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PYRROLIZIDINE AND PYRIDINE MONOTERPENE ALKALOIDS FROM TWO *CASTILLEJA* PLANT HOSTS OF THE PLUME MOTH, *PLATYPTILIA PICA*[1]

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ABSTRACT.—*Castilleja rhexifolia* was found to contain senecionine and its *N*-oxide as major alkaloids of the stems and leaves, while a new pyridine monoterpene alkaloid (rhexifoline) was the major alkaloid of the blossoms and seeds. A purported hybrid of *C. rhexifolia* with *Castilleja miniata* was found to contain sarracine and three new alkaloids, (7-angelylplatynecine, 9-angelylplatynecine, and its *N*-oxide) in stems and leaves. A trace of senecionine was found. The major alkaloid of the whole plant, but particularly concentrated in the seeds, was rhexifoline. Deoxyrhexifoline was also identified from the seeds.

Both larvae and adults of the plume moth (*Platyptilia pica*, Lepidoptera), which is hosted by *C. rhexifolia*, contain alkaloids. An extract of adult moths was shown by gc-ms to contain rhexifoline.

Our report (1) on the presence of the pyrrolizidine alkaloid senecionine in *Castilleja rhexifolia* Rydberg (a Rocky Mountain Indian Paint Brush species) was the first report of a pyrrolizidine in the Scrophulariaceae and the first reported alkaloid in *Castilleja*. This was followed by the finding (2) of quinolizidine (but no pyrrolizidine) alkaloids in *Castilleja miniata*. The present paper reports complete alkaloid analyses of *C. rhexifolia* and of a purported hybrid of the two species, designated "*C. rhexifolia* aff. *miniata*." All three taxa are hosts to the plume moth, *Platyptilia pica*, whose larvae and adults we have found to sequester alkaloids. Alkaloid analysis of a fourth host, *Penstemon whippleanus* (also of the Scrophulariaceae), has also been accomplished and reported (2).

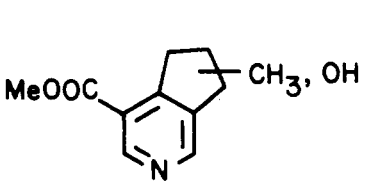
RESULTS

C. RHEXIFOLIA.—The major alkaloid of stems and leaves (as previously reported) was senecionine. The content was 0.05% in the stems and 0.13% in the leaves, with 40% of the total occurring as senecionine *N*-oxide. Small amounts (<0.01%) occurred in the blossom heads and only a trace could be identified in the seeds. The stems and leaves also contained traces of other pyrrolizidines, of which sarracine and indicine (or one of their stereoisomers) were identified.

The major alkaloid of the seeds (0.04%) and a minor component of the other parts was a new pyridine monoterpene alkaloid, dubbed rhexifoline. The molecular formula of $C_{11}H_{13}NO_3$ (MW 207) for rhexifoline was established by hrms, which also revealed a base peak of *m/z* 174 (loss of H_2O and CH_3). The uv spectrum (λ max 225, 273) and the presence of singlets at 9.04 and 8.67 ppm in the pmr spectrum were consistent with a 3,4,5-trisubstituted pyridine structure. The pmr spectrum also showed an alkyl methyl doublet at 1.34 ppm and a methyl singlet at 4.00 ppm. A 1715 cm^{-1} conjugated ester band was present in the ir spectrum. These data were best accommodated by part structure **1** for rhexifoline, and this structure was supported by noise and off-resonance decoupled cmr spectra. The pmr spectrum showed a one proton doublet of doublets downfield at 5.61 ppm, while the resonance for the proton coupled to the alkyl methyl was established (by double resonance) to be a one proton multiplet at 3.59 ppm. These two one-proton resonances were not themselves coupled; this would establish rhexifoline as either **2** or **3**. Structure **2** would be preferred on biogenetic grounds (3),

[1]Paper 3 in the series "Chemistry of the Scrophulariaceae." For paper 2, see J.W. McCoy and F.R. Stermitz, *J. Nat. Prod.*, **46**, 902 (1983).

because the C-7 methyl (R_2) corresponds to a methyl of the geranylpyrophosphate precursor. A similar compound, leptorhabine **4**, was identified (4) from *Leptorhabdos parviflora*, also of the Scrophulariaceae. In **4**, the C-5 proton was observed at 5.06 ppm, while it occurs at 5.61 ppm in rhexifoline. The extra downfield shift is attributable to the neighboring carbomethoxy group. Such a downfield shift of the C-5 proton has been observed in similar carbomethoxy-bearing pyridine monoterpenes such as cantleyine (5). Other pyridine monoterpenes lacking the carbomethoxy group exhibit C-5 proton chemical shifts similar to **4** (6,7). Thus, **2** is the preferred structure.



