

Application of Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry to the Differentiation of Stereoisomeric Diterpenoid 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 seven stereoisomeric diterpenoid alkaloids at position 1 or 12. 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 the major fragment ion, corresponding to the $[M+H-H_2O]^+$ ion or the $[M+H-CH_3COOH]^+$ ion, and comparison of the APCI spectra showed that the abundance of fragment ions was significantly higher for C-1 β -form alkaloids than for C-1 α -form alkaloids, and for C-12 β -form alkaloids than for C-12 α -form alkaloids. The characteristic fragment ions were formed due to the loss of an acetic acid or a water molecule at position 12. 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 CH_3COOD or D_2O from the precursor ion gave the fragment ion. This result indicated that the proton of protonation was included in the leaving acetic acid and water molecule, respectively. The peak intensity ratio for $R=[M+H]^+/[M+H-H_2O]^+ + [M+H-CH_3COOH]^+$ manifested the stereochemical differentiation of alkaloids at position 1 or 12.

Key words atmospheric pressure chemical ionization; diterpenoid alkaloid; 12-*epi*-lucidusculine 12,15-diacetyl-1-*epi*-lucidusculine; lucidusculine; mass spectrometry

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, 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, 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 alkaloids⁶⁾ and eburnane-type alkaloids,⁷⁾ and indoloquinolizine⁸⁾ and indole⁹⁾ alkaloids by electrospray ionization have appeared in recent years.

Various *Aconitum* (Ranunculaceae) plants produce norditerpenoid and diterpenoid alkaloids.¹⁰⁾ Structure studies of diterpenoid alkaloids involving mass spectrometry have been carried out essentially by electron impact (EI) ionization and conventional analysis.¹¹⁻¹⁷⁾ EI spectra of *Aconitum* alkaloids showed a molecular ion together with many fragment ions. In general, atmospheric pressure chemical ionization (APCI) has a wider range of applications for elucidation of structures of organic compounds and generates protonated molecules ($[M+H]^+$) with remarkable ease and efficiency. We applied an APCI-MS method to the analysis of *Aconitum* alkaloids,¹⁸⁻²⁰⁾ and the APCI mass spectra of *Aconitum* alkaloids showed predominantly the $[M+H]^+$ ion together with major

fragment ions. We previously reported that high-performance liquid chromatography (HPLC)-APCI-MS was useful for the structural elucidation of six stereoisomeric norditerpenoid neoline-type alkaloids at position 6,²¹⁾ corresponding to neoline, 14-acetylneoline, chasmanine, subcusine, subcumine and 6-*epi*-chasmanine; and for the structural elucidation of ten stereoisomeric norditerpenoid neoline-type alkaloids, corresponding to neoline, 14-acetylneoline, 8-acetyl-14-benzoylneoline, 1-*epi*-neoline, 14-acetyl-1-*epi*-neoline and 8-acetyl-14-benzoyl-1-*epi*-neoline; and delcosine-type alkaloids, corresponding to delcosine, 14-acetyldelcosine, 1-*epi*-delcosine and 14-acetyl-1-*epi*-delcosine, at position 1.²²⁾ Comparison of APCI spectra of these alkaloids showed that the abundance of fragment ions was significantly higher for C-6 β -form alkaloids, corresponding to subcusine, subcumine and 6-*epi*-chasmanine, than for C-6 α -form alkaloids, corresponding to neoline, 14-acetylneoline and chasmanine, and for C-1 β -form alkaloids, corresponding to 1-*epi*-neoline, 14-acetyl-1-*epi*-neoline, 8-acetyl-14-benzoyl-1-*epi*-neoline, 1-*epi*-delcosine and 14-acetyl-1-*epi*-delcosine, than for C-1 α -form alkaloids, corresponding to neoline, 14-acetylneoline, 8-acetyl-14-benzoylneoline, delcosine and 14-acetyldelcosine.

In the present paper, we report the results of an HPLC-APCI-MS study of diterpenoid alkaloids, 12,15-diacetyl-lucidusculine (1),²³⁾ 12,15-diacetyl-1-*epi*-lucidusculine (2), 1,12,15-triacetyl-lucidusculine (3),²³⁾ 1,12,15-triacetyl-1-*epi*-lucidusculine (4), lucidusculine (5),²³⁾ 12-*epi*-lucidusculine (6),²⁴⁾ and 1,12,15-triacetyl-12-*epi*-lucidusculine (7), for resolving structural problems related to the differentiation of stereoisomers. These alkaloids differ in the stereochemistry at position 1 or 12, alkaloids 2, 4, 6 and 7 being characterized by a β -form and alkaloids 1, 3 and 5 by an α -form.

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

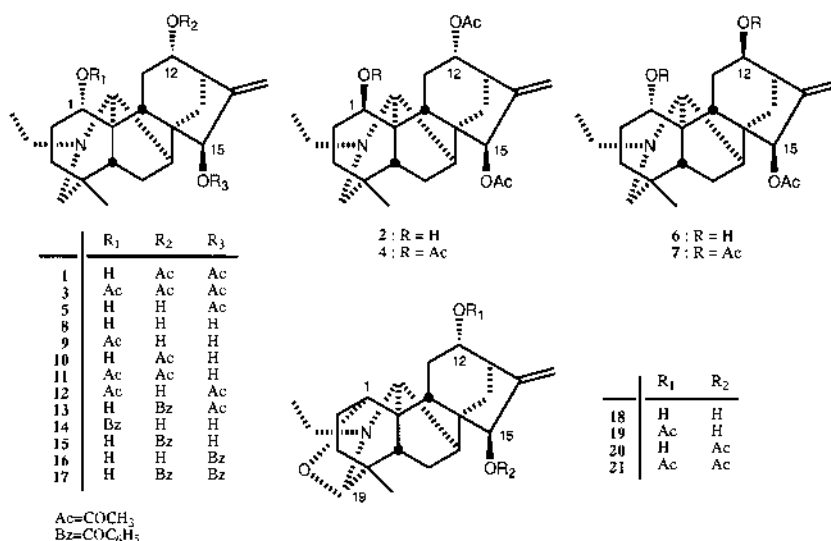


Chart 1

Results and Discussion

We applied an HPLC-APCI-MS method to the investigation of stereochemical differentiation of diterpenoid alkaloids **1**–**4**, which contain a C-1 α - or β -form, and alkaloids **5**–**7**, which contain a C-12 α - or β -form. At first, the APCI mass spectra obtained by HPLC-APCI-MS of alkaloids **1**–**4** were examined (Fig. 1). When APCI mass spectra of alkaloids **1**–**4** were recorded 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 **1** and **2** showed a very intense ion peak at m/z 444, corresponding to the $[M+H]^+$ ion, and fragment ions at m/z 426 and 384, which were formed due to the loss of a water and an acetic acid molecule, respectively. Drift collision-induced dissociation (drift-CID) analysis^{25,26} of **1** and **2** (Fig. 2) clearly showed that the abundance of fragment ions increased. Comparison of the two spectra showed a remarkable increase in the relative abundance of the ion peaks at m/z 426 and 384 in the case of **2**. The spectra of **3** and **4** revealed a strong $[M+H]^+$ ion at m/z 486 and a fragment ion at m/z 426, which was formed due to the loss of an acetic acid molecule (Table 1). Drift-CID analysis of **3** and **4** showed that the abundance of the fragment ion increased. Comparison of these spectra indicated that the abundance of the m/z 426 ion for **4** was greater than that for **3**. These results indicated that the relative abundance of the characteristic fragment ions for C-1 β -form alkaloids, which contain **2** and **4**, was greater than that for C-1 α -form alkaloids, which contain **1** and **3**.

