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The Unexpected Isolation of CTP-431, a Novel Thiopyrone from the Sponge *Cacospongia mycofijiensis*

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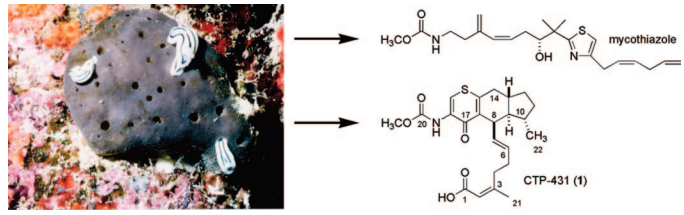
Received May 21, 2008



A reinvestigation of a Fijian collection of *Cacospongia mycofijiensis* has yielded the known mycothiazole and a novel heterocyclic, CTP-431 (**1**). Its structure including absolute configuration as 8*R*,9*R*,10*S*,13*S* was established using NMR data, calculated DFT ^{13}C chemical shifts and results from X-ray crystallography. It is possible that the tricyclic skeleton of CTP-431 (**1**) is biosynthetically related to the macrolide latrunculin A, however the thiopyrone moiety of **1** has no previous precedent in natural products chemistry.

Introduction

The Indo Pacific marine sponge *Cacospongia mycofijiensis* seems to be a rich source of structurally diverse as well as biologically active secondary metabolites. We have found it possible to recognize and then collect this organism from a variety of habitats. After many years of study, it appears that four distinct biosynthetic products (structures in Figure S1, Supporting Information) can be repeatedly isolated from this sponge.[1] They include ordinary terpenoids accompanied by more distinctive scaffolds produced by polyketide synthases (PKS) or assembled by mixed polyketide nonribosomal peptide synthetases (PKS-NRPS). While the sesquiterpene product, dendrolasin,[2] is indistinctive, the other compounds such as fijianolides[3] A and B (syn. isolaulimalide and laulimalide)[4] are striking PKS-derived 20-membered ring macrolides. Both have been shown to stabilize microtubules[5] at a similar but distinct site relative to that of paclitaxel,[6] and fijianolide B demonstrates in vivo efficacy against HCT-116 tumor-bearing SCID mice.[1] The third group is headed by latrunculin A and B[2,7] which are of PKS-NRPS origin with 16- or 14-membered macrolides, respectively, and both have a pendant thiazolidinone moiety. These substances exhibit potent destabilizing affects on microfilament assembly[8,9] and are now the most widely used molecular probes to investigate actin polymerization and dynamics.[10] The final class consists of the PKS-NRPS-derived mycothiazole[11,12] containing a thiazole ring imbedded between two acyclic polyketide chains. Although limited bioactivity data

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(1) Johnson, T. A.; Tenney, K.; Cichewicz, R. H.; Morinaka, B. I.; White, K. N.; Amagata, T.; Subramanian, B.; Media, J.; Mooberry, S. L.; Valeriote, F. A.; Crews, P. *J. Med. Chem.* **2007**, *50*, 3795–3803.
(2) Kakou, Y.; Crews, P.; Bakus, G. J. *J. Nat. Prod.* **1987**, *50*, 482–484.
(3) Quinoa, E.; Kakou, Y.; Crews, P. *J. Org. Chem.* **1988**, *53*, 3642–3644.
(4) Corley, D. G.; Herb, R.; Moore, R. E.; Scheuer, P. J.; Paul, V. J. *J. Org. Chem.* **1988**, *53*, 3644–3646.
(5) Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653–660.
(6) Pryor, D. E.; O'Brate, A.; Bilcer, G.; Diaz, J. F.; Wang, Y. F.; Wang, Y.; Kabaki, M.; Jung, M. K.; Andreu, J. M.; Ghosh, A. K.; Giannakakou, P.; Hamel, E. *Biochemistry* **2002**, *41*, 9109–9115.
(7) Groweiss, A.; Shmueli, U.; Kashman, Y. *J. Org. Chem.* **1983**, *48*, 3512–3516.
(8) Spector, I.; Shochet, N. R.; Kashman, Y.; Groweiss, A. *Science* **1983**, *219*, 493–495.
(9) Ayscough, K. R.; Stryker, J.; Pokala, N.; Sanders, M.; Crews, P.; Drubin, D. G. *J. Cell Biol.* **1997**, *137*, 399–416.
(10) The latrunculins A and B are widely used molecular tools for the study of actin biology, and a survey of the literature from 1984 to the present shows more than 500 publications on this subject.

