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RED-SQUILL POWDERS AS RATICIDES
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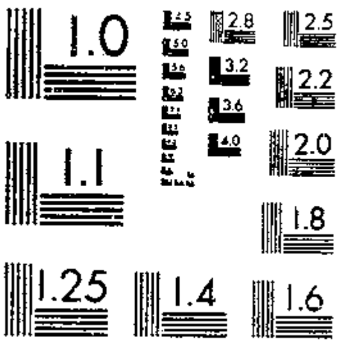

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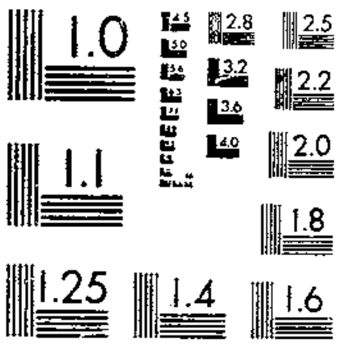

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TECHNICAL BULLETIN No. 134 NOVEMBER, 1929

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WASHINGTON, D. C.

RED-SQUILL POWDERS AS RATICIDES

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PURPOSE OF INVESTIGATION

The principal requirements for an ideal rat poison are safety, effectiveness, and economy. The rat poisons in most general use to-day (arsenic, barium carbonate, phosphorus, and strychnine) have toxic properties that seriously menace the safety not only of wild and domestic animals, but also of human beings. Red squill is relatively safer and more nearly approaches the ideal rat poison. Animals other than rats usually refuse to eat red squill in the concentrations used for rat baits. The difficulty of obtaining a uniformly toxic preparation of red squill has retarded its development for this purpose. To contribute to the information needed for the production of a potent red-squill preparation for the efficient destruction of rats, the investigation here reported was undertaken.

Acknowledgment: The work here reported was a cooperative study by members of the Bureaus of Chemistry, Biological Survey, and Plant Industry. The writers wish to express their thanks to the following for assistance in various ways during the four years of the investigation: Bureau of Chemistry—N. G. Barbella, R. M. Hann, D. B. Jones, G. L. Keenan, B. A. Linden, E. W. Schwartze, K. A. Smith, and C. G. Spencer; Bureau of Biological Survey—C. C. Carr, F. N. Jarvis, and M. A. Stewart; Bureau of Plant Industry—O. F. Black, J. W. Kelley, A. F. Sievers, and W. W. Stockberger.

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SOURCE AND STRUCTURE OF SQUILL

Squill, *Urginea maritima* (L) Baker, also called *U. scilla* Steinheil, is a perennial plant growing wild on the coast of southern Italy, Sicily, and Sardinia, and elsewhere along the Mediterranean Sea. The bulbs produced are pear shaped, usually from 3 to 6 inches in diameter and from 300 to 2,000 grams in weight (pl. 1), and are composed of closely overlapping fleshy scales. The outer scales are dry, brittle, and reddish brown; the inner scales vary from light yellowish white to deep cherry or mahogany; and the central ones (core) are usually white.

There are two commercial varieties of squill—white squill, which is marketed as dry, white scales and is the product official in various pharmacopœias, and red squill, which until recently was obtainable only in the fresh state. Both the viscous, mucilaginous juice and the dry scales of each variety are irritating to the skin. This stinging effect upon the skin, as well as the acrid prickly taste, is commonly attributed to calcium-oxalate raphides. White squill is used in human medicine as a heart tonic, emetic, diuretic, and nauseant expectorant. Various European investigators have recommended red squill as a rat poison.

Microscopic examination of squill was made by George L. Keenan, of the Food, Drug, and Insecticide Administration, who states:

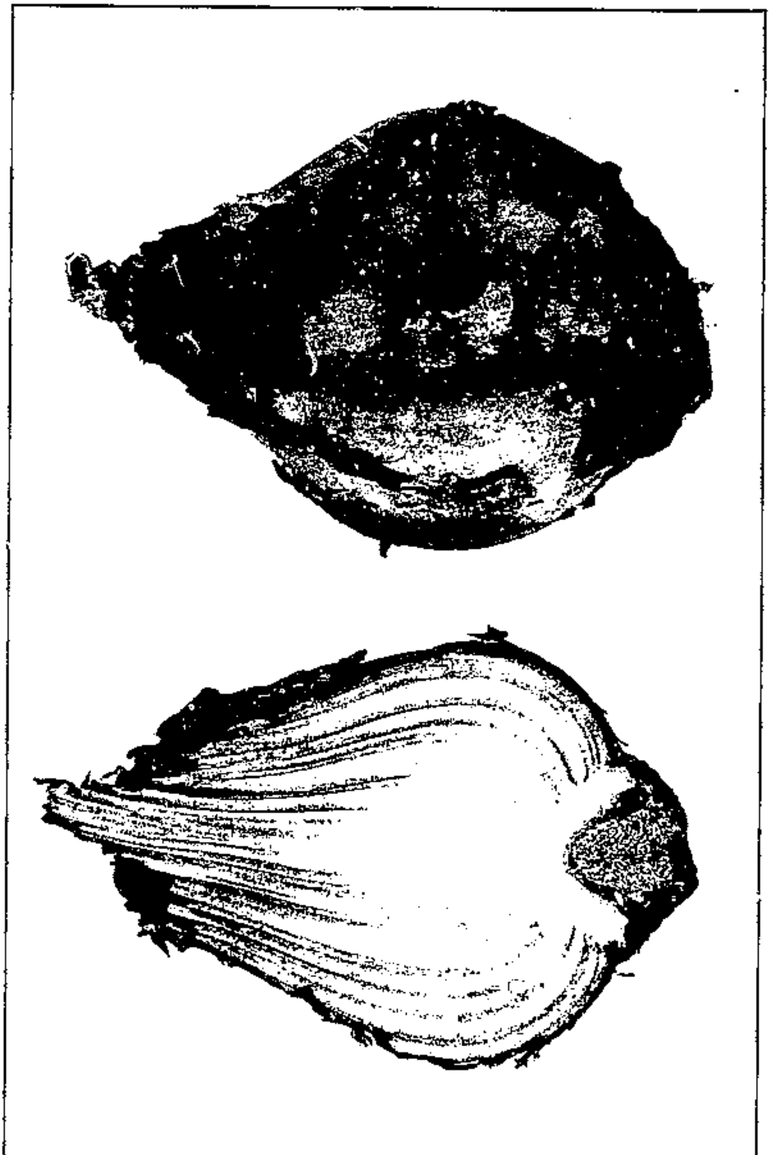
> A microscopic examination of red-squill powder shows that the anatomical structure is similar to that of white squill. The powdered bulb of both varieties consists of fragments of parenchyma cells, spiral vessels, and long raphides of calcium oxalate, some of which are surrounded by dried masses of transparent refractive material, the mucilage sheath. The longest acicular crystals are approximately 1 millimeter long. Short raphides are present also. The greatest diagnostic difference is the presence of pigment cells in the red-squill scales and their absence in the white. The red pigment cells are numerous in the scales of the red squill and are readily found in the powdered material.
>
> To facilitate its identification, the red squill was reduced to a fine powder and examined microscopically in a drop of menstruum consisting of an acidified chloral-hydrate-glycerol solution (made by dissolving 45 grams of crystals of chloral hydrate in 25 cubic centimeters of dilute hydrochloric acid (1 : 8) and 10 cubic centimeters of glycerol). The slide was not warmed. The red coloring matter gradually diffused out of the cells, giving the pigment masses a reddish appearance. Long raphides of calcium oxalate were found in the dried mucilaginous material, many in close proximity to the red masses.
>
> When a powdered dry bait containing 5 per cent of red squill was examined according to the method just described, the pigment cells were readily found.

RESULTS OF PREVIOUS INVESTIGATIONS

CHEMICAL

An exhaustive review of the chemical studies on squill since 1741 is given by George (*12*[1]). The active principle has not been definitely isolated and identified. Many of the products obtained by extraction with various solvents have proved to be impure mixtures. In many instances it is not plain whether red or white squill was studied. George states that the active constituents of squill are (1) scillitin, $C_{17}H_{25}O_6$, a yellow, very bitter, nitrogen-free glucoside, slightly soluble in water (0.18 per cent) and soluble in alcohol, which was isolated

[1] Reference is made by italic numbers in parentheses to "Literature cited," p. 35.



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RED-SQUILL BULB (URGINEA MARITIMA)

by Kopaczewski (*18*) as the toxic principle of squill; (2) scillidiuretin, a strong diuretic, also isolated by Kopaczewski; (3) xanthoscillide, a yellow crystalline glucoside, insoluble in chloroform, but soluble in boiling alcohol, isolated by Buschmann (*2*); and (4) a water-soluble toxic substance isolated, but not identified, by Ewins (*8*). George obtained a red glucoside from African squill, *Urginea burkei*, which corresponded in properties to Merck's scillitoxin. (Scillitoxin has not been chemically identified as a definite chemical entity.) Galavielle and Cristol (*11*) found the same chemical principle in *Scilla autumnalis* L. grown in the neighborhood of Montpellier, France, as in *S. maritima*. A patent has been granted to Stoll and Suter (*27*) for a process of isolating a physiologically active glucoside, "Scillaren," from white squill. The chemical composition and formula are not given in the patent.

PHYSIOLOGICAL[1]

Lewin (*21*) reported that animals receiving squill showed a slower pulse, dyspnea, and pyrexia, and died on account of respiratory failure. Danysz and Kopaczewski (*6*) stressed the paralysis of the hind limbs, convulsions, and characteristic gyrations of rats following the consumption of squill. They state that the respiration is greatly depressed and that after several gasps the animal dies. Gunn and Heathcote (*14*) found that the subcutaneous minimum lethal dose of the glucoside prepared by Ewins's process (*8*) was 5 milligrams per kilo of body weight for the rabbit and 150 milligrams per kilo for the rat; also, that much stronger concentrations were required to stop the isolated rat heart than to stop the isolated rabbit heart in the same space of time. They concluded:

> It is, of course, still possible that the rat may be more susceptible than other domestic animals to squill taken by the mouth; but this, if true, must be due, say, to some constituent of squill other than the glucoside or to some difference in action than the action on the heart. The possibility that this alleged hypersusceptibility of the rat may be due merely to the fact that the rat is the only domestic animal that will eat squill has not been sufficiently excluded * * *. The rat is markedly less susceptible than the rabbit.

