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# Himalensines A and B, Alkaloids from *Daphniphyllum himalense*

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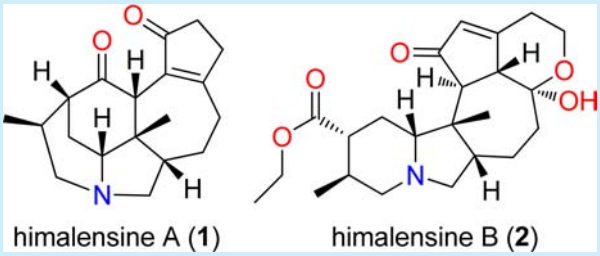
Supporting Information

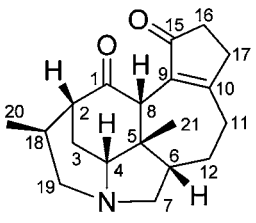
**ABSTRACT:** Chemical investigation into the alkaloidal constituents of the Nepalese *Daphniphyllum himalense* has returned two new compounds, himalensines A (**1**) and B (**2**), with unprecedented carbon skeletons. Structures of the two alkaloids have been characterized on the basis of spectroscopic methods, especially via 2D NMR data analysis. Himalensine B (**2**) showed marginal inhibitory activities against two kinases, PTP1B and IKK-$\beta$.



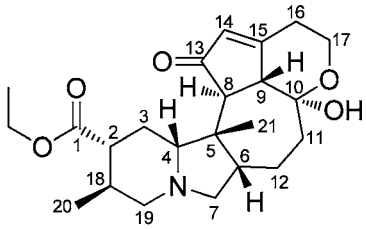

*Daphniphyllum* alkaloids, with highly complex structures and various bioactivities, are a class of natural products produced by the Daphniphyllaceae plants. Due particularly to their diverse and intriguing carbon skeletons, these alkaloids have been investigated intensively since the 1960s, with over 200 members reported to date.[1] Although studies from natural products chemists slowed down a bit after the first prosperous decade of this century, efforts from synthetic chemists have accelerated because of increasing understanding of the chemistry of this structure family.[2−10]

*Daphniphyllum himalense* (Benth.) Muell.-Arg. only grows in the southern lower regions of the Himalaya Mountains[11] and has been studied less than other species[12−14] owing to difficulties in the collection of plant material. Recently, we carried out a project aiming to explore bioactive molecules from Nepalese medicinal plants and reported a series of new hydroxylated *Daphniphyllum* alkaloids from *D. himalense*.[15] As a continuation of that early work, we have obtained from the less polar fractions two new additional alkaloids, himalensines A (**1**) and B (**2**), whose structures were assigned via detailed spectroscopic data analyses. Alkaloid **1** features a 13,14,22-trinorcalyciphylline A type backbone, while **2** incorporates a 22-nor-1,13-secodaphnicyclidin framework with the ethyl group being likely introduced during the extraction process. The two compounds were screened in a panel of kinase inhibitory assays [protein tyrosine phosphatase 1B (PTP1B), aurora kinase A, histone deacetylase 6 (HDAC6), and inhibitor of $\kappa$ light polypeptide gene enhancer in B-cells, kinase $\beta$ (IKK-$\beta$)]. We herein present the isolation, structure elucidation, and biological evaluation of the two novel alkaloids.

The crude alkaloids (12.3 g) were prepared from the ethanolic percolation of the twigs and leaves of *D. himalense* via a standard acid−base procedure.[15] Twelve fractions were obtained from a subsequent fractionation of this alkaloid extract over silica gel, eluted with a trinary solvent system of petroleum ether−EtOAc−$HNEt_2$. Himalensines A (**1**, 1.6 mg) and B (**2**, 2.1 mg) were isolated from the fifth and sixth fractions, respectively, via the same process of silica gel column chromatography and semipreparative HPLC.



