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MOLLINEDINE, A NEW ALKALOID FROM *MOLLINEDIA COSTARICENSIS*

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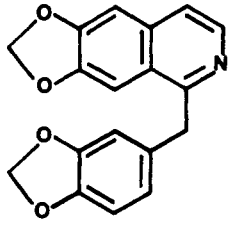
ABSTRACT.—*Mollinedia costaricensis* is a plant indigenous to Central America, where it has been used as a medicinal. Chromatography of the alkaloidal fraction of an EtOH extract of the roots over Si gel afforded mollinedine, which was characterized as a new benzylisoquinoline alkaloid by a consideration of physicochemical and spectral data, as well as by conversion to escholamine and preparation from papaverine. Although mollinedine failed to exhibit any significant inhibitory activity against the in vitro growth of a series of standard bacteria and fungi, preliminary pharmacological testing in rats and guinea pigs demonstrated an inhibitory effect against ouabain-induced and $CaCl_2$-induced myocardial arrhythmias.

Mollinedia costaricensis Donn. Sm. (Monimiaceae) is a plant indigenous to Central America where it has been used as a medicinal.[1] An infusion of the boiled leaves of the related species *Mollinedia guatemalensis* has been employed in the therapy of stomachache and is known by the common name of "sac-e-yen" (1). After an EtOH extract of *M. costaricensis* tested positive for alkaloids in our laboratories utilizing common screening techniques (2), it was decided to undertake a phytochemical investigation of this plant. This investigation was prompted by the knowledge that the family Monimiaceae is a rich source of benzylisoquinoline-derived alkaloids (3,4), coupled with the absence of phytochemical literature references to the genus *Mollinedia*.

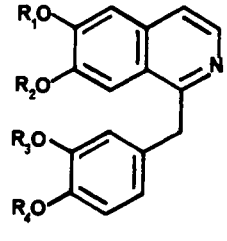
RESULTS AND DISCUSSION

The wood of *M. costaricensis* was dried, ground, and extracted with EtOH. The concentrated EtOH extract was suspended in H_2O, acidified with dilute HCl, and extracted with Et_2O (fraction A). The acidic layer was alkalinized with NH_4OH and extracted with Et_2O (fraction B). Chromatography of fraction B over Si gel in $CHCl_3$ and



1

2 **1**-MeI salt



3 $R_1=R_2=R_3=R_4=Me$
4 $R_1=Me$, $R_2=R_3=R_4=H$
5 $R_1=R_2=R_3=R_4=H$

[1]Personal communication, Dr. Jose A. Lopez, Facultad de Farmacia, Universidad de Costa Rica, Costa Rica.

elution with $CHCl_3$ followed by a $CHCl_3$/MeOH gradient, afforded alkaloid-positive fractions. Rechromatography of those fractions eluted with $CHCl_3$-MeOH (95:5) over Si gel and elution with $CHCl_3$-MeOH (49:1) afforded mollinedine [**1**] as tan needles from MeOH, mp 158–160°. The uv spectrum showed maxima at 329 nm (log ε 3.52), 315 (3.41), 285 (3.65), and 237 (4.56) with a bathochromic shift in acidic medium to 341 nm (log ε 3.61), 327 (3.64), 308 (3.67), 295 (3.64), and 247 (4.56), and was, thus, characteristic of a benzylisoquinoline alkaloid (5).

The ir spectrum (KBr) of **1** showed strong aromatic absorption bands at 1585 and 1500 cm^{-1}. The ^{1}H-nmr spectrum (300 MHz) (TFA-*d*) indicated the presence of one biaryl methylene group as a two-proton singlet at δ 4.68, two methylenedioxy groups as a pair of two-proton singlets at δ 5.96 and 6.26, and seven aromatic protons. The aromatic protons were observed as a broad one-proton singlet at 6.75, two sharp one-proton singlets at 7.35 and 7.66, and a pair of two-proton double doublets, the first of which was found at 6.78 and 6.88 ($J = 7.9$ Hz) while the second was observed at 7.89 and 7.99 ($J = 6.3$ Hz). The ^{13}C-nmr spectrum (75.46 MHz; $CDCl_3$ + traces TFA-*d*; ^{1}H decoupled) indicated the presence of a biaryl methylene carbon at δ 37.4, two methylenedioxy methylene carbons at 101.7 and 102.6, four methylenedioxy aromatic carbons at 148.9, 152.2, 154.4, and 156.5, and eleven other aromatic carbons in the range of 103.9 and 160.6.

