Toxic Piperidine Alkaloids from Pine (*Pinus*) and Spruce (*Picea*) Trees. New Structures and a Biosynthetic Hypothesis

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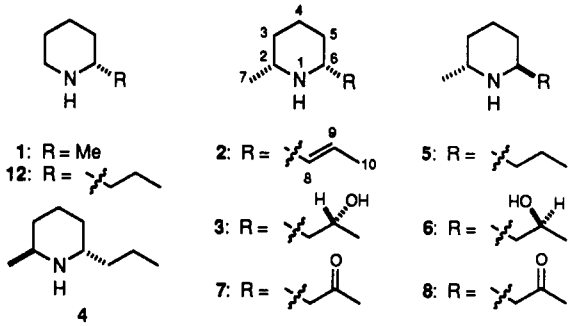
A series of 2,6-disubstituted piperidine alkaloids have been isolated from several *Pinus* (pine) and *Picea* (spruce) species and characterized structurally. The pines appear to contain only *cis*-disubstituted piperidines, while the spruces contain both *cis*- and *trans*-disubstituted piperidines. The structural relationships among the alkaloids suggest a plausible biosynthetic scheme and a reason why previous attempts to elucidate the biosynthesis of pinidine failed beyond establishing its polyketide origin. A mixture of alkaloids from needles of *Pinus ponderosa* proved to be highly teratogenic. The alkaloids might therefore be involved in so-called pine needle abortion which occurs in pregnant range cows which feed on Ponderosa pine needles.

Introduction

The plant family Pinaceae is represented throughout North America by four major genera, *Abies* (true fir), *Picea* (spruce), *Pinus* (pine), and *Pseudotsuga* (Douglas fir), with the additional genera *Larix* (larch, tamarack) and *Tsuga* (hemlock) more restricted to the northern U.S. and Canada. Chemically, the family has been characterized mainly by the presence of resins, tannins, terpenes, phenylpropanoid-derived lignans, flavonoids, and a few stilbenes. Many of these economically-important conifers are susceptible to disease or insect attack or to damage by vertebrate herbivores.[1] The nature of many of these chemical components as defensive (or potential defensive) substances has also been studied.[2] Pine needles or bark are said[3] to make a pleasant tea, and there is extensive data on Native American medicinal use of various conifer plant parts.[4] On the other hand, toxicity and premature parturition or fetus abortion has been a long recognized problem in pregnant range cows which consume the needles of *Pinus ponderosa* (Ponderosa pine).[5] In spite of over 20 years of research,[6] the cause of such abortions is still undetermined.

Alkaloids were unknown from the family Pinaceae until α-pipecoline, **1**, was isolated[7] from *Pinus sabiniana* along with another alkaloid, (−)-pinidine, later shown[8] to be **2**.



Alkaloid **2** was also found in two other rare California pines, *Pinus jeffreyi* and *Pinus torreyana*. The absolute configuration was established,[9] and total syntheses have been performed.[10] Leete and co-workers proved the polyketide nature of **2** biosynthesis by showing incorporation of labeled acetate and malonate but failed in attempts to find more proximate precursors to **2**.[10a,11,12]

No further alkaloids were reported from the Pinaceae until the discovery[13] of (−)-pinidinol, **3**, from *Picea engelmannii*, which was followed by X-ray crystal determination[14] of its absolute configuration. Finally, the first *trans*-disubstituted piperidine, epidihydropinidine (either **4** or **5**), was isolated from *P. engelmannii* and shown to

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be present, along with 3, in six other *Picea* species.[15] The alkaloids were detected in the needles, wood, and roots. Thus, piperidine alkaloids are now recognized as common constituents of spruce trees and at least some pine trees.

In a continuation of this work, we have identified additional new alkaloids from *Pinus* and *Picea* species. From the structural relationships among these alkaloids, a biosynthetic hypothesis is emerging which suggests why the earlier biosynthetic work[11,12] faltered beyond establishing a polyketide origin for the alkaloids. In addition, preliminary toxicity and teratogenicity testing has been accomplished on piperidine alkaloids from *Pinus ponderosa*.

Results

Crude base fractions were prepared by differential pH separation of methanol extracts of *P. jeffreyi* Grev. & Balf. (Jeffrey pine), *Pinus edulis* Engelm. (piñon pine), *P. ponderosa* Laws. (Ponderosa pine), *Pinus nigra* J. F. Arnold (Austrian pine), *Pinus sylvestris* L. (Scot's pine), *Picea abies* (L.) Karsten. (Norway spruce), and *Picea pungens* Engelm. (Colorado blue spruce). Total alkaloid content was in the 0.03%–0.08% fresh weight range, depending upon the species or plant part. The alkaloid fractions were subjected to GCMS analysis, and most proved to be complex mixtures of up to 10 alkaloids depending upon the plant part extracted. Generally, only three or four alkaloids were major, with minor traces of others visible only when the GC column was purposely overloaded. There was some variation in alkaloid content among each of the taxa studied. A series of volatile alkaloids, mostly *m/z* 139 and 141, eluted at about 3 min under the conditions employed (see Experimental Section), while a series of less volatile compounds (*m/z* 153–169) appeared between 3.8 and 4.5 min. The major alkaloids in these fractions were separated and purified by chromatography.

