Application of Liquid Chromatography–Atmospheric Pressure Chemical Ionization Mass Spectrometry to the Differentiation of Stereoisomeric C_{19}-Norditerpenoid Alkaloids

Koji WADA,* Takao MORI, and Norio KAWAHARA

Hokkaido College of Pharmacy, 7–1, Katsuraoka-cho, Otaru 047–0264, Japan.
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High-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (HPLC–APCI-MS) was successfully applied to stereoisomeric C_{19}-norditerpenoid alkaloids at position 1. APCI-MS allowed the easy and precise control of the energy deposition by varying the drift voltage. Comparison of the breakdown curves, observed by changing the potential difference between the first electrode and the second electrode of the APCI ion source, revealed stereochemical dependence of different fragmentations. The APCI spectra of alkaloids were predominantly the $[M+H]^+$ ion and major fragment ion, corresponding to the $[M+H-H_2O]^+$ ion or the $[M+H-CH_3COOH]^+$ ion, and comparison of the spectra showed that the abundance of fragment ions was significantly higher for C-1 β-form alkaloids than for C-1 α-form alkaloids. The characteristic fragment ions were formed by the loss of a water, acetic acid or methanol molecule at position 8. The fragmentation mechanisms depending on the stereochemistry of the precursor ion could be discerned by recording the spectra in a deuterated solvent system of 0.05 M ammonium acetate in D_2O–acetonitrile–tetrahydrofuran. Loss of D_2O from the precursor ion gave the fragment ion. This result indicated that the proton of protonation was included in the leaving water molecule. The peak intensity ratio $R=[M+H]^+/[M+H-H_2O]^+$ manifested the stereochemical differentiation of alkaloids at position 1.

Key words atmospheric pressure chemical ionization; C_{19}-norditerpenoid alkaloid; 1-*epi*-neoline; 1-*epi*-delcosine

The structural elucidation of organic compounds of natural and synthetic origin has been one of the major analytical applications of mass spectrometry (MS). In general, the mass spectra provide useful information on the stereochemistry of the compound under investigation, and the stereochemical information arises from sterically controlled ionic fragmentations.[1] The fragmentation pattern manifested in a conventional mass spectrum is a direct reflection of the internal energy distribution of precursor ions. Consequently, the stereochemical differentiation, which generally depends on one particular fragmentation pathway among various dissociation channels, is very sensitive to the experimental conditions of ionization. Several analytical methods have been developed in order to control the amount of internal energy deposited on the precursor ion of interest with regard to the fragment ion yield.[2—4] A number of reports on the stereochemistry of indoloquinolizine alkaloids,[5] quinolizine alkaloids,[6] eburnane-type alkaloids,[7] and indoloquinolizine[8] and indole[9] alkaloids by electrospray ionization have appeared in recent years.

Various *Aconitum* (Ranunculaceae) plants produce C_{19}-norditerpenoid and C_{20}-diterpenoid alkaloids.[10] Previous structure studies of diterpenoid alkaloids involving MS have been carried out essentially by electron impact (EI) ionization and conventional analysis.[11—16] In particular, the fragmentation pathways of the presence of a C-6(OCH_3)–C-7(OH)–C-8(OH) grouping in C_{19}-norditerpenoid alkaloids have been extensively investigated, the structures of the fragment ions being supported by deuterium labeling at C-6 (OCD_3).[17] To our knowledge, previous investigation of the stereochemical differentiation of diterpenoid alkaloids has been performed using only EI.[18] Earlier reports have shown the optimum conditions for determination of C_{19}-norditerpenoid and C_{20}-diterpenoid alkaloids in plant extracts using high-performance liquid chromatography–atmospheric pressure chemical ionization MS (HPLC–APCI-MS).[19—21] In a previous study,[22] we demonstrated that HPLC–APCI-MS could be used in experiments on the energy-dependence of ion abundances with a view to differentiating stereoisomeric pairs of C_{19}-norditerpenoid alkaloids at position 6.

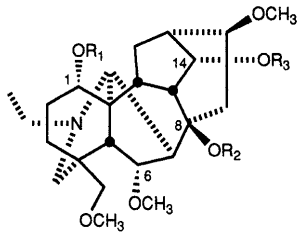
In the present paper, we report the results of an HPLC–APCI-MS study of C_{19}-norditerpenoid neoline-type alkaloids, neoline (**1**), 1-*epi*-neoline (**2**),[23] 14-acetylneoline (**3**), 14-acetyl-1-*epi*-neoline (**4**),[24] 8-acetyl-14-benzoylneoline (**5**) and 8-acetyl-14-benzoyl-1-*epi*-neoline (**6**), and delcosine-type alkaloids, delcosine (**13**), 1-*epi*-delcosine (**14**), 14-acetyldelcosine (**15**) and 14-acetyl-1-*epi*-delcosine (**16**), for resolving structural problems related to the differentiation of stereoisomers. These alkaloids differ only in the stereochemistry at position 1, alkaloids **2**, **4**, **6**, **14** and **16** being characterized by a β-hydroxyl group and alkaloids **1**, **3**, **5**, **13** and **15** by an α-hydroxyl group.

Results and Discussion

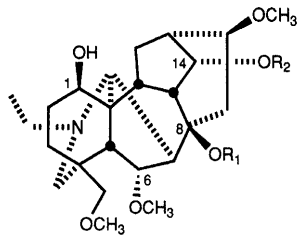
In our previous studies,[19—21] it was demonstrated that HPLC–APCI-MS permitted the observation of clearly protonated molecule ($[M+H]^+$) species of *Aconitum* alkaloids. The APCI mass spectra of *Aconitum* alkaloids showed predominantly the $[M+H]^+$ ion together with most major fragment ions. We previously reported that HPLC–APCI-MS was useful for the structural elucidation of six stereoisomeric C_{19}-norditerpenoid alkaloids at position 6.[22] Comparison of the APCI spectra showed that the abundance of fragment ions was significantly higher for C-6 β-form alkaloids than for C-6 α-form alkaloids.

In the present study, we applied an HPLC–APCI-MS method to the investigation of stereochemical differentiation of C_{19}-norditerpenoid neoline-type alkaloids **1**—**6** and delcosine-type alkaloids **13**—**16**. At first, the APCI mass spectra obtained in the HPLC–APCI-MS of neoline-type alkaloids

* To whom correspondence should be addressed. e-mail: kowada@hokuyakudai.ac.jp



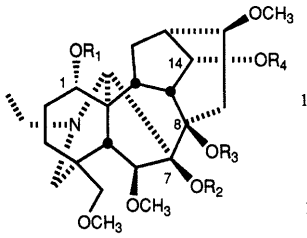
	R_1	R_2	R_3
Neoline (**1**)	H	H	H
14-Acetylneoline (**3**)	H	H	Ac
8-Acetyl-14-benzoylneoline (**5**)	H	Ac	Bz
1-Acetylneoline (**7**)	Ac	H	H
1,14-Diacetylneoline (**8**)	Ac	H	Ac
1-Benzoylneoline (**9**)	Bz	H	H
14-Benzoylneoline (**10**)	H	H	Bz
1,14-Dibenzoylneoline (**11**)	Bz	H	Bz
1-Chloroacetylneoline (**12**)	$COCH_2Cl$	H	H



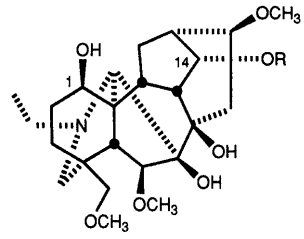
	R_1	R_2
1-*Epi*-neoline (**2**)	H	H
14-Acetyl-1-*epi*-neoline (**4**)	H	Ac
8-Acetyl-14-benzoyl-1-*epi*-neoline (**6**)	Ac	Bz

Ac=$COCH_3$
Bz=COC_6H_5

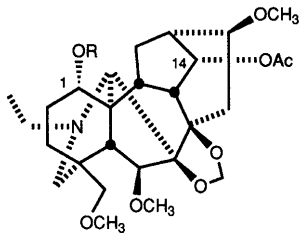
Chart 1



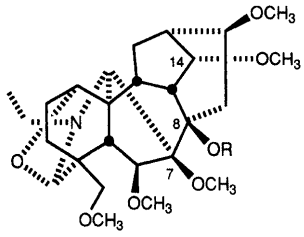
	R_1	R_2	R_3	R_4
Delcosine (**13**)	H	H	H	H
14-Acetyldelcosine (**15**)	H	H	H	Ac
1-Acetyldelcosine (**17**)	Ac	H	H	H
1,14-Diacetyldelcosine (**18**)	Ac	H	H	Ac
14-Acetyl-1-chloroacetyldelcosine (**19**)	$COCH_2Cl$	H	H	Ac
Browniine (**20**)	CH_3	H	H	H
14-acetylbrowniine (**21**)	CH_3	H	H	Ac
Delsoline (**22**)	H	H	H	CH_3
Delphatine (**23**)	CH_3	H	H	CH_3
7,14-Di-*O*-methyldelcosine (**24**)	H	CH_3	H	CH_3
1,7,8,14-Tetra-*O*-methyldelcosine (**25**)	CH_3	CH_3	CH_3	CH_3



