Hetisine-Type Diterpenoid Alkaloids from the Bhutanese Medicinal Plant *Aconitum orochryseum*

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Investigation of *Aconitum orochryseum*, a Bhutanese traditional medicine ("gSo-ba Rig-pa") plant locally known as "Bong-kar", resulted in the isolation of three new hetisine-type diterpenoid alkaloids, named orochrine (**1**), 2-*O*-acetylorochrine (**2**), and 2-*O*-acetyl-7α-hydroxyorochrine (**3**), together with the previously reported alkaloids atisinium chloride (**4**) and virescenine (**5**). The structures of the new alkaloids were elucidated by spectroscopic data analysis.

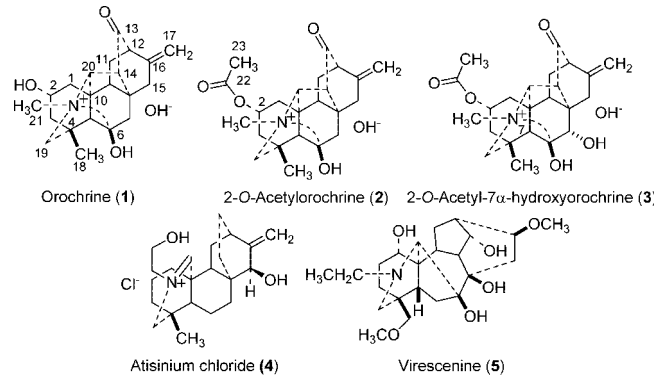
The plant genus *Aconitum* (Ranunculaceae) has over 100 species that are native to temperate regions of the northern hemisphere. Crude preparations from *Aconitum* plants were popularly used in Asia, Alaska, and Europe[1] in folklore and traditional medicine for the treatment of traumatic injury,[2] as a febrifuge and bitter tonic,[3] and as ingredients in intoxicating liquors.[4] Even in modern medicine, aconitine-containing liniments have been used for the treatment of rheumatism, neuralgia, and sciatica.[5] In Bhutan, three species of the genus, *Aconitum lacinatum* Stapf, *Aconitum violaceum* Jacq., and *Aconitum orochryseum* Stapf, are used in the formulation of more than 25 traditional medicines.[6,7]

Due to the widespread use of *Aconitum* plants in folk medicine[8] and also due to the presence of biologically active diterpenoid alkaloids,[9] the genus has been the subject of extensive phytochemical and pharmacological investigations. However, no work has been reported on the Bhutanese *Aconitum* species. In this context, one of these species, *Aconitum orochryseum* Stapf, was analyzed for alkaloids.

A. orochryseum is a herbaceous perennial plant that grows to a height of 40–100 cm.[10] It is endemic to Bhutan and bordering areas. Analysis of the alkaloids from this plant resulted in the isolation of three new hetisine-type C_{20}-diterpenoid alkaloids, which have been named orochrine (**1**), 2-*O*-acetylorochrine (**2**), and 2-*O*-acetyl-7α-hydroxyorochrine (**3**), together with two known alkaloids, atisinium chloride (**4**) and virescenine (**5**). The details are reported in this paper.

Orochrine (**1**) was obtained as needle-like crystals from acetone/diethyl ether. Interestingly, this alkaloid formed a translucent gel in the presence of chloroform. The alkaloid was optically active and had a high melting point. The LRCIMS indicated a molecular ion peak at *m/z* 342 (quaternary ammonium ion), and the HRCIMS supported the molecular formula, $C_{21}H_{28}NO_3$, for this alkaloid.

No signals for aromatic protons were evident in the ^{1}H NMR spectrum of **1** (Table 1). Two broad singlets (δ 5.04, 1H and δ 4.94, 1H) indicated the presence of an exocyclic methylene group, which strongly suggested that this alkaloid belongs to the hetisine type of C_{20}-diterpenoid alkaloids. A sharp singlet resonating at δ 1.48 (3H) was assigned to the C-18 methyl group. The signals ascribed to H-20 and H-12 (1H each, br s) were observed at δ 4.27 and 2.97, respectively. The H-19 proton signals resonated at δ 3.35 (d, 1H, J = 11.5 Hz) and 4.30 (d, 1H, J = 11.5 Hz). Since almost all hetisine-type alkaloids display these four characteristic proton signals,[8] it was concluded that **1** indeed belongs to this compound class; this skeletal assignment was also substantiated by the subsequent quaternary carbon designations.



