

ALKALOID SCREENING OF CATALONIA (SPAIN) PLANTS, I.

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ABSTRACT.—Of higher plants collected from various parts of Catalonia, 127 species were screened for alkaloids. Of these, 97 had not been reported previously in the literature as being alkaloid-bearing plants, and only one reference on the presence or absence of unknown alkaloids has been found for each of the other 30 plants. The presence of alkaloids in 13 plant species is reported for the first time.

A search for alkaloid-containing species of flora in Catalonia, Spain, was initiated by us (1) in 1979 in order to find new alkaloid-containing plants and eventually new alkaloids that could later be studied chemically and pharmacologically. It is remarkable that no information is available concerning the occurrence of alkaloids in the species of our flora.

Of about 400 samples that we have tested, we repeat those for which we have not found any bibliographic reference (97 species), in order to contribute to the knowledge of new alkaloid-containing plants. In addition, species for which we have found only one reference relative to the presence or absence of alkaloidal compounds (without specifying their structure) are also included (30 species).

In order to determine whether the plants included in this work had previously been reported to serve as a source of alkaloids or of compounds giving alkaloid-positive reactions, we have consulted the comprehensive reference works of Willaman and Li (2), Smolenski, *et al.* (3-9), Fong, *et al.* (10), Hartley, *et al.* (11), Raffauf (12), as well as the Specialist Periodical Reports "The Alkaloids" (13, 14).

EXPERIMENTAL

In our selection of methods for extraction and purification of alkaloids from plant material, as well as the alkaloid-precipitating reagents, we took into account the observations of Farnsworth (15) and Euler and Farnsworth (16) on the advantages and disadvantages of the known procedures.

The samples were collected by us and verified by the Botanical Institute of Barcelona, where voucher specimens for each plant have been deposited. Plant material was first air-dried and then subjected to further drying in an oven with a hot-air current at approximately 50°.

Because application of the usual alkaloid-precipitating reagents to crude plant extracts will result in some false-positive reactions (17), a procedure was developed in which an initial test was carried out on the crude extract, followed by a confirmatory test on extracts giving a positive reaction.

PRELIMINARY TEST.—Powdered plant material (20 g) was extracted by boiling 96% ethanol for 5-10 min and then macerating at room temperature for 24 h. Filtration of the mixture provided a marc and an extract. The extract was evaporated to dryness *in vacuo* at 35-40°, and the residue was dissolved with 2 N HCl (5 ml). The acidic solution was clarified by mixing with Hyflo Super Cel and filtering. The filtrate was tested with a few drops of the following alkaloid-precipitating reagents: Wagner's (18), Dragendorff's (18), Scheibler's (18), Sonnenschein's (19), Bertrand's (20), Valser's (21) and Hager's (18).

CONFIRMATORY TEST.—For the plant extracts giving a positive reaction in the preliminary test, a confirmatory test using 100 g of dried and powdered plant material was made as described above, up to the point following filtration of the Hyflo-Super-Cel-treated extract. The filtrate was brought to pH 10-11 with ammonium hydroxide, and this alkalized solution was shaken with chloroform. The chloroformic layer (nonquaternary alkaloids) was evaporated to dryness and the residue dissolved in 2 N HCl (2 ml). The aqueous solution (quaternary alkaloids, including *N*-oxides) was extracted with chloroform-ethanol (3:2 v/v), and the organic layer was evaporated to dryness and then the residue dissolved in 2 N HCl (2 ml).

A portion of both tertiary and quaternary alkaloid solutions was tested with the alkaloid-precipitating reagents mentioned above. These results were compared with standard alkaloids according to the criteria of Martello and Farnsworth (17).

Another portion of each solution was chromatographed on Silica Gel G plates with a mixture of *n*-butanol-acetic acid-water (4:1:1 v/v) as a developing solvent (22), and alkaloid spots were revealed with the Munier and Macheboeuf modification of Dragendorff's reagent (23) and with the iodoplatinate reagent (24). This allowed us to estimate the approximate number of alkaloids in each extract.

