Communications to the Editor

Chem. Pharm. Bull.
35(2) 928—931 (1987)

FOUR NEW BISABOLENE-TYPE AMINOSESQUITERPENES FROM AN OKINAWAN MARINE SPONGE, *THEONELLA* SP. (THEONELLIDAE)

Isao Kitagawa,*,[a] Norihito Yoshioka,[a] Chikayo Kamba,[a]
Masayuki Yoshikawa,[a] and Yoshihiro Hamamoto[b]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1-6,
Yamada-oka, Suita, Osaka 565, Japan and Suntory Institute
for Biomedical Research,[b] Shimamoto-cho, Mishima-gun, Osaka
618, Japan

Four new aminosesquiterpenes, aminobisabolene (1), aminobisabolenol (2), isoaminobisabolenol-a (3), and isoaminobisabolenol-b (4), were isolated from an Okinawan marine sponge, *Theonella* sp. (Theonellidae) and their absolute configurations were determined.

KEYWORDS —— aminobisabolene; aminobisabolenol; isoaminobisabolenol-a; isoaminobisabolenol-b; aminosesquiterpene; *Theonella* sp.; marine sponge; allyl benzoate chirality method; sesquiterpene X-ray analysis

In a continuing study in search of bioactive constituents from marine organisms,[1)] we recently isolated five peptide-lactones, theonellapeptolides Ia, Ib, Ic, Id, and Ie, from an Okinawan marine sponge of *Theonella* sp. (Theonellidae) collected on the coral reef of Zamami-jima, Okinawa and determined the chemical structure of the major constituent theonellapeptolide Id.[2)]

For comparison, we have investigated another sponge specimen of *Theonella* sp. collected in July on the coral reef of Hatoma-jima, Okinawa.[3)] This specimen does not contain peptide-lactone but instead contains four new aminosesquiterpenes, aminobisabolene (1), aminobisabolenol (2), isoaminobisabolenol-a (3), and isoaminobisabolenol-b (4). This paper deals with their structure.[4)]

The cold acetone extract of the fresh sponge was partitioned into an AcOEt-water mixture. The AcOEt soluble portion was purified by column chromatography (SiO_2, $CHCl_3$-MeOH) to afford aminobisabolene (1, 9.6% from the AcOEt ext.). The water-soluble portion was extracted with 1-BuOH and the 1-BuOH extract was purified by column chromatography (SiO_2: $CHCl_3$-MeOH, $CHCl_3$-MeOH-aq. NH_4OH; SiO_2-$AgNO_3$: $CHCl_3$-MeOH-H_2O) to furnish aminobisabolenol (2) and a mixture of isoaminobisabolenol -a and -b (both 2.0% from the 1-BuOH ext.). The latter mixture was treated with 2,4-dinitrofluorobenzene in aq. Na_2CO_3-acetone (r.t.) and the DNP derivatives were separated by HPLC (Zorbax ODS, MeOH-H_2O=7:1). Removal of the DNP residue with Dowex 1x2 (OH^- form) in aq. acetone furnished isoaminobisabolenol-a (3, 0.8%) and -b (4, 0.8% from the 1-BuOH ext.).

Aminobisabolene (1), colorless oil, $C_{15}H_{27}N$,[5)] $[\alpha]_D$ +39° (MeOH), IR ($CHCl_3$): 3480-3100, 1579 cm^{-1}, colored yellowish orange with the Ninhydrin reagent. The ^1H

NMR spectrum[6)] of 1 showed signals due to one *tert.* methyl (δ 1.05, s, 8-CH_3), three olefinic methyls [δ 1.62, 1.63, 1.67, all 3H s, 1-CH_3, 13-$(CH_3)_2$], and two olefinic protons (δ 5.11, t, J=7.0 Hz, 12-H; δ 5.38, br s, 2-H). Acetylation of 1 afforded an N-acetate (1a), oil, $C_{17}H_{29}NO$, IR ($CHCl_3$): 3450, 1667, 1500 cm^{-1}, and SeO_2 oxidation of 1a in dioxane at 32°C gave a conjugated aldehyde (5), oil, $C_{17}H_{27}NO_2$, IR ($CHCl_3$): 3436, 1670. Detailed comparisons of ^1H and ^{13}C NMR data (Table I) for 1, 1a, and 5 indicate that 1 has a bisabolene structure with an amino group at C-8. To determine the structure, the bromobenzoyl derivative (1b), mp 114-116°C, $C_{17}H_{28}NOBr$, was subjected to X-ray analysis.[7)] As depicted in Fig. 1, the absolute configuration of 1b has been confirmed. Thus, the absolute stereostructure of aminobisabolene (1) with 4*R*, 8*S* configuration has been established.

