New Secodaphnane-Type Alkaloids with Cytotoxic Activities from Daphniphyllum angustifolium Hutch

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Abstract
One new Daphniphyllum alkaloid, daphnioldhanol A (1), together with three known ones, were isolated from the stem part of Daphniphyllum angustifolium Hutch. Their structures were elucidated by spectroscopic methods and comparing with the literature data. Compound 2 is a new natural product, but known by synthesis as a racemate. Compound 1 exhibited weak cytotoxic activity against Hela cell line with IC50 of 31.9 μM.

1 Introduction
Alkaloids are a class of compounds with significant activities (with a variety of novel skeletons) that are widely found in nature [1–7]. Daphniphyllum alkaloids are a structurally diversified group of complex polycyclic natural products isolated from the Daphniphyllum genus [8]. Since these unique, versatile and complex nitrogen heterocyclic compounds exhibit a wide range of biological activities and are extremely challenging, they have aroused great interest in total synthesis and biosynthetic studies [9–16]. In recent years, quite a number of new Daphniphyllum alkaloids have been isolated and identified, and some of them possessed novel skeletons [17–20]. In our continued search for Daphniphyllum alkaloids with interesting skeletons [21–24], one new Daphniphyllum alkaloid, daphnioldhanol A (1), together with three known ones, were isolated from the stems of Daphniphyllum angustifolium Hutch (Fig. 1).
Herein, the isolation, structural elucidation, and bioactivities of these compounds are reported.

2 Results and Discussion

2.1 Structure Elucidation of the Compounds

Daphnioldhanol A (1) was obtained as white amorphous powder. Its molecular formula, C_{32}H_{48}NO_{5}, was established by positive HRESIMS at m/z 526.3534 [M+ H]^+ (calcld 526.3534), with 10 degrees of unsaturation. The IR absorptions implied the presence of hydroxyl (3441 cm\(^{-1}\)), and an imine moiety (1631 cm\(^{-1}\)). The 13C NMR and DEPT data of 1 displayed 32 carbon signals (Table 1), due to three tetrasubstituted sp\(^2\) carbon atoms at lower field and 29 sp\(^3\) carbon atoms (5 × C, 6 × CH, 11 × CH\(_2\), 6 × CH\(_3\)) at higher field. According to the molecular formula and relative NMR data, one CH group (δ\(_{C}\) 65.4, δ\(_{H}\) 2.95) was ascribed to those bearing an N-atom, while one quaternary C-atom (δ\(_{C}\) 84.4) were attributed to those bearing an O-atom. Additionally, three sp\(^2\) quaternary carbons were attributable to one lactone carbonyl (δ\(_{C}\) 180.2), one ester carbonyl (δ\(_{C}\) 172.2), and one iminium group (δ\(_{C}\) 168.4), while taking into account the three degrees of unsaturation. The remaining seven degrees of unsaturation were accounted for the presence of the heptacyclic system of 1.

The \(^1\)H and \(^{13}\)C NMR spectra of 1 were closely related to those of the known compound Daphnioldhanine I [25], with the exception of the loss of signal for a CH in the latter and the addition of signal for quaternary carbon with a hydroxyl (δ\(_{C}\) 84.4), which were supported by the HMBC correlations of H-11/C-9, H-13/C-9, H-15/C-9, H-16a/C-9, H-17/C-9.

To determine the orientation of the hydroxyl at C-9, we compared the 13C NMR data of both 1 and daphnioldhanine I, which revealed that 9-OH substituent significantly shields the C-21 (5 ppm decrease) in the former. This indicated that the 9-OH in 1 should take a β-orientation. Moreover, the remaining relative configuration of 1 was elucidated from ROESY correlations as shown in computer-generated 3D drawing, which was the same as that of the daphnioldhanine I (Fig. 2).