The present experiments tended to confirm the conclusions of Gunn and Heathcote. Death following squill is apparently produced by respiratory rather than cardiac action.

TOXICITY

The toxicity of squill (whether of the red or of the white variety is not always stated) has been studied by a number of investigators since the first experiments by Orfila in 1818 (*22, v. 2, pp. 99-102*). The reports are summarized in Table 1.

The great apparent variation in toxicity may be attributed to differences in products, in methods of preparation, and in administration. Relatively enormous quantities of squill are apparently required to injure animals other than rats.

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[1] While this bulletin was being prepared two articles by Winton (*28, 29*) were published. Winton concluded that "the cardiac glucoside and the rat-poisoning 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."

TABLE 1.—*Effect of squill preparations upon animals (reported in literature)*

Reference	Animals	Product	Dose	Result
Lewin (*21*)	Rats	Dried bulb	1 gram	Death.
		Extract	6 grams	Do.
Kopaczewski (*18*)	do	Dried bulb	830 mg./kg	Do.
		Scillitin	1 mg./kg	Do.
Claremont (*5*)	do	Red-squill powder	260 to 1,800+mg./kg	Do.
		White-squill powder.	9,000 mg./kg	Survived.
		Urginea burkei powder.	2,000 mg./kg	Death.
Dujardin-Beaumetz (*7*).	do	Squill	10 mg	Do.
Howarth (*16*, pp. 16–67).	do	Scillitin	0.02 to 0.05 mg	Do.
		Squill	32 mg	Do.
Orfila (*22*, v. 2, pp. 99–102).	Dogs	Fresh bulb	2½ ounces	Do.
Lewin (*21*)	Guinea pigs	Scillitoxin	0.5 mg	Do.
Hertwig (*10*, p. 190)	Dogs and rabbits	do	100 to 500 mg	Do.
	Dogs, cats, and hogs	Fresh bulb	0.25 to 2 grams	Vomiting, diarrhea, diuresis.
	do	do	45 grams	Death.
	Horse and cow	do	30 grams	Diarrhea.
	2 horses	do	60 grams	Death.
Kopaczewski (*18*)	Guinea pigs	Scillitin	1 mg./kg	Do.
Claremont (*4*)	Dogs	do	do	Do.
	Cats	do	2 mg./kg	Do.
	Rabbits	do	2½ mg./kg	Do.

USE OF SQUILL AS RAT POISON

The Danish antirat law of 1907 forbade the use of strychnine, arsenic, and sulphurous preparations in rat control but permitted various squill preparations. Aumann (*1*) lists nine commercial squill preparations offered for sale in Germany in 1912; he also states that practice had confirmed the laboratory finding that squill preparations are twice as efficient as raticides as are the bacterial poisons. Smith (*26*) found that only one of five chemical extracts on the English market in 1921 killed rats. Aqueous, alcoholic, and acetic-acid extracts of dried white squill failed to kill rats, although each of three alcoholic extracts of dried red squill proved fatal.

Claremont (*4, 5*) reported an intensive study of squill as a rat poison. The lethal doses of various red-squill powders ranged from 260 to more than 1,800 milligrams per kilo of body weight to rats and did not seem to bear any relation to the chemical characteristics of the preparations. The reducing sugars ranged from 3.32 to 10.45 per cent, and the total sugar after inversion from 6.94 to 73.73 per cent. Hydrochloric acid intensified the red color of the extract. Some powders and pastes on the British market were found to be toxic to rats. White-squill powders failed to kill in doses of 9,000 milligrams per kilo of body weight. South African squill, *Urginea burkei*, gave more mucilage than the ordinary varieties, and only one of three powders killed, the lethal dose of it being 2,000 milligrams per kilo of body weight. A drying temperature of 60° C. appeared to produce a satisfactory powder, although the lethal principle was not destroyed in preparing squill biscuits at temperatures around 100°. The powder preserved its toxicity when stored in air-tight containers. Cold and hot water extracts were toxic, but alcohol failed to extract the toxic principle. Wild rats were rather more susceptible than tame rats. Microscopic examination showed no specific characteristic except the large number of raphides. Claremont concluded that the toxicity of squill preparations can not be inferred from analytical results but must be determined by feeding experiments on rats.

Kobert (*17*) quotes Kunkel's findings that red squill is more potent than white, that the outer scales act more intensely than the inner scales, and that the bulbs gathered in summer are more potent than those collected in the spring.

Lantz (*19*), Lereboullet (*20*), George (*12*), Howarth (*16*), and Schlupp (*23*) report the suitability of red squill as a rat poison under various conditions. Howarth states further that "it is, moreover, regarded as comparatively harmless to domestic animals." W. K. Tonkin, rat officer of Middlesex County, England, reports[4] that

> phosphorus pastes, barium carbonate, and one or two proprietary poisons have on occasion been used, but freshly extracted squill, both as a lethal rat poison and by reason of its being comparatively harmless to domestic animals, has been found of greater use than any other poison.

Boulenger (*3*) found that squill solution was the most effective rat poison, being three times as toxic as barium carbonate to rodents and also less harmful to domestic animals.

Finally, the British Ministry of Agriculture and Fisheries (*13*) recommended the use of squill:

> Baits containing barium carbonate or red squill * * * are recommended by the ministry, and if used as directed, and with proper precautions, are less dangerous than baits containing such poisons as arsenic, strychnine, and phosphorus * * *. Red squill is the safer, and it is advisable to use it in preference to other toxic agents for application on farms and in places where special care is necessary owing to the presence of poultry, livestock, domestic animals, or stored food supplies * * *. Owing to the peculiar chemical nature of red squill in its various forms, as sold for the destruction of rats and mice, users of these preparations should satisfy themselves that they obtain guaranteed toxic red-squill raticides.

Recapitulating, the literature indicates that red squill is toxic to rats whether fed as the fresh bulb or as a powder, that it is essentially nontoxic to animals other than rats, and that white squill is nontoxic to rats (*25*). Apparently, the toxic principle has not been consistently isolated by chemical methods.

EXPERIMENTAL PROCEDURE

PREPARATION OF POWDER

In the preliminary experiments, 24 powders were prepared from samples taken from a 900-pound keg of squill bulbs imported from Cagliari, Sardinia, in the fall of 1923 and kept in storage at 4° to 5° C. This entire series of powders was prepared by Otis F. Black and James W. Kelley, of the Office of Drug, Poisonous, and Oil Plants, Bureau of Plant Industry, United States Department of Agriculture.

The outer, dry, reddish-brown husks, which were found to be less toxic than the inner scales, were stripped from all bulbs. The bulbs were sliced and, in most instances, air-dried for several hours and placed in long glass tubes. A current of air was passed over the surface. The temperature was increased over a period of several days from room temperature to a given maximum, which was maintained until constancy in weight indicated that the products were thoroughly dried. They were then removed and ground in a drug mill until the powder passed through a 40-mesh sieve. These powders contained 1 to 2 per cent of moisture. After being ground, all powders were placed in tightly closed screw-top vials. Details of time and temperature are given in Table 2.

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[4] Personal communication.

TABLE 2.—*Method of preparation of red-squill powders*

P. C. No.	Date made	Bulbs from which powder was made [1]	Previous treatment	Condition when used	Preliminary exposure to air at room temperature	Drying conditions					Remarks
						Electric oven	Vacuum oven	Current of hot air	Steam oven	Temperature	
Series 1:	1923					*Hours*	*Hours*	*Hours*	*Days*	°*C.*	
11	August	C	Just received from Sardinia.	Fresh	Yes	8				60-80	
	1924										
16	Jan. 15	C	Stored at room temperature since August, 1923.	Sprouting; no decomposition.	7 days	8				60	
17	do	C	do	do	do		8			80-95	
18	do	C	do	do	do			8		60-80	
19	do	C	do	do	No	72				70	
20	do	C	do	do	No	8		72		60-60	Dried in current of hot air, then in oven at 60° C.
21	do	C	do	do	No		72			80	
22	do	C	do	do	Yes	8				70	Husks.
23	do	C	do	do	No	8				70	Core.
24	Feb. 19	C	Stored at 5° C. since August, 1923.	Slight decomposition	5 days	8		16		80, 20-65	Dried in current of hot air, then in oven at 80° C.
25	do	C	do	do	do	48				60	
26	do	C	do	do	do	48				80	
27	do	C	do	do	do	24				100	
28	do	C	do	do	do	8				110	
29	do	C	do	do	do	8				120	
30	Feb. 27	C	do	do	do		16			80	
31	Mar. 1	C	do	do	10 days		12			100	
32	do	C	do	do	do		8			110	
33	do	C	do	do	do		8			120	
34	do	C	do	do	do		8			140	
35	Apr. 7	C	do	do	45 days	12		8		85	Dried in current of hot air, then in oven at 85° C.
36	do	C	do	do	do		12			100	
37	Apr. 12	S	do	Sprouting; slight decomposition.	3 days	8		8		80, 20-65	Dried in current of hot air, then in oven at 80° C.
38	do	S	do	do	do	8		8		80, 20-65	Do.