APCI mass spectra of alkaloids **1**–**4** showed characteristic fragment ions, which were formed due to the loss of a water and an acetic acid molecule. Therefore, evidence of fragmentation mechanisms depending on the precursor ion was provided by the study of fragmentation behaviors of luciculine (**8**), 1-acetylliculine (**9**), 12-acetylliculine (**10**), 1,12-diacetylliculine (**11**), 1,15-diacetylliculine (**12**), 12-benzoyllucidusculine (**13**), 1-benzoyllucidusculine (**14**), 12-benzoyllucidusculine (**15**), 15-benzoyllucidusculine (**16**), 12,15-dibenzoyllucidusculine (**17**), dehydrolucidusculine (**18**), 12-acetyldehydrolucidusculine (**19**), dehydrolucidusculine (**20**) and 12-

acetyldehydrolucidusculine (**21**) (Table 1). The spectra of **10**, **11**, **19** and **21**, which contain an acetyl group at C-12, showed a fragment ion peak corresponding to the $[M+H-CH_3COOH]^+$ ion. Similarly, the spectra of **15** and **17**, which contain a benzoyl group at C-12, showed a fragment ion peak corresponding to the $[M+H-C_6H_5COOH]^+$ ion. The spectra of **13**, which contains a benzoyl group at C-12, showed a major fragment ion peak at m/z 384 corresponding to the $[M+H-C_6H_5COOH]^+$ ion. The spectra of **8**, **9**, **12**, **14**, **16**, **18** and **20**, which contain an OH group at C-12, showed a fragment ion peak corresponding to the $[M+H-H_2O]^+$ ion. These results indicated that the characteristic fragment ions were formed due to the loss of a water, an acetic acid or a benzoic acid molecule at position 12.

In order to compare the stabilities of the $[M+H]^+$ ions of **1** and **2** towards the fragmentation processes, we proceeded to the study of energy dependence of the ion abundances. This experiment was carried out in a simple manner: the drift voltage was increased by 15 V every three scans in the range 0–240 V, and the ion peak intensity values were calculated by averaging the signals measured in each set of three scans.²¹⁾

The results for the ions at m/z 426 and 384 are shown in Fig. 3. The ion appearing at m/z 384 was of very similar abundance for the two isomers. In contrast, the conditions for the formation of the ion at m/z 426 appear to be closely related to the stereochemistry of the compounds under investigation and were sensitive to energy variation. The curve of the ion at m/z 426 was completely different. In the case of **2**, the loss of a water molecule remained the major fragmentation pathway over the whole energy range. The situation is completely reversed for **1**, in favor of the m/z 384 fragment ion, whose abundance reaches a maximum around 140 V. For comparison, energy dependence of the fragmentation pathway in relation to the stereochemistry of the protonated molecules was examined (Fig. 4). The formation of the ion at m/z 426 clearly required a greater amount of energy in the case of **1** than in the case of **2**. The two curves obtained were separated from 40–50 V, evidently corresponding to the stability difference between the $[M+H]^+$ ions of **1** and **2** towards this

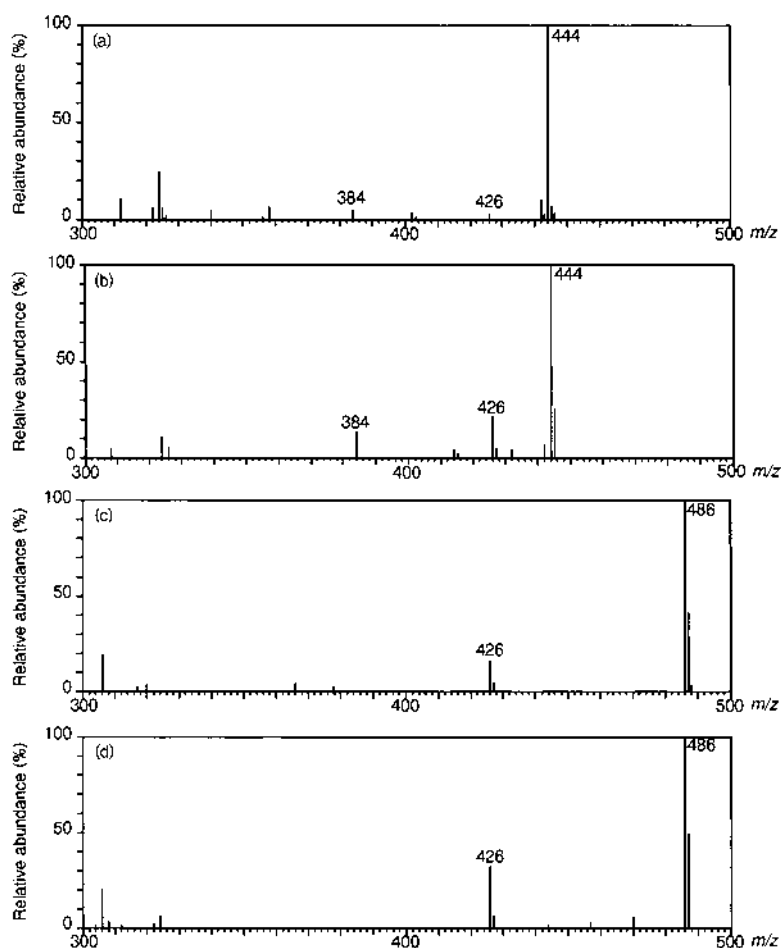


Fig. 1. APCI Mass Spectra of Alkaloids

(a) 12,15-Diacetyluciculine (**1**), (b) 12,15-diacetyl-1-*epi*-luciculine (**2**), (c) 1,12,15-triacetyluciculine (**3**), (d) 1,12,15-triacetyl-1-*epi*-luciculine (**4**). The drift voltage between the first and the second electrodes was 140 V.

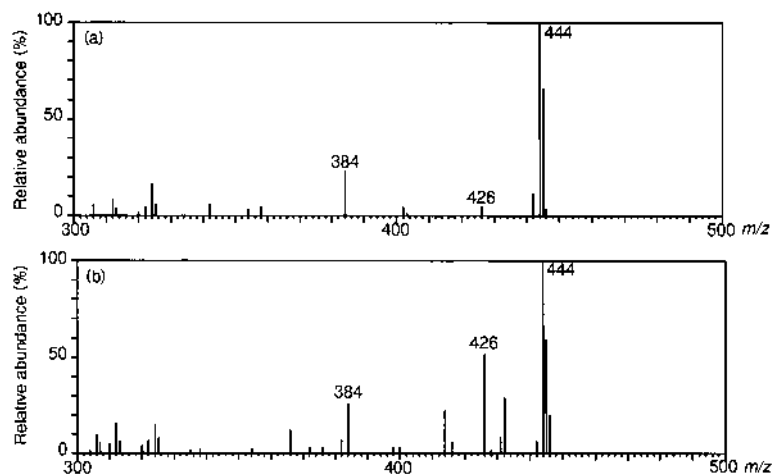


Fig. 2. APCI Mass Spectra of Alkaloids 12,15-Diacetyluciculine (**1**; a) and 12,15-Diacetyl-1-*epi*-luciculine (**2**; b)

The drift voltage between the first and the second electrodes was 160 V.

fragmentation pathway. Also, the formation of the ion at m/z 384 appeared in the decreasing parts of curves that were identical for the two compounds. The two curves obtained were separated from about 40 V, evidently corresponding to the stability difference between the $[M+H]^+$ ions **1** and **2** towards this fragmentation pathway. These results indicated

that the formation of the fragment ions at m/z 426 and 384 clearly required a greater amount of energy in the case of **1**, which contains a C-1 α -form, than in the case of **2**, which contains a C-1 β -form.

The fragmentation mechanisms depending on the stereochemistry of the precursor ion could be discerned by record-

Table 1. m/z and Relative Abundances (%) of the Mass Spectral Fragments of Diterpenoid Alkaloids

	$[M+H]^+$	$[M+H-H_2O]^+$	$[M+H-RCOOH]^+$	$[M-d_n+D]^+$	$[M-d_n+D-D_2O]^+$	$[M-d_n+D-CH_3COOD]^+$
1	444 (100%)	426 (3%)	384 (6%)	446 (100%)	426 (6%)	385 (1%)
2	444 (100%)	426 (22%)	384 (12%)	446 (100%)	426 (14%)	385 (4%)
3	486 (100%)	—	426 (15%)	487 (100%)	—	426 (32%)
4	486 (100%)	—	426 (32%)	—	—	—
5	402 (100%)	384 (10%)	342 (7%)	405 (100%)	385 (2%)	344 (4%)
6	402 (100%)	384 (12%)	342 (24%)	405 (100%)	385 (8%)	344 (13%)
7	486 (100%)	—	426 (28%)	—	—	—
8	360 (100%)	342 (3%)	—	364 (100%)	344 (6%)	—
9	402 (100%)	384 (4%)	342 (29%)	—	—	—
10	402 (100%)	—	342 (27%)	—	—	—
11	444 (68%)	—	384 (14%)	—	—	—
12	444 (100%)	426 (3%)	384 (45%)	—	—	—
13	506 (100%)	488 (9%)	446 (10%), 384 (68%)	—	—	—
14	464 (100%)	446 (3%)	342 (53%)	—	—	—
15	464 (100%)	—	342 (23%)	—	—	—
16	464 (100%)	446 (8%)	342 (9%)	—	—	—
17	568 (100%)	—	446 (20%)	—	—	—
18	358 (100%)	340 (39%)	—	—	—	—
19	400 (100%)	—	340 (11%)	—	—	—
20	400 (100%)	382 (1%)	340 (18%)	—	—	—
21	442 (100%)	—	382 (2%)	—	—	—

