myocardium, lung, liver and kidney, upon which were superimposed the effects of circulatory failure incident to myocardial damage, and, in many instances, secondary lobular pneumonia.

Lethal and slightly sublethal doses produced cloudy swelling, loss of cross striations and sometimes coagulation necrosis of the fibers of the myocardium, with swelling and pyknosis of the nuclei, edema and slight leucocytic infiltration of interstitial tissue, and edema of the vascular endothelium.

Similar effects, varying only in degree and in accord with the architecture of the organ, were seen in the lung, liver and kidney. Acute passive congestion was the most striking picture in the spleen, central degeneration (often of the fatty type) or necrosis was the most prominent lesion in the liver, while generalized cloudy swelling and degeneration of the tubal epithelium characterized the kidney changes.

Such damage as occurred in the stomach and small intestine resulted in edema and superficial desquamation of the mucosa, with some evidences of congestion and acute exudative reaction in the submucosa.

The animals to which repeated doses of vanillin and ethyl vanillin were administered or fed in dosages above 20 mg. per Kg. of body weight showed definite toxic changes of the foregoing type in their tissues. The rats given daily doses of 20 mg./Kg. for $4^1/_2$ months failed to develop pathologic processes of greater severity or frequency than those commonly found in control animals as the result of spontaneous disease or other unrecognized environmental influences.

Discussion.—The daily ingestion of 20 mg./Kg. of vanillin and ethyl vanillin by rats over a period of 126 days was found to be harmless. Higher doses produced histopathological changes of varying degrees of severity in the myocardium, kidney, liver, lungs, spleen and stomach of rats and rabbits.

It is evident that these compounds are capable of causing fatal poisoning but their relative insolubility in cold water or in cold milk and the bitter taste of concentrated extracts make the possibility of such an event very remote. Concentrations up to 0.5 Gm. of vanillin or 0.15 Gm. of ethyl vanillin per quart are added to foods, and in view of current dietary usage or even extreme individual predilections for flavored foods, injurious effects from prolonged consumption of these compounds are not to be anticipated.

Most of the vanillin is oxidized in the animal body to vanillic acid, which is excreted with the urine as conjugated sulfate. Traces of vanillin and vanillic acid are excreted as such (Preusse (1)).

CONCLUSION

When ingested in high concentration vanillin and ethyl vanillin induce an acute toxemia with death by circulatory failure. Experimental observations on rabbits and rats indicate that the quantities which are employed in foods as flavoring materials are harmless.

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A Study of Red Squill*

By Guilford C. Gross†

The use of red squill as a rat poison has become very popular in recent years. A great share of this popularity is due, no doubt, to the fact that while red squill is extremely toxic to rodents, it is relatively non-toxic to other wild and domestic animals and human beings. The other rodent poisons in general use to-day (arsenic, barium carbonate, phosphorus and strychnine) have the disadvantage of being toxic to all forms of animal life.

The development of red squill as a specific rat poison has been greatly retarded until recent years because of the difficulty which was experienced in obtaining a uniformly toxic preparation. However, recently there has been considerable investigation conducted with reference to the toxic nature of the rat-killing principle in red squill and as a result this difficulty has been largely overcome.

* Kilmer Paper, 1939.
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It is interesting to note that while red squill is of the same species of *Urginea* as white squill, the latter is not effective as a rat poison. It is thus apparent that red squill contains an additional principle which is toxic to rats. In this respect, Winton (1) states that the cardiac glucoside and rat-killing principle in red squill are distinct substances. Both of these substances

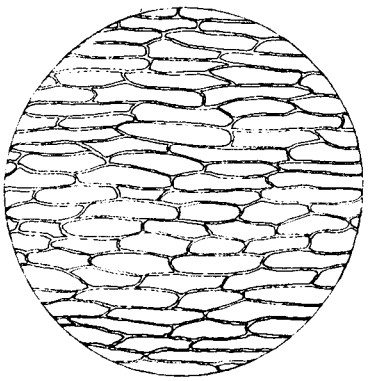

Fig. 1.—Red Squill Bulb Scale. Surface Section.

