The Fortification of Red Squill (*Urginea maritima*) by Means of an Extract of Red Squill*

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Red squill powder of sufficient toxicity is an ideal poison for use by the general public for the control of rats. Due to its strong emetic action, the danger to other animals including humans is very slight, while toxic red squill properly handled will remove or reduce to a minimum a rat infestation. Since commercial red squill powder is prepared merely by drying and grinding the whole bulb, which may come from a number of sources, it is hard to obtain a powder of uniform toxicity. This difficulty has increased in recent years until at the present time it is almost impossible to procure on the market a satisfactory product of this type. Squill powders bioassayed within the last two years in this laboratory have varied in killing power from 400 mg./Kg. to approximately 3000 mg./Kg. The price has never been an index of toxicity and red squill powder practically useless for rat control has often been more expensive than better material. Consequently, the desirability of having available for use a standardized red squill powder of sufficient potency to be highly effective in rat-control work is apparent. This led to the development of a method of "fortification" by which weaker squills can be made more highly toxic and still retain the safety emetic factor possessed by the original powder. Recently also, the problem of importing red squill from the Mediterranean area where it grows in the wild state has become increasingly difficult, and the necessity for utilizing squill stock of low toxicity has made the "fortification" process imperative.

EXPERIMENTAL

The Extraction Method and Its Operation.—The extraction process is carried out in three stages and takes place in three crocks supplied with drain spigots at the bottom of wells which consist of U-shaped sheet metal partitions 6 in. wide and 4 in. deep with metal screens along the open side. Each screen has a removable cover, and the well extends from the bottom to the top of the crock (Fig. 1).

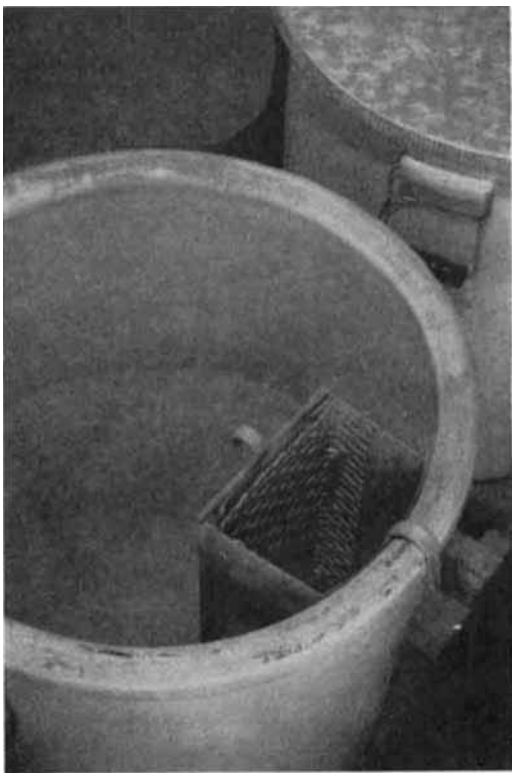

Figure 1.

The crocks are mounted on rollers so that they may be shifted easily from one stage to another when the process is once under way. Fresh solvent (80% ethyl alcohol) is introduced at the left end of the series of crocks, and raw squill powder at the right end of the system. To start the process, crock No. 1 is charged with squill powder and covered with 80% ethyl alcohol, using approximately $^1/_3$ of a gallon per pound of powder. The contents of the crock are stirred and allowed to stand over night, at which time the extract is drained and placed in crock No. 2 on a fresh charge of squill which has been moistened with fresh solvent. Enough fresh solvent is added to crock No. 1 to bring it to its original volume, and the contents of both crocks are stirred and allowed to stand over night. This procedure is repeated on the following day, at which

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time crock No. 3 is added to the line when the extract from crock No. 2 is placed on a fresh charge of squill previously moistened with solvent. Also at this time the extract from crock No. 1 is placed on the drained marc in crock No. 2, and fresh solvent added to the drained marc in crock No. 1.

