H-I-2). 6.20 (2 H, *s*, H-I-6,8), 6.30 (1 H, *s*, H-II-6), 6.50 (1 H, *s*, H-II-3), 6.60 (2 H, *d*, $J = 8$ Hz, H-II-3′, 5′), 6.98 (1H, *d*, $J = 8$ Hz, H-I-5′), 7.10–7.40 (4H, *m*, H-I-2′, 6′; H-II-2′, 6′).

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REFERENCES

1. Perry, L. M. and Metzger, J. (eds) (1980) *Medicinal Plants of East and South-East Asia*, p. 175. MIT Press, London.
2. Iwu, M. M. (1985) *Experientia* **41**, 699.
3. Yates, P. and Stout, G. H. (1958) *J. Am. Chem. Soc.* **80**, 1691.
4. Karanjagaokan, C. G., Rama Rao, A. V., Venkataraman, K., Yemul, S. S. and Palmer, K. J. (1973) *Tetrahedron Letters* 4977.
5. Jackson, B., Locksley, H. D., Scheinmann, F. and Wolstenholme, W. A. (1971) *J. Chem. Soc. C* 3791.
6. Ampofo, S. A. and Waterman, P. G. (1986) *Phytochemistry* **10**, 2351.
7. Locksley, H. D. (1973) *The Chemistry of Biflavanoid Compounds in Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H. and Kirby, G. W., eds) Vol. 30, p. 275. Springer, New York.
8. Markham, K. R. and Mohanchari, V. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 19. Chapman & Hall, London.
9. Karanjagaokan, C. H., Radhakrishnan, P. V. and Venkataraman, K. (1967) *Tetrahedron Letters* 3195.
10. Crichton, E. G. and Waterman, P. G. (1979) *Phytochemistry* **18**, 1553.

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ALKALOIDS OF *THALICTRUM LANKESTERI*

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Key Word Index—*Thalictrum lankesteri*; Ranunculaceae; above-ground portion; bisbenzylisoquinoline alkaloid; hernandezine; protoberberine alkaloids.

Abstract—The isolation and identification of six alkaloids from an extract of the above-ground parts of *Thalictrum lankesteri* Standl. (Ranunculaceae) are described. The alkaloids isolated are the bisbenzylisoquinoline hernandezine, and the protoberberines berberine, palmatine, thalifendine, columbamine, and jatrorrhizine.

INTRODUCTION

Thalictrum lankesteri Standl. (Ranunculaceae), a perennial herb indigenous to Central America with a history of folkloric use as a medicinal plant, is a close botanical relative of other medicinally useful *Thalictrum* species [1]. Crude extracts of numerous species of *Thalictrum*, as well as pure alkaloids from these plants, have been used for centuries in the treatment of various ailments, some of which include cancer, jaundice, snakebite, rheumatism, and leprosy, as well as many other infections [2]. The genus *Thalictrum* is a well-known source of benzylisoquinoline-derived alkaloids, with over 220 of these bases (many possessing definable biological activities) having been isolated and identified [2]. It was decided to undertake a phytochemical investigation of the alkaloids of the above-ground parts of *Thalictrum lankesteri* because of the absence of reports in the literature concerning this species and because of the proclivity of the genus as a source of biologically active benzylisoquinoline-derived alkaloids. This paper reports the isolation of one non-quaternary and five quaternary alkaloids from an extract of the above-ground parts of *T. lankesteri*, a species indigenous to Costa Rica. These alkaloids include the bisbenzylisoquinoline hernandezine and the protoberberines berberine, palmatine, thalifendine, columbamine, and jatrorrhizine.