10.1021/jo801096m CCC: $40.75
Published on Web 08/21/2008

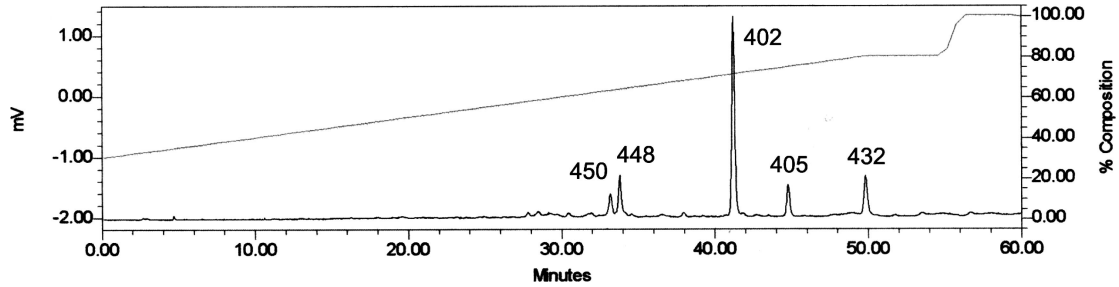
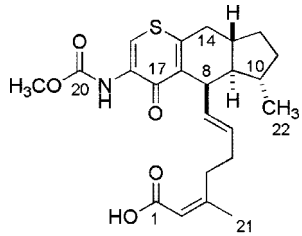


FIGURE 1. ELSD analysis of Coll. No. 89126 XFD fraction with annotations including *m/z* ions.

has been accumulated to date for mycothiazole, it exhibits impressive selectivity in the National Cancer Institute (NCI) 60 cell line screen against tumor lines such as DMS 114 (small cell lung cancer) and NCI-H23 (non small cell lung cancer).[12] We now extend this fascinating record by disclosing the isolation and properties of yet one more unique chemotype, CTP-431, unexpectedly isolated from a 1989 Fijian collection of *C. mycofijiensis* archived in our repository.

Results and Discussion

The desire to obtain additional mycothiazole and its analogues for further experimental therapeutic investigation was the motivation to survey unextracted *C. mycofijiensis* samples. One attractive specimen proved to be a large collection of *C. mycofijiensis*[13] (coll. no. 89126, 5.8 kg wet wt) obtained from the Beqa Lagoon, Fiji. This material was collected and preserved according to our standard laboratory procedures[14] and then stored in CH_3OH at +4 °C for 19 years! The workup, via accelerated solvent extraction (ASE), involved exposure of 2.3 kg of the material to three successive solvents of hexanes (sample coded as XFH = 2.1 g), dichloromethane (sample coded as XFD = 2.6 g), and methanol (sample coded as XFM = 1.7 g). An ELSD-LCMS survey of the XFD sample provided the surprising results of Figure 1, including the following: (a) mycothiazole (MH^+ 405) as one major component (isolated amount = 8.7 mg), which had previously been isolated from Vanuatu and not Fiji collections, (b) the absence of latrunculin A (M − H_2O + H 404) normally seen from Fiji collections, and (c) the conclusion that four masses present in the LC−MS trace (at *m/z* 450, 448, 402, 432) represented new compounds. The ^1H NMR data of semipure fractions indicated that the first three were latrunculin A derivatives; however, they were not investigated further. All of our attention was devoted to the last compound, designated as CTP-431 (**1**, isolated amount = 8.3 mg) because it appeared to be unrelated to the former set. CTP-431 plus each of the other four metabolites were also observed by LC−MS in the crude extracts of sponge material (3.5 kg) separately processed by a traditional extraction and Kupchan-like solvent partitioning[15] (Chart S2, Supporting Information).