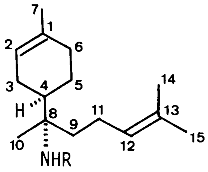
Aminobisabolenol (2), colorless oil, $C_{15}H_{27}NO$, $[\alpha]_D$ +29° (MeOH), IR ($CHCl_3$): 3580, 3320 (br) cm^{-1}, colored yellowish orange with the Ninhydrin reagent. The ^1H NMR spectrum of 2 showed signals due to three *tert.* methyls [δ 1.08, 3H s, 8-CH_3; δ 1.28, 6H s, 13-$(CH_3)_2$], one olefinic methyl (δ 1.63, s, 1-CH_3), and three olefinic protons (δ 5.38, br s, 2-H; δ 5.66, dt, J=15.5, 6.7 Hz, 11-H; δ 5.70, d, J=15.5 Hz, 12-H). Ordinary acetylation of 2 provided an N-acetate (2a), oil, $C_{17}H_{29}NO_2$, IR ($CHCl_3$): 3591, 3443, 1663, 1498 cm^{-1} and an N,O-diacetate (2b), oil, $C_{19}H_{31}NO_3$, IR ($CHCl_3$): 3442, 1718, 1666, 1495, 1238 cm^{-1}, in a 2:1 ratio. Treatment of 2 with 9% HCl-dry MeOH (r.t.) gave isomeric methoxyl derivatives: 2c (50%), oil, $C_{16}H_{29}NO$ and 6 (15%)(a mixture of C-11 isomers). Acetylation of 2c yielded an N-acetate (2d), oil, $C_{18}H_{31}NO_2$, IR ($CHCl_3$): 3450, 1665, 1498 cm^{-1}. Comparisons of the physical data for 2, 2a - 2d, and 6 revealed the plane structure of 2.

Catalytic hydrogenation (5% Pd-C) of 2 (r.t.) and subsequent treatment of the product with $SOCl_2$-pyridine (0°C, 5 min, N_2) provided 7. Acetylation of 7 followed by catalytic hydrogenation (5% Pd-C, r.t.) afforded 8 (63% from 2, a mixture of C-1 isomers in a 5:2 ratio).[8)] On the other hand, catalytic hydrogenation (5% Pd-C) of N-acetylaminobisabolene (1a) yielded 8 (a diastereomeric mixture in a 7:2 ratio). Finally, constant-current electrolysis [Pt electrode, $(PhSe)_2$, Et_4NBr, 1 mA/cm^2, 1 h, 0-5°C] of 1a in MeOH furnished 2d (conversion 50%, yield 80%). Thus, the absolute configuration of aminobisabolenol (2) has been determined.

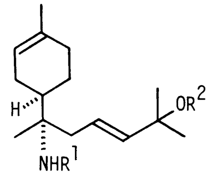
Isoaminobisabolenol-a (3), colorless oil, $C_{15}H_{27}NO$, $[\alpha]_D$ +34° (MeOH), IR ($CHCl_3$): 3665, 3360 (br), 1598 cm^{-1} and isoaminobisabolenol-b (4), colorless oil, $C_{15}H_{27}NO$, $[\alpha]_D$ +40° (MeOH), IR ($CHCl_3$): 3665, 3365 (br), 1608 cm^{-1}, are diastereomers. The ^1H NMR spectra (in $CDCl_3$) of both compounds showed signals due to one *tert.* methyl (3: δ 1.21; 4: δ 1.26; each s), exomethylene protons (3: δ 4.82, 4.98; 4: δ 4.83, 4.98; all 1H, br s), two olefinic methyls (3: δ 1.64, 1.73; 4: δ 1.64, 1.71; all 3H s), and one olefinic proton (3, 4: δ 5.36; both 1H, br s).

Acetylation followed by NaOMe-MeOH treatment (r.t.) of 3 or 4 provided 3a, oil, $C_{17}H_{29}NO_2$, IR ($CHCl_3$): 3660, 3600, 3450, 1664, 1493 cm^{-1} or 4a, oil, $C_{17}H_{29}NO_2$, IR ($CHCl_3$): 3660, 3600, 3450, 1661, 1494 cm^{-1}. CrO_3 oxidation of 3a or 4a in pyridine yielded the same conjugated ketone (9), oil, $C_{17}H_{27}NO_2$, IR ($CHCl_3$): 3450, 1666, 1497 cm^{-1}. Thus, isoaminobisabolenol-a (3) and -b (4) have been shown to be C-12 configurational isomers.

