Tetrahedron Letters, Vol.28, No.46, pp 5673-5676, 1987 Printed in Great Britain 0040-4039/87 \$3.00 + .00 Pergamon Journals Ltd.

STOLONIDIOL, A NEW MARINE DITERPENOID WITH A STRONG CYTOTOXIC ACTIVITY FROM THE JAPANESE SOFT CORAL

Kenichiro Mori, Kazuo Iguchi, Nobuko Yamada, and Yasuji Yamada* Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan

Yoshinobu Inouye

Department of Chemistry, University of Tsukuba, Sakura-mura, Niihari-gun, Ibaraki 305, Japan

<u>Abstract</u>: Structures of stolonidiol (<u>1</u>) and stolonidiol monoacetate (<u>2</u>), new marine diterpenoids with a strong cytotoxic activity from the Japanese soft coral <u>Clavularia</u> sp., were established by means of spectroscopic analyses, chemical reactions, and X-ray crystallographic analysis.

During the course of our investigation¹ on bioactive substances from the Japanese soft coral, two new diterpenoids, named stolonidiol (<u>1</u>) and stolonidiol monoacetate (<u>2</u>), have been isolated from the <u>Clavularia</u> sp.² as the major secondary metabolites. Both compounds showed a markedly strong cytotoxic activity against P388 leukemia cells <u>in vitro</u> (the IC₅₀ value of each; 0.015 μ g/ml), and ichthyotoxic activity toward a killifish <u>Oryzias latipes</u> (minimum lethal concentration; 10 μ g/ml for <u>1</u> and 17 μ g/ml for <u>2</u>). This paper describes the isolation and structure elucidation of 1 and <u>2</u>.

The ethyl acetate extract (73 g) of the freeze-dried organisms (1 kg) was subjected to repeated silica gel column chromatography (hexane-ethyl acetate as an eluent) by monitoring the ichthyotoxic activity, to give stolonidiol $(\underline{1})^3$ [colorless viscous oil, 1.5 g (2.1% yield based on the ethyl acetate extract), $C_{\underline{20}}H_{\underline{32}}O_4$, $[\alpha]_{\underline{D}}$ -31.0° (c 1.4, CHCl₃)] and stolonidiol monoacetate ($\underline{2}$)⁴ [colorless viscous oil, 2.3 g (3.2% yield), $C_{\underline{22}}H_{\underline{34}}O_5$, $[\alpha]_{\underline{D}}$ -26.8° (c 0.38, CHCl₃)].

The IR spectrum (CHCl₃) of <u>1</u> showed the presence of hydroxy group (3430 cm⁻¹) and <u>exo</u>-methylene group (1645, 910 cm⁻¹). The ¹H-NMR (400 MHz, CDCl₃) and ¹³C-NMR (100 MHz, CDCl₃) spectra of <u>1</u> showed the signals due to two trisubstituted epoxides, two quaternary carbons (one of which bears a hydroxy group), a hydroxymethyl, an <u>exo</u>-methylene and three methyls, seven methylenes, and a methine in addition (data shown below in Table 1). These spectral data and the degree of five unsaturation suggest that <u>1</u> is a bicarbocyclic diterpenoid with two epoxy moieties and two hydroxy groups. Acetylation of <u>1</u> with acetic anhydride in pyridine at room temperature gave the monoacetate <u>2</u>, which was identical to the natural product in all respects, including optical rotation. The partial structure of $-CH-C(CH_2OH)-CH_2-CH-C \leq$ in <u>1</u> was elucidated by



the following chemical reactions. Treatment of $\underline{1}$ with methanolic potassium hydroxide under reflux gave a triol $\underline{3}$,⁵ which was then oxidized with sodium metaperiodate in methanol at room temperature in the presence of 5% aqueous sodium hydrogen carbonate to give a conjugated enone 4.⁶

Results of the following two-dimensional NMR measurements of <u>1</u>, coupled with the above-mentioned findings, led to the plane structures of <u>1</u> and <u>2</u>. The ¹H-¹H homonuclear shift correlation spectrum (¹H-¹H COSY), the ¹H J-resolved spectrum, and the ¹H-¹³C heteronuclear shift correlation spectrum (¹H-¹³C COSY) of <u>1</u> clarified the chemical shifts and coupling patterns of the overlapped ¹H signals. These measurements also afforded the full correlation of the ¹H signals with the ¹³C signals as shown in Table 1. The ¹³C-¹³C homonuclear shift correlation spectrum (INADEQUATE) of <u>1</u> exhibited the cross peaks of twenty-one pairs of carbons as shown in Fig. 1. Connection of each carbon pair clarified unambiguously the sequence of carbon atoms giving the structure <u>1</u> for stolonidiol and thus <u>2</u> for stolonidiol monoacetate.