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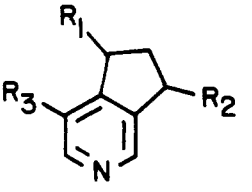


2: R_1=OH, R_2=CH_3, R_3=COOMe
3: R_1=CH_3, R_2=OH, R_3=COOMe
4: R_1=OH, R_2=CH_3, R_3=H (leptorhabine)

It remained to establish the relative stereochemistry for rhexifoline. None had been suggested (4) for leptorhabine. This was accomplished by difference nOe studies. Irradiation at 5.61 ppm (H-5) gave 4% enhancement of a 2.03 ppm resonance attributable to H-6β, but no enhancement of the 2.43 ppm resonance attributable to H-6α. Irradiation of H-6β (2.03 ppm) gave an 8.9% enhancement of H-5, while H-7 was not enhanced. This established H-6β *syn* to H-5 and *anti* to H-7. Therefore, H-5 and H-7 must be *anti* to each other and so must also be the OH and CH_3 substituents. As confirmation, irradiation of H-6α (2.43 ppm) gave a 5.4% enhancement of H-7 and no enhancement of H-5. The final structure of rhexifoline is therefore **5**. Additional proof resulted from conversion of the known iridoid penstemonoside to rhexifoline (8). The literature does not provide many ^{13}C-analyses of pyridine monoterpenes, and hence, we have done a complete assignment as given in the Experimental section. The detailed analysis, along with more details on the pmr spectrum, is available in a thesis (9).

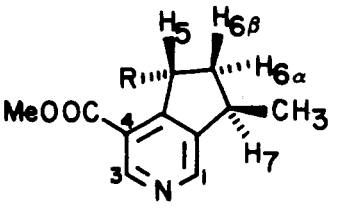
Rhexifoline was isolated in similar yields from procedures using either NH_3 or NaOH for basification, and hence, **5** is not an artifact arising from iridoid reaction with NH_3. Such artifactual syntheses of pyridine monoterpenes have been known to occur in isolations from some plant species.

C. RHEXIFOLIA AFF. *MINIATA*.—This taxon was collected not far from the collection site of *C. rhexifolia*. Morphological considerations (10) suggested that the collection showed possible hybridization with *C. miniata*. *C. miniata* does not presently grow within 50 km of the collection site.

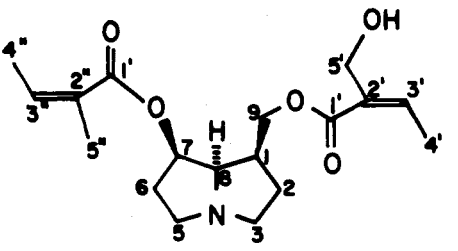
The major alkaloid of the whole plant was rhexifoline (0.01%). It was most concentrated in the seeds and flowers (0.09%), while minor amounts were found in the bracts and traces in stems and leaves. The seeds also contained small amounts of another new pyridine monoterpene alkaloid, deoxyrhexifoline (**6**). The identification followed simply from spectral comparisons with rhexifoline (Experimental section).

Pyrrolizidine alkaloids were again found (0.01% in the combined stems, leaves, and bracts plus traces in seeds and flowers). Interestingly, only trace amounts of senecionine were present. The major pyrrolizidines, found in approximately equal amounts, were sarracine (**7**) and three new alkaloids: 7-angelylplatynecine (**8**), 9-angelylplatynecine (**9**), and 9-angelylplatynecine *N*-oxide.

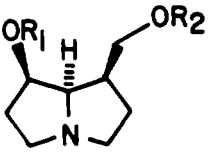
7-Angelylplatynecine was identified by pmr, cmr, and mass spectra. The high re-

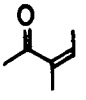


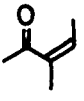
5: R=OH (rhexifoline)
6: R=H (deoxyrhexifoline)



7 (sarracine)



8: R_1=  ; R_2=H (7-angelylplatynecine)

9: R_1=H; R_2=  (9-angelylplatynecine)

solution mass spectrum established the molecular formula of $C_{13}H_{21}NO_3$. The base peak at *m/z* 82 is characteristic of saturated pyrrolizidines, while major fragments at *m/z* 156 and 139 result from the necic acid fragmentation. In the pmr spectrum, a group of resonances at 1.89, 2.02, and 6.10 ppm were shown by double resonance to be coupled to each other but were independent of the rest of the molecule. The observed chemical shifts were consistent with an angelate moiety. The olefinic proton resonance at 6.10 ppm distinguished the angelate from the tiglate, where this proton is normally at 6.50-7.30 ppm (11). The point of attachment of the angelate to the necine base was clear from the chemical shifts of the H-7 and H-9 protons. The former was a broad triplet at 5.34 ppm, while the latter geminal pair occurred as a two-proton doublet at 3.74. The H-9 resonances are not known to occur upfield at 4.00 ppm if the H-9 hydroxyl is esterified. The necine base could be identified as platynecine by comparison of the 360 MHz pmr spectrum to that of sarracine, for which a standard sample was available. The H-8 proton was at 3.55 ppm, close to the sarracine value of 3.69 ppm. Coupling constants were $J_{1,8}$=8.0Hz and $J_{7,8}$=3.7Hz for sarracine, and $J_{1,8}$=8.0Hz and $J_{1,8}$=3.4Hz for **8**. The sum of the coupling constants in H-7 as measured by width at half height was 6.5 for sarracine and 6.4 for **8**. The ΣJ_7 has been used previously (12, 13) for establishing configurations in saturated pyrrolizidines. Complete analysis of the 360 MHz pmr spectrum was accomplished by double resonance. Both this and the cmr spectrum were in complete accord with the structural assignment and the detailed data are given in the Experimental section.

