BIOLOGICALLY ACTIVE XENICANE DITERPENOIDS FROM THE GORGONIAN *ACALYCIGORGIA* SP.

Masamitsu Ochi,* Kumi Kataoka, Akira Tatsukawa, Hiyoshizo Kotsuki, and Kozo Shibata†*

Faculty of Science, Kochi University, Akebono-cho, Kochi 780, Japan

†Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan

Abstract-----Five new biologically active diterpenoids, acalycigorgins A-E, have been isolated from the gorgonian *Acalycigorgia* sp. The structures and relative stereochemistry of acalycigorgins were determined by extensive 2D-nmr studies.

The gorgonian corals have been shown to possess a wide variety of secondary metabolites, including terpenoids, steroids, and lipids. Many of these metabolites exhibit interesting biological activities, e. g. ichthyotoxicity, cytotoxicity, and insecticidal and antifouling activities.[1] In the course of our investigation on biologically active constituents of marine invertebrates, the methanol extract of a gorgonian *Acalycigorgia* sp. collected in Sukumo Bay, Shikoku, Japan, was found to show lethality to brine shrimp and inhibitory activity on the cell division of fertilized ascidian eggs. Bioassay directed fractionation of the extract has led to the isolation of nine diterpenoids of the xenicane class, four previously known compounds, waixenicin A (**1**),[2] acalycixeniolides B' (**2**) and C (**3**),[3] and ginamallene (**4**),[4] and five new biologically active congeners, designated acalycigorgins A-E. A part of this work has been described in a preliminary communication.[5] The present paper gives a full account of the isolation and structure determination of these new compounds.

RESULTS AND DISCUSSION

Specimens of *Acalycigorgia* sp. were collected by using SCUBA at Sukumo Bay in June 1990. Freshly collected animals were stored frozen and subsequently extracted with methanol. The methanol solution was concentrated to an aqueous suspension which was then extracted with hexane. Fractionation of the hexane extract by

sequential application of Sephadex LH-20, silica gel, and reverse phase high-performance liquid chromatographies gave waixenicin A, acalycixeniolides B' and C, ginamallene, and acalycigorgins A-E.

Acalycigorgin A (**5**) was isolated as an optically active colorless oil, $[\alpha]_D^{20}$ +82.3° (*c* 0.40, $CHCl_3$). The molecular formula $C_{25}H_{34}O_7$ was established by high resolution ms (*m/z* 446.2311, M^+, Δ +0.7 mmu) in combination with the ^{13}C nmr data. The ir spectrum of **5** showed prominent peaks due to acetoxyl (1735 and 1230 cm^{-1}) and conjugated ester (1720 cm^{-1}) groups. The close similarity between **5** and waixenicin A (**1**)[2] was revealed by the comparison of their spectral data. The ^{13}C nmr data of **5** included twenty signals compatible with the carbon frame work of **1**. Variances noted were in observations of signals due to a methoxycarbonyl group [δ_H 3.78 (3H, s); δ_C 51.76 and 168.03] in **5**. A combination of the ^1H-^1H and ^1H-^{13}C COSY spectra together with partial spin decoupling studies allowed a complete assignment of all the proton and carbon resonances, leading to a gross structure (**5**) for acalycigorgin A. The E-geometry of the C_{14} double bond was evident from the observation of an nOe between 13-H and 16-Me and the appearance of the ^{13}C nmr signal due to 16-Me at rather higher field (δ 12.83).[6]

Table 1. ^1H Nmr Data (400 MHz, $CDCl_3$) for Acalycigorgins A (**5**), B (**6**), C (**7**), D (**8**), and E (**9**).