From the gCOSY spectrum of **1**, a distinct geminal coupling was observed between the proton signals at δ 2.69 (d, 1H, J = 17 Hz, H-15) and 2.52 (d, 1H, J = 17.5 Hz, H-15) (Table S1, Supporting Information). These geminal protons were coupled to the exocyclic methylene protons H-17 (δ 5.04, br s, 1H and δ 4.94, br s, 1H). The gCOSY spectrum also confirmed strong coupling between the H-19 proton signals at δ 4.30 and 3.35.

The ^{13}C NMR spectrum of **1** indicated the presence of an alkene group with a signal at δ 142.6 (C-16) and another signal at δ 112.3 (C-17) (Table 2). A ketone carbonyl group (C-13) was assigned to the resonance signal at δ 208.7. Generally, for the hetisine-type C_{20} diterpenoid alkaloids, the signal assigned[13] to C-2 with a α-hydroxyl group resonates in the range δ_C 66.1–67.2 (or at δ_C 64.5 for deacetylheterophylloidine[12]) with its respective proton signal between δ_H 4.02 and 4.31 as a broad singlet. On the basis of this evidence, the carbon signal at δ 65.5, with an associated proton signal (broad singlet) at δ 4.14 (H-2β), was assigned to C-2 with a hydroxyl group attached. The DEPT analysis showed 15 protonated carbons; seven of these were methylene carbon atoms (including the exocyclic methylene group), six were methine carbons, and two were methyl carbons. On the basis of this DEPT, gCOSY, and TOCSY as well as gHSQC spectroscopic analysis, complete carbon–proton connectivity, proton–proton correlations, and group assignments were achieved for **1**.

The gHSQC correlations confirmed that the signal assigned to C-17 had a chemical shift of δ 112.3 and the C-18 (methyl carbon) was represented by the signal at δ 30.3. Using the scaffold of the hetisine-type diterpenoid alkaloids, the cross carbon peaks based on the gHMBC (carbon to proton and proton to carbon correlation) and gNOESY spectra were assigned (Table S1, Supporting Information). From the gHMBC correlation, the C-4, C-6, C-8, C-10, C-13, and C-16 quaternary carbons were established. The chemical

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Table 1. ^{1}H NMR Spectroscopic Data (CD_3OD) for Orochrine (**1**), 2-*O*-Acetylorochrine (**2**), and 2-*O*-Acetyl-7α-hydroxyorochrine (**3**)

	orochrine (**1**)	2-*O*-acetylorochrine (**2**)	2-*O*-acetyl-7α-hydroxyorochrine (**3**)
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	1.59, d (15)	1.65, d (17.5), 2H	1.65, d (16.5)
	1.75, d (15)		1.74, d (15.5)
2	4.14, br s	5.11, d (3)	5.21, s
3	1.60, d (15)	1.66, d (15.5)	1.65, d (17)
	1.93, t (15)	1.92, d (15.5)	1.93, d (16.5)
5	2.15, s	2.17, s	2.12, s
7	2.23, d (12.5)	2.23, d (14.5)	4.35, s
	2.31, d (15)	2.27, d (14.5)	
9	2.20, s	2.17, s	2.20, d (7.5)
11	1.86, d (14)	1.84, m, 2H	1.86, d (6.5), 2H
	2.03, d (14.5)		
12	2.97, br s	2.89, d (3.5)	2.95, s
14	2.98, br s	2.97, d (2)	3.00, s
15	2.52, d (17.5)	2.47, d (17)	2.48, d (17.5)
	2.69, d (17)	2.62, d (17.5)	2.96, d (17.5)
17	5.04, br s	4.86, br s	4.92, br s
	4.94, br s	4.95, br s	5.00, br s
18	1.48, s	1.43, s, 3H	1.52, s, 3H
19	3.35, d (11.5)	3.37, d (12)	3.42, d (11.5)
	4.30, d (11.5)	3.79, d (12)	3.61, d (10.0)
20	4.27, s	3.76, s	3.63, s
21	2.90, s	2.90, s, 3H	3.12, s
23		2.06, s, 3H	2.01, s, 3H