The ei mass spectrum of **1** showed the molecular ion at *m/z* 307 (48%) (observed 307.0767, calculated 307.0766 for $C_{18}H_{13}O_4N$) and the base peak at *m/z* 306, with other smaller fragment ions at *m/z* 278 (8%), 248 (18), 191 (6), and 190 (7). These spectral data were characteristic of a papaverine-like benzylisoquinoline alkaloid containing one methylenedioxy group at the C-6–C-7 position and a second methylenedioxy group at the C-3′–C-4′ position (5). Treatment of mollinedine with MeI afforded mollinedine methiodide (*N*-methylmollinedine iodide), which was found to be identical to escholamine iodide [**2**] (6) by direct comparison (uv, ir, mp, mmp). Finally, a consideration of the 300-MHz ^{1}H-^{1}H homonuclear two-dimensional correlation spectrum (2D-COSY) (TFA-*d*; Figure 1) confirmed the structure as expected.

The quaternary alkaloid escholamine was first isolated from an *Eschscholtzia* sp., most likely *Eschscholtzia oregana* Greene (Papaveraceae), in 1966 (6) and apparently has not been reported in any other plant to date. To our knowledge, this is the first reported isolation of the tertiary parent alkaloid of escholamine, and we have assigned the trivial name of mollinedine to this tertiary base.

Mollinedine was synthesized to corroborate further the assigned structure and to prepare an additional amount of alkaloid for potential pharmacological screening. Papaverine [**3**] was first demethylated to 6-methoxy-7,3′,4′-trihydroxypapaverine [**4**] by refluxing for 1 h with 48% HBr (7). An additional 8 h of refluxing of the triphenol **4** with 48% HBr afforded papaveroline [**5**] (7). Finally, methylenation of papaveroline with CH_2Br_2 in DMF in the presence of excess CsF (8) afforded mollinedine [**1**], mp 179–180° (after three recrystallizations from MeOH) [other mp reported in the literature included 168–170° (C_6H_6) (9), 168–169° (petroleum ether-Et_2O) (10), and 170–172° (C_6H_6) (11)].

The benzylisoquinoline alkaloids are of two types, either the more commonly occurring benzyltetrahydroisoquinoline type (1,2,3,4-tetrahydro-) or the less commonly occurring benzylisoquinoline type (fully aromatic). Eleven benzylisoquinoline alkaloids have been isolated from seven genera within six families. These alkaloids include berbithine (6,7,3′,4′,5′-pentaoxygenated tertiary base) from *Berberis actinacantha* Mart. ex Schult. (Berberidaceae) (12), cristadine (6,7,3′,4′-tetraoxygenated tertiary base) from *Erythrina crista-galli* L. (Leguminosae) (13), escholamidine (6,7,3′,4′-tetraoxygenated quaternary base) from *Eschscholtzia oregana* Greene

