4-Hydroxylated Piperidines and N-Methyleuphococcinine (1-Methyl-3-granatanone) from Picea (Spruce) Species. Identification and Synthesis

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4-Hydroxylated Piperidines and *N*-Methyleuphococcinine (1-Methyl-3-granatanone) from *Picea* (Spruce) Species. Identification and Synthesis

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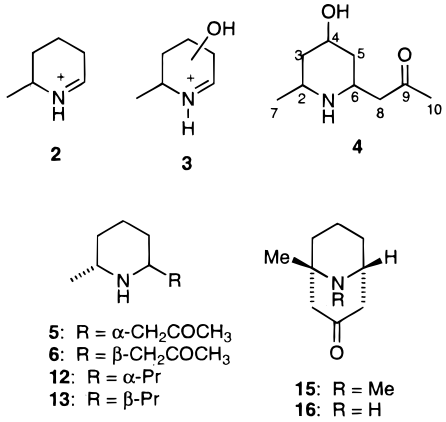
Three trace alkaloids from Colorado blue spruce, *Picea pungens*, were identified by synthesis and GC−MS comparisons as 4α-hydroxy-*cis*-2-methyl-6-(2-oxopropyl)piperidine (**1**), 4α-hydroxy-*cis*-2-methyl-6-propylpiperidine (**11**), and 1-methylgranatanone (**15**) (*N*-methyl-9-aza-1-methylbicyclo[3.3.1]nonane or *N*-methyleuphococcinine). Alkaloids **1** and **11** are the first among numerous known pine and spruce piperidines to contain a ring-oxygenated substituent.

Conifers have yielded a number of 2,6-disubstituted piperidines (currently numbering 12[1]), and several of these have also been identified from beetles.[2] Although oxygen functionalities have been found on the propyl side chain of these alkaloids, none has been reported that bears oxygen on the piperidine ring. This seems unusual since the biosynthesis proceeds from an acetate pathway[3] (presumably through a polyketide). The major alkaloids from several spruce species have been structurally characterized[1], but GCMS analysis of the crude alkaloid mixtures indicated the presence of a number of trace alkaloids. Analysis of the mass spectra suggested that two of these might possess piperidine ring oxygenation. The MS fragmentation pattern of another unknown suggested it contained an *N*-methyl group, a functionality so far not observed among the conifer 2,6-disubstituted piperidines. Rather than attempt to obtain these trace unknowns (less than 1% of the total alkaloid content) from large amounts of plant material, we elected to synthesize alkaloids whose structures were suggested by the mass spectral fragmentation patterns.

One unknown trace alkaloid, (**1**), *m*/z 171 in the mass spectrum, showed a base peak at *m*/*z* 114. Many of the conifer alkaloids show an *m*/*z* 98 base peak, due to fragment **2**. This suggested that the base peak of the unknown could be represented by fragment **3**. If the unknown contained a hydroxyl group in the piperidine ring, it was likely to be at C-4, in accordance with a polyketide precursor. This would leave a C_3H_5O fragment for the C-6 side chain and, in accord with an acetate origin, suggested structure **4** for the unknown. In support of this hypothesis, both **5** and **6** have been found in *Picea pungens*, Colorado blue spruce.

A diastereoselective total synthesis (Scheme 1) provided **1.** This alkaloid gave an identical retention time and fragmentation pattern in the GCMS to the *m*/*z* 171 unknown and coeluted with that unknown in a partially purified alkaloid mixture from *P. pungens*. The key step in the Scheme 1 synthesis was the Na/EtOH reduction of **9**, which produced **10** as the major diastereomer. GC−MS showed 94% of the total alkaloid content as **10** and 6% as a combination of three other diastereomers. In 2,6-disubstituted piperidines, the cis relationship of the substituents is assured by the ^{13}C resonances of δ 50−60 for C-2 and C-6, since a trans relationship typically results in resonances at δ 40−50.[4] Assignment of the C-4 OH stereochemistry was based upon an NOE enhancement of the H-4 δ 3.65 resonance for **1** when either H-2 or H-6 was irradiated. All the conifer 2,6-disubstituted alkaloids have the identical absolute configuration for the methyl at C-2, except for euphococcine (see below). Structure **1** therefore probably represents the absolute as well as relative stereochemistry at C-2, C-4, and C-6. GCMS analysis of the crude **10** to **1** oxidation reaction product (Scheme 1) also showed small amounts of a diketone and a 4-keto-9-hydroxypiperidine. Both of these might be expected to occur naturally, but neither has as yet been encountered.



