Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry of Solanidine, Leptinidine, and Acetylleptinidine. Steroidal Alkaloids from *Solanum chacoense* Bitter

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Solanidine, leptinidine, and acetylleptinidine from *Solanum chacoense* Bitter were structurally characterized by high-resolution MS and high-field ^{1}H-NMR and ^{13}C-NMR. ^{1}H-NMR and ^{13}C-NMR chemical shifts were assigned for solanidine and acetylleptinidine using DEPT, HMBC, and HMQC. Complete carbon chemical shift assignments are presented for all three alkaloids. The orientation of the C-23 acetoxyl group of acetylleptinidine was determined to be axial.

Keywords: *NMR-MS spectroscopy; solanidine; leptinidine; acetylleptinidine*

INTRODUCTION

Solanum chacoense Bitter contains leptines, leptinines (Kuhn and Löw, 1957, 1961), α-solanine, and α-chaconine (Figure 1). The leptines are natural antifeedants to the Colorado potato beetle (*Leptinotarsa decemlineata* Say; Carter, 1987; Sinden et al., 1986; Sturckow and Löw, 1961). Since the glycoalkaloid component of insect resistance is considered to be so significant, it has been suggested that selection and breeding for insect resistance be based on specific deterrent glycoalkaloid content (Sindent et al., 1986; Tingey, 1984). Further, it has even been suggested that it may be possible to screen for deterrent glycoalkaloid content in lieu of resistance testing (Deahl and Sinden, 1987). Because of the potential ecological and toxicological significance of certain steroidal glycoalkaloids (McMillan and Thompson, 1979; Friedman, 1992), several methods have been developed to assay (glyco)-alkaloid content in leaves and tubers of *Solanum* species (Coxon, 1984; Coxon and Jones, 1981; Crabbe and Fryer, 1980; Deahl and Sinden, 1987; Jellema et al., 1980, 1981; King, 1980; Lawson et al., 1992; Morris and Lee, 1981; Van Gelder, 1985).

Unfortunately, neither the leptines, the leptinines, nor their aglycons, acetylleptinidine and leptinidine, respectively, can be purchased commercially. Since analytical/chromatographic standards of these alkaloids must be purified by the analyst, spectral information should be readily available in order to confirm the structural identity of isolates. Although spectral data have been published for many steroidal alkaloids, a thorough search of the chemical literature (CA Search, 1967−1991; Chemical Abstracts, 1957−1966 and 1992) failed to retrieve specific publications regarding mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy of either acetylleptinidine or leptinidine. Also, whereas ^{13}C-NMR spectra have been reported for solanidine (Radeglia et al., 1977), the aglycon of α-solanine and α-chaconine, neither high-field ^{1}H-NMR nor high-resolution MS (HR-MS) data were found to be reported for this alkaloid. Fast atom bombardment MS of solanidine has been performed (Price et al., 1985), but only the $[M - H]^+$ and m/z 204 species was reported. In this paper, HR-MS and high-field ^{1}H- and ^{13}C-NMR data for acetylleptinidine, leptinidine, and solanidine are reported.

MATERIALS AND METHODS

Acetylleptinidine. Acetylleptinidine was isolated and purified from *S. chacoense*, accession PI 458310-1, as previously described (Lawson et al., 1992). Briefly, freeze-dried leaves were homogenized in 2% acetic acid methanol using a Waring blender. The homogenate was filtered, and the filtrate was evaporated to near dryness at 50 °C *in vacuo*. The residue was redissolved in 1 N HCl in methanol, capped under nitrogen, and hydrolyzed at 70 °C for 4 h. After cooling, the hydrolysate was raised to pH 10 with concentrated ammonium hydroxide and centrifuged at 12000*g* for 6 min. The supernatant was partitioned against chloroform, and the chloroform phase was evaporated to near dryness at 40 °C *in vacuo*. The residue was redissolved in chloroform, and acetylleptinidine was purified by flash chromatography using silica gel columns and mobile phases of hexane and ethyl acetate/hexane. Thin-layer chromatography (TLC; silica gel, choroform/methanol 7:3 v/v) was used to determine the composition of collected fractions. Acetylleptinidine was recrystallized from methanol.

Leptinidine. Leptinidine (21 mg) was prepared by hydrolysis of (70 °C for 30 min) recrystallized acetylleptinidine (22 mg) in 0.83 N sodium hydroxide in methanol/water. After cooling, the hydrolysate was partitioned twice against benzene; the benzene phase contained leptinidine, which was recrystallized from methanol.

