A Benzylisoquinoline Alkaloid from *Doryphora sassafras*

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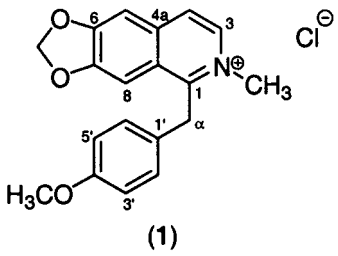
Chemical investigation of the Australian rainforest plant *Doryphora sassafras* has resulted in the isolation of a new natural product, 2-methyl-1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinolinium chloride (**1**). The iodide salt of compound **1** has previously been synthesized but only partially characterized. This paper reports the full spectroscopic characterization of **1** by MS, IR, UV, and NMR data.

The κ opioid receptor appears to play an important role in sensory integration including visual, auditory, olfactory, and nociceptive processing. Our interest in finding novel treatments for various pain conditions led us to screen extracts against the κ opioid receptor. As part of our continuing HTS drug discovery program, an extract from *Doryphora sassafras* was targeted for chemical evaluation, as it displayed κ opioid receptor affinity. Benzylisoquinolines have been reported from many primitive flowering plant families, such as the Annonaceae, Lauraceae, and Monimiaceae. The genus *Doryphora* (Monimiaceae) has been the source of several isoquinolines, aporphines, and bisbenzylisoquinolines.[1,2] To date, reticuline is the only benzylisoquinoline that has been isolated from a *Doryphora* species. This paper reports on the isolation and structural elucidation of the plant alkaloid 2-methyl-1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinolinium chloride (**1**). The alkaloid showed only weak binding to the κ opioid receptor. The demethyl analogue of **1** had previously been isolated from *Ocotea* sp. (Lauraceae) and in a later paper had been *N*-methylated using MeI and MeOH under refluxing conditions to afford **1** as its iodide salt.[3,4] This is the first reported isolation of compound **1** from a natural source.



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The CH_2Cl_2 extract from the dried leaves of *D. sassafras* was initially chromatographed on a C_{18} bonded silica flash column using a H_2O/MeOH gradient. Further purification on a silica flash column using CH_2Cl_2 and increasing amounts of MeOH followed by partitioning between $CHCl_3$ and aqueous HCl afforded the benzylisoquinolinium alkaloid **1**. Compound **1** was obtained as a stable brown gum. The formula $C_{19}H_{18}NO_3$ was assigned to the molecular ion at *m*/*z* 308.1274 (Δ 2.3 ppm) in the (+)-HRESIMS for **1**.

A strong absorption band in the UV spectrum at 253 nm in conjunction with strong absorption bands in the IR spectrum at 1607, 1521, and 1466 cm^{-1} indicated that **1** was an oxygenated aromatic alkaloid. Since all 18 protons were visible in the ^1H NMR spectrum, compound **1** was established to be a quaternary alkaloid. Analysis of the COSY and ^1H NMR spectra suggested that the molecule contained a *p*-methoxy benzyl moiety as well as a two-proton spin system, which was indicative of the α and β protons of pyridine. Downfield singlets at δ 7.39 and 7.56 suggested two more aromatic protons.

Correlations observed in the HMQC and HMBC spectra indicated that the molecule contained 17 carbon signals (two sets of two carbons were coincident), including seven aromatic quaternary carbons (three were oxygenated aromatic carbons at δ 152.3, 155.6, and 159.3). Eight protonated aromatic carbons were identified: two were α to oxygenated aromatic carbons and attached to proton singlets (δ 104.1 and 102.9), two were α- and β-pyridine carbons (δ 137.7 and 123.7), and the remaining four were assigned to the protonated carbons of the *p*-methoxy phenyl group. A HMQC correlation from the three-proton singlet at δ 3.76 to a carbon at δ 55.4 indicated an aromatic methoxyl in the molecule. A methylenedioxy group was supported by a HMQC correlation from the two-proton singlet at δ 6.28 to a carbon at δ 103.7. An *N*-methyl group was assigned since correlations from a three-proton singlet at δ 4.54 were observed to a carbon at δ 46.6.

A *p*-methoxy benzyl group was supported by HMBC correlations from the methoxyl singlet (δ 3.76) and the two proton aromatic doublets at δ 6.92 and 6.83 to an oxygenated aromatic carbon at (δ 159.3). The benzylic proton signal at δ 4.87 showed HMBC correlations to three carbons (C-1′, C-2′, and C-6′) of the *p*-methoxy phenyl group.

The methylenedioxy proton singlet, δ 6.28, showed HMBC correlations to two downfield oxygenated aromatic carbons (δ 155.6 and 152.3). Correlations from the two aromatic singlets (δ 7.39 and 7.56) to the same oxygenated aromatic carbons indicated that these protons were *ortho* to the methylenedioxy group and *para* to each other. The singlet at δ 7.39 also showed a correlation to the β-pyridine carbon (δ 123.7), thus indicating an isoquinoline was present in the molecule. This assignment was further supported by correlations from the *N*-methyl proton at δ 4.54 to the α pyridine carbons at δ 155.2 and 137.7.

The *p*-methoxybenzyl moiety was attached to the isoquinoline at C-1 since correlations were observed from the benzylic proton δ 4.87 to the α- and β-pyridine carbons δ 155.2 and 126.8. Hence, structure **1** was assigned as 2-methyl-1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinolinium chloride. The previously published ^{13}C NMR data for the synthesized benzylisoquinoline compound **1** was in

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close agreement with our data.[4] However, the ^{13}C NMR chemical shifts for the quaternary carbons C-1 and C-7 had been misassigned for the synthetic metabolite. HMBC data obtained for the natural product were used to definitively assign these two carbons as resonating at δ 155.1 (C-1) and 152.1 (C-7).