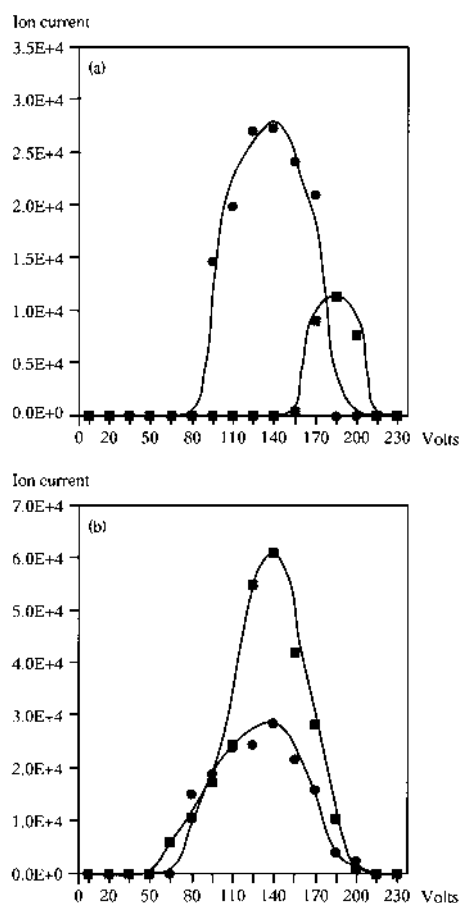


Fig. 3. Ion Currents of the Fragment Ions at m/z 426 (■) and m/z 384 (●) Arising from Alkaloids 12,15-Diacetyluciculine (1; a) and 12,15-Diacetyl-1-*epi*-luciculine (2; b) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

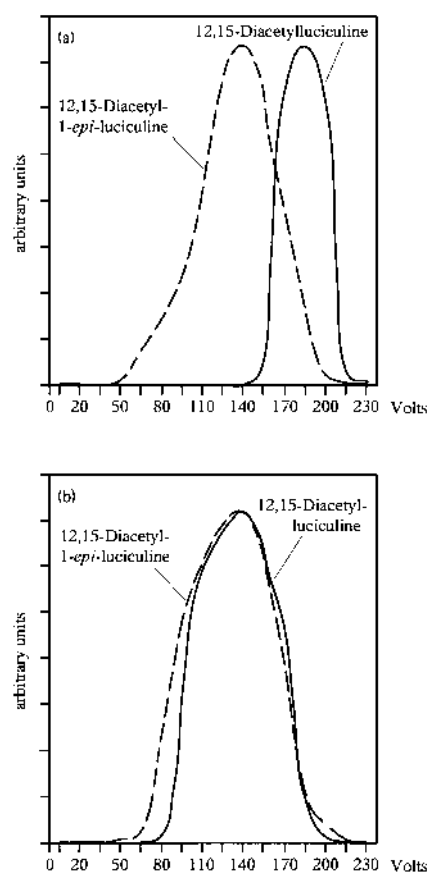


Fig. 4. Ion Currents of the Fragment Ions at m/z 426 (a) and m/z 384 (b) Arising from Alkaloids 12,15-Diacetyluciculine (1, Solid Line) and 12,15-Diacetyl-1-*epi*-luciculine (2, Dashed Line) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

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

ing the spectra of alkaloids 1 and 2 in a deuterated solvent system of 0.05 M ammonium acetate in D_2O –acetonitrile–tetrahydrofuran. These spectra showed a major ion peak at

m/z 446 corresponding to the $[M-d+D]^+$ ions formed by deuterium exchange of hydroxyl hydrogen and addition of D^+ on the molecules (Fig. 5). Loss of D_2O and CH_3COOD

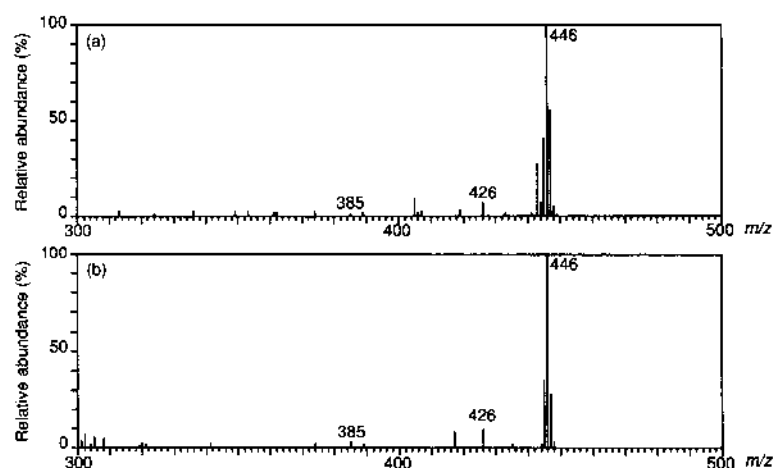


Fig. 5. APCI Mass Spectra of Alkaloids Recorded by Solvent System of 0.05 M Ammonium Acetate in D_2O -Acetonitrile-Tetrahydrofuran (a) 12,15-Diacetyl-luciculine (1), (b) 12,15-diacetyl-1-*epi*-luciculine (2). The drift voltage between the first and the second electrodes was 140 V.

from this precursor ion gave the fragment ions at m/z 426 and 385, respectively (Table 1). This result indicated that the proton of protonation was included in the leaving water molecule and acetic acid molecule, irrespective of the stereochemistry at position 1. As we have reported,²¹⁾ the site of protonation in the norditerpenoid alkaloids was at a nitrogen atom, and proton chelation occurred between the amino group and the C-1 hydroxyl group. Similarly, the site of protonation in the diterpenoid alkaloids was at a nitrogen atom, and proton chelation occurred between the amino group and the C-1 hydroxyl group (Chart 2a). The fragment ion at m/z 384 was formed so that the proton of protonation could be transferred to the C-12 acetyl group and fragmented as an acetic acid molecule.

The stabilization of the $[M+H]^+$ ion of **1** relative to the stereoisomer **2** towards the loss of a water molecule is thought to be due to an intramolecular H-bonded system (Chart 2a). Therefore, in the case of **2**, it was presumed that proton chelation cannot occur (Chart 2b), and proton transfer occurred more easily in alkaloid **2** than in **1**, and that the formation of the ion at m/z 384 was easier in the case of **2** than in the case of **1**. Also, the formation of the ion at m/z 426 was due to the elimination of a water molecule at position 1. In the case of **2**, it was presumed that proton chelation cannot occur, and the axial positions of the corresponding substituents (C-1 β hydroxyl group and the hydrogen at C-3, C-5 and C-9) suggested a 1,3-diaxial interaction effect of the fragmentation (Chart 2b). In fact, the abundance of the fragment ion m/z 426 increased more in the case of **2** than in the case of **1**.

We considered the peak intensity ratio for alkaloids **1**–**4** to be $R = [M+H]^+ / [M+H-H_2O]^+ + [M+H-CH_3COOH]^+$. The R values of alkaloids **1** and **3**, which contain a C-1 α -hydroxyl group, were 6.7–11.1, respectively, whereas those of alkaloids **2** and **4**, which contain a C-1 β -hydroxyl group, were 2.9–3.1, respectively. These results indicated that the R value showed stereochemical differentiation of alkaloids at position 1.