After the three-stage process has been put in operation through the several steps just outlined, our pilot plant functions as follows: three 30-gal. crocks have been, in turn, charged with 60 lbs. of dry red squill powder and covered with 20 gal. of 80% alcohol. At 9 a. m. all the crocks are drained from the bottom. The extract from crock No. 2 is placed upon the marc in crock No. 3, and the extract from crock No. 1 is placed on the marc in crock No. 2. The contents of both crocks are stirred and allowed to stand until 4 p. m. of the same day. The marc from crock No. 1 is taken out and put through a press to remove all of the residual extract possible, and the exhausted marc from which the rat-killing principle has been extracted is discarded. The extract expressed from the marc from crock No. 1, together with that drained from crock No. 3, is concentrated for use in subsequent "fortification" procedures. This is accomplished by removing the alcohol under reduced pressure in a recovery type still. A fresh charge of 60 lbs. of red squill powder is then placed in crock No. 1, moistened with 5 gal. of solvent, where it remains in this condition until 4 p. m., and the crock moved to the original position of crock No. 3 in the system. Crock No. 3 goes to stage 2, while crock No. 2 moves to stage 1. At 4 p. m. the extracts in crocks No. 2 and No. 3 are drained and moved to the right on the production line, the extract from No. 3 being placed on the fresh charge of squill in crock No. 1, and the extract from crock No. 2 being transferred into crock No. 3. Fresh 80% alcohol is placed on the drained marc in crock No. 2. The contents of all crocks are stirred and allowed to remain without further treatment until the next morning when the whole procedure is repeated. As described, three days are required to set the three-stage process into operation, after which one fresh batch of red squill is added, one exhausted marc discarded and one distillation and "fortification" carried out daily. At this stage of the process each fresh batch of powder is extracted five times and remains under extraction for a total of 65 hrs.

The rat-killing principle of red squill which has been concentrated in the vacuum still is added to a calculated amount of raw powder to be "fortified," thoroughly mixed, placed in trays with perforated bottoms and dried in an oven at 80° C. When thoroughly dry it is ground to a 20-mesh powder in a hammer mill. The capacity of this pilot plant is 60 lbs. of raw red squill powder extracted per day, and the daily production may be either 6 lbs. of the dried extract or as much as 66 lbs. of a red squill powder of a 1:1 "fortification," which is made by adding the extract from 60 lbs. of squill to 60 lbs. of the original red squill powder.

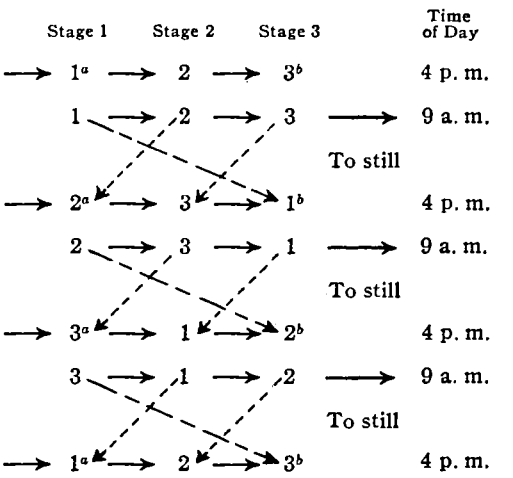

Fig. 2.—Diagram of the Extraction Process in Operation.

1, 2, 3 Crock numbers.
——→ Solvent movement.
- - -→ Movement of crocks with contents.
- - -→ Movement of empty crocks.

[a] Fresh solvent added.
[b] Fresh charge of squill added.

Advantages of the Method.—The method consists of a multiple extraction with 80% alcohol (in the case described, five extractions) of a dry red squill powder. The process incorporates three well-known methods of drug extraction—agitation, maceration and percolation. Subsequently the rat-killing principle is concentrated under reduced pressure and the recovered alcohol is used again in the process. This extraction is carried out in a counter-current system whereby the solvent becomes saturated with inert material in the first stage of the process, and in the following stages becomes increasingly potent with respect to the rat-killing principle, thus making the concentrated dry extract many times more toxic on a weight basis than can be obtained by a series of separate extractions of a single lot of squill. By this multiple extraction and counter-current system, with subsequent evaporation and concentration, a dried product has been prepared which killed male rats at 125 mg./Kg., when the powder from which it was obtained required 2500 mg./Kg. to kill. This procedure is in contrast with the methods of extracting a single lot of red squill powder the same number of times with fresh solvent, which produces a concentrate having approximately twice the killing power of the powder from which it was prepared.

