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Dehydrozaluzanin C: a potent plant growth regulator with potential use as a natural herbicide template ☆

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Abstract

The natural product dehydrozaluzanin C (former DHZ) is a sesquiterpene lactone obtained from different weeds of the Compositae family. Its potential as a plant growth regulator has been evaluated by using a phytotoxic allelopathic bioassay, where the commercial herbicide Logran® is used as internal reference. The evaluation is made based on their effects on germination and growth over several dicotyledon and monocotyledon species. The activity was tested in the range of 1000–0.001 μM. In almost all cases, DHZ was more active than the internal reference at 1000 μM, and its activity fell below the level of the internal reference at 100 μM. These results confirm DHZ as a potent plant growth regulator and a good candidate for the development of new herbicide models.

Keywords: Sesquiterpene lactones; Guaianolides; Dehydrozaluzanin C; Logran®; Plant growth regulator; Phytotoxicity; Natural herbicides

1. Introduction

Modern agricultural techniques heavily rely on the use of pesticides and herbicides to get control of pests and weeds. However, the economic and environmental costs of such procedures are rapidly increasing, and sometimes, these compounds become ineffective due to the appearance of resistance and cross-resistance phenomena. Natural phytotoxins constitute a large reservoir of underdeveloped compounds with high agricultural potential, and which might target receptor sites not yet exploited by synthetic herbicides (Duke and Abbas, 1995; Duke et al., 1997). Nowadays, microbial compounds have rendered many active natural products that should be used as new herbicides (Amagasa et al., 1994). The study of the chemical interactions among plants could be another useful approach for the development of new classes of environmentally soft herbicides (Duke and Abbas, 1995). Allelopathic compounds are, in fact, potential sources of phytotoxins that could lead to design of new chemotypes of herbicides. Surprisingly, their potential is still relatively untapped compared to the microbial compounds (Hay, 1999).

Sesquiterpene lactones constitute a numerous group of compounds with several biological activities, including the allelopathic ones (Picman and Picman, 1984; Macías et al., 1993, 1996). Dehydrozaluzanin C (**3**, former DHZ) is a sesquiterpene lactone with a guaianolide skeleton that has been isolated from different members of the Compositae family (Bohlman and Le Van, 1977; Bohlman et al., 1978, 1980, 1980a, 1980b, 1982; Bohlman and Zdero, 1982). It has also been reported to inhibit root growth (Asakawa and Takemoto, 1979; Kalsi et al., 1984), and recently, there was a first study relative to their mode of action (Galindo et al., 1999), however, no further work has been reported. We are actually conducting a systematic

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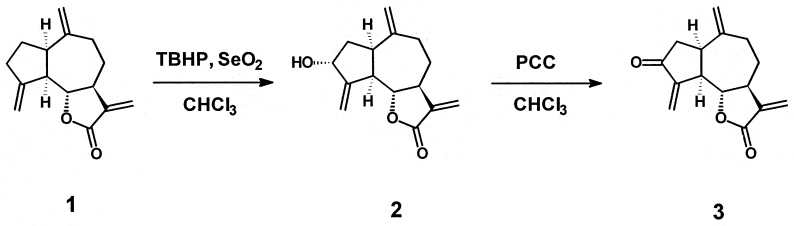

Fig. 1. Semi-synthesis of dehydrozaluzanin C (**3**) from dehydrocostuslactone (**1**).

evaluation of the phytotoxic activity of sesquiterpene lactones looking for the structural requirements needed for the activity (Macías et al., 1992, 1999). Based on these previous results, we have selected DHZ as one of the most promising candidate for further studies.

In the present paper, biological data of the phytotoxic activity of DHZ on eight different plant species is presented, regarding three main macroscopic parameters: germination, and root and shoot elongation. The commercial herbicide Logran® (59.4% terbumetrine and 0.6% triasulfuron), previously selected as internal standard for this type of bioassay (Macías et al., 2000), is used, aiming at two different objectives: (a) to validate the results with a well-known active compound or formulation and (b) to establish the relative levels of activity of the natural product with respect to commercial herbicides.

