ALKALOIDS OF *MAHONIA REPENS* WITH A BRIEF REVIEW OF PREVIOUS WORK IN THE GENUS *MAHONIA*

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ABSTRACT.—The alkaloid content of *Mahonia repens* (Lindl.) G. Don was examined. The leaves were found to contain the tertiary aporphines isocorydine (**1**), corydine (**2**), thaliporphine (**3**), and glaucine (**4**). The alkaloid content of the stems and of the roots were similar. The bisbenzylisoquinolines oxyacanthine (**9**), obamegine (**12**), thalrugosine (**11**), and obaberine (**10**) were present as were the protoberberines, berberine (**5**), jatrorrhizine (**6**), columbamine (**7**), and palmatine (**8**). The quaternary aporphine magnoflorine (**13**) was also in the stems and roots. One nonalkaloidal component, syringaresinol (**16**), was obtained from the roots. Inconsistent literature ^{1}H nmr spectral data for obamegine were corrected.

The family Berberidaceae contains nine genera and approximately 590 species native to the northern hemisphere and South America. The two largest genera in the Berberidaceae are *Berberis*, containing about 450 species, and *Mahonia*, containing about 70 species. A monumental (410 page) taxonomic revision of *Berberis* and *Mahonia* is available (1) which delineates the individual species and discusses differences between the two genera.

Both genera are widely used in folk medicine and contain many alkaloids with physiological activity. The genus *Berberis* has been the most extensively studied of the two, although selected *Mahonia* species (particularly from Asia) have been investigated. We have compiled the literature reports on alkaloids from *Mahonia* in table 1. In no case have we attempted to assess the correctness of the genus and species assignments. Table 1 shows the common occurrence of three protoberberines (berberine, jatrorrhizine, and palmatine), three bisbenzylisoquinolines (berbamine, oxyacanthine, and isotetrandrine), and the quaternary aporphine magnoflorine. These alkaloids are also common in *Berberis* species.

A *Mahonia* species of the Rocky Mountains, *Mahonia repens* (Lindl.) G. Don, was used by the Ramah Navajo Indians (2) as an emetic, as a treatment for diarrhea, and to counteract scorpion bites. The plant has been touted in the press (3a) and elsewhere (3b) as a "nerve tonic". The genus *Mahonia* is divided into two groups: Orientales and Occidentales. Of the 18 species previously studied, 15 belong to the Orientales group. Each group is divided into sections and subsections. *M. repens* and *M. aquifolia* belong to section Aquifoliatae, subsection *Euaquifoliatae*, and no other previously investigated species belong to this section or subsection. As part of a search for antitumor substances or other physiologically active compounds, we report here the results of our analysis of *Mahonia repens*.

RESULTS AND DISCUSSION

LEAVES.—The leaves were found to contain the aporphines isocorydine (**1**), corydine (**2**), thaliporphine (**3**), and glaucine (**4**). In the course of this work, we discovered that the reverse side of silica gel tlc plates, 24 hrs after having been sprayed with iodoplatinic acid reagent, showed spots with colors characteristic of each alkaloid. Thus, isocorydine gave a bright green spot, corydine a gray-green one, thaliporphine an orange spot, and glaucine a yellow color. The first two alkaloids are 10,11-substituted and the latter two are 9,10-substituted, but not enough data is available to determine whether or not these colors could be used as a distinguishing feature. Some of the column chromatographic separations were much more easily followed by observation of the reverse side of the plates used for tlc analysis rather than the sprayed side.

Isocorydine and corydine are common alkaloids previously found in *Mahonia*.

TABLE 1. Alkaloid content of *Mahonia* species.

	1	2	3	4	5	6	7	8	9	10
M. acanthifolia G. Don[a,b]	x	x	x						x	
M. aquifolium (Pursh.) Nutt.[c]	x	x		x	x	x		x	x	
M. borealis Takeda[d,e]	x	x	x						x	
M. fortunei (Lindl.) Fedde[f]	x	x	x				x	x	x	
M. griffithii Takeda[g]	x	x	x					x	x	
M. japonica (Thundb.) DC.[h]	x	x	x					x		x
M. leschenaultii (Wall) Takeda[i]	x	x					x		x	
M. lomariifolia Takeda[j]	x	x	x				x	x		x
M. manipurensis Takeda[d,i]	x	x	x						x	
M. morrisonensis Takeda[j]	x	x	x				x	x		x
M. napaulensis DC.[a]	x	x								
M. nepalensis Fedde[k]	x	x								
M. philippinensis Takeda[l]	x	x	x					x		x
M. sikkimensis Takeda[i]	x	x							x	
M. simonsii Takeda[d,e]	x	x	x						x	
M. swaseyi (Buckley) Fedde[m]	x							x		
M. thunbergii DC.[n]		x					x	x		
M. trifoliolata (Moric.) Fedde[m]	x									

