# Amphidinolides O and P, Novel 15-Membered Macrolides from the Dinoflagellate *Amphidinium* sp.: Analysis of the Relative Stereochemistry and Stable Solution Conformation

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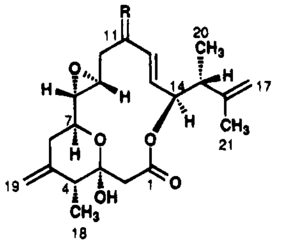
Amphidinolides O (**1**) and P (**2**), two novel cytotoxic 15-membered macrolides with unprecedented backbone frameworks, were isolated from the cultured marine dinoflagellate *Amphidinium* sp. Their gross structures were elucidated by extensive spectroscopic studies, and their relative stereochemistries as well as stable solution conformations were analyzed on the basis of NMR methods and molecular mechanics calculations.

Marine dinoflagellates of the genus *Amphidinium* have been recognized as a source of novel bioactive secondary metabolites with unprecedented structures.[1–3] During our studies on bioactive substances from Okinawan marine organisms,[4] we have investigated extracts of the laboratory-cultured marine dinoflagellate *Amphidinium* sp.,[5] which was a symbiont of the Okinawan marine flatworm *Amphiscolops* sp.,[6] to isolate a series of cytotoxic macrolides, named amphidinolides, with various backbone skeletons and biogenetically unusual structural features.[7] Here we describe isolation and structure elucidation of two novel 15-membered macrolides, amphidinolides O (**1**) and P (**2**), which were obtained by further examination of the cytotoxic fraction of the extract of this microalga and exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro (IC$_{50}$ values: **1**, 1.7 and 3.6 $\mu$g/mL, respectively; **2**, 1.6 and 5.8 $\mu$g/mL, respectively). Compounds **1** and **2** possess closely related structures; the C-11 ketone group of the former is replaced by an exomethylene group for the latter. Both **1** and **2** have seven chiral centers; the relative configurations of five of them located on a dihydropyran and an epoxide moiety (C-3, C-4, C-7, C-8, and C-9) were elucidated as 3*S**, 4*R**, 7*S**, 8*S**,[8] and 9*S** for **1** and 3*S**, 4*R**, 7*S**, 8*R**,[8] and 9*S** for **2** by the NMR data (coupling constants and NOE correlations), while the remaining two positions (C-14 and C-15) were assigned as 14*R** and 15*R** on the basis of analysis using molecular mechanics calculations.



**1** R = O
**2** R = CH$_2$
22

The harvested algal cells (878 g, wet weight, from 3240 L of culture) were extracted with MeOH/toluene (3:1), and the extracts were partitioned between toluene and water. The toluene-soluble fraction was subjected to a silica gel column (CHCl$_3$/MeOH, 95:5), and the cytotoxic fractions were subsequently separated by gel filtration on Sephadex LH-20 (CHCl$_3$/MeOH, 1:1) and flash chromatography on ODS (85% MeOH). Fractions whose $^1$H NMR spectra exhibited significantly the exomethylene signals[9] were finally purified by reversed-phase HPLC (ODS, 75% CH$_3$CN) to afford amphidinolides O (**1**, 0.0001% yield, wet weight) and P (**2**, 0.0002% yield).