2. Results and discussion

Treatment of dehydrocostuslactone (**1**) with SeO_2 and *terc*-butyl-hydroperoxide, as previously described (Kalsi et al., 1984; Macías et al., 1992), afforded a major product *iso*zaluzanin C (**2**), which was easily oxidized to DHZ (**3**) using a chloroformic solution of pyridinium chlorochromate (PCC) (Fig. 1).

The effects produced by DHZ and the internal reference (Logran®) on the germination, and root and shoot length of different monocotyledon and dicotyledon species are presented in Tables 1 and 2 and Figs. 2 and 3. The tested concentrations ranged between 1000 and 0.001 μM. Plant species were selected as representatives of main monocotyledon and dicotyledon weeds and important commercial crops (Macías et al., 2000).

2.1. Dicotyledon species

The species assayed were lettuce (*Lactuca sativa* cv. nigra and cv. roman), tomato (*Lycopersicon esculentum* L. cv. Tres Cantos), carrot (*Daucus carota* L. cv. Coral) and cress (*Lepidium sativum* L.). From Table 1 and Fig. 2, no relevant activities below 10 μM were observed for Logran®, or for DHZ in almost all cases. Thus, discussion will focus on the activities at 100 and 1000 μM.

The effect of DHZ (1000 μM) on germination is higher than that obtained with the same concentration

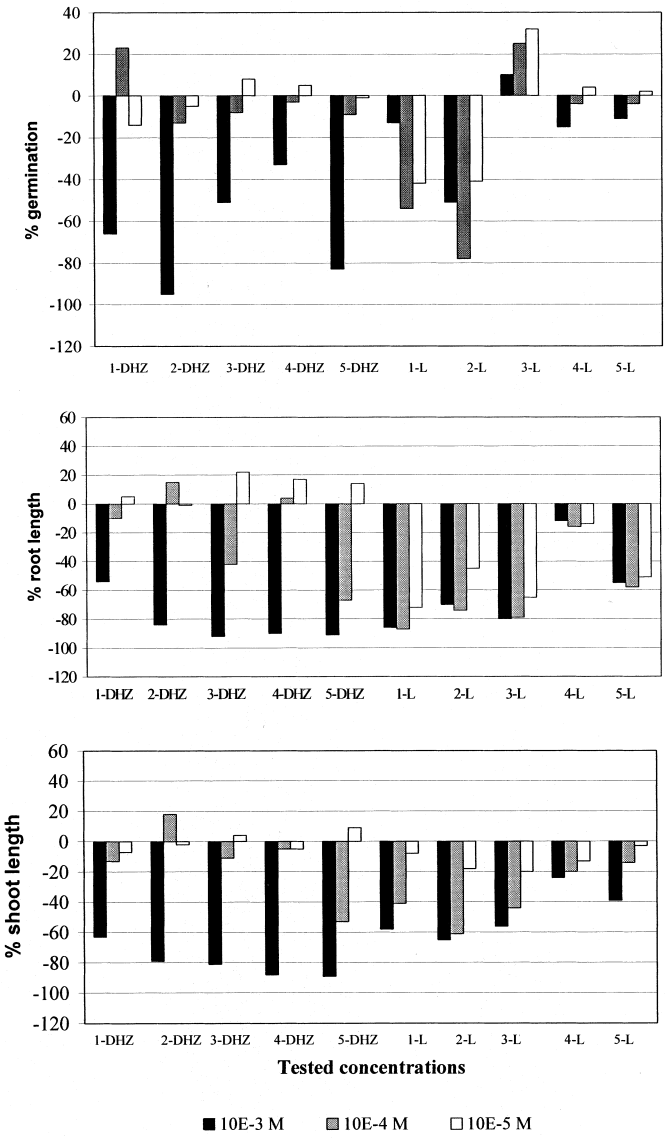

Fig. 2. Selected effects of DHZ and Logran® on germination and growth development. **1**: *Lactuca sativa* cv. nigra; **2**: *Lactuca sativa* cv. roman; **3**: *Lycopersicon esculentum* L.; **4**: *Daucus carota* L.; **5**: *Lepidium sativum* L.

