# Structures and Absolute Configurations of Three 7,8-Secolabdane Diterpenes from the Chinese Liverwort *Pallavicinia ambigua*

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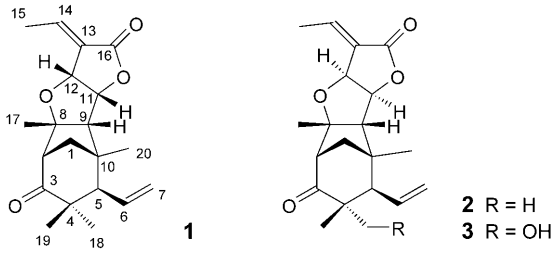
The known 7,8-secolabdane type diterpenoids neopallavicinin (**1**), pallavicinin (**2**), and 18-hydroxypallavicinin (**3**) were isolated from *Pallavicinia ambigua*, and their structures were determined. The X-ray crystal structure of **1** was solved, and, in combination with CD and NMR studies, the absolute configurations of **1**–**3** were established. A possible biogenetic pathway for **1** and **2** from a single labdane precursor is proposed.

**Introduction.** – Many sesqui- and diterpenoids as well as aromatic compounds isolated from liverworts show interesting biological activities such as antifungal, antimicrobial, cytotoxic, and insect-antifeedant effects [1][2]. During our search for biologically active substances from bryophytes, the three secolabdane diterpenoids **1**–**3** were isolated from the Chinese liverwort *Pallavicinia ambigua* (MITT.) STEPH. [3–6]. These compounds have been isolated before, and named neopallavicinin (**1**), pallavicinin (**2**), and 18-hydroxypallavicinin (**3**) [7–9]. Here, we report their absolute configurations, as determined on the basis of NMR and circular-dichroism (CD) analyses.




**Results and Discussion.** – Neopallavicinin (**1**) was obtained as colorless prisms. It had the molecular formula $C_{20}H_{26}O_4$, as shown by high-resolution mass spectrometry (HR-MS), the molecular ion being observed at *m/z* 330.1830 ($M^+$; calc. 330.1831), consistent with 20 observed $^{13}$C-NMR signals. Complete NMR data analysis disclosed the structure of a 7,8-secolabdane diterpenoid. The relative configuration of **1** was confirmed by X-ray analysis (*Fig. 1*).

Pallavicinin (**2**) was found to be a stereoisomer of **1** by comparison of its NMR data with those reported earlier [7]. Compound **3** was determined to be a congener of **2**, with an additional OH group at C(18), as revealed by comparing its $^1$H- and $^{13}$C-NMR spectroscopic data with those of **1** and **2**.

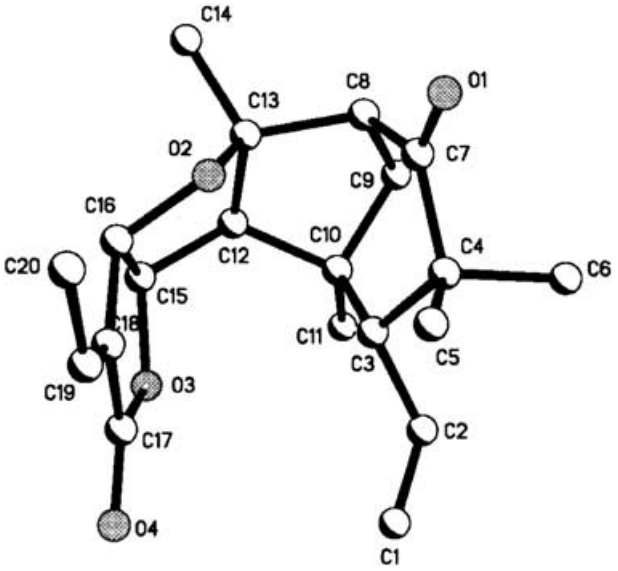



Fig. 1. *X-Ray crystal structure of neopallavicinin* (**1**). ORTEP drawing.

In the CD spectra of **1** and **2**, uniform negative *Cotton* effects were observed, with a maximum at 304 nm due to the n → $\pi^*$ transition of the non-conjugated C=O group in 3-position (*Fig. 2*). This corroborated that both **1** and **2** had the same configuration with respect to the cyclohexanone ring as that reported for compound **3** [8]. The $\pi \rightarrow \pi^*$ transition of the lactone moiety gave rise to a negative *Cotton* effect at *ca.* 225 nm for **1**, but positive effects at 230 nm for both **2** and **3**. This implied that the lactone rings in **2** and **3** had an inverted fusion geometry relative to **1** [10]. Furthermore, the positive *Cotton* effect with a maximum near 250 nm (n → $\pi^*$) supported a *cis*-fused $\gamma$-lactone for compound **1** [11]. From these data, the absolute configurations of all

