Tandem Mass Spectrometry for Structural Identification of Sesquiterpene Alkaloids from the Stems of *Dendrobium nobile* Using LC-QToF*

Authors Yan-Hong Wang[1], Bharathi Avula[1], Naohito Abe[1], Feng Wei[2], Mei Wang[1], Shuang-Cheng Ma[2], Zulfiqar Ali[1], Mahmoud A. Elsohly[1,3], Ikhlas A. Khan[1,4,5]

Affiliations The affiliations are listed at the end of the article

Key words
- *Dendrobium nobile*
- Orchidaceae
- *shi hu*
- dendrobine
- nobilonine
- UHPLC-QToF/MS
- tandem mass
- sesquiterpene alkaloids

Abstract

Dendrobium nobile is one of the fundamental herbs in traditional Chinese medicine. Sesquiterpene alkaloids are the main active components in this plant. Due to weak ultraviolet absorption and low content in *D. nobile*, these sesquiterpene alkaloids have not been extensively studied using chromatographic methods. Herein, tandem mass spectrometry combined with liquid chromatography separation provides a tool for the identification and characterization of the alkaloids from *D. nobile*. A total of nine sesquiterpene alkaloids were characterized by ultrahigh-performance liquid chromatography tandem mass spectrometry. These alkaloids can be classified into two subgroups that are represented by dendrobine and nobilonine. Tandem mass spectrometric studies revealed the fragmentation pathways of these two subgroup alkaloids that were used for the identification and characterization of other alkaloids in *D. nobile*. Characterization of these alkaloids using accurate mass and diagnostic fragments provided a reliable methodology for the analysis of *D. nobile* by ultrahigh-performance liquid chromatography tandem mass spectrometry. The limit of detection was defined as the signal-to-noise ratio equal to 3 : 1. Limits of detection of dendrobine and nobilonine were less than 30 ng/mL. The developed method was applied for the analysis of various *Dendrobium* species and related dietary supplements. Alkaloids were identified from *D. nobile*, but not detected from commercial samples including 13 other *Dendrobium* species and the 7 dietary supplements.

Introduction

Dendrobium nobile Lindl. (Orchidaceae) is also known as Noble Dendrobium or *jin-chai-shi-hu*. The stems of *D. nobile* are one of the fundamental herbs in traditional Chinese medicine (TCM) used for the treatment of chronic gastritis to lower cholesterol levels, regulate blood pressure, promote the secretion of saliva, reduce fever, and strengthen the immune system [1]. Phytochemical investigations revealed alkaloids, aromatic compounds, sesquiterpenoids, and polysaccharides in *D. nobile* [2]. The polysaccharides, alkaloids, and aromatics were reported as the main components responsible for the biological activities of *Dendrobium* [3]. Fourteen dendrobine-type sesquiterpene alkaloids have been isolated from *D. nobile*. Dendrobine, dendramine, nobilonine, dendroxine, and 6-hydroxydendroxine are some of the major alkaloids [4].

In recent years, some pre-workout dietary supplements advertise *D. nobile* and claim that dendrobine-type alkaloids can improve bodybuilding. In 2014, the USFDA sent a warning letter to the manufacturer of one of these products concerned that the product had led to the misuse of dendrobium extract [5]. However, there is no literature or analytical method which emphasized the characterization of dendrobine-type alkaloids from *D. nobile*. Development of a rapid method for the determination of dendrobine-type alkaloids will be useful for quality control and quality assurance of dietary supplement products claiming to contain *D. nobile*.

Previous studies mainly focused on the analysis of bibenzyls, phenanthrenes, fluorenones, and coumarins from *Dendrobium* species using HPLC [6–

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Correspondence
Ikhlas A. Khan
National Center for Natural Products Research
University of Mississippi
University, MS 38677
United States
Phone: + 1 66 29 15 78 21
Fax: + 1 66 29 15 79 89
ikhan@olemiss.edu

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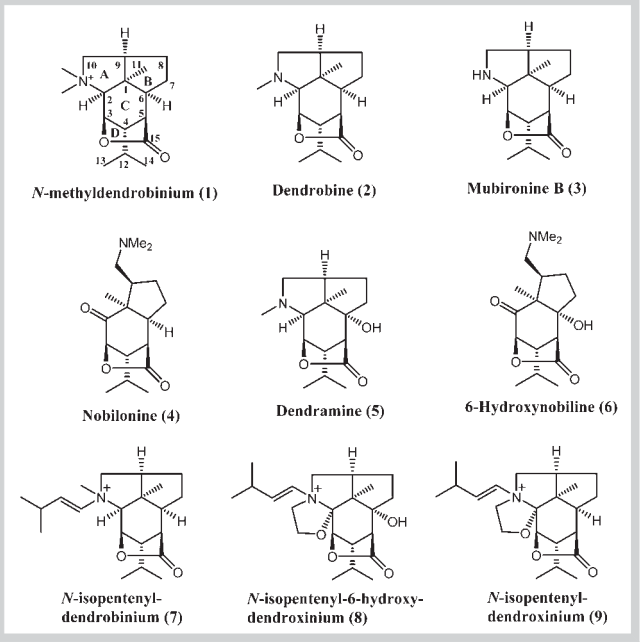

Fig. 1 Structures of sesquiterpene alkaloids: **1** *N*-methyldendrobinium, **2** dendrobine, **3** mubironine B, **4** nobilonine, **5** dendramine, **6** 6-hydroxynobiline, **7** *N*-isopentenyldendrobinium, **8** *N*-isopentenyl-6-hydroxydendroxinium, and **9** *N*-isopentenyldendroxinium.

