regulation effects are compatible with the inhibition of cellular division, resulting in severe limitations in the enlargement of the meristematic tissue, as seen in transverse cuts of radicles and shoots.

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