**1**



**2**

Himalensine A (**1**) was obtained as white amorphous powder. The molecular formula of $C_{19}H_{25}NO_2$ was established by $^{13}C$ NMR (Table 1) and (+)-HRESIMS (*m*/*z* 300.1959 [M + H]$^+$, calcd 300.1964) data, indicative of eight degrees of unsaturation (DOU). The IR absorption bands at 1696 and 1641 cm$^{-1}$ suggested the presence of carbonyl and olefinic functionalities, respectively. Analysis of the NMR data (Table 1) for **1** revealed 19 carbon signals that were attributable to two methyls, seven sp$^3$ methylenes, five sp$^3$ methines, and five quaternary ones (two olefinic and two carbonyls), as evidenced by DEPT and HSQC experiments (Figures S2 and S4, Supporting Information). One double bond and two carbonyl groups occupied three DOU, and a pentacyclic architecture was considered to be responsible for the remaining five DOU.

Further examination of $^1H-^1H$ COSY data enabled the establishment of four structural fragments as shown in Figure 1: (a) $H_2$-19 to $H_3$-20 via H-18, (b) $H_2$-2 to H-4, (c) $H_2$-7 to $H_2$-11 via H-6 and $H_2$-12, and (d) $H_2$-16 to $H_2$-17. These partial structures were further connected to accomplish the full assignment of the planar skeleton for **1** by inspection of HMBC

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Table 1. $^{1}H$ and $^{13}C$ NMR Data for Alkaloids 1 and 2

| | 1[a] | | 2[b] | | 2[c] | |
|---|---|---|---|---|---|---|
| no. | $\delta_C$ | $\delta_H$, mult ($J$, Hz) | $\delta_C$ | $\delta_H$, mult ($J$, Hz) | $\delta_C$ | $\delta_H$, mult ($J$, Hz) |
| 1 | 214.4 | | 177.3 | | 176.0 | |
| 2 | 46.1 | 2.04, brs | 51.7 | 2.09, ddd (11.3, 11.3, 3.2) | 51.4 | 2.24, ddd (11.1, 11.1, 3.2) |
| 3 | 20.3 | a 2.14, dd (14.8, 3.5) | 27.7 | $\beta$ 1.94, ddd (12.4, 3.2, 3.2) | 28.1 | $\beta$ 2.59, m |
| | | b 2.08, brdd (14.8, 4.9) | | $\alpha$ 1.48, ddd (12.8, 12.4, 11.3) | | $\alpha$ 1.88, m |
| 4 | 65.1 | 3.52, brs | 69.3 | 2.91, dd (12.8, 3.2) | 69.6 | 3.05, dd (11.8, 2.9) |
| 5 | 48.3 | | 48.4 | | 48.0 | |
| 6 | 48.8 | 2.30, m | 52.2 | 1.89, m | 52.0 | 1.84, m |
| 7 | 54.9 | $\beta$ 2.92, m | 57.7 | $\beta$ 3.09, dd (9.5, 8.2) | 58.1 | $\beta$ 3.03, m |
| | | $\alpha$ 2.80, m | | $\alpha$ 2.62, dd (9.5, 6.4) | | $\alpha$ 2.62, m |
| 8 | 51.7 | 3.65, s | 51.1 | 2.90, d (3.9) | 50.6 | 3.43, d (4.3) |
| 9 | 138.5 | | 53.5 | 2.84, brd (3.9) | 53.1 | 3.02, m |
| 10 | 179.9 | | 99.1 | | 98.8 | |
| 11 | 28.1 | a 2.60, m | 43.3 | $\alpha$ 1.88, m | 43.8 | $\alpha$ 2.18, m |
| | | b 2.23, m | | $\beta$ 1.69, m | | $\beta$ 1.93, m |
| 12 | 22.9 | 1.80, m, 2H | 30.71[d] | $\alpha$ 1.77, m | 30.35[e] | $\alpha$ 2.17, m |
| | | | | $\beta$ 1.51, m | | $\beta$ 1.45, m |
| 13 | | | 211.8 | | 209.3 | |
| 14 | | | 128.9 | 5.84, brs | 128.7 | 6.02 dd, (1.9, 1.9) |
| 15 | 208.7 | | 178.7 | | 176.3 | |
| 16 | 34.6 | a 2.49, ddd (18.9, 6.5, 2.5) | 30.73[d] | 2.68, m, 2H | 30.40[e] | a 2.63, m |
| | | b 2.43, ddd (18.9, 6.5, 2.5) | | | | b 2.57, m |
| 17 | 32.0 | a 2.71, m | 59.9 | a 4.15, m | 59.2 | a 4.40, ddd (12.0, 10.9, 3.5) |
| | | b 2.64, m | | b 3.84, m | | b 3.91, ddd (10.9, 7.0, 1.3) |
| 18 | 31.8 | 2.74, m | 27.3 | 2.02, m | 27.9 | 2.20, m |
| 19 | 49.6 | $\alpha$ 2.82, dd (14.1, 7.1) | 56.3 | $\alpha$ 2.95, dd (13.6, 4.0) | 56.6 | $\alpha$ 2.95, dd (13.0, 3.8) |
| | | $\beta$ 2.56, dd (14.1, 10.3) | | $\beta$ 2.43, dd (13.6, 11.4) | | $\beta$ 2.36, dd (13.0, 10.8) |
| 20 | 19.2 | 0.99, d (6.8) | 17.2 | 0.81, d (6.1) | 17.5 | 0.82, d (6.0) |
| 21 | 26.3 | 1.12, s | 25.7 | 1.01, s | 26.1 | 1.25, s |
| OEt | | | 14.7 | 1.26, t (7.1) | 14.7 | 1.09, t (7.1) |
| | | | 61.7 | 4.14, m, 2H | 60.6 | 4.13, dq (10.2, 7.1) |
| | | | | | | 4.06, dq (10.2, 7.1) |
| OH | | | | | | 7.77 (s) |

