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Structure elucidation of 21,22-dihydroxyonnamides A_1–A_4 from the marine sponge *Theonella swinhoei*: an empirical rule to assign the relative stereochemistry of linear 1,5-diols

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ABSTRACT

A polar cytotoxic fraction of the marine sponge *Theonella swinhoei* was analyzed to be a mixture of four isomeric compounds, 21,22-dihydroxyonnamides A_1, A_2, A_3, and A_4. They were separated after conversion to the isopropyridene derivatives. The structures of the 21*S*,22*S*- and 21*R*,22*R*-isomers were determined by comparison of their spectral data with those prepared from onnamide A by asymmetric dihydroxylation. During the analysis of NMR data of these derivatives, an empirical rule was implied to assign the relative stereochemistry of linear 1,5-diol. This rule was applied to assign the stereochemistry of the remaining congeners.

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Onnamide A (**1**) was first isolated from the Okinawan marine sponge *Theonella swinhoei* as an antiviral constituent.[1] Several congeners of onnamide A were isolated from *T. swinhoei* and *Discodermia* sp. as cytotoxins.[2–4] Onnamide A exhibits cytotoxicity by inhibiting protein synthesis in eukaryotes as does structurally related pederin, isolated from the blister beetle *Paederus fuscipes*.[5] The putative biosynthetic gene cluster of onnamide A has been cloned from the metagenome of *T. swinhoei* and traced to a prokaryotic genome.[6] We have noticed the presence of a more polar and abundant cytotoxic fraction that contained congeners of onnamides, which had not been fractionated further because of its complexity. A renewed interest of this class of metabolites by the discovery of psymberin/irciniastatin[7,8] prompted us to study the constituents of the polar cytotoxic fraction.

An HPLC fraction eluted in the reverse-phase HPLC prior to onnamide B[2] was subjected to two rounds of HPLC using ODS and phenylhexyl stationary phases and a mixture of MeOH and phosphate buffer as the mobile phase to afford a cytotoxic peak. Because this material gave a sharp peak in a variety of HPLC conditions, we carried out structure elucidation of this material.[9] The HRESIMS (*m/z* 828.4592 $[M+H]^+$) gave a molecular formula of $C_{39}H_{65}N_5O_{14}$. Interpretation of 2D NMR data allowed the gross structure of this material to be assigned as 21,22-dihydroxyonnamide A: the Δ^{21}-olefinic signals in onnamide A[1] were replaced by signals assignable to 1,2-diol [δ_H 3.53 m (H-21) and 4.06 m (H-22)]; δ_C 75.2 (C-21) and 76.1 (C-22)] and Δ^{23}-olefinic signals [δ_H 6.18 (H-23) and 6.43 (H-24)]; δ_C 142.2 (C-23) and 130.3 (C-24)] were perturbed as a result of dihydroxylation. Even though the remaining NMR signals appeared homogeneous, H-23 and H-24 signals appeared as complex multiplets implying the presence of a heterogeneity near Δ^{23}-olefin.[10,11]

Because we reasoned that the heterogeneity arose from the isomerism in the diol portion and because it was reported that the assignment of the relative stereochemistry of 1,2-diol is impossible by standard spectroscopic methods,[12] we converted the mixture to the isopropylidene derivatives by treatment with 2,2-dimethoxypropane in DMF in the presence of PPTS. Fortunately, the product was separated by ODS HPLC into four peaks in a ratio of 2:2:1:1 to afford isopropyridene derivatives of 21,22-dihydroxyonnamides A_1, A_2, A_3, and A_4 (**2**–**5**, respectively).

Interpretation of COSY and HSQC data indicated that **2**–**5** had the same gross structure (Tables 1 and 2). In their ^{1}H NMR spectra, the isopropylidene methyls resonated separately in **2** and **3**, whereas they coalesced in **4** and **5**, suggesting that **2** and **3** were *cis*-acetonides and that **4** and **5** were *trans*-acetonides. This was supported by the ROESY spectra of **2** and **3**, in which one acetonide methyl signal was correlated to both H-21 and H-22, whereas the other methyl signal was correlated to neither H-21 nor H-22.

