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Absolute Stereochemistry of Ulapualide A

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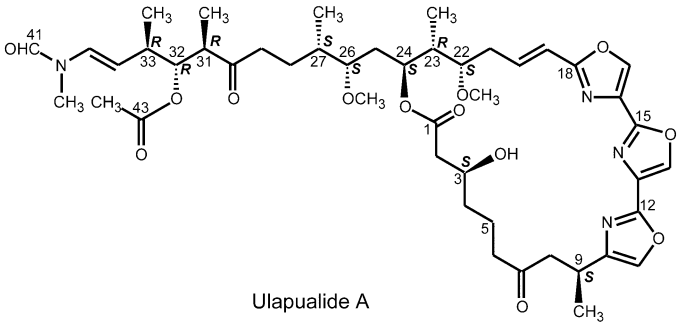
ABSTRACT



The structure of ulapualide A (1) has been solved by X-ray crystallography in a complex with G-actin. The stereochemical configuration was assigned as 3*S*,9*S*,22*S*,23*R*,24*S*,26*S*,27*S*,31*R*,32*R*,33*R*.

In 1986, ulapualides A (**1**) and B,[1] as well as kabiramide C (**2**),[2] were reported as cytotoxic constituents from the egg masses of marine nudibranches (sea slugs) that afforded them with virtual immunity from predation. These bioactive secondary metabolites became the first members of trisoxazole macrolide family, a characteristic class of marine macrolides, having three consecutive oxazoles in the macrolide ring and an extended aliphatic tail.[3] For some of these molecules, it was later discovered that their cytotoxic properties included an ability to depolymerize F-actin,[4−6] thereby interfering with actin filament dynamics. Recently, an investigation of the binding site of two of these molecules, kabiramide C (**2**) and halichondramide (**3**), on G-actin using X-ray crystallography and fluorescence spectroscopy has revealed their exact mechanism of action. Briefly, trisoxazole macrolides (1) bind to subdomains 1 and 3 of G-actin in an irreversible manner, (2) cap the G-actin/ligand complex at the plus-end of F-actin, and (3) behave as molecular mimetics to the actin-severing protein, gelsolin.[7,8] These studies also provided the absolute stereochemistry of kabiramide C (**2**) and halichondramide (**3**) or its hydroxylated product jaspisamide A (**4**). After the elaborate work by Matsunaga and Panek showing the absolute stereochemistry of mycalolides (e.g., **5**) by chemical synthesis of key degradation products,[9]

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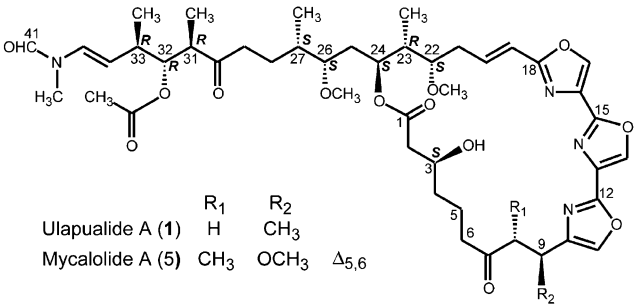
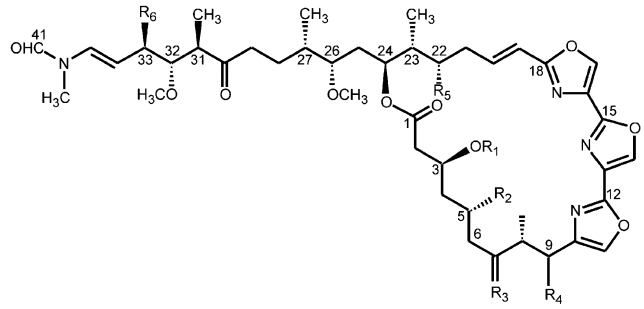
our results represented the first stereochemical configuration assignment for trisoxazole macrolides by X-ray crystallography. In a continuation of this study, we report the absolute configuration of ulapualide A (**1**), which has eluded description for 17 years.



