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ACALYCIXENIOLIDES, NOVEL NORDITERPENES WITH ALLENE FUNCTIONALITY FROM TWO GORGONIANS OF THE GENUS ACALYCIGORGIA[1]

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Abstract – Acalycixeniolide B' (**4**), C (**5**) and the previously reported ginamallene (**3**) have been isolated from the gorgonian of the genus Acalycigorgia as inhibitors of cell division of fertilized sea urchin eggs. The structures of **4** and **5** have been determined by spectroscopic study and by comparison of spectral data with those of acalycixeniolide A (**1**) and B (**2**) isolated from A. inermis.

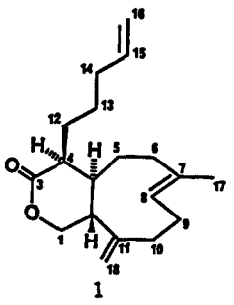
Coelenterates, particularly soft corals and gorgonians are a rich source of diterpenoids which include cembranoids, asbestinins, briareins, pseudopterosins, and xenicanes.[2] These diterpenes possess a variety of biological activity ranging from cytotoxicity to anti-inflammatory activity. Xenicane diterpenoids are a conspicuous group of gorgonian and soft coral metabolites.[3,4] As part of our continuing search for bioactive metabolites from Japanese marine invertebrates, we isolated two norditerpenes designated acalycixeniolide A (**1**) and B (**2**) as inhibitors of cell division of fertilized starfish eggs from the gorgonian Acalycigorgia inermis.[5] Acalycixeniolide B was a xenicane-type norditerpene with a terminal allene. Subsequently, we isolated three active norditerpenes from another species of Acalycigorgia as inhibitors of cell division of fertilized sea urchin eggs. The major active compound **3** was a xenicin-type norditerpene with a terminal allene.[4] Two minor compounds, **4** and **5**, were xenicane-type norditerpenes with a terminal allene. The major compound **3** was also isolated from three species of Acalycigorgia from Okinawan waters by the Ryukyu group and designated ginamallene.[6] In this paper, we report the isolation and structure determination of the two minor active constituents of Acalycigorgia sp.

Two novel norditerpenes, acalycixeniolide A (**1**) and B (**2**) were isolated from Acalycigorgia inermis (Hedlund). The animals (500 g, wet weight) collected by using SCUBA in the Gulf of Suruga at a depth of 15m were extracted with ethanol and the extract was partitioned between ether and water. The ether fraction was successively purified on a SiO_2 column (benzene/ethyl acetate, stepwise elution), followed by HPLC on SiO_2 (n-hexane/ethyl acetate/acetonitrile, 94.5:5:0.5), and on ODS (methanol/water, 9:1) to yield acalycixeniolide A (10 mg) and B (6 mg). The structures of

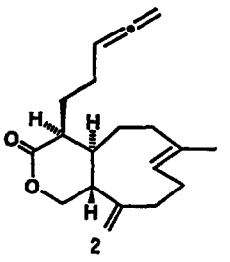
these two compounds were determined by spectroscopic analyses including extensive NMR experiments. Relative stereochemistry was deduced by NOE difference spectroscopy (NOEDS) and decoupling defference spectroscopy(DDS).[5]

Subsequently, three novel norditerpenes were isolated from another species of Acalycigorgia . The specimen (380 g) collected by using SCUBA (-15 to -20 m) in the Gulf of Suruga was extracted with ethanol, and the extract was separated by solvent partition and silica gel flash chromatography (n-hexane/ethyl acetate). The active fractions were chromatographed by HPLC on ODS (methanol/water, 9:1) to give pure ginamallene (3) (124 mg) and a mixture of 4 and 5. The mixture was further purified by HPLC on ODS (acetonitrile/water, 3:1) and then on silica gel (n-hexane/ether, 4:1) to yield 4 (5 mg) and 5 (4 mg).

Acalycixeniolide B'(4) is a colorless oil, $[\alpha]_D$ +15°(c 0.25, $CHCl_3$). The molecular formula of $C_{19}H_{26}O_2$, which was identical with that of acalycixeniolide B, was established by an $(M+H)^+$ ion peak at m/z 287 in FABMS and by NMR data (Table 1, 2). Its IR spectrum (2950, 1960, 1740, 1640 cm^{-1}) was also similar to that of acalycixeniolide B. In the UV region, it was transparent above 200 nm. The gross structure was deduced by ^1H and ^{13}C NMR, H-H and C-H COSY, and NOE difference spectroscopy. Relative streochemistry was secured by NOE difference spectroscopy as shown in Fig.1.