1-*Epi*-delcosine (**14**) : R=H
14-Acetyl-1-*epi*-delcosine (**16**) : R=Ac



14-Acetyldelbrunine (**26**) : R=H
1,14-Diacetyldelbrunine (**27**) : R=Ac



7,14-Di-*O*-methyl-18-methoxygadesine (**28**) : R=H
7,8,14-Tri-*O*-methyl-18-methoxygadesine (**29**) : R=CH_3

Chart 2

1—**6** were examined (Fig. 1). When HPLC–APCI-MS of alkaloids **1**—**6** were measured at the drift voltage of 140 V between the first electrode and the second electrode of the APCI ion source, the $[M+H]^+$ ion and a characteristic product ion were obtained (Table 1). The spectra of neoline (**1**)[22] and 1-*epi*-neoline (**2**) showed a very intense ion peak at *m/z* 438, corresponding to the $[M+H]^+$ ions, and a fragment ion at *m/z* 420, which was formed by the loss of a water molecule. Similarly, the spectra of 14-acetylneoline (**3**)[22] and 14-acetyl-1-*epi*-neoline (**4**) revealed a strong $[M+H]^+$ ion at *m/z* 480 and a fragment ion at *m/z* 462, which was formed by the loss of a water molecule. The spectra of both 8-acetyl-14-benzoylneoline (**5**) and 8-acetyl-14-benzoyl-1-*epi*-neoline (**6**) exhibited an ion at *m/z* 584, corresponding to an $[M+H]^+$ ion, and a fragment ion at *m/z* 524. The fragment ion at *m/z* 524 was attributed to the loss of an acetic acid molecule from the $[M+H]^+$ ion. The $[M+H]^+$ ion of these alkaloids clearly appeared to be more stable, undergoing a much more limited fragmentation. As we have reported,[22] the site of protonation in the alkaloids was at a nitrogen atom, and proton chelation

Table 1. *m/z* and Relative Abundance (%) of the Mass Spectral Fragments of Norditerpenoid Neoline-Type Alkaloids

Compd.	$[M+H]^+$	$[M+H-H_2O]^+$	$[M+H-RCOOH]^+$	$[M\text{-}d_n+D]^+$	$[M\text{-}d_n+D-D_2O]^+$	$[M\text{-}d_n+D-CH_3COOD]^+$
1	438 (100%)	420 (10%)	—	442 (100%)	422 (12%)	—
2	438 (100%)	420 (18%)	—	442 (100%)	422 (28%)	—
3	480 (100%)	462 (6%)	—	483 (100%)	463 (8%)	—
4	480 (100%)	462 (14%)	—			
5	584 (100%)	—	524 (23%)	587 (100%)	—	525 (7%)
6	584 (100%)	—	524 (72%)			
7	480 (100%)	462 (8%)	420 (11%)	483 (84%)	463 (9%)	422 (18%)
8	522 (100%)	504 (17%)	462 (8%)	524 (100%)	504 (10%)	463 (18%)
9	542 (100%)	524 (5%)	420 (5%)			
10	542 (100%)	524 (14%)	—			
11	646 (100%)	628 (9%)	524 (35%)			
12	514 (19%)	496 (10%)	420 (38%)			

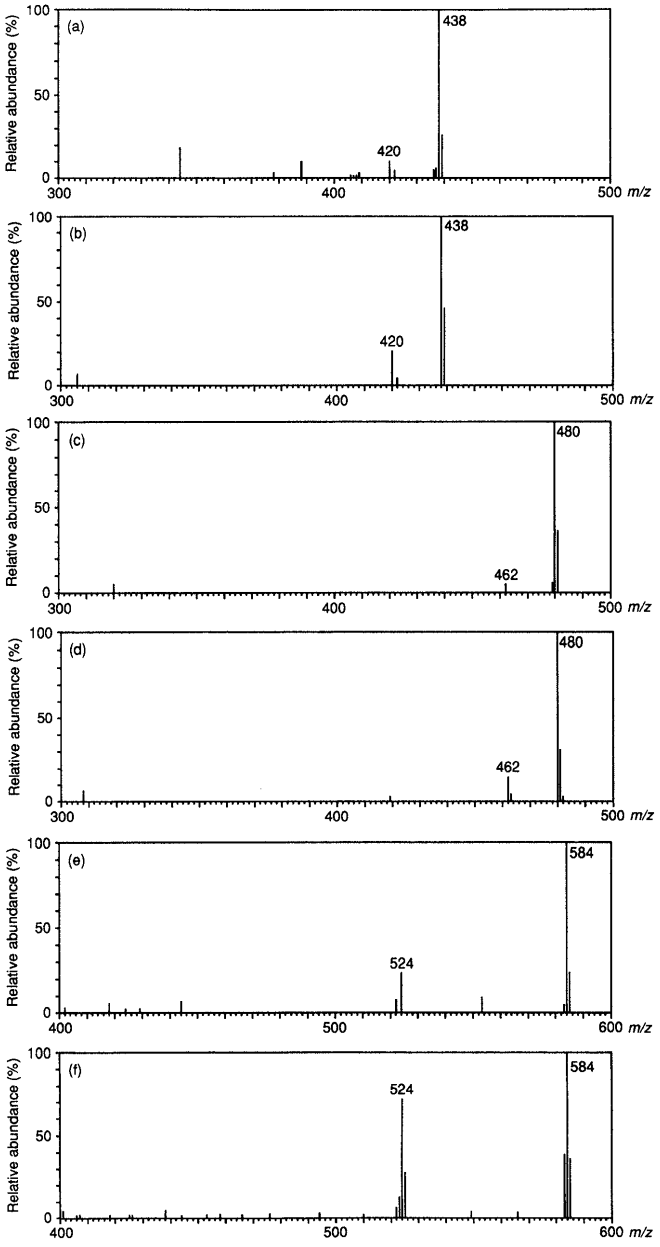


Fig. 1. APCI Mass Spectra of Alkaloids

(a) Neoline (**1**), (b) 1-*epi*-neoline (**2**), (c) 14-acetylneoline (**3**), (d) 14-acetyl-1-*epi*-neoline (**4**), (e) 8-acetyl-14-benzoylneoline (**5**) and (f) 8-acetyl-14-benzoyl-1-*epi*-neoline (**6**). The drift voltage between the first and the second electrodes was 140 V.

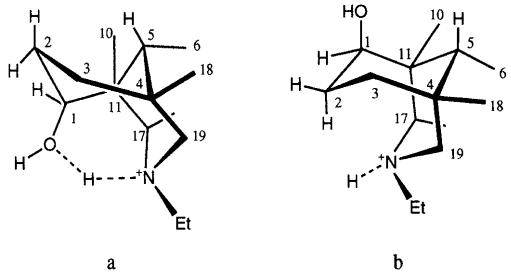


Chart 3

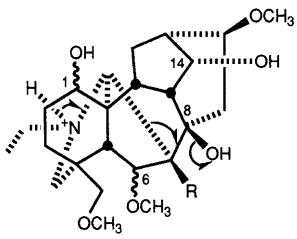


Chart 4

occurred between the amino group and the C-1 hydroxyl group (Chart 3a), with the proton being transferred to the C-8 hydroxyl group and fragmented as a water molecule (Chart 4).

In order to compare the stabilities of $[M+H]^+$ ions **1** and **2** towards the fragmentation processes, we proceeded to the study of energy dependence of the ion abundances.[22] The results for the ions at *m/z* 438 and 420 are shown in Fig. 2. Conditions for the formation of the ion at *m/z* 420 appeared to be closely related to the stereochemistry of the alkaloids under investigation and were sensitive to energy variation. The curves of the ion at *m/z* 420 were completely different. The top of the m/z 420 ion curves corresponding to **2** seemed to be located at voltage values lower than **1**. For comparison, energy dependence of the fragmentation pathway in relation to the stereochemistry of the protonated molecules was examined (Fig. 3). The formation of the ion at *m/z* 420 clearly required a greater amount energy in the case of 1 than 2. The two curves obtained were separated by 30—35 V, evidently corresponding to the stability difference between the $[M+H]^+$ ions **1** and **2** towards the fragmentation pathway.

