CONSTITUENTS OF *MAGNOLIA GRANDIFLORA*. III. TOXIC PRINCIPLE OF THE WOOD[1]

KOPPAKA V. RAO* and TERRY L. DAVIS

Department of Medicinal Chemistry, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610

ABSTRACT.—The alcoholic extract of the wood of *Magnolia grandiflora* exhibited toxicity to mice when injected by the ip route. The toxic principle was found to be a phenolic quaternary alkaloid and was obtained as a colorless crystalline solid. Analytical and spectral data showed that it was identical with menisperine (also called chakranine and isocorydinium cation) which has not been previously isolated from the genus *Magnolia*.

Much of the impetus for research on *Magnolia grandiflora* has been its long history of use in folk medicine. The bark, wood and other parts of the plant have been featured in American Indian medicine and listed in the United States Pharmacopoeia and pharmacognosy texts as bitter tonics, antimalarials and diaphoretics (2, 3, 4). A number of biologically active alkaloids such as magnoflorine, magnocurarine and salicifoline (5, 6) and sesquiterpene lactones such as parthenolide and costunolide (7, 8) have been isolated from this or related species of *Magnolia*, although the activities of many of these were recognized only after their isolation. The toxicity of the extracts of *Magnolia grandiflora* has not been recognized in the literature. In our studies on the biologically active components of *Magnolia grandiflora*, we have observed that the alcoholic extract of the heart wood was toxic to mice when given by the ip route at 250–400 mg/kg. Isolation of the toxic principle and elucidation of its structure form the subject of this paper.

Fractionation of the concentrate by partition between water (pH 2) and ethyl acetate showed that only the aqueous phase was toxic. Since the aqueous layer gave a positive test for alkaloids, the alkaloidal fraction was separated from the nonalkaloidal components by the use of Mayer's reagent and both fractions tested. Only the alkaloidal fraction was found to be toxic. Since extraction at pH 9 did not transfer the activity into the solvent layer, the toxic alkaloid was considered to be quaternary.

For the isolation of the toxic principle, the alkaloidal fraction of the concentrate was separated by precipitation with Mayer's reagent, converted to the chloride (hydrochloride) by ion-exchange and extracted at pH 9 with chloroform. The solvent layer contained an alkaloidal component which was not toxic and it was purified as the crystalline hydrochloride (Alkaloid A). Extraction of the aqueous layer with *n*-butanol gave nearly 50–60% of the toxic alkaloid (Alkaloid B) into the solvent layer.

Purification of alkaloid B by adsorption chromatography on silica gel or alumina was not possible because of its highly polar nature. The alkaloid from either the *n*-butanol layer or the aqueous layer could be purified by chromatography on Sephadex LH–20 by the system ethyl acetate/ethanol (4:1), or by chromatography on a partition column with cellulose as the support and the system, ethyl acetate-*n*-butanol (3:1)-water. Three components were resolved: fraction 1, which was identical with alkaloid A, fraction 2, the major component (toxic alkaloid, alkaloid B) and fraction 3, a minor component, which was identified as magnoflorine. The toxic alkaloid was converted to the iodide salt which was obtained as a colorless crystalline solid.

Elemental analysis of alkaloid B agreed with the molecular formula $C_{21}H_{26}NO_4I$. Its uv spectrum indicated its phenolic nature and general similarity to that of

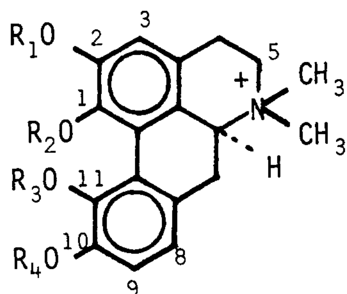
[1]For paper II in this series. See ref. 1.

