One milliliter of the separated acid extract was treated with a few drops of Mayer's reagent, and a second 1-ml. portion was treated with silicotungstic acid reagent as previously described. Turbidity or precipitation after the addition of either of these reagents was taken as a confirmed positive test for the presence of alkaloids in the extract. The results of these tests are presented in Table I.

Each plant sample was also screened for alkaloids using thin-layer chromatography according to the method of Farnsworth and Euler (3). This procedure was modified only in that the final volume of fraction I (chloroform extract) applied to each thin-layer plate was 30 μl. The results from this test are also presented in Table I.

Saponins—Since all saponins, whether steroidal or triterpenoid, will hemolyze red blood cells, utilization of this property is advantageous for detecting this class of compounds in plant material. A red blood cell suspension was prepared and standardized against digitonin according to the protocol of Wall *et al.* (2). One milliliter of each plant extract was mixed with 10 ml. of the red blood suspension and the mixtures were allowed to stand for 1 hr. before observing the results. Complete hemolysis of the red blood cells in any instance was taken as evidence for a positive test, the results of which are presented in Table I.

Tannins—Twenty milliliters of the original 80% ethanol extract from each plant sample was evaporated to dryness on a steam bath and the residue was stirred with 5 ml. of distilled water and filtered. Two milliliters of the filtrate was treated with a few drops of gelatin–salt reagent (1), and precipitation was taken as evidence for the presence of tannins. The addition of ferric chloride reagent to extracts of plants giving positive gelatin–salt tests served to further categorize the tannin present as to hydrolyzable (blue, blue-black), or condensed types (green, blue-green). These results are presented in Table I.

SUMMARY

Of the 50 species screened for alkaloids, 24 showed the presence of tertiary alkaloids, representing 14 families of plants. Only two species exhibited the presence of quaternary alkaloids, one in the *Annonaceae*, and the other in the *Pedaliaceae*. Sixteen species, distributed in 13 families, contained saponins. The 43 species giving positive tests for tannins were in 29 different families.

REFERENCES

(1) Wall, M. E., Krider, M. E., Krewson, C. F., Eddy, C. R., Willaman, J. J., Corell, D. S., and Gentry, H. S., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1(1954).

(2) Wall, M. E., Eddy, C. R., McClennan, M. L., and Klumpp, M. E., *Anal. Chem.*, **24**, 1337(1952).

(3) Farnsworth, N. R., and Euler, K. L., *Lloydia*, **25**, 186(1962).

Isolation and Characterization of Alkaloids from *Caulophyllum thalictroides*

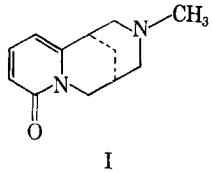
By MICHAEL S. FLOM, RAYMOND W. DOSKOTCH, and JACK L. BEAL

The lupin alkaloids, methylcytisine, baptifoline, and anagyrine, and the aporphine alkaloid, magnoflorine, were isolated from the roots and rhizomes of *Caulophyllum thalictroides*. Separation of the tertiary lupin alkaloids was by partition column chromatography while magnoflorine was obtained after chromatography on alumina.

THE PRESENCE of methylcytisine (I) in the roots and rhizomes of *Caulophyllum thalictroides* (L.) Michx., (family, *Berberidaceae*), commonly called blue cohosh, has been known for many years (1). On re-examination of this source for alkaloids, a thin-layer chromatographic analysis of the tertiary alkaloid fraction revealed at least six spots reacting with Dragendorff's spray reagent. The quaternary alkaloid fraction indicated only one spot when tested by the same spray reagent.

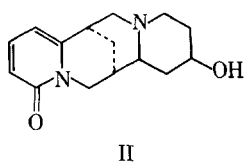
Partition column chromatography on diatomaceous earth with Skellysolve B–ethylene dichloride–methanol–water (10:6:2.5:0.5) as solvent system separated the tertiary alkaloids. The three major alkaloids from this fraction were obtained crystalline or as crystalline salts and were identified as methylcytisine (I), baptifoline (II), and anagyrine (III).

