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Stereostructure Reassignment and Determination of the Absolute Configuration of Pericosine D_o by a Synthetic Approach

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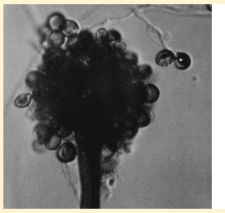
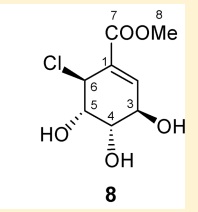
Supporting Information

ABSTRACT: A combination of chemical synthesis and NMR methods was used to reassign the structure of pericosine D_o (**8**), a cytotoxic marine natural product produced by the fungus *Periconia byssoides* OUPS-N133 that was originally derived from the sea hare *Aplysia kurodai*. Chemical synthesis was used to prepare pericoisne D_o (**8**) from a known chlorohydrin that was in turn derived from (−)-quinic acid. The absolute configuration of natural pericosine D_o (**8**) was determined to be methyl (3*R*,4*S*,5*S*,6*S*)-6-chloro-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate. HPLC analyses using a chiral-phase column indicated that pericosine D_o (**8**) exists in an enantiomerically pure form in nature.





8

Pericosines A−E (**1**−**5**) are a family of cytotoxic metabolites isolated from *Periconia byssoides* OUPS-N133, a fungus that was collected from the sea hare *Aplysia kurodai*.[1] We report herein our ongoing efforts to elucidate the structures of members of this family of carbasugars. Interest in this family of molecules arises in part due to their unique and highly functionalized C-7 cyclohexenoid structures, significant bioactivity, and unknown absolute configurations. The latter poses an interesting challenge for asymmetric synthesis.[2−7] Through our synthetic studies, we elucidated the absolute configuration of naturally occurring **1** and also prepared the compound that corresponds to the structure originally reported as pericosine D (**4**). However, the data for synthetic **4** did not agree with those appearing in the literature.[1] In contrast, deprotection of acetonide **4a**, which was supplied by the original authors, provided a product whose spectroscopic data matched satisfactorily with synthetic **4**. The structure of sample **4a** used in the deprotection experiment was reconfirmed by comparison of ^{1}H NMR data with those reported, eliminating the possibility that there was a mix-up of samples. The fact that **4a** was originally derived from a natural compound indicates that the structure of **4** does represent a natural product, but the data originally reported for pericosine D actually correspond to an undefined diastereomer of pericosine D. Therefore, the determination of the correct structure for the original pericosine D is still needed. We have designated this unassigned molecule pericosine D_o to indicate the natural product originally named pericosine D. This compound should be another diastereomer of **1** and **4**. It is important to elucidate the structure of pericosine D_o because of its significant cytotoxicity to the murine P388 leukemia cell line (ED_{50} value: 3.0 ug/mL). In this paper we describe the revision of the relative configuration and the determination of the absolute configuration of pericosine D_o by a synthetic approach. This is significant for natural product chemistry both in the discovery of new drug lead compounds and in understanding the biosynthetic pathway of these natural products.

All possible diastereomers of C-6 chlorinated pericosines (**1**, **4**, **6**−**11**) are shown in Figure 1. In prior studies[2−4] we completed the synthesis of the four diastereomers (**1**, **4**, **6**, and **7**) containing a *cis*-configuration between C-3 and C-4 and noted that each of these isomers did not correspond to pericosine D_o. Therefore, pericosine D_o must have a *trans*-configuration between C-3 and C-4, and the correct structure must be either compound **8**, **9**, **10**, or **11**. Among the four possible 3,4-*trans*-diastereomers, we also synthesized compound **9**[2] and found it to be different from pericosine D_o as well. The three remaining diastereomers **8**, **10**, and **11** were potential targets for synthesis. There are few reports of highly functionalized cyclohexenoids possessing such relative configurations as **10** or **11** in natural compounds.[8−12] Because we had a sample of precursor **8a** from our previous work, **8** was the most accessible molecule. The synthesis of **8** is summarized in Scheme 1. An inseparable mixture of epoxides **14** and **15** (ca. 3:2) was obtained through a five-step synthesis from (−)-quinic acid (**12**) via unstable diene **13**.[4] Treatment of the mixture of **14** and **15** with HCl afforded a mixture of **6a**, **4a′**, **8a**, and **16a**. As mentioned in our previous work,[4] the relative configuration of **8a** was determined by comparing its ^{1}H NMR coupling constants with those of structurally related compounds. The NOESY spectrum of **8a** did not give any crucial cross-peaks. Chlorohydrins **6a**, **4a′**, and **8a** should be derived from epoxide **14**. The configuration of **14** was confirmed as described in our previous paper.[4] This was also supported by the fact that **14** is different from the diastereomeric isomer **17** that appeared in another recent work of ours.[6] Therefore, the configuration of C-3 in **8a** was assigned as *R*, and the

