Thalictrum Alkaloids I

Thalicarpine, A New Hypotensive Alkaloid from *Thalictrum dasycarpum*

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A preliminary study of the roots of *Thalictrum dasycarpum* Fisch. and Lall from Wisconsin yielded magnoflorine and a new alkaloid, thalicarpine. Evidence is presented for assignment to thalicarpine of the empirical formula $C_{41}H_{48}O_8N_2$. Thalicarpine was evaluated in a variety of pharmacologic procedures and found to possess a modicum of biological activity.

THALICTRUM TOURN. EX LINN. (*Ranunculaceae*), commonly known as "meadow rue," is a widely distributed genus. Many medicinal uses of *Thalictrum* species in folk remedies have been recorded. *T. foliosum* DC. is found throughout the Himalayas and taken as a tonic, aperient, purgative, diuretic, febrifuge, a remedy for atonic dyspepsia, and used as an application for ophthalmia (1). *T. thunbergii* DC. is used in Japan as a home remedy against stomach ache and diarrhea (2). *T. collinum* Walbr., *T. angustifolium* L., and *T. silvaticum* Koch. have been used in Ukrainian folk medicine as diuretics (3). *T. fendleri* Engelm. was prepared by the Indians of Nevada as a tea to cure gonorrhea; a decoction of the root was used against colds (4). *T. minus* L. is used in south Africa to treat fevers (5). Intravenous injection of the hydrochlorides of the extract of the total alkaloids from *T. minus* L. has recently been shown to exert an effect on the blood pressure and pulse of frogs, cats, and dogs (6).

In the course of a continuing screening program for alkaloid-bearing plants, the roots of *Thalictrum dasycarpum* Fisch. and Lall[1] from Wisconsin were found to afford substantial yields of alkaloids. Pharmacological evaluation of the nonquaternary alkaloid fraction demonstrated potent hypotensive activity in the anesthetized cat after intravenous administration.[2] The present report describes a preliminary study of the alkaloids of *T. dasycarpum* and the isolation and characterization of thalicarpine, a new hypotensive tertiary base. While our work was in progress, the isolation from *T. dasycarpum* of the quaternary alkaloids magnoflorine and berberine was reported (7). However, no work on the nonquaternary alkaloids has appeared to date.

Coarsely ground plant was extracted successively with methanol and 1.5% triethylamine in methanol. The extracts were processed for alkaloid content by the procedure summarized in Fig. 1, whereby an 0.35% yield of crude alkaloids was obtained. Fraction E, the quaternary alkaloid fraction, yielded magnoflorine as the only isolatable component. Study of the nonquaternary phenolic alkaloid Fractions B and D is in progress and will be reported in due course.

The nonquaternary nonphenolic alkaloid Fractions A and C were combined on the basis of their paper chromatographic patterns. Chromatographic fractionation led to isolation of the major alkaloid of the plant (0.07% of the dried roots). The compound showed m.p. 160–161°, $[\alpha]_D^{25}$ + 133° (methanol); + 89° (chloroform), and ultraviolet, infrared, and N.M.R. spectral characteristics which indicated that the material is a new compound. The name *thalicarpine*, reflecting the botanical origin, is proposed for the alkaloid.

The molecular formula $C_{41}H_{48}O_8N_2$ was assigned for thalicarpine on the basis of elemental analysis and molecular weight determination by nonaqueous titration. Analysis showed the presence of seven O-methyl groups and two N-methyl groups. The N.M.R. spectrum in deuterated chloroform solution supports the formula, showing six N-methyl protons, 21 O-methyl protons, 14 aliphatic protons, and seven aromatic protons. The infrared spectrum indicates the presence of aromatic rings and aromatic O-methyl groups, but absence of hydroxyl, carbonyl, and isolated double-bonds.

A sequence of derivatives was prepared to seek confirmation of the empirical formula as well as to provide conversion products useful for structure elucidation. Treatment of thalicarpine with methyl iodide afforded a product which resisted all attempts at crystallization. However, treatment of the amorphous methiodide with alkali yielded a Hofmann methine which was converted to a crystalline methiodide. Analysis afforded results which support a $C_{45}H_{58}O_8N_2I_2$ formula for the Hofmann

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[1] Air dried roots, collected in southern Wisconsin during the summers of 1958, 1959, and 1960. We thank Professor H. H. Iltis, University of Wisconsin, for confirming the identity of the plant. A voucher specimen is deposited in the University of Wisconsin Herbarium.