The quaternary alkaloid fraction was obtained *via* the reineckate salt which when converted to the chloride salt and chromatographed on alumina yielded crystalline magnoflorine chloride (IV).



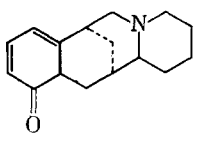
I

(−) Methylcytisine



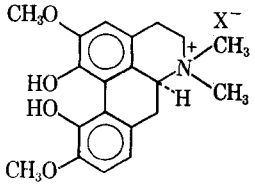
II

(−) Baptifoline



III

(−) Anagyrine



IV

(+) Magnoflorine

Received April 14, 1967, from the College of Pharmacy, Ohio State University, Columbus, OH 43210

Accepted for publication July 24, 1967.

Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.

This work was supported by research grant GM-05640 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

EXPERIMENTAL[1]

Plant Material—The roots and rhizomes of *Caulophyllum thalictroides* (L.) Michx. used in this investigation were obtained from S. B. Penick and Co. (lot No. 668 BA W-80576).

Extraction and Initial Separation—The plant material (13.7 Kg.) was extracted by percolation at room temperature with ethanol U.S.P. requiring about 74 L. of solvent to completely exhaust the material of alkaloids as evidenced by a negative Valser's test. Evaporation of the solvent at reduced pressure at 40° left 2.24 Kg. of residue. This was partitioned between chloroform and 2% aqueous citric acid in 100-Gm. quantities requiring 300 ml. of each phase. The citric acid phase was extracted three times with equal volumes of chloroform and then brought to pH 9 with concentrated ammonium hydroxide and then extracted with chloroform. This extract was dried over anhydrous sodium sulfate and on evaporation of the solvent left 30.6 Gm. of crude tertiary alkaloids.

Thin-layer chromatography of the tertiary alkaloids on Silica Gel G[2] with chloroform–methanol–ammonium hydroxide (200:20:1) indicated the presence of six tertiary alkaloids when detected with Dragendorff's reagent (2).

The ammonium hydroxide solution after removal of the tertiary alkaloids was acidified with 10% hydrochloric acid producing a gelatinous precipitate which was collected by filtration. From this precipitate it was possible to isolate caulosaponin, m.p. 257–259° [lit. values m.p. 250–255° (1), 254–255° (3)] by the procedure of Power and Salway (1). Hydrolysis of caulosaponin with aqueous alcoholic hydrochloric acid yielded hederagenin, m.p. 333–335°, $[\alpha]_D^{29}$ + 80° (c, 0.10% in ethanol), ultraviolet end absorption, λ = 210 mμ (ϵ = 4380); the literature values (3) are m.p. 332–333°, $[\alpha]_D^{20}$ + 78° (c, 0.10% in ethanol), ultraviolet absorption $\lambda_{max.}$ = 210 mμ (ϵ = 2860). Refluxing hederagenin in acetic anhydride for 1.5 hr. gave the acetate, m.p. 172–174° [lit. value (3) m.p. 173–174°]. The acidic filtrate was treated with a saturated aqueous solution of ammonium reineckate (2 Gm. reagent/100 ml. solution) while stirring. The precipitate of alkaloid reineckate was collected by suction filtration, sucked dry, and then washed with ether giving a yield of 285 Gm. TLC examination of the crude quaternary chloride (see below) on Silica Gel G with *n*-propanol–ammonium hydroxide–water (4:1:1) as solvent system showed one spot (R_f 0.55) when sprayed with Dragendorff's reagent.

Isolation of Magnoflorine Chloride—The alkaloid reineckate (190 Gm.) was dissolved in 3 L. of acetone–water (1:1) and the solution passed through a column containing 1700 ml. of Amberlite IRA-410 (Cl) resin. The crude quaternary alkaloid chloride (81 Gm.) from the effluent was chromatographed on Woelm neutral alumina,[3] activity V. The mixture was placed on the column as a methanol solution and eluted with methanol. The effluent was examined by TLC and the alkaloid fraction collected. Crystallization of the alkaloid fraction (72 Gm.) from ethanol gave magnoflorine chloride, m.p. 240–241° dec., $[\alpha]_D^{27}$ + 208° (c, 0.144% in methanol). The reported values (4) are m.p. 242–243° dec. and $[\alpha]_D^{31}$ + 213°. The infrared spectrum of the isolated substance and that of an authentic sample of magnoflorine chloride were superimposable. Mobility of the two samples was the same on TLC plates.