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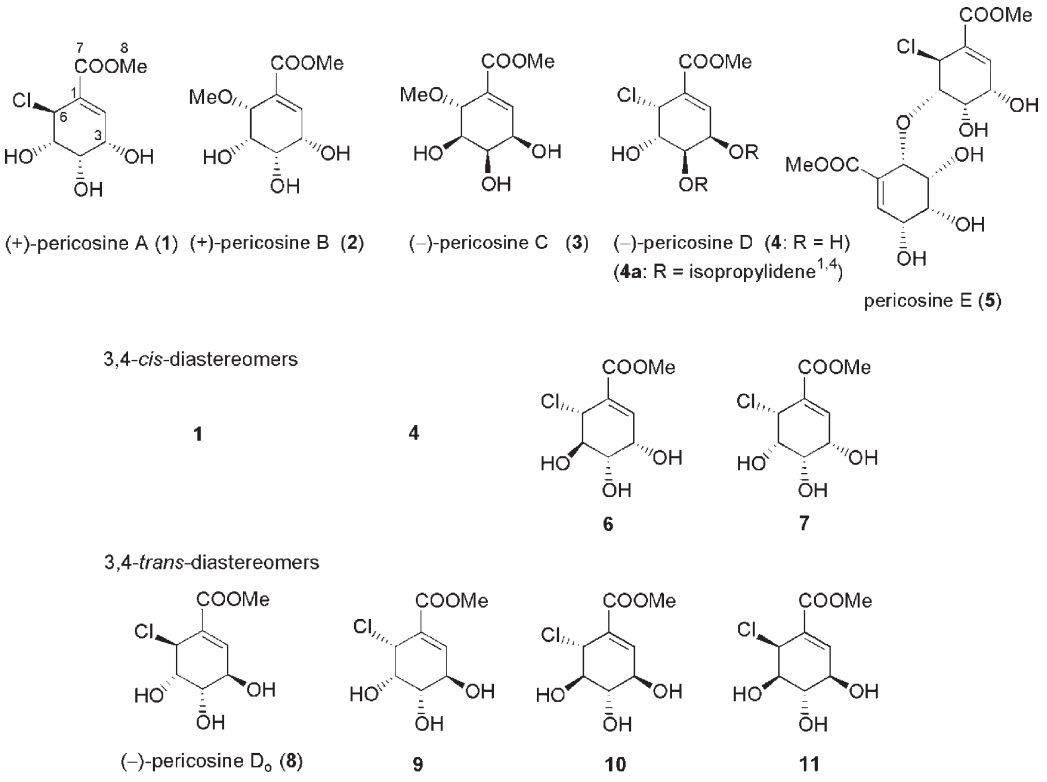
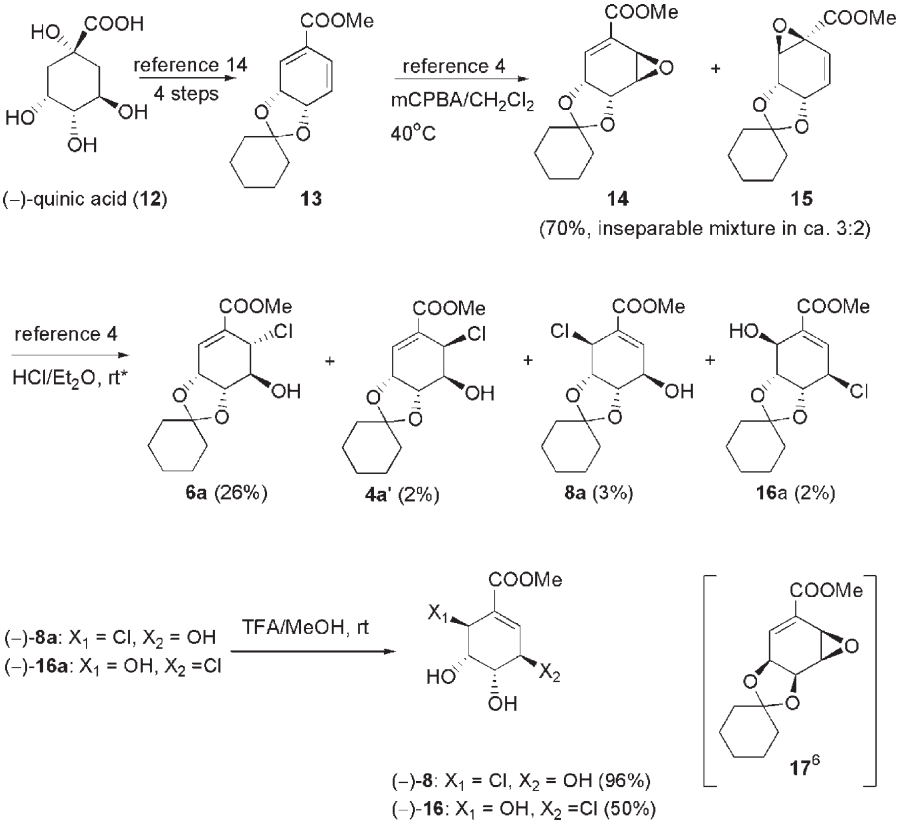


