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Daphneodorins A–C, Anti-HIV Gnidimacrin Related Macrocyclic Daphnane Orthoesters from *Daphne odora*

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Abstract

Three novel gnidimacrin related macrocyclic daphnanes (GMDs), daphneodorins A–C (**2–4**), were isolated from *Daphne odora* Thunb., together with gnidimacrin (**1**). Their structures were established by extensive physicochemical and spectroscopic analyses. Compounds **2** and **3** potently inhibited HIV-1 replication at subnanomolar concentrations (EC₅₀ 0.16 and 0.25 nM, respectively). Compounds **2–4** represent a novel type of GMDs that are highly oxygenated on the macrocyclic ring, suggesting good potential for anti-HIV drug development by further chemical modification.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b03539.

Experimental details; 1D and 2D NMR, HRESIMS, IR, UV, and ECD spectra of compounds **2–4** (PDF)

The authors declare no competing financial interest.

Daphnane diterpenoids, which feature a *trans*-fused 5/7/6-tricarbocyclic ring system, are biosynthetically related to tiglane-type diterpenoids and distributed in plants of the Thymelaeaceae and Euphorbiaceae families.¹ This class of natural products exhibits excellent biological effects, such as anticancer, anti-HIV, and analgesic activities.² For example, resiniferatoxin, a potent TRPV1 agonist is scheduled for a Phase III clinical trial to treat knee pain due to osteoarthritis.³

Gnidimacrin, which was initially isolated from *Gnidia subcordata* (Thymelaeaceae),⁴ has been highlighted as an anticancer drug candidate due to its potent and selective antiproliferative activities against a number of cancer cell lines, as well as excellent *in vivo* antitumor effects.⁵ On the other hand, our studies have further demonstrated the potential of gnidimacrin as a novel anti-HIV drug candidate based on the “Shock and Kill” strategy, since it showed extremely potent dual effects of inhibition on HIV-1 replication and reactivation of latent HIV-1, mechanistically by selective activation of PKC β II.⁶

Structurally, gnidimacrin is a 1-alkyldaphnane with a macrocyclic ring spanning the diterpene skeleton. This macrocyclic ring greatly enhances the biological potency, in comparison with nonmacrocyclic ring daphnanes.⁷ Even when compared with the major HIV latency-reversing agents (LRAs), such as prostratin (PKC activator), vorinostat, or romidepsin (HDACI), gnidimacrin showed superior ability to reduce the latent HIV-1 reservoirs in an *ex vivo* model.^{6a,8} Moreover, gnidimacrin did not show irritant and tumor-promoting activities, which are considered as the key reasons for serious side effects caused by daphnane- and tiglane-type diterpenoids such as phorbol esters.⁶

Although excellent biological activities have been demonstrated, to date, the identification of gnidimacrin related macrocyclic daphnanes (GMDs) from the plant kingdom has been quite limited. To our knowledge, only dozens of GMDs have been reported from some genera of Thymelaeaceae, such as *Daphne*, *Gnidia*, *Pimelea*, *Stellera*, and *Wikstroemia*.^{2a,7,9} Moreover, due to the structural complexity, the total synthesis of GMDs has not yet been achieved, although it has been greatly challenged.¹⁰ Therefore, unraveling the biological and chemical diversity of GMDs through a phytochemical approach remains a crucial issue.

Daphne odora Thunb. is an evergreen shrub belonging to the family Thymelaeaceae. It is native to China and distributed in Indochina and Japan. Various parts, including flowers,

roots, and leaves, have been used in traditional Chinese medicines for the treatment of pain and chronic dermatitis.¹¹ Previous chemical investigations have resulted in the isolation of daphnane-type diterpenoids, as well as bioflavonoids, coumarins and phenols.¹² In the present study, a phytochemical investigation was carried out on *D. odora* and resulted in the isolation and structural elucidation of three GMDs with a highly modified macrocyclic ring; their anti-HIV activities were also evaluated.