Comparison of the spectra of **1** and **2** showed a remarkable increase in the relative abundance of the ion peak at *m/z* 420 in the case of **2**. Also, the abundance of the fragment ion at

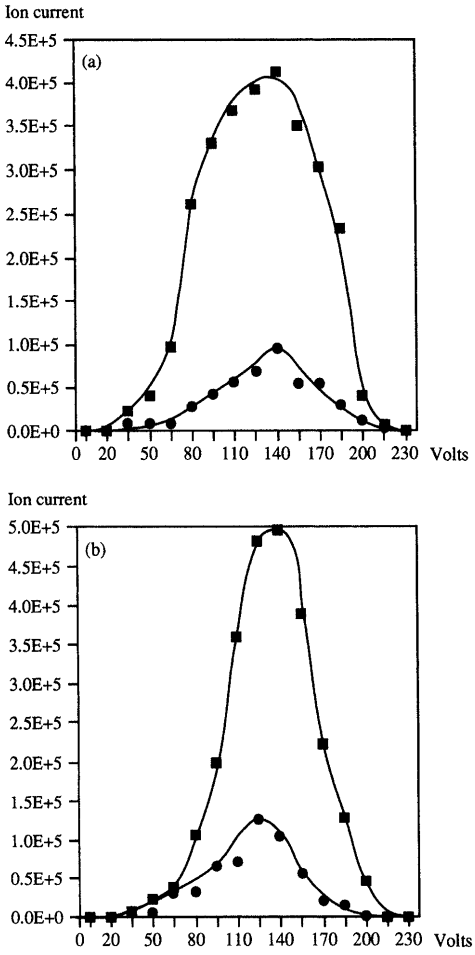


Fig. 2. Ion Currents of the Protonated Molecule at *m/z* 438 (■) and the Fragment Ion at *m/z* 420 (●) Arising from Alkaloids Neoline (**1**; a) and 1-*Epi*-neoline (**2**; b) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

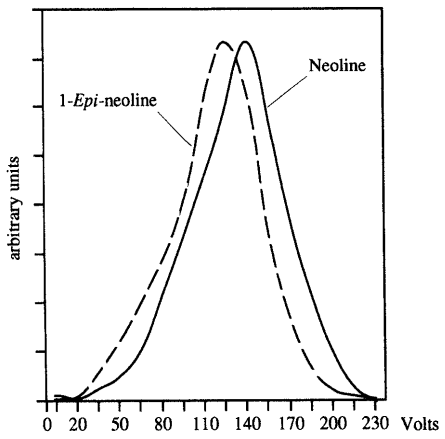


Fig. 3. Ion Currents of the Fragment Ion *m/z* 420 Arising from Alkaloids Neoline (**1**, Solid Line) and 1-*Epi*-neoline (**2**, Dashed Line) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

For a better comparison, the tops of the curves were equalized in the figure.

m/z 462, similar to that of the *m/z* 420 fragment ion in **2**, was larger for **4** than for its epimer **3**. Clearly, the abundance of the ion peak at *m/z* 524 was significantly higher for **6** than for its epimer **5**. These results appeared as a stereospecific fragmentation process. Examination of drift collision-induced

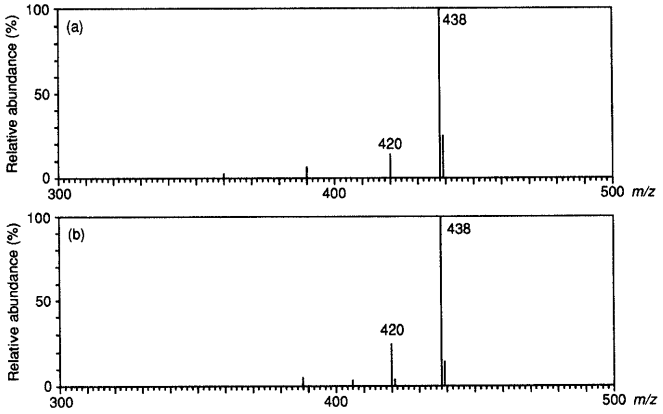


Fig. 4. APCI Mass Spectra of Alkaloids Neoline (**1**; a) and 1-*Epi*-neoline (**2**; b)

The drift voltage between the first and the second electrodes was 180 V.

dissociation (CID) analysis[25,26] (drift voltage of 180 V) of **1**—**4** clearly showed that there was a loss of water from the $[M+H]^+$ ion and that the abundance of the $[M+H-H_2O]^+$ ion increased (Fig. 4). In the drift CID spectra of alkaloids **5** and **6**, the abundance of the $[M+H-CH_3COOH]^+$ ion increased. The spectra of alkaloids **5** and **6** did not show a fragment ion at *m/z* 566 corresponding to the $[M+H-H_2O]^+$ ion. Because of an intramolecular H-bonded system between the amino group and the C-1 hydroxyl group (Chart 3a), the $[M+H]^+$ ion of alkaloid **5** was stabilized. Similarly, the $[M+H]^+$ ions of alkaloids **1** and **3** were stabilized. In the case of alkaloids **2**, **4** and **6**, proton chelation cannot occur (Chart 3b), and proton transfer therefore occurred more easily in alkaloids **2**, **4** and **6** than in alkaloids **1**, **3** and **5**. We considered that the peak intensity ratio for alkaloids **1**—**4** is $R=[M+H]^+/[M+H-H_2O]^+$ and that for alkaloids **5** and **6** is $R'=[M+H]^+/[M+H-CH_3COOH]^+$. The R values of alkaloids **1** and **3**, which contain C-1 α-hydroxyl groups, were 10—17, whereas those of alkaloids **2** and **4**, which contain C-1 β-hydroxyl groups, were 5.6—7.1. The R' value of alkaloid **5**, which contains a C-1 α-hydroxyl group, was 4.4, whereas that of alkaloid **6**, which contains a C-1 β-hydroxyl group, was 1.4. These results indicated that the R and R' values showed stereochemical differentiation of alkaloids at position 1.

The fragmentation mechanisms depending on the stereochemistry of the precursor ion could be discerned by recording the spectra of alkaloids **1** and **2** in a deuterated solvent system of 0.05 M ammonium acetate in D_2O–acetonitrile–tetrahydrofuran.[22] These spectra showed a major ion peak at *m/z* 442 corresponding to the $[M\text{-}d_3+D]^+$ ions formed by deuterium exchange of hydroxyl hydrogens and addition of D^+ on the molecules (Fig. 5). Loss of D_2O from this precursor ion gave the fragment ion at *m/z* 422. This result indicated that the proton of protonation was included in the leaving water molecule, irrespective of the stereochemistry at position 1. Furthermore, the fragment ion at *m/z* 525 in **5** was formed by the loss of CH_3COOD from the precursor ion at *m/z* 587 corresponding to the $[M\text{-}d+D]^+$ ion. These results revealed that the site of the leaving hydroxyl or acetyl group was position 8.

The study of fragmentation behavior of alkaloids 1-acetylneoline (**7**),[22] 1,14-diacetylneoline (**8**),[22] 1-benzoylneoline

(**9**),[27] 14-benzoylneoline (**10**),[27] 1,14-dibenzoylneoline (**11**)[27] and 1-chloroacetylneoline (**12**) showed an $[M+H]^+$ ion peak and a characteristic fragment ion peak corresponding to the $[M+H-H_2O]^+$ and the $[M+H-R_1OH]^+$ ions. The common substitution feature of alkaloids **7**—**9** and **11**—**12** corresponds to the presence of an ester group at C-1 and a hydroxyl group at C-8. The spectra of **7** and **8** showed a fragment ion peak corresponding to the $[M+H-CH_3COOH]^+$ ion, and the spectra of **9** and **11** showed a fragment ion peak corresponding to the $[M+H-C_6H_5COOH]^+$ ion. Also, the spectra of **12** showed a major fragment ion at *m/z* 420 corresponding to the $[M+H-ClCH_2COOH]^+$ ion. In the spectra of **10**, fragment ion peaks other than that of the $[M+H-H_2O]^+$ ion were not observed. These results indicated that the characteristic fragment ion $[M+H-H_2O]^+$ was formed by the loss of a water molecule at the above-mentioned position 8. In addition, in the case of the presence of an ester group at C-1, the characteristic fragment ions were formed by the loss of R_1OH at position 1. Furthermore, the deuterated spectra of **7** and **8** showed fragment ions at *m/z* 422 and 463, respectively, corresponding to the $[M-d_n+D-CH_3COOD]^+$ ion from the $[M-d_n+D]^+$ ion. This result revealed that the site of the leaving acetyl group was the above-mentioned position 1.

Next, the APCI mass spectra obtained in the HPLC–APCI-MS of delcosine-type alkaloids **13**—**16** were examined and were first recorded at the drift voltage of 140 V (Fig. 6). The spectra of delcosine (**13**) and 1-*epi*-delcosine (**14**) showed a highly intense ion peak at *m/z* 454, corre-

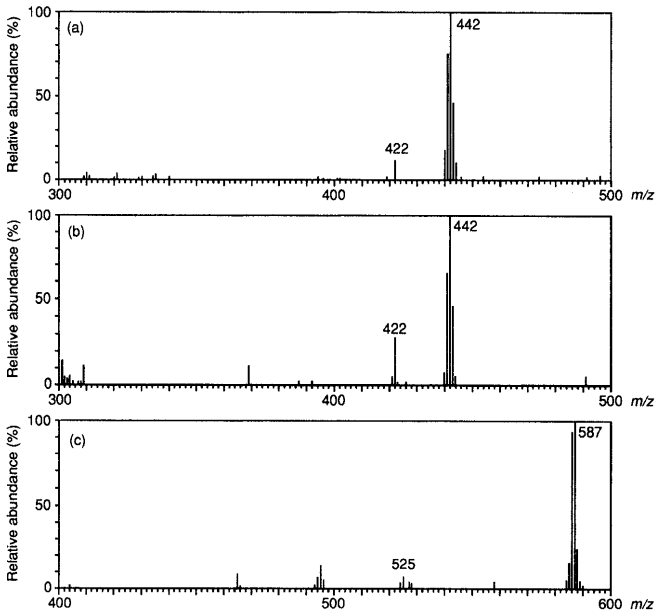


Fig. 5. APCI Mass Spectra of Alkaloids Recorded by the Solvent System of 0.05 M Ammonium Acetate in D_2O–Acetonitrile–Tetrahydrofuran

(a) Neoline (**1**), (b) 1-*epi*-neoline (**2**) and (c) 8-acetyl-14-benzoylneoline (**5**). The drift voltage between the first and the second electrodes was 140 V.

