Detecting Potential Teratogenic Alkaloids from Blue Cohosh Rhizomes Using an in Vitro Rat Embryo Culture

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The novel alkaloid thalictroidine (**1**), as well as the known alkaloids taspine (**2**), magnoflorine (**3**), anagyrine (**4**), baptifoline (**5**), 5,6-dehydro-α-isolupanine (**6**), α-isolupanine (**7**), lupanine (**8**), *N*-methylcytisine (**9**), and sparteine (**10**), were identified from an extract of *Caulophyllum thalictroides* rhizomes. *N*-Methylcytisine exhibited teratogenic activity in the rat embryo culture (REC), an in vitro method to detect potential teratogens. The structure of **1** was elucidated using various spectroscopic methods, primarily by NMR techniques. Thalictroidine, anagyrine, and α-isolupanine were not teratogenic in the REC at tested concentrations. Taspine (**2**) showed high embryotoxicity, but no teratogenic activity, in the REC.

The rhizomes of blue cohosh, *Caulophyllum thalictroides* (L.) Michx. (Berberidaceae), are used in certain dietary supplement products, but the safety and efficacy of this plant have not been studied extensively. Previous researchers identified three quinolizidine alkaloids in the rhizomes of this plant, including anagyrine (**4**), baptiflorine (**5**), and *N*-methylcytisine (**9**), along with the quaternary aporphine alkaloid magnoflorine (**3**).[1,2] The triterpenoid glycoside, caulosaponin, has also been isolated from the rhizomes of this plant.[2] Quinolizidine alkaloid levels in selected blue cohosh-containing dietary supplement products have been determined previously by gas chromatography, and the following ranges were found: *N*-methylcytisine, 5−850 ppm; anagyrine, 2−390 ppm; and baptifoline, 9−900 ppm, with the lower concentrations found in liquid-extract products.[3] Magnoflorine was measured in *C. thalictroides* rhizomes at a level of 11 000 ppm.[4] This investigation was undertaken because of public health concerns arising from the knowledge that anagyrine is a suspected teratogen,[5,6] and blue cohosh-containing dietary supplements are used by women of reproductive age.[7]

Limited information is available regarding the toxicity of blue cohosh. However, several cases of adverse birth outcomes have been associated with maternal ingestion of blue cohosh-containing products. In one case, profound neonatal congestive heart failure was attributed to the maternal ingestion of blue cohosh extract.[8] The male infant was hospitalized for 31 days, and upon follow-up at age 2, the child had persistent cardiomegaly, with reduced left ventricular function, and remained on digoxin therapy.[8] In another case, a combination of blue cohosh and black cohosh [*Cimicifuga racemosa* (L.) Nutt.] was administered at about 42 weeks gestation by a midwife.[9] The neonate was not able to breathe spontaneously, and two midwives administered cardiac massage and oxygen. The neonate was transferred to a hospital where she reportedly experienced seizures and acute tubular necrosis. Mechanical ventilation was required, and the computerized tomograph showed basal ganglia and parasagittal hypoxic injury.[9] Certain information presented in this report, however, has been disputed.[10] Additionally, two cases of adverse events associated with ingestion of blue cohosh-containing dietary supplements appear in the FDA's Special Nutritionals Adverse Event Monitoring System database.[11] Ingestion of the bitter-tasting seeds of blue cohosh have also resulted in episodes of acute intoxication, including vomiting and diarrhea.[12,13]

In this study, the rhizomes of blue cohosh were extracted, and using activity-guided fractionation with the REC system to detect potential teratogens, a number of alkaloids were obtained. Selected extracts and purified blue cohosh alkaloids were tested in the REC.

