rather than repression of the ionization of sodium hydroxide as has been reported in the literature.

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A STUDY OF THE TOXIC PRINCIPLES OF RED SQUILL.*,[1]

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It is estimated that rats do damage to the amount of many millions of dollars annually, and at times carry disease. For these reasons the extermination of rats is a big problem and one to which the government gives much attention.

For the past several years powdered red squill has been widely used as a raticide. Its popularity is due, perhaps, to the fact that it is acceptable to rats and relatively non-toxic to domestic animals. Its success in rat control work has led to much speculation as to the nature and character of its killing principles.

Our study of red squill was begun in 1931, the object being to isolate and identify the toxic constituent or constituents. A very extended number of extractions have been made using a large variety of solvents. The extracts which were obtained were all tested upon rats to determine their potency. The nature of the squill has made the isolation of its constituents a difficult task. While the chemical nature of the rat-killing principle of squill is as yet unsolved we are able to report that a highly toxic, reasonably stable non-crystallizable product has been obtained and will be briefly described under the experimental part of this paper.

Red and White Squill.—There are two varieties of squill, Urginea maritima, designated as red and white squill. White squill is official in the United States Pharmacopœia XI. The red squill is not official.

White squill is generally preferred for medicinal use although red squill has been used in some countries. There is, however, very little information to be had concerning the medicinal differences in these two drugs.

Red squill is native to those countries bordering on the Mediterranean. It is a perennial herb with fibrous roots proceeding from the base of a large, tunicated, pear-shaped bulb. The bulb is from four to six inches long, often weighing as much as four pounds and usually grows about half immersed in the sand.

Claremont (1) found no essential chemical differences between the red and white squills, but observed that the latter had no action on rats. Winton (2) says, "The cardiac glucoside and the rat killing principle in red squills are distinct substances. The former occurs in about equal amounts in red and white squills. The rat poisoning substance, however, is present in significant amounts in red squills only."

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[1] This paper has been abstracted from a thesis submitted to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June 1938.

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In our work we have observed that red squill will kill rats but that white squill will not. This fact has been repeatedly reported by other workers. It would seem, therefore, that red squill contains some toxic principles which are not present in white squill.

Franklin (3) says, "There appears to be a very considerable difference in the white squill used in medicine and the red squill used for poisoning rats." He also suggests that it is important that they not be confused because of the irritating and paralyzing property of red squill. Winton (2) suggests that, "the properties of the rat poisoning substance have escaped recognition on account of its confusion with the cardiac glucoside."

The Active Constituents.—A variety of names has been applied to the constituents of squill by many investigators. This has led to much confusion. Those names most frequently found in literature are as follows: scillitin, skuline, scillapicrin, scillin, scilladiuretin, scillinine, scillitoxin, scillosterol, scillain, xanthoscillide, scillamarine, scillaren, scillaren-A, scillaren-B and an unnamed compound with the possible formula of $C_{15}H_{20}O_6$. In addition to these, sinistrin, mucilage, sugar, calcium oxalate and a trace of volatile oil have been reported. The presence of a pigment is indicated and we found traces of a fixed oil.

Of the constituents the only one reported as being toxic to rats is the one with the possible formula of $C_{15}H_{20}O_6$.

EXPERIMENTAL.

The red squill used in this investigation was supplied by the K R O Company of Springfield, Ohio. It was a fine powder and in all respects the same as that sold to the trade under the name of K R O.

The several batches of red squill received from this source were all uniformly alike in appearance and toxicity.

The Feeding of Red and White Squill.—A small group of white rats were fed increasing doses of white squill each day for eight days. On the eighth day they received 8000 mg. per kilo. They showed no hesitancy in eating the bait and no symptoms of poisoning of any kind were indicated.

The approximate toxicity of K R O red squill powder was determined by feeding it to both male and female rats. It was found to be fatal to male rats in doses of about 500 mg. per kilo and in doses of about 200 mg. per kilo for the females. Samples of red squill powder from other sources were tested also but none were as toxic as the K R O powder. They likewise varied greatly in potency.

Extraction Studies.—Inasmuch as we were interested in the active constituent of red squill it was necessary to isolate it in some manner. A large number of solvents were tried upon the squill and the extractives carefully tested by feeding. It was found that 80% alcohol was the solvent best suited for extracting the potent principle. This is the solvent which was used in all of our subsequent extractions.

The detailed results of the whole series of solvents which were studied need not be given except to say that acetone and methyl alcohol dissolved a portion of the rat-killing principle. The addition of hydrochloric acid to the menstruum destroyed the toxic substance.

Extraction Procedure.—The procedure for obtaining the potent extract of red squill will now be described.

Twenty-two hundred and fifty grams of the powdered squill are placed in a four-gallon container and macerated with 7000 ml. of 80% alcohol for 24 hours. The mass should be stirred, preferably with a mechanical mixer, at half-hour intervals each two hours in so far as possible. After being allowed to settle, 2500 ml. of the clear superantant liquid are decanted. 3000 ml. more of the menstruum are then added and the maceration and stirring continued for a second 24-hour period. After settling, the clear supernatant liquid is again decanted and the mass transferred to a filter and allowed to drain. The filter is thoroughly washed with 80% alcohol. The combined alcoholic extracts are next concentrated to a thick syrup at a temperature not exceeding 80°.

