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Preparation and properties of red squill extracts

Robert Edward Buck
University of Massachusetts Amherst

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I. INTRODUCTION AND REVIEW OF LITERATURE

The rat is the world's most destructive pest, and is probably one of man's worst enemies in the animal world. It has been estimated that these pests effect approximately two hundred millions of dollars of damage yearly. This great loss includes not merely destruction of food and property, but also losses due to fires started, buildings undermined and chickens killed. Also these rodents are instrumental in the spread of disease, such as the plague, and thus are a menace to the health of the population. Although many thousands of dollars have been spent in the attempt to eradicate these pests, they are present most everywhere.

The most effective methods of rat control are (8): rat-proofing of all buildings, preventing the animals from securing food and shelter, poisoning, and trapping. The rat-proofing of all buildings is the fundamental step after which those rats which are present within can be exterminated and means taken for the prevention of the entrance of more of the pests. Although measures for the permanent riddance of rats should be taken wherever practicable, they usually must be combined with rat destruction, and in many places continual rat

killing is the only practical method of control. The most effective means of destroying rats is by poisoning.

The principal requirements for an ideal rat poison are safety, effectiveness and economy. The rat poisons generally used a few years ago were arsenic, barium carbonate, phosphorus, thallium, and strychnine. These poisons possess toxic properties which not only destroy the rats but also seriously menace the safety of not only wild and domestic animals, but also that of human beings. These poisons have declined in use and have largely been replaced by red squill.

1. Red Squill as a Raticide

Red squill is comparatively safe and might be considered as the ideal rat poison. It has the distinct advantage of being relatively harmless to human beings and other animals. This is partly due to its acrid taste, which is objectionable to most animals, and also to the fact that it usually acts as an emetic when taken in dangerous quantities. Rats, however, usually take it readily and cannot vomit, so that red squill is unique in that it is a poison specific to rats.

Red squill belongs to the genus Urginea, and botanically is known as Urginea maritima and also as

U. scilla (11). It is also called scilla or sea-onion and is a perennial plant belonging to the lily family, and bears a flowering stem and leaves at different periods of the year. It grows wild in southern Italy, Sicily, Sardinia, and other countries bordering on the Mediterranean Sea. The onion-like bulbs are pear-shaped, from 15 to 30 centimeters in diameter, and weighing from 300 to 2000 grams. The bulbs are usually gathered during the dormant period in the summer and in the early fall. The scilla is a slow growing plant usually taking from 5 to 7 years to reach its maximum size. The bulb is composed of closely overlapping fleshy scales. The outer scales are dry, brittle, and reddish brown; the inner scales vary from a light yellowish to a deep mahogany color, while the central part of the bulb or core is usually white.

There are two commercial varieties of squill - white and red squill. The greatest difference is the presence of pigment cells in the red squill while these cells are absent in the white squill. White squill is used in human medicine as a heart tonic, emetic, diuretic, and nauseant expectorant. Red squill has all of the properties of white squill and in addition contains active toxic constituents that have not been

chemically isolated and identified.

Both the red and the white squill contain needle-like crystals (calcium oxalate raphides) that are irritating to the skin and cause a stinging sensation. These raphides also give the red squill its acrid prickly taste which makes it objectionable to humans and most animals, except rats and mice. It is probably this objectionable taste combined with its emetic effect which renders the squill relatively harmless.

In the preparation of the oven-dried red squill powder, the bulbs are sliced thin after the dry outer husks have been removed (9) and then are placed in a previously heated drying oven and held at a constant heat of 60° C. until thoroughly dried. Red squill dried at higher or lower temperatures has been found less toxic than when dried at approximately 60° C. After being dried the squill is ground fine and packed in air-tight containers. When thoroughly dried, squill does not deteriorate, and if kept in air-tight containers it will retain its original toxicity indefinitely. Most of the oven-dried product is prepared in this country, from the fresh bulbs which have been imported.

In making the sun-dried product (11), the bulbs after collection are deprived of their roots and dry membranous outer scales, cut into slices transversely, dried in the sun and packed in casks for shipment. The drug is imported from Leghorn, Italy and Amsterdam, Holland. The toxicity of the powder which is prepared by sun-drying is considerably less and more variable than the powder which is prepared under controlled conditions.

Red squill has been used successfully as a raticide for several years and recently has greatly displaced the so-called chemical poisons, such as strychnine and arsenic. The Danish anti-rat law of 1907 forbade the use of these chemical poisons, but it did permit the use of squill preparations. Aumann (1) lists nine commercial squill preparations offered for sale in Germany in 1912. Many extracts are on the market at the present time but most of them are relatively inert. In 1933, a glycerine extract was manufactured on a commercial scale at Barnstable, Massachusetts, but the results obtained with this preparation were not very satisfactory. At the present time the use of red squill as a rat poison is officially sanctioned by the Public Health and Agriculture Departments of both the United States and Great Britain.

2. Chemical Properties of Red Squill

Considerable work has been done on the chemical study of the active constituent of red squill but it has never been isolated or identified. Many of the products which have been obtained by extractions with various solvents have been found to be impure mixtures. In 1925, George (5) made a review of the chemical studies which have been made on red squill.

George states in his review that there are four active constituents of squill which have been found. Kopaczewski isolated scillitin, $C_{17}H_{25}O_6$, a yellow, very bitter, nitrogen free glucoside, very slightly soluble in water and soluble in alcohol, which he considered as the toxic principle of squill. Scillidiuretin, a strong diuretic was also isolated by Kopaczewski. Buschman (2) isolated xanthoscillide, a yellow crystalline glucoside, which is insoluble in chloroform, but soluble in boiling alcohol. A water-soluble toxic substance was also isolated by Ewin (4), but not identified.

Scillitin, the glucoside which was isolated by Kopaczewski is readily hydrolyzed, yielding dextrose and amorphous products. Smith (10) prepared an aqueous extract which was not toxic to rats and from Kopaczewski's

observations, he concluded that scillitin was absent from the extract as scillitin is practically insoluble in water. He also prepared an alcoholic extract, which proved very effective, although the whole of the toxic principle was not extracted.

Merck (6) has prepared two toxic substances, scillipicrine and scillitoxin, the first of which is soluble in water, and the second soluble in alcohol. However, scillitoxin has not been identified as a definite chemical entity. Merck has also prepared scillitin which was isolated by Kopaczewski.

Since calcium oxalate was universally recognized as being present in squill, Munch, Silver, and Horn (7) fed calcium oxalate to rats in doses as large as 1000 milligrams per kilo of body weight, which is equivalent to 33,000 milligrams of squill per kilo, without producing any evident effect. From these results, and since calcium oxalate is found in white as well as red squill, they concluded that the calcium oxalate could not be the primary cause in the death of the rats.

3. Physiological Effect of Red Squill on Rats

Rats that have eaten a fatal dose of red squill, usually become somewhat lethargic. This period may last from 4 to 14 hours, during which time the animals do not appear to be in any pain. After this period, they usually exhibit characteristic tremors and depression in the hind legs. This is followed by progressive paralysis of the trunk and forelegs, breathing becomes difficult and the respiratory rate increases. Also during this period, the animal rolls over in a peculiar and characteristic manner. This rolling motion continues at frequent intervals until death. The animal appears to be in considerable pain during this period, and whenever these symptoms are observed, the rat rarely recovers. Post-mortem examinations (7) have indicated that death following squill is apparently produced by respiratory rather than cardiac action. It has been observed that the heart continues to beat after breathing has stopped. Irritation of the digestive tract also is pronounced, but not to an extent that would prove fatal.