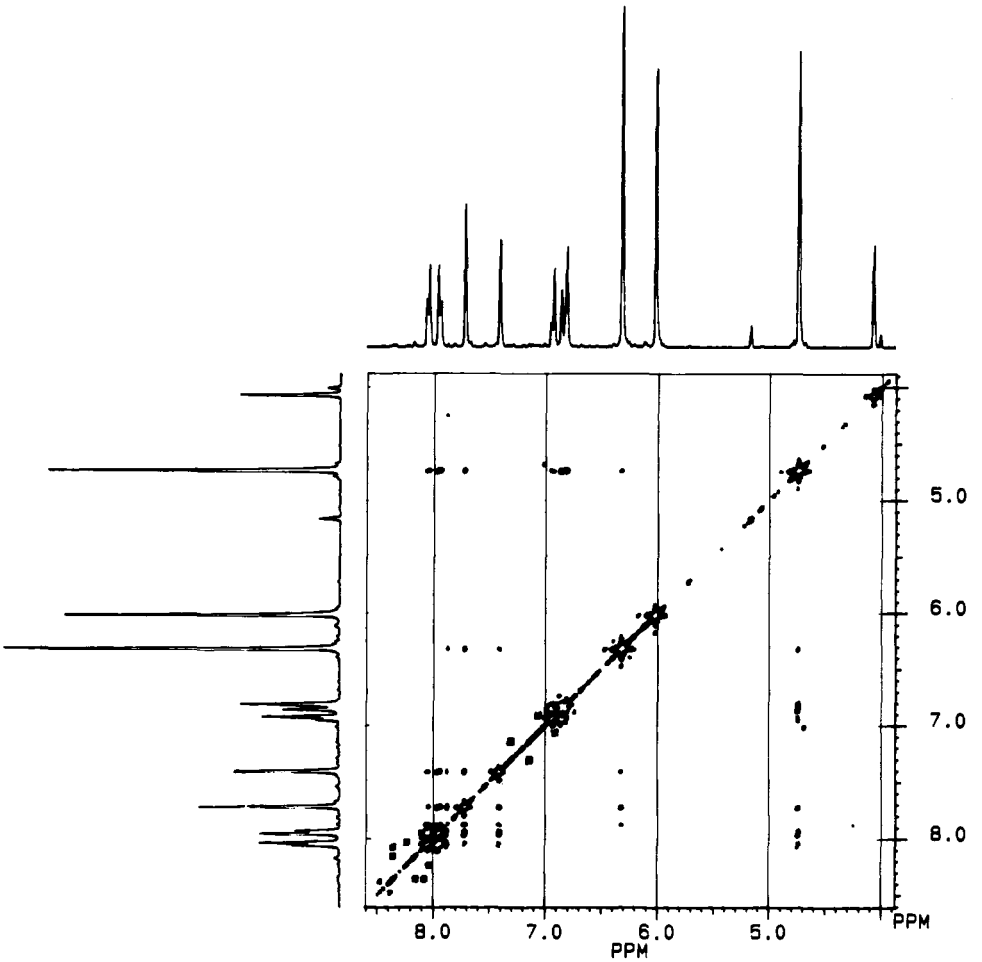


FIGURE 1. 2D-COSY Spectrum of mollinedine [1].

(Papaveraceae) (14), escholamine (6,7,3′,4′-tetraoxygenated quaternary base) from *E. oregana* Greene (Papaveraceae) (14), glycomarine (also known as sevanine-β-D-glucoside) (6,7,3′,4′-tetraoxygenated tertiary base) from *Papaver arenarium* (Papaveraceae) (15), isosevanine (6,7,3′,4′-tetraoxygenated tertiary base) from *Hedycarya angustifolia* A. Cunn. (Monimiaceae) (16), *N*-methylpalaudinium salt (6,7,3′,4′-tetraoxygenated quaternary base) from *Thalictrum polygamum* Muhl. (Ranunculaceae) (17), palaudine (6,7,3′,4′-tetraoxygenated tertiary base) from *Papaver somniferum* L. (Papaveraceae) (18), sevanine (6,7,3′,4′-tetraoxygenated tertiary base) from *Papaver macrostomum* Boiss. et Huet (Papaveraceae) (19), takatonine (5,6,7,4′-tetraoxygenated quaternary base) from *Thalictrum thunbergii* DC. (Ranunculaceae) and *Thalictrum minus* L. var. *microphyllum* Boiss. (Ranunculaceae) (20), and yuzirine (6,7,4′-trioxygenated tertiary base) from *Zizyphus jujuba* (Rhamnaceae) (21). In addition, it is also interesting to note that at least four benzylisoquinolone alkaloids have been isolated to date from higher plants. These alkaloids include gandharamine (6,7,4′-trioxygenated quaternary base) from *Berberis baluchistanica* Ahrendt (Berberidaceae) (22) and from *Thalictrum fendleri* Engelm. ex Gray (Ranunculaceae) (22), *N*-methylpapaveraldine (6,7,3′,4′-tetraoxygenated quaternary base) from *Stephania sasakii* Hayata (Menispermaceae) (23), rugosinone (6,7,2′,3′,4′-pentaoxygenated tertiary base) from *Thalictrum rugosum* Ait. (Ranunculaceae) (24), and thalmicrinone (5,6,7,4′-

tetraoxygenated tertiary base) from *Thalictrum minus* L. var. *microphyllum* Boiss. (Ranunculaceae) (25).