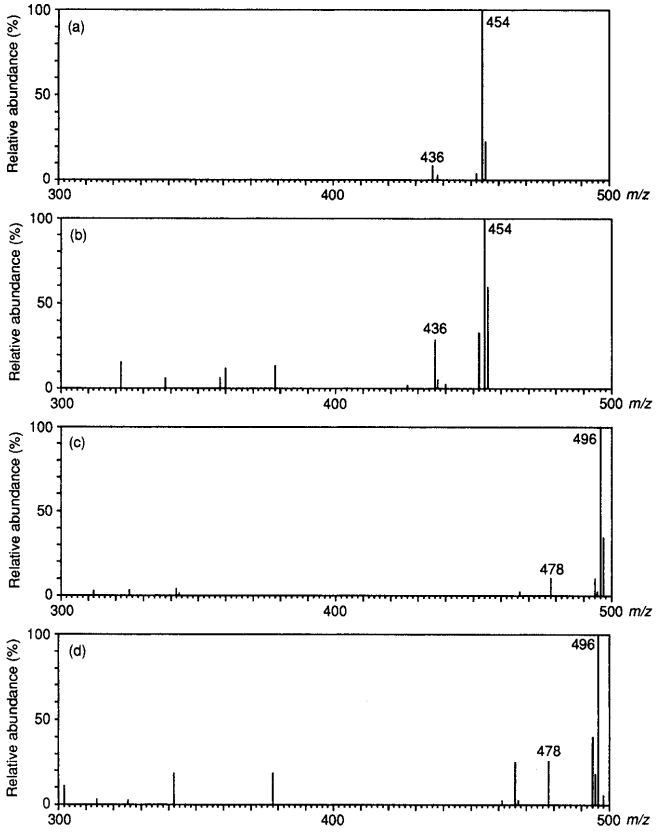


Fig. 6. APCI Mass Spectra of Alkaloids

(a) Delcosine (**13**), (b) 1-*epi*-delcosine (**14**), (c) 14-acetyldelcosine (**15**) and (d) 14-acetyl-1-*epi*-delcosine (**16**). The drift voltage between the first and the second electrodes was 140 V.

Table 2. *m/z* and Relative Abundance (%) of the Mass Spectral Fragments of Norditerpenoid Delcosine-Type Alkaloids

Compd.	$[M+H]^+$	$[M+H-H_2O]^+$	$[M+H-CH_3OH]^+$	$[M+H-CH_3COOH]^+$
13	454 (100%)	436 (9%)	—	—
14	454 (100%)	436 (29%)	—	—
15	496 (100%)	478 (10%)	—	—
16	496 (100%)	478 (26%)	—	—
17	496 (100%)	478 (18%)	—	—
18	538 (100%)	520 (5%)	—	—
19	572 (100%)	554 (11%)	—	—
20	468 (100%)	450 (26%)	—	—
21	510 (100%)	492 (7%)	—	—
22	468 (100%)	450 (16%)	—	—
23	482 (100%)	464 (27%)	—	—
24	482 (100%)	464 (57%)	—	—
25	510 (21%)	—	478 (100%)	—
26	508 (100%)	—	—	—
27	550 (100%)	—	—	490 (30%)
28	480 (77%)	462 (100%)	—	—
29	494 (9%)	—	462 (100%)	—

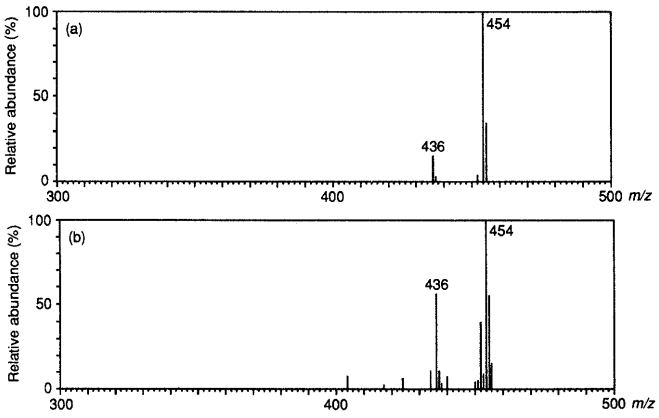


Fig. 7. APCI Mass Spectra of Alkaloids Delcosine (**13**; a) and 1-*Epi*-delcosine (**14**; b)

The drift voltage between the first and the second electrodes was 180 V.

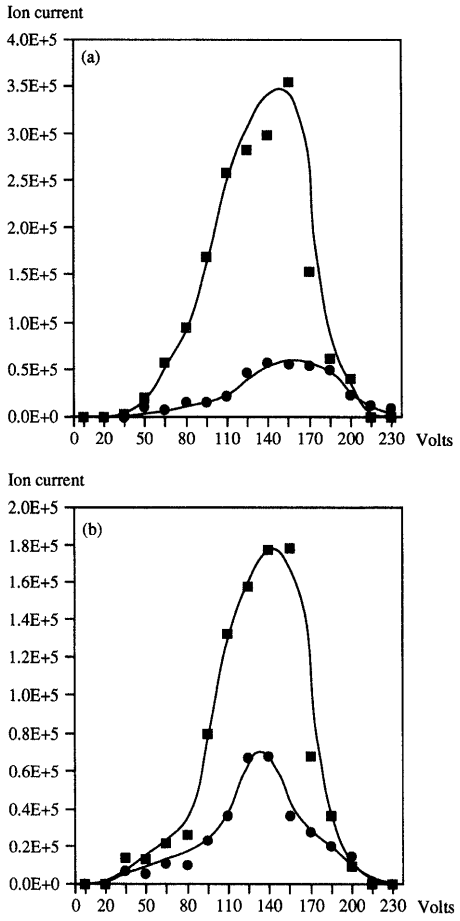


Fig. 8. Ion Currents of the Protonated Molecule at *m/z* 454 (■) and the Fragment Ion at *m/z* 436 (●) Arising from Alkaloids Delcosine (**13**; a) and 1-*Epi*-delcosine (**14**; b) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

sponding to the $[M+H]^+$ ions, and a fragment ion at *m/z* 436, which was formed by the loss of a water molecule. Similarly, the spectra of 14-acetyldelcosine (**15**) and 14-acetyl-1-*epi*-delcosine (**16**) exhibited a strong $[M+H]^+$ ion at *m/z* 496 and a fragment ion at *m/z* 478, which was formed by the loss of a water molecule.

Evidence of fragmentation mechanisms depending on the precursor ion was provided by the study of fragmentation behavior of alkaloids 1-acetyldelcosine (**17**),[28] 1,14-diacetyldelcosine (**18**),[29] 14-acetyl-1-chloroacetyldelcosine (**19**), browniine (**20**),[30] 14-acetylbrowniine (**21**),[30] delsoline (**22**),[31] delphatine (**23**),[31] 7,14-di-*O*-methyldelcosine (**24**), 1,7,8,14-tetra-*O*-methyldelcosine (**25**), 14-acetyldelbrunine (**26**),[32] 1,14-diacetyldelbrunine (**27**), 7,14-di-*O*-methyl-18-methoxygadesine (**28**) and 7,8,14-tri-*O*-methyl-18-methoxygadesine (**29**). The common substitution feature of alkaloids **17**—**24** corresponds to the presence of a hydroxyl group at C-8 and **28** corresponds to only the presence of a hydroxyl group at C-8. The spectra of **17**—**24** and **28** showed a major fragment ion peak corresponding to the $[M+H-H_2O]^+$ ion. Also, the spectra of **25** and **29**, in the presence of a methoxyl group at C-8, showed a major fragment ion peak corresponding to the $[M+H-CH_3OH]^+$ ion. The spectra of **27**, in the presence of acetyl group at C-1, showed a major fragment ion peak corresponding to the $[M+H-CH_3COOH]^+$ ion. These results indicated that the characteristic fragment ions were formed by the loss of H_2O or CH_3OH at position 8, as previously reported for neoline-type alkaloids[22] (Chart 4), and by the loss of CH_3COOH at position 1, as in the above-mentioned neoline-type alkaloids.