4. Purpose of the Investigation

The purpose of this investigation was that of extracting the toxic principle from powdered red squill,

both oven-dried and sun-dried. Various solvents have been used and both maceration and percolation methods have been employed. Since there is no chemical test for the toxic principle, the toxicity of the various extracts was determined by means of biological-assays, white rats being used. Claremont (3) and Silver (7) have observed that wild (brown) rats are more susceptible to squill poisoning than white rats, and since wild rats were not readily obtainable, white rats were used in these assays. The extracts were concentrated and dried on various substances in an attempt to make them more easily handled and to render them more palatable to rats.

II. EXPERIMENTAL WORK

1. Methods of Extraction

Three methods of extraction were used in this investigation. The extracts were prepared by Soxhlet extraction, by shaking, and by stirring. Each method will be discussed separately with the data of the feeding tests which were conducted to determine the toxicity of the various extracts. Table I. shows the toxicity of the red squill powder from which all of the extracts have been prepared. This test was conducted for purposes of comparison between the extract and the original powder from which the extracts were prepared.

A. Soxhlet Extraction

In this method of extraction, a Soxhlet extraction apparatus was used. In all cases 15 grams of red squill powder and 100 cc. of the solvent were used. Numerous solvents were tried in an effort to determine the best and the most practical solvent. After the liquid extracts had been prepared, they were concentrated on driers and the solvent evaporated.

(1) Preparation of Extracts for Feeding Tests

After the liquid extracts had been prepared, these solutions were concentrated on different substances in an attempt to make them more easily handled and to render them more palatable to the rats. In this investigation, two drying agents have been used:- bran and B-lactose.

The extract was placed in an evaporating dish and the drying agent was added in amounts depending on the strength of the finished product which was desired. If the extract was weak, only a small amount of the drying agent was added, so that the rat would not have to eat an excessive amount of the material to get a toxic dose. Then the extract was evaporated on an electric hot plate nearly to dryness. At this point, the extract was placed in a drying oven and

left there until all of the solvent had been driven off. This insured the complete removal of the solvent, leaving the toxic constituent deposited on the drying agent, so that a dry product was obtained, and also any undesirable flavor which might be caused by the solvent would be removed.

Then this dried product was weighed and the relation of its weight to the weight of the original squill powder from which the extract was made, was determined and the baits computed from this relationship. For example, suppose that 9 grams of dried extract was obtained from 15 grams of red squill powder, then .6 grams of the dried extract would be equivalent to 1 gram of the powder. Therefore, in making the baits only .6 as much of the dried extract would have to be used, as of the powder. The rats would not have to consume as much of the dried extract as of the powder to obtain the same amount of toxic constituent, i.e., assuming that all of the toxic constituent has been extracted from the powder.

The following data show the relationship between the original amount of red squill powder from which the extracts were made, and the amount of dried extract which was obtained. In all cases, the extracts were dried on bran except where indicated (LS-1A and

LS-2A). These two extracts were dried on B-lactose in an attempt to determine which was more palatable to rats.

LS-19 1 hour extraction with methyl alcohol

15 grams powder = 9.280 grams dried extract

1 gram powder = 0.618 grams dried extract

LS-15 2 hour extraction with methyl alcohol

15 grams powder = 9.810 grams dried extract

1 gram powder = 0.654 grams dried extract

LS-11 4 hour extraction with methyl alcohol

15 grams powder =11.670 grams dried extract

1 gram powder = 0.778 grams dried extract

LS-12 4 hour extraction with synthetic methanol

15 grams powder =10.405 grams dried extract

1 gram powder = 0.693 grams dried extract

LS-2 8 hour extraction with methyl alcohol

A. Dried on B-lactose

7.5 grams powder =13.000 grams dried extract

1 gram powder = 1.730 grams dried extract

B. Dried on bran

7.5 grams powder = 5.350 grams dried extract

1 gram powder = 0.710 grams dried extract

LS-9 16 hour extraction with methyl alcohol

15 grams powder = 8.720 grams dried extract

1 gram powder = 0.581 grams dried extract

LS-20 1 hour extraction with ethyl alcohol

15 grams powder = 8.510 grams dried extract

1 gram powder = 0.567 grams dried extract

LS-16 2 hour extraction with ethyl alcohol

15 grams powder = 10.030 grams dried extract

1 gram powder = 0.668 grams dried extract

LS-1 8 hour extraction with ethyl alcohol

A. Dried on B-lactose

7.5 grams powder = 11.770 grams dried extract

1 gram powder = 1.550 grams dried extract

B. Dried on bran

7.5 grams powder = 4.110 grams dried extract

1 gram powder = 0.550 grams dried extract

LS-5 4 hour extraction with Cellosolve (Ethylene glycol mono-ethyl ether)

15 grams powder = 8.520 grams dried extract

1 gram powder = 0.568 grams dried extract

LS-8 8 hour extraction with Cellosolve

15 grams powder = 13.725 grams dried extract

1 gram powder = 0.915 grams dried extract

LS-7 4 hour extraction with water

15 grams powder = 7.485 grams dried extract

1 gram powder = 0.499 grams dried extract

LS-10 4 hour extraction with ethyl ether

15 grams powder = 8.935 grams dried extract

1 gram powder = 0.596 grams dried extract

LS-17 4 hour extraction with ethylene dichloride

15 grams powder = 8.610 grams dried extract

1 gram powder = 0.574 grams dried extract

LS-18 4 hour extraction with acetone

15 grams powder = 8.100 grams dried extract

1 gram powder = 0.540 grams dried extract

(2) Method of Making Feeding Tests

All of the toxicity determinations were made on white rats which were essentially uniform, and in all cases normal and healthy animals were used. The animals were first weighed and then placed in individual cages. These rats were then deprived of food for 16-18 hours, but were given plenty of water. This procedure was carried out to insure a somewhat constant appetite and to favor the rapid consumption of the experimental diet.

The product to be tested was weighed out and mixed with the ordinary laboratory rat food, which consists of 66 per cent ground whole wheat, 33 per cent powdered whole milk, and 1 per cent salt. The extract and the rat food were mixed in the proportion of 1 part of extract to 9 parts of rat food, so that the extract was 10 per cent of the total weight of the bait. This ratio was adhered to in all of the

feeding tests made in this investigation, and in practically all of the tests, the baits were readily and completely eaten. The weighed baits were placed in the cages in shallow glass containers and frequent inspections were made to determine the time at which all of the bait was consumed. The dish was then removed and the rat left without food and water until the next morning.

Frequent inspections were then made to determine the onset of the symptoms of squill poisoning. On the following day the animals, which did not exhibit the characteristic symptoms of squill poisoning, were offered the regular diet and water. All of the rats that died within three days after being fed the bait, and in which the squill symptoms were produced were considered as having been killed by squill.

The amount of extract which was fed was based upon the amount of red squill powder from which the extract was made. In this manner, a direct comparison of the extract and the powder could be made and the value of the extract determined.