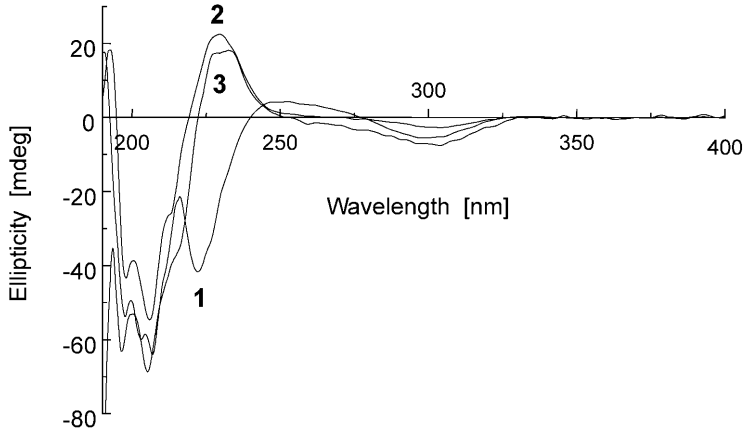
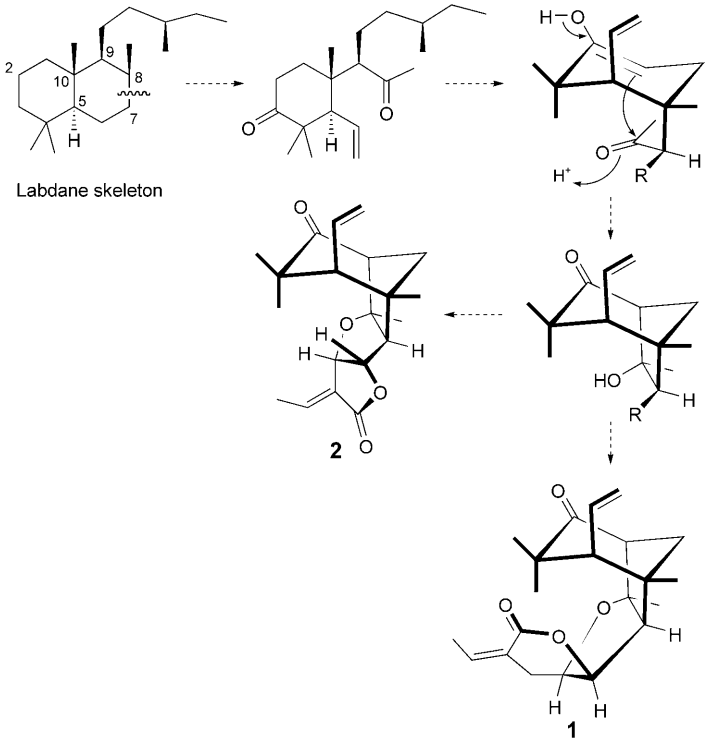



Fig. 2. *Circular dichroism spectra of compounds* **1**–**3**

three compounds could be established (see the systematic names in the *Exper. Part*). They may be derived from the same labdane precursor; and a possible biogenetic pathway is shown in the *Scheme*.

Scheme. *Possible Biosynthetic Pathway to Compounds* **1** *and* **2**




## Experimental Part

*General*. All solvents were of anal. grade (*Tianjin No. 1 Chemical Reagent Factory*), and petroleum ether (PE) had a b.p. of 60–90°. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical, Inc.*, China) or *Sephadex LH-20* (*Pharmacia*). Melting points (m.p.): *X*-6 melting-point apparatus (*Beijing TECH Instrument Co., Ltd*). CD Spectra: *JASCO J-810* spectropolarimeter; $\lambda$ [nm] ($\Delta\varepsilon$). IR Spectra: *Nexus 470* FT-IR spectrometer; in $cm^{-1}$. NMR Spectra: *Bruker Avance-600* spectrometer, at 600 ($^1H$) or 150 MHz ($^{13}C$); $\delta$ in ppm rel. to $Me_4Si$, *J* in Hz. HR-MS: *Finnigan MAT-95* mass spectrometer; in *m/z*.

*Plant Material. Pallavicinia ambigua* was collected in July 2003 in Mount Wuyi, Fujian province, P.R. China, and identified by Prof. *Chien Gao*, Shenyang Institute of Applied Ecology, Chinese Academy of Sciences.

*Extraction and Isolation.* The mechanically ground, air-dried plant material (1.25 kg) was successively extracted with $Et_2O$ (4×) and MeOH (4×) at r.t. The combined $Et_2O$ extracts were evaporated (30 g), and then subjected to CC ($SiO_2$; PE/acetone gradient) to furnish six fractions (Fr.). *Fr. 3* and *Fr. 4*, eluted with PE/acetone 100:2, were further purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford **2** (52 mg) and **1** (39 mg), resp. *Fr. 6*, eluted with PE/acetone 100:3, was re-subjected to CC ($SiO_2$; PE/acetone 100:4), and the resulting major fraction was further purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford **3** (20 mg).

