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(–)-Axinyssene: A Novel Cytotoxic Diterpene from a Japanese Marine Sponge *Axinyssa* sp

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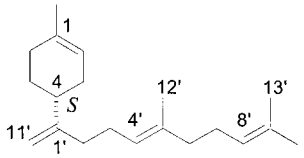
ABSTRACT



(-)-axinyssene (**1**)

A novel diterpene, (–)-axinyssene, was isolated from the Japanese marine sponge *Axinyssa* sp. The structure of (–)-axinyssene was determined on the basis of spectroscopic and synthetic evidence to be 1-methyl-4-[(4*E*)-5′,9′-dimethyl-1′-methylene-4′,8′-decadienyl]-(4*S*)-cyclohexene. (–)- and (+)-axinyssene showed mild cytotoxicity against acute promyelocytic leukemia, HL-60 cells.

Marine sponges have proved to be a rich source of secondary metabolites with unusual structures as well as interesting biological activities. In the last few decades, it is not unusual to suggest that most of the bioactive compounds might be produced by symbiotic microorganisms.[1] Sponges of the genus *Axinyssa* have been reported to yield a variety of sesquiterpene, isocyanides, thiocyanates, isothiocyanates, formamides, and carbonimide dichlorides.[2] In the course of our continuing research on biologically active compounds from Japanese marine invertebrates, we have investigated the isolation for the cytotoxic principle from the marine sponge *Axinyssa* sp. We report here the isolation and structure elucidation of a new diterpene, (−)-axinyssene (**1**). *Axinyssa* sp. (wet weight = 289 g) was collected by hand at depths of 10 m off Tsutsumi Island, Fukuoka prefecture, Japan, in September, 1996.[3] The *n*-hexane-soluble fraction (2.02 g) of the acetone extract obtained from *Axinyssa* sp. showed cytotoxicity against HL-60 cells at 33 μg/mL. Bioassay guided the separation of the active fraction by Sephadex LH-20, silica gel chromatography (*n*-hexane/EtOAc), and reversed-phase HPLC to give an active compound, (−)-axinyssene (**1**, 1.4 mg), together with four known sesquiterpenes, isothiocyanate[4] (**2**, 10.1 mg), (+)-axisothiocyanate 2[5] (**3**, 9.8 mg), and epipolasin A[6] (**4**, 7.4 mg).

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(1) Faulkner, D. J. *J. Nat. Prod. Rep*. **2002**, *19*, 1−48 and previous reports in this series.

(2) (a) Marcus, A. H.; Molinski, T. F.; Fahy, E.; Faulkner, D. J. *J. Org. Chem*. **1989**, *54*, 5184. (b) He, H.-Y.; Salvá, J.; Catalos, R. F.; Faulkner, D. J. *J. Org. Chem*. **1992**, *57*, 3191. (c) Patil, A. D.; Freyer, A. J.; Reichwein, R.; Bean, M. F.; Faucette, L.; Johnson, R. K.; Haltiwanger, R. C.; Eggleston, D. S. *J. Nat. Prod*. **1997**, *60*, 507. (d) Hirota, H.; Okino, T.; Yoshimura, E.; Fusetani, N. *Tetrahedron* **1998**, *54*, 13971. (e) Li, C.-J.; Schmitz, F. J.; Kelly, M. *J. Nat. Prod*. **1999**, *62*, 1330. (f) Petrichtcheva, D. V.; Duque, C.; Duenas, A.; Zea, S.; Hara, N.; Fujimoto, Y. *J. Nat. Prod*. **2002**, *65*, 851.

(3) *Axinyssa* sp.: Class Demospongiae, order Astrophorida, family Ancorinidae. A voucher specimen was deposited in the Zoological Museum in University of Amsterdam (ZMA Por 17060).

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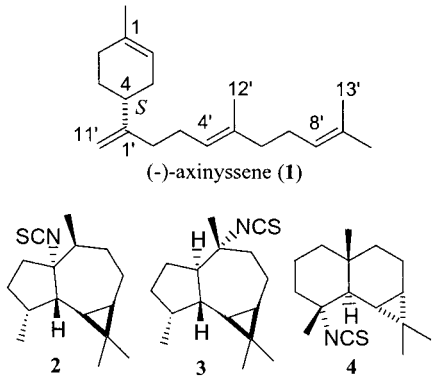

Figure 1. Structure of (−)-axinyssene (**1**) and sesquiterpenes.