Table 1
Germination and growth effects of DHZ (**3**) and Logran® on different dicotyledoneous plant species[a]

Concentration	DHZ (**3**)					Logran				
	Lactuca sativa cv. four seasons	*Lactuca sativa* cv. roman	*Lycopersicum esculentum*	*Daucus carota*	*Lepidium sativum*	*Lactuca sativa* cv. four seasons	*Lactuca sativa* cv. roman	*Lycopersicum esculentum*	*Daucus carota*	*Lepidium sativum*
Germination										
10^{-3} M	−66	−95	−51	−33	−83	−13	−51	10	−15	−11
10^{-4} M	23	−13	−8	−3	−9	−54	−78	25	−4	−4
10^{-5} M	−14	−5	8	5	−1	−42	−41	32	4	2
10^{-6} M	25	−2	10	7	1	−35	−19	21	−3	−3
10^{-7} M	5	−13	2	5	−5	−11	−5	27	7	6
10^{-8} M	0	−2	6	1	−2	−13	−10	5	−13	−2
10^{-9} M	−5	−6	−6	12	−10	−6	4	13	4	−11
Root length										
10^{-3} M	−54	−84	−92	−90	−91	−86	−70	−80	−12	−55
10^{-4} M	−10	15	−42	4	−67	−87	−74	−79	−16	−58
10^{-5} M	5	−1	22	17	14	−72	−45	−65	−14	−51
10^{-6} M	−2	−15	6	14	14	−59	−29	−32	−19	−27
10^{-7} M	11	8	1	4	16	−44	−19	4	16	−21
10^{-8} M	29	11	23	12	0	−40	−16	3	29	−13
10^{-9} M	53	8	5	12	−17	−17	−6	13	1	−12
Shoot length										
10^{-3} M	−63	−79	−81	−88	−89	−58	−65	−56	−24	−39
10^{-4} M	−13	18	−11	−5	−53	−41	−61	−44	−20	−14
10^{-5} M	−7	−2	4	−5	9	−8	−18	−20	−13	−3
10^{-6} M	−12	−8	7	3	−2	9	−2	−3	−15	8
10^{-7} M	15	3	1	6	8	16	−3	3	−11	15
10^{-8} M	16	−2	5	−4	−10	9	−2	5	−40	10
10^{-9} M	38	0	−8	5	−16	5	8	4	−26	12

[a] Values are presented as percentage differences from the control (e.g., 16% means 116% compared with the control). Values are significantly different from the control with $P > 0.05$ for the Welch's test. a: $P < 0.01$; b: $0.01 < P < 0.05$.

of Logran® (Fig. 2). Moreover, DHZ is active on species like carrot and cress where the internal standard shows no activity. On the other hand, 100 μM treatments of the herbicide maintain the levels of activity for both species of lettuce around 50%, while DHZ (100 μM) has no activity.

Root growth is strongly inhibited by DHZ (1000 μM) in all the tested species (Fig. 2). Lettuce and tomato are similarly inhibited by both treatments; cress is more sensitive to Logran® but the herbicide is still less active than DHZ at this concentration; carrot remains insensitive to herbicide. Treatment with DHZ (100 μM) again changes the situation: Logran® maintains the activity in lettuce, tomato and cress, while DHZ inhibitory effects are lower than Logran® levels in lettuce and tomato, cress is equally inhibited by DHZ and the internal standard. It is also remarkable that though the level of inhibition obtained in tomato fall below that of the herbicide, it is still significant to reach at the 40% value.

A similar situation is observed for the shoot length parameter (Fig. 2). The main difference is the lower sensitivity of tomato to DHZ at 1000 μM, and of cress to Logran® at 100 and 1000 μM, the activity of the herbicide being lower than that showed by DHZ (100 μM).