The known compounds were identified as (−)-nitrone 17 (2) [26], daphnilactone A (3) [27], dapholdhamine B (4) [28], respectively, by comparison of their spectroscopic data with those reported in the literature (Fig. 1). Compound 2 was obtained as white amorphous powder. MS analysis of 2 revealed a [2 M + H]^+ peak at m/z 747. By comparison of its \(^1\)H and \(^{13}\)C NMR data with those of (±)-nitrone 17 in the literature, high similarity between them indicated that 2 shared the same structure as the latter. However, the compound 2 is a new chiral natural product with OR at −31.75°, but known by synthesis is racemate.

Table 1 \(^1\)H and \(^{13}\)C NMR spectroscopic data for compound 1\(^a\) (δ in ppm and J in Hz)

| No. | δ\(_{C}\) | δ\(_{H}\) (Mult. J) | No. | δ\(_{C}\) | δ\(_{H}\) (Mult. J) |
|-----|---------|------------------|-----|---------|------------------|
| 1   | 168.4   | –                | 16a| 54.1    | 3.67 (dt, 14.4, 3.6) |
| 2   | 40.5    | 1.47 (o)         | 16b| 2.50 (d, 15) |
| 3a  | 33.7    | 2.09 (m)         | 17a| 43      | 2.89 (m)         |
| 3b  | 1.08    | (m)              | 17b| 2.23 (d, 4.2) |
| 4a  | 38.4    | 2.06 (m)         | 18 | 32.5    | 1.68 (m)         |
| 4b  | 1.61    | (m)              | 19 | 21.9    | 0.96 (d, 6.6)    |
| 5   | 38.7    | –                | 20 | 22.4    | 1.02 (d, 6.6)    |
| 6   | 55.2    | 2.73 (t, 9.0)    | 21 | 27.3    | 1.13 (s)         |
| 7   | 65.4    | 2.95 (s)         | 22 | 58.1    | 1.86 (m)         |
| 8   | 54.3    | –                | 23 | 51.6    | –                |
| 9   | 84.4    | –                | 24 | 18.8    | 1.20 (s)         |
| 10  | 54.2    | –                | 25 | 180.2   | –                |
| 11a | 35      | 2.64 (dd 12, 4.8)| 26 | 72.1    | 4.77 (d, 4.8)    |
| 11b | 1.10    | (m)              | 27a| 26.9    | 1.85 (o)         |
| 12a | 30.3    | 1.85 (o)         | 27b| 1.61 (o) |
| 12b | 1.52 (dd, 11.4, 5.4)| 28a| 27    | 1.85 (o) |
| 13a | 28.2    | 1.95 (m)         | 28b| 1.61 (o) |
| 13b | 1.69    | (m)              | 29 | 87.7    | –                |
| 14a | 27.9    | 1.77 (o)         | 30 | 24.8    | 1.47 (o)         |
| 14b | 1.17    | (m)              | 31 | 171.2   | –                |
| 15  | 44.4    | 1.77 (o)         | 32 | 22      | 2.13 (s)         |

\(^a\)Recorded in Methanol-\(d_4\) at 800 MHz (\(^1\)H) and 200 MHz (\(^{13}\)C)

Fig. 1 The structures of compounds 1–4

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A plausible biogenetic pathway for 1 and 2 was proposed as shown in Scheme 1. Biogenetically, both 1 and 2 should be the derivatives of secodaphnane-type alkaloid [13, 29], which might be originated from sequalene, as proposed from Heathcock [26]. Then, 1 and 2 might be formed via different pathway.

2.2 Cytotoxic Activity

Both compounds 1 and 2 have been tested for their cytotoxicity against Hela, MCF-7, A549, MGC-803 and COLO-205 human cancer cell lines in vitro. The results indicated that 1 exhibited weak cytotoxic activity against Hela cell line with IC$_{50}$ of 31.9 μM (Table 2).

| Human cancer cell lines | Compound 1 | Doxorubicin |
|-------------------------|------------|-------------|
| Hela                    | 31.9       | 0.77        |
| MCF-7                   | $>$ 76     | 1.57        |
| A549                    | 52.2       | 1.92        |
| MGC-803                 | 69.7       | 1.05        |
| COLO-205                | 71.8       | 2.23        |