Amphidinolide O (**1**) had a molecular formula of C$_{21}$H$_{28}$O$_6$ as revealed by HREIMS (*m/z* 376.1887, M$^+$, $\Delta$ +0.1 mmu). The UV and IR absorption spectra were indicative of the presence of enone ($\lambda_{max}$ 231 nm; $\nu_{max}$ 1700 cm$^{-1}$) and ester ($\nu_{max}$ 1720 cm$^{-1}$) functionalities. The $^1$H and $^{13}$C NMR data (Table 1) suggested that a molecule of **1** contains two exomethylenes, one $\alpha,\beta$-unsaturated ketone, one ester (or lactone), one hemiketal, one epoxide, two oxymethines, two unoxygenated methines, three sp$^3$ methylenes, and three methyl groups. Interpretation of the $^1$H–$^1$H COSY spectrum of **1** revealed the proton connectivities of four partial structural units, (i) from OH-3 to H$_2$-19, (ii) from H$_2$-6 to H$_2$-10, (iii) from H-12 to H$_3$-20, and (iv) from H$_2$-17 to H$_3$-21, which are designated by bold lines in Figure 1. Connections of these four partial structural units i–iv and remaining three carbons (C-1, C-2, and C-10) were clearly indicated by the $^1$H–$^{13}$C long-range correlations observed in the HMBC spectrum of **1**: H$_2$-2/C-1, H$_2$-2/C-3, H$_2$-19/C-6, H$_2$-6/C-5, H$_2$-

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[2] (a) Bauer, I.; Maranda, L.; Shimizu, Y.; Peterson, R. W.; Cornell, L.; Steiner, J. R.; Clardy, J. *J. Am. Chem. Soc.* **1994**, *116*, 2657–2658. (b) Bauer, I.; Maranda, L.; Young, K. A.; Shimizu, Y.; Fairchild, C.; Cornell, L.; MacBeth, J.; Huang, S. *J. Org. Chem.* **1995**, *60*, 1084–1086. (c) Bauer, I.; Maranda, L.; Young, K. A.; Shimizu, Y.; Huang, S. *Tetrahedron Lett.* **1995**, *36*, 991–994.

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[4] Takeuchi, S.; Kikuchi, T.; Tsukamoto, S.; Ishibashi, M.; Kobayashi, J. *Tetrahedron* **1995**, *51*, 5979–5986 and references cited therein.

[5] This dinoflagellate *Amphidinium* sp. (strain number Y-5) was used for previous studies to obtain amphidinolides A–E, J, K, M, and N and amphidinin A.[1,6]

[6] Kobayashi, J.; Ishibashi, M. *Chem. Rev.* **1993**, *93*, 1753–1769.

[7] Kobayashi, J.; Takahashi, M.; Ishibashi, M. *J. Chem. Soc., Chem. Commun.* In press.

[8] The relative stereochemistry of C-8 of compounds **1** and **2** is the same, but the expression (*S**/*R**) is different due to the change of functional group at C-11 (oxygen and methylene).

[9] Previously isolated amphidinolides all contain exomethylene groups,[6,7] which were detected as sharp singlets around 5 ppm in the $^1$H NMR spectra of crude fractions in the latter stage of the isolation process.

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**Table 1.** $^1H$ and $^{13}C$ NMR Data of Amphidinolide O (1) in $C_6D_6$