Position	**5**	**6**	**7**	**8**	**9**
1	5.88 (d,1.8)	5.96 (d,2.1)		5.50 (d,8.8)	3.99 (dd,11.3,3.1)
					4.16 (dd,11.3,3.8)
3	6.51 (d,1.8)	6.53 (d,1.8)	6.27 (brs)	5.05 (s)	
4					2.15 (m)
4a	2.16 (brs)	2.22 (m)	2.37 (m)	2.75 (m)	1.68 (m)
8	5.34 (brt,8.5)	2.94 (dd,10.7,3.7)	5.33 (brt,7.9)	5.39 (dd,9.4,7.3)	5.34 (brdd,9.2,6.4)
11a	1.98 (brs)	2.40 (brs)	2.73 (brs)	2.16 (brs)	1.80 (ddd,4.0,3.8,3.1)
12	5.39 (t,7.3)	5.39 (t,7.3)	2.02 (m)	5.47 (t,6.7)	1.54 (m)
			2.08 (m)		1.91 (m)
14	6.62 (tq,7.0,1.2)	6.60 (tq,7.3,1.2)	5.08 (m)	5.10 (m)	5.13 (brt,5.8)
16	1.88 (brs)	1.85 (brs)	1.62 (s)	1.65 (s)	1.62 (s)
17			1.70 (s)	1.70 (s)	1.70 (s)
18	1.66 (s)	1.31 (s)	1.74 (s)	1.72 (s)	1.68 (s)
19	4.77 (brs)	4.98 (brs)	4.90 (s)	4.80 (s)	4.75 (brs)
	4.78 (brs)	5.00 (brs)	4.93 (s)	4.85 (s)	4.89 (brs)
OAc	2.04 (x2)	2.03, 2.08		2.01	
OMe	3.78	3.73		3.40	

Figures in parenthese are coupling constants in Hz.

Acalycigorgin B (**6**) was obtained as a colorless viscous oil, $C_{25}H_{34}O_8$, $[\alpha]_D^{21}$ +70.8° (*c* 0.13, $CHCl_3$), and displayed the spectral data quite similar to those of **5**. The only significant difference clarified by the comparison of ^1H and ^{13}C nmr data was the replacement of the trisubstituted double bond at C_7-C_8 in **5** by an epoxide [δ_H 2.94 (1H, dd, *J*=10.7 and 3.7 Hz); δ_C 59.69 and 62.52]. The observation of nOes between 18-Me and the β-proton at C_{11a} and between 8-H and the α-proton at C_{4a} defined the configuration of the epoxide to be α-oriented. Thus, the structure (**6**) is assigned to acalycigorgin B.

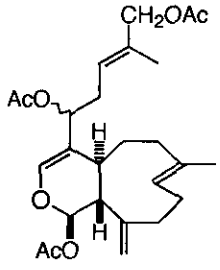
Table 2. ^{13}C Nmr Data (100 MHz, $CDCl_3$) for Acalycigorgins A (**5**), B (**6**), C (**7**), D (**8**), and E (**9**).

Carbon	**5**	**6**	**7**	**8**	**9**
1	91.83	91.29	168.88	94.87	67.05
3	140.94	141.26	134.55	105.55	175.33
4	115.77	115.37	119.06	137.37	44.72
4a	37.07	36.98	42.57	41.36	46.10
5	30.54	29.79	30.68	37.46	35.33
6	40.10	39.73	39.97	40.66	39.97
7	135.63	59.69	135.43	135.76	135.35
8	124.57	62.52	125.18	124.38	124.55
9	25.06	24.39	25.01	25.57	25.06
10	35.43	32.65	35.87	34.88	35.42
11	151.13	148.44	152.34	152.50	152.79
11a	49.27	47.24	52.07	53.88	49.27
12	73.56	73.15	30.05	129.03	29.60
13	31.99	31.96	26.50	26.72	26.44
14	136.18	135.86	123.38	121.60	123.82
15	130.04	130.22	132.52	132.59	132.54
16	12.83	12.81	17.83	17.83	17.77
17	168.03	167.94	25.66	25.63	25.66
18	16.75	16.59	16.91	16.90	16.40
19	113.30	114.66	112.79	112.98	113.39
OAc	20.94	20.91		20.97	
	21.32	21.25		169.64	
	169.49	169.36			
	170.16	170.12			
OMe	51.76	51.80		55.40	