The 9-angelylplatynecine structure **9** was established on the basis of the mass and 360 MHz pmr spectra. The mass spectrum was essentially identical to that for **8**. As opposed to the pmr spectral results for **8**, **9** showed the H-7 resonance at 4.34 ppm (not esterified) and the H-9 geminal pair as pairs of doublets at 4.62 and 4.48 ppm (esterified). The characteristic angelate resonances were at 1.89, 2.02, and 6.09 ppm. The necine base was again identified by $J_{1,8}$ and $J_{7,8}$ coupling constants as well as the half

height width (ΣJ_7) of the H-7 resonance. Complete spectral data are in the Experimental section.

The structure of 9-angelylplatynecine *N*-oxide was determined by mass spectrum and nmr data comparisons with the other pyrrolizidine isolates described above. Ring proton resonances were shifted downfield, with the greatest shifts occurring at positions next to nitrogen. The ^{13}C-resonance for C-8 at 89.7 ppm was diagnostic for the *N*-oxide (14, 15) as was a mass spectrum fragment at *m/z* 238 corresponding to the loss of OH (16). Complete spectral data are in the Experimental section. In confirmation, reduction converted the *N*-oxide to **9**.

PRELIMINARY ANALYSIS OF *PLATYPTILIA PICA*.—Tlc tests showed the presence of alkaloids in the larvae and adult moths raised on both collections of *Castilleja*. No alkaloids were detected in the larvae or moth frass. Adult moths from *C. rhexifolia* were extracted and the extract purified by preparative tlc. An alkaloid-positive band was removed and subjected to gc-ms analysis. One peak was positively identified as rhexifoline by retention time and fragmentation. Although all data have not as yet been analyzed, we could find no spectra with typical pyrrolizidine characteristics.

DISCUSSION

Our results need to be viewed from two aspects: (a) the alkaloid content and relationships among *Castilleja* species, and (b) the plant relationships to the plume moth for which they are hosts.

One major finding is the occurrence of unsaturated pyrrolizidines in *C. rhexifolia*, saturated pyrrolizidines in *C. rhexifolia* aff. *miniata*, and quinolizidines in *C. miniata* (2). The only overlap in content was the finding of a trace of unsaturated pyrrolizidines in *C. rhexifolia* aff. *miniata* and the probable identification of a trace of the saturated pyrrolizidine sarracine in *C. rhexifolia*. Because of the lack of *C. miniata* near the *C. rhexifolia* aff. *miniata* collection site, we hypothesize that the latter taxon may simply be a chromosomal variety of *C. rhexifolia*, rather than a hybrid. As pointed out previously (2), the suggested (17) involvement of polyploidy with speciation in *Castilleja* and the large variation in both chromosome number and morphology within each species of *Castilleja* makes taxonomy difficult in this genus.

Environmental effects on alkaloid production have not been assessed. *C. rhexifolia* occurs mainly in swampy areas, the *C. rhexifolia* aff. *miniata* population was from a nearby disturbed, sandy, dry roadbed bank. *Castilleja* are also known to be facultative parasites. Where work has been done in the Scrophulariaceae (18), the parasite apparently recognizes a chemical from the host, but there is little or no evidence that the secondary metabolite composition of the parasite is affected by the host. For now, our hypothesis is that the alkaloid composition effects we observed are genetically based; chromosome counts, to be conducted, should provide useful evidence.

Our original finding that the senecionine-containing *C. rhexifolia* was host to the plume moth, *Platyptilia pica*, suggested an addition to the known pyrrolizidine-Lepidoptera interactions, but complete lack of such alkaloids in *C. miniata* and *Penstemon whippleanus* (which are also hosts) (2) was not consistent with this hypothesis. Our identification of pyridine monoterpene alkaloids in all of the host species, as well as finding rhexifoline in adult moths from *C. rhexifolia*, suggests that these alkaloids may be involved in the interaction.[2] The major larval food is indeed the seeds, where rhexifoline is in high concentration.

Bowers has implicated (19) iridoid content among species of the Scrophulariaceae as a larval feeding stimulant and defense strategy of checkerspot butterflies. Because

[2]We have also found rhexifoline in moths raised from *C. sulphurea*: G. Harris, unpublished results.

iridoids are the precursors of pyridine monoterpenes, the presence of such alkaloids in the plume moth might be due to conversion of the precursor iridoid to the alkaloid in the larvae or moths, rather than to direct alkaloid sequestration from consumed plant material. Such a possibility cannot be discounted at this time and is a viable alternate answer (if a single one exists) to a chemical basis for the plant-insect interactions observed in this family. We have found penstemonoside, the probable iridoid precursor of rhexifoline, in high concentration in *C. rhexifolia* blossom heads, leaves, and seeds (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Pmr and cmr spectra were taken on JEOL FX-100 or Nicolet NT-360 spectrometers. Chemical shifts are reported as parts per million downfield from TMS unless otherwise specified. Ir spectra were recorded on a Beckman Acculab 3 spectrometer or a Nicolet MX-1 spectrometer as a thin film on KBr plates. Uv spectra were recorded on a Varian Techtron model 635 spectrometer. Optical rotations were measured on a Perkin Elmer 241 polarimeter. Melting points are uncorrected. Percentages are reported on a dry weight basis.