CTP-431 (**1**)

The total structure elucidation of CTP-431 (**1**) could not be completed on the basis of the NMR data, but was eventually finalized via X-ray analysis. The proof began by establishing the formula as $C_{23}H_{29}NO_5S$, based on the $[MH]^+$ ion *m/z* 432.1839 (calcd for $C_{23}H_{30}NO_5S$ 432.1844), which differed from that of latrunculin A by +C and −2H and indicated 10 degrees of unsaturation. The ^{13}C NMR spectrum of **1** contained resonances that could be assigned to all 23 carbons. The DEPT and HMQC data of Table 1 showed that 27 of the hydrogen atoms were attached to carbons (3 < CH_3, 5 < CH_2, 8 < CH, 7 < C) and required that two protons were affixed to heteroatoms N, O, or S. Further, the 1D and 2D NMR data recorded in both C_6D_6 and $CDCl_3$ from Table 1 provided the basis to draft four substructures **A**−**D** that were merged into **E** as summarized in Figure 2 (and discussed in the experimental procedures of the Supporting Information). There were two possibilities for incorporating the O and S atoms into substructure **E**, as a thioic acid−pyrone, substructure **F**, or as a carboxylic acid−thiopyranone **G**. Unfortunately, the mass spectral data collected (including MS−MS) did not provide enough definitive *m/z* ion peaks to make a clear decision between **F** and **G**. Using the ^{13}C NMR shifts in the two key regions where the structures differed was explored next. The experimental δ_C data (in $CDCl_3$) of **1** at C-1 to C-3 and C-21 were virtually identical to values (in $CDCl_3$) at analogous positions for latrunculeic acid (Figure 3)[16] and made **G** an obvious first choice. However, an exhaustive literature search failed to reveal appropriate model data to rigorously rule out possibility **F**.

Evidence that alternative **G** and not **F** was the best match for **1** was obtained from the density functional theory GIAO (gauge independent atomic orbital[17]) method to obtain predictions of the ^{13}C δ values. Our laboratory has previously found that excellent agreement can be obtained between experimental and predicted ^{13}C shifts using the B3LYP/6-31G*//B3LYP/6-31G* basis sets[18] with an acceptable fit based on two parameters: % score > 85 and MAE (mean absolute error) < 2.2. The δ_C results tabulated in Figure 3 focused on two structural regions, defined as the lower core **F′/G′** and upper core **F″/G″**.

(11) Crews, P.; Kakou, Y.; Quinoa, E. *J. Am. Chem. Soc.* **1988**, *110*, 4365–4368.
(12) Sonnenschein, R. N.; Johnson, T. A.; Tenney, K.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2006**, *69*, 145–7.
(13) Sanders, M. L.; van Soest, R. W. M. *Biologie* **1996**, *88*, 117–122.
(14) Rodriguez, J.; Nieto, R. M.; Crews, P. *J. Nat. Prod.* **1993**, *56*, 2034–2040.
(15) Thale, Z.; Johnson, T.; Tenney, K.; Wenzel, P. J.; Lobkovsky, E.; Clardy, J.; Media, J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *J. Org. Chem.* **2002**, *67*, 9384–9391.
(16) Vilozny, B.; Amagata, T.; Mooberry, S. L.; Crews, P. *J. Nat. Prod.* **2004**, *67*, 1055–1057.
(17) Atalay, Y.; Basoglu, A.; Avci, D. *Spectrochim. Acta, Part A* **2008**, *69*, 460–466.