Results and Discussion

The MeOH extract of the rhizomes of *C. thalictroides* was made alkaline and initially extracted exhaustively with EtOAc, and subsequently with 1-butanol. The original MeOH extract of blue cohosh and the resulting EtOAc and 1-butanol extracts were each tested in an in vitro REC. At concentrations of 500 μg/mL, the EtOAc and 1-BuOH extracts were lethal to the embryos (Table 1), and at a concentration of 250 μg/mL, the EtOAc extract was still lethal to the rat embryo. This EtOAc extract also tested positive for alkaloids in the Dragendorff test and was subjected to flash chromatography over Si gel with a gradient of MeOH in $CHCl_3$, yielding 12 fractions. Further chromatography of these fractions gave nine alkaloids, including the novel compound thalictroidine (**1**). The eight known constituents identified include the aporphine alkaloid taspine (**2**) and the quinolizidine alkaloids, anagyrine (**4**), baptifoline (**5**), 5,6-dehydro-α-isolupanine (**6**), α-isolupanine (**7**), lupanine (**8**), *N*-methylcytisine (**9**), and sparteine (**10**). Taspine (**2**), α-isolupanine (**7**), lupanine (**8**), and sparteine (**10**) had not been reported previously from blue cohosh. In addition, the quaternary amine, magnoflorine (**3**), was obtained from the 1-butanol extract in this study (Figure 1).

The EIMS of thalictroidine (**1**) displayed a molecular ion at *m*/*z* 233, shown by a high-resolution measurement to be $C_{14}H_{19}NO_2$. The ^1H NMR spectrum for **1** revealed four aromatic protons at δ 7.81 (2H, d, J = 8.8 Hz, H-10/14)

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Table 1. Effects of Extracts and Purified Components of *C. thalictroides* on the Developmental Score[a] of Cultured Rat Embryos

	concentration of test material (μg/mL								
fraction/component	0[b]	0.1	1.25	5	20	50	80	250	500
methanol extract	30.1	[c]		29.8		29.5			30.2
1-butanol extract	30.1			33.0		27.5			lethal
ethyl acetate extract	30.1			32.2	24.2	28.2	27.0	lethal	lethal
anagyrine (**4**)	30.1			28.2	26.5		28.2	15.5	
α-isolupanine (**7**)	30.1			29.5	29.2		30.5		
magnoflorine (**3**)	30.1		28.2	29.0	27.8		28.2		
N-methylcytisine (**9**)	30.1			29.0	29.8[d]	26.2[d]	24.0[d]	22.9[d]	
taspine (**2**)	30.1	27.6	27.7	lethal	lethal		lethal	lethal	
thalictroidine (**1**)	30.1			30.8	29.5		29.2		

[a] The developmental score[27] is an assessment of the overall growth and morphogenesis of organogenesis-staged rodent embryos. A score lower than the control indicates retardation of overall growth and development. The presence of a specific major malformation(s) in the absence of significant overall growth inhibition is indicative of a teratogenic response. [b] Solvent (DMSO−EtOH, 1:1) control. Pooled controls across experiments. [c] Not tested at that concentration. [d] Treatments where one embryo or more had major malformations (e.g., unfused regions of the anterior neural tube, poor or absent eye development, or severe twists in tail flexure).