Because the *trans*-acetonides originate from the 21,22-*cis*-diols, they were considered to be accessible through the asymmetric dihydroxylation of onnamide A.[13] Onnamide A was oxidized with

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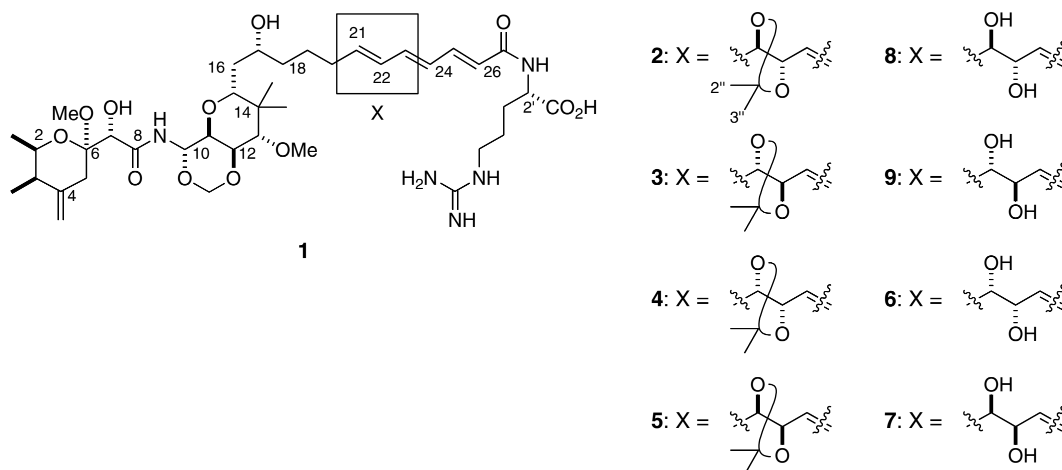

Table 1
Selected ^{1}H NMR data for **2–9** in CD_3OD at 500 MHz[a]

Number	2	3	4	5	6	7	8	9
H-7	4.30 s	4.24 s	4.25 s	4.30 s	4.27 s	4.29 s	4.29 s	4.27 s
H-15	3.54 (6.1, 6.1)	3.48 (6.1, 6.1)	3.48 (4.6, 7.6)	3.52 (6.1, 6.1)	3.48 (5.2, 7.0)	3.48 m	3.55 (3.4, 8.9)	3.47 (4.2, 7.6)
H-16ab	4.57 m	1.52 m	1.52 m	1.57 m	1.52 m	1.52 m	1.53 m	1.53 m
H-17	3.68 m	3.61 m	3.63 m	3.68 m	3.63 m	3.66 m	3.66 m	3.62 m
H-18a	1.35 m	1.28 m	1.29 m	1.37 m	1.23 m	1.30 m	1.30 m	1.24 m
H-18b	1.50 m	1.47 m	1.47 m	1.52 m	1.42 m	1.42 m	1.45 m	1.53 m
H-19a	1.49 m	1.28 m	1.34 m	1.53 m	1.32 m	1.48 m	1.48 m	1.36 m
H-19b	1.49 m	1.60 m	1.63 m	1.53 m	1.64 m	1.48 m	1.48 m	1.67 m
H-20a	1.51 m	1.43 m	1.53 m	1.63 m	1.36 m	1.38 m	1.39 m	1.35 m
H-20b	1.55 m	1.43 m	1.62 m	1.63 m	1.54 m	1.49 m	1.54 m	1.59 m
H-21	4.28 m	4.22 (6.4, 6.4, 6.4)	3.72 (3.7, 8.2, 8.2)	3.79 (4.3, 7.9, 7.9)	3.53 m	3.49 m	3.52 m	3.47 m
H-22	4.68 m	4.63 m	4.13 (7.6, 7.6)	4.19 (7.9, 7.9)	4.06 (5.8, 5.8)	4.07 (5.0, 5.0)	4.02 (5.8, 5.8)	4.04 (5.3, 5.3)
H-23	6.09 (7.3, 15.3)	6.03 (7.3, 15.3)	6.04 (7.0, 15.3)	6.10 (7.3, 15.3)	6.15 (5.8, 15.3)	6.17 (5.8, 15.3)	6.20 (6.1, 15.3)	6.21 (6.1, 15.3)
H_3-2″	1.39 (3H, s)	1.37 (3H, s)	1.37 (3H, s)	1.43 (3H, s)				
H_3-3″	1.50 (3H, s)	1.44 (3H, s)	1.37 (3H, s)	1.43 (3H, s)				

[a] Chemical shifts for the remaining portion differ from those of the corresponding positions of onnamide A by less than 0.02 ppm.

AD-mix α or AD-mix β to afford **6** or **7**, respectively, as the major product (Tables 1 and 2). Conversion of **6** to the isopropylidene derivative afforded a compound which gave the ^{1}H NMR spectrum that was indistinguishable from that of **4**. Similarly, the isopropylidene derivative of **7** was indistinguishable from that of **5**. By considering the stereoselectivity of the reaction,[14] the stereochemistry of **6** and **7** was determined as 21*S*,22*S* and 21*R*,22*R*, respectively.

