ISOLATION, SEMI-SYNTHESIS, AND NMR SPECTRAL STUDIES OF LOLINE ALKALOIDS[1]

R.J. Petroski, S.G. Yates, D. Weisleder, and R.G. Powell*

*USDA, Agricultural Research Service, Northern Regional Research Center,
1815 N. University Street, Peoria, Illinois 61604*

ABSTRACT.—Loline, a saturated pyrrolizidine-type alkaloid, was isolated from tall fescue (*Festuca arundinacea*) seed infected with the endophytic fungus *Acremonium coenophialum*. Procedures are described for the efficient conversion of loline to derivatives also known to occur naturally: norloline, N-formylnorloline, N-acetylnorloline, N-methylloline, N-formylloline, and N-acetylloline. The loline alkaloids are of interest as they are suspected contributors to several disease syndromes in cattle that consume endophyte-infected tall fescue. The structure of hydroxy-chlorololine, a reaction product of loline with HCl, was determined, and complete [1]H- and ^{13}C-nmr assignments for all the lolines are reported.

Occurrence of the loline alkaloids, a group of pyrrolizidine alkaloids, has so far been restricted to the genera *Lolium* (1,2) and *Festuca* (3) (Graminae), and *Adenocarpus* (4) (Leguminosae). Naturally occurring loline alkaloids include loline (festucine) [1], N-formylloline [2], N-acetylloline (lolinine) [3], N-methylloline [4], norloline [5], N-formylnorloline [6], N-acetylnorloline [7], and N-propionylnorloline (decorticasine) [8]. Alkaloids 1–7 have been isolated from *Lolium cuneatum*, alkaloids 1–3, 7 have been reported as constituents of *Festuca arundinacea* (tall fescue), and 8 occurs in *Adenocarpus decorticans*. The absolute configuration of 1 was determined by X-ray crystallography (5), and a total synthesis of 1 has been reported (6). Partial [1]H-nmr assignments have been reported for 1 (7–9), 2 (7), 3 (7) and 5 (10), but detailed [1]H-nmr spectra have not appeared and ^{13}C spectra of the loline alkaloids are entirely absent in the literature.

Tall fescue (*F. arundinacea*) is a major forage crop of the southeastern region of the
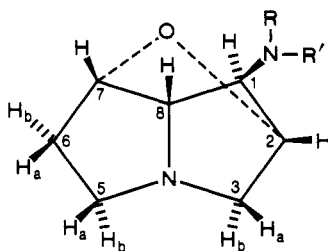
1	R=Me, R'=H
2,2'*	R=Me, R'=CHO
3	R=Me, R'=Ac
4	R=R'=Me
5	R=R'=H
6	R=H, R'=CHO
7	R=H, R'=Ac
8	R=H, R'=EtC=O

*60:40 mixture of rotamers 2 and 2', respectively in CDCl₃ at ambient temperature.

United States. At least 35 million acres are under cultivation in this region (11). Over 90% of the tall fescue grown in the United States is infected, to various degrees, with *Acremonium coenophialum*, an endophytic fungus of the Clavicipitaceae (12).

Studies have revealed a positive association between endophyte infection, loline alkaloid production, and resistance to insect pests such as sod webworm, *Crambus* spp. (13); fall armyworm, *Spodoptera frugiperda* (14); and aphids, *Rhopalosipum pali* and *Schizaphis graminum* (15). Yates *et al.* (16) demonstrated that **2** is toxic to the large milkweed bug, *Oncopeltus fasciatus*. Loline alkaloid levels in endophyte-infected tall fescue vary widely due to seasonal and environmental factors, but it is not unusual for the total of **2** and **3** to exceed 2 g/kg (17). Unfortunately, presence of the endophyte in tall fescue is also associated with several disease syndromes in cattle (18) and is responsible for losses to cattle producers of an estimated $50 to $200 million annually (11).

We report preparation of the naturally occurring loline alkaloids **2–7** from **1** isolated from tall fescue seed; their nmr spectral assignments are given. The structure of hydroxychlorololine [**9**], a product of reaction of **1** with HCl, is also reported for the first time.