TABLE 1. ^{1}H and ^{13}C NMR[a] Data of 1 in C_6D_6 and $CDCl_3$

	C_6D_6						$CDCl_3$					
position	δ_H (J in Hz)	δ_C	type[b]	COSY	HMBC	1D NOE	δ_H (J in Hz)	δ_C	type[b]	COSY	HMBC	1D NOE
1		171.5	C					170.6	C			
2	5.69 (d, 1.2)	116.7	CH	21	1, 3, 4		5.67 (d, 1.2)	115.9	CH		1, 3, 4, 21	
3		163.0	C					163.1	C			
4a	2.67 (ddd, 14.4, 12.6, 7.5)	33.0	CH_2	5	2, 3, 5, 6		2.65 (ddd, 15.0, 12.6, 7.3)	33.0	CH_2	5	2, 3, 5, 6, 21	
4b	2.73 (ddd, 14.3, 12.3, 7.4)			5	2, 3, 5, 6		2.72 (ddd, 15.0, 12.4, 7.6)			5	2, 3, 5, 6, 21	
5	2.15 (m)	31.5	CH_2	4, 6	3, 4, 6		2.20 (m)	31.2	CH_2	4, 6	3, 6, 7	
6	5.62 (dt, 15.2, 7.3)	132.6	CH	5, 7	5, 8		5.35 (ddd, 15.2, 11.7, 6.0)	132.2	CH			
7	5.35 (dd, 15.2, 8.1)	127.9*	CH	6, 8	6, 8, 16		5.36 (dd, 15.2, 6.4)	127.9	CH			5, 8, 10, 13
8	4.16 (dd, 8.1, 5.4)	38.8	CH	7, 9	7, 9, 16		3.96 (dd, 5.7, 3.5)	38.8	CH	9	6, 7, 9, 13, 15, 16, 17	
9	0.70 (td, 11.4, 5.0)	52.2	CH	8, 10	8, 16	8, 14a, 22	1.10 (dt, 11.6, 5.3)	52.5	CH	8, 10	7, 8, 10, 11, 14, 22	
10	1.70 (m)	35.1	CH	9, 11, 22			1.75 (m)	34.9	CH	9, 11	21	
11a	1.15 (m)	32.0	CH_2	10, 12a			1.25 (m)	31.8	CH_2			
11b	1.80 (m)			10			1.93 (m)					
12a	0.92 (m)	29.6	CH_2	11a	11		1.25 (m)	29.4	CH_2			
12b	1.95 (m)						1.95 (m)					
13	1.62 (m)	34.7	CH	14a, 14b			1.88 (m)	34.4	CH			
14a	1.92 (dd, 16.9, 11.2)	37.9	CH_2	13	12, 13, 15, 16		2.50 (dd, 17.1, 11.2)	38.2	CH_2	13	12, 13, 15, 16	
14b	2.41 (dd, 16.9, 5.5)			13	9, 13, 15, 16		2.96 (dd, 17.1, 5.5)				9, 13, 15, 16	
15		148.3	C					149.3	C			
16		136.6	C					136.5	C			
17		172.1	C					172.1	C			
18		133.9	C					133.4	C			
19	8.60 (s)	114.6	CH				8.41 (s)	115.4	CH		15, 17, 18	
20		154.5	C					154.5	C			
21	1.89 (d, 1.2)	24.9	CH_3		2, 3, 4		1.87 (d, 1.2)	25.4	CH_3	2	2, 3, 4	
22	0.98 (d, 6.4)	18.2	CH_3	10	9, 10, 11		1.03 (d, 6.4)	18.1	CH_3	10	9, 10, 11	
OMe	3.36 (s)	52.0	CH_3		20		3.78 (s)	52.5	CH_3		20	
NH	8.72 (s)				17, 18, 20		8.35 (s)				17, 18, 19, 20	

[a] Measured at 600 MHz (^{1}H) and 125 MHz (^{13}C). [b] Carbon type determined by DEPT and HMQC experiments. * Assignment from HMQC.

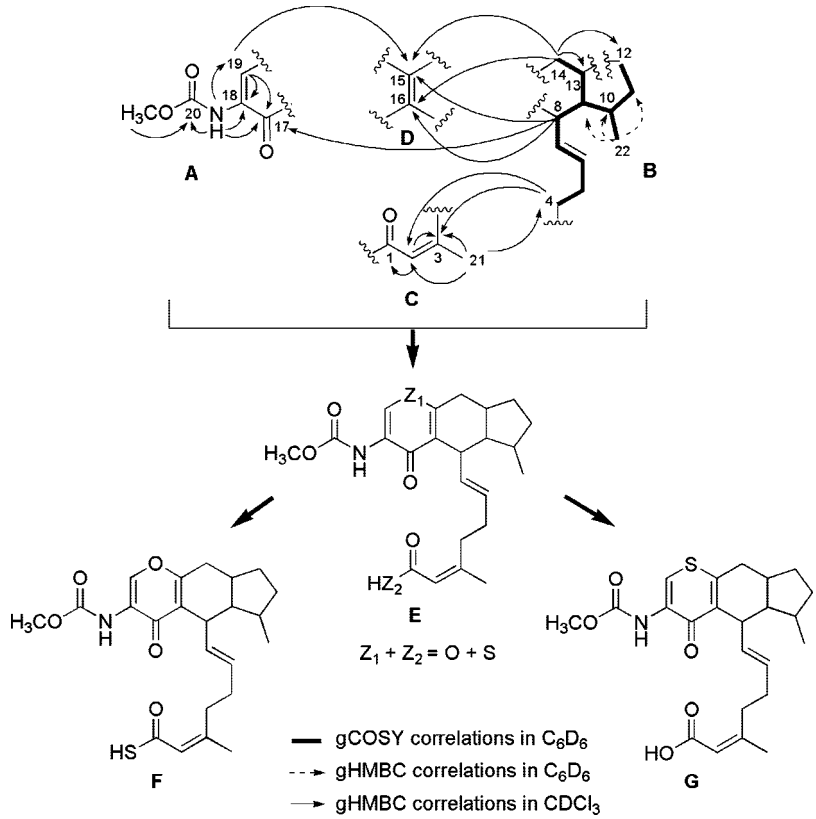


FIGURE 2. Substructures and significant 2D NMR correlations for CTP-431 (**1**).

