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ALKALOIDS OF *PICEA BREWERIANA*

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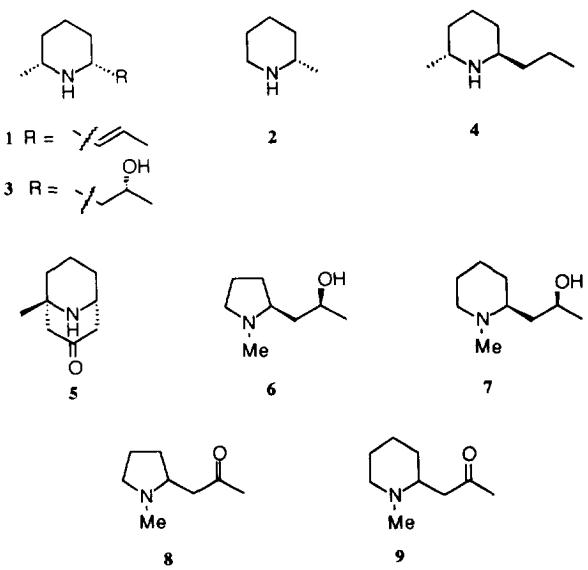
Key Word Index—*Picea breweriana*; Pinaceae; spruce; piperidine alkaloids; pyrrolidine alkaloids; hygroline; hygrine; *N*-methylsedridine; *N*-methylpelletierine; euphococcinine.

Abstract—Alkaloids found in *Picea breweriana* include hygrine, hygroline, *N*-methylsedridine and *N*-methylpelletierine. This represents the first isolation of these alkaloids from the Pinaceae. Euphococcinine, an alkaloid previously found in *Picea* and *Pinus* species, is also present. The presence of alkaloids characteristic of two different biosynthetic pathways in *P. breweriana* highlights the apparent uniqueness of this species.

INTRODUCTION

Isolation of alkaloids from the Pinaceae was, until recent years, limited to several species of *Pinus* containing pinidine (**1**) and, in some cases, α-pipecoline (**2**) [1–3]. Pinidinol (**3**) and epidihydropinidine (**4**) were then isolated from *Picea engelamannii* [4–6]. A survey of eight representative *Picea* species indicated the presence of **3** and **4** to be widespread in the genus [6]. Subsequently, additional *cis*- and *trans*-2,6-disubstituted piperidine alkaloids, as well as euphococcinine (**5**), have been isolated from *Picea* and *Pinus* species [7, 8].

Picea breweriana is a rare spruce, endemic to the Siskiyou mountains (northern California–Oregon). It was unusual in the original *Picea* survey [6] in that it did not contain **3** and **4**, but contained other unknown alkaloids. We now report the isolation and identification of these alkaloids from *P. breweriana*.



RESULTS AND DISCUSSION

Alkaloids were extracted from *P. breweriana* needles via steam distillation followed by acid–base extraction of both the distillate and distillation residue. Analysis of the resultant alkaloid mixtures by GC-mass spectrometry showed the distillate contained two alkaloids (A and B), while the residue contained three alkaloids (A, B and C). The alkaloid mixture from the distillation residue was separated by thermal conductivity gas chromatography and the individual alkaloids were analysed by GC-mass spectrometry, ^{1}H and ^{13}C NMR.

Alkaloid A was collected from the gas chromatograph as a solid. Mass spectrometry showed a base peak at *m/z* 84, suggesting either a piperidine- or methyl pyrrolidine-type structure. A methyl singlet at δ 2.35 suggested a *N*-methlypyrrolidine structure. The ^{1}H NMR spectrum showed a methyl doublet at δ 1.15 and a CH at δ 4.16 characteristic of a $-CH_2CH(OH)Me$ fragment. ^{13}C and ^{13}C DEPT NMR showed two methine, four methylene, and two methyl carbons. Together, this information led to the general structure of hygroline (**6**), or its diastereomer, pseudohygroline. Confirmation of this general structure for alkaloid A was obtained by inde-

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pendent synthesis of the diastereomeric mixture [9]. Alkaloid A was identical with one of the diastereomers in the mixture by ^{1}H and ^{13}C NMR. Specifically, GC-mass spectrometry showed alkaloid A to be identical in retention time and fragmentation pattern with the lowest retention time diastereomer of the synthetic mixture. Alkaloid A was identified as hygroline (**6**) rather than pseudohygroline, by comparison of its ^{1}H NMR spectrum with the spectra reported for each of the two diastereomers [10].