[2] The authors thank Mr. Edward Macko, Smith Kline and French Laboratories, Philadelphia, Pa., for the pharmacological results reported herein.

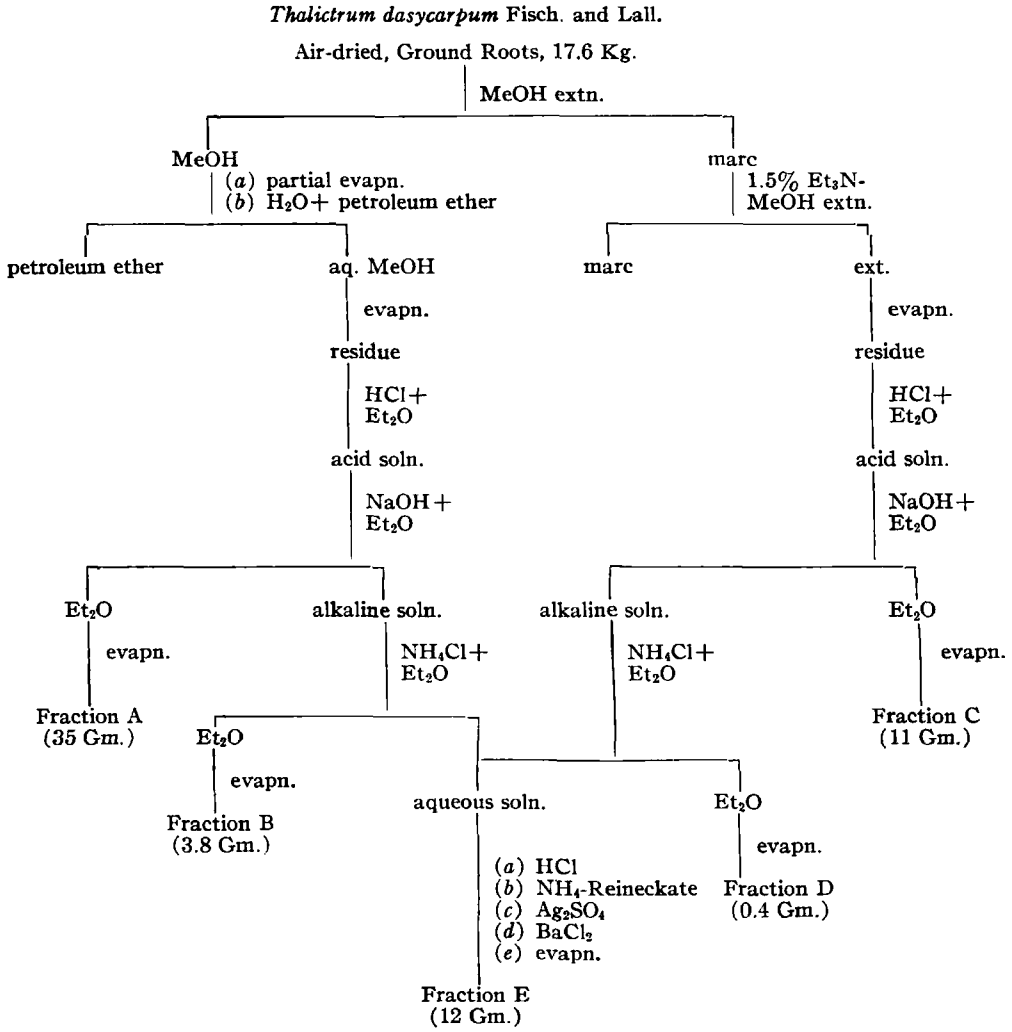

Fig. 1.—Flow sheet for separation of alkaloids of *Thalictrum dasycarpum* Fisch. and Lall.

methine methiodide. A second Hofmann degradation yielded a des-N-methine, which afforded analytical results indicative of a $C_{39}H_{38}O_8$ empirical formula. The formulas of both methine derivatives support the $C_{41}H_{48}O_8N_2$ formula for thalicarpine. Further structural studies are in progress and will be reported at a later date.