RESULTS AND DISCUSSION

Isolation of loline [**1**] from tall fescue and subsequent conversion of **1** to loline derivatives were achieved using published procedures (1,7) or slight modifications thereof. However, separation problems were encountered in the preparation of norloline [**5**] from **1**. Alkaloid **1** was separated from **5** by gc after conversion to their N-formyl derivatives. Preparative-scale separation of **1** and **5** was accomplished by cc on activity-grade III neutral alumina.

Two-dimensional homonuclear COSY and ^{1}H/^{13}C heteronuclear correlation nmr experiments were conducted on the series of loline alkaloids (**1–7**, **1**·2HCl, and **9**·2HCl). The ^{13}C and ^{1}H assignments are reported in Tables 1 and 2.

The signal at δ 33.5 was assigned to C-6 because all other carbons of loline have an adjacent heteroatom. The carbon signal at δ 34.7 and proton signal at δ 2.19 were assigned to the N-Me group. Protons on C-6 were assigned to δ 1.74 ddd and δ 1.65 dddd on the basis of 2D ^{1}H/^{13}C heteronuclear correlation experiments. Both protons were coupled to the protons on C-5: δ 2.77 ddd and δ 2.62 ddd. C-5 was assigned to the signal at δ 54.0. DEPT experiments confirmed that only C-3, -5, and -6 have triplet multiplicity, corresponding to two attached protons. Because C-5 and C-6 were previously assigned, the signal at δ 60.6 was assigned to C-3. The C-6 proton signal at δ 1.65, designated 6a on the structure, was coupled to the proton on C-7 (δ 4.10 dd, $J = 4.3$ Hz). The signal at δ 81.0 in the carbon spectrum was assigned to C-7. H-6a was also coupled to H-6b (δ 1.74, $J = 14.3$ Hz), H-5a (δ 2.62, $J = 9.8$ Hz), and H-5b (δ 2.77, $J = 3.8$ Hz). H-5a was also coupled to H-5b ($J = 12.3$ Hz).

The H-7 also couples to H-8 (δ 2.84, $J = 1.9$ Hz), and H-8 is further coupled to H-
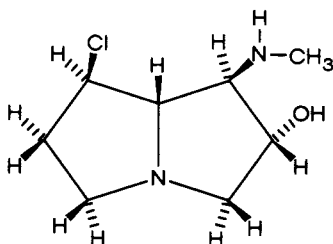

9

TABLE 1.   ¹H-nmr Chemical Shifts (δ) and Proton Couplings (J, Hz) for Loline [1], N-Formylloline (Rotamers 2 and 2'), N-Acetylloline [3], N-Methylloline [4], Norloline [5], N-Formylnorloline [6], N-Acetylnorloline [7], Hydroxychlorololine·2HCl (9·2HCl), and Loline·2HCl (1·2HCl).ª

Proton	Multiplicity	Compound									
		1	2	2'	3	4	5	6	7	9·2HCl	1·2HCl
		Chemical Shifts									
H-1	dd	3.03	3.68	3.82	4.05	2.53	3.48	4.52	4.41	3.77	4.23
H-2	dd	3.71	4.05	4.56	4.38	3.82	3.72	4.29	4.17	4.66	4.79
H-3a	dd	3.10	3.09	3.10	3.03	3.36	3.38	3.43	3.29	3.92	4.15
H-3b	dᵇ	2.10	2.33	2.29	2.26	2.18	2.29	2.53	2.42	3.37	3.55
H-5a	ddd	2.62	2.83	2.83	2.84	2.78	2.80	3.04	2.90	3.52	3.73
H-5b	ddd	2.77	2.91	2.91	2.93	2.89	2.98	3.17	3.10	3.92	3.73
H-6a	dddd	1.65	1.80	1.80	1.81	1.75	1.84	2.07	1.98	2.34	2.28
H-6b	ddd	1.74	1.93	1.94	1.92	1.86	1.94	2.18	2.08	2.74	2.37
H-7	dd	4.10	4.34	4.27	4.29	4.25	4.29	4.50	4.44	4.80	4.72
H-8	dd	2.84	3.30	3.34	3.10	3.01	2.92	3.32	3.09	4.48	4.79
NH	bm	—	—	—	—	—	—	7.39	6.21	—	4.76
NMe	s	2.19	2.76	2.94	3.04	2.12	—	—	—	2.79	2.79
NMe	s	—	—	—	—	2.12	—	—	—	—	—
HC=O	s	—	7.25	8.23	—	—	—	8.17	—	—	—
MeC=O	s	—	—	—	1.96	—	—	—	1.97	—	—