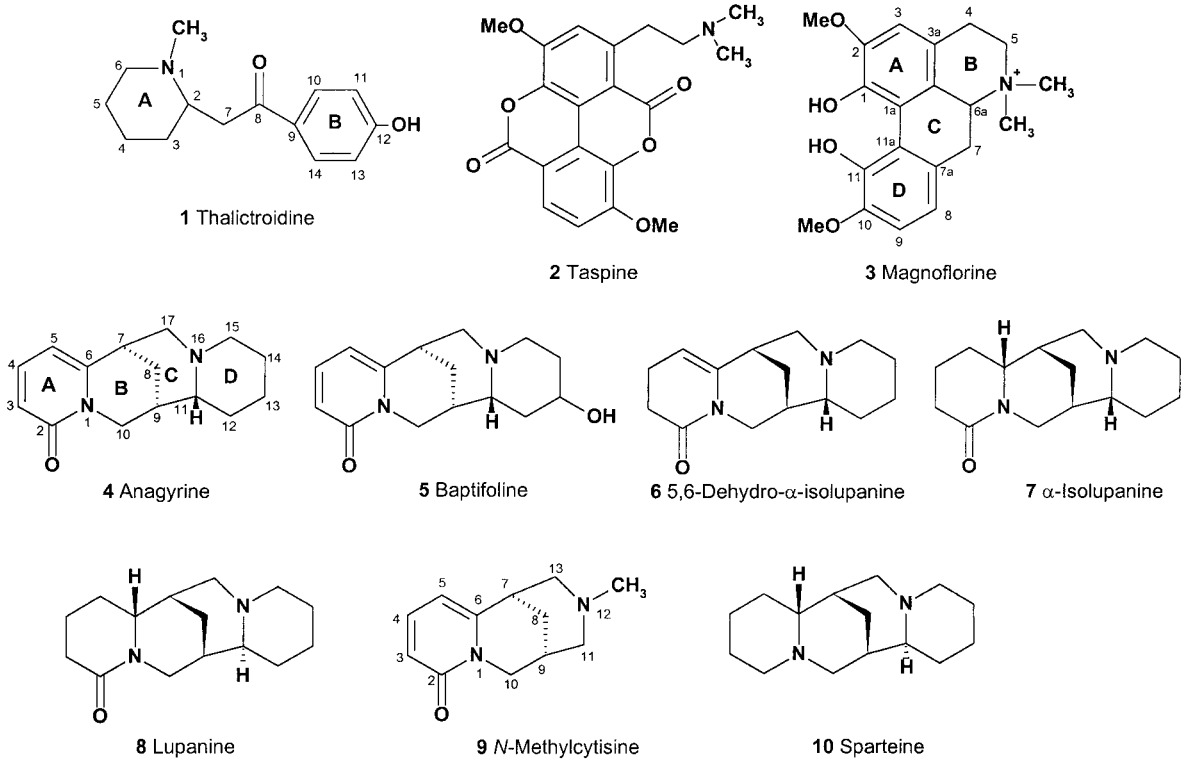

Figure 1. Alkaloids identified from *C. thalictroides*.

and 6.81 (2H, d, J = 8.8 Hz, H-11/13), suggesting a para-substituted phenol. The ^{13}C NMR spectrum displayed 12 distinct resonances (Table 2), including a ketone at δ 196.4 (s) and an *N*-methyl functional group at δ 42.7 (q). An HMBC NMR experiment helped to establish the NMR assignments for the A-ring, with the methylene protons at 3.03 and 2.39 ppm displaying long-range correlations to three carbons, including the NCH_3 at δ 42.7, C-2 at δ 59.9, and C-4 at δ 23.4. The position of the ketone in relation to the A- and B-rings was also established by the HMBC NMR experiment, in which aromatic H-10/14 (δ 7.81) were correlated to carbonyl C-8 (δ 196.4). Thalictroidine (**1**) is a novel piperidyl−acetophenone compound. This carbon skeleton is not common in the plant kingdom.[14] Thalictroidine did not show any teratogenic activity in the REC at the concentrations tested (Table 1).

The ^{1}H and ^{13}C NMR spectra of taspine (**2**) obtained from blue cohosh were consistent with published values.[15] Furthermore, by EIMS, the base peak of **2** is *m*/*z* 58, a fragment corresponding to a dimethyl methylene iminium ($[CH_2{=}NMe_2]^+$), characteristic of phenanthrene-type alkaloids.[16] In the REC, taspine (**2**) was lethal, but not teratogenic, to the embryos at 5 μg/mL (Table 1). Previous researchers have found that taspine is cytotoxic at concentrations as low as 0.5 μg/mL.[15] Taspine has been isolated before from the rhizomes and leaves of *Caulophyllum robustum* Maxim. as well as from the sap and leaves of *Croton* species.[17,18] Other phenanthrene alkaloids such as morphine, heroin, and codeine are highly cytotoxic and are suspected human teratogens.[6] The aporphine alkaloid, magnoflorine (**3**), a probable biosynthetic precursor to taspine, was isolated from the 1-butanol extract of blue cohosh, but it was not active in the REC (Table 1).

The known quinolizidine alkaloids, anagyrine (**4**), baptifoline (**5**), 5,6-dehydro-α-isolupanine (**6**), α-isolupanine (**7**), lupanine (**8**), *N*-methylcytisine (**9**), and sparteine (**10**), were identified from the EtOAc extract. Their identity was confirmed by ^{1}H and ^{13}C NMR measurements and/or by GC−MS comparisons with standard compounds. Quinolizidine alkaloids are known to possess pharmacological

