A Novel Alkaloid from *Melodinus henryi*

by **Hua Zhou**[a])[b]), **Hong-Ping He**[b]), **Yue-Hu Wang**[b]), and **Xiao-Jiang Hao***[b])

[a]) Department of Food Science and Engineering, Jinan University, Guangzhou 510632, P. R. China
[b]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China
(phone: +86-871-5223263; fax: +86-871-5219684; e-mail: haoxj@mail.kib.ac.cn)

The isolation and structure elucidation of a novel alkaloid, namely of the 14-*O*-ethyl-substituted (3α,14α,16α)-2,7-secoeburnamine derivative **1** from the leaf of *Melodinus henryi* is reported. Ten known alkaloids were also isolated. Their structures were determined spectroscopically. The isolates were evaluated for their cytotoxicity.

Introduction. – Plants of the genus *Melodinus* are being used in Chinese folk medicine for the treatment of meningitis in children and rheumatic heart diseases [1]. Many indole alkoids have been isolated from related plants [2–4], but there has been no previous work on chemical components of *Melodinus henryi*. To discover the active compounds in this species, studies on the alkaloids of *Melodinus henryi* were carried out. The present article deals with the isolation and structure elucidation of a novel alkaloid, namely of the 14-*O*-ethyl-substituted (3α,14α,16α)-2,7-secoeburnamine derivative **1** (*Fig. 1*) together with ten known compounds: (+)-eburnamine ((3α,14α,16α); **2** *Fig. 1*) [5], 14-epieburnamine (=(−)-isoeburnamine; (3α,14β,16α)) [5], (±)-condylocarpine [6], (±)-isocondylocarpine [6], rhazinilam [7], vincamenine [8], akuammicine [9], norfluorocurarine [9], 10,22-dioxokopsane [10], and stemmadenine [11].

1
2,7-Seco derivative of (+)-eburnamine

2
(+)-Eburnamine (3α,14α,16α)

Fig. 1. *Alkaloids* **1** *and* **2** *isolated from* Melodinus henryi

Results and Discussion. – *Structure Elucidation.* Alkaloid **1** was shown to have the molecular formula $C_{21}H_{28}N_2O_3$ on the basis of HR-ESI-MS data (*m*/*z* 379.1993 ([*M* + Na]$^+$)), which indicated nine degrees of unsaturation. The ^{13}C-NMR and DEPT data

displayed signals of two Me, eight CH_2, and six CH groups, and five quaternary C-atoms. The H-atom signals at δ(H) 7.64 (*dd*, $J = 7.8$, 1.0 Hz, H–C(9)), 7.44 (*dt*, $J = 7.8$, 1.0 Hz, H–C(10)), 7.47 (*dt*, $J = 7.8$, 1.0 Hz, H–C(11)), and 7.29 (*dd*, $J = 7.8$, 1.0 Hz, H–C(12)), and C-atom signals at δ(C) 138.2 (C(8)), 124.8 (C(9)), 128.1 (C(10)), 131.5 (C(10)), 126.4 (C(11)), and 139.8 (C(13)) in the ^{1}H- and ^{13}C-NMR spectra are characteristic for the presence of an *ortho*-substituted benzene moiety. Downfield signals in the ^{13}C-NMR spectrum at δ(C) 204.1 and 172.8 suggested the presence of a ketone C=O and amide functionality, respectively. ^{1}H,^{1}H-COSY Cross-peaks were observed between CH_2(20) and Me(21), and between CH_2(1′) and Me(2′) which allowed the assignment of two Et side chains. The HMBC data (*Fig. 2*) revealed correlations of δ(H) 2.92 and 3.05 (CH_2(5)) with δ(C) 44.8 (C(6)) and 204.1 (C(7)) and of δ(H) 1.85 and 1.32 (CH_2(18)) and δ(C) 56.3 (C(19)), which indicated the presence of the fragment: C(7)–C(6)–C(5)–N(4)–C(19)–C(18), which was further supported by the ^{1}H,^{1}H-COSY cross-peaks δ(H) 2.86/2.41 (CH_2(19)) and δ(H) 1.85/1.32 (CH_2(6)). A quaternary C-atom at δ(C) 172.8 (C(2)) showed HMBC cross-peaks to δ(H) 2.66 (H–C(3)) and allowed the assignment of the fragment N(4)–C(3)–C(2)–N(1).

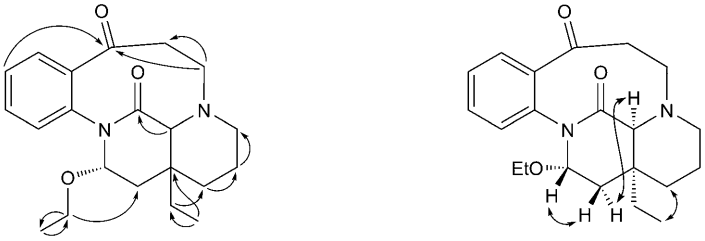

Fig. 2. *Selected HMBC* (H → C) *and ROESY correlations* (H ↔ H) *of* **1**

The relative configuration of alkaloid **1** was determined through a 2D-ROESY NMR experiment. Some selected ROESY correlations are shown in *Fig. 2:* H–C(14)/H_β–C(15), H_α–C(15)/H–C(3), and CH_2(17)/Me(21).