Table 1.   Continued

Proton	Compound									
	1	2	2'	3	4	5	6	7	9·2HCl	1·2HCl
	Proton Couplings									
$J_{1,2}$	1.6	1.5	1.4	2.7	1.2	<2	2.1	2.5	4.9	<2
$J_{2,3a}$	<2	<2	<2	<2	1.2	1.6	<2	1.0	5.6	1.0
$J_{2,3b}$	—	—	—	—	—	—	—	—	5.6	—
$J_{3a,3b}$	11.6	11.9	11.8	11.7	10.9	11.7	12.0	11.8	13.1	13.9
$J_{5a,5b}$	12.3	13.1	13.1	12.8	12.8	12.8	12.9	12.8	12.1	12.8
$J_{5a,6a}$	9.8	9.3	9.3	9.1	9.3	9.3	9.3	9.3	7.1	8.2
$J_{5a,6b}$	7.3	7.1	7.1	7.4	7.2	7.5	7.5	7.4	5.0	7.7
$J_{5b,6a}$	3.8	4.4	4.4	4.2	4.2	3.8	4.0	3.6	5.3	5.0
$J_{5b,6b}$	8.3	7.8	7.8	8.4	8.4	8.3	8.6	8.3	6.4	9.6
$J_{6a,6b}$	14.3	14.3	14.3	14.2	14.0	14.3	14.3	14.4	15.0	14.6
$J_{6a,7}$	4.3	4.3	4.3	4.3	4.4	4.4	3.9	4.3	4.6	4.8
$J_{6b,7}$	—	—	—	—	—	—	—	—	4.9	—
$J_{7,8}$	1.9	1.8	1.8	<2	1.8	1.9	2.2	1.9	3.2	2.2
$J_{8,1}$	1.8	2.0	2.0	<2	1.3	1.8	<2	1.6	6.1	1.9

[a]Spectra were recorded at 300 MHz. Chemical shifts are reported relative to TMS with CDCl₃, solutions for all compounds except 9·2HCl and 1·2HCl. For compounds 9·2HCl and 1·2HCl, spectra were recorded with D₂O solutions, and chemical shifts are relative to internal Me₂CO (δ 2.12).
[b]In the case of 9·2HCl, the H-2 is also coupled to H-3b.

TABLE 2. ^{13}C-nmr Assignments for Loline [1], N-Formylloline (Rotamers 2 and 2'), N-Acetylloline [3], N-Methylloline [4], Norloline [5], N-Formylnorloline [6], N-Acetylnorloline [7], Hydroxychlorololine·2HCl (9·2HCl), and Loline·2HCl (1·2HCl).ᵃ