Finally, although mollinedine failed to exhibit any significant in vitro inhibitory activity on the growth of a series of standard bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Mycobacterium smegmatis*) and fungi (*Cryptococcus neoformans, Saccharomyces cerevisiae, Aspergillus flavus, Aspergillus fumigatus, Trichophyton mentagrophytes, Candida albicans*), preliminary pharmacological testing in rats and guinea pigs demonstrated an inhibitory effect against ouabain-induced and $CaCl_2$-induced myocardial arrhythmias.

An extract of the leaves of *M. costaricensis* failed to demonstrate the presence of alkaloids but resulted in the isolation of the flavone vitexin, mp 260°, identical by direct comparison (mp, ir) with an authentic sample and by comparison (uv, optical rotation) with literature values (26).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Methods and equipment used for chromatography and spectrometry have been described previously (27,28).

PLANT MATERIAL.—The plant material used in this study was collected at the shore of Laguna Rio Cuarto, Heredia, in the late summer of 1986, and was identified by Dr. Jorge Gómez Laurito. A herbarium specimen is on deposit at the herbarium of the National Museum, San José, Costa Rica.

EXTRACTION AND ISOLATION.—Powdered, dried wood (2.15 kg) of *M. costaricensis* was extracted by percolation with EtOH (95%) to exhaustion. The extract residue (ca. 200 g) was suspended in H_2O (1 liter), acidified with HCl, and extracted with Et_2O (4 × 300 ml). The Et_2O layers were combined and evaporated to a residue (fraction A; 18 g). The acidic layer was alkalinized with NH_4OH and extracted with Et_2O (4 × 300 ml). The Et_2O layers were combined and evaporated to a residue (fraction B; 0.5 g).

CHROMATOGRAPHY OF FRACTION B.—Fraction B was dissolved in $CHCl_3$ (10 ml) and chromatographed over Si gel (25 g) in $CHCl_3$. Elution was with $CHCl_3$ followed by increasing proportions of MeOH in $CHCl_3$, and 25-ml fractions were collected.

ISOLATION OF MOLLINEDINE [**1**].—Two fractions eluted with $CHCl_3$-MeOH (95:5) were combined and rechromatographed over Si gel (10 g) in $CHCl_3$. Elution with $CHCl_3$-MeOH (95:5) afforded a residue, which on treatment with MeOH gave mollinedine [**1**] (13 mg) as cream-colored needles, mp 158–160°; R_f 0.76 in C_6H_6-Me_2CO-NH_4OH (1:1:0.1); uv λ max (MeOH) 329 nm (log ε 3.52), 315 (3.41), 285 (3.65), and 237 (4.56) with a bathochromic shift in 0.01 N methanolic HCl to 341 nm (log ε 3.61), 327 (3.64), 308 (3.67), 295 (3.64), and 247 (4.56). Ir ν max (KBr) 1632 cm^{-1} (w), 1620 (w), 1605 (w), 1585, 1500, 1492, 1469, 1443, 1421, 1379, 1359, 1335, 1300, 1275, 1255, 1230, 1219, 1195, 1179, 1030, 940, 920, 880, 860, 852, 835, 819, 809, 778, 755, 660; ^{1}H nmr (300 MHz, TFA-*d*), δ 4.68 (2H, s, CH_2, H-α), 5.96 (2H, s, CH_2O_2), 6.26 (2H, s, CH_2O_2), 6.75 (1H, s, H-2′), 6.78 (1H, d, J = 7.9 Hz, H-6′), 6.88 (1H, d, J = 7.9 Hz, H-5′), 7.35 (1H, s, H-5), 7.66 (1H, s, H-8), 7.89 (1H, d, J = 6.3 Hz, H-4), and 7.99 (1H, d, J = 6.3 Hz, H-3); ^{13}C nmr (75.46 MHz, $CDCl_3$ + traces of TFA-*d*, ^{1}H-decoupled), δ 37.4 (C-α), 101.7 (CH_2O_2), 102.6 (CH_2O_2), 148.9 + 152.2 + 154.4 + 156.5 (2 $ArCH_2O_2$), 103.9–160.6 (11 *Ar*C); hrms 307.0767, $C_{18}H_{13}O_4N$ requires 307.0766.