Diacetylleptinidine. Acetylleptinidine (10 mg) was dissolved in a minimum amount of pyridine and combined with 2 mL of acetic anhydride. The solution was then incubated at 90 °C for 90 min. After the reaction mixture cooled to room temperature, 5 mL of water was added to destroy excess acetic anhydride. The solution was again cooled before partitioning three times against 1 mL portions of dichloromethane. The reaction was quantitative according to TLC. White needles from methanol: mp 196−210 °C; ^{1}H NMR (500 MHz) 3-H (δ 4.80, m, pentet in appearance), 6-H (d), 18-H_3 (s), 19-H_3, 21-H_3 (δ 1.04, d, J = 6.6 Hz), 26-H_β, (dd, J = 10.3 and 3.1 Hz), 27-H_3 (d, J = 6.7 Hz, EI-MS (70 eV); m/z (rel intensity) 497 [$C_{31}H_{47}NO_4$] (11), 482 [$C_{30}H_{44}NO_4$] (4), 454 [$C_{29}H_{44}NO_3$] (8), 437 [$C_{29}H_{43}NO_2$] (100), 262 [$C_{16}H_{24}NO_2$] (11), 208 [$C_{12}H_{18}NO_2$] (6), 148 [$C_{10}H_{14}N$] (13).

Solanidine and Solasodine. Solanidine and solasodine were purchased (Sigma Chemical Co., St. Louis, MO) and

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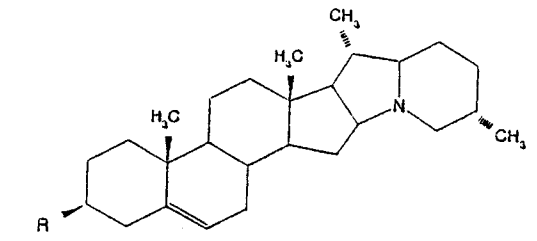
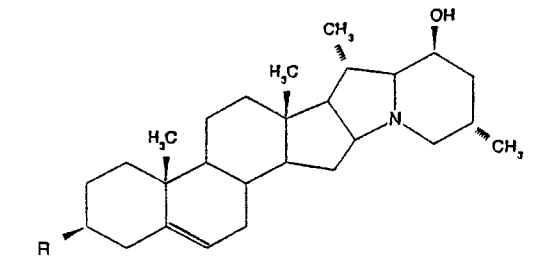


Solanidine R = OH

Solanine R = Galactose—Glucose
Rhamnose

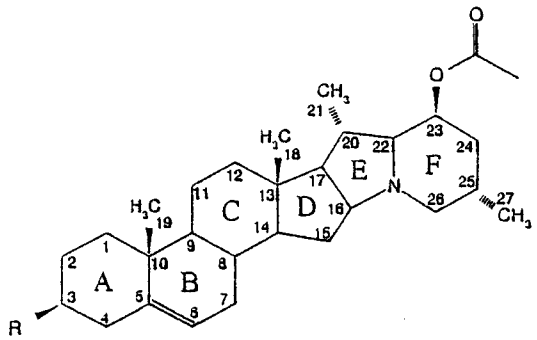
Chaconine R = Glucose—Rhamnose
Rhamnose



Leptinidine R = OH

Leptinine I R = Glucose—Rhamnose
Rhamnose

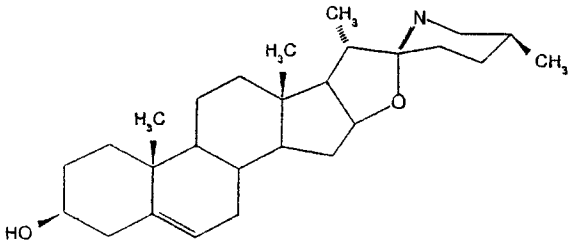
Leptinine II R = Galactose—Glucose
Rhamnose



Acetylleptinidine R = OH

Leptine I R = Glucose—Rhamnose
Rhamnose

Leptine II R = Galactose—Glucose
Rhamnose



Solasodine

Figure 1. *S. chacoense* C27-steroidal alkaloids and solasodine.

recrystallized from methanol. Solanidine was also isolated and purified from freeze-dried leaves of *S. chacoense* as previously described (Lawson et al., 1992).