Separation of the Tertiary Alkaloids—The mixture of tertiary alkaloids, which on TLC indicated six components, was separated on a partition chromatographic column according to the procedure of Brown and Kupchan (5). The solvent system was Skellysolve B–ethylene dichloride–methanol–water (10:6:2.5:0.5). A column, 3.7 cm. (i.d.) × 47 cm., was prepared by mixing 182 ml. of the stationary phase (upper phase) with 300 Gm. of diatomaceous earth[4] and packing the material in 1-cm. segments with the aid of a ramrod. The mobile phase was passed through the packed column till all of the air had been removed (about 2 days). The holdup volume of the column was 395 ml.

The tertiary alkaloid mixture of 1.32 Gm. in 20 ml. of the mobile phase was passed into the column. The flow rate was 30 ml./hr. Fractions of effluent (20 ml.) after passage of the holdup volume were collected and analyzed by TLC. A summary of the separation obtained is given in Table I along with the tubes pooled according to the materials present.

Of the fractions examined only fractions 2, 3, and 8 yielded crystalline product or a crystalline salt. All the other fractions remained as heavy oils. These latter fractions account for only about 9% of the crude tertiary alkaloids. (Table I.)

Methylcytisine (I)—From pooled fraction No. 3, the 343 mg. of crude material yielded 194 mg. of crystalline product from benzene–Skellysolve B, m.p. 137° [lit. value (1) m.p. 137°]. The perchlorate salt was prepared by the usual procedure, m.p. 280–281° [lit. value (6) m.p. 282°] as well as the crystalline picrate, m.p. 221–222° [lit. value (1) m.p. 228°]. The specific rotation was $[\alpha]_D^{30}$ − 224° (c, 1.05% in water) [lit. value (1) $[\alpha]_D$ − 221.6°]. The ultraviolet absorption spectrum showed $\lambda_{max.}$ = 309 mμ (ϵ = 7,560); 234(6,860). In addition, a known sample of cytisine was methylated by the Eschweiler-Clarke procedure of the Leuckart reaction (7) to give a product identical with the isolated material (I.R., U.V., and mixed melting point determination).

Baptifoline (II)—The pooled column fraction No. 8 (241 mg.) was crystallized from benzene to yield 118 mg. of product, m.p. 210°. [Baptifoline lit. value (8) m.p. 210°.] The crystalline perchlorate salt, m.p. 286–287°, and the picrate which sinters at 145° then melts at 252° were prepared by the usual methods. The values in the literature are m.p. 286–287° (8) and m.p. 145° (sinters), then 256° (6). The optical rotation was $[\alpha]_D^{27}$ − 133° (c,

[1] Melting points were determined with a Thomas-Hoover Uni-Melt capillary melting point apparatus in sealed evacuated capillaries and were corrected. The infrared spectra were taken in chloroform and as KBr pellets on a Perkin-Elmer model 237 infrared spectrophotometer. The ultraviolet spectra were determined on a Cary model 15 recording spectrophotometer, in ethanol.

[2] Made by E. Merck (Darmstadt, West Germany), obtained from Brinkmann Instruments, Inc., Westbury, N. Y.

[3] Obtained from Alupharm Chemicals, New Orleans, La.

[4] Celite 545, a product of Johns-Manville Corp., New York, N. Y., which was purified by dispersing in 6 *N* HCl, three times, then filtering by suction and washing successively with distilled water, methanol, benzene, Skellysolve B, and methanol. Skellysolve B is a petroleum ether fraction, b.p. 60–80°. Ethylene dichloride, C.P., was from Union Carbide Co., and methanol A.R. was from Mallinckrodt Chemicals.

