PHYTOCHEMICAL SCREENING OF SOME ROCKY MOUNTAIN PLANTS

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ABSTRACT.—Fifty-four species of plants from 36 genera and 21 families were collected in the mountains and high plains of northern Colorado. Extracts were tested for alkaloids, cytotoxicity, antitumor activity and IP toxicity in mice. A few were tested for insect attractant or deterrent properties. Alkaloids were found in 23 species, 15 showed cytotoxicity, and 17 exhibited IP toxicity. Four extracts showed insect deterrent properties, and three proved to be attractant. None of the extracts showed useful antitumor activity. High alkaloid content and/or activity in several of the screens can be used to identify species and genera worthy of detailed phytochemical investigation.

In the months of June and July, plant growth in the subalpine areas of the Rocky Mountains is almost tropical in diversity of species. Snow melt gives rise to a rapid development of annual plants whose luxurious growth is contrasted with plants of the higher alpine region. The latter remain generally small and stunted. The subalpine area is, therefore, a place for easy collection of numerous species for our program in discovery of new alkaloids and other physiologically active compounds.

RESULTS AND DISCUSSION

Table 1 gives the results of our plant extract screens which included tests for alkaloids, cytotoxicity, antitumor activity, lethality in mice by intraperitoneal injection, and insect feedant studies.

TABLE 1. Phytochemical screening results on Rocky Mountain plants.[a]

	Alkaloids[b]	Cytotoxicity[c]	Toxicity[d]	Insect Feedant Studies[e]
ASTERACEAE				
Chrysothamnus viscidoflorus (Hook.) Nutt.	0	20	200	
Haplopappus amerioides (Nutt.) Gray	++		200	
Senecio dimorphophyllus Greene	0	0	0	Attr.
Senecio hydrophyllus Nutt.	+	0	0	
BRASSICACEAE				
Arabis holbelii Hornem.	0	0	0	Deter.
Cardamine cordifolia A. Gray	+	0	0	
Thalaspi montanum L.	0	0	0	0
BORAGINACEAE				
Cryptantha virgata (Porter) Payson	0	65	0	
Mertensia ciliata (James) G. Don	0	0	0	
CARYOPHYLLACEAE				
Cerastium arvense L.	+	0	0	0
CRUCIFERAE				
Physaria vitalifera Rydb.	+++		400	
CRASSULACEAE				
Sedum rhodanthum A. Gray	0	0	0	
GENTIANACEAE				
Frasera speciosa Griseb.	+	0	400	
HYDROPHYLLACEAE				
Phacelia sericea (Graham) Gray	0	88	0	Deter.
IRIDACEAE				
Iris missouriensis Nutt.	0	0	0	
LEGUMINOSAE				
Thermopsis montana Nutt. ex T. & G.	+++	0	200	
LILIACEAE				
Allium brevistylum A. Wats.	0	0	0	
Erythronium grandiflorum Persh.	+++	66	0	Attr.
Zigadenus paniculatus (Nutt.) S. Watts	+++		50	

TABLE 1. *Continued*

	Alkaloids[b]	Cytotoxicity[c]	Toxicity[d]	Insect Feedant Studies[e]
LOASACEAE				
Mentzelia decapetala (Pursh.) Urb. & Gilg...	0	0	400	
ORCHIDACEAE				
Habenaria dilata (Persh.) Hook.....	++	0	0	0
ONAGRACEAE				
Epilobium angustifolium L.....	0	30	200	
Epilobium hornemanii Rchb.....	0	59	100	Deter.
POLEMONIACEAE				
Gilia aggregata (Pursh.) Spreng.....	0	45	200	
Phlox multiflora A. Nels.....	+		400	
Polemonium pulcherimum ssp. *delicatum* (Rydb.) Brand.....	0	66	37.5	Deter.
POLYGONACEAE				
Eriogonum alatum Torr.....	+		100	
Eriogonum annum Nutt.....	++		0	
Eriogonum campanulatum Nutt.....	+	18	12.5	
Eriogonum flavum Nutt.....	0		0	
Eriogonum inflatum Torr. & Frem.....	+		0	
Eriogonum arcuatum Greene.....	0		0	
Eriogonum pauciflorun Pursh. var. *graphaloides*.....	0		0	
Eriogonum umbellatum Torr.....	0	31	0	
Oxyria digyma (L.) Hill.....	0	0	0	0
Polygonum bistortoides Pursh.....	0	0	0	0
RANUNCULACEAE				
Ranunculus adoneus A. Gray.....	+	66	0	0
Ranunculus alismaefolius Geyar ex Benth....	0	66	0	Attr.
Trollius laxus Salisb. var. *albiflorus* A. Gray.....	+++	0	0	Deter.
ROSACEAE				
Potentilla diversifolia Lehm.....	0	0	0	0
Potentilla fissa Nutt.....	0	30	200	
Potentilla fruticosa L.....	0	38	400	
SCHROPHULARIACEAE				
Castilleja flava Wats.....	+	0	0	
Castilleja rhexifolia Rydb.....	++	0	0	
Castilleja sulphurea Rydb.....	0	0	0	0
Pedicularis bracteosa Benth. var. *paysonia* Pennel.....	++	0	0	
Pedicularis crenulata Benth.....	+		0	
Pedicularis groenlandica Retz.....	+	0	0	
Pedicularis racemosa Dougl. ex Hook.....	0	0	0	0
Penstemon virens Pennel.....	0			
Penstemon whippleanus A. Gray.....	++	0	0	
Veronica wormskjoldii R. & S.....	+	0	0	0