Table 2. ^{13}C NMR Data (CD_3OD) for Orochrine (**1**), 2-*O*-Acetylorochrine (**2**), and 2-*O*-Acetyl-7α-hydroxyorochrine (**3**)

	orochrine (**1**)	2-*O*-acetylorochrine (**2**)	2-*O*-acetyl-7α-hydroxyorochrine (**3**)
position	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	34.5, CH_2	31.6, CH_2	31.2, CH_2
2	65.5, CH	69.0, CH	66.9, CH
3	41.4, CH_2	38.5, CH_2	37.9, CH_2
4	36.6, qC	36.5, qC	35.9, qC
5	59.1, CH	58.5, CH	55.8, CH
6	106.2, qC	106.6, qC	105.7, qC
7	38.2, CH_2	38.0, CH_2	71.7, CH
8	43.9, qC	44.2, qC	45.3, qC
9	49.4, CH	49.3, CH	46.7, CH
10	47.4, qC	47.4, qC	47.7, qC
11	23.3, CH_2	23.3, CH_2	22.1, CH_2
12	53.5, CH	53.3, CH	51.6, CH
13	208.7, qC	208.7, qC	206.7, qC
14	56.3, CH	56.2, CH	52.5, CH
15	32.6, CH_2	32.4, CH_2	28.2, CH_2
16	142.6, qC	142.3, qC	139.1, qC
17	112.3, CH_2	112.4, CH_2	113.1, CH_2
18	30.3, CH_3	30.1, CH_3	29.7, CH_3
19	70.5, CH_2	70.5, CH_2	70.4, CH_2
20	75.1, CH	75.1, CH	73.5, CH
21	37.3, CH_3	37.3, CH_3	40.4, CH_3
22		171.3, qC	169.1, qC
23		21.3, CH_3	21.4, CH_3

shifts of C-4 (36.6) and C-10 (47.4) closely matched the range of chemical shifts reported in the literature for hetisine-type diterpenoid alkaloids.[8]

The gHMBC and gNOESY cross-peak analysis of **1** supported the N–C-6 and C-14–C-20 assignments, and the NOESY results (Figure 1) were consistent with the spatial arrangements of protons and functional groups observed in a computer-generated 3D model (Spartan software; AM1). The presence of a quaternary nitrogen (attached to C-19, C-20, C-6, and the C-21 methyl group) and the quaternary carbon at C-6 (attached to C-5, C-7, N-CH_3, and a hydroxyl group) indicated its relationship to vakhmadine, which was isolated as a quaternary hydroxide salt from *Aconitum palmatum*.[14] Thus, the structure of **1** was confirmed by comparing its structure with the structure of vakhmadine and also with the

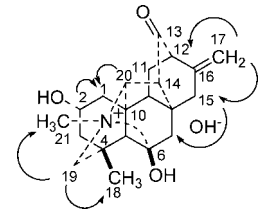

Figure 1. Selected NOESY NMR correlations seen for orochrine (**1**).

general range of chemical shifts reported in the literature on C_{20} hetisine-type diterpenoid alkaloids.[8] From this comparative analysis, the ^{13}C and ^{1}H NMR chemical shifts for **1** corresponded closely with the values for vakhmadine. In both cases, the presence of a quaternary nitrogen atom caused weak deshielding effects on nearby protons in the N-CH_3 group (δ 2.90), H-19 (δ 3.35 and 4.30), and H-20 (δ 4.27). Similarly, the hydroxyl group at C-6 caused the protons of C-18 to shift slightly downfield to δ 1.48 and the *N*-methyl carbon and protons to be shifted upfield at δ_C 37.3 (γ effect)[8] and δ_H 2.90,[15] respectively.