Next, the APCI mass spectra of alkaloids **5** and **6** exhibited at m/z 402 a highly intense ion peak corresponding to the $[M+H]^+$ ion (Fig. 6). The spectra of **5** and **6** showed fragment ions at m/z 384 and 342, and the fragment ions were

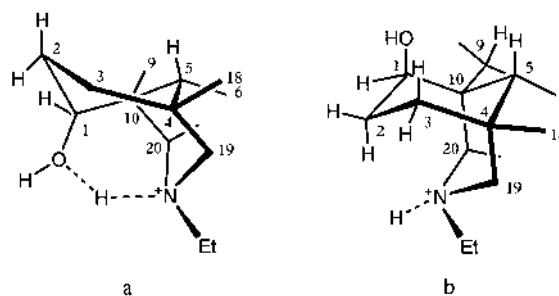


Chart 2

formed from the $[M+H]^+$ ion due to the loss of a water and an acetic acid molecule, respectively (Table 1). The drift-CID spectra of **5** and **6** indicated that the abundance of fragment ions at m/z 384 and 342 increased, and the abundance of the ion at m/z 342 was greater for **6**, which contains a C-1 β -form, than for its epimer **5**, which contains a C-1 α -form (Fig. 7).

In order to compare the stabilities of the $[M+H]^+$ ion of **5** and **6** towards the fragmentation processes, we proceeded to the study of energy dependence of the ion abundances. The results for the ions at m/z 342 and 384 are shown in Fig. 8. The ion appearing at m/z 384 was of very similar abundance for the two isomers. In contrast, the conditions for the formation of the ion at m/z 342 appear to be closely related to the stereochemistry of the compounds under investigation and were sensitive to energy variation. The curves of the ions at m/z 342 and 384 were completely different. In the case of **6**, the loss of an acetic acid molecule remained the major fragmentation pathway over the whole energy range. The situation is completely reversed for **5**, in favor of the m/z 384 fragment ion, whose abundance reaches a maximum at around 140 V. The top of the m/z 342 ion curves corresponding to **6** seemed to be located at voltage values lower than **5**.

For comparison, energy dependence of the fragmentation pathway in relation to the stereochemistry of the protonated molecules was examined (Fig. 9). In the m/z 384 ion curves, even though the increasing part of the curve was shifted to higher energies at about 70 V in the case of **5**, it was noteworthy that the m/z 384 ion current became strong in the case

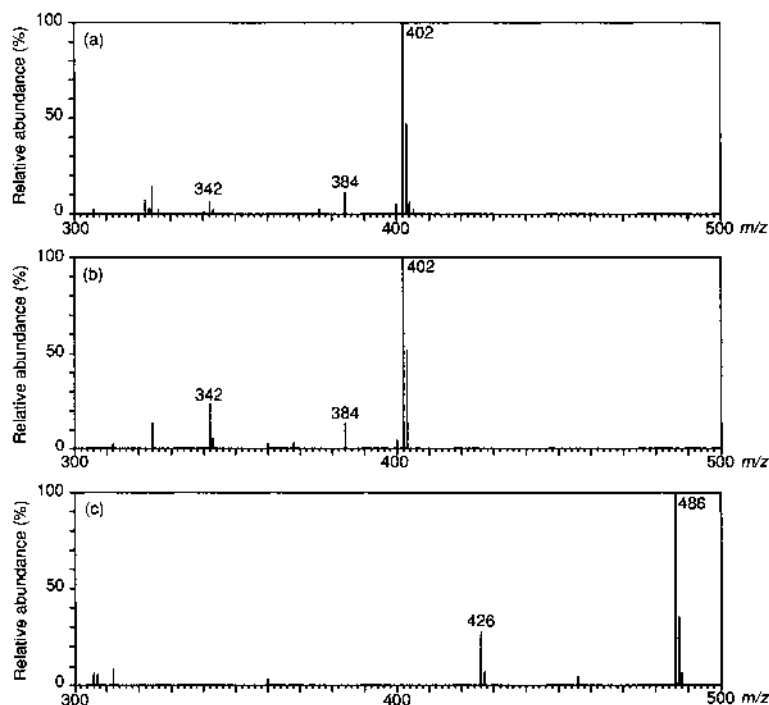


Fig. 6. APCI Mass Spectra of Alkaloids

(a) Lucidusculine (**5**), (b) 12-*epi*-lucidusculine (**6**), (c) 1,12,15-triacetyl-12-*epi*-luciculine (**7**). The drift voltage between the first and the second electrodes was 140 V.

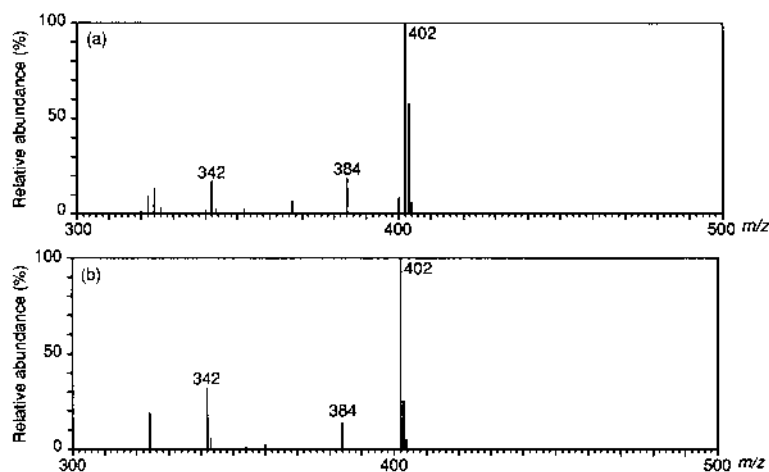


Fig. 7. APCI Mass Spectra of Alkaloids Lucidusculine (**5**; a) and 12-*Epi*-lucidusculine (**6**; b)

The drift voltage between the first and the second electrodes was 160 V.

of **6**. In contrast, the formation of the ion at m/z 342 clearly required a greater amount of energy in the case of **5** than in the case of **6**. The two curves obtained were separated by 65–70 V, evidently corresponding to the stability difference between the $[M+H]^+$ ions **5** and **6** towards this fragmentation pathway. These results indicated that the formation of the fragment ions at m/z 342 and 384 clearly required a greater amount of energy in the case of **5**, which contains a C-12 α -form, than the case of **6**, which contains a C-12 β -form.

The spectrum of alkaloid **7** revealed a strong $[M+H]^+$ ion at m/z 486. A fragment ion was observed at m/z 426. This ion could be explained by the loss of an acetic acid molecule from the $[M+H]^+$ ion. Drift-CID analysis of **7** showed that the abundance of the fragment ion increased. Comparison of

7 and **3** spectra indicated that the abundance of the m/z 426 ion for **7** was greater than that for **3**.

In the spectra of **3** and **5**–**7**, the abundance of the fragment ion was greater for C-12 β -form alkaloids, which correspond to **6** and **7**, than for its epimer C-12 α -form alkaloids, which correspond to **3** and **5**. Comparison of spectra of **5** and **6** showed a remarkable increase in the relative abundance of the fragment ion peaks at m/z 342 and 384 in the case of **6**. Similarly, the m/z 426 ion abundance was higher for **7** than for **3**. These results suggested that in the case of alkaloids **6** and **7**, it was caused by the steric interactions of the substituents (C-9 hydrogen, C-12 β -hydroxyl, C-15 β -acetyl group) (Chart 3).

The fragmentation mechanisms depending on the stereochemistry of the precursor ion could be discerned by record-

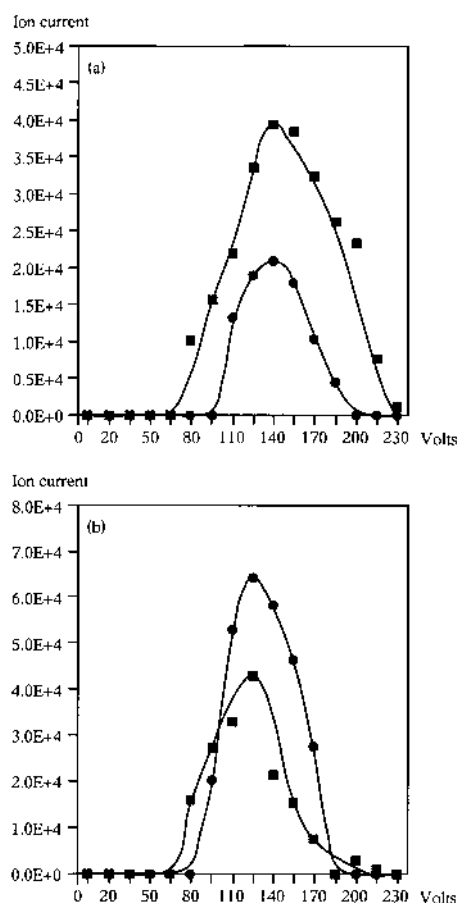


Fig. 8. Ion Currents of the Fragment Ions at m/z 384 (■) and m/z 342 (●) Arising from Alkaloids Lucidusculine (**5**; a) and 12-*Epi*-lucidusculine (**6**; b) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

