Phytochemistry Letters 21 (2017) 179–182

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Melohenryines A and B, two new indole alkaloids from *Melodinus henryi*

Ling-Li Guo[a], Yu-Xi Yuan[b], Hong-Ping He[b], Shun-Lin Li[a], Yu Zhang[a,*], Xiao-Jiang Hao[a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China

[b] Yunnan University of TCM, Kunming, 650500, Yunnan, People's Republic of China

ARTICLE INFO

Keywords:
Monoterpenoid indole alkaloids
Melohenryines A and B
Melodinus henryi

ABSTRACT

Two new monoterpenoid indole alkaloids, melohenryines A and B (**1** and **2**), along with six known indole alkaloids, were isolated from the twigs and leaves of *Melodinus henryi*. Structures of the new alkaloids were established by extensive spectroscopic techniques including NMR spectroscopy and mass spectrometry. Melohenryine A (**1**) represents the first example of monoterpenoid indole alkaloids characterized an ester carbonyl group at C-19 position. All of the new compounds were evaluated for in vitro cytotoxicity against several human cancer cell lines.

1. Introduction

Monoterpenoid indole alkaloids are one of the major groups of alkaloids in plants and elaborated mainly by plants of Apocynaceae, Loganiaceae, and Rubiaceae families (Dewick, 2009). Many of these alkaloids, such as vincristine and its derivatives, are quite outstanding due to their famous antitumor activity (Newman and Cragg, 2016). The genus *Melodinus* (Apocynaceae) comprises about 50 species, and is distributed mainly over the tropical or subtropical Asia and Australia (Li et al., 1995). Previous study demonstrated that the genus is rich in monoterpenoid indole alkaloids and a series of alkaloids characterized with structural complexity and promising biological activities have been isolated and identified from this genus (Liu et al., 2012, 2016). As a part of our ongoing research into bioactive indole alkaloids (Guo et al., 2012; Fu et al., 2014a, 2014b; Zhang et al., 2015), two new monoterpenoid indole alkaloids, melohenryines A and B (**1** and **2**), were isolated from the twigs and leaves of *Melodinus henryi*. Reported herein are the isolation and structure elucidation of the new alkaloids **1** and **2**.

2. Results and discussion

Melohenryine A (**1**) had the molecular formula of $C_{21}H_{24}N_2O_4$, as established by HRESIMS (m/z = 369.1798, $[M + H]^+$; calcd. 369.1809), with 11 ° of unsaturation. The IR absorption bands at 1731 and 1632 cm^{-1} implied the presence of ester carbonyl and amide functionalities, respectively. The ^1H and ^{13}C NMR spectra (Table 1) showed 21 carbon signals including two methyls (one methoxy), four sp^3 methylenes, nine methines (six sp^2 carbon atoms), and six quaternary carbon atoms (four sp^2 carbon atoms). Detailed analysis of the NMR data of **1** (Table 1) and the characteristic NMR signals of two methylenes (δ_H 4.14, 4.20 (each 1H), δ_C 61.6; δ_H3.54, 3.80 (each 1H), δ_C 67.8) germinal to nitrogen and one methine (δ_H 4.65 (1H); δ_C 97.0) bonded to nitrogen indicated that **1** was a scandine-type monoterpenoid indole alkaloid and was analogous to those of scandine N_b-oxide (He et al., 1992). The major difference was the absence of a quaternary carbon atom and a monosubstituted double bond in **1**. The key HMBC correlations of CH_3-18 (δ_H 1.28, d, .2 Hz) and H-19 (δ_H 2.62, q, J = 7.2 Hz) with the ester carbonyl (δ_C 173.7) indicated the ester carbonyl was located at C-19. ^1H-^1H COSY correlations of H-16 (δ_H 2.71, dd, J = 14.4, 6.6 Hz, 1H) and H_2-17 (δ_H 2.13, dd, J = 12.6, 7.2 Hz; 2.28, dd, J = 12.6, 6.6 Hz, each 1H), and of CH_3-18 and H-19 verified the above elucidation. Detailed analysis of the 2D NMR spectra (HSQC, ^1H-^1H COSY, and HMBC) confirmed that the other parts of the molecule were the same as those of scandine N_b-oxide. Thus, the planar structure of melohenryine A was assigned as **1** (Fig. 2A), which represents the first example of monoterpenoid indole alkaloids characterized with an ester carbonyl group at C-19 position.