1) berberine; 2) jatrorrhizine; 3) palmatine; 4) isocorydine; 5) corydine; 6) isoboldine; 7) magnoflorine; 8) berbamine; 9) oxyacanthine; 10) isotetrandine.

[a]R. Chatterjee and M. P. Guha, *Science and Culture*, **15**, 163 (1949); *Chem. Abstr.*, **44**, 2706e (1950).

[b]R. Chatterjee and M. P. Guha, *J. Am. Pharm. Assoc.*, **39**, 577 (1950); *Chem. Abstr.*, **44**, 10821g (1950).

[c](1) L. P. Naidovich, D. A. Fesenko and B. K. Rostotskii, *Khim. Prir. Soedin.*, **6** (6), 775 (1970). (2) P. P. Panov, N. M. Mollov and L. N. Panova, *Dokl. Bolg. Akad. Nauk*, **24** (5), 675 (1971); *Chem. Abstr.*, **75**, 148465z (1971). (3) H. Ripperger, *Pharmazie*, **34**, 435 (1979).

[d]R. Chatterjee, M. P. Guha and S. K. Sen, *J. Am. Pharm. Assoc.*, **40**, 36 (1950); *Chem. Abstr.*, **45**, 3565 (1951).

[e]R. Chatterjee, M. P. Guha and S. K. Sen, *Science and Culture*, **16**, 321 (1951); *Chem. Abstr.*, **47**, 5636h (1953).

[f](1) M. Tomita and T. Abe, *J. Pharm. Soc. Japan*, **72**, 773 (1952); *Chem. Abstr.*, **47**, 33234 (1953). (2) M. Tomita and H. Ishu, *Yakugaku Zasshi*, **77**, 213 (1957); *Chem. Abstr.*, **51**, 8366i (1957).

[g]R. Chatterjee and M. P. Guha, *J. Am. Pharm. Assoc.*, **39**, 181 (1950); *Chem. Abstr.*, **44**, *4636*f (1950).

[h](1) M. Tomita and T. Abe, *J. Pharm. Soc. Japan*, **72**, 735 (1952); *Chem. Abstr.*, **47**, 3323c (1953). (2) M. Tomita, Y. Inubushi and N. Mizoguchi, *J. Pharm. Soc. Japan*, **73**, 776 (1953); *Chem. Abstr.*, **47**, 10805f (1953). (3) M. Tomita, Y. Inubushi and N. Mizoguchi, *Pharm. Bull. (Japan)*, **1**, 53 (1953); *Chem. Abstr.*, **49**, 13600b (1955). (4) M. Tomita and M. Sugamoto, *Yakugaku Zasshi*, **81**, 1090 (1961); *Chem. Abstr.*, **56**, 3564f (1962).

[i]R. Chatterjee and M. P. Guha, *J. Am. Pharm. Assoc.*, **40**, 229 (1951); *Chem. Abstr.*, **45**, 9068d (1951).

[j](1) T. H. Yang, *Yakugaku Zasshi*, **80**, 1304 (1960); *Chem. Abstr.*, **55**, 3005b (1961). (2) H. Kondo and M. Tomita, *J. Pharm. Soc. Japan*, **50**, 309 (1930); *Chem. Abstr.*, **24**, 3512 (1930).

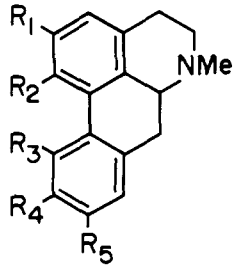
[k](1) R. Chatterjee, *J. Am. Pharm. Assoc.*, **33**, 205 (1944); *Chem. Abstr.*, **38**, 5259 (1944). (2) R. Chatterjee, *J. Am. Pharm. Assoc.*, **33**, 210 (1944); *Chem. Abstr.*, **38**, 5259 (1944). (3) T. R. Govindachari, B. R. Pai, S. Rajadurai and U. R. Rao, *Proc. Indian Acad. Sci.*, **47A**, 41 (1958); *Chem. Abstr.*, **52**, 14630e (1958).