[a]Where no results are given, the particular test was not conducted. [b]The rating +, ++, or +++ are subjective estimates based upon relative densities of iodoplatinate visualized spots on tlc. [c]National Cancer Institute KB cell culture screen. Data are ED_{50} in μg/ml. [d]Extracts are injected IP in mice for the National Cancer Institute P388 screen, usually at a maximum dose of 400 mg/kg. Zero means that no lethality was observed at the highest dose tested. The data are for the lowest dose in mg/kg which exhibited some lethality. [e]A grasshopper antifeedant or attractant screen was used. Extracts were attractive (attr.), deterrent (deter.) or inactive (zero).

Antitumor testing (PS screen) gave no results which reached the active level (T/C 125) and, hence, these results are omitted from the Table. The first PS screen on an *Eriogonum campanulatum* extract gave a T/C of 132 at 0.78 mg/kg, but retesting did not confirm this and gave a maximum of T/C 118 at 6.25 mg/kg. Testing at higher doses was hampered by extreme IP toxicity. Since *E. campanulatum* extracts were effectively cytotoxic and contained alkaloids, the genus *Eriogonum* appears a good candidate for further studies.

A remarkably large percentage of plants proved alkaloid positive (23 of 54 tested species). Hamon and co-workers recently (1) tested 53 species of plants from the South Saskatchewan river area and found only seven positives. Our

sample included significant numbers of positive species from the Polygonaceae, Ranunculaceae, and Scrophulariaceae, none of which were represented in the Hamon sample. The Hamon sample also included relatively large numbers of Cruciferae and Legominosae genera, most of which proved negative. As far as we are aware, only *Thermopsis montana* and *Zygadenus paniculatus* were previously known to contain alkaloids.

Our current work is focussing on *Eriogonum* and *Castilleja* species (2) and *Trollius laxus*. The data of Table 1 should provide leads for others interested in phytochemical studies.

EXPERIMENTAL

PLANT MATERIALS.—Plants were identified by Dr. Dieter H. Wilkin of the Department of Botany and Plant Pathology, Colorado State University (CSU). Voucher specimens were deposited in the Colorado State University herbarium and were keyed to collection numbers of F. R. Stermitz and T. R. Suess (3). In general, the dried, whole, above-ground plant was used.

EXTRACT PREPARATION.—The dried, ground plant material (300 g.) was extracted (Soxhlet) with hexane and then methanol or ethanol. The alcoholic solutions were evaporated to a paste and tested directly and/or partitioned between chloroform and water prior to testing of the chloroform soluble portion (3). For most of the alkaloid tests, the alcohol extract was dissolved in butanol-benzene (1:1) and extracted with dil H_2SO_4. The acid layer was made basic with NH_4OH and extracted with chloroform, and the chloroform residue was tested by tlc on Si gel with iodoplatinate visualizing reagent.

BIOASSAYS.—Extracts were tested for cytotoxicity and antitumor activity by contractors for the National Cancer Institute (4). Cytotoxicity was measured *in vitro* against human nasopharynx carcinoma (KB) and antitumor activity against murine lymphocytic leukemia (P388) *in vivo* in mice (5). The P388 (PS) test involved injection of six infected mice at three different doses. Lethality was reported as deaths occurring prior to the standard death time due to the murine leukemia. Insect feedant effects were measured by John L. Capinera of the Colorado State University Department of Zoology and Entomology according to a published (6) procedure.

ACKNOWLEDGMENT

This work was supported in part by grant CA19243 from the National Cancer Institute, National Institutes of Health.

Received 7 April 1981

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