TLC–SPECTROPHOTOMETRIC ASSAY OF THE MAIN GLYCOSIDES OF RED SQUILL, A SPECIFIC RODENTICIDE

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Abstract.—Red squill bulbs, with reported specific rodenticidal properties, have been assayed for their content of the two main glycosides, scilliroside and scillaren A, by a method depending on the separation of the glycosides from purified plant extracts by tlc followed by spectrophotometric (uv and visible) determination of the individual glycosides in the eluates. The method was found convenient for assessment of the potency of red squill bulbs.

Red squill (*Urginea maritima* var. *pancratium*, Stein Baker) has been known since immemorial times as an effective rodenticide; however, it was not until the late 1940's that its specificity to kill rats, but not poultry and domestic animals, was reported (1). Selective action and the decreased hazard to other animals is due to its inducing vomiting in most animals when ingested, however, rats can not vomit and hence are susceptible (2). The French Pharmacopoeia Codex (3) includes a raticidal paste composed of red squill bulb power, flour, and sugar. The bulbs were reported to contain the glycosides scilliroside (4, 5) and scillaren A (6, 7) of which the first was reported to be responsible for the selective rodenticidal effect (8).

Scientific literature lacks any reference to a specific method for assay of the active glycosides in red squill bulbs. Thompson reported (2) that the active ingredients of this selective rodenticide have never been determined. On the other hand, a few references (9–12) could be traced which dealt with the chromatographic separation and quantification of active constituents of white squill, a completely different species. Consequently, it was desirable to establish a rapid and sensitive method to help in the assessment of the value of red squill bulbs based on their content of active glycosides.

In this paper, a method is reported for the assay of scilliroside and scillaren A individually in red squill bulbs. The method involves separation of the glycosides on silca gel G plates followed by estimation of the individual glycoside eluates by spectrophotometry (uv, or visible after treatment with a color reagent). The method was found feasible and precise.

EXPERIMENTAL[3]

Plant material.— The bulbs of red squill used in this work were collected during September 1976 from the "El-Maktala" region near the Western Mediterranean coast (427 km west Alexandria) in Egypt. The material was identified by Professor Vivi Tackholm, Professor of Plant Taxonomy, Faculty of Science, Cairo University. The bulbs were sliced, dried in hot air at 60°C to constant weight, powdered, and kept in well closed amber colored containers.

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[3]Standard scilliroside and scillaren A were kindly supplied by Sandoz A. G., Basel, Switzerland. Glass plates (20 x 20 cm) for tlc were coated with 0.25 mm layers of methanol-washed silica gel G (Merck) and activated at 110°C for 30 minutes; the developing system was chloroform/methanol/formamide (80:19:1); the spray reagent was saturated solution of antimony trichloride in chloroform; the color reagent was pre-cooled sulphuric acid (20 ml) added gradually to 80 ml of pre-cooled acetic anhydride with gentle agitation.

QUANTITATIVE TLC RECOVERY OF STANDARD SCILLIROSIDE AND SCHILLAREN A.—Silica gel G plates were spotted with volumes of ethanolic solutions of scilliroside and scillaren A corresponding to amounts ranging between 20 and 300 μg of each. Pilot spots were loaded on the periphery of each plate. After the plates were developed at 20°C to about 12 cm, they were dried and the pilot spots located by spraying with antimony trichloride reagent followed by heating at 105°C for 5 minutes. The non-sprayed central spot areas corresponding to glycoside spots were separately scraped and eluted with 5, 3, and 2 ml of ethanol; the eluants were separated by centrifugation. The eluants of each spot area were combined and adjusted to 10 ml with ethanol. Control glycoside-free areas of plates developed in the same jar were treated in the same manner and used as blanks.

DETERMINATION OF STANDARD GLYCOSIDES IN THE ELUATES.—a. *Direct uv:* Absorbances of the individual ethanolic eluates were determined on a Jean and Constant uv spectrophotometer (Prolabo, Paris) at an optimum of 300 nm.

b. *Colorimetric estimation:* Aliquots of the individual spot eluates were evaporated to dryness. The residue was dissolved in 5 ml of glacial acetic acid and then 5 ml of the color reagent was added. The developed color was measured at an optimum of 20 minutes, and of 670 nm and 660 nm for scilliroside and scillaren A, respectively.

PREPARATION OF PURIFIED EXTRACTS OF RED SQUILL BULBS.—The plant material was extracted by a method based on the general methods described in the literature (4, 12) for extraction of glycosides. Two gm of dry material were boiled with 20 ml of ethanol for 2 minutes, then 10 ml water was added and the mixture boiled for an additional 5 minutes and filtered. The residue was re-extracted by boiling with 2 x 10 ml 60% ethanol for 5 minutes. The combined filtered extracts were concentrated to about 20 ml under reduced pressure at 60°C then treated with 2 ml of 10% lead acetate solution. The liquid was allowed to stand for 10 minutes, with occasional shaking, and then filtered. The filtrate was de-leaded with 2 ml of a 10% solution of sodium phosphate and re-filtered. The filtrate was extracted with 3 x 10 ml chloroform/butanol (8:2); the extractive was then evaporated to dryness under reduced pressure at 60°C.