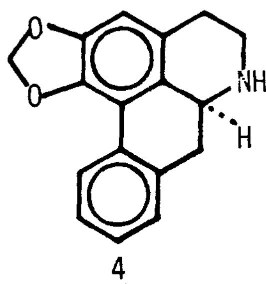
aporphine alkaloids. Mass spectrum of the iodide gave a molecular ion at *m/e* 341 (nor base, $M^+ - CH_3I$) together with the ion *m/e* 142 (CH_3I) with fragment ions being shown at *m/e* 326, 310 and 298 corresponding to the loss of CH_3, OCH_3 and $CH_2{=}NCH_3$ respectively. Methylation gave the monomethyl ether while acetylation with acetic anhydride/pyridine did not proceed. Acid-catalyzed acetylation did give the monoacetate.

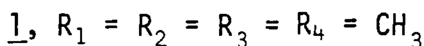
Although the molecular formula cannot be used to distinguish between an aporphine skeleton with methoxyl and hydroxyl groups or a benzyltetrahydroisoquinoline system with a methylene-dioxy group, the spectral data: uv maxima (9) and absence of a prominent 3,4-dihydroisoquinolinium ion in the mass spectrum (10) pointed to an aporphine structure. In support of this, the nmr spectrum clearly indicated the absence of a methylenedioxy group but presence of three methoxyls and one phenolic hydroxyl and, hence, an aporphine skeleton.

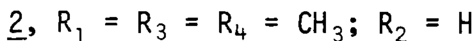
Of the two biogenetically consistent tetraoxygenated aporphine systems, the 1, 2, 10, 11 and 1, 2, 9, 10, the choice of the former for the toxic alkaloid was made on the basis of the uv spectrum (9), and the mass spectrum (11). As a final proof, the monomethyl ether of the alkaloid was found to be identical with the dimethyl ether of magnoflorine (**1**).

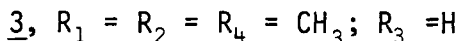
Although four possibilities exist for the location of the phenolic hydroxyl, representatives of only two of these have been isolated, one with the hydroxyl at C–1 (*N*-methylcorydinium cation **2**) and the other at C–11 (*N*-methylisocorydinium cation **3**). Samples of neither could be obtained and comparison of published spectral data was the only means available. The difficulty with which it underwent acetylation suggested possible steric hindrance and, hence, location of OH at C–10 or C–11. This was further supported by the hydroxyl frequency of 3200 cm^{-1} which was consistent with that of aporphines and the phenanthrenes derived from them with a phenolic hydroxyl at C–1 or C–11 (11). Likewise, the chemical shifts of the methoxyls indicated that the methoxyl groups are at C–2 and C–10, in conformity with the published data (13, 14).






1, $R_1 = R_2 = R_3 = R_4 = CH_3$


2, $R_1 = R_3 = R_4 = CH_3$; $R_2 = H$


3, $R_1 = R_2 = R_4 = CH_3$; $R_3 = H$

The nmr spectrum of alkaloid B was not sufficiently similar to that of either *N*-methylcorydinium or *N*-methylisocorydinium iodides as published in the literature to allow for a choice. The critical factor is the observation of an AB-quartet for the assignment of the other resonances. Not only has a range of values been given for these resonances, but also they vary with the solvents used (15, 16). Although protons at C–8 and C–9 are expected to show an AB quartet,

the published spectra showed only singlets: *N*-methylcorydinium (17), δ 7.33 and 7.27 for H-8 and H-9 and δ 7.05 for H-3; *N*-methylisocorydinium (18), δ 6.96 for both H-8 and H-9 and δ 7.05 for H-3. As the chemical shift differences become small, the outer peaks of the AB-quartet are often small and overlooked. When only singlets appear, the assignments are questionable. Based on the AB-quartets observed with both the *Magnolia* alkaloid and its methyl ether, the following assignments may be made for alkaloid B: H-3, δ 6.96; H-8 (or H-9) δ 7.11d or 6.98d.

The choice between the two was made on the basis of the ability of the phenolic alkaloids to couple with a diazonium salt. Of the two possibilities only **3** has an unsubstituted para position and is expected to undergo coupling. Formation of an azo dye was observed with alkaloid B and magnoflorine but not with their respective methyl ethers. The dye also exhibited a pronounced shift in its λ max of 375 nm to 516 nm upon basification, thus indicating the presence of a phenolic function conjugated with an azo group. Alkaloid B was therefore assigned structure **3**, identical with that of *N*-methylisocorydinium iodide.

