

## 산호로부터 2개의 푸란노세스키테르펜의 분리와 구조 결정

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### Isolation and Structure Determination of Two Furanosquiterpenes from the Soft Coral *Sinularia lochmodes*

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**요 약.** 하와이 Pohnpei에서 채집한 soft coral *Sinularia lochmodes*로부터 2개의 furanosquiterpene인 (5'E)-5-(2',6'-dimethylocta-5',7'-dienyl) furan-3-carboxylic acid (1)와 (1'E,5'E)-5-(2',6'-dimethylocta-1',5',7'-trienyl) furan-3-carboxylic acid (2)가 정출되었다. 이들의 구조를 <sup>1</sup>H, <sup>13</sup>C NMR, Homo-COSY, <sup>1</sup>H-<sup>13</sup>C (1 bond) Heteronuclear Multiple Quantum Coherence Spectroscopy (HMQC), <sup>1</sup>H-<sup>13</sup>C (2 and 3 bond) Heteronuclear Multiple Bond Coherence Spectroscopy (HMBC), Electron Impact Mass Spectroscopy (EI-ms) 및 Infrared Spectroscopy (IR)에 의해 밝혔다.

**ABSTRACT.** Two furanosquiterpenes<sup>3</sup>, (5'E)-5-(2',6'-dimethylocta-5',7'-dienyl) furan-3-carboxylic acid (1) and (1'E,5'E)-5-(2',6'-dimethylocta-1',5',7'-trienyl) furan-3-carboxylic acid (2), were isolated from soft coral *Sinularia lochmodes* collected from Palikir pass at Pohnpei Micronesia, June, 1990 in Hawaii. Their structures were elucidated by <sup>1</sup>H, <sup>13</sup>C NMR, Homo-COSY, <sup>1</sup>H-<sup>13</sup>C (1 bond) Heteronuclear Multiple Quantum Coherence Spectroscopy (HMQC), <sup>1</sup>H-<sup>13</sup>C (2 and 3 bond) Heteronuclear Multiple Bond Coherence Spectroscopy (HMBC), Electron Impact Mass Spectroscopy (EI-ms) 및 Infrared Spectroscopy (IR).

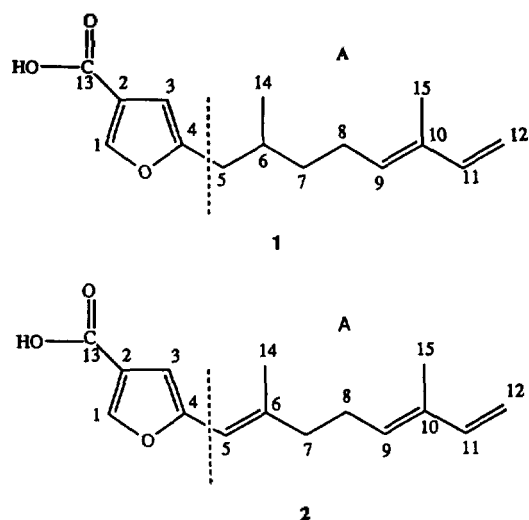
#### INTRODUCTION

Marine organisms, especially invertebrates such as soft corals, sponges, and molluscs, produce many new and unusual secondary metabolites which are unprecedented among terrestrial natural products. The field of marine natural products is becoming ever more sophisticated. Instead of simply searching for new metabolites, the research has become much more applied, targeting compounds which exhibit pharmacologically useful biological activities.

Furanosquiterpenes have been isolated from Sinularian soft coral in Australia<sup>1-3</sup>. We now report the isolation and structure elucidation of previously reported furanosquiterpenes<sup>3</sup> 1 and 2 from the CH<sub>2</sub>Cl<sub>2</sub>-isopropyl alcohol (IPA) (1 : 1) extract of the soft coral *Sinularia lochmodes* collected from Palikir pass at Pohnpei, June, 1990.

B. F. Bowden *et al*<sup>3</sup> separated compounds 1 and 2 as their methyl esters and characterized them after hydrolysis. We isolated them the first time as carboxylic acid and assigned their structures readily by Heteronuclear Multiple Bond Coherence (HMBC) NMR techniques.

