ACALYCIGORGINS A, B, AND C, THREE NEW BIOLOGICALLY ACTIVE DITERPENOIDS FROM THE GORGONIAN *ACALYCIGORGIA* SP.

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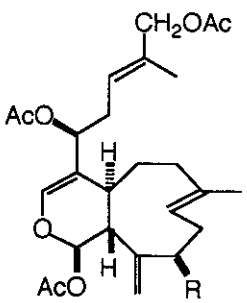
Abstract-----Three new biologically active diterpenoids, acalycigorgins A, B, and C, have been isolated from the gorgonian *Acalycigorgia* sp. and their structures were fully characterized by extensive 2D-nmr studies.

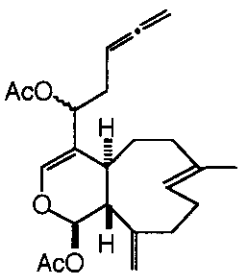
The gorgonian corals have been shown to possess a wide variety of secondary metabolites, including terpenoids, steroids, and lipids. Many of these metabolites exhibit interesting biological activities, e. g. ichthyotoxicity, cytotoxicity, and antifouling activity.[1] In the course of our investigation on biologically active constituents of marine invertebrates, the methanol extract of a gorgonian *Acalycigorgia* sp. collected in Sukumo Bay 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 three new diterpenoids together with three previously known diterpenoids of xenicane class, waixenicin A (**1**),[2] ginamallene (**2**),[3] and acalycixeniolide C (**3**).[4] The present paper deals with the structural determination of these new compounds, designated acalycigorgins A, B, and C.

Acalycigorgin A (**4**)[5] was isolated as an optically active colorless oil (0.0014%, wet weight), $[\alpha]_D^{20}$ +82.3° (c 0.40, $CHCl_3$), from the dichloromethane soluble fraction of the methanol extract of frozen specimens through Sephadex LH-20 (MeOH/CH_2Cl_2 1:1) and silica gel (hexane/AcOEt) column chromatography, followed by reverse phase hplc (ODS column, MeOH/H_2O). 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 **4** showed prominent peaks due to acetoxyl (1735 and 1230 cm^{-1}) and conjugated ester (1720 cm^{-1}) groups. The

close similarity between **4** and waixenicin A (**1**) was revealed by the comparison of their spectral data. The ^{13}C nmr data of **4** 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 51.77 and 168.03]. 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,[5] leading to a gross structure (**4**) for acalycigorgin A. The stereochemistry at C_{12} was deduced as shown in structure (**4**) from comparison of ^1H and ^{13}C nmr data of **4** with those of waixenicin B (**7**),[2] the stereostructure of which had been established by single-crystal X-ray analysis. The coupling pattern of the ^1H nmr signal due to 12-H (t, J=7.3 Hz) of **4** was consistent with that of **7**. The ^{13}C nmr values for the carbons in the row C_3-C_5 in **4** were in good agreement with those of the equivalent carbons in **7**. 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 a rather higher field (δ 12.83).



1 R=H
7 R=OH



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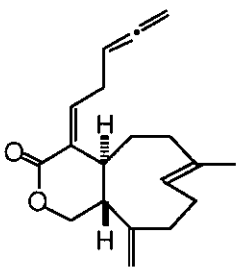

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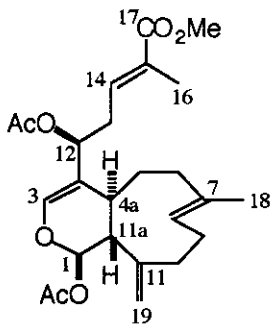

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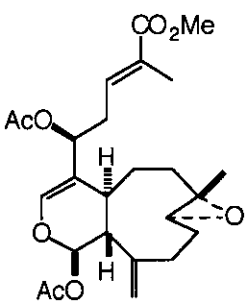

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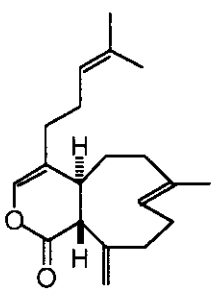

6

Acalycigorgin B (**5**)[6] was obtained as a colorless viscous oil (0.00072%, wet weight), $C_{25}H_{34}O_8$, $[\alpha]_D^{21}$ +70.8° (c 0.13, $CHCl_3$), and displayed the spectral data quite similar to those of **4**. The only significant difference in their ^1H and ^{13}C nmr data was the replacement of the trisubstituted double bond at C7-C8 in **4** 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 β-protons at C_5, C_{10}, and C_{11a} defined the configuration of the epoxide to be α-oriented. Thus, the structure (**5**) is assigned to acalycigorgin B.

Acalycigorgin C (**6**),[7] colorless oil (0.00052%, wet weight), $C_{20}H_{28}O_2$, $[\alpha]_D^{21}$ +40.3° (c 0.28, $CHCl_3$), had the spectroscopic properties somewhat different from those of **4** and **5**. The ^1H and ^{13}C nmr spectra of **6** do not show the presence of acetoxyl and methoxycarbonyl groups present in **4** and **5** and, instead, show the presence of a isopropylidene group [δ_H 1.62 and 1.70 (3H each, s); δ_C 17.83 and 25.66] and an enol lactone [δ_H 6.27 (1H, br s); 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 at C_1-C_3. From the evidence outlined above, we proposed the structure (**6**) for acalycigorgin C.