The relative stereochemistry of **1** was elucidated from a ROESY experiment (Fig. 2B). The cross-peaks of H-21/H-19, H-21/CH_3-18 in the ROESY spectrum revealed that H-21 and H-19 are co-facial and were defined arbitrarily as α-orientation. Thus, H-16 was designated as β-orientation due to the tortuous rigidity ring system, which was confirmed by the observation of correlations of H-16/H-5b and H-16/H-6a.

The ECD spectrum of **1** showed positive Cotton effects at 252 ($\Delta\varepsilon$ +5.20), and 207 ($\Delta\varepsilon$ +11.24) and negative Cotton effects at 227 ($\Delta\varepsilon$ −3.71), which were contributed mainly by the quinolin chromophore.

* Corresponding authors at: State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China.

E-mail addresses: zhangyu@mail.kib.ac.cn (Y. Zhang), haoxj@mail.kib.ac.cn (X.-J. Hao).

http://dx.doi.org/10.1016/j.phytol.2017.07.001
Received 9 November 2016; Received in revised form 7 April 2017; Accepted 4 July 2017
Available online 10 July 2017
1874-3900/

Table 1
^{1}H and ^{13}C NMR Data of melohenryines A (**1**) and B (**2**).

	1[a]		**2**[b]	
No.	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2	–	169.4	–	168.2
3a	4.14 (dd, 15.6, 6.0)	61.6	4.10 (d, 17.5)	64.4
3b	4.20 (dt, 15.6, 3.0)		4.17 (dd, 17.5, 3.5)	
5a	3.54 (m)	67.8	3.41 (td, 11.0, 3.5)	69.0
5b	3.80 (ddd, 14.4, 10.8, 5.4)		3.48 (t, 11.0)	
6a	2.13 (dd, 15.0, 6.6)	37.7	2.21 (dd, 14.5, 7.5)	36.1
6b	2.78 (dd, 15.0, 13.2, 5.4)		2.35 (ddd, 14.5, 11.0, 7.5)	
7	–	58.3	–	56.3
8	–	124.4	–	128.4
9	8.05 (d, 7.8)	129.6	7.74 (dd, 7.5, 1.5)	127.2
10	7.21 (td, 7.8, 1.2)	125.2	6.76 (td, 7.5, 1.5)	124.3
11	7.26 (td, 7.8, 1.2)	128.9	6.96 (td, 7.5, 1.5)	129.4
12	6.80 (dd, 7.8, 1.2)	115.7	6.68 (d, 7.5)	116.8
13	–	134.6	–	137.2
14	6.17 (ddd, 10.8, 6.6, 4.8)	125.0	5.65 (ddd, 11.0, 5.0, 3.5)	122.8
15	6.09 (dd, 10.8, 3.0)	134.8	5.79 (d, 11.0)	124.4
16	2.71 (dd, 14.4, 6.6)	48.1	–	68.3
17a	2.13 (dd, 12.6, 7.2)	42.3	1.86 (d, 12.5)	35.0
17b	2.28 (dd, 12.6, 6.6)		2.18 (d, 12.5)	
18	1.28 (d, 7.2)	14.6	0.99 (d, 7.0)	12.0
19	2.62 (q, 7.2)	49.9	1.85 (q, 7.0)	52.5
20	–	51.2	–	50.8
21	4.65 (s)	97.0	3.72 (s)	84.7
22	–	173.7	–	208.1
OCH_3	3.49 (s)	52.1		

[a] ^{1}H NMR measured at 400 MHz, ^{13}C NMR measured at 100 MHz in $CDCl_3$.
[b] ^{1}H measured at 500 MHz, ^{13}C NMR measured at 125 MHz in $CDCl_3$–CD_3OD (3:1).

Moreover, the agreement of ECD curve of **1** with those of melohemsine I (Zhang et al., 2016) allowed the absolute configuration of **1** as 7*R*, 16*S*, 19 *R*, 20*R*, 21*S* (Figs. 1 and 2).