Table 2. Thalictroidine (**1**) ^{1}H and ^{13}C NMR Data[a]

position	^{1}H	^{13}C	*n*-H	^{1}H HMBC connectivities
2	3.07 m	59.9	1	1.53, 1.75, 2.38, 2.85, 3.03, 3.38
3α	1.75 dt (11.3, 4.1)	31.2	2	1.40, 1.73, 2.85, 3.07, 3.38
3*β*	1.53 dq (11.3, 2)			
4α	1.40 tq (11.3, 4.7)	23.4	2	1.53, 1.75, 3.03, 3.07
4*β*	1.73 m			
5α	1.70 m	24.5	2	1.53, 1.75, 3.03
5*β*	1.68 m			
6α	2.39 dt (11, 5)	56.4	2	1.73, 2.38
6*β*	3.03 dt (11.5, 3.4)			
7A	3.38 dd (16.6, 5.4)	41.2	2	1.53, 1.75
7B	2.85 dd (16.6, 6.3)			
8		196.4	0	2.85, 3.38, 7.81
9		127.8	0	6.81
10/14	7.81 d (8.8)	131.0	1	7.81
11/13	6.81 d (8.8)	116.4	1	6.81
12		164.0	0	7.81
NCH_3	2.38 s	42.7	3	2.39, 3.03

[a] Chemical shifts referenced to $CDCl_3$ at 7.25 ppm (^{1}H) and 77.0 ppm (^{13}C); coupling constants in parentheses.

and toxicological properties ranging from antiinflammatory to mutagenic.[19] *N*-Methylcytisine, a tricyclic *N*-methyl-containing quinolizidine alkaloid (**9**), was the most active of the quinolizidines tested in the REC. *N*-Methylcytisine (**9**) caused major malformations (e.g., open anterior neural tube, poor or absent eye development, twisted tail) at a medium concentration, 20 μg/mL, that did not significantly inhibit overall growth and development. Higher treatment levels caused similar malformations as well as inhibition of overall growth and morphogenesis. *N*-Methylcytisine (**9**) has pharmacological activity similar to that of nicotine.[20] The compound stimulates the ganglion cells of the cardiac vagus in frogs, paralyzes the ganglia of the cardiac vagus in dogs, and produces hyperglycemia in rabbits.[20] A recent study examined the binding of various quinolizidine alkaloids to the nicotinic and muscarinic acetylcholine receptors.[21] Of the 14 quinolizidine alkaloids tested, *N*-methylcytisine (**9**) had the greatest affinity for the nicotinic receptor ($IC_{50} = 0.05$ μM), and it showed the least binding affinity to the muscarinic acetylcholine receptor.[21] Conversely, anagyrine (**4**) displayed little binding affinity to the nicotinic receptor ($IC_{50} = 2096$ μM) and greater binding affinity to the muscarinic acetylcholine receptor ($IC_{50} = 132$ μM).[21]

Anagyrine (**4**) is a well-known toxic constituent of certain legumes and is associated with crooked-calf disease.[5] In the REC, anagyrine did not display teratogenic effects, except at very high concentrations, which also severely inhibited overall morphogenesis (Table 1). Anagyrine's inactivity in the REC is consistent with the fact that the REC is not scored for skeletal development. Furthermore, it is thought that anagyrine may need to be metabolized by rumen microflora in order for the alkaloid to have a teratogenic effect. The related saturated quinolizidine alkaloid, α-isolupanine (**7**), also did not inhibit the developmental score or induce malformations of the cultured rat embryos at the concentrations tested (Table 1).

In this study, *N*-methylcytisine (**9**) displayed the most distinct teratogenic effects in the REC. In previous work, *N*-methylcytisine has been found at various levels in blue cohosh-containing dietary supplement products, from a low of 3 ppm to a high of 850 ppm.[3] In addition, this compound is found in other plants used as ingredients in dietary supplements, such as fenugreek (*Trigonella foenum-graecum* L.). The phenanthrene alkaloid taspine (**2**) was found to be extremely toxic to the RECs, but this compound was obtained in low yield, 0.00013% (w/w), from blue cohosh rhizomes. The novel compound thalictroidine (**1**) was not active in this assay at tested concentrations.