(−)-Axinyssene (**1**)[7] was obtained as a colorless oil. The EIMS showed the molecular ion peak at *m*/*z* 272. The ^{1}H and ^{13}C NMR and HSQC spectral data suggested the presence of four olefinic methyls, eight methylenes, one methine, three trisubstituted olefines, and one vinyl. From the above data, the molecular formula of **1** was suggested to be $C_{20}H_{32}$. The ^{1}H−^{1}H COSY and HMBC spectra afforded partial structures as shown in Figure 2. Furthermore, these

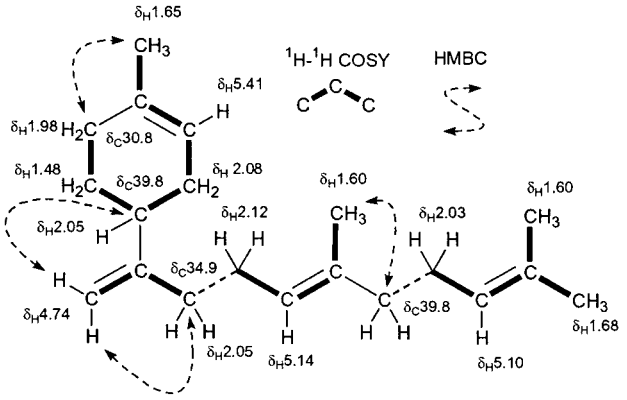

Figure 2. Significant correlations in DQF-COSY and PFG-HMBC spectra of **1**.

three partial structures were merged by the aid of the analysis of EIMS fragmentation as shown in Figure 3.

To determine the absolute configuration of **1**, (+)- and (−)-axinyssene were synthesized from the commercially available limonene and geraniol. The C-10 position of (+)- and (−)-limonene was alkylated with *sec*-BuLi-tetamethyl-

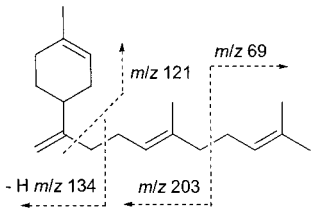

Figure 3. Intensive fragment peaks in EIMS of **1**.

ethylenediamine (TMEDA) complex and geranyl bromide,[8] and (+)- and (−)-axinyssene were obtained in 68.2 and 66.4% yields, respectively.[9]

The specific rotation of **1** showed an unreliable value due to the small quantity of material available; therefore, optical resolution with a chiral HPLC was applied for determination of the absolute configuration of **1**.[10] The synthesized (+)- and (−)-axinyssenes were subjected to chiral HPLC and showed retention times at 7.31 min for (+)-axinyssene and at 7.08 min for (−)-axinyssene, respectively. The retention time at 7.06 min for natural axinyssene was in good agreement with that of (−)-axinyssene. Accordingly, the structure of **1** was determined to be (−)-axinyssene (1-methyl-4-[(4*E*)-5′,9′dimethyl-1′-methylene-4′,8′-decadienyl]-(4*S*)-cyclohexene).

The cytotoxic activity of axinyssenes was evaluated by the MTT method. The IC_{50} values of axinyssenes against HL-60 cells were 16.9 μg/mL (**1**, natural), 12.1 μg/mL [(+)-axinyssene], and 9.6 μg/mL [(−)-axinyssene]. The (+)- and (−)-axinyssenes did not differ significantly in their cytotoxicity.

The carbon framework of (−)-axinyssene (**1**) has not been described and may be biosynthesized via geranyl-geranyl-diphosphate directly.