[l](1) E. R. Castro, A. C. Santos and P. Valenzuela, *Univ. Philippines Nat. and Applied Sci. Bull.*, **2**, 401 (1932); *Chem. Abstr.*, **27**, 2251 (1933). (2) S. Villareal-Sulit and G. Aguilar-Santos, *Philippine J. Sci.*, **92**, 35 (1963); *Chem. Abstr.*, **60**, 3265d (1964).

[m](1) G. A. Greathouse and G. M. Watkins, *Am. J. Botany*, **25**, 743 (1938); *Chem. Abstr.*, **33**, 2939 (1939). (2) G. A. Greathouse and N. E. Rigler, *Plant Physiol.*, **15**, 563 (1940).

Glaucine, is, likewise, of relatively common occurence but has not been found in other *Mahonia* as yet. Thaliporphine is a relatively uncommon aporphine alkaloid, whose literature mp and optical rotation values showed lack of purity. We found mp 185–186° and $[\alpha] = +55$ (EtOH) for our isolated material.

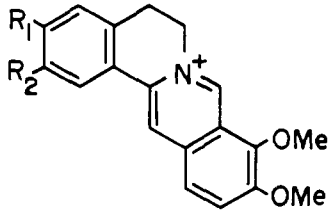
STEMS AND ROOTS.—*Mahonia repens* spreads by means of underground stems, which then develop true roots. Distinguishing between the roots and underground stems is difficult. Separate analyses were done on the above-ground stems and on root samples, which probably also contained some underground stem material. We detected little or no difference in the alkaloid content of the above-ground



1 (isocorydine): $R_1=R_2=R_4=OMe$; $R_3=OH$; $R_5=H$.
2 (corydine): $R_1=R_3=R_4=OMe$; $R_2=OH$; $R_5=H$.
3 (thaliporphine): $R_1=R_4=R_5=OMe$; $R_2=OH$; $R_3=H$.
4 (glaucine): $R_1=R_2=R_4=R_5=OMe$; $R_3=H$.

stem sample and the root (plus underground stem) sample. In terms of actual isolation, some of the alkaloids described here were obtained from the stem sample and others from the "root" sample, but all alkaloids were present in both sources.

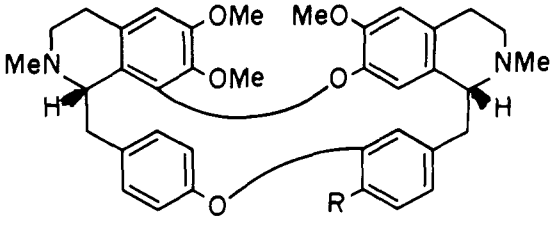
Previous *Mahonia* analyses (table 1) and the brilliant yellow color of *Mahonia* roots and stem bark indicated that protoberberines should be expected. Two different isolation schemes were used so that a fraction rich in these could be obtained as well as a fraction in which they were absent. Protoberberines give positive tests in the National Cancer Institute *in vitro* KB cell cytotoxicity screen, but none of those to be expected in *Mahonia* have significant activity in *in vivo* antitumor screens.



5 (berberine): $R_1=R_2=OCH_2O$.
6 (jatrorrhizine): $R_1=OH$; $R_2=OMe$.
7 (columbamine): $R_1=OMe$; $R_2=OH$.
8 (palmatine): $R_1=R_2=OMe$.

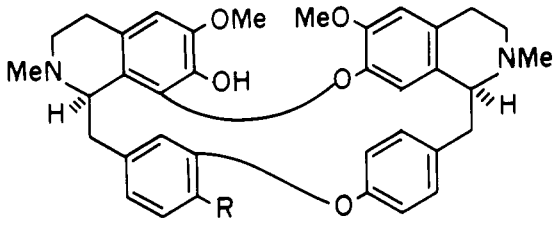
From one isolation scheme, we isolated and identified berberine (**5**), jatrorrhizine (**6**), and columbamine (**7**) and obtained ms and tlc evidence for palmatine (**8**). Thus, the protoberberine content of *Mahonia repens* is unexceptional (table 1).