12], capillary electrophoresis (CE) [13], and gas chromatography (GC) [14]. In two LC-UV methods, one or two alkaloids were used in the analysis of *Dendrobium* species [9, 10]. UV detection is considered inappropriate for dendrobine-type alkaloids because these compounds have extremely weak ultraviolet absorption due to the absence of chromophore in the core skeleton. Mass spectrometry detection, especially high-resolution mass spectrometric techniques such as quadrupole time-of-flight (QToF), is a useful tool for the identification of sesquiterpene alkaloids since it can provide accurate mass and structural information.

As a part of a USFDA research program for the science based authentication of dietary supplements, an ultrahigh-performance liquid chromatograph (UHPLC) coupled with an electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI/QToF MS) method was developed. The UHPLC coupled with tandem mass spectrometric method was designed to identify and structurally characterize sesquiterpene alkaloids from *Dendrobium* species and dietary supplements claiming to contain *D. nobile*.

Results and Discussion

In a preliminary test for the characterization of dendrobine-type alkaloids, different columns were applied for the analysis of *D. nobile* extracts (NCNPR #13021). The columns tested were Acquity UPLC BEH Shield RP18 (100 × 2.1 mm i.d., 1.7 µm), UPLC BEH C18 (100 × 2.1 mm i.d., 1.7 µm), and UPLC HSS T3 (100 × 2.1 mm i.d., 1.8 µm). The best separation and peak shape were achieved on the UPLC BEH Shield RP18 column. Different solvent systems such as acetonitrile/water and methanol/water were investigated to determine the best separation conditions for the sesquiterpene alkaloids. Optimal chromatographic separation was observed by using acetonitrile with 0.05% formic acid (v/v) and 0.05% formic acid in water as the mobile phase. Authentic plant sample #13021 was extracted with 100% methanol and 70% methanol (methanol/water = 70 : 30, v/v), respectively. The peak areas of the major alkaloids (compounds **2, 4, 5**, and **7–9**) did not differ significantly. However, considering the higher solubility of alkaloids' salts in aqueous solution, 70% methanol was used as the extraction solvent.

Dendrobine-type sesquiterpene alkaloids from *D. nobile* have a basic skeleton that consists of one picrotoxan-type sesquiterpenoid combined with a five-membered C2–C9-linked N-heterocycle and a C3–C5-linked lactone ring (**Fig. 1**).

The characterization of the sesquiterpene alkaloids in *D. nobile* authentic plant sample #13021 was based on two approaches: (1) unambiguous identification by comparing with reference standards (dendrobine and nobilonine) in terms of the retention time, accurate mass, and MS/MS fragment ions, and (2) tentative characterization of compounds without reference by analyzing accurate mass and MS/MS product ions, comparing key fragments (diagnostic fragments) with the fragmentation pattern of dendrobine and nobilonine, and surveying the literature. As a result, a total of nine alkaloids were identified or tentatively characterized. Structural information including the retention time, precursor ions ($[M]^+$/$[M + H]^+$), MS/MS product ions, and the composition of fragments for these compounds is summarized in **Table 1**. The base peak ion (BPI) chromatogram of the extracts of *D. nobile* and extracted ion chromatograms (EIC) of the individual alkaloids are shown in **Fig. 2**. The MS/MS spectra of nine alkaloids are presented in **Fig. 3**.

In the developed UHPLC-MS method, the standard compound dendrobine (compound **2** in **Fig. 2**) produced a protonated molecular ion at *m/z* 264.1956 ($[M + H]^+$, $C_{16}H_{26}NO_2^+$, *calcd.* 264.1958). The limit of detection (LOD) was defined as the signal-to-noise ratio equal to 3 : 1. The LOD of dendrobine was less than 30 ng/mL. Collision-induced dissociation (CID) of the $[M + H]^+$ ions led to the cleavage of the lactone ring between C3 and C5. After the neutral losses of H_2O from C-3 and CO from C-5, product ions at *m/z* 218.1901 ($C_{15}H_{24}N^+$) were generated. The successive cleavages of C4-C12 and C1–C11 generated ions at *m/z* 176.1433 $[218\text{-}C_3H_6]^+$ and 160.1111 $[176\text{-}CH_4]^+$, respectively. The fragment $[176\text{-}CH_4]^+$ consecutively yielded ions at *m/z* 145.0878 and 133.1003 corresponding to fragments $[160\text{-}CH_3]^+$ and $[160\text{-}HCN]^+$, respectively. Product ions at *m/z* 119.0848 and 105.0690 came from fragment $[160\text{-}HCN]^+$ corresponding to the losses of CH_2 and C_2H_4, respectively. The proposed fragmentation pathway of dendrobine is shown in **Fig. 4**. The neutral losses of 28, 18, and 42 Da corresponding to the CO, H_2O, and C_3H_6 moieties, respectively, are diagnostic for the characterization of the core skeleton of dendrobine-type alkaloids, which are also observed in the MS/MS spectra of compounds **2, 3, 5**, and **7–9** in **Table 1**.

From the methanolic extract of *D. nobile*, compound **2** was unambiguously identified as dendrobine. Compound **3** produced ions at *m/z* 250.1802 ($[M + H]^+$, $C_{15}H_{24}NO_2^+$, *calcd.* 250.1802), and the characteristic fragment ions at *m/z* 232.1702, 204.1756, and 190.1229 were found in accordance with the fragmentation pattern of dendrobine-type alkaloids. According to the molecular formula of the $[M + H]^+$ ion, compound **3** was 14 Da less than that of dendrobine **2**, indicating a loss of a methylene moiety in **2**. Based on the aforementioned information, compound **3** could be tentatively identified as mubironine B, which has been reported

Table 1 Positive ESI/QToF/MS/MS data of alkaloid compounds identified from *D. nobile*: **1** *N*-methyldendrobinium, **2** dendrobine, **3** mubironine B, **4** nobilonine, **5** dendramine, **6** 6-hydroxynobiline, **7** *N*-isopentenyldendrobinium, **8** *N*-isopentenyl-6-hydroxydendroxinium, and **9** *N*-isopentenyldendroxinium.