This study describes the first reported isolation of (+)-*N*-methylisocorydinium salt from any species of *Magnolia*. This alkaloid, also known as menisperine and chakranine, was isolated originally from *Xanthoxylum brachyanthum* F. Muell. and *X. verificum* F. M. Bail. (19), subsequently from *Bragantia wallachii* R. Br. (12), and later was shown to be widespread in *Annonaceae, Lauraceae, Menispermaceae, Papaveraceae, Berberidaceae* and *Rutaceae* (13, 14).

The acute LD_{50} of alkaloid B-iodide was found to be 10 mg/kg (21 μM/kg) by the ip route in mice. For comparison, LD_{50} values of 2.2 mg/kg (5.9 μM/kg) by the iv route for the chloride (20) and 12 mg/kg (25 μM/kg) by the ip route for the iodide (6) have been provided. Magnoflorine showed LD_{50} by the ip route of 19.6 mg/kg (38 μM/kg) (6). A behavioral profile was studied by use of the procedure of Campbell and Richter (21) in mice which involves observation of sixteen signs thirty minutes following the injection. The only positive sign observed was that of midriasis. At doses near the LD_{50}, the onset of effects was observed in five minutes with marked depression of activity and labored breathing. Moribund animals became increasingly cyanotic and breathing more convulsive. Just prior to death, the animal usually reared and/or scampered frantically. At LD_{50}, death occurred in 15–20 minutes.

The second crystalline alkaloid (A) gave elemental analysis consistent with the molecular formula $C_{17}H_{15}NO_2$ (M^+ 265). The uv spectrum was characteristic of the aporprhine nucleus (9) and the spectrum was unchanged in base. The nmr spectrum indicated the presence of a methylenedioxy group, five aromatic H and other signals characteristic of an aporphine system (18). The presence of one proton singlet at δ 2.87 which was shifted by acid, and the lack of an N-methyl function indicated that the nitrogen was secondary. This was confirmed by the formation of a crystalline acetamide. The nmr spectral characteristics agreed with those described for the aporphine alkaloids by Johns *et al.*, (22). The mass spectrum showed the expected intense molecular ion (*m/e* 265, 63%), an even stronger M-1 (m/e 264, 100%) characteristic of the aporphines (10, 11) and other fragment ions at *m/e* 236 and *m/e* 235 resulting from the loss of HCO and H_2CO, respectively, generated from a retro-Diels Alder process. Based on the analytical and spectral data, alkaloid A was identified as (−)-anonaine **4**. This alkaloid was originally isolated from *Annona reticulata* L. (23) and later found in several species of *Magnolia* (24, 25).

EXPERIMENTAL[2]

[2]Melting points were obtained on Fisher-Johns hot stage apparatus and were uncorrected. The spectra reported in this study have been obtained on the following instruments: uv, Beckman 25; ir, Beckman Acculab 3; nmr, Varian T60 with tetramethylsilane as internal standard; mass spectrum, DuPont 490 chemical ionization mass spectrometer. Thin-layer chromatography was performed on micro slides 1 x 3 and 2 x 3 inches coated with silica gel (Merck HF 254+366) and visualized by quenching of uv light and iodoplatinic chloride spray.

ISOLATION OF ALKALOIDS A AND B.—Shavings from trunk wood of *Magnolia grandiflora* collected in Gainesville, Florida, (4.9 kg) were extracted with ethanol (12 liters) at 20° for two days. A combination of three such extracts was concentrated to a syrup (48 g), at which stage it showed an LD_{50} value of 500 mg/kg. The concentrate was partitioned between water (pH 2, 1 liter) and ethyl acetate (1 liter). The aqueous layer, which contained 26 g of total solids with LD_{50} of 350 mg/kg, was concentrated to 200 ml and treated with Mayer's reagent until precipitation was complete. Celite was used to filter the precipitate, and the cake was washed with 0.01 *N* HCl. The solid was stirred with acetone-water (1:1, 100 ml) and filtered. The filtrate and washings were passed through a column of Dowex-1 (50 g, Cl^- form). The column was washed with acetone-water (1:1). The effluent and wash (total solids 7.65 g, LD_{50} 70 mg/kg) were concentrated to remove acetone, adjusted to pH 9 and extracted twice with chloroform.