Tables II - XVIII give the results of the feeding tests in determining the toxicity of the various extracts. A summary of the results is given in Table A.

Discussion of Results

The results of these feeding tests indicate that methyl and ethyl alcohol are the best solvents to use as the extracting agent for the toxic principle of red squill. The methyl alcohol extracts slightly more than the ethyl alcohol with the one and two hour extractions. These two solvents extract practically all of the toxic principle, since the baits were computed on the basis of the original squill powder from which the extracts were made, and these extracts killed at the same feeding levels as the powder itself. Cellosolve also gave good results, but not as good as methyl or ethyl alcohol. Such solvents as water, ethyl ether, acetone, and ethylene dichloride extracted only traces of the toxic principle.

The results also indicate that the four hour extraction with methyl alcohol is the shortest extraction which will remove the maximum amount of the toxic constituent of red squill powder.

In the extracts which did not contain any of the active principle, no color was extracted; but in the toxic extracts there was a considerable amount of red color, which seemed to denote the complete removal of the toxic constituent.

Table A. Summary of Tables II-XVIII
Toxicity Determinations of Extracts Prepared Using Various Solvents
Soxhlet Extraction Method

	2000 mg/kilo	1000 mg/kilo	900 mg/kilo	800 mg/kilo	700 mg/kilo	600 mg/kilo	500 mg/kilo	400 mg/kilo
Red Squill Powder					5/5	5/5	5/5	3/5
CH_3OH - 1 hour				2/4			0/4	
CH_3OH - 2 hour				4/4		4/4	2/4	
CH_3OH - 4 hour				2/2		2/2	4/4	
CH_3OH - 8 hour - dried on B-lactose	2/2	2/2	2/2	2/2		4/4	4/4	
CH_3OH - 8 hour	2/2	2/2	2/2	2/2		4/4	7/8	
CH_3OH - 16 hour				4/4		4/4	4/4	3/4
Synthetic CH_3OH - 4 hour				2/2		2/2	4/4	
C_2H_5OH - 1 hour				3/4			0/4	
C_2H_5OH - 2 hour				4/4		3/4	0/4	
C_2H_5OH - 8 hour	2/2	2/2	2/2	2/2		4/4	4/4	
C_2H_5OH - 8 hour - dried on B-lactose	1/1	2/2	2/2	2/2		4/4	4/4	
Cellosolve - 4 hour	2/2	6/6	4/4	4/4	4/4	3/4	3/4	
Cellosolve - 8 hour				4/4			2/4	
Water - 4 hour	0/2	0/2						
Ethyl Ether - 4 hour		0/2						
$C_2H_4Cl_2$ - 4 hour		0/4						
Acetone - 4 hour		0/4						

The numerator of each fraction indicates the number of rats dying within three days; the denominator indicates the total number of rats fed.

In extracts LS-1A and LS-2A, the results of the feeding tests (Tables VI and XI) show that the rats eat the extracts which were dried on bran as well as those dried on B-lactose. For this reason all of the extracts which have been prepared from this point on, have been concentrated on bran as the drying agent.

B. Shaking

The red squill powder from which the extract was to be made, was weighed out and placed in an 8 ounce glass jar, the solvent was added, the jar sealed, and placed in the shaking machine for different periods of time. The mixture was then filtered by means of suction. Then these extracts were concentrated on bran as a dryer and the solvent evaporated. In all of these extracts, methyl alcohol was used as the solvent and the amounts of powder and solvent which were used were in the proportion of 1 part of powder to 6 parts of solvent, i.e., 20 grams of powder and 150 cc. (120 grams) of methyl alcohol. The speed of the shaking machine was approximately 250 agitations per minute.

The following data show the relationship between the original amount of red squill powder from which the extracts were made, and the amount of dried extract which was obtained.

LS-31 15 minute shake

20 grams powder = 15.030 grams dried extract

1 gram powder = 0.751 grams dried extract

LS-32 30 minute shake

20 grams powder = 16.845 grams dried extract

1 gram powder = 0.842 grams dried extract

LS-33 45 minute shake

20 grams powder = 15.840 grams dried extract

1 gram powder = 0.792 grams dried extract

LS-34 60 minute shake

20 grams powder = 15.185 grams dried extract

1 gram powder - 0.759 grams dried extract

LS-35 90 minute shake

20 grams powder = 16.480 grams dried extract

1 gram powder = 0.824 grams dried extract

LS-36 120 minute shake

20 grams powder = 16.320 grams dried extract

1 gram powder = 0.816 grams dried extract

LS-37 180 minutes shake

20 grams powder = 14.175 grams dried extract

1 gram powder = 0.709 grams dried extract

LS-38 240 minute shake

17.7 grams powder = 21.590 grams dried extract

1 gram powder = 1.219 grams dried extract

LS-39 360 minute shake

18.6 grams powder = 18.080 grams dried extract

1 gram powder = 0.972 grams dried extract

Tables XIX - XXVII give the results of the feeding tests in determining the toxicity of the various extracts. A summary of the results is given in Table B.

Discussion of Results

The results of this series of experiments show that the shaking method of extraction is not as efficient as the Soxhlet extraction method, even though the mixture of powder and menstruum was shaken for as long as six hours. This method of extraction only extracts about 30 per cent of the toxic principle from the squill powder at the six hour shake, while the Soxhlet extraction method extracts practically 100 per cent.

Table B

Summary of Tables XVIII-XXVII
Toxicity Determination of Methyl Alcohol Extracts
Prepared by Shaking

	700 mg/kilo	600 mg/kilo	500 mg/kilo
Red squill powder	5/5	5/5	5/5
15 minute shake			0/8
30 minute shake			2/8
45 minute shake			1/8
60 minute shake			1/8
90 minute shake		2/4	2/8
120 minute shake			3/8
180 minute shake	6/10	2/4	2/8
240 minute shake	7/10	2/4	2/8
360 minute shake	7/10		3/8

C. Stirring

In this method of extraction, a Read electric Mixonette was used. The squill powder and the solvent were placed in the bowl of the Mixonette and were stirred for varying lengths of time. In all of the extracts prepared in this manner, the squill powder and the solvent were mixed in the proportion of 1 part of powder to 6 parts of solvent, i.e., 40 grams of powder and 240 grams (300 cc.) of the solvent. In all of these extractions, methyl alcohol was used as the solvent. These liquid extracts were then concentrated on bran as a dryer and the solvent evaporated.

The following data show the relationship between the original amount of red squill powder from which the extracts were made, and the amount of dried extract which was obtained.

LS-40 15 minute stir

40 grams powder = 31.827 grams dried extract

1 gram powder = 0.795 grams dried extract

LS-41 30 minute stir

40 grams powder = 30.328 grams dried extract

1 gram powder = 0.758 grams dried extract

LS-42 45 minutes stir

40 grams powder = 30.717 grams dried extract

1 gram powder = 0.768 grams dried extract

LS-43 60 minute stir

40 grams powder = 31.745 grams dried extract

1 gram powder = 0.794 grams dried extract

LS-44 90 minute stir

40 grams powder = 27.340 grams dried extract

1 gram powder = 0.684 grams dried extract

LS-45 120 minute stir

40 grams powder = 30.322 grams dried extract

1 gram powder = 0.758 grams dried extract

Tables XXVIII - XXXIII give the results of the feeding tests in determining the toxicity of the various extracts. A summary of the results is given in Table C.