| position | $\delta_H$ | | $\delta_C$ | HMBC correlations | NOESY correlations[a] |
|---|---|---|---|---|---|
| 1 | | | 172.3 | $H_2$-2 | |
| 2 (a) | 2.40 | d | 43.7 | | 3-OH, H-4, $H_3$-18 |
| 2 (b) | 2.38 | d | | | 3-OH, H-4, $H_3$-18 |
| 3 | | | 99.1 | $H_2$-2, $H_3$-18, H-4 | |
| OH-3 | 4.78 | d | | | $H_2$-2, H-7 |
| 4 | 1.98 | br q | 44.94 | OH-3, H-6a, $H_3$-18, $H_2$-19 | H-2a, H-6b, $H_3$-18 |
| 5 | | | 143.1 | $H_2$-6, $H_3$-18, $H_2$-19 | |
| 6 (a) | 2.50 | dd | 39.6 | $H_2$-19 | H-6b, H-7, H-19a |
| 6 (b) | 2.08 | dd | | | H-4, H-6a, H-8 |
| 7 | 3.47 | ddd | 73.8 | H-6b | 3-OH, H-6a, H-9, H-19a |
| 8 | 2.37 | dd | 61.1 | H-6b, H-10b, H-12 | H-6b, H-10b, H-12 |
| 9 | 2.23 | dt | 52.9 | $H_2$-10 | H-7, H-10a |
| 10 (a) | 2.96 | dd | 46.2 | H-9 | H-9, H-10b |
| 10 (b) | 1.86 | dd | | | H-10a, H-12 |
| 11 | | | 194.0 | $H_2$-10, H-12, H-13 | |
| 12 | 7.00 | dd | 126.4 | H-10a, H-14 | H-8, H-10b |
| 13 | 7.06 | dd | 141.7 | H-14 | H-14, H-15, $H_3$-20 |
| 14 | 5.53 | dt | 75.8 | H-12, H-13, $H_3$-20 | H-13, H-15, $H_3$-20, $H_3$-21 |
| 15 | 2.35 | quint | 44.92 | H-14, $H_3$-20, $H_3$-21 | H-13, H-14, H-17b, $H_3$-20 |
| 16 | | | 145.5 | $H_2$-17, $H_3$-20, $H_3$-21 | |
| 17 (a) | 4.83 | t | 112.9 | $H_3$-21 | $H_3$-21 |
| 17 (b) | 4.81 | br s | | | H-14, H-15, $H_3$-20 |
| 18 (3H) | 1.01 | d | 12.1 | | $H_2$-2, H-4 |
| 19 (a) | 4.89 | d | 110.2 | H-4, $H_2$-6 | H-6a, H-7 |
| 19 (b) | 4.87 | d | | | $H_3$-18 |
| 20 (3H) | 0.93 | d | 15.5 | | H-13, H-14, H-15, H-17b |
| 21 (3H) | 1.59 | s | 20.2 | $H_2$-17 | H-14, H-17a |

[a] Clearly observed cross-peaks only. J(H/H) in Hz: 2a/2b = 12.7; OH-3/4 = 1.8; 4/18(3H) = 6.8; 6a/6b = 12.9; 6a/7 = 2.6; 6b/7 = 11.4; 7/8 = 8.5; 8/9 = 2.0; 9/10a = 2.0; 9/10b = 10.8; 10a/10b = 16.0; 12/13 = 15.4; 12/14 = 1.6; 13/14 = 2.5; 14/15 = 7.4; 15/17a = 1.4; 15/20(3H) = 7.0; 17a/17b = 1.4; 19a/19b = 1.4.

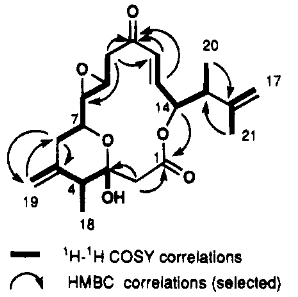

**Figure 1.** $^1H-^1H$ COSY and HMBC data of amphidinolide O (1).

6/C-19, $H_2$-10/C-11, H-10a[10]/C-12, H-12/C-11, H-13/C-11, $H_3$-20/C-16, and $H_3$-21/C-15 (arrows in Figure 1). The C-1 ester carbonyl was deduced to be connected to the C-14 oxymethine from the low-field resonance of H-14 ($\delta_H$ 5.53) as well as the HMBC correlation for H-14/C-1 to give rise to a 15-membered lactone ring. Seven out of eight unsaturations were thus accounted for, and the remaining one was ascribed to an ether linkage between the C-3 hemiketal and the C-7 oxymethine to construct a tetrahyrdopyran (THP) ring, which was corroborated by the NOESY correlation for OH-3/H-7. The planar structure of amphidinolide O was therefore deduced as 1; the $\Delta^{12}$-olefin was *E* on the basis of the coupling constant ($J_{12,13}$ = 15.4 Hz). The coupling constants ($J_{6a,7}$ = 2.6 Hz and $J_{6b,7}$ = 11.4 Hz) as well as the NOESY correlations for OH-3/H-7, H-4/H-6b, and H-6a/H-7 indicated that the THP ring adopts a chair conformation; the H-7 and OH-3 were oriented α-axially and the C-18 methyl group on C-4 was α-equatorial. The NOESY cross-peaks for H-7/H-9 and H-6b/H-8 along with the *J*-value ($J_{8,9}$ = 2.0 Hz) implied that the 8,9-epoxide was *trans*,[11] and a distinct NOESY correlation was observed between H-8 and H-12, which suggested that the H-8 was located inside of the macrocycle and, therefore, the oxygen of the epoxide was oriented outside. From these results, the relative configurations of five chiral centers contained in the THP and epoxide ring portion were elucidated as 3*S**, 4*R**, 7*S**, 8*S**, and 9*S**.