Figure 1. Structures of pericosines A−E (**1**−**5**) and possible chlorine-containing diastereomers (**1**, **4**, and **6**−**11**).

Scheme 1. Synthesis of (−)-Pericosine D$_o$ (8)



*Racemic **6a**, **4a′**, **8a**, and **16a** were obtained via the same procedure from a racemic mixture of **14** and **15** (ca.3:1)[13] in 47, 1, 4, and 7% yields, respectively. See Experimental Section.

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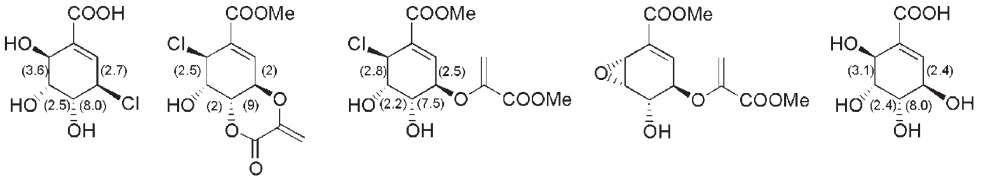
Table 1. NMR Spectroscocic Data of Synthesized and Natural Pericosine D_o (8) (^{1}H 500 MHz, ^{13}C 125 MHz, acetone-d_6)

	synthesized pericosine D_o (8)			natural pericosine D_o (8)[a]	
position	δ_C, mult.	δ_H, (J in Hz)	HMBC	δ_C, mult.	δ_H, (J in Hz)
1	129.1, C			129.06, C	
2	144.8, CH	6.91, d (2.5)	1, 4, 6, 7	144.78, CH	6.90, d (2.5)
3	69.8, CH	4.47, br dd (8.5, 2.5)	1, 2, 4	69.79, CH	4.46, ddd (8.5, 6.2,[b] 2.5)
4	71.1, CH	3.99, dd (8.5, 2.6)	3	71.10, CH	3.99, ddd (8.5, 6.0,[b] 2.6)
5	75.2, CH	4.20, dd (3.0, 2.5)	1, 3, 4	75.19, CH	4.21, dt (3.1,[b] 2.6)
6	56.1, CH	4.82, dd (3.0, 0.8)	1, 2, 4, 5, 7	56.02, CH	4.83, dd (2.6, 1.1)
7	165.8, C			165.86, C	
8	52.4, CH_3	3.78, s	1, 7	52.38, CH_3	3.77, s

[a] Reference 1. [b] Coupling with −OH.



16 natural product 18 cyathiformine C (19) cyathiformine B (20) 21

1. Numbers in parentheses represent coupling constants in ^{1}H-NMR spectra measured in acetone-d_6 for **16** and 18.
2. Numbers in parentheses represent coupling constants in ^{1}H-NMR spectra measured in $CDCl_3$ for **19**, and methanol-d_4 for **21**, respectively.