[a]In $CDCl_3$. [b]In $CD_3OD$. [c]In $C_5D_5N$. [d,e]Interchangeable assignments

data (Figure 1). In detail, the HMBC correlations from $H_3$-20 to C-18 and C-2, from $H_2$-19 to C-4 and C-7, and from $H_2$-7 to C-4 facilitated the construction of ring B as drawn, while those from H-18 and H-8 to C-1 and from $H_3$-21 to C-4, C-5, C-6 and C-8 established rings A and C. Finally, the correlations from H-8 to C-9, C-10, and C-15, from H-11 and $H_2$-17 to C-10, and from $H_2$-16 to C-15, fulfilled the linkage of rings D and E.

The relative configuration of **1** was assigned on the basis of NOESY data (Figure 1). The strong NOESY correlations of $H_3$-21 with H-4, H-6, and H-8 were supportive of their cofacial relationship, and they were determined to be $\beta$-oriented as those in calyciphylline A type alkaloids.[1] Subsequent observation of the correlations for H-8 with H-3a and H-3b with H-19$\beta$ suggested a boatlike conformation for rings A and B, and then the remarkable NOESY correlations of $H_3$-20 with H-19$\beta$ and H-2 confirmed the relative configurations at C-2 and C-18 as shown. The structure of himalensine A (**1**) was thus unambiguously characterized to possess a novel 13,14,22-trinorcalyciphylline A type skeleton. To the best of our knowledge, it is the first natural $C_{19}$ *Daphniphyllum* alkaloid reported so far.

Himalensine B (**2**) was assigned a molecular formula of $C_{23}H_{33}NO_5$ based on $^{13}C$ NMR data (Table 1) and the (+)-HRESIMS ion at $m/z$ 404.2443 ($[M + H]^+$, calcd 404.2437), incorporating eight DOU same as **1**. The IR spectrum indicated the presence of carbonyl (1729 and 1700 $cm^{-1}$) and olefinic (1628 $cm^{-1}$) groups. Analysis of the NMR data in $CD_3OD$ (Table 1) for **2** revealed diagnostic resonances for an ethoxyl ($\delta_C$ 61.7 and 14.7), an ester carbonyl ($\delta_C$ 177.3), and an $\alpha,\beta$-conjugated ketone ($\delta_C$ 211.8, 178.7, and 128.9). The remaining carbon signals were attributable to two methyls, seven $sp^3$ methylenes (one oxygenated), six $sp^3$ methines, and two $sp^3$ quaternary carbons by DEPT and HSQC experiments (Figures S11 and S13, Supporting Information). A hydroxyl of a hemiketal group ($\delta_C$ 99.1) was also deducible from the aforementioned analyses, which was further supported by the $^{1}H$ NMR data measured in $C_5D_5N$ (Table 1) with a singlet proton signal resolved at $\delta_H$ 7.77. Similar to **1**, these observations also required **2** to bear a pentacyclic ring system.