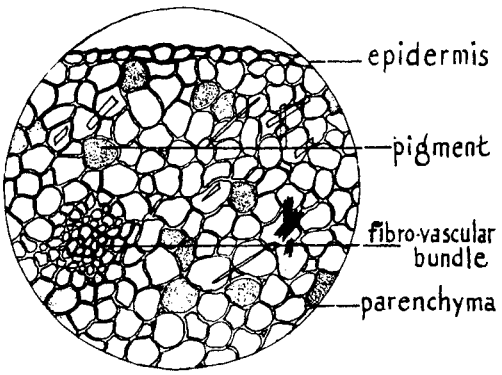

Fig. 2.—Red Squill Bulb Scale. Cross Section.

are found in red squill while white squill does not contain significant amounts of the rat-killing principle.

Both squills have essentially the same medicinal action, but differ in regard to rat-killing properties.

EXPERIMENTAL

HISTOLOGICAL STUDY

A microscopical study of red squill reveals the same structures found in the white variety, but, in addition, red pigment cells are present in the red variety. G. Keenan (2) of the Food, Drug and Insecticide Administration has made a microscopical comparison of red and white squill powders. He states that the greatest diagnostic difference is the presence of pigment cells in the red squill scales and their absence in the white.

Sections of the scales of the fresh bulb of red squill were cut and studied microscopically. The following structures were noted:

Epidermis: Epidermis of thin-walled cells, stomata present.

Mesophyll: Mesophyll of thin-walled cells, irregular in shape, parenchymatous in nature.

Fibrovascular Bundles: Fibrovascular bundles scattered throughout mesophyll. Spiral tracheæ were observed.

Pigment Cells: Cells containing a red pigment were found scattered throughout the mesophyll.

Crystals: Raphides of calcium oxalate were numerous. The crystals varied greatly in length.

Drawings of the bulb scales of red squill, Figs. 1, 2, 3 and 4, show these structures.

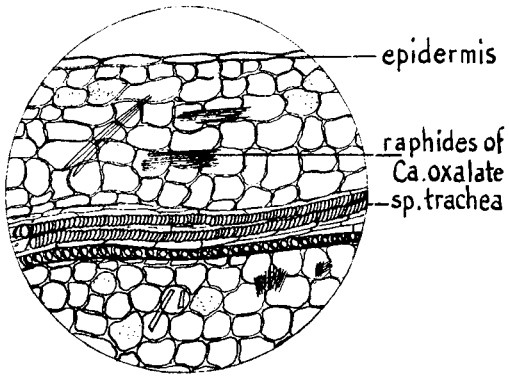

Fig. 3.—Red Squill Bulb Scale. Longitudinal Section.

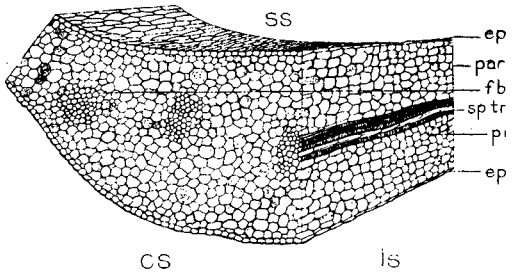

Fig. 4.—Red Squill Bulb Scale.

ss = surface section, *cs* = cross section, *ls* = longitudinal section, *ep* = epidermis, *pi* = pigment, *par* = parenchyma, *sp* = spiral trachea, *fb* = fibro-vascular bundle.

A microscopical examination for comparison of red squill and white squill powders was made. Other than the presence of red pigment cells in red

squill powder, no important differences in the appearance of the two powders could be detected as is shown in Figs. 5 and 6.

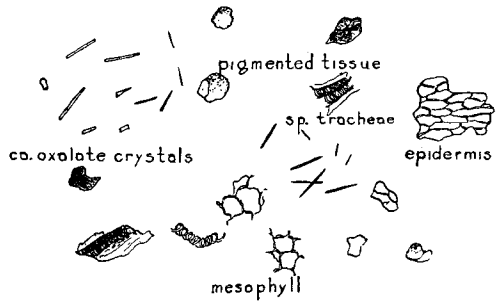

Fig. 5.—Red Squill Powder.