The alkaloid content of the plants was also estimated from the chloroformic and chloroform-ethanolic extracts by titration with 0.01 N *p*-toluenesulfonic acid in chloroform using dimethyl yellow (0.5% in hexane) as indicator (11). However, as the molecular weight of every one of these possible alkaloids is unknown, the values are reported in mEq/100 g dry weight.

RESULTS AND DISCUSSION

We have tested 127 species representing 102 genera, belonging to 43 families. These are presented in Appendix 1, arranged alphabetically, according to plant family, together with their botanical names, date of collection, plant parts, and results of the screening tests, along with references to earlier investigations.

Samples have been classified as positive, negative, or doubtful. An extract was considered as containing alkaloids when it presented an average result of "+" with the alkaloid-precipitating reagents, a minimum of 0.03 mEq/100 g dry weight in the titration, and at least one spot on the chromatograms. Sometimes, even though one of these three conditions failed (*i.e.*, tlc), a sample was considered as alkaloid-containing, if the other evaluation criteria were strongly positive.

Of the 127 plant species, 89 (70%) gave preliminary positive tests for tertiary and/or quaternary alkaloids, including primary and secondary amines, as well as *N*-oxides. There are 18 species (14 of the total) that gave confirmatory positive tests for alkaloids, and 13 of them have not been reported previously in the literature. The new alkaloid-containing species are found in the families Caryophyllaceae, Compositae, Cruciferae, Dipsacaceae, Leguminosae, Linaceae, Orchidaceae, Plantaginaceae, Scrophulariaceae, and Umbelliferae.

APPENDIX 1. Results of alkaloid screening of plant samples.

Family—Botanical name	Month/year of collection	Part of plant	Preliminary test	Confirmatory test						Final conclusion	Previous works
				Tertiary alkaloids			Quaternary alkaloids				
				Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)	Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)		
AQUIFOLIACEAE											
<i>Ilex aquifolium</i> L.	6/80	Lf	-							N	None
ASPIDACEAE											
<i>Polystichum setiferum</i> Woyne	5/80	Lf	+	-	0.000	-	-	0.011	-	N	None
BETULACEAE											
<i>Alnus glutinosa</i> (L.) Gaertner	6/79	Lf,if	-							N	N (19)
BORAGINACEAE											
<i>LitospERMUM fruticosum</i> L.	5/79	Px	+	(+)	0.000	-	(+)	0.043	-	N	None
<i>LitospERMUM officinale</i> L.	9/79	Pl	-							N	None
<i>Myosotis arvensis</i> (L.) Hill	5/80	Pl	+	(+)	0.022	-	(+)	0.022	-	N	None
CAMPANULACEAE											
<i>Campanula rapunculosa</i> L.	6/79	Px	+	(+)	0.022	-	++(+)	0.053	-	D	None
CAPRIFOLIACEAE											
<i>Lonicera implexa</i> Aiton	5/79	Lf,bd	+	+	0.033	0.10	++(+)	0.098	0.03 0.22 0.41	P	N (9)
CARYOPHYLLACEAE											
<i>Dianthus pyrenaicus</i> Pourret	8/79	Px	+	+(+)	0.022	-	++	0.043	0.08	P	None

APPENDIX 1. Continued.