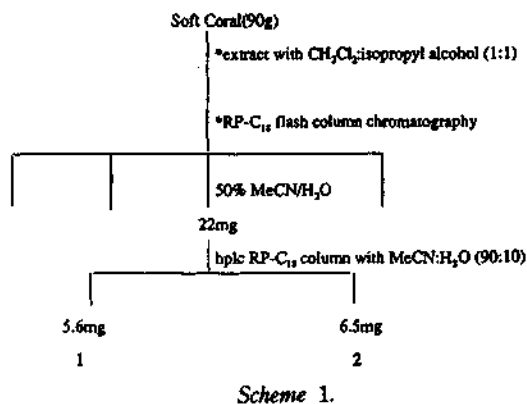
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## EXPERIMENTALS

**General experimental procedures.** All solvents were distilled from glass before use. TLC plates were viewed under shortwave UV light and then developed by 20%  $\text{H}_2\text{SO}_4$  or vanillin solution spray reagent followed by heating at approximately  $130^\circ\text{C}$ . Infrared spectra were recorded on a Perkin-Elmer model 1420 spectrophotometer. NMR spectra were measured on a General Electric QE-300 or GN OMEGA 500 at 500 MHz ( $^1\text{H}$ ) and 125 MHz ( $^{13}\text{C}$ ).  $^1\text{H}$  NMR chemical shifts are reported in ppm with the chemical shift of the residual protons of the solvent used as internal standard.  $^{13}\text{C}$  NMR chemical shifts are reported in ppm by using the natural abundance  $^{13}\text{C}$  of the solvent as an internal standard. Mass spectra were measured on a VG-70SE magnetic sector mass spectrometer. YMC RP- $\text{C}_{18}$  gel (particle size  $40\sim 65\ \mu$ ) was used for flash column chromatography. For hplc separation, a YMC ODS (particle size  $5\sim 20\ \mu$ ,  $10.0\ \text{mm}\times 30\ \text{cm}$ )  $\text{C}_{18}$  column was used in reversed-phase mode with Waters (Model 501) solvent metering pumps and Waters Associates Model 441 UV detection at 254 nm; attenuator 32x, chart speed 12CM/HR.

HMBC condition of 1: concentration of sample, 5.6 mg/ $\text{CDCl}_3$ , 0.6 ml, 256 of blocks $\times$ 2048 data matrix with 96 of scan per  $t_1$  increment, 3.0 delay period for long-range couplings,  $100^\circ\text{s}$  shifted sine



bell squared filtering for  $t_1$  and 3900 Hz line broadening for it.

HMBC condition of 2: concentration of sample, 6.5 mg/ $\text{DMSO-d}_6$ , 0.6 ml, the other parameters is the same as 1.

**Collection and isolation.** The soft coral was collected from Palikir pass at Pohnpei, June 1990. The freeze-dried soft coral (90 g) was extracted twice with 500 ml of  $\text{CH}_2\text{Cl}_2$ -*i*-PrOH (1:1) for 2 days, and the residue (660 mg) was subjected to RP- $\text{C}_{18}$  flash column chromatography using 50% MeCN/ $\text{H}_2\text{O}$ , yielding 30 mg of a mixture of furanosesquiterpenes. The mixture (30 mg) was separated by reversed-phase hplc [Phenomenex, MeCN/ $\text{H}_2\text{O}$  (90:10), 1.8 mL/min] to give 5.6 mg of pure 1 and 6.5 mg of pure 2 as white solids (Scheme 1).

**(5'E)-5-(2',6'-dimethylocta-5',7'-dienyl) furan-3-carboxylic acid (1).** EI-*ms*,  $M^+$  248.1416, 230 [ $M^+ - \text{H}_2\text{O}$ , 9%], 215 [ $M^+ - \text{H}_2\text{O}$ ,  $\text{CH}_3$ , 5%], 203 [ $M^+ - \text{CO}_2\text{H}$ , 6%], 125 [ $M^+ - \text{C}_9\text{H}_{15}$ , 83%], 152 [ $M^+ - \text{C}_7\text{H}_{12}$ , 31%], 81 [ $M^+ - \text{C}_9\text{H}_{11}\text{O}_2$ , 100%]; IR  $\nu_{\text{max}}$  3200~2810, 1660, 1580, 1420, 1215, 1130, 985, 972, 925, 840, 773  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR see Table 1.

**(1'E,5'E)-5-(2',6'-dimethylocta-1',5',7'-trienyl) furan-3-carboxylic acid (2).** EI-*ms*,  $M^+$  246, 165 [ $M^+ - \text{C}_8\text{H}_9$ , 58%], 147 [ $M^+ - \text{C}_9\text{H}_{11}\text{O}$ , 100%], 119 [ $M^+ - \text{C}_7\text{H}_{11}\text{O}_2$ , 12%], 81 [ $M^+ - \text{C}_9\text{H}_9\text{O}_3$ , 45%]; IR  $\nu_{\text{max}}$  3300~2600, 1670, 1570, 1440, 1250, 1160, 990, 970, 930, 900, 765  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR see Table 2.