The mass spectrum of alkaloid B showed a base peak at *m/z* 98, suggesting a methylpiperidine structure and a $[M]^+$ at *m/z* 157. The ^{1}H NMR of alkaloid B, like that for alkaloid A, exhibited a methyl doublet (δ 1.15) and a downfield CH (δ 4.22) with splitting appropriate for $CH_2CH(OH)Me$. A methyl singlet was also present at δ 2.34, characteristic of a *N*-methylpiperidine structure. The ^{13}C and ^{13}C DEPT NMR spectra of alkaloid B showed two methine, five methylene, and two methyl carbons. These results led to the general structure of *N*-methylsedridine (**7**), or its diastereomer, *N*-methylallosedridine. Confirmation of this general structure for alkaloid B was obtained by independent synthesis of the diastereomeric mixture of *N*-methylsedridine and *N*-methylallosedridine [9]. Alkaloid B was identical with one of the diastereomers in the mixture by ^{1}H and ^{13}C NMR. GC-mass spectrometry showed alkaloid B to be identical in retention time and fragmentation pattern with the lowest retention time diastereomer of the synthetic mixture. The diastereomeric identity of alkaloid B was determined from the relative boiling point of the two diastereomers and ^{13}C NMR data. *N*-Methylsedridine is reported to have a lower boiling point than *N*-methylallosedridine (103–105°/24 mm and 118–120°/24 mm, respectively) [11]. Partial Kugelrohr distillation of the synthetic diastereomeric mixture gave a distillate with a ^{1}H NMR spectrum showing enrichment in the diastereomer corresponding to alkaloid B, indicating that alkaloid B is more volatile *N*-methylsedridine. Furthermore, while ^{13}C NMR data are not available for *N*-methylsedridine, there are ^{13}C NMR values for the three carbons of *N*-methylallosedridine [12]. This ^{13}C NMR data matched three carbon signals in the synthetic diastereomeric mixture corresponding to the diastereomer which is not alkaloid B. Thus, alkaloid B is *N*-methylsedridine (**7**), rather than *N*-methylallosedridine.

The mass spectrum of alkaloid C showed a base peak at *m/z* 110 and a $[M]^+$ at *m/z* 153. The ^{1}H NMR spectrum showed a methyl singlet at δ 1.18 but, unlike alkaloids A and B, there was no indication of a *N*-methyl group. The ^{13}C and ^{13}C DEPT NMR spectra indicated the presence of one carbonyl, one quaternary carbon, one methine, five methylene and one methyl carbon, and led to the structure of 1-methyl-9-nor-3-granatanone, or euphococcinine (**5**). The structure was confirmed by ^{1}H and ^{13}C NMR comparison of alkaloid C with an authentic sample of **5**. GC-mass spectrometry of alkaloid C and **5** showed identical retention times and fragmentation patterns.

In addition to the steam distillation method, alkaloids could be isolated from needles or wood via methanol extraction followed by acid–base extraction. This milder method gave an alkaloid mixture which contained the three alkaloids (**5–7**), along with at least three additional alkaloids (D–F), as detected by GC-mass spectrometry.

The GC retention time of alkaloid D was very close to that of **6**. In addition, its mass fragmentation pattern was very similar to that of **6**, with a base peak at *m/z* 84, and a $[M]^+$ at *m/z* 141, 2 mu less than that of **6**. These data suggested the presence of the ketone corresponding to **6**, i.e. hygrine (**8**). The identity of alkaloid D as hygrine was confirmed by comparison with a synthetic sample. GC retention times and fragmentation patterns were identical.

The GC retention time of alkaloid E was very close to that of **7**. In addition, its mass fragmentation pattern was very similar to that of **7**, with a base peak at *m/z* 98, and a $[M]^+$ at *m/z* 155, 2 mu less than that of **7**. These data suggested the presence of the ketone corresponding to **7**, *N*-methylpelletierine (**9**). The identity of alkaloid E as *N*-methylpelletierine (**9**) was confirmed by comparison with a synthetic sample of **9**. GC retention times and mass fragmentation patterns were identical.