One major alkaloid from the nonvolatile fraction of most of the extracts proved to be (−)-pinidinol, **3**. Compound **3** was a major alkaloid of *P. jeffreyi* even though it had not been reported in previous work on this conifer.[7,8,10–12] (−)-Pinidine, **2**, was a major component of needles of *P. jeffreyi*, as was previously shown,[7,8] and *P. ponderosa*. Structural assignments of other alkaloids were made as discussed in the following. A detailed report on alkaloid patterns and plant part variations among the various taxa will be reported elsewhere.

(+)-6-Epidihydropinidine or (2*R*,6*R*)-2-Methyl-6-propylpiperidine (5). A major volatile alkaloid of *P. abies* and *P. pungens* was (+)-epidihydropinidine which had previously been shown[15] to be a *trans*-disubstituted piperidine, either **4** or **5**. The structure was resolved in favor of **5** by a single-crystal X-ray structure on the hydrochloride salt. Interestingly, there were two conformationally different molecules in the asymmetric unit. In one, the methyl at C-2 was axial and the propyl at C-6 was equatorial, while the reverse was true for the other molecule.

(+)-6-Epi-9-epipinidinol or (2*R*,6*S*)-2-Methyl-6-[(2*S*)2-hydroxypropyl]piperidine (6). A second alkaloid of major concentration in the nonvolatile alkaloid fraction of *P. abies* and *P. pungens* eluted slightly later than **3** in the GC experiment and had an essentially identical mass spectrum (M^+ *m/z* 157). In most of the isolates the ratio was about 4:1 or 3:2 in favor of **3**, and initially separation of the isomers was not successful. A screening of nine individual blue spruce trees revealed one in which twigs contained instead a 4:1 ratio in favor of the unknown isomer. From this, a pure sample of the isomer was obtained via flash chromatography. The ^1H and ^{13}C NMR spectra of the isomer were very similar to, but distinct from, those of **3**. Keys to the basic structure assignment were the ^{13}C resonances at δ 46.1 and 47.5 for C-2 and C-6 (assigned by HETCOR and ^1H NMR decoupling experiments). These are typical[16] for a *trans*-2,6-disubstituted piperidine, rather than a *cis* compound[13] (δ 52.5 for C-2 and δ 54.9 for C-6 of **3**). Long-term crystallization of the hydrochloride salt eventually yielded a crystal of the unknown isomer whose structure was shown to be **6** by an X-ray experiment.

(−)-Pinidinone or (−)-(2*R*,6*R*)-2-Methyl-6-(2-oxopropyl)piperidine (7) and (+)-6-Epipinidinone or (+)-(2*R*,6*S*-2-Methyl-6-(2-oxopropyl)piperidine (8). Two trace components in extracts of *P. pungens* had virtually identical mass spectra (M^+ *m/z* 155), but different retention times. Analysis of the mass spectrum suggested that these components might be the ketone analogs of **3** and **6**. Jones oxidation of **3** and **6** yielded ketones whose ^1H and ^{13}C NMR spectra were consistent with those expected for **7** and **8** and whose GC retention times and mass spectra were identical with those of the isolated components. Alkaloid **7** had been previously isolated[17] from coccinellid ladybird beetles (*Cryptolaemus montrouzieri*) and identified by NMR and GCMS comparison with a prepared synthetic. Our spectra were essentially identical with those reported for **7**.[17] The tentative structure **8** had been assigned to an additional ladybird beetle component which was reported[17] to isomerize to **7** under some GC conditions, but not others. We observed no GC isomerization of **8** to **7**, but pure **8** gradually converted to a mixture of **7** and **8** in methanol. Alkaloid **7** did not change under the same conditions. Such a solution epimerization was reported[18] for lobeline (which contains a 6-(2-oxophenethyl)piperidine part structure) and for other piperidine and pyrrolidine alkaloids of similar structure.[19]

(+)-Euphococcinine or (+)-1-Methyl-9-nor-3-granatanone or (+)-9-Aza-1-methylbicyclo[3.3.1.]nonan-3-one (9). An *m/z* 153 peak was seen in the GCMS of several extracts, and this alkaloid, **9**, was essentially the only component of mature *P. edulis* needles. Bud needles, wood, bark, and twigs contained appreciable amounts of **3** and **2**. Compound **9** was a major alkaloid of *P. pungens* new needle buds, the frass of western spruce budworms (*Choristoneura occidentalis*) which were feeding on those buds, and needles of *P. ponderosa*, *P. nigra*, and *P. sylvestris*. Sufficient material was isolated for ^1H and ^{13}C NMR and ORD spectra, which established the structure as **9**. This alkaloid was previously isolated[20] from *Euphorbia atoto* of the Euphorbiaceae and from the ladybird beetle.[17] The absolute configuration was originally proven