Ei and ci mass spectra were taken on a VG MM16 mass spectrometer with a Systems Industries interface and a Digital PDP8-A computer. Gc-ms data were collected using a Perkin Elmer Sigma 2 Chromatograph coupled to the mass spectrometer via a modified VG capillary interface.

Tlc was performed on 0.25 mm Si gel 60 F-254 (Merck) or aluminum oxide F-254 type T (Merck) plates. Preparative tlc was run on the same adsorbents, of 2 mm and 1.5 mm thickness, respectively. Visualization was with uv light, iodoplatinic acid, Ehrlich's and *o*-chloranil/Ehrlich's spray reagents. Rf values refer to tlc on 5×10 cm silica gel plates developed with $CHCl_3$-MeOH (1:1).

Flash chromatography and mplc were used on a preparative scale with silica gel 60 (MCB) or aluminum oxide (Merck). Hplc was run on a Waters Model 6000 Chromatograph with a UK-6 injector. A Hamilton PRP-1 XAD analytical column was employed throughout.

ISOLATION PROCEDURES *(C. RHEXIFOLIA).*—*C. rhexifolia*, Colorado State University (CSU) Herbarium #65242, was collected from a swampy meadow during bloom at 10,000 feet altitude west of Fort Collins, CO.

Whole plant material (1664 g) was allowed to air dry and was then ground, defatted with hexane (Soxhlet), and extracted with MeOH for 48 h. The MeOH extract was split into two portions, and each evaporated to near dryness under reduced pressure at a temperature not exceeding 40°. Portion A (684 g) was taken up in 1M H_2SO_4, washed with 4×250 ml $CHCl_3$, and the combined organic layers back extracted with 1M H_2SO_4. The combined acidified aqueous phases were then allowed to stand overnight with stirring in the presence of an excess of zinc dust. The mixture was filtered, made basic to pH 9 with NH_4OH and extracted with 5×200 ml $CHCl_3$. The organic layers were combined, filtered through anhydrous Na_2SO_4, and evaporated to a semicrystalline residue.

Crystallization of this residue from EtOH gave senecionine. Flash column chromatography of the mother liquor with EtOAc-iPrOH-NH_4OH (12:7:1) gave rhexifoline, senecionine, and mixed lower Rf pyrrolizidines. The total amount of senecionine recovered from this procedure was 342 mg (0.05%). Portion B (980 g) was treated similarly with the omission of the zinc reduction. The amount of senecionine recovered from the unreduced fraction was 294 mg (0.03%).

All fractions containing rhexifoline were combined and purified by a small-scale acid/base procedure to obtain spectroscopic data. Pure rhexifoline was isolated as a viscous yellow oil that decomposed slowly to a dark purple residue over a period of several days.

Fractions containing lower Rf alkaloids were combined and chromatographed by preparative tlc (Si gel) with EtOAc-iPrOH-MeOH-NH_4OH (12:6:3:1). One band was shown to contain sarracine as the major component by ms. A lower Rf band was of insufficient purity for further characterization.

Dried stems and leaves (560 g) were defatted with hexane, extracted with MeOH in a Soxhlet apparatus, and subjected to the previously described acid/base procedure with zinc reduction. The crude base fraction was crystallized from Me_2CO to yield senecionine (62 mg) and the mother liquor fractionated by mplc (Si gel) employing a stepwise gradient of 250 mls each: $CHCl_3$, MeOH-$CHCl_3$ (1:9), MeOH-$CHCl_3$ (1:3). MeOH-$CHCl_3$ (1:1), MeOH, and a 5% HCl methanolic wash. Twenty-five fractions were collected. Fractions 15 and 16 gave traces of impure rhexifoline. Fractions 17 through 21 gave predominantly senecionine (160 mg). The remaining fractions contained mixed low Rf alkaloids.

The latter fractions were combined and chromatographed on an alumina column with MeOH-$CHCl_3$ mixtures of increasing polarity. The principal alkaloid-containing fraction (16 mg) was further purified by preparative tlc (Si gel) with MeOH-$CHCl_3$ (6:4). The low Rf alkaloid band was removed and chromatographed by hplc to yield an indicine-type pyrrolizidine (pmr and ms). A Hamilton PRP-1 XAD column was used with H_2O-CH_3CN (7:3) and a flow rate of 0.7 ml/min. The indicine type pyrrolizidine was found in the fraction eluting from 4.0 to 7.0 min.

