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Anti-insect Activity of Bufadienolides from *Urginea maritima*

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Squill [*Urginea maritima* (L.) Baker, Liliaceae] is a native medicinal and ornamental plant from the Mediterranean area. The bulbs were an ancient source of rodenticide products replaced later on by warfarin and modern anticoagulant raticides. Since rats have developed resistance to such products there is now renewed interest in the species.

In the 1950s, attempts were made in the United States to introduce this new crop for arid lands. A collection of bulbs are still maintained at Gentry Experimental Farm, Murrieta, California. Some limitations concerning variability in toxicity were previously discussed (Verbiscar et al. 1986a; Gentry et al. 1987). Scilliroside, a high toxicity bufadienolide glycoside, is the main active principle. Other glycosides and aglycones have also been isolated from the bulb (Verbiscar et al. 1986b).

We tested this plant in our research program to screen botanicals for insecticidal activity at "Centro de Investigación y Desarrollo Agroalimentario" in Murcia, Spain. In Spain squill is a wild plant of coastal areas (Fig. 1). Ethanolic extracts of bulbs have been active against stored product pests (Pascual-Villalobos and Fernández 1999), although such anti-insect effects could not be attributed to specific compounds in that study. The objective of this work was to test pure bufadienolides isolated from *U. maritima.*

METHODOLOGY

Compounds

Five different bufadienolides (proscillaridin A, scillaren A, scillirosid, gammabufotalin, and scillirosidin) were obtained as pure substances (see previous work of Krenn et al. 1994) from *U. maritima* bulbs and they were provided by B. Kopp from the Institute of Pharmacognosy in Vienna (Austria).

Insects

The effects of compounds were studied on the stored product pest *Tribolium castaneum* (Coleoptera: Tenebrionidae). Larvae were obtained from same age cultures kept on an artificial diet of white wheat flour and beer yeast (95:5) at a constant temperature of 30°C in the dark.

Fig. 1. Wild *Urginea maritima* plants in "La Unión," Murcia, Spain.

Bioassays

Topical Application Bioassay. Substances (dissolved in acetone) were applied topically (using a micropipete) to 25 day old larvae at doses of 10, 20, 30, and 40 µg/insect. Larvae were placed singly inside glass tubes and kept at a constant temperature of 30°C in darkness; 24 replications per treatment. Mortality (%) was recorded after one and seven days. If a response to increasing doses of the products was obtained, probit analyses were performed and LD_{50} calculated when the fit was good.

Insect Growth Inhibition and Fertility Bioassay. Substances were incorporated into the standard insect diet at a dose of 2%. The experimental unit was a 4 ml glass vial with 100 mg of diet and one newly hatched larvae. Twelve replications were set up for each compound. The experiment was kept in the dark at a constant temperature of 30ºC. Length (mm) and mortality (%) of larvae were measured after 14 days. Emerged adults from each treatment were paired during 4 days on the same diet and the number of fertile laid eggs were counted. The comparison between treatments and control was done using the non parametric Mann-Whitney U test.

RESULTS

Proscillaridin A, scilliroside, and scillirosidin were active by topical application (Table 1). The aglycone, scillirosidin was more lethal, causing over 50% mortality in *Tribolium* larvae at 10 µg/insect, than its glycoside scilliroside. Scillaren A and gammabufotalin were less toxic on insects and a response to increasing doses was not obtained. Only data from scilliroside applications fitted to the probit model being LD_{50} of 25.5 and 17.1 µg/insect for mortality after one and seven days respectively.

Intake of bufadienolides at 2% in the diet caused a statistically significant larvae growth inhibition and adult fertility reduction (Table 2). Ingestion of scillirosidin or proscillaridin A was most deleterious (larvae of 4.36 mm and 4.73 mm respectively in comparison with 6.9 mm of control); in these treatments a delay to reach pupae stage from 21 to 29.2 days was also obtained. The number of eggs laid per female was clearly reduced if the compounds were added to the diet. Again, scillirosidin and proscillaridin A were more active by completely inhibiting the fertility (Table 2). Also, scillirosidin, the aglycone appears to be more active than the glycoside (scilliroside).

Table 1. Mortality[z] (%) of *T. castaneum* larvae (25 day old) caused by topical application of bufadienolides (n=24).

Compound	Days after application	Dose (µg/larvae) 10	20	30	40	Probit fit χ^2(df=2)		LD_{50}
Proscillaridin A	1	41.7	29.2	41.7	70.8	23.9	poor	--
	7	45.8	50	87.5	91.7	17.3	poor	
Scilliroside	1	20.8	37.5	66.7	75	2.2	good	25.5
	7	29.2	62.5	83.3	83.3	3.0	good	17.1
Scillirosidin	1	54.2	41.7	58.3	95.8	47.1	poor	
	7	52.3	45.8	79.2	100	46.3	poor	
Scillaren A	1	33.3	25	4.2	41.7	--		--
	7	41.7	33.3	33.3	50	--		--
Gammabufotalin	1	20.8	25	41.7	29.2	--		--
	7	20.8	29.2	50	37.5	--		--

[z]Mortality of control (application of the solvent, acetone) was 0% at day 1 and 8.3% at day 7.

Table 2. Insect growth inhibition and influence on fertility of bufadienolide intake (n=12).

Compound[z]	Larvae length[y] after 14 days (mm)	Mortality (%)	Fertility[yx] (no. eggs/pair)
Proscillaridin A	4.73 ***	25.0	0.0 ***
Scilliroside	6.00 ***	16.7	11.0 ***
Scillirosidin	4.36 ***	8.3	0.2 ***
Scillaren A	5.42 ***	8.3	6.3 ***
Gammabufotalin	5.96 *	25.0	5.8 ***
Control	6.9	0.0	26.1 ***

[z]Mixed at 2% in the diet.
[y]Mean values and significance of Mann-Whitney U test for comparison between treatment and control (ns=non significant, * $0.01<p<0.05$, *** $p<0.001$).
[x]During 4 days.

We report for the first time that *Urginea maritima* bufadienolides induce anti-insect effects on *Tribolium castaneum*. This suggests that squill should be investigated for activity against other insects and pests.

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