Phytochemistry, 1965, Vol. 4, pp. 425 to 433. Pergamon Press Ltd. Printed in England

ALKALOID-SCREENING OF SWEDISH PLANTS

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(*Received* 23 *November* 1964)

Abstract—A method is given for a semi-quantitative determination of the alkaloid concentration in dried plant material. In a screening of two hundred plants in Sweden, wild and cultivated, the following plants not previously known to contain alkaloids gave a positive reaction corresponding to an alkaloid concentration of more than 0·05 per cent of the dried plant: *Linnea borealis* and *Valeriana sambucifolia*; and, additionally, the following gave a positive reaction corresponding to at least 0·01 per cent: *Armeria maritima*, *Bartonia aurea*, *Cobaea scandens*, *Euphrasia frigida*, *Galium aparine*, *Hemerocallis conspicua*, *Lonicera periclymenum*, *Melampyrum pratense*, *Odontites litoralis*, *Pedicularis palustris*, *Pedicularis silvatica*, *Phragmites communis*, *Picea abies*, *Pinguicula vulgaris*, *Platanthera bifolia*, *Plantago media*, *Polygala vulgaris*, *Polygonum viviparum*, *Rhamnus cathartica*, *Rhamnus frangula*, *Silene rupestris*, *Sorbus intermedia*, *Spergula arvensis*, *Spergula marginata*, and *Veronica officinalis*. The following plants, previously reported to contain alkaloids, gave a positive reaction corresponding to more than 0·05 per cent: *Ligustrum vulgare*, *Succisa pratensis*, and *Valeriana officinalis*. In the plant family Scrophulariaceae ten out of nineteen species investigated gave a positive reaction corresponding to at least 0·01 per cent of alkaloids. Earlier reports indicating the presence of alkaloids in the following plants were not confirmed: *Alnus glutinosa*, *Anthemis tinctoria*, *Butomus umbellatus*, *Calla palustris*, *Chamaenerion angustifolium*, *Corylus avellana*, *Euphorbia peplus*, *Geranium molle*, *Glaux maritima*, *Herniaria glabra*, *Odontites verna*, *Pyrola rotundifolia*, and *Scirpus Tabernaemontani*. The present results are compared with earlier reports available from the literature.

INTRODUCTION

EUROPEAN plants have been investigated extensively, and information on alkaloid-bearing plants is available in several monographs.[1–10] The result of investigations of some plants which gave no reaction with alkaloid reagents may not have been published, so that it is not generally known if they contain alkaloids; and obviously many plants have not as yet been investigated for alkaloid content.

The purpose of the present study was to obtain more thorough information on Swedish plants as potential sources of new alkaloids. The various species included in the present screening were not collected randomly. We chose some because it was not known whether or not alkaloids occur in the family or genus; we chose others because alkaloids are known to occur in some but not in all species of the family or genus. However, plants belonging to families

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[1] A. HUSEMANN, A. HILGER and T. HUSEMANN, *Die Pflanzenstoffe in chemischer, physiologischer, pharmacologischer und toxicologischer Hinsicht*, Springer, Berlin (1882-1884).
[2] G. DRAGENDORFF, *Die Heilpflanzen der verschiedenen Völker und Zeiten*, Enke, Stuttgart (1898).
[3] F. CZAPEK, *Biochemie der Pflanzen*, Fischer, Jena (1921).
[4] C. WEHMER, *Die Pflanzenstoffe*, Fischer, Jena (1929–1935).
[5] G. KLEIN, *Handbuch der Pflanzenanalyse*, Springer, Wien (1933).
[6] T. A. HENRY, *The Plant Alkaloids*, Churchill, London (1949).
[7] R. H. F. MANSKE and H. L. HOLMES, *The Alkaloids, Chemistry and Physiology*, Academic Press, N.Y. (1950–1960).
[8] V. S. SOKOLOV, *Alkaloidnocyne Rasteniya SSSR*, Izdatel'stvo Akad. Nauk SSSR, Moscow (1952).
[9] H. G. BOIT, *Ergebnisse der Alkaloid-Chemie bis 1960*, Akademie Verlag, Berlin (1961).
[10] J. J. WILLAMAN and B. G. SCHUBERT, *U.S. Dept. Agr. Tech. Bul.* No. 1234 (1961).

well-known for their alkaloid content, such as Leguminosae and Solanaceae, were not included. For systematic reasons we extended the screening to include some small plants not easily available in quantities. A few plants were investigated because of contradictory reports in the literature or because an early report has neither been confirmed nor denied.