Carbon	Multiplicity	Compound									
		1	2	2'	3	4	5	6	7	9·2HCl	1·2HCl
C-1	d	67.8	65.3	62.4	64.1	74.1	60.5	55.8	57.6	68.2	65.9
C-2	d	73.4	73.9	73.0	73.4	74.2	76.2	73.6	73.8	73.6	73.9
C-3	t	60.6	60.4	60.8	61.0	61.2	60.8	60.8	60.9	60.4	64.2
C-5	t	54.0	54.4	54.4	54.7	54.3	54.5	53.7	54.6	57.6	58.1
C-6	t	33.5	32.8	32.6	33.0	33.4	34.1	31.7	33.9	36.1	31.6
C-7	d	81.0	81.8	80.0	80.6	82.0	81.7	80.0	80.9	60.8	83.2
C-8	d	69.0	67.4	67.8	67.8	69.2	71.9	69.7	69.6	77.5	72.2
NMe	q	34.7	33.4	29.8	33.9	44.4	—	—	—	34.8	36.5
NMe	q	—	—	—	—	44.4	—	—	—	—	—
HC=O	d	—	162.1	163.5	—	—	—	161.8	—	—	—
MeC=O	s	—	—	—	171.5	—	—	—	170.2	—	—
MeC=O	q	—	—	—	22.3	—	—	—	23.1	—	—

ᵃSpectra were recorded at 75.5 MHz. Chemical shifts are reported relative to CDCl₃, (δ 77.0) for all compounds except 9·2HCl and 1·2HCl. For compounds 9·2HCl and 1·2HCl, spectra were recorded with D₂O solutions relative to internal Me₂CO at δ 30.5.

1 (δ 3.03, *J* = 1.8 Hz). Irradiation of H-8 changes the H-7 signal (dd) to a sharp doublet showing only coupling of H-6a with H-7. The H-1 signal (apparent triplet, but actually a doublet of doublets) also changes to a sharp doublet showing only coupling of H-1 with H-2 (δ 3.71, *J* = 1.6 Hz). $^1H/^{13}C$ heteronuclear correlation experiments showed signals for C-8, -1, and -2 are at δ 69.0, δ 67.8, and δ 73.4, respectively. The H-2 is coupled to H-3a (δ 3.10, *J*<2 Hz). Irradiation of H-2 resulted in changing the H-3a signal (dd) to a sharp doublet showing only the H-3,3a coupling of 11.6 Hz. Abnormally low vicinal coupling constants found in loline have been reported previously by Aasen and Culvenor (9). Using a Dreiding model of loline, the authors reported approximate dihedral angles of 45° for 2,3a and 75° for 2,3b. The coupling of H-2 with H-3b is too small to be observed. In loline, cis vicinal couplings are larger than trans vicinal couplings.

   Two rotational isomers (2 and 2′) were evident in the ^{13}C- and ^1H-nmr spectra of *N*-formylloline, but only one rotational isomer was apparent in the spectrum of *N*-formylnorloline (6). LaPlanche and Rogers (20) studied the phenomenon of rotational isomerism in compounds such as *N*-isopropyl-*N*-methylformamide. The appearance of rotamers is only evident in compounds having a substituent that inhibits free rotation. Assignments of ^1H and ^{13}C signals for each of the rotamers were relatively easy because the minor rotamer (40%) nmr peaks were only ⅔ the size of those for the major rotamer (60%). Two rotamers were previously observed in the ^1H-nmr spectrum of *N*-formylloline by Robbins *et al.* (7).

   A comparison of **1**·2HCl with **9**·2HCl shows a dramatic difference in the signals for C-7 (δ 83.2 vs. δ 60.8). This observation is consistent with the chlorine being on C-7. A slight shift, from δ 73.9 to δ 73.6, for the C-2 signal is consistent with a hydroxyl group residing on C-2. The hydroxyl group at C-2 should be trans to the amine group at C-1. In the opening of the ether bridge, stereochemistry at C-2 should remain unchanged. Loline is regenerated by the action of base on **9**·2HCl (3), strongly suggesting SN-2 displacement of Cl⁻ by O⁻ from C-2. For this to occur, the stereochemistry of C-7 should be *R* in **9**·2HCl.

