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ABSTRACT: Red squill has been used in rodent control for several hundred years but in the United States its use has decreased since the 1950s. However, there is now a recognized need for rodenticides with different kinds of toxic activity. Red squill is being investigated as an acute rodenticide and an economic crop for the southwestern states. Clones from a prior USDA collection have been assayed by high-performance liquid chromatography and selections are being propagated in California and Arizona. The major toxicant, scilliroside, is relatively fast acting, causing convulsions and death to rats and mice. This glycoside is also strongly emetic to humans, cats and dogs, affording a safety factor uncommon to high-toxicity rodenticides. Our chemical, processing, agronomic, and toxicological studies are a technical basis for further developing this potentially superior rodenticide.

INTRODUCTION

Red squill, Urginea maritima, is a large onion-like plant that grows wild in the coastal lands around the Mediterranean Sea (Crabtree 1947). The bulb extracts and dried powders are highly toxic and have been used for rodent control since the 13th century (Chitty 1954, Marsh and Howard 1975). Although 1.36 million pounds of red squill powder were imported into the U.S. in 1944, representing 7.5 million pounds of fresh bulbs (Markwood 1957), this declined substantially in the 1950s and has ceased in the current decade. There is a need for rodenticides with different kinds of toxicity other than anticoagulent activity. This has prompted our investigation of red squill.

Red squill, a member of the Liliaceae family, grows through autumn, winter and spring when it is moist and cool, looking like a field of corn, Figure 1. In late May and June it stops growing completely, the leaves dry up, fall off, and the bulb is dormant for the hot dry summer months. The first flowering occurs in the sixth year as tall attractive spikes rise from the bare bulbs during August and September. With rain or irrigation the leaves reappear in October and the bulb begins to grow again. The plant is harvested after 5 or 6 years of growth, when the individual bulbs weigh anywhere from 4 to 3 lbs. The weight of the bulb depends on the individual clone and cultivation methods.

Figure 1. Red squill growing in the field. One bulb shown on top of the ground.

In 1944, a collection of 1500 red squill bulbs were brought to North America by the U.S. Foreign Economic Administration. In 1946, after an initial planting in Mexico by Charles Gilly, these bulbs were moved to the USDA Torrey Pines Experimental Station, La Jolla, Southern California. Here they were given clone numbers and were propagated vegetatively by wedge sections. Each new bulblet was then a replicate of every other member of the clone. Seed propagation was also tried but this is much slower and gives bulbs with mixed genetics and varying toxicity. Samples of the clones were tested for toxicity at the Fish and Wildlife Service, Denver Wildlife Research Center, using a rat bioassay. A goal was to develop genetic strains with high toxicity. In 1960, the USDA abandoned the red squill project

and distributed the higher toxicity bulbs to several parties. Of the four recipients, the Gentry Experimental Farm was the only one to preserve, maintain and propagate most of the clones received. These clones are the basis for our current studies.

In 1980, Anver Bioscience Design received a grant from the National Science Foundation to begin to establish a technical basis for developing red squill as a rodenticide in the U.S. A final report was completed on initial studies (Verbiscar et al. 1984), and we are now developing process and formulation procedures (Verbiscar et al. 1985). More detailed articles have been prepared on composition, analysis, toxicity (Verbiscar et al. 1986), and domestication of red squill (Gentry et al. 1986).

PROPAGATION AND PRODUCTION

Red squill has never before been cultivated in the U.S. or abroad to our knowledge. Red squill grows without irrigation on the dry farm grainlands of southern California where annual rainfall is 12 to 20 inches occurring principally from autumn to spring. Trial plantings in Arizona have been successful in well-drained soils, although some irrigation may be desirable for improved growth in certain semi-arid winter areas. Irrigation and fertilization increase bulb growth rate in a predictable manner. The plant withstands temperature extremes of 20 to 115°F. without injury. This affords a wide range of areas for production of red squill in the southwestern United States.

The plant is propagated by wedge sections or scale sections containing part of the root pad. A larger bulb can be easily cut into 12 wedge sections which are planted in May or June. Scale sections can also be planted; and even though 40 to 70 propagules can be obtained from a single bulb, these grow more slowly than the wedge sections. Propagation from seeds has several disadvantages. Growth from seeds is very slow, and the toxicity and mature bulb size of the seedling propagule will be variable. The most desirable strain will be fast growing with a high percent scilliroside.

