A New Bioactive Triene Aldehyde from the Marine Sponge *Leucetta microraphis*

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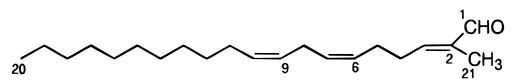
A new triene aldehyde, (2*E*,6*Z*,9*Z*)-2-methyl-2,6,9-icosatrienal (**1**), was isolated from a MeOH extract of the Okinawan marine sponge *Leucetta microraphis*. The structure of **1** was determined by spectroscopic analysis. Three imidazole alkaloids, leucettamine B, preclathridine A, and (9*E*)-clathridine 9-*N*-(2-sulfoethyl)imine, were also obtained and identified. Compound **1** showed moderate growth-inhibitory activity toward HeLa S3 cells.

Marine calcareous sponges of the genus *Leucetta* are rich sources of structurally unique and biologically active compounds.[1−14] Imidazole alkaloids such as naamidines,[12,13] isonaamidines,[12,13] and leucettamines[7] are major compounds present in *Leucetta* sponges. Interesting zinc complexes of naamidines and isonaamidins have also been found.[1,5,9] Among the imidazole alkaloids, pyronaamidines have been shown to be cytotoxic[11] and leucettamine A and leucettamidine, to possess leukotriene B_4 receptor binding activity.[7] The antimicrobial polyenes, leucettamols A and B,[8] and cytotoxic polyene, rhapsamine,[2] were previously obtained from sponges of the genus *Leucetta* as nonalkaloidal constituents. These compounds were found in sponges that inhabit the seawaters of Micronesia, the Red Sea, Bermuda, Fiji, the Great Barrier Reef, New Caledonia, and Antarctica. No studies have been conducted to date on constituents from *Leucetta* sponges in Okinawan seawater.

During investigation on constituents from Okinawan marine invertebrates, a new bioactive triene aldehyde (**1**) was obtained from an Okinawan specimen of *Leucetta microraphis* (Haeckel) (Leucettidae) along with three known imidazole alkaloids. The isolation and structure determination of **1** are discussed herein.



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The methanolic extract of the sponge was subjected to solvent-partition to obtain EtOAc-, BuOH-, and H_2O-soluble portions. From the EtOAc-soluble portion, compound **1** (colorless oil, 3.4 mg) and leucettamine B[7] were isolated. Preclathridine A[9] and (9*E*)-clathridine 9-*N*-(2-sulfoethyl)imine[10] were also obtained from the BuOH-soluble portion. The structures of three known imidazole alkaloids were identified by the comparison of spectral data with those in the literature.

Compound **1** was found to have the molecular formula, $C_{21}H_{36}O$, based on HREIMS; found 304.2776 [$(M)^+$, $C_{21}H_{36}O$ requires 304.2766]. The presence of an α,β-unsaturated carbonyl group was suggested by UV (230 nm) and IR (1693 cm^{-1}) absorptions. The ^{13}C NMR spectrum of **1** (Table 1) showed the following 21 carbon signals: one aldehyde, five olefinic methines, one olefinic quaternary carbon, 12 methylenes, and two methyls. The ^1H NMR spectrum of **1** (Table 1) indicated one aldehyde proton, one olefinic proton on a conjugated system, four olefinic protons on two nonconjugated carbon−carbon double bonds, 24 methylene protons, and two methyl groups.

Table 1. ^{13}C and ^1H NMR Data (100 and 400 MHz, in $CDCl_3$) of Compound **1**

