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# Hitorins A and B, Hexacyclic $C_{25}$ Terpenoids from *Chloranthus japonicus*

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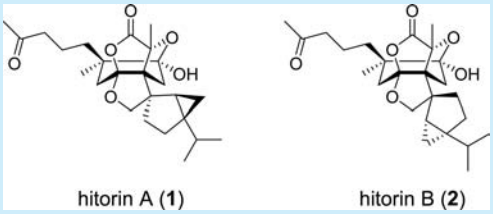
Supporting Information

**ABSTRACT:** Two novel $C_{25}$ terpenoids with a 6/5/5/5/5/3 hexacyclic skeleton including one γ-lactone ring and two tetrahydrofuran rings, hitorins A (**1**) and B (**2**), were isolated from the aerial parts of *Chloranthus japonicus*. The structures of **1** and **2** were elucidated on the basis of spectroscopic analyses as well as TDDFT ECD calculations. Hitorins A (**1**) and B (**2**) might be biogenetically derived from eudesmane sesquiterpene and thujane monoterpene.




The plants belonging to the genus *Chloranthus* (Chloranthaceae) are recognized as a rich source of sesquiterpenoids. Among them, dimers of lindenane sesquiterpene possessing a 18-membered ring are structurally unique and exhibited intriguing biological activities such as inhibition of cell adhesion,[1] delayed rectifier $K^+$ current inhibition,[2] tyrosinase inhibition,[3] hepatoprotective activity,[4] and anti-HIV activity.[5]

*Chloranthus japonicus* (hitorishizuka in Japanese) has been used as a traditional medicine by the Ainu people, an ethnic minority living in Hokkaido, Japan, for the treatment of gastrointestinal disorders. In our search for structurally interesting natural products from *Chloranthus* plants, we have previously reported the isolation of some dimers of lindenane sesquiterpene from the roots of *C. spicatus*.[6] As part of this program, the constituents of the aerial parts of *C. japonicus* were investigated, resulting in the isolation of two novel $C_{25}$ terpenoids, hitorins A (**1**) and B (**2**). In this paper, we describe the isolation and structure elucidation of **1** and **2**.

The dried aerial parts of *C. japonicus* (962 g) were extracted with MeOH to give a crude extract, which was partitioned with EtOAc and $H_2O$. Repeated chromatographic separations of the EtOAc-soluble material using Toyopearl HW-40C, silica gel, and MCI-gel CHP20/P120 columns afforded a terpenoid-containing fraction. The fraction was purified by ODS HPLC and silica gel preparative TLC to give hitorins A (**1**, 0.00042%) and B (**2**, 0.00037%).

Hitorin A (**1**)[7] was obtained as an optically active colorless amorphous solid {$[\alpha]_D$ +90.5 (*c* 0.20, MeOH)}. The molecular formula of **1** was established to be $C_{25}H_{36}O_6$ by HRESIMS (*m*/*z* 455.24074 $[M + Na]^+$, Δ −0.22 mmu), corresponding to eight degrees of unsaturation. The $^1H$ NMR spectrum displayed the signals of one $D_2O$-exchangeable proton, three tertiary methyls, and one isopropyl group (Table 1), while the $^{13}C$ NMR spectrum revealed the presence of 25 carbons including one ketone carbonyl carbon, one ester carbonyl carbon, two acetal carbons, five $sp^3$ quaternary carbons, two $sp^3$ methines, nine $sp^3$ methylenes, and five methyls (Table 1). These findings were indicative of hitorin A (**1**) to be a $C_{25}$ terpenoid.

The planar structure of **1** consisting of two partial units (units A and B) was elucidated by analysis of the 2D NMR spectra including the $^1H-^1H$ COSY, HSQC, and HMBC spectra (Figure 1). In unit A, the presence of a cyclohexane ring (C-5−C-10) was suggested by HMBC correlations for $H_2$-6 to C-5, C-7, C-8, and C-10, $H_2$-9 to C-8, and $H_3$-14 to C5, C9, and C-10. The existence of a 2-pentanone moiety was revealed by $^1H-^1H$ COSY cross-peaks of $H_2$-2 to $H_2$-1 and $H_2$-3 and HMBC correlations for $H_3$-15 to C-3 and C-4 as well as an IR absorption at 1703 $cm^{-1}$.