Series 2:											
719 A	1925 Dec. 7	C	Sardinian bulbs; held at room temperature since October, 1925.	Fresh	No	68				80	Composite scales; cores removed.
B	do	C	do	do	68 hours	48				80	Do.
C	do	C	do	do	do	48				80	Cores only.
AF	Dec. 18	C	do	Alcoholic fermentation	No	74				80	
AAF	1926 Jan. 7	C	do	Acetic acid fermentation.	No	216				80	
720 A	1925 Dec. 12	C	Sardinian bulbs; stored at 5° C. since October, 1925.	Slight fermentation	No	46				80	Whitish-pink bulbs.
B	do	C	do	do	46 hours	93				80	Do.
721 A	do	C	do	do	No	46				8	Pink-red bulbs.
B	do	C	do	do	46 hours	93				80	Do.
722 A	do	C	do	Slight decomposition	No	46				0	Deep-red bulbs.
B	do	C	do	do	46 hours	93				80	Do.
723 A	do	C	do	do	No	46				80	Nearly red bulbs.
B	do	C	do	do	46 hours	93				80	Do.
724 A	1926 Jan. 8	C	do	Fresh	No				4	70	Whitish-pink and pink-red bulbs.
B	do	C	do	do	8 days				4	70	Do.
725 A	do	C	do	do	No				4	70	Nearly red and deep-red bulbs.
B	do	C	do	do	8 days				4	70	Do.
727 A	do	C	do	do	No	96				80	All colors; mixture 724 and 725 composite.
760	Feb. 25	C	do	do	No				12	70–80	All colors.
762	Mar. 3	C	do	do	No	40				120	From composite all colors as P. C. 761.
763	do	C	do	Frozen	No	63				100	Do.
764	Mar. 8	C	do	do	No	41				80	Do.
765	Mar. 11	C	do	do	No	64				60	Do.
766	Mar. 3	C	do	Fresh	No					Room.	From composite all colors as P. C. 761; 14 days in desiccator over $CaCl_2$.
767	Mar. 10	C	do	Acetic acid fermentation.	No				10	80	Rest of 722 and 723, combined.
768	do	C	do	do	No				10	80	Rest of 720 and 721, combined.
770	Mar. 4	C	do	Slight fermentation	No				25	40–50, 80	First 3 days at 40–50° C., then at 80° C.
774	Mar. 17	C	do	Frozen	No	47				120	From composite P. C. 761; duplicate of P. C. 762.
775	Mar. 10	C	do	Fresh	No				10	80	Rest of P. C. 770.
782	Mar. 30	C	do	Frozen	No	67				80	From composite P. C. 761; duplicate of P. C. 764.

[1] Composite (C) or single (S).

TABLE 2.—*Method of preparation of red-squill powders*—Continued

P. C. No.	Date made	Bulbs from which powder was made	Previous treatment	Condition when used	Preliminary exposure to air at room temperature	Drying conditions					Remarks
						Electric oven	Vacuum oven	Current of hot air	Steam oven	Temperature	
Series 2—Con.	1926					*Hours*	*Hours*	*Hours*	*Days*	*°C.*	
785	Apr. 12	[2] S	Sardinian bulbs; stored at 5° C. since October, 1925.	Frozen	No	120				80	
786	do	[2] S	do	do	No	120				80	
787	do	[2] S	do	do	No	120				80	
788	do	[3] S	do	do	No	120				80	
789	do	[3] S	do	do	No	120				80	
790	do	[3] S	do	do	No	120				80	
817	June 7	C	Stored at 5° C. since October, 1925.	Fresh; not frozen	No	48				120	From composite P. C. 816.
818	do	C	do	do	No	48				100	Do.
819	do	C	do	do	No	48				80	Do.
820	do	C	do	do	No	48				60	Do.
841	Aug. 16	C	do	P. C. 840 used; fresh, not frozen.	No	168				80	From composite P. C. 840.
842	do	C	do	P. C. 840 used; frozen 2 days.	No	72				60	Do.
843	do	C	do	do	No	72				80	Do.
844	do	C	do	do	No	72				100	Do.
845	do	C	do	do	No	72				120	Do.

[2] Large bulbs. [3] Small bulbs.

As the results of these preliminary experiments indicated that squill is of value as a rat poison, the investigational work was continued, material from a second shipment of approximately a ton of squill bulbs from Sardinia being used. Upon reaching the United States in the fall of 1925, the 200-pound bags of squill were stored at 4° to 5° C. Samples were drawn from time to time for the preparation of powders.

Essentially the same method of preparation was used as in the preliminary experiments. As previous investigation had indicated the desirability of using high temperatures in order to check fermentation or enzyme action quickly, an electric oven that could be set to within 1° was used for drying most of the powders in this series. In some instances it was desirable to duplicate commercial conditions. For such drying a commercial steam drier was used. Dried material was ground to pass through a 40-mesh sieve and packed in jars. The approximately ¼-inch-thick slices obtained in the commercial runs by using a hand-operated slicing machine proved to be too thin, as a large quantity of mucilaginous juice was exuded, facilitating fermentation and materially retarding drying. Slices half an inch thick were found to be more satisfactory.

By using the method finally adopted for the preparation of squill powders (p. 33), it has been possible to reproduce powders having the same toxicity.

METHOD OF FEEDING

Most of the toxicity determinations were made on white rats. Wherever feasible, results on wild (brown) rats were also obtained. All rats were held in cages for about a week to make the experimental animals essentially uniform. No rats weighing less than 100 grams each, and when possible only those that weighed between 150 and 250 grams each, were used. None of the rats received food for 18 hours, but all were given water freely, to insure a somewhat constant appetite and to favor the rapid consumption of the experimental diet. As previous experiments had indicated that rats readily consume 1 per cent of their body weight, efforts were made to feed approximately this quantity of total food.

Animals were weighed and placed in separate cages. The ordinary laboratory rat food—99 per cent of a mixture of whole-milk powder (one-third) and whole wheat flour (two-thirds) and 1 per cent of salt—was passed through a 40-mesh screen. The sample of squill to be fed was thoroughly mixed with this food, so that approximately 1 per cent of the animal's body weight would be fed in giving the desired dose of squill. The weighed squill-rat-food mixtures were placed in individual glass sponge cups. The time when these cups were placed in the cages was noted, and frequent inspections were made to determine the time at which all food had been consumed. The dish was then removed and the rat left without food and water until the next morning.

Frequent inspections were made to determine the onset of symptoms of squill poisoning. Several times the fatal dose of fresh scales or of food containing squill powder has been readily eaten. Concentrations up to 40 per cent of squill powder in rat food have been rapidly and completely devoured, indicating that the presence of calcium-oxalate raphides does not deter a starved rat from eating these products. On the day following the feeding of an experimental diet, animals that were not in an obviously depressed condition or that were not gyrating viciously were offered the regular diet and water. All rats that died within five days after having been fed squill, and in which squill symptoms had been produced, were considered to have been killed by squill.

The detailed results obtained from about 3,000 white and 400 wild (brown) rats are reported in Tables 3, 4, and 5. The preparations as made were given "P. C." (pharmacology card) numbers to identify them. As these experiments were carried on over several years, the date of each series of feeding tests is given to show that there was at least a general agreement in susceptibility of test animals throughout the entire investigation. The quantities of dried squill powder fed are given in grams. The numerator of each fraction shows the number of rats dying from the indicated dose within a period of five days; the denominator shows the total number of rats fed the indicated dose. The MLD (minimum lethal dose) was selected as the smallest dose of a dried squill powder, in milligrams per kilogram body weight, that killed all, or practically all, the rats within five days.

EFFECTS OF SQUILL ON RATS

Rats that have eaten a toxic dose of squill soon become somewhat lethargic. From 4 to 14 hours later they usually exhibit characteristic tremors and sensory depression in the hind legs. This is followed by progressive paralysis of the trunk and forelegs. Respiration increases in rate and becomes labored. The animal next starts a peculiar, extremely characteristic rotation on its long axis. These gyrations have continued, at intervals, for half an hour and sometimes for 24 hours. The animal rolls over and over for some time, and then stops, apparently through fatigue. After resting, it resumes this gyration. Stimuli, such as a current of air, cause prompt resumption of gyrations. Few rats that showed this symptom recovered. Post-mortem examinations of a number of animals indicated acute cardiac dilatation, and hyperemia of the gastrointestinal tract and omentum. Although no cause for death other than acute cardiac dilatation was observed, in some instances the heart beat was detected after the cessation of respiration, indicating that respiratory paralysis played a part in producing death by squill.

RESULTS OF INVESTIGATION

During the progress of this investigation a number of individual problems were studied. For clarity of presentation, the results obtained in each separate study are reported under separate headings. The data given in Tables 6 to 13 are drawn from the results in Tables 2, 3, 4, and 5.

TABLE 3.—*Toxicity of red-squill powders to rats* [1]

WHITE RATS

P. C. No.	Month of feeding	Minimum lethal dose	Toxicity of powder in quantities of milligrams per kilo of body weight																							
			25	50	75	100	150	200	250	300	350	400	500	625	750	1,000	1,250	1,500	1,750	2,000	2,500	3,000	3,500	4,000	5,000	6,000
	1924	*Mg. per kgm. of body weight*																								
11	February	500							0/3	0/1		1/2	3/3													
16	do	750										1/3	1/3		6/6	3/3		3/3								
17	do	625											4/6	2/2	4/4											
18	April	500				0/1		0/3	3/5	1/3	0/3	3/4	7/8	2/2	6/6											
19	February	2,000+											0/2		1/4	0/1				1/2						
20	do	1,000										0/1	0/2	0/1	1/4	4/4	2/2			3/3						
21	do	1,000											0/2		1/2	2/2				2/3						
22	do	3,000+						0/1				0/1	0/1		0/2	0/1	0/1			0/1		0/3				
23	do	3,000+											0/2		0/2	0/4				0/2		0/3				
24	April	3,000+							1/6				2/6		2/6	1/6		0/3		0/3		1/3				
25	March	1,500													0/2	3/9	1/7	2/2								
26	do	1,000											0/1		1/5	5/5										
27	do	2,000						0/1			0/2		3/11		2/9	4/9	0/3	3/6		2/2						
28	do	2,500+														1/4		1/3		4/9	2/6					
29	do	3,000+											0/1			0/3				1/7	0/5	2/3				
30	do	750							1/1		0/2		2/7		4/4	1/1										
31	do	1,500+							0/3				1/5		0/6	2/11	1/3	4/6								
32	do	750						0/1			0/2		2/5		3/3	3/3										
33	do	750									0/2	1/1	2/4		3/3	2/2										
34	do	2,000											0/1			0/2		1/4		2/2						
35	April	1,750											0/3			1/3		2/5	3/3	5/6						
36	do	2,000											0/3			0/3		1/3	1/2	4/4	1/1					
37	do	3,000											0/3			0/3		0/3	3/3	0/5	1/3	2/2				
38	May	250				4/13	4/5	4/6	6/6			3/3	2/3			3/3		3/3		3/3						
	1925																									
714	October	4,000											1/6			2/6		2/6		1/5	3/3	2/5		5/6		1/1
715	September	6,000+											0/6			0/6		0/5		0/5		0/3		0/5		0/5

[1] Numerator of each fraction shows number of rats dying within five days; denominator shows total number of rats fed indicated dose.