Melohenryine B (**2**) was isolated as a light yellow amorphous powder, the ^{13}C NMR data (Table 1) and its positive HRESIMS signal at *m/z* 337.1538 ($[M + H]^+$, calcd. 337.1547) indicated its molecular formula of $C_{20}H_{20}N_2O_3$. All of the 20 carbon signals were well resolved in the DEPT and ^{13}C NMR spectrum (Table 1), and were classified as one methyl, four methylenes, eight methines, and seven quaternary carbon atoms. Detailed analysis of the NMR data of **2** indicated that **2** shared the same basic skeleton with those of meloscandonine (Plat et al., 1970). The striking differences were the chemical shifts of carbon atoms which are bonded to nitrogen in **2** (C-3, C-5, and C-21) were downshifted to 17.2, 14.2, and 14.8 ppm, respectively, by comparison with those of meloscandonine. The above data demonstrated that **2** was N-oxide form of meloscandonine, which was further confirmed by its molecular formula was larger than that of meloscandonine by 16 mass units. Detailed analysis of 2D NMR (HSQC, ^{1}H-^{1}H COSY, HMBC, and ROESY) data confirmed that the other parts were the same as those of meloscandonine. Thus, melohenryine B (**2**) was assigned as shown in Figs. 3A and B.

Furthermore, the ECD spectrum of **2** showed the similar Cotton effects as **1** and melohemsine I. Thus, its absolute configuration was defined as 7*S*, 16*R*, 19*R*, 20*R*, 21*S* (Fig. 1).

All of the known alkaloids were identified as meloscandonine (**3**) (Plat et al., 1970), 19-epimeloscandonine (**4**) (Guo and Zhou, 1993), 10-hydroxyscandine (**5**) (Zhou et al., 1988), scandine (**6**) (Daudon et al., 1975), scandine N_b-oxide (**7**) (He et al., 1992), and meloscine [8] (Daudon et al., 1975) by comparing their NMR data with the literatures, respectively.

The findings of melodinus-type alkaloids (**1–8**), otherwise known as meloquinolines, are representative secondary metabolites of the *Melodinus* genus, and are in agreement with the previous works on the constituents of the genus (Guo and Zhou, 1993; He et al., 1992; Liu et al., 2012,,2016). They could be considered as a chemotaxonomic marker of *Melodinus* genus from a chemotaxonomic point of view.

The new compounds, **1** and **2**, were evaluated for their cytotoxic activities against five human cancer cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480 by using the MTT method (Reed and Muench, 1938), with cisplatin as a positive control. The results indicated that **1** and **2** were inactive against the above cancer cells (IC_{50} > 40 μM). Although other melodinus-type alkaloids (**3–8**) did not show cytotoxic activities against several human cancer cell lines (Feng et al., 2010; Guo and Zhou, 1993; Liu et al., 2012; Zhang et al., 2016) as well, further studies still need to elucidate their promising bioactivity.

3. Experimental section

3.1. General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC. IR spectra were measured with a Bio-Rad FTS-135 spectrometer from KBr pellets,