Discussion of Results

The results of this series of experiments show that stirring is the poorest method of extraction which has been tried in this investigation. The extract which was prepared by stirring for two hours, extracted less than 10 per cent of the toxic constituent from the squill powder.

Table C Summary of Tables XXVIII-XXXIII
Toxicity Determinations of Methyl Alcohol Extracts
Prepared by Stirring

	600 mg/kilo	500 mg/kilo
Red squill powder	5/5	5/5
15 minute stir	1/8	0/4 (1 sick)
30 minute stir	0/8	0/4
45 minute stir	1/8	0/4 (1 sick)
60 minute stir	2/8	0/4 (1 sick)
90 minute stir	4/12	0/4 (2 sick)
120 minute stir	4/12	0/4 (2 sick)

2. Effect of Soaking on Extraction

The suggestion was made that soaking the powder in the menstruum before making the extraction might aid in extracting the toxic principle from the squill powder. A series of tests were made to determine if soaking did have any effect on the extraction.

The squill powder was placed in a half-pint glass jar and the solvent (methyl alcohol) was added and the powder allowed to soak for varying lengths of time. The amounts of powder and methyl alcohol which were used were in the ratio of 6 parts of alcohol to 1 part of squill powder, by weight. After soaking, the mixture of powder and alcohol was placed in a very rapid motion shaking machine and shaken for two hours. In all of these tests the same period of shaking was used. After the two hour shake, the mixture was filtered by means of suction, and the liquid extract was dried down on bran. Toxicity determinations were then conducted on these dried extracts.

The following data show the relationship between the original amount of redsquill powder from which the extracts were made, and the amount of dried extract which was obtained.

2 hour soak - 2 hour shake

LS-25	20 grams powder	=	12.085 grams dried extract
	1 gram powder	=	0.604 grams dried extract
LS-26	20 grams powder	=	14.265 grams dried extract
	1 gram powder	=	0.713 grams dried extract

4 hour soak - 2 hour shake

LS-27	20 grams powder	=	16.280 grams dried extract
	1 gram powder	=	0.814 grams dried extract
LS-28	20 grams powder	=	16.935 grams dried extract
	1 gram powder	=	0.847 grams dried extract

6 hour soak - 2 hour shake

LS-29	20 grams powder	=	16.995 grams dried extract
	1 gram powder	=	0.850 grams dried extract
LS-30	20 grams powder	=	14.330 grams dried extract
	1 gram powder	=	0.716 grams dried extract

Tables XXXIV - XXXVI give the results of the feeding tests which were conducted to determine the toxicity of the various extracts. A summary of the results is given in Table D.

Discussion of Results

The results of this series of tests show that soaking the squill powder previous to extraction does not have any appreciable effect on the total

Table D Summary of Tables XXXIV-XXXVI
Toxicity Determinations of Methyl Alcohol Extracts
Prepared by Soaking before Shaking

	500 mg/kilo
2 hour soak - 2 hour shake	2/4
" " " "	1/4 (1 sick)
4 hour soak - 2 hour shake	2/4
" " " "	2/4
6 hour soak - 2 hour shake	2/4
" " " "	1/4 (2 sick)

amount of thetoxic constituent which is extracted from the red squill powder.

3. Effect of Particle-size of Powder on Extraction

It was thought that the particle-size of the powder from which the extract was made, might have an effect on the amount of the toxic principle which was extracted from the powder. A portion of the red squill powder was screened and the following table gives the percentage composition of the powder, according to particle-size.

50 mesh -------	30.2%
50-100 mesh -------	21.2%
100-150 mesh -------	9.2%
150-200 mesh -------	7.5%
200-250 mesh -------	4.5%
250-300 mesh -------	14.5%
300 mesh -------	12.9%

The two sizes of powder which were used in this experiment were the 50 mesh and the 300 mesh. In the first run which was made, the two lots of powder were extracted in the Soxhlet extraction apparatus for 40 minutes. In the second run, a 30 minute extraction was employed. Methyl alcohol was

used as the solvent in both cases. After the extracts had been prepared, they were concentrated on bran as a drying base. Then toxicity determinations were conducted on these prepared extracts.

The following data show the relationship between the original amount of red squill powder from which the extracts were made, and the amount of dried extract which was obtained.

Extracts prepared from 50 mesh powder

LS-13 40 minute extraction

15 grams powder = 8.930 grams dried extract

1 gram powder = 0.595 grams dried extract

LS-23 30 minute extraction

15 grams powder = 10.970 grams dried extract

1 gram powder = 0.731 grams dried extract

Extracts prepared from 300 mesh powder

LS-14 40 minute extraction

15 grams powder = 8.290 grams dried extract

1 gram powder = 0.552 grams dried extract

LS-24 30 minute extraction

15 grams powder = 9.530 grams dried extract

1 gram powder = 0.635 grams dried extract

Tables XXXVII - XXXVIII give the results of the feeding tests in determining the toxicity of the various extracts. A summary of the results is given in Table E.

Discussion of Results

The results of this series of experiments were rather unusual in that the extracts which were prepared from the coarse powder were more toxic than those which were prepared from the fine powder. This may be due to the fact that the particles of the fine powder are packed together so closely that the menstruum cannat come into immediate contact with all of these particles, while in the case of the coarse powder, which is more loosely packed, the menstruum can permeate the powder more readily.

The 30 minute extract which was prepared from the coarse powder had approximately the same toxicity as the extract which was prepared from the unscreened powder and extracted for two hours.

It was also noticed that during the extraction process, more color was extracted in the case of the coarse powder, and also there was a more rapid extraction of the red color. The final

Table E

Summary of Tables XXXVII-XXXVIII
Toxicity Determinations of Methyl Alcohol Extracts
Prepared from 50 mesh and 300 mesh powder

Mesh	50			300		
Feeding Level	800	600	500	800	600	500
40 minute extraction	4/4	4/4	3/4	3/4	0/4 (2 sick)	0/4
30 minute extraction	4/4	4/4		0/4	0/4	

extract from the coarse powder was much more red than that prepared from the fine. This bears out the fact as reported previously, that the extraction of the red color can be used as an indicator to show whether or not there has been a complete extraction of the toxic principle.

III. SUMMARY

1. Toxic extracts can be prepared from red squill powder and these extracts can be used as efficient raticides.

2. Methyl and ethyl alcohols are the most efficient solvents for the extraction of the toxic principle from red squill.

3. The Soxhlet extraction method is the best method of those tried for the extraction of the toxic principle. A four hour Soxhlet extraction with methyl alcohol extracts practically all of the active constituent from the squill. A six hour shaking with methyl alcohol only extracts about 30 per cent of the toxic constituent, while a two hour stirring extracts less than 10 per cent.

4. The extraction of the red color from the red squill powder may be used as an indicator to show whether or not there has been a complete extraction of the toxic principle.

5. Bran is a convenient and suitable carrier for the dried extract.

6. Soaking the red squill powder in the menstruum previous to extraction does not have any apparent effect on the total amount of the toxic constituent which is extracted.

7. An extract which is prepared from the coarse powder is more toxic than one prepared from fine powder, in a short period of extraction, both being prepared in the same manner and under the same conditions.

