The distribution of sarcophytol-A in the Sarcophyton genus was investigated in seven samples belonging to S. glaucum (3 samples), S. infundibuliferum (2 samples), S. crassocaule (1 sample) and S. trocheliophorum (1 sample) that were collected on Ishigaki Island in Okinawa Prefecture. Sarcophytol-A was present in one sample each of S. glaucum and S. infundibuliferum. This study indicates that the composition of ceramabroids in the Sarcophyton genus is not related with the respective species, but with the individual samples collected.

Key words: sarcophytol-A; sarcophytolin-A; 2-[(E, E, E)-7, 8’-epoxy-4’, 8’, 12’-trimethylcyclotetradeca-1’, 3’, 11’-triényl]propan-2-ol; Sarcophyton glaucum; anti-tumor-promoter

The abundant production and accumulation of ceramabroids in soft corals is intriguing, but it is not yet clear whether these metabolites simply serve as repellents of predators. Among the various soft corals, Sarcophyton glaucum is one of the most intensively studied species because of its wide distribution in the coral reefs of Indo-Pacific coastal waters, but its major ceramabroids have been found to vary according to the locality where each sample was collected. A cerambrane-type lactone, sarcophine (I), and its 16-deoxo derivative (II) had been found in S. glaucum in the Red Sea1,2 (Fig. 1). On the other hand, Kobayashi et al.3,4 have found that S. glaucum collected off Ishigaki Island in Okinawa Prefecture did not reveal the presence of compounds corresponding to I and II. They further indicated that the lipid extracts contained sarcophytol-B (IIIa), sarcophytol-A (IVA), sarcophytol-A acetate (IVb), and sarcophytonin-A (V) (Fig. 1), in which IVA was the major component, constituting about two thirds of the total cerambrane-type diterpenes.

Later, Fujiki and co-workers5-8 found that IIIa and IVa inhibited the activity of the powerful tumor-promoter, teleocidin, in a two-stage carcinogenesis experiment on mouse dorsal skin by using dimethylbenzanthracene as an initiator. Effects on the growth and average number of tumors were observed even with an equimolar amount of IVa with respect to teleocidin, in contrast to other known natural anti-tumor-promoters (flavonoids, pentagalloyl glucose, dihydroxyceramabroids from tobacco leaves, etc.) which generally required the application of one thousand times or more of the promoter quantity. The powerful activity of sarcophytol-A has also been confirmed by other researchers9,10.

These results would indicate that IIIa or IVa could be medically used as an anti-tumor promoter. Although IVA can be chemically synthesized,11 its application by industry may be difficult owing to the complicated synthetic process. At present, therefore, it is considered that the extraction of IVA from S. glaucum is practical for large-scale production by industry. The present study was thus undertaken to investigate whether the presence of IVA as the major component of ceramabroids is common in S. glaucum, or whether IVA is also present in the other species of the Sarcophyton genus.

We collected seven samples of four species belonging to the Sarcophyton genus off Ishigaki Island in Okinawa Prefecture in September 1996. The classification and identification was made by Dr. Yehuda Benayahu of University of Israel, the data being summarized in Table 1.

Wet and ground material (500–800 g) from each sample was exhaustively extracted with methanol and then with dichloromethane. The combined extract was concentrated in vacuo, and mixed with dichloromethane. The precipitated material was removed by filtration, and the resulting filtrate was concentrated to afford a crude extract (20-35 g).

The presence of IVA in the crude extract from each of the seven samples was primarily examined by a
Sarcohypol A in the Sarcohypyon Genus

![Structures of Sarcohypol (I) and Its 16-Deoxo Derivative (II), Sarcohypol-B (IIIa) and Its Acetate (IIIb), Sarcohypol-A (IVa) and Its Acetate (IVb), Sarcohypolton-A (V), and 2-{[(E, E)-7'-8'-Epoxy-4',8',12'-trimethylcyclooctadeca-1',3',11'-triienyl]propan-2-ol (VI).](image)

**Fig. 1.** Structures of Sarcohypol (I) and Its 16-Deoxo Derivative (II), Sarcohypol-B (IIIa) and Its Acetate (IIIb), Sarcohypol-A (IVa) and Its Acetate (IVb), Sarcohypolton-A (V), and 2-{[(E, E)-7'-8'-Epoxy-4',8',12'-trimethylcyclooctadeca-1',3',11'-triienyl]propan-2-ol (VI).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Identification</th>
<th>Main components of cambranoids</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. glaucum</td>
<td>IVa</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>S. glaucum</td>
<td>V</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>S. glaucum</td>
<td>not detected**</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>S. infundibuliforme</td>
<td>IVa</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>S. infundibuliforme</td>
<td>VI</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>S. trochoeliophorum</td>
<td>V</td>
<td>7.6</td>
</tr>
<tr>
<td>7</td>
<td>S. crassocele</td>
<td>not detected**</td>
<td></td>
</tr>
</tbody>
</table>

* Weight content (%) in the lipid extract.