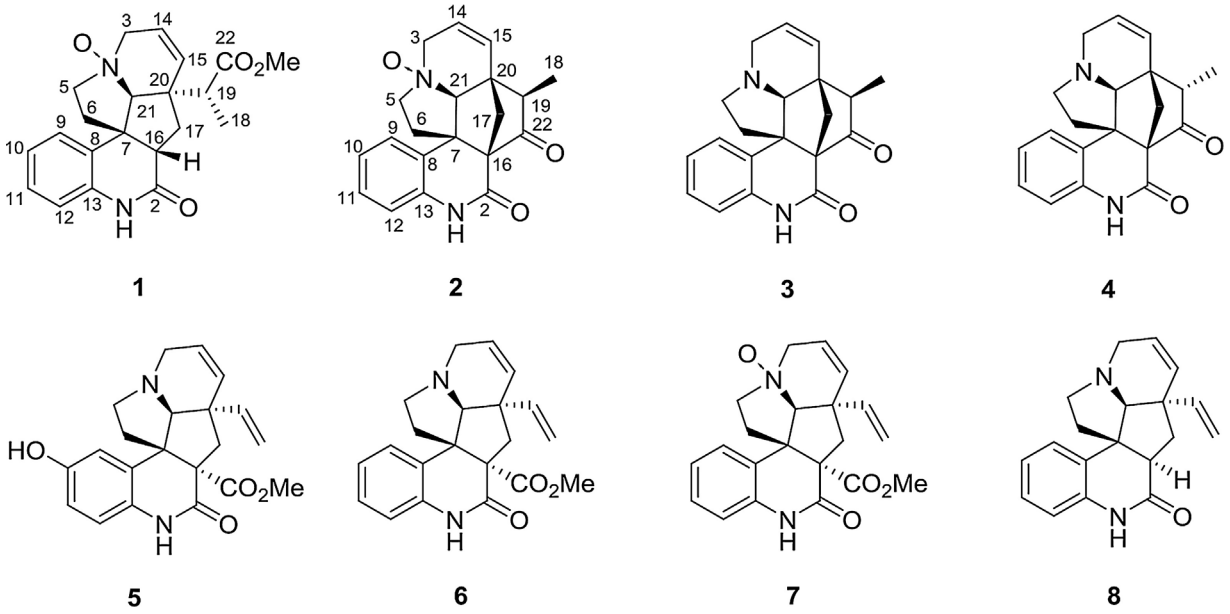

Fig. 1. Structures of compounds **1**-**8**.

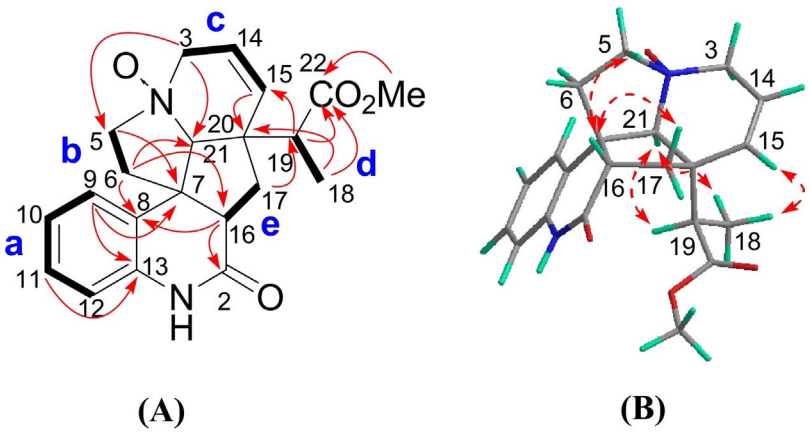

Fig. 2. (A) ^{1}H–^{1}H COSY (bold) and HMBC (arrow, H → C) correlations of **1**. (B) ROESY correlations of **1**.

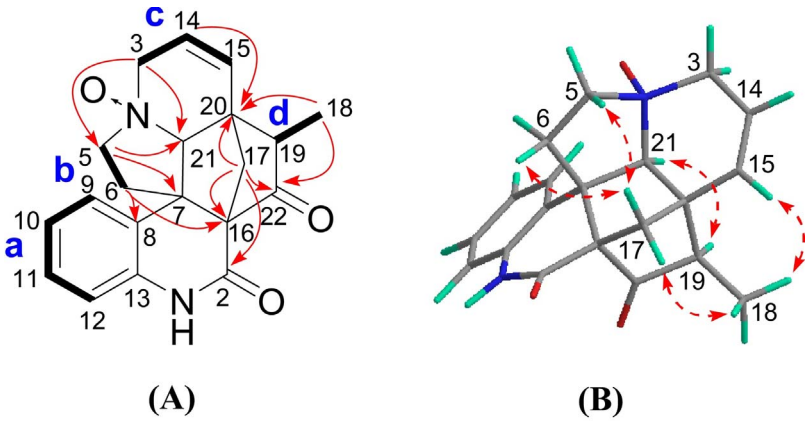

Fig. 3. (A) ^{1}H–^{1}H COSY (bold) and HMBC (arrow, H → C) correlations of **2**. (B) ROESY correlations of **2**.

whereas ECD spectra were recorded with an Applied Photophysics Chirascan spectrometer. ESI and HRESIMS were measured on a Waters Xevo TQ-S instrument and Aglient 1290 UPLC/6540 Q-TOF spectrometer, respectively. 1D and 2D NMR spectra were measured on AM-400, ADVANCE III-500 and AV-600 spectrometers, using TMS as internal standard, and chemical shifts were recorded as δ values. Silica gel (300–400 mesh, Qingdao Marine Chemical Inc., China), Lichroprep RP-18 gel (40–63 μm; Merck, Darmstadt, Germany), and Sephadex LH-20 (40–70 μm, Amersham Biosciences, Sweden), were used for column chromatography.