The molecular formula of amphidinolide P (2) was determined as $C_{22}H_{30}O_5$ by HREIMS (*m/z* 374.2092, $M^+$, $\Delta$ −0.2 mmu), which corresponds to the composition that one oxygen atom of 1 is replaced by a $CH_2$ unit. The spectral data of 2 were almost analogous to those of compound 1 except for the following observations: (i) two IR absorption bands due to carbonyl groups were observed for 1, but one ($\nu_{max}$ 1730 $cm^{-1}$) for 2; (ii) the UV absorption of 2 underwent a blue-shift ($\lambda_{max}$ 225 nm), assignable to a diene chromophore; (iii) the $^{13}C$ NMR of 2 showed no signal due to a conjugated ketone, but NMR signals for another exomethylene groups were observed [$\delta_C$ 118.1 (C-22) and 142.3 (C-11); $\delta_H$ 4.98 (1H, br s) and 4.85 (1H, br s) for $H_2$-22]. From these findings, amphidinolide P was suggested to possess the structure 2; the C-11 ketone group of amphidinolide O (1) was substituted by an exomethylene group, which was fully consistent with the 2D NMR data ($^1H-^1H$ COSY, HOHAHA, HMQC, HMBC, and NOESY spectra). The relative configurations of five chiral centers (C-3, C-4, C-7, C-8, and C-9) were inferred as the same as those of 1[8] from the $^1H$ NMR coupling constants and NOESY correlation data (Table 2).

Amphidinolides O (1) and P (2) possess seven chiral centers; the relative configurations of five chiral centers contained in the THP and epoxide ring portion were both elucidated on the basis of the NMR data (vide supra). The relative stereochemistries of remaining two chiral

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(10) For the assignment of nonequivalent methylene protons, lower field methylene protons were suffixed by a (*e.g.*, H-2a), while higher field ones were suffixed by b (*e.g.*, H-2b).

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**Table 2. $^1$H and $^{13}$C NMR Data of Amphidinolide P (2) in $C_6D_6$**