Table 1. NMR data for **1** in CDCl<sub>3</sub>

Position	<sup>1</sup> H NMR	<sup>13</sup> C NMR	HMBC
1	7.97, 1H, d, <i>J</i> =0.72 Hz	147.58	2, 3
2		118.94	
3	6.36, 1H, d, <i>J</i> =0.72 Hz	105.96	1, 2, 4, 5
4		157.17	
5	2.63, 1H, dd, <i>J</i> =15.11, 5.99 Hz 2.46, 1H, dd, <i>J</i> =15.11, 5.99 Hz	35.09	3, 4, 6, 7, 14
6	1.88~1.80, 1H, m	32.05	4, 5, 7, 8, 14
7	1.46~1.39, 1H, m 1.29~1.22, 1H, m	36.05	5, 6, 8, 9, 14
8	2.23~2.09, 2H, m	25.66	6, 7, 9
9	5.45, 1H, t, <i>J</i> =7.20 Hz	132.78	7, 8, 11, 15
10		134.07	
11	6.35, 1H, dd, <i>J</i> =17.26, 10.8 Hz	141.48	9, 10
12	5.07, 1H, d, <i>J</i> =17.27 Hz 4.92, 1H, d, <i>J</i> =10.5 Hz	110.55	10, 11
13		168.19	
14	0.92, 3H, d, <i>J</i> =6.4 Hz	19.35	5, 6, 7
15	1.73, 3H, s	11.61	9, 10, 11

s: singlet, d: doublet, dd: doublet of doublets, m: multiplet.

## RESULTS AND DISCUSSION

The CH<sub>2</sub>Cl<sub>2</sub>-IPA (1 : 1) extract of freeze-dried soft coral, showed cytotoxicity against KB cell line<sup>4</sup> (MIC of 1 μg/mL, 3<sup>+</sup>). The extract was subjected to C<sub>18</sub> flash column chromatography (50% MeCN/H<sub>2</sub>O) and followed by high performance liquid chromatography using RP-C<sub>18</sub> column with MeCN : H<sub>2</sub>O (90 : 10) to give two furanosesquiterpenes **1** and **2**. The compounds **1** and **2** were identified as furanosesquiterpenes by comparison of their spectral data with literature values<sup>3</sup>.

A molecular formula of C<sub>15</sub>H<sub>20</sub>O<sub>3</sub> for **1** was established by EI-ms. The IR spectrum of **1** indicated the presence of carboxylic acid (3200~2810 cm<sup>-1</sup>) and conjugated carbonyl (1660 cm<sup>-1</sup>) group. The

Table 2. NMR data for **2** in DMSO-d<sub>6</sub>

Position	<sup>1</sup> H NMR	<sup>13</sup> C NMR	HMBC
1	8.05, 1H, s	146.81	2, 3, 13
2		126.86	
3	6.53, 1H, s	107.96	1, 2, 4, 5, 13
4		155.46	
5	6.11, 1H, s	114.47	3, 4, 7, 14
6		141.12	
7	2.27, 1H, t, <i>J</i> =8.1 Hz	40.77	5, 6, 8, 9, 14
8	2.36, 1H, m	27.32	6, 7, 9, 10
9	5.52, 1H, t, <i>J</i> =6.66 Hz	132.71	7, 8, 11, 15
10		135.22	
11	6.36, 1H, dd, <i>J</i> =17.58, 10.93 Hz	142.34	9, 10, 15
12	5.07, 1H, d, <i>J</i> =17.58 Hz 4.89, 1H, d, <i>J</i> =10.92 Hz	110.98	10, 11
13		164.51	
14	1.99, 3H, s	18.70	5, 6, 7
15	1.74, 3H, s	11.71	9, 10, 11

<sup>1</sup>H and <sup>13</sup>C NMR spectra (CDCl<sub>3</sub>) of **1** showed the presence of one carboxylic acid group (δ<sub>c</sub> 168.19), four double bonds (δ<sub>H</sub> 7.97, 6.36, 5.45, 6.35, 5.07, 4.92); δ<sub>c</sub> 147.58, 118.94, 105.96, 157.17, 132.78, 134.07, 141.48, 110.55, and two methyl groups (δ<sub>H</sub> 0.92 and 1.73; δ<sub>c</sub> 19.35 and 11.61). Analyses of the <sup>1</sup>H-<sup>1</sup>H COSY spectrum of **1** allowed assignment of all protons and revealed the proton connectivities for two partial structures, C-5 to C-9, C-11 to C-12. COSY correlations were observed between H3 (δ 6.36) and H5 (δ 2.63 and 2.46); between H5 and H3, H6 (δ 1.88~1.80); between H8 (δ 2.23~2.09) and H9 (δ 5.45); between H11 (δ 6.35) and H12 (δ 5.07 and 4.92). The assignment of all protonated carbons was established by a HMQC<sup>5</sup> experiment (Table 1). Connection of four quaternary carbons (C-2, C-4, C-10, and C-13) and furan ring (C-1 to C-4) with partial structure (A part) was achieved by the <sup>1</sup>H-<sup>13</sup>C long-range correlations observed in the HMBC<sup>6</sup> spectrum (Table 1).