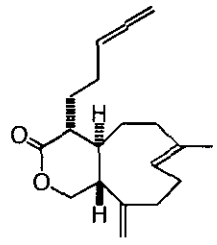
Acalycigorgin C (**7**), colorless oil, $C_{20}H_{28}O_2$, $[\alpha]_D^{21}$ +40.3° (*c* 0.28, $CHCl_3$), had the spectroscopic properties somewhat different from those of **5** and **6**. The ^1H and ^{13}C nmr spectra of **7** do not show the presence of acetoxyl and methoxycarbonyl groups present in **5** and **6** and, instead, show the presence of a 2-methyl-1-propenyl group [δ_H 1.62, 1.70 (3H each, s) and 5.08 (1H, m); δ_C 17.83, 25.66, 123.38, and 132.52] and an enol lactone [ν_{max} 1755 and 1685 cm^{-1}; δ_H 6.27 (1H, brs); δ_C 119.06, 134.55, and 168.88]. Observations of a ^1H-^1H long-range coupling between the signal at δ 6.27 and that of 4a-H and a ^1H-^{13}C long-range correlation between the signal of 11a-H and the carbonyl carbon signal at δ 168.88 established the closure of the lactone ring between C_1 and C_3 positions. From the evidence outlined above, we proposed the structure (**7**) for acalycigorgin C.

Acalycigorgin D (**8**) was isolated as an optically active colorless oil, $C_{23}H_{34}O_4$, $[\alpha]_D^{20}$ -51.3° (*c* 0.18, $CHCl_3$). The ^1H and ^{13}C nmr data revealed the presence of one acetylated hemiacetal [δ_H 2.01 (3H, s) and 5.50 (1H, d, *J*=8.8 Hz); δ_C 20.97, 94.87, and 169.64] and one methylated hemiacetal [δ_H 3.40 (3H, s) and 5.05 (1H, s); δ_C 55.40 and 105.55] groups, together with one 2-methyl-1-propenyl group, one vinylic methyl group, one exocyclic methylene group, and two trisubstituted double bond. These findings were strongly reminiscent of the nine-membered ring segment and the side chain of caraxeniolide C' (**10**).[7] Moreover, examination of the proton connectivity, assisted with COLOC experiments, elucidated the locations of the trisubstituted double bonds at C_4-C_{12} and C_7-C_8, the acetylated hemiacetal group at C_1, and the methylated hemiacetal group at C_3, the last two forming a six-membered ether ring. The observation of nOes between 1-H and 3-H, between 1-H and 4a-H, and between 3-H and 12-H established the β-orientation of both the acetoxyl and methoxyl groups and the E-geometry of the C_4-C_{12} double bond. Therefore, acalycigorgin D must be represented by the structure (**8**). Acalycigorgin D would be an artifact which was derived from the corresponding acetate (**11**) by the S_N2' syn-reaction[8] in isolation processes using methanol as the solvent.

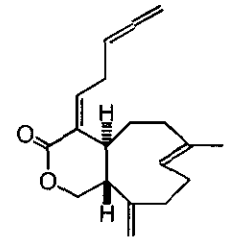
Acalycigorgin E (**9**) was obtained as an amorphous solid, $C_{20}H_{30}O_2$, $[\alpha]_D^{21}$ +51.0° (*c* 0.16, $CHCl_3$), and displayed spectral data quite similar to those of acalycixeniolide B' (**2**).[3] The only significant difference in their ^1H and ^{13}C nmr data was the replacement of the allenyl group in the side chain of **2** by a 2-methyl-1-propenyl group [δ_H 1.62, 1.70 (3H each, s) and 5.13 (1H, brt, *J*=5.8 Hz); δ_C 17.77, 25.66, 123.82, and 132.54] in **9**. The observation of nOes among 1β-H (δ 4.16), 4-H, and 11a-H and between 11a-H and 18-Me defined the stereochemistry at C_4 as shown in the structure (**9**). Based on these data together with the pertinent ^{13}C nmr data, we assigned the structure (**9**) for acalycigorgin E.