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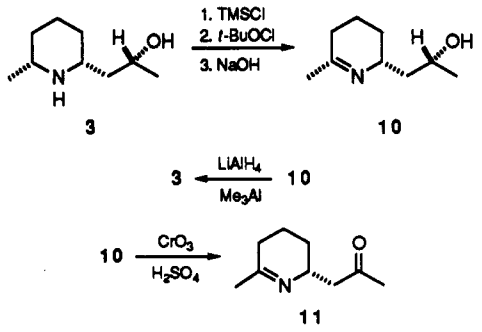
(20) Hart, N. K.; Johns, S. R.; Lamberton, J. A. *Aust. J. Chem.* **1967**, *20*, 561.

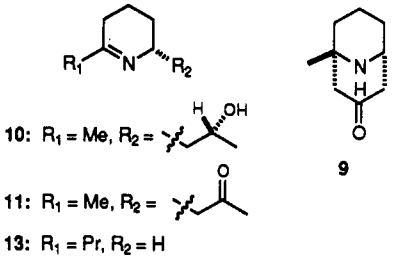
Scheme I. Chemical Interconversions of Isolated Alkaloids



by ORD[21] and recently confirmed by total synthesis.[22] Alkaloid **9** was also isolated as a defensive chemical from the blood of the Mexican bean beetle *Epilachna varivestis* and named euphococcinine.[23] Piñon pine nuts, a common food in the southwest U.S., did not contain any of the alkaloids reported in this work.

(+)-1,2-Dehydropinidinol (10) and 1,2-Dehydropinidinone (11). A major alkaloid component of *P. nigra* and *P. sylvestris* needles and a moderate component of *P. ponderosa* cones and needles showed an M^+ *m/z* 155 peak in the GCMS as did **7**, but the fragmentation pattern and retention time were different from those of **7**. The ^{13}C NMR spectrum showed the presence of two methyls, four methylenes, two methines, and a quaternary sp^2 carbon resonance at δ 169.5. The ^1H NMR spectrum showed a methyl doublet (J = 6.4 Hz), whose coupled proton was at δ 4.09, consistent with a $-CH(OH)CH_3$ moiety as in **3**. The second methyl (δ 1.92) was also a doublet, but with J = 2.0 Hz. The latter was typical for a long-range coupling between a methyl and a proton cis to the methyl across a double bond, here the NH proton. These were key resonances which suggested the structure **10**. All carbon and proton resonances could be assigned in a HETCOR spectrum. That **10**, (+)-1,2-dehydropinidinol, was indeed the structure was proven by its synthesis from **3** and reduction to **3** (Scheme I). No 1,6-imine was detected in the oxidation reaction, and the reduction appeared to be completely stereospecific.

A major, somewhat unstable component of *P. pungens* needles, frass of spruce budworm feeding on *P. pungens*, cones and needles of *Pinus ponderosa*, and needles of *P. sylvestris* and *P. nigra* showed an M^+ *m/z* 153 peak in the GCMS as did **9**, but with a different retention time and fragmentation pattern. The base peak in the mass spectrum was *m/z* 110, which could represent loss of $COCH_3$ from the molecular ion and the ^1H NMR spectrum showed the expected singlet methyl (δ 2.07). A doublet methyl (δ 1.80, J = 1.9 Hz) was assigned to protons on C-7 and also significant were the ^1H NMR peaks for the C-8 methylene: δ 2.38 (dd, J = 16.1, 7.9 Hz) and δ 2.72 (dd, 16.0, 5.9 Hz). Insufficient pure material was available for a ^{13}C NMR spectrum, and attempts to purify the compound resulted in its decomposition. A mixture of the unknown with **10** was, however, amenable to ^{13}C NMR, and after subtraction of resonances due to **10**, the unknown was found to exhibit an aliphatic carbonyl (δ 207.8) and



an imine carbon (δ 168.8), along with resonances for two methyl, four methylene, and one methine carbons. These data were consistent with structure **11** (1,2-dehydropinidinone), which was assured by synthesis from **3** (Scheme I) and comparisons by ^1H NMR and GCMS. Although the product from **3** would have a known absolute configuration at C-6, the instability problems precluded obtaining optical rotations of the isolate and the semisynthetic material. Since all pine alkaloids found so far have the same absolute configuration at C-6, this is presumed (but not proven) for **11**.