Almost all wild plants were collected in the vicinity of Stockholm or on the Swedish west coast near Fiskebäckskil. A few were collected in the province of Härjedalen. Horticultural plants were grown in Stockholm. The names used for the wild plants are those given by Hylander.[11] The dried plant material was extracted with methanol. An aqueous solution of the acid soluble portion of the methanol extract was made basic with ammonia and subjected to differential extraction with chloroform followed by chloroform-ethanol. The two extracts so obtained were then tested with six alkaloid reagents: Mayer's reagent (mercuric chloride and potassium iodide), Wagner's reagent (potassium triiodide), Dragendorff's reagent (bismuth nitrate and potassium iodide) Sonnenschein's reagent (phosphomolybdic acid), silicotungstic acid, and Hager's reagent (picric acid).

The precipitates formed by alkaloid reagents vary in amount with different alkaloids.[12] Hence, any estimate of alkaloid concentration from the amount of the precipitate with one reagent is only approximate. The accuracy can be improved by using several reagents. A semi-quantitative method for estimating the alkaloid concentration in dried plant material was worked out for the present screening. The method is based on comparing the amount of the precipitates obtained with six different alkaloid reagents, as compared with the precipitates with strychnine solutions of known concentrations. Abisch and Reichstein[13] have also published a micromethod, using this principle for estimating the alkaloid concentration in a dried methanolic extract of plant material.

RESULTS

The presence of alkaloids was considered to be demonstrated only if the reaction was positive with the six reagents.[13] However, one of the reagents, picric acid, has so much lower sensitivity than the others[12] that if the reactions with all other reagents used was weak but positive, this was considered to indicate the presence of alkaloids even if no precipitate was obtained with picric acid. Nevertheless, picric acid was included because it appeared to be one of the most discriminating reagents for the assay. Sonnenschein's reagent sometimes gave strong precipitates when borderline reactions were obtained with the other reagents, as was also the case to a lesser extent with silicotungstic acid.

The efficiency of the method was tested in a few experiments. Known amounts of gentianine, pyridine, and strychnine were added to plant material (*Knautia arvensis*) which in previous analyses had given no alkaloid reactions. The amounts of gentianine and strychnine added were recovered, but the pyridine could not be detected. It may be concluded that volatile alkaloids are not detected by our method.

Some substances give false-positive reactions with alkaloid reagents,[14–19] and this pro-

[11] N. HYLANDER, *Förteckning över Nordens växter utgiven av Lunds botaniska förening*, Gleerups, Lund (1955).
[12] R. MARTELLO and N. R. FARNSWORTH, *Lloydia* **25**, 176 (1962).
[13] E. ABISCH and T. REICHSTEIN, *Helv. Chim. Acta* **43**, 1844 (1960).
[14] R. F. RAFFAUF, *Econ. Botany* **14**, 276 (1960).
[15] O. SCHMIEDEBERG, *Arch. Exp. Pathol. Pharmakol.* **16**, 149 (1883).
[16] W. MARMÉ, *Ztschr. f. rat. Med.* [3] **26**, 1 (1866).
[17] L. H. BRIGGS and R. H. LOCKER, *J. Chem. Soc.* 2157 (1949).
[18] N. R. FARNSWORTH and K. L. EULER, *Lloydia* **25**, 186 (1962).

blem has recently been investigated by Farnsworth, Pilewski and Draus.[19] Mayer's and Hager's reagents appear to us to be particularly discriminating for alkaloids. Choline[20] does not give a positive reaction with all of the reagents used, and hence does not interfere in this analysis. Repeated analyses of the same plant material indicated that our estimate of alkaloid concentrations was reproducible within the accuracy noted.

The results from the screening of the plants chosen are presented in Table 1 together with references to earlier investigations. Because of our planned selection of plants, the fraction giving a positive alkaloid reaction was considerably higher than usual. Almost one-quarter of these plants gave a precipitation reaction corresponding to at least 0·01 per cent; work on plants with less concentration is considered impractical.[14] Twenty-seven of these are apparently new alkaloid sources. Traces of alkaloids were found in a second quarter of the plants. Almost one-sixth of the plants gave a positive reaction for such alkaloids only which are not extractable with chloroform from alkaline solution.

A general conclusion from this and earlier investigations is that the alkaloid concentration in most Nordic plants is low, and that relatively few plants have alkaloids in sufficient concentrations to warrant further investigation.