8. Rats which do not receive a lethal dose of the extract in the initial feeding, will repeatedly consume more baits prepared from extracts. This overcomes one drawback of the powder, i.e. after rats become sick from squill powder, they will not eat it again.

IV. BIBLIOGRAPHY

1. Aumann
1912. Vergleichende Untersuchungen uber die Wirksamkeit Bakterieller und Chemischer Rattenvertilgungsmittel. Centbl. Bakt. (etc.) 63: 212-221, illus.

2. Buschmann, E.
1919. Untersuchungen uber die Chemischen Bestandteile von Bulbus Scillae. Arch. Pharm. 257: 79-86.

3. Claremont, C. L.
1921-22. National Research on Rat Destruction. Journ. Roy. Sanit. Inst. 42: 311-318.

4. Ewins, A. J.
1911-12. A New Water Soluble Active Constituent of Squills. Journ. Pharmacol. and Expt. Ther. 3: 155-160.

5. George, E.
1925. The Active Principles of the African Squill, Urginea Burkei (Slangkop) and its Suitability or Otherwise as a Substitute for Urginea Maritima in Commerce. Journ. So. African Chem. Inst. 8: 14-27.

6. Merck Index
1930. Fourth edition, Rahway, N. J.

7. Munch, J. C., Silver, J. and Horn, E. E.
1929. Red Squill Powders as Raticides. U.S.D.A. Technical Bulletin No. 134.

8. Silver, James
1930. Rat Control. U.S.D.A. Farmer's Bulletin No. 1533.

9. Silver, J. and Munch, J. C.
1931. Red Squill Powder in Rat Control. U.S.D.A. Leaflet No. 65

10. Smith, W. F.
1921. Liquid Extract of Red Squill (Scilla Maritima) as a Rat Poison. Analyst 46: 178-180.

11. U. S. Dispensatory. 21st edition. p. 965.

V. ACKNOWLEDGEMENT

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TABLES

TABLE I - Toxicity of red squill powder from which extracts were made.

Rat No.	Rat weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
			700 mg. per kilo of body weight			
1	300	2100	210	2100	210	Dead
2	245	1720	172	1720	172	Dead
3	235	1650	165	1650	165	Dead
4	180	1260	126	1260	126	Dead
5	235	1650	165	1650	165	Dead
			600 mg. per kilo of body weight			
6	265	1590	159	1590	159	Dead
7	230	1380	138	1380	138	Dead
8	235	1410	141	1410	141	Dead
9	225	1350	135	1350	135	Dead
10	180	1080	108	1080	108	Dead
			500 mg. per kilo of body weight			
11	180	900	90	900	90	Dead
12	235	1180	118	1180	118	Dead
13	210	1050	105	1050	105	Dead
14	235	1180	118	1180	118	Dead
15	225	1130	113	1130	113	Dead

400 mg. per kilo of body weight

16	195	780	78	780	78	Dead
17	245	980	98	980	98	Dead
18	255	1020	102	1020	102	Sick
19	250	1000	100	1000	100	Dead
20	230	920	92	920	92	Sick

TABLE II - LS-19 - Toxicity of methyl alcohol extract (1 hour extraction).

800 mg. per kilo of body weight

Rat No.	Rat weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	110	540	54	540	54	Dead
2	90	450	45	450	45	Sick
3	95	470	47	470	47	Alive
4	110	540	54	540	54	Dead
		500 mg. per kilo of body weight				
5	105	330	33	330	33	Alive
6	109	340	34	340	34	Alive
7	96	300	30	300	30	Alive
8	100	310	31	310	31	Sick

TABLE III - LS-15 - Toxicity of methyl alcohol extract (2 hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		800 mg. per kilo of body weight				
1	110	580	58	580	58	Dead
2	130	680	68	680	68	Dead
3	140	740	74	740	74	Dead
4	165	860	86	860	86	Dead
		600 mg. per kilo of body weight				
5	140	550	55	550	55	Dead
6	150	590	59	590	59	Dead
7	120	470	47	470	47	Dead
8	140	550	55	550	55	Dead
		500 mg. per kilo of body weight				
9	175	570	57	570	57	Dead
10	115	380	38	380	38	Alive
11	155	510	51	510	51	Dead
12	165	540	54	540	54	Sick

TABLE IV - LS-11 - Toxicity of methyl alcohol extract (4hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		800 mg. per kilo of body weight				
1	110	690	69	690	69	Dead
2	110	690	69	690	69	Dead
		600 mg. per kilo of body weight				
3	130	810	81	810	81	Dead
4	180	1020	102	1020	102	Dead
		500 mg. per kilo of body weight				
5	145	570	57	570	57	Dead
6	140	550	55	550	55	Dead
7	135	530	53	530	53	Dead
8	185	720	72	720	72	Dead

TABLE V - LS-12 - Toxicity of synthetic methanol extract (4 hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		800 mg. per kilo of body weight				
1	185	1020	102	1020	102	Dead
2	185	1020	102	1020	102	Dead
		600 mg. per kilo of body weight				
3	185	770	77	770	77	Dead
4	145	600	60	600	60	Dead
		500 mg. per kilo of body weight				
5	125	430	43	430	43	Dead
6	130	450	45	450	45	Dead
7	125	430	43	430	43	Dead
8	110	380	38	380	38	Dead

TABLE VI - L3-2A - Toxicity of methyl alcohol extract (8 hour extraction). Dried on β-lactose.

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		2000 mg. per kilo of body weight				
1	210	7270	727	3025	302.5	Dead
2	220	7810	781	4540	454	Dead
		1000 mg. per kilo of body weight				
3	185	3200	320	3200	320	Dead
4	225	3890	389	3030	303	Dead
		900 mg. per kilo of body weight				
5	250	3820	382	3820	382	Dead
6	220	3360	336	3360	336	Very sick
		800 mg. per kilo of body weight				
7	145	1970	197	1970	197	Dead
8	150	2040	204	2040	204	Dead
		600 mg. per kilo of body weight				
9	135	1380	138	1380	138	Dead
10	135	1380	138	1380	138	Dead
11	145	1480	148	1480	148	Dead
12	140	1430	143	1430	143	Dead

500 mg. per kilo of body weight

13	145	1240	124	1240	124	Dead
14	125	1070	107	1070	107	Dead
15	155	1330	133	1330	133	Dead
16	115	990	99	990	99	Dead

TABLE VII - LS-2B - Toxicity of methyl alcohol extract (8 hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		2000 mg. per kilo of body weight				
1	260	3690	369	2900	290	Dead
2	225	3200	320	1690	169	Dead
		1000 mg. per kilo of body weight				
3	283	2000	200	2000	200	Dead
4	220	1560	156	1560	156	Very sick
		900 mg. per kilo of body weight				
5	140	900	90	900	90	Dead
6	175	1120	112	1120	112	Dead
		800 mg. per kilo of body weight				
7	220	1250	125	870	87	Dead
8	230	1300	130	1300	130	Dead
		600 mg. per kilo of body weight				
9	125	530	53	530	53	Dead
10	135	580	58	580	58	Dead
11	130	550	55	550	55	Dead
12	125	530	53	530	53	Dead