Dried blossoms (280 g) were extracted by repeated MeOH percolation. The extract was purified using the previously described acid/base procedure without zinc reduction to yield 160 mg crude base. To recover any *N*-oxides not extractable from the basic aqueous phase, it was reduced in volume, acidified, and reduced with zinc dust. After filtration, the solution was worked up in the usual manner to yield 30 mg additional pyrrolizidines. The crude base fractions were chromatographed by mplc (Si gel) using MeOH-$CHCl_3$ mixtures of increasing polarity in a similar fashion to chromatography of the stems and leaves extract. Fractions 5 and 6 gave nearly pure rhexifoline (50 mg, 0.02%). Fractions 13 through 17 gave small amounts of senecionine (25 mg, 0.01%). Fractions 37 through 39 gave senecionine *N*-oxide (12 mg, 0.004%), and the remaining fractions gave a mixture of lower Rf alkaloids.

Dried seeds (35 g) were extracted by MeOH percolation. The combined extract was purified by the usual acid/base procedure with zinc reduction. The small amount of crude base (30 mg) was chromatographed by preparative tlc (alumina) with MeOH-$CHCl_3$ (1:1). The highest band gave nearly pure rhexifoline (12.5 mg, 0.04%). The only other band spraying alkaloid-positive (1 mg) was found to contain trace amounts of rhexifoline, senecionine, and two other lower Rf alkaloid positives.

DATA FOR ISOLATED COMPOUNDS (*C. RHEXIFOLIA*).—Senecionine was identical to that previously isolated (1) by mp, ir, pmr and cmr spectra, and mass spectrum.

Senecionine *N*-oxide was synthesized as follows: to 34.5 mg senecionine in 10 ml cooled MeOH was added dropwise 1.0 ml 30% H_2O_2. After allowing to stand overnight, a small amount of PtO_2 was added to the reaction mixture. This was stirred for 2 h, filtered, and evaporated to dryness. Purification on Si gel with MeOH-$CHCl_3$ (1:1) gave 17.0 mg senecionine *N*-oxide (47%), identical to the isolate by 360 MHz pmr and cmr spectra (9, 14).

Rhexifoline: Rf 0.90; $[\alpha]^{23}D$ +18 (c=.82, $CHCl_3$); uv (EtOH) λ max 225, 273 nm; ir (cm^{-1}) 3370, 2915, 1715, 1450, 1285; high resolution eims *m/z* (rel. int.) M^+ 207.0895 (16), calcd. 207.0892, 192 (8), 179 (97), 174 (100), 164 (42), 160 (18), 146 (17), 132 (15), 91 (13), 77 (18); cmr (25 MHz, $CDCl_3$) 20.6 (q, C-7 methyl), 35.6 (d, C-7), 41.6 (t, C-6), 52.6 (q, OCH_3), 73.7 (d, C-5), 122.1 (s, C-4), 144.7 (s, C-7a), 149.1 (dd, C-3), 149.6 (dd, C-1), 154.8 (s, C-4a), 166.8 (s, C=O); pmr (360 MHz, $CDCl_3$) δ 1.34 (3H, d, J=7.0 Hz), 2.03 (1H, ddd, J=6.3, 7.4, 13.4 Hz), 2.43 (1H, ddd, J=3.7, 6.9, 13.4 Hz), 3.59 (1H, m), 4.00 (3H, s), 5.61 (1H, dd, J=7.4, 3.7 Hz), 8.67 (1H, s), 9.04 (1H, s). Detailed discussion of pmr and cmr assignments is the result of double resonance and nOe experiments and is available in the thesis (9), as are spectral data for acetate and 3,4-dinitrobenzoate derivatives.

Indicine-type Pyrrolizidine: Rf 0.15-0.18; eims *m/z* (rel. int.) M^+ 299 (5), 156 (8), 139 (39), 138 (100), 120 (12), 95 (22), 94 (29), 93 (72), 80 (17); NH_3-cims M+1 300 (100), 139 (12), 138 (32), 136 (11), 93 (10); pmr of mixed isolate (360 MHz, CD_3OD) δ 0.92 (3H, d), 0.94 (3H, d), 1.16 (3H, d, J=6.3 Hz, H-4′), 1.97 (2H, m, H-6), 2.15 (1H, m, H-5′), 2.75 (1H, m, H-5u), 3.87 (1H, m, H-3d), 4.05 (1H. q, H-3′), 4.20 (1H, m, H-8), 4.37 (1H, m, H-7), 5.86 (1H, br.s, H-2), other signals obscured by solvent and impurity peaks.

Sarracine-type Pyrrolizidine: Rf 0.21; eims *m/z* (rel. int.) M^+ 337 (3), 238 (22), 154 (33), 138 (84), 137 (84), 120 (30), 98 (78), 93 (75), 82 (100), 80 (32); CH_4-cims *m/z* M+1 338 (32), 238 (33), 156 (8), 138 (53), 137 (12), 120 (18), 29 (100).