Experimental Section

General Experimental Procedures. All NMR spectra were recorded on a Varian VXR-400S spectrometer. IR spectra were recorded on a Bio-Rad model FTS-60A spectrometer, Digilab Division (Cambridge, MA). EIMS was carried out on a Micromass AutospecQ. Analytical TLC was carried out on Merck Si gel 60 F_{254} 250-μm TLC plates and visualized by a UV lamp and by Dragendorff reagent (Sigma Scientific, St. Louis, MO). Flash chromatography was performed using a Biotage flash chromatography system, Flash 40 + SIM, with Si gel cartridges (230−400 mesh). Centrifugally enhanced radial chromatography was carried out on a Harrison Chromatotron with Si gel as the stationary phase. Sparteine was obtained from Sigma Scientific (St. Louis, MO); taspine was a generous gift of Dr. Walter H. Lewis (Washington University, St. Louis, MO); and *N*-methylcytisine was a gift of the Drug Synthesis & Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD).

Plant Material. The dried whole rhizomes of *C. thalictroides* (13.5 kg) were obtained from American Botanicals (Eolia, MO) in February 1997. The identity of the plant material was verified by comparison with authentic rhizome material. In addition, a MeOH extraction of the rhizomes was analyzed by TLC and GC−MS and appeared consistent, both qualitatively and quantitatively, with the previously reported pattern of alkaloids from the plant.[1]

Rat Embryo Culture Assay. Time-pregnant rats were purchased for this study from Zivic-Miller Laboratories (Zelienople, PA). The rats were received on gestation days 1, 2, or 8. The animals were housed in plastic shoe box cages containing hardwood-chip bedding. Food (Purina no. 5002C) and water were available to the animals ad libitum. The female rats were euthanized on gestation day 9 with CO_2 using the Euthanex System.

The in vitro embryo culture system used has been previously described.[22] On gestation day 9.5, rat embryos were dissected from the dams at developmental stages 11a−11c,[23] and cultured as previously described. The medium consisted of 2.0 mL rat serum and 0.5 mL Waymouth's MB 752/1 medium containing penicillin−streptomycin (250 units and 250 μg/mL). Selected blue-cohosh extracts and purified alkaloids were dissolved in a solution of EtOH−DMSO (1:1) and then added to the culture medium (10 μL) at the beginning of culture. After 45 h in culture, embryonic development was assessed as previously described.[24]

Extraction and Isolation. The whole rhizomes (13.5 kg) were powdered using a Retsch Mill, SR3 (Retsch GmbH, 5657 Haan, Germany), and extracted three times with 80% AR-grade MeOH and 20% Milli-Q-grade water. The combined extracts were concentrated under reduced pressure to obtain a dark brown viscous mass. The concentrated extract was diluted with about 4 L of Milli-Q grade water while being mechanically stirred. The pH of this nonhomogenous aqueous solution was adjusted to approximately pH 9 by adding concentrated ammonium hydroxide to the vessel while it was being stirred. This basic extract was partitioned with H_2O-saturated EtOAc three times, and the EtOAc extract was concentrated under reduced pressure to dryness (50.24 g). The aqueous extract was then partitioned three times with H_2O-saturated 1-BuOH. The combined 1-BuOH extracts were concentrated using a rotary evaporator, and final drying took place under a high vacuum pump to yield 726.3 g of 1-BuOH extract.

A portion of the EtOAc extract (10 g) was separated over Si gel by flash chromatography using a gradient of $CHCl_3$−MeOH (1:0 to 1:1). Fractions were pooled based on TLC profile, resulting in a total of 12 fractions, designated A−L, all of which