A second isolation scheme provided a colorless crude alkaloid fraction which was free from protoberberine alkaloids and whose nmr indicated it to be a mixture of bisbenzylisoquinoline alkaloids. This mixture (NSC B838442) was cytotoxic



9 (oxyacanthine): R = OH.
10 (obaberine): R = OMe.

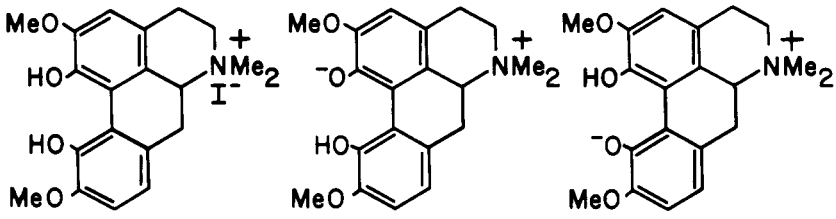
in the NIH KB screen (ED_{50} 13 μg/ml) but inactive in the P388 *in vivo* screen. From the mixture, we were able to isolate and identify the bisbenzylisoquinoline alkaloids oxyacanthine (**9**), obaberine (**10**), thalrugosine (**11**), and obamegine (**12**). Sufficient amounts were not isolated so that individual components could be tested, but we were informed (4) that our major component, oxyacanthine, had previously been tested and found to have a KB cell cytotoxicity level similar to that of our crude extract.



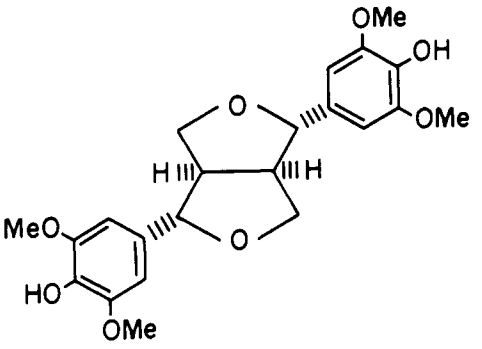
11 (thalrugosine): R=OMe.
12 (obamegine): R=OH.

Oxyacanthine is a common constituent of *Mahonia* (table 1), but the other bisbenzylisoquinolines are new to the genus. We had some difficulty establishing the identity of obamegine. The mass spectral fragmentation and extensive nmr analysis at 360 MHz (5) indicated that the unknown should be either obamegine, its enantiomer (*N*-methyl-7-*O*-demethylpeinamine), atherospermoline or its enantiomer (krukovine). Our nmr values were not consistent with those of atherospermoline or krukovine, but were nearly identical to those of *N*-methyl-7-*O*-demethylpeinamine (6). Since obamegine is the enantiomer of *N*-methyl-7-*O*-demethylpeinamine, both should have identical nmr spectra. The nmr spectrum reported (7) for obamegine listed OMe resonances at 3.60 and 3.79 ppm, while we found 3.79 and 3.94 ppm. The corresponding peinamine derivative (6) gave 3.75 and 3.85 ppm values. A sample of obamegine isolated from *Berberis zabeliana* was obtained (8); it had OMe resonances at 3.79 and 3.93, in good agreement with our material. Other peaks checked as well, so there seems little doubt that our isolated material is obamegine. During our literature work in this area, we also discovered that a recent review of bisbenzylisoquinoline alkaloids (9) quoted an early report (10) that the N-Me resonances of atherospermoline were coincidental, but not a later one (11) which showed that they were indeed different: 2.31 and 2.62 ppm.