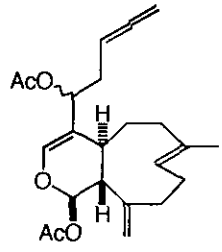
1



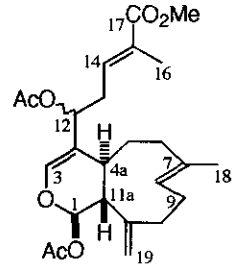
2



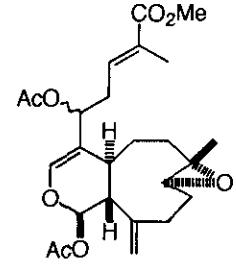
3



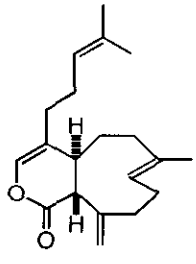
4



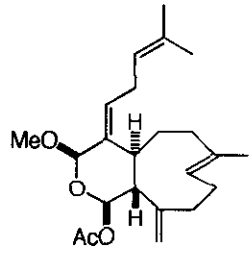
5



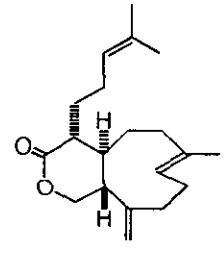
6



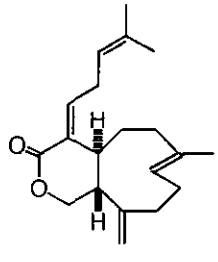
7



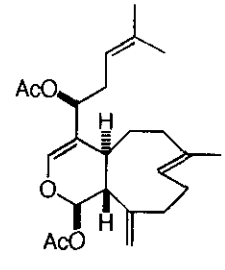
8



9



10



11

Acalycigorgins A, B, D, and E inhibited the cell division of fertilized ascidian (*Styela partita*) eggs with IC_{50} values of 10, 5.0, 8.0, and 8.0 μg/ml,[9] respectively, while acalycigorgins C, D, and E displayed toxicity in the brine shrimp lethality bioassay (LC_{50}=7.6, 20, and 1.5 μg/ml, respectively).[10]

EXPERIMENTAL

General: The ir spectra were recorded on a JASCO model A-302 spectrophotometer. The ^1H (400 MHz) and ^{13}C (100 MHz) nmr spectra were taken on a JEOL JNM-GX400 instrument in deuteriochloroform solutions, with TMS as the internal standard. Mass spectra as well as high resolution mass spectra were measured with a JEOL JMS-D300 spectrometer at 70 eV of ionization energy. A Union Gikken apparatus, model PM-101, was used for measuring the rotations. High-performance liquid chromatographic separation was performed on a Waters Associates HPLC model 6000A, with a TSK-GEL LS-410KG (ODS) column.

Extraction and Isolation; Specimens of *Acalycigorgia* sp. were collected by divers, using SCUBA, at depths of 10-20 m at Sukumo Bay in June 1990. Freshly collected organisms were kept frozen until just prior to extraction. Thawed material (2.40 kg) was homogenized with methanol (8 l) and left at room temperature for a few hours. After filtration, the methanol solution was concentrated *in vacuo* to an aqueous suspension (1.5 l) and extracted with hexane (0.5 l x3). The hexane layer was subsequently dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue (23 g) was permeated through a Sephadex LH-20 column and eluted with methanol/dichloromethane (1:1); five fractions were collected. The third fraction (9.50 g) was subjected to chromatography on a silica gel column; elution with hexane/ethyl acetate mixtures of increasing polarity (9:1 → 7:3) gave seven fractions, A-G. Rechromatography of fraction F (2.28 g) on silica gel using hexane/diethyl ether (9:1→2:1) as the eluent, followed by reverse phase HPLC (MeOH/H_2O 7:3) yielded acalycigorgin A (**5**) (255 mg), waixenicin A (**1**) (13 mg), and acalycigorgin B (**6**) (42 mg). Fraction E (3.13 g) was rechromatographed on silica gel with hexane/ethyl acetate (9:1→4:1) and subsequently purified by reverse phase HPLC (MeOH/H_2O 75:25) to obtain acalycixeniolide C (**3**) (75 mg), acalycixeniolide B' (**2**) (58 mg), and ginamallene (**4**) (511 mg). Purification of fractions B (175 mg), C (526 mg), and D (909 mg) by silica gel column chromatography (hexane/benzene 1:3, benzene, and hexane/EtOAc 19:1, respectively) followed by reverse phase HPLC (MeOH/H_2O 8:2) afforded acalycigorgins C (**7**) (12 mg), D (**8**) (27 mg), and E (**9**) (24 mg), respectively.