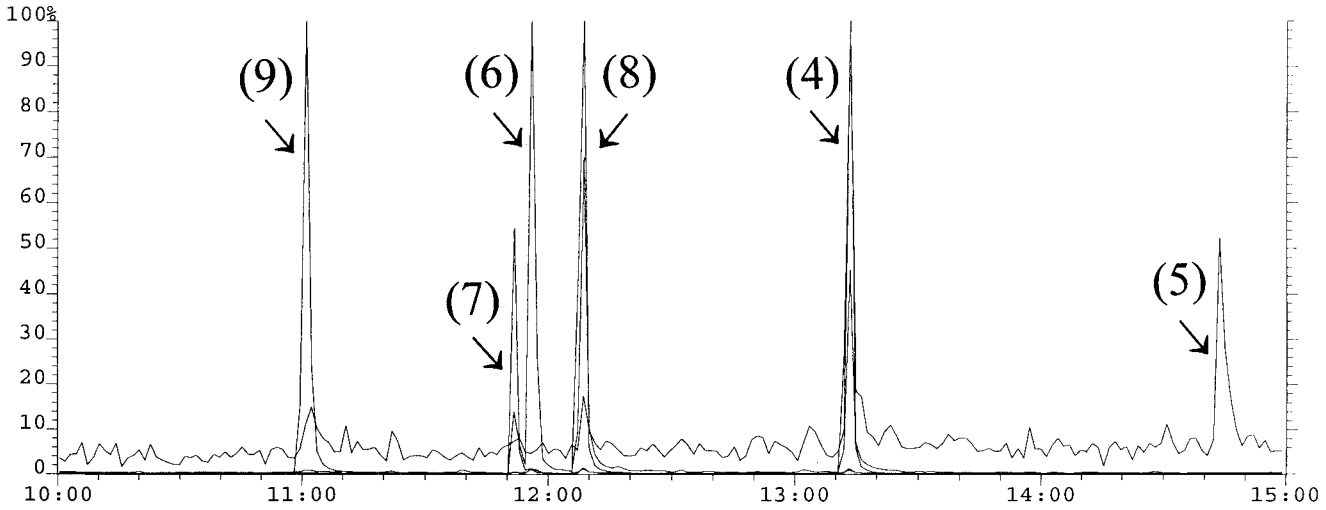

Figure 2. GC−MS analysis of basic materials isolated from an ethyl acetate extract of blue cohosh. Overlying ion profiles on *m/z* 204, 244, 246, 248, and 260 indicate locations of (**9**) *N*-methylcytisine at 11:01 min, (**7**) α-isolupanine (11:52), (**6**) 5,6-dehydro-α-isolupanine (11:56), (**8**) lupanine (12:07), (**4**) anagyrine (13:13), and (**5**) baptifoline (14:44) in the data.

tested positive for alkaloids in the Dragendorff test. Fraction I (49 mg), eluted with $CHCl_3$−MeOH (11:7), was separated over Si gel first by flash chromatography using an isocratic solvent system, $CHCl_3$−MeOH−NH_4OH (9:1:0.1). Further purification was achieved using centrifugally enhanced radial chromatography with a solvent gradient of $CHCl_3$−MeOH−NH_4OH (19:1:0.2 to 9:1:0.1), to yield the purified compound thalictroidine (**1**). Fraction G (57 mg), eluted with $CHCl_3$−MeOH (7:3), was purified over Si gel using centrifugally enhanced radial chromatography with a gradient of $CHCl_3$−MeOH (97:3 to 43:7) to give the purified alkaloid taspine (**2**). Fraction E (537 mg), eluted with $CHCl_3$−MeOH (4: 1), was separated over Si gel using centrifugally enhanced radial chromatography with a solvent gradient of $CHCl_3$−MeOH (97:3 to 4:1) to yield baptifoline (**5**) and α-isolupanine (**7**). Fraction C, eluted with $CHCl_3$−MeOH (9: 1), was separated over Si gel to yield anagyrine (**4**) and *N*-methylcytisine (**9**). The quaternary aporphine, magnoflorine (**3**), was isolated from the 1-BuOH extract by flash chromatography over Si gel using MeOH−H_2O−NH_4OH (9:0.5:0.5).

Alkaloid Analysis by GC−MS. Extracts and purified alkaloids were analyzed by GC−MS with a 30 m × 0.2 mm i.d. fused-silica methyl silicone capillary column (Hewlett−Packard Ultra-1, 0.33 m film). The head pressure of the hydrogen carrier gas was 25 psi for a measured linear velocity of 40 cm/s. The column oven was temperature programmed 1 min after splitless injection from 50 °C to a final of 270 °C at a rate of 20 °C/min with a 30-min hold at 270 °C. The injection port temperature was 220 °C, and the transfer line temperature was 280 °C. The Autospec Q mass spectrometer was operated in EI mode with 70 eV electron energy, 400 mA emission current, 250 °C ion source temperature, as it was repetitively scanned from 522 to 48 daltons at 1 s/decade and 2200 resolution for the GC−MS analyses. HREIMS data were recorded at 10 000 resolution. A representative chromatogram is reported in Figure 2, recorded for an EtOAc extract found to contain *N*-methylcytisine (t_R 11:01 min), α-isolupanine (t_R 11:52 min), 5,6-dehydro-α-lupanine (t_R 11:56 min), lupanine (t_R 12:07 min), anagyrine (t_R 13:13 min), and baptifoline (t_R 14:44 min).

