# STUDIES ON NATIVE MEDICINAL PLANTS, I. THE QUATERNARY ALKALOIDS OF *THALICTRUM JAVANICUM*

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ABSTRACT.—In the stem and roots of *Thalictrum javanicum* Bl. six quaternary alkaloids, palmatine, berberine, columbamine, jatrorrhizine, magnoflorine and demethylene berberine were isolated and identified. The structure of the new alkaloid demethylene berberine was proved by means of uv, ir, nmr and mass spectrometry and by its chemical conversion into palmatine.

*Thalictrum* (Meadow Rue), a genus of the Ranunculaceae (crow-foot) family, includes a wide range of herbs which are mostly dispersed in temperate and tropical regions of the world at elevations 4000 to 10,000 feet (1). Since *Thalictrum* species are known to produce a variety of alkaloids of chemical and biological interest, it was thought desirable to investigate the alkaloidal constituents of *Thalictrum javanicum*, which has a repute in Indian folklore medicine and remains uninvestigated to date.

*Thalictrum javanicum* Bl. is an erect, perennial herb, two to three feet tall. The leaves of the plants are ternately compound and are fixed on a glabrous stem, while the roots are fibrous and yellowish brown in color resembling liquorice but extremely bitter. During the flowering season, terminal panicles bear white flowers. The plant is found in the temperate Himalayas from Kashmir to Sikkim and in the Khasi Hills and Nilgiri Hills in Tamil Nadu (2).

## RESULTS AND DISCUSSION

The air-dried plant material was extracted and chromatographed as described in the experimental section. The fractions collected from column chromatography when purified by repeated preparative tlc on silica gel afforded the pure alkaloids. By this process, six major quaternary alkaloids, palmatine, berberine, columbamine, jatrorrhizine, magnoflorine and demethylene berberine were isolated in sufficient quantities to allow their complete identification. Work is in progress on the isolation and structure elucidation of the remaining tertiary alkaloids. The structure of the new alkaloid demethylene berberine is given in fig. 1.

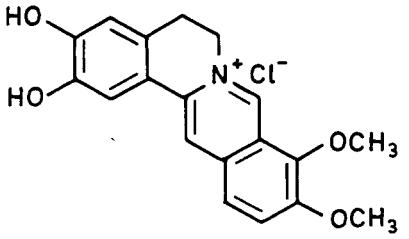


FIGURE 1. Structure of the new alkaloid demethylene berberine.

Palmatine, berberine, columbamine, jatrorrhizine, magnoflorine, and demethylene berberine were separated by repeated preparative tlc of the various column chromatographic fractions (table 1) and were characterized by the comparison of their physical constants with those reported in the literature. The identification was confirmed in each case by direct comparison with an authentic sample (mixed mp, co-tlc, ir, uv, nmr and mass spectral data).

TABLE 1. Results of chromatography of quaternary alkaloid fraction.

| Fractions | Solvent | Weight of residue (mg) | Remarks |
|---|---|---|---|
| 1–11 | 1% $CH_3OH$–$CHCl_3$ | 122 | Nonalkaloidal residue. |
| 12–20 | 5% $CH_3OH$–$CHCl_3$ | 475 | Palmatine and berberine with minor impurities. |
| 21–35 | 10% $CH_3OH$–$CHCl_3$ | 135 | Complex alkaloidal mixture containing columbamine as major alkaloid. |
| 36–42 | 10% $CH_3OH$–$CHCl_3$ | 150 | Mixture of alkaloid jatrorrhizine and magnoflorine with some impurities. |
| 43–55 | 20% $CH_3OH$–$CHCl_3$ | 220 | Complex alkaloidal mixture with alkaloid magnoflorine as major alkaloid. |
| 56–70 | 30% $CH_3OH$–$CHCl_3$ | 390 | Mixture of alkaloid magnoflorine (minor) and demethylene berberine (major) with minor impurities. |
| 71–95 | 50% $CH_3OH$–$CHCl_3$ | 630 | Nonalkaloidal residue with magnoflorine and demethylene berberine as minor alkaloids. |
| 96–110 | MeOH | 400 | Dark brown non-alkaloidal residue. |

Fractions 56 to 70, eluted with 30% methanol in chloroform, yielded the alkaloid demethylene berberine. The alkaloid gave a molecular ion at *m/e* $324^+$ analyzing for $C_{19}H_{18}O_4N$. The uv spectrum in conjugation with ir spectrum suggested the presence of a protoberberine nucleus. The alkaloid exhibited a shift in uv absorption peaks, on addition of acid as well as base, suggesting the presence of hydroxyl groups in the molecule. On acetylation of the alkaloid a diacetyl derivative was obtained establishing the presence of two hydroxyl groups.