1



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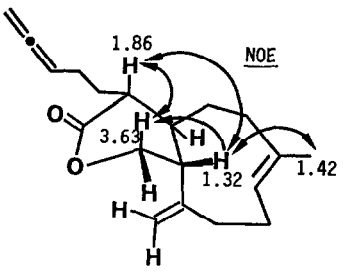
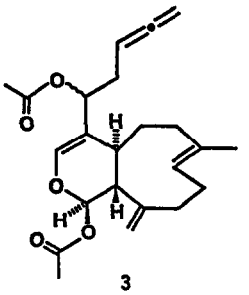


Fig.1



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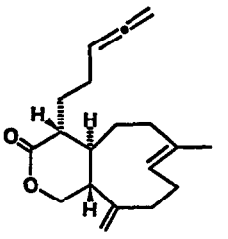

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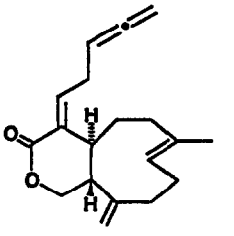

5

Acalycixeniolide B' (**4**) differed from **2** in the vicinal coupling pattern of the C-1 protons (δ 3.59, 1H, dd, J=11.2, 3.0 Hz; δ 3.63, 1H, dd, J=11.5, 2.0 Hz), which reflect difference in the relative stereochemistry at C-4 together with conformational change of the lactone ring. Thus, acalycixeniolide B' (**4**) was the C-4 epimer of acalycixeniolide B (**2**).

Acalycixeniolide C (**5**) was also related to acalycixeniolide B, judging from the IR spectrum (2950, 1960, 1740, 1640 cm^{-1}) and NMR spectra. It showed a UV absorption at 206 nm (ε1100), indicative of a conjugated system, thus designated acalycixeniolide C. The molecular formula of $C_{19}H_{24}O_2$ was deduced by the FABMS spectrum [$(M+H)^+$ m/z 285] and ^{13}C NMR data. The ^1H NMR spectrum revealed that **5** has a sidechain different from that of **4**. The C-4a proton appeared in relatively lower field at δ2.76 in **5**. This proton was coupled to three protons, the C-11a methine at δ1.88 and the C-5 methylene at δ1.48 and 1.88. On the other hand, in the sidechain portion, an allenyl

Table 1. ^1H NMR Spectral Data for **1-5**

C	1[a]	2[a]	3[b]	4[bc]	5[bc]
1	3.91 dd	3.92 dd	6.27 brs	3.59(dd,11.5,3.0)	3.37(dd,12.0,11.5)
	4.14 dd	4.17 dd		3.63(dd,11.5,2.0)	3.79(dd,11.5,6.0)
3			6.61 d		
4	2.78 brq	2.92 brq		1.86 m	
4a	2.09 ddd	2.08 m	2.35 m	1.49(ddd,12.0,10.0,2.0)	2.76(brdd,6.0,4.0)
5	1.08 tdd	1.08 tdd	1.65 m	0.98(tdd,14.0,10.0,4.0)	1.48 m
	1.69 dt	1.65 m	2.17 m	1.41(dt,14.0,4.0)	1.88 m
6	1.97 ddd	1.95 m	2.17 m	1.80 m	1.36 m
	2.19 dt	2.20 dt		1.92(dt,12.5,4.0)	1.95 m
8	5.34 brt	5.34 brt	5.45 brt	5.20(brt,8.0)	5.28(brt,8.0)
9	2.08 m	2.08 m	2.00 m	1.85 m	1.86 m
	2.47 m	2.47 m	2.35 m	2.22 m	2.25 m
10	2.10 m	2.10 m	2.12 m	1.73(dt,12.0,9.0)	1.75 m
	2.33 m	2.33 m		2.00(dd,14.0,9.0)	1.98 m
11a	1.96 ddd	1.99 ddd	2.12 m	1.34 m	1.88 m
12	1.48 m	1.52 m	5.55 t	1.56(tt,6.5,2.0)	6.54(t,8.0)
	1.92 m	2.02 m		2.05 m	
13	1.38 m	2.10 m	2.35 m	2.22 m	2.55 m
	1.52 m		2.45 m	2.42 m	2.63 m
14	2.12 m	5.12 quint	4.98 quint	5.19(quint,6.5)	4.95(quint,6.5)
16	4.98 dq	4.70 m	4.64 m	4.70 m	4.63 m
	5.04 dq				
17	1.68 d	1.68 brs	1.59 d	1.41(d,1.0)	1.41(d,1.0)
18	4.95 brs	4.96 brs	4.88 brs	4.80 brs	4.63 brs
	4.96 brs	4.98 brs	5.03 brs	4.97 brs	4.68 brs
			1-$C\underline{H}_3CO_2$ 1.71 s		
			12-$C\underline{H}_3CO_2$ 1.60 s		

a: 500MHz; $CDCl_3$ as internal reference = 7.25 ppm
b: 400MHz; C_6D_6 as internal reference = 7.20 ppm
c: Coupling constants in hertz