*Neopallavicinin* (=*(1*S*,2*S*,3*R*,6*E*,7*R*,9*R*,10*S*,13*R*)-13-Ethenyl-6-ethylidene-1,9,12,12-tetramethyl-4,8-dioxatetracyclo[8.3.1.0$^{2,9}$.0$^{3,7}$]tetradecane-5,11-dione*; **1**). Colorless prisms. M.p. 190.5–192.4°. $[\alpha]_D^{20} = -209$ (*c* =

1.71 mg/ml, $CHCl_3$). CD ($CHCl_3$, $c = 9.2$ mg/ml): 206.9 (−5.62), 216.2 (−1.89), 222.1 (−3.66), 249.5 (+0.35), 298.4 (−0.49). EI-MS (70 eV): 331 (13), 330 (64), 315 (54), 220 (79), 167 (86), 107 (85), 83 (100). HR-MS: 330.1830 ($M^+$, $C_{20}H_{26}O_4^+$; calc. 330.1831).

*Pallavicinin* (= *(1S,2S,3S,6E,7S,9R,10S,13R)-13-Ethenyl-6-ethylidene-1,9,12,12-tetramethyl-4,8-dioxatetracyclo[8.3.1.0$^{2,9}$.0$^{3,7}$]tetradecane-5,11-dione*; **2**). Colorless prisms. M.p. 219–221°. $[\alpha]_D^{20} = +12$ ($c = 1.56$ mg/ml, $CHCl_3$). CD ($CHCl_3$, $c = 8.9$ mg/ml): 206 (−4.79), 230 (+2.04), 304 (−0.25). HR-MS: 330.1833 ($M^+$, $C_{20}H_{26}O_4^+$; calc. 330.1831).

*18-Hydroxypallavicinin* (= *(1S,2S,3S,6E,7S,9R,10S,12R,13R)-13-Ethenyl-6-ethylidene-12-(hydroxymethyl)-1,9,12-trimethyl-4,8-dioxatetracyclo[8.3.1.0$^{2,9}$.0$^{3,7}$]tetradecane-5,11-dione*; **3**). Colorless granular crystal ($CHCl_3$). $[\alpha]_D^{20} = -5$ ($c = 1.26$ mg/ml, $CHCl_3$). CD ($CHCl_3$, $c = 6.6$ mg/ml): 205 (−2.73), 230 (+0.72), 304 (−0.3). $^1$H-NMR (600 MHz, $CDCl_3$): 7.03 (*dq*, $J = 7.2$, 1.7, H–C(14)); 5.85 (*dt*, $J = 10.2$, 16.8, H–C(6)); 5.66 (*d*, $J = 6.2$, H–C(12)); 5.20 (*dd*, $J = 1.5$, 9.9, $H_a$–C(7)); 5.05 (*dd*, $J = 1.1$, 16.8, $H_b$–C(7)); 5.0 (*d*, $J = 6.4$, H–C(11)); 3.77 (*d*, $J = 11.1$, $H_a$–C(18)); 3.24 (*d*, $J = 11.1$, $H_b$–C(18)); 2.72 (*d*, $J = 4.4$, H–C(2)); 2.38 (*s*, 18-OH); 2.36 (*s*, H–C(9)); 2.31 (*d*, $J = 10.5$, $H_a$–C(1)); 1.96 (*d*, $J = 7.2$, Me(15)); 1.74 (*m*, $H_b$–C(1), H–C(5)); 1.38 (*s*, Me(17)); 1.27 (*s*, Me(20)); 1.25 (*s*, Me(19)). $^{13}$C-NMR (150 MHz, $CDCl_3$): 214.6 (C(3)); 169.1 (C(16)); 144.2 (C(14)); 135.6 (C(6)); 129.1 (C(13)); 118.1 (C(7)); 95.1 (C(8)); 81.3 (C(11)); 80.3 (C(12)); 70.1 (C(9)); 66.7 (C(18)); 60.1 (C(2)); 50.3 (C(5)); 44.0 (C(4)); 43.3 (C(10)); 40.2 (C(1)); 31.9 (C(17)); 22.7 (C(19)); 25.0 (C(20)); 15.8 (C(15)). EI-MS (70 eV): 346 (20), 316 (100), 287 (45), 219 (55). HR-MS: 346.1800 ($M^+$, $C_{20}H_{26}O_5^+$; calc. 346.1780).

*Crystallographic Data for Compound* **1**[1]). Formula, $C_{20}H_{26}O_4$; crystal system, tetragonal; space group, $P4_32_12$; lattice parameters: $a = 10.7648(17)$, $b = 10.7648(17)$, $c = 30.823(16)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 3572(2)$ Å$^3$, $Z = 8$; crystal size, $0.46 \times 0.41 \times 0.38$ mm. A total of 1900 unique reflections were collected, using graphite-monochromated Mo$K_\alpha$ radiation (0.71073 Å) on a *Bruker P4* diffractometer. The structure was solved by direct methods (SIR-97), and refined by full-matrix-least-squares techniques based on $F^2$, with $R = 0.0611$, $wR_2 = 0.1355$.

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[1]) The crystallographic data of **1** have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication number CCDC-253300. Copies of the data can be obtained, free of charge, *via* internet (http://www.ccdc.cam.ac.uk/data_request/cif), e-mail (data_request@ccdc.cam.ac.uk) or fax (+44-1223-336033).