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RESULTS AND DISCUSSION

This is the first report of alkaloids in this species. Hernandezine has been shown to possess hypotensive, anti-inflammatory, hypothermic, and *in vitro* antimicrobial activities. In addition, the alkaloid was observed to produce an inhibition of conditioned avoidance reactions and motor-conditioned reflexes in rodents [2]. Berberine, palmatine, columbamine and jatrorrhizine have been shown to be inhibitors of *in vitro* microbial growth against a wide variety of organisms, some of which include *Mycobacterium smegmatis*, *Candida albicans*, *Saccharomyces carlsbergensis*, *Staphylococcus aureus*, and

Klebsiella pneumoniae [2]. Berberine, jatrorrhizine and columbamine are weak inhibitors of butyrylcholinesterase activity in human serum [2]. Parenteral administration of jatrorrhizine failed to protect rodents from drug-induced hypoxia but did inhibit chloroform-induced myocardial arrhythmias [2]. Berberine and palmatine decreased the adrenal concentration of ascorbic acid in rats after parenteral administration and inhibited the action of rabbit spleen cholinesterase and horse serum pseudocholinesterase [2]. Berberine was noted to have choleretic properties, to inhibit immune hemolysis, and to act as a myocardial stimulant, with vagal blocking effects [2]. Finally, berberine was observed to possess hypotensive, ileal spasmogenic, sedative, alpha-adrenergic blocking, and anti-curariform properties [2].

Hernandezine has been isolated from at least six other species of *Thalictrum* but has not been found outside of this genus [2–5]. Berberine has been isolated from at least 33 other species of *Thalictrum* [2, 6, 7] while palmatine, columbamine and jatrorrhizine have been isolated from 12–16 additional *Thalictrum* species [2, 6, 7].

EXPERIMENTAL

General. Methods, equipment and chemicals have been described in ref. [8].

Plant material. The plant material used in this study was collected by Dr Luis J. Poveda in Tejar, Cartago, Costa Rica in 1986. A herbarium specimen is on deposit at the Museo Nacional, San Jose, Costa Rica.

Extraction and fractionation. Powdered, dried above-ground parts (0.9 kg) were extracted in a Soxhlet apparatus with petrol for 20 hr. The dried, defatted plant material was subsequently exhaustively extracted by percolation with EtOH. The EtOH extract was concd to a viscous residue (*ca* 70 g) and partitioned between aq. citric acid (2%) (1 l.) and EtOAc (300 ml) (× 4). The acidic layer was basified with NH_4OH to pH 9–10 and extracted with Et_2O (300 ml) (× 4). The Et_2O extracts were combined, dried (dry Na_2SO_4), filtered and evapd to afford a residue (0.5 g) (fraction A). The remaining aq. layer was acidified to pH 2–3 with HCl and treated with Mayer's Reagent until pptn ceased. The ppt. was filtered by suction, washed with H_2O, suspended in MeOH (200 ml), and shaken with anion exchange resin (IRA-400 [I]) (50 g) for 48 hr. The mixture was filtered and the remaining resin rinsed with MeOH. The filtrate and the MeOH rinsings were combined and evapd to afford a dark yellow residue (2 g) (Fraction B).

Chromatography of fraction A. Fraction A (0.5 g) was dissolved in $CHCl_3$ (30 ml) and placed on a column of silica gel G 60 (230–400 mesh) (30 g) in $CHCl_3$. Elution was begun with $CHCl_3$ (100 ml), followed by a MeOH gradient (2, 4, 6, 10 and 20%), and finally MeOH. Five fractions of 20 ml each were collected for each eluent.

Isolation of hernandezine. Elution with $CHCl_3$–MeOH (49:1) and (24:1) afforded fractions which were combined to afford a crystalline residue. Treatment of this residue with MeOH afforded white needles of hernandezine (15 mg), which was recrystallized from Me_2CO as fine white needles, mp 120–122°, $[\alpha]_D^{23} = +234°$ ($CHCl_3$; *c* 0.195), identical by direct comparison (UV, IR, mp, $[\alpha]_D$) with an authentic sample. Only trace amounts of other alkaloids were detected in this and subsequent column fractions.