500 mg. per kilo of body weight

13	130	460	46	460	46	Dead
14	135	480	48	480	48	Alive
15	135	480	48	480	48	Dead
16	125	450	45	450	45	Dead
17	120	430	43	430	43	Dead
18	155	550	55	550	55	Dead
19	110	390	39	390	39	Dead
20	155	550	55	550	55	Dead

TABLE VIII - LS-9 - Toxicity of methyl alcohol extract (16 hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		800 mg. per kilo of body weight				
1	295	1370	137	1370	137	Dead
2	250	1160	116	1160	116	Dead
3	280	1300	130	1300	130	Dead
4	195	900	90	900	90	Dead
		600 mg. per kilo of body weight				
5	190	660	66	660	66	Dead
6	260	900	90	900	90	Dead
7	260	900	90	900	90	Dead
8	260	900	90	900	90	Dead
		500 mg. per kilo of body weight				
9	120	350	35	350	35	Dead
10	115	340	34	340	34	Dead
11	135	400	40	400	40	Dead
12	170	490	49	490	49	Dead

400 mg. per kilo of body weight

13	160	370	37	370	37	Sick
14	105	240	24	240	24	Dead
15	135	310	31	310	31	Dead
16	115	270	27	270	27	Dead

TABLE IX - LS-20 - Toxicity of ethyl alcohol extract (1 hour extraction).

800 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	110	500	50	500	50	Alive
2	95	430	43	430	43	Dead
3	100	460	46	460	46	Dead
4	100	460	46	460	46	Dead
		500 mg. per kilo of body weight				
5	120	340	34	340	34	Very sick
6	98	280	28	280	28	Sick
7	105	300	30	300	30	Alive
8	115	330	33	330	33	Alive

TABLE X - L3-16 - Toxicity of ethyl alcohol extract (2 hour extraction).

800 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	125	670	67	670	67	Dead
2	200	1070	107	1070	107	Dead
3	110	590	59	590	59	Dead
4	120	640	64	640	64	Dead
		600 mg. per kilo of body weight				
5	105	420	42	420	42	Dead
6	140	560	56	560	56	Sick
7	115	460	46	460	46	Dead
8	135	540	54	540	54	Dead
		500 mg. per kilo of body weight				
9	92	310	31	310	31	Sick
10	105	350	35	350	35	Alive
11	108	360	36	360	36	Alive
12	105	350	35	350	35	Alive

TABLE XI - LS-1A - Toxicity of ethyl alcohol extract (8 hour extraction) .
Dried on β-lactose

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		2000 mg. per kilo of body weight				
1	180	5580	558	0	0	Did not eat bait
2	208	6440	644	2785	278.5	Dead
		1000 mg. per kilo of body weight				
3	185	2860	286	2860	286	Dead
4	180	2790	279	2790	279	Dead
		900 mg. per kilo of body weight				
5	150	2160	216	2160	216	Dead
6	125	1800	180	1800	180	Dead
		800 mg. per kilo of body weight				
7	155	1980	198	1980	198	Dead
8	150	1920	192	1920	192	Dead
		600 mg. per kilo of body weight				
9	125	1200	120	1200	120	Dead
10	180	1720	172	1720	172	Dead
11	185	1770	177	1770	177	Dead
12	135	1300	130	1300	130	Dead

500 mg. per kilo of body weight

13	130	1010	101	1010	101	Dead
14	140	1090	109	1090	109	Dead
15	140	1090	109	1090	109	Dead
16	135	1050	105	1050	105	Dead

TABLE XII - LS-13 - Toxicity of ethyl alcohol extract (8 hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
			2000 mg. per kilo of body weight			
1	210	2310	231	2310	231	Dead
2	220	2420	242	2420	242	Dead
			1000 mg. per kilo of body weight			
3	186	1020	102	1020	102	Dead
4	245	1350	135	1350	135	Dead
			900 mg. per kilo of body weight			
5	220	1090	109	1090	109	Dead
6	170	840	84	840	84	Dead
			800 mg. per kilo of body weight			
7	165	670	67	670	67	Dead
8	255	1120	112	1120	112	Dead
			600 mg. per kilo of body weight			
9	135	450	45	450	45	Dead
10	140	460	46	460	46	Dead
11	135	450	45	450	45	Dead
12	190	630	63	630	63	Dead

500 mg. per kilo of body weight

13	130	360	36	360	36	Dead
14	130	360	36	360	36	Dead
15	145	400	40	400	40	Dead
16	195	540	54	540	54	Dead

TABLE XIII - LS-5 - Toxicity of Cellosolve extract (4 hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		2000 mg. per kilo of body weight				
1	200	2280	228	2280	228	Dead
2	325	3700	370	2040	204	Dead
		1100 mg. per kilo of body weight				
3	175	1090	109	1090	109	Dead
4	165	1030	103	1030	103	Dead
5	170	1060	106	1060	106	Dead
6	185	1160	116	1160	116	Dead
		1000 mg. per kilo of body weight				
7	235	1340	134	1340	134	Dead
8	225	1280	128	1280	128	Dead
9	245	1400	140	1400	140	Dead
10	220	1250	125	1250	125	Very sick
11	190	1080	108	1080	108	Dead
12	175	1000	100	1000	100	Dead

		900 mg. per kilo of body weight				
13	240	1230	123	1230	123	Very sick
14	295	1510	151	1510	151	Dead
15	195	1000	100	540	54	Dead
16	250	1280	128	1280	128	Dead
		800 mg. per kilo of body weight				
17	225	1030	103	1030	103	Dead
18	230	1050	105	1050	105	Dead
19	185	840	84	840	84	Dead
20	205	920	92	920	92	Dead
		700 mg. per kilo of body weight				
21	170	680	68	680	68	Dead
22	195	780	78	780	78	Dead
23	165	660	66	660	66	Dead
24	125	500	50	500	50	Dead
		600 mg. per kilo of body weight				
25	200	680	68	680	68	Dead
26	210	720	72	720	72	Dead
27	140	480	48	480	48	Dead
28	195	670	67	670	67	Alive

500 mg. per kilo of body weight

29	175	500	50	500	50	Dead
30	175	500	50	500	50	Dead
31	160	450	45	450	45	Alive
32	190	540	54	540	54	Dead

TABLE XIV - LS-8 - Toxicity of Cellosolve extract (8 hour extraction).

800 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	120	880	88	880	88	Dead
2	125	920	92	920	92	Dead
3	200	1470	147	1470	147	Dead
4	195	1430	143	1430	143	Dead
		500 mg. per kilo of body weight				
5	170	770	77	770	77	Alive
6	170	770	77	770	77	Dead
7	195	900	90	900	90	Sick
8	200	920	92	920	92	Dead

TABLE XV - LS-7 - Toxicity of water extract (4 hour extraction).

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
		2000 mg. per kilo of body weight				
1	245	2450	245	2450	245	Alive
2	360	3600	360	3600	360	Alive
		1000 mg. per kilo of body weight				
3	265	1320	132	1320	132	Alive
4	250	1250	125	1250	125	Alive

TABLE XVI - LS-10 - Toxicity of ethyl ether extract (4 hour extraction).

1000 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	125	750	75	750	75	Alive
2	115	690	69	690	69	Alive

TABLE XVII - LS-17 - Toxicity of ethylene dichloride extract (4 hour extraction).