In the nmr spectrum, the alkaloid exhibited signals for two methoxy groups, attached to an aromatic ring, at $\delta$ 6.13 and 5.99, and were assigned to $C_9$ and $C_{10}$, respectively, on the basis of mass fragmentation pattern (ions due to ring D). The aromatic region of the spectrum integrated for six protons, of which ortho coupled $C_{11}$ and $C_{12}$ protons were present as doublets at 1.88 ($J=9$ Hz, 1H) and 2.09 (d, $J=9.5$ Hz, 1H), respectively. The characteristic low-field signal at 0.34 was assigned to $C_8$ proton. The singlets at 1.30, 2.39 and 3.59 were assigned to C–13, C–1 and C–4 protons, respectively.

On methylation, the resulting *O-O*-dimethyl derivative was found to be almost identical with the alkaloid palmatine. Further, identification of the *O-O*-dimethyl derivative was confirmed by direct comparison with an authentic sample of palmatine (mixed mp and co-tlc). This established the position of substituents (2,3 and 9,10) and configuration of the alkaloid.

In the mass spectrum of the tetrahydro derivative of the alkaloid, the molecular ion peak was observed at *m/e* 327. The other significant peaks were at *m/e* 326 ($M^+$–1), 162 (isoquinolium ion) 164 and 149 (base peak), suggesting the possible positions of the two hydroxyl groups at $C_2$ and $C_3$.

The positions of the hydroxyl groups at $C_2$ and $C_3$ was further confirmed by the study of the mass spectrum of tetrahydro-*O-O*-diethyl derivative of the alkaloid. The peak for the isoquinolium ion at 218 established the structure of demethylene berberine. This is the first report of the isolation of this alkaloid from a natural source.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES—Melting points were taken on a Kofler Hot Stage apparatus and are uncorrected. The ir spectra were recorded on a Perkin-Elmer 137 or 577 grating infrared spectrometer. The uv spectra were measured in a Perkin-Elmer 202 automatic recording spectrometer. The nmr spectra were taken on a Varian A60 and a Perkin-Elmer R-30 spectrometer with TMS as an internal standard. The mass spectra were run on a JEOL JMS-D300 instrument fitted with a direct inlet system. Silica gel-G was used for preparative tlc (1 mm thickness, 20 g for each 20 cm x 20 cm glass plate) and spots were visualized either in $I_2$ vapor or by Dragendorff's reagent.

PLANT MATERIAL.—The plant material used in this study was collected from four different different locations in a diameter of one km in Simla, Himachal Pradesh, during July 1979 and identified by Dr. S. L. Kapoor, Asst. Director, N.B.R.I., Lucknow. A herbarium specimen is on deposit in the herbarium of the Institute.

EXTRACTION AND FRACTIONATION.—Preliminary investigation of the four samples of plants by tlc of their alkaloid fractions revealed that all the four were qualitatively identical, hence they were mixed for combined extraction.

The air dried powdered material (7 kg) was extracted by percolation to exhaustion with ethanol (10 liters x 5). The total alcoholic extract was concentrated under reduced pressure and below 40°. The resulting greenish residue was dissolved in 400 ml of chloroform and was shaken with an equal volume of 5% aqueous hydrochloric acid solution. The extraction with dilute HCl was repeated twice. Removal of chloroform by evaporation at reduced pressure left a residue containing neutral and acidic constituents.

The combined acidic aqueous extract was made alkaline to pH 8–9 with conc ammonium hydroxide solution and extracted four times with equal volumes of chloroform. The pooled and dried (over anhydrous $Na_2SO_4$) chloroform extract on evaporation under reduced pressure left a residue (A) (3 g) of the crude tertiary bases which is under investigation for its constituents and will be reported later.

The aqueous alkaline solution, after removal of tertiary bases, was acidified to pH 5 with citric acid (solid) and then treated with 2% ammonium reineckate solution until precipitation of the alkaloidal reineckates was complete. After cooling overnight the precipitate was collected by suction filtration. It was dissolved in methyl alcohol (20 ml), and the solution was passed through an anion exchange resin column (Amberlite IRA-410, 10 gs) to convert reineckates to the chlorides. Removal of the solvent by evaporation at reduced pressure and below 40° left a dark yellowish brown residue B (4 g) containing quaternary alkaloids.

