ACTA CHEMICA SCANDINAVICA 19 (1965) 1297–1300

Alkaloid-screening of Plants from Boyce Thompson Southwestern Arboretum*

ESKIL HULTIN**

Department of Pharmacology, University of California Medical Center, Los Angeles, Calif. 90024, U.S.A.

In a semi-quantitative screening of twenty-five plants, cultivated in Arizona, alkaloid concentrations larger than 0.01 % of the dried material were estimated to be present in the following species in which alkaloids have not knowingly been reported previously to occur: *Cneorum tricoccon*, *Condalia lycioides*, *Larrea divaricata*, *Olea europaea*, *Santolina Chamaecyparissus*, *Porlieria angustifolia*, and *Zizyphus Jujuba*. Sonnenschein's reagent and silicotungstic acid reagent may give false-positive reactions.

A method has recently been published for the semiquantitative screening of plants for alkaloids by which one can estimate not only the chloroform-extractable alkaloids but also any others.[1] In these tests, the amounts of precipitates of crude alkaloid fractions of plants treated with alkaloid reagents are compared with similarly treated strychnine solutions. The amount of precipitate obtained with different alkaloids does not show larger variation than what may be accepted for a semiquantitative screening method;[2] and in the method used for this study the variation is decreased by the use of several reagents.

In the present investigation, plants from Boyce Thompson Southwestern Arboretum were screened as potential sources of new alkaloids. Some of the species included were selected because no alkaloid-bearing plants have been reported in the same family or genus; others were selected because alkaloids are known to occur in some species of the family or genus, but not in all of them.

The following six alkaloid reagents were used: Mayer's (mercuric chloride and potassium iodide), Wagner's (potassium triiodide), Dragendorff's (bismuth nitrate and potassium iodide), Sonnenschein's (phosphomolybdic acid), silicotungstic acid, and Hager's reagent (picric acid). Their relative tendency to give false-positive reactions was studied with a few plant extracts, from

* Since May, 1965 Desert Biology Station, University of Arizona.
** On leave of absence from University of Stockholm, Sweden.

Table 1. Reagents tested for false-positive reactions. The reactions are graded by comparison of the intensity of the reaction between strychnine solutions and Mayer's (M), Wagner's (W), Dragendorff's (D), Sonnenschein's (S), silicotungstic acid (Si-W), and Hager's reagent. 0 designates no precipitate; other figures give the order of magnitude expressed in mg per 100 g of dried material. Part of plant: l = leaf, s = stem or twig.

Species	Plant part	M	W	D	S	Si-W	H
Artemisia tridentata Nutt.[a]	l	0	1	1	1	1	0
Baccharis sarothroides A. Gray	s	0	0	10	30	30	0
Terminalia australis Cambess.	l	0	0	10	30	30	0
Tarchonathus minor Less.	s	0	0.3	1	10	10	0

[a] Alkaloids have been found in *Artemisia tridentata.*[5]

which alkaloids had previously been extracted with chloroform and with chloroform and alcohol;[3,4] the material extractable from the residue with acetone-methanol was transferred to water solution. The results are given in Table 1. Sonnenschein's reagent, silicotungstic acid reagent and, to somewhat lesser extent, Dragendorff's reagent have a larger tendency to give false-positive reactions than do Mayer's and Hager's.

The results from the screening are given in Table 2, together with references to closely related, alkaloid-bearing species.

EXPERIMENTAL

The method previously reported was followed with only minor modifications.[1] The plant material was dried at a temperature not exceeding 50°C and ground. Five grams of the plant material were extracted overnight with 50 ml of methanol at room temperature and subsequently at 50°C for 5 h; heavily wax-coated leaves were previously defatted with pentane. The mixture was filtered, and the residue was washed with hot methanol. The solvent was evaporated *in vacuo*. The extract was taken up in 2 ml of methanol, and 12 ml of 1 % hydrochloric acid were added. The mixture was shaken vigorously and decanted through a filter. The extraction was repeated with 8 ml of 1 % hydrochloric acid and the mixture filtered. The combined filtrates were made alkaline with concentrated ammonia and extracted three times with 20 ml of chloroform, and the extracts were washed with 5 ml of a half saturated water solution of sodium sulfate (Fraction A). The aqueous solution was half-saturated with sodium sulfate, the solution with which the chloroform extracts were washed was added, and the extraction was continued with 50 ml of a mixture of chloroform and ethyl alcohol 3:2 v/v three times. The extracts so obtained were also washed with 5 ml of half-saturated sodium sulfate solution (Fraction B).