1000 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	190	1080	108	1080	108	Alive
2	95	540	54	540	54	Alive
3	110	630	63	630	63	Alive
4	115	650	65	650	65	Alive

TABLE XVIII - LS-18 - Toxicity of acetone extract (4 hour extraction).

1000 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	120	650	65	650	65	Alive
2	115	620	62	620	62	Alive
3	115	620	62	620	62	Alive
4	115	620	62	620	62	Alive

TABLE XIX - LS-31 - Toxicity of methyl alcohol extract prepared by shaking for 15 minutes.

500 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	140	530	53	530	53	Alive
2	160	600	60	600	60	Alive
3	130	490	49	490	49	Alive
4	125	470	47	0	0	Did not eat bait
5	105	390	39	390	39	Very sick
6	110	410	41	410	41	Alive
7	105	390	39	390	39	Alive
8	115	430	43	430	43	Very sick

TABLE XX + LS-32 - Toxicity of methyl alcohol extract prepared by shaking for 30 minutes.

500 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	130	550	55	550	55	Dead
2	205	860	86	860	86	Very sick
3	155	650	65	650	65	Sick
4	145	620	62	620	62	Dead
5	135	570	57	570	57	Alive
6	115	480	48	480	48	Very sick
7	110	460	46	460	46	Alive
8	110	460	46	460	46	Very sick

TABLE XXI - LS-33 - Toxicity of methyl alcohol extract prepared by shaking for 45 minutes.

500 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	135	530	53	530	53	Alive
2	125	500	50	500	50	Alive
3	120	480	48	480	48	Alive
4	140	550	55	550	55	Alive
5	110	440	44	440	44	Alive
6	110	440	44	440	44	Dead
7	110	440	44	440	44	Alive
8	105	420	42	420	42	Sick

TABLE XXII - LS-34 - Toxicity of methyl alcohol extract prepared by shaking for 60 minutes.

500 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	205	780	78	780	78	Sick
2	215	820	82	820	82	Sick
3	110	420	42	420	42	Dead
4	185	700	70	700	70	Sick
5	135	510	51	510	51	Alive
6	95	360	36	360	36	Alive
7	110	420	42	420	42	Alive
8	110	420	42	420	42	Very sick

TABLE XXIII - LS-35 - Toxicity of methyl alcohol extract prepared by shaking for 90 minutes.

600 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	135	680	68	680	68	Dead
2	120	590	59	590	59	Dead
3	115	570	57	570	57	Alive
4	105	520	52	520	52	Alive
		500 mg. per kilo of body weight				
5	160	660	66	660	66	Alive
6	190	780	78	780	78	Dead
7	160	660	66	660	66	Alive
8	205	840	84	840	84	Dead
9	100	410	41	410	41	Alive
10	95	390	39	390	39	Alive
11	100	410	41	410	41	Alive
12	115	470	47	470	47	Alive

TABLE XXIV - LS-36 - Toxicity of methyl alcohol Extract prepared by shaking for 120 minutes.

500 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	95	390	39	390	39	Alive
2	120	490	49	490	49	Alive
3	90	370	37	370	37	Dead
4	100	410	41	410	41	Alive
5	115	470	47	470	47	Dead
6	115	470	47	470	47	Alive
7	120	490	49	490	49	Alive
8	120	490	49	490	49	Dead

TABLE XXV - LS-37 - Toxicity of methyl alcohol extract prepared by shaking for 180 minutes.

700 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	135	670	67	670	67	Dead
2	125	620	62	620	62	Dead
3	135	670	67	670	67	Dead
4	145	720	72	720	72	Sick
5	140	690	69	690	69	Sick
6	125	620	62	620	62	Dead
7	130	650	65	650	65	Sick
8	135	670	67	670	67	Dead
9	110	550	55	550	55	Sick
10	115	570	57	570	57	Dead
		600 mg. per kilo of body weight				
11	135	570	57	570	57	Alive
12	135	570	57	570	57	Alive
13	105	450	45	450	45	Dead
14	125	530	53	530	53	Dead

500 mg. per kilo of body weight

15	155	550	55	550	55	Alive
16	195	690	69	690	69	Dead
17	140	500	50	500	50	Alive
18	180	640	64	640	64	Alive
19	110	390	39	390	39	Alive
20	115	410	41	410	41	Alive
21	125	440	44	440	44	Alive
22	120	430	43	430	43	Dead

TABLE XXVI - L3-38 - Toxicity of methyl alcohol extract prepared by shaking for 240 minutes.

700 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	125	1070	107	1070	107	Dead
2	125	1070	107	1070	107	Dead
3	100	850	85	850	85	Sick
4	120	1020	102	1020	102	Sick
5	125	1070	107	1070	107	Dead
6	110	940	94	940	94	Sick
7	130	1110	111	1110	111	Dead
8	115	980	98	980	98	Dead
9	115	980	98	980	98	Dead
10	150	1280	128	1280	128	Dead
		600 mg. per kilo of body weight				
11	120	880	88	880	88	Dead
12	110	800	80	800	80	Sick
13	140	1020	102	1020	102	Dead
14	135	990	99	990	99	Alive

500 mg. per kilo of body weight

15	140	850	85	850	85	Alive
16	120	730	73	730	73	Sick
17	145	880	88	880	88	Dead
18	145	880	88	880	88	Alive
19	150	910	91	910	91	Sick
20	120	730	73	730	73	Dead
21	145	880	88	880	88	Sick
22	140	850	85	850	85	Alive

TABLE XXVII - LS-39 - Toxicity of methyl alcohol extract prepared by shaking for 360 minutes.

700 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	135	920	92	920	92	Dead
2	145	980	98	980	98	Sick
3	135	920	92	920	92	Dead
4	135	920	92	920	92	Sick
5	145	980	98	980	98	Dead
6	140	950	95	950	95	Dead
7	160	1090	109	1090	109	Dead
8	140	950	95	950	95	Dead
9	125	850	85	850	85	Dead
10	140	950	95	950	95	Sick
		500 mg. per kilo of body weight				
11	195	950	95	950	95	Alive
12	140	680	68	680	68	Alive
13	175	850	85	850	85	Alive
14	190	920	92	920	92	Alive

15	115	560	56	560	56	Dead
16	115	560	56	560	56	Dead
17	130	630	63	630	63	Dead
18	130	630	63	630	63	Very sick

TABLE XXVIII - L5-40 - Toxicity of methyl alcohol extract prepared by stirring for 15 minutes.

600 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	145	690	69	690	69	Alive
2	155	740	74	740	74	Alive
3	110	520	52	520	52	Dead
4	140	670	67	670	67	Sick
5	115	550	55	550	55	Alive
6	130	620	62	0	0	Did not eat bait
7	160	760	76	760	76	Alive
8	130	620	62	620	62	Alive
		500 mg. per kilo of body weight				
9	170	680	68	680	68	Alive
10	170	680	68	680	68	Very sick
11	120	480	48	480	48	Alive
12	155	620	62	620	62	Alive

TABLE XXIX - LS-41 - Toxicity of methyl alcohol extract prepared by stirring for 30 minutes.