HMBC correlations for $H_3$-14 to C-1, C-5, C-9, and C-10 indicated that the 2-pentanone moiety and a methyl group (Me-14) were connected to C-10. The connectivities among C-7, C-12, and C-13 via C-11 were disclosed by HMBC cross-peaks of $H_3$-13 to C-7, C-11, and C-12. The chemical shifts of C-11 ($\delta_C$ 82.2) implied the presence of an oxygen functionality at C-11, whereas C-5 ($\delta_C$ 107.4) and C-8 ($\delta_C$ 117.2) were assigned as hemiacetal and acetal carbons, respectively, by their chemical shifts and an HMBC correlation for 5-OH to C-5. The presence of a γ-lactone ring (C-7, C-8, C-11, and C-12) was deduced by an IR absorption at 1794 $cm^{-1}$. Thus, the planar structure of unit A was assigned as shown in Figure 1, corresponding with 4,5-*seco*-eudesmane sesquiterpene.

In unit B, three spin systems ($H_2$-2′/$H_2$-3′, H-5′/$H_2$-6′, and $H_3$-8′/H-7′/$H_3$-9′) were revealed by interpretation of the $^1H-^1H$ COSY spectrum. The presence of a bicyclo[3.1.0]-

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Table 1. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR Data for Hitorins A (1) and B (2) in $CDCl_3$

| | 1 | | 2 | |
|---|---|---|---|---|
| position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 1.46, 1.19 (each 1H, m) | 33.9 | 1.47, 1.21 (each 1H, m) | 33.6 |
| 2 | 1.55, 1.49 (each 1H, m) | 18.4 | 1.58 (2H, m) | 18.3 |
| 3 | 2.41 (2H, t, $J$ = 7.2) | 44.3 | 2.40 (2H, t, $J$ = 7.3) | 44.2 |
| 4 | | 209.4 | | 209.7 |
| 5 | | 107.4 | | 107.0 |
| 6 | 2.15, 1.84 (each 1H, d, $J$ = 12.5) | 36.4 | 2.36, 2.03 (each 1H, d, $J$ = 13.0) | 37.0 |
| 7 | | 64.1 | | 62.8 |
| 8 | | 117.2 | | 117.8 |
| 9 | 2.24 (1H, d, $J$ = 15.5), 1.48 (1H, m) | 41.6 | 2.30, 1.61 (each 1H, d, $J$ = 15.4) | 40.5 |
| 10 | | 40.5 | | 40.5 |
| 11 | | 82.2 | | 82.6 |
| 12 | | 172.6 | | 172.9 |
| 13 | 1.49 (3H, s) | 17.3 | 1.45 (3H, s) | 18.8 |
| 14 | 1.00 (3H, s) | 23.8 | 1.04 (3H, s) | 23.9 |
| 15 | 2.11 (3H, s) | 30.0 | 2.10 (3H, s) | 29.8 |
| 1′ | | 38.1 | | 30.7 |
| 2′ | 1.89, 1.54 (each 1H, m) | 31.9 | 1.69 (1H, dd, $J$ = 12.3, 7.7), 1.57 (1H, m) | 26.8 |
| 3′ | 1.53, 1.14 (each 1H, m) | 26.4 | 1.47 (1H, m), 1.28 (1H, dd, $J$ = 12.8, 7.5) | 25.7 |
| 4′ | | 52.2 | | 52.4 |
| 5′ | 1.13 (1H, dd, $J$ = 8.3, 3.3) | 28.5 | 1.08 (1H, m) | 30.7 |
| 6′ | 0.51 (1H, dd, $J$ = 8.3, 5.4), 0.07 (1H, dd, $J$ = 5.4, 3.3) | 8.0 | 0.43, 0.43 (each 1H, m) | 12.1 |
| 7′ | 1.88 (1H, m) | 29.7 | 1.36 (1H, sept, $J$ = 6.8) | 31.9 |
| 8′ | 0.93 (3H, d, $J$ = 6.7) | 17.9 | 0.90 (3H, d, $J$ = 6.8) | 19.8 |
| 9′ | 0.56 (3H, d, $J$ = 6.7) | 21.3 | 0.87 (3H, d, $J$ = 6.8) | 19.5 |
| 10′ | 3.97, 3.58 (each 1H, d, $J$ = 8.8) | 78.2 | 3.73, 3.57 (each 1H, d, $J$ = 9.0) | 77.5 |
| 5-OH | 2.58 (1H, s) | | 2.50 (1H, s) | |