GC and GC/MS. GC-flame ionization detector (FID) analysis of *S. chacoense* leaf extracts was performed as described previously (Lawson et al., 1992). GC/MS was performed under the same conditions as for GC-FID analysis of leaf extracts, but using an HP 5890 GC interfaced with an HP 5970 quadrapole mass selective detector (70 eV).

High-Resolution Mass Spectrometry. A VG 70-250S high-resolution mass spectrometer was used to obtain electron impact mass spectra. The mass spectrometer ionization was set at 70 eV, and the source temperature was 180 °C.

Nuclear Magnetic Resonance Spectroscopy. ^{1}H- and ^{13}C-NMR spectra of leptinidine, diacetylleptinidine, solasidine, and 3-acetylsolasidine were acquired using a Bruker WM-500 spectrometer at 500.13 and 125.76 MHz, respectively. Samples were dissolved in either $CDCl_3$ or C_5D_5N with 0.3% $SiMe_4$ as the internal standard.

All two-dimensional NMR spectra of solanidine and acetylleptinidine were collected on a Varian UNITY *plus*-600 spectrometer equipped with Ultrashims, a pulsed-field gradient (PFG) accessory, and a 5 mm ^{1}H/^{13}C/^{15}N triple-resonance gradient probe. Two-dimensional NMR spectra were obtained from 20 mg of solanidine and 2 mg of acetylleptinidine dissolved in 0.7 mL of C_5D_5N, without sample spinning, at 30 ± 1 °C, and with ^{1}H and ^{13}C 90° pulse widths of 10.7 and 18.0 μs. All two-dimensional spectra were processed on Sun Workstations using Vnmr software.

The PFG-HMQC (Hurd and John, 1991) spectra were obtained using the following parameters: 8000.0 and 26603.3 Hz spectral windows in the f_2 and f_1 dimensions, respectively, 1.0 s relaxation delay, 0.054 s acquisition time with GARP (Shaka et al., 1985) decoupling applied during the acquisition period, delays optimized for C−H one-bond coupling constants of J = 140 Hz. The first, second, and third gradient pulses had duration of 2.0 ms with magnitudes of 0.110, 0.110, and −0.052 T/m, respectively. The spectra were obtained by averaging 8 transients for each of the1024 t_1 increments. Fourier transformation (FT) was performed on a 2048 × 2048 matrix, after sinebell weight in both dimensions.

The PFG-HMBC (Rinaldi et al., 1995) spectra were obtained with the following parameters: 8000.0 and 26603.3 Hz spectral windows in the f_2 and f_1 dimensiions, respectively, 1.0 s relaxation delay, 0.256 s acquisition time. The first and second gradient pulses had duration of 2.0 ms with magnitudes of 0.110 and 0.082 T/m, respectively. Delays (taumb = $1/J_{C-H}$) were optimized for long-range coupling constants of J = 10.0

Table 1. Principal EI-MS Fragment Ions of *S. chacoense* Steroidal Alkaloids

alkaloid	fragment ions, *m/z* (relative abundance, %)
solanidine	397 (19.18), 382 (11.12), 368 (1.63), 354 (0.96), 341 (1.02), 326 (0.86), 272 (0.69), 259 (0.58), 204 (25.87), 178 (4.26), **150** (100.00), 136 (7.13), 124 (4.50), 98 (7.84), 79 (3.79), 67 (3.62), 55 (6.34)
leptinidine	413 (15.65), 398 (5.29), 369 (3.38), 354 (2.62), 342 (3.45), 326 (1.57), 312 (0.86), 294 (1.32), 267 (1.15), 236 (0.87), 220 (25.97), 207 (3.06), 194 (4.22), 181 (4.15), **166** (100.00), 148 (5.28), 137 (9.59), 122 (4.87), 105 (7.44), 91 (9.78), 82 (8.59), 67 (8.45), 55 (12.95)
acetylleptinidine	455 (6.23), 440 (5.48), 412 (13.48), **395** (100.00), 380 (6.54), 368 (2.65), 354 (1.72), 340 (1.73), 326 (1.32), 262 (21.97), 249 (1.82), 236 (3.42), 223 (2.51), 208 (11.68), 162 (11.64), 148 (47.90), 134 (8.54), 119 (5.23), 105 (8.64), 91 (11.20), 79 (0.31), 67 (9.62), 55 (13.42), 43 (22.33)

Hz (taumb = 0.05 s). The spectra were obtained by averaging 16 transients for each of the 1024 t_1 increments. FT was performed after zero-filling to a 4096 × 2048 matrix and weighting with a shifted Gaussian in f_2 and sinebell in f_1 dimensions, respectively.