Table 2. ^{13}C NMR Spectral Data for 1-5

C	1[a]	2[a]	3[b]	4[b]	5[b]
1	70.5 t	70.5 t	92.3 d	66.1 t	70.5 t
3	175.3 s	175.3 s	141.7 d	174.1 s	169.7 s
4	45.3 d	44.9 d	115.9 s	44.2 d	136.4 s
4a	42.2 d	41.1 d	37.1 d	46.4 d	43.9 d
5	30.1 t	30.1 t	31.0 t	35.0 t	34.7 t
6	39.9 t	39.7 t	40.3 t	40.0 t	40.4 t
7	135.8 s	135.8 s	135.7 s	135.1 s	135.4 s
8	123.9 d	123.8 d	124.7 d	124.7 d	124.4 d
9	24.9 t	24.9 t	25.3 t	25.1 t	25.2 t
10	35.5 t	35.5 t	35.6 t	35.5 t	37.9 t
11	153.0 s	153.0 s	151.8 s	152.9 s	152.7 s
11a	49.7 d	49.8 d	49.9 d	49.4 d	49.9 d
12	26.9 t[c]	26.2 t[d]	74.0 d	29.0 t	134.7 d
13	26.7 t[c]	25.8 t[d]	32.2 t	27.0 t	26.6 t
14	33.6 t	89.0 d	85.9 d	90.2 d	87.6 d
15	138.3 d	208.6 s	209.8 s	209.1 s	209.1 s
16	114.8 t	75.3 t	75.3 t	75.3 t	76.2 t
17	16.4 q	16.3 q	16.7 q	16.2 q	16.2 q
18	112.1 t	112.1 t	113.3 t	113.5 t	112.7 t
			$1\text{-}\underline{CH_3}CO_2$ 20.8 q		
			$12\text{-}\underline{CH_3}CO_2$ 20.3 q		
			$1\text{-}CH_3\underline{CO_2}$ 169.5 s[e]		
			$12\text{-}CH_3\underline{CO_2}$ 168.8 s[e]		

a: 125MHz; $CDCl_3$ as internal reference = 77.0 ppm
b: 100MHz; C_6D_6 as internal reference = 128.0 ppm
c-e: Assignments may be interchanged

methine at δ4.95 (1H, quint, J=6.5 Hz) was coupled to methylene signals at δ2.55 and 2.63 which were further coupled to the olefin proton at δ6.54 (1H, t, J=8.0 Hz). These results placed the double bond [δ_C 134.7d, 136.4s] at the junction of the sidechain portion and δ-lactone ring. The E-geometry of this double bond was assigned on the basis of NMR signals reported by Schwartz et al.[7] Interpretation of the COSY spectrum led to assign the gross structure for 5 (Table 1). Trans-configuration of the ring juncture of 5 was similarly deduced by the NOE difference spectrum.

Norditerpenes with allene functionality have only been known from five species of Acalycigorgia,[5,6] indicating that allene biosynthesis may be specific for Acalycigorgia. It is conceivable that the allene moiety is derived via oxidative fission of a methyl group. Acalycixeniolide A (1) found in A. inermis may be an intermediate of the allenes. Acalycixeniculin A (3), acalycixeniolide B' (4), and C (5) were not only cytotoxic against P388 leukemia cells with IC_{50} of 0.27, <2.5, and 2.5 μg/mL, respectively, but also active in the sea urchin Hemicentrotus pulcherrimus egg assay with IC_{50} of 1.0, 15.0, and 50.0 μg/mL, respectively. They also inhibit the growth of fungi, Mortierella ramannianus and Penicillium chrysogenum at 25 μg/5mm disk.

EXPERIMENTAL

UV spectra were taken on a Hitachi 330 spectrophotometer. IR spectra were measured on a JASCO A-202 infrared spectrophotometer. FAB mass spectra were recorded on a JEOL JMS-DX 303 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GX 400 and a Bruker AM-500 spectrometer. Optical rotations were determined on a JASCO DIP-4 polarimeter.