ISOLATION PROCEDURES.—*C. rhexifolia aff. miniata* (voucher FRS 206-CSU Herbarium #65241) was collected in bloom along a gravelly roadbank near the *C. rhexifolia* location. Dried and powdered stems and leaves (700 g) were extracted by repeated MeOH percolation. The wet residue recovered from solvent evaporation was taken up in 1 M H_2SO_4 (200 ml), washed with $CHCl_3$, made basic to pH 9 with NH_4OH, and extracted with $CHCl_3$. After drying with Na_2SO_4, the combined $CHCl_3$ phases were evaporated to yield 130 mg crude base fraction. The aqueous phase was reduced in volume, acidified to pH 1 with 25% H_2SO_4, and stirred overnight in the presence of zinc dust. After filtration, the aqueous phase was treated as just described to afford additional pyrrolizidines (308 mg).

The combined crude base fractions were fractionated by mplc on Si gel with a stepwise gradient of equal volumes $CHCl_3$, $CHCl_3$-MeOH (3:1 and 1:1), MeOH, and a 5% NH_4OH in MeOH wash. Most pyrrolizidines were recovered in several wash fractions. Preparative tlc (alumina), EtOAc-iPrOH (1:1), of the first wash fraction afforded 9-angelylplatynecine (9-AP) *N*-oxide (25 mg, 0.004%). The remaining alkaloid fractions were combined and rechromatographed by preparative tlc on alumina with $CHCl_3$-MeOH (9:1). This afforded 7-angelylplatynecine (7-AP) (10 mg, 0.002%) and mixtures of sarracine (8 mg, 0.001%), 9-AP *N*-oxide, 9-AP, 7-AP, an indicine-type pyrrolizidine, and trace amounts of other unidentified alkaloids (20 mg).

The bracts (150 g) were treated similarly to the stems and leaves to yield 240 mg of crude base. Mplc on Si gel with the previously described $CHCl_3$-MeOH gradient and 5% NH_4OH wash gave 33 fractions. Early fractions afforded rhexifoline (13 mg, 0.009%) and trace amounts of senecionine. The latter fractions afforded sarracine (14 mg, 0.009%) and mixtures of 7-AP, 9-AP *N*-oxide, 9-AP, and other unidentified alkaloids (12 mg, 0.008%). Treatment of a small amount of the bract extract, using NaOH instead of

NH_4OH, yielded rhexifoline in amounts similar to those produced with the NH_4OH treatment indicating that rhexifoline is not an artifact.

Seeds and flowers (99 g) were extracted by MeOH percolation and the extract purified by the usual acid/base procedure with zinc reduction. Mplc of the crude base (256 mg) on neutral alumina with a stepwise elution gradient of hexane-$CHCl_3$ (8:2, 6:4, and 3:7), $CHCl_3$, MeOH-$CHCl_3$ (1:1) and MeOH afforded rhexifoline, a mixture of pyridine monoterpenes, and a mixed pyrrolizidine fraction (2 mg, 0.002%). The mixed pyridine monoterpene fraction was purified by preparative tlc on alumina with hexane-$CHCl_3$ (6:4) to yield rhexifoline and deoxyrhexifoline (2 mg, 0.002%). The total amount of rhexifoline recovered was 92 mg (0.09%).

Partial purification of fractions containing trace amounts of other pyrrolizidines was achieved, and incomplete data for these are available in a thesis (9). One is an indicine-type, and a second may be 9-angelylretronecine.

DATA FOR ISOLATED ALKALOIDS (*C. RHEXIFOLIA* AFF. *MINIATA*).—*7-Angelylplatynecine*: Rf 0.12; $[\alpha]^{23.5}D$ −98 (c=0.004, EtOH); uv (EtOH) λ max 239 nm; ir (cm^{-1}) 3330, 2954, 1714, 1231, 1154, 1038, 837; eims *m/z* (rel. int.) M^+ 239.1530 (2) calcd. for $C_{13}H_{21}NO_3$ 239.1516, 221 (6), 156 (23), 140 (8), 139 (24), 138 (7), 96 (16), 95 (44), 83 (9), 82 (100); cmr ($CDCl_3$, 90 MHz) δ 167.0 (s, C-1′), 139.4 (d, C-3′), 127.2 (s, C-2′), 75.1 (d, C-7)*, 69.2 (d, C-8)*, 62.7 (t, C-9), 55.6 (t, C-3)*, 53.6 (t, C-5)*, 43.9 (d, C-1), 35.2 (t, C-6)*, 28.7 (t, C-2)*, 20.8 (q, C-4′), 15.8 (q, C-5′); [The asterisked assignments are not unambiguous. Assignments are based on the arguments of Molyneux, *et al.* (15)]; pmr ($CDCl_3$, 360 MHz) δ6.10 (1H, qq, *J*=7.2, 1.4 Hz, H-3′), 5.34 (1H, br.t, H-7), 3.74 (2H, d, *J*=7.5 Hz, H-9), 3.54 (1H, dd, *J*=8.0, 3.4 Hz, H-8), 3.29 (1H, m, H-5), 3.18 (1H, m, H-3), 2.78 (1H, m, H-3), 2.72 (1H, m, H-5), 2.63 (1H, m, H-1), 2.06 (2H, m, H-6), 2.02 (3H, dq, *J*=7.2, 1.5 Hz, H-4′), 1.89 (3H, dq, *J*=1.5, 1.4 Hz, H-5′), 1.86 (1H, m, H-2), 1.76 (1H, m, H-2).