| position | $\delta_H$ | | $\delta_C$ | HMBC correlations | NOESY correlations[a] |
|---|---|---|---|---|---|
| 1 | | | 172.4 | $H_2$-2 | |
| 2 (a) | 2.41 | d | 45.01 | | H-2b H-4 $H_3$-18 |
| 2 (b) | 2.31 | d | | | H-2a 3-OH $H_3$-18 |
| 3 | | | 99.2 | $H_2$-2, $H_3$-18 | |
| OH-3 | 4.31 | d | | | H-2b H-7 |
| 4 | 1.99 | br q | 45.2 | | H-2a, H-6b, H3-18 |
| 5 | | | 143.7 | $H_2$-6, $H_3$-18 | |
| 6 (a) | 2.56 | dd | 39.4 | | H-6b, H-19a |
| 6 (b) | 2.14 | t | | | H-4, H-6a |
| 7 | 3.51 | ddd | 73.5 | H-6b | 3-OH, H-9 |
| 8 | 2.66 | dd | 62.8 | $H_2$-10 | H-13 |
| 9 | 2.52 | d | 58.2 | $H_2$-10 | H-7, H-10a |
| 10 (a) | 2.72 | d | 36.4 | | H-9, H-10b, H-22b |
| 10 (b) | 2.21 | dd | | | H-10a, H-13 |
| 11 | | | 142.3 | $H_2$-10, H-13 | |
| 12 | 6.24 | d | 133.6 | H-10a, H-14, $H_2$-22 | H-14, $H_3$-20 H-22a |
| 13 | 5.64 | dd | 129.1 | | H-8, H-10b |
| 14 | 5.34 | dd | 78.5 | H-12, H3-20 | H-12, $H_3$-20, $H_3$-21 |
| 15 | 2.47 | qd | 45.04 | | H-17a, $H_3$-20 |
| 16 | | | 146.5 | $H_3$-21 | |
| 17 (a) | 4.93 | br s | 112.3 | $H_3$-21 | H-15, $H_3$-20 |
| 17 (b) | 4.92 | br s | | | $H_3$-21 |
| 18 (3H) | 0.96 | d | 16.1 | | $H_2$-2, H-4, H-19b |
| 19 (a) | 4.86 | br s | 110.0 | $H_2$-6 | H-6a |
| 19 (b) | 4.81 | br s | | | $H_3$-18 |
| 20 (3H) | 0.95 | d | 11.8 | $H_3$-21 | H-12, H-14, H-15, H-17a, $H_3$-21 |
| 21 (3H) | 1.71 | s | 19.5 | $H_2$-17 | H-14, H-17b, $H_3$-20 |
| 22 (a) | 4.98 | br s | 118.1 | $H_2$-10, H-18 | H-12, H-22b |
| (b) | 4.85 | br s | | H-10b, H-22a | |

[a] Clearly observed cross-peaks only. *J*(H/H) in Hz: 2a/2b = 12.0; OH-3/4 = 1.5; 4/18(3H) = 6.9; 6a/6b = 12.7; 6a/7 = 2.5; 6b/7 = 11.7; 7/8 = 8.3; 8/9 = 1.4; 9/10a = ~0; 9/10b = 9.5; 10a/10b = 13.9; 12/13 = 16.2; 12/14 = ~0; 13/14 = 7.5; 14/15 = 9.3; 15/20(3H) = 7.3

**Table 3. Summary Data of the Molecular Mechanics Calculations for Diastereomers 1a–d and 2a–d**

| diastereomer | the C-11–C-13 conformation of the major cluster | population of the major cluster (%) | calculated average of $J_{14,15}$ (Hz)[a] | distance[b] H-8/H-12 (Å) | distance[b] H-8/H-13 (Å) | distance[b] H-12/H-14 (Å) | distance[b] H-13/H-14 (Å) |
|---|---|---|---|---|---|---|---|
| **1a** (14*R**15*R**) = **1** | *S*-cis | 76.6 | 9.2 | 3.023 | 5.811 | 3.773 | 2.390 |
| **1b** (14*R**15*S**) | *S*-cis | 84.9 | 2.5 | 3.043 | 5.878 | 3.713 | 2.420 |
| **1c** (14*S**15*R**) | *S*-cis | 89.5 | 4.8 | 2.986 | 5.777 | 2.747 | 2.873 |
| **1d** (14*S**15*S**) | *S*-cis | 94.1 | 10.3 | 3.030 | 5.796 | 2.985 | 2.821 |
| **2a** (14*R**15*R**) = **2** | *S*-trans | 76.7 | 9.5 | 5.340 | 2.737 | 2.377 | 3.130 |
| **2b** (14*R**15*S**) | *S*-trans | 83.9 | 3.7 | 5.382 | 2.719 | 2.401 | 3.110 |
| **2c** (14*S**15*R**) | *S*-cis | 70.1 | 6.3 | 3.090 | 5.726 | 2.585 | 2.938 |
| **2d** (14*S**15*S**) | *S*-cis | 68.3 | 10.4 | 3.176 | 5.546 | 2.631 | 2.973 |

