

MASS SPECTROMETRY OF METASTABLE IONS FROM LAGOCHILINE-TYPE DITERPENOIDS

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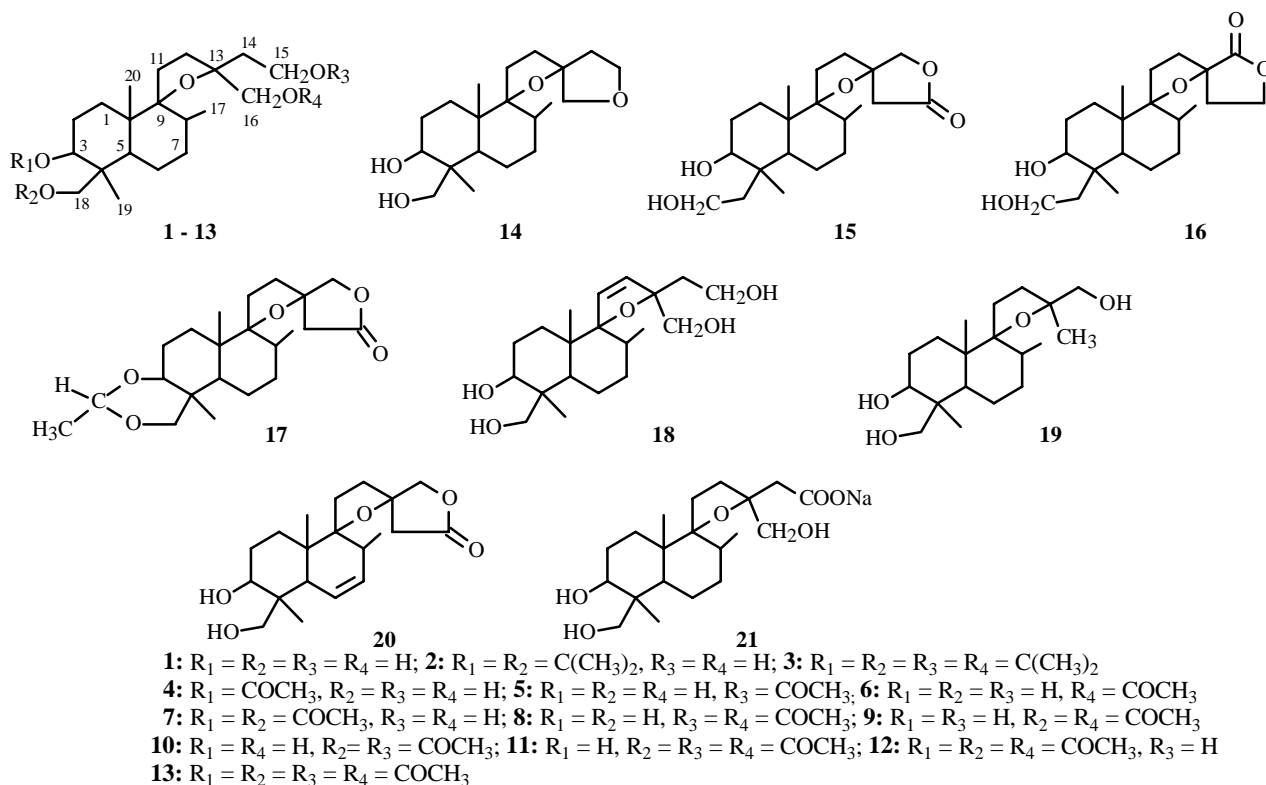
UDC 543.51+547.914.6

Low- and high-resolution mass spectra and spectra of metastable ions were studied using direct analysis of daughter ions (DADI) and metastable defocusing (MD) of lagochiline-type diterpenoids. Patterns in their fragmentation were found.

Key words: metastable ions, diterpenoid, lagochiline, lagochiline acetates, lagochirsine.

The high hemostatic activity of extracts from *Lagochilus inebrians* stimulated comprehensive studies of the structures and synthesis of lagochiline-type diterpenoids. More than 20 new compounds were discovered, among which the sodium salt of the diterpene lactone lagochiline called Lagoden has been incorporated into medical practice [1] as an effective hemostatic agent.