Figure 2. Structures of reference compounds.

remaining undefined configuration of C-6 in **8** must be *S*. If C-6 in **8** had the *R*-configuration, **8a** should be converted into known compound **9**.[2] Chlorohydrin **8a** was treated with TFA in MeOH at room temperature for deprotection of the 3,4-*O*-cyclohexylidene moiety to afford a product in 96% yield. This product was different from **9**.

The spectroscopic data of synthesized **8** satisfactorily agreed with the reported data of pericosine D_o[1] except for the specific rotation. The assignments of the ^{1}H and ^{13}C NMR signals for synthesized **8** were confirmed by 2D NMR experiments. Similar to **8a**, the NOESY spectrum of **8** did not yield any crucial information on the relative configuration. Details of the NMR data of **8** are provided in Table 1.

Interestingly, synthesized (−)-**8** corresponds to the core part of both the chlorine-containing natural product **18** and cyathiformine C (**19**) (Figure 2). Compounds **18** and **19** are metabolites of other fungi.[15,16] The fact that **19** was synthesized from cyathiformine B (**20**)[17,18] implies the possibility of a similar epoxide precursor in the biosynthetic pathway of the pericosines.[6]

The similarity of the ^{1}H NMR coupling constants between **8** and **18** measured with acetone-d_6 also supported our assignment of the relative configuration of **8**. Another non-natural regioisomer of pericosine, (−)-**16**, which was derived from **16a**, also showed comparable coupling constants in the same solvent. In addition, the coupling constants for **19**[15] and (6*S*)-6-hydroxy-5-epishikimic acid (**21**)[12] were also similar to those for **8**, despite having NMR data recorded in $CDCl_3$ and methanol-d_4, respectively. This implies that **19** and **21**, which possess the same relative configuration as **8**, take similar conformations in different solvents.

The specific rotation of synthesized **8**, which was derived from **12**, had the opposite sign ($[\alpha]^{25}_D$ −6.5) of the reported datum of **8** ($[\alpha]_D$ +1.9).[1] Because of the difference in specific rotation values between synthesized (−)-**8** and natural **8**, in addition to the difference in their signs, we questioned whether or not natural **8** also exists as an enantiomeric mixture, similar to **2**, **3**, and **5**.[1,13] Therefore, we synthesized racemic **8** starting from the Diels−Alder adducts derived from the reaction of furan and methyl acrylate via racemic **13**. According to the procedures from our previous work,[5,6,13] an approximately 3:1 mixture of racemic **14** and **15** was obtained from *rac*-**13** by the oxidation with DMDO (dimethyldioxirane). The mixture of *rac*-**14** and *rac*-**15** was similarly treated with HCl in dry ether to afford *rac*-**6a** (47%), *rac*-**4a**′ (1%), *rac*-**8a** (4%), and *rac*-**16a** (7%). The improved yields of the chlorohydrins were due to the improved ratio of **14**:**15**.[13] From *rac*-**8a**, *rac*-**8** was prepared in the same way as described above. Synthesized *rac*-**8** was subjected to chiral-phase HPLC to determine a suitable column and conditions that would yield clear enantioseparation in a 50:50 ratio. After many trials, CHIRALPAK IA (Daicel) was selected.[13] Natural **8** and synthesized (−)-**8** derived from **12** were subjected to HPLC analysis with CHIRALPAK IA under the optimum conditions. To our surprise, they were identical, suggesting that natural **8** exists in an enantiomerically pure form. Both HPLC samples of synthetic and natural **8** had the same retention time and showed positive optical rotation at 426 nm. Then, the specific rotation (at 589 nm) of natural **8** was remeasured using the same conditions, and an acceptable $[\alpha]^{25}_D$ value of −6.6 was obtained. From these experiments, the absolute configuration of natural pericosine D_o was assigned as methyl (3*R*,4*S*,5*S*,6*S*)-6-chloro-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate (**8**).