ISOLATION OF QUATERNARY ALKALOIDS FROM RESIDUE (B).—The crude quaternary alkaloidal residue (B) (4 g) was deposited on a column of silicic acid, packed in a slurry with chloroform, and eluted with the following succession of solvents, 1% methanol in chloroform, 5% methanol in chloroform, 10% methanol in chloroform, 20% methanol in chloroform, and 30% methanol in chloroform and, finally, with methanol, each in fractions of 20 ml. The results are presented in table 1.

SEPARATION AND CHARACTERIZATION OF ALKALOIDS.—The alkaloids were separated from the chromatographic fractions by repeated thin layer chromatography with chloroform-methanol-10% ammonia (9:1:1) as the developing solvent.

Palmatine, berberine, columbamine, jatrorrhizine and magnoflorine were compared with authentic samples and were identical in mp, ir, uv, and exhibited no depression in mixture mp and agreed with the data reported in the literature (3, 4, 5).

DEMETHYLENE BERBERINE.—Demethylene berberine exhibited the following spectral data: mp 225°; uv λ max (MeOH) 235(log ε 4.63), 270(4.52), 325(4.49); λ max (MeOH-HCl) 225(4.68), 263(4.56), 340(4.51) nm; λ max (MeOH-NaOH) 238(4.61), 274(4.48), 330(4.54) nm; ir max (KBr) 3450, 2900, 1600, 1510, 1390, 1350, 1250, 1200 $cm^{-1}$; nmr 6.13 (s, 3H, $OCH_3$), 5.99 (s, 3H, $OCH_3$), 3.59 (s, 1H, $C_4$-*H*), 2.39 (s, 1H, $C_1$-*H*), 2.09 (d, J=8.5, 1H, $C_{12}$-*H*), 1.88 (d, J=9.0, 1H, $C_{11}$-*H*), 1.30 (s, 1H, $C_{13}$-*H*), 0.34 (s, 1H, $C_8$-*H*); ms (tetrahydro deriv.) *m/e* 328 (4), 327 ($M^+$, 72), 326(61), 310(2), 165(26), 164(92), and 149 (100%).

*O-O*-DIMETHYL-DEMTHYLENE BERBERINE.—To a solution of demethylene berberine (50 mg) in methanol was added an ethanol solution of $CH_2N_2$; the mixture was left at room temperature for 36 hours. The solvent from the resulting mixture was removed, and the product was purified by thin layer chromatography [plates: silica gel-G; solvent: chloroform-methanol (3:1)]. The *O-O*-dimethyl derivative (80 mg) thus obtained was identical to palmatine (mp, uv, ir, nmr and ms).

ACETYLATION OF ALKALOID DEMETHYLENE BERBERINE.—A mixture of demethylene berberine (40 mg), pyridine (2 ml) and $Ac_2O$ (2 ml) was left at room temperature for 24 hrs. The resulting mixture when washed up in the usual manner, furnished an acetyl derivative as an oil. The acetate gave the following spectral data: ir ν max (neat) 1720 $cm^{-1}$ ($COCH_3$); nmr ($CCl_4$) 7.93 (s, 6H, 2xO.CO.$CH_3$).

TETRAHYDRODERIVATIVE OF *O-O*-DIETHYL ALKALOID DEMETHYLENE BERBERINE.—A solution of demethylene berberine (30 mg) and an excess of diazoethane ether azeotrope in methanol (10 ml) was kept at 0° for several days; the *O-O*-diethyl derivative was obtained. The crude product (40 mg) obtained after the removal of the solvent was dissolved in methanol. $NaBH_4$ was added to this product, which was then kept for 12 hrs. The product, when purified by preparative thin layer chromatography [plates; silica gel G, solvent chloroform-methanol (80:20), yielded 25 mg of pure tetrahydroderivative of *O-O*-diethyl demethylene berberine. It gave a mass spec of: 383 ($M^+$, 100%), 382 ($M^+$-1, 45), 364 (41), 218 (10), 217 (12), 164 (83), 149 (49).

REDUCTION OF ALKALOID PALMATINE, BERBERINE, JATRORRHIZINE COLUMBAMINE AND DEMETHYLENE BERBERINE.—Pure alkaloids (50 mg each) were dissolved in methanol (25 ml), and $NaBH_4$ was added until the reduction was completed (followed by tlc). The solvent was removed, and the respective residues were dissolved separately in basic diethyl ether and filtered. The basic diethyl ether layer, when taken to dryness, gave the tetrahydro derivative of the alkaloid taken. Each derivative was used for mass spectral analysis.

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