	R_1	R_2	
Ulapualide A (**1**)	H	CH_3	
Mycalolide A (**5**)	CH_3	OCH_3	$\Delta_{5,6}$



	R_1	R_2	R_3	R_4	R_5	R_6	
Kabiramide C (**2**)	$CONH_2$	CH_3	β-OH	α-OCH_3	OCH_3	CH_3	
Halichondramide (**3**)	H	H	O	β-OCH_3	H	H	$\Delta_{5,6}$
Jaspisamide A (**4**)	H	OH	O	β-OCH_3	H	H	

Since the initial isolation studies of ulapualide A (**1**), considerable effort has been devoted to determining the stereostructure of this complex molecule. Pattenden et al. first proposed the relative stereochemistry of **1** on the basis of a molecular mechanics study under the premise that it incorporates a metal inside the macrolide ring.[10] Later they succeeded in the total synthesis of a "putative" ulapualide A;[11] however, they recognized that there were some discrepancies between the ^{13}C NMR data of synthetic and those of natural ulapualide A at C26, C27Me, and C28. Given that **1**−**3** are structurally related, it is also notable that there is a discrepancy between our structural studies and the model proposed by Pattenden et al., such that the chiral centers from C1 to C26 are a mirror image of **2** and **3**. To resolve this question, we initiated a crystallographic study on ulapualide A.

Ulapualide A (**1**) was isolated from *Hexabranchus sanguineus* eggs collected at Pupukea, O'ahu, as previously described.[1] Its identity was confirmed by NMR and mass spectroscopy. **1** was crystallized as a complex with purified

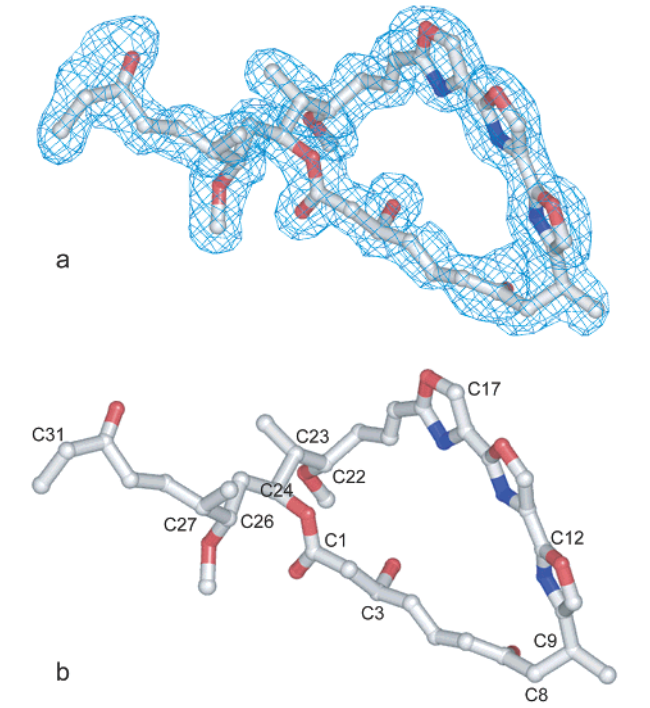


Figure 1. Structure of Ulapualide A (**1**). (a) Electron density map calculated with σ_a-weighted coefficients of the form $2mF_o - dF_c$ and contoured at 1σ. (b) **1** with labeled chiral centers.

G-actin from rabbit muscle[12] by mixing **1** at a 1.1−1.2 molar excess. Crystals of the actin−ulapualide A complex were grown in a small-scale batch by mixing 5 μL of the complex in a 1:1 ratio with 100 mM MES pH 6.0, 15% methyl ether poly(ethylene glycol) 5000, 75 mM $CaCl_2$, 1 mM sodium azide, and 1 mM TCEP.[13] The colorless crystals grew as thin plates of dimensions ~0.3 × 0.1 × 0.02 mm over 2−3 days at 20° C.

The structure of the Ca−ATP form of G−actin in a complex with ulapualide A (**1**) was solved by molecular replacement to a resolution of 1.6 Å. The perspective view of the X-ray model for the toxin is shown in Figure 1.

Crystallographic information is summarized in Table 1.

The data allow unequivocal assignment of atoms found in the macrolide ring and extending to C31 of the tail section to the electron density. Beyond C31, the electron density for the rest of the tail of ulapualide A (**1**) was disordered within the complex. Overall, the chiral centers that are well-ordered for the structure are nearly identical to those of mycalolide A (**5**).[9,14] Comparing ^1H NMR spectral data of **1** and those of **5** shows it is likely that they have the same stereochemical arrangement for the remainder of the tail section.[15] Taken together, we have defined the absolute stereochemistry of the ulapualide A (**1**) chiral centers as 3*S*,9*S*,22*S*,23*R*,24*S*,26*S*,27*S*,31*R*,32*R*,33*R*. Similar to the stereochemical configuration of kabiramide C (**2**), halichondra-