About 12,500 wedge section propagules can be planted per acre. This planting would require about 1040 bulbs weighing a total of about 4000 lbs. In 5 to 6 years each of these bulbs will weigh about 5 to 6 lbs., or a total of 60,000 to 70,000 lbs. At 18% dry weight this is equivalent to 12,000 lbs of red squill powder. At 0.10% scilliroside this is enough dry powder to kill about 5 million male rats, or 15 million female rats. Of course, this assumes 100% efficiency in the use of the bait. With the rodents adeptness at learning to avoid toxic baits, with subsequent bait loss, this is not a practical assumption. However, the potential of this product as a rodenticide is apparent. At present there are 12 acres under cultivation at the Gentry Experimental Farm, mainly for experimental purposes. There seems to be a need for several thousand acres of red squill for the commercial market.

ASSAY METHODS

The major toxicant in red squill was found to be scilliroside, (Stoll and Renz 1942) Figure 2. We confirmed this using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) to identify the scilla glycosides and aglycones in extracts of the plant parts. TLC was used for qualitative identification of the compounds and HPLC provided a quantitative assay. All of the assays in this report are on powder samples of the bulb and other plant parts where the moisture level has been brought to 5 to 8% by drying in an oven at 65°C. Experiments comparing these dry powder extract assays with assays of the fresh bulbs (82% water) showed that short-term drying had little or no effect on the scilliroside. Although scilliroside is the major toxicant in red squill (Stoll and Renz 1942), varieties contain lesser amounts of other scilla glycosides including sciilaren A, scilliglaucoside, scillirubroside and scillarenin β-D-glucoside (Stoll et al. 1943, Wartburg and Renz 1959, Wartburg 1966). We also found small amounts of scillirosidin, desacetylscilliroside and a new glucosyl scilliroside in one clone (Verbiscar et al. 1986).

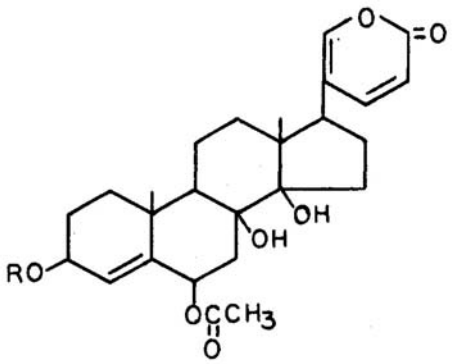

Figure 2. Scilliroside R = Glucose
Scillirosidin R = H

Our HPLC system included two 3.2 X 250 mm Lichrosorb S160 5u columns protected by a 3.2 X 40 mm Porasil A precolumn. Acetonitrile: water (97:3) was used as an eluant at a flow rate of 0.9 ml/min., with the variable wavelength detector set at 295-300nm. Peak areas were quantitated with an Integrating data processor. As an example, in clone 333-28 two scilla glycosides were detected including scilliroside at 12 min. and desacetylscilliroside at 16.5 min. A phenolic glucoside occurred as an interfering peak at 10.1 min. in all of the bulb extracts and had to be removed by precipitation with lead acetate prior to HPLC assay.

The purpose in developing a chemical analysis was to get away from the expensive rat bioassay. We found that wedge sections of the bulb could be taken for assay. These consist in a vertical slice including skin, scales, pad and core. This representative sampling conserved bulb tissue for propagation of high scilliroside content clones. We are now able to evaluate and select clones for propagation based on their scilliroside content and growth rate.

SCILLIROSIDE CONTENT OF CLONES AND PLANT PARTS

One of the problems encountered by importers of red squill powders in the past was the variation in toxicity as determined in a rat bioassay. This variation in toxicity can now be understood in terms of a number of factors.

Table 1 shows the scilliroside content of 10 clones among 60 that we assayed. In this group the scilliroside content of the dry powders ranges from 0.03 to 0.26%. Among the 60 clones assayed the range for scilliroside was 0.01 to 0.53%. The age of the bulb and the irrigation and fertilization conditions had no effect on scilliroside percent, although more total scilliroside is present in larger bulbs, of course. In one experiment the scilliroside content of 42 bulbs of the same age, 22 years, grown from seeds from a single clone #333 were assayed. Scilliroside content ranged from a low of 0.01% to a high of 0.35% with an average of 0.10%. Three of these clones are noted in Table 1. This clearly demonstrated the genetic basis for scilliroside level in a bulb. The differences in toxicity among clones may partially explain some of the great differences in toxicity for imported red squill dry powders which derived from wild plants. The wild plants propagate from windblown seeds. The weight of bulbs from different clones also varies (Gentry et al. 1986). The total scilliroside content of a bulb based on percent and bulb size is the best basis for selecting clones for propagation.