A methanol extract of the branches of *D. odora* was partitioned between EtOAc and H₂O. The EtOAc-soluble fraction was fractionated by Diaion HP-20 column chromatography (CC) to yield a crude diterpenoid fraction. Separation of this fraction using a combination of silica gel CC, ODS CC, and preparative HPLC afforded gnidimacrin (**1**) and three GMDs (**2–4**, Figure 1). Gnidimacrin was previously reported from *Gnidia subcordata*, *Pimelea ligustrina*, *P. prostrata*, and *Stellera chamaejasme*^{4,7} and for the first time from *D. odora*. GMDs (**2–4**) were named daphneodorins A–C, and their structures were elucidated by detailed interpretation of spectroscopic and physiochemical data.

Daphneodorin A (**2**) was isolated as a colorless solid, $[\alpha]_D^{23} +21.6$ (*c* 0.10, MeOH). Its molecular formula of C₅₃H₆₀O₁₆ was determined from the positive-ion HRESIMS peak at *m/z* 975.3769 [M + Na]⁺ (calcd for C₅₃H₆₀O₁₆Na, 975.3779). The IR absorption revealed the presence of hydroxy (3448 cm⁻¹) and carbonyl moieties (1719 cm⁻¹). In the ¹H NMR spectrum, the characteristic resonances for an isopropenyl moiety at δ_H 4.92 (H_a-16), 5.14 (H_b-16) and 1.79 (H₃-17) indicated the presence of a daphnane skeleton (Table S1). In the ¹³C NMR spectrum, a typical quaternary carbon resonance at δ_C 118.2 (C-1') was assigned to an orthoester group. In a comparison of the ¹H and ¹³C NMR spectra of **2** and gnidimacrin (**1**), superimposable resonances were observed for the daphnane part. Namely, the resonances due to polyoxygenated functionalities of the daphnane backbone in **2** were observed for an epoxy group at δ_H 3.35 (H-7), δ_C 60.7 (C-6), and 63.5 (C-7); three oxygenated methines at δ_H 4.88 (H-3), 4.04 (H-5), and 4.37 (H-14); two oxygenated methylenes at δ_H 4.07 (H_a-18), 4.93 (H_b-18), and 3.81 (H₂-20); and three oxygenated quaternary carbons at δ_C 79.4 (C-4), 81.6 (C-9), and 84.6 (C-13). The angular proton resonances characteristic of the *trans*-fused A/B/C ring junction in daphnanes were observed at δ_H 2.95 (H-10) and 3.00 (H-8).⁷ Furthermore, the resonance of a methine proton at δ_H 3.03 (H-1) was characteristic for 1-alkyldaphnane. The functionalities were further confirmed by comprehensive analysis of the 2D NMR data, especially the DQF-COSY and HMBC spectra (Figure 2).

Compound **2** revealed quite different NMR resonances assignable to the macrocyclic ring part from gnidimacrin (**1**) (Table S2). In particular, two oxymenthine resonances at δ_H 5.38 (H-6') and 5.63 (H-7'), and δ_C 72.2 (C-6') and 74.8 (C-7') were observed instead of two methylene resonances in **1**. Interpretation of the ¹H–¹H COSY correlations from H-6' and H-7' led to the determination of two spin systems, one from H-6' to H-2' and the other from H-7' to H₃-10' (Figure 2). Although H-6' and H-7' were not coupled with each other in the DQF-COSY spectrum, observation of the HMBC correlations from H-6' to C-4' and C-7', from H-7' to C-6', and from H-8' to C-6' and C-7' led to the determination of their vicinal positions. The ¹H–¹H COSY correlation between H-9' and H-1 was also not observed, which is a common feature of 1-alkyldaphnanes.¹³ The connection of C-9' and C-1 was

spectroscopically defined by the HMBC correlations from H-1 to C-10' and from H-10 to C-9'. In addition, the position of a hydroxy at C-2' was defined by the HMBC correlations from H-14 and H-2' to C-1'.

Four esterified carbonyl carbon resonances were assigned to three benzoyloxy moieties and one acetyloxy moiety, respectively. The connections of the benzoyloxy moieties attached to C-3, C-6', and C-7' and the acetyloxy moiety attached to C-18 were determined by the HMBC correlations from each corresponding proton to the carbonyl carbons (Figure 2).