3.2. Plant material

The twigs and leaves of *Melodinus henryi* were collected in Jinghong, Yunnan Province, People's Republic of China, in October 2010. The material was identified by Dr. Zhi Wang, Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany (KIB). A specimen (No. H20101005) was deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences (CAS).

3.3. Extraction and isolation

The powdered twigs and leaves of *M. henryi* (22.0 kg) were extracted three times with EtOH (95:5, v/v). The combined extracts were concentrated under reduced pressure, and adjusted to pH = 2 ~ 3 with 3% tartaric acid. The acidic mixture was defatted with petroleum ether (PE) and ethyl acetate (EtOAc) and then basified to pH = 9 ~10 with saturated Na_2CO_3. The aqueous phase was subsequently extracted with $CHCl_3$ to give crude alkaloids (60.0 g). The crude alkaloid was then subjected to a silica gel CC (300–400 mesh) using $CHCl_3$-MeOH gradient (1:0 → 0:1) to obtain five fractions (I–V). Fr. II was subjected to silica gel CC eluting with PE-Me_2CO (25:1–5:1) to give **4** (25 mg) and **6** (120 mg). Fr. III was purified by reversed phase chromatography on a C_{18} column (MeOH-H_2O, 30:70–100:0, v/v) to afford three subfractions (C1-C3). Subfraction C2 was further purified by silica gel CC eluting with PE-Me_2CO (15:1-5:1) to give **3** (40 mg) and **7** (120 mg). Fr. IV (11.0 g) was subjected to silica gel CC eluting with PE-Me_2CO-Et_2NH (15:1:0.1-5:1:0.1, v/v), then followed by Sephadex LH-20 (MeOH) to afford **1** (1.6 mg) and **5** (406 mg). Fraction V (9.0 g) was further purified by reversed phase chromatography on a C_{18} column (MeOH-H_2O, 20:80–100:0, v/v) to give four subfractions (D1-D4). Subfraction D2 (1.8 g) was further purified by silica gel CC eluting with $CHCl_3$-MeOH (19:1-8:2, v/v), and then Sephadex LH-20 (MeOH) to yield **2** (14.0 mg) and **8** (3.0 mg).

3.3.1. Melohenryine A (1)

Light yellow amorphous powder; $[\alpha]_D^{25}$ - 51.2 (*c* 0.15, MeOH); UV (MeOH) λmax(log ε) 253 (3.80), 208 (4.33) nm; ECD (0.00015 M, MeOH) $\lambda_{max}(\Delta\varepsilon)$ 207 (+11.24), 227 (-3.71), 252(+5.20) nm; IR (KBr) ν_{max} 3432, 2980, 1731, 1632, and 1223 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; ESIMS *m/z*: 369 $[M + H]^+$; HRESIMS *m/z* 369.1798 $[M + H]^+$ (calcd. for $C_{21}H_{25}N_2O_4$, 369.1809).

3.3.2. Melohenryine B (2)

Light yellow amorphous powder; $[\alpha]_D^{25}$ +57.5 (*c* 0.34, MeOH); UV (MeOH) λmax(log ε) 253 (3.81), 208 (4.27) nm; ECD (0.000133 M, MeOH) λmax($\Delta\varepsilon$) 197 (+17.90), 217 (−10.00), 230 (−7.21), 256 (+13.85) nm; IR (KBr) ν_{max} 3426, 2985, 1750, 1680, and 1383 cm^{-1}; ^{1}H ($CDCl_3$:CD_3OD (3:1), 500 MHz) and ^{13}C NMR ($CDCl_3$:CD_3OD (3:1), 125 MHz) data, see Table 1; ESIMS *m/z*: 337 $[M + H]^+$; HRESIMS *m/z* 337.1538 $[M + H]^+$ (calcd. for $C_{20}H_{21}N_2O_3$, 337.1547).

