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CHLOROVULONES, NEW HALOGENATED MARINE PROSTANOIDS WITH AN ANTITUMOR ACTIVITY FROM THE STOLONIFER CLAVULARIA VIRIDIS QUOY AND GAIMARD[1]

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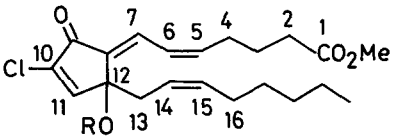
Abstract: New halogenated marine prostanoids, chlorovulone I, II and III were isolated from the stolonifer Clavularia viridis Quoy and Gaimard. The structure elucidation and the antitumor activity of chlorovulones were described.

The marine prostanoids clavulones[2] isolated from the stolonifer Clavularia viridis Quoy and Gaimard have been received much attention because of their unique structural features and remarkable antitumor activities.[3] The total synthesis[4] of clavulones and the antileukemic effect[5] of clavulones on human promyelocytic leukemia (HL-60) cells have been also studied. Recently the structures of punaglandins,[6] the halogenated prostanoids with the related structures to those of clavulones, isolated from the octocoral Telesto riisei, have been reported. Punaglandins showed a stronger antitumor activity than that of clavulones.[3b,6] Our continuing investigation on clavulone congeners from Clavularia viridis has resulted in the isolation of a series of new halogenated prostanoids chlorovulones, which showed a very strong antitumor activity. This paper describes the isolation and the structure elucidation of chlorovulone I (1), II (2) and III (3) on the basis of spectral analysis, and the antitumor activity of chlorovulone I (1), which is a major component, in human promyelocytic leukemia (HL-60) cells. The presence of chlorovulone IV (4) is also described.

The freeze-dried organisms (210 g) of Clavularia viridis, collected at the coral reef of Ishigaki Island (Okinawa, Japan) in April, 1985, were extracted with ether. The extract (7.68 g) was chromatographed on a silica gel column using n-hexane-ethyl acetate (5:1) as an eluent to give a mixture of chlorovulones (100 mg),[7] whose polarities were very close to each other, in addition to clavulones.[2a] Repeated high pressure liquid chromatography (HPLC)[silica gel (Lichrosorb), n-hexane-ether (2:1)] of the mixture gave chlorovulone I (1)[8,9] (colorless oil, 20 mg, $C_{21}H_{29}ClO_4$), III (3)(colorless oil, 2 mg, $C_{21}H_{29}ClO_4$)



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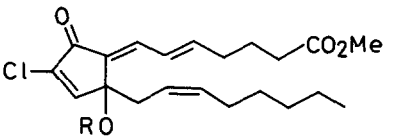

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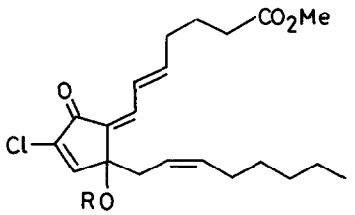

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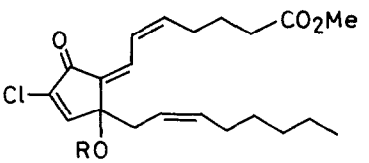

4 R = H
8 R = Ac

and II (2)(colorless oil, 4 mg, $C_{21}H_{29}ClO_4$) in a ratio of 10:1:2 in order of increasing polarity. Although chlorovulone IV (4) was not isolated from the chlorovulone mixture as a pure state, its presence was suggested by the isolation of the corresponding acetate 8, when the mixture was subjected to acetylation (acetic anhydride, pyridine) to give an easily separable acetate mixture (5-8) [HPLC, silica gel (Lichrosorb), n-hexane-ether (3:1)].

The physical data of 1-3 are summarized in the Table 1. The ^{1}H-NMR spectra of 1, 2 and 3 are closely related to those of clavulone I, II and III, respectively, except for the lack of the signals due to the two acetyl groups and the olefinic proton at C-10 present in clavulones, indicating that chlorovulones have the related structures to those of clavulones.