To assign the relative configurations of **2**, the NOESY correlations were interpreted in detail. The NOESY correlations between H-1/H₃-19, H-7/H-8, H-7/H-14, H-7/H₂-20, H-8/H-11, and H-8/H-14 indicated these protons or their inclusive functional moieties have β -orientations. On the other hand, the NOESY correlations between H-2/H-3, H-3/H-5, and H-5/H-10 indicated that H-2, H-5, and H-10 have α -orientations. In the macrocyclic ring part, the NOESY correlation between H-9'/H_b-18 suggested that H-9' has a β -orientation. The chemical shift of CH₃-10' at δ_C 18.3 was in agreement of the α -orientation, whereas that of the β -orientation was reported at δ_C 12.¹⁴ The NOESY correlation between H₃-10'/H₃-19 offered further evidence for this assignment. Then, the two protons at C-8' were distinguished configurationally from the NOESY correlations of H-8' α with H-9' and H-10 and of H-8' β with H-2. The orientations of H-6' and H-7' were defined as β , based the NOESY correlations observed between H-6'/H-9', H-6'/H₃-10', H-7'/H-8' β , H-7'/H₃-10', and H-6'/H-7'. The NMR chemical shifts of H-2' and C-2' and the coupling constants between H-2'/H_{a,b}-3' were same as those of gnidimacrin (**1**), suggesting an α -orientation for H-2' (Figure 3).

As aforementioned, H-6' and H-7' were not coupled with each other ($J_{6',7'} = 0$ Hz), suggesting that the dihedral angle of H6'-C6'-C7'-H7' is about 90°, which was in good agreement with the result from molecular modeling. Since the positional relationship of the two benzoyl groups bound to C-6' and C-7' was favorable to apply the "dibenzoate chirality rule",¹⁵ the ECD spectrum was measured. As shown in Figure 4, a positive split Cotton effect was observed around 230 nm, indicative of the electrical dipole transition moments having a right-handed screw relation and thus 6'*R*,7'*S*. The conclusion was in good agreement with natural daphnanes. Thus, the structure of daphneodorin A (**2**) was established as shown in Figure 1.

Daphneodorins B (**3**) and C (**4**) were isolated as a pair of structural isomers with the same molecular formula of C₅₅H₆₂O₁₈, determined from the positive-ion HRESIMS data. Both **3** and **4** revealed ¹H and ¹³C NMR resonances characteristic for macrocyclic 1-alkyldaphnane orthoesters, like those found for **2**. The NMR resonances assignable to the daphnane parts of **1**, **2**, and **3** were superimposable. However, the ¹H NMR spectrum of **4** showed an upfield shifted resonance for H-3 and downfield shifted resonances for H_{a,b}-20, suggesting that **4** has a C-3 deacylated and C-20 acylated daphnane structure. This conclusion was confirmed by observation of the HMBC correlations from H_{a,b}-20 to a carbonyl carbon at δ_C 166.5 (20-Bz-CO).

Compounds **3** and **4** have the same structure in the macrocyclic part, likely originating biosynthetically from a polyoxygenated fatty acid. In both compounds, the NMR resonances for an oxymethine were observed instead of the methylene resonances found in **2**. Furthermore, the NMR resonances for an additional acetyl moiety were also observed. Since the molecular weight of **3** and **4** is 58 Da higher than that of **2**, one more acetyloxy group is likely present in **3** and **4**. Interpretation of the ^1H - ^1H COSY correlations led to the construction of two spin systems from $\text{H}_3\text{-10}'$ to $\text{H-7}'$ and from $\text{H-2}'$ to $\text{H-5}'$ (Figure 2). Although, no obvious correlations were observed between the oxygenated methine protons of $\text{H-5}'$, $\text{H-6}'$, and $\text{H-7}'$ in the DQF-COSY spectra, the connection of $\text{C-5}'/\text{C-6}'/\text{C-7}'$ was established based on the HMBC correlations from $\text{H-5}'$ to $\text{C-3}'$ and $\text{C-7}'$ and from $\text{H-8}'$ to $\text{C-6}'$. Regarding the locations of the acyl moieties, in the HMBC spectrum of **4**, the correlations from $\text{H-5}'$ to the carbonyl carbon of a benzoyl moiety and from $\text{H-6}'$ to the carbonyl carbon of an acetyl moiety suggested that benzoyloxy and acetyloxy moieties were attached at $\text{C-5}'$ and $\text{C-6}'$, respectively. The identical NMR data for $\text{H-7}'$ in **2–4** suggested that the remaining benzoyloxy moiety was present at $\text{C-7}'$.