EXPERIMENTAL

   ISOLATION OF LOLINE [**1**].—Endophyte-infected tall fescue seed (*F. arundinacea*, Lambert Seed Co., Camden, AL) was defatted with hexane (ca. 1.5 liter/kg seed), air-dried, ground in a milling machine to 2-mm mesh, and extracted with MeOH (3 × 10 ml/g seed). The MeOH extract was concentrated in vacuo ca. fiftyfold and diluted with 1% aqueous citric acid (5 volumes). The acidic solution was extracted with CHCl₃ (3 ×, with equal volumes) to give a "CHCl₃ I" fraction. The remaining aqueous solution was adjusted to pH 11 with NaOH and carefully extracted with CHCl₃ (5 ×, with equal volumes) using thorough but gentle mixing to avoid emulsions. The CHCl₃ extract was concentrated in vacuo to ¹⁄₁₀ volume and extracted with 0.2 N aqueous HCl (3 ×, ¼ volume). The CHCl₃ phase "CHCl₃ II" contained only small amounts of alkaloids. The aqueous HCl phase was basified with NaOH to pH 11 and extracted with CHCl₃ (5 ×, with equal volumes) to afford a "CHCl₃ III" fraction which contained loline-type alkaloids. The "CHCl₃ III" fraction was concentrated in vacuo to ¹⁄₁₀ volume, dried over anhydrous Na₂SO₄, and filtered, and the loline-type alkaloids were precipitated by bubbling anhydrous HCl gas through the CHCl₃ solution. Approximately 8 g of mixed loline alkaloid dihydrochloride salts were obtained per 5 kg of endophyte-infected tall fescue seed. The alkaloid mixture was completely hydrolyzed to alkaloid **1** and trace amounts of **4** and **5** by heating for 3 h at 80° in 1 N HCl (10 ml/g mixed loline·2HCl). After cooling, the reaction mixture was washed with CHCl₃ (3 ×, with ½ volumes), adjusted to pH 11 with NaOH, and then extracted with CHCl₃ (5 ×, with equal volumes). The CHCl₃ extract was concentrated to ⅕ the original volume, dried over anhydrous Na₂SO₄, and filtered, and dry HCl gas was bubbled into the CHCl₃ to precipitate crude **1**·2HCl which was recrystallized from EtOH to give 4.73 g pure **1**·2HCl: mp 243–248°; ^1H nmr see Table 1; ^{13}C nmr see Table 2. Loline-free base [**1**] was prepared by dissolving salt **1**·2HCl in water, basifying to pH 10 with NaOH, extracting into CHCl₃, and evaporating the dried solvent to afford a clear viscous oil: ir ν max (CHCl₃) 2793, 2715, 1476, 1432, 1367, 1311, 1293, 1251, 1218, 1134, 1090, 1044, 1025, 991, 956 cm⁻¹; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims (70 eV) *m/z* (rel. int.) [M]⁺ 154 (3.5), 123 (12), 110 (34), 95 (31), 82 (100).

INSTRUMENTAL METHODS.—^{1}H- and ^{13}C-nmr spectra were recorded with a Bruker WM 300 spectrometer operating at 75.47 MHz for ^{13}C and 300.13 MHz for ^{1}H from either $CDCl_3$ or D_2O solutions; $CDCl_3$ served as the internal lock as well as an internal reference standard at 77 ppm for ^{13}C. For spectra in D_2O (**1**·2HCl and **9**·2HCl), chemical shifts are relative to internal Me_2CO, δ 2.12 and 30.5 for ^{1}H and ^{13}C, respectively.

Mass spectra were recorded in the eims mode at 70 eV in a Finnigan model 4600 TSQ spectrometer with sample introduction through a gas chromatograph. Ir spectra were recorded on a Mattson Cygnus 25 FTIR; melting points were determined on a Fischer-Johns block and are uncorrected.