However, unlike vakhmadine, orochrine (**1**) possesses a carbonyl group at C-13 (δ 208.7) instead of a hydroxyl group and did not contain a C-3 hydroxyl group. Because of the C-13 carbonyl, the chemical shifts of C-9 (δ 49.4), C-11 (δ 23.3), and C-12 (δ 53.5) shifted downfield compared to the shifts for the same carbons assigned in vakhmadine. When the NMR data for orochrine was compared with that for panicudine, which has a C-13 carbonyl but no C-3 hydroxyl group, the chemical shifts of C-13 and its neighboring carbons (C-12 and C-14) in **1** were generally consistent with the chemical shifts of the signals for the carbons of panicudine. Panicudine was isolated from *Aconitum paniculatum* Lam.[8] The counterion for the structure of orochrine (**1**) was assigned as the hydroxide ion in view of the aqueous extraction process used.

The compound 2-*O*-acetylorochrine (**2**) was obtained as a white solid. LRCIMS gave a peak at *m/z* 384 (MH^+ peak), and HRCIMS supported a molecular formula of $C_{23}H_{30}NO_4$ (42 amu greater than that of **1** and corresponding to a C_2H_2O unit). The structure of **2** differed from orochrine (**1**) only in the C-2 substituent, with an acetate rather than a hydroxyl at the C-2 position. Such biosynthetically reasonable acetylations are not unusual in these types of alkaloids. Like orochrine (**1**), no signals for aromatic protons were evident in the ^{1}H NMR spectrum of 2-*O*-acetylorochrine (**2**) (Table 1), and so the index of hydrogen deficiency was indicative of saturated rings or double bonds. However, the presence of an exocyclic methylene group was indicated by two broad singlets (δ 4.86 and 4.95; 1H each). These protons corresponded to the carbon peak at δ 112.4 from the gHSQC correlation. The proton signals in this case shifted upfield (+0.09), but the signal for carbon shifted downfield (+0.15) in comparison to the equivalent signals in orochrine (**1**).

In the ^{13}C NMR spectrum of **2**, the N-CH_3 carbon (C-21) was assigned to the signal at δ 37.3 (Table 2), while in the ^{1}H NMR spectrum the methyl group was assigned to a singlet signal at δ 2.90 (Table 1). In addition, another three-proton singlet for the acetate protons was present at δ 2.06, which correlated with a carbon signal at δ 21.3. A new ^{13}C NMR peak at δ 171.3 was consistent with the ester carbonyl group. The C-13 carbonyl group signal resonated at δ 208.7. The signal ascribed to C-6 (δ 106.6) was assigned on the basis of the gHMBC data (Table S2, Supporting Information).

From the ^{1}H NMR and gCOSY spectra (Table S2), the H-15 geminal protons [δ 2.47 (1H, d, J = 17 Hz) and δ 2.62 (1H d, J = 17.5 Hz)] were coupled to H-17 in an exocyclic methylene group. As with other hetisine-type alkaloids,[8] a singlet [δ 3.76 (1H)] ascribed to H-20 and a doublet [δ 2.89 (1H, J = 3.5 Hz)] for H-12 were present. Unlike the case with orochrine (**1**), the signal ascribed

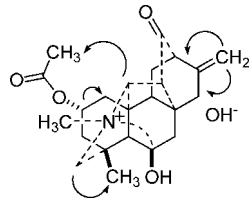

Figure 2. Selected NOESY NMR correlations seen for 2-*O*-acetylorochrine (**2**).

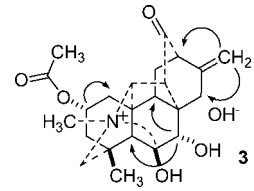

Figure 3. Selected NOESY NMR correlations seen for 2-*O*-acetyl-7α-hydroxyorochrine (**3**).

to H-11 (2H) appeared as a multiplet and the signal ascribed to H-14 was a doublet [δ 2.97 (1H, $J = 2$ Hz)]. A doublet signal for H-2β was observed slightly downfield (compared to the signal for H-2β in orochrine) at δ 5.11, consistent with the presence of the adjacent 2α-acetate group.[8]