The NOESY (Jeener et al., 1979) spectra were obtained with the following parameters: 5999.7 Hz spectral windows in both f_1 and f_2, 1.0 s relaxation delay, 0.341 s acquisition time, and 0.75 s mixing time. The spectra were obtained by averaging 16 transients for 380 complex t_1 increments. FT was performed after zero-filling to a 4096 × 2048 matrix and weighted with a shifted Gaussian in both dimensions.

RESULTS AND DISCUSSION

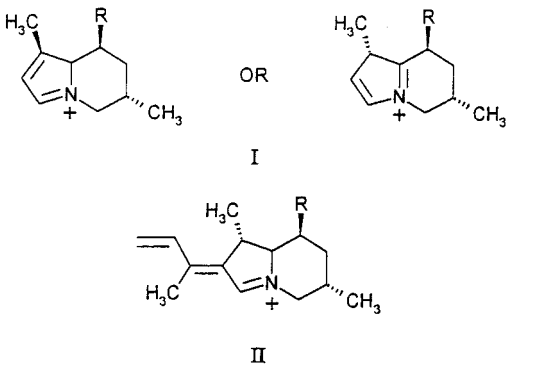
Solanidine. The presence of solanidine in *S. chacoense* leaf extracts was tentatively identified by TLC. The solute had an R_f of 0.64 in chloroform/methanol (7:3 v/v), comigrated with purchased solanidine, and reacted positively with Dragendorff's reagent (Krebs et al., 1969).

GC/MS of leaf extracts revealed a parent ion at *m/z* 397 for a peak having the same retention time as authentic solanidine. Also, the fragmentation patterns from both GC/MS and HR-MS (Table 1) were identical to that of authentic solanidine and included fragments at *m/z* 204 ($C_{14}H_{22}N$; Figure 2) and *m/z* 150 ($C_{10}H_{16}N$; Figure 2), which are diagnostic of solanidane-based alkaloids (Budzikiewicz, 1964). HR-MS (Table 1) provided a parent peak at 397.3345, supporting the molecular formula ($C_{27}H_{43}NO$ requires a molecular weight of 397.3345) for solanidine.

^{1}H-NMR assignments were established through HMBC and HMQC. A multiplet at δ 3.87 corresponding to the 3-H alpha to the 3-β-hydroxyl (Ripperger and Porzel, 1992) was observed. Other recognizable signals included 6-H (δ 5.45, bd, J = 5.1 Hz, H_2), 18-H_3 (δ 0.98, s), 19-H_3 (δ 1.07, s) 21-H_3 (δ 1.00, d, J = 6.3 Hz), 26-H_β (δ 2.93, dd, J = 10.2 and 3.6 Hz), and 27-H_3 (δ 0.86, d, J = 6.5 Hz).

^{13}C-NMR chemical shift assignments (Table 2) were established rigorously through DEPT, HMBC, and HMQC experiments and compared well with literature values for solanidine (Radeglia et al., 1977). In addition, the interchangeable signals for C-24 and C-25 reported in Radeglia et al. (1977) were rigorously assigned.

Leptinidine. The presence of leptinidine in leaf extracts was initially indicated by GC/MS, which revealed a peak having a parent ion at *m/z* 413 and was thus consistent with the molecular formula ($C_{27}H_{43}NO_2$) which requires 413.3294 for leptinidine. Purified sample



Alkaloid	Structure	Mass	Fragment Formula
Solanidine	R = H	I = 150 II = 204	$C_{10}H_{16}N$ $C_{14}H_{22}N$
Leptinidine	R = OH	I = 166 II = 220	$C_{10}H_{16}NO$ $C_{14}H_{22}NO$
Acetylleptinidine	R = OC(O)CH3	I = 208 II = 262	$C_{12}H_{18}NO_2$ $C_{16}H_{24}NO_2$

Figure 2. Diagnostic fragment ions from EI-MS of solanidine, leptinidine, and acetylleptinidine.