ANALYTICAL CHROMATOGRAPHY.—Synthetic reaction mixtures and isolated compounds were examined by gc. All isolated alkaloid-free bases were homogeneous by gc. Retention times in min for the various alkaloids were: **1**, 14.6; **2**, 24.2; **3**, 24.7; **4**, 14.8; **5**, 14.3; **6**, 27.2; **7**, 27.8. Gc was accomplished with a Bendix Model 2600 gas chromatograph equipped with a 2 mm × 6 ft glass column packed with 3% Poly-A 101A, 100–120 mesh, on Gas-Chrom Q (Applied Science Laboratories). Carrier gas (He, 40 psig) was supplied at a rotameter setting of 30–40 ml/min. H_2 (20 psig) was supplied at 30–40 ml/min, and air (30 psig) was introduced at 600 ml/min. Temperatures were set at 225 and 250° at inlet and detector, respectively; electrometer was normally operated at 3 × 10^{-10} amp. Temperature was programmed to hold 0.5 min at 60°, then 6°/min to 180°, hold 0.5 min, then 3°/min to 220° and hold 0.5 min. Tlc separation of **1** and **5** was accomplished using silanized Si gel 60 5 × 20 cm plates (EM Reagents); developing solvent was 0.5% Et_3N in MeOH. The R_f values for **1** and **5** were 0.43 and 0.30, respectively. Alkaloids were detected using potassium iodoplatinate spray reagent.

N-FORMYLLOLINE [**2**].—A solution of 154 mg (1 mmol) of **1** in 2 ml of ethyl formate was allowed to stand 96 h at room temperature in a closed vial. Solvent was evaporated to afford **2** as a clear viscous oil: ir ν max ($CHCl_3$) 2937, 2880, 1671, 1473, 1386, 1353, 1084, 1050, 1024, 962 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims (70 eV) m/z (rel. int.) [M − 28]$^+$ 154 (11), 123 (9), 110 (9), 95 (24), 82 (100).

N-ACETYLLOLINE [**3**].—Acetyl chloride (0.71 ml, 10 mmol) was added to a solution of **1** (1.54 g, 10 mmol) on 10 ml of $CHCl_3$ and stirred at room temperature overnight. The reaction solution was extracted with 0.1 N HCl (3 × 10 ml), and the combined aqueous acid phase was adjusted to pH 10 with NaOH. The alkaline solution was extracted with $CHCl_3$ (5 × 10 ml), combined extracts were dried over anhydrous Na_2SO_4, and the $CHCl_3$ was removed by evaporation in vacuo to yield **3** as a clear viscous oil: ir ν max ($CHCl_3$) 2937, 2878, 1652, 1472, 1400, 1348, 1023, 957 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims (70 eV) m/z (rel. int.) [M]$^+$ 196 (2.1), 167 (5), 153 (8), 123 (23), 95 (43), 82 (100), 42 (42).

N-METHYLLOLINE [**4**].—Loline (2.75 g, 15 mmol) was added to a mixture of HCO_2H (1.0 ml) and formaldehyde (1.6 ml of formalin) and heated at reflux for 4 h. The reaction mixture was cooled, made acid with 1 N HCl to pH 1–2, and washed with Et_2O (3 × 5 ml); the pH was adjusted to 10 with NaOH, and the alkaline solution was extracted with $CHCl_3$ (7 × 5 ml). The $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and then evaporated to afford an oily residue. Gc analysis showed a mixture of 10% **2** and 90% **4**. The mixture was separated on a Si gel column (50 g) using 5% MeOH in $CHCl_3$ (300 ml), then 20% MeOH in $CHCl_3$ (100 ml). Compound **2** eluted within the first 100 ml of solvent. Compound **4** eluted just after **2**; solvent was evaporated in vacuo. Compound **4** was converted to its dihydrochloride salt by addition of two equivalents of HCl in EtOH and recrystallized from EtOH to yield 1.9 g of product, mp 212–216°. The salt was converted to free base **4** by the same methods used to obtain **1**: ir ν max ($CHCl_3$) 2942, 2875, 2818, 2768, 1600, 1466, 1367, 1314, 1272, 1184, 1094, 1042, 960 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims (70 eV) m/z (rel. int.) [M]$^+$ 168 (1.7), 123 (42), 95 (68), 82 (100).