position	δ_C	δ_H
1	195.2 (CH)	9.39 (*s*)
2	139.7 (C)	
3	153.8 (CH)	6.48 (*tq*, $J = 7.1, 1.3$ Hz)
4	29.0 (CH_2)	2.43 (*q*, $J = 7.1$ Hz)
5	26.0 (CH_2)	2.28 (*q*, $J = 7.1$ Hz)
6	127.9 (CH)	5.41 (*m*)
7	129.8 (CH)	5.45 (*m*)
8	25.7 (CH_2)	2.79 (*t*, $J = 6.8$ Hz)
9	127.3 (CH)	5.32 (*m*)
10	130.7 (CH)	5.41 (*m*)
11	27.3 (CH_2)	2.04 (*q*, $J = 6.8$ Hz)
12	29.63 (CH_2)[a]	1.34 (*m*)
13−17	29.33−29.63[a]	1.25−1.30 (*m*, 10 H)
18	31.9 (CH_2)	1.26 (*m*)
19	22.7 (CH_2)	1.26 (*m*)
20	14.0 (CH_3)	0.88 (*t*, $J = 6.8$ Hz)
21	9.3 (CH_3)	1.75 (*d*, $J = 1.3$ Hz)

[a] There are two methylene carbons at δ 29.33, one at δ 29.55, and three at δ 29.63.

$^1H-^1H$ COSY demonstrated the $^1H-^1H$ correlations shown in Figure 1, thus indicating partial structures A, B, and C. Following the clarification of ^1H and ^{13}C NMR signal correlations based on the $^1H-^{13}C$ HMQC spectrum (Table 1), a $^1H-^{13}C$ HMBC experiment was performed to confirm partial structures A, B, and C and indicate that the aldehyde was connected to the trisubstituted double bond. Thus, based on the above, the remaining five methylenes are clearly shown to be situated between positions C-13 and C-19. Thus, the gross structure of **1**, except for the stereochemistry of the three carbon−carbon double bonds, was established.

(2*E*,6*Z*,9*Z*)-Configurations of the three double bonds in **1** were clarified based on NOE correlations. $^1H-^1H$ NOESY correlations between the aldehyde proton at δ 9.39 (H-1) and the olefinic proton at δ 6.48 (H-3) and between the methyl protons at δ 1.75 (H-21) and the methylene protons at δ 2.43 (H-4) showed the C-2 (3) double bond to have the *E* configuration. Methylene protons at δ 2.79 (H-8) correlated not only with those of δ 2.28 (H-5) but also with δ 2.04 (H-11), thus indicating the C-6 (7) and C-9 (10) double

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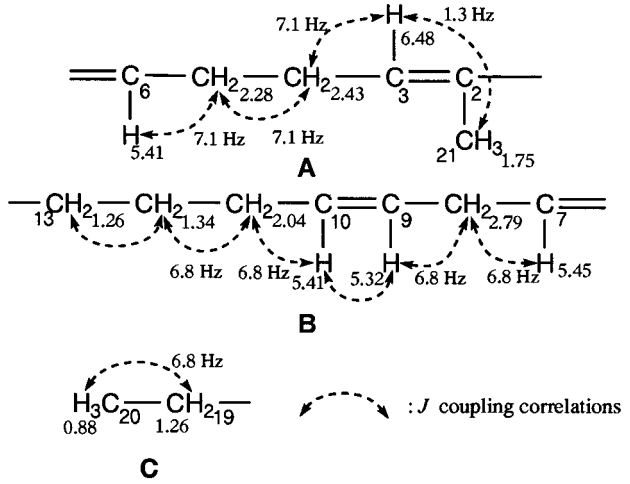

Figure 1. $^1H-^1H$ correlations and partial structures of **1**.

bonds to both have the *Z* configuration. The structure of **1** was thus concluded to be (2*E*,6*Z*,9*Z*)-2-methyl-2,6,9-icosatrienal.