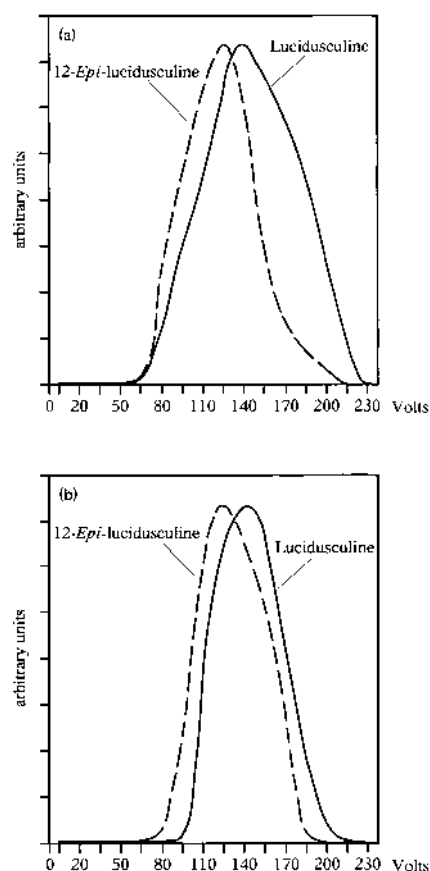


Fig. 9. Ion Currents of the Fragment Ions at m/z 384 (a) and m/z 342 (b) Arising from Alkaloids Lucidusculine (**5**, Solid Line) and 12-*Epi*-lucidusculine (**6**, Dashed Line) as a Function of the Drift Voltage between the First and the Second Electrodes of the APCI Ion Source

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

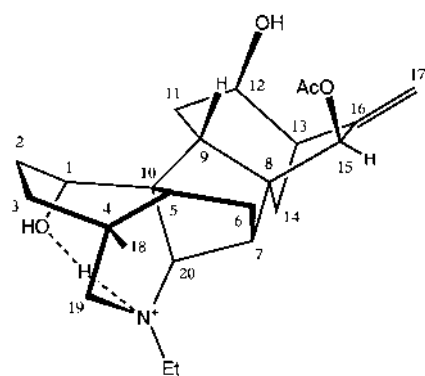


Chart 3

ing the spectra of alkaloids **5** and **6** in a deuterated solvent system of 0.05 M ammonium acetate in D_2O -acetonitrile-tetrahydrofuran. These spectra showed a major ion peak at m/z 405 corresponding to the $[M-d_2+D]^+$ ions formed by deuterium exchange of hydroxyl hydrogen and addition of D^+ on the molecules (Fig. 10). Loss of CH_3COOD and D_2O from this precursor ion gave the fragment ions at m/z 344 and 385, respectively. This result indicated that the proton of protonation was included in the leaving water or acetic acid molecule, irrespective of the stereochemistry at position 12.

We considered the peak intensity ratio for alkaloids **3** and

5—**7** to be $R = [M+H]^+ / [M+H-H_2O]^+ + [M+H-CH_3COOH]^+$. The R values of alkaloids **3** and **5**, which contain a C-12 α -form, were 5.9—6.7, whereas those of alkaloids **6** and **7**, which contain a C-12 β -form, were 2.8—3.6. These results indicated that the R value showed stereochemical differentiation of alkaloids at position 12.

In conclusion, the APCI spectra of diterpenoid alkaloids were remarkably simple and very similar with respect to characteristic fragments. APCI-MS was successfully applied to seven stereoisomeric diterpenoid alkaloids at position 1 or 12. APCI-MS was useful for the structural elucidation of seven stereoisomeric diterpenoid alkaloids. Comparison of the APCI spectra of these alkaloids showed that the abundance of fragment ions was significantly higher for C-1 β -form alkaloids than for C-12 α -form alkaloids, and for C-12 β -form alkaloids than for C-12 α -form alkaloids. The peak intensity ratio for $R = [M+H]^+ / [M+H-H_2O]^+ + [M+H-CH_3COOH]^+$ manifested the stereochemical differentiation of alkaloids at position 1 or 12. We are considering a future APCI-MS study of diterpenoid alkaloids **8**—**21** to resolve the structural problems related to the differentiation of stereoisomers. APCI-MS experiments can be useful for detecting subtle structural characteristics of organic molecules, including stereochemical differentiation.

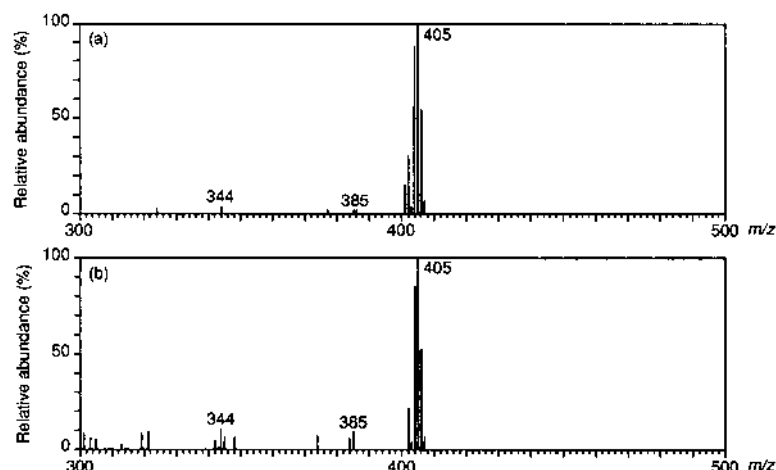


Fig. 10. APCI Mass Spectra of Alkaloids Recorded by Solvent System of 0.05 M Ammonium Acetate in D_2O -Acetonitrile-Tetrahydrofuran

(a) Lucidusculine (5), (b) 12-*epi*-lucidusculine (6). The drift voltage between the first and the second electrodes was 140 V.

Experimental

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

Materials 12,15-Diacetyluciculine (1), lucidusculine (5), luciculine (8), 1-acetyluciculine (9), dehydro-lucidusculine (20) and 12-acetyldehydro-lucidusculine (21) were purified from *Aconitum yesoense* var. *macrocysoense* roots and identified as described previously.²³ 1,12,15-Triacetyluciculine (3),²³ 12-acetyluciculine (10),²⁷ dehydro-lucidusculine (18)²³ and 12-acetyldehydro-lucidusculine (19)²⁷ were prepared as described previously. 12,15-Diacetyl-1-*epi*-luciculine (2), 1,12,15-triacetyl-1-*epi*-luciculine (4), 12-*epi*-lucidusculine (6),²⁴ 1,12,15-triacetyl-12-*epi*-luciculine (7), 1,12-diacetyluciculine (11), 1,15-diacetyluciculine (12),²⁸ 12-benzoyllucidusculine (13), 1-benzoylluciculine (14), 12-benzoylluciculine (15), 15-benzoylluciculine (16) and 12,15-dibenzoylluciculine (17) were synthesized from 12,15-diacetyluciculine (2), dehydro-lucidusculine (20), 1-acetyluciculine (9), 1,12,15-triacetyluciculine (3), lucidusculine (5) and luciculine (8), respectively. Ammonium acetate of reagent grade was purchased from Kanto Chemicals (Tokyo, Japan), and acetonitrile and tetrahydrofuran of HPLC grade were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of 12,15-Diacetyl-1-*epi*-luciculine (2) 1) Oxidation of 12,15-Diacetyluciculine: A solution of 12,15-diacetyluciculine (1: 30.6 mg) in dichloromethane (9 ml) was mixed with pyridinium dichromate (PDC; 78 mg). The mixture was stirred at 0 °C for 4 h 50 min. The reaction mixture was passed through a short column of florisil and then purified by column chromatography on silica gel (5–1% hexane-ether saturated with 28% ammonia) to give 12,15-diacetyl-1-dehydro-lucidusculine (19.2 mg, 63%) and the starting material (15.8 mg). Amorphous. IR (KBr) cm^{-1} : 1742, 1696, 1232, 1164, 907. 1H -NMR ($CDCl_3$) δ : 0.80 (3H, s, 18- CH_3), 1.08 (3H, t, $J=7.0$ Hz, $N-CH_2CH_3$), 2.02, 2.11 (each 3H, s, $OCOCH_3$), 4.63 (1H, dd, $J=7.0, 9.7$ Hz, 12 β -H), 4.98, 5.29 (each 1H, s, 17- H_2), 5.49 (1H, s, 15 α -H). HR-EI-MS m/z : 441.2491 (Calcd for $C_{26}H_{35}NO_5$: 441.2514). EI-MS m/z : 441 (M^+), 398 ($M^+ - COCH_3$, base peak), 382 ($M^+ - OCOCH_3$).