Peak	t_R (min)	CE (ev)	Precursor Ion (*m/z*)	Experimental MS/MS (*m/z*)	Calculated MS/MS (*m/z*)	Elemental composition	Error (ppm)	Structural elucidation
1	3.50	40	278.2120	278.2120	278.2115	$C_{17}H_{28}NO_2^+$	3.10	*N*-methylden-drobinium
				190.1621	190.1596	$C_{13}H_{20}N^+$	13.14	
				145.0878	145.0886	$C_{10}H_{11}N^{+\cdot}$	− 6.01	
				119.0846	119.0855	$C_9H_{11}^+$	− 8.41	
				105.0692	105.0699	$C_8H_9^+$	− 2.50	
2	4.12	40	264.1956	264.1956	264.1958	$C_{16}H_{26}NO_2^+$	− 0.76	dendrobine
				246.1865	246.1852	$C_{16}H_{24}NO^+$	4.96	
				236.2000	236.2009	$C_{15}H_{26}NO^+$	− 3.83	
				218.1901	218.1903	$C_{15}H_{24}N^+$	− 0.91	
				176.1433	176.1434	$C_{12}H_{18}N^+$	− 0.48	
				160.1111	160.1121	$C_{11}H_{14}N^+$	− 5.20	
				145.0878	145.0886	$C_{10}H_{11}N^{+\cdot}$	− 4.49	
				133.1003	133.1012	$C_{10}H_{13}^+$	− 5.48	
				119.0848	119.0855	$C_9H_{11}^+$	− 2.26	
				105.0690	105.0699	$C_8H_9^+$	− 3.08	
3	4.38	30	250.1802	250.1802	250.1802	$C_{15}H_{24}NO_2^+$	0.00	mubironine B
				232.1702	232.1696	$C_{15}H_{22}NO^+$	2.71	
				204.1756	204.1747	$C_{14}H_{22}N^+$	4.43	
				190.1229	190.1226	$C_{12}H_{16}NO^+$	1.58	
				175.0989	175.0992	$C_{11}H_{13}NO^{+\cdot}$	− 1.71	
				162.1263	162.1277	$C_{11}H_{16}N^+$	− 8.64	
				145.0882	145.0886	$C_{10}H_{11}N^{+\cdot}$	− 2.28	
				133.1016	133.1012	$C_{10}H_{13}^+$	2.48	
				119.0845	119.0855	$C_9H_{11}^+$	− 7.51	
				105.0699	105.0699	$C_8H_9^+$	0.00	
4	5.17	30	294.2067	294.2067	294.2064	$C_{17}H_{28}NO_3^+$	0.90	nobilonine
				249.1486	249.1485	$C_{15}H_{21}O_3^+$	0.38	
				221.1538	221.1536	$C_{14}H_{21}O_2^+$	0.81	
				203.1434	203.1430	$C_{14}H_{19}O^+$	1.83	
				175.1480	175.1481	$C_{13}H_{19}^+$	− 0.57	
				161.0960	161.0961	$C_{11}H_{13}O^+$	− 0.62	
				133.1011	133.1012	$C_{10}H_{13}^+$	− 0.84	
				121.0643	121.0648	$C_8H_9O^+$	− 4.13	
				107.0498	107.0491	$C_7H_7O^+$	6.54	
5	5.42	30	280.1909	280.1909	280.1907	$C_{16}H_{26}NO_3^+$	0.53	dendroamine
				263.1876	263.1880	$C_{16}H_{25}NO_2^{+\cdot}$	− 1.30	
				262.1801	262.1802	$C_{16}H_{24}NO_2^+$	− 0.34	
				235.1927	235.1931	$C_{15}H_{25}NO^{+\cdot}$	− 1.53	
				220.1337	220.1332	$C_{13}H_{18}NO_2^+$	2.03	
				207.1983	207.1982	$C_{14}H_{25}N^{+\cdot}$	0.43	
				192.1387	192.1383	$C_{12}H_{18}NO^+$	1.98	
				178.1226	178.1226	$C_{11}H_{16}NO^+$	0.00	
				164.1435	164.1434	$C_{11}H_{18}N^+$	0.34	
				136.1121	136.1121	$C_9H_{14}N^+$	0.00	
6	6.77	30	310.2024	310.2024	310.2013	$C_{17}H_{28}NO_4^+$	4.01	6-hydroxynobi-line
				292.1931	292.1907	$C_{17}H_{26}NO_3^+$	9.08	
				249.1484	249.1485	$C_{15}H_{21}O_3^+$	− 0.38	
				221.1536	221.1536	$C_{14}H_{21}O_2^+$	0.00	
				203.1428	203.1430	$C_{14}H_{19}O^+$	− 0.81	
				175.1483	175.1481	$C_{13}H_{19}^+$	0.81	
				161.0952	161.0961	$C_{11}H_{13}O^+$	− 4.11	
				133.1018	133.1012	$C_{10}H_{13}^+$	2.92	
				107.0482	107.0491	$C_7H_7O^+$	− 5.11	
7	10.1	50	332.2593	332.2593	332.2584	$C_{21}H_{34}NO_2^+$	2.71	*N*-isopentenyl-dendrobinium
				264.1965	264.1958	$C_{16}H_{26}NO_2^+$	2.65	
				246.1865	246.1852	$C_{16}H_{24}NO^+$	5.28	
				218.1900	218.1903	$C_{15}H_{24}N^+$	− 1.37	
				176.1434	176.1434	$C_{12}H_{18}N^+$	0.00	
				160.1116	160.1121	$C_{11}H_{14}N^+$	− 3.12	
				119.0856	119.0855	$C_9H_{11}^+$	0.84	
				105.0709	105.0699	$C_8H_9^+$	9.52	

continued next page

Table 1 Positive ESI/QToF/MS/MS data of alkaloid compounds identified from *D. nobile*: **1** *N*-methyldendrobinium, **2** dendrobine, **3** mubironine B, **4** nobilonine, **5** dendramine, **6** 6-hydroxynobiline, **7** *N*-isopentenyldendrobinium, **8** *N*-isopentenyl-6-hydroxydendroxinium, and **9** *N*-isopentenyldendroxinium. *continued*

