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BUFADIENOLIDES IN DIFFERENT CHROMOSOMAL RACES OF INDIAN SQUILL

SUMITA JHA and SUMITRA SEN

Chromosome Research Centre, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Calcutta-700019, India

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Abstract—Scilliphaeoside and anhydroscilliphaeosidin have been isolated from tetraploid Indian squill. The absence of scilliphaeoside in all populations of diploids and hexaploids and anhydrophaeosidin in diploids, triploids and hexaploids might be one of the reasons why they were not detected in previous studies.

INTRODUCTION

About a dozen bufadienolides have been isolated from the European squill, *Urginea maritima* Baker (syn. *Scilla maritima*) [1–5]. Commercial samples of Indian squill are reported [6] to be mixtures of *Urginea indica* Kunth and *Scilla indica* Roxb. and most of the phytochemical investigations on Indian squill have been carried out on a mixture of these two species [7–9]. It may be noted that *U. indica* and *S. indica* are not synonyms. Recently it has been shown than *S. indica* does not contain scillaren A [10], the principal bufadienolide of *U. maritima* [11] and the only bufadienolide to have been isolated from *U. indica* [12]. In *U. indica*, a polyploid series having different cytotypes has been recorded [13–15]. The present investigation has been undertaken to isolate and compare the distribution of bufadienolides in twelve different cytotypes belonging to four ploidy levels of *U. indica*.

RESULTS AND DISCUSSION

Proscillaridin A and scillaren A, the major glycosides of *U. maritima*, are present in bulbs of all the twelve cytotypes of *U. indica*. Besides these, two minor components known to occur in European squill [5]: scilliphaeoside (a glycoside) and anhydroscilliphaeosidin (an aglycone), are present in the tetraploid cytotypes of Indian squill and scilliphaeoside in the triploids. But they were absent in the diploid and hexaploid cytotypes studied (Table 1). The leaves do not contain bufadienolides whereas the roots of all the plants contain proscillaridin A and scillaren A. Glucoscillarenin [1] and scilliglaucosid [2], present in the white variety of *U. maritima*, and scillirosid [3], present in the pink variety of *U. maritima*, could not be detected in any of the cytotypes. The approximate concentrations (% dry wt) of proscillaridin A, scillaren A, scilliphaeoside and anhydroscilliphaeosidin in bulbs were found to be: 0.18, 0.10, 0.10 and 0.7 respectively.

This is the first report of the isolation of a glycoside other than scillaren A from Indian squill. The very fact that the cytotypes have been found to differ with respect to the presence or absence of two of the glycosides may be the reason for them not having been detected in previous studies.

EXPERIMENTAL

Materials. Bulbs of *U. indica* from the northern and southern states of India were grown under identical conditions in the experimental garden. The twelve cytotypes taken for analysis represented four chromosomal levels; diploid, triploid, tetraploid and hexaploid. The cytotypes differ with respect to details of karyotype [14]. Bulbs, roots and leaves were collected during the active growth period of the plant (for 3 successive years), washed and then dried.

Extraction of bufadienolides. The dried plant material (bulbs, 100–200 g; roots and leaves, 15–20 g) was defatted with petrol (bp 60–80°) and extracted with EtOH. The extract was concd, purified [4] and the glycosides were extracted following the method used in previous studies [4, 9]. Suitable precautions were taken to prevent hydrolysis of the glycosides in the course of the extraction [16].

TLC. The glycoside extracts were screened for the presence of bufadienolides by TLC on Si gel G developed with CH_2Cl_2–MeOH–formamide (80:9:1) (system 1), CH_2Cl_2–MeOH–H_2O (87:12:1) (system 2), $CHCl_3$–MeOH–H_2O (40:9:1) (system 3) or $CHCl_3$–EtOAc (1:1) (system 4) [17–20]. The reagent used to detect the bufadienolides [25% TCA in $CHCl_3$–aq. 3% chloramine T (4:1)] was sensitive down to $<0.5\,\mu g$/spot. Standard samples of scillaren A, proscillaridin A, scillirosid, scilliglaucosid and glucoscillarenin were used for detecting their presence or absence in glycoside extracts.

Isolation and identification of bufadienolides. The glycoside extract of each race was separately chromatographed on a column of Si gel developed with a $CHCl_3$–EtOAc–MeOH gradient. The material eluted with $CHCl_3$ was crystallized from

Table 1. Distribution of bufadienolides in bulbs of different cytotypes

Cytotype	Location of collection	Ploidy	Proscillaridin A	Scillaren A	Scilliphaeoside	Anhydroscilliphaeosidin
1	Almora (5494′)	2n	+	+	−	−
2	Coimbatore (900′)	2n	+	+	−	−
3	Bangalore (3113′)	2n	+	+	−	−
4	Jodhpur	2n	+	+	−	−
5	Tuticorin	2n	+	+	−	−
6	Bangalore	3n	+	+	+	−
7	Pune (1850′)	3n	+	+	+	−
8	Bangalore	4n	+	+	+	+
9	Pune	4n	+	+	+	+
10	Orissa	4n	+	+	+	+
11	Tuticorin	6n	+	+	−	−
12	100 km NW of Tuticorin	6n	+	+	−	−

+, present; −, absent.