TABLE 3.—*Toxicity of red-squill powders to rats*—Continued

WHITE RATS—Continued

P. C. No.	Month of feeding	Minimum lethal dose	Toxicity of powder in quantities of milligrams per kilo of body weight																							
			25	50	75	100	150	200	250	300	350	400	500	625	750	1,000	1,250	1,500	1,750	2,000	2,500	3,000	3,500	4,000	5,000	6,000
		Mg. per kgm. of body weight																								
719 A	1925 December	1,000								0/2		1/3	1/3	0/2	2/3	1/1			1/1							
	1926 March								0/1					0/1	3/5	3/3		4/5								
	August													5/5	4/5	4/5										
	September												1/5		0/5											
	October												4/5	4/5	5/5	5/5										
	November											1/2	5/5	3/3												
719 B	1925 December	625				0/1		0/3	1/2	0/2		3/6	5/7	1/1	1/1	1/1										
	1926 August													5/5	5/5	5/5										
719 C	1925 December	1,000						0/2					0/2		1/2	1/1				1/1						
719 AF	1926 February	2,000						0/1			0/3		0/4		0/3	3/9	0/4	8/15		1/1	1/1					
719 AAF	do	2,000							0/6					0/2	0/3	0/5	3/8	7/9		5/5	1/1					
720 A	1925 December	250				0/6	6/10	5/7		1/1			1/1		1/1	1/1										
	1926 June						2/5	12/15	4/5																	
720 B	1925 December	250				0/18	11/16	5/6		2/2																
	1926 June						0/5	13/15	4/5																	

721	A	1925 December	350				0/1		1/6	5/9	1/1			1/1		1/1											
		1926 June								5/5	4/5	11/11															
		November					0/1	0/2		1/4	7/8	4/5															
	B	1925 December	400					0/2	0/1	2/4		6/6		5/6													
		1926 February							3/5	3/5	4/5					5/5											
		June									1/5	8/10	5/5														
722	A	1925 December	500+				0/2		0/2	2/2	1/1			1/1		1/1	1/1										
		1926 July								0/5	0/5	3/10		5/10													
		November					0/3		3/5					2/5													
	B	1925 December	350					0/1	0/1	1/3																	
		1926 February								5/5			4/5														
		March							0/5	5/5	5/5		5/5														
		July										1/6															
		October								4/5	4/5	5/5															
		December							0/10																		
723	A	1925 December	1,000+						0/2	1/4	1/1			1/1		1/1	1/1										
		1926 February										2/5															
		March										0/5	2/5	2/5	1/3	2/3	2/2										
		July								0/5					2/5	3/5											
		September														3/15	1/5										
		November														0/1	2/4										
	B	1925 December	500						0/1																		
		1926 February							1/3		3/3																
		March							0/2		1/3		1/7	5/5													
		July								1/5				5/5	4/5	5/5											
		December						1/5	0/5	0/5	0/5					5/5	5/5										
724	A	March	500										3/5	5/5													
		May										1/3		2/3		3/3	3/3										
		July												4/5	5/5	3/3											
		September												5/5													
	B	April	750						0/3	4/6			2/3	3/3		3/3											
		September												1/5	2/5	8/10											

TABLE 3.—*Toxicity of red-squill powders to rats*—Continued

WHITE RATS—Continued

P. C. No.	Month of feeding	Minimum lethal dose	Toxicity of powder in quantities of milligrams per kilo of body weight																						
			25	50	75	100	150	200	250	300	350	400	500	625	750	1,000	1,250	1,500	1,750	2,000	2,500	3,000	3,500	4,000	5,000	6,000
		Mg. per kgm. of body weight																								
	1926																									
725 A	April	500							2/3			1/3	3/3	2/3	2/3											
	May										0/3		2/3		3/3	3/3										
	September											4/5	5/5	5/5	5/5											
	October												4/5													
	November						0/2		1/3	6/6			2/2													
	December									5/5																
	1927																									
	January							5/13	6/12																	
	1926																									
725 B	April	625							2/3			3/3	7/11	3/3	3/3											
	September											3/5	5/5	4/5	4/5											
	November							3/6		7/9																
727A	March	500							0/5			0/4	3/4	2/2		2/2										
	September											2/5	11/11	5/5	5/5											
748	March	5,000+							0/2							0/1		0/1		0/1		0/1			0/5	
749	do	200					1/3	3/3	3/3				3/3		1/1	4/4		1/1								
750	do	4,000+							0/3				0/3			0/3		0/3		0/3	0/6	0/1		0/1		
751	do	3,500														0/3				0/4			2/2		3/3	
752	April	5,000+																0/3				0/3			0/3	
753	March	5,000+														0/3									1/3	
754	do	750								0/3			2/5		5/5	3/3		4/4		1/1	1/1	2/2				
	November													4/5												
756	February	625							0/3	2/3		1/3	5/8	3/3	3/3	3/3										
	April								3/3			0/3	3/3	6/6												
	November												4/5													
759	February	1,500				0/6			0/3				0/3		2/9	3/3		1/1								
	April															1/3	2/3	3/3								
	November																4/5									
760	March	500						2/5	2/6	6/14	1/5		3/3		3/3											
762	do	750							0/3			3/3	4/9		6/6	3/3	1/1	3/3		2/2						
763	do	750							0/3			3/3	5/9		6/6	3/3	3/3			3/3						
764	do	2,000						0/5		1/5		0/5	3/8		2/5	1/3		2/4		4/5						
765	December	1,250										0/5	1/5		2/5	2/3	5/5	3/3		3/3						
	do															3/5										

766	March	1,250											6/6		2/3	0/3	3/3	3/3								
767	do	750						0/1			1/1	3/8	3/4		3/3											
768	do	750								2/3		3/8	7/8		5/5			3/3								
770	do	750											0/3	2/3	4/4			2/2								
774	do	1,250							0/3			0/5	0/4		2/5	1/3	5/5	2/3								
	October															5/5		5/5								
775	March	625							0/3			2/5	2/3		3/3			2/3								
	October									2/2			3/5	3/3												
782	March	1,250											0/6			1/3	3/3	6/6			3/3					
785	April	1,500							0/3			1/3	5/6			3/3										
786	do	2,000											0/3			0/3				1/3	2/3				3/3	
787	do	500							1/3			2/12	6/6													
788	do	250				0/3		0/3	3/3				6/6		1/1	2/2										
789	do	250				0/3		0/3	3/3			3/3				3/3										
790	May	5,000+											0/3			0/3				0/3		0/3			1/3	
817	June	2,500							0/3				2/3							0/5	8/8	2/2			3/4	
818	1927 January	3,000+							0/4				1/15													
	1926 June								1/3				0/3			2/3				0/6	7/15	4/5				
	1927 January								0/3				6/13													
	February														2/10	1/5										
819	1926 June	2,500							0/3				1/6			0/3				0/5	5/5	5/5				
	January								2/3		0/5		2/13		2/13	1/6	0/1			0/3						
820	1926 June	2,500							0/3				0/3			1/3				0/6	5/5	4/5				
	December															2/5		4/5		4/4						
	1927																									
841	March	1,500											3/5		3/5	12/15		4/5								
842	do	1,000											1/5		3/5	9/10	5/5									
843	do	750											2/5		5/5	5/5										
844	do	750											1/5		5/5	5/5										
845	do	2,000											3/5		1/5	1/5		1/5		5/5	4/5					

TABLE 3.—*Toxicity of red-squill powders to rats*—Continued

WILD RATS

P. C. No.	Month of feeding	Minimum lethal dose	Toxicity of powder in quantities of milligrams per kilo of body weight																							
			25	50	75	100	150	200	250	300	350	400	500	625	750	1,000	1,250	1,500	1,750	2,000	2,500	3,000	3,500	4,000	5,000	6,000
		Mg. per kgm. of body weight																								
	1924																									
16	March	250						0/1				1/1				2/2										
	April							1/1	2/2			2/2														
18	March	500							2/3		1/1		2/3		1/1	3/3										
	April					1/2	3/3	1/1	3/3																	
24	March	250											1/1	2/2												
	April							0/1	2/2			2/2														
38	May	200						1/1																		
	1926																									
719A F	February	500+						0/2	1/3	0/1	0/3	1/1	1/4													
720A	January	250	0/2	3/5	0/1	3/6	5/5	5/8	8/8	3/3																
720B	do	150	0/1			1/3	3/3	5/5		5/5																
721A	do	350	0/3	1/4		1/6	1/1	3/4	4/6	1/6	4/4	1/1	6/6													
722A	do	500		1/5		1/4		1/4	4/6		3/4															
	September										0/2		5/5		2/3											
723A	January	500	0/1	0/1		0/3	0/3	1/1	3/6	5/9	2/3	1/1	1/2													
724A	do	500			0/1	0/1	7/10	2/7	8/13	6/7	3/4		3/3													
724B	do	500								0/2		1/4	3/3													
725A	do	300+					0/1	1/4	7/8	4/6																
725B	do	500							0/2	0/1		3/5	2/2													
727A	do	500+					0/3	2/5		4/5		3/4	5/8													
	March					0/1	0/1		2/3	2/4																

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TABLE 4.—*Toxicity of calcium oxalate and white squill to white rats*[1]

Product	Minimum lethal dose	Toxicity in quantities of milligrams per kilo of body weight											
		100	250	500	625	1,000	3,000	4,000	5,000	7,500	10,000	15,000	18,000
	Mg. per kilo of body weight												
Calcium oxalate	1,000+	0/3	0/3	0/3	0/3	0/3							
White squill	18,000+						0/3	0/1	0/6	1/3	1/3	0/2	0/1

[1] Numerator of each fraction shows number of rats dying within 5 days; denominator shows total number of rats fed indicated dose.