Peak	t_R (min)	CE (ev)	Precursor Ion (*m/z*)	Experimental MS/MS (*m/z*)	Calculated MS/MS (*m/z*)	Elemental composition	Error (ppm)	Structural elucidation
8	10.59	50	376.2482	376.2482	376.2482	$C_{22}H_{34}NO_4^+$	0.00	*N*-isopentenyl-6-hydroxydendroxinium
				308.1850	308.1856	$C_{17}H_{26}NO_4^+$	− 1.95	
				290.1746	290.1751	$C_{17}H_{24}NO_3^+$	− 1.72	
				275.1520	275.1516	$C_{16}H_{21}NO_3^{+\cdot}$	1.45	
				262.1811	262.1802	$C_{16}H_{24}NO_2^+$	3.43	
				246.1120	246.1125	$C_{14}H_{16}NO_3^+$	− 2.03	
				232.1329	232.1332	$C_{14}H_{18}NO_2^+$	− 1.29	
				202.1229	202.1226	$C_{13}H_{16}NO^+$	1.48	
9	11.42	50	360.2535	360.2535	360.2533	$C_{22}H_{34}NO_3^+$	0.56	*N*-isopentenyldendroxinium
				292.1911	292.1907	$C_{17}H_{26}NO_3^+$	1.37	
				274.1805	274.1802	$C_{17}H_{24}NO_2^+$	1.09	
				264.1958	264.1958	$C_{16}H_{26}NO_2^+$	0.00	
				262.1818	262.1802	$C_{16}H_{24}NO_2^+$	6.10	
				250.1442	250.1438	$C_{14}H_{20}NO_3^+$	1.60	
				248.1997	248.2009	$C_{16}H_{26}NO^+$	− 4.83	
				246.1857	246.1852	$C_{16}H_{24}NO^+$	2.03	
				219.1247	219.1254	$C_{13}H_{17}NO_2^{+\cdot}$	− 3.19	
				205.1454	205.1461	$C_{13}H_{19}NO^{+\cdot}$	− 3.41	
				176.1077	176.1070	$C_{11}H_{14}NO^+$	3.97	

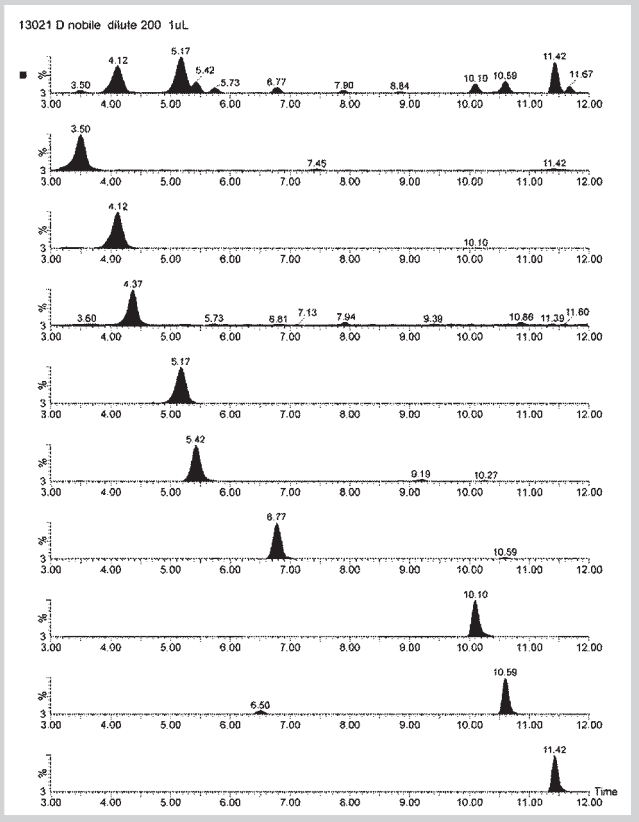

Fig. 2 Base peak ions (BPI) chromatogram of #13021 *D. nobile* and extracted ion chromatograms (EIC) of individual sesquiterpene alkaloids: **1** *N*-methyldendrobinium, **2** dendrobine, **3** mubironine B, **4** nobilonine, **5** dendramine, **6** 6-hydroxynobiline, **7** *N*-isopentenyldendrobinium, **8** *N*-isopentenyl-6-hydroxy-dendroxinium, and **9** *N*-isopentenyldendroxinium.

from the whole plant of *Dendrobium* Snowflake "Red Star", a cultivar of *D. nobile* [15].

Compound **1** produced an $[M]^+$ ion at *m/z* 278.2120 ($C_{17}H_{28}NO_2^+$, *calcd.* 278.2115). Since the concentration of compound **1** was much lower than that of dendrobine **2** and compound **3**, intensities of the fragment ions of compound **1** were not observed. On the basis of an elemental analysis, compound **1** was 14 Da heavier than that of dendrobine **2**, indicating an additional methylene moiety in **2**. Overall, considering the identification of mubironine B (**3**) and dendrobine **2** from this sample as well as a previous report from the literature [16], compound **1** could be tentatively identified as *N*-methyldendrobinium.