NORLOLINE [**5**].—Loline (1.54 g, 10 mmol) was dissolved in 20 ml of 20% aqueous H_2SO_4 and cooled to 0–4° in an ice bath. A solution of $KMnO_4$ (630 mg, 4.0 mmol) in 20 ml of cold H_2O was added slowly. The reaction mixture was stirred for 3 h, warmed to room temperature, and filtered through Whatman #42 ashless paper to remove solids, and the clear acidic filtrate was evaporated at 60° under reduced pressure to remove excess H_2O and HCHO. The solution (⅓ original volume) was adjusted to pH 10–11 with 12 ml of 6 N NaOH and extracted exhaustively (10 × 2 vol) with $CHCl_3$. After drying and evaporation of $CHCl_3$, an oily residue (1.4 g) was obtained which consisted primarily of **1** and **5** in near equal amounts. The gc method (see Analytical Chromatography) used to measure reaction products did not readily separate **1** from **5** but easily separated the corresponding N-formyl derivatives (prepared by adding a drop of the oily residue to 1 ml of ethyl formate and allowing the reaction to proceed overnight). Resolution of the oily residue was accomplished by applying the sample (4.4 g from several preparations) to an activity grade III neutral alumina column (220 g, 36 cm × 2.8 cm) and eluting with 2% MeOH in $CHCl_3$. Fractions of 25 ml each were collected; fractions 12–15 contained mostly **1** (1.2 g), fractions 16 and 17 contained a mixture (0.9 g) of **1** and **5**, and fractions 18–26 contained **5** (1.3 g). Identification of alkaloids **1** and **5** in fractions was accomplished by tlc as described under Analytical Chromatography. The mixture

from fractions 16 and 17 (0.9 g) was rechromatographed in the manner described above to yield an additional 76 mg of **5**. Norloline [**5**] was converted to its dihydrochloride salt by the addition of two equivalents of HCl in EtOH, and the salt was recrystallized from EtOH/H_2O to give 0.87 g of crystalline flakes, mp 271–274°. The free base was prepared by dissolving 210 mg in 1 ml of H_2O, basifying with 6 N NaOH, extracting **5** into $CHCl_3$ (10 × 2 ml), drying over anhydrous Na_2SO_4, and evaporating to yield 80 mg of **5** as a clear viscous oil. Alkaloid **5** was quite H_2O-soluble and also quite volatile. Spectral properties: ir ν max (CHCl₃) 3376, 3293, 3181, 2964, 2937, 2876, 1616, 1473, 1293, 1249, 1216, 1174, 1041, 1000, 998, 974 cm⁻¹; ¹H nmr see Table 1; ¹³C nmr see Table 2; eims (70 eV) *m/z* (rel. int.) [M]⁺ 140 (4.3), 123 (24), 111 (15), 97 (22), 95 (15), 82 (100), 69 (26).

N-FORMYLNORLOLINE [**6**].—Norloline (140 mg, 1 mmol) was formylated in 2 ml of ethyl formate overnight. Evaporation of solvent yielded **6** as a clear viscous oil: ir ν max (CHCl₃) 3190, 2972, 2880, 2790, 1681, 1600, 1541, 1388, 1339, 1247, 1105, 1007 cm⁻¹; ¹H nmr see Table 1; ¹³C nmr see Table 2; mass spectrum (70 eV) *m/z* (rel. int.) [M]⁺ 168 (0.3), 140 (3), 123 (5), 95 (17), 82 (100), 69 (26).

N-ACETYLNORLOLINE [**7**].—Norloline (140 mg, 1 mmol) was added to 3 ml of phenyl acetate and kept at room temperature for 96 h. The resulting mixture was extracted with 2 ml of 1 N HCl. The aqueous acid phase was washed with $CHCl_3$ (3 × 2 ml), then basified with 1 ml of 6 N NaOH. The basified solution was extracted with $CHCl_3$ (4 × 3 ml), the pooled $CHCl_3$ extracts were dried over anhydrous Na_2SO_4, and $CHCl_3$ was evaporated to yield 85 mg of **7** as a clear viscous oil: ir ν max (CHCl₃) 3284, 3182, 2967, 2939, 2878, 1673, 1542, 1473, 1436, 1374, 1294, 1006, 962 cm⁻¹; ¹H nmr see Table 1; ¹³C nmr see Table 2; eims (70 eV) *m/z* (rel. int.) [M − 29]⁺ 153 (2.6), 139 (1), 123 (7), 95 (21), 82 (100), 69 (34).