A second trace unknown alkaloid (**11**) (*m*/*z* 157) also showed an MS fragment (*m*/*z* 114) consistent with **3**. If this alkaloid was also a 4-hydroxylated piperidine, the side chain at C-6 would be a simple propyl group. This is reasonable, since both **12** and **13** have been isolated from conifers. Alkaloid **11** was prepared by a two-step diastereoselective total synthesis (Scheme 2), and its identity with the natural alkaloid was again proven by GCMS (fragmentation and retention time) and co-injection with an isolate mixture containing the unknown. The ^{13}C NMR spectra of **14** and **11** showed (via the chemical shifts of the C-2 and C-6 resonances) that they were 2,6-cis-disubstituted. The reaction producing **11** gave a 96:4 ratio of the cis to trans isomers. The synthesis was based on that of Daly and co-workers,[5] who reported an 80/1 cis/trans ratio for a similar one-step, 25% yield preparation of 2-methyl-4-keto-6-nonylpiperidine. The reduction of **14** with sodium borohydride gave **11** as the predominant diastereomer (99:1

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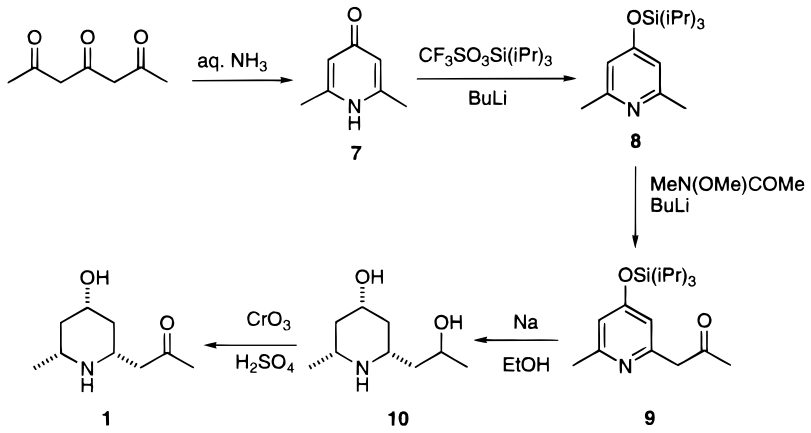
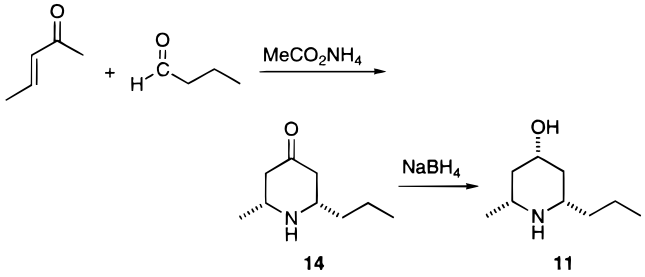
Scheme 1



Scheme 2



by GC−MS). An NOE enhancement established the C-4 OH stereochemistry as for **1**. Again, since all the known conifer piperidines have the same stereochemistry at C-2, **11** probably depicts absolute as well as relative stereochemistry.

Although the literature contains numerous enantioselective syntheses of 2,6-disubstituted piperidines, those leading to C-4 oxygenated analogues are lacking. Recently, however, an enantioselective synthesis of enantio-**5** passed through the corresponding 4-keto analogue, with the 4-keto group subsequently being reduced.[6] This would appear easily adaptable to enantioselective synthesis of both **1** and **11**.

Trace occurrence of the biosynthetically expected 4-oxygenated piperidines in conifers could mean either that they are rapidly converted to the corresponding nonoxygenated piperidines or that such a reaction precedes cyclization from the presumed polyketide precursor.[1e] An anonymous reviewer suggested that they may be artifacts produced during isolation.