Compound **5** produced ions with *m/z* 280.1909 ($[M + H]^+$, $C_{16}H_{26}NO_3^+$, *calcd.* 280.1907), and the characteristic fragment ions at *m/z* 262.1801, 220.1337, and 192.1387 were found in agreement with traits of the core skeleton of dendrobine-type alkaloids. The elemental composition showed that the $[M + H]^+$ ion of compound **5** was 16 Da heavier than that of dendrobine **2**, which indicated that compound **5** was an oxygenated derivative of dendrobine. Therefore, compound **5** was tentatively identified as dendramine, a previously reported compound in *D. nobile* [17]. In the MS/MS spectra of compound **5**, fragment ions at *m/z* 263.1876, 235.1927, and 207.1983 could be radical ions corresponding to fragments $C_{16}H_{25}NO_2^{+\cdot}$ ($[M + H - OH]^{+\cdot}$), $C_{15}H_{25}NO^{+\cdot}$ ($[263\text{-}CO]^{+\cdot}$), and $C_{14}H_{25}N^{+\cdot}$ ($[235\text{-}CO]^{+\cdot}$), respectively. For dendrobine-type alkaloids, radical ions are likely formed when C-6 is oxidized.

A group of compounds (t_R, 10.10, 10.59, and 11.42 min) had the common feature that a 68 Da fragment was dissociated from each compound, which corresponded to the loss of an isoamyl alkynyl moiety (C_5H_8). Compound **7** (t_R, 10.10 min) was identified at *m/z* 332.2593 ($[M]^+$, $C_{21}H_{34}NO_2^+$, *calcd.* 332.2584). After application of a collision energy of 50 eV, a product ion at *m/z* 264.1965 ($C_{16}H_{26}NO_2^+$) corresponding to the loss of the C_5H_8 moiety from the molecular ion of compound **7** was generated. This product ion had the same elemental composition as the dendrobine $[M + H]^+$ ion. Interestingly, fragment ions at *m/z* 264.1965, 246.1865, 218.1900, 176.1434, 160.1116, 119.0856, and 105.0709 were also

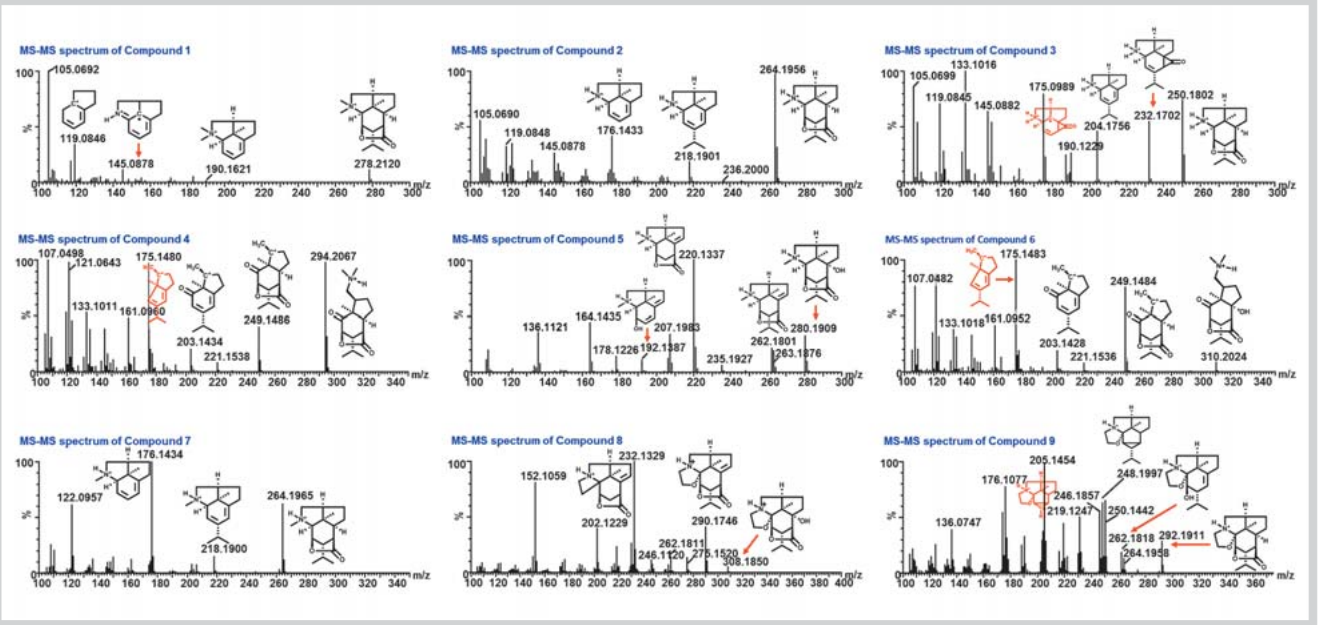

Fig. 3 MS/MS spectra of identified sesequiterpene alkaloids in *D. nobile*: **1** *N*-methyldendrobinium, **2** dendrobine, **3** mubironine B, **4** nobilonine, **5** dendramine, **6** 6-hydroxynobiline, **7** *N*-isopentenyl-dendrobinium, **8** *N*-isopentenyl-6-hydroxy-dendroxinium, and **9** *N*-isopentenyldendroxinium (Color figure available online only).

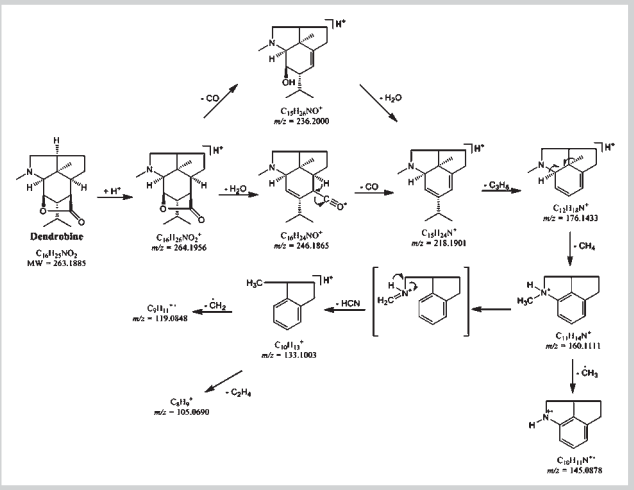

Fig. 4 Proposed fragmentation pathway of dendrobine (**2**).