TABLE 1. Pmr Double Resonance (45 db)

Signal irradiated	Signal exhibiting coupling
6.10	2.02, 1.89
5.34	3.54, 2.06, 1.86
3.74	2.63
3.54	5.34, 2.63
3.29	2.72, 2.06
3.18	2.78, 1.86, 1.76
2.78	3.18, 1.86, 1.76
2.72	3.29, 2.06
2.63	3.74, 3.54, 1.86, 1.76
2.06	5.34, 3.29, 2.72
2.02	6.10, 1.89
1.89	6.10, 2.02
1.86	3.18, 2.78, 2.63, 1.76
1.76	3.18, 2.78, 2.63, 1.86

Sarracine: Rf 0.29; eims *m/z* (rel. int.) M^+ 337 (1), 237 (19), 222 (17), 139 (27), 138 (100), 122 (37), 96 (29), 95 (43), 83 (38), 82 (78); pmr ($CDCl_3$, 360 MHz) δ 6.38 (1H, q, *J*=7.2 Hz, H-3′), 6.13 (1H, qq, *J*=7.3, 1.4 Hz, H-3″), 5.36 (1H, br.t, H-7), 4.43 (1H, dd, *J*=7.6, 11.0 Hz, H-9), 4.25 (1H, dd, *J*=7.0, 11.0 Hz, H-9), 4.24 (2H, s, H-5′), 3.69 (1H, dd, *J*=3.7, 8.0 Hz, H-8), 3.40 (1H, br.t, H-5), 3.25 (1H, m, H-3), 2.85 (1H, m, H-1), 2.80 (1H, m, H-3), 2.76 (1H, m, H-5), 2.00-2.14 (2H, m, H-2, H-6), 2.05 (3H, d, *J*=7.3 Hz, H-4′), 2.02 (3H, dq, *J*=7.3, 1.5 Hz, H-4″), 1.80-1.98 (2H, m, H-2, H-6), 1.91 (3H, dq, *J*=1.5, 1.4 Hz, H-5″).

9-Angelylplatynecine N-oxide: Rf 0.29; uv (EtOH) δ max 237; ir (cm^{-1}) 3150 br., 2920, 1703, 1455, 1230, 1150, 792; eims *m/z* (rel. int.) 238 (20), 137 (36), 119 (54), 106 (49), 95 (60), 82 (100), 80 (39), 55 (84); NH_3-cims M+1 240 (14), 238 (51), 156 (37), 138 (100), 136 (26), 131 (17); cmr (CH_3OD, 25 MHz) δ 168.9 (s, C-1′), 139.1 (d, C-3′), 128.8 (s, C-2′), 89.7 (d, C-8), 72.4 (d, C-7), 71.7 (t, C-3)*, 69.9 (t, C-5)*, 64.1 (t, C-9), 39.4 (d, C-1), 35.7 (t, C-6)*, 30.1 (t, C-2)*, 20.8 (q, C-5), 16.1 (q, C-4′); [The asterisked assignments are not unambiguous. Assignments are based on the arguments of Molyneux, *et al.* (15)]. Pmr ($CDCl_3$, 360 MHz) δ 6.10 (1H, qq, *J*=7.2, 1.4 Hz, H-3′), 4.66 (1H, dd, *J*=11.0, 8.2 Hz, H-9), 4.63 (1H, br.s, H-7), 4.43 (1H, dd, *J*=11.9, 6.9 Hz, H-9), 3.95-3.83 (2H, m, H-5, H-3), 3.80 (1H, dd, *J*=4.9, 8.4 Hz, H-8), 3.71 (1H, m, H-5), 3.62 (1H, m, H-3), 3.26 (1H, m, H-1), 2.64

(1H, m, H-6), 2.34 (1H, m, H-2), 2.00-2.13 (2H, m, H-2, H-6), 1.98 (3H, dq, J=7.2, 1.5 Hz, H-4′), 1.87 (3H, dq, J=1.5, 1.4 Hz, H-5′).

9-Angelylplatynecine: Rf 0.07; uv (EtOH) λ max 232; eims *m/z* (rel. int.) M^+ 239 (4), 221 (8), 156 (19), 139 (18), 96 (22), 95 (51), 83 (29), 82 (100), 80 (10), 55 (30); NH_3-cims *m/z* (rel. int.) M+1 240 (100), 241 (16), 177 (16), 140 (69), 138 (16), 122 (12), 95 (13), 82 (29); pmr ($CDCl_3$, 360 MHz) δ 6.09 (1H, qq, J=7.2, 1.4 Hz, H-3′), 4.62 (1H, dd, J=11.0, 7.6 Hz, H-9), 4.48 (1H, dd, J=11.0, 7.0 Hz, H-9), 4.34 (1H, br.t, H-7), 3.52 (1H, dd, J=8.0, 2.7 Hz, H-8), 3.44 (1H, m), 3.26 (1H, m), 2.85-2.95 (2H, m), 2.73 (1H, m), 1.70-2.2 (4H, m), 1.99 (3H, dq, J=7.2, 1.5 Hz, H-4′), 1.89 (3H, dq, J=1.5, 1.4 Hz, H-5′).