One fraction which had yielded protoberberine alkaloids also showed the presence of an additional substance which was purified by plc. The material isolated had λmax 323, 280, 230, and showed no change upon addition of base. Addition of acid changed the uv to λmax 303, 269, and 225. The unknown showed tlc behavior in an ammonia-containing solvent identical to that of a standard sample of magnoflorine iodide (**13**). The uv data was puzzling in that the spectrum of the isolate was identical to that of the standard sample of magnoflorine *after the addition of base*, while the spectrum of the unknown in acid was



13 **14** **15**



16 syringaresinol.

identical with that of standard magnoflorine. The ^1H and ^{13}C nmr spectra (D_2O) of the isolate showed differences from standard magnoflorine (see Experimental). When standard magnoflorine was dissolved in D_2O and several drops of conc NaOD was added, the ^1H nmr spectrum obtained was identical with that of our isolate. These data indicate that magnoflorine had been isolated as the zwitterion which could be represented as **14** or **15**. A key difference in the ^1H nmr of the isolate was the presence of an aromatic AA'BB' system (6.40 doublet $J=7.5$ Hz and 6.72 ppm doublet $J=7.5$ Hz). This change in the "normal" spectrum of magnoflorine indicated that the zwitterion structure is probably that of **15**. Verpoorte and co-workers have also commented (12) on the magnoflorine zwitterion.

Finally, from one fraction of extracted root material we were also able to isolate a small amount of the lignan syringaresinol (**16**).

Table 2 summarizes the data from *Mahonia repens*. Yields are based on isolated material, along with estimates from tlc comparisons of the crude fractions and partially purified fractions. They should be considered as an approximation.

TABLE 2. Components isolated from *Mahonia repens*.

	Percent from leaves	Percent from stems and roots
isocorydine	0.15	—
corydine	.08	—
thaliporphine	.03	—
glaucine	.007	—
oxyacanthine	—	0.14
obamegine	—	.04
thalrugosine	—	.03
obaberine	—	.007
berberine	—	.18
jatrorrhizine	—	.07
magnoflorine	—	.04
columbamine	—	.02
palmatine	—	trace
syringaresinol	—	.008

As was the case with *M. aquifolium* (table 1), we have found essentially only tertiary aporphines in the leaves, with the two major alkaloids being the same. How far this corresponds or differs from other *Mahonia* species cannot be determined since rarely are the leaves studied. Thus the lack of tertiary alkaloids appearing in table 1 should not be construed as indicating their absence, but merely the fact that leaf alkaloids were probably not investigated. Roots and stems contained none of these tertiary aporphines nor their quaternary derivatives.

The bisbenzylisoquinoline alkaloid content was somewhat surprising in that

the very commonly occurring berbamine was not found. Obamegine, thalrugosine, and obaberine are all firsts for the genus *Mahonia*.

It is clear from our work and the results listed in table 1 that neither the protoberberines nor magnoflorine will be useful for phylogenetic studies in the genus. Occurrence of individual bisbenzylisoquinoline alkaloids is somewhat more scattered, but their absence in several species (or the occurrence of only oxyacanthine) may be a function of incomplete investigation rather than true absence. More complete investigation of some of the species of table 1 or thorough study of other species is necessary.

Gottlieb (13) and Torres and Marini-Bettolo (14), who recently found syringaresinol and berbamine together in *Berberis chilensis*, have commented on the possible phylogenetic meaning of co-occurrence of benzylisoquinoline alkaloids and phenylpropanoids. Phenylpropanoids have not commonly been reported from *Mahonia* species, but it is difficult to assess whether or not this was because they are not there or simply because no effort was made to find them.

A recent paper has confirmed (**15**) the strong antidiarrhaeal effect of berberine and its use in gastroenteritis.

EXPERIMENTAL SECTION

Complete spectral data and details of procedures not described below are available in a thesis (5). *Mahonia repens* (Lindl.) G. Don was collected west of Fort Collins, Colorado, in August 1978. A voucher specimen with complete collection data was deposited in the Colorado State University Herbarium.

LEAVES.—Dried, finely ground leaves (925 g) were extracted (Soxhlet, 24 hrs) with 2.5 liters of hexane, followed by ethanol. The hexane residue (36 g) yielded only a trace of crude bases and was not investigated further. The ethanol residue (329 g of viscous green oil) yielded 8.23 g of crude bases after being dissolved in 1M HCl, adjustment of the pH to 9 with NaOH, and extraction with chloroform. Of this, 6 g was adsorbed onto 12 g of basic Al_2O_3 (Baker) and chromatographed on a 5 x 13 cm Al_2O_3 column. Elution was with toluene/methanol (15:1; 600 ml; 5 fractions taken) followed by 150 ml of methanol (1 fraction). Fractions 1 and 2 yielded 1.0 g of mainly one alkaloid which, upon crystallization from ethanol gave (+)-*isocorydine*, mp 183–185°, identified by ^{1}H nmr, uv, and uv base shift, ms, and optical rotation.