We studied the mass spectra and spectra of metastable ions of lagochiline (**1**) [2], 3,18-*O*-isopropylidene lagochiline (**2**) [3], 3,18,15,16-di-*O*-isopropylidene lagochiline (**3**) [3], acetyl derivatives of lagochiline (**4-13**) [4], anhydrolagochiline (**14**) [5], lagochirsine (**15**) [6], isolagochirsine (**16**) [5], lagochirsidine (**17**) [5], dehydration products of lagochiline 11,12-dehydrolagochiline (**18**) [6], 9,13-epoxy-3,18,16-trihydroxy-15-norlabdane (**19**) [7], acid-hydrolysis products of lagoden 6,7-dehydrolagochirsine (**20**) [6], and a mixture of acetyl derivatives from acetylation of lagochiline by acetic anhydride [8].



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TABLE 1. m/z Values and Relative Intensities of Characteristic Ions A^+ , B^+ , and C^+ and the Ion with m/z 83 for **1-19**, %

Compound	M^+	A^+	B^+	C^+	m/z 83
1	356 (44)	198 (16)	185 (100)	172 (20)	86
2	396 (36)	198 (5)	185 (100)	172 (10)	96
3	436 (28)	238 (12)	225 (100)	212 (19)	40
4	398 (19)	198 (10)	185 (100)	172 (28)	82
5	398 (20)	240 (18)	227 (100)	214 (24)	68
6	398 (16)	240 (15)	227 (100)	214 (22)	96
7	440 (6)	198 (12)	185 (100)	172 (16)	93
8	440 (4)	282 (8)	269 (100)	256 (14)	80
9	440 (7)	240 (13)	227 (100)	214 (18)	92
10	440 (6)	240 (7)	227 (96)	214 (6)	100
11	482 (7)	282 (9)	269 (88)	256 (19)	100
12	482 (9)	240 (8)	227 (92)	214 (13)	100
13	524 (2)	282 (6)	269 (48)	256 (4)	100
14	338 (48)	180 (9)	167 (100)	154 (10)	96
15	352 (19)	194 (100)	181 (80)	168 (21)	60
16	352 (4)	194 (34)	181 (100)	168 (9)	33
17	378 (24)	180 (2)	167 (70)	154 (14)	100
18	354 (41)	196 (18)	183 (100)	170 (21)	
19	326 (16)	168 (9)	155 (100)	142 (18)	15

Mass spectra of **1-20** typically had weak peaks for the molecular ion and ions corresponding to cleavage of the functional groups on C_3 , C_{15} , C_{16} , and C_{18} as radicals, which is characteristic of labdane terpenoids of the grindelene group [9, 10].

The principal splitting pattern for $[M]^+$ of these compounds by electron impact involved cleavage of the C_9 - C_{10} bond to form its oxonium species, further decomposition of which formed the key fragments A^+ , B^+ , and C^+ (Table 1 gives their m/z values and relative intensities).

Mass spectra of all compounds had a very strong B^+ peak whereas the other peaks (A^+ and C^+) were weak. The presence of peaks for these key fragments (A^+ , B^+ , and C^+) was very valuable for analytical purposes because their m/z values clearly indicated the nature and degree of substitution in this part of the molecule.

Mass spectra of mono-, di-, tri-, and tetraacetate lagochiline derivatives are especially interesting because these compounds often occur in plants of the genus *Lagochilus* and the probability that the acetates will be found in different combinations hinders their unambiguous identification.