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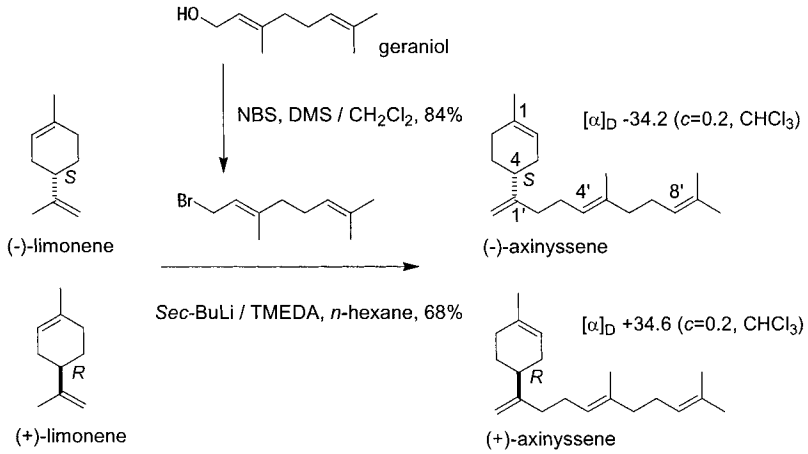
(5) Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C.; Sica, D. *Tetrahedron* **1975**, *31*, 269.
(6) Capon, R.; MacLeod, J. *Aust. J. Chem.* **1988**, *41*, 979.

(7) Axinyssene (natural, **1**): colorless oil; IR ($CHCl_3$, cm^{-1}) 2927, 1456, 1377, 891; EIMS (*m*/*z*) 272(M^+), 257, 204, 187, 69 (base peak); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48(1H, m, H5), 1.60(6H, s, H10′, H12′), 1.65(3H, s, H7), 1.68(3H, s, H13′), 1.81(1H, m, H3), 1.94(2H, m, H6′), 1.98(2H, m, H6), 2.03(2H, m, H7′), 2.05(1H, m, H4), 2.08(2H, m, H3), 4.74(2H, d, J = 6.9, H11′), 5.10(1H, t, J = 6.2, H8′), 5.14(1H, t, J = 6.4, H4′), 5.41(1H, br.s, H2); ^{13}C NMR (125.0 MHz, $CDCl_3$) δ 133.7(s, C1), 120.8(d, C-2), 31.4(t, C3), 39.8(d, C4), 28.4(t, C5), 30.8(t, C6), 23.4(q, C7), 154.3(s, C1′), 34.9(t, C2′), 26.8(t, C3′), 124.2(d, C4′), 135.1(s, C5′), 39.7(t, C6′), 26.8(t, C7′), 124.4(d, C8′), 131.3(s, C9′), 17.7(q, C10′), 107.1(t, C11′), 16.0(q, C12′), 25.7(q, C-13′).
(8) Suemune, H.; Kawahara, T.; Sakai, K. *Chem. Phram. Bull.* **1986**, *34*, 550.
(9) (+)-Axinyssene: colorless oil; $[\alpha]_D$ +34.6° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 87.74; H, 12.35. EIMS (*m*/*z*) 272(M^+); ^{13}C NMR (125.0 MHz, $CDCl_3$) δ 133.7(C1), 120.9(C2), 31.5(C3), 39.9(C4), 28.3(C5), 30.8(C6), 23.4(C7), 154.3(C1′), 34.9(C2′), 26.8(C3′), 124.3(C4′), 135.1(C5′), 39.7(C6′), 26.8(C7′), 124.4(C8′), 131.2-(C9′), 17.7(C10′), 107.2(C11′), 16.0(C12′), 25.7(C13′).
(10) (−)-Axinyssene: colorless oil; $[\alpha]_D$ −34.2° (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 87.78; H, 12.25. EIMS (*m*/*z*) 272(M^+); ^{13}C NMR (125.0 MHz, $CDCl_3$) δ 133.7(C1), 120.8(C2), 31.5(C3), 39.8(C4), 28.4(C5), 30.8(C6), 23.4(C7), 154.3(C1′), 34.9(C2′), 26.8(C3′), 124.2(C4′), 135.1(C5′), 39.7(C6′), 26.7(C7′), 124.4(C8′), 131.3-(C9′), 17.7(C10′), 107.2(C11′), 16.0(C12′), 25.7(C13′); for ^{1}H NMR data, see Supporting Information.

Scheme 1. Synthesis of (+)- and (−)-Axinyssenes



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Supporting Information Available: ^{1}H NMR spectra of natural, synthesized (+)- and (−)-axinyssenes and two-dimensional NMR spectra of (−)-axinyssenes. This material is available free of charge via the Internet at http://pubs.acs.org.

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