TABLE 1. SPECIES TESTED FOR ALKALOID CONTENT, AND REACTION OBTAINED

Family	Species	Plant* part	Fraction† A	Fraction† B	Reference‡
Acanthaceae	*Thunbergia alata* Hook.	w	0	1	+[21]
Adoxaceae	*Adoxa moschatellina* L.	w	0	3	
Alismataceae	*Alisma plantago-aquatica* L.	w	0	0	−[22], −[23]§
	Sagittaria sagittifolia L.	w	1	1	[24]
Araceae	*Calla palustris* L.	w	0	0	+[22]
Aristolochiaceae	*Asarum europaeum* L.	w	10	30	−[22], +[26], +[27]
Asclepiadaceae	*Cynanchum vincetoxicum* (L.) Pers.	w	10	0	+[22,23,28,29]

* Part of plant: b = bark, f = fruit, l = leaf, r = root, rb = root bark, s = stem or twig, t = tuber, and w = whole plant.

† Fraction A: extracted with chloroform from an ammoniacal solution; fraction B: after addition of sodium sulfate, subsequently extracted with chloroform:ethanol (3:2). The reaction was graded by comparison with the intensity of the reaction between strychnine and the reagents used. 0 designates less than 1 mg per 100 g of dried material; other figures give the order of magnitude expressed in mg per cent.

‡ References for earlier investigations: + and − designate positive and negative reaction, respectively, reported for the same species; parentheses show that a previous investigation was run on a closely related alkaloid-bearing plant.

§ Massagetov[23] got a positive alkaloid reaction from the seeds.

¶ This may be a false-positive reaction.[25]

[19] N. R. FARNSWORTH, N. A. PILEWSKI and F. J. DRAUS, *Lloydia* **25**, 312 (1962).
[20] G. KLEIN and A. ZELLER, *Österr. botan. Z.* **79**, 40 (1930).
[21] H. R. ARTHUR, *J. Pharm. Pharmacol.* **6**, 66 (1954).
[22] A. I. BANKOVSKII, M. P. ZARUBINA and L. A. SERGEEVA, *Trudy Vsesoyuz. Nauchn-Issledovatel. Inst. Lekarstv. Rast.* **9**, 119 (1947).
[23] P. S. MASSAGETOV, *Trudy Vsesoyuz. Nauchn-Issledovatel. Inst. Lekarstv. Rast.* **9**, 3 (1947).
[24] M. E. WALL, J. W. GARVIN, J. J. WILLAMAN, O. JONES and B. G. SCHUBERT, *J. Pharm. Sci* **50**, 1001 (1961).
[25] M. PAILER, Personal communication.
[26] A. ORECHOFF, *Arch. Pharm.* **272**/**44**, 673 (1934).
[27] M. A. ABDUL'MENEV, *Farmatsiya* **8**, No. 4, 39 (1945).
[28] L. J. WEBB, *Australia, Commonwealth Sci. Ind. Res. Organization Bull.* No. 241 (1949).
[29] L. J. WEBB, *Australia, Commonwealth Sci. Ind. Res. Organization Bull.* No. 268 (1952).

TABLE 1—*continued*

Family	Species	Plant part	Fraction A	Fraction B	Reference
Betulaceae	*Alnus glutinosa* (L.) Gaertn.	b, rb	0	0	+[22]
	Alnus incana (L.) Moench	b, rb	0	1	+[22]
	Betula nana L.	l	0	0	
	Betula verrucosa Erh.	rb	3	1	+[22], +[30], +[31]
Butomaceae	*Butomus umbellatus* L.	w	0	0	+[31]
Campanulaceae	*Campanula latifolia* L.	w	0	0	−[26]
	Campanula persicifolia L.	w	0	0	
	Campanula rapunculoides L.	w	0	0	−[22], −[35]
	Campanula rotundifolia L.	w	3	0	+[32]
	Campanula trachelium L.	w	0	0	
	Jasione montana L.	w	10	3	+[22], +[32]
	Platycodon grandiflorum A. DC.	r	3	10	
	Platycodon grandiflorum A. DC.	s, l	0	3	+[32]
Caprifoliaceae	*Linnea borealis* L.	w	30	30	
	Lonicera periclymenum L.	b	10	10	(29, 33, 34)
	Viburnum opulus L.	b	3	3	+[31], −[35]
Caryophyllaceae	*Cerastium holosteoides* Fr.	w	1	1	
	Dianthus deltoides L.	w	1	3	−[22], (26, 33, 36)
	Herniaria glabra L.	w	0	0	+[37]
	Lychnis flos-cuculi L.	w	—	3	−[22], +[26], +[38]
	Melandrium rubrum (Weig.) Garcke	w	0	3	(33)
	Minuartia peploides (L.) Hiern	w	0	3	
	Saponaria officinalis L.	r	0	0	
	Saponaria officinalis L.	s, l	0	3	+[22], −[35]
	Scleranthus annuus L.	w	0	0	
	Silene acaulis (L.) Jacq.	w	0	0	(33)
	Silene maritima With.	s, l	1	1	(33)
	Silene maritima With.	r	3	3	
	Silene rupestris L.	w	10	10	(33)
	Spergula arvensis L.	w	3	10	
	Spergula marginata (DC.) Murb.	w	10	30	
	Spergula rubra (L.) D. Dietr.	w	0	1	−[35]
	Stellaria graminea L.	w	0	0	(39)
	Stellaria media (L.) Vill.	w	1	3	(39)
Chenopodiaceae	*Atriplex litoralis* L.	w	0	3	+[33]
	Salicornia europaea L.	w	0	0	
	Salsola kali L.	w	0	10	+[28], +[31], +[40], +[41]
	Suaeda maritima (L.) Dum.	w	0	0	(28, 33, 35)