Table 1. Scilliroside content of selected clones.

Clone-bulb	Plant part	Plant age, yrs.	Scilliroside % in dry powders
297	wedge	7	0.13
324	wedge	6	0.03
638-B	wedge	8	0.11
843-A	wedge	10	0.10
841-H	wedge, 5 bulbs	7	0.20
868-C	wedge, 5 bulbs	unknown	0.14
871-B	whole bulb	3	0.15
333-12	wedge	22	0.03
333-28	wedge	22	0.26
333-42	wedge	22	0.13

Table 2 shows scilliroside occurring in all of the plant parts. Fresh leaves, skins and flowering stalks have low scilliroside levels, which are even lower when dry. It seems that the scilliroside as well as the nutrients in the leaves transport down into the bulb just prior to summer dormancy. This is the probable reason why summer has been the traditional time to harvest red squill bulbs. The core, pad and roots contain the highest quantity of scilliroside and should remain with the bulbs during harvesting and processing. One dry shriveled planting wedge attached to a healthy growing bulb had an exceedingly high scilliroside content, see 466-G at 1.25% in Table 2.

Table 2. Scilliroside content of plant parts.

Clone-bulb	Plant part	Plant age, yrs.	Scilliroside % in dry powders
466-K	wedge, 5 bulbs	5	0.09
466-B	scales	7	0.04
"	flowering stalk	"	0.04
"	red skins	"	0.05
"	core	"	0.17
"	roots	"	0.22
466-D	fresh leaves	8	0.03
466-E	dry flower stalk	>6	<0.01
466-G	dry planting wedge	unknown	1.25

Table 3 shows an interesting phenomena that can influence harvest time. After about 6 years red squill shoots up a thick flower spike as tall as 6 feet from the leafless plant. The flower is attractive horticulturally and is sold in the California market. The stalk contains low scilliroside on a percent basis. However, during this flowering period of about 2 to 4 weeks the bulbs themselves show a very high percent scilliroside. This is apparently due to the fact that the fast-growing stalk utilized nutrients from the bulb leaving the scilliroside behind. The flowering period is, therefore, probably the most economically productive time for harvesting both the flower and bulb, each of which has commercial value.

Table 3. Scilliroside assays of flowering bulbs.

	Scilliroside, %	
Clone	Prior range	Flowering period*
466	0.08 - 0.12	0.30
841	0.04 - 0.28	0.27
843	0.07 - 0.10	0.21
868	0.08 - 0.53	0.50

*Composite wedge sections of 5 bulbs, >6 years old, September 1984.

Table 4 demonstrates that red squill powders stored in bottles or in open beakers generally had lower scilliroside levels. Degradation of scilliroside can occur on storage and when exposed to the atmosphere. Of the five powders assayed only #1372 maintained its high scilliroside content of 0.10%. The other four lost 50 to 70% of their scilliroside to some form of decomposition. One of these degradation reactions could be hydrolysis of the 6-acetyl group which occurs readily. The resulting 60 desacetylscilliroside has low toxicity. Microscopic examination of dry red squill powders prepared by hammer mill comminution showed they were not homogeneous, with large variations in particle size. The small hard granules may protect occluded scilliroside against degradation by moisture or oxygen in the air. Cruder grinding will provide larger granules and could improve stability of the product. The variability in size of the granule may also affect formulation requirements and bioavailability of the toxicants in the rodent stomach.

Table 4. Scilliroside content of stored powders.

	Scilliroside, %		
Clone-bulb	1st Assay	2nd Assay†	3rd Assay††
466-A	0.09*	0.05	0.06
871-A	0.10*	0.10	0.06
1372	0.10*	0.09	0.10
333-4	0.16**	0.07	0.06
333-10	0.17**	0.05	0.05

* June 1, 1980; ** July 1, 1981; † December 9, 1982; †† March 31, 1983, after 110 days in open beakers.