[a] The observed values of $J_{14,15}$: **1**, 7.4 Hz; **2**, 9.3 Hz. [b] Distances for the lowest energy confomers.

centers (C-14 and C-15) were then investigated by the combination of the $^1$H NMR data and molecular mechanics calculations. We considered four diastereomers (**1a**–**d** and **2a**–**d**) for each compound (**1** and **2**), respectively (**1a**/**2a**, 14*R**15*R**; **1b**/**2b**, 14*R**15*S**; **1c**/**2c**, 14*S**15*R**; **1d**/**2d**, 14*S**15*S**), and systematic conformational searching[12] was carried out using the Macromodel program[13] for each diastereomer. For the calculation of the diastereomer **1a**, 1000 randomly-generated structures were evaluated, yielding 26 unique conformations in the 0–10 kJ/mol energy region[14] which could be classified into three clusters. Conformers belonging to the same cluster (1, 2, or 3) possess approximately identical conformations as to the 15-membered macrocycle portion, and they are assignable to rotamers around the bonds to the methyl groups or those in the side chain moiety. The lowest energy conformers belonging to these three clusters were represented as **1a**$_1$ (58.897 kJ/mol), **1a**$_2$ (62.776 kJ/mol), and **1a**$_3$ (67.120 kJ/mol), respectively; the conformers **1a**$_1$ and **1a**$_3$ possess the macrocycle conformation with *S*-cis for the C-11–C-13 enone part,[15] while the conformer **1a**$_2$ adopts the *S*-trans conformation. The calculated populations of these three clusters were 76.6%, 21.1%, and 2.3%, respectively, and the average value of $J_{14,15}$ of **1a** was 9.2 Hz.[16] For the diastereomers **1b**–**d** and **2a**–**d**, the calculations were carried out by essentially the same procedures, and the results were summarized in Table 3. The distance H-8/H-12 of the conformers having the *S*-cis enone or diene (*ca.* 3.0 Å) is much shorter than the distance H-8/H-13 (*ca.* 5.7 Å), and conversely, for those having *S*-trans conformation the distance H-8/H-13 (*ca.* 2.7 Å) is significantly shorter than that of H-8/H-12 (*ca.* 5.3 Å).

The NOESY spectrum of amphidinolide O (**1**) in $C_6D_6$

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(13) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.

(14) See Table 4 in the supporting information. Conformers with steric energies beyond 10 kJ/mol were all disregarded for the calculations of the populations as well as the average *J*-values in all eight cases (**1a**–**d** and **2a**–**d**), since the contributions of such conformers are negligibly small (<0.8%).

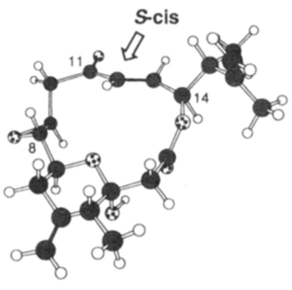
(15) The conformer **1a**$_1$ has the *S*-cis conformation with the hydrogen on C-12 (H-12) oriented to the $\beta$-side with regard to the average plane of the macrocycle, whereas the H-12 of the conformer **1a**$_3$ is directed to the α-side. See Figure 4 in the supporting information.

(16) The *J*-values were calculated according to the equation for H–C(sp$^3$)–C(sp$^3$)–H system in the Macromodel program (Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792), and also see footnote e of Table 4 (supporting information).