Family—Botanical name	Month/year of collection	Part of plant	Preliminary test	Confirmatory test						Final conclusion	Previous works
				Tertiary alkaloids			Quaternary alkaloids				
				Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)	Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)		
<i>Moebringia trinervia</i> (L.) Clairv.	5/80	Pl	+	(+)	0.011	—	+(+)	0.033	0.39	D	None
<i>Saponaria ocyroides</i> L.	6/80	Px	+	(+)	0.011	0.02	+	0.043	—	D	None
<i>Silene alba</i> Miller	5/80	Px	+	(+)	0.022	—	+(+)	0.250	—	P	None
<i>Stellaria holostea</i> L.	6/80	Px	+	—	0.000	—	(+)	0.022	—	N	None
CISTACEAE											
<i>Cistus albidus</i> L.	5/79	Px	—	—	—	—	—	—	—	N	None
<i>Cistus crispus</i> L.	6/80	Px	—	—	—	—	—	—	—	N	None
<i>Cistus monspeliensis</i> L.	5/79	Pl	—	—	—	—	—	—	—	N	None
<i>Cistus salviaefolius</i> L.	5/79	Pl	—	—	—	—	—	—	—	N	None
<i>Helianthemum apenninum</i> (L.) Miller	6/80	Px	—	—	—	—	—	—	—	N	None
<i>Helianthemum guttatum</i> (L.) Miller	6/80	Pl	+	(+)	0.000	—	—	0.011	—	N	None
<i>Helianthemum nummularium</i> (L.) Miller	6/80	Px	—	—	—	—	—	—	—	N	P (2)
COMPOSITAE											
<i>Anacyclus valentinus</i> L.	6/79	Px	+	(+)	0.011	—	—	0.022	—	N	None
<i>Andryala integrifolia</i> L.	6/79	Px	—	—	—	—	—	—	—	N	None
<i>Centaurea aspera</i> L.	6/79	Px	+	(+)	0.022	—	+	0.065	—	D	None
<i>Centaurea nigra</i> L.	8/79	Px	+	(+)	0.022	—	+	0.043	—	D	None
<i>Centaurea paniculata</i> L.	8/79	Px	+	(+)	0.022	—	+	0.011	—	D	None
<i>Centaurea pectinata</i> L.	6/80	Px	+	(+)	0.054	—	++	0.098	0.07	P	None
									0.23		
<i>Cirsium monspessulanum</i> (L.) Hill	8/79	Pl	+	(+)	0.000	—	+	0.022	—	N	None
<i>Crepis vesicaria</i> L.	5/80	Px	+	(+)	0.011	—	+	0.033	0.04	D	None
<i>Doronicum pardalianches</i> L.	5/80	Px	+	(+)	0.022	—	(+)	0.054	—	N	None
<i>Galactites tomentosa</i> Moench.	5/80	Px	+	—	0.000	—	(+)	0.033	—	N	None
<i>Helichrysum stoechas</i> (L.) Moench.	6/79	Px	+	—	0.011	—	(+)	0.022	—	N	None
<i>Matricaria inodora</i> L.	8/79	Px	+	(+)	0.011	—	+	0.043	—	D	N (25)
<i>Picris echioides</i> L.	6/79	Px	—	—	—	—	—	—	—	N	None
<i>Santolina chamaecyparissus</i> L.	6/80	Px	+	(+)	0.011	—	+(+)	0.022	0.19	P	P (2)
<i>Tolpis barbata</i> (L.) Gaertner	6/80	Px	+	(+)	0.000	—	—	0.033	—	N	None
<i>Urospermum dalechampii</i> (L.) Scop.	5/79	Pl	+	—	0.011	—	(+)	0.033	—	N	None
CONVOLVULACEAE											
<i>Convolvulus althaeoides</i> L.	5/79	Px	+	(+)	0.033	—	(+)	0.033	—	D	None
CORIARIACEAE											
<i>Cortaria myrtifolia</i> L.	5/80	Lf	—	—	—	—	—	—	—	N	None
CRASSULACEAE											
<i>Sedum sediforme</i> (Jacq.) Pam	7/79	Px	—	—	—	—	—	—	—	N	N (4)
CRUCIFERAE											
<i>Biscutella laevigata</i> L.	7/79	Pl	+	(+)	0.011	—	+	0.043	0.24	D	None
<i>Erucastrum nasturtiifolium</i> Schulz	5/80	Px	+	(+)	0.022	—	+(+)	0.054	0.19	P	None
<i>Erysimum grandiflorum</i> Desf.	6/80	Pl	+	(+)	0.043	—	(+)	0.033	—	D	None
<i>Hirschfeldia incana</i> (L.) Lagreze-Fossat	5/79	Px	+	(+)	0.000	—	+	0.043	—	D	None
CUCURBITACEAE											
<i>Bryonia cretica</i> L.	6/79	Px	+	—	0.000	—	(+)	0.054	—	N	None
CUPRESACEAE											
<i>Juniperus oxycedrus</i> L.	5/79	Px	—	—	—	—	—	—	—	N	None
CYPERACEAE											
<i>Carex pendula</i> Hudson	5/80	Px	+	(+)	0.011	—	++	0.022	—	D	None
DIPSACACEAE											
<i>Knautia arvensis</i> (L.) Coult	5/79	Px	+	+	0.000	—	+	0.065	—	D	N (19)
<i>Scabiosa maritima</i> L.	6/79	Px	+	++	0.043	—	++	0.076	0.05	P	None
ERICACEAE											
<i>Arbutus unedo</i> L.	5/79	Lf	—	—	—	—	—	—	—	N	N (6)
<i>Erica arborea</i> L.	5/79	Px	—	—	—	—	—	—	—	N	None
EUPHORBIACEAE											
<i>Euphorbia characias</i> L.	5/79	Px	+	(+)	0.011	—	—	0.033	—	N	P (6)
<i>Euphorbia segetalis</i> L.	7/79	Px	—	—	—	—	—	—	—	N	None