TOXICITY TO RATS

Toxicity testing was carried out at the Toxicology Program, Northeastern University, under the direction of Jeffrey B. Blumberg and Robert A. Schatz. Mature Charles River rats were housed by sex, three to a cage, and fasted from food 10 to 14 hours before dosing. For the lethal dose (LD) studies the red squill preparations were suspended in 0.25% agar and homogenized to assure optimal uniformity and bioavailability. Gavage volumes of 10 ml/kg were administered orally at several dose levels and the rats were observed for 48 hours. Animals surviving after 2 days were monitored for another 12 days. The dose/toxicity curve for the lethal preparations was very steep, and for some preparations the LD_{100} values were not much higher than the LD_{50} values, Table 5.

Table 5. Toxicity of red squill preparations to Charles River rats.

Preparation	Scilla cmpd., %	LD_{50}, mg/kg	Ratio male/female
Scilliroside, prep.	82		3.8
Male		5.3	
Female		1.4	
Scillirosidin	100		
Male		4.2	
Desacetylscillirosidin	100		
Female		>5.0	
Clone #1372 powder	0.10*		3.5
Male		436	
Female		125	
Clone #871 powder	0.10*		2.7
Male		446	
Female		165	

*Scilliroside

The data show that scilliroside and red squill powders are 3 to 4 times more toxic to female rats than to males. Higher toxicity to female rats has previously been noted by several groups (Winton 1927, Stoll and Renz 1942, Dybing et al. 1952). Rothlin and Schalch (1952) using Glaxo rats found LD_{50} values of 1.35 mg/kg for males and 0.43 mg/kg for females. The male/female toxicity ratio of 3.1 for Glaxo rats approximates the toxicity ratio for our Charles River rats. Rothlin and Schalch also reported the toxicity of scilliroside to 11 other laboratory animals and wild rodents. The variations in toxicity were very great, ranging from 0.17 mg/kg for a grey house mouse, 6 mg/kg for cats, to 37 mg/kg for a field mouse. Only a few species in addition to the Glaxo rats demonstrated a sex difference in response to scilliroside. Large differences in toxicity of red squill powder among chickens, rabbits and guinea pigs has been noted by others (Lubitz and Fellers 1941).

Lethality is reduced substantially when the 6-acetyl group is removed as in 6-desacetylscillirosidin. When only the glucose is removed, the aglycone scillirosidin shows lethality equal to or greater than scilliroside on a molar basis (Rothlin and Schalch 1952). The data in our continuing studies indicate that scillirosidin is the active metabolite of scilliroside. Although scilliroside itself may have cardiovascular activity, it is unlikely that it will transport into the brain to cause convulsions, paralysis and death typical of red squill.

It seems likely that a digestive enzyme or some microorganism in the gastrointestinal tract produces a β-glucosidase that can split the glucose from scilliroside releasing scillirosidin. Many animals and organisms contain such an enzyme used for splitting sucrose and other carbohydrates as sources of carbon and energy. Differences in gut bacteria in different species and in individual animals may partly explain the great differences in lethality for scilliroside and red squill preparations. Cats, for example, show large differences in their response to scilliroside as an emetic, ranging from 0.5 to 7 hours (Gold et al. 1947, 1950). The minimum dose for emesis to occur in cats was found to be 0.05 mg/kg, compared to a lethal dose of about 6 mg/kg. Our current work with pigeons found the aglycone scillirosidin administered orally in diets, evoked an emetic response, generally within 10 minutes (Verbiscar and Marsh 1986).

Red squill preparations are emetic to humans, and this is the principal safety factor in red squill (Belt 1944). A young woman reportedly ingested 1.5 teaspoons of red squill powder as an emetic. Assuming a scilliroside content of 0.1% in the dry powder, this dose is equivalent to about 0.1 mg/kg of scilliroside. The woman experienced depression, dizziness, nightmare, diarrhea and vomiting but no pain, and recovered on day 3. Another person ingested 1 gram of red squill with no effects. He then took 2.6 grams which caused nausea and vomiting in 15 minutes but nothing else. Assuming 0.1% scilliroside level and a 70-kg person, the dose of scilliroside in the latter case is about 0.04 mg/kg. These emetic doses are well below the toxic doses, and encourage the further development of red squill as a superior rodenticide.

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