PREPARATION OF MOLLINEDINE METHIODIDE (*N*-METHYLMOLLINEDINE IODIDE) [**2**].—To mollinedine (5 mg) in Me_2CO (5 ml) was added MeI (0.1 ml), and the reaction mixture was allowed to stand at room temperature for 12 h. The solvent was evaporated and the resulting residue dissolved in $CHCl_3$-MeOH (9:1) and chromatographed over Si gel (1 g) in $CHCl_3$. Elution with $CHCl_3$-MeOH (9:1) and evaporation of the solvent afforded a residue which was crystallized from MeOH to afford mollinedine methiodide (*N*-methylmollinedine iodide) as pale yellow needles, mp 273–274°, which was identical to escholamine iodide (6) by direct comparison (uv, ir, mp).

PREPARATION OF MOLLINEDINE [**1**].—Papaverine [**3**] (5 g) was dissolved in HBr (48%, 50 ml) and refluxed for 1 h. The resulting precipitate was filtered by suction, washed with H_2O, and dried to afford 6-methoxy-7,3′,4′-trihydroxypapaverine HBr [**4**] (4.76 g, 95%), mp 248°; ^{1}H nmr (100 MHz, DMSO-d_6, free base), δ 4.04 (3H, s, OMe), 4.55 (2H, s, ArCH_2Ar, H-α), 6.47–6.73 (3H, m, H-2′, H-5′, H-6′), 7.67 (1H, s, H-5 or H-8), 7.70 (1H, s, H-5 or H-8), 8.10 (1H, d, J = 6.6 Hz, H-4), 8.35 (1H, d, J = 6.6 Hz, H-3), 8.92 (br, OH), identical to an authentic reference sample by direct comparison (mp, mmp, ir). Triphenol **4** (4.76 g) was dissolved in 48% HBr (850 ml) and refluxed for 8 h. The resulting solution was

cooled to room temperature and allowed to stand for 12 h at refrigerator temperature. The resulting crystals were filtered by suction, washed with H_2O, and dried under vacuum at 50° to afford papaveroline HBr [**5**] as pale brown crystals (3.66 g, 80% yield), mp 300° after charring at 270°; ^{1}H nmr (300 MHz, MeOH-d_4, free base), δ 4.61 (2H, s, ArCH_2Ar, H-α), 6.58 and 6.60 (1H, dd, J = 2.1 and 8.1 Hz, H-6′), 6.67 (1H, d, J = 1.98 Hz, H-2′), 6.75 (1H, d, J = 8.1 Hz, H-5′), 7.40 (1H, s, H-5), 7.72 (1H, s, H-8), 7.95 (1H, d, J = 6.6 Hz, H-4), 8.09 (1H, d, J = 6.6 Hz, H-3), identical to an authentic reference sample (7) by direct comparison (mp, mmp, ir). To papaveroline [**5**] (1 g, 0.0035 mol) in anhydrous DMF (30 ml) was added anhydrous CsF (19 g, 0.125 mol), and the mixture was shaken for 15 min (8). The reaction was allowed to cool to room temperature, and dibromomethane (0.8 g, 0.004 mol) was added. The resulting mixture was heated at 110–120° for 1.5 h and cooled to room temperature. The mixture was treated with ice H_2O (30 ml) and extracted with Et_2O (4 × 60 ml). The Et_2O extracts were pooled and extracted first with H_2O (200 ml) and then with aqueous NaOH (1%, 200 ml). The remaining Et_2O extract was washed with H_2O (200 ml), dried (anhydrous $MgSO_4$), filtered, and evaporated to a crystalline residue. Treatment of the residue with C_6H_6/Et_2O afforded mollinedine [**1**] (0.35 g, 32% yield), mp 179–180° (3 recrystallizations from MeOH) identical with the natural product by direct comparison (uv, ir, ^{1}H nmr, ms).

ACKNOWLEDGMENTS

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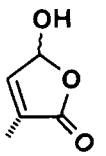
ERRATUM

Peter G. Waterman has requested the following correction for the paper entitled "Limonoids, Alkaloids, and a Coumarin from the Root and Stem Barks of *Tetradium glabrifolium*," *J. Nat. Prod.*, **50**, 1160 (1987).

On page 1161 the structure numbers **2** and **3** should be transposed and should appear as follows:

2 R=



3 R=