Table 2. Carbon Chemical Shifts[a] for Solanidine, Leptinidine, and Acetylleptinidine

carbon	solanidine[b]	leptinidine[b]	acetylleptinidine[b]
1	38.0	37.9	37.9[c]
2	32.8	32.4	32.7
3	71.4	71.3	71.4
4	43.6	43.5	43.6
5	142.1	142.1	142.1
6	121.4	121.1	121.3
7	32.5	32.6	32.5
8	32.2	31.1	32.2
9	50.7	50.6	50.6
10	37.0	37.1	37.1
11	21.4	21.2	21.3
12	40.2	41.0	40.0
13	40.8	41.1	41.1
14	57.9	57.9	57.9
15	31.7	31.2	31.8
16	69.5	69.8	69.0
17	63.5	62.4	62.6
18	17.1	16.9	16.6
19	19.7	19.6	19.5
20	37.0	32.0	31.2
21	18.7	18.3	18.8
22	74.9	78.6	76.5
23	29.8	64.9	67.7
24	33.8	40.1	37.8[c]
25	31.5	25.5	26.4
26	60.7	60.4	59.8
27	19.8	19.3	19.2
COCH$_3$			170.9
COCH$_3$			21.1

[a] δ values in ppm downfield from $SiMe_4$. [b] In C_5D_5N. [c] The assignments are interchangeable.

(white needles from methanol) had a mp of 238−241 °C, which was similar to that reported for leptinidine by Kuhn and Löw (1957, 1961; 239−240 °C), and had an R_f of 0.55 in chloroform/methanol (7:3 v/v). TLC of leaf extracts revealed a compound that comigrated with leptinidine derived from base hydrolysis of purified acetylleptinidine (see below) and reacted positively with Dragendorff's reagent (Krebs et al., 1969).

GC/MS and HR-MS exhibited diagnostic fragments at *m/z* 166 and 220 (Table 1; Figure 2), which were 16

amu greater than the corresponding fragments (*m/z* 150 and 204) from solanidine. These data indicated the presence of a hydroxyl group in ring E or F. GC/MS and HR-MS (Table 1) of the base hydrolysate of purified acetylleptinidine (see below) had a fragmentation pattern identical to that of leptinidine found in *S. chacoense* leaf extracts and included the major fragments reported previously (Osman et al., 1987). HR-MS (Table 1) of the base hydrolysate of acetylleptinidine produced a parent peak at *m/z* 413.3294.

The ^{1}H-NMR spectrum also supported the presence of a hydroxyl at C-23, by exhibiting a signal at δ 3.78 (d, J = 7.5 Hz), which, according to 2D-NMR CH-correlation, was coupled to C-23 (Table 2). This assignment is consistent with that previously given to the 23α-H of dihydroleptinidine (Krishna Kumari et al., 1985). The ^{1}H-NMR spectrum further revealed signals that were assigned to 3-H (δ 3.51, m, pentet in appearance), 6-H (δ 5.35, d, J = 5.0 Hz), 18-H$_3$ (δ 1.02, s), 19-H$_3$ (δ 0.87, s), 21−H$_3$ (δ 0.97, d, J = 6.8 Hz), 26-H$_\beta$ (δ 2.87, d, J = 10.6 Hz), and 27-H$_3$ (δ 0.86, d, J = 8.8 Hz).

Acetylleptinidine. GC/MS of leaf extracts from *S. chacoense* also revealed a peak having a parent ion at *m/z* 455, which is consistent with the molecular formula ($C_{29}H_{45}NO_3$ requires 455.3399) for acetylleptinidine. Purified sample (white needles from methanol) had an R_f of 0.78 with choroform/methanol (7:3 v/v) and reacted positively with Dragendorff's reagent (Krebs et al., 1969). The mp (194−199 °C) was slightly higher than the 191−196 °C reported by Kuhn and Löw (1961) for x-acetylleptinidine.

GC/MS and HR-MS (*m/z* [M]$^+$ 455.3385; Table 1) of the purified sample produced fragmentation patterns identical to that produced by the *m/z* [M]$^+$ 455 peak identified by GC/MS of leaf extracts. Fragments were also observed at *m/z* 208 ($C_{12}H_{18}NO_2$) and *m/z* 262 ($C_{16}H_{24}NO_2$) (Figure 2), which corresponded to the acetoxyl analogs of the *m/z* 150 and 204 fragments, respectively, of solanidine and indicated substitution of the acetoxyl in ring E or F. MS also revealed a base peak at *m/z* 395 ($C_{27}H_{41}NO$), which is consistent with the loss of acetic acid [M − CH_3CO_2H]$^+$.