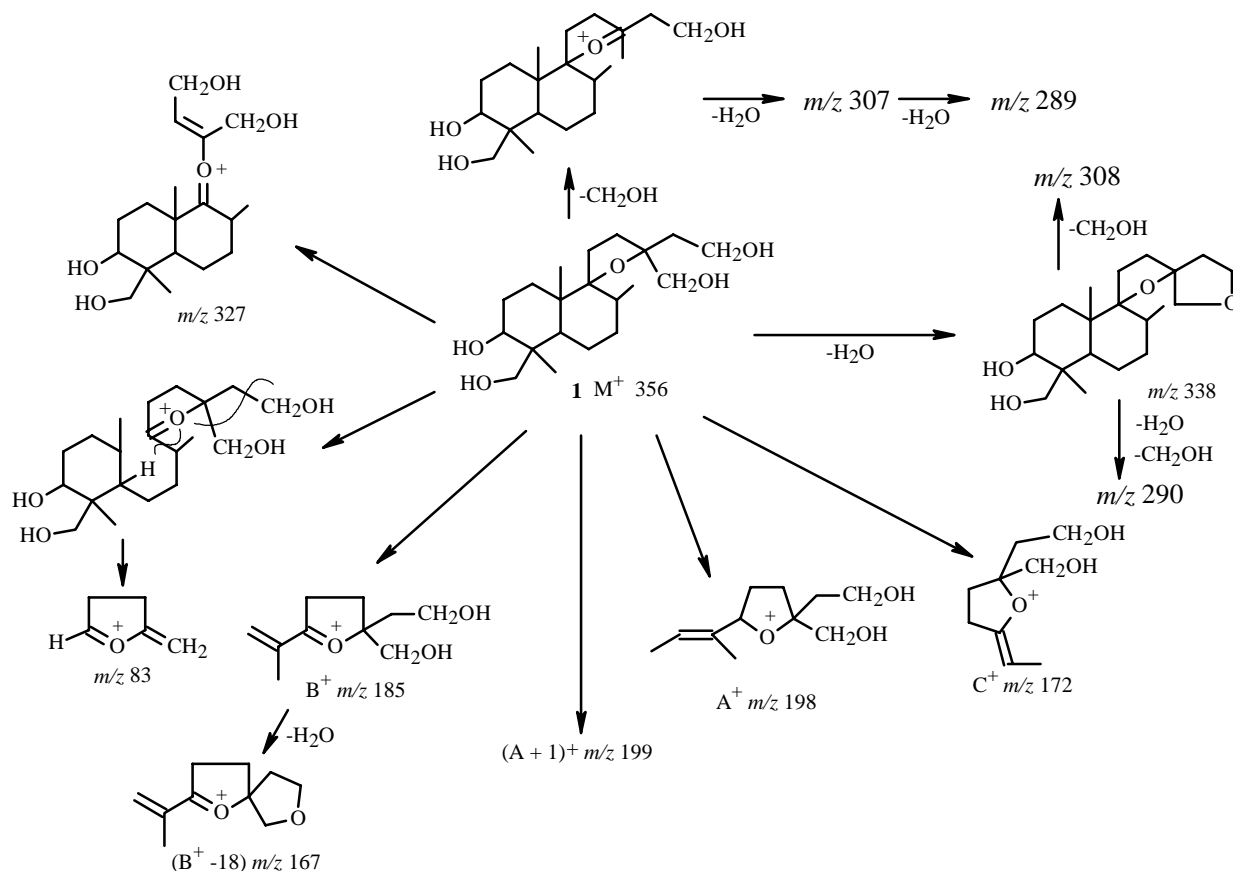
Peaks for the key ions (A^+ , B^+ , and C^+) in spectra of the C_{15} - and C_{16} -monoacetates of **5** and **6** had m/z values 240, 227, and 214. The spectra of these acetates differed in that the spectrum of the C_{16} -monoacetate of **6** had a strong peak for the $[M - 73]^+$ ion, which corresponded to loss of a CH_2OCOCH_3 group, although it was absent in the spectrum of the C_{15} -monoacetate of **5**.

A comparison of the mass spectra of the lagochiline derivatives revealed the following. If the acetyl was located in the 15-position, then the principal ions in the mass spectrum were ions with m/z 398, 380, 240, 227, 214, and 83 whereas an acetyl in the 3-position gave ions with m/z 398, 198, 185, and 172. However, the ion with m/z 380 was not observed because a water molecule was not lost from the molecular ion $[M - 18]^+$. If the acetate was located in the C_{16} -position, then the principal ions were those with m/z 398, 240, 227, and 214.

The mass spectrum of the C_{15} and C_{16} diacetate **8** had peaks for A^+ , B^+ , and C^+ with m/z 282, 269, and 214; for the C_{16} - C_{18} and C_{15} - C_{18} diacetates **9** and **10**, these ions had m/z 240, 227, and 214, respectively.

A peak for the $[M - 73]^+$ ion was also in mass spectra of certain diacetates and triacetates of lagochiline with acetyls in analogous positions. The difference lay in the m/z value for the molecular ions. Mass spectra of these compounds also typically had a strong peak with m/z 83.

Key fragments in the mass spectrum of **1** were formed according to Scheme 1.