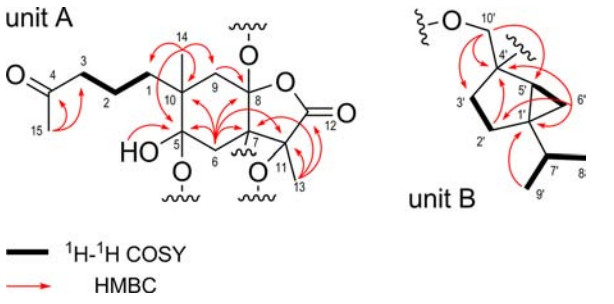


Figure 1. Selected 2D NMR correlations for two partial structures (units A and B) of hitorin A (1).

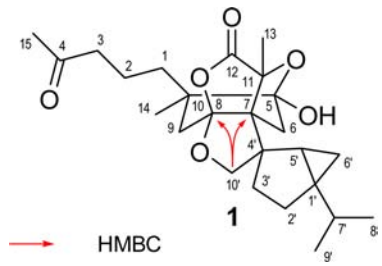


Figure 2. Connectivity between units A and B and the planar structure of hitorin A (1).

hexane moiety was disclosed by HMBC correlations for $H_2$-2′ to C-4′ and $H_2$-6′ to C-1′, C-2′, and C-4′.

An isopropyl group at C-1′ and a hydroxymethyl group at C-4′ were suggested by HMBC cross-peaks of $H_3$-9′ to C-1′ and $H_2$-10′ to C-3′, C-4′, and C-5′. Therefore, unit B was assigned as shown in Figure 1, corresponding with thujane monoterpene.

The connectivity between units A and B through a direct C−C bond (C-7 to C-4′) and an ether linkage (C-8 to C-10′) was revealed by HMBC correlations for $H_2$-10′ to C-7 and C-8, while the degree of unsaturation and molecular formula suggested the presence of a tetrahydrofuran ring (C-5−C-7 and C-11) connected C-5 to C-11 via an ether linkage. Therefore, the planar structure of hitorin A (1) was established as shown in Figure 2.

The relative configuration of hitorin A (1) was elucidated as follows. A steric restriction of the fused tetracyclic core of 1 implied the 11$R$* configuration and the boat conformation of the cyclohexane ring (C-5−C-10). A ROESY correlation for H-6$\alpha$ to $H_3$-14 supported the conformation, and indicated the $R$* configuration of C-10. ROESY correlations for $H_3$-13 to H-5′ and $H_3$-8′ disclosed that these protons were located on the same side of the molecule. Therefore, the relative configurations for C-1′, C-4′, and C-5′ were assigned as $R$*, $R$*, and $S$*, respectively, which were underpinned by a ROESY correlation for H-6′$\alpha$ to H-10′$\beta$. Thus, the relative configuration of hitorin A (1) was concluded as shown in Figure 3.

Hitorin B (2)[8] was isolated as an optically active colorless amorphous solid {$[\alpha]_D$ + 63.7 ($c$ 0.19, MeOH)}. A pseudomolecular ion peak at $m/z$ 455.24155 ($[M + Na]^+$, Δ + 0.60 mmu) in the HRESIMS was indicative of the molecular formula of 2 to be $C_{25}H_{36}O_6$, which was identical to that of 1. Though the 1D NMR spectra of 2 were similar to those of 1, subtle differences were found for the chemical shifts of $H_2$-6′ and isopropyl group (Table 1), suggesting 2 to be a diastereomer of 1. This was supported by analysis of the 2D NMR spectra (Figure S15).