Alkaloid F, as yet unidentified, had a GC-mass spectral fragmentation pattern very similar to that of euphococcinine (**5**); it may represent an isomeric structure.

Isolation of euphococcinine (**5**) from *P. breweriana* is not unusual, given its prior isolation from *Pinus* and *Picea* [7, 8]. GC-mass spectroscopic reinvestigation of our earlier *Picea* extracts [6] suggests that **5** is present in *Picea mariana*, *P. chihuahuana*, *P. engelmannii*, *P. brachytyla*, *P. likiangensis*, *P. glauca* and *P. pungens*, as well. It, like previously reported alkaloids of the Pinaceae, is presumably biosynthesized via a polyketide pathway. In addition to *Pinus* and *Picea*, **5** has been isolated from *Euphorbia atoto* [13], and from the beetles, *Cryptolaemus montrouzieri* [14] and *Epilachna varivestis* [15]. Euphococcinine was found to have antifeedant activity against spiders and ants [15], and was weakly active against Gram-negative bacteria [7].

The presence of alkaloids **6–9** in *P. breweriana*, however, appears to be quite unusual. *N*-Methylsedridine (**7**) has not previously been isolated as a natural product. While hygroline (**6**) and hygrine (**8**) have been isolated from a number of sources [16], and *N*-methylpelletierine (**9**) has been isolated from plants such as *Punica granatum* [17] and *Sedum sarmentosum* [18], this is the first report of alkaloids of this type from the Pinaceae. These alkaloids are presumably formed via an amino acid pathway. Hygroline and hygrine would be derived from ornithine and *N*-methylpelletierine and *N*-methylsedridine, from lysine.

Picea breweriana has been relegated by Rushforth to its own subgroup within *Picea* [19]. The absence of pinidinol and epidihydropinidine, the presence of alkaloids characteristic of two different biosynthetic pathways, and the presence of alkaloids new to the Pinaceae highlight the apparent uniqueness of this species.

EXPERIMENTAL

Instrumentation. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) chemical shifts are given in δ with TMS (^{1}H, δ 0) or $CDCl_3$ (^{13}C, δ 77.0) as int. standard. GC-MS (70 eV) utilized a 30 m × 0.25 mm i.d. × 0.25 μm film Carbowax column with a temp. prog.: 40° (2 min), heating rate 10° min^{-1} to 200°. Gas chromatography utilized a 1.8 m × 2 mm o.d., 3% OV-17 column at 150°, with a thermal conductivity detector.

Plant material. Samples of *P. breweriana* were from the Arnold Arboretum of Harvard University (voucher #1218-79A) or the Hoyt Arboretum in Portland, Oregon (identification confirmed by F. Nilsen, Hoyt Arboretum). Fresh branches were stored at 4° until needed.

Steam distillation and isolation of alkaloids. The general method of ref. [20] was followed. Needles (54.5 g) were ground with 8% Na_2CO_3 (250 ml). The mixt. was diluted with H_2O to 1 l and steam-distilled. After ~250 ml of distillate was collected, the mixt. began to foam and distillation was discontinued.

Alkaloid isolation from distillate. The distillate was basified with 50% KOH and then extracted with $CHCl_3$. The $CHCl_3$ extracts were combined and extracted with 2 M HCl. The combined, ice-cooled aq. HCl extracts were basified with 50% NaOH and then extracted with $CHCl_3$. The $CHCl_3$ extracts were dried (Na_2SO_4), filtered and evapd to give 17 mg of alkaloids. GC-MS indicated the presence of two alkaloids. Alkaloid A (hygroline): GC-MS R_t 13.0 min; *m/z* (rel. int.): 143 $[M]^+$ (1), 98 $[M - 45]^+$ (2), 84 $[M - CH_2CH(OH)\ Me]^+$(100), 82 $[M - 61]^+$ (7), 70 $[M - 73]^+$ (5). Alkaloid B (*N*-methylsedridine): GC-MS R_t 15.2 min; *m/z* (rel. int.): 157 $[M]^+$ (1), 142 $[M - Me]^+$ (1), 112 $[M - 45]^+$ (1), 98 $[M - CH_2CH(OH)\ Me]^+$ (100), 96 $[M - 61]^+$ (2), 84 $[M - 73]^+$ (2), 70 $[M - 87]^+$ (13).