3 Experimental

3.1 General Experimental Procedures

Optical rotations were measured with a Jasco P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for IR spectra as KBr pellets. 1D and 2D NMR spectra were recorded on Bruker spectrometer with TMS as internal standard. HRESIMS was performed on a triple quadrupole mass spectrometer. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Waters X-Bridge Prep Shield RP18 (10 × 150 mm) column. Column chromatography (CC) was performed using silica gel (100–200 mesh and 300–400 mesh, Qingdao Marine Chemical, Inc., Qingdao, P. R. China) and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden).
3.2 Plant Material

The stems of *Daphniphyllum Angustifolium* used in this study was collected from Jinfo mountain, Chongqing, P. R. China, in October 2013, and botanically authenticated by professor Deng Hong-ping. A voucher specimen (KIB-HAO2014012) was deposited at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

Air-dried stems of *Daphniphyllum Angustifolium* (20 kg) were powdered and extracted with MeOH (24 h × 3) at room temperature, and the solvent was evaporated in vacuo. The MeOH extract was then partitioned between EtOAc and TFA/H2O at pH 3.0. Water-soluble materials, after being adjusted at pH 10.0 with saturated Na2CO3, were partitioned with CHCl3. CHCl3-soluble materials (100.6 g) was subjected to silica gel column chromatography (CC) and eluted with gradient CHCl3/MeOH to yield five fractions F-1–F-5. F-4 was repeatedly submitted to silica gel CC and Sephadex LH-20, then purified by HPLC to afford compounds 1 (18.0 mg) and 2 (2.0 mg). Accordingly, 3 (18.0 mg) was obtained from F-1; 4 (2.5 mg) was obtained from F-5.

3.4 Daphnioldhanol A (1)

Daphnioldhanol A (1): White amorphous powder; C32H47NO5; Positive HR-ESI-MS at m/z 526.3534 [M + H]+ (calcd. for C32H48NO5, 526.3527); [α]D20 = +9.88° (c = 0.54, MeOH); UV (MeOH) λmax (log ε) 265 (3.43) nm, 242 (3.56) nm, 215 (3.93) nm; IR: νmax (KBr) cm–1: 3440, 2928, 2869, 1772, 1743, 1714, 1661, 1383, 1226, 1028. 

3.5 Nitrone 17 (2)

(−)-Nitrone 17 (2): Colorless oil; C23H35NO3; ESI-MS (positive): m/z 747 [2 M + H]+; 1H NMR (CDCl3, 400 MHz) δH: 3.72 (3H, s), 1.58 (3H, s), 1.02 (1H, d, 6.16), 0.94 (3H, s), 0.85 (3H, d, 6.48); 13C NMR (CDCl3, 100 MHz) δC: 157.2 (C-1), 48.8 (C-2), 27.0 (C-3), 39.0 (C-4), 51.7 (C-5), 52.5 (C-6), 84.1 (C-7), 50.9 (C-8), 52.5 (C-9), 52.9 (C-10), 33.4 (C-11), 22.7 (C-12), 26.2 (C-13), 31.6 (C-14), 25.7 (C-15), 37.0 (C-16), 38.9 (C-17), 31.5 (C-18), 21.0 (C-19), 20.6 (C-20), 23.3 (C-21), 174.1 (C-22), 51.9 (C-23).

3.6 Cytotoxicity Assays

Cytotoxic activity of compound 1 against Hela, MCF-7, A549, MGC-803, and COLO-205 human cancer cell lines in vitro were measured using methylthiazol tetrazolium (MTT) assay [30]. Doxorubicin was used as a positive control.

4 Concluding Remarks

In conclusion, one new *Daphniphyllum* alkaloid, daphnioldhanol A (1), together with three known ones, were isolated from the stem part of *D. angustifolium* Hutch. Compound 1 exhibited week cytotoxic activity against Hela cell line.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13659-021-00309-w.

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Declarations

Conflict of interest Authors declare that there is no conflict of interest.

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