Compound **1** may be regarded as a fatty acid-related aldehyde having a methyl branch on C-2. Although a number of branched fatty acids and related compounds have been found,[15−22] those that are methyl-branched on C-2 are rare.[20−22] Compound **1** showed growth-inhibitory activity toward HeLa S3 cells at 10 μg/mL.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a JASCO V-520 spectrophotometer and IR spectra with a Perkin-Elmer FT-IR 1600 spectrophotometer. NMR spectra were measured with a Bruker DPX-400 spectrometer (^1H; 400 MHz, ^{13}C; 100 MHz). DEPT, $^1H-^1H$ COSY, $^1H-^1H$ NOESY, $^1H-^{13}C$ HMQC, and $^1H-^{13}C$ HMBC were measured based on standard Bruker pulse sequences. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^1H 7.26 ppm; ^{13}C 77.0 ppm) as the internal standard, and C-multiplicities were assigned based on DEPT experiments. EIMS and HREIMS were obtained with a Micromass Auto Spec spectrometer. Open column chromatography was carried out on Si gel 60 (63−200 μm, Merck) for normal phase; Si gel 60 silanized (C_2 Si gel, 63−200 μm, Merck) for reversed-phase; and Sephadex LH-20 (Pharmacia) for gel chromatography. Flash column chromatography was performed on Si gel 60 (40−63 μm, Merck). Medium-pressure liquid chromatography (MPLC) was carried out on an Ultra Pack Si gel column (40 μm, 250 × 20 i.d., Yamazen, SI-40-B) for normal-phase and an Ultra Pack DIOL column (40 μm, 250 × 20 i.d., Yamazen, DIOL-40-B) for reversed-phase. HPLC was performed on YMC-Pack ODS-AM column (5 μm, 250 × 20 i.d., SH-343-5AM, YMC).

Animal Material. The sponge *Leucetta microraphis* was taken from the coral reef of Ishigaki Island (Okinawa Prefecture, Japan) in June 1996, at a depth of 10 m. A voucher specimen (no. 96-S-9) is presently on deposit at this laboratory, Tokyo University of Pharmacy and Life Science (Tokyo, Japan).

Extraction and Isolation. Wet specimens (1.25 kg) were extracted three times with MeOH (5 L, each). After filtration, the MeOH solution was concentrated under reduced pressure to give the MeOH extract (total, 48.2 g), which was then partitioned between H_2O and EtOAc. After removal of the EtOAc solution, the remaining aqueous layer was extracted with BuOH. Each fraction was concentrated under reduced pressure to give EtOAc- (3.4 g), BuOH- (3.2 g), and H_2O- (38.9 g) soluble portions.

The EtOAc-soluble portion was chromatographed on a Si gel column (200 g). Stepwise elution with *n*-hexane−EtOAc (9:1, 8:2 and 1:1, each 1 L), EtOAc (1 L), and MeOH (1 L) afforded eight fractions. On the first fraction [511 mg, eluted with *n*-hexanes−EtOAc (9:1)], normal-phase flash column chromatography [Si gel 60, *n*-hexanes−EtOAc (97:3)] and normal-phase MPLC [Ultra Pack Si gel, *n*-hexanes−EtOAc (98:2)] were carried out to give crude compound **1**, which was purified by reversed-phase HPLC (ODS, CH_3CN) to give **1** (3.4 mg). For the seventh fraction (318 mg, eluted with EtOAc), normal-phase flash column chromatography [Si gel 60, *n*-hexane−dioxane (1:1)] and recrystallization from MeOH were conducted to obtain leucettamine B (20.8 mg).[7]

The BuOH-soluble portion was chromatographed on a silanized Si gel column (60 g). Stepwise elution with H_2O, H_2O−MeOH (3:1, 1:1, and 1:3), MeOH, and dioxane gave six fractions. For the second fraction [186 mg, eluted with H_2O−MeOH (3:1)], gel chromatography (Sephadex LH-20, MeOH) and reversed-phase MPLC [DIOL, $CHCl_3$−MeOH (19:1)] were carried out to afford preclathridine A (45 mg).[9] The third fraction [125 mg, eluted with H_2O−MeOH (1:1)] was subjected to gel chromatography (Sephadex LH-20, $CHCl_3$, and MeOH), normal-phase column chromatography [Si gel 60, $CHCl_3$−MeOH (19:1 and 9:1)], and recrystallization from MeOH to obtain (9*E*)-clathridine 9-*N*-(2-sulfoethyl)imine (23.6 mg).[10]

Compound 1: colorless oil (3.4 mg), UV λ_{max} (EtOH) 230 (4.27) nm; IR (film) 2925, 2854, 1693 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; HREIMS *m/z* 304.2776 (calcd for $C_{21}H_{36}O$ 304.2766); EIMS $[M]^+$ 304 (8), 275 (6), 220 (95), 191 (7), 177 (14), 163 (18), 149 (25), 135 (30), 121 (29), 109 (45), 95 (78), 79 (86), 67 (100), 55 (63).