$CHCl_3$–MeOH to give *ca* 35 mg of wedge-shaped crystals (mp 218–222°). TLC (system 4), R_f 0.5 (scillaridin, >0.5; other glycosides, <0.5). UV λ_{max}^{EtOH} nm: 225, 233, 241.5, 298 (log ε 4.38, 4.37, 4.16, 3.78); IR ν_{max}^{KBr} cm^{-1}: 3875 and 3600 (OH), 1710 and 1635 (α-pyrone). ^1H NMR (90 MHz, DMSO): δ 7.92 (1H, *d*, $J_1 = 9.5, J_2 = 2$ Hz, C-22), 7.50 (1H, $J = 2$ Hz, C-21), 6.32 (1H, *d*, $J = 9.5$ Hz, C-23), 0.86 (3H, *s*, Me_3-19), 0.58 (3H, *s*, Me-18). The above properties were the same as those that had been reported for anhydroscilliphaeosidin [5].

The material eluted with $CHCl_3$–EtOAc (1:9) gave 60 mg of crystals (MeOH, mp 213°) which were identified as proscillaridin A by mmp, co-chromatography on TLC and IR. The fractions eluted with EtOAc yielded 50 mg of white crystals (MeOH, mp 248–250°). TLC (system 3), R_f 0.4 (material did not run with any of the standard glycosides); UV λ_{max}^{EtOH} 298 nm; IR ν_{max}^{KBr} cm^{-1}: 3600 (OH), 1705 (α-pyrone). ^1H NMR (90 MHz, DMSO): δ 7.90 (*d*, $J_1 = 9$, $J_2 = 2$ Hz, C-22), 7.52 (1H, fine *d*, $J = 2$ Hz, C-21), 6.35 (1H, *d*, $J = 9$ Hz, C-23), 5.30 (1H, *br. s*, C-4), 1.17 (3H, *d*, $J = 6$ Hz, rhamnose-Me), 1.05 (3H, *s*, Me-C-19), 0.57 (3H, Me-C-18). The UV, IR and NMR data indicated that the compound was scilliphaeoside [5].

The functions eluted with MeOH–EtOAc (1:49) gave 25 mg of a crystalline residue (MeOH, mp 270°) which was identified as scillaren A by direct comparison with an authentic sample. The identities of scilliphaeoside and anhydroscilliphaeosidin were further confirmed by direct comparisons with standard samples.

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REFERENCES

1. Stoll, A. and Kreis, W. (1951) *Helv. Chim. Acta* **34,** 1431.
2. Stoll, A., Wartburg, A. von and Renz, J. (1953) *Helv. Chim. Acta* **36,** 1531.
3. Wartburg, A. von and Renz, J. (1959) *Helv. Chim. Acta* **42,** 1620.
4. Wartburg, A. von (1966) *Helv. Chim. Acta* **49,** 30.
5. Wartburg, A. von, Kohn, M. and Huber, K. (1968) *Helv. Chim. Acta* **51,** 1317.
6. (1976) *Wealth of India—Raw Materials*, Vol. X, p. 416. C.S.I.R., New Delhi.
7. Seshadri, T. R. and Subramaniam, S. (1950) *J. Sci. Ind. Res. Sect. B* **9,** 114.
8. Rangaswami, S. and Subramaniam, S. (1954) *J. Sci. Ind. Res. Sect. B* **13,** 150.
9. Krishna Rao, R. V. and Rangaswami, S. (1967) *Tetrahedron Letters* 4563.
10. Jha, S. and Sen, S. (1980) *Curr. Sci.* **7,** 273.
11. Stoll, A., Suter, H., Kreis, W., Bussemaker, B. and Hofmann, A. (1933) *Helv. Chim. Acta* **16,** 703.
12. Rangaswami, S. and Subramaniam, S. (1956) *J. Sci. Ind. Res. Sect. C* **15,** 80.

13. Raghavan, T. S. and Venkatasubban, K. R. (1940) *Cytologia* **11,** 71.
14. Sen, S. (1973) *Folia Biol* **21,** 183.
15. Jha, S. and Sen, S. (1980) *Cell Chromosome News l.* (in press).
16. Trim, A. R. (1955) in *Modern Methods of Plant Analysis* (Paech, K. and Tracey, M. V., eds.) Vol. 11, p. 295. Springer, Berlin.
17. Stahl, E. and Kaltenbach, U. (1961) *J. Chromatogr.* **5,** 458.
18. Fauconnet, L. and Waldesbühl, M. (1963) *Pharm. Acta Helv.* **38,** 423.
19. Alfermann, H., Boy, M., Doller, P. C., Hagedorn, W., Heins, M., Wahl, J. and Reinhard, E. (1977) in *Plant Tissue Culture and its Biotechnological Applications.* (Barz, W., Reinhard, E. and Zenk, M. H., eds.) Springer, Berlin.
20. Gorlich, B. (1960) *Arzneim.-Forsch.* **10,** 770.