Comparison of the spectra of **13** and **14** showed a remarkable increase in the relative abundance of the ion peak at *m/z* 436 in the case of **14**. Also, the abundance of the fragment ion at *m/z* 478 was larger for **16** than for its epimer **15**. These results appeared as a stereospecific fragmentation process. The drift CID spectrum of **13**—**16** clearly showed a characteristic fragment ion, and the abundance of the $[M+H-H_2O]^+$ ion increased (Fig. 7). We considered that the peak intensity ratio for alkaloids **13**—**16** is $R=[M+H]^+/[M+H-H_2O]^+$. The *R* values of alkaloids **13** and **15**, which contain C-1 α-hydroxyl groups, were 10—11, whereas those of alkaloids **14** and **16**, which contain C-1 β-hydroxyl groups, were 3.5—3.9. These results indicated that the *R* value showed stereochemical differentiation of alkaloids at position 1.

In order to compare the stabilities of the $[M+H]^+$ ions of **13** and **14** towards the fragmentation processes, we proceeded to the study of energy dependence of the ion intensities.[22] The results for the ions at *m/z* 454 and 436 are shown in Fig. 8. The curves of the ion at *m/z* 436 were completely different. For the sake of comparison, energy dependence of the fragmentation pathway in relation to the stereochemistry of the protonated molecules was examined (Fig. 9). The formation of the ion *m/z* 436 clearly required a greater amount energy in the case of **13** than **14**. The two curves obtained were separated by 10—15 V, evidently corresponding to the stability difference between $[M+H]^+$ ions of **13** and **14** towards this fragmentation pathway.

The fragmentation mechanisms depending on the stereochemistry of the precursor ion could be perceived by recording the spectra of alkaloids **13** and **14** in a deuterated solvent system of 0.05 M ammonium acetate in D_2O–acetonitrile–tetrahydrofuran.[22] These spectra showed a major ion peak at *m/z* 459 corresponding to the $[M\text{-}d_4+D]^+$ ions formed by deuterium exchange of hydroxyl hydrogens and addition of D^+ on the molecules (Fig. 10). The fragment ion at *m/z* 439 was attributed to the loss of D_2O from this precursor ion. This result indicated that the proton of protonation was included in the leaving water molecule, irrespective of the

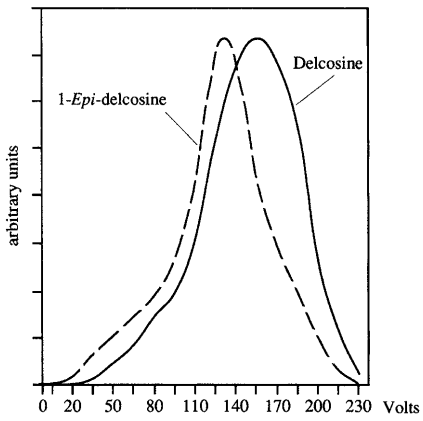


Fig. 9. Ion Currents of the Fragment Ion *m/z* 436 Arising from Alkaloids Delcosine (**13**, Solid Line) and 1-*Epi*-delcosine (**14**, Dashed Line) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

For a better comparison, the tops of the curves were equalized in the figure.

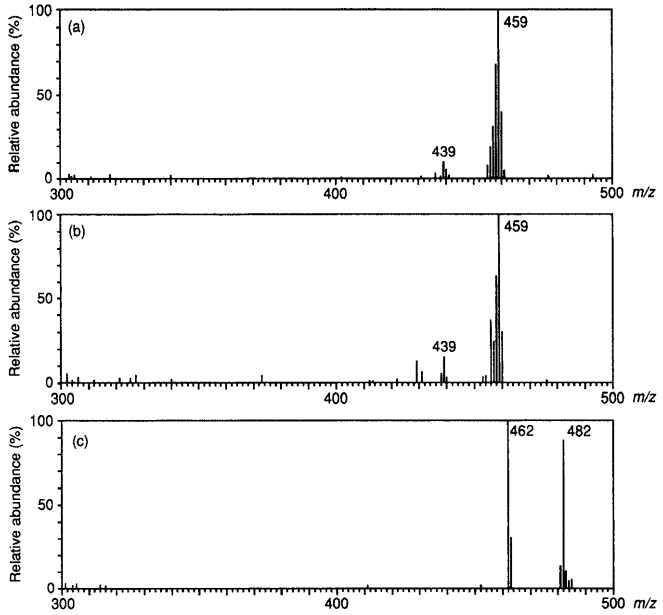


Fig. 10. APCI Mass Spectra of Alkaloids Recorded by the Solvent System of 0.05 M Ammonium Acetate in D_2O–Acetonitrile–Tetrahydrofuran

Delcosine (**13**; a), 1-*epi*-delcosine (**14**; b) and 7,14-di-*O*-methyl-18-methoxygadesine (**28**; c). The drift voltage between the first and the second electrodes was 140 V.

stereochemistry at position 1. Furthermore, the fragment ion at *m/z* 462 in **28** was formed by the loss of D_2O from the precursor ion at *m/z* 482 corresponding to the $[M\text{-}d+D]^+$ ion. These results revealed that the site of the leaving hydroxyl group was position 8, as in neoline-type alkaloids. Proton chelation occurred between the amino group and the C-1 hydroxyl group (Chart 3a), and the proton was transferred to the C-8 hydroxyl group and fragmented as a water molecule (Chart 4). In the case of alkaloids **14** and **16**, proton chelation cannot occur (Chart 3b), and proton transfer therefore occurred more easily in alkaloids **14** and **16** than in alkaloids **13** and **15**.

In conclusion, the APCI mass spectra of the C_{19}-norditerpenoid neoline-type and delcosine-type alkaloids were remarkably simple and very similar with respect to characteristic fragments. In the APCI mass spectra of the C_{19}-norditerpenoid neoline-type and delcosine-type alkaloids, the characteristic fragment ions were formed by loss of substituents at position 8. In addition, in the presence of an ester group at C-1, the site of elimination of ester molecules was position 1. Comparison of the characteristic fragment ion abundance showed a complementarity for structural investigations of stereochemical differentiation. Information obtained from energy dependence experiments can be very valuable for the detection of subtle structural characteristics of organic molecules, including stereochemical differentiation.

Experimental

All melting points were measured on a Yanagimoto micromelting points apparatus without correction. IR spectra were recorded using a model FT/IR 7000 spectrometer (Jasco, Tokyo, Japan). ^{1}H-NMR spectra were measured in deuterated trichloromethane solution with a model GX-270 spectrometer (JEOL, Tokyo, Japan) using tetramethylsilane (TMS) as an internal standard. MS was performed with a model M-2000 mass spectrometer (Hitachi, Tokyo, Japan).

Materials Neoline (**1**), delcosine (**13**), 14-acetyldelcosine (**15**), browniine (**20**) and 14-acetylbrowniine (**21**) were purified from *Aconitum japonicum* roots[27] and *Aconitum yesoense* var. *macroyesoense* (Nakai) Tamura roots,[30] respectively, and identified as described previously. 14-Acetylneoline (**3**),[22] 1,14-diacetylneoline (**8**),[22] 1-benzoylneoline (**9**),[27] 14-benzoylneoline (**10**),[27] 1,14-dibenzoylneoline (**11**)[27] and 1-acetyldelcosine (**17**)[28] were synthesized as described previously. 1-*Epi*-neoline (**2**),[23] 14-acetyl-1-*epi*-neoline (**4**),[24] 8-acetyl-14-benzoylneoline (**5**), 8-acetyl-14-benzoyl-1-*epi*-neoline (**6**), 1-acetylneoline (**7**), 1-chloroacetylneoline (**12**), 1-*epi*-delcosine (**14**), 14-acetyl-1-*epi*-delcosine (**16**), 1,14-diacetyldelcosine (**18**),[29] 14-acetyl-1-chloroacetyldelcosine (**19**), delsoline (**22**),[31] delphatine (**23**),[31] 7,14-di-*O*-methyldelcosine (**24**), 1,7,8,14-tetra-*O*-methyldelcosine (**25**), 14-acetyldelbrunine (**26**),[32] 1,14-diacetyldelbrunine (**27**), 7,14-di-*O*-methyl-18-methoxygadesine (**28**) and 7,8,14-tri-*O*-methyl-18-methoxygadesine (**29**) were synthesized from neoline (**1**), 14-acetylneoline (**3**), 14-acetyl-1-*epi*-neoline (**4**), 14-benzoylneoline (**10**), delcosine (**13**), 14-acetyldelcosine (**15**), browniine (**20**), delsoline (**22**) and 14-acetyldelbrunine (**26**), respectively. Ammonium acetate of reagent grade was purchased from Kanto Chemicals (Tokyo, Japan), and acetonitrile and tetrahydrofuran of HPLC grade and deuterium oxide (99.9%) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of 14-Acetyl-1-*epi*-neoline (4) 1) Oxidation of 14-acetylneoline. A solution of 14-acetylneoline (**3**: 134.8 mg) in dichloromethane (39 ml) was mixed with pyridinium dichromate (PDC; 317.7 mg). The mixture was stirred at room temperature for 1 h. The reaction mixture was passed through a short column of florisil to give 14-acetyl-1-dehydroneoline (83.3 mg, 62%) and starting material (19.4 mg). Amorphous. IR ($CHCl_3$) cm^{-1}: 3588, 1744, 1212, 1135. ^{1}H-NMR δ: 1.10 (3H, t, J=7.0 Hz, *N*-CH_2CH_3), 2.06 (3H, s, $OCOCH_3$), 3.23, 3.29, 3.37 (each 3H, s, OCH_3), 4.07 (1H, d, J=6.3 Hz, 6β-H), 4.88 (1H, t, J=4.6 Hz, 14β-H). HR-EI-MS *m/z*: 477.2718 (Calcd for $C_{26}H_{39}NO_7$: 477.2725). EI-MS *m/z*: 477 (M^+), 462 (M^+-CH_3, base peak), 446 (M^+-OCH_3).