By comparison with (+)-eburnamine (**2**) [5], the difference was that both C(2) and C(7) have been transformed into C=O groups in **1** accompanying the fracture of the former bond, besides the replacement of the OH by an EtO group at C(14). To the best of our knowledge, alkaloid **1** is a novel natural product and was assigned as '(3α,14α,16α)-14-*O*-ethyl-2,7-dioxo-2,7-secoeburnamine'.

Biological Studies. All alkaloids were evaluated for cytotoxicity by using the WT cell. None of the alkaloids showed a significant effect. Only 2,7-secoeburnamine derivative **1** exhibited moderate cytotoxic activity.

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Experimental Part

General. Solvents were distilled before use. TLC and column chromatography (CC): precoated plates with silica gel F_{254} and silica gel *H* (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China), resp. Optical rotations: *Horiba-SEAP-300* spectropolarimer. UV Spectra: *Shimadzu-210A* double-beam spectrometer; λ_{max} in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AM-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI- and HR-ESI-MS: *VG-AUTO-spec-3000* spectrometer; in *m*/*z* (rel. %).

Plant Material. The leaves of *Melodinus henryi* were collected in Xishuangbanna (Yunnan Province of China) in February 2004 and were air-dried. The dried leaves (6.0 kg) were ground and extracted with 95% acetone (4 × 2 l during 4, 3, 2 and 1 h, resp.). The extract was filtered and concentrated and the residue extracted with $CHCl_3$. The $CHCl_3$ extract (30 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 1:0, 9:1, 8:2, 7:3, and 1:1): *Fractions 1–4*. *Fr. 1* mainly contained **1** (10 mg), rhazinilam (15 mg), and vincamenine (21 mg). *Fr. 2* was subjected to repeated CC (SiO_2, AcOEt/petroleum ether 2:5): (±)-condylocarpine (25 mg), (±)-isocondylocarpine (12 mg), and akuammicine (25 mg). *Fr. 3* was purified further by CC (SiO_2, AcOEt/MeOH 5:1): norfluorocurarine (10 mg), 10,22-dioxokopsane (35 mg), (+)-eburnamine (**2**; 15 mg), and 14-epieburnamine (25 mg). Stemmadenine (18 mg) was isolated from *Fr. 4* by CC (SiO_2, $CHCl_3$/MeOH 5:2).

(3α,14α,16α)-14-Ethoxy-14,15-dihydro-2,7-secoeburnamenine-2,7-dione (=*(6a*S,*7*S,*1b*S*)-16-Ethoxy-7-ethyl-7,8,9,10,12,13-hexahydro-14*H-*5,7-ethanopyrido[2,1-*c*][1,4]benzodiazonine-6,14(6a*H*)-dione*; **1**): White powder. $[\alpha]_D^{27.6} = +126.6$ ($c = 0.65$, $CHCl_3$). UV: 330, 317, 302, 241. IR (KBr): 3435, 2924, 2852, 1691, 1632, 1599. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.64 (*dd*, *J* = 7.8, 1.0, H–C(9)); 7.47 (*dt*, *J* = 7.8, 1.0, H–C(11)); 7.44 (*dt*, *J* = 7.8, 1.0, H–C(10)); 7.29 (*dd*, *J* = 7.8, 1.0, H–C(12)); 5.12–5.14 (*m*, H–C(14)); 3.55–3.57 (*m*, 1 H–C(1′)); 3.42–3.44 (*m*, 1 H–C(1′)); 3.05 (*m*, 1 H–C(5)); 2.92–2.94 (*m*, 1 H–C(5)); 2.86 (*m*, 1 H–C(19)); 2.82 (*m*, 1 H–C(6)); 2.67 (*m*, 1 H–C(6)); 2.66 (*m*, H–C(3)); 2.41 (*t*, *J* = 17.0, 1 H–C(19)); 1.85–1.87 (*m*, 1 H–C(18)); 1.75–1.77 (*m*, H–C(15)); 1.68–1.69 (*m*, H–C(17); 1.58–1.60 (*m*, 2 H–C(20)); 1.32–132 (*m*, 1 H–C(18)); 1.28 (*t*, *J* = 2.0, Me(2′)); 1.25 (*m*, 1 H–C(15)); 1.09 (*m*, 1 H–C(17)); 0.91 (*t*, *J* = 7.5, Me(21)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 172.8 (*s*, C(2)); 74.4 (*d*, C(3)); 53.4 (*t*, C(5)); 44.8 (*t*, C(6)); 204.1 (*s*, C(7)); 138.2 (*s*, C(8)); 124.8 (*d*, C(9)); 128.1 (*d*, C(10)); 131.5 (*d*, C(11)); 126.4 (*d*, C(12)); 139.8 (*s*, C(13)); 89.5 (*d*, C(14)); 30.1 (*t*, C(15)); 36.2 (*s*, C(16)); 32.8 (*t*, C(17)); 31.7 (*t*, C(18)); 56.3 (*t*, C(19)); 22.4 (*t*, C(20)); 7.3 (*q*, C(21)); 63.5 (*t*, C(1′)); 15.1 (*q*, C(2′)). EI-MS: 356 (40, M^+), 327 (100), 283 (65), 241 (40). HR-ESI-MS: 379.1993 ($[M + Na]^+$, $C_{21}H_{28}N_2NaO_3^+$; calc. 379.1997).

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