HYDROXYCHLOROLOLINE·2HCl.—Compound **9**·2HCl was prepared from **1**·2HCl by the method of Yates and Tookey (3): ir ν max (KBr) 3306, 2969, 2896, 2765, 2696, 2419, 1630, 1566, 1472, 1341, 1268, 1103, 1008, 956 cm⁻¹; ¹H nmr see Table 1; ¹³C nmr see Table 2; eims (70 eV) *m/z* (rel. int.) [M − HCl]⁺ 154 (7.5), 137 (4), 118 (1), 104 (3), 86 (5), 82 (100).

ACKNOWLEDGMENTS

LITERATURE CITED

1. S.Y. Yunosov and S.T. Akramov, *J. Gen. Chem. USSR (Engl. Transl.)*, **25**, 1965 (1955).
2. G. Dannhardt and L. Steindl, *Planta Med.*, 212 (1985).
3. S.G. Yates and H.L. Tookey, *Aust. J. Chem.*, **18**, 53 (1965).
4. M. Ribas-Barceló and I. Ribas-Marqués, *An. Quim.*, **64**, 637 (1968); *Chem. Abstr.*, **69**, 87265m (1968).
5. R.B. Bates and S.R. Morehead, *Tetrahedron Lett.*, **17**, 1629 (1972).
6. J.J. Tufariello, H. Meckler, and K. Winzenberg, *J. Org. Chem.*, **51**, 3356 (1986).
7. J.D. Robbins, J.G. Sweeny, S.R. Wilkinson, and D. Burdick, *J. Agric. Food Chem.*, **20**, 1040 (1972).
8. H.L. Tookey and S.G. Yates, *Quimica*, **68**, 921 (1972).
9. A.J. Aasen and C.C.J. Culvenor, *Aust. J. Chem.*, **22**, 2021 (1969).
10. I. Ribas-Marqués and M. Pazo-Carracedo, *An. Real Soc. Fis. Quim.*, Ser. B, **63**, 915 (1967); *Chem. Abstr.*, **68**, 39878w (1968).
11. M.R. Siegel, M.C. Johnson, D.R. Varney, W.C. Nesmith, R.C. Buckner, L.P. Bush, P.B. Burrus II, T.A. Jones, and J.A. Boling, *Phytopathology*, **74**(8), 932 (1984).
12. G. Morgan-Jones and W. Gams, *Mycotaxon*, **15**, 311 (1982).
13. C.R. Funk, P.M. Halisky, M.C. Johnson, M.R. Siegel, A.V. Stewart, S. Ahmad, R.H. Hurley, and I.C. Harvey, *Biotechnology*, **1**(2), 189 (1983).
14. T.D. Hardy, K. Clay, and A.M. Hammond Jr., *Environ. Entomol.*, **15**, 1083 (1986).
15. M.C. Johnson, D.L. Dahlman, M.R. Siegel, L.R. Bush, G.C.M. Latch, D.A. Potter, and D.R. Varney, *Appl. Environ. Microbiol.*, **49**, 568 (1985).
16. S.G. Yates, J.C. Fenster, and R.J. Bartelt, *J. Agric. Food Chem.*, **37**(2), 354 (1989).
17. T.A. Jones, R.C. Buckner, and P.B. Burrus II, *Can. J. Plant Sci.*, **65**(2), 317 (1985).
18. D. Sanchez, *Agric. Res.*, **35**(8), 12 (1987).
19. J.C. Sheehan and V.J. Grenda, *J. Am. Chem. Soc.*, **84**, 2417 (1962).
20. L.A. LaPlanche and M.T. Rogers, *J. Am. Chem. Soc.*, **85**, 3728 (1963).