solution clearly revealed cross-peaks due to H-8/H-12 and H-10b/H-12 with no correlations for H-8/H-13 or H-10b/H-13 observed. On the other hand, the NOESY spectra of amphidinolide P (**2**) in $C_6D_6$ showed substantial correlations for H-8/H-13, H-10b/H-13, and H-12/H-22a, but no cross-peaks due to H-8/H-12, H-10b/H-12, or H-13/H-22a were visible. These observations implied that, in the $C_6D_6$ solution states, the C-11−C-13 enone moiety of **1** is abundantly *S*-cis while the *S*-trans conformation is predominant for the C-11−C-13 diene moiety of **2**. This result appears interesting and was consistent with the calculation data of 14*R** diastereomers (**1a**/**1b** and **2a**/**2b**, Table 3); for **1a** and **1b** the conformers belonging to the major cluster comprise the *S*-cis enone and those for **2a** and **2b** have the *S*-trans diene. In the NOESY spectrum of **1** a cross-peak due to H-13/H-14 was intensely observed, but that for H-12/H-14 appeared indistinct.[17] On the contrary, the NOESY spectrum of **2** showed a significant cross-peak for H-12/H-14 while the H-13/H-14 correlation was obscure.[17] These findings also coincided with the calculation data of the **1a**/**1b** and **2a**/**2b** diastereomers (Table 3); the calculated distance H-13/H-14 of the lowest conformer of **1a**/**1b** (*ca.* 2.4 Å) is much shorter than that of H-12/H-14 (*ca.* 3.7 Å), whereas the latter (*ca.* 2.4 Å) is considerably shorter than the former (*ca.* 3.1 Å) for **2a**/**2b** diastereomers. In addition, for the 14*S** diastereomers (**1c**/**1d** and **2c**/**2d**), the calculated distances of H-12/H-14 and H-13/H-14 are not significantly different from each other (*ca.* 2.6−2.9 Å, Table 3), which appears to be inconsistent with the NOESY data of **1** and **2** described above. From all of these results, the 14*R** configuration was strongly suggested for both **1** and **2**.

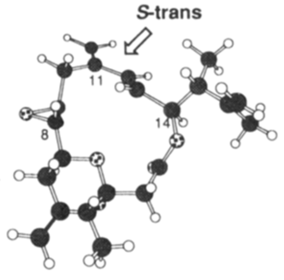
For the remaining one chiral center at C-15, two diastereomers [**1a** and **2a** (15*R**); **1b** and **2b** (15*S**)] now had to be considered for **1** and **2**, respectively, and the relative stereochemistry of C-15 was analyzed on the basis of comparison of the proton−proton coupling constant ($J_{14,15}$) between the observed and the calculated values. The observed $J_{14,15}$-values were 7.4 and 9.3 Hz for **1** and **2**, respectively. The calculated average values of $J_{14,15}$ are shown in Table 3, and those for the 15*R** diastereomers (**1a**, 9.2 Hz; **2a**, 9.5 Hz) corresponded somewhat better than those of 15*S** diastereomers (**1b**, 2.5 Hz; **2b**, 3.7 Hz). Thus, in conclusion, the structures of amphidinolides O and P were deduced as **1** (=**1a**) and **2** (=**2a**), respectively, including the relative configurations (3*S**, 4*R**, 7*S**, 8*S**,[8] 9*S**, 14*R**, and 15*R** for **1** and 3*S**, 4*R**, 7*S**, 8*R**,[8] 9*S**, 14*R**, and 15*R** for **2**) and predominant solution conformation as represented in Figures 2 (**1a**) and 3 (**2a**), respectively. The absolute stereochemistries of **1** and **2** remain undetermined.

Amphidinolides O (**1**) and P (**2**) are novel macrolide natural products possessing unique 15-membered macrocyclic lactones, and their backbone frameworks are hitherto unprecedented. Both **1** and **2** contain unique structural features characteristic of the amphidinolides group.[7] (i) odd-numbered lactone, (ii) the presence of exomethylene units, and (iii) the presence of vicinally located one-carbon branches (*viz.*, methyl or exomethylene) at C-4/C-5 and C-15/C-16 positions. The structural difference between **1** and **2** is found only at the C-11 position, *viz.*, the ketone group for **1** and the exomethylene group for **2**. To the best of our knowledge, natural product analogs with this type of structural difference (ketone/exomethylene) are quite rare. Compounds **1** and **2** are likely to be biogenetically related to each other; one may be a precursor of the other, but it is unknown which one preceded the other.