Acalycigorgin A (5): A colorless oil, $[\alpha]_D^{20}$ +82.3° (*c* 0.40, $CHCl_3$); ir (CCl_4) 3080, 1735, 1720, 1665, 1230, and 900 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; HRms *m/z* 446.2311 (M^+, $C_{25}H_{34}O_7$, Δ +0.7 mmu).

Acalycigorgin B (6): A colorless oil, $[\alpha]_D^{21}$ +70.8° (*c* 0.13, $CHCl_3$); ir (CCl_4) 3080, 1740, 1720, 1665, 1615, 1230, and 905 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; HRms *m/z* 402.2063 (M^+-AcOH, $C_{23}H_{30}O_6$, Δ +2.0 mmu).

Acalycigorgin C (7): A colorless oil, $[\alpha]_D^{21}$ +40.3° (*c* 0.28, $CHCl_3$); ir (CCl_4) 3080, 1755, 1685, 1640, and 900 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; HRms *m/z* 300.2090 (M^+, $C_{20}H_{28}O_2$, Δ 0.0 mmu).

Acalycigorgin D (8): A colorless oil, $[\alpha]_D^{21}$ -51.3° (*c* 0.18, $CHCl_3$); ir (CCl_4) 3080, 1755, 1640, 1225, and 900 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; HRms *m/z* 374.2450 (M^+, $C_{23}H_{34}O_4$, Δ -0.7 mmu).

Acalycigorgin E (9): An amorphous solid, $[\alpha]_D^{21}$ +51.4° (*c* 0.16, $CHCl_3$); ir (CCl_4) 3060, 1745, 1640, and 900 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; HRms *m/z* 302.2238 (M^+, $C_{20}H_{30}O_2$, Δ -0.8 mmu).

ACKNOWLEDGEMENTS

We are grateful to Dr. Y. Imahara, Wakayama Prefectural Museum of Natural History, for the identification of the *Acalycigorgia* species. We are also indebted to Dr. K. Kawamura, Department of Biology, Kochi University, for his kind cooperation in the fertilized ascidian (*Styela partita*) egg assay. The present work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (No. 63470022 to M. O.).

REFERENCES

1. For recent reviews see: D. J. Faulkner, *Nat. Prod. Chem.*, 1986, **3**, 1; 1987, **4**, 539; 1988, **5**, 613; 1990, **7**, 269, 1991, **8**, 97.
2. S. J. Coval, P. J. Scheuer, G. K. Matsumoto, and J. Clardy, *Tetrahedron*, 1984, **40**, 3823.
3. N. Fusetani, M. Asano, S. Matsunaga, and K. Hashimoto, *Tetrahedron*, 1989, **45**, 1647.
4. S. Hokawa, J. Tanaka, T. Higa, N. Fusetani, M. Asano, S. Matsunaga, and K. Hashimoto, *Chem. Lett.*, **1988**, 855.
5. M. Ochi, K. Kataoka, A. Tatsukawa, H. Kotsuki, and K. Shibata, *Heterocycles*, 1993, **36**, 41.
6. J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York (1972), pp.80-85.

7. R. E. Schwartz, P. J. Scheuer, W. Zabel, and W. H. Watson, *Tetrahedron*, 1981, **37**, 2725.

8. W.-D. Stohrer, *Angew. Chem., Int. Ed. Engl.*, 1983, **22**, 613, and references cited therein.

9. K. Kawamura, H. Fujita, and M. Nakauchi, *Develop. Growth Differ.*, 1987, **29**, 627.

10. T. Miyauchi, *Ecol. Chem.*, 1986, **8**, 41 and references cited therein.

Received, 25th August, 1993