Biological Activity. Some piperidine alkaloids such as coniine, **12**, and γ-coniceine, **13**, are known livestock teratogens.[24] Because of this and the above-mentioned pine needle abortion syndrome, we submitted a purified alkaloid mixture from *P. ponderosa* to a frog embryo teratogenesis assay *Xenopus* (FETAX).[25] The mixture, as the HCl salts, was a *P. ponderosa* needle isolate and contained (by GCMS analysis) **2** (24%), **9** (25%), and **11** (31%), along with two minor unidentified alkaloids. Embryo toxicity was high, but in diluted samples 100% of survivors showed malformations. A cytochrome P-450 metabolic activation system increased the toxicity and teratogenicity. A sample of pure (±)-pinidine hydrochloride was a more potent toxicant and teratogen than the alkaloid mixture, and the potency was again greatly increased with a metabolic activation system. These results will be reported quantitatively and in detail elsewhere. The malformations were severe and involved all organ systems. The same test has recently been used to explore structural relationships for teratogenicity among a number of toxic potato alkaloids.[26]

Compound **3** showed no antimicrobial activity against several Gram-positive, Gram-negative, and yeast organisms in our laboratories. Alkaloid **9** was weakly active against the Gram-negative bacteria *Bacillus subtilis* (MIC 1 mg/mL) and *Micrococcus luteus* (MIC 10 mg/mL) but inactive against yeasts and Gram-positive bacteria. One report[15] of a preliminary study stated that a crude alkaloid mixture (mostly **3** and **5**) from Engelmann spruce showed moderate to high antifeedant activity with eastern spruce budworm.

Discussion

The continued identification of new alkaloids from the Pinaceae and expansion of knowledge of their broad occurrence in the family seems remarkable for common trees whose chemistry has been probed for many decades.[27] Although the alkaloid content of the trees is low, the

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(22) Yue, C.; Royer, J.; Husson, H.-P. *J. Org. Chem.* **1992**, *57*, 4211.
(23) Eisner, T.; Goetz, M.; Aneshansley, D.; Ferstandig-Arnold, G.; Meinwald, J. *Experientia* **1986**, 204.
(24) Keeler, R. F. In *Handbook of Natural Toxins*; Keeler, R. F., Tu, A. T., Eds.; Marcel Dekker: New York, 1983; Vol. 1, pp 167–168.
(25) Fort, D. J.; Bantle, J. A. *Fund. Appl. Toxicology* **1990**, *14*, 720.
(26) Friedman, M.; Rayburn, J. R.; Bantle, J. A. *J. Agric. Food Chem.* **1992**, *40*, 1617.
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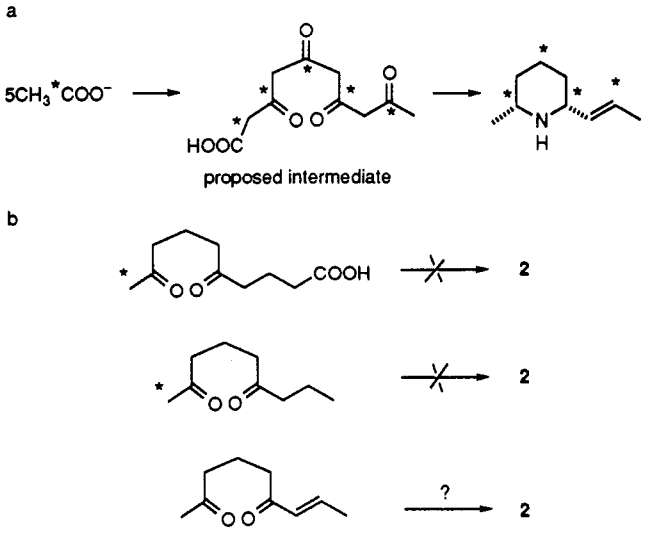
Scheme II. (a) Original[11] Pinidine Biosynthesis Study and Proposed Polyketide Intermediate. (b) Subsequent[10a,12] Labeled Precursor Feeding Experiments and Alternate Hypothesis



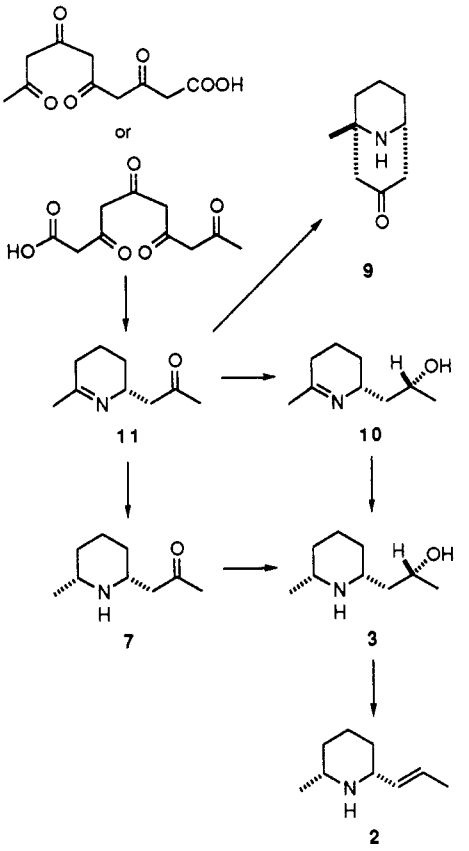
biomass availability is enormous. The Pinaceae may furnish the ecosystem with the largest total mass of alkaloids of any plant family. Whether or not these compounds play a direct, significant role in the biology or ecology of spruce and pines is yet to be determined. The FETAX results show that one or more of the alkaloids are embryotoxic and teratogenic. The calf abortions caused by *Pinus ponderosa* needle consumption are only very rarely accompanied by malformations, but it has been suggested[28] that abortions and other reproductive consequences can be produced by teratogens which could be teratogenic at a level just below that which interrupts pregnancy. Pine needle teas have occasionally been employed in the western U.S. in folk or home remedies, but such preparations are now clearly contraindicated during pregnancy.