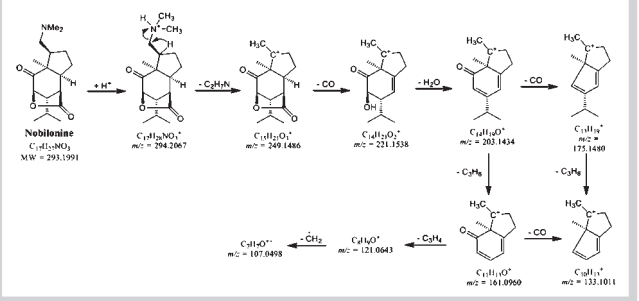

Fig. 5 Proposed fragmentation pathway of nobilonine (**4**).

identical to the fragment ions of dendrobine. Hence, compound **7** was tentatively characterized as *N*-isopentenyldendrobinium [18].

Compound **9** (t_R 11.42 min) yielded ions at *m/z* 360.2535 ($[M]^+$, $C_{22}H_{34}NO_3^+$, *calcd.* 360.2533). The characteristic fragment ions at *m/z* 292.1911, 274.1805, 264.1958, and 250.1442 indicated that compound **9** had a core skeleton of dendrobine-type alkaloids. The product ion at *m/z* 292.1911 ($C_{17}H_{26}NO_3^+$) corresponded to the loss of the C_5H_8 moiety from the molecular ion of compound **9**. The elemental composition $C_{17}H_{26}NO_3^+$ of the product ion at *m/z* 292.1911 was the same as the dendroxine $[M + H]^+$ ion. Dendroxine has been previously reported in *D. nobile* [19]. Thus, compound **9** was tentatively characterized as *N*-isopentenyldendroxinium, which was previously isolated from *D. nobile, D. friedricksianum*, and *D. hildebrandii* [16, 20].

Compound **8** (t_R 10.59 min) produced ions at *m/z* 376.2482 ($[M]^+$, $C_{22}H_{34}NO_4^+$, *calcd.* 376.2482). The product ion at *m/z* 308.1850 and fragment ions at *m/z* 290.1746 and 262.1811 indicated that compound **8** possessed an isopentenyl moiety and a core skeleton of dendrobine-type alkaloids. The elemental composition of compound **8** was 16 Da heavier than that of compound **9**, indicating that compound **8** was an oxygenated derivative of compound **9**. Furthermore, in the MS/MS spectra of compound **8**, the peak at *m/z* 275.1520 was a radical ion corresponding to the loss of a $CH_3\cdot$ moiety from the fragment ion at *m/z* 290.1746. Hence, compound **8** was tentatively identified as *N*-isopentenyl-6-hydroxy-dendroxinium, previously reported in *D. friedricksianum*, and *D. hildebrandii* [20].

Another subgroup of dendrobine alkaloids is nobilonine-type sesquiterpene alkaloids (**4** and **6** in ◉ **Fig. 1**), a group of compounds derived from dendrobine. In this subgroup, the C2–C9 N-heterocycle ring (A ring) is opened between C2 and N, and a ketone is formed at C-2.

Compound **4** was unambiguously identified as nobilonine from the methanolic extract of *D. nobile* from comparison with reference standards. The LOD of nobilonine was less than 30 ng/mL. Nobilonine (compound **4** in ◉ **Fig. 2**) was eluted from the column at 5.17 min, and produced a protonated molecular ion at *m/z* 294.2067 ($[M + H]^+$, $C_{17}H_{28}NO_3^+$, *calcd.* 294.2064). The CID of the $[M + H]^+$ ions produced fragment ion $C_{15}H_{21}O_3^+$ at *m/z* 249.1486 after the neutral loss of the C_2H_7N moiety from C-10. Following that, the lactone ring was opened, and the $C_{15}H_{21}O_3^+$ fragment successively yielded $C_{14}H_{21}O_2^+$ ($[249\text{-}CO]^+$) and $C_{14}H_{19}O^+$ ($[221\text{-}H_2O]^+$) at *m/z* 221.1538 and 203.1434, respectively. The fragment $C_{14}H_{19}O^+$ yielded key fragments at *m/z* 175.1480 ($C_{13}H_{19}^+$) and 161.0960 ($C_{11}H_{13}O^+$), respectively, corresponding to the losses of moieties CO at C-2 and C_3H_6 at C-3. From ion 161.0960 ($C_{11}H_{13}O^+$), product ions corresponding to fragments $C_{10}H_9O^+$ ($[161\text{-}CH_4]^+$), $C_{10}H_{13}^+$ ($[161\text{-}CO]^+$), $C_8H_9O^+$ ($[161\text{-}C_3H_4]^+$), and $C_7H_7O^+$ ($[121\text{-}CH_2{\cdot}]^{+\cdot}$) were produced with *m/z* 145.0651, 133.1011, 121.0643, and 107.0498, respectively. A proposed fragmentation pathway of nobilonine is illustrated in ◉ **Fig. 5**. In the MS/MS spectra of nobilonine, the key fragments at *m/z* 249, 203, 175, 161, and 121 are important for the characterization of nobilonine. The neutral losses of 18, 28, and 45 Da corresponding to the H_2O, CO, and C_2H_7N moieties, respectively, are characteristic for the determination of nobilonine-type alkaloids.