In the time course of piperidine alkaloid synthesis from germination to seedlings,[1i,7] the concentration of an unknown alkaloid of *m*/*z* 167 (GCMS) increased with growth time, paralleling the accumulation of 1-methyl-9-nor-3-granatanone (euphococcinine), **16**. A molecular weight of 167 would be 14 mass units greater than that of **16**. The *N*-methyl derivative **15** was a likely candidate for the unknown, based upon the MS fragmentation and GC retention time. Indeed, N-methylation of natural **16** produced **15**, as confirmed by spectral data. This is the first N-methylated 2,6-disubstituted piperidine among the alkaloids reported from conifers. A few *N*-methyl monosubstituted piperidines were uniquely identified in *Picea breweriana*.[1h] The absolute configuration of **16** (and hence that of **15**) is known. Although this is opposite at C-2 to all the other conifer piperidines, **15** and **16** probably arise from a precursor whose cyclization inverts that stereochemistry.[1e]

Experimental Section

General Experimental Procedures. NMR spectra were obtained at 300 (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$ (^{1}H, δ 7.24; ^{13}C, δ 77.0). FTIR spectra were as NaCl films. GCMS: 15 m HP-1 capillary column (30m for **15** analysis); 80 °C (1 min), ramped at 5 °C/min to 145° (0.1 min), followed by a second ramp at 50 °C/min to 290 °C (4 min). Alkaloid purifications were by differential pH extractions: dissolution in dilute aqueous HCl, extraction with $CHCl_3$, basification with aqueous NaOH, extraction with $CHCl_3$, drying (Na_2SO_4), and evaporating in vacuo. Where presented, NMR assignments were assured by DEPT, NOE, and 2D experiments.

2,6-Dimethyl-4-pyrone (7). A 10% solution of acetylacetone (3.0 mL, 0.069 mol) in anhydrous THF was added dropwise to a stirring mixture of diisopropylamine (9.0 mL, 0.069 mol) and BuLi (2.2 M, 32.0 mL, 0.070 mol) in 60 mL of anhydrous THF at −30 °C. The mixture was stirred for 1.5 h at −5 °C, and a 10% solution of MeN(OMe)COMe (3.0 g, 0.029 mol) in anhydrous THF was added. The solution was stirred for 18 h, the THF was evaporated, 100 mL of anhydrous Et_2O was added, and then 50 mL of 20% aqueous HCl was added slowly. The organic layer was separated and the aqueous layer extracted 3× with 30 mL of Et_2O. The combined organic layers were dried and evaporated, and the residue was vacuum distilled at 80−90 °C to yield 2.94 g (0.021 mol, 71%) of 2,4,6-trioxoheptane as a yellow solid, which was carried on directly. The triketone (2.20 g, 15.5 mmol) was dissolved in 90 mL of aqueous NH_3 (29%) and heated at 130 °C in an oil bath for 3 h. About two-thirds of the solvent was evaporated in vacuo, and 70 mL of aqueous NH_3 was added. The solvent was evaporated completely, and the light yellow residue was dissolved in 10 mL of anhydrous EtOH. Cold Et_2O (40 mL) was added, and the precipitated white solid was filtered, rinsed with cold Et_2O, and allowed to dry to yield **7** (1.78 g, 14.5 mmol, 93%; mp 228−229 °C (lit.[8] mp 227.5−229 °C)).

***cis,cis*-2-Methyl-4-hydroxy-6-(2-hydroxypropyl)piperidine (10).** To **7** (0.500 g, 4.1 mmol) was added 250 mL of anhydrous THF and the mixture dissolved by sonication. The solution was cooled to −70 °C, and BuLi (2.0 mL, 4.4 mmol) was added slowly under Ar. The mixture was stirred for 2.5 h until −15 °C was reached and recooled to −60 °C, and $CF_3SO_3Si(^iPr)_3$ (1.10 mL, 4.1 mmol) was added slowly. The solution was stirred for 12 h and cooled to −70 °C, and BuLi (2.0 mL, 4.4 mmol) was added slowly. The soln was stirred until −40 °C was reached (2 h), MeN(OMe)COMe (0.66 g, 6.4 mmol) was added dropwise, and the mixture was stirred until it reached room temperature. The solution was evaporated in vacuo and the residue treated with 100 mL of Et_2O and 70 mL of 23% aqueous NH_4Cl. The layers were separated, and the aqueous layer was washed with 2 × 70 mL of Et_2O. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated to leave 1.37 g of crude **9**, which was carried on without purification. To the crude **9** was added 240 mL of anhydrous EtOH, and the solution was heated to reflux while being stirred under Ar. Sodium (9.4 g) was added in small incre-