The chloroform extract was concentrated to 5 ml, diluted with ether (10 ml) and treated with ethanolic HCl until no more precipitation occurred. The solid was filtered, washed with ethanol-ether (1:19) and crystallized from methanol twice to give alkaloid A as a colorless crystalline solid; yield 1.2 g; mp 273–275° dec (lit 277° dec (22); $[\alpha]_D -73°$ (c 1.1 ethanol); uv, λ max 235, 271 and 315 nm; log ε 4.21, 4.21 and 3.75 respectively; nmr (D_6–DMSO): δ 6.00, d and δ 5.87, d, O–CH_2–O; δ 8.00, m, 1H, δ 7.13, 7.02, m, 3H; δ 6.50, s, 1H; δ 4.1–3.7, , 1H and δ 3.5–2.4, m, 6H.

Anal. calc. for $C_{17}H_{15}NO_2$·HCl: C, 67.66; H, 5.34; N, 4.64; Cl, 11.75. Found: C, 67.48; H, 5.13; N, 4.69; Cl, 11.51.

The aqueous layer, after the chloroform extraction, was readjusted to pH 7 and extracted twice with *n*-butanol. The solvent layer yielded 3.3 g of a solid, (LD_{50}, 80 mg/kg), leaving 3.4 g in the aqueous layer (LD_{50} 50 mg/kg).

The butanol-extractable fraction (3.3 g) was chromatographed on Sephadex LH20 (65 g) in 10% ethanol-ethyl acetate. Elution with this solvent gave an additional 100 mg of anonaine. Elution with 20% ethanol-ethyl acetate gave the major fraction (Alkaloid B, 0.55 g, LD_{50} 17 mg/kg). It was converted to the iodide by passage through a Dowex-1 (iodide) column and crystallized from methanol twice to yield alkaloid B as a colorless crystalline solid; yield, 0.35 g; mp 225–28° dec (lit. 225–30° dec (14), $[\alpha]_D +212°$ (c, 1.27 ethanol; uv, λ max 220, 270 and 302 nm, log ε 4.76, 4.21 and 3.82 respectively; in basic solution: 250 and 354 nm; nmr (D_6–DMSO): δ 8.67, broad, exchangeable, ArOH; δ 7.00, s, 1H; δ 7.11, 6.98, AB-q, J=8 Hz, 2H; δ 3.93, 3.85, 3.72, singlets.

Anal. calc. for $C_{21}H_{26}NO_4I$: C, 52.18; H, 5.42; N, 3.00; I, 26.26. Found: C, 52.01; H, 5.18; N, 2.85; I, 25.95.

The nonextractable fraction was purified by partition chromatography performed with cellulose (200 g) and the system ethyl acetate-*n*-butanol (3:1) equilibrated with water. Elution with this solvent gave three bands, alkaloid A, alkaloid B (major) and magnoflorine. Alkaloid B was processed via the iodide salt as before; yields 0.6 g.

METHYLATION OF (+)-N-METHYL ISOCORYDINIUM SALT.—A sample of **3** (0.1 g) in acetone (50 ml) was stirred with dimethyl sulfate (0.1 ml) and anhydrous potassium carbonate (1 g) at 25° for 30 hours. The mixture was filtered; the filtrate was concentrated to dryness, dissolved in water and passed through a column of Dowex-1 I^-. The iodide salt was recovered by concentration and crystallized from methanol; yield, 100 mg., mp 256–258° dec (18); nmr δ 3.92, 3H; δ 3.90, s, 3H; δ 3.71, s, 6H; M^+ 369.

ACETYLATION OF (+)-N-METHYLISOCORYDINIUM SALT.—A solution of **3** (0.1 g) in acetic anhydride (5 ml) containing one drop of phosphoric acid was heated at 100° for two hours. The cooled solution was diluted with water and passed through a column of Dowex-1-acetate to remove the phosphoric acid. The product was converted to the iodide salt, as before, and crystallized from methanol, yield 0.08 g; mp 263–265° dec (lit. 260–265° (18)); ν 1770 cm^{-1} and δ 210, s, 3H.