Compound **6** produced ions at *m/z* 310.2024 ($[M + H]^+$, $C_{17}H_{28}NO_4^+$, *calcd.* 310.2013). Based on the analysis of elemental composition, compound **6** was 16 Da heavier than that of nobilonine **4**. This indicated that compound **6** was an oxygenated derivative of **4**. In the MS/MS spectra of compound **6**, the product ion (*m/z* 292.1931), corresponding to the loss of H_2O from the protonated ion $[M + H]^+$, further proved that compound **6** contained a hydroxyl group. Moreover, characteristic fragment ions at *m/z* 249.1484, 221.1636, 203.1428, 175.1483, and 161.0952 were found and indicated that compound **6** had a skeleton of nobilonine-type alkaloids. Based on the aforementioned information, compound **6** could be tentatively identified as 6-hydroxynobilonine, which has been previously isolated from *D. hildebrandii* and *Dendrobium moniliforme* (L.) Sw. [21,22].

Sample source and results for the identification and characterization of the nine sesquiterpene alkaloids from various samples are listed in ◉ **Table 2**. A total of 26 samples including 19 plant samples and 7 dietary supplements were analyzed using the developed UHPLC-MS/MS method. As shown in ◉ **Table 2**, only sample #13021 was an authentic plant sample, the rest of the 18 plant samples were commercial samples purchased either from local markets in China or from the Internet. Six of the *D. nobile* plant samples were analyzed. Among the five *D. nobile* commercial samples (#8270, 10876, 10882, 10883, and 13034), two samples (#8270 and #10876) were found to contain eight of the nine alkaloids (*N*-isopentenyl-6-hydroxydendroxinium **8** was not detected). The other thirteen *Dendrobium* plant samples did not contain any of these nine sesquiterpene alkaloids. Seven dietary supplements that were purchase from the Internet and claimed to contain either dendrobine alkaloids or *D. nobile* plant or both terms were tested. From these samples, none of dendrobine-type alkaloids were identified.

Materials and Methods

UHPLC chromatographic conditions

The UHPLC analyses were performed on a Waters Acquity UPLC™ system including binary solvent manager, sampler manager, column compartment, and a PDA detector (Waters Acquity model code UPD). The separation was carried out on a Waters Acquity UPLC™ BEH Shield RP18 column (100 × 2.1 mm i.d., 1.7 µm) that was equipped with a guard column (Vanguard 2.1 × 5 mm). The sample temperature and column temperature were maintained at 15 °C and 30 °C, respectively. The mobile phase consisted of water containing 0.05% formic acid (v/v) (A) and acetonitrile with 0.05% formic acid (B). The analysis was performed using the following gradient elution at a flow rate of 0.30 mL/min: 0–8 min, 5% B to 13% B; 8–10 min, 13% B to 20% B; 10–12 min, 20% B to 35% B; and 12–18 min, 35% B to 100% B. Each run was followed by a 4-min wash with 100% B and an equilibration period of 3.5 min with the initial conditions. Strong needle wash (90/10; acetonitrile/water, v/v) and weak needle wash solution (10/90; acetonitrile/water, v/v) were used. The total run time for analysis was 18 min.

ESI/ToF/MS/MS

The high-resolution ESI-MS experiments were carried on a Waters ACQUITY™ XEVO QToF mass spectrometer that was connected to the UHPLC system via an ESI interface. The ESI source was operated in the positive ionization mode with the capillary voltage at 2.5 kV. The source and desolvation temperatures were set at 150 and 350 °C, respectively. The cone and desolvation gas flows were 50 and 900 L/h, respectively. The cone voltage was set at 35 V. All data collected in the centroid mode were acquired using MassLynx™ NT 4.1 software. Mass accuracy of the parent and major fragments in this study was limited within 5 ppm, but few minor fragment ions were tolerated up to 10 ppm when considering its limited peak intensity in the analysis. Leucine-enkephalin was used for the lock mass at a concentration of 2 ng/mL and flow rate of 5 µL/min. Ions $[M + H]^+$ (*m/z* 556.2771 Da) and a fragment ion (*m/z* 278.1141 Da) of leucine-enkephalin were employed to ensure mass accuracy during the MS analysis. The lock spray interval was set at 30 s, and the data were averaged over three scans. The mass spectrometer was programmed to step between low (20 eV) and elevated (30–50 eV) collision energies on the gas cell, using a scan time of 0.1 s per function over a mass range of 50–900 *m/z*. When data were acquired with MS^E, two interleaved scan functions were used. The first scan function acquired a wide mass range using low collision energy. This scan function collected precursor ion information for the sample. The second scan function acquired data over the same mass range; however, the collision energy was ramped from low to high. This scan function allowed for the collection of a full-scan accurate mass fragments with precursor ion information. The mass spectrometer under the MS/MS tandem mode was programmed to use a scan time of 1 s per function scanned 50–500 *m/z* and the collision energy applied to every precursor (◉ **Table 1**) was optimized for the best profile of the product ions. MS^E and MS/MS data independent analysis provide accurate mass measurements of all detectable precursor and product ions, which are achieved by post-acquisition and lock mass corrections.

Table 2 Identification of sesquiterpene alkaloids from *Dendrobine* species and dietary supplements: **1** *N*-methyldendrobinium, **2** dendrobine, **3** mubironine B, **4** nobilonine, **5** dendramine, **6** 6-hydroxynobiline, **7** *N*-isopentenyl-dendrobinium, **8** *N*-isopentenyl-6-hydroxydendroxinium, and **9** *N*-isopentenyldendroxinium (+ = identified; – = not detected).