The mother liquor from the recrystallization was combined with fractions 3–5, evaporated and rechromatographed [Si gel (mplc); chloroform-methanol (99:1), 15 ml fractions]. The separation was analyzed by tlc with an iodoplatinic acid spray and viewing the reverse of the tlc plate after 24 hours. Fractions 66–97 showed a bright green alkaloid spot [R_f 0.38, chloroform-methanol (20:1)] which was additional isocorydine. Fractions 111–160 yielded 0.5 g of an alkaloid [gray-green spot R_f 0.35, chloroform-methanol (20:1)] which was identified as (+)-corydine (^{1}H nmr, optical rotation uv, and uv base shift). Fractions 170–230 yielded 20 mg of an alkaloid (orange spot, R_f 0.27, 20:1 chloroform-methanol), mp 185–186° d., $[\alpha]^{25}D$ +55° (c 0.0046, EtOH). The ^{1}H nmr and uv were identical with those reported (16) for *thaliborphine*, mp 170–172°. Fractions 98–108 yielded 50 mg of an alkaloid [yellow spot, R_f 0.28, chloroform-methanol (20:1)] which was identified as (+)-glaucine by optical rotation, ^{1}H nmr and uv.

STEMS.—Dried, ground stems (1013 g) were extracted (Soxhlet, 24 hrs) with 2.5 liters of hexane, followed by ethanol. The hexane residue showed no alkaloids and was not investigated further. Evaporation of the ethanol yielded 137 g of viscous residue. Of this, 45 g was dissolved in 2% citric acid, extracted with chloroform, made basic to pH 9 (NH_4OH), and extracted with diethyl ether and then with chloroform. The chloroform solution yielded 0.72 g of residue. The ether solution was extracted with 5% NaOH and then evaporated to yield 0.16 g of "nonphenolic" alkaloid residue. The aqueous solution was brought to pH 9 and extracted with ether. The ether was evaporated to yield 0.54 g of crude "phenolic" alkaloids. Each of the residues was investigated as follows.

The 0.16 g ether "nonphenolic" residue was chromatographed on neutral Al_2O_3 with chloroform followed by increment additions of methanol. Fractions 9–11 (chloroform eluant) yielded 25 mg of an alkaloid shown to be *obaberine* (optical rotation, ^{1}H nmr, uv, mass spectrum) (17). Further fractions showed no separation so they were combined and evaporated, and the residue was purified by plc [Si gel; ethyl acetate-methanol-ammonium hydroxide solution (17:2:1)]. Removal of a band at R_f 0.3 yielded 15 mg of *thalrugosine*, which was identified by ^{1}H nmr, ms, and optical rotation (18). The 0.54 g of phenolic alkaloid residue was purified by plc [Si gel; ethyl acetate-2-propanol-ammonium hydroxide solution (12:7:1)]. Further purification of one eluted band by hplc [two μ-Porasil columns in series, ethyl acetate 2-propanol-ammonium hydroxide solution (9:10:1)] yielded oxyacanthine which was identified by ^{1}H nmr, ms, uv, and optical rotation. A second eluted band from the plc was repurified by mplc (ethyl acetate-2-propanol-ammonium hydroxide solution (12:7:1), 5 ml fractions]. Fractions 30–40 yielded 35 mg of a single alkaloid: ms, M^- 594 (21), 579 (2), 471 (1), 403 (6), 381 (57), 367 (17), 192 (100), 191 (57), 174 (31), 168 (19); nmr ($CDCl_3$, 360 MHz) 2.33 and 2.50 (NMe), 3.79 and 3.94 (OMe), 6.07 (1H, s), 6.24 (1H, d, J=2.2), 6.37 (1H, s), 6.44 (1H, dd, J=2.9 and 8.6), 6.62 (1H, dd, J=2.2 and 8.6), 6.75 (1H, d, J=8.6), 6.77 (1H, s), 6.84 (1H, dd, J=2.9 and 8.6), 7.11 (1H, dd,

J=2.9 and 8.6), 7.33 (1H, dd, J=2.9 and 8.6). The optical rotation was $[\alpha]^{19}D = +225°$ (c 0.013, EtOH), Lit.: +99° (Ref. 7, $CHCl_3$), +273° (Ref. 19). These data are discussed in the Results section. Along with a standard sample ^1H nmr (8), they established the unknown as *obamegine*.