The preliminary results[15] on spruce budworm antifeedant activity of a crude alkaloid fraction (mostly **3** and **5**) from Engelman spruce suggest that these alkaloids may partially deter such predators. When disturbed, Mexican bean beetles secrete blood which contains **9**, and the alkaloid was shown[23] to be a deterrent against spiders and ants at the blood concentration. Alkaloid **9** is present in nearly all of the conifer needles we have tested and could be another candidate as a conifer defensive substance. Piperidine alkaloids exhibit a wide variety of pharmacological activity[19] which could affect other animal herbivores.

At the time of the biosynthesis investigations by the Leete group, compounds **1** and **2** were the only conifer alkaloids known. In the first report,[11] carboxyl-labeled acetate was fed to *P. jeffreyi* seedlings, alkaloid **2** was isolated, and degradation showed label incorporation into alternate carbons. The Scheme IIa proposal was made, and it was stated that "loss of the carboxyl group from the intermediate poly-β-keto acid, reaction with a nitrogen source, and plausible reductions and dehydrations could afford pinidine". Thus, the biogenetic sequence **7** to **3** to **2** was postulated. Further feeding experiments (Scheme IIb) were, however, based on the alternate hypothesis that

(28) Keeler, R. F. *Clin. Toxicol.* **1972**, *5*, 529–565.

Scheme III. Possible Pathways in Pinidine Biosynthesis As Suggested by Structural Relationships of Isolates



nitrogen incorporation was a late event and that the propenyl side chain was formed from the saturated precursor.[10a,12] When these experiments failed, the last hypothesis[12] (not tested) was that the polyketide precursor of **2** might already have the requisite unsaturation. Since we have now found the postulated intemediates **7** and **3**, it seems more likely that the original hypothesis of Leete and Juneau was correct and that piperidine ring formation takes place before reactions which modify the side chain (Scheme III). The imine **11** appears to be a key precursor since it would be formed in the piperidine ring cyclization step and is also a likely precursor to **9**. Although **11** was not detected in *P. jeffreyi*, it was found in major concentrations in *P. ponderosa*, along with **9**. Scheme III would represent the basic scheme in the genus *Pinus*, since we encountered only *cis*-2,6-disubstituted piperidines and no *trans*-2,6-disubstituted piperidines in any of five pine species studied. Compounds **2**, **3**, and **9** represented over 95% of the alkaloid content in *P. jeffreyi*, for example. Needles from *P. ponderosa* contained over 80% of the alkaloid content as **2**, **9**, and **11**. No **5** or **6**, which would have been easily seen even in trace amounts, could be detected in any of the pines. Alkaloid **8** isomerizes slowly to **7**, but it is unlikely that this accounts for the sole occurrence of cis compounds in pines since several trans isomers are isolable from the spruce species.

In the *Picea* (spruce) species, the *trans*-2,6-disubstituted piperidines **5** and **6** were encountered along with **3**.[13,15] It is also notable that in all spruce species, the major volatile alkaloid is **5**, not **2** as it is in the pines. The carbonyl reductions of **7** and **8** must proceed with opposite stereoselectivity if, as seems most likely, they are the precursors of **3** and **6**, respectively. The genesis of the large

stereochemical variety among the spruce alkaloids, as well as the validity of the overall pathway in the pines (Scheme III) will need to be probed through labeled precursor feeding experiments.

The similarity of alkaloid patterns among woody gymnosperms (Pinaceae), a latex-bearing angiosperm (*Euphorbia*) and insects (the coccinellid beetles) represents a remarkable biochemical example of "analogical or adaptive resemblances"[29] (now termed convergent evolution[30]) in biologically disparate organisms. The name euphococcinine for **9** was indeed coined[23] to "allude to its dual botanical and insectan origin". The botanical occurrence of **9** has now been extended to representatives of the relatively primitive plant order Gymnospermae.

Experimental Section

General Experimental Details. NMR spectra were run at 300 (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$. Organic extracts were dried over Na_2SO_4. GCMS experiments utilized a 12-m × 0.2-mm i.d., 100% dimethylpolysiloxane column connected to a mass selective detector.

