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QUATERNARY ALKALOIDS OF *THALICTRUM CULTRATUM*

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Key Word Index—*Thalictrum cultratum*; Ranunculaceae; roots; quaternary alkaloids; protoberberine alkaloids; aporphine alkaloid.

Abstract—The isolation and identification of seven alkaloids from the quaternary alkaloid fraction of a root extract of *Thalictrum cultratum* are described. The alkaloids isolated are the protoberberines berberine, palmatine, jatrorrhizine, columbamine, thalifendine, and (+)-thalidastine; and the aporphine (+)-magnoflorine.

INTRODUCTION

Thalictrum cultratum Wall is a perennial herb indigenous to Pakistan [1] and China [2] with a history of folkloric use as a medicinal plant in therapy of fever, acute enteritis and dysentery, conjunctivitis, pyogenic dermatitis, glossitis, and oropharyngitis [2]. Several detailed reports have recently appeared describing the isolation and identification of 30 nonquaternary bisbenzylisoquinoline and aporphine-benzylisoquinoline dimeric alkaloids, 13 of which were novel, from extracts of the Pakistani whole plant [1, 3, 4]. The absence of knowledge of the composition of the quaternary alkaloids of this species coupled with its folkloric uses as a medicinal, prompted us to undertake a study of this group of alkaloids. This paper reports the isolation of seven quaternary alkaloids from an extract of the roots of *T. cultratum* indigenous to southwestern China. These alkaloids include the protoberberines berberine, palmatine, jatrorrhizine, columbamine, thalifendine and (+)-thalidastine, as well as the aporphine alkaloid (+)-magnoflorine.

RESULTS AND DISCUSSION

Berberine, columbamine, jatrorrhizine and palmatine all possess *in vitro* antimicrobial properties while both berberine and palmatine are also *in vitro* inhibitors of cholinesterase [5]. Jatrorrhizine and berberine are active inhibitors of drug-induced myocardial arrhythmias and berberine is known to possess sedative, hypotensive, alpha-adrenergic blocking, ileal spasmogenic, and anticurariform properties [5].

This is the first report of the presence of quaternary alkaloids in this species. Palmatine, jatrorrhizine, columbamine, and thalifendine have been previously isolated from 10–14 other *Thalictrum* species while berberine and magnoflorine have been isolated from 31 *Thalictrum* species [5]. Thalidastine, however, has been isolated from only two other species of *Thalictrum*, namely *T. fendleri* Engelm. ex Gray and *T. foliolosum* DC. and has not been found in any other genera of higher plants [5]. The genus *Thalictrum* remains a uniquely prolific source of benzylisoquinoline-derived alkaloids of varying biological activities, with approximately 220 different alkaloids having been described [1, 2, 5].

EXPERIMENTAL

General. Methods, equipment and chemicals have been previously described [6].

Plant material. The plant material used in this study was acquired by Professor Z.-C. Lou, Department of Pharmacognosy, School of Pharmacy, Beijing Medical University, Beijing, 100083, People's Republic of China, from a collection in the mountainous areas of the Yunnan province of China. A herbarium specimen is on deposit at the Department of Pharmacognosy, School of Pharmacy, Beijing Medical University, Beijing, 100083, People's Republic of China.

Extraction and fractionation. Powdered, dried roots (970 g) were extracted by percolation with petrol (6.6 l) followed by EtOH (80 l). The EtOH extract was concd to a viscous residue (113 g) and partitioned between Et_2O (2 l.) and aqueous tartaric acid (1%) (2 l) (×2). The acidic layer was basified with NH_4OH to pH 8–9 and extracted with Et_2O (4 l) (×4). The remaining aqueous layer was acidified to pH 2–3 with HCl and treated with a saturated solution of ammonium reineckate (Reinecke Salt) until pptn ceased. The precipitate was filtered by suction, washed with H_2O (500 ml), and stirred with MeOH (3 l) for 3 h. The resulting suspension was filtered and and the filtrate passed through an anion exchange resin (IRA-401S[Cl]) (300 g) to afford a dark residue (29.6 g) (Fraction A). The insoluble yellow solid remaining from the filtration was suspended in MeOH (2 l), shaken with anion exchange resin (IRA-401S[Cl]) (200 g) for 96 hr, filtered, and the filtrate evapd to afford a yellow residue (2.9 g) (Fraction B).

Chromatography of fraction A. Fraction A (29.6 g) was dissolved in MeOH (100 ml), adsorbed onto silicic acid (30 g) and chromatographed over a column of silicic acid (200 g) (Column A). Elution with $CHCl_3$–MeOH (19:1) (5 l) afforded a dark residue (1.66 g) and was followed by elution with $CHCl_3$–MeOH (19:12) (1.8 l) to yield a dark, semi-solid mass (3.78 g) (Fraction C).