Acalycigorgins A and B inhibited the cell division of fertilized ascidian (*Styela partita*) eggs at 10 μg/ml and 5 μg/ml,[8] respectively, and acalycigorgin C displayed toxicity in the brine shrimp lethality bioassay (LC_{50}=7.6 μg/ml).[9]

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5. **4**: Ir (CCl_4) 3080, 1735, 1720, 1665, 1230, and 900 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 1.66 and 1.88 (3H each, s, 18- and 16-H_3), 1.98 (1H, br s, 11a-H), 2.04 (6H, s, 2Ac), 2.16 (1H, m, 4a-H), 3.78 (3H, s, OCH_3), 4.77 and 4.78 (1H each, br s, 19-H_2), 5.34 (1H, br t, J=8.5 Hz, 8-H), 5.39 (1H, t, J=7.3 Hz, 12-H), 5.88 (1H, d, J=1.8 Hz, 1-H), 6.51 (1H, d, J=1.8 Hz, 3-H), and 6.62 (1H, tq, J=7.0 and 1.2 Hz, 14-H); ^{13}C nmr (100 MHz, $CDCl_3$) δ 12.83 (C_{16}), 16.75 (C_{18}), 20.94 and 21.32 (2Ac), 25.06 (C_9), 30.54 (C_5), 31.99 (C_{13}), 35.43 (C_{10}), 37.07 (C_{4a}), 40.10 (C_6), 49.27 (C_{11a}), 51.77 (OCH_3), 73.56 (C_{12}), 91.83 (C_1), 113.30 (C_{19}), 115.77 (C_4), 124.57 (C_8), 130.04 (C_{15}), 135.63 (C_7), 136.18 (C_{14}), 140.94 (C_3), 151.13 (C_{11}), 168.03 (C_{17}), 169.49 and 170.16 (2Ac); HRms m/z 446.2311 (M^+, $C_{25}H_{34}O_7$, Δ +0.7 mmu).

6. **5**: Ir (CCl_4) 3080, 1740, 1720, 1665, 1615, 1230, and 905 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 1.31 and 1.85 (3H each, s, 18- and 16-H_3), 2.03 and 2.08 (3H each, s, 2Ac), 2.22 (1H, m, 4a-H), 2.40 (1H, br s, 11a-H), 2.94 (1H, dd, J=10.7 and 3.7 Hz, 8-H), 3.73 (3H, s, OCH_3), 4.98 and 5.00 (1H each, br s, 19-H_2), 5.39 (1H, t, J=7.3 Hz, 12-H), 5.96 (1H, d, J=2.1 Hz, 1-H), 6.53 (1H, d, J=1.8 Hz, 3-H), and 6.60 (1H, tq, J=7.3 and 1.2 Hz, 14-H); ^{13}C nmr (100 MHz, $CDCl_3$) δ 12.81 (C_{16}), 16.59 (C_{18}), 20.91 and 21.25 (2Ac), 24.39 (C_9), 29.79 (C_5), 31.96 (C_{13}), 32.65 (C_{10}), 36.98 (C_{4a}), 39.73 (C_6), 47.24 (C_{11a}), 51.80 (OCH_3), 59.69 (C_7), 62.52 (C_8), 73.15 (C_{12}), 91.29 (C_1), 114.67 (C_{19}), 115.37 (C_4), 130.22 (C_{15}), 135.86 (C_{14}), 141.26 (C_3), 148.44 (C_{11}), 167.94 (C_{17}), 169.36 and 170.12 (2Ac); HRms m/z 402.2063 (M^+-AcOH, $C_{23}H_{30}O_6$, Δ +2.0 mmu).

7. **6**: Ir (CCl_4) 3080, 1755, 1685, 1640, and 900 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 1.62, 1.70, and 1.74 (3H each, s, 16-, 17-, and 18-H_3), 2.37 (1H, m, 4a-H), 2.73 (1H, br s, 11a-H), 4.90 and 4.93 (1H each, s, 19-H_2), 5.08 (1H, m, 14-H), 5.33 (1H, br t, J=7.9 Hz, 8-H), and 6.27 (1h, br s, 3-H); ^{13}C nmr (100 MHz, $CDCl_3$) δ 16.91 (C_{18}), 17.83 (C_{16}), 25.01 (C_9), 25.66 (C_{17}), 26.50 (C_{13}), 30.05 (C_{12}), 30.68 (C_5), 35.87 (C_{10}), 39.97 (C_6), 42.57 (C_{4a}), 52.07 (C_{11a}), 112.79 (C_{19}), 119.06 (C_4), 123.38 (C_{14}), 125.18 (C_8), 132.52 (C_{15}), 134.55 (C_3), 135.43 (C_7), 152.34 (C_{11}), and 168.88 (C_1); HRms m/z 300.2090 (M^+, $C_{20}H_{28}O_2$, Δ 0.0 mmu).

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