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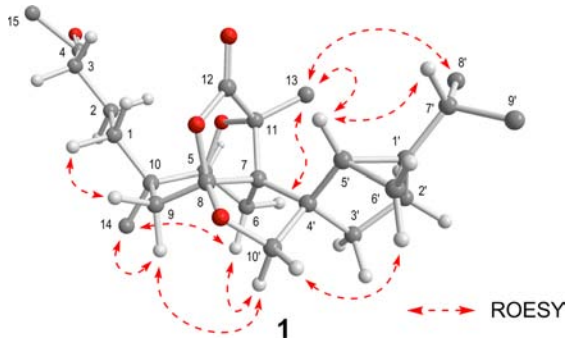



**Figure 3.** Key ROESY correlations and the relative configuration for hitorin A (**1**) (protons of methyl groups were omitted).

In the ROESY spectrum of **2**, correlations for $H_3$-14/H-6$\alpha$, $H_3$-14/H-9$\alpha$, H-6$\beta$/$H_3$-13, $H_2$-10′/H-5′, and $H_2$-10′/$H_3$-8′ were observed like **1**. Therefore, **2** was concluded to be 4′-epimer of **1**, which was supported by ROESY cross-peaks of H-5′/ H-6$\alpha$ and H-5′/ H-9$\alpha$ (Figure 4).

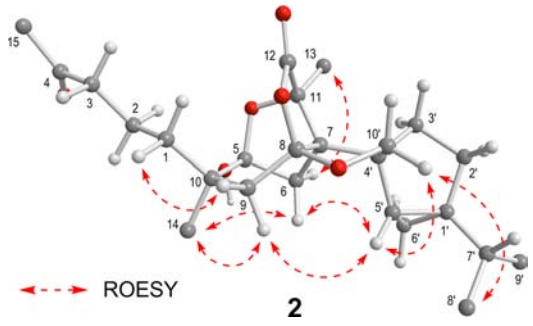



**Figure 4.** Key ROESY correlations and the relative configuration for hitorin B (**2**) (protons of methyl groups were omitted).

The absolute configurations of **1** and **2** were deduced by theoretical calculations of electronic circular dichroism (ECD) spectra predicted from time dependent density functional theory (TDDFT).[9] The ECD curves for two pair of enantiomers were calculated on the basis of the relative configurations established by ROESY experiments (5*S*,7*R*,8*S*,10*R*,11*R*,1′*R*,4′*R*,5′*S* and 5*R*,7*S*,8*R*,10*S*,11*S*,1′*S*,4′*S*,5′*R* for **1** and 5*S*,7*R*,8*S*,10*R*,11*R*,1′*R*,4′*S*,5′*S* and 5*R*,7*S*,8*R*,10*S*,11*S*,1′*S*,4′*R*,5′*R* for **2**). In EDC spectra of **1** and **2**, negative Cotton effects were observed at 232 (Figure 5) and 233 nm (Figure S16), respectively. The calculated ECD curves of 5*S*,7*R*,8*S*,10*R*,11*R*,1′*R*,4′*R*,5′*S* and 5*S*,7*R*,8*S*,10*R*,11*R*,1′*R*,4′*S*,5′*S* matched well with the experimental ECD curves of **1** and **2**, respectively.

Though a variety of $C_{25}$ sesterterpenoids have been reported from natural sources,[10] hitorins A (**1**) and B (**2**) are structurally unique $C_{25}$ terpenoids possessing an unprecedented 6/5/5/5/5/3 hexacyclic skeleton including one $\gamma$-lactone ring and two tetrahydrofuran rings. Possible biogenetic pathways of hitorins A (**1**) and B (**2**) are proposed as shown in Scheme 1. After condensation of atractylenoide III,[11] a known eudesmane sesquiterpene, and sabinene,[12] a known thujane monoterpene, the oxidative cleavage of $\Delta^{4(5)}$ may give possible biogenetic intermediate (**X**) with a 4,5-*seco*-eudesmane sesquiterpene moiety. Successive intermolecular cyclization might give hitorins A (**1**) and B (**2**).