Thalictroidine (1): obtained as a colorless glassy material; IR (film) ν_{max} 2942, 1665, 1601, 1581, 1167, 848 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 2; EIMS (70 eV) *m/z* (rel int) [M^+] 233 (15), 218 (5), 163 (16), 149 (18), 136 (45), 121 (80), 98 (100), 65 (28); HREIMS found *m/z* 233.14156, calcd $C_{14}H_{19}NO_2$, 233.14158; yield 0.00045% (w/w).

Taspine (2): ^1H NMR, ^{13}C NMR, and EIMS data consistent with published data;[15] yield 0.00013% (w/w).

Baptifoline (5): ^1H NMR (δ, $CDCl_3$) 6.40 (1H, dd, *J* = 9, 1 Hz, H-3), 7.26 (1H, dd, *J* = 9, 7 Hz, H-4), 5.97 (1H, dd, *J* = 7, 1.3 Hz, H-5), 2.95 (1H, m, H-7), 1.67 (1H, brd, *J* = 13 Hz, H-8*R*), 1.97 (1H, brd, *J* = 13 Hz, H-8*S*), 2.08 (1H, m, H-9), 3.88 (1H, dd, *J* = 15.4, 6.6 Hz, H-10α), 4.06 (1H, brd, *J* = 15.4, H-10*β*), 3.44 (1H, brdt, *J* = 13, 2, 2, H-11), 2.10 (1H, brdt, *J* = 14, 2.5, H-12α), 1.25 (1H, brd, *J* = 14, H-12*β*), 4.26 (1H, m, 13α), 1.85 (1H, dt, *J* = 13.5, 13.5, 2, H-14α), 1.31 (1H, brd, *J* = 13.5, H-14*β*), 2.37 (1H, brd, *J* = 14, H-15α), 3.19 (1H, dt, *J* = 14, 14, 2.8, H-15*β*), 3.33 (1H, brd, *J* = 10.7, H-17α), 2.45 (1H, brd, *J* = 10.7, H-17*β*); ^{13}C NMR data, (δ, $CDCl_3$) 163.6 (s, C-2), 116.5 (d, C-3), 138.9 (d, C-4), 105.0 (d, C-5), 151.8 (s, C-6), 35.2 (d, C-7), 20.4 (t, C-8), 31.8 (d, C-9), 51.5 (t, C-10), 65.3 (d, C-11), 29.0 (t, C-12), 55.8 (d, C-13), 25.7 (t, C-14), 47.8 (t, C-15), 52.1 (t, C-17); EIMS data, consistent with baptifoline standard.

α-Isolupanine (7): ^1H NMR (δ, $CDCl_3$) 1.17 (1H, m, H-13B), 1.30 (1H, m, H-12A), 1.45 (2H, m, H-14), 1.56 (1H, m, H-9), 1.59 (1H, m, H-4B), 1.60 (1H, m, H-7), 1.62 (1H, m, H-12A), 1.68 (1H, m, H-13A), 1.68 (1H, m, H-15B), 1.76 (2H, m, H-5), 1.78 (2H, m, H-8), 1.80 (1H, m, H-4A), 1.93 (1H, m, H-11), 2.13 (1H, m, H-17B), 2.23 (1H, m, H-3B), 2.36 (1H, m, H-3A), 2.54 (1H, m, H-10B) 2.67 (1H, m, H-15A), 2.97 (1H, m, H-17A), 3.45 (1H, d, H-6), 4.86 (1H, brd, H-10A); ^{13}C NMR data (δ, $CDCl_3$) 169.0 (s, C-2), 32.9 (t, C-3), 19.8 (t, C-4), 27.6 (t, C-5), 58.7 (s, C-6), 34.4 (d, C-7), 35.4 (t, C-8), 34.2 (d, C-9), 42.2 (t, C-10), 65.8 (d, C-11), 30.4 (t, C-12), 24.8 (t, C-13), 25.6 (t, C-14), 57.3 (t, C-15), 56.7 (t, C-17); ^1H NMR and ^3C NMR data, revised from data previously published for α-isolupanine;[25] EIMS, consistent with published data.[26]