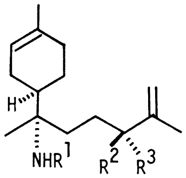
Treatment of N-acetylaminobisabolene (1a) with *m*-chloroperbenzoic acid in



1 : R = H
(aminobisabolene)
1a : R = Ac
1b : R = $\underset{\mathrm{O}}{\underset{\|}{\mathrm{C}}}\mathrm{CH_2Br}$



2 : $R^1 = R^2 = H$
(aminobisabolenol)
2a : $R^1 = Ac$, $R^2 = H$
2b : $R^1 = R^2 = Ac$
2c : $R^1 = H$, $R^2 = Me$
2d : $R^1 = Ac$, $R^2 = Me$



3 : $R^1 = R^3 = H$, $R^2 = OH$
(isoaminobisabolenol-a)
3a : $R^1 = Ac$, $R^2 = OH$, $R^3 = H$
3b : $R^1 = Ac$, $R^2 = OBz$, $R^3 = H$
4 : $R^1 = R^2 = H$, $R^3 = OH$
(isoaminobisabolenol-b)
4a : $R^1 = Ac$, $R^2 = H$, $R^3 = OH$
4b : $R^1 = Ac$, $R^2 = H$, $R^3 = OBz$

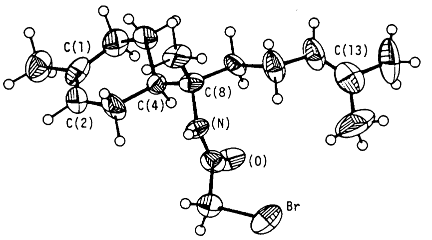
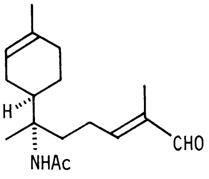
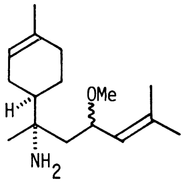


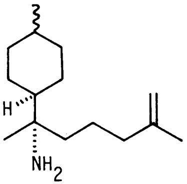
Fig 1 : **1b**



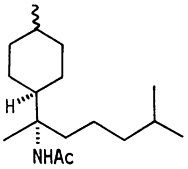
5



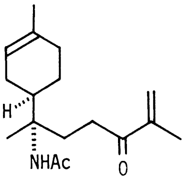
6



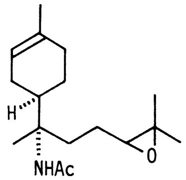
7



8



9



10

Table I. ^{13}C NMR Data$^{a)}$

	1	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**	**5**	**9**
1	133.4	133.9	133.9	134.0	134.0	134.1	133.8	134.1	134.1	134.1
2	120.1	120.7	119.7$^{c)}$	120.5	120.0	120.5	120.0	120.5	120.3	120.4
3	25.8$^{b)}$	26.9$^{b)}$	25.9$^{b)}$	26.4$^{b)}$	26.2$^{b)}$	26.6$^{b)}$	26.0$^{b)}$	26.6$^{b)}$	26.4$^{b)}$	26.6$^{b)}$
4	41.6	40.9	41.1	40.2	42.4	40.9	40.5	40.8	40.5	40.8
5	23.5$^{b)}$	24.3$^{b)}$	23.6$^{b)}$	24.4$^{b)}$	23.7$^{b)}$	24.4$^{b)}$	23.7$^{b)}$	24.4$^{b)}$	24.3$^{b)}$	24.4$^{b)}$
6	30.8	31.4	30.7	31.2	30.9	31.3$^{c)}$	30.8	31.3$^{c)}$	31.2	31.2
7	22.9	23.0	23.2	23.2	23.2	23.3	23.2	23.3	23.2	23.2
8	55.6	59.0	59.0	58.9	57.3	58.8	58.2	58.8	58.7	58.6
9	38.4	36.2	39.4	38.6	29.2	29.4	28.7	29.1	34.5	29.7
10	21.9	20.9	21.5	20.8	22.2	21.1	22.1	21.0	20.7	20.8
11	23.3$^{b)}$	22.7$^{b)}$	119.4$^{c)}$	122.2	33.7	31.9$^{c)}$	33.4	31.5$^{c)}$	23.9$^{b)}$	32.7
12	123.8	124.7	144.7	141.9	75.6	76.1	75.0	75.9	139.3	202.7
13	131.3	131.3	70.7	70.7	147.3	147.6	146.9	147.5	154.8	144.4
14	17.3	17.5	29.3	30.1	110.9	111.1	111.1	111.1	9.2	124.9
15	25.3	25.5	29.3	30.1	18.2	17.6	18.3	17.8	195.1	17.7

a) Measured at 22.5 MHz in $CDCl_3$ with a JEOL FX-90Q NMR spectrometer.
b,c) The assignments for these signals within the same column may be interchanged.