[30] FROMME; from CAESAR and LORETZ, *Just's Botanischer Jahresbericht*, **25** II, 19 (1897).
[31] P. S. MASSAGETOV, *Farmatsiya* 9 No. 3, 22 (1946).
[32] H. GERTIG, *Dissertationes Pharm.* **14**, 527 (1962).
[33] S. Y. ZOLOTNITSKAYA, *Izvest. Akad. Nauk Armyan. SSR, Biol. i Sel'skokhoz. Nauki* 7, 27 (1954).
[34] G. V. LAZUR'EVSKII and A. S. SADYKOV, *Tr. SAGU, nov. ser.* **2** No. 1 (1945), from V. S. SOKOLOV, *loc. cit.*, p. 263.
[35] M. E. WALL, C. S. FENSKE, J. W. GARVIN, J. J. WILLAMAN, Q. JONES, B. G. SCHUBERT and H. S. GENTRY, *J. Am. Pharm. Assoc. Sci. Ed.* **48**, 695 (1959).
[36] M. N. VARLAKOV, *Khim.-Farm. Prom.* (1932) No. 2, from V. S. SOKOLOV, *loc. cit.*, p. 196
[37] SCHNEEGANS, *J. Pharm. Alsace-Lorraine* 206 (1890), from C. WEHMER, *loc. cit.*, p. 305.
[38] O. STEPPUHN, *Arch. Exp. Pathol. Pharmakol.* **147**, 79 (1930).
[39] M. A. NAUMENKO, *Nekotorye Voprosy Farm. (Kiev) Sbornik* 242 (1956) from *Chem. Abstr.* **52**, 3044e (1958).
[40] B. BORKOWSKI, K. DROST and B. PASICHOWA, *Acta Polon. Pharm.* **16**, 57 (1959).
[41] K. DROST, *Dissertationes Pharm.* **13**, 167 (1961).

TABLE 1—*continued*

Family	Species	Plant part	Fraction A	Fraction B	References
Compositae	*Antennaria dioeca* (L.) Gaertn.	w	10	10	−[22], +[26]
	Anthemis arvensis L.	w	3	3	−[35], +[42*]
	Anthemis tinctoria L.	w	0	0	+[26]
	Centaurea cyanus L.	w	3	3	+[22]
	Centaurea jacea L.	s, l	10	30	+[22], +[26], +[43]
	Centaurea jacea L.	r	0	0	+[22]
	Centaurea scabiosa L.	s, l	10	10	+[22], +[44]
	Chrysanthemum segetum L.	w	0	0	(26, 45)
	Cirsium palustre (L.) Scop.	w	3	3	(35, 46)
	Cirsium vulgare (Savi) Ten.	w	1	0	−[35]
Convolvulaceae	*Calystegia sepium* (L.) R. Br. (*Convolvulus sepium* L.)	w	1	1	+[22], ±[23], −[35]
	Convolvulus arvensis L.	s, l	3	1	+[23], −[26], −[35]
	Cuscuta europaea L.	w	1	0	(28)
Cornaceae	*Cornus suecica* L.	w	0	0	(35)
Corylaceae	*Corylus avellana* L.	rb	0	0	+[22]
Cupressaceae	*Juniperus communis* L.	rb	0	3	(23, 47)
Cyperaceae	*Carex acuta* L.	w	1	0	(33, 48)
	Carex echinata Murr. (*C. stellulata* Good.)	w	0	0	(33, 48)
	Carex nigra (L) Reich. (*C. Goodenowii* Gay.)	w	3	0	(33, 48)
	Eleocharis uniglumis (Link) Schult.	w	1	0	
	Eriophorum angustifolium Honck.	w	0	0	
	Scirpus maritimus L.	w	1	1	
	Scirpus silvaticus L.	w	0	0	
	Scirpus Tabernaemontani C. C. Gmel.	r	0	0	+[23]
Dipsacaceae	*Knautia arvensis* (L.) Coult.	w	0	0	(49)
	Succisa pratensis Moench (*Scabiosa succisa* L.)	w	100	10	+[50], +[51]
Droseraceae	*Drosera rotundifolia* L.	w	0	0	
Elaeagnaceae	*Hippophaë rhamnoides* L.	l	0	0	
	Hippophaë rhamnoides L.	b	0	3	+[52], +[23]
	Hippophaë rhamnoides L.	rb	0	30	
Empetraceae	*Empetrum nigrum* L.	w	0	0	
Ericaceae	*Andromeda polifolia* L.	w	0	0	

* Pattone's[42] alkaloid can hardly be identical with the alkaloid detected here because his alkaloid was insoluble in alcohol.