DETERMINATION OF SCILLIROSIDE AND SCILLAREN A IN PURIFIED EXTRACTS OF THE BULBS.—Aliquots of the purified extracts of red squill bulbs were subjected to quantitative tlc followed by uv as well as chlorimetric determination as described above for the standards.

RESULTS AND DISCUSSION

The results for the assay of scilliroside and scillaren A in the red squill bulbs collected for this study, both before and after standard additions, are presented in table 1.

TABLE 1. Results of assay of scilliroside and scillaren A in red squill bubls before and after standard additions.

Method	Scilliroside (ppm)*			Scillaren A (ppm)*		
	In RSB**	In RSB+100 ppm scilliroside	In RSB+100 ppm scillaren A	In RSB**	In RSB+100 ppm scilliroside	In RSB+100 ppm scillaren A
TLC-UV method	44.8 (+0.18)	144.4 (+0.26)	44.6 (−0.22)	36.6 (+0.16)	36.2 (+0.24)	136.4 (+0.88)
TLC-colorimetric method	46.4 (−0.48)	147.0 (+0.78)	45.8 (+0.40)	38.2 (+0.66)	37.8 (+0.42)	138.4 (+0.62)

*Mean of 5 determinations + SD.
**RSB: red squill bulb, dry powder.

A rapid and sensitive chemical assay of the main effective glycosides of red squill is a prerequisite to its extensive use as a rodenticide. Because the bulbs are collected from wild plants, samples collected at different seasons or from different localities may vary considerably in potency.

Successful separation of scilliroside (hR_f 42) from scillaren A (hR_f 63), as well as other contituents of the plant extract, was achieved by tlc as described.

Both glycosides could be quantitatively eluted from the corresponding spot areas on the plates by extraction with 3 massive portions of ethanol totaling 10 ml.

Spot eluates could be directly subjected to uv spectrophotometry. However, most commercially available silica gel G was found to interfere with this direct uv measurement due to the presence of interfering substances which were not identified. This difficulty was overcome by washing the absorbent twice with boiling methanol. This treatment eliminated the interference. Recoveries of standard glycosides were $96.2 \pm 1.69\%$ and $96.9 \pm 2.1\%$ for scilliroside and scillaren A, respectively.

The use of the color reaction resulted in greater precision and higher recoveries, and eliminated the need for purifying the silica gel. The optimum conditions for the colorimetric assay were determined by systematic variation of the ratio of the constituents of the reagent, which was originally described (13) as a spray reagent for certain steroid glycosides, based on the Liebermann-Burchard reagent (14). The selected conditions are given in the experimental section. Recoveries were $98.7 \pm 1.02\%$ and $99.1 \pm 0.89\%$ for scilliroside and scillaren A, respectively.

As a result of the present work, it should now be possible to determine scilliroside and scillaren A in red squill samples from different sources, which will help in their use as a standardized rodenticide.

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LITERATURE CITED

1. J. M. Watt and M. Gerdina, "Medicinal and Poisonous Plants of Southern and Eastern Africa", Livingstone, London (1962).
2. W. T. Thompson, "Agricultural Chemicals, Vol. III, Fumigants, Growth Regulators, Repellents and Rodenticides", Thompson Publications, Fresno, California (1977).
3. French Pharmacopoeia Codex, 8th ed. (1965).
4. A. Stoll and J. Renz, *Bull. Sci. Pharmacol.* **47**, 54 (1940).
5. A. Stoll, J. Renz and H. Helfenstein, *Helv. Chim. Acta*, **26**, 648 (1943).
6. C. W. Shoppee, "Chemistry of the Steroids", 2nd ed. Butterworths, London (1964).
7. A. V. Wartburg, *Helv. Chim. Acta*, **49**, 30 (1966).
8. A. Stoll and J. Renz, *Helv. Chim. Acta*, **25**, 42 (1942).
9. W. Steidle, *Planta Med.*, **9**, 435 (1969).
10. M. A. El-Keiy, M. F. Hashem, M. S. Darwish, S. M. Abdel-Wahab and S. A. About-Tabl, *J. Pharm. Sci.* (UAR), **6**, 217 (1965).
11. L. Nover, *J. Chromatog.*, **39**, 450 (1969).
12. M. S. Karawya and T. Khalifa, *Planta Med.*, **21**, 226 (1973).
13. E. Stahl, "Thin Layer Chromatography", Springer-Verlag, New York (1969).
14. D. Liebermann and H. Burchard, *Chem. Zantr.*, **61**, 1 (1890).