Deoxyrhexifoline: ir (cm^{-1}) 2872, 1724, 1480, 1307, 1213, 1141; eims *m/z* (rel. int.) M^+ 191.0948 (80), calcd. for $C_{11}H_{13}NO_2$ 191.0943, 190 (15), 176 (100), 160 (24), 144 (29), 132 (24), 130 (19), 117 (35), 116 (23), 77 (14); NH_3-CI MS M+1 192 (100), 176 (17), 160 (4), 132 (4), 117 (5); pmr ($CDCl_3$, 360 MHz) δ 8.99 (1H, s, H-1), 8.54 (1H, s, H-3), 3.93 (3H, s, H-10), 3.38 (1H, ddd, J=18.5, 8.8, 4.1 Hz, H-5), 3.30 (1H, br.q, H-7), 3.13 (1H, m, H-5), 2.38 (1H, m, H-6), 1.67 (1H, m, H-6), 1.34 (3H, d, J=6.9 Hz, H-8).

Analysis of Plume Moth Extract: 317 mixed male and female adult moths (~770 mg) raised from harvested *C. rhexifolia* were stored at 5° in 50 ml MeOH. The solution was then allowed to stand at 25° for 24 h and macerated. The mixture was filtered and the solid residue washed with several portions of MeOH (50 ml total). Following evaporation of the solvent, the residue was triturated with MeOH and filtered. This was repeated until the entire residue (~50 mg) could be dissolved in 250 μl MeOH. The residue was separated by preparative tlc with $CHCl_3$-MeOH (1:1). The alkaloid positive band (11 mg) was removed and analyzed by gc-ms. Gc conditions were as follows: 30 m J & W DB-5 fused-silica bonded-phase capillary column. Temperature programmed at 2°/min from 170° to 300°, 10 to 1 split with helium at 1 ml/min. An early peak having the retention time of rhexifoline gave an identical mass spectrum. Visual inspection of spectra from peaks where pyrrolizidines would elute as well as computer searching for typical pyrrolizidine fragments gave no positive results for the presence of these alkaloids. Data from all peaks have not been completely analyzed. The general procedures have not been optimized for detection of more volatile components. Additional work on the gc-ms data as well as localization of rhexifoline in the adult moth is continuing.

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LITERATURE CITED

1. F.R. Stermitz and T.R. Suess, *Phytochemistry*, **17**, 2142 (1978).
2. J. McCoy and F.R. Stermitz, *J. Nat. Prod.*, **46**, 902 (1983).
3. G.A. Cordell, *The Alkaloids*, **16**, 470 (1977).
4. Kh. A. Kadyrov, V.I. Vinogradova, A. Abdusamatov, and S. Yu. Yunusov, *Khim. Prir. Soedin.*, **10**, 683 (1974).
5. T. Sevenet, B.C. Das, J. Parello, and P. Potier, *Bull. Soc. Chim.*, 3120 (1970).
6. H.R. Arthur, S.R. Johns, J.A. Lamberton, and S.N. Loo, *Aust. J. Chem.*, **20**, 2505 (1967).
7. F. Rulko and K. Witkiewicz, *Pol. J. Pharmacol. Pharm.*, **26**, 561 (1974).
8. M.R. Roby and F.R. Stermitz, *J. Nat. Prod.*, **47**, 854 (1984).
9. M.R. Roby, Ph.D. Thesis, Colorado State University, 1983.
10. L. Heckard, University of California, Berkeley, private communication, 1981.
11. C.C.J. Culvenor, S.R. Johns, J.A. Lamberton, and L.W. Smith, *Aust. J. Chem.*, **23**, 1279 (1970).
12. A.J. Aasen, C.C.J. Culvenor, and L.W. Smith, *J. Org. Chem.*, **34**, 4137 (1969).
13. M.A. Siddiqu, K.A. Suri, O.P. Suri, and C.K. Atal, *Phytochemistry*, **18**, 1413 (1979).
14. H.J. Segall and J.L. Dallas, *Phytochemistry*, **22**, 1271 (1983).
15. R.J. Molyneux, J.N. Roitman, M. Benson, and R.E. Lundin, *Phytochemistry*, **21**, 439 (1982).
16. Ya. V. Rashkets, V.A. Abdullaev, and S. Yu. Yunusov, *Khim. Prir. Soed.*, 153 (1978).
17. L.R. Heckard and T. Chuang, *Brittonia*, **29**, 159 (1977).
18. J.C. Steffens, J.L. Roark, D.G. Lynn, and J.L. Riopel, *J. Am. Chem. Soc.*, **105**, 1669 (1983) and references therein.
19. M.D. Bowers, *J. Chem. Ecol.*, **9**, 475 (1983).

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