[42] PATTONE, *J. pharm. chim.* **35**, 198 (1859).
[43] D. E. DIONIS'EV and E. E. CHERNOMAZ, *Uchenye Zapiski Rostovsk. na Donu Gos. Univ.* **9** (1938), from V. S. SOKOLOV, *loc. cit.*, p. 272.
[44] B. V. KURMAZ and V. K. YASHCHENKO, *Sbornik Nauch. Trudov Dnepropetrovsk. Med. Inst.* **6**, 333 (1958), from *Chem. Abstr.* **55**, 8769i (1961).
[45] A. F. GAMMERMAN and M. D. SHUPINSKAYA, *Farmatsiya i Farmakol.* No. 3–4 (1937), from V. S. SOKOLOV, *loc. cit.*, p. 273.
[46] N. M. ISMAILOV, *Izvest. Akad. Nauk Azerbaidzhan. SSR. Ser. Biol. i Sel'skokhoz. Nauk* No. 4, 11 (1958), from *Chem. Abstr.* **53**, 3597a (1959).
[47] G. V. LAZUR'EVSKII and A. S. SADYKOV, *Trudy Uzbeksk. Gos. Univ.* **15** (1939), from V. S. SOKOLOV, *loc. cit.*, p. 162.
[48] I. V. TERENT'EVA and G. V. LAZUR'EVSKII, *Zh. Obshch. Khim.* **27**, 3170 (1957).
[49] N. M. ISMAILOV and R. YA.RZAZADE, *Dokl. Akad. Nauk. Azerb. SSR* **10**, 197 (1954).
[50] V. S. SOKOLOV, *loc. cit.*, p. 132.
[51] K. TORSSELL, *Arkiv Kemi* **21**, 93 (1963).
[52] P. S. MASSAGETOV, *Zh. Obshch. Khim.* **16**, 775 (1946).

TABLE 1—*continued*

Family	Species	Plant part	Fraction A	Fraction B	References
Ericaceae	*Arctostaphylos uva-ursi* (L.) Spreng.	w	0	0	
	Erica tetralix L.	w	0	0	
	Ledum palustre L.	w	0	0	
	Vaccinium myrtillus L.	w	0	10	+[33]
	Vaccinium oxycoccus L.	w	0	0	
	Vaccinium uliginosum L.	w	0	0	
	Vaccinium vitis-idaea L.	w	1	0	−[53]*
Euphorbiaceae	*Euphorbia cyparissias* L.	w	0	0	(33)
	Euphorbia palustris L.	s, l	0	0	
	Euphorbia palustris L.	rb	0	0	
	Euphorbia peplus L.	w	0	0	+[28], +[54]
	Euphorbia polychroma A. Kern	w	0	0	
	Mercurialis perennis L.	w	0	1	+[22]
Fagaceae	*Fagus silvatica* L.	rb	0	0	(35)
	Quercus robur L.	rb	0	0	
	Quercus petraea (Matt.) Liebl.	rb	0	0	
Geraniaceae	*Geranium lucidum* L.	w	1	0	
	Geranium molle L.	w	0	0	+[55]
	Geranium Robertianum L.	w	3	3	
	Geranium silvaticum L.	w	3	0	+[22], +[33]
Gramineae	*Bromus hordeaceus* L. (*B. mollis* L.)	w	3	3	
	Bromus secalinus L.	w	3	0	
	Dactylis glomerata L.	w	3	3	
	Holcus mollis L.	w	1	3	
	Phragmites communis Trin.	r	10	3	−[35]
Hippocastanaceae	*Aesculus hippocastanum* L.	rb	0	0	
Hippuridaceae (Halorrhagaceae)	*Hippuris vulgaris* (L.) Hiern	s, l	3	10	+[23]
Hydrophyllaceae	*Phacelia tanacetifolia* Benth.	w	1	3	
Isoëtaceae	*Isoëtes echinospora* Dur.	w	1	0	
	Isoëtes lacustris L.	w	0	0	
Juncaceae	*Juncus articulatus* L.	w	0	0	
	Juncus conglomeratus L.	w	3	—	
	Juncus effusus L.	w	0	1	
	Juncus squarrosus L.	w	3	0	
	Juncus subnodulosus Schrank	w	0	0	
	Juncus tenuis Willd.	w	0	0	
	Luzula multiflora (Retz.) Lej.	w	0	0	
	Luzula pilosa (L.) Willd.	w	0	0	
Juncaginaceae	*Triglochin maritimum* L.	w	0	1	
Lemnaceae	*Lemna minor* L.	w	0	0	
Lentibulariaceae	*Pinguicula vulgaris* L.	w	10	3	
	Utricularia minor L.	w	3	3	
Liliaceae	"*Hemerocallis conspicua*"†	r	3	10	(22, 56)
Loasaceae	*Bartonia aurea* Lindl.	w	3	30	
Loganiaceae	*Buddleia Davidii* Franch.	b	0	3	(29)