Among the dicotyledon species tested, cress was most sensitive to DHZ, while all the other species were not sensitive to DHZ at 100 μM, cress growth (root and shoot parameters) is still strongly inhibited. Tomato root length is the other parameter inhibited by DHZ at this concentration.

2.2. Monocotyledon species

The species assayed were onion (*Allium cepa* L. cv. Valenciana), wheat (*Triticum aestivum* L. cv. Cortex), barley (*Hordeum vulgare* L. cv. Wellam) and maize (*Zea mays* L. cv. Oropesa). The situation is similar to that observed for dicotyledon species (Fig. 3): DHZ has a higher level of activity than Logran® at 1000 μM, but its activity is lowered at 100 μM below the levels of the herbicide.

Regarding germination, Logran® showed no relevant activities on onion, or maize, while wheat shows minor effects; barley is strongly affected both by Log-

Table 2
Germination and growth effects of DHZ (**3**) and Logran on different monocotyledoneous plant species[a]

Concentration	DHZ (3)				Logran			
	Allium cepa	*Triticum aestivum*	*Hordeum vulgare*	*Zea mays*	*Allium cepa*	*Triticum aestivum*	*Hordeum vulgare*	*Zea mays*
Germination								
10^{-3} M	−36	−63	−96	−16	−18	−36	−82	−15
10^{-4} M	−3	−1	−11	−4	−4	−14	−58	15
10^{-5} M	−6	3	−5	1	0	−58	−20	−12
10^{-6} M	1	13	−11	−4	29	−30	−2	11
10^{-7} M	−14	−7	−7	−4	22	−9	−9	−8
10^{-8} M	−2	0	−11	−17	−40	0	−16	−2
10^{-9} M	3	5	18	−16	−9	−14	−38	−15
Root length								
10^{-3} M	−90	−82	−40	−50	−80	−28	−54	−81
10^{-4} M	−19	−4	−13	12	−72	−31	−46	−60
10^{-5} M	−12	−1	−25	19	−66	−24	4	−37
10^{-6} M	9	2	−8	8	−63	−5	41	0
10^{-7} M	2	−2	−33	45	−49	−3	42	17
10^{-8} M	14	8	−16	−12	−41	−5	43	31
10^{-9} M	0	−1	9	−17	−25	2	40	34
Shoot length								
10^{-3} M	−79	−55	−15	−19	−66	−26	−55	−71
10^{-4} M	−10	−4	−7	−9	−66	−25	−20	−15
10^{-5} M	−12	2	−14	−5	−61	−26	−13	7
10^{-6} M	−5	−7	−3	−7	−59	0	−1	−9
10^{-7} M	3	0	−12	8	−44	−6	−2	16
10^{-8} M	−6	−6	−13	−16	−42	0	−4	17
10^{-9} M	−1	−4	−13	−16	−21	9	−3	20

[a] Values are presented as percentage differences from the control (e.g., 16% means 116% compared with the control). Values are significantly different from the control with $P > 0.05$ for the Welch's test. a: $P < 0.01$; b: $0.01 < P < 0.05$.

ran® and DHZ (1000 μM). In germination, the higher sensitivity to the treatments was shown by barley, followed by wheat and onion. Maize remained insensitive.

Wheat root length development was more affected by DHZ than Logran®, while onion root inhibition exhibited the same levels for both treatments; barley and maize had slightly lower activities with DHZ (1000 μM) than with the internal standard. However, while the levels of activity of Logran® were still important with the two following dilutions, DHZ (100 μM) showed no activity.

Shoot length is strongly inhibited by DHZ at 1000 μM in onion and wheat, while barley and maize are not affected. Logran® shows no activity with wheat, while onion, barley and maize are strongly inhibited at 1000 μM. A dose-response effect was obtained with the herbicide for all the tested species.