2) 14-Acetyl-1-*epi*-neoline. 14-Acetyl-1-dehydroneoline (83.3 mg), dissolved in MeOH–absolute EtOH (1 : 1, 16 ml), was treated with $NaBH_4$ (20 mg). The resulting solution was stirred at room temperature for 2 h 15 min. Water was added, and the mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness after being washed with 5% sodium hydrogencarbonate and brine and then dried with anhydrous magnesium sulfate and purification by column chromatography on silica gel (1% hexane–ethyl acetate) gave 14-acetylneoline (**3**; 17.1 mg, 21%) and 14-acetyl-1-*epi*-neoline (**4**; 50.0 mg, 60%).

4: Amorphous. IR (KBr) cm^{-1}: 3454, 1742, 1247, 1096. ^{1}H-NMR δ: 1.05 (3H, t, J=7.0 Hz, *N*-CH_2CH_3), 2.06 (3H, s, $OCOCH_3$), 3.26, 3.31, 3.34 (each 3H, s, OCH_3), 3.88 (1H, br s, 1α-H), 4.07 (1H, d, J=6.6 Hz, 6β-H), 4.88 (1H, t, J=4.6 Hz, 14β-H). HR-EI-MS *m/z*: 479.2857 (Calcd for $C_{26}H_{41}NO_7$: 479.2880). EI-MS *m/z*: 479 (M^+), 464 (M^+-CH_3, base peak), 462 (M^+-OH), 448, 446.

Preparation of 1-*Epi*-neoline (2) A mixture of 14-acetyl-1-*epi*-neoline (**4**: 20.0 mg) and 5% KOH–aq.MeOH (5 ml) was kept at room temperature for 15.5 h. The resulting solution was evaporated and after the addition of water, the solution was extracted with $CHCl_3$. Then the usual work-up afforded an amorphous powder (18.1 mg, 99%). Amorphous. IR (KBr) cm^{-1}: 3450, 1100. ^{1}H-NMR δ: 1.46 (3H, t, J=7.3 Hz, *N*-CH_2CH_3), 3.30, 3.33, 3.34 (each 3H, s, OCH_3), 3.83 (1H, br s, 1α-H), 4.14 (1H, d, J=6.5 Hz, 6β-H),

4.24 (1H, t, J=4.6 Hz, 14β-H). HR-EI-MS m/z: 437.2763 (Calcd for $C_{24}H_{39}NO_6$: 437.2775). EI-MS m/z: 437 (M^+), 422 (M^+-CH_3, base peak), 420 (M^+-OH), 406, 404.

Preparation of 8-Acetyl-14-benzoylneoline (5) and 8-Acetyl-14-benzoyl-1-*epi*-neoline (6) 1) Oxidation of 14-benzoylneoline. A solution of 14-benzoylneoline (**10**: 239.3 mg) in dichloromethane (68 ml) was mixed with PDC (499.4 mg). The mixture was stirred at room temperature for 1 h. The reaction mixture was passed through a short column of florisil and then purified on column chromatography of silica gel (25—10% hexane–ether saturated with 28% ammonia) to give 14-benzoyl-1-dehydroneoline (67.4 mg, 28%) and starting material (20.8 mg). Amorphous. IR ($CHCl_3$) cm^{-1}: 3622, 1717, 1218, 1133. ^{1}H-NMR δ: 1.11 (3H, t, J=7.0 Hz, N-CH_2CH_3), 3.22, 3.30, 3.36 (each 3H, s, OCH_3), 4.09 (1H, d, J=6.5 Hz, 6β-H), 5.21 (1H, t, J=4.6 Hz, 14β-H), 7.43 (2H, t, J=7.0 Hz, aromatic-H), 7.55 (1H, t, J=7.3 Hz, aromatic-H), 8.00 (2H, d, J=6.8 Hz, aromatic-H). HR-EI-MS m/z: 539.2894 (Calcd for $C_{31}H_{41}NO_7$: 539.2882). EI-MS m/z: 539 (M^+), 522 (M^+-OH), 509, 494, 423, 105 ($[COC_6H_5]^+$, base peak).

2) Acetylation of 14-benzoyl-1-dehydroneoline. A mixture of 14-benzoyl-1-dehydroneoline (67.4 mg), acetic anhydride (1 ml) and p-toluenesulfonic acid monohydrate (36.7 mg) was kept at 90—100 °C for 2.5 h. The usual work-up and purification by column chromatography on silica gel (40% hexane–ether saturated with 28% ammonia) afforded 8-acetyl-14-benzoyl-1-dehydroneoline (48.0 mg, 66%). Amorphous. IR ($CHCl_3$) cm^{-1}: 1719, 1222, 1135. ^{1}H-NMR δ: 1.11 (3H, t, J=7.0 Hz, N-CH_2CH_3), 1.42 (3H, s, $OCOCH_3$), 3.22, 3.28, 3.36 (each 3H, s, OCH_3), 4.00 (1H, d, J=7.5 Hz, 6β-H), 5.12 (1H, t, J=4.2 Hz, 14β-H), 7.43 (2H, t, J=7.0 Hz, aromatic-H), 7.55 (1H, t, J=7.3 Hz, aromatic-H), 8.05 (2H, d, J=7.0 Hz, aromatic-H). HR-EI-MS m/z: 581.3005 (Calcd for $C_{33}H_{43}NO_8$: 581.2987). EI-MS m/z: 581 (M^+), 566 (M^+-CH_3, base peak), 550 (M^+-OCH_3), 522 ($M^+-OCOCH_3$).

3) Reduction of 8-acetyl-14-benzoyl-1-dehydroneoline. 8-Acetyl-14-benzoyl-1-dehydroneoline (48.0 mg), dissolved in MeOH–absolute EtOH (1 : 1, 10 ml), was treated with $NaBH_4$ (11 mg). The resulting solution was stirred at room temperature for 2 h. Water was added, and the mixture was extracted with $CHCl_3$. Then the usual work-up and purification by column chromatography on silica gel (50% hexane–ethyl acetate) gave 8-acetyl-14-benzoylneoline (**5**; 10.2 mg, 21%) and 8-acetyl-14-benzoyl-1-*epi*-neoline (**6**; 25.0 mg, 52%).

5: Amorphous. IR (KBr) cm^{-1}: 3438, 1725, 1280, 1118. ^{1}H-NMR δ: 1.16 (3H, t, J=7.0 Hz, N-CH_2CH_3), 1.42 (3H, s, $OCOCH_3$), 3.20, 3.31, 3.39 (each 3H, s, OCH_3), 3.74 (1H, s, 1β-H), 4.06 (1H, d, J=5.6 Hz, 6β-H), 5.09 (1H, t, J=4.6 Hz, 14β-H), 7.44 (2H, t, J=7.3 Hz, aromatic-H), 7.56 (1H, t, J=7.3 Hz, aromatic-H), 8.05 (2H, d, J=7.0 Hz, aromatic-H). HR-EI-MS m/z: 583.3141 (Calcd for $C_{33}H_{45}NO_8$: 583.3143). EI-MS m/z: 583 (M^+), 568 (M^+-CH_3), 566 (M^+–OH, base peak), 524 ($M^+-OCOCH_3$), 105 ($[COC_6H_5]^+$).

6: mp 169—171.5 °C. IR (KBr) cm^{-1}: 3516, 1723, 1278, 1164. ^{1}H-NMR δ: 1.06 (3H, t, J=7.0 Hz, N-CH_2CH_3), 1.43 (3H, s, $OCOCH_3$), 3.19, 3.29, 3.37 (each 3H, s, OCH_3), 3.93 (1H, br s, 1α-H), 4.03 (1H, d, J=6.5 Hz, 6β-H), 5.13 (1H, t, J=4.6 Hz, 14β-H), 7.43 (2H, t, J=7.0 Hz, aromatic-H), 7.55 (1H, t, J=7.5 Hz, aromatic-H), 8.05 (2H, d, J=7.0 Hz, aromatic-H). HR-EI-MS m/z: 583.3168 (Calcd for $C_{33}H_{45}NO_8$: 583.3143). EI-MS m/z: 583 (M^+), 568 (M^+-CH_3), 566 (M^+-OH), 524 ($M^+-OCOCH_3$, base peak), 105 ($[COC_6H_5]^+$).