				Retention time (min) and MS (*m*/*z*) of sesquiterpene alkaloids								
				1	2	3	4	5	6	7	8	9
				3.50 min	4.12 min	4.37 min	5.17 min	5.42 min	6.77 min	10.10 min	10.59 min	11.42 min
Sample Type	NCNPR Code	Sample Name	Plant Sample Source/ Products Label Claims	*m*/*z* 278.2120	*m*/*z* 264.1956	*m*/*z* 250.1802	*m*/*z* 294.2067	*m*/*z* 280.1909	*m*/*z* 310.2024	*m*/*z* 332.2593	*m*/*z* 376.2482	*m*/*z* 360.2535
Plant samples	13021	*D. nobile*	Authentic plant, Univ. of Mississippi	+	+	+	+	+	+	+	+	+
	8270	*D. nobile*	Commercial sample, purchased online	+	+	+	+	+	+	+	–	+
	10876	*D. nobile*	Commercial sample, purchased online	+	+	+	+	+	+	+	–	+
	10882	*D. nobile*	Commercial sample, purchased online	–	–	–	–	–	–	–	–	–
	10883	*D. nobile*	Commercial sample, purchased online	–	–	–	–	–	–	–	–	–
	13034	*D. nobile*	Commercial sample, purchased online	–	–	–	–	–	–	–	–	–
	13022	*D. fimbriatum*	Commercial sample, purchased online	–	–	–	–	–	–	–	–	–
	14990	*D. devonianum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14991	*D. denneanum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14992	*D. polyanthum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14993	*D. aphyllum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14994	*D. transparens*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14995	*D. wardianum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14996	*D. heterocarpum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14997	*D. falconeri*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14998	*D. chrysotoxum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	14999	*D. crepidatum*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	15000	*D. trigonopus*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
	15001	*D. officinale*	Commercial sample, Yunnan, China	–	–	–	–	–	–	–	–	–
Dietary supplements	12910	DS-1	Alkaloid including Dendrobine, Dendroxine, Dendromine	–	–	–	–	–	–	–	–	–
	15730	DS-2	*D. nobile*; Dendrobine, Dendramine, Dendroxine, 3-hydroxy-2-oxydendrobine, 6-hydroxydendroxine, 8-hydroxydendroxine, Nobiline, Dendrine	–	–	–	–	–	–	–	–	–
	15731	DS-3	*D. nobile*; Dendrobine, Dendroxine, Dendramine, nobilonine, 6-hydroxydendrobine	–	–	–	–	–	–	–	–	–
	15732	DS-4	*D. nobile* (stem)	–	–	–	–	–	–	–	–	–
	15733	DS-5	Dendrobium extract (stem)	–	–	–	–	–	–	–	–	–
	15734	DS-6	*D. nobile*	–	–	–	–	–	–	–	–	–
	15737	DS-8	Dendrobium extract (stem); alkaloid including Dendrobine, Dendroxine, Dendramine	–	–	–	–	–	–	–	–	–

Chemicals and reagents

The reference compound dendrobine (**2**) was purchased from Quality Phytochemicals LLC with a purity greater than 98%. Standard compound nobilonine (**4**) was isolated at the National Center for Natural Products Research (NCNPR), The University of Mississippi, University, MS, USA. The identity of nobilonine was determined by analysis of the spectral data (^{1}H- and ^{13}C-NMR and HR-ESIMS) [23], and the purity of nobilonine was confirmed to be above 95% by chromatographic methods.

HPLC grade methanol, acetonitrile, and formic acid were purchased from Fisher. Water for the HPLC mobile phase was purified using a Milipore Synergy Water Purification System.

Plant materials

Stems of *D. nobile* (NCNPR #13021) were obtained from the cultivated, living collection of the Maynard W. Quimby Medicinal Plant Garden, The University of Mississippi. The plant material was identified by Dr. Aruna Weerasooriya of the Maynard W. Quimby Medicinal Plant Garden, The University of Mississippi, University, Mississippi, USA. Stems of *Dendrobium devonianum* (#14990), *Dendrobium denneanum* Kerr (#14991), *Dendrobium polyanthum* Wall. ex Lindl. (#14992), *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch. (#14993), *Dendrobium transparens* Wall. ex Lindl. (#14994), *Dendrobium wardianum* R.Warner (#14995), *Dendrobium heterocarpum* Wall. ex Lindl. (#14996), *Dendrobium falconeri* Hook. (#14997), *Dendrobium chrysotoxum* Lindl. (#14998), *Dendrobium crepidatum* Lindl. & Paxton (#14999), *Dendrobium trigonopus* Rchb.f. (#15000), and *Dendrobium officinale* Kimura & Migo (#15001) were purchased from local markets in Yunnan Province, People's Republic of China and identified by Dr. Feng Wei of the Institute for Control of Traditional Chinese Medicine and Ethnic Medicine, National Institutes for Food and Drug Control, Beijing, China. Five plant samples (stems) of *D. nobile* (#8270, 10876, 10882, 10883, and 13034), *Dendrobium fimbriatum* Hook. (#13022), and seven dietary supplements (#12910, #15730–15734, and #15737) claiming to contain *D. nobile* or dendrobine-type alkaloids were purchased online. Specimens of all samples are deposited at the Repository of Botanicals, NCNPR, The University of Mississippi, University, Mississippi, USA.

Sample preparation

Dry plant material was ground as fine powder. Five to ten tablets or capsules were weighed. The tablets (or capsule contents) were combined and ground with a mortar and pestle.

Dry powder of the plant samples (0.5 g) or an adequate amount of capsule/tablet/powder/pill contents were weighed and sonicated in 2.5 mL of 70% methanol (methanol : water = 70 : 30, v/v) for 30 min, followed by centrifugation at 959 × *g* for 15 min. The supernatant was transferred to a 10-mL volumetric flask. The procedure was repeated three more times with 2.5 mL 70% methanol and the respective supernatants were combined. The final volume was adjusted to 10 mL with 70% methanol and mixed thoroughly. Prior to injection, an adequate volume of sample was passed through a 0.45-µm PTFE filter and collected in an LC sample vial. For plant samples where a few constituents were highly concentrated, the sample solutions were further diluted up to 200 times.

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Conflict of Interest

The authors declare no conflict of interest.

Affiliations

[1] National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS, USA
[2] Institute for Control of Traditional Chinese Medicine and Ethnic Medicine, National Institutes for Food and Drug Control, Beijing, People's Republic of China
[3] ElSohly Laboratories, Incorporated, Oxford, MS, USA
[4] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS, USA
[5] Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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