EXPERIMENTAL

Melting points have been corrected for stem exposure. Values of $[\alpha]_D$ have been approximated to the nearest degree. Infrared spectra were determined on a Beckmann IR-5A infrared spectrophotometer. Ultraviolet spectra were determined in methanol on a Cary recording spectrophotometer. The N.M.R. spectra were taken on a Varian A-60 N.M.R. spectrometer. Paper chromatography was conducted by the descending technique on Whatman No. 4 paper pretreated with buffer at pH 3.5.

Extraction of Alkaloids from Thalictrum dasycarpum. Separation into Main Fractions

Coarsely ground *T. dasycarpum* (air dried roots, 17.6 Kg., from middle and southern Wisconsin) was continuously extracted with methanol in a Soxhlet-type extractor. The extraction was continued with a fresh charge of solvent at the end of 3 days. When the extraction was stopped after a total of 10 days, the extract returning to the pot yielded a residue which did not give a positive Mayer's test upon evaporation. The methanol extract was concentrated under reduced pressure to a dark brown semisolid concentrate. The 1 L. concentrate was diluted with 2 L. of water and extracted with 3 L. of petroleum ether (Skellysolve B, b.p. 60–68°) to remove fat. The aqueous methanolic solution was further evaporated to remove most of the methanol, then triturated with 1.5% hydrochloric acid. After rejecting acid insoluble material, the acidic solution was washed with 6 L. of ether, made alkaline with sodium hydroxide, and extracted with 6 L. of ether. The ether extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to yield a glass-like alkaloidal residue (Fraction A, 35 Gm.; see Fig. 1). The alkaline solution re-

maining from ether extraction was made more weakly basic to pH 8.5 by adding ammonium chloride and extracted with 6 L. of ether. The ether extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to yield a semisolid alkaloidal residue (Fraction B, 3.8 Gm.). The aqueous solution remaining from ether extraction was reserved for study of the quaternary alkaloids.

Next, the marc remaining from the methanol extraction was extracted continuously with 1.5% trimethylamine in methanol for 12 days. When extraction was stopped, evaporation of the extract returning to the pot yielded a residue which did not give a positive test with Mayer's reagent. The trimethylamine-methanol extract was evaporated to dryness, and the residue was triturated with 1.5% hydrochloric acid (1 L.). After rejecting acid-insoluble material, the acidic solution was washed with ether, made alkaline with sodium hydroxide, and extracted with 2 L. of ether. The ether extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to yield 11 Gm. of alkaloidal residue (Fraction C). The basicity of the aqueous solution remaining from ether extraction was adjusted to pH 8.5 by adding ammonium chloride. The alkaline solution was then extracted with 2 L. of ether. The ether extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to yield a semisolid alkaloidal residue (Fraction D, 0.4 Gm.). The aqueous alkaline solution was combined with the corresponding aqueous solution from the methanol extract. The combined solution was re-acidified with concentrated hydrochloric acid and treated with a saturated Reinecke salt solution. The dried precipitate was dissolved in acetone, the solution was clarified by filtration, and treated with a saturated aqueous silver sulfate solution to complete precipitation. Silver Reineckate was removed by filtration; the filtrate was treated with a barium chloride solution to complete precipitation. After removal of barium sulfate by centrifugation, the supernatant solution was evaporated to dryness under reduced pressure to yield 12 Gm. of quaternary chloride mixture (Fraction E).

Isolation of Alkaloids

Fractions A and C, constituting the nonquaternary nonphenolic alkaloids, were combined on the basis of the similarity of their paper chromatographic patterns. Similarly, Fractions B and D, constituting the nonquaternary phenolic alkaloids, were combined.