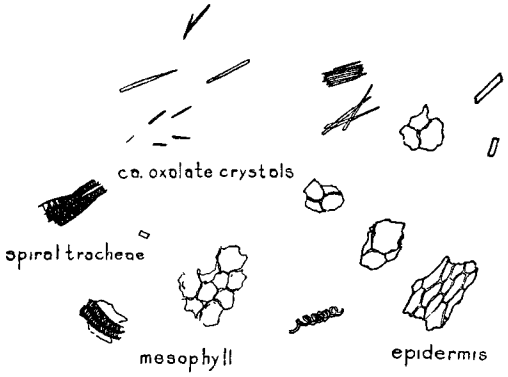

Fig. 6.—White Squill Powder.

EXTRACTION STUDIES AND TOXICITY EXPERIMENTS

Experimental evidence has shown that the rat-killing principle is present in significant quantities only in the red variety of squill. The isolation of this principle was conducted according to the method suggested by LeBlanc (3) and which, in his work, gave a product approximately 100 times as potent as the original powder.

Before extraction studies were begun, a commercial sample of red squill was fed to 8 rats to determine its approximate toxicity. The product used contained 10% of red squill powder in what appeared to be a base of corn meal. It was fed to both male and female rats. In all cases, the animals were starved for 24 hours prior to feedings. The powder was further diluted with corn meal at the time of administration. Results of the experiment are shown in Table I.

From the above table, it may be noted that, while only in one instance did the product prove fatal to male rats when fed at the rate of 100 mg. of red squill powder per Kg., the product did appear to be quite toxic since male rats receiving doses of 50 mg./Kg. became very sick. Female rats appeared to be more susceptible. All of those receiving doses of 100 mg. of red squill powder per Kg. died and one receiving a dose of 50 mg./Kg. also succumbed.

EXTRACTION OF THE RAT-KILLING PRINCIPLE

LeBlanc (3) has suggested the use of 80% ethyl alcohol in the extraction of the rat-killing principle from powdered red squill. The extract thus obtained is further purified using animal charcoal and petroleum ether. The procedure used here was essentially the same. The following steps were involved:

(*a*) *Extraction of the Active Principle Using 80% Ethyl Alcohol as the Solvent.*—The extract thus obtained was dried at a temperature not exceeding 80° C. and powdered.

(*b*) *Purification of the Extract Using Animal Charcoal.*—An aqueous solution of the extract obtained above was shaken with animal charcoal. The animal charcoal adsorbed the rat-killing principle, and it is removed from the charcoal by agitation with 80% ethyl alcohol. The product thus obtained is dried at 80° C and powdered.

(*c*) *Further Purification of the Potent Extract Using Petroleum Ether.*—The extract obtained above was dissolved in 80% alcohol and to this an equal volume of ethyl ether was added and the mixture shaken. A small amount of material was thrown out of solution. The mixture was filtered and the filtrate evaporated to dryness at 80° C. The extract thus obtained was shaken with petroleum ether for several minutes to remove a small amount of a fixed oil present as an impurity. The mixture was then filtered and the potent principle allowed to dry spontaneously. The product thus obtained represents a concentrated form of the rat-killing principle.

Table I.—Results of Toxicity Tests

Rat Number	Sex	Weight, Gm.	Amount Powder mg./Kg.	Dilution	Amount Squill, mg./Kg.	Total mg. Squill Fed per Rat	Results
1	M	325	325.0	1:10	100	32.5	Killed
2	M	285	142.5	1:10	50	14.2	Sick, recovered
3	M	185	185.0	1:10	100	18.5	Sick, recovered
4	M	132	66.0	1:10	50	6.6	Sick, recovered
5	F	170	85.0	1:10	50	8.5	Killed
6	F	143	143.0	1:10	100	14.3	Killed
7	F	285	285.0	1:10	100	28.5	Killed
8	F	142	71.0	1:10	50	7.1	Sick, recovered

POTENCY OF THE VARIOUS EXTRACTS

The extract obtained under (*b*) was fed in varying quantities to previously starved rats. Prior to feed-

ing, the product was mixed with powdered oatmeal and moistened slightly. The results are shown in Table II.