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References and Notes

(1) Flom, M. S.; Doskotch, R. W.; Beal, J. L. *J. Pharm. Sci.* **1967**, *56*, 1515−1517.
(2) Power, F. B.; Salway, A. H. *J. Chem. Soc.* **1913**, *103*, 191−209.
(3) Betz, J. M.; Andrzejewski, D.; Casey, R. E.; Woldemariam, T. Z. *Phytochem. Anal.* **1998**, *9*, 232−236.
(4) Woldemariam, T. Z.; Betz, J. M.; Houghton, P. J. J. *Pharm. Biomed. Anal.* **1997**, *15*, 839−843.
(5) Keeler, R. F. In *Handbook of Natural Toxins: Plant and Fungal Toxins*, Keeler, R. F., Tu, A. T., Eds.; Marcel Dekker: New York, 1983; Vol. 1, pp 161−199.
(6) Kilgore, W. W.; Crosby, D. G.; Craigmill, A. L.; Poppen, N. K. *Calif. Agric.* **1981**, *35*, 6−10.
(7) Gladstar, R. *Herbal Healing for Women: Simple Home Remedies for Women of All Ages*; Simon & Schuster: New York, 1993; pp 178−179, 234−235.

(8) Jones, T. K.; Lawson, B. M. *J. Pediatr.* **1998**, *132*, 550−552.
(9) Gunn, T. R.; Wright, I. M. R. *N. Z. Med. J.* **1996**, *109*, 410−411.
(10) Baillie, N.; Rasmussen, P. *N. Z. Med. J.* **1997**, *110*, 20−21.
(11) http://vm.cfsan.fda.gov/~dms/aems.html (accessed July 15, 1998).
(12) Hardin, J. W.; Arena, J. M. *Human Poisoning from Native and Cultured Plants*, 2nd ed.; Duke University Press: Durham, 1974; pp 60−61.
(13) Lempe, K. F. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 4th ed.; Amdur, M. O., Doull, J., Klaassen, C. D., Eds.; McGraw-Hill: New York, 1991; p 808.
(14) Al-Shamma, A.; Drake, S. D.; Guagliardi, L. E.; Mitscher, L. A.; Swayze, J. K. *Phytochemistry* **1982**, *21*, 485−487.
(15) Pieters, L.; De Bruyne, T.; Claeys, M.; Vlietinck, A.; Calomme, M.; Vanden Berghe, D. *J. Nat. Prod.* **1993**, *56*, 899−906.
(16) Achenbach, H.; Frey, D.; Waibel, R. *J. Nat. Prod.* **1991**, *54*, 1331−1336.
(17) Safronich, L. N. *Tr. Vsees. Nauch.-Issledovatel. Inst. Lekarstv. I Aromat. Rast.* **1959**, *11*, 30−37; *Chem. Abstr.* **1961**, *55*, 18892.
(18) Persinos Perdue, G.; Blomster, R. N.; Blake, D. A.; Farnsworth, N. R. *J. Pharm. Sci.* **1979**, *68*, 124−126.
(19) Kinghorn, A. D.; Balandrin, M. F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; John Wiley & Sons: New York, 1984; Vol. 2, pp 105−148.
(20) Scott, C. C.; Chen, K. K. *J. Pharm. Exp. Therap.* **1943**, *79*, 334−339.
(21) Schmeller, T.; Sauerwein, M.; Sporer, F.; Wink, M.; Müller, W. E. *J. Nat. Prod.* **1994**, *57*, 1316−1319.
(22) New, D. A. T. *Biol. Rev.* **1978**, *53*, 81−122.
(23) Fujinaga, M.; Brown, N. A.; Baden, J. M. *Teratology* **1992**, *46*, 183−190.
(24) Cockroft, D. L. In *Postimplantation Mammalian Embryos: A Practical Approach*; Copp, A. J., Cockroft, D. L., Eds.; Oxford University Press: New York, 1990; pp 15−40.
(25) Bohlmann, F.; Zeisberg, R. *Chem. Ber.* **1975**, *108*, 1043−1051.
(26) Wink, M. In *Methods in Plant Biochemistry: Alkaloids and Sulfur Compounds*; Waterman, P. G., Dey, P. M., Eds. (Harborne, J. B., Series Ed.); Academic Press: New York, 1993; Vol. 8, pp 197−239.
(27) Brown, N. A.; Fabro, S. *Teratology* **1981**, *24*, 65−78.

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