Grayanotoxin I. Occurrence in Additional *Ericaceae* Species

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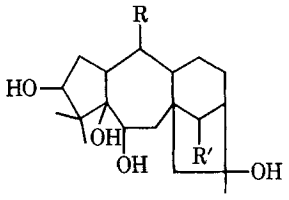
The title compound was identified in five of seven ericaceous species not previously investigated but listed as being poisonous. Thin-layer chromatography and spectral analyses established the presence of grayanotoxin (acetylandromedol) in *Rhododendron macrophyllum*. Comparative chromatography and U.V. spectral analysis indicated that the compound was present in four additional species.

THE GRAYANOTOXINS (I) are known to occur only in the family *Ericaceae* (1). Several members of this family, which occur in the Pacific Northwest and are reported to be poisonous (2–4), have never been investigated for the presence of these compounds. This study was undertaken to determine if the grayanotoxins could be identified in certain native species thereby verifying, at least in part, the agent responsible for the reported toxicity.



	R	R′
Grayanotoxin I	CH_3, OH	OAc
Grayanotoxin II	CH_2	H, OH
Grayanotoxin III	CH_3, OH	H, OH

EXPERIMENTAL

Chromatographic Screening—The plants were collected in Oregon during the summer of 1966.[1] The leaves were dried in a forced-air dryer, ground to a coarse powder, and stored in plastic bags until time for extraction.

One hundred-gram samples of each plant were defatted with petroleum ether in a Soxhlet apparatus and then exhaustively extracted with ethanol U.S.P. The ethanol extracts were concentrated *in vacuo* to a lesser volume (about 50 ml.) and diluted with an equal volume of distilled water. These mixtures were subjected to liquid–liquid extraction with chloroform U.S.P. The chloroform extracts were concentrated to dryness *in vacuo*, redissolved in a minimum amount of chloroform, and examined chromatographically.

Thin-layer chromatography on Silica Gel G plates, prepared according to Stahl, was employed as the preliminary screening procedure. Three solvent systems were found to separate authentic samples of the grayanotoxins.[2] The results of the co-spotting experiments are recorded in Table I. Grayanotoxin I (G-I) was verified as being present in the following: *Rhododendron albiflorum* Hook, *R. macrophyllum* D. Don., *R. occidentale* (T. and G.) Gray, *Kalmia polifolia* Wang. var. *microphylla* Hook, and *K. polifolia* Wang. var. *polifolia*. The toxic principle could not be detected in either *Ledum glandulosum* Nutt or *Menziesea ferruginea* Smith.

A vacuum-zone extractor was used to remove samples from unsprayed chromatoplates for U.V. analyses. Areas corresponding in R_f to G-I for each of the five plants, as well as G-I, were then removed from the extractor with boiling methanol (spectral grade). Aliquots were scanned in a Beckman DB recording spectrophotometer and all gave a characteristic maximum at 255 mμ.

TABLE I—CO-SPOTTING OF PLANT EXTRACTS

Compd.	Solvent Systems[a] CAW	EIW	TEF
1, Grayanotoxin I	0.86[b]	0.73	0.18
2, Grayanotoxin II	0.90	0.79	0.22
3, Grayanotoxin III	0.80	0.69	0.12
4, *R. albiflorum*	0.86	0.73	0.18
5, Co-spot (*1* and *4*)	0.86	0.73	0.18
6, *R. macrophyllum*	0.85	0.73	0.18
7, Co-spot (*1* and *6*)	0.85	0.73	0.18
8, *R. occidentale*	0.87	0.71	0.17
9, Co-spot (*1* and *8*)	0.87	0.70	0.17
10, *K. poli.* var. micro.	0.86	0.71	0.19
11, Co-spot (*1* and *10*)	0.86	0.71	0.20
12, *K. poli.* var. poli.	0.87	0.72	0.17
13, Co-spot (*1* and *13*)	0.87	0.70	0.18
14, *L. glandulosum*	...	...	...
15, Co-spot (*1* and *14*)	0.86	0.71	0.18
16, *M. ferrugenea*	...	...	...
17, Co-spot (*1* and *16*)	0.88	0.74	0.18

[a] Solvent systems: CAW, chloroform–acetic acid–water (35:50:17.5); EIW, ethyl acetate–isopropanol–water (65:24:11); TEF, toluene–ethyl acetate–water (5:4:1). [b] Detection: sprayed with $SbCl_3$ (10% w/w in $CHCl_3$), visualized under U.V. light, and sprayed again with Godin's reagent (1% vanillin in EtOH, 3% H_2SO_4, equal parts.) Heated to 110° for 10 min. after each spray. $SbCl_3$ gives a red-pink coloration, U.V. a red fluorescence, and Godin's reagent a characteristic blue coloration.