TABLE 5.—*Toxicity of fresh squill bulbs to rats*[1]

WHITE RATS

P. C. No.	Month of feeding	Minimum lethal dose	Toxicity of bulbs in quantities of milligrams per kilo of body weight[2]																
			75	100	150	200	250	300	350	500	625	750	1,000	1,250	1,500	2,000	2,500	3,000	3,500
		Mg. per kgm. of body weight																	
	1924																		
16A[3]	April	1,250					0/6			2/6	1/1	0/3	3/5	6/6	3/3	3/3			
	1925																		
711[3]	October	150(?)		2/3	2/2														
712[3]	do	150+	0/2	0/2	0/1														
713[4]	do	150+		0/4	0/1														
719[3]	December	1,000								0/1	1/1	0/1	1/1						
720[5]	do	1,000								1/1		0/2	1/1						
721[6]	do	500								1/1		1/1	2/2						
722[7]	do	750				0/1				0/1	0/1	1/1							
723[8]	do	350+		0/2				0/1	0/1										
	1926																		
761[9]	March	750		0/3				0/3		1/3	4/6	3/3	3/3						
816[10]	June	3,000									1/5	1/5	1/5		0/4	1/4	2/4	3/3	
840[10]	August	2,000					0/1			0/6		0/3	2/12		3/5	3/4			1/1

WILD RATS

P. C. No.	Month of feeding	Minimum lethal dose	75	100	150	200	250	300	350	500	625	750	1,000	1,250	1,500	2,000	2,500	3,000	3,500
	1924																		
16A	March	150		3/3		1/1		2/2											
	April			2/3	3/3		2/2												

[1] All fresh squill results have been recalculated to dry basis, assuming fresh bulb to contain 80 per cent of moisture.
[2] Numerator of each fraction shows number of rats dying within 5 days; denominator shows total number of rats fed indicated dose.
[3] Sardinian, red.
[4] Sardinian, white.
[5] Sardinian, whitish pink
[6] Sardinian, pink.
[7] Sardinian, deep red.
[8] Sardinian, nearly red.
[9] Sardinian, all colors.
[10] Red squill.

EFFECT OF TEMPERATURE ON TOXICITY

Powders made at 80° to 100° C. were more toxic than those made at other temperatures. Apparently they were more toxic than the fresh bulbs used in their manufacture, even after an 80 per cent allowance had been made for the moisture content of the bulbs. The powders made at room temperature (25° C.) and at 60° C. were less toxic than the fresh bulbs (on a dry basis), and also less toxic than powders made at 80° C. Temperatures above 100° C. seemed to decompose the active principle. Somewhat similar findings have been reported by Claremont (*5*). Although heating rarely increases toxicity, such a result is not unknown, as in the conversion of quinine into quinotoxin. Further investigation is necessary to determine the factors underlying this apparent increase in toxicity.

It was concluded that the most toxic powders were produced at a drying temperature of 80° to 100° C. (Table 6.)

TABLE 6.—*Effect of temperature on toxicity of red-squill powders*

[Mg. per kgm. of body weight]

Series No.	Equivalent minimum lethal dose of undried fresh bulb	Minimum lethal dose of squill dried at—						
		25° C.	60° C.	80° C.	100° C.	110° C.	120° C.	140° C.
1 (hot-air oven)		--------	750	400	--------	--------	--------	--------
2 (hot-air oven)	1,250	--------	1,500	1,000	2,000	2,500+	3,000+	--------
3 (vacuum oven)	1,250	--------	--------	750	1,500+	750	750	2,000
4 (electric oven)	750	1,250	1,250	500	750	--------	750	--------

DIRECT OVEN DRYING VERSUS PRELIMINARY AIR DRYING

One portion of each of seven pairs of samples prepared from the same composite lots of squill bulbs was weighed and placed directly in the drying oven at the desired temperature. The other portions were exposed on open watch glasses in the air for five days, after which they were dried at the same temperatures as the first set. (Table 7.)

TABLE 7.—*Effect of type of drying on toxicity*

Sample No.	Minimum lethal dose of squill powder—		Sample No.	Minimum lethal dose of squill powder—	
	Direct oven dried	Preliminary air dried		Direct oven dried	Preliminary air dried
	Mg. per kg. of body weight	*Mg. per kg. of body weight*		*Mg. per kg. of body weight*	*Mg. per kg. of body weight*
P. C. 719	1,000	625	P. C. 723	1,000+	500
P. C. 720	250	250	P. C. 724	500	750
P. C. 721	350	400	P. C. 725	500	625
P. C. 722	500+	350			

No difference was found in one set, but the variations in toxicity in the other sets suggested that other factors played more significant rôles in the development of toxicity. Direct oven drying is a simpler and more convenient procedure. It was concluded that direct oven drying gives as good results as preliminary air drying in the production of toxic squill powders.

EFFECT OF FERMENTATION ON TOXICITY AND YIELD OF POWDER

The 1925 experiments showed that sliced composites of fresh squill bulb fermented rapidly upon exposure to the air. Accordingly, an experiment was conducted to determine the effect of fermentation on the toxicity of squill powders, and incidentally to determine whether the fermentation was conducted upon the glucose of the toxic glucoside (or glucosides).

Portions of chopped composite taken from a fermenting lot and dried formed series 1. (Table 8.) One lot (P. C. 719A) was made by directly drying the unfermented squill in an electric oven at 80° C. Another (P. C. 719AF) was made from the same composite, which had stood in a closed fruit jar for 11 days at room temperature (25° C.). By this time the fermentaion had reached the alcoholic stage, that is, the odor of alcohol was apparent when the jar was opened. From 500,000,000 to 800,000,000 bacteria, from 5,000,000 to 18,000,000 yeasts, and 10,000 molds per gram were found in different parts of the material.[5] After fermentation had reached the acetic-acid stage, sample P. C. 719AAF was taken.

Table 8.—*Effect of fermentation on yield and toxicity of red-squill powders*

Series No.	Yield			Toxicity		
	Unfermented	Alcoholic	Acetic acid	Unfermented	Alcoholic	Acetic acid
	Per cent	*Per cent*	*Per cent*	*Mg. per kgm. of body weight*	*Mg. per kgm. of body weight*	*Mg. per kgm. of body weight*
1	23.2	17.8	12.0	1,000	2,000	2,000
2				250–350		750
3				500–1,000		750

For series 2, a mixture of bulbs comprising the whitish-pink (P. C. 720) and the pink-red (P. C. 721), and for series 3 a mixture of the deep-red (P. C. 722) and the nearly red (P. C. 723) color groups of bulbs (p. 21) were allowed to rot to the slimy, oozy stage and were then sliced and dried in a steam-heated commercial drying oven. Samples P. C. 767 and P. C. 768 were produced.

In series 1 the yield and the toxicity decreased during fermentation. The sample taken during the stage of acetic-acid fermentation gave only half as much powder as the unfermented bulb, and it was only half as toxic to rats. A similar loss in activity was noted in series 2. Incomplete data were obtained in series 3, owing to lack of material.

It was concluded that chopped bulbs should be dried immediately to avoid decreases in yield and toxicity following fermentation.

EFFECT OF VARIABILITY OF SQUILL BULBS ON TOXICITY

Variations in the toxicity of squill preparations have been reported in the literature. Origin, climatic conditions during growth, and season of harvesting are reported to be factors in the toxicity of squill.

[5] These determinations were made by B. A. Linden, of the Food, Drug, and Insecticide Administration.

In the 1924 experiments, the minimum lethal dose to white rats of the powders made simultaneously from two individual bulbs was found to be 250 and 3,000 milligrams per kilo of body weight. In the 1925 experiments six powders were made at the same time, and dried in the same oven at 80° C. simultaneously. Each powder was made from a single bulb, three from large bulbs (weighing around 750 to 1,000 grams each), and three from small bulbs (weighing around 200 grams each). The lethal doses to white rats were found to be 500, 500, and 2,500 milligrams per kilo of body weight for the powders from the large bulbs, and 250, 250, and over 5,000 milligrams per kilo of body weight for powders made from the small bulbs. No characteristic differences in the appearance of the bulbs or powders were apparent.

A sample of red-squill bulbs from Sardinia was found somewhat more toxic than one grown in Sicily during the same year.

Powders were prepared from composites of various portions of squill bulbs to see if material differences in toxicity could be found. The minimum lethal dose to white rats of the powder from the outer dry husks and scales was over 3,000 milligrams per kilo of body weight, that of the middle fleshy scales was 500 and 750 milligrams per kilo of body weight, and that of the central core was 1,000 and over 3,000 milligrams per kilo of body weight.

The readily available outer dry husks were stripped from all bulbs before the bulbs were sliced, in the preparation of squill powders. Although the core was less toxic than the middle fleshy scales, it constituted so small a portion of the bulb that it was not deemed necessary to remove it.

It was concluded that powders should be made from composites of a number of bulbs, and that the outer dry husks should be rejected.

EFFECT OF INTENSITY OF COLOR OF SQUILL BULBS ON TOXICITY

The color of the middle, fleshy scales of squill varies in intensity from a light whitish pink to a deep mahogany. A sackful of bulbs was arbitrarily divided into four color groups, and powders were prepared from six bulb composites of each group by two methods: (1) One portion of the chopped composite was directly oven-dried at 80° C. for 45 hours to constant weight; (2) the remainer of the chopped composite was exposed to the air for 46 hours at room temperature (25° C.) and then dried in the electric oven at 80° C. for 93 hours to constant weight. The quantities of bulbs in each color class, and the minimum lethal doses to white rats are given in Table 9.

TABLE 9.—*Effect of intensity of color of squill bulbs on toxicity*

Sample No.	Color of bulbs	Quantity of bulbs		Minimum lethal dose of powder made by—	
				Method 1	Method 2
		Kgm.	*Per cent*	*Mg. per kgm. of body weight*	*Mg. per kgm. of body weight*
P. C. 720	Whitish pink	3.0	3.9	250	250
P. C. 721	Pink red	9.0	11.7	350	400
P. C. 723	Nearly red	35.0	45.6	1,000+	500
P. C. 722	Deep red	29.8	38.8	500+	350

The powders made by one method were about as toxic as those by the other in all but the nearly red group. The whitish-pink bulbs gave the most toxic powder and the nearly red ones the least, although still satisfactorily potent. As nearly red bulbs comprised almost half the shipment, it was not deemed desirable to reject them, even though they were less toxic than those of the other groups. To facilitate the application of experimental results to commercial conditions, all powders thereafter were made from run-of-shipment bulbs, without regard to color. Properly selected light-colored bulbs, however, might give more toxic powders.

TOXICITY OF WHITE SQUILL TO RATS

White squill is reported to be botanically identical with red squill and to have essentially the same microscopic appearance, except for the lack of pigment cells. The minimum lethal dose of three samples of white-squill powder fed to rats could not be determined, but it was more than 18,000 milligrams per kilo of body weight. White squill is apparently nontoxic to rats.