To our disappointment, the first proof-of-concept DFT calculation comparison for the lower core region (**F′/G′**), involving analysis of the isostructural atoms of latrunculeic acid, was unrewarding as evidenced by the MAE = 5.0 and % score = 33 for **G′**. Alternatively, the DFT data for **F′** illustrated that the predicted shifts were sensitive to heteroatom changes. The DFT calculations were next carried out on simple models (see Scheme S2, Supporting Information) and provided excellent results for γ-pyrone[19] [MAE = 0.1, % score = 100] and γ-thiopyrone[20]

(18) White, K. N.; Amagata, T.; Tenney, K.; Oliver, A. G.; Wenzel, P.; Crews, P., unpublished results.

(19) Pouchert, C. J.; Behnke, J. *The Aldrich Library of 13C and 1H FT NMR Spectra*, I ed.; Aldrich: Milwaukee, WI, 1993; Vol. 1, p 723 B.

(20) Detty, M. R.; Luss, H. R. *Organometallics* **1992**, *11*, 2157–2162.

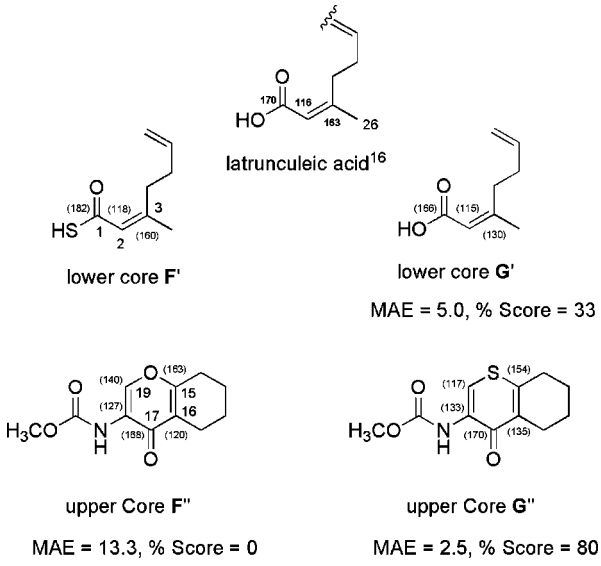


FIGURE 3. Experimental δ_C values (in bold) and DFT calculation results (in parenthesis) for lower core (**F′** and **G′**) and upper core (**F″** and **G″**).

[MAE = 1.0, % score = 100]. A second round of calculations was informative; the fit of **F″** was unacceptable whereas the agreement for **G″** was nearly excellent.

A final push to complete the structure elucidation for CTP-431 (**1**), concluded above to be planar structure **G**, focused on defining its conformational and configuration properties. A trans relationship could be assigned for H-9 and H-13 based on the $^3J_{9,13}$ = 11.4 Hz. The six-membered ring appeared to adopt a twist-chair conformation as evidenced by the large $\Delta\delta_H$ = 0.46 ppm ($CDCl_3$) for the diastereotopic H-14's and a trans diaxial coupling (J = 11.2 Hz) between H-14a and H-13. The same relative facial stereochemistry shown in **1** for the side chain at C-8, H-13, and H-10 was deduced from a series of 1D NOE correlations including those from H-7 to H-13 and to H-10. Similarly, the additional 1 D NOEs from H-9 to H-14a and to H_3-22 also indicated that these three groups were in on the same side of the molecular plane. It became possible to define the absolute configurations once columnar crystals were obtained using a vapor diffusion method. This material was subjected to X-ray analysis, and the outcome of that examination is shown in Figure 4. The diffraction pattern verified the gross structure as **1** (R factor = 0.0580) while also providing evidence for the 8*R*,9*R*,10*S*,13*S* designations through a comparison of the intensities of the Friedel pairs of reflections with the Flack parameter = 0.02 (6).[21,22] Additional inspection of the X-ray structure indicates a similar conformation of **1** exists in solution vs the solid state.

