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RAT POISON FROM WHITE SQUILL

BY

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It is generally known that white squill contains little, if any, rat poison (for literature, see Gold, Modell, Catell, Benton, and Cotlove, 1947). However, as early as 1938, Dr. J. O. Pinkston, former chairman of this department, discovered that, whereas white squill bulbs collected from the coastal areas were inactive, those from the Lebanon mountains showed such activity that he used white squill powder to exterminate wild rats in and around the campus of the University. We have later obtained white squill from another part of the Lebanon mountains which occasionally showed as much activity as red squill. In general, however, the bulbs we have used yielded a dry squill powder that was lethal to male rats in an oral dose of 1.5–2 g./kg. body weight.

Attempts to isolate the active principle from white squill were begun in 1942. In 1944 a small quantity of crystalline material was obtained with an oral MLD in male rats of about 1.5 mg./kg. Owing to the war, the isolation of scilliroside from red squill by Stoll and Renz (1942) became known to us only in 1945. Although the two methods of preparation had nothing in common, the products appeared to be identical, judged by the results of elementary analysis, crystal form, solubility, and the Liebermann colour reaction.

In 1950 the Sandoz firm in Basle kindly supplied us with a sample of crystalline scilliroside, and a new batch of white squill was used to prepare material for comparison. It was soon found that the 1944 success had been dependent on the use of a brand of Merck (Darmstadt) charcoal which had stood in the laboratory for many years. The active substance was quantitatively adsorbed by the old charcoal and was eluted in a relatively pure form by treatment with 50% *iso*propyl alcohol. The available specimens of charcoal—Darco G 60, Mallinckrodt, and a new specimen from Merck—adsorbed the substance so tenaciously that it could not be eluted by 50% *iso*propyl alcohol, and the procedure had therefore to be modified from that step onwards. An amorphous colourless product, 30–35% as toxic to rats as Stoll's scilliroside, was obtained, and proved to be a mixture of scilliroside and a closely related product. This paper describes the properties of both compounds.

MATERIALS AND METHODS

Preparation of Rat Poison From White Squill.—The squill bulbs were collected during the months of June and July from a spot in Southern Lebanon *ca.* 1,800 ft. above sea level and about 15 miles from the Mediterranean coast. Activity was not lost if the bulbs were stored for a few months in a cool and shady place. Six kg. of intact scales was treated with 18 l. of a solution made by diluting 11.4 l. of 95% alcohol with 6.6 l. of water and kept standing at room temperature for a week. This solution contained maximum activity and a minimum of residue. The filtered solution was treated with lead subacetate until no more turbidity was produced on further addition. It was allowed to stand for not more than five hours, when the upper clear layer was siphoned off and the residue centrifuged. The clear fluid was immediately treated with enough sodium acid phosphate to remove the lead. The clear filtrate was then evaporated at a temperature of about 15° C. to approximately 1.5 l., and enough (*ca.* 40–60 g.) decolorizing charcoal added to adsorb all active material; it was kept in the refrigerator for 24 hours and shaken occasionally. The charcoal was filtered and washed with water and 95% alcohol. The alcohol-moist charcoal kept indefinitely in a glass-stoppered bottle.

Elution.—The old procedure need not be given in detail, since it used a sample of charcoal that is no longer available. It involved extraction of the charcoal five to six times with 50% *iso*propyl alcohol, evaporation of the extract to a small volume and repeated extraction of the aqueous residue with chloroform; evaporation of the chloroform extract to a small volume and adsorption on a column of alumina (Merck, standardized according to Brockmann); extrusion of the column and extraction with ice-cold 95% alcohol. The alcohol extract was evaporated to dryness and the residue crystallized from aqueous methanol in the form of irregular prisms.

(Found: C, 62.15; H, 7.84. Scilliroside, $C_{32}H_{44}O_{12}$, requires C, 61.92; H, 7.15%).

According to the new procedure the charcoal adsorbate was shaken for one hour with 500 ml. of a solution containing 75 volumes 95% alcohol, 20 volumes chloroform, and 5 volumes water. The extraction was repeated four or five times and the combined extracts evaporated at a low temperature to a volume of 80 ml. The brown and turbid residue was extracted several times with a total of 100 ml. benzene. From the benzene extract a crystalline aglucone was isolated which will be described elsewhere, but which possessed no lethal action on rats. The aqueous layer was evaporated to remove the benzene and then extracted four times with a total of 500 ml. of a chloroform solution containing 5% (v/v) butanol. This solution, according to Stoll and Renz (1942), extracts scilliroside but not scillaren. The combined extracts, which contained 85% of the activity, were evaporated to dryness under reduced pressure, dissolved in 25 ml. acetone, and adsorbed on a column of acid-washed alumina (Merck, Rahway, N.J.). The column was washed with 200 ml. acetone, and elution was accomplished with a total of 500 ml. 50% alcohol-acetone mixture. On evaporation the alcohol-acetone extract gave an amorphous, slightly coloured powder, which was then dissolved in ethyl acetate. This solution was evaporated until it became turbid and was then treated, while shaking, with an excess of petroleum ether. Ethyl acetate was removed by repeated decantations and replacement with petroleum ether. The product was filtered and dried. Repeated adsorption on alumina and elution with other fluid mixtures, such as 50% alcohol-ethyl acetate, or 10% methanol in chloroform, gave the same product and did not contribute to an increase in activity. The yield was about 1.5 g. from 6 kg. fresh scales.