ROOTS.—Dried and ground roots (1411 g) were extracted (Soxhlet) 24 hrs each with hexane, ethanol, and methanol. The residue from the ethanol extraction (174 g) was dissolved in 2% citric acid and extracted with ethyl acetate. The aqueous was brought to pH 9 with NH_4OH and extracted with diethyl ether; 12.8 g of crude alkaloids were obtained. The aqueous layer was extracted with chloroform (2.04 g of residue resulting) and then acidified to pH 5 with HCl. When treated three times with 5% Reinecke salt, the solution gave a total (after drying) of 37.5 g of Reineckate ppt. The ppt was dissolved in 1.8 liters of 50% aq acetone, and 365 g of Dowex 1-X8 (Cl^- form) added. The mixture was stirred for three days and the resin removed by filtration. Evaporation of the solution left 15 g of dark yellow solid. The solid was adsorbed onto 30 g of activity I acidic alumina, and this was placed on a column of alumina (900 g). Elution was with two liters each of 2, 3.5, 6, 10, 18, 35, and 60% methanol in chloroform; 750 ml fractions were collected. Fractions 2 and 3 yielded 0.5 g of material which when rechromatographed by flash column (20) (Si gel, 2% methanol in chloroform), yielded 0.12 g of *syringaresinol*, identical by ^1H nmr and tlc in three solvents with a standard sample (21). Combining fractions 6–9 yielded 2.0 g of *berberine*, identical to ir, uv, and tlc with a previously isolated sample (22). Fractions 15–20 contained two alkaloids [R_f 0.30 and 0.53 in methanol-water-ammonium hydroxide (15:3:1)]. The residue was rechromatographed (flash column). Early fractions yielded *jatrorrhizine*, which was crystallized from methanol to which a few drops of conc HCl had been added. The structure was proven by mp (204–207°), uv, ^1H nmr and ir (23, 24). Later fractions of the flash column gave an alkaloid which was purified by plc (Si gel, [methanol-water-ammonium hydroxide solution (15:3:1) as solvent]; *magnoflorine* was obtained as a zwitterion. Uv λmax 230, 280, 323; OH^- no change; H_3O^+ λmax 225, 269, 303; ^1H nmr (D_2O) 2.58 and 3.06 (N-Me), 3.73 and 3.83 (OMe), aromatic protons at 6.50 (1H, s), 6.40 (1H, d, J=7.5 Hz), 6.72 (1H, d, J=7.5 Hz); ^{13}C nmr 24.61t, 31.59t, 43.53q, 53.79q, 55.95q, 56.29q, 62.08t, 70.76d, 109.19d, 110.33d, 115.56s, 116.78d, 120.86s, 123.32s, 125.85s, 149.63s, 150.58s, 151.43s, 152.72s; ms 342(1), 327(6), 312(3), 298(6), 284(18), 271(64), 255(30), 58(100). The R_f of the compound on tlc was identical with that of magnoflorine (25) and the identity was proven by dissolving a standard sample of magnoflorine in D_2O, and adding several drops of NaOD, and then comparing the ^1H nmr spectra.

Fraction 12 contained an alkaloid which was purified by plc [methanol-water-ammonium hydroxide solution (15:3:1)]; 0.04 g of *columbamine* was obtained (26). Uv λmax 227, 265, 272, 350, 433; OH^- 225, 242, 274, 330, 368; ^1H nmr ($CDCl_3$, d_6-DMSO) 3.90, 4.10 and 4.16 (OMe); 6.90, 7.83, 8.03, 8.66, and 9.86 (aromatic protons); the uv and tlc in three solvent systems were identical with those of a standard sample. Since berberine, columbamine, and jatrorrhizine often occur along with palmatine, we prepared a sample of palmatine by methylation of our isolated jatrorrhizine. Tlc indicated that the isolated 2.0 g of berberine contained a trace impurity of R_f value identical with that of the synthesized palmatine. Extensive plc purification (cyclohexane:diethylamine solvent) of the isolated berberine yielded authentic *palmatine*, (ci-ms and tlc).

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