The syrupy concentrate is next dissolved in 5000 ml. of water and two pounds of animal charcoal added. This mixture is stirred occasionally for two hours and then filtered. The filter is finally washed carefully with 500 ml. of cold water. This is done for the purpose of removing as much of the soluble inert substances as possible without dissolving out the potent principle which has been absorbed by the charcoal.

The moist charcoal is next transferred to a large bottle and 2000 ml. of 80% alcohol are added. The mass is shaken at ten-minute intervals for two hours. The potent principle is removed from the charcoal by the menstruum. The mixture is then filtered, the filter being carefully washed with 1000 ml. of 80% alcohol. The alcoholic filtrates are then evaporated to a syrupy consistency at 80° and spread in thin layers on pill tiles. The tiles are placed in an oven, kept at a temperature of 80°. When the extract has dried, it is carefully scraped from the tiles, powdered, weighed and carefully preserved in a tightly stoppered bottle.

Care should be taken in scraping the dried, irritating, potent extract from the tiles. The wearing of a mask during this part of the procedure is advised.

The extract obtained in this manner generally killed male rats in doses of 10 mg. or less per kilo body weight. The females were killed with about half this dose, hence the extract was approximately fifty times more potent than the squill powder.

Purification of the Extract.—A purified, highly potent product was obtained by dissolving the extract, just described, in 80% alcohol. Upon the addition of an equal amount of anhydrous ether, the mixture became cloudy which upon being shaken deposited a brownish, resinous mass. The alcohol-ether solution was filtered and evaporated to dryness. The resinous precipitate which remained behind was redissolved in 80% alcohol and also carefully evaporated to dryness. These two residues were brownish, resinous masses, the alcohol-ether soluble portion accounting for about forty per cent of the original extract while the insoluble precipitate accounted for the remaining sixty per cent.

Both of these products were fed to rats. The results obtained are given in Table I, following. "Extract A" is that described above as the alcohol-ether soluble extract. "Extract B" is that described as the alcohol-ether insoluble extract.

TABLE I.

No.	Product Fed.	Sex.	Weight Gm.	Dose Mg. per Kilo.	Dose Mg. per Rat.	Results.
1	Extract A	M	470	5	2.350	Dead
2	Extract A	M	498	5	2.450	Dead
3	Extract A	M	404	5	2.020	Dead
4	Extract A	M	386	5	1.930	Dead
5	Extract A	M	218	5	1.090	Dead
6	Extract A	M	224	5	1.120	Dead
7	Extract A	M	344	5	1.720	Sick. Recovered
8	Extract A	M	428	3	1.284	Sick. Recovered
9	Extract A	M	370	3	1.110	Sick. Recovered
10	Extract A	F	336	5	1.680	Dead
11	Extract A	F	318	3	0.954	Dead
12	Extract A	F	284	3	0.852	Dead
13	Extract A	F	270	3	0.810	Dead
14	Extract A	F	238	3	0.714	Dead
15	Extract A	F	217	3	0.636	Sick. Recovered
16	Extract A	F	218	3	0.654	Dead
17	Extract A	F	234	3	0.702	Dead
18	Extract A	F	230	3	0.690	Dead
19	Extract A	F	248	2	0.596	Dead
20	Extract A	F	228	2	0.456	Sick. Recovered
21	Extract B	F	208	10	2.080	No symptoms
22	Extract B	F	270	50	13.500	No symptoms
23	Extract B	F	210	50	10.500	No symptoms
24	Extract B	F	200	50	10.000	No symptoms
25	Extract B	F	314	50	15.700	No symptoms

It will be seen from the results indicated in Table I, that the alcohol-ether soluble extract is a very potent extract being approximately one hundred times as toxic as the original red squill powder, 2 mg. to 3 mg. per kilo body weight being fatal to female rats and about twice that amount being fatal to males.

The potent extract, just described, was non-crystalline. The usual qualitative tests for carbon, hydrogen, oxygen, phosphorus, sulfur, nitrogen and the halogens were made and carbon, hydrogen and oxygen were found to be present. Further purification procedures are contemplated with a view to obtaining the toxic principle in a more highly refined state.

SUMMARY.

1. Temperatures up to 100° are not injurious to the toxic principles of red squill.

2. The rat-killing principle of red squill is extracted from red squill by a menstruum comprised of 80% alcohol.

3. Both animal charcoal and activated charcoal adsorb the rat-killing principle from an aqueous solution. It may be recovered from the charcoal by treating the latter with 80% alcohol.

4. A highly potent product has been obtained which possesses a toxicity one hundred times that of red squill powder from which it is obtained.

5. It has been shown that female rats are killed with about half the dose of red squill powder and squill extracts that is required to kill male rats.

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