Sources. Fresh branches of *Picea abies* (L.) Karsten. for needle and branch analyses were supplied from a Bavarian forest by Prof. Dr. E.-D. Schulze, Lehrstuhl für Pflanzenökologie, University of Bayreuth. Other samples were from commercially purchased trees in 5 gallon pots or from a living tree in Fort Collins. *P. jeffreyi* Grev. & Balf. was supplied by Dr. Robert F. Scharpf, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA. *P. ponderosa* Dougl. ex Laws & P. Laws. was collected in Fort Collins City Park and identified by Tim Buchanan, Fort Collins City Forester. *P. edulis* Engelm. was collected in northern Colorado and identified by R. D. Moench, Colorado State Forest Service, Fort Collins. *P. pungens* Engelm. and *P. nigra* J. F. Arnold were from a commerical tree farm north of Fort Collins and from the Colorado State University campus. They were identified by R. D. Moench. *P. sylvestris* L. was a commercial sample.

Isolation of Alkaloid Fractions and GCMS Analysis. In a typical isolation, fresh (or dried, ground) plant material was extracted several times at room temperature with methanol, and the extracts were combined and evaporated to an oily residue. This was distributed between chloroform and 10% aqueous HCl, the layers were separated, and the HCl was back-extracted with chloroform. The acidic layer was made strongly basic with sodium hydroxide and then extracted three to five times with equal volumes of chloroform. The chloroform solution was dried (sodium sulfate), a portion was partially evaporated, and the solution analyzed by GCMS. This ensured that the more volatile alkaloids, which were lost or partially lost upon complete evaporation, would be detected along with the nonvolatile alkaloids.

In a typical GCMS run to screen the entire spectrum of alkaloids, injection was at 70 °C, followed by a column temperature ramp at 20° per minute to 280 °C. As an example of such a typical analysis, the base fraction from an extract of *P. abies* needles showed the following results [**compound** (fraction of the total alkaloids), retention time, *m/z* (rel intensity) of major MS peaks]: **2** (2%), 2.9 min, 139 (35), 124 (74), 96 (100), 82 (75); **5** (26%), 3.1 min, 141 (7), 126 (18), 98 (100), 81 (6); **7** (10%), 3.8 min, 153 (11), 140 (15), 112 (17), 98 (100), 82 (42); **3** (56%), 4.3 min, 157 (13), 142 (28), 98 (100); **6** (6%), 4.5 min, 157 (2), 142 (8), 98 (100).

(+)-Epidihydropinidine (5). The HCl salts of a crude alkaloid isolate from *P. abies* were chromatographed on C_{18} silica gel under vacuo (VLC), using a gradient of MeOH (0–100%) in water. Pure **5** (whose physical and spectral data corresponded to those of the literature[15]) was obtained from the 20% MeOH fraction and crystallized from ethyl acetate. Absolute configuration was determined by single-crystal X-ray crystallography of the HCl salt. Crystals were orthorhombic, space group $C222$, a = 9.852 (2) Å, b = 33.663 (7) Å, c = 14.147 (3) Å, Z = 16, T = 120 K. Diffraction data were measured with a Siemens P4/R diffractometer and rotating anode source, Ni-filtered Cu Kα radiation, (λ = 1.541 78 Å). The structure was based on 2004 unique reflections, and solution and refinement were via the SHELXTL-plus system.[31] The asymmetric unit contained two molecules of different conformations, and the crystal had channels of unidentified solvent. Main atoms were refined with anisotropic U's, H atoms in idealized, riding positions, with fixed, isotropic U's. Absolute configuration was determined by refining a multiplier for f''(found 1.01(13), 1.00 corresponds to an ideally correct assignment, −1.00 to the opposite enantiomer). Final R = 0.073 for 1478 reflections with $I > 3\sigma(I)$, R = 0.095 for all data.[32]

(+)-6-Epi-9-epipinidinol (6). Flash chromatography (silica gel, MeOH eluent) of a crude basic extract from *P. pungens* afforded **6**. To the pure base was added ethereal HCl and then evaporated to give **6**·HCl, which eventually crystallized from the neat oil, mp 159–161 °C. Absolute configuration was determined by X-ray crystallography. Crystals were orthorhombic, space group $P2_12_12_1$, a = 7.517 (2) Å, b = 9.173 (2) Å, c = 16.067 Å, Z = 4, T = 119 K. Data collection, structure solution, and refinement were as for **5**. The absolute configuration indicator was 1.15(6). Final R = 0.0324 for 769 reflections with $I > 3\sigma(I)$, R = 0.0343 for all 813 measured.[32]

Spectral data for the base **6**: $[\alpha]^{25}_D$ = +4.2° ($CHCl_3$, c 6.4); MS M$^+$ *m/z* 157 (3), 142 (8), 124 (3), 98 (100), 82 (10), 70 (15), 55 (19), 44 (39); ^{1}H NMR δ 4.04 (1H, m, H-9), 3.46 (1H, bs, NH or OH), 3.29 (1H, m, H-6), 3.10 (1H, m, H-2), 1.84 (1H, ddd, 3.8, 9.0, 14 Hz, H-8), 1.63 (1H, m, H-3), 1.59 (2H, m, H-4), 1.50 (1H, m, H-5), 1.35 (1H, m, H-5), 1.25 (1H, ddd, 3.4, 6.7, 14 Hz, H-8), 1.21 (1H, m, H-3), 1.16 (3H, d, 6.3 Hz, H-10), 1.07 (3H, d, 6.7 Hz, H-7); ^{13}C NMR δ 65.7 C-9, 47.5 C-6, 46.1 C-2, 40.0 C-8, 32.3 C-3, 31.2 C-5, 23.3 C-10, 20.0 C-7, 19.4 C-4.