ACETYLATION OF ANONAINE.—A sample of **4** (0.1 g) was stirred in a mixture of acetic anhydride and acetic acid (3 ml, 1:1) at 25° for 15 hours and then at 70° for 30 minutes. The mixture was diluted with water (25 ml), the solution neutralized with sodium bicarbonate and the solid filtered. Crystallization from methanol gave the acetamide as a colorless crystalline solid, mp 229–231° (lit 229–30°, 26); yield, 0.1 g; ν 1645 cm^{-1}; nmr ($CDCl_3$) δ 2.21, s, 3H.

Received 31 July 1981

LITERATURE CITED

1. K. V. Rao and T. L. Davis, *Planta Med.*, In press.
2. F. G. Speck, Jr., "American Ethnobotany", D. E. Moerman, Ed., Garland Publishing Co., New York, 1883, p. 113.
3. W. W. Wood, Ed., "Pharmacopoeia of the United States of America" (6th Decennial Revision), New York, p. 215.
4. E. P. Klaus, "Pharmacognosy", Lea and Febiger, Philadelphia, 1956, p. 222.
5. T. Nakano, *Pharm. Bull. (Japan)*, **2**, 321 (1954)., *Chem. Abs.*, **50**, 6475e (1956).
6. K. Inoue, *Nippon Yakurigaku Zasshi*, **53**, 797 (1957)., *Chem. Abs.*, **52**, 18870b (1958).
7. R. W. Doskotch and F. S. El-Feraly, *J. Pharm. Sci.*, **58**, 877 (1969).
8. R. M. Wiedhopf, M. Young, E. Bianchi and J. R. Cole, *J. Pharm. Sci.*, **62**, 345 (1973).
9. M. Shamma and W. A. Slusarchyk, *Chem. Rev.*, **64**, 59 (1964).
10. H. Budzikiewicz, D. Djerassi and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. I, Holden-Day Inc., San Francisco, 1964, p. 174.

11. M. Ohashi, J. M. Wilson, H. Budzikiewicz, M. Shamma, W. A. Slusarchyk and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2807 (1963).
12. A. R. Katritzky, R. A. Y. Jones and S. S. Bhatnagar, *J. Chem. Soc.*, 1950 (1960).
13. H. Guinaudeau, M. Leboeuf and A. Cavé, *Lloydia*, **38**, 275 (1975).
14. H. Guinaudeau, M. Leboeuf and A. Cavé, *J. Nat. Prod.*, **42**, 325 (1979).
15. D. S. B. Bhakuni, S. Tewari and M. M. Dhar, *Phytochemistry*, **11**, 1819 (1972).
16. S. R. Johns and J. A. Lamberton, *Aust. J. Chem.*, **20**, 1277 (1967).
17. A. M. Kuck, *Chem. Ind.* (London 118) (1966).
18. A. Groebel, D. Lenoir and R. Pernet, *Planta Med.*, **18**, 66 (1970).
19. J. R. Cannon, G. M. Hughes, E. Ritchie and W. C. Taylor, *Aust. J. Chem.*, **6**, 86 (1953).
20. V. N. Kamat, A. Vaz, P. V. Divekar, F. Fernandez and S. S. Bhatnagar, *Indian J. Med. Research*, **46**, 418 (1958).
21. D. E. S. Campbell and W. Richter, *Acta Pharmacol. Toxicol.*, **25**, 345 (1967).
22. S. R. Johns, J. S. Lamberton and A. A. Sioumis, *Aust. J. Chem.*, **21**, 1388 (1968).
23. A. C. Santos, *Phillippine J. Sci.*, **43**, 561 (1930).
24. K. Ito and A. Yoshida, *J. Pharm. Soc. Japan*, **86**, 124 (1966)., Chem. Abs., **64**, 16285f (1966).
25. M. Tomita and M. Kozuka, *&. Pharm. Soc. Japan*, **87**, 1134 (1967)., Chem. Abs., **68**, 10233W (1968).
26. C. D. Hufford, *Phytochemistry*, **15**, 1169 (1976).