Scheme 1

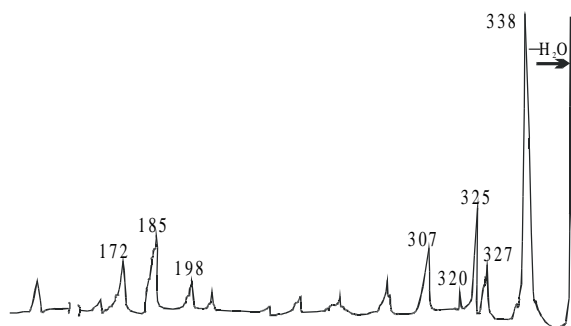


Fig. 1. DADI spectrum of **1**.

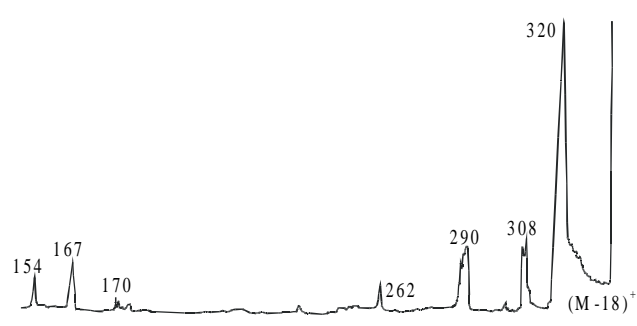


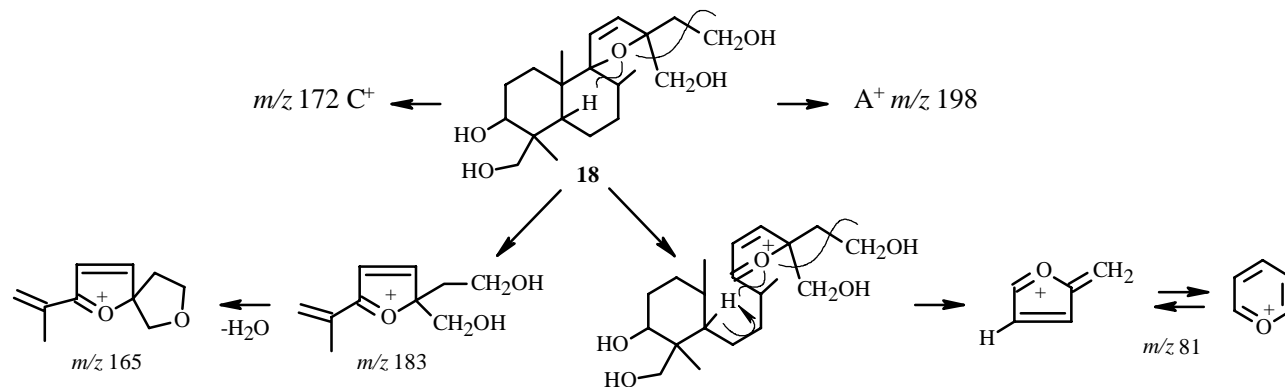
Fig. 2. DADI spectrum of **14**.

The fragmentation pattern of **1** was confirmed by its mass spectrum and the direct analysis of daughter ions (DADI) spectrum of its molecular ion (Fig. 1).

The DADI spectrum of $[M]^+$ for **1** exhibited strong peaks at high mass numbers that were either absent or very weak in its ordinary mass spectrum. The peak for the dehydrated ion in the DADI spectrum of $[M]^+$ for **1** was the base peak. Furthermore, it contained peaks for ions indicating that loss of two waters (m/z 320) and a water together with a CH_2OH (m/z 307) were occurring simultaneously.

Peaks for ions with m/z 185 and 172 corresponding to decomposition of ring B in the DADI spectrum of $[M]^+$ for **1** were strong whereas that for the ion with m/z 198 was weak. The spectrum exhibited a peak for the ion with m/z 167 $[B - 18]^+$ arising directly from $[M]^+$ through simultaneous loss of a water and a $C_9H_{19}O_2$ group (Fig. 1, Scheme 1).

A distinguishing feature of the DADI spectrum of $[M]^+$ for **14** (anhydrolagochiline) was the presence of a peak for the ion with m/z 154 that was absent in the DADI spectrum of **1** (Fig. 2).