CH_2Cl_2 (r.t.) furnished a diastereomeric mixture of 12,13-epoxides (10) (conversion 45%, yield 67%), which, on treatment with *p*-TsOH·H_2O in CH_2Cl_2, provided 3a and 4a in a 4:3 ratio (conversion 49%, yieid 45%). Thus, the structures of 3a and 4a have been elucidated except for their C-12 configurations which have been determined by application of the allylic benzoate chirality method.[9)]

Benzoylation (benzoyl chloride-pyridine, 0°C, 1 h) of 3a or 4a afforded 3b, oil, $C_{24}H_{33}NO_3$ or 4b, oil, $C_{24}H_{33}NO_3$. The CD spectra (in MeOH) of 3b and 4b respectively showed a first positive maximum: $[\theta]_{226}$ +2100 and a first negative maximum: $[\theta]_{226}$ -3500. Consequently, the 12*S* and 12*R* configurations of 3 and 4 respectively have been determined.

Two bisabolene sesquiterpenes having an isothiocyanate group or a formamide group at C-1 were isolated from a marine sponge of *Theonella* sp. (collected in Kerama Rettō, Okinawa).[10)] The present bisabolene sesquiterpenes characteristically have an amino group at C-8. This may be of interest from the chemotaxonomical viewpoint.

REFERENCES AND NOTES

1) A recent paper on these subjects: I. Kitagawa, Z. Cui, B. W. Son, M. Kobayashi, and Y. Kyogoku, Chem. Pharm. Bull., 35, 124 (1987), and the preceding papers of this series.
2) I. Kitagawa, M. Kobayashi, N. K. Lee, H. Shibuya, Y. Kawata, and F. Sakiyama, Chem. Pharm. Bull., 34, 2664 (1986). This sponge species was abbreviated Theo-83-ZM-1 in our laboratory.
3) This marine sponge (abbreviated Theo-84-HM-1) looks very similar to, but differs from, the one collected in Zamami-jima (Theo-83-ZM-1). It was identified as *Theonella* sp. (Theonellidae) by Dr. T. Hoshino, Mukaijima Biological Station, Hiroshima University, to whom the authors are grateful.
4) I. Kitagawa, M. Kobayashi, N. K. Lee, H. Shibuya, C. Kamba, M. Yoshikawa, Y. Kawata, and F. Sakiyama, presented at the 28th Symposium on the Chemistry of Natural Products, held at Sendai, Oct. 1986. Symposium Papers, p. 144.
5) The molecular compositions of the new compounds, with their chemical formulae, were determined by high resolution mass spectrometry.
6) The ^1H NMR spectra were measured at 500 MHz in CD_3OD (unless specified otherwise) with a JEOL GX-500 NMR spectrometer.
7) The crystals were recrystallized from n-hexane: prisms, orthorhombic, space group $P2_12_12_1$, a=9.7655(9), b=19.987(2), c=9.0470(7) Å, v=1765.8(3) $Å^3$, z=4, D_C= 1.29 g/cm^3, F(000)=152, μ(Cu Kα)=31.476. The X-ray diffraction intensity data from the crystal (0.1x0.2x0.3 mm) were obtained on a Rigaku AFC diffractometer equipped with a rotating anode X-ray generator (50 kV-200 mA), using graphite-monochromated Cu Kα radiation (λ=1.5418 Å). A total of 1663 independent reflections with $2\theta \leq 126°$ were collected by the ω scanning mode ($2\theta<45°$) and the $\omega/2\theta$ scanning mode ($2\theta \geq 45°$). The structure was solved by the direct method MULTAN80 (Main, *et al.*, 1980). The refinement of the structure using 1584 reflections with $|Fo| \geq 3\sigma(Fo)$ was carried out by the block-diagonal least-squares method with anisotropic thermal parameters and the R factor was reduced to 0.065. Computations were performed on the PANAFACOM U-1200 II of RIGAKU RASA-5RP system. The determination of the absolute structure was carried out on a microcomputer NEC PC-9801 VM2 using the unpublished program "CHIRAL" developed by Prof. Y. Katsube, *et al.* The authors are grateful to Profs. Y. Katsube and N. Tanaka for providing them with the program.
8) Two diastereomers: 8a, oil, $C_{17}H_{34}NO$, IR ($CHCl_3$): 3670, 3450, 1664, 1497 and 8b, oil, $C_{17}H_{34}NO$, IR ($CHCl_3$): 3670, 3440, 1661, 1493 cm^{-1}, were separated by HPLC (Zorbax SIL, n-hexane-AcOEt). However, the C-1 configuration of each compound has not yet been clarified.
9) a) N. Harada and K. Nakanishi, "Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry," Tokyo Kagaku Dojin, Tokyo, 1982; b) N. Harada, Kagaku to Seibutsu, 22, 329 (1984).
10) H. Nakamura, J. Kobayashi, Y. Ohizumi, and Y. Hirata, Tetrahedron Lett., 25, 5401 (1984).

(Received January 14, 1987)