* Klein[5] erroneously states that Laland found an alkaloid; but Laland[53] only reported that he did not find narcotine in lingonberries.

† Originating from Messrs. Perry's Hardy Plant Farm, Enfield, Middlesex, England.

53 P. LALAND, *Z. Physiol. Chem.* **204**, 112 (1932).
54 A. E. A. SHARAF, *Brit. Vet. J.* **105**, 128 (1949).
55 J. L. VAN EIJK, *Pharm. Weekblad.* **87**, 70 (1952).
56 G. KLEIN and G. POLLAUF, *Österr. Botan. Z.* **78**, 251 (1929).

TABLE 1—*continued*

Family	Species	Plant part	Fraction A	Fraction B	Reference
Lythraceae	*Lythrum salicaria* L.	s, l	3	0	−[22], +[23], −[24]
Myricaceae	*Myrica gale* L.	l, rb	3	0	
Oleaceae	*Fraxinus excelsior* L.	rb	0	1	−[57], (23, 26, 33, 35)
	Ligustrum vulgare L.	b	100	3	
	Ligustrum vulgare L.	l	30	30	+[23]
Onagraceae	*Clarkia* sp.	w	0	0	
	Chamaenerion angustifolium (L.) Scop.	w	0	0	+[22], +[26], −[35]
	Epilobium Hornemanni Rchb.	w	0	0	
	Epilobium montanum L.	w	0	0	
	Epilobium roseum Schreb.	w	0	0	+[23]
Orchidaceae	*Orchis maculata* L.	r	0	3	
	Platanthera bifolia (L.) L. C. Rich	w	3	10	
Pinaceae	*Larix decidua* Mill.	b, l	0	0	(22)
	Picea abies (L. H. Karst.	l	30	10	(58)
	Picea abies (L.) H. Karst.	rb	3	0	
	Pinus silvestris L.	l	3	3	+[22], +[31]
	Pinus silvestris L.	rb	0	0	
Plantaginaceae	*Plantago lanceolata* L.	w	3	3	+[23]
	Plantago maritima L.	w	3	3	(59, 60, 61)
	Plantago media L.	w	1	10	(59, 60, 61)
Plumbaginaceae	*Armeria maritima* (Mill.) Willd. (*Statice armeria* L.)	w	10	10	(23, 26, 62)
	Limonium vulgare Mill.	s, l, r	0	0	(33)
Polemoniaceae	*Cobaea scandens* Cav.*	s, l	0	10	
	Phlox paniculata hybr.	s, l	1	1	−[22]
Polygalaceae	*Polygala vulgaris* L.	w	10	3	(24)
Polygonaceae	*Polygonum aviculare* L.	w	0	0	±[61]
	Polygonum hydropiper L.	w	0	1	+[22], −[24], +[29]
	Polygonum viviparum L.	w	0	10	
	Rumex acetosa L.	w	0	0	(29, 64 †)
	Rumex longifolius DC	w	0	0	
Polypodiaceae	*Pteridium aquilinium* Kuhn	l	0	0	+[65]
	Pteridium aquilinium Kuhn	r	1	0	
Portulacaceae	*Montia fontana* L.	w	0	0	
	Portulaca grandiflora Lindl.	w	1	1	(29)
Potamogetonaceae	*Potamogeton natans* L.	f	1	1	
	Potamogeton polygonifolius Pourr.	w	3	0	
Primulaceae	*Glaux maritima* L.	w	0	0	+[23]
	Lysimachia thyrsiflora L.	w	0	0	
	Lysimachia vulgaris L.	w	0	0	−[22], −[26], −[35]
	Primula veris L.	w	3	0	
	Trientalis europaea L.	w	0	0	
Pyrolaceae	*Pyrola rotundifolia* L.	w	0	0	+[22], −[26]

* Hutchinson[63] lists *Cobaea* in the family Cobaeaceae.

† *Rumex obtusifolius* contains α-picoline[10] which is not detected by our method.