Acetylation of the White Squill Mixture.—The usual method, utilizing acetic anhydride and pyridine at room temperature, was employed (Stoll and Renz, 1942). The product was purified on the alumina column.

Oxidation of the Acetylated Product.—The method of Stoll, Renz, and Helfenstein (1943) was used.

Separation of the Mixture by Paper Chromatography.—Water was used as the stable phase and a mixture of cyclohexane and cyclohexanol as the mobile phase.

Paper Electrophoresis.—This was kindly performed by Dozent Dr. Kurt Wallenfels, Boehringer & Sons, Mannheim, Germany. The paper was sprayed with perchloric and sulphuric acids and heated prior to inspection under the ultra-violet lamp.

Acid Hydrolysis of the White Squill Mixture.—The method of Mannich and Siewert (1942) was used, utilizing hydrochloric acid and acetone at room temperature for two days. The details of the purification of the two crystalline aglucones obtained will be published elsewhere.

Results

Analyses.—1. *White squill mixture* dried in vacuum at 80° C. Found:—Batch I: C, 62.0; H, 7.47. Batch II: C, 62.64; H, 7.57. Scilliroside ($C_{32}H_{44}O_{12}$) requires C, 61.92; H, 7.15%.

2. *Acetylated product* dried in high vacuum at 80° C., m.p. 131–133° C. Found: C, 60.54; H, 6.61. Tetraacetyl-scilliroside ($C_{40}H_{52}O_{16}$) requires C, 60.90; H, 6.65%.

3. *Oxidized acetylated product,* m.p. 233–234° C. No depression with compound prepared from crystalline scilliroside. Found: C, 61.33; H, 6.47. Dehydrotetraacetyl-scilliroside ($C_{40}H_{50}O_{16}$) requires C, 61.04; H, 6.41%.

4. *Hydrolysis products of white squill mixture.* Aglucone I—needles, from methanol, m.p. 223–225° C. Found: (i) C, 73.66; H, 7.76; (ii) C, 73.41; H, 7.45%. Aglucone II—leaflets, from ethanol, m.p. 232–234° C. Found: C, 76.33; H, 8.01%.

Paper Chromatography.—This revealed one zone for scilliroside and two contiguous zones for the white squill mixture, one of which moved at the same rate as scilliroside.

Paper Electrophoresis.—This also revealed one band for scilliroside and two for the white squill mixture.

Ultra-violet Absorption Spectra.—The spectrum of the white squill mixture and that of scilliroside are identical. Both show a maximum at 297 mμ and a molecular extinction coefficient of 3.71 at that wavelength. (For complete curve see Stoll and Renz (1942)).

Liebermann Colour Reaction.—Stoll's scilliroside: violet→blue→blue-green. White squill mixture: violet→blue→blue-green.

Toxicity to Male Rats.—It can be seen from Table I that the white squill mixture is 30–35% as active as scilliroside.

TABLE I

COMPARATIVE TOXICITIES OF SCILLIROSIDE AND WHITE SQUILL RAT POISON BY INTRAVENOUS INJECTION TO MALE RATS

Scilliroside			White Squill Product		
Dose mg./kg.	No. of Rats	No. Died with Convulsions	Dose mg./kg.	No. of Rats	No. Died with Convulsions
0·8	6	0	1·8	6	0
0·9	12	6	2·5	6	2
1·0	18	14	2·7	12	5
			3·0	12	10

Cardiotoxic Activity (Table II).—Dog heart-lung preparations were used and the glycosides were infused at the optimal rate according to Farah (1946). Before the infusion was started the hearts were made hypodynamic by pentobarbitone; the initial systemic output was decreased to one half or less. The therapeutic effect was assessed by a decrease in right atrial pressure and a simultaneous increase in systemic output. It will be noticed that scilliroside is less active than ouabain but more active than digoxin, digitoxin, and oleandrin, as judged by the data obtained by Farah and Maresh (1948). It will also be seen from Tables I and II that although the white squill glycoside mixture contains only 30–35% scilliroside it is about 55% as toxic to the heart, indicating that the rat-inactive product is cardiotoxic but less so than scilliroside. The ratio of the therapeutic dose to the lethal dose is slightly higher for the squill glycosides than that obtained by Farah and Maresh for other heart poisons. This may be due to the fact that we have used a big dose of pentobarbitone to induce failure, and in most experiments have started the infusion of the glycosides while the atrial pressure was still rising. Where the therapeutic dose is left out it means that the turning point was not sharp or that no definite therapeutic activity was observed.