Isolation of Grayanotoxin I—A 1-Kg. sample of *R. macrophyllum* was extracted in a similar manner as previously described. The resulting hydroalcoholic solution was then extracted with ethyl acetate in a liquid–liquid extractor for 48 hr. The ethyl acetate fraction on drying *in vacuo* yielded 50 Gm. of a dark green residue which was initially

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[1] Plant voucher specimens have been placed in the Oregon State University Herbarium.

[2] Supplied through the courtesy of Professor H. Kakisawa, Tokyo Kyoiku University, Otsuka, Japan.

TABLE II—COLUMN CHROMATOGRAPHY OF *Rhododendron* EXTRACT

Solvent	Fractions	Vol., ml.	R_f Value[a]
1, EtOAc	4	250	...
2, *n*-Propanol (10%) in EtOAc	4	250	0.33[b]
3, *n*-Propanol (50%) in EtOAc	4	250	0.33
4, *n*-Propanol	4	250	...
Grayanotoxin I	...	...	0.33

[a] Solvent system: TEFF, toluene–ethyl formate–formic acid (5:4:1). [b] Detection: see Table I.

TABLE III—COLUMN CHROMATOGRAPHY OF *Rhododendron* FRACTION

Solvent	Fractions	Vol., ml.	R_f Value[a]
1, Benzene	1	500	...
2, EtOAc	6	250	0.34[b]
3, *n*-Propanol (5%) in EtOAc	4	250	0.34
4, *n*-Propanol (10%) in EtOAc	4	250	...
Grayanotoxin I	...	...	0.34

[a] Solvent system: TEFF, toluene–ethyl formate–formic acid (5:4:1). [b] Detection: see Table I.

purified on three identical 450-Gm. alumina columns (5 × 50 cm.).[3] The column was eluted first with twelve 250-ml. fractions of ethyl acetate followed by six 250-ml. fractions of 10% methanol in ethyl acetate.

The progress of the column was monitored by using the solvent system TEF (see Table I). The third and fourth fractions of the second solvent clearly demonstrated the presence of G-I. The combined fractions yielded 4.5 Gm. of greenish residue which was placed upon another 450-Gm. alumina column and eluted as shown in Table II.

The G-I containing fractions thus collected were combined, concentrated to dryness, washed with boiling *n*-hexane, and dissolved in ethyl acetate–*n*-hexane (1:1) in an attempt to precipitate the compound. This procedure yielded a dark green oil which was placed upon a 100-Gm. alumina column (2.5 × 25 cm.) and eluted as shown in Table III.

The positive fractions were combined, dried, and crystallized from *n*-hexane–acetone to yield a green-white precipitate. Two recrystallizations from *n*-hexane–ethyl acetate yielded 5 mg. of pure white needles, m.p. 256–258°.[4] [Reported m.p. 240–243° (5), 259–260° (6), and 264–266° (7).]

Co-chromatography using known G-I and the isolated compound was employed as an additional verification procedure. The co-spot did not separate in the same three solvent systems as in Table I and had an R_f value equivalent to both controls.

The I.R. (KBr pellet) and U.V. spectra (spectral grade methanol) of the isolated as well as the reference G-I were identical.

SUMMARY

Seven species of reportedly poisonous ericaceous plants were screened by thin-layer chromatography in order to detect the presence of the grayanotoxins. Grayanotoxin I was isolated and identified from one of the species. Five of the plants screened were found to contain grayanotoxin I whereas grayanotoxins II and III were not detected. Poisoning reportedly caused by *Ledum glandulosum* and *Menziesea ferruginea* cannot be attributed to the grayanotoxins because of their apparent absence in these species.

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[3] Alumina, activated, chromatographic grade; Matheson, Coleman and Bell, East Rutherford, N. J.

[4] Kofler micro-melting point apparatus, C. Reichert A. G., Vienna, Austria; melting point was uncorrected.