[57] E. Z. Asoeva, *Uchenye Zapiski Pyatigorskii Farm. Inst.* **4**, 69 (1959), from *Chem. Abstr.* **55**, 18894c (1961).
[58] T. Kariyone, M. Takahashi, A. Nitta and Y. Tsunehisa, *J. Pharm. Soc., Japan* **76**, 611 (1956).
[59] V. M. Pobedina, *Izv. Azerb. Filiala Akad. Nauk SSSR* No. 2 (1938), from V. S. Sokolov, *loc. cit.*, p. 262.
[60] A. V. Danilova and R. A. Konovalova, *Zh. Obshch. Khim.* **22**, 2237 (1952).
[61] A. V. Danilova, *Zh. Obshch. Khim.* **26**, 2069 (1956).
[62] F. A. Dalpe, *Am. J. Pharm.* **56**, 361 (1884).
[63] J. Hutchinson, *The Families of Flowering Plants*, p. 387, Clarendon Press, Oxford (1959).
[64] S. Wickinson, *Nature (London)* **181**, 636 (1958).
[65] S. Toda, *J. Biochem. (Tokyo)* **2**, 429 (1923).

TABLE 1—*continued*

Family	Species	Plant part	Fraction A	Fraction B	Reference
Ranunculaceae	*Actaea spicata* L.	s, l	1	0	+[26]
	Actaea spicata L.	r	0	0	−[22]
	Caltha palustris L.	r	3	0	+[22], +[26], +[66], +[67]
Rhamnaceae	*Rhamnus cathartica* L.	rb	1	10	−[22] (49)
	Rhamnus frangula L.	rb	10	0	−[68] (49)
Rosaceae	*Cotoneaster intergerrimus* Med.	b, l	0	0	(23)
	Crataegus monogyna Jacq.	rb	0	3	[69]
	Potentilla plaustris (L.) Scop. (*Comarum palustre* L.)	w	0	0	
	Sorbus aucuparia L.	rb	3	0	
	Sorbus intermedia (Erh.) Pers. (*S. suecica* (L.) Krok)	rb.	10	0	
Rubiaceae	*Galium aparine* L.	w	0	30	
	Galium mollugo L.	w	0	3	+[22], −[26]
	Galium verum L.	w	1	30	+[22], +[23], −[26]
Salicaceae	*Populus tremula* L.	rb	1	0	
Saxifragaceae	*Hydrangea petiolaris* Sieb et Zucc.	b, r	0	0	(70, 71)
	Parnassia palustris L.	r	1	0	
	Ribes alpinum L.	b	0	0	
	Ribes alpinum L.	rb	1	0	
	Saxifraga granulata L.	w	0	0	
Scheuchzeriaceae	*Scheuchzeria palustris* L.	w	1	3	
Scrophulariaceae	*Euphrasia officinalis* L.	w	3	3	(23)
	Euphrasia frigida Pusgl.	w	10	10	
	Linaria vulgaris Mill.	w	10	0	+[22], +[26]
	Melampyrum cristatum L.	w	10	30	+[26]
	Melampyrum nemorosum L.	2	3	3	+[26]
	Melampyrum pratense L.	w	10	10	
	Odontites litoralis Fr.	w	10	30	
	Odontites verna (Bell.) Dum. (*Odontites serotina* (Lam.) Rchb.)	w	0	0	+[23]
	Pedicularis palustris L.	w	10	30	(33)
	Pedicularis silvatica L.	w	10	10	(23)
	Rhinanthus minor L.	w	3	30	+[72*]
	Rhinanthus serotinus (Schönh.) Sch. et Th.	w	1	10	+[72*]
	Scrophularia nodosa L.	s, l	1	1	[73]
	Scrophularia nodosa L.	t	0	0	+[74]
	Verbascum nigrum L.	r	3	1	(23)
	Verbascum thapsus L.	s, l†	1	3	−[68]
	Verbascum thapsus L.	rb	10	10	
	Veronica beccabunga L.	w	3	3	(22, 23, 59)
	Veronica officinalis L.	w	0	10	

* *Rhinanthus crista-galli* L.