Alkaloid isolation from distillation residue. The residue was filtered through gauze, extracted with $CHCl_3$ and the combined $CHCl_3$ layers extracted with 2 M HCl. The combined, ice-cooled aq. HCl extracts were basified with 50% KOH and then extracted with $CHCl_3$. Evapn of the $CHCl_3$ extracts gave 23 mg alkaloids. GC-MS indicated that three alkaloids were present. Alkaloid A (hygroline): GC-MS R_t 13.2 min; fragmentation pattern as above. Alkaloid B (*N*-methylsedridine): GC-MS R_t 15.4 min; fragmentation pattern as above. Alkaloid C (euphococcinine): GC-MS R_t 19.6 min; fragmentation pattern matched authentic sample and ref. [13]. The three alkaloids were separated by GC and characterized by ^{1}H and ^{13}C NMR spectra. Alkaloid A (hygroline): ^{1}H NMR spectrum matched ref. [10]; ^{13}C NMR ($CDCl_3$): δ64.8 (*d*, C-7), 64.6 (*d*, C-2), 57.1 (*t*, C-5), 40.5 (*q*, *N*-Me), 36.9 (*t*, C-6), 28.2 (*t*, C-3), 23.7 (*q*, C-8), 23.4 (*t*, C-4). Alkaloid B (*N*-methylsedridine): ^{1}H NMR: δ1.15 (3H, *d*, J = 6.0 Hz, H-9), 1.20–1.34(2H, *m*, H-7, H-4), 1.45–1.62(3H, *m*, H-3, H-5, H-5), 1.67–1.79 (2H, *m*, H-3, H-4), 1.90–2.02 (2H, *m*, H-6, H-7), 2.11–2.20 (1H, *m*, H-2), 2.34 (3H, *s*, *N*-Me), 2.90(1H, *br d*, J = 11.3 Hz, H-6), 4.22(1H, *dqd*, J = 11, 6, 3 Hz, H-8). ^{13}C NMR ($CDCl_3$) δ65.2(*d*, C-8), 62.9(*d*, C-2), 57.4(*t*, C-6), 44.1(*q*, *N*-Me), 38.7(*t*, C-7), 29.7(*t*, C-3), 25.8(*t*, C-5), 24.5(*t*, C-4), 23.6(*q*, C-9). Alkaloid C (euphococcinine): ^{1}H NMR spectrum matched ref. [13] and authentic sample; ^{13}C NMR spectrum matched ref. [7].

Extraction and isolation of alkaloids. Needles (41.7 g) were ground under liq. N_2. MeOH was added to give a vol. of ~400 ml and the mixt. allowed to stand at room temp. After 4 days, the mixt. was filtered and fr. MeOH added to the needle residue. After 3 days at room temp., the mixt. was filtered, fr. MeOH added and the mixt. then filtered again after 3 days. Filtrates were combined and evapd. H_2O was added and the mixt. extracted with Et_2O and then $CHCl_3$. The resultant aq. phase was basified with K_2CO_3, extracted with $CHCl_3$ and the combined extracts evapd. The resultant crude alkaloid extract was further purified by addition to 0.1 M HCl and extraction with $CHCl_3$. The aq. phase was basified with K_2CO_3 and then extracted with $CHCl_3$. The combined $CHCl_3$ extracts were evapd to give 37 mg of alkaloids. GC-MS indicated the presence of six alkaloids. Alkaloid A (hygroline): GC-MS R_t 13.0 min, fragmentation pattern as above. Alkaloid D (hygrine): GC-MS R_t 13.2 min; *m/z*(rel int): 141 $[M]^+$ (3), 98 $[M - 43]^+$ (6), 84 $[M - CH_2CH(OH)\ Me]^+$ (100), 82 $[M - 59]^+$ (12), 70 $[M - 71]^+$ (9). Alkaloid B (*N*-methylsedridine): GC-MS R_t 15.2 min, fragmentation pattern as above. Alkaloid E (*N*-methylpelletierine): GC-MS R_t 15.5 min; *m/z* (rel. int.) 155 $[M]^+$ (3), 140 $[M - Me]^+$ (1), 112 $[M - 43]^+$ (4), 98 $[M - CH_2CH(OH)\ Me]^+$ (100), 96 $[M - 59]^+$ (5), 84 $[M - 71]^+$ (3), 82 $[M - 73]^+$ (3), 70 $[M - 85]^+$ (29). Alkaloid F: GC-MS R_t 17.2 min; *m/z* (rel. int.): 153 $[M]^+$ (2), 110 $[M - 43]^+$ (100), 98 $[M - 55]^+$ (7), 96 $[M - 57]^+$ (14), 95 $[M - 58]^+$ (7), 94 $[M - 59]^+$ (8), 83 $[M - 70]^+$ (19). Alkaloid C (euphococcinine): GC-MS R_t 19.6 min, fragmentation pattern as above.