Chromatography of fraction B. Fraction B (2 g) was dissolved in MeOH (50 ml), adsorbed on to Si gel (5 g), and placed on a column of acid-washed silica gel (60 g) in $CHCl_3$. Elution was begun with $CHCl_3$ (75 ml), followed by $CHCl_3$–MeOH mixtures (5, 10, 15, 20 and 50%) (75 ml each) and MeOH (75 ml). Combination of the 15 and 20% MeOH fractions afforded a residue (138 mg) which was dissolved in $CHCl_3$–MeOH–NH_4OH (30:10:1) (5 ml) and chromatographed over kieselgel (70–230 mesh) (20 g) in the same solvent.

Isolation of berberine. Elution with the same solvent (25 ml) afforded a yellow residue which was dissolved in MeOH (10 ml) and passed over an anion exchange resin (IRA-400 [Cl]) (10 g) in MeOH to afford yellow needles of berberine chloride (6 mg), mp 204–205°, identical by direct comparison (UV, IR, mp) with an authentic sample [6].

Isolation of palmatine. Continued elution with the same solvent (25 ml) afforded a mixture of berberine and a second alkaloid. Further elution with this solvent (25 ml) gave a yellow residue which was dissolved in MeOH (10 ml) and passed over an anion exchange resin (IRA-400 [Cl]) (10 g) in MeOH to afford yellow needles of palmatine chloride (12 mg), mp 247–249°, identical by direct comparison (UV, IR, mp) with an authentic sample [6].

Isolation of thalifendine. Continued elution with the same solvent (25 ml) afforded a yellow residue which was dissolved in MeOH (10 ml) and passed over an anion exchange resin (IRA-400 [Cl]) (10 g) to yield yellow needles of thalifendine chloride (3 mg), mp 231–233°, identical by direct comparison (UV, IR, mp) with an authentic sample [6].

Isolation of columbamine. Continued elution with the same solvent (25 ml) afforded a mixture of thalifendine and a second alkaloid. Further elution (25 ml) gave a yellow residue which was dissolved in MeOH (10 ml) and passed over an anion exchange resin (IRA-400 [Cl]) (10 g) to afford yellow needles of columbamine chloride (8 mg), mp 240–243°, identical by direct comparison (UV, IR, mp) with an authentic sample [6].

Isolation of jatrorrhizine. Continued elution with the same solvent (50 ml) afforded a mixture of columbamine and a second alkaloid. Further elution (100 ml) yielded a red-orange residue which was dissolved in MeOH (10 ml) and passed over an anion exchange resin (IRA-400 [Cl]) (10 g) to afford orange needles of jatrorrhizine chloride (6 mg), mp 204–206°, identical by direct comparison (UV, IR, mp) with an authentic sample [6].

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REFERENCES

1. Marroquin, H. F. (1957) *Enfermedades De Los Conquistadores*, p. 115. Ministerio De Cultura Departamento Editorial, San Salvador, El Salvador.
2. Schiff, P. L., Jr. (1987) in *Alkaloids: Chemical and Biological Perspectives* (Pelletier, S. W., ed.), pp. 271–637. Wiley, New York.
3. Guha, K. P., Mukherjee, B. and Mukherjee, R. (1979) *J. Nat. Prod.* **42**, 1.
4. Schiff, P. L., Jr. (1983) *J. Nat. Prod.* **46**, 1.
5. Schiff, P. L., Jr. (1987) *J. Nat. Prod.* **50**, 529.
6. Gao, C.-Y., Lou, Z.-C., Lin, F.-T., Lin, M. C. and Schiff, P. L., Jr. (1987) *Phytochemistry* **26**, 3003.
7. Lou, Z.-C., Gao, C.-Y., Lin, F.-T., Lin, M.-C., Zhang, J., Slatkin, D. J. and Schiff, P. L., Jr. (1988) *Planta Med.* **53**, 498.
8. Chattopadhyay, S. K., Ray, A. B., Slatkin, D. J. and Schiff, P. L., Jr. (1983) *Phytochemistry* **22**, 2607.