Extraction and Isolation

Acalycigorgia inermis (Hedlund) was collected in the Gulf of Suruga by SCUBA(-15 m). The specimens were immediately frozen with Dry Ice, transferred to our laboratory, and kept frozen at -20°C until processed. The frozen specimens (500 g, wet weight) were extracted with ethanol(3x2 L) in a Waring Blendor. The extracts were concentrated under reduced pressure and partitioned between ether and water. The ether-soluble portion was fractionated on a silica gel column (4.5 x 45 cm, BW-200 Fuji Davison) with stepwise elution using benzene (0.5 L), benzene/ethyl acetate [39:1(1 L), 19:1(2 L), 9:1(2 L), 4:1(2 L)]. The activity was eluted in the benzene/ethyl acetate(19:1) fractions, which were further purified by HPLC on SI 60-5 (2 x 30 cm, Merck) with n-hexane/ethyl acetate/acetonitrile (94.5:5:0.5), then on Develosil ODS-5 (2 x 30 cm, Nomura Chemical) with methanol/water(9:1) , to yield acalycixeniolide A (**1**) (10 mg) and B (**2**) (6 mg).

Colonies of another gorgonian of the genus Acalycigorgia were collected in the Gulf of Suruga by SCUBA (-15 to -20 m) . The specimens were immediately frozen with Dry Ice, and transferred to our laboratory, and kept frozen at -20°C until extracted. The frozen specimens (380 g) were extracted with ethanol (4x1.5 L) in a Waring Blendor. The combined extracts were concentrated under reduced pressure and partitioned between ether and water. The ether soluble materials were then partitioned between n-hexane and methanol/water (9:1). The active aqueous methanol layer (2.2 g) was subjected to silica gel (300 g, Kieselgel 60, Merck) flash column chromatography. The column was eluted successively with n-hexane (1 L), n-hexane/ethyl acetate [9:1 (3 L), 8:1 (2 L), 3:1 (2 L), 3:2 (1.5 L), 1:1 (1 L)] and ethyl acetate (1 L). Activity was eluted in the n-hexane/ethyl acetate (9:1) and (8:1) fractions (500 mg), which were further purified by HPLC on YMC-Pack AM-ODS-5 (2 x 25 cm) with methanol/water (9:1) to afford ginamallene(**3**) (124 mg, 0.032% wet weight) and a mixture of **4** and **5**. The mixture of **4** and **5** was separated by HPLC on YMC-Pack AM-ODS-5 (2 x 25 cm) with acetonitrile/water (3:1) and on YMC A024 SIL (1 x 25 cm) with n-hexane/ether (4:1) to yield **4** (5 mg, 0.01 %) and **5** (4 mg, 0.01 %).

Acalycixeniolide A (1)

Colorless amorphous powder; $[\alpha]_D$ +143°(c 0.31, CH_2Cl_2); IR (film) 3050, 2950, 2650, 1750, 1640, 1460, 1390, 1320, 1260, 1170, 1110, 1040, 1025, 910 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HREIMS M^+ m/z 288.2085, $C_{19}H_{28}O_2$ Δ -0.5 mmu.

Acalycixeniolide B (2)

Colorless amorphous powder; IR (film) 3050, 2950, 2650, 1960, 1750, 1640, 1440, 1380, 1340, 1255, 1220, 1155, 1100, 1020, 940 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HREIMS M^+, m/z 286.1888, $C_{19}H_{26}O_2$ Δ -4.4 mmu.

Ginamallene (3)

Colorless oil; $[\alpha]_D$ +53°(c 1.05, $CHCl_3$); IR (film) 3050, 2900, 1960, 1730, 1655, 1430, 1360, 1230, 1140, 1010, 940 cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 1, 2, and ref.2); FABMS m/z 387 $(M+H)^+$, 327 $(M-CH_3COOH+H)^+$, 267 $(M-2CH_3COOH+H)^+$.

Acalycixeniolide B' (4)
Colorless oil; $[\alpha]_D$ +15°(c 0.25, $CHCl_3$); IR (film) 3050, 2950, 2650, 1960, 1740, 1640, 1440, 1380, 1340, 1255, 1220, 1150, 1100, 1050, 970 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); FABMS m/z 287 $(M+H)^+$.

Acalycixeniolide C (5)
Colorless oil; $[\alpha]_D$ +208°(c 0.20, $CHCl_3$); UV (EtOH) 206nm (ε 1100); IR (film) 3050, 2950, 2650, 1960, 1740, 1640, 1450, 1380, 1310, 1255, 1230, 1170, 1125, 1040, 1020 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 2); FABMS m/z 285 $(M+H)^+$

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