The effort to evaluate the bioactivity of **1** was primarily based on the past history of positive results for *C. mycofijiensis* metabolites. In contrast to the robust antiactin activity of the latrunculins, CTP-431 (**1**) displayed no signs of microfilament disrupting effects at 50 μM, and these data were obtained using a previously employed cytoskeletal assay. Similarly, **1** showed only mild cytotoxicity (IC_{50} = 18 μM) against human colon carcinoma HCT-116. By contrast, synthetic structures possessing

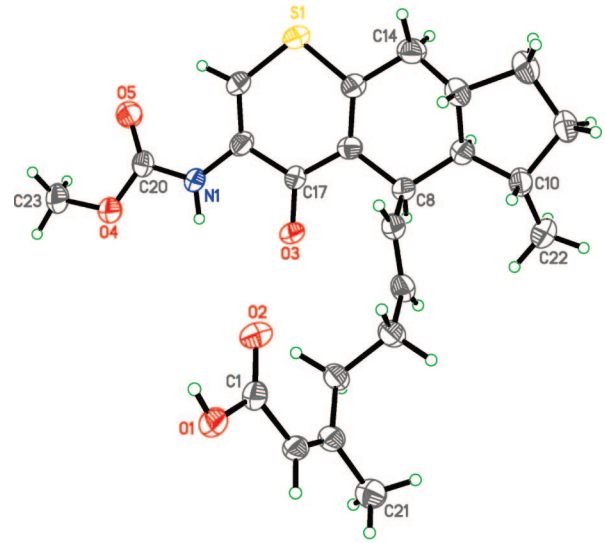


FIGURE 4. X-ray crystal structure of CTP-431 (**1**).

a thiopyrone core have been shown to be potent inhibitors (IC_{50} ~0.2 μM) of DNA-dependent protein kinase.[23]

The most structurally distinct portion of CTP-431 (**1**) consists of the thiopyrone functionality not previously observed in a natural product. Alternatively, **1** possesses several familiar structural features including an α,β-unsaturated carboxylate that is a signature residue of all members of the latrunculin A and B families. The spatial relationship between C-21 and C-22 of **1** is similar to a conserved feature for the latrunculins as all analogues have the two pendant C-methyl groups separated by four or six carbons. The configuration of the aliphatic methyl bearing carbon in the latrunculins is often shown as *S*, the same as depicted in **1**. Finally, the S and N atoms appear to be a masked cysteine residue analogous to that observed in most latrunculins and also present in mycothiazole. Outlined in Figure 5 is a plausible scheme that shows how **1** and latrunculin A could be derived from the same mixed PKS-NRPS biogenetic precursor. A major difference in the formation of the former vs the latter is in the cyclization steps shown as solid arrows vs dashed arrows, accompanied by further modification of the cysteine N or S atoms.

Experimental Section

Biological Material, Collection, and Identification. Specimens of *Cacospongia mycofijiensis* (coll. no. 89126) (5.8 kg wet weight) were collected using scuba in 1989 from the Beqa Lagoon, Fiji, at depths of 15−20 m. Taxonomic identification was based on comparison of the biological features to other samples in our repository and physical features of those previously published.[13] Voucher specimens and underwater photos are available upon request. This sample is identical to the voucher (BMNH 1986-9-18-1,2) we have deposited previously in the Natural History Museum (London).[13]

Extraction and Isolation. Samples were preserved in the field according to our standard laboratory procedures[14] and stored in a cold room at +4 °C until extraction was performed. Initially, a 2.3 kg sample of the sponge was rapidly extracted with hexanes (XFH), dichloromethane (XFD), and methanol (XFM) using an accelerated

(21) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.
(22) Dounay, A. B.; Humphreys, P. G.; Overman, L. E.; Wrobleski, A. D. *J. Am. Chem. Soc.* **2008**, *130*, 5368–5377.

(23) Hollick, J. J.; Rigoreau, L. J. M.; Cano-Soumillac, C.; Cockcroft, X.; Curtin, N. J.; Frigerio, M.; Golding, B. T.; Guiard, S.; Hardcastle, I. R.; Hickson, I.; Hummersone, M. G.; Menear, K. A.; Martin, N. M. B.; Matthews, I.; Newell, D. R.; Ord, R.; Richardson, C. J.; Smith, G. C. M.; Griffin, R. J. *J. Med. Chem.* **2007**, *50*, 1958–1972.