Preparation of 1-Acetylneoline (7) A mixture of neoline (**1**: 30.5 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) was kept at 0 °C for 2 h. The usual work-up and purification by column chromatography on silica gel (ether saturated with 28% ammonia) afforded 1-acetylneoline (20.7 mg, 62%). Amorphous. IR (KBr) cm^{-1}: 3444, 1738, 1247, 1112. ^{1}H-NMR δ: 1.10 (3H, t, J=7.0 Hz, N-CH_2CH_3), 2.01 (3H, s, $OCOCH_3$), 3.30, 3.31, 3.35 (each 3H, s, OCH_3), 4.09 (1H, t, J=4.8 Hz, 14β-H), 4.21 (1H, d, J=6.8 Hz, 6β-H), 4.81 (1H, dd, J=6.8, 10.5 Hz, 1β-H). HR-EI-MS m/z: 479.2879 (Calcd for $C_{26}H_{41}NO_7$: 479.2882). EI-MS m/z: 479 (M^+), 462 (M^+-OH), 437, 420 ($M^+-OCOCH_3$, base peak).

Preparation of 1-Chloroacetylneoline (12) A mixture of neoline (**1**: 51.0 mg), pyridine (0.8 ml) and chloroacetic anhydride (60.4 mg) was kept at room temperature for 12.5 h. The usual work-up afforded 1-chloroacetylneoline (37.5 mg, 63%). Amorphous. IR (KBr) cm^{-1}: 3432, 1750, 1243, 1112. ^{1}H-NMR δ: 1.10 (3H, t, J=7.0 Hz, N-CH_2CH_3), 3.30, 3.31, 3.35 (each 3H, s, OCH_3), 4.02 (2H, s, $OCOCH_2Cl$), 4.11 (1H, t, J=4.6 Hz, 14β-H), 4.22 (1H, d, J=6.8 Hz, 6β-H), 4.90 (1H, dd, J=6.8, 10.2 Hz, 1β-H). HR-EI-MS m/z: 513.2427 (Calcd for $C_{26}H_{40}ClNO_7$: 513.2491). EI-MS m/z: 515 (M^++2), 513 (M^+), 498, 496 (M^+-OH), 420 ($M^+-OCOCH_2Cl$, base peak).

Preparation of 1-*Epi*-delcosine (14) 1) Oxidation of 14-acetyldelcosine. A solution of 14-acetyldelcosine (**15**: 200.3 mg) in dichloromethane (60 ml) was mixed with PDC (457.2 mg). The mixture was stirred at 0 °C for 5 h. The reaction mixture was passed through a short column of florisil and afforded 14-acetyl-1-dehydrodelcosine (101.5 mg, 51%) and the starting material (10.4 mg). Amorphous. IR ($CHCl_3$) cm^{-1}: 3622, 1736, 1218, 1135. ^{1}H-NMR δ: 1.01 (3H, t, J=7.0 Hz, N-CH_2CH_3), 2.04 (3H, s, $OCOCH_3$), 3.29 (6H, s, OCH_3), 3.37 (3H, s, OCH_3), 3.98 (1H, s, 6α-H), 4.78 (1H, t, J=4.6 Hz, 14β-H). HR-EI-MS m/z: 493.2676 (Calcd for $C_{26}H_{39}NO_8$: 493.2674). EI-MS m/z: 493 (M^+), 478 (M^+-CH_3, base peak), 475, 460.

2) 1-*Epi*-delcosine. 14-acetyl-1-dehydrodelcosine (56.9 mg), dissolved in tetrahydrofuran (20 ml), was treated with $LiAlH_4$ (60 mg). The resulting solution was stirred at room temperature for 2 h. Water was added, and the mixture was filtrated with a glass filter. Then the solution was evaporated, and then purification by column chromatography on silica gel (0.3% MeOH–$CHCl_3$ saturated with 28% ammonia) gave delcosine (**13**; 19.0 mg, 36%) and 1-*epi*-delcosine (**14**; 24.2 mg, 46%).

14: Amorphous. IR (KBr) cm^{-1}: 3454, 1091. ^{1}H-NMR δ: 1.02 (3H, t, J=7.0 Hz, N-CH_2CH_3), 3.31, 3.36, 3.38 (each 3H, s, OCH_3), 3.81 (1H, br s, 1α-H), 3.95 (1H, s, 6α-H), 4.14 (1H, dd, J=4.6, 8.5 Hz, 14β-H). HR-EI-MS m/z: 453.2707 (Calcd for $C_{24}H_{39}NO_7$: 453.2714). EI-MS m/z: 453 (M^+), 438 (M^+-CH_3), 436 (M^+-OH), 422, 420 (base peak).

Preparation of 14-Acetyl-1-*epi*-delcosine (16) 14-Acetyl-1-dehydrodelcosine (44.6 mg), dissolved in MeOH (8 ml), was treated with $NaBH_4$ (10 mg). The resulting solution was stirred at room temperature for 1 h 50 min. Water was added, and the mixture was extracted with $CHCl_3$. Then the usual work-up and purification by column chromatography on silica gel (ethyl acetate) gave 14-acetyldelcosine (**15**; 20.4 mg, 46%) and 14-acetyl-1-*epi*-delcosine (**16**; 19.7 mg, 44%).

16: mp, 112 °C (dec.). IR (KBr) cm^{-1}: 3452, 1738, 1253, 1091. ^{1}H-NMR δ: 1.02 (3H, t, J=7.0 Hz, N-CH_2CH_3), 2.06 (3H, s, $OCOCH_3$), 3.30, 3.32, 3.37 (each 3H, s, OCH_3), 3.87 (1H, br s, 1α-H), 3.94 (1H, s, 6α-H), 4.83 (1H, t, J=4.6 Hz, 14β-H). HR-EI-MS m/z: 495.2851 (Calcd for $C_{26}H_{41}NO_8$: 495.2830). EI-MS m/z: 495 (M^+, base peak), 480 (M^+-CH_3), 478 (M^+-OH), 462.

Preparation of 1,14-Diacetyldelcosine (18) A mixture of delcosine (**13**: 200 mg), pyridine (3 ml) and acetic anhydride (3 ml) was kept at 80 °C for 1 h. The usual work-up and purification by column chromatography on silica gel (20% hexane–ether saturated with 28% ammonia) afforded 1,14-diactyldelcosine (89.1 mg, 38%). mp, 83 °C (dec.). IR (KBr) cm^{-1}: 3450, 1725, 1245. ^{1}H-NMR δ: 1.08 (3H, t, J=7.2 Hz, N-CH_2CH_3), 2.04, 2.05 (each 3H, s, $OCOCH_3$), 3.28, 3.31, 3.41 (each 3H, s, OCH_3), 3.92 (1H, s, 6α-H), 4.71 (1H, t, J=4.6 Hz, 14β-H), 4.72 (1H, dd, J=8.2, 9.8 Hz, 1β-H). HR-EI-MS m/z: 537.2909 (Calcd for $C_{28}H_{43}NO_9$: 537.2935). EI-MS m/z: 537 (M^+), 522 (M^+-CH_3), 479 (base peak).

Preparation of 14-Acetyl-1-chloroacetyldelcosine (19) A mixture of 14-acetyldelcosine (**15**: 31.0 mg), pyridine (0.5 ml) and chloroacetic anhydride (34.5 mg) was kept at room temperature for 1.5 h. The usual work-up and purification by column chromatography on silica gel (5% hexane–ether saturated with 28% ammonia) afforded 14-acetyl-1-chloroacetyldelcosine (20.3 mg, 57%) and the starting material (11.4 mg). Amorphous. IR (KBr) cm^{-1}: 3460, 1738, 1251, 1093. ^{1}H-NMR δ: 1.08 (3H, t, J=7.0 Hz, N-CH_2CH_3), 2.05 (3H, s, $OCOCH_3$), 3.29, 3.31, 3.41 (each 3H, s, OCH_3), 3.93 (1H, s, 6α-H), 4.05 (2H, s, $OCOCH_2Cl$), 4.71 (1H, t, J=4.8 Hz, 14β-H), 4.81 (1H, dd, J=7.8, 9.7 Hz, 1β-H). HR-EI-MS m/z: 571.2537 (Calcd for $C_{28}H_{42}ClNO_9$: 571.2545). EI-MS m/z: 573 (M^++2), 571 (M^+), 556 (M^+-CH_3), 553 (M^+-OH), 540, 538, 478 ($M^+-OCOCH_2Cl$, base peak).

Preparation of Delsoline (22) A mixture of delcosine (**13**: 50.8 mg), dioxane (5 ml), sodium hydride (NaH; 54.1 mg) and methyl iodide (0.14 ml) was kept at 0 °C for 1 h and then at room temperature for 16 h. The solution was filtrated, and then purification by column chromatography on silica gel (3% MeOH–$CHCl_3$ saturated with 28% ammonia) afforded delsoline (28.6 mg, 54%). mp 216—219 °C. IR (KBr) cm^{-1}: 3458, 1102. ^{1}H-NMR δ: 1.09 (3H, t, J=7.0 Hz, N-CH_2CH_3), 3.33, 3.41 (each 3H, s, OCH_3), 3.36 (6H, s, OCH_3), 4.06 (1H, s, 6α-H). HR-EI-MS m/z: 467.2854 (Calcd for $C_{25}H_{41}NO_7$: 467.2882). EI-MS m/z: 467 (M^+), 452 (M^+-CH_3, base peak), 450 (M^+-OH), 436 (M^+-OCH_3).