The extracts were dried with anhydrous sodium sulfate and each was evaporated *in vacuo* and tested for alkaloids. The residue was taken up in 1 ml of 1 % hydrochloric acid and 1 ml of chloroform, and the mixture was shaken vigorously. The chloroform phase was discarded, and the water phase taken out with a dropper pipet and filtered through cotton. The filtrate was divided into 6 portions, each of which was poured into a small test tube. The presence of alkaloids was tested with one drop of the following five reagents: Mayer's, Wagner's, Dragendorff's, Sonnenschein's and silicotungstic acid reagent; and with an equal volume of Hager's reagent. The reagents were prepared according to Cromwell [18] except for Sonnenschein's, which was prepared as a 10 % solution of phosphomolybdic acid in dilute nitric acid, 1:9 v/v.

Table 2. Species tested for alkaloid content, and reaction obtained. Part of plant: b = bark, f = fruit, l = leaf, rb = root bark. The reaction was graded by comparison with the intensity of the reaction between strychnine sulfate solutions and the reagents used, and the average intensity given. 0 designates less than 1 mg per 100 g of dried material; other figures give the order of magnitude expressed in mg per 100 g. References are given if a species listed belongs to a genus in which alkaloid-bearing species have been reported. Alkaloids have not knowingly been reported in the species listed except as indicated. Fraction A: extracted with chloroform from an ammoniacal solution; fraction B: after addition of sodium sulfate to half saturation extracted with chloroform-ethanol 3:2.

Family	Species	Plant part	Fraction A	Fraction B	References
Anacardiaceae	*Rhus lancea* L.	l	0	0	6
Buxaceae	*Simmondsia chinensis* (Link) C. K. Schneid.	l	3	30	7[a]
Capparidaceae	*Isomeris arborea* Nutt. var. *globosa* Cov.	s	1	3	
Celastraceae	*Maytenus phyllanthoides* Benth.	l	1	3	8
	Mortonia scabrella A. Gray	b, l	0	0	
Chenopodiaceae	*Atriplex nummularia* Lindl.	s	0	3	9
Cneoraceae	*Cneorum tricoccon* L.	b	1	10	
	Cneorum tricoccon L.	l	1	30	
Combretaceae	*Terminalia australis* Cambess.	l	1	3	
	Terminalia australis Cambess.	rb	0	0	
Compositae	*Baccharis sarothroides* A. Gray	s	0	3	10, 11
	Santolina Chamaecyparissus L.	s	10	30	
	Tarchonanthus minor Less.	s	0	1	12
Ebenaceae	*Diospyros Kaki* L.	l, rb	0	0	11, 13
Meliaceae	*Melianthus comosus* Vahl	s	0	1	
Myrtaceae	*Feijoa Sellowiana* Berg	l	0	1	
Oleaceae	*Olea europaea* L.				11, 14
	var. *oleaster* DC.	l	30	10	
	var. *sativa* (Hoffm. et Link) Rouy.	rb	10	0	
	Phillyrea latifolia L.	l	0	0	15
	Phillyrea latifolia L.	rb	3	0	
Phytolaccaceae	*Phytolacca dioica* L.	l, rb	0	0	11, 16
Pittosporaceae	*Pittosporum phillyraeoides* DC.	s, rb	0	0	11[b], 13
Rhamnaceae	*Condalia lycioides* Weberbauer	l	100	3	
	Karwinskia Humboldtiana Zucc.	l, rb	0	0	
	Zizyphus Jujuba Lam.	l	1	0	17
	Zizyphus Jujuba Lam.	rb	10	3	
Sapindaceae	*Dodonaea viscosa* Jacq.	l	0	0	11 [b]
Scrophulariaceae	*Leucophyllum texanum* Benth.	s	3	10	
Zygophyllaceae	*Larrea divaricata* Cav.	b	1	100	
	Larrea divaricata Cav.	f, l	0	0	
	Larrea divaricata Cav.	rb	3	30	
	Porlieria angustifolia A. Gray	b	10	300	
	Porlieria angustifolia A. Gray	l	1	0	