Wood (98.7 g) was immersed in liq. N_2 and then pulverized. The resultant mixt. was soaked × 3 in MeOH over a period of 7 days, and worked-up following the general procedure described above to give 20 mg of alkaloids. GC-MS showed the same six alkaloids as in needles, by R_t and fragmentation pattern. ^{1}H NMR spectrum also matched that of the alkaloid mixt. from needles.

Synthesis of hygroline (**6**) and pseudohygroline. The general procedure of ref. [9] was followed, utilizing the partial $LiAlH_4$ reduction of *N*-methylpyrrolidinone, reaction with acetoacetate to give hygrine and then reduction with $NaBH_4$ to give hygroline and pseudohygroline. ^{1}H and ^{13}C NMR spectra of the isolated hygrine matched those given in refs [21, 22]. The isolated hygroline and pseudohygroline mixt. had a ^{1}H NMR spectrum with peaks corresponding to lit. values for the two diastereomers [10]. ^{13}C NMR ($CDCl_3$) spectrum of the diastereomeric mixt. had peaks corresponding to hygroline (see above) and pseudohygroline: δ 67.1(*d*, C-7), 65.4 (*d*, C-2), 55.4 (*t*, C-5), 42.8 (*t*, C-6), 42.8 (*q*, *N*-Me), 30.4 (*t*, C-3), 24.1 (*q*, C-8), 22.6 (*t*, C-4). Two peaks having fragmentation patterns identical with alkaloid B were present in the GC-MS: R_t 13.1 min (hygroline), R_t 14.8 min (pseudohygroline).

Synthesis of N-*methylsedridine* (7) *and* N-*methylallosedridine*. The general procedure of ref. [9] was followed, utilizing the partial $LiAlH_4$ reduction of *N*-methyl-2-piperidone, reaction with acetoacetate to give *N*-methylpelletierine and then reduction with $NaBH_4$ to give *N*-methylsedridine and *N*-methylallosedridine. ^{1}H NMR spectrum of the isolated *N*-methylpelletierine matched ref. [21]. ^{13}C NMR spectrum of the isolated *N*-methylpelletierine included three peaks given in ref. [12]. ^{13}C NMR($CDCl_3$): δ207.7 (*s*, C = 0), 58.9 (*d*, C-2), 56.1 (*t*, C-6), 47.5 (*t*, C-7), 43.3 (*N*-Me), 32.0 (*t*, C-3), 30.8 (*q*, C-9), 25.7 (*t*, C-5), 23.6 (*t*, C-4). ^{1}H NMR ($CDCl_3$) spectrum of the isolated *N*-methylsedridine and *N*-methylallosedridine contained peaks corresponding to *N*-methylsedridine (see above) and *N*-methylallosedridine. *N*-methylallosedridine partial ^{1}H NMR ($CDCl_3$): δ1.18 (3H, *d*, J = 6 Hz, H-9), 2.42 (3H, *s*, *N*-Me), 3.00 (1H, *m*, H-6), 3.95 (1H, *dqd*, J = 10, 6, 2 Hz, H-8). ^{13}C NMR ($CDCl_3$) spectrum of the isolated *N*-methylsedridine and *N*-methylallosedridine mixt. contained peaks corresponding to *N*-methylsedridine (see above) and *N*-methylallosedridine, including three peaks given in the lit. for *N*-methylallosedridine [12]. *N*-Methylallosedridine ^{13}C NMR ($CDCl_3$): δ68.2 (*d*, C-8), 60.8 (*d*, C-2), 51.8 (*t*, C-6), 40.0 (*q*, *N*-Me), 39.3 (*t*, C-7), 26.2 (*t*, C-3), 24.1(*q*, C-9), 22.6, 20.8 (*tt*, C-4, 5).

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