No other phytotoxic carbonyl containing guaianolides have been described yet, but some allelopathic isoguaianolides with an additional carbonyl group have been reported. Ambrosin and its 11,13-dihydro derivative damsin (isolated from *Parthenium hysterophorus*) have been reported to inhibit germination and growth (Kanchan and Jayachandra, 1980); confertiflorin (*Ambrosia confertiflora*) (Fischer and Mabry, 1985) strongly inhibits the germination of *Amaranthus palmeri* (49%, 100 μM) and *A. retroflexus* (21%, 10 μM); and partenin (*P. hysterophorus*) also exerts an inhibitory effect on the germination of *A. palmeri* (40%, 1 μM) and carrot (24%, 100 μM) (Fischer et al., 1989). The effects obtained with DHZ are higher than

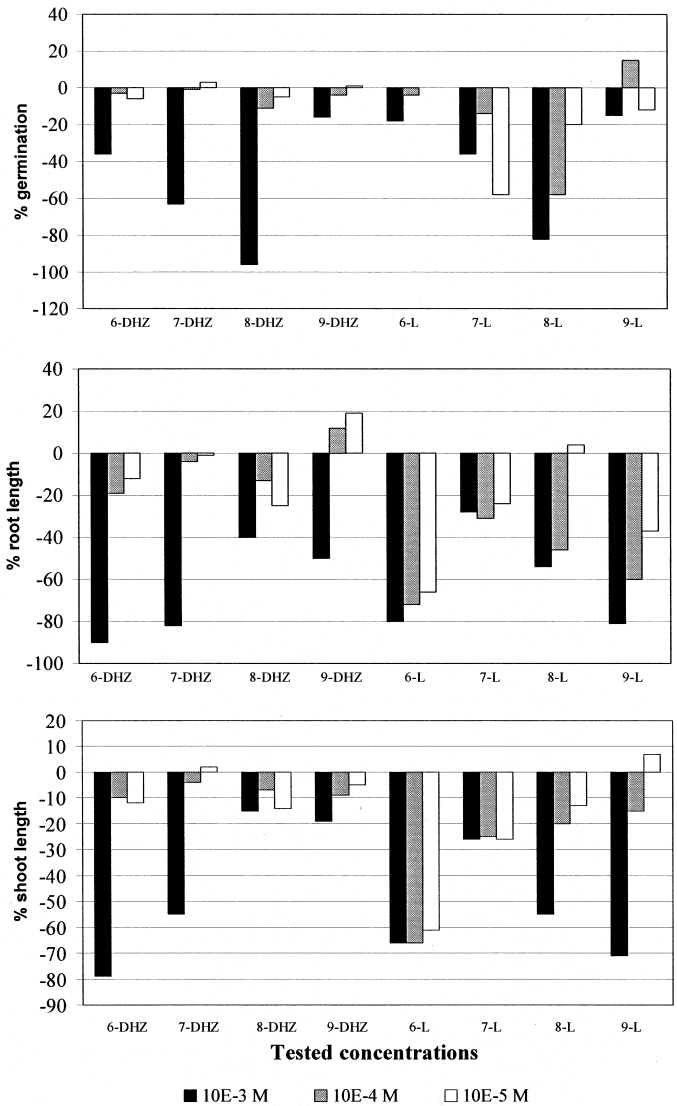

Fig. 3. Selected effects of DHZ and Logran® on germination and growth development. **6**: *Allium cepa* L.; 7: *Triticum aestivum* L.; **8**: *Hordeum vulgare* L.; **9**: *Zea mays* L.

those reported with the above mentioned compounds. DHZ presented a 40% inhibition on the less sensitive carrot, while confertiflorin is not active and partenin is slightly active. Thus, comparison with other similar active structures reveals DHZ as a very active and promising lead product to be developed.

It is also important to note the different profiles of activity showed by DHZ and the internal reference, DHZ presenting a wider spectrum of activity. Though DHZ could not maintain the activity with the dilution at the same level as Logran®, DHZ presented activity at 1000 μM in species where the herbicide showed no activity (tomato, carrot, cress and wheat). It is also noticeable that DHZ can affect some parameters when Logran® is inactive (e.g., carrot germination). This could be indicative of a different mode of action, but due to the nature of this study where only macroscopic parameters, such as germination and growth, are measured, this cannot be assured and needs to be studied.