Isolation of magnoflorine. Elution of column A with $CHCl_3$–MeOH (19:10) (2.4 l) yielded a brown, semi-crystalline

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residue (15.29 g), which on treatment with hot MeOH deposited pale brown needles. Several crystallizations from MeOH furnished magnoflorine chloride as white needles (247 mg), mp 219–221°; mp 248–250° (iodide salt); $[\alpha]_D^{23} = 203°$ (MeOH; *c* 0.92) (iodide salt), identical by direct comparison (UV, IR, mp, MS, $[\alpha]_D$ with an authentic sample [6].

Chromatography of fraction C. Fraction C (3.78 g) was dissolved in $CHCl_3$–MeOH–NH_4OH (17:4:1) (20 ml) and chromatographed over a column of Si gel (300 g) (Column B). Elution with the same solvent (600 ml) afforded a residue (165 mg). Continued elution (1.2 l.) gave a dark brown residue (418 mg) which was rechromatographed over Si gel (50 g) (Column C) in $CHCl_3$–MeOH–NH_4OH (60:20:1).

Isolation of palmatine. Continued elution with $CHCl_3$–MeOH–NH_4OH (60:20:1) (100 ml) afforded a yellow residue which was dissolved in MeOH (20 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (20 g) in MeOH to afford palmatine chloride (27 mg) as yellow needles, mp 248–250°, identical by direct comparison (UV, IR, mp) with an authentic sample prepared from the iodide salt [6] via passage over anion exchange resin (IRA-401S[Cl]).

Isolation of berberine. Continued elution with the same solvent (150 ml) afforded a yellow crystalline residue which was dissolved in MeOH (50 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (50 g) in MeOH to afford yellow needles of berberine chloride (242 mg), mp 203–205°, identical by direct comparison (UV, IR, mp) with an authentic sample prepared from the iodide salt [6] via passage over anion exchange resin (IRA-401S[Cl]).

Isolation of columbamine. Continued elution of Column C with the same solvent (100 ml) gave a yellow mass which was dissolved in MeOH (20 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (20 g) in MeOH to afford yellow needles of columbamine chloride (27 mg), mp 240–242°, identical by direct comparison (UV, IR, mp) with an authentic sample prepared from the iodide salt [6] via passage over anion exchange resin (IRA-401S[Cl]).

Isolation of thalifendine. Continued elution of Column C with the same solvent (150 ml) afforded a dark residue which was dissolved in MeOH (20 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (20 g) in MeOH to afford yellow needles of thalifendine chloride (14 mg), mp 230–233°, identical by direct comparison (UV, IR, mp) with an authentic sample [6].

Isolation of jatrorrhizine. Continued elution of Column B with $CHCl_3$–MeOH–NH_4OH (17:4:1) (600 ml) afforded a dark residue (350 mg) which was rechromatographed over Si gel (50 g) (Column D) in $CHCl_3$–MeOH–NH_4OH (280:80:1). Elution of column D with the same solvent (100 ml) afforded an orange crystalline residue which was dissolved in MeOH (20 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (20 g) in MeOH to give jatrorrhizine chloride (15 mg), mp 205–206°, identical by direct comparison (UV, IR, mp) with an authentic sample prepared from the iodide salt [6] via passage over anion exchange resin (IRA-401S[Cl]).

Isolation of thalidastine. Continued elution of column D with same solvent (100 ml) gave a dark residue which was dissolved in MeOH (20 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (20 g) in MeOH to afford yellow needles of (+)-thalidastine chloride (23 mg), mp 236–238° dec., $[\alpha]_D^{23} + 173°$ (MeOH; *c* 0.17); identical by direct comparison (UV, IR, mp) with authentic sample [6].

Chromatography of fraction B. Fraction B (2.9 g) was dissolved in MeOH (20 ml), adsorbed onto Si gel (5 g), and chromatographed over a column (Column E) of Si gel (50 g) in $CHCl_3$.

Isolation of berberine. Elution of Column E with $CHCl_3$–MeOH (49:1) (5 l) afforded an alkaloid-negative residue (34 mg). Elution with $CHCl_3$–MeOH (19:1) (7 l) afforded a bright yellow residue which was dissolved in MeOH (75 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (75 g) in MeOH to give yellow needles (847 mg) of berberine chloride identified in the same manner as previously described.

Isolation of thalfifendine. Elution of Column E with $CHCl_3$–MeOH (90:1) (2 l) afforded a dark residue which was dissolved in MeOH (10 ml) and passed over an anion exchange resin (IRA-401S[Cl]) (10 g) in MeOH to give thalifendine chloride as yellow needles (6 mg), identified in the same manner as previously described.

Isolation of magnoflorine. Elution of Column E with $CHCl_3$–MeOH (9:5) (2.5 l) gave a semicrystalline brown mass which on treatment with MeOH–HCl (50:1) finished pale needles of magnoflorine chloride (203 mg), identified in the same manner as previously described.

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