ments over 1 h, and the solution was heated at reflux for 3 h. Then, 100 mL of H_2O was added, and the EtOH in the solution was evaporated in vacuo. The remaining aqueous layer was extracted with $CHCl_3$ (4 × 40 mL), and the combined $CHCl_3$ layers were dried and evaporated to yield 0.357 g of residue. This was purified by VLC (15 g silica gel 60, $CHCl_3$/MeOH gradient elution). Evaporation of the 80−100% MeOH fractions yielded **10** (70 mg, 0.41 mmol, 10% overall from **7**) as an oil, which was a 1:1 mixture of isomers (at C-9) as evidenced by the 18 ^{13}C NMR resonances, with four in the COH region (d 64.8−68.6).

***cis,cis*-2-Methyl-4-hydroxy-6-(2-oxopropyl)piperidine, (1).** A diol (**10**) mixture (44.1 mg, 0.25 mmol) was dissolved in 10 mL of acetone, 4.5 mL of Jones reagent (CrO_3/H_2SO_4, 0.051 M, 0.23 mmol) was added, and the reaction mixture was stirred overnight. The acetone was evaporated and the residue partially purified by differential pH extraction. The resulting residue was chromatographed on basic alumina (acetone eluent) to yield 11.3 mg, 0.066 mmol, 26% yield) of **1** as an oil. ^1H NMR δ 1.05 (3H, d, J = 6.3 Hz, H-7), 1.05 (1H, m, H-3α), 1.10 (1H, m, H-5α), 1.81 (1H, dp, J = 11.9, 2.2 Hz, H-5β), 1.89 (1H, dp, J = 12.0, 2.2 Hz, H-3β), 2.11 (3H, s, H-10), 2.55 (2H, dd, J = 7.5, 2.7 Hz, H-8), 2.71 (1H, m, H-2), 3.01 (1H, m, H-6), 3.64 (1H, m, H-4); ^{13}C NMR δ 22.3 (C-7), 30.5 (C-10), 41.0 (C-5), 43.3 (C-3), 49.8 (C-8), 49.9 (C-2), 50.1 (C-6), 68.7 (C-4), 208.1 (C-9); HRMS for $C_9H_{17}NO_2$ calcd 171.1259, found 171.1254; EIMS 171 (12), 156 (20), 128 (22), 114 (100), 96 (17), 84 (20), 80 (25), 70 (93), 44 (39), 43 (60); GC−MS t_R 7.26 min.

***cis*-2-Methyl-4-oxo-6-propylpiperidine (14).** A mixture of *n*-butanal (10.78 g, 149.5 mmol), 3-penten-2-one (19.35 g; Aldrich Chemical Co., technical grade), and NH_4OAc (11.52 g, 149.5 mmol) in 600 mL of MeOH was stirred under Ar for 3 days. The solvent was evaporated to leave a brown residue (32.21 g). The residue was purified by column chromatography (Sephadex LH-20; 30 g; 3:1:1 hexane/toluene/MeOH) using 1−3 g of residue per separation and collecting 15−20 fractions of 15−20 mL each. In general, fractions 3−15 were combined. Of the partially purified mixture, 5.5 g was chromatographed (flash silica gel; EtOAc; 21 fractions of 50 mL each and 7 fractions of 125 mL each), with the last fraction being combined and evaporated to leave 1.53 g (9.87 mmol, 6.6% yield) of nearly pure **14** (96:4 of **14**/6-*epi*-**14**). This was recrystallized three times in EtOAc to give 349 mg of pure **14**: ^1H NMR δ 0.85 (3H, t, J = 7.2 Hz), 1.13 (3H, d, J = 6.3 Hz), 1.39 (4H, m), 1.99 (2H, m), 2.28 (2H, m), 2.74 (1H, m), 2.91 (1H, m); ^{13}C NMR δ 14.0, 18.9, 22.6, 39.1, 48.1, 50.1, 52.0, 56.2, 209.5; HR-MS (FABH$^+$) for $C_9H_{18}NO$ calcd 156.1388, found 156.1391; EI-MS 155 (3), 140 (2), 112 (67), 98 (20), 70 (100), 56 (11), 43 (28), 42 (27); FTIR 2962, 1716, 1558, 1455, 1407 cm^{-1}; GC−MS t_R 4.70 min.