[a] The presence of alkaloids in *Simmondsia californica (= S. chinensis)* was reported by Martin-Sans.[7]

[b] The presence of alkaloids in *Pittosporum phillyraeoides* (fruit) and in *Dodonaea viscosa* was reported by Webb.[11]

The presence of alkaloids was considered demonstrated only if a precipitate appeared with all reagents, unless the precipitates with the five first-mentioned reagents were small; then the reaction was considered positive without a precipitate with Hager's

reagent, as this reagent has a considerably lower sensitivity than the others.[2] The densities of the precipitates were compared to the densities of precipitates obtained with standard strychnine solutions in 1 % hydrochloric acid, made up to the following amounts: 0.028, 0.089, 0.28, 0.89, ... mg/ml.

To test for false-positive reactions, the aqueous phase remaining after the extraction with chloroform-ethanol was evaporated *in vacuo* almost to dryness, and then extracted with acetone-methanol 1:1, and the extract was treated as before.

Acknowledgements. The author wishes to express his sincere thanks to Mr. J. E. Thompson, Jr. for permission to collect material in *Boyce Thompson Southwestern Arboretum* in Arizona, and to Mrs. Ingegerd Appelgren and Mr. Darrell E. Zinn for skilful assistance. This work was supported by *U.S. Army* Contract No. DA 118—108—CHL—6613(A) CPI—15190.

REFERENCES

1. Hultin, E. and Torssell, K. *Phytochemistry* **4** (1965) 425.
2. Martello, R. and Farnsworth, N. R. *Lloydia* **25** (1962) 176.
3. Farnsworth, N. R. and Euler, K. L. *Lloydia* **25** (1962) 186.
4. Farnsworth, N. R., Pilewski, N. A. and Draus, F. J. *Lloydia* **25** (1962) 312.
5. Kinney, C. R. and Sugihara, J. *J. Org. Chem.* **8** (1943) 290.
6. Karaev, A. I., Aliev, R. K., Guseinov, G. A. and Gasanov, G. *Dokl. Akad. Nauk Azerb. SSR* **10** No. 3 (1954) 189; from *Chem. Abstr.* **50** (1956) 490f.
7. Martin-Sans, E. *Compt. Rend.* **191** (1930) 625.
8. Perrot, E., Millat, L. and Colas, R. *Bull. Sci. Pharmacol.* **44** (1937) 325.
9. Zolotnitskaya, S. Y. *Izv. Akad. Nauk Arm. SSR, Biol. i Sel'skokhoz. Nauki* **7** No. 5 (1954) 27.
10. Arata, P. N. *Pharm. J.* [3] **10** (1879) 6.
11. Webb, L. J. *Australia, Commonwealth Sci. Ind. Res. Organ. Bull.* No. 241 (1949).
12. Canzoneri, F. and Spica, G. *Gazz. Chim. Ital.* **12** (1882) 227.
13. Webb, L. J. *Australia, Commonwealth Sci. Ind. Res. Organ. Bull.* No. 268 (1952).
14. Boorsma, P. A. *Mededel. s'Lands Plantentuin* **18** (1897) 29; **31** (1899) 132; from Wehmer, C. *Die Pflanzenstoffe*, Zweite Auflage. Gustav Fischer, Jena 1931, p. 953.
15. Jachelli, *J. chim. med.* [2] **4** (1848) 93; from *Pharmaceutisches Central-Blatt* **19** (1848) 415.
16. Arnold, H. L. *Poisonous plants of Hawaii*, Tongg, Honolulu 1944; from Webb, L. J. *Australia, Commonwealth Sci. Ind. Res. Organ. Bull.* No. 232 (1948).
17. Ismailov, N. M. and Rzazade, R. Ya. *Dokl. Akad. Nauk Azerb. SSR* **10** (1954) 197; from Willaman, J. J. and Schubert, B. G. *Alkaloid-bearing plants and their contained alkaloids*, US Dept. Agr. Techn. Bull. 1234, Washington 1961.
18. Cromwell, B. T. In Paech, K. and Tracey, M. V. (Eds.) *Modern methods of plant analysis*, Springer-Verlag, Berlin, Göttingen, Heidelberg 1955, Vol. 4, p. 367.

Received April 5, 1965.