600 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	160	730	73	730	73	Alive
2	140	640	64	640	64	Alive
3	150	680	68	680	68	Alive
4	165	750	75	750	75	Alive
5	125	570	57	570	57	Alive
6	120	550	55	550	55	Alive
7	145	660	66	660	66	Alive
8	125	570	57	570	57	Alive
		500 mg. per kilo of body weight				
9	165	630	63	630	63	Alive
10	130	490	49	490	49	Alive
11	155	590	59	590	59	Alive
12	120	450	45	450	45	Sick

TABLE XXX - LS-42 - Toxicity of methyl alcohol extract prepared by stirring for 45 minutes.

600 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	115	530	53	530	53	Alive
2	170	780	78	780	78	Alive
3	140	650	65	650	65	Alive
4	150	690	69	690	69	Alive
5	155	710	71	710	71	Alive
6	150	690	69	690	69	Alive
7	100	460	46	460	46	Dead
8	115	530	53	530	53	Alive
		500 mg. per kilo of body weight				
9	115	440	44	440	44	Alive
10	130	500	50	500	50	Alive
11	130	500	50	500	50	Very sick
12	120	460	46	460	46	Alive

TABLE XXXI - LS-43 - Toxicity of methyl alcohol extract prepared by stirring for 60 minutes.

600 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	140	670	67	670	67	Alive
2	155	740	74	740	74	Alive
3	140	670	67	670	67	Alive
4	120	560	56	560	56	Alive
5	125	600	60	600	60	Alive
6	155	740	74	740	74	Alive
7	125	600	60	600	60	Dead
8	105	500	50	500	50	Dead
		500 mg. per kilo of body weight				
9	145	580	58	580	58	Alive
10	135	540	54	540	54	Alive
11	120	480	48	480	48	Alive
12	125	500	50	500	50	Sick

TABLE XXXII - LS-44 - Toxicity of methyl alcohol extract prepared by stirring for 90 minutes.

600 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	115	470	47	470	47	Alive
2	120	490	49	490	49	Alive
3	150	610	61	610	61	Alive
4	120	490	49	490	49	Alive
5	145	590	59	590	59	Dead
6	150	610	61	610	61	Dead
7	140	570	57	570	57	Dead
8	160	660	66	660	66	Dead
9	135	550	55	550	55	Alive
10	135	550	55	550	55	Sick
11	135	550	55	550	55	Alive
12	130	530	53	530	53	Alive
		500 mg. per kilo of body weight				
13	155	530	53	530	53	Alive
14	150	510	51	510	51	Sick
15	110	370	37	370	37	Alive
16	120	410	41	410	41	Very sick

TABLE XXXIII - LS-45 - Toxicity of methyl alcohol extract prepared by stirring for 120 minutes.

600 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	115	520	52	520	52	Alive
2	170	770	77	770	77	Dead
3	155	700	70	700	70	Dead
4	130	590	59	590	59	Dead
5	140	640	64	640	64	Alive
6	120	550	55	550	55	Alive
7	96	430	43	430	43	Alive
8	140	640	64	640	64	Alive
9	130	590	59	590	59	Dead
10	135	610	61	610	61	Alive
11	150	680	68	680	68	Alive
12	140	640	64	0	0	Did not eat bait
		500 mg. per kilo of body weight				
13	135	510	51	510	51	Very sick
14	165	630	63	630	63	Sick
15	135	510	51	510	51	Alive
16	120	450	45	450	45	Alive

TABLE XXXIV

Toxicity of extract prepared by soaking for 2 hours before shaking.

500 mg. per kilo of body weight

LS-25

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	130	390	39	390	39	Dead
2	145	440	44	0	0	Did not eat bait
3	130	390	39	390	39	Alive
4	125	380	38	380	38	Dead
		LS-26				
5	165	590	59	590	59	Alive
6	140	500	50	500	50	Sick
7	95	340	34	340	34	Alive
8	145	520	52	520	52	Dead

TABLE XXXV

Toxicity of extract prepared by soaking for 4 hours before shaking.
500 mg. per kilo of body weight
LS-27

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	135	550	55	550	55	Alive
2	135	550	55	550	55	Alive
3	140	570	57	570	57	Dead
4	245	1000	100	1000	100	Dead
			LS-28			
5	220	930	93	930	93	Alive
6	225	950	95	950	95	Dead
7	170	720	72	720	72	Alive
8	165	700	70	700	70	Dead

TABLE XXXVI

Toxicity of extract prepared by soaking for 6 hours before shaking.

500 mg. per kilo of body weight

LS-29

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	170	720	72	720	72	Alive
2	125	530	53	530	53	Dead
3	180	770	77	770	77	Dead
4	140	600	60	600	60	Alive
		LS-30				
5	180	640	64	640	64	Sick
6	220	790	79	790	79	Alive
7	195	700	70	700	70	Sick
8	105	380	38	380	38	Dead

TABLE XXXVII

Toxicity of extracts prepared from >50 mesh powder

LS-13 - 40 minute extraction

800 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	145	680	68	680	68	Dead
2	120	580	58	580	58	Dead
3	180	860	86	860	86	Dead
4	200	960	96	960	96	Dead
		600 mg. per kilo of body weight				
5	160	580	58	580	58	Dead
6	165	600	60	600	60	Dead
7	150	540	54	540	54	Dead
8	140	500	50	500	50	Dead
		500 mg. per kilo of body weight				
9	105	320	32	320	32	Dead
10	105	320	32	320	32	Dead
11	105	320	32	320	32	Sick
12	100	300	30	300	30	Dead

LS-23 - 30 minute extraction

800 mg. per kilo of body weight

1	165	960	96	960	96	Dead
2	140	820	82	820	82	Dead
3	150	880	88	880	88	Dead
4	150	880	88	880	88	Dead

600 mg. per kilo of body weight

5	150	660	66	660	66	Dead
6	135	590	59	590	59	Dead
7	115	500	50	500	50	Dead
8	145	640	64	640	64	Dead

TABLE XXXVIII

Toxicity of extracts prepared from 300 mesh powder.

LS - 14 - 40 minute extraction

800 mg. per kilo of body weight

Rat No.	Rat Weight grams	Bait Fed mgs.	Squill Fed mgs.	Bait Eaten mgs.	Squill Eaten mgs.	Results
1	140	620	62	620	62	Dead
2	115	510	51	510	51	Dead
3	145	640	64	640	64	Sick
4	140	620	62	620	62	Dead
		600 mg. per kilo of body weight				
5	155	510	51	510	51	Sick
6	165	550	55	550	55	Sick
7	155	510	51	510	51	Alive
8	150	500	50	500	50	Alive
		500 mg. per kilo of body weight				
9	106	290	29	290	29	Alive
10	95	260	26	260	26	Alive
11	96	260	26	0	0	Did not eat bait
12	102	280	28	280	28	Alive

LS-24 - 30 minute extraction

800 mg. per kilo of body weight

1	155	790	79	790	79	Alive
2	140	710	71	710	71	Alive
3	165	840	84	840	84	Alive
4	145	740	74	740	74	Alive

600 mg. per kilo of body weight

5	165	630	63	630	63	Alive
6	145	550	55	550	55	Alive
7	180	690	69	690	69	Alive
8	125	480	48	480	48	Alive

Aproved by

C. R. Fellers

R. J. Bouma

Edw. B. Holland

Graduate Committee

Date May 24, 1934