Fraction E.—The mixture of quaternary chlorides was converted into a mixture of quaternary iodide salts with potassium iodide in aqueous solution. The mixture of iodides was fractionated by successive extraction with chloroform, acetone, and methanol. The methanol extract deposited a crude crystalline solid upon concentration. Repeated recrystallization from methanol gave crystalline magnoflorine iodide as pale-yellow prisms, m.p. 251–252°; $[\alpha]_D^{20}$ + 198° (c 0.23, methanol). The melting point was not depressed on admixture with an authentic sample of magnoflorine iodide.[3] The paper chromatographic behavior and infrared spectrum (Nujol mull) were identical with those of the authentic magnoflorine iodide sample.[4]

The Nonquaternary Nonphenolic Fraction.—The crude alkaloid mixture was fractionated by column chromatography on 1 Kg. Florisil. The column was developed by successive elution with benzene, 10, 30, and 60% chloroform-benzene, chloroform, 20% acetone-chloroform, acetone, and methanol. The 30 and 60% chloroform-benzene eluates, on treatment with ethyl acetate, gave 12-Gm. colorless needles, m.p. 160–161°, $[\alpha]_D^{25}$ + 133° (c 0.83, methanol), + 89° (c 0.88, chloroform), $\lambda_{max.}$ 282 mμ, (ε 17,000), 302 mμ (ε 13,000). The N.M.R. spectrum ($CDCl_3$) showed τ = 7.55, 7.52 (6H, N-methyl), 6.40, 6.29, 6.21, 6.19, 6.17, 6.09, 6.05 (21H, O-methyl), 3.79, 3.47, 3.40, 3.37, 3.32, 1.77 (7H aromatic).

Anal.—Calcd. for $C_{41}H_{48}O_8N_2$: C, 70.67; H, 6.94; N, 4.02; 7 (OCH_3), 31.17; 2 (NCH_3), 8.33. Found: C, 70.72; H, 6.72; N, 4.07; (OCH_3), 27.40; (NCH_3), 7.48.

Hofmann Degradation of Thalicarpine

About 3 mg. of anhydrous potassium carbonate was added to a 3-Gm. solution of thalicarpine (4) in 20 ml. methanol and 10 ml. methyl iodide. The solution was heated under reflux on a steam bath for 6 hours. The reaction mixture was then brought to dryness under reduced pressure to yield a semisolid residue. All attempts to crystallize the residue failed. The amorphous thalicarpine dimethiodide (4.29 Gm.) was dissolved in 25 ml. of methanol, and 10 Gm. of potassium hydroxide was added to the solution in small portions to obtain a clear solution. The reaction mixture was heated on a steam bath for 2 hours under magnetic stirring. The mixture was cooled in an ice bath and then evaporated to dryness under reduced pressure. The dried residue was triturated with 50 ml. of water and 50 ml. of ether. The aqueous layer was washed with another portion of 50 ml. ether. The ether layer and wash were combined, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to yield 3.36 Gm. of semisolid residue. The Hofmann methine thus obtained was also uncrystallizable. The amorphous Hofmann methine was treated with 10 ml. methyl iodide in 20 ml. methanol at the boiling point of the solution for 6 hours. The reaction mixture was then brought to dryness to leave a 4.05-Gm. brown solid. The residue was treated with methanol to give colorless micro prisms, m.p. 275–276° dec., $[\alpha]_D^{25}$ + 0° (c 1.24, methanol), $\lambda_{max.}$ 266 mμ (ε 62,000), 309 mμ (ε 27,000), 323 mμ (ε 28,000), 346 mμ (ε 20,000).

Anal.—Calcd. for $C_{45}H_{58}O_8N_2I_2 \cdot H_2O$: C, 52.64; H, 5.89; N, 2.73; I, 24.72; 7 (OCH_3), 21.16; 4 (NCH_3), 11.31. Found: C, 52.25; H, 5.59; N, 2.91; I, 25.04; (OCH_3), 21.33; (NCH_3), 11.47.

Thalicarpine dimethylmethine dimethiodide (1.2 Gm.) was converted into methochloride by the action of freshly prepared silver chloride to obtain 1.06 Gm. of the glass-like dimethochloride. A 10-Gm. quantity of potassium hydroxide was added in small portions to a solution of the methochloride in 20 ml. of water and 10 ml. of methanol. The reaction mixture was then heated on a steam bath for 3 hours under

[3] The authors thank Professor E. Fujita, Institute for Chemical Research, Kyoto University, Kyoto, Japan, for an authentic sample of magnoflorine iodide.