Preparation of Delphatine (23) A mixture of browniine (**20**: 57.5 mg), 1,2-dimethoxyethane (6 ml), NaH (31.0 mg) and methyl iodide (0.077 ml) was kept at 0 °C for 1 h and then at room temperature for 16 h. The solution was filtrated, and then purification by column chromatography on silica gel ($CHCl_3$ saturated with 28% ammonia) afforded delphatine (31.2 mg, 51%). Amorphous. IR (KBr) cm^{-1}: 3456, 1091. ^{1}H-NMR δ: 1.04 (3H, t, J=7.0 Hz, N-CH_2CH_3), 3.24, 3.30, 3.33, 3.40, 3.41 (each 3H, s, OCH_3), 4.08 (1H, s,

6α-H). HR-EI-MS *m/z*: 481.3014 (Calcd for $C_{26}H_{43}NO_7$: 481.3037). EI-MS *m/z*: 481 (M^+), 466 (M^+-CH_3), 464 (M^+-OH), 450 (M^+-OCH_3, base peak).

Preparation of 7,14-Di-*O*-methyldelcosine (24) and 7,14-Di-*O*-methyl-18-methoxygadesine (28) A mixture of delsoline (**22**: 15.5 mg), 1,2-dimethoxyethane (2 ml), NaH (16.4 mg) and methyl iodide (0.04 ml) was kept at 0 °C for 1 h and then at room temperature for 20 h. The solution was filtrated, and then purification by column chromatography on silica gel (20% hexane–ether saturated with 28% ammonia) afforded 7,14-di-*O*-methyldelcosine (**24**, 8.8 mg, 55%) and 7,14-di-*O*-methyl-18-methoxygadesine (**28**, 2.0 mg, 13%).

24: mp 208—209.5 °C. IR (KBr) cm^{-1}: 3486, 1114, 1094. ^{1}H-NMR δ: 1.06 (3H, t, *J*=7.0 Hz, *N*-CH_2CH_3), 3.36, 3.37, 3.39, 3.41, 3.59 (each 3H, s, OCH_3), 4.16 (1H, s, 6α-H). HR-EI-MS *m/z*: 481.3015 (Calcd for $C_{26}H_{43}NO_7$: 481.3037). EI-MS *m/z*: 481 (M^+), 466 (M^+-CH_3, base peak), 464 (M^+-OH).

28: Amorphous. IR ($CHCl_3$) cm^{-1}: 3452, 1135, 1104. ^{1}H-NMR δ: 0.99 (3H, t, *J*=7.0 Hz, *N*-CH_2CH_3), 3.34, 3.35, 3.40, 3.43, 3.67 (each 3H, s, OCH_3), 3.85 (1H, s, 19-H), 4.10 (1H, s, 6α-H). HR-EI-MS *m/z*: 479.2881 (Calcd for $C_{26}H_{41}NO_7$: 479.2881). EI-MS *m/z*: 479 (M^+), 464 (M^+-CH_3, base peak).

Preparation of 1,7,8,14-Tetra-*O*-methyldelcosine (25) and 7,8,14-Tri-*O*-methyl-18-methoxygadesine (29) A mixture of delcosine (**13**: 81.0 mg), 1,2-dimethoxyethane (8 ml), NaH (87.5 mg) and methyl iodide (0.24 ml) was kept at 0 °C for 1 h and then at room temperature for 2 d. The solution was filtrated, and then purification by column chromatography on silica gel (30% hexane–ether saturated with 28% ammonia) afforded 1,7,8,14-tetra-*O*-methyldelcosine (**25**, 44.2 mg, 44%) and 7,8,14-tri-*O*-methyl-18-methoxygadesine (**29**, 8.2 mg, 9%).

25: Amorphous. IR ($CHCl_3$) cm^{-1}: 1098. ^{1}H-NMR δ: 1.00 (3H, t, *J*=7.0 Hz, *N*-CH_2CH_3), 3.21, 3.34, 3.36, 3.43, 3.59 (each 3H, s, OCH_3), 3.37 (6H, s, OCH_3), 4.00 (1H, s, 6α-H). HR-EI-MS *m/z*: 509.3366 (Calcd for $C_{28}H_{47}NO_7$: 509.3350). EI-MS *m/z*: 509 (M^+), 494 (M^+-CH_3), 478 (M^+-OCH_3, base peak).

29: Amorphous. IR ($CHCl_3$) cm^{-1}: 1135, 1104. ^{1}H-NMR δ: 1.03 (3H, t, *J*=7.0 Hz, *N*-CH_2CH_3), 3.37, 3.39, 3.41, 3.68 (each 3H, s, OCH_3), 3.38 (6H, s, OCH_3), 3.95 (1H, s, 19-H) , 4.19 (1H, s, 6α-H). HR-EI-MS *m/z*: 493.3042 (Calcd for $C_{27}H_{43}NO_7$: 493.3038). EI-MS *m/z*: 493 (M^+), 478 (M^+-CH_3, base peak), 446.

Preparation of 14-Acetyldelbrunine (26) A mixture of 14-acetyldelcosine (**15**: 200 mg), water (0.08 ml), paraformaldehyde (400 mg), acetic acid (8 ml) and conc-H_2SO_4 (0.2 ml) was kept at 100 °C for 25 h. Water was added. The usual work-up and purification by column chromatography on silica gel (5% hexane–ether saturated with 28% ammonia) afforded 14-acetyldelbrunine (24.7 mg, 55%) and the starting material (68.1 mg). Amorphous. IR (KBr) cm^{-1}: 3454, 1740, 1247, 1094. ^{1}H-NMR δ: 1.11 (3H, t, *J*=7.0 Hz, *N*-CH_2CH_3), 2.06 (3H, s, $OCOCH_3$), 3.29 (6H, s, OCH_3), 3.38 (3H, s, OCH_3), 3.80 (1H, s, 6α-H), 4.86 (1H, t, *J*=4.6 Hz, 14β-H), 5.05, 5.10 (each 1H, d, *J*=1.0 Hz, OCH_2O). HR-EI-MS *m/z*: 507.2836 (Calcd for $C_{27}H_{41}NO_8$: 507.2830). EI-MS *m/z*: 507 (M^+), 492 (M^+-CH_3), 490 (M^+-OH), 474 (base peak).

Preparation of 1,14-Diacetyldelbrunine (27) A mixture of 14-acetyldelbrunine (**26**: 17.2 mg), pyridine (0.5 ml) and acetic anhydride (0.5 ml) was kept at 70 °C for 15 h. The usual work-up and purification by column chromatography on silica gel (10% hexane–ether saturated with 28% ammonia) afforded 1,14-diacetyldelbrunine (8.8 mg, 47%). Amorphous. IR ($CHCl_3$) cm^{-1}: 1731, 1251, 1133. ^{1}H-NMR δ: 1.09 (3H, t, *J*=7.0 Hz, *N*-CH_2CH_3), 2.05, 2.06 (each 3H, s, $OCOCH_3$), 3.27, 3.29, 3.35 (each 3H, s, OCH_3), 3.73 (1H, s, 6α-H), 4.75 (1H, t, *J*=9.2 Hz, 1β-H), 4.79 (1H, t, *J*=4.6 Hz, 14β-H), 5.08 (2H, s, OCH_2O). HR-EI-MS *m/z*: 549.2934 (Calcd for $C_{29}H_{43}NO_9$: 549.2935). EI-MS *m/z*: 549 (M^+), 534 (M^+-CH_3), 518 (M^+-OCH_3), 504, 490 ($M^+-OCOCH_3$, base peak).

HPLC–APCI-MS Conditions A model M-2000 mass spectrometer (Hitachi, Tokyo, Japan) through an APCI interface was used as the HPLC–APCI-MS system. The HPLC system consisted of a Model L-6200 chromatographic pump (Hitachi, Tokyo, Japan) and a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector with a 20-μl loop. Direct injection analysis was performed without HPLC columns. The eluent was transferred at the flow rate of 0.8 ml/min directly to the APCI interface. The solvent consisted of 0.05 M ammonium acetate–acetonitrile–tetrahydrofuran (60 : 25 : 15, v/v). The mass spectrometer interface consisted of nebulizing and vaporizing units. The temperature of the nebulizer was set to 280 °C to give optimum abundance of the target ions. The desolvation temperature was set to 400 °C. The vaporized sample and solvent molecules were passed into the ion source of the APCI-MS system. The solvent molecules were ionized by corona discharge, and then the sample molecules and ionized solvent molecules underwent ion-molecule reactions.

Deuterium Exchange Studies Sample solution dissolved in deuterated methanol. The solvent consisted of 0.05 M ammonium acetate in D_2O–acetonitrile–tetrahydrofuran (60 : 25 : 15, v/v). The conditions were the same as those stated above.

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