2) Reduction of 12,15-Diacetyl-1-dehydro-lucidusculine: 12,15-Diacetyl-1-dehydro-lucidusculine (23.0 mg), dissolved in MeOH-absolute EtOH (1:1, 4 ml), was treated with $NaBH_4$ (5 mg). The resulting solution was stirred at room temperature for 50 min. Water was added, and the mixture was extracted with $CHCl_3$. The combined extracts were washed with 5% aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and evaporated in vacuum to give a residue, which was separated by column chromatography on silica gel (10% hexane-ether saturated with 28% ammonia) to give 12,15-diacetyl-1-*epi*-luciculine (2; 5.6 mg, 24%) and 12,15-diacetyluciculine²³ (1; 13.5 mg, 58%).

2) Amorphous. IR (KBr) cm^{-1} : 3450, 1738, 1647, 1236, 1027, 900. 1H -NMR ($CDCl_3$) δ : 0.74 (3H, s, 18- CH_3), 1.03 (3H, t, $J=7.0$ Hz, $N-CH_2CH_3$), 2.04, 2.12 (each 3H, s, $OCOCH_3$), 4.10 (1H, brs, 1 α -H), 4.68 (1H, dd, $J=7.3, 10.2$ Hz, 12 β -H), 4.97, 5.28 (each 1H, s, 17- H_2), 5.50 (1H, s, 15 α -

H). HR-EI-MS m/z : 443.2678 (Calcd for $C_{26}H_{37}NO_5$: 443.2671). EI-MS m/z : 443 (M^+ , base peak), 425 ($M^+ - H_2O$), 400 ($M^+ - COCH_3$), 384 ($M^+ - OCOCH_3$).

Preparation of 1,12,15-Triacetyl-1-*epi*-luciculine (4) Acetic anhydride (0.7 ml) was added to a solution of 12,15-diacetyl-1-*epi*-luciculine (2; 10.3 mg) in pyridine (0.7 ml), and the mixture was then kept at 70 °C for 3 h. The usual work-up and purification by column chromatography on silica gel (15% hexane-ether saturated with 28% ammonia) afforded 1,12,15-triacetyl-1-*epi*-luciculine (4; 7.3 mg, 65%). Amorphous. IR (KBr) cm^{-1} : 1738, 1649, 1243, 1162, 901. 1H -NMR ($CDCl_3$) δ : 0.75 (3H, s, 18- CH_3), 1.02 (3H, t, $J=7.0$ Hz, $N-CH_2CH_3$), 2.03, 2.07, 2.16 (each 3H, s, $OCOCH_3$), 4.63 (1H, dd, $J=7.0, 10.0$ Hz, 12 β -H), 4.99, 5.30 (each 1H, s, 17- H_2), 5.14 (1H, brs, 1 α -H), 5.50 (1H, s, 15 α -H). HR-EI-MS m/z : 485.2770 (Calcd for $C_{28}H_{39}NO_6$: 485.2777). EI-MS m/z : 485 (M^+ , base peak), 442 ($M^+ - COCH_3$), 426 ($M^+ - OCOCH_3$).

Preparation of 12-*Epi*-lucidusculine (6) 1) Oxidation of Dehydro-lucidusculine: A solution of dehydro-lucidusculine (15: 197 mg) in dichloromethane (56 ml) was mixed with PDC (743.2 mg). The mixture was stirred at room temperature for 3 h. The reaction mixture was passed through a short column of florisil and then purified by column chromatography on silica gel (20% hexane-ether saturated with 28% ammonia) to give 12-dehydro-dehydro-lucidusculine (41.6 mg, 21%) and the starting material (69.7 mg). mp 132–135 °C. IR (KBr) cm^{-1} : 1742, 1723, 1680, 1234, 1178, 898. 1H -NMR ($CDCl_3$) δ : 0.84 (3H, s, 18- CH_3), 1.03 (3H, t, $J=7.0$ Hz, $N-CH_2CH_3$), 2.15 (3H, s, $OCOCH_3$), 3.71 (1H, s, 19-H), 3.99 (1H, d, $J=5.3$ Hz, 1-H), 4.96, 5.28 (each 1H, s, 17- H_2), 5.64 (1H, s, 15 α -H). HR-EI-MS m/z : 397.2240 (Calcd for $C_{24}H_{31}NO_4$: 397.2251). EI-MS m/z : 397 (M^+ , base peak), 354 ($M^+ - COCH_3$), 341 ($M^+ - C_2H_5O$).

2) Reduction of 12-Dehydro-dehydro-lucidusculine: $NaBH_4$ (15 mg) was added to the stirred solution of 12-dehydro-dehydro-lucidusculine (70.6 mg) in MeOH-absolute EtOH (1:1, 12 ml) at room temperature. After 1 h 30 min, water was added. Work-up of the reaction mixture in the usual manner resulted in a residue, which was separated by column chromatography on silica gel ($CHCl_3$ saturated with 28% ammonia) to give 12-*epi*-lucidusculine (6; 37.1 mg, 52%). mp 204 °C (dec.). IR (KBr) cm^{-1} : 3374, 1742, 1661, 1234, 1127, 899. 1H -NMR ($CDCl_3$) δ : 0.79 (3H, s, 18- CH_3), 1.13 (3H, t, $J=7.0$ Hz, $N-CH_2CH_3$), 2.13 (3H, s, $OCOCH_3$), 3.90 (1H, dd, $J=6.3, 8.3$ Hz, 1 β -H), 4.18 (1H, brs, 12 α -H), 5.17, 5.23 (each 1H, s, 17- H_2), 5.59 (1H, s, 15 α -H). HR-EI-MS m/z : 401.2542 (Calcd for $C_{24}H_{35}NO_4$: 401.2564). EI-MS m/z : 401 (M^+ , base peak), 384 ($M^+ - OH$), 358 ($M^+ - COCH_3$), 342 ($M^+ - OCOCH_3$).

Preparation of 1,12,15-Triacetyl-12-*epi*-luciculine (7) Acetic anhydride (0.6 ml) and pyridine (0.6 ml) were added to 12-*epi*-lucidusculine (6; 13 mg), and the mixture was kept at 70 °C for 2 h. The usual work-up afforded 1,12,15-triacetyl-12-*epi*-luciculine (7; 15.0 mg, 96%). mp 153 °C (dec.). IR (KBr) cm^{-1} : 1734, 1247, 1170, 911. 1H -NMR ($CDCl_3$) δ : 0.73 (3H, s, 18- CH_3), 1.05 (3H, t, $J=7.0$ Hz, $N-CH_2CH_3$), 1.92, 2.05, 2.11 (each 3H, s, $OCOCH_3$), 4.89, 5.04 (each 1H, s, 17- H_2), 5.01 (1H, ddd, $J=8.7, 6.1, 1.4$ Hz, 12 α -H), 5.09 (1H, dd, $J=6.5, 10.7$ Hz, 1 β -H), 5.59 (1H, s, 15 α -H). HR-EI-MS m/z : 485.2780 (Calcd for $C_{28}H_{39}NO_6$: 485.2777). EI-MS m/z :

485 (M^+), 442 ($M^+ - \text{COCH}_3$), 426 ($M^+ - \text{OCOCH}_3$, base peak).

Preparation of 1,12-Diacetyluciculine (11) A mixture of 1-acetyluciculine (**9**; 16 mg), pyridine (1 ml) and acetic anhydride (1 ml) was kept at 0 °C for 1 h 20 min. The usual work-up and purification by column chromatography on silica gel (50% hexane–ether saturated with 28% ammonia) afforded 1,12,15-triacetyluciculine²³ (**3**; 1.6 mg, 12%) and 1,12-diacetyluciculine (**11**; 6.1 mg, 34%).