Now that the structure of pericosine D_o has been established, we must discuss the possibility of a transformation between **8** and

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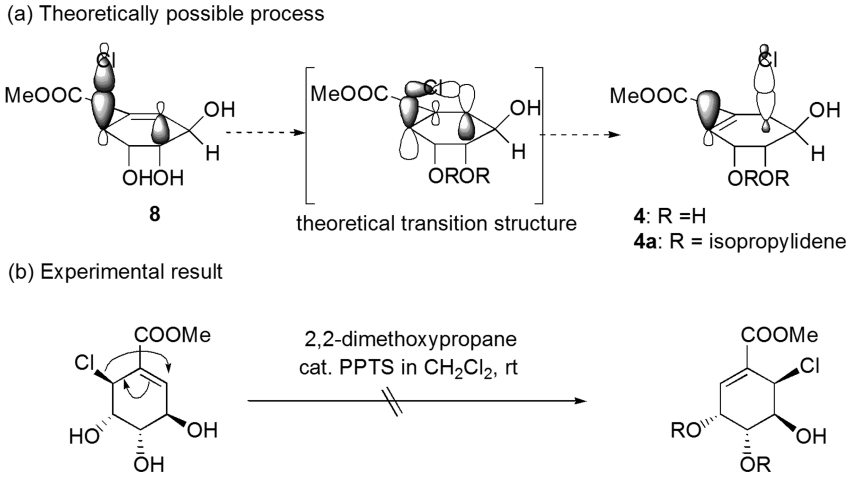

Figure 3. Theoretical possibility of conversion of **8** into **4a** via a [1a,3s]-sigmatropic rearrangement and the experimental result.

acetonide **4a** as described in the literature.[1] Theoretically, it is possible via a [1a,3s]-sigmatropic rearrangement under thermal conditions according to the Woodward−Hoffmann rules.[18] Important orbitals in the hypothetical [1a,3s]-sigmatropic process are illustrated in Figure 3. When acetonide formation from **8** was re-examined with 2,2-dimethoxypropane in dry CH_2Cl_2 at room temperature in the presence of a catalytic amount of pyridinium *para*-toluenesulfonate (PPTS),[1] no reaction occurred. The same reaction at higher temperature (60−80 °C) resulted in the formation of a complex mixture that did not include **4a**. Therefore, the formation of **4a** as originally reported[1] is still unexplained. It is possible that the starting compound as presented in the prior work was not actually **4**, but rather **8**. However, it is clear from our studies that pericosine D_o (**8**) and pericosine D (**4**) are independent compounds.

In conclusion, we have elucidated the relative configuration and the absolute configuration of pericosine D_o (**8**) to be methyl (3*R*,4*S*,5*S*,6*S*)-6-chloro-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate through this synthetic approach. Chiral-phase HPLC analysis proved that natural **8** exists as a pure enantiomer. We also conclude that pericosine D_o is a compound distinct from the structure originally assigned as pericosine D.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotation was measured on a JASCO DIP-1000 polarimeter. IR spectra were obtained with a JEOL FT/IR-680 Plus spectrometer. NMR spectra were recorded at 27 °C on a Varian UNITY INOVA-500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) in acetone-d_6 with tetramethylsilane (TMS) as the internal reference. HRMS values were determined using a JEOL JMS-700 (2) mass spectrometer. Liquid column chromatography was conducted over silica gel (Silicycle, SiliaFlash F60, 230−400 mesh). Analytical TLC was performed on precoated Merck aluminum sheets (DC-Alufolien Kieselgel 60 F_{254}), and the compounds were viewed by spraying an EtOH solution of phosphomolybdic acid, followed by heating. Chiral-phase HPLC analysis was carried out with a Shimadzu Prominance equipped with a Shimadzu CTO-20AC (50 μL sample loop) and a Shimadzu SPD-20A UV−vis detector. Chiralpak IA (Daicel Chemical Industries, Japan) was used as the analytical column.