TABLE II

THERAPEUTIC AND LETHAL DOSES OF SQUILL GLYCOSIDES IN DOG HEART-LUNG PREPARATIONS

In all except the first four experiments of the first series, pentobarbitone was added to reduce the initial systemic output to one half or less, before infusion of the glycosides was started

Exp. No.	Glycoside	Rate of Administration. Micromoles/min	Therapeutic Dose in % of Lethal Dose	Lethal Dose. Micromoles/kg. Heart	Duration of Infusion of Glycoside in Min.
1A	Scilliroside	0·002	—	3·41	80
2A	,,	0·002	—	4·10	88
3A	,,	0·002	—	3·54	79
4A	,,	0·002	30·4	3·51	102
5A	,,	0·002	—	3·89	96
6A	,,	0·002	23·6	2·94	102
7A	,,	0·002	32·6	3·95	92
8A	,,	0·002	22·7	2·79	88
9A	,,	0·002	13·8	2·38	94
10A	,,	0·002	13·4	2·62	112
			Av. 22·7	3·31	93·3
1B	,,	0·001	20·1	3·42	174
2B	,,	0·001	20·2	2·1	148
3B	,,	0·001	15·1	3·3	165
4B	,,	0·001	20·8	3·96	231
			Av. 19	3·19	179
	White squill mixture product M1b				
1C	,, ,,	0·004	23·1	5·86	108
2C	,, ,,	0·004	—	4·77	85
3C	,, ,,	0·004	27·8	4·40	90
4C	,, ,,	0·004	22·2	6·92	108
5C	,, ,,	0·004	—	8·45	97
			Av. 24·4	6·08	98
1D	,, ,,	0·002	21·65	6·30	162
2D	,, ,,	0·002	29·20	5·24	137
3D	,, ,,	0·002	26·00	5·88	154
4D	,, ,,	0·002	—	5·84	158
			Av. 25·6	5·81	153
	White squill mixture product M1c				
1E	,, ,,	0·002	24	6·94	150
2E	,, ,,	0·002	28	5·0	160
3E	,, ,,	0·002	27·2	7·73	184
4E	,, ,,	0·002	18·2	5·67	165
5E	,, ,,	0·002	30·6	6·23	163
6E	,, ,,	0·002	24·8	3·93	121
			Av. 25·4	5·92	157

DISCUSSION

The amorphous product from white squill appears to be a mixture of two substances. This is indicated by the results of paper chromatography as well as paper electrophoresis experiments, which revealed only two zones. One of these products is clearly scilliroside, for oxidation of the acetylated mixture with chromic acid or lead tetraacetate according to Stoll, Renz, and Helfenstein (1943) gave, in a yield of 30%, a crystalline dehydrotetraacetyl-scilliroside which was indistinguishable from the product obtained from pure scilliroside. Since the amorphous product is only 30–35% as toxic to rats as scilliroside, it is difficult to avoid the conclusion that the rodenticidal activity of white squill is due exclusively to its scilliroside content, as Gold *et al.* (1947) had suspected. However, tested on the dog heart-lung preparation, the white squill mixture is about 55% as toxic as scilliroside, and we prefer to explain this by suggesting that the rat-inactive principle is toxic to the heart but less so than scilliroside. There are, however, alternative explanations, but we consider these to be less plausible. Assuming always that the methods of bioassay are sufficiently accurate, one could suspect, for instance, that both the rodenticidal and the cardiotoxic activities of the white squill mixture are due to its scilliroside content which might be, say, 55%. One would then have to assume that the preparative losses reduce the yield of dehydrotetraacetyl-scilliroside to 30% and that the rat-inactive principle interfered with the rat assay.

The nature of the rat-inactive principle is not known with certainty yet. But it must be closely related to scilliroside: the two products behave similarly on the alumina column, and have almost identical elementary compositions and molecular extinction coefficients. Acid hydrolysis of the amorphous product according to Mannich and Siewert (1942) yields two crystalline aglucones

which must be derived from the rat-inactive glycoside, since this method of hydrolysis yields no crystalline products from pure scilliroside. The chemical structure of these aglucones is being further investigated and will be published elsewhere. In the meantime further attempts are being made, by the use of paper chromatography, to isolate the rat-inactive principle on a preparative scale.

It is not known why the squill that grows in the coastal areas is almost devoid of lethal action on rats while that which grows in the mountains is active. The two are botanically indistinguishable. Three factors may be responsible for this difference—the soil, the altitude, and the nearness to the sea. Attempts are being made to solve this interesting problem by transplanting squill bulbs from the coast to the mountain area and vice-versa, both with and without the surrounding native soil.

SUMMARY

1. Wild white squill that grows in some parts of the Lebanon mountains contains sufficient rat poison to be of use in exterminating rats. The white squill that grows in the coastal areas is almost inactive.

2. The active principle has been shown to be identical with scilliroside, the substance obtained from red squill grown in North Africa.

3. Scilliroside was tested, on the dog heart-lung preparation, for its cardiotoxic activity and was found to be less active than ouabain but more active than digoxin, digitoxin, and oleandrin.

4. White squill also contains another glycoside, hitherto undescribed, which bears a close chemical relationship to scilliroside, but which is not toxic to rats and is less cardiotoxic than scilliroside.

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