11: Amorphous. IR (KBr) cm^{-1} : 3375, 1730, 1640, 1245, 900. $^1\text{H-NMR}$ (CDCl_3) δ : 0.74 (3H, s, 18- CH_3), 1.07 (3H, t, $J=7.3$ Hz, $N\text{-CH}_2\text{CH}_3$), 2.01, 2.04 (each 3H, s, OCOCH_3), 4.16 (1H, s, 15 α -H), 4.40 (1H, dd, $J=6.3$, 10.0 Hz, 12 β -H), 5.04 (1H, dd, $J=6.8$, 10.7 Hz, 1 β -H), 5.21, 5.33 (each 1H, s, 17- H_2). HR-EI-MS m/z : 443.2697 (Calcd for $\text{C}_{26}\text{H}_{37}\text{NO}_5$: 443.2671). EI-MS m/z : 443 (M^+), 384 ($M^+ - \text{OCOCH}_3$, base peak).

Preparation of 1,15-Diacetyluciculine (12) A mixture of 1,12,15-triacetyluciculine (**3**; 145.3 mg) and K_2CO_3 -aqueous MeOH (16 ml, pH 9.0) was refluxed for 16 h. The usual work-up and purification by column chromatography on silica gel (CHCl_3 saturated with 28% ammonia) afforded 1,15-diacetyluciculine²⁶ (**12**; 28.1 mg, 21%) and 1-acetyluciculine²³ (**9**; 48.1 mg, 40%).

12: Amorphous. IR (KBr) cm^{-1} : 3400, 1720, 1640, 1230, 910. $^1\text{H-NMR}$ (CDCl_3) δ : 0.74 (3H, s, 18- CH_3), 1.11 (3H, t, $J=7.3$ Hz, $N\text{-CH}_2\text{CH}_3$), 2.08, 2.10 (each 3H, s, OCOCH_3), 3.55 (1H, dd, $J=6.3$, 9.5 Hz, 12 β -H), 4.93, 5.11 (each 1H, s, 17- H_2), 5.05 (1H, dd, $J=6.9$, 10.5 Hz, 1 β -H), 5.49 (1H, s, 15 α -H). HR-EI-MS m/z : 443.2691 (Calcd for $\text{C}_{26}\text{H}_{37}\text{NO}_5$: 443.2671). EI-MS m/z : 443 (M^+), 384 ($M^+ - \text{OCOCH}_3$, base peak).

Preparation of 12-Benzoyluciculine (13) A mixture of lucidusculine (**5**; 50.4 mg), pyridine (1 ml) and benzoyl chloride (0.059 ml) was kept at 0 °C for 1 h. The usual work-up and purification by column chromatography on silica gel (2% hexane–ether saturated with 28% ammonia) afforded 12-benzoyluciculine (**13**; 60.6 mg, 95%). mp 122–123 °C. IR (KBr) cm^{-1} : 3456, 1740, 1717, 1560, 1278, 1236, 907. $^1\text{H-NMR}$ (CDCl_3) δ : 0.78 (3H, s, 18- CH_3), 1.08 (3H, t, $J=7.0$ Hz, $N\text{-CH}_2\text{CH}_3$), 2.13 (3H, s, OCOCH_3), 3.95 (1H, dd, $J=6.1$, 7.5 Hz, 1 β -H), 4.85 (1H, t, $J=8.3$ Hz, 12 β -H), 5.04, 5.36 (each 1H, s, 17- H_2), 5.55 (1H, s, 15 α -H), 7.43 (2H, t, $J=7.5$ Hz, COC_6H_5), 7.55 (1H, t, $J=7.5$ Hz, COC_6H_5), 8.54 (2H, dd, $J=1.4$, 8.5 Hz, COC_6H_5). HR-EI-MS m/z : 505.2834 (Calcd for $\text{C}_{31}\text{H}_{39}\text{NO}_5$: 505.2827). EI-MS m/z : 505 (M^+ , base peak), 488 ($M^+ - \text{OH}$), 462 ($M^+ - \text{COCH}_3$), 446 ($M^+ - \text{OCOCH}_3$), 384 ($M^+ - \text{OCOC}_6\text{H}_5$).

Preparation of 1-Benzoyluciculine (14), 12-Benzoyluciculine (15) and 12,15-Dibenzoyluciculine (21) 1) A mixture of luciculine (**8**; 200 mg), pyridine (4 ml) and benzoyl chloride (0.45 ml) was kept at room temperature for 3 h 10 min. The usual work-up and purification by column chromatography on silica gel (50% hexane–ether saturated with 28% ammonia) afforded 12-benzoyluciculine (**15**; 73.8 mg, 29%), 1,12-dibenzoyluciculine (60.2 mg, 19%), 12,15-dibenzoyluciculine (**17**; 80.1 mg, 25%) and 1,12,15-tribenzoyluciculine (99.2 mg, 27%).

15: Amorphous. IR (KBr) cm^{-1} : 3410, 1715, 1603, 1584, 1278, 903. $^1\text{H-NMR}$ (CDCl_3) δ : 0.73 (3H, s, 18- CH_3), 1.03 (3H, t, $J=7.0$ Hz, $N\text{-CH}_2\text{CH}_3$), 3.90 (1H, dd, $J=5.8$, 7.5 Hz, 1 β -H), 4.17 (1H, d, $J=7.8$ Hz, 15 α -H), 4.70 (1H, t, $J=8.5$ Hz, 12 β -H), 5.21, 5.37 (each 1H, s, 17- H_2), 7.37 (2H, t, $J=7.8$ Hz, COC_6H_5), 7.49 (1H, t, $J=7.5$ Hz, COC_6H_5), 8.00 (2H, dd, $J=1.4$, 8.3 Hz, COC_6H_5). HR-EI-MS m/z : 463.2707 (Calcd for $\text{C}_{29}\text{H}_{37}\text{NO}_4$: 463.2720). EI-MS m/z : 463 (M^+), 446 ($M^+ - \text{OH}$), 342 ($M^+ - \text{OCOC}_6\text{H}_5$, base peak), 105 ($[\text{COC}_6\text{H}_5]^+$).

1,12-Dibenzoyluciculine: Amorphous. IR (KBr) cm^{-1} : 3420, 1710, 1603, 1584, 1276, 905. $^1\text{H-NMR}$ (CDCl_3) δ : 0.79 (3H, s, 18- CH_3), 1.17 (3H, t, $J=7.0$ Hz, $N\text{-CH}_2\text{CH}_3$), 4.22 (1H, d, $J=7.8$ Hz, 15 α -H), 4.52 (1H, dd, $J=6.5$, 10.5 Hz, 12 β -H), 5.26, 5.43 (each 1H, s, 17- H_2), 5.37 (1H, dd, $J=7.0$, 10.5 Hz, 1 β -H), 7.18 (2H, t, $J=7.5$ Hz, COC_6H_5), 7.38 (2H, t, $J=8.5$ Hz, COC_6H_5), 7.46 (3H, t, $J=8.7$ Hz, COC_6H_5), 7.59 (1H, t, $J=7.3$ Hz, COC_6H_5), 8.08 (2H, d, $J=8.5$ Hz, COC_6H_5). HR-EI-MS m/z : 567.2976 (Calcd for $\text{C}_{36}\text{H}_{41}\text{NO}_5$: 567.2982). EI-MS m/z : 567 (M^+), 446 ($M^+ - \text{OCOC}_6\text{H}_5$, base peak), 105 ($[\text{COC}_6\text{H}_5]^+$).

17: mp 128–130 °C. IR (KBr) cm^{-1} : 3372, 1717, 1624, 1578, 1272, 903. $^1\text{H-NMR}$ (CDCl_3) δ : 0.61 (3H, s, 18- CH_3), 1.02 (3H, t, $J=7.0$ Hz, $N\text{-CH}_2\text{CH}_3$), 3.96 (1H, dd, $J=6.1$, 8.0 Hz, 1 β -H), 4.86 (1H, t, $J=8.5$ Hz, 12 β -H), 5.05, 5.32 (each 1H, s, 17- H_2), 5.76 (1H, s, 15 α -H), 7.34–7.55, 7.98–8.04 (10H, m, COC_6H_5). HR-EI-MS m/z : 567.2974 (Calcd for $\text{C}_{36}\text{H}_{41}\text{NO}_5$: 567.2982). EI-MS m/z : 567 (M^+), 462 ($M^+ - \text{COC}_6\text{H}_5$), 444 (base peak), 105 ($[\text{COC}_6\text{H}_5]^+$).