**Table 1. Identification of Seven Samples of the Soft Coral Sarcohypyon Genus and Their Main Components of Cambranoids**

$^{13}$C-NMR study, comparing the results with the reported data. The results indicated that samples No. 1 and No. 4 contained IVa. Spectroscopy was performed by a JEOL JNM-LA400 spectrometer, using deuterated chloroform (CDCl$_3$) as the solvent, and peaks are reported in ppm downfield from TMS.

To ascertain the presence of IVa in the extract of sample No. 1 (S. glaucum), column chromatography of the crude extract (5 g) was undertaken by using hexane-dichloromethane (1:4), dichloromethane, and dichloromethane-methanol mixtures of increasing polarity. Wako-gel C-100 was used, this being obtained from Wako Pure Chemical Ind. (Osaka, Japan). The column size was 2.5 × 6 cm, and the fraction volume was 200 ml. Elution by hexane-dichloromethane (1:4) and dichloromethane (200 ml each) afforded fractions (4.06 g) containing IVa as the main component, this being checked by TLC. A plate of silica gel 60F$_{254}$ obtained from E. Merck (Darmstadt, Germany) was used, with chloroform as the solvent. Rechromatography was performed on 2.5 g of the IVa-rich fraction. An almost pure sample (0.68 g) by TLC ($R_f$ 0.32) was obtained by eluting with a hexane-dichloromethane mixture (1:2 and 1:3). The content of IVa in this crude extract was calculated as 22%. IVa was identified by $^{13}$C-NMR. The other kinds of cambranoids in Fig. 1 were not detected in the respective fractions from the foregoing chromatography by the $^{13}$C-NMR study, in which sample concentration was 5 mg/400 μl of CDCl$_3$ and the number of accumulation times was 4096.

A similar experiment was performed on the crude extract of sample No. 4 (S. infundibuliforme), and the result indicated that this sample also contained compound IVa as the main component of cambranoids; the weight content of IVa in the crude extract was 28%. No other kinds of cambranoids were detected by the above-mentioned NMR study.

In respect of the other samples, an experiment was undertaken to confirm the absence of compound IVa. Column chromatography of sample No. 2 (S. glaucum) was carried out in a similar way to that already described, the crude extract (5 g) being used as the starting material. The NMR study of all the chromatographic fractions confirmed that compound IVa was absent, but that the fractions (0.4 g) eluted with dichloromethane and dichloromethane-methanol (98:2) contained an almost pure sample of compound V by TLC ($R_f$ 0.38), in which chloroform was used as...
the solvent. The weight content of V in the crude extract was 8.0%.

A similar experiment was performed on sample No. 6 (S. trocheliophorum), and the result indicated that it did not contain compound IVa, but likewise contained V as the main component of the cembranoids; the content of V in the crude extract was 7.6%.

Column chromatography of sample No. 5 (S. infundibilisforme) was conducted as already described, the crude extract (8 g) being used as the starting material. The NMR study of all the chromatographic fractions indicated that compound IVa was absent. Elution with dichloromethane and dichloromethanemethanol (98:2; 200 ml each) afforded a kind of cembranoid (2.63 g). Two grams of the sample was rechromatographed. Elution with dichloromethanemethanol (98:2 and 95:5) afforded 1.48 g of an almost pure sample by TLC, using chloroform as the solvent (R; 0.58). The NMR study indicated that this compound was VI (Fig. 1), which had also been found in the lipid extract of a Sarcophyton sp. by Coll et al. The weight content of VI in the crude extract was 24%.

A similar experiment was carried out on samples No. 3 (S. glaucum) and No. 7 (S. crassocaule). The respective fractions in the chromatography were similarly checked by an NMR study, but all the fractions contained negligible amounts of cembranoids. The main components of the cembranoids in the seven samples of the Sarcophyton genus and their weight contents (%) in the crude extracts are summarized in Table 1.

Our present study indicates that three samples from different individuals of S. glaucum contained different kinds of cembranoids; No. 1 sample contained compound IVa, No. 2 contained compound V, and No. 3 contained negligible amounts of cembranoids. Kobayashi et al. have shown that the predominant component of S. glaucum was IVa, which was associated with minor components such as III, IVb, and V. The difference between their results and those from the present study can be explained. Our study was performed on a lipid extract which had been prepared from an individual of S. glaucum, in which the average weight of the extract was less than 50 g. The experiment by Kobayashi et al. used 400 g of a lipid extract for fractionating various kinds of cembranoids. The sample used by Kobayashi et al. might therefore have been a mixture of many individuals of S. glaucum, rather than one individual. If this were the case, our present result does not conflict with the observations of Kobayashi et al.

The sample of S. infundibilisforme (No. 4) also contained compound IVa as the major component of the cembranoids, while another of the same species (sample No. 5) contained compound VI, which had been isolated from the lipid extract of a species of the Sarcophyton genus collected from the sea off Australia. The sample of S. trocheliophorum (No. 6) contained compound V as the major component of the cembranoids, similarly to sample No. 2 of S. glaucum.

These results lead to the conclusion that the composition of cembranoids (sarcophytol-A, sarcophytin-A, etc.) is not always related to the respective species of the Sarcophyton genus, but rather to the individuals collected.

Acknowledgments

We thank Dr. Yehuda Benyahu of University of Israel who identified the samples belonging to the Sarcophyton genus.

References