Compound **1** was only weakly active in the κ opioid receptor binding assay, displaying 50 ± 9% inhibition of binding of the κ opioid agonist [^{3}H]U69593 to membranes from guinea pig cerebellum at 100 μM.

Experimental Section

General Experimental Procedures. The ^1H and 2D NMR spectra were recorded on a Varian 600 MHz Unity INOVA at 599.926 MHz for ^1H and 149.982 MHz for ^{13}C. The ^{13}C NMR spectrum was recorded on a Varian 500 MHz Unity INOVA at 499.923 MHz for ^1H and 124.981 MHz for ^{13}C. The ^1H and ^{13}C chemical shifts are expressed in ppm (δ) relative to TMS, but were referenced to residual proto-deutero solvent signals for $CDCl_3$. An open glass column loaded with either Alltech Davisil 30−40 μm 60 Å C_{18} or silica was used for the flash chromatography. All other general experimental procedures have been reported elsewhere.[5]

Plant Material. The leaves of *Doryphora sassafras* Endlicher (Monimiaceae) were collected during January of 1994 from Wilson's Peak, Queensland, Australia. A voucher specimen (No. 600632) has been lodged at the Queensland Herbarium.

Extraction and Isolation. The air-dried and ground leaves of *D. sassafras* (10 g dry wt) were exhaustively extracted with CH_2Cl_2 (2 × 200 mL), then evaporated to dryness under vacuum to yield a dark green gum (0.617 g). This material was chromatographed on a C_{18} bonded silica flash column (40 × 40 mm) using 10% stepwise elutions from 20% MeOH/80% H_2O to 100% MeOH to afford 9 fractions. Both fractions 7 and 8 were combined (0.110 g) and further purified on a silica flash column (40 × 40 mm) using 2% stepwise elutions from 100% CH_2Cl_2 to 20% MeOH/80% CH_2Cl_2. The fraction that eluted from the column in 15% MeOH/85% CH_2Cl_2 was dissolved in $CHCl_3$ and partitioned with aqueous HCl. The organic layer contained pure 2-methyl-1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinolinium chloride (**1**, 6.5 mg, 0.065% dry wt).

2-Methyl-1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinolinium chloride (**1**): brown gum; UV (MeOH) λ_{max} (log ϵ) 228 (4.18), 253 (4.43), 283 (3.60), 313 (3.78), 333 sh (3.70), 348 sh (3.60) nm; IR (NaCl) ν_{max} 1607, 1521, 1466, 1280, 1248, 1185, 1124, 1032, 934, 668 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.98 (1H, d, J = 6.6 Hz, H3), 8.09 (1H, d, J = 6.6 Hz, H4), 7.56 (1H, s, H8), 7.39 (1H, s, H5), 6.92 (2H, d, J = 9.0 Hz, H2′, H6′), 6.83 (2H, d, J = 9.0 Hz, H3′, H5′), 6.28 (2H, s, OCH_2O), 4.87 (2H, s, αH), 4.54 (3H, s, N-CH_3), 3.76 (3H, s, O−CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.3 (s, C-4′), 155.6 (s, C-6), 155.2 (s, C-1), 152.3 (s, C-7), 138.4 (s, C-4a), 137.7 (d, C-3), 129.0 (d, C-2′, C-6′), 126.2 (s, C8a), 124.9 (s, C-1′), 123.7 (d, C-4), 115.1 (d, C-3′, C5′), 104.1 (d, C-5), 103.7 (t, OCH_2O), 102.9 (d, C-8), 55.4 (q, OCH_3), 46.6 (q, NCH_3), 34.6 (t, C-α); (+)-LRESMS m/z (rel int) 308 (100%) [M, $C_{19}H_{18}NO_3$]$^+$; (+)-HRESMS m/z 308.1274 (calcd for $C_{19}H_{18}NO_3$ [M]$^+$ 308.1281, Δ −2.3 ppm).

κ Opioid Receptor Binding Assay. Membranes were prepared by rehydrating guinea pig brains (Institute of Medical and Veterinary Sciences, South Australia) in 50 mM Tris·HCl (pH 7.4 at 4 °C), and the cerebellum was dissected out. Cerebellum tissue was homogenized in a 7-fold volume of 50 mM Tris·HCl (pH 7.4 at 4 °C) and centrifuged at 20000*g* (JA-17 rotor, J2MI Beckman centrifuge) for 20 min at 4 °C. Pellets were resuspended in buffer, incubated at 37 °C for 45 min to remove endogenous ligands, and then washed twice in buffer. Pellets were pooled and resuspended at 200 mg/mL wet weight of tissue and stored at −70 °C until used. Incubations were performed in 210 μL total volume containing 1 nM [^{3}H]U69593 and 10 μM naloxone for determination of nonspecific binding and reactions started by the addition of 190 μg of membrane protein. Incubation was for 90 min at 23 °C. Reactions were stopped by rapid filtration (Tomtec Cell Harvester 96) onto GF/B filter mats presoaked with PEI (0.01%). Filter mats were dried and then counted in a Betaplate liquid scintillation counter (1205 Wallac). The potency of compound **1** was estimated from concentration response curves performed in duplicate with six treatment levels and a final DMSO concentration of 2%. Compounds were solubilized in 100% DMSO. Potency was determined by nonlinear least-squares analysis and model testing by analysis of variance (GraphPad Inplot 4.02).

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References and Notes

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