TOXICITY OF CALCIUM OXALATE TO RATS

The presence of calcium oxalate in squill powders has been universally recognized. Flückiger and Hanbury (*9, p. 691*) report the presence of 3.07 per cent of $CaC_2O_4.3\ H_2O$ in a sample of dried white squill. A dose of 1,000 milligrams per kilo of body weight of calcium oxalate (equivalent to 33,000 milligrams per kilo of squill, calculated from Flückiger's assay) was fed to rats without producing any apparent effect. Since calcium oxalate is present in white as well as in red squill, and since the squill powders that kill rats in doses around 200 milligrams per kilo would contain only 6 milligrams per kilo of calcium oxalate in the lethal dose, it was concluded that calcium oxalate itself could not be the primary cause of death in rats dying after eating red-squill powders.

PREPARATION OF RED-SQUILL POWDER ON A SEMICOMMERCIAL BASIS

From 10 to 30 kilograms of squill bulbs were dried in a 65 by 150 by 200 centimeter (25 by 60 by 80 inches, approximately) drier, holding six trays, each 27.5 by 90 centimeters (12 by 36 inches), and heated by closed steam coils. The temperature was kept constant within 4° or 5° C. during a given run. The outer scales were stripped off and the bulbs were sliced by hand or by slicing machines onto tared trays, until the trays were covered to a depth of 2 to 3 inches. The trays were placed in the oven, which had previously been heated to the desired temperature. Weights were taken from time to time until constancy of weight indicated that the bulbs had dried to equilibrium. The dried bulbs were then mixed and ground in an electric mill until the product passed through a 40-mesh sieve. The ground powder, after being again thoroughly mixed, was placed in screw-top cardboard mailing tubes for protection from the air. After six months to a year, the powder in these cardboard tubes solidified into a hard cake, but no change in toxicity could be detected. (Table 10.)

TABLE 10.—*Effect of semicommercial preparation on toxicity of red-squill powders*

Run No.	Sample No.	Color of bulbs	Fermentation	Minimum lethal dose
	P. C.			*Mg. per kgm. of body weight*
1	724A	Deep red and nearly red	None	500
	724B	do	Slight	750
	725A	Whitish pink and pink red	None	500
	725B	do	Slight	625
2	760	Mixed	None	500
3	770	do	do	750
4	775	do	do	625
	767	Whitish pink and pink red	Acetic acid	750
	768	Deep red and nearly red	do	750

For the first trial run, the outer scales were removed from about 150 kilograms of fresh bulbs, which were then arbitrarily divided into two groups, deep red and nearly red (P. C. 724), and whitish pink and pink red (P. C. 725). One portion of each group was sliced, weighed, placed directly in the oven, and dried, at temperatures ranging from 68° to 75° C., the average being 72°. The remainder was sliced, weighed, and left exposed to the air for four days. Slight fermentation had started when the material was loaded into the oven and dried at the same temperature as the first group.

For the second run (P. C. 760) a composite of 250 kilograms of bulbs, irrespective of color, was used. The outer husks were removed, and the bulbs were sliced into large chunks. The weighed material was dried at once at a temperature ranging from 70° to 80° C.

For the third run (P. C. 770) a composite was made from 2 sackfuls of bulbs. A machine was employed to make uniform slices approximately a quarter of an inch thick. This brought out much of the mucilaginous material from the bulbs and reduced them to a more slimy consistency before drying. Conditions were therefore unintentionally made more favorable for fermentation. The use of sharp instruments in slicing the bulbs is advisable to avoid bringing out an excess of the mucilaginous juices. During the first three days of drying the oven temperature was maintained at 40° to 50° C. On the fourth day it was brought up to 80°, where it was held during the rest of the drying period.

One portion of the fourth run (P. C. 775) consisted of the remainder of the bulbs used in the preparation of P. C. 770. They were also finely sliced. In addition, bulbs that had been stored at 4° to 5° C. for three months were used. Fermentation during storage had been marked. The bulbs were soft and spongy, with the leaves separating, and were dripping mucilaginous slime. Two composites were made. The whitish pink (P. C. 720) and pink red (P. C. 721) groups were consolidated as P. C. 768; the deep red (P. C. 722) and nearly red (P. C. 723) groups were consolidated as P. C. 767. The spongy bulbs were sliced onto a tray and placed directly in the oven to dry. During the fourth run the oven temperature was held at approximately 80° C.

Although the temperature was not held so constant as in the electric oven, the products obtained in the steam oven corresponded reasonably well in toxicity with corresponding samples from the same composite dried in the electric oven. Thus, the minimum lethal dose of P. C. 727A, dried in an electric oven, was 500 milligrams per kilo of body weight, and that of P. C. 724A and of P. C. 725A, dried in a steam oven, was the same.

Sample P. C. 760 in the second run and P. C. 775 in the fourth run were made at 80° C., and both were more toxic than P. C. 770 in the third run, which was held at 40° to 50° for three days before being heated to 80°. This suggests that a rapid rise to 80° for the fresh bulb tends to produce the most toxic squill powder.

The rate of drying was followed only until uniform weight was indicated. At 80° C. it seems that drying would be complete in about three days.

Although food containing 40 per cent of squill powder was readily eaten, 10 per cent concentrations were used in most instances. With this concentration, a rat consuming 1 per cent of its body weight of food would consume 0.1 per cent of its body weight of squill, or 1,000 milligrams of squill per kilo. Accordingly, squill preparations having a lethal dose at or below 1,000 milligrams per kilo appear to be sufficiently toxic for commercial purposes. Most of the experimental powders killed in materially smaller doses.

The production under semicommercial conditions on a semicommercial scale of squill powders having similar toxicities indicates the possibility of successfully manufacturing squill powders of a satisfactory degree of toxicity on a commercial basis.

RELATIVE SUSCEPTIBILITY OF WHITE RATS AND OF WILD (BROWN) RATS TO SQUILL POWDERS

From the literature it would seem that wild (brown) rats are more susceptible to squill than white rats. The lethal doses found in the investigations here reported, when the same powder was fed to the two forms, are given in Table 11.

TABLE 11.—*Relative susceptibility of white rats and of wild (brown) rats to squill powders*

Sample No.	Minimum lethal dose for—		Ratio	Sample No.	Minimum lethal dose for—		Ratio
	White rats	Wild rats			White rats	Wild rats	
P. C.	*Mg. per kgm. of body weight*	*Mg. per kgm. of body weight*		*P. C.*	*Mg. per kgm. of body weight*	*Mg. per kgm. of body weight*	
16	750	250	3.0	723A	1,000+	500	(2.0+)
18	500	500	1.0	724A	500	500	1.0
38	250	200	1.25	724B	750	500	1.5
719A F	2,000	500+	(4.0−)	725A	500	300+	(1.7−)
720A	250	250	1.0	725B	625	500	1.3
720B	250	150	1.7	727A	500	500+	(1.0−)
721A	350	350	1.0				
722A	500+	500	(1.0+)	Average			1.5

In some series no difference in susceptibility was noted; in others the wild rats appeared to be about three times as sensitive. Lack of material and the difficulty of obtaining a fairly large supply of wild rats for feeding purposes at any definite time prevented more accurate determinations of differences in susceptibility of the two forms.

Results obtained in these 14 series confirm Claremont's findings (*5*) that, on the average, less squill powder is required to kill wild rats than is required to kill white rats.

EFFECT OF RED-SQUILL POWDERS ON DOMESTIC AND OTHER ANIMALS

Previous investigators have stated that squill, although toxic to rats, is not toxic to cats, dogs, chickens, and other domestic animals. As no data showing the minimum lethal dose of red squill to these animals were found in the literature, a series of experiments was undertaken to determine the toxicity of squill to animals other than rats.

CATS

Squill powders were mixed with whole-milk powder, and various proportions were added to 100 grams of lean hashed meat, which was then fed to cats. In food containing 10 to 25 parts of squill powder per million the unusual flavor was detected, and the food was eaten slowly but completely. The quantities of food containing higher concentrations of squill that cats consumed within 24 hours were then determined. (Table 12.)

TABLE 12.—*Relative percentages of squill-meat baits eaten by cats in a 24-hour period*

Concentration of squill (parts per million)	Percentage eaten	Concentration of squill (parts per million)	Percentage eaten
100	100	2,500	8
200	90	5,000	20
500	84	10,000	7
1,000	75		

As rat baits are customarily exposed in concentrations of 10 per cent, or 100,000 parts per million, cats probably will not eat such food.

DOGS

Dogs refused ground meat containing 1,000, 2,500, and 5,000 parts of squill powder per million of meat. When squill powder suspended in water was injected into the stomach by stomach tube, emesis and diarrhea followed in several instances after doses of 100 milligrams per kilo of body weight. As dogs refused food containing a small concentration of squill powder, such as 1,000 parts per million, it does not seem that they would be hurt by squill. Two separate instances have been noted where dogs gulped down squill rat baits containing 10 per cent of squill mixed with sausage. Emesis was the only effect noted.

CHICKENS

No effect was observed following the injection into chickens' crops of quantities less than 2,000 milligrams of squill powder per kilo of body weight. Diarrhea followed the injection of 2,000 and 3,000 milligrams per kilo. Squill powder was mixed with cracked corn and with laying mash in 10 per cent concentrations. After one or two picks, chickens refused to eat such food. Two chickens were placed on a diet containing 10 per cent of squill powder in growing mash.

After refusing to eat for several days, they consumed the mash without much hesitation. This food was continued for two weeks, during which time the chickens apparently grew as well as controls fed on untreated mash. Odom Stewart, formerly of the Bureau of Biological Survey, in experiments with baby chicks at Raleigh, N. C., concluded that chicks are not likely to eat enough feed containing 10 per cent of powdered red squill in 1, 2, or 3 feedings to cause death, even when fed it to the exclusion of other foods.

PIGEONS

After a few picks, pigeons refused to eat mash containing 10 per cent of squill powder. Injection of 2,000 milligrams of squill powder per kilo of body weight into the crop failed to produce any noticeable effect. The injection of 3,000 and 4,000 milligrams per kilo resulted in emesis, but no other untoward effect could be noted.