(−)-Pinidinone (7) from 3. To 0.5 mL of Jones reagent (1.36 g of CrO_3, 13.6 mmol, in 5 mL of 3 M sulfuric acid) was added a solution of **3** (23 mg, 0.15 mmol) in 0.5 mL of acetone. The solution was stirred for 30 min at rt and then quenched with $NaHSO_3$. The reaction mixture was made basic with aqueous NaOH, extracted with $CHCl_3$, dried, and carefully evaporated. The product had the same GCMS fragmentation pattern and retention time and coeluted with the *m/z* 155 compound in a natural isolate mixture: $[\alpha]^{25}_D$ = −4.0° (MeOH, c 3.5); MS M$^+$ *m/z* 155 (8), 140 (21), 112 (22), 98 (100), 82 (46), 70 (27), 55 (16), 43 (70); ^{1}H NMR δ 3.01 (1H, m, H-6), 2.69 (1H, m, H-2), 2.56 (2H, dd, 4.5, 7.5 Hz, H-8), 2.12 (3H, s, H-10), 1.76–1.28 (6H, m, H-3, H-4, H-5), 1.05 (3H, d, 6.3 Hz, H-7); ^{13}C NMR δ 208.4, 52.4, 52.2, 50.2, 33.6, 31.6, 30.6, 24.5, 22.8.

(+)-6-Epipinidinone (8) from 6. **8** was similarly prepared from **6** (17 mg, 0.11 mmol). The GC retention time for **8** was the same as that for a *m/z* 155 compound in a *P. pungens* alkaloid mixture isolate, and the MS fragmentation patterns were essentially identical. Freshly prepared **8** also contained some **7** and, upon standing in MeOH, the proportion of **7** increased over a period of several days. $[\alpha]^{25}_D$ for **8** was calculated to be +8.0°, from the known optical rotation of **7** and the optical rotation of a mixture of **7** and **8**: MS M$^+$ *m/z* 155 (6), 140 (19), 112 (14), 98 (100), 82 (47), 70 (22), 55 (19), 43 (63); ^{1}H NMR δ 3.46 (1H, m, H-6), 3.06 (1H, m, H-2), 2.75 (1H, dd, 8.4, 17 Hz, H-8), 2.51 (1H, dd, 4.8, 17 Hz, H-8), 2.13 (3H, s, H-10), 1.70–1.20 (6H, m, H-3, H-4, H-5), 1.10 (3H, d, 6.5 Hz, H-7); ^{13}C NMR δ 208.4, 47.2, 47.0, 46.1, 32.1, 30.6, 30.5, 20.5, 19.3.

(+)-1-Methyl-9-nor-3-granatanone (9). **9** was obtained nearly pure as the total alkaloid isolate directly from *P. edulis* needles. Pure **9** was obtained by sublimation. The CD, MS, and ^{1}H NMR spectra were essentially the same as those previously

(29) Darwin, C. *On The Origin of Species*; Murray: London, 1869; Facsimile Edition, Harvard University: Cambridge, 1964; p 427.

(30) Mayr, E. *The Growth of Biological Thought*; Harvard University: Cambridge, 1982; p 212.

(31) Sheldrick, G. M. SHELXTL-plus, Siemens Analytical X-ray Instruments, Inc., Madison, WI, 1990.

(32) Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

reported.[20,21] ^{13}C NMR (not previously reported): δ 211.1 C-3, 53.4 C-2, 52.2 C-1, 49.7 C-5, 46.1 C-4, 38.5 C-8, 31.5 C-10, 31.1 C-6, 18.0 C-7.

(+)-1,2-Dehydropinidinol (10). Vacuum liquid chromatography (VLC) (silica gel, methanol 0–100% in water containing a trace of aqueous NH_3) of a crude basic extract from *P. ponderosa* yielded **10** in the 30–100% MeOH fractions. **10** was stable in $CHCl_3$ but decomposed when neat: CD ($CHCl_3$) = +1.21 (ε) at 247 nm; $[\alpha]^{25}_D$ = +20° ($CHCl_3$, *c* 0.7); IR 3362, 1654 cm^{-1}; MS M^+ *m/z* 155 (8), 140 (39), 111 (39), 110 (62), 97 (100), 96 (83), 82 (64), 55 (34), 42 (73); ^1H NMR δ 4.09 (1H, m, H-9), 3.66 (1H, m, H-6), 2.13 (2H, m, H-3), 1.92 (3H, d, 2.0 Hz, H-7), 1.69 (1H, m, H-4), 1.66 (2H, dd, 4.7, 6.7 Hz, H-8), 1.60 (1H, m, H-4), 1.60 (1H, m, H-5), 1.25 (3H, d, 6.4 Hz, H-10), 1.21 (1H, m, H-5); ^{13}C NMR δ 169.5 C-2, 66.2 C-9, 55.0 C-6, 43.2 C-8, 30.1 C-3, 27.7 C-5, 27.2 C-7, 23.0 C-10, 18.9 C-4.