Scheme 2

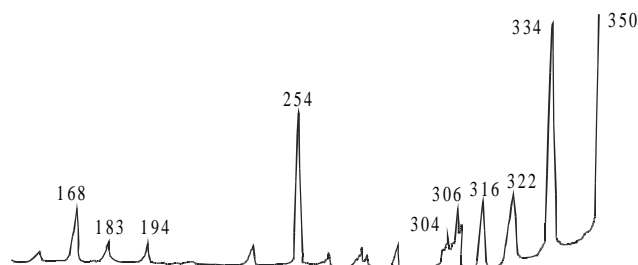


Fig. 3. DADI spectrum of **15**.

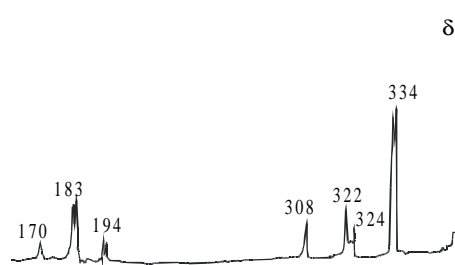


Fig. 4. DADI spectrum of **16**.

Dehydration of **1** produced two products, 11,12-dehydrolagochiline (**18**) and 9,13-epoxy-3,18,16-trihydroxy-15-norlabdane (**19**).

The mass spectrum of **18** differed from that of **1** in the peak intensities of the principal ions. The peak for the $[M - 18]^+$ ion of **18** was slightly stronger than the peak for the analogous ion of **1**. The main differences in the spectra of **1** and **18** were the m/z values for all characteristic ions of **18** that were lower by two mass units than those of **1** (Table 1, Scheme 2). The low-resolution mass spectra and elemental analyses of the molecular and characteristic ions revealed that **18** and **1** differed in the structure of ring C by the presence of a double bond between C_{11} and C_{12} . If the double bond was at the C_7 – C_8 position, then ring B should decompose by a retrodiene mechanism. The spectrum did not contain peaks for such ions and did have peaks for A^+ , B^+ , C^+ , and $[B - 18]^+$ with m/z 196, 183, 170, and 165, which were indicative of the similarity and difference of **1** and **18**. The peak for the ion with m/z 165 in the spectrum of **18** was stronger than the analogous peak in the mass spectrum of **1** (m/z 167). The C_{11} – C_{12} position for the double bond in **18** agreed well with the intensity of the peak for the ion with m/z 81 compared with that for the peak of the ion with m/z 83 in the spectrum of **1**.

Lagoden (**21**), the only water-soluble diterpenoid with hemostatic properties, is the sodium salt of lagochirsinic acid, which is synthesized from **1**. It is not volatile under mass spectrometric conditions. Acid hydrolysis of lagoden produces lagochirsinic acid, which quickly cyclizes under the hydrolysis conditions into lagochirsine (**15**) via loss of water. Moreover, the hydrolysis product may be its isomer, isolagochirsine (**16**), with a C_{16} carbonyl. The DADI spectra of lagochirsine and isolagochirsine were studied in order to demonstrate that isolagochirsine was present in the hydrolysis products.

The DADI spectra of $[M]^+$ (352) for **15** (Fig. 3) and $[M]^+$ for **16** (Fig. 4) had different characteristic peaks. A strong peak for a dehydrated ion and peaks for ions corresponding to loss of two waters (m/z 316), CH_2O (m/z 322), and simultaneous loss of water and CH_2O (m/z 304) were common to both. A strong peak for an ion with m/z 254 in the DADI spectrum of $[M]^+$ for **15** was absent in the DADI spectrum of $[M]^+$ for **16**. The formation of this ion in the DADI spectrum of $[M]^+$ for **15** is clearly related to loss of atoms from rings C and D.

The DADI spectrum of $[M]^+$ for **16** (Fig. 4) exhibited peaks for ions corresponding to loss of CO (m/z 324) and CO_2 (m/z 308). The DADI spectrum of $[M]^+$ for **15** did not show these peaks (Fig. 3). Simultaneous loss of water and CH_2O was typical for **15** and **16**. A peak for an ion with m/z 254, corresponding to decomposition of ring C, was typical only for **15**.