A comparison of NMR data of **4–7** in the H-15 to H-23 region disclosed a clear trend in chemical shifts, which well reflected the relative stereochemistry of C-17 and C-21 (Table 1). The most significant difference between the ^{1}H NMR spectra of **4** and **5** was the chemical shifts of H_2-19: they were separated by 0.29 ppm in the 17,21-*syn* isomer **4**, whereas they coalesced in the 17,21-*anti* isomer **5**. This trend was conserved in the *syn*-diol **6** and the *anti*-diol **7**. This phenomenon was interpreted as follows. In the 17,21-*syn* isomer, when the relevant portion adopts an extended conformation, one of C-19 methylene protons is close to both oxygen atoms, whereas another methylene proton is distant from both of the oxygens, rendering the two methylene protons nonequivalent.[15] In the 17,21-*anti* isomers, each C-19 methylene proton is close to either of the oxygen atoms, thereby rendering these protons magnetically nearly equivalent (Fig. 1).

Against these backgrounds, we noticed the distinct equivalence and nonequivalence of H_2-19 signals in **2** and **3**, respectively, indicating the 17,21-*anti* and 17,21-*syn* stereochemistry for **2** and **3**, respectively. Then, we prepared the diols **8** and **9** from **2** and **3**,

Table 2
Selected ^{13}C NMR data for **2-9** in CD_3OD at 125 MHz[a]

Number	2	3	4	5	6	7	8	9
C-15	78.8	78.9	78.7	78.8	78.5	78.5	78.5	78.5
C-16	36.8	37.1	37.2	37.1	37.2	36.8	37.0	37.0
C-17	71.2	71.4	71.1	71.1	71.2	71.2	71.2	71.2
C-18	36.8	37.1	37.2	37.1	37.0	36.8	37.0	37.2
C-19	22.9	23.2	23.0	22.9	22.6	22.8	22.6	22.5
C-20	31.5	31.7	33.0	33.0	33.7	33.5	33.9	33.9
C-21	79.7	79.9	82.2	82.2	75.2	75.4	75.6	75.3
C-22	79.9	79.9	82.8	82.9	76.1	76.0	76.2	76.2
C-2″	25.7	25.8	27.0	27.5				
C-3″	28.3	28.3	27.4	27.5				

[a] Chemical shifts for the remaining portion differ from those of the corresponding positions of onnamide A by less than 0.3 ppm.

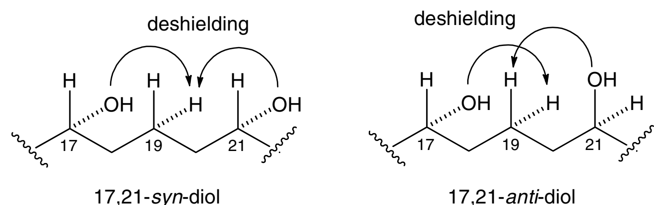

Figure 1. Effects of hydroxyl groups on the chemical shifts of methylene protons at 3-position in 1,5-diol.

respectively, by treatment with PPTS and ethylene glycol in MeOH.[16] It was found that H_2-19 signals were equivalent in **8** and nonequivalent in **9** (Tables 1 and 2). Therefore, the stereochemistry of **2** and **8** was assigned as 21*R*,22*S*, while that of **3** and **9** was assigned as 21*S*,22*R*.

Our rule is also exemplified by the ^{1}H NMR data of amphidinol 3, which has one 1,5-*syn*-diol substructure. In this compound, the methylene protons at 3-position were nonequivalent resonating at δ 1.43 and 1.60.[17] Our empirical rule may by useful to assign the relative stereochemistry of natural products with linear 1,5-diol substructures.

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9. The fraction [yellowish solids; $[\alpha]_D^{22}$ +73 (*c* 0.050, MeOH); UV (MeOH) λ_{max} 263 nm (ε 2290); HRESIMS (positive) *m/z* 828.4592 $[M+H]^+$ (calcd for $C_{39}H_{65}N_5O_{14}$, 828.4606)] exhibited an IC_{50} value of 40 ng/mL against P388 cells. Because the fraction was composed of four isomers in a ratio of 2:2:1:1 (vide infra), none of the isomers exhibits very potent cytotoxicity.
10. It was not possible to decipher H-21 and H-22 signals, because they were both broad multiplets.
11. ^{1}H chemical shifts of the fraction coincided with those of onnamide A with an error of less than 0.02 ppm except for H-7 and those placed between H_2-18 and H-26. Chemical shifts of 10-O-CH (δ 5.48) and H-13 (3.62) should be corrected as 5.20 and 3.64 ppm, respectively. The influence of the oxidation at C-21 and C-22 on H-7 indicated the spatial vicinity of these portions.
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