(+)-1,2-Dehydropinidinol (10) from 3. A solution of trimethylchlorosilane (35 mg, 0.33 mmol) in 1 mL of benzene was slowly added to a stirred solution of **3** (35 mg, 0.35 mmol) and triethylamine (103 mg, 1 mmol) in benzene (5 mL). The mixture was stirred for 30 min at rt, and the solvent and excess triethylamine were then evaporated in vacuo. The residue was suspended in benzene (2 mL) and passed through a Celite filter to remove triethylamine hydrochloride. The filter was washed with benzene (10 mL × 2 times). The benzene was evaporated, and the residue was dissolved in Et_2O (5 mL). *tert*-Butyl hypochlorite (48 mg, 0.45 mmol) was added to the Et_2O solution, and the reaction mixture was stirred in the dark for 15 min. The solvent was evaporated, the residue was dissolved in absolute EtOH (5 mL), and the solution was heated at reflux with added NaOH (20 mg, 0.5 mmol) for 45 min. Solvent was evaporated, the residue was suspended in water, and the solution was extracted with $CHCl_3$ to yield 25 mg (0.25 mmol; 55%) of **10**. VLC was used as above for final purification of **10**, whose NMR spectral data were identical with those of the isolate.

(+)-1,2-Dehydropinidinol (10) to (−)-Pinidinol (3). To 6 mg (0.04 mmol) of **10** in 1 mL of dry THF was added an excess of LAH, immediately followed by the addition of Me_3Al (0.8 mL, 9 mmol). Under argon, this solution was allowed to stir for 2.5 h at −78 °C. The reaction was quenched with four drops of concd aqueous NaOH and stirred for 10 min at rt. The solution was extracted with $CHCl_3$ (4 × 2 mL), dried, and carefully evaporated under nitrogen to yield 3.4 mg (0.02 mmol; 50%) of **3**. The ^1H NMR spectrum of the product was identical to that of isolated **3**.

1,2-Dehydropinidinone (11). A preliminary screening of fresh new buds of *P. ponderosa*, collected on May 12, 1992, and treated by the general procedure, yielded 2 mg of alkaloid residue which was nearly pure **11**, although GCMS analysis showed small amounts of **9** and **10** as well. This yielded a useful ^1H NMR spectrum for **11**. To obtain further material, 208 g of fresh buds with emerging needles were collected on May 28, 1992, and 49 mg of alkaloid material was isolated. This proved to be an approximte 3:2 mixture of **11** and **10**, from which a ^{13}C NMR spectrum for **11** was obtained by subtracting out the resonances from **10**. Further attempts to obtain **11** pure as an isolate failed since it did not survive separation procedures. The resultant ^1H and ^{13}C NMR resonances and GCMS were identical with those of synthetic **11** prepared from **10** as follows. Several drops of Jones reagent (see above) were added to a stirred solution of **10** (44 mg, 0.28 mmol) in 2 mL of acetone until the color became yellow. The reaction mixture was quenched in 15 min by addition of 0.2 mL of a saturated aqueous solution of $NaHSO_3$. The solution was extracted with $CHCl_3$ and dried. TLC (C_{18} silica gel, acetone/benzene (4:1)) yielded 22 mg (0.14 mmol; 51%) of **11**, from which spectral data were obtained, but which did not survive purification for optical rotation measurements: MS M^+ *m/z* 153 (11), 110 (100), 96 (27), 82 (20), 71 (14), 43 (77); ^1H NMR δ 2.72 (1H, dd, 5.8, 16 Hz, H-8), 2.38 (1H, dd, 7.9, 16 Hz, H-8), 2.07 (3H, s, H-10), 2.00 (1H, m, H-6), 1.80 (3H, d, 1.9 Hz, H-7), 1.70–1.40 (6H, m, H-3, H-4, H-5); ^{13}C NMR δ 207.8 C-9, 168.8 C-2, 53.8 C-6, 50.9 C-8, 29.9 C-10, 29.8 C-3, 27.0 C-7, 26.7 C-5, 18.3 C-4.

Mature needles of *P. ponderosa* contained considerable pinidine, **2**, which was lacking from new buds or emerging needles of those buds. For the FETAX experiment, a combination of needle extracts was used to obtain a requisite 146 mg (as HCl salts). GCMS analysis showed this mixture to contain 24% **2**, 25% **9**, 31% **11**, and the remainder 10% each of two unknown MW 153 alkaloids.

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Supplementary Material Available: Spectra and figures showing structures of **5**·HCl and **6**·HCl computer drawn from the X-ray data (21 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.