The relative and absolute configurations of $\underline{1}$ and $\underline{2}$ were obtained by an X-ray analysis on a single crystal of p-bromobenzoate $\underline{6}$, ⁷ which was obtained as a crystalline product together with non-crystalline $\underline{5}$ in the reaction of $\underline{1}$ with p-bromobenzoyl chloride in pyridine. The result of the X-ray analysis of $\underline{6}$ is shown in Fig. 2. The crystallographic data are as follow: $C_{27}H_{36}BrClO_5$, F.W. 555.93, $D_m = 1.33$ g cm⁻³, orthorhombic, space group $P2_12_12_1$, a = 12.780 (3), b = 18.724(3), c = 11.351(4) Å, and Z = 4. The intensities were measured on a Rigaku AFC-5 diffractometer with graphite monochromated Mo Kot radiation up to $2\theta = 55^{\circ}$. The structure was solved by a heavy atom method and refined by full-matrix least squares⁸ using 1625 independent structure factors with $|Fo| \ge 3\alpha(Fo)$. Measurement of 30 Bijovoet pairs of the S value, $[|Fc(hkl)| - |Fc(-h-k-1)l]/\alpha(Fo)$, larger than ± 4.9 , determined the absolute stereochemistry. The final refinement with anisotropic temperature factors for all non-hydrogen atoms under the fixed geometries and B_{150} 's of hydrogens except those of the hydroxyls converged the R factor to 0.045.⁹ Thus the absolute configurations of six chiral centers in $\underline{1}$ and $\underline{2}$ were elucidated as 1<u>S</u>, 7<u>S</u>, 8<u>S</u>, 10<u>R</u>, 11<u>R</u>, and 12<u>S</u>. The absolute configuration at C-10 of $\underline{1}$ and $\underline{2}$ was also supported by the

Table 1. ¹³C- and ¹H-NMR data of 1

δ ^C ppm	$\delta^{ m H}$ ppm (J in Hz)
44.5	_
37.9	1.29(brdd, 9.2, 14.6)
29.3	1.92(dd, 10.0, 16.2) 2.16(td, 9.1, 16.2)
148.7	
31.4	2.26(dtd, 1.4, 8.4, 14.1) 2.47(brddd, 6.1, 7.7, 13.8)
24.8	1.65(tdd, 6.4, 8.4, 16.4) 1.76(m)
58.0	3.16(dd. 6.4, 7.5)
63.7	
26.9	2.13(dd, 2.0, 15.9) 2.48(dd, 8.0, 15.9)
56.6	3.12(dd, 2.0, 8.0)
75.9	
48.3	2.29(dd, 3.8, 9.8)
27.2	1.61(m)
	1.95(m)
36.9	1.68 (m)
	1.70(m)
23.5	0.85(s)
111.3	4./2(brs)
65.5	4.81(brs) 3.62(d, 12.4) 3.77(d, 12.4)
74.6	
29.6	1.19(s)
26.1	1.29(s)
	δ ^C ppm 44.5 37.9 29.3 148.7 31.4 24.8 58.0 63.7 26.9 56.6 75.9 48.3 27.2 36.9 23.5 111.3 65.5 74.6 29.6 26.1

Fig. 1. ¹³C-¹³C homonuclear shift correlation two-dimensional NMR spectrum of <u>1</u> (100 MHz, CDCl₃)

CD measurement of the p-bromobenzoate $\frac{8}{7}^{11}$ derived from an allylic alcohol $\frac{7}{7}^{12}$ which was obtained by the reaction of $\frac{2}{2}$ with phosphorous oxychloride in pyridine. The CD spectrum (EtOH) of $\frac{8}{2}$ [UV 243 nm (ε 10700)] showed a positive Cotton effect at 247 nm ($\Delta\varepsilon$ +10.8), 13 indicating the positive chirality between the two chromophores (the diene and p-bromobenzoyl groups). 14 This CD data revealed the <u>R</u> configuration at C-10 in <u>8</u>, and thus the 10<u>R</u> configuration in 1 and 2.

The structures of $\underline{1}$ and $\underline{2}$ are characterized by a new bicyclo[9.3.0]-



Fig. 2. Perspective view (ORTEP)¹⁰ of the molecule 6

tetradecane skeleton having the <u>cis</u> geometry between the methyl group at C-1 and the alkyl group at C-12, being diastereomeric to that of the dolabellane-type bicyclic diterpenoids.¹⁵

<u>Acknowledgement</u> We thank Dr. S. Mizobuchi, Kirin Brewery Co. Ltd., for measurement of cytotoxic activity, and Dr. K. Muzik, Harvard University, for identification of the soft coral.