Cytotoxic Activity. In a 25-well plate, HeLa S3 cells (1×10^5 cells) were inoculated in 2 mL of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The tested compounds were added to each well at an adequate concentration in MeOH (2 μL) at the inoculation time. The cells were maintained at 37 °C in 5% CO_2. Seventy-two hours after inoculation, the cells were stained with crystal violet, and the cell growth was determined by microscopic observation.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Fu, X.; Schmitz, F. J.; Tanner, R. S.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 384−386.
(2) Jayatilake, G. S.; Baker, B. J.; McClintock, J. B. *Tetrahedron Lett.* **1997**, *38*, 7507−7510.
(3) Plubrukarn, A.; Smith, D. W.; Cramer, R. E.; Davidson, B. S. *J. Nat. Prod.* **1997**, *60*, 712−715.
(4) Fu, X.; Barnes, J. R.; Do, T.; Schmitz, F. J. *J. Nat. Prod.* **1997**, *60*, 497−498.
(5) Mancini, I.; Guella, G.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1995**, *78*, 1178−1184.
(6) Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 1229−1234.
(7) Chan, G. W.; Mong, S.; Hemling, M. E.; Freyer, A. J.; Offen, P. H.; DeBrosse, C. W.; Sarau, H. M.; Westley, J. W. *J. Nat. Prod.* **1993**, *56*, 116−121.
(8) Kong, F.; Faulkner, D. J. *J. Org. Chem.* **1993**, *58*, 970−971.
(9) Alvi, K. A.; Peters, B. M.; Hunter, L. M.; Crews, P. *Tetrahedron* **1993**, *49*, 329−336.

(10) He, H.-Y.; Faulkner, D. J.; Lee, A. Y.; Clardy, J. *J. Org. Chem*. **1992**, *57*, 2176−2178.
(11) Akee, R. K.; Carroll, T. R.; Yoshida, W. Y.; Scheuer, P. J.; Stout, T. J.; Clardy, J. *J. Org. Chem*. **1990**, *55*, 1944−1946.
(12) Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193−2200.
(13) Carmely, S.; Kashman, Y. *Tetrahedron Lett*. **1987**, *28*, 3003−3006.
(14) Cardellina, J. H., II; Meinwald, J. *J. Org. Chem*. **1981**, *46*, 4782−4784.
(15) Yu, C.-M.; Curtis, J. M.; Wright, J. L. C.; Ayer, S. W.; Fathi-Afshar, Z. R. *Can. J. Chem*. **1996**, *74*, 730−735.
(16) Carballeira, N. M.; Negron, V. *J. Nat. Prod*. **1991**, *54*, 305−309.
(17) Carballeira, N. M.; Shalabi, F. *Lipids* **1990**, *25*, 835−840.
(18) Carballeira, N.; Thompson, J. E.; Ayanoglu, E.; Djerassi, C. *J. Org. Chem*. **1986**, *51*, 2751−2756.
(19) Fish, W. R.; Holz, G. G., Jr.; Beach, D. H. *Mol. Biochem. Parasitol*. **1982**, *5*, 1−18.
(20) Duncan, W. R. H.; Lough, A. K.; Garton, G. A.; Brooks, P. *Lipids* **1981**, *9*, 669−673.
(21) Jacob, J. *Hoppe-Seyler's Z. Physiol. Chem*. **1975**, *356*, 1823−1825.
(22) Karlsson, H.; Odham, G. *Ark. Kemi* **1969**, *31*, 143−158.

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