The relative configurations for the daphnane part of **3** and **4** were the same as those of **2**. On the macrocyclic ring, $\text{H-6}'$, $\text{H-7}'$, and $\text{H-9}'$ have β -orientations, since these protons showed the same NOESY correlations found in **2**, namely, $\text{H-6}'/\text{H-9}'$, $\text{H-6}'/\text{H}_3\text{-10}'$, $\text{H-7}'/\text{H-8}' \beta$, $\text{H-7}'/\text{H}_3\text{-10}'$, $\text{H-6}'/\text{H-7}'$, and $\text{H-9}'/\text{H}_\text{b}\text{-18}$. $\text{H-5}'$ and $\text{H-2}'$ have α -orientation based on the NOESY correlations between $\text{H-5}'/\text{H-8}' \alpha$, $\text{H-5}'/\text{H-9}'$, and $\text{H-5}'/\text{H-2}'$ (Figure 5).

The reasonableness of these assignments was also supported by the behaviors of related proton resonances. First, $\text{H-5}' \alpha$, $\text{H-6}'$, and $\text{H-7}'$ were not coupled with each other like those in **2**. Second, the aromatic proton resonances belonging to the benzoyloxy moieties at $\text{C-7}'$ were shifted upfield from those in **2**; this shift is due to the shielding effect resulting from the diamagnetic anisotropy of the stacked aromatic ring in the benzoyloxy moiety at $\text{C-5}'$. Furthermore, the proton resonance of $\text{H-2}'$ was shifted downfield from that in **2**, which may be due to the deshielding effect of the benzoyloxy moiety at $\text{C-5}'$. Thus, the structures of compounds **3** and **4** were established as shown in Figure 1.

Since compounds **2–4** share a high structural similarity to gnidimacrin (**1**), they were evaluated for anti-HIV activity against HIV-1 infection of MT4 cells (Table 1).⁷ Compounds **2** and **3** potently inhibited HIV-1 replication at subnanomolar concentrations. Compound **4** was less potent than **3**, which is consistent with the structure–activity relationship (SAR) correlation that a benzoyloxy group at C-20 instead of C-3 led to reduced activity.¹³

Despite the great potential of gnidimacrin (**1**) as a novel anti-HIV and anticancer drug candidate, its limited natural distribution and structural complexity present high barriers for drug development. Therefore, the discovery of new GMDs either through isolation from natural resources or chemical modification of known compounds is crucial for continued structural optimization and mechanism of action studies. Our previous studies demonstrated that the macrocyclic part plays an important role in the biological function of gnidimacrin (**1**). The $2'$ -OH moiety located at the right side of the macrocycle is critical for the potent anti-HIV activity, and chemical modification of this position led to the identification of new GMDs with comparable anti-HIV activity to that of gnidimacrin (**1**).¹³ The new compounds

2–4 represent a novel type of GMDs that are highly oxygenated on the left side of macrocyclic ring, providing the potential for further chemical modification. A scalable separation and chemical modification of GMDs **2–4** are currently in progress.

Supplementary Material

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Figure 1.
Structures of compounds **1–4**.

Figure 2.
 ^1H - ^1H COSY and HMBC correlations of compounds 2–4.

Figure 3.
NOESY correlations of **2**: (a) daphnane part and (b) macrocyclic ring part.

Figure 4.
ECD and UV spectra of **2**.

Figure 5.
NOESY correlations of the macrocyclic ring part of **3**.

Table 1.

Anti-HIV Activity of 1–4

compd	anti-HIV (NL4–3) EC ₅₀ (nM)	cytotoxicity (MT4) IC ₅₀ (nM)
1	0.061 ± 0.023	>25
2	0.16 ± 0.060	>25
3	0.25 ± 0.061	>25
4	2.9 ± 0.76	>25