The presence of an acetoxyl was also supported by ^{1}H-NMR spectroscopy, which revealed a singlet at δ 2.18 corresponding to the methyl alpha to the carbonyl, and ^{13}C-NMR spectroscopy (Table 2), which exhibited both a singlet at δ 170.9 and a quartet at δ 21.1. These signals were assigned to the carbonyl carbon and the methyl carbon alpha to the carbonyl, respectively. Substitution of the acetoxyl at C-23 was further indicated by a proton resonating at δ 5.32, which is similar to that reported for the 23α-H for dihydroleptinidine diacetate (Murakami et al., 1985; Schreiber and Ripperger, 1967), and was confirmed by a long-range 2D-NMR CH-correlation between this proton and the carbonyl carbon (Table 3).

The position of the acetoxyl group was also verified by direct comparison of the ^{13}C-NMR chemical shifts of diacetylleptinidine with those of 3-acetylsolasodine and solasodine (Bird et al., 1979). Since rings A−D of these compounds have the same structure (see Figure 1), such a comparison would indicate whether the acetoxyl was substituted at C-3. The data (Table 4) show that the chemical shifts for C-1 through C-6 of acetylleptinidine do not agree with those of 3-acetylsolasodine but are nearly identical to those reported for solasodine. However, acetylation of acetylleptinidine brought the chemical shifts of these carbons into excellent agreement with those reported for 3-acetylsolasodine and thus indicated that the acetoxyl group of acetylleptinidine was not at C-3.

Table 3. NMR Data for Acetylleptinidine

position	δ_H (multiplicity, J_{HH})	δ_C	HMBC correlations (C-no.)
1[a]	1.10 (m)	37.9	
	1.88 (m)		
2	1.82 (m)	32.7	C-3, 4, 10
	2.12 (m)		
3	3.88 (m)	71.4	
4	2.64 (m)	43.6	C-2, 3, 5, 6, 10
5		142.1	
6	5.45 (bd, 5.2)	121.3	C-4, 7, 8, 10
7	1.60 (m)	32.5	
	2.80 (m)		
8	2.04 (m)	32.2	
9	0.99 (m)	50.6	C-1, 7, 8, 10, 11, 19
10		37.1	
11	1.50 (m)	21.3	
	2.16 (m)		
12	1.12 (m)	40.0	C-11, 13, 17, 18
	1.73 (m)		C-9, 14
13		41.1	
14	1.15 (m)	57.9	
15	1.15 (m)	31.8	
	1.83 (m)		
16	2.64 (m)	69.0	C-13, 26
17	1.49 (m)	62.6	C-16, 18, 20, 21
18	1.01 (s)	16.6	C-12, 13, 14, 17
19	1.08 (s)	19.5	C-1, 5, 9, 10
20	1.99 (m)	31.2	
21	1.06 (d, 6.4)	18.8	C-17, 20
22	1.79 (m)	76.5	
23	5.32 (m)	67.7	$\underline{C}OCH_3$, C-25
24[a]	1.10 (m)	37.8	
	1.88 (m)		
25	2.07 (m)	26.4	
26	1.43 (m)	59.8	C-16, 22, 24, 27
	2.96 (dd, 3.3, 11.0)		C-22, 24, 25
27	0.83 (d, 7.5)	19.2	C-24, 25, 26
$\underline{C}OCH_3$		170.9	
$CO\underline{C}H_3$	2.18 (s)	21.1	$\underline{C}OCH_3$

[a] The assignments are interchangeable.

Table 4. ^{13}C-NMR Chemical Shifts[a] of Solasodine, 3-Acetylsolasodine, Acetylleptinidine, and Diacetylleptinidine

alkaloid	C-2	C-3	C-4	C-5	C-6
solasodine[b]	31.6	71.7	42.3	141.0	121.5
3-acetylsolasodine[b]	27.8	73.9	38.1	139.8	122.4
acetylleptinidine[c]	32.7	71.4	43.6	142.1	121.3
3,23-diacetylleptinidine[c]	28.1	74.0	38.6	140.0	122.8

[a] δ values in ppm downfield from $SiMe_4$. [b] In $CDCl_3$. [c] In C_5D_5N.

In summary, we have reported high-field ^{1}H- and ^{13}C-NMR and HR-MS data for the *S. chacoense* steroidal alkaloids solanidine, leptinidine, and acetylleptinidine. Complete carbon chemical shift assignments have been presented for all three alkaloids. In addition, the axial orientation of the C-23 acetyoxyl group of acetylleptini-

dine was established. These data should assist in routine structural verification of these compounds and may allow analysis of these compounds in crude extracts or intact leaves.

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