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).

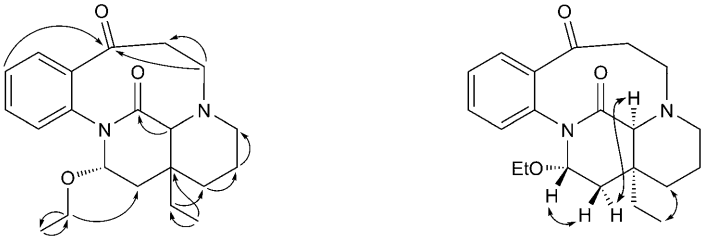

Fig. 2. *Selected HMBC* (H → C) *and ROESY correlations* (H ↔ H) *of* **1**

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.

This work was financially supported by the *NSFC* of China to *X.-J. H.* (No. 39525025) and the *Project of University Science Achievement of Guangdong Province* (cgzhzd0709). All spectra were recorded by the analytical group of the Laboratory of Photochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, and the cytotoxicity tests of all compounds by using the WT cell were performed by Mr. *Quan Chen* at the Institute of Zoology (IOZ), Chinese Academy of Sciences (CAS).

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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Received January 17, 2010