References and Notes

- a) K.Iguchi, S.Kaneta, K.Mori, Y.Yamada, A.Honda, and Y.Mori, <u>Tetrahedron Lett.</u>, <u>26</u>, 5787 (1985) and other references cited therein; b) H.Nagaoka, K.Iguchi, T.Miyakoshi, N.Yamada, and Y.Yamada, <u>ibid</u>, <u>27</u>, 223(1986); c) K.Iguchi, K.Mori, M.Suzuki, H.Takahashi, and Y.Yamada, <u>Chem.Lett</u>., 1789(1986).
- 2. The present soft coral, collected at the coral reef of Ishigaki Island (Okinawa, Japan), was identified as <u>Clavularia viridis</u> (Order <u>Stolonifera</u>) by Dr.K.Muzik. Our previous studies^{la} on <u>C.viridis</u> exhibited that it contained a large amount of the marine prostanoids, clavulones and their congeners. However no such prostanoid was found from the present collection. We currently assume that the present soft coral is a variant of <u>C.viridis</u>.
- 3. All new compounds gave satisfactory data in combustion analysis or high resolution mass measurement.
- 4. <u>2</u>: IR(CHCl₃) 3450, 1730 cm⁻¹; ¹H-NMR(400 MHz,CDCl₃) 0.85(3H,s), 1.18(3H,s), 1.29(3H,s), 2.11 (3H,s), 3.01(1H,t,J=7.0 Hz), 3.14(1H,dd,J=1.5, 7.9 Hz), 3.91(1H,d,J=12.2 Hz), 4.28(1H,d,J= 12.2 Hz), 4.72(1H,brs), 4.80(1H,brs).
- 5. 3: mp 88-90°C; $[\alpha]_D$ -0.9°(c 0.67,CHCl₃). The stereochemistry of the methoxy group at C-7 in 3 and 4 is tentatively assigned as an α configuration.
- 6. <u>4</u>: np 146-8°C; $[\sigma]_D$ -52.4°(c 0.58,CHCl₃); UV(EtOH) 238 nn(ϵ 6470); ¹H-NMR(400 MNz,CDCl₃) 6.68(1H,d,J=15.2 Hz), 6.70(1H,d,J=15.2 Hz).
- 7. $\underline{6}$: mp 163-4°C; $[\alpha]_D$ +35.9°(c 0.44,CHCl₃). The compound $\underline{6}$ was reconverted to $\underline{1}$ by treatment with methanolic potassium carbonate.
- L.W.Finger, "A System of Fortran IV Computer Programs for Crystal Structure Computations, RFINE 2", Geophysical Laboratory, Carnegie Institute, Washington, DC(1979). The program modified by H.Horiuchi was used.
- 9. All crystallographic calculations were done on a FACOM M-380 computer at the Science Information Processing Center, University of Tsukuba. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.
- 10. C.K.Johnson, ORTEPII, Report ORNL-5138, Oak Ridge National Laboratory, Tennessee, USA(1976).
- 11. 8: [a]_D +77.3°(c 0.44,CHCl₃); ¹H-NMR(400 MHz,CDCl₃) 1.02(3H,s), 1.88(3H,brs,H-20), 1.94(3H, s), 4.73(1H,brs), 4.77(1H,brs), 5.03(1H,brs,H-19), 5.21(1H,brs,H-19), 5.74(1H,t,J=4.3 Hz, H-10).
- 12. $\underline{7}$: $[a]_{D}$ +22.2°(c 0.09,CHCl₃); UV(EtOH) 226 nm(ε 6900).
- 13. The second Cotton effect was observed at 228 nm ($\Delta \varepsilon$ -1.1). Such weak (or virtually unobservable second Cotton effect was frequently encountered in our experiences in the case of the compounds having a relatively large wave length difference between the UV absorptions of the two chromophores. See also the following reference; M.Koreeda, N.Harada, and K.Nakanishi, J.Am.Chem.Soc., 96, 266(1974).
- 14. N.Harada, J.Iwabuchi, Y.Yokota, H.Uda, and K.Nakanishi, J.Am.Chem.Soc., 103, 5590(1981).
- 15. For example; C.Ireland, D.J.Faulkner, J.Finer, and J.Clardy, <u>J.Am.Chem.Soc.</u>, <u>98</u>, 4664(1976); A.G.González, J.D.Martín, M.Norte, R.Pérez, V.Weyler, S.Rafii, and J.Clardy, <u>Tetrahedron</u> <u>Lett.</u>, <u>24</u>, 1075(1983); S.De Rosa, S.De Stefano, S.Macula, E.Trivellone, and N.Zavochik, <u>Tetrahedron</u>, <u>40</u>, 4991(1984); A.Matsuo, K.Yoshida, K.Uohama, S.Hayashi, J.D.Connolly, and G.A.Sim, <u>Chem.Lett.</u>, 935(1985).

(Received in Japan 19 May 1987)