APPENDIX I. Continued.

Family—Botanical name	Month/year of collection	Part of plant	Preliminary test	Confirmatory test						Final conclusion	Previous works
				Tertiary alkaloids			Quaternary alkaloids				
				Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)	Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)		
<i>Euphorbia serrata</i> L.	5/79	Pl	+	-	0.022	-	-	0.043	-	N	None
FAGACEAE											
<i>Castanea sativa</i> Miller	6/80	Lf	-	-	-	-	-	-	-	N	None
<i>Corylus avellana</i> L.	6/80	Lf	-	-	-	-	-	-	-	N	None
<i>Quercus coccifera</i> L.	5/80	Px	-	-	-	-	-	-	-	N	None
<i>Quercus ilex</i> L.	5/80	Lf	-	-	-	-	-	-	-	N	N (7)
<i>Quercus pubescens</i> Willd.	5/79	Lf	-	-	-	-	-	-	-	N	None
GERANIACEAE											
<i>Erodium malacoides</i> (L.) L'Her.	5/80	Pl	+	+	0.000	-	+(+)	0.043	0.03	P	P (26)
<i>Geranium rotundifolium</i> L.	5/79	Pl	-	-	-	-	-	-	-	N	N (27)
GRAMINEAE											
<i>Ampelodesmos mauritanica</i> Durand & Schinz	5/79	Px	+	-	0.000	-	+	0.000	-	N	None
<i>Avena sativa</i> L.	6/80	Pl	+	-	0.000	-	(+)	0.022	-	N	None
LABIATAE											
<i>Lavandula stoechas</i> L.	5/79	Pl	-	-	-	-	-	-	-	N	None
<i>Melitis melissophyllum</i> L.	6/80	Px	+	(+)	0.011	-	(+)	0.022	-	N	None
<i>Mentha longifolia</i> (L.) Hudson	7/79	Pl	-	-	-	-	-	-	-	N	N (4)
<i>Mentha rotundifolia</i> (L.) Hudson	6/79	Px	+	+(+)	0.076	0.14	+(+)	0.054	0.18	P	N (28)
<i>Prunella vulgaris</i> L.	6/79	Px	-	-	-	-	-	-	-	N	None
<i>Salvia pratensis</i> L.	6/80	Px	-	-	-	-	-	-	-	N	N (4)
<i>Satureja calaminta</i> (L.) Scheele	5/80	Px	+	(+)	0.011	-	+	0.043	-	D	None
<i>Sideritis hirsuta</i> L.	6/80	Px	+	-	0.022	-	+	0.033	-	D	None
LEGUMINOSAE											
<i>Dorycnium hirsutum</i> (L.) Ser.	5/80	Px	-	-	-	-	-	-	-	N	None
<i>Dorycnium pentaphyllum</i> Scop.	5/79	Px	+	-	0.000	-	-	0.022	-	N	None
<i>Dorycnium rectum</i> (L.) Ser.	6/79	Px	-	-	-	-	-	-	-	N	P (8)