***cis,cis*-2-Methyl-4-hydroxy-6-propylpiperidine, 11**. Ketopiperidine **14** (115 mg, 0.74 mmol) in 20 mL of MeOH was cooled to 0 °C, and $NaBH_4$ (40 mg, 1.1 mmol) was added slowly over 5 min. The solution was stirred for 20 min and then 30 min at room temperature. To the solution was added 15 mL of H_2O and the MeOH evaporated in vacuo. The aqueous layer was extracted with $CHCl_3$ (3 × 20 mL), and the $CHCl_3$ solutions were combined, dried (Na_2SO_4), and evaporated to yield a white solid (69 mg, 0.44 mmol, 59% yield). The solid was recrystallized from EtOAc to give **11** (45 mg, 0.29 mmol, 39% yield): mp 88−89 °C; ^1H NMR δ 0.88 (3H, app t, H-10), 0.92 (1H, m, H5α), 0.98 (1H, m, H3α), 1.09 (3H, d, J = 6.3 Hz, H-7), 1.34 (4H, m, H-8, H-9), 1.92 (2H, m, H-3β, H-5β), 2.55 (1H, m, H-6), 2.64 (1H, m, H-2), 3.62 (1H, m, H-4); ^{13}C NMR δ 14.1 (C-10), 19.1 (C-9), 22.4 (C-7), 38.9 (C-8), 41.6 (C-5), 43.8 (C-3), 50.1 (C-2), 54.5 (C-6), 69.0 (C-4); HR-MS (FABH$^+$) for $C_9H_{20}NO$ calcd 158.1545, found 158.1556; EI-MS 157 (1), 156 (1), 142 (5), 114 (100), 98 (14), 96 (16), 70 (77), 55 (10), 44 (40), 43 (30); FTIR (NaCl film) 3254, 2958, 1456, 1378, 1318, 1117, 1030 cm^{-1}; GC−MS t_R 4.94 min.

1-Methyl-3-granatanone (*N*-Methyleuphococcinine; *N*-Methyl-9-aza-1-methylbicyclo[3.3.1]nonan-3-one) (15). To 37 mg (0.25 mmol) of **16** (isolated from *P. pungens*[1i]) in 15 mL of MeCN was added, while stirring, 1.5 mL of 37% aqueous CH_2O. After the mixture was stirred 10 min, 50 mg (0.81 mmol) of $NaCNBH_3$ and three drops of glacial HOAc were added. After the solution was stirred for 1 h, 10 drops of 1 M NaOH was added, and the solution was extracted with hexane (3 × 20 mL). The hexane extracts were combined, dried (Na_2SO_4), and evaporated to yield almost pure (NMR) **15** (11 mg, 30% yield). A final pure sample was obtained by differential pH extraction: ^1H NMR δ 1.15 (3H, s, H-7), 1.51 (5H, m, H-3, H-4, 1/2 of H-5), 1.88 (1H, m, 1/2 of H-5), 2.17 (2H, d, J = 16.8 Hz, 1/2 of H-8 and 1/2 of H-10), 2.40 (1H, d, J = 16.8, 1/2 of H-10), 2.52 (3H, s, N-Me), 2.66 (1H, dd, J = 16.8, 6.9, 1/2 of H-8), 3.33 (1H, bs, H-6); ^{13}C NMR δ 18.2 (C-4), 29.0 (C-7), 29.5 (C-5), 36.6 (N-Me), 36.9 (C-3), 41.4 (C-8), 48.1(C-10), 55.9 (C-2), 58.1 (C-6), 211.1 (C-9); HR-MS FABH$^+$ for $C_{10}H_{18}NO$ calcd 168.1388, found 168.1396; EI-MS 167 (40), 152 (8), 124 (100), 110 (94), 97 (26), 71 (32), 56 (81); GC−MS (30 m column) t_R 13.32 min; $[\alpha]^{23}_D$ = +22° (*c* 0.20, $CHCl_3$).

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