Besides the ethyl group described above, four additional structural moieties were observed and are depicted below by inspection of $^{1}H-^{1}H$ COSY data in $CD_3OD$ (Figure 2): (b) H-2 to H-4 and to $H_2$-19 via H-18($H_3$-20), (c) $H_2$-7 to $H_2$-11 via H-6 and $H_2$-12, (d) $H_2$-16 to $H_2$-17, and (e) H-8 to H-9. The planar structure of **2** was then fully established via careful examination of HMBC data in $CD_3OD$ (Figure 2), which successfully linked these structural fragments. The ethoxycarbonyl fragment was attached to C-2 as supported by the HMBC correlations from $CH_3CH_2$ and H-2 to the ester



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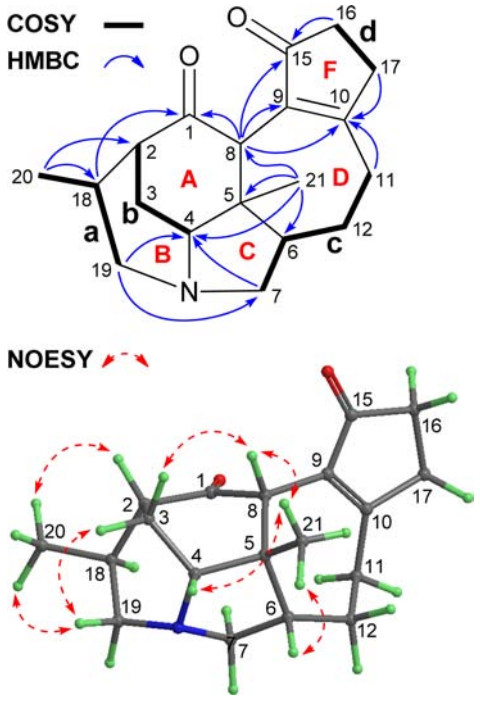