The gHMBC and gNOESY correlations (Table S2) provided further confirmation of carbon–proton connectivities and spatial arrangements. The carbon to proton and proton to carbon cross-peak analysis was in accord with structure **2**, and the gNOESY spectrum confirmed the relative configuration of the alkaloid; selected key NOESY interactions are shown in Figure 2. From the gHMBC correlation, C-4, C-6, C-8, C-10, C-13, and C-16 were assigned as quaternary carbons, and the chemical shifts for C-4, C-10, and C-5 of δ 36.5, 47.4, and 58.5, respectively, were consistent with the normal range noted for these carbon signals in related alkaloids.[8] The orientation of protons and functional groups in the 3D model of the structure generated by the Spartan computer program (AM1) was consistent with the gNOESY correlations and the stereochemistry assigned to **2**.

2-*O*-Acetyl-7α-hydroxyorochrine (**3**) was isolated as a pale green solid, and the HRCIMS indicated a molecular formula of $C_{23}H_{30}NO_5$. Like **1** and **2**, the ^{1}H NMR spectrum of **3** had no signals for aromatic protons (Table 1). However, the exocyclic methylene peaks were clearly present [δ 4.92 and δ 5.00 (each 1H, brs)], which suggested that this alkaloid also belonged to the hetisine-type C_{20}-diterpenoid alkaloids. In the ^{13}C NMR spectrum of **3** (Table 2), the C-18 carbon was assigned to a peak at δ 29.7, and its protons resonated at δ 1.52 (s, 3H). The *N*-methyl carbon (C-21) was assigned to the signal at δ 40.4. A three-proton singlet for the ester methyl protons was present at δ 2.01, and the corresponding carbon signal at δ 21.4. A carbon peak for the ester carbonyl was present at δ 169.1. The C-13 carbonyl group resonated at δ 206.7. The signal for the C-6 carbon resonated at δ 105.7. The N-C6 bond was established from the HMBC spectrum (Table S3, Supporting Information).

The ^{1}H NMR and gCOSY (Table S3) spectra of **3** revealed that the H-15 geminal protons (δ 2.48 and 2.96) were coupled to the H-17 exocyclic methylene protons. The signal at δ 2.12 (1H s) was assigned to the H-5 proton, while the signal at δ 2.20 (1H, d) was assigned to the H-9 proton. The signal for H-2β (δ 5.21,1H, br s) was consistent with the presence of the C-2 acetoxy group.

The DEPT spectrum of **3** revealed seven methine, six methylene, and three methyl groups. Relative to **2**, alkaloid **3** had gained one methine and lost one methylene carbon. The complete carbon–proton correlations for these protons and carbons were established from analysis of the gHMBC, gCOSY, and TOCSY spectra.

From the gHMBC and gNOESY correlations (Table S3, Supporting Information), proton–carbon and carbon–proton connectivities as well as the relative configuration of the alkaloid was established. The cross-peaks observed in the gHMBC spectrum were assigned on the basis of the scaffold of the hetisine-type alkaloids, and this established C-4, C-6, C-8, C-10, C-13, and C-16 as quaternary carbons. The peaks at δ 35.9, 47.7, and 55.8 were assigned to C-4, C-10, and C-5, respectively, as the chemical shifts were within the normal range noted for these carbon signals in related alkaloids.[8]

The signal at δ_C 71.7 was assigned to C-7, consistent with hydroxyl group substitution, while H-7 was assigned to the signal at δ_H 4.35. Literature precedent indicated that this C-7 hydroxyl group normally has the α-orientation and the signal for H-7β should be within the range of chemical shifts of δ 3.87–4.50.[8] The chemical shift for H-7 was within this range, and the gNOESY correlations (Figure 3) of H-7 to H-5 and H-9 (both of these last two protons being β) were consistent with H-7 also being in the β-orientation; these observed interactions were also consistent with those predicted from an AM-1 based model (Spartan program) of 2-*O*-acetyl-7α-hydroxyorochrine (**3**). Thus a 7α-OH was assigned in the structure of **3**.