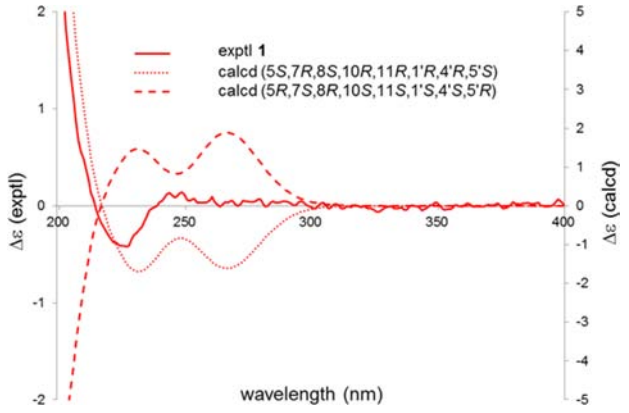
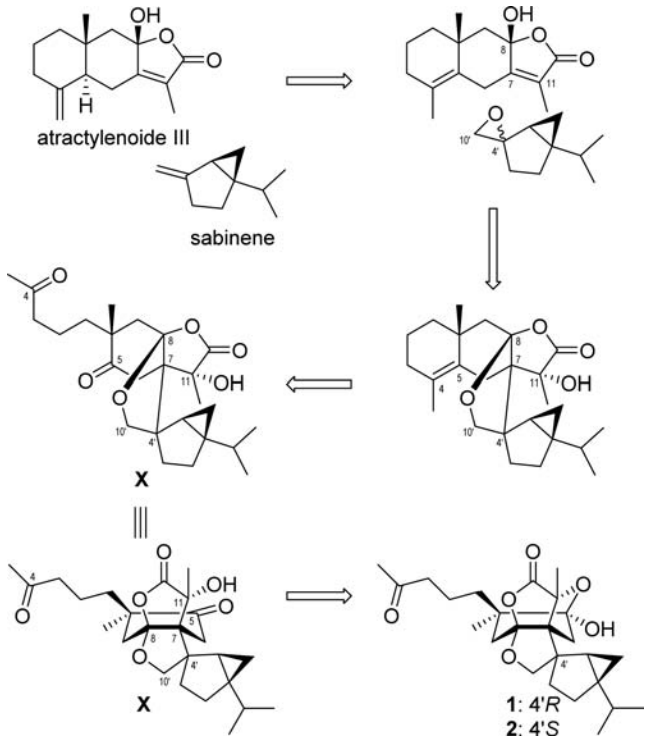



**Figure 5.** Calculated and experimental ECD spectra of hitorin A (**1**).

**Scheme 1. Possible Biogenetic Pathways of Hitorins A (1) and B (2)**




To the best of our knowledge, hitorins A (**1**) and B (**2**) are the first examples of $C_{25}$ terpenoids consisting of 4,5-*seco*-eudesmane sesquiterpene and thujane monoterpene. Biological activities of hitorins A (**1**) and B (**2**) are under investigation.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02842.

Experimental section; 1D and 2D NMR spectra of **1** and **2** (PDF)

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### Notes

The authors declare no competing financial interest.

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(7) Hitorin A (**1**): colorless amorphous solid; $[\alpha]_D$ +90.5 (*c* 0.20, MeOH); IR (KBr) $\nu_{max}$ 3520, 1794, and 1703 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (Table 1); HRESIMS *m*/*z* 455.24074 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_6Na$, 455.24096); CD (MeOH; $4.39 \times 10^{-5}$ M, $\Delta\varepsilon$) $\lambda_{max}$ 232 (−1.7).
(8) Hitorin B (**2**): colorless amorphous solid; $[\alpha]_D$ + 63.7 (*c* 0.19, MeOH); IR (KBr) $\nu_{max}$ 3677, 1782, and 1708 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (Table 1); HRESIMS: *m*/*z* 455.24155 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_6Na$, 455.24096); CD (MeOH; $4.39 \times 10^{-5}$ M, $\Delta\varepsilon$) $\lambda_{max}$ 233 (−1.1).
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