TABLE I—SEPARATION OF TERTIARY ALKALOID MIXTURE

Fraction No.	Tube No., 20 ml.	TLC R_f	Pooled Wt., mg.	Crystalline Product, mg.	% Yield[a]	Compd.
1	12–14	0.74	35	...	...	...
2	16–19	0.67	144	109[b]	0.012	Anagyrine
3	21–28	0.60	343	194	0.033	Methylcytisine
4	43–50	0.38	25	...	...	...
5	54–58	0.28	14	...	...	...
6	71–85	0.27	22	...	...	...
7	91–104	0.24	22	...	...	...
8	105–165	0.28	241	118	0.020	Baptifoline

[a] Based on dry plant material. [b] As perchlorate salt.

0.325% in ethanol). [Lit. value $[\alpha]_D^{18}$ − 147.7° (6).] The ultraviolet absorption spectrum showed $\lambda_{max.}$ = 309 mμ (ε = 7,540); 234(7,150). The infrared spectrum of authentic baptifoline[5] taken in a Nujol mull was identical to that of our compound determined in the same way.

Anagyrine (III)—The oil (144 mg.) in the pooled fraction No. 2 provided a crystalline perchlorate from methanol, m.p. 315°, and a picrate from methanol, m.p. 247–249°. Anagyrine perchlorate melts at 315° (8) and the picrate at 249–251° (6). The ultraviolet absorption spectrum of the perchlorate salt showed $\lambda_{max.}$ = 308 mμ (ε = 7,460); 234(6,900) and an infrared absorption spectrum with peaks at $\nu_{max.}$ 1650, 1570, and 1560 cm.$^{-1}$ when taken in a Nujol mull, and are consistent with the reported values for anagyrine (9).

DISCUSSION

C. thalictroides has been known for over 50 years to contain methylcytisine. With the application of modern isolation and analytical methods this source has been found to contain many more alkaloids of which three not previously recognized have been isolated in pure form and identified.

The three lupin tertiary alkaloids, methylcytisine, baptifoline, and anagyrine, were readily separated by partition column chromatography and are the first example of the isolation of the latter two alkaloids from the family *Berberidaceae*. The other alkaloids (at least three) in this fraction were present in very small amounts, remained as oils, and could not be induced to crystallize or to form crystalline salts, although separated by the column method so as to show only one spot reacting with Dragendorff's reagent. Further identification of the minor tertiary alkaloids must await the processing of large amounts of plant material. Undoubtedly they will also be of the lupin type.

The presence of magnoflorine in *Caulophyllum robustum* Maxim., has been reported by Tomita and Takahashi (10). The presence of this ubiquitous alkaloid has now been confirmed in *C. thalictroides*.

[5] Kindly provided by Professor Shigenobu Okuda, University of Tokyo, Tokyo, Japan.

REFERENCES

(1) Power, F. B., and Salway, A. H., *J. Chem. Soc.*, **103**, 191(1913).
(2) Block, R. J., Durrum, E. L., and Zweig, G., "A Manual of Paper Chromatography and Paper Electrophoresis," 2nd ed., Academic Press Inc., New York, N. Y., 1958, p. 361.
(3) McShefferty, J., and Stenlake, J. B., *J. Chem. Soc.*, **1956**, 2314.
(4) Winek, C. L., Beal, J. L., and Cava, M. P., *Lloydia*, **27**, 111(1964).
(5) Brown, K. S., Jr., and Kupchan, S. M., *J. Chromatog.*, **9**, 71(1962).
(6) Marion, L., and Cockburn, W. F., *J. Am. Chem. Soc.*, **70**, 3472(1948).
(7) Moore, M. L., in "Organic Reactions," vol. V, Adams, R., ed., John Wiley & Sons, Inc., New York, N. Y., 1949, p. 301.
(8) Marion, L., and Turcotte, F., *J. Am. Chem. Soc.*, **70**, 3253(1948).
(9) Okuda, S., Murakoshi, I., Kamata, H., Kashida, Y., Haginawa, J., and Tsuda, K., *Chem. Pharm. Bull. (Japan)*, **13**, 482(1965).
(10) Tomita, M., and Takahashi, J., *Yakugaku Zasshi*, **78** 680(1958).