The other NMR data for 2-*O*-acetyl-7α-hydroxyorochrine (**3**) were consistent with the general range of chemical shifts reported in the literature for these diterpenoid alkaloids.[8,16] A comparison of the spectroscopic data for this alkaloid with that for orochrine (**1**) and 2-*O*-acetylorochrine (**2**) indicated all three had a similar scaffold.

The major alkaloid in *A. orochryseum* was confirmed as the known compound atisinium chloride (**4**) from the spectroscopic data analysis and comparison with literature data.[17–19]

Another known alkaloid, virescenine (**5**), was also obtained. The alkaloid (**5**) was identified by ^{1}H NMR, ^{13}C NMR, DEPT, gCOSY, gNOESY, gHSQC, TOCSY, and gHMBC spectroscopic analysis and a comparison of melting point, optical rotation, and ion fragmentation pattern with the corresponding data for virescenine reported in the literature.[16,20,21] The spectroscopic data matched those reported for virescenine, apart from a difference in the chemical shifts of the signals ascribed to C-10 (δ 43.3) and C-13 (δ 39.4) compared to those noted in the literature[16] (δ 39.9 and 43.6, respectively). The assignment of the signals to the C-10 and C-13 position for **5** was supported by gCOSY and gHMBC correlations (Supporting Information).

Experimental Section

General Experimental Procedures. Melting points were determined using a Reichert hot-stage apparatus and were corrected. The optical rotations were measured with a JASCO Dip-370 digital polarimeter using a sodium lamp; an average of 10 optical readings were taken to obtain the observed rotation value. ^{1}H NMR (at 499.91 MHz), gCOSY, ^{13}C NMR (at 125.71 MHz), DEPT, NOESY, gHSQC, gHMBC, and TOCSY were obtained on solutions in CD_3OD using a nanoprobe on a Varian Unity Inova-500 MHz NMR spectrometer. LRCIMS (isobutane as the carrier gas) and LREIMS (at 70 eV) were obtained on a Shimadzu QP-5000 by the direct insertion technique. HRCIMS were run on a VG Autospec oa-TOF mass spectrometer (methane as the carrier gas). ESMS was performed on a Micromass Q-Tof-2 with acetonitrile as solvent. Preparative TLC plates were made using Merck Kieselgel 60 PF_{254} (0.3 mm thickness) on glass plates (20 × 20 cm). Premade aluminum-backed silica plates (0.2 mm silica thickness) supplied by Merck were used for separating isolates of smaller quantities. Visualizations of the separated bands on preparative TLC plates were done under short- and long-wavelength UV light (254 and 366 nm, respectively). Solvent ratios were vol/vol. Organic solvents were either used as is if AR grade or otherwise distilled before use. Dragendorff's reagent and Mayer's reagent were used for the detection of alkaloids in the plant extracts. Mayer's reagent was prepared by dissolving mercuric chloride (1.4 g) and potassium iodide (5 g) in water (100 mL). Dragendorff's reagent was prepared using the Munier and

Mache-Boeuf method.[22] For computational modeling, the semiempirical program AM1[23] was used on a PC Spartan Pro (Wavefunction, Irvine, CA).

Plant Material. Aerial parts of *Aconitum orochryseum* Stapf were collected from Lingshi Dungkhag (Bhutan) in June and August 2002. The plant material was air-dried in the drying unit at Lingshi and was then transported to the Pharmaceutical and Research Unit (Bhutan). The voucher specimen (No. 83) of this plant, which was identified by Mr. Samten (Pharmaceutical and Research Unit, Institute of Traditional Medicine Services, Ministry of Health, Thimphu, Bhutan), is deposited in the collection at the Pharmaceutical and Research Unit, Thimphu, Bhutan.