**Figure 1.** Key 2D NMR correlations for **1**.

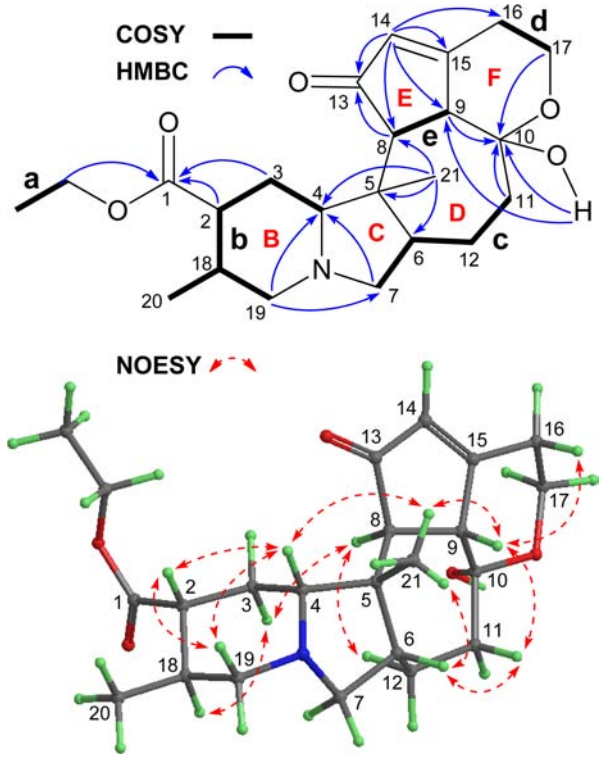


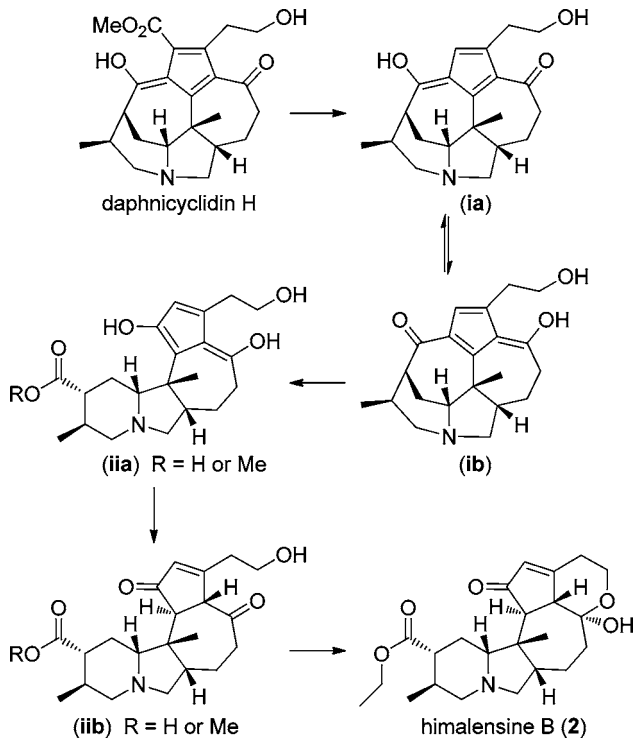

**Figure 2.** Key 2D NMR correlations for **2**.

carbonyl carbon at $\delta_C$ 177.3, and ring A was then constructed via the correlations from $H_2$-19 to C-4 and C-7 and from $H_2$-7 to C-4. Furthermore, ring B with a methyl group at C-5 was confirmed by the HMBC correlations from $H_3$-21 to C-4, C-5, C-6, and C-8. Subsequent observation of correlations from the hydroxyl proton to C-9 and C-10 and from H-9 and $H_2$-11 to C-10 facilitated the construction of ring C as shown, which also supported the location of the hemiketal at C-10. Lastly, the establishment of rings D and E was accomplished by the HMBC correlations from $H_2$-17 to C-10, from H-8 to C-13, and from H-14 to C-8, C-9, C-13, C-15, and C-16.

The relative configuration of **2** was assigned mainly via analyses of proton couplings and NOESY data in $C_5D_5N$ (Figure 2). H-4, H-6, and Me-21 were considered to be coplanar and $\beta$-directed as those in **1** based on the strong NOESY correlations of $H_3$-21 with H-4 and H-6. Then the correlation pairs of H-4/H-2, H-2/H-19$\beta$, and H-19$\beta$/H-4 supported that ring A adopted a typical chairlike conformation and that all three protons were $\beta$-axially located. Similarly, the chairlike conformation of ring C and the $\beta$-positions of H-9 and H-11$\beta$ were determined via the correlation pairs of H-6/H-11$\beta$, H-11$\beta$/H-9, H-9/$H_3$-21, and $H_3$-21/H-6. The $\alpha$-axial orientations of H-3$\alpha$ and H-18 were inferred from their large coupling constants with H-2 ($J_{2,3\alpha}$ = $J_{2,18}$ = 11.1 Hz), while the NOESY correlations of H-8 with both H-3$\alpha$ and H-12$\alpha$ indicated an $\alpha$-direction for H-8. The $\alpha$-orientation of 10-OH was indicated by a weak correlation with H-12$\alpha$ and was further confirmed by the pyridine-induced chemical shift effect of H-8 ($\Delta\delta_H$ 0.53) and H-12$\alpha$ ($\Delta\delta_H$ 0.40) as compared with the data measured in $CD_3OD$ (Table 1).[16] Therefore, the structure of **2** was elucidated to bear a 22-nor-1,13-secodaphnicyclidin skeleton.

Considering the presence of the ethyl group, himalensine B (**2**) might be a solvolysis product during the percolating process of the plant material, and originally, it could exist as a carboxylic acid or the corresponding methyl ester. The biosynthetic origin of **2** could be a daphnicyclidin-type alkaloid such as daphnicyclidin H[17] (Scheme 1), which after decarboxylation would yield a pair of key tautomers (**ia** and **ib**). The tautomer **ib** could undergo Baeyer–Villager oxidation and ring opening to return the new skeleton in the form of a carboxylic acid or its methyl ester (**iib**), and subsequent

**Scheme 1. Proposed Biosynthetic Pathway for 2**



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esterification or ester interchange reaction in the extracting solvent EtOH would yield alkaloid **2**.

The in vitro inhibitory effects of the two alkaloids against four kinases, PTP1B, aurora A, HDAC6, and IKK-$\beta$, were screened at a preliminary concentration of 20 $\mu$g/mL (Table S1, Supporting Information), whereas only **2** exhibited weak activities against PTP1B and IKK-$\beta$ with inhibitory rates of 31% and 29%, respectively.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00362.

Experimental section and raw spectroscopic data including IR, MS, and NMR spectra for alkaloids **1** and **2** (PDF)

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

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