A one-proton doublet at δ 7.97 showed correla-

tions to two carbon signals at  $\delta$  118.94 (C-2) and 105.96 (C-3). One-proton doublet at  $\delta$  6.36 showed correlation to three carbon signals at  $\delta$  147.57 (C-1), 118.94 (C-2) and 157.17 (C-4), in addition to correlation to the methylene carbon at  $\delta$  35.09 (C-5). Two-protons at  $\delta$  2.63 and 2.46 showed correlations to two carbon signals of furan ring at  $\delta$  105.96 (C-3) and 157.17 (C-4), and to carbon signals at  $\delta$  32.05 (C-6), 36.05 (C-7) and 19.35 (C-14). This H-C correlation firmly established a connecting the furan ring and A part. One-proton triplet at  $\delta$  5.45 showed a correlation to two methylene carbons [ $\delta$  36.05 (C-7) and 25.66 (C-8)] and to methyl carbon signals at  $\delta$  11.61 (C-15) and to double bond carbon signal  $\delta$  141.48 (C-11). One-proton doublet at  $\delta$  5.07 (*trans*) and the other proton doublet at  $\delta$  4.92 (*cis*) showed correlations to two carbons at  $\delta$  134.08 (C-10) and 141.48 (C-11). This connection confirmed the structure of the diene.

EI-ms data of **2** ( $M^+$  246) coincided with the molecular formula  $C_{15}H_{18}O_3$ . The  $^1H$  and  $^{13}C$ -NMR spectrum of **2** were similar to those of **1**. No proton signal at  $\delta$  6.11 was observed in the  $^1H$  NMR spectrum of **1**, which suggested that **2** was a furan conjugated to part A. The  $^1H$  and  $^{13}C$  NMR spectra (DMSO- $d_6$ ) of **2** were indicative of five double bonds and methyl groups;  $\delta_H$  8.05 (1H, s), 6.53 (1H, s), 6.11 (1H, s), 6.36 (1H, dd,  $J=17.58, 10.93$  Hz), 5.52 (1H, brt,  $J=6.66$  Hz), 5.07 (1H, d,  $J=17.58$  Hz), 4.89 (1H, d, 10.9 Hz), 1.99 (3H, s), 1.74 (3H, s);  $\delta_C$  155.46, 146.81, 142.34, 141.12, 135.22, 132.71, 126.86, 114.47, 110.98, 107.96, 18.70, 11.71.

The assignment of all protonated carbons was established by a HMQC experiment (Table 2). Connection of furan carbons and carbons of part A

was achieved by the  $^1H$ - $^{13}C$  long-range correlations observed in the HMBC spectrum (Table 2).

One-proton singlet at  $\delta$  6.53 showed correlation to a carbonyl carbon signal at  $\delta$  164.51 and two carbon signals at  $\delta$  146.81 (C-1), 126.86 (C-2), in addition to correlations to two carbon signals at  $\delta$  155.46 (C-4) and 114.47 (C-5). One-proton singlet at  $\delta$  6.11 showed correlations to two carbon signals of furan ring at  $\delta$  107.96 (C-3), 155.46 (C-4) and to methylene and methyl carbon signal at  $\delta$  40.77 (C-7), 18.70 (C-14), thus connecting the furan ring and A part which conjugated with furan ring. One-proton triplet at  $\delta$  5.52 showed correlation to two methylene carbons [ $\delta$  40.77 (C-7) and 27.32 (C-8)], methyl carbon signal at  $\delta$  11.71 (C-15), and double bond carbon signal  $\delta$  142.34 (C-11). One-proton doublet at  $\delta$  5.07 and the other proton doublet at 4.89 showed correlations to two carbon signals at  $\delta$  135.22 (C-10) and 142.34 (C-11).

## REFERENCES

1. Coll, J. C.; Liyanage, N.; Stokie, G. J.; Van Altena, I. A.; Nemorin, J. N. E.; Sternhele, S.; Kazlauskas, R. *Aust. J. Chem.* **1978**, *31*, 157.
2. Coll, J. C.; Mitchell, S. J.; Stokie, G. J. *Tetrahedron Lett.* **1977**, 1539.
3. Bowden, B. F.; Coll, J. C.; de Silva, E. D.; de Costa, M. S. L.; Djura, P. J.; Mahendran, M.; Tapiolas, D. M. *Aust. J. Chem.* **1983**, *36*, 371.
4. Cook, K. M.; Friedberg, E. C. *Biochemistry* **1978**, *17*, 850.
5. Otting, G.; Wuthrich, K. *J. Magn. Reson.* **1988**, *76*, 569.
6. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.