The major component chlorovulone I (1) showed the molecular ion peaks at m/z 380 and 382 (3:1) in the mass spectrum, showing that 1 possesses one chlorine atom. The monocyclic structure for 1 was indicated by the degree of unsaturation (seven) and the ^{13}C-NMR spectrum[10](the signals due to four olefins and two carbonyls). The presence of a conjugated α-chlorocyclopentenone moiety was indicated by the UV [243(ε 14,600)nm], IR (1705 cm^{-1}), ^{1}H-NMR [δ 7.21(1H, d,J=0.6 Hz, H-11)ppm] and ^{13}C-NMR [δ 187.7(s,C=O)ppm] spectra. The UV absorption at 315(ε15,100)nm in 1 showed that the cyclopentenone carbonyl also conjugates with a diene group forming a cross-conjugated system. The following moieties were also deduced by the spectral data of 1; a methoxycarbonyl group [IR 1720 cm^{-1}, ^{1}H-NMR 3.68(3H,s), ^{13}C-NMR 173.6(s), 51.6(q)], a disubstituted double bond [^{1}H-NMR 5.54(1H,ttd,J=1.4,8.7,10.9 Hz, H-15), 5.22(1H,ttd,J=1.5, 7.7,10.9 Hz, H-14)] and a tertiary hydroxyl group [IR 3560, 3350 cm^{-1}, ^{13}C-NMR 77.7(s)]. Decoupling experiments[11] in the ^{1}H-NMR of 1 clarified the following partial structures; -CO-C(OH)=CH-CH=CH-CH_2-CH_2-CH_2-CO_2CH_3 and -C(OH)-CH_2-CH=CH-CH_2-CH_2- . The

Table 1. Physical Data of Chlorovulone I (1), II (2) and III (3)

	1	2	3
$[\alpha]_D(CHCl_3)$	-1.2°(c 0.17)	+22.7°(c 0.075)	+27.3°(c 0.033)
UV λ_{max}^{EtOH}(nm)	243(ε14,600) 315(15,100)	237(10,000) 312(10,100)	238(13,200) 315(11,900)
^{1}H-NMR (400 MHz) $\delta_{ppm}^{CDCl_3}$ (J in Hz)	0.88(3H,t,J=7.2) 1.30(6H,m) 1.80(2H,quint.J=7.4) 1.97(2H,brq,J=7.0) 2.35(2H,t,J=7.4) 2.42(2H,m) 2.67(1H,ddd,J=0.5,7.8,14.2) 2.82(1H,ddd,J=0.5,7.5,14.2) 3.68(3H,s) 5.22(1H,ttd,J=1.5,7.7,10.9) 5.54(1H,ttd,J=1.4,8.7,10.9) 6.11(1H,tdd,J=7.9,0.9,10.9) 6.77(1H,tdd,J=1.5,10.9,12.6) 7.21(1H,d,J=0.6) 7.33(1H,brd,J=12.6)	0.88(3H,t,J=7.1) 1.30(6H,m) 1.82(2H,quint.J=7.4) 1.96(2H,brq,J=7.5) 2.30(2H,brq,J=7.5) 2.35(2H,t,J=7.4) 2.68(1H,brdd,J=7.9,14.3) 2.81(1H,brdd,J=7.5,14.3) 3.67(3H,s) 5.23(1H,ttd,J=1.4,7.7,10.9) 5.55(1H,brtd,J=7.5,10.9) 6.28(1H,td,J=7.4,15.1) 6.77(1H,tdd,J=1.3,11.9,15.1) 7.03(1H,d,J=11.9) 7.20(1H,s)	0.88(3H,t,J=7.1) 1.30(6H,m) 1.81(2H,quint.J=7.5) 2.00(2H,brq,J=6.9) 2.30(2H,brq,J=7.5) 2.35(2H,t,J=7.5) 2.55(1H,brdd,J=7.6,14.4) 2.67(1H,brdd,J=8.3,14.4) 3.68(3H,s) 5.29(1H,ttd,J=1.7,7.6,10.9) 5.38(1H,brtd,J=7.4,10.9) 6.19(1H,td,J=7.1,15.7) 6.68(1H,d,J=11.4) 7.15(1H,s) 7.56(1H,tdd,J=1.4,11.4,15.7)

location of the tertiary hydroxyl group at C-12 was indicated by the low field shift of H-11 [7.39(1H,s)] in the ^{1}H-NMR spectrum of chlorovulone I acetate (5).[12] These findings established the structure of chlorovulone I as shown in 1 except for stereochemistry.

The Z-configuration of the carbon-carbon double bonds at both C-5 and C-14 were shown by the coupling constants between the olefinic protons, $J_{5,6}$ =10.9 Hz and $J_{14,15}$ =10.9 Hz, respectively. The E-configuration of the double bond at C-7 was indicated by the low field shift of H-7 (7.33 ppm) due to the anisotropy effect of the cyclopentenone carbonyl group.

The spectral data of chlorovulone II (2) and III (3) (the corresponding acetates 6[12] and 7[12]) are closely related to those of 1 (the acetate 5) except for the ^{1}H-NMR signals due to the olefinic protons of the dienone moiety, showing that 1-3 are the geometrical isomers each other. This was supported by the photoisomerization of 1 (fluorescent lamp, benzene, 40 hr) to give a mixture of 1, 2 and 3 in a ratio of 1:2:1. The stereochemistry of the three carbon-carbon double bonds [(5E,7E,14Z) for 2 and (5E,7Z,14Z) for 3] were determined by the coupling constants of the olefinic protons and the low field shifts of H-7 (7.03 ppm) for 2 and H-6 (7.56 ppm) for 3 due to the anisotropy effect of the cyclopentenone carbonyl group. The structure of chlorovulone IV (4), having the stereochemistry of 5Z,7Z,14Z, was suggested by the analysis of the spectral data of its acetate 8.[12] The absolute stereochemistry at C-12 in 1-3 is currently under investigation.