<i>Dorycnopsis gerardii</i> Boiss.	6/80	Px	+	-	0.000	-	-	0.022	-	N	None
<i>Hedysarum spinosissimum</i> L.	5/79	Pl	+	-	0.000	-	-	0.065	-	N	None
<i>Lathyrus cicera</i> L.	5/80	Px	+	(+)	0.022	-	-	0.022	-	N	None
<i>Lathyrus cirrhosus</i> Sering	8/79	Px	+	+	0.011	-	+(+)	0.065	-	D	None
<i>Melilotus neapolitana</i> Ten.	5/79	Pl	+	++	0.043	-	+(+)	0.011	0.11	P	None
<i>Onobrychis supina</i> DC in Laun	6/80	Px	-	-	-	-	-	-	-	N	None
<i>Psoralea bituminosa</i> L.	5/79	Px	+	+	0.011	-	+	0.043	-	D	N (3)
<i>Trifolium angustifolium</i> L.	6/80	Px	+	-	0.011	-	+	0.032	-	D	N (29)
<i>Trifolium pratense</i> L.	6/79	Px	+	(+)	0.000	-	(+)	0.033	-	N	N (4)
<i>Trifolium rubens</i> L.	6/80	Px	+	(+)	0.011	-	(+)	0.033	-	N	None
<i>Vicia tetrasperma</i> Moench.	6/80	Px	+	(+)	0.022	-	+	0.033	-	D	N (4)
<i>Vicia villosa</i> Roth.	6/80	Px	+	-	0.011	-	(+)	0.033	-	N	N (30)
LILIACEAE											
<i>Allium roseum</i> L.	5/80	Pl	+	(+)	0.000	0.62	-	0.130	-	D	None
<i>Aphyllanthes monspeliensis</i> L.	5/79	Pl	+	(+)	0.022	-	(+)	0.022	-	N	None
<i>Ornithogalum umbellatum</i> L.	6/80	Px	+	(+)	0.043	-	(+)	0.043	-	N	N (4)
LINACEAE											
<i>Linum angustifolium</i> Hudson	6/80	Px	+	-	0.022	-	+	0.022	-	D	None
<i>Linum narbonense</i> L.	6/80	Px	+	(+)	0.011	-	(+)	0.022	-	N	None
<i>Linum salsoides</i> Lam.	6/80	Px	+	++(+)	0.065	0.03	+(+)	0.097	-	P	None
OLEACEAE											
<i>Fraxinus angustifolia</i> Vahl.	6/79	Lf	+	(+)	0.011	-	+	0.022	-	D	None
ONAGRACEAE											
<i>Epilobium parviflorum</i> Schreber	6/79	Px	-	-	-	-	-	-	-	N	None
<i>Oenothera rosea</i> L'Her. ex Aiton	5/79	Px	-	-	-	-	-	-	-	N	None
ORCHIDACEAE											
<i>Limodorum abortivum</i> (L.) Swartz	5/79	Pl	+	(+)	0.011	-	++	0.076	0.09	P	None
<i>Orchis simia</i> Lam.	6/80	Px	+	++(+)	0.076	0.05 0.24	++(+)	0.282	0.03 0.08	P	None

APPENDIX 1. Continued.