[4] Experiment by Dr. B. Dasgupta.

magnetic stirring. The vigorous evolution of trimethylamine gas from the reaction mixture was observed during the early part of the reaction. The reaction mixture was then brought to dryness under reduced pressure. The dried residue was triturated with 50 ml. of water and 200 ml. of ether. The aqueous layer was washed with 100 ml. of ether. The ethereal layer was combined with the washing, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to leave a 0.75-Gm. yellow oil. The des-*N*-methine crystallized from ether as colorless fine prisms (0.64 Gm.), m.p. 170–172°, $[\alpha]_D^{25} \pm 0°$ (c 2.05, chloroform); $\lambda_{max.}$ 267 mμ (ε 55,000), 317 mμ (ε 31,000); N.M.R. spectrum ($CDCl_3$), τ = 5.76, 5.83, 5.88, 5.91, 6.05, 6.07, 6.19 (21H, O-methyl).

Anal.—Calcd. for $C_{39}H_{38}O_8$: C, 73.80; H, 6.04. Found: C, 73.54; H, 6.20.

PHARMACOLOGICAL RESULTS[2]

Thalicarpine was evaluated in a variety of pharmacologic procedures and found to possess a modicum of biological activity. In acute dose range or toxicity studies, oral doses of 300 mg./Kg. failed to produce discernible gross behavioral changes in the mouse. Intraperitoneal injection of 25 mg./Kg. of thalicarpine to a cat produced sensitivity of the forepaws, rubbing of the neck, and emesis.

The principal action of thalicarpine on blood pressure was depressor in nature. In the cat anesthetized with chloralose, mean arterial blood pressure was lowered transiently following acute intravenous doses ranging from 0.5 to 5 mg./Kg. Lethality, due to respiratory arrest, occurred at a dose of 10 mg./Kg. Bradycardia, respiratory depression, and andrenergic blocking action accounted for the weak hypotensive activity. Anticoagulant, hypoglycemic, and anticonvulsant properties were not observed after oral doses of 100 mg./Kg. in the rat, guinea pig, or mouse, respectively. An oral dose of 50 mg./Kg. caused a slight antidiuretic response in rats hydrated with saline. In the Randall and Selitto test for anti-inflammatory activity oral doses of 50 mg./Kg. of thalicarpine produced a very low order of analgetic activity (25%) and no significant antipyretic action.

In summary, weak hypotensive activity of a transient nature was the principal action of thalicarpine when injected intravenously into the anesthetized cat. Respiratory toxicity and weak adrenolytic activity accompanied this action. Thalicarpine failed to exhibit significant biological activity as an anti-inflammatory, anticoagulant, hypoglycemic, or diuretic agent.

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Gas Chromatographic Analysis of Oil of Nutmeg

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Four samples of commercially available oil of nutmeg were analyzed by gas chromatography. Of a number of stationary liquid phases used, a 20 per cent Reoplex 400 on Dichromite gave the best separation. The composition of the oils was determined on the basis of retention times and enrichment.

ESSENTIAL OILS contain volatile compounds representing many classes of organic substances. One such volatile oil is the well known oil of nutmeg (*Myristica*), an important spice used for the flavoring of numerous food products. It is also used as a component of certain types of perfumes and as a flavoring agent for dentifrices (1). The literature lists two oils of myristica—oil of nutmeg and oil of mace. Both are derived from the fruit of *Myristica fragrans* Houtt. (*fam. Myristicaceae*) (2).

The dried seeds of nutmeg contain from 5 to 15% of the volatile oil, as well as from 25 to 40% of a fixed oil, and from 5 to 15% of ash. The rest consists of moisture, fiber, and starch (3, 4).

There are two principal types of nutmeg which are recognized today, and these depend primarily on geographical origin. "Banda nutmegs" or East Indian variety are the finest; the other variety comes from the West Indies (5).

The West Indian type of oil has a lower specific gravity, lower refractive index, and a lower residue on evaporation, but has a higher

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