Based on the results of these experiments, DHZ can be considered as a potent growth regulator, specially for dicotyledon species. Consequently, it can be proposed as a lead compound for further studies in the development of new herbicides, since it presents activity levels which are comparable (or even higher) with those of a commercial herbicide and has an apparently different mode of action. Future research should aim to enhance its activity, to modify the molecule in order to maintain the activity with dilution, and to determine its specific site of action and the processes affected at the molecular level. Another basic aspect is to evaluate the toxicological activity of DHZ, which should be preliminary investigated.

3. Methods and materials

3.1. Selenium dioxide (SeO_2)–terc-butylhydroperoxide (TBHP) oxidation

533 mg of **1** was dissolved in chloroform (0.03 M) and 60 mg of SeO_2 and 2 ml of TBHP (1:0, 5:2) were added while stirring. After 10 min, the reaction mixture was filtered through silica gel and then purified by Column Chromatography (CC) (*n*-hexane:EtOAc, 6:4) to yield 55% of *iso*zaluzanin C (**2**), 5α-hydroxy-dehydrocostuslactone (28%) and 5α-hydroxy-*iso*zaluzanin C (13%).

3.2. Oxidation of isozaluzanin C

260 mg of **2** was dissolved in 25 ml of chloroform and then stirred with 465 mg of PCC (1:2). After 1 h, the reaction mixture was filtered through silica gel and then purified by CC (*n*-hexane:EtOAc, 7:3) to yield dehydrozaluzanin C (53%) and 2,3-dehydro-14-oxo-dehydrocostuslactone (18%).

3.3. Seed germination and growth bioassays

Seeds of *L. sativa* cv. nigra and cv. roman, *L. esculentum* L. cv. Tres Cantos, *D. carota* L. cv. Coral, *L. sativum* L., *A. cepa* L. cv. Valenciana, and *Z. mays* L. cv. Oropesa were obtained from FITÓ, S.L. (Barcelona, Spain). *Hordeum vulgare* L. cv. Wellam and *T. aestivum* L. cv. Cortex were obtained from Rancho La Merced, Junta de Andalucía, Jerez, Cádiz, Spain. All undersized or damaged seeds were discarded, and the assayed seeds of uniform size were selected. Bioassays were carried out in plastic Petri dishes of 9 cm diameter lined with Whatman No. 1 filter paper.

Germination and growth bioassays were as follows: *L. sativum*, 25 seeds per dish, 5 ml test solution, 4 days dark, 25°C and four replicates of each concentration; *L. sativa* cv. roman and cv. four seasons, *L. esculentum*, and *A. cepa*, 25 seeds per dish, 5 ml test solution, 5 days dark, 25°C and four replicates of each concentration; *D. carota*, 25 seeds per dish, 5 ml test solution, 7 days dark, 25°C and four replicates of each concentration; *T. aestivum*, *H. vulgare*, and *Z. mays*, 10 seeds per dish, 5 ml test solution, 5 days dark, 25°C and 10 replicates of each concentration (Macías et al., 2000).

Test solutions (1000 μM) were prepared using H_2O–MES (2-[*N*-morpholino]ethanesulfonic acid, 10 mM), and the other solutions were obtained by dilution. Parallel controls were performed. All pH values were adjusted to 6.0 before bioassay with MES. Osmotic pressure values were measured on a vapor pressure osmometer (WESCOR 5500) and ranged between 30 and 38 mOsmolar.

Data is presented as percentage differences from control in graphics and tables (Figs. 2 and 3 and Tables 1 and 2). Thus, zero represents the control; positive values represent stimulation of the studied parameter and negative values represent inhibition.

3.4. Statistical treatment

Germination and root and shoot length values were tested by the Welch's test; differences between test solutions and controls were significant ($P = 0.01$) (Tables 1 and 2).

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