Family—Botanical name	Month/year of collection	Part of plant	Preliminary test	Confirmatory test						Final conclusion	Previous works
				Tertiary alkaloids			Quaternary alkaloids				
				Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)	Alkaloid-precipitating reactions ^a	Titration	Thin-layer chromatography (Rf)		
PINACEAE											
<i>Pinus halepensis</i> Miller	5/80	Lf	+	-	0.032	0.03	+(+)	0.032	0.06 0.52	P	P(7)
PLANTAGINACEAE											
<i>Plantago holostemum</i> Scop.	8/79	Pl	+	+	0.043	-	++	0.054	0.16	P	None
POLIGALACEAE											
<i>Polygala calcarea</i> Schultz	6/80	Px	+	-	0.011	-	++	0.032	0.21	D	None
RANUNCULACEAE											
<i>Helleborus foetidus</i> L.	6/79	Px	+	(+)	0.011	-	+(+)	0.022	-	D	None
<i>Ranunculus acris</i> L.	6/79	Px	+	(+)	0.022	-	+(+)	0.032	-	D	None
<i>Ranunculus bulbosus</i> L.	5/80	Pl	+	(+)	0.011	-	+	0.043	-	D	P(2)
RHAMNACEAE											
<i>Rhamnus alaternus</i> L.	6/79	Px	+	-	0.022	-	(+)	0.043	-	N	None
ROSACEAE											
<i>Filipendula ulmaria</i> (L.) Maxim.	7/79	Px	-	-	-	-	-	-	-	N	P(2)
<i>Geum urbanum</i> L.	6/79	Pl	-	-	-	-	-	-	-	N	N(8)
<i>Rosa canina</i> L.	6/79	Lf, if	-	-	-	-	-	-	-	N	None
<i>Rubus caesius</i> L.	6/79	Lf, if	+	(+)	0.000	-	-	0.011	-	N	N(5)
<i>Rubus ulmifolius</i> Schott	6/79	Px	+	(+)	0.000	-	+	0.011	-	D	None
RUBIACEAE											
<i>Galium lucidum</i> All	6/79	Px	+	(+)	0.011	-	+(+)	0.043	-	D	None
<i>Rubia perigrina</i> L.	5/79	Px	+	(+)	0.022	-	(+)	0.022	-	D	None
SALICACEAE											
<i>Populus nigra x canadensis</i> Moench	9/79	Lf	-	-	-	-	-	-	-	N	None
<i>Salix alba</i> L.	6/79	Lf, bd	+	(+)	0.000	-	(+)	0.022	-	N	None
SAXIFRAGACEAE											
<i>Saxifraga granulata</i> L.	6/80	Pl	+	-	0.000	-	-	0.043	-	N	N(19)
SCROPHULARIACEAE											
<i>Rhinanthus mediterraneus</i> Adam.	6/80	Px	+	+	0.032	0.04	+	0.054	-	P	None
<i>Scrophularia umbrosa</i> Dumort	6/79	Pl	+	(+)	0.022	-	+	0.043	-	D	None
<i>Verbascum boerbavii</i> L.	6/80	Px	+	++	0.022	-	+++	0.097	0.03	P	None
<i>Verbascum sinuatum</i> L.	7/79	Px	-	-	-	-	-	-	-	N	None
<i>Veronica chamaedrys</i> L.	6/80	Px	+	-	0.022	-	+	0.022	-	D	None
TILIACEAE											
<i>Tilia platyphyllos</i> Scop.	9/79	Lf	+	(+)	0.032	-	(+)	0.011	-	N	N(5)
ULMACEAE											
<i>Ulmus minor</i> Miller	6/79	Lf	+	-	0.000	-	-	0.011	-	N	None
UMBELLIFERAE											
<i>Angelica silvestris</i> L.	9/79	Pl	+	(+)	0.022	-	(+)	0.011	-	N	None
<i>Apium nodiflorum</i> (L.) Lag.	6/79	Px	+	(+)	0.022	-	+	0.032	-	D	None
<i>Eryngium campestre</i> L.	6/79	Px	+	(+)	0.011	-	(+)	0.032	-	N	None
<i>Ligusticum lucidum</i> Miller	7/79	Px	+	+	0.032	0.52	++	0.152	0.06	P	None
<i>Petroselinum sativum</i> Hoffm.	9/79	Pl	+	-	0.032	0.53	+(+)	0.022	-	D	None
<i>Torilis arvensis</i> (Hudson) Link.	6/79	Px	+	+	0.000	-	+	0.032	-	D	None
VIOLACEAE											
<i>Viola buvanii</i> Timb-Lagr.	6/80	Px	-	-	-	-	-	-	-	N	None

^aCopies of specific results with each reagent can be obtained by writing to Dr. F. Viladomat.

Lf=leaves; if=inflorescences; bd=buds; Px=aerial part; Pl=whole plant.

P=positive; N=negative; D=doubtful.

None=species not listed in the literature cited.

(+)=<0.01%; +=0.01-0.05%; ++=0.05-0.1%; +++=>0.1%.

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