PIGS

A dose of 250 milligrams of squill powder per kilo was given in a gelatin capsule at 10.45 a. m. to a 4-month-old pig weighing about 16 kilograms. No evidence of squill action was noticeable until about 4 p. m., when signs of gastric distress developed. The pig vomited several times during the night. At 9 o'clock the next morning it was unable to stand without definite ataxia, and consistently refused food, but drank water freely. Some food was eaten at noon but it was vomited about 3 p. m. The pig's tail was limp and straight, although at the time of feeding it was tightly curled. On the second day after the injection the pig had practically recovered.

After a 9-day intermission, to permit complete recovery from the first dose, a mixture of cracked corn and middlings containing 10 per cent of the same squill powder was offered. The pig took only one or two bites; then grunted, refused further food, and vigorously rubbed its snout on the cage walls and floor. This reaction was probably due to the sting of the calcium-oxalate raphides. Half an hour later, and again the next morning, the same squill food was refused, but untreated food was readily consumed.

Post-mortem examination showed nothing abnormal, except some evidence of irritation in the stomach. When the body weight of a pig is considered, it is readily apparent that the quantity of squill bait required to cause toxic effects would need to be very large. For this particular animal, the quantity of squill power given would be sufficient to kill 16 kilograms (35 pounds) of rats. Under ordinary conditions of rat baiting, it hardly seems possible that a pig would consume enough poisoned bait to cause trouble.

WOODCHUCKS

Injected in aqueous suspension into the stomach of woodchucks, the minimum lethal dose of sample P. C. 18 was found to be 500 milligrams per kilo of body weight (the same as for rats). Vomiting, however, frequently followed even smaller doses. Baits containing squill were refused. Squill does not seem to be suitable for use in controlling woodchucks.

PRAIRIE DOGS AND POCKET GOPHERS

Prairie dogs and pocket gophers refused to eat freshly exposed squill baits. After rains had washed off the squill, the remaining baits were readily consumed.

From these feeding and stomach-tube experiments, it seems safe to conclude that squill mixtures containing 5 to 10 per cent of squill powder either will not be eaten by animals other than rats, or will produce emesis with direct removal of the poison. Probably, therefore, squill powders deserve the reputation accorded them in the literature of being generally harmless to farm animals. Of course, failure to produce death may be due to the animal's failure to eat and retain enough squill powder, rather than to relatively high species resistance or insusceptibility.

YIELDS OF TOXIC SQUILL POWDERS ("RAT UNITS")

Generally speaking, squill bulbs lose 80 per cent of their weight during drying. The yields of powder obtained when samples were dried under stated conditions are given in Table 13. The weight of powder obtained in following a given process is not significant of the efficiency of the process unless the toxicity of the powder obtained is also considered. Two kilograms of a highly toxic powder may represent a better yield than 4 kilograms of a relatively nontoxic preparation. It is necessary to know both the yield and the toxicity in judging the efficiency of producing a squill powder under a given set of conditions.

TABLE 13.—*Rate of loss in weight of squill bulbs during drying*

P. C. No.	Bulb		Drying temperature	Drying oven	Loss in weight after drying for—									
	Part used and color	Condition			18 hours	1 day	2 days	3 days	4 days	5 days	6 days	8 days	10 days	15 days
					P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
766	All	Fresh	20–25	None [1]	9.0	25.2	60.0	69.5		76.0		76.5	76.5	76.0
765	do	Frozen	60	Electric	61.5	71.5	76.0	76.5						
724A	Deep red and nearly red	Fresh	72	Steam					78.5					
725A	Whitish pink and pink red.	do	72	do					79.4					
770	All	do	[3] 40–80	do										76.0
820	do	do	60	Electric	73.3	76.8	78.0							
842	do	Frozen 2 days	60	do			[illegible].4	82.1						
719A	do	Fresh	80	do			76.8	76.8						
719B	do	do	[3] 80	do		49.0	70.2	75.2	78.5	78.5				
719C	Core only	do	[3] 80	do				83.0	85.0					
719AF	All	Alcoholic fermentation	80	do		82.0		83.2						
719AAF	do	Acetic-acid fermentation	80	do			87.6				88.0	88.0		
720A	Whitish pink	Slight fermentation	80	do			88.0							
720B	do	do	[3] 80	do			76.0	89.0	80.5	89.9	89.9			
721A	Pink red	do	80	do			87.1							
721B	do	do	[3] 80	do			74.0	87.5	87.6	88.1	88.1			
722A	Deep red	do	80	do			78.5							
822B	do	do	[3] 80	do			55.2	79.0	79.1	79.3	79.3			
723A	Nearly red	do	80	do			75.6							
723B	do	do	[3] 80	do			55.0	77.3	77.5	77.7	77.7			
727A	All [4]	Fresh	80	do							76.1			
760	do	do	80	Steam			48.3						78.0	
764	do	Frozen	80	Electric	73.5	78.0	78.0							
767	Mixture of No. 722 and No. 723.	Acetic-acid fermentation	80	Steam									79.2	
768	Mixture of No. 720 and No. 721.	do	80	do									79.9	
775	All	Fresh	80	do									79.0	
782	do	Frozen	80	Electric	75.0	75.0	79.0							
785	Nearly red	do	80	do						72.4				
786	do	do	80	do						79.2				
787	do	do	80	do						74.3				
788	do	do	80	do						76.1				
789	do	do	80	do						73.0				
790	do	do	80	do						76.7				

819	All	Fresh	80	do	76.3	76.8	78.0							
841	do	do	80	do		76.5	81.1			81.1	82.5			
843	do	Frozen 2 days	80	do		81.8	82.2	82.4						
763	do	Frozen	100	do	72.5	76.0	77.5	77.5						
818	do	Fresh	100	do	75.3	78.0	78.3							
844	do	Frozen 2 days	100	do		81.5	82.1	82.3						
762	do	Fresh	120	do	67.5	80.5	80.5							
774	do	Frozen	120	do		77.5	77.5							
817	do	Fresh	120	do	78.1	78.7	78.8							
845	do	Frozen 2 days	120	do		80.5	82.8	83.0						

[1] Dried in desiccator over calcium chloride.
[2] 3 days at 40 to 50° C.; then at 80°.
[3] First at room temperature; then at 80°.
[4] From composite used in No. P. C. 724 and No. P. C. 725 dried in steam oven.

In an attempt to express in a single figure the results of combining the two variables, yield and toxicity, the so-called "rat units" of powders have been calculated. A "rat unit" is the yield in grams from 1 kilogram of fresh squill bulbs divided by the minimum lethal dose in grams per kilo of body weight. It is the number of kilograms of rats that should be killed by the powder obtained from a kilogram of fresh squill bulbs treated by the method under consideration. For example, if a 20 per cent yield was obtained by a certain method, and the powder had a minimum lethal dose of 500 milligrams per kilo of body weight, the rat unit for the method would be calculated as follows:

Yield $=20$ per cent$\times 1$ kilogram of fresh bulbs$=200$ grams
Toxicity$=500$ milligrams per kilo$=0.5$ grams

$$\text{Rat units}=\frac{\text{Yield}}{\text{Toxicity}}=\frac{200}{0.5}=400.$$

Another method for making a powder with 200 rat units would obviously be less valuable, being but half as efficient. The rat units determined for a number of series of samples are given in Table 14.

TABLE 14.—*Yields of toxic squill powders*

Sample No.	Method of drying	Yield	Minimum lethal dose	Rat units	Sample No.	Method of drying	Yield	Minimum lethal dose	Rat units
P. C.		*Per cent*	*Mg. per kgm. of body weight*		*P. C.*		*Per cent*	*Mg. per kgm. of body weight*	
761	Fresh bulb		4,000	250	723B	Electric oven	22.3	500	450
719A	Electric oven	23.2	1,000	230	727A	do	23.9	500	480
719AF	do	17.8	2,000	90	762	do	19.5	750	200
719AAF	do	12.0	2,000	60	774	do	22.5	1,250	180
760	Desiccator	23.5	1,250	190	763	do	22.5	750	300
719A	Electric oven	23.2	1,000	230	764	do	22.0	2,000	110
720A	do	12.0	250	480	782	do	21.0	1,250	170
721A	do	12.9	350	370	785	do	23.5	1,250	190
722A	do	21.5	500+	430—	724A	Steam oven	21.5	500	430
723A	do	24.4	1,000+	240—	725A	do	20.6	500	410
719B	do	21.5	625	350	766	do	20.4	500	410
720B	do	10.1	250	400	770	do	22.2	750	300
721B	do	11.9	400	300	775	do	21.2	625	340
722B	do	20.7	350	590					

The rat unit for the fresh bulb (P. C. 761) was 250. Essentially the same value was obtained for P. C. 719A in the unfermented stage. Fermentation to the alcoholic stage reduced the rat unit to 90, and further fermentation to the acetic-acid stage caused a drop to 60, showing the loss in activity during fermentation to be progressive. Freezing caused a decrease to about 200 rat units. The rat units of powders prepared under laboratory conditions ranged from 110 to 590, while the values for powders made under semicommercial conditions ranged from 300 to 430. Attention to manufacturing details would be expected to raise the rat units for commercial powders above 500. Indeterminate values for two of the P. C. No. 719 series prevent careful comparison, but the A series averaged less than 350, whereas the B series averaged 420 rat units. This would suggest that preliminary air-drying treatment given the B series preserved the toxicity or aided in its development. The fact that many powders have rat units greater than 250, the value for fresh squill bulbs, also suggests that the toxic principle in squill bulbs may be linked to some plant ingredient in a thermolabile condition, and that the toxic principle is liberated by heating to 80° to 100° C.

STABILITY OF SQUILL POWDERS

Two red-squill powders stored in screw-top vials in the light for 15 months showed no change in lethal dose. A 10 per cent squill biscuit that had been baked was as toxic a year later as the squill content would require. A number of powders tested over a period of a year have shown no evidence of loss of activity.