Table II.—Potency of Extracts

Rat Number	Sex	Weight, Gm.	Amount Extract, mg./Kg.	Total mg. Extract Fed per Rat	Results
9	M	287	100	28.7	Died
10	M	190	25	4.7	Died
11	M	244	15	3.7	Died
12[a]	M	352	10	3.5	Lived
13	M	329	5	1.6	Died

[a] Rat No. 12 ate less than one-half of the bait.

To determine whether or not the red squill extract obtained above is toxic to rats when fed at less than 5 mg./Kg., four rats were weighed, starved and fed graduated doses of the extract. A 1–5 dilution of extract was used in feeding and this was further diluted with oatmeal at the time of administration. The diluent used in the first case was lactose. Results of the experiment are shown in Table III.

Table III.—Potency of Lactose Dilutions

Rat Number	Sex	Weight Gm.	Amount of Dilution Fed mg./Kg.	Dilution	Amount Extract mg./Kg.	Total mg. Extract per Rat	Results
17	F	317	25	1:5	5	1.58	Died
18	M	272	20	1:5	4	1.08	Lived
19	M	243	15	1:5	3	0.73	Lived
20	M	232	10	1:5	2	0.46	Lived

From the foregoing experiments it may be estimated that the minimum lethal dose of the extract for male rats is somewhere in the vicinity of 5 mg./Kg.

In order to determine the toxicity of the purified extract obtained under (*c*), 4 male rats were weighed, starved and fed graduated doses of the extract. The extract was diluted 1–10 with lactose. At the time of administration, it was further diluted with oatmeal. Results of the feeding are shown in Table IV.

Table IV.—Potency of Dilutions of Purified Extract

Rat Number	Sex	Weight, Gm.	Amount of Dilution Fed, mg./Kg.	Dilution	Amount Extract, mg./Kg.	Total Mg. Extract per Rat	Results
21	M	280	50	1:10	5	1.40	Died
22	M	224	40	1:10	4	0.89	Died
23	M	241	30	1:10	3	0.72	Lived
24	M	225	20	1:10	2	0.45	Lived

The results shown in Table IV indicate that the minimum lethal dose for male rats lies between 3 and 5 mg./Kg. However, the small number of experiments does not warrant arriving at any definite conclusion. The toxicity to female rats of the extract obtained under (*c*) was not determined but it would apparently be somewhat below the above figures.

PHYSIOLOGICAL ACTION OF THE RAT-KILLING PRINCIPLE ON RATS

The physiological action of the extract (*b*) was observed on the rats fed in Table II. Within 2 hours after feeding the red squill extract, rats Nos. 9, 10 and 11 (Table II) exhibited symptoms of poisoning. They became lethargic, with rat No. 9 exhibiting marked inflammation of the area of the nose and mouth due to the irritant action of the squill. This inflammation was not so noticeable on the 10th and 11th rats. The rats did not readily respond to stimuli although, when the cages were tapped, they jerked slightly. They sneezed considerably. Within 4 hours, all rats except No. 12 were very sick. They were slightly more excitable but remained quiet when not disturbed. Respiration increased in rate and appeared to be labored. The characteristic gyrating symptoms were observed 6 hours after administration in rats Nos. 9, 10 and 11. Gyrations occurred at short intervals. The application of a slight stimulus, such as tapping the cage, caused a resting rat to promptly resume its gyrating movements. At times the rats went through the motions of vomiting but were unable to do so. Rat No. 13 showed symptoms of poisoning but these were not as marked as in the first three cases. Rat No. 12 appeared to be in fairly healthy condition.