† Green parts peeled off from supporting woody structure. This plant may give a false-positive reaction.[68]

[66] O. KELLER, *Arch. Pharm.* **248**, 463 (1910).
[67] E. POULSSON, *Arch. Exp. Pathol. Pharmakol.* **80**, 173 (1917).
[68] K. L. EULER and N. R. FARNSWORTH, *Lloydia* **25**, 296 (1962).
[69] R. NEU and P. HAGEDORN, *Deut. Apoth. Ztg.* **96**, 19 (1956).
[70] C. S. JANG, F. Y. FU, K. C. HUANG and C. Y. WANG, *Nature* **161**, 401 (1948).
[71] F. ABLONDI, S. GORDON, J. MORTON, II, and J. H. WILLIAMS, *J. Org. Chem.* **17**, 14 (1952).
[72] A. P. ORekhov, *Khim. farm. prom.* **1** (1935), from V. S. SOKOLOV, *loc. cit.*, p. 261.
[73] F. KOCH, *Arch. Pharm.* **233**, 48 (1895).
[74] V. D. MOER, *Ned. Tijdschr. Pharm.* 1, (1896), from C. WEHMER, *loc. cit.*, p. 1122.

TABLE 1—*continued*

Family	Species	Plant part	Fraction A	Fraction B	Reference
Scrophulariaceae	*Veronica scutellata* L.	w	3	3	
	Veronica spicata L.	w	1	0	−[22]
Sparganiaceae	*Sparganium ramosum* Huds.	w	1	0	+[33]
Tamaricaceae	*Myricaria germanica* (L.) Desv.	rb	0	0	
Typhaceae	*Typha latifolia* L.	w	0	0	−[33], −[35], [35]
Ulmaceae	*Ulmus glabra* Huds.	rb	1	0	[22]
Umbelliferae	*Heracleum spondylium* L.	r	0	0	[23]
	Ligusticum scoticum L.	s, l	0	0	[33, 75]
Valerianaceae	*Valeriana officinalis* L.	r	30	30	+[76], +[77]
	Valeriana sambucifolia Mikan	s, l, r	10	100	
Violaceae	*Viola riviniana* Rchb.	w	0	0	[78]
	Viola tricolor L.	w	1	3	−[22], +[79]
Zosteraceae	*Zostera marina* L.	w	0	0	

EXPERIMENTAL

A 4 g sample of carefully dried (under 50°), ground plant material was allowed to stand overnight with 40 ml of methanol and was then warmed for 4 hr at 50°. The mixture was filtered, the residue washed with 20 ml of methanol and the combined extracts evaporated *in vacuo*. The residue was suspended in methanol (2 ml), and 12 ml of 1% HCl added. The mixture was shaken and filtered, using an additional 8 ml of 1% HCl to wash the residue. The filtrate was made basic with conc. NH_3 and extracted with three 20 ml portions of chloroform (fraction A). The aqueous solution was next made half-saturated with sodium sulfate and extracted three times with 20 ml of chloroform: ethanol (3:2 v/v) (fraction B). The organic phases were washed with 5 ml of half-saturated sodium sulfate solution and dried with anhydrous sodium sulfate.

The two extracts were evaporated separately *in vacuo*, and 1·0 ml of HCl and 1·0 ml of chloroform were added to each with vigorous shaking. The aqueous phase from each was pipetted off, filtered through cotton, and divided into six portions. These samples were tested with the following alkaloid reagents, prepared according to Cromwell[80] except where indicated: Mayer's, Wagner's, Dragendorff's, Sonnenschein's (prepared as 10% solution of phosphomolybdic acid in dilute nitric acid (1:9 v/v)), silicotungstic acid, and Hager's reagents. The amounts of precipitate formed were compared to those resulting from strychnine solutions of known concentrations.

Acknowledgements—This investigation was supported by Statens naturvetenskapliga forskningsråd, Helge Axelson Johnsons Stiftelse, Fonden för biträdeshjälp åt docenter, and Hahns fond. Cultivated plants were obtained from W. Weibull, AB. We wish to thank Professor Folke Fagerlind and Dr. K. S. Suneson for help and advice in finding some of the wild plants, and Dr. Charlotte D. Hultin for help with the editing of the manuscript. Part of this work was carried out at Kristinebergs zoologiska station, Fiskebäckskil. Mrs. L. Bäckbom, Mr. V. Selberg and Mrs. K. Wahlberg, F.K., have been helpful in carrying out most of the analyses.

[75] Y.-T. KU and K.-F. TSENG, *Hua Hsüen Hsüen Pao* **23**, 246 (1957), from *Chem. Abstr.* **52**, 15828b (1958).
[76] S. WALISZEWSKY, *L'Union pharmaceutique*, from *Apotheker-Ztg.* 6, 186 (1891).
[77] B. BORKOWSKI, *Pharmazie* **15**, 568 (1960).
[78] M. PAILER and K. NOWOTNY, *Naturwissenschaften* **45**, 419 (1958).
[79] O. LINDE, *Apotheker-Ztg.* **34**, 37 (1919).
[80] B. T. CROMWELL, in *Modern Methods of Plant Analysis* (Edited by K. PAECH and M. V. TRACEY) Vol. 4, p. 367, Springer-Verlag, Berlin (1955).