ACCEPTANCE TESTS

To determine the palatability of 10 per cent squill biscuit as compared with that of commercial samples of 5 per cent arsenious-oxide biscuit and 30 per cent barium-carbonate biscuit, several dishes were placed in a cage containing a starved white rat. Each dish contained more poisoned food than would be necessary to cause death by itself. After exposure from one to five hours, the dishes were removed, and the quantities of food consumed were determined. A number of tests were run simultaneously. With the weight of squill biscuit eaten considered as unity in each case, the ratio of control biscuit to 10 per cent squill biscuit was 2.6 : 1.0; that of 5 per cent arsenious-oxide biscuit to 10 per cent squill was 0.17 : 1.0; and that of 30 per cent barium-carbonate biscuit to 10 per cent squill biscuit was 0.93 : 1.0. The absolute quantities of poisons consumed would then be in the ratio of 250 squill to 750 barium carbonate to 20 arsenious oxide. These figures are roughly proportional to the minimum lethal doses of the poisons under consideration. Barium carbonate is approximately one-third as toxic as squill powder; fed in three times the concentration, about equal quantities of bait are consumed. As the toxicity of arsenious oxide depends upon its degree of fineness, closer comparisons are not available.

A field-acceptance test in comparison with phosphorus baits is reported by Teall.[6] At Vienna, Austria, an organized 2-day rat-killing campaign was held in January, 1927, phosphorus baits being used exclusively. Of 1,460,000 baits exposed, about 20 per cent were taken. In March, 1927, another 2-day campaign was undertaken, during which only squill baits were used. Of 1,260,000 baits exposed, more than 50 per cent were taken. It was noted that squill was taken in many houses where phosphorus bait had not been touched.

The degree of acceptance of squill baits varies with the relative palatability to rats of the foods with which the squill is mixed. Directions for preparing and distributing red-squill baits have recently been published by the Department of Agriculture (*24, p. 8, 9*).

COMMERCIAL SQUILL RAT POISONS

Three liquid squill extracts that had been offered for sale as rat poisons were tested. One was one-fourth and the other two were less than one-tenth as toxic as their labels would indicate. Two squill biscuits and three squill powders were found to correspond in toxicity to the labeled claims for squill content.

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[6] TEALL, G. ORGANIZATION AND RESULTS OF THE FIRST VIENNA RAT-KILLING CAMPAIGN. (Second supplementary report to Report No. 6025 dated May 20, 1925, in reply to department's unnumbered instruction of March 28, 1925, File No. 1027-1848.) [1927.] [Original on file in Department of State, not seen. Copy on file in U. S. Dept. Agr., Bur. Biol. Survey.]

EXTRACTION OF TOXIC PRINCIPLES BY VARIOUS SOLVENTS

To obtain information regarding the chemical nature of the toxic principle of red squill, a mixture of two powders (P. C. 724A and P. C. 725A) was taken for intensive study. Seventy-five-gram portions in Soxhlet thimbles were extracted with water, with 95 per cent ethyl alcohol, with acetone, and with chloroform, until there was no further appearance of color. The solutions were concentrated in a current of warm air, and the resulting solid extracts were dried in an electric oven at 80° C. and finally in a vacuum desiccator over calcium chloride. The residues were removed from the Soxhlet thimbles and dried in the electric oven at 80°. Feeding tests were made with the original mixed powder and with some of the extracts and residues.

The water extraction, started on January 20, 1926, was run from 8.30 a. m. until 5 p. m. daily for 19 days. The dark-red mucilaginous extract, possessing an odor of molasses, was concentrated on a steam bath, then dried in an oven, and finally allowed to stand over calcium chloride in a desiccator until constant in weight. It was ground to pass through a 40-mesh sieve. Both the extract (P. C. 751) and the exhausted residue (P. C. 750) were very low in toxicity, indicating that the active principle is decomposed by this treatment.

The alcoholic extraction for 19 days removed a much smaller quantity of mucilage, but apparently as much color as did the water extraction. The residue caked and became very hard upon exposure to the air. The solid extract (P. C. 749) had a rather oily feel, and was the most toxic extract in this series. The lethal dose of the residue (P. C. 748) exceeded 5,000 milligrams per kilo of body weight. The residue therefore is inert (nontoxic).

Acetone extraction gave a small yield of lemon-yellow crystals (P. C. 755) and no mucilage or color. Feeding tests showed that the very fluffy residue (P. C. 754) was two-thirds as toxic as the original powder, indicating that a portion of the toxic principle is destroyed during the treatment with acetone.

Chloroform gave the same result as acetone. The solid extract (P. C. 757) was small and oily. The fluffy residue (P. C. 756) was somewhat less toxic than the original powder. The acetone and the chloroform extracts were too small to be used for feeding tests.

Distilled water was repercolated through another 75-gram charge. Channels formed, producing incomplete extraction. So much mucilage dissolved that after seven days further repercolation was found to be impossible. The thick, molasseslike percolate had a sweetish odor and reduced Fehling's solution. Evaporated to a very dark-brown, tough, solid extract (P. C. 759), it was readily eaten by rats. The lethal dose was 1,500 milligrams per kilo of body weight. The incompletely extracted residue was not fed to rats.

To study further the effect of water in extracting the toxic principle, 75 grams of squill powder were placed in a beaker with about 800 cubic centimeters of water and warmed on a steam bath. Twice a day the solvent was removed in a Buchner funnel, and a fresh charge of water was added. After 22 days the extraction appeared complete. The aqueous solution was concentrated to a solid extract (P. C. 753). The residue (P. C. 752) was a dark-brown, tough sheet, heavily laden with glittering calcium-oxalate crystals. Both the extract and the residue were nontoxic, showing that the active principle is totally destroyed by this treatment.

Table 15 shows the yield, the lethal doses, and the proportion of toxic principle from the original powder that appeared in the various extracts and in the residues.

TABLE 15.—*Effect of method of extraction on yield and toxicity of squill*

Method of extraction	Solvent used	Yield		Minimum lethal dose		Total toxicity	
		Solid extract	Residue	Solid extract	Residue	Solid extract	Residue
		Per cent	*Per cent*	*Mg. per kg. of body weight*	*Mg. per kg. of body weight*	*Per cent*	*Per cent*
Original powder			100		500		100
Percolation	Water	18.5	81.5	1,500		6.2	
Exhaustion	do	82.6	17.4	5,000+	5,000+	0.8−	1.7−
Soxhlet	do	44.3	55.7	3,500	4,000+	6.3	7.0−
Do	Alcohol (95 per cent)	14.0	86.0	200	5,000+	35.0	8.6−
Do	Acetone	1.3	98.7		750		66.0
Do	Chloroform	1.3	98.7		625		80.0

Exhaustion with water on the steam bath, or in a Soxhlet thimble at 100° C., caused almost total destruction of the toxic principle in the extracts as well as in the residues. As dry heat at 100° C. did not appear to cause any decrease in toxicity, it seems evident that this loss in potency is related to the hydrolysis of the toxic glucoside. The alcoholic residue was nontoxic, and the acetone and choroform residues were somewhat less potent than the original powder. Even though the boiling point of each of these three solvents is less than 80°, it seems that any method of extraction tried caused some destruction of the toxic principle. The alcoholic extract possessed one-third the activity of the original powder.

Apparently the toxic principle of red squill is soluble in alcohol, but not in water, acetone, or chloroform. Water and alcohol extracted appreciable quantities of mucilage and reducing sugars. Extraction of the toxic principle suggests itself in the study of the chemistry of the active principle. It seems evident, however, that the cost of undertaking this on a commercial scale would far outweigh any possible benefit of marketing a more toxic preparation. Therefore no further experiments in this direction were conducted.

SUGGESTED METHOD OF PREPARATION OF TOXIC SQUILL POWDERS

Based upon the results obtained during a 3-year series of experiments in the manufacture of red-squill powders, the following method is offered as furnishing the most toxic product:[7]

Remove the outer dry husks from fresh red-squill bulbs obtained as soon as feasible after digging, and slice the bulbs transversely into sections one-fourth to one-half inch thick. Place the sliced composite as soon as possible in a drying oven, which has been previously heated to 80° C., and dry to constant weight at that temperature. Grind the dried material so that it will pass through a 40-mesh sieve. Pack the powder in hermetically sealed containers.

[7] It is possible that other manufacturing conditions might yield products of equal toxicity.

Powders made by this process should kill white rats in doses approximating 250 to 500 milligrams per kilo of body weight, and should be stable for several years.

Because of the variations in toxicity of different lots of squill, the minimum lethal dose of every lot of squill powder should be determined by feeding it to rats in the laboratory before it is marketed. It should be fed in 10 per cent concentrations in ordinary rat food to white rats that have been deprived of all food for 18 hours. The minimum lethal dose is the smallest dose that kills all the rats within five days. At least five rats should be fed with each dose tested. This is necessary to insure essentially uniform toxicity in squill powders distributed commercially. As a result of feeding tests, great variations in the potency of successive lots of powders may be reduced by properly mixing powders of higher degrees of toxicity with less-potent preparations, to produce the same standard potency. Such procedure would put a premium on the production of the most toxic squill powders, but would also permit the utilization of less toxic preparations.

As a standard of toxicity it is suggested that commercial squill powders having a minimum lethal dose of 1,000 milligrams per kilo of body weight be marketed, and that commercial squill baits having a minimum lethal dose of 10 grams per kilo be prepared. Such baits would contain 10 per cent of the standard squill powder (that is, a squill powder with a minimum lethal dose of 1,000 milligrams per kilo). If this standard bait is used, it would be necessary that a rat eat only 1 per cent of its body weight to obtain enough poison to kill it. Many of the wild rats studied during this investigation weighed between 250 and 400 grams (8 to 14 ounces). From 2.5 to 4 grams (one-twelfth to one-seventh of an ounce) of such a squill bait would be necessary to kill them. These experiments indicated that rats will readily eat much larger quantities of such baits.

CONCLUSIONS

Powdered red squill is toxic to rats; white squill is not.

Powders prepared by directly drying unfermented, sliced red squill bulbs in an oven at 80° C. are usually more toxic than those prepared under other conditions. The lethal dose of squill powders prepared by this method is usually about 250 milligrams per kilo of body weight for white rats; wild (brown) rats are killed by somewhat smaller doses.

Cats, dogs, chickens, and pigeons were not seriously harmed by squill powder. Food poisoned with squill either was not eaten or, if eaten, was promptly vomited. Consequently it has been indicated that red squill is nontoxic to these animals under normal conditions and when exposed in the concentration recommended for rat poisons.

Because of variations in toxicity, squill powders should be tested before being marketed and adjusted so that 10 grams of 10 per cent squill bait will kill a minimum of 1 kilogram of rat. (One ounce will kill 7 pounds of rats.)

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