(−)-Pericosine D_o: Methyl (3*R*,4*S*,5*S*,6*S*)-6-Chloro-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate (8). Chlorohydrin **8a** (7.4 mg, 0.024 mmol) was dissolved in MeOH (0.5 mL) and trifluoroacetic acid (0.5 mL). After stirring for 1 h at room temperature, the solvent was removed under reduced pressure directly to give a crude product, which was purified by silica gel column chromatography (EtOAc/hexane, 1:1 to 2:1) to afford (−)-**8** (5.2 mg, 96%). (−)-**8**: Colorless oil, $[\alpha]^{25}_D$ −6.5 (*c* 0.56, EtOH); IR (liquid film) ν_{max} 3409 (OH), 1716 (C=O), 1644 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR data are provided in Table 1; HRMS *m*/*z* 223.0365 $[M + H]^+$ (calcd for $C_8H_{12}O_5{}^{35}Cl$, 223.0372). Reported data[1] of natural **1** (designated as pericosine D in the original paper): $[\alpha]_D$ +1.9 (*c* 1.05, EtOH) (remeasured specific rotation in this study; $[\alpha]_D$ −6.6 (*c* 0.51, EtOH)); oil; IR (liquid film) ν_{max} 3332 (OH), 1720 (CO), 1635 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR data are provided in Table 1; HRMS *m*/*z* 223.0365 $[M + H]^+$ (calcd for $C_8H_{12}{}^{35}ClO_5$, 223.0372).

Synthesis of Racemic Chlorohydrins 4a′, 6a, 8a, and 16a. To a mixture of *rac*-epoxides **14** and **15** (ca. 3:1, 285.2 mg, 1.1 mmol) in dry Et_2O (1 mL), which was prepared following the procedure in a previous report,[13] was added 1 M HCl in Et_2O (1.2 mL, 1.2 mmol) at 0 °C. After stirring overnight, the reaction mixture was evaporated directly to afford a crude mixture, which was purified by column chromatography (EtOAc/hexane/MeOH, 2:7:1) and preparative TLC (EtOAc/hexane/MeOH, 2:7:1) to give *rac*-**6a** (153.0 mg, 47%), *rac*-**4a′** (2.9 mg, 1%), *rac*-**8a** (14.3 mg, 4%), *rac*-**16a** (23.7 mg, 7%), and recovered *rac*-**15** (12.9 mg, 5%). *rac*-**8** was prepared from *rac*-**8a** in the same way as described above.

Chiral-Phase HPLC Analysis of Synthetic Racemic 8, (−)-8, and Natural 8. HPLC enantioseparation of **8** was carried out with Chiralpak IA as the analytical column (0.46 cm i.d. × 25 cm L) at 40 °C under isocratic elution using *n*-hexane/EtOH (70:30%, v/v) at a flow rate of 1.0 mL/min and UV detection at 216 nm. Analytical samples of **8** were injected in 30 μL portions (500 ppm in *n*-hexane/EtOH, 70:30 (v/v)). Optical rotations were monitored at the same time at 426 nm by LED (light-emitting diode).

Methyl (3*R*,4*S*,5*S*,6*S*)-3-Chloro-4,5,6-trihydroxy-1-cyclohexene-1-carboxylate (16). Chlorohydrin **16a** (12.0 mg, 0.040 mmol) was dissolved in MeOH (0.45 mL) and trifluoroacetic acid (0.5 mL). After stirring for 3 h at rt, the solvent was removed under reduced pressure directly to give a crude product that was purified by preparative TLC (eluent: EtOAc) to afford (−)-**16** (4.4 mg, 50%). (−)-**16**: colorless oil; $[\alpha]^{25}_D$ −8.1 (*c* 0.44, EtOH); IR (liquid film) ν_{max} 3377 (OH), 1700 (C=O), 1644 (C=C) cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.76 (1H, d, *J* = 2.7 Hz, H-2), 4.71 (1H, ddd, *J* = 8.0, 2.7, 0.6 Hz, H-3), 4.55 (1H, dd, *J* = 3.6, 0.8 Hz, H-6), 4.04 (1H, dd, *J* = 8.0, 2.5 Hz, H-4), 4.01 (1H, dd, *J* = 3.6, 2.5 Hz, H-5), 3.76 (3H, s, $COOCH_3$); ^{13}C NMR (acetone-d_6, 125 MHz) δ 166.8 (C, C-7), 139.2 (CH, C-2), 132.9 (C, C-1), 74.7 (CH,

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C-5), 72.7 (CH, C-4), 68.7 (CH, C-6), 60.4 (CH, C-3), 52.2 (CH_3, C-8); HRMS m/z 223.0371 $[M + H]^+$ (calcd for $C_8H_{12}{}^{35}ClO_5$, 223.0372); m/z 225.0334 $[M + H]^+$ (calcd for $C_8H_{12}{}^{37}ClO_5$, 225.0334).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Copies of ^1H and ^{13}C NMR spectra of natural and synthesized **8** and compound **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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