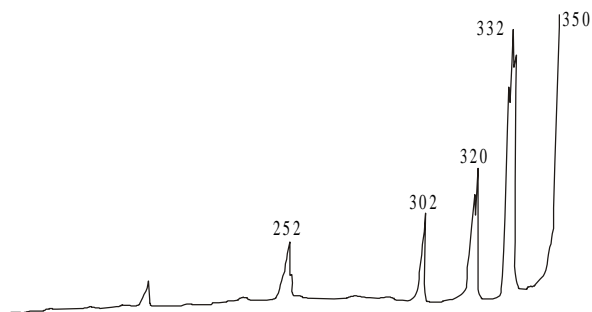


Fig. 5. DADI spectrum of **20**.

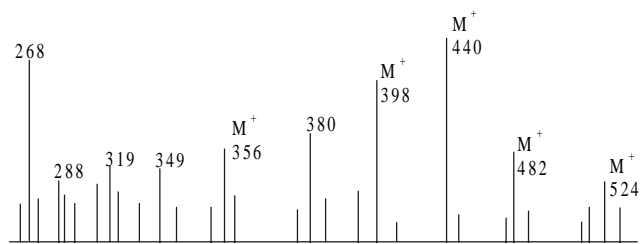


Fig. 6. Mass spectrum of a mixture of lagochiline acetyl derivatives.

Isolagochirsine was not a product of acid hydrolysis of lagoden. However, there was another pure compound (**20**) besides lagochirsine with a molecular ion with m/z 350 in the mass spectrum that differed by two mass units from the molecular ion of **15**.

The DADI spectrum of $[M]^+$ with m/z 350 (**20**) typically had a peak for the dehydrated ion with m/z 332 and peaks for ions arising from decomposition of ring B. In the spectrum of **20**, the m/z value for this ion was 252 or two mass units less than the same ion of lagochirsine with m/z 254. Peaks for ions corresponding to decomposition of ring B and formation of A^+ , B^+ , and C^+ were not found in the DADI spectrum of $[M]^+$ for **20** (Fig. 5). This indicated that **20** differed from **15** in the structure of ring B. Of the three possible positions for the double bond (C_5-C_6 , C_6-C_7 , and C_7-C_8), the most probable was C_6-C_7 because this position for the double bond prevents subsequent decomposition of ring B.

A fragmentation pattern for formation of daughter ions from the molecular ions was devised from the study of the mass spectra and spectra of the metastable ions of lagochiline, anhydrolagochiline, lagochirsine, isolagochirsine, and various acetyl derivatives of lagochiline. A detailed investigation of the mass spectra of the mono-, di-, and triacetates of lagochiline derivatives made it possible to study the mass spectra of a mixture of lagochiline acetates without separating them into pure compounds.

Acetylation by acetic anhydride of lagochiline formed the mono-, di-, tri-, and tetraacetyl derivatives at the four hydroxyls in it.

The mass spectrum of lagochiline acetates (Fig. 6) had ions with m/z 398 for the mono-; m/z 440, di-; m/z 482, tri-; and m/z 524, tetraacetate of lagochiline. These ions were of medium strength and conformed to all rules for formation and decomposition.

Mass spectra of the acetylation products showed that the ion with m/z 356 corresponded to the molecular ion of lagochiline itself and was of low overall concentration. The concentration of each component in the mixture could be estimated by measuring the intensities of the molecular ions of lagochiline and its acetylation products. The percent content of each component in the product mixture was calculated as follows: lagochiline, 6%; lagochiline monoacetate, 22; diacetate, 35; triacetate, 25; tetraacetate, 10; other volatile organic compounds, 2.

Thus, a comparison of the mass spectra of lagochiline and its acetyl derivatives could find the quantitative content of each acetyl compound in the product mixture from acetylation. The results show promise for use in quantitative determination of lagochiline and its acetates in extracted substances from *Lagochilus* that are the main component of several medicinal preparations.

EXPERIMENTAL

Mass spectra were recorded in MX-1331, MX-1320, and MAT 311 (Varian, USA) instruments by direct sample introduction into the ion source of the ionization chamber at 100-120°C and ionizing potential 70 eV with vaporizer temperature 40-80°C.

DADI and MD spectra were recorded in the MAT 311 instrument under the aforementioned conditions.

Synthesis of Lagochiline Acetates. Lagochiline (1.4 g) was treated with freshly distilled acetic anhydride (4 mL) and calcined sodium acetate (0.3 g) and heated at reflux on a water bath for 1 h. The resulting viscous liquid was washed repeatedly with distilled water and dried in vacuo. The yield of mixed lagochiline acetates was 1.23 g.

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