Another departure from previous work on methods of increasing the potency of squill is the way in which the powder is "fortified" with the concentrated material of the extract. Early attempts along this line involved the complete drying of the squill concentrate and the addition of the dried and powdered material to the raw powder for the purpose of increasing its toxicity. This proved to be an expensive method due to the difficulties involved in the drying of the gummy concentrate, which is

produced in quantity when squill is extracted repeatedly with fresh solvent. Our system of handling this phase of the problem is to concentrate the extracts to a thick syrup, dissolve in water and blend this mixture with the amount of raw red squill powder needed for a desired degree of "fortification." The "fortified" squill is then dried (only simple drying is involved here) in an oven at 80° C. and ground in a hammer mill to a 20-mesh powder. This yields a uniform product resembling commercial type red squill powder in both appearance and physical properties.

By means of this process it is possible to "fortify" red squill powder to a desired toxicity (within limits), utilizing squill stocks now available, and still retain the advantages of safety which this material possesses for rat control work. The product yielded by this method has proved to be equal in efficiency and safety to the untreated red squill powder of a comparable toxicity. This system of "fortification" may be used for either large or small scale operation, providing the necessary adaptation of commercial scale equipment to the processes involved is made.

TABLE I

Bioassay of Squill Powder before Treatment, Lethal Dose in Mg./Kg. for Male Rats	Amount of Squill in Lbs., the Extract from Which Is Added to 1 Lb. of the Same Powder	Degree of Fortification	Theoretical Potency of the Fortified Powder with Respect to the Original Powder	Bioassay of the Product of Fortification, Lethal Dose in Mg./Kg. for Male Rats
2000	2	2:1	3	600
	4	4:1	5	400
	8	8:1	9	200
	9	9:1	10	200
2000	2	2:1	3	500
1500	2	2:1	3	400
1000	1	1:1	2	400
500	1	1:1	2	200
	6	6:1	7	150

Degree of Fortification.—A red squill powder which, on bioassay, kills male rats at from 200 mg./Kg. to 400 mg./Kg. is an effective toxic agent for field use in rat control. The production of a "fortified" red squill powder killing in this range has been made the objective of this investigation. A squill of this toxicity can be used in relatively less quantity in rat baits, a fact which increases the efficiency of the bait. This also helps to overcome the aversion shown by rats to food containing a high percentage of squill powder—an important factor in squill usage. The red squill powder which is to be "fortified" is first bioassayed, using male rats, to determine its potency which in turn indicates the degree of "fortification" necessary to make it a useful product. The squill is then processed on this basis. Table I shows the results of "fortification" carried out with five different lots of red squill powder.

DISCUSSION

The "fortified" powder (Table I) in the lower degrees of "fortification," was found to possess a higher degree of toxicity than could be accounted for by simple summation of the amounts of the toxic principle (based on the bioassay of the original powder) contained in the amount of squill represented by the final product. The same held true for the squill which, untreated, killed male rats at 500 mg./Kg. However, at a 6:1 degree of "fortification" the toxicity of the resulting product was only 150 mg./Kg., which indicates that another step is needed in the process to purify the extract before addition to the powder in order to attain a toxicity of seven times that of the starting material. Since a 200 mg./Kg. squill can be produced by a single extract, the method has not been designed to include further purification. Studies with squills of variable particle size have proved 20-mesh powder to be more satisfactory for use in this process than the finer powders, although 40-mesh powders have been successfully treated. Utilizing this process of "fortification" more than 6000 lbs. of fortified squill powder have been prepared from commercially available powder which varied widely in toxicity and particle size.

CONCLUSION

1. An efficient method for the "fortification" of red squill powder, using the countercurrent principle of extraction, has been developed. This makes it possible to increase the potency of weak red squill powder to the point where it can be used efficiently in rat-control operations.

2. "Fortified" squill retains all of the safety factors inherent in red squill powder.

3. The solvent is recovered and used again in the process.

4. The method may easily be adapted to large or small scale operations.