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Table 1. Summary of Crystallographic Statistics for Actin−Ulapualide A

data collection/refinement	actin-ulapualide A
space group	$P2_1$
unit cell dimensions	$a = 40.8$ Å, $b = 75.2$ Å, $c = 67.5$ Å; $\beta = 100.2°$
resolution (Å)	30−1.6
reflections: total, unique	216 388, 52 947
average I/σ	36.5 (5.2)
completeness (%)	99.7 (99.9)
R_{merge} (%)	6.6 (34.7)
R_{work} (%)	15.2 (19.1)
R_{free} (%)	17.9 (23.8)
Wilson B-value (Å^2)	26.2
average B-factors (Å^2)	
actin	18.9
toxin	20.8
CaATP	13.3
solvent	31.7
ramachandran (%)	
most favored	93.5
additionally allowed	6.1
generously allowed	0.3
disallowed	0.0
rms deviations	
bond length (Å)	0.015
bond angle (°)	1.67

Data in parentheses represent highest-resolution shell (1.66−1.6 Å). R_{work} refers to the R_{factor} for the data utilized in the refinement and R_{free} refers to the R_{factor} for the 5% of the data that were excluded from the refinement.

mide (**3**), and jaspisamide A (**4**), the configurations of the chiral centers for the biological ulapualide A (**1**) differ at C3, C22, C23, C24, and C26 from those described in the total synthesis of ulapualide A.[16] Thus, ulapualide A constructed by chemical synthesis was in fact nearly a mirror image of the natural molecule for the portion from C-1 to C-26.

The structure of the actin−ulapualide A complex is shown in Figure 2. In an identical manner to kabiramide C (**2**) and jaspisamide A (**4**), ulapualide A (**1**) binds to actin in the cleft between subdomains 1 and 3. Furthermore, the trisoxazole moiety of the macrolide ring is not planar but is bent slightly, which likely serves to maximize the hydrophobic interactions of this portion of the toxin and the hydrophobic patch on actin (residues Ile341, Ile345, Ser348, and Leu349). For kabiramide C (**2**) and jaspisamide A (**4**), it appeared that

(15) Ulapualide A in $CDCl_3$ showed δ 1.06 d (31-Me), 5.14 dd (H-32), 1.04 d (33-Me), 4.97 dd (H-34), 6.50 d (H-35), 3.03 s (N-Me), and 8.29 (N−CHO). Mycalolide A in $CDCl_3$ showed δ 1.07 d (31-Me), 5.15 dd (H-32), 1.05 d (33-Me), 4.98 dd (H-34), 6.50 d (H-35), 3.01 s (N-Me), and 8.29 s (N−CHO).

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(17) Coordinates and structure factors for **1** have been deposited in the RSCB with accession number 1S22.

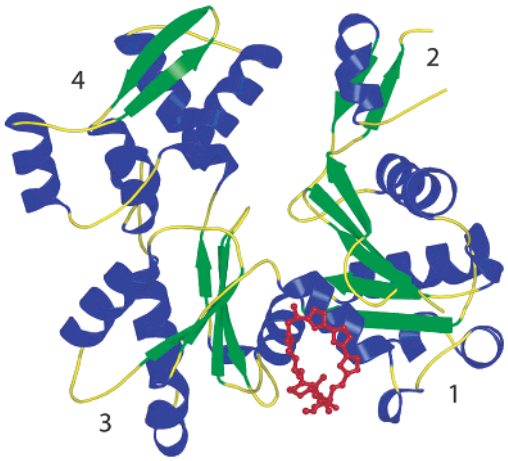

Figure 2. Structure of the actin−ulapualide A complex. Actin is depicted in a ribbon representation, and the toxin is shown as a ball-and-stick model colored in red. Actin subdomains 1−4 are labeled.

their aliphatic tail participated in the most extensive interactions with actin and therefore was suggested to be the primary determinant for toxin binding.[7] However, the lack of electron density for the tail portion beyond C31 of ulapualide A indicates that this region may be free to rotate within the complex. We believe that this effect is created by the presence of the acetate moiety at C32. We also observed that the macrolide ring of ulapualide A is less circular than those of kabiramide C (**2**) or jaspisamide A (**4**). It is possible that this slightly altered ring shape generates a stronger hydrophobic interaction with actin, compensating for weaker binding at the tail.

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Supporting Information Available: Toxin and actin purification, crystallization conditions, data processing, structure solution and refinement, and crystallographic data for **1**.[17] This material is available free of charge via the Internet at http://pubs.acs.org.

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