Extraction and Isolation. The plant material (1 kg) was chopped into small pieces and was extracted with methanol. The methanol extract was filtered, and the process was repeated four times (over 4 days). Rotary evaporation of the solvent then afforded a crude methanol extract (101.7 g). The LREIMS of this initial crude extract revealed ion peaks consistent with the presence of the compounds later isolated. The methanol extract (101 g) was acidified with 0.1 M H_2SO_4 and then extracted with $CHCl_3$ (3 × 60 mL). The acidic aqueous extract was basified (pH 8–10) with 20% aqueous Na_2CO_3 solution and then extracted with $CHCl_3$ (4 × 60 mL). The combined chloroform extract was washed with H_2O and dried with Na_2SO_4, and the solvent was evaporated under reduced pressure at 40 °C to afford a crude alkaloid extract (561 mg). The crude alkaloid (494 mg) was dissolved in 10% MeOH/90% CH_2Cl_2 and was separated using preparative TLC [silica gel; mobile phase 10% MeOH/89% CH_2Cl_2/conc aqueous NH_3 solution (1 mL, 28%)]. Eleven bands were obtained on initial separation, and each band contained different compounds with LRCIMS peaks at *m/z* 344 (atisinium ion), 342, 384, 400, 358, 424, 440, 406, 314, 448/466, and 298. Repeated preparative TLC on silica gel then afforded three new alkaloids: orochrine (**1**) [14.9 mg; solvent system was 10% MeOH/89% CH_2Cl_2/conc aqueous NH_3 solution (1 mL, 28%)], 2-*O*-acetylorochrine (**2**) [5.2 mg; solvent system was 8% MeOH/91% $CHCl_3$/conc aqueous NH_3 solution (1 mL, 28%)], and 2-*O*-acetyl-7α-hydroxyorochrine (**3**) [8.1 mg; solvent system was 10% MeOH/89% CH_2Cl_2/conc aqueous NH_3 solution (1 mL, 28%)]. A further fraction was recrystallized from methanol/diethyl ether to afford atisinium chloride (**4**)[18,19] (16.9 mg). Another known[16] alkaloid, virescenine (**5**) (4.6 mg), was also isolated by preparative TLC [silica gel; developing solvent: 6% MeOH/93% CH_2Cl_2/conc aqueous NH_3 solution (1 mL, 28%)].

Orochrine (1): colorless needles; mp 210–214 °C; $[\alpha]_D^{23}$ −65.2 (*c* 0.62, MeOH); ^{1}H and ^{13}C NMR (500 MHz, nanoprobe, CD_3OD) data, see Tables 1 and 2, respectively; LREIMS *m/z* 341 $[M]^+$ (37), 326 (28), 192 (17), 122 (27), 91 (17), 84 (32), 55 (31), 44 (100%); HRCIMS *m/z* 342.2077 $[MH]^+$ (calcd for $C_{21}H_{28}NO_3$, 342.2070).

2-*O*-Acetylorochrine (2): amorphous solid; mp 148–150 °C; $[\alpha]_D^{22}$ −82.5 (*c* 0.82, $CHCl_3$); ^{1}H and ^{13}C NMR (500 MHz, nanoprobe, CD_3OD) data, see Tables 1 and 2, respectively; LREIMS *m/z* 383 $[M]^+$ (39), 368 (34), 344 (17), 324 (51), 173 (25), 148 (18), 122 (33), 105 (18), 91 (26), 84 (35), 58 (68), 44 (100), 43 (100); HRCIMS *m/z* 384.2170 $[MH]^+$ (calcd for $C_{23}H_{30}NO_4$, 384.2174).

2-*O*-Acetyl-7α-hydroxyorochrine (3): pale green solid; mp 110–112 °C; $[\alpha]_D^{25}$ −31.8 (*c* 0.34, MeOH); ^{1}H and ^{13}C NMR (500 MHz, nanoprobe, CD_3OD) data, see Tables 1 and 2, respectively; LREIMS *m/z* 399 $[M]^+$ (37), 382 (73), 340 (35), 174 (17), 173 (16), 122 (26), 105 (18), 91 (27), 84 (43), 55 (38), 44 (100), 43 (100); HRCIMS *m/z* 400.2129 $[MH]^+$ (calcd for $C_{23}H_{30}NO_5$, 400.2124).

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Supporting Information Available: 2D NMR data for compounds **1**–**3** (Tables S1, S2, and S3), LREIMS and HRESMS data for atisinium chloride (**4**), and ^{1}H and ^{13}C NMR data plus LRCIMS, HREIMS, and LREIMS data for virescenine (**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

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