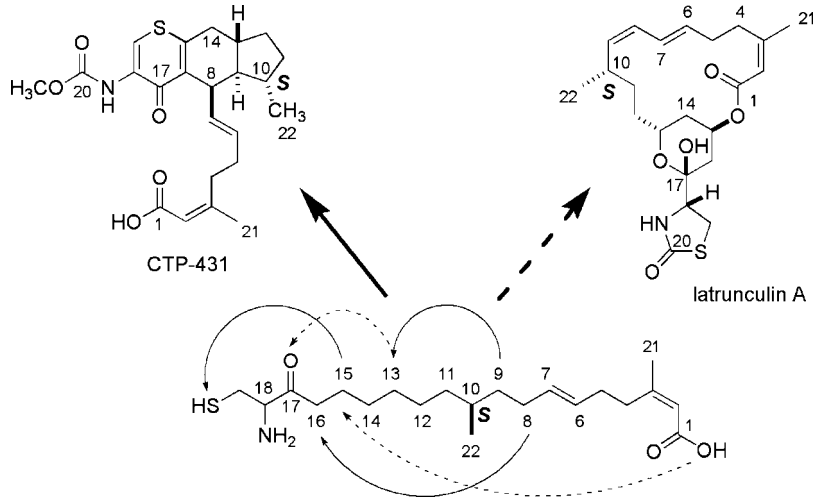


FIGURE 5. Proposed biogenetic precursor and mechanism to generate: CTP-431 (**1**) (solid arrows) and latrunculin A (dashed arrows).

solvent extraction system under high pressure (1700 psi) at 110 °C. Pure compounds were obtained as follows: A 75 mg portion of the 89126 dichloromethane extract (XFD) was fractionated using repeated semipreparative reversed-phase gradient HPLC (30:70 CH_3CN/H_2O up to 80:20 over 50 min) to give seven fractions. Fractions H5 (8.7 mg) and H6 (8.3 mg) afforded pure mycothiazole and CTP 431 (**1**) (see Chart S1, Supporting Information). The remaining 3.5 kg of sponge was subsequently extracted using a modified Kupchan-like solvent partition scheme[15] (see Chart S2, Supporting Information).

Mycothiazole: viscous oil; LRESITOFMS *m*/*z* 405.1 $[M + H]^+$; ^1H and ^{13}C NMR data in Table S1 and Figures S2 and S3 (see the Supporting Information) and were in agreement with literature values.[12]

CTP-431 (1): white amorphous powder and colorless crystal; UV (MeOH) λ_{max} 226 nm (ϵ 7369) 245 nm (ϵ 5454) 317 nm (ϵ 3385); $[\alpha]^{28}_D + 48$ (*c* 0.5, $CHCl_3$); mp 114−122 °C; HRESITOFMS *m*/*z* 432.1839 $[M + H]^+$ (calcd for $C_{23}H_{30}NO_5S$ 432.1844); NMR spectral data, see Table 1.

Crystallization Method. Suitable crystals were obtained using a vapor diffusion method: 3 mg of **1** was dissolved in 300 μL of $CDCl_3$ surround by 3 mL of hexanes, sealed, and stored at −4 °C for 3 weeks. The sample crystallizes as colorless columnar crystals (mp 114−122 °C).

For further information regarding the X-ray crystallography data of **1**, see the experimental procedures in the Supporting Information.

Acknowledgment. This work was supported by the National Institutes of Health (RO1 CA 47135). The single-crystal X-ray diffraction data in this work were recorded on an instrument supported by the National Science Foundation, Major Research Instrumentation (MRI) Program under Grant No.CHE-0521569. We are grateful to Dr. S. L. Mooberry and D. LeBouef of the Southwestern Foundation for Biomedical Research for evaluating CTP-431 (**1**) using their cytoskeletal assays. We also give special thanks to Prof. W. Aalbersberg, University of the South Pacific, Fiji, and A. Raiwalui, Permanent Secretary for the Director of Fisheries, Ministry of Agriculture, Fisheries and Forests Division, for obtaining administrative approvals to carry out research in Fiji.

Supporting Information Available: Eight tables and 16 figures are provided which include the experimental procedures, NMR, and X-ray crystallography data of CTP-431 (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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