Chlorovulone I (1) showed the strong antiproliferative and cytotoxic activities in human promyelocytic leukemia (HL-60) cells *in vitro* as shown in the Table 2. The IC_{50} value of 1 in the HL-60 cells is 0.03 μM (0.01 μg/ml), which

is about 13 times stronger than that of clavulone I.[5]

Table 2. Effect of Chlorovulone I and Clavulone I on the Cell Growth of Human Promyelocytic Leukemia (HL-60) Cells[5]

	IC_{50}	Cytotoxic effect
Chlorovulone I	0.03 μM(0.01 μg/ml)	>0.3 μM(0.1 μg/ml)
Clavulone I	0.4 μM(0.2 μg/ml)	>1 μM(0.5 μg/ml)

References and Notes

1. This paper constitutes Part XII of "Studies on Marine Natural Products."
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7. Chlorovulones were eluted prior to the elution of clavulones.
8. All new compounds gave satisfactory high resolution mass measurement.
9. Chlorovulone I, II, III and IV have the stereochemistry of the carbon-carbon double bonds, (5Z,7E,14Z), (5E,7E,14Z), (5E,7Z,14Z) and (5Z,7Z,14Z), respectively, which correspond to those of clavulone I, II, III and IV, respectively.
10. 1: ^{13}C-NMR(100 MHz,$CDCl_3$) δ ppm 13.9(q), 22.4(t),24.3(t), 27.1(t), 27.3(t), 29.0(t), 31.4(t), 33.2(t), 33.6(t), 51.6(q), 77.7(s), 121.7(d), 123.8(d), 127.9(d), 134.8(d), 136.4(s), 137.9(s), 143.6(d), 154.0(d), 173.6(s), 187.7(s).
11. The result of the ^{1}H-NMR decoupling experiment of 1 is summarized as follows;

irradiated proton (δ_{ppm})	observed protons and changes (J in Hz)
H-2(2.35)	H-3(1.80,quint.)→t J=7.4
H-4(2.42)	H-3(1.80,quint.)→t J=7.4 H-5(6.11,tdd)→dd J=0.9,10.9 H-6(6.77,tdd)→dd J=10.9,12.6
H-6(6.77)	H-5(6.11,tdd)→changed H-7(7.33,brd)→brs
H-14(5.22)	H-13(2.67,ddd)→dd J=0.5,14.2 H-13(2.82,ddd)→dd J=0.5,14.2 H-15(5.54,ttd)→brt J=8.7
H-16(1.97)	H-14(5.22,ttd)→td J=7.7,10.9 H-15(5.54,ttd)→td J=1.4,10.9

12. 5: colorless oil, $[\alpha]_D$ -9.7°(c 0.041,$CHCl_3$). ^{1}H-NMR(400 MHz,$CDCl_3$) δ_{ppm} 2.03(3H,s), 3.69(3H, s), 6.10(1H,td,J=8.0,10.6 Hz), 6.53(1H,brdd,J=11.0,12.6 Hz), 7.33(1H,d,J=12.6 Hz), 7.39 (1H,s).
    6: colorless oil, $[\alpha]_D$ -20.4°(c 0.24,$CHCl_3$).
    7: colorless oil, $[\alpha]_D$ -3.5°(c 0.085,$CHCl_3$). The HPLC analysis of the ether extract of C.viridis showed the presence of the acetates 5, 6 and 7 as the natural products.
    8: colorless oil, $[\alpha]_D$ -12°(c 0.025,$CHCl_3$), ^{1}H-NMR(400 MHz,$CDCl_3$) δ_{ppm} 0.88(3H,t,J=7.2 Hz), 1.79(2H,quint.J=7.4 Hz), 1.97(2H,brq,J=7.4 Hz), 2.04(3H,s), 2.35(2H,t,J=7.4 Hz), 2.70 (1H,brdd,J=7.1,14.3 Hz), 2.94(1H,brdd,J=7.4,14.3 Hz), 3.67(3H,s), 5.21(1H,brq,J=10.8 Hz), 5.54(1H,brtd,J=7.4,10.8 Hz), 6.02(1H,brtd,J=8.8,10.8 Hz), 6.98(1H,d,J=12.0 Hz), 7.44 (1H,s), 7.55(1H,brt,J=12.0 Hz).

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