**1a** (14R*15R*) = **1**

**Figure 2.** Three-dimensional structures of the lowest energy conformer of amphidinolide O (**1**).



**2a** (14R*15R*) = **2**

**Figure 3.** Three-dimensional structures of the lowest energy conformer of amphidinolide P (**2**).

## Experimental Section

**General Methods.** For each 2D NMR experiment a total of 256 increments of 1K data points were collected. The COSY and HMBC spectra were recorded in the absolute mode, while the NOESY, HOHAHA, and HMQC spectra were in the phase-sensitive mode. Computer modeling was carried out with the Macromodel program (version 4.0) using a Silicon Graphics Personal IRIS workstation.

**Isolation.** The harvested cells of the cultured dinoflagellate *Amphidinium* sp. (878 g, wet weight, from 3240 L of culture) were extracted with MeOH/toluene (3:1; 1 L × 3). After addition of 1 M NaCl (1.5 L), the mixture was extracted with toluene (500 mL × 5). The toluene-soluble fraction was evaporated under reduced pressure to give a residue (40 g), which was partially (26 g) subjected to a flash column chromatography on silica gel (4.5 × 40 cm) eluted with $CHCl_3$/MeOH (95:5). The fraction eluting from 330 to 540 mL (3.6 g) was then separated by gel filtration on Sephadex LH-20 (3.5 × 108 cm; $CHCl_3$/MeOH, 1:1), and the fraction eluting from 350 to 400 mL (2.1 g) was subsequently separated by flash chromatography on ODS (YMC-GEL ODS 60 Å, I-40/60, 25 × 330 mm; 85% MeOH) to give a fraction (95 mg) containing a mixture of **1** and **2** in the 200−400 mL fraction, which was finally purified by reversed-phase HPLC (Develosil ODS-5, Nomura Chemical, 10 × 250 mm; flow rate, 2.5 mL/min; detection, RI (refractive index) and UV at 220 nm; eluant, 75% $CH_3CN$) to afford amphidinolides O (**1**, 0.9 mg, $t_R$ 12.7 min) and P (**2**, 1.4 mg, $t_R$ 22.0 min).

**Amphidinolide O (1)**: colorless needles; $[\alpha]^{20}_D$ +65° (*c* 0.12, MeOH); UV (MeOH) $\lambda_{max}$ 231 nm ($\epsilon$ 6400); IR (film) $\nu_{max}$ 3480, 1720, 1700, and 1640 cm$^{-1}$; $^1H$ and $^{13}C$ NMR (Table 1); EIMS *m/z* 376 ($M^+$); HREIMS *m/z* 376.1887, calcd for $C_{21}H_{28}O_6$ (M) 376.1886.

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[17] The F1 slice spectra of the NOESY spectra of **1** and **2** at the resonance of H-14 ($\delta_H$ 5.53 and 5.34, respectively) are shown in Figures 10 and 16 (supporting information), respectively, which indicated that the intensity ratio of the NOESY cross-peaks for H-12/H-14 and H-13/H-14 of **1** was 0.43:1 while that of **2** was 1:0.24.

**Amphidinolide P (2)**: colorless amorphous solid; $[\alpha]^{20}_{D}$ +31° ($c$ 0.098, MeOH); UV (MeOH) $\lambda_{max}$ 225 nm ($\epsilon$ 7300); IR (film) $\nu_{max}$ 3500 and 1730 cm$^{-1}$; $^1H$ and $^{13}C$ NMR (Table 2); EIMS $m/z$ 374 ($M^+$); HREIMS $m/z$ 374.2092, calcd for $C_{22}H_{30}O_5$ (M) 374.2094.

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**Supporting Information Available:** 2D and $^{13}C$ NMR spectra of compounds **1** and **2** and tables of the calculation data and three-dimensional structures of **1a$_1$**−**1a$_3$** and the lowest energy conformers of the diastereomers of **1a**−**d** and **2a**−**d** (27 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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