Acknowledgements

The work was financially supported by the National Natural Science Foundation of China [81473122], the Youth Innovation Promotion Association of CAS[2015323], CAS "Light of West China" Program (grant to Yu Zhang), the Young Academic and Technical Leader Raising Foundation of Yunnan Province (to Yu Zhang), and State Key Laboratory of Phytochemistry and Plant Resources in West China (P2016-ZZ08).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.phytol.2017.07.001.

References

Daudon, M., Mehri, M.H., Plat, M.M., 1975. Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances: XXXIV. Monomeric quinolinic Melodinus alkaloids. J. Org. Chem. 40, 2838–2839.

Dewick, P.M., 2009. Alkaloids. Medicinal Natural Products: a Biosynthetic Approach. John Wiely & Sons Ltd., Chichester, pp. 369–380.

Feng, T., Li, Y., Wang, Y.Y., Cai, X.H., Liu, Y.P., Luo, X.D., 2010. Cytotoxic indole alkaloids from *Melodinus tenuicaudatus*. J. Nat. Prod. 73, 1075–1079.

Fu, Y.H., Di, Y.T., He, H.P., Li, S.L., Zhang, Y., Hao, X.J., 2014a. Angustifonines A and B, cytotoxic bisindole alkaloids from *Bousigonia angustifolia*. J. Nat. Prod. 77, 57–62.

Fu, Y.H., Li, S.L., Li, S.F., He, H.P., Di, Y.T., Zhang, Y., Hao, X.J., 2014b. Cytotoxic eburnamine-aspidosperpermine type bisindole alkaloids from *Bousigonia mekongensis*. Fitoterapia 98, 45–52.

Guo, L.W., Zhou, Y.L., 1993. Alkaloids from *Melodinus hemsleyanus*. Phytochemistry 34, 563–566.

Guo, L.L., He, H.P., Di, Y.T., Li, S.F., Cheng, Y.Y., Yang, W., Li, Y., Yu, J.P., Zhang, Y., Hao, X.J., 2012. Indole alkaloids from *Ervatamia chinensis*. Phytochemistry 74, 140–145.

He, X., Zhou, Y.L., Huang, Z.H., 1992. Study on the alkaloids of *Melodinus fusiformis*. Huaxue xuebao 50, 96–101.

Li, P.T., Leeuwenberg, A.J.M., Middleton, D.J., 1995. Flora of China 16, 147–150.

Liu, Y.P., Li, Y., Cai, X.H., Li, X.Y., Kong, L.M., Cheng, G.G., Luo, X.D., 2012. Melodinines M-U, cytotoxic alkaloids from *Melodinus suaveolens*. J. Nat. Prod. 75, 220–224.

Liu, Y.P., Yue, G.G.L., Lee, J.K.M., Feng, T., Zhao, Y.L., Li, Y., Lau, C.B.S., Luo, X.D., 2016. Melodinine V, an antitumor bisindole alkaloid with selective cytotoxicity from *Melodinus henryi*. Bioorg. Med. Chem. Lett. 26, 4895–4898.

Newman, D.J., Cragg, G.M., 2016. Natural products as sources of new drugs from 1981 to 2014. J. Nat. Prod. 79, 629–661.

Plat, M., Hachem-Mehri, M.K., Scheidegger, U., Potier, P., 1970. Structure et stereochimie de la meloscandonine, alcaloide du *Melodinus scandens* forst. Tetrahedron Lett. 39, 3395–3398.

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent end points. Am. J. Hyg. 27, 493–497.

Zhang, Y., Guo, L.L., Yang, G.M., Guo, F., Di, Y.T., Li, S.L., Chen, D.Z., Hao, X.J., 2015. New vobasinyl-ibogan type bisindole alkaloids from *Tabernaemontana corymbosa*. Fitoterapia 100, 150–155.

Zhang, J., Ding, Y., Huang, X.J., Jiang, R.W., Wang, Y., Sun, P.H., Fan, R.Z., Zhang, X.Q., Ye, W.C., 2016. Melohemsines A-I, melodinus-type alkaloids from *Melodinus hemsleyanus*. RSC Adv. 6, 92218–92224.

Zhou, Y.L., Ye, J.H., Li, Z.M., Huang, Z.H., 1988. Study on the alkaloids of *Melodinus tenuicaudatus*. Planta Med. 54, 315–317.