When it was definitely established that rats Nos. 9, 10 and 11 would not live much longer, the following experiment was carried out, the purpose being to determine, if possible, whether or not the rat-killing principle is absorbed. One cc. of blood was taken from the heart of rat No. 9 and injected intraperitoneally into a healthy rat (rat No. 14). Likewise, $2^1/_2$ cc. of blood were drawn from rat No. 10 and $1^1/_2$ cc. from rat No. 11 and injected into 2 healthy rats (rats Nos. 15 and 16, respectively). All of the injected rats lived, indicating that there is no absorption of the rat-killing principle.

PHYSIOLOGICAL ACTION OF RAT-KILLING PRINCIPLE ON THE DOG

A dog weighing 15.9 Kg. was fed red squill extract (*b*) at the rate of 48.4 mg./Kg. It was mixed with ground dog food and fed late one afternoon. The dog took several bites of the treated food and then refused to eat the remainder. No symptoms of poisoning other than salivation were noted immediately following administration. The next morning, how-

ever, when the dog was removed from the cage, it was observed that he staggered slightly as he walked. At this time the same dose of the extract previously given was administered by means of a stomach tube. The first symptoms of poisoning noted were intense salivation and vomiting. These appeared shortly after the administration of the poison. The heartbeat appeared to become somewhat slower and irregular, but it was difficult to make an accurate count. The dog attempted to vomit on several occasions but was unable to do so. At 27 minutes after administration, the animal experienced severe convulsions and died within several minutes. Postmortem examination revealed the heart stopped in systole. It is believed that heart action and respiration ceased almost simultaneously.

SUMMARY

1. The structure of the inner scale of the bulb of red squill was studied microscopically.

2. Powdered red squill and powdered white squill were compared under the microscope. The presence of fragments of red pigmented tissue in the red squill powder served as the only diagnostic difference between the two powders.

3. The active rat-killing principle was extracted using 80% ethyl alcohol as the solvent. This, on subsequent purification with animal charcoal, ether and petroleum ether, yielded a product which caused death in a male rat when fed at the rate of 4 mg./Kg.

4. The physiological action of the rat-killing principle on rats and dogs was observed.

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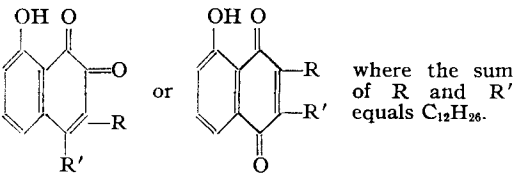
Wilhelm Ostwald (1853–1932) was awarded the Nobel Prize for Chemistry in 1909 in recognition of his work on catalysis and for his investigations in the fields of chemical equilibria and reaction velocities.

The Constitution of Celastrol—Part III

*By Ole Gisvold**

In previous publications (1) it was shown that celastrol, a pigment found in the outer bark of the root of *Celastrus Scandens*, has the formula $C_{22}H_{30}O_3$. One oxygen was reported present as a hydroxyl group and the remaining two oxygens as possibly being in the form of an ortho quinone. Subsequent investigations on the constitution of this pigment indicate that it might possibly be an alkyl substituted 5-hydroxy-1, 4-naphthoquinone with the following tentative formulas



where the sum of R and R' equals $C_{12}H_{26}$.

Permanganate oxidation of celastrol yielded a small quantity of a crystalline material that was best characterized as 3-hydroxy phthalic acid. Attempts to obtain more of this compound or its methoxy derivative by oxidation with chromic acid did not prove successful. However, all crude oxidation materials gave a positive fluorescein test, with resorcinol when tested for phthalates.

Celastrol does not form a water-soluble bisulfite addition product and only a minute quantity of a phenazine. It can be reduced with sulfurous acid and reoxidized by atmospheric oxygen after the removal of sulfur dioxide.

Celastrol is orange and, according to Hooker, (2) 2 hydroxy naphthoquinones which have a side chain in position, 3 are yellow if a double bond is present in the β,γ-position and red to orange if the double bond is in the α,β-position. Ultimate analysis of celastrol indicates that it does not have a double bond in the side chain. This is also strengthened by the fact that celastrol when reduced with Raney nickel at 190° in alcohol is converted to its original color upon

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