1,12,15-Tribenzoyluciculine: Amorphous. IR (KBr) cm^{-1} : 1717, 1603, 1584, 1270, 895. $^1\text{H-NMR}$ (CDCl_3) δ : 0.67 (3H, s, 18- CH_3), 1.17 (3H, t, $J=7.0$ Hz, $N\text{-CH}_2\text{CH}_3$), 4.67 (1H, dd, $J=6.3$, 9.2 Hz, 12 β -H), 5.11, 5.40 (each 1H, s, 17- H_2), 5.45 (1H, dd, $J=7.3$, 10.5 Hz, 1 β -H), 5.81 (1H, s, 15 α -

H), 7.16–8.12 (15H, m, COC_6H_5). HR-EI-MS m/z : 671.3259 (Calcd for $\text{C}_{43}\text{H}_{45}\text{NO}_6$: 671.3245). EI-MS m/z : 671 (M^+), 566 ($M^+ - \text{COC}_6\text{H}_5$), 550 ($M^+ - \text{OCOC}_6\text{H}_5$), 105 ($[\text{COC}_6\text{H}_5]^+$, base peak).

2) Hydrolysis of 1,12-Dibenzoyluciculine: A mixture of 1,12-dibenzoyluciculine (60.2 mg) and 28% ammonia–MeOH– CHCl_3 (4–5.2–2 ml) was stirred at 70 °C for 10 d. The reaction mixture was evaporated, and then purification by column chromatography on silica gel (33% hexane–ether saturated with 28% ammonia) afforded 1-benzoyluciculine (**14**; 17.3 mg, 35%) and the starting material (24.5 mg). Amorphous. IR (KBr) cm^{-1} : 3452, 1700, 1584, 1276, 907. $^1\text{H-NMR}$ (CDCl_3) δ : 0.78 (3H, s, 18- CH_3), 1.15 (3H, t, $J=7.0$ Hz, $N\text{-CH}_2\text{CH}_3$), 3.36 (1H, m, 12 β -H), 4.18 (1H, d, $J=7.8$ Hz, 15 α -H), 5.14 (2H, s, 17- H_2), 5.34 (1H, dd, $J=7.0$, 10.4 Hz, 1 β -H), 7.45 (2H, t, $J=7.5$ Hz, COC_6H_5), 7.53 (1H, t, $J=7.5$ Hz, COC_6H_5), 8.05 (2H, dd, $J=1.4$, 8.3 Hz, COC_6H_5). HR-EI-MS m/z : 463.2722 (Calcd for $\text{C}_{29}\text{H}_{37}\text{NO}_4$: 463.2720). EI-MS m/z : 463 (M^+), 342 ($M^+ - \text{OCOC}_6\text{H}_5$, base peak), 105 ($[\text{COC}_6\text{H}_5]^+$).

Preparation of 15-Benzoyluciculine (16) A mixture of 12,15-dibenzoyluciculine (**17**; 80.1 mg) and 28% ammonia–MeOH– CHCl_3 (4–5.2–2 ml) was stirred at 70 °C for 4 d. The reaction mixture was evaporated, and then purification by column chromatography on silica gel (10% MeOH–ether saturated with 28% ammonia) afforded 15-benzoyluciculine (**16**; 25.9 mg, 40%) and the starting material (35.3 mg). mp 126–129 °C. IR (KBr) cm^{-1} : 3384, 1719, 1603, 1584, 1272, 895. $^1\text{H-NMR}$ (CDCl_3) δ : 0.65 (3H, s, 18- CH_3), 1.07 (3H, t, $J=7.0$ Hz, $N\text{-CH}_2\text{CH}_3$), 3.72 (1H, t, $J=7.8$ Hz, 12 β -H), 3.99 (1H, dd, $J=6.1$, 8.3 Hz, 1 β -H), 4.99, 5.13 (each 1H, s, 17- H_2), 5.77 (1H, s, 15 α -H), 7.48 (2H, t, $J=7.3$ Hz, COC_6H_5), 7.60 (1H, t, $J=7.3$ Hz, COC_6H_5), 8.06 (2H, dd, $J=1.4$, 8.3 Hz, COC_6H_5). HR-EI-MS m/z : 463.2720 (Calcd for $\text{C}_{29}\text{H}_{37}\text{NO}_4$: 463.2720). EI-MS m/z : 463 (M^+), 358 ($M^+ - \text{COC}_6\text{H}_5$, base peak), 105 ($[\text{COC}_6\text{H}_5]^+$).

HPLC-APCI-MS Conditions²¹ 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. 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 Studies²¹ 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.

References

- 1) Splitter J. S., Turecek F. (eds.), "Application of Mass Spectrometry of Organic Stereochemistry," VCH, New York, 1994, pp. 83–671.
- 2) Fetterolf D. D., Yost R. A., *Int. J. Mass Spectrom. Ion Phys.*, **44**, 37–50 (1982).
- 3) McLuckey S. A., Cooks R. G., "Tandem Mass Spectrometry," ed. by McLaffey F. W., Wiley-Intersciences, New York, 1983, pp. 303–320.
- 4) Hayes R. N., Gross M. L., *Methods Enzymol.*, **193**, 237–263 (1990).
- 5) Beckett A. H., Dwuma-Badu D., Haddock R. E., *Tetrahedron*, **25**, 5961–5969 (1969).
- 6) Fujisawa H., *Chem. Pharm. Bull.*, **36**, 4136–4143 (1988).
- 7) Czira G., Tamás J., Kalaus G., *Org. Mass Spectrom.*, **19**, 555–562 (1984).
- 8) Laprévote O., Ducrot P., Thal C., Serani L., Das B. C., *J. Mass Spectrom.*, **31**, 1149–1155 (1996).
- 9) Laprévote O., Serani L., Das B. C., *J. Mass Spectrom.*, **32**, 339–340 (1997).
- 10) Amiya T., Bando H., "The Alkaloids," Vol. 34, ed. by Brossi A., Academic Press, San Diego, 1988, pp. 95–179.
- 11) Edwards O. E., "The Alkaloids," Vol. 1, ed. by Saxton J. E., The Chemical Society, London, 1971, pp. 343–381.
- 12) Pelletier S. W., Page S. W., "The Alkaloids," Vol. 3, ed. by Saxton J. E., The Chemical Society, London, 1973, pp. 232–257.
- 13) Pelletier S. W., Page S. W., "The Alkaloids," Vol. 8, ed. by Grudon M. F., The Chemical Society, London, 1978, pp. 219–245.

- 14) Pelletier S. W., Page S. W., "The Alkaloids," Vol. 10, ed. by Grudon M. F., The Chemical Society, London, 1981, pp. 211—226.
- 15) Yunusov M. S., *Natural Product Reports*, **8**, 499—526 (1991).
- 16) Yunusov M. S., *Natural Product Reports*, **10**, 471—486 (1993).
- 17) Yunusov M. S., Rashkes Ya. V., Yunusov S. Yu., *Khim. Prir. Soedin.*, **8**, 85—87 (1972).
- 18) Wada K., Bando H., Kawahara N., *J. Chromatogr.*, **644**, 43—48 (1993).
- 19) Wada K., Bando H., Kawahara N., Mori T., Murayama M., *Biol. Mass Spectrom.*, **23**, 97—102 (1994).
- 20) Wada K., Bando H., Kawahara N., *Natural Medicines*, **51**, 37—39 (1997).
- 21) Wada K., Mori T., Kawahara N., *J. Mass Spectrom.*, **35**, 432—439 (2000).
- 22) Wada K., Mori T., Kawahara N., *Chem. Pharm. Bull.*, **48**, 660—668 (2000).
- 23) Bando H., Wada K., Amiya T., *Heterocycles*, **26**, 2623—2637 (1987).
- 24) Takayama H., Wu F. E., Eda H., Aimi N., Sakai S., *Chem. Pharm. Bull.*, **39**, 1644—1646 (1991).
- 25) Kambara H., Kanomata I., *Anal. Chem.*, **49**, 270—275 (1977).
- 26) Sakairi M., Kambara H., *Anal. Chem.*, **60**, 774—780 (1988).
- 27) Wada K., Ishizuki S., Mori T., Bando H., Murayama M., Kawahara N., *Biol. Pharm. Bull.*, **20**, 978—982 (1997).
- 28) Takayama H., Tokita A., Miyuki I., Sakai S., Kurosaki F., Okamoto T., *Yakugaku Zasshi*, **102**, 245—257 (1982).