Biosynthesis of Sterols from Mevalonate in a Starfish, 
*Coscinasterias acutispina*

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This study deals with the biosynthesis of sterols from mevalonate in a starfish, *Coscinasterias acutispina*. After injection of mevalonate-2-14C, the metabolites were investigated by using thin-layer, column, and gas-liquid chromatographic techniques. The detailed investigation of radioactive desmethylsterols showed that radioactivity was mainly associated with cholest-7-enol. However, there was no evidence for the incorporation of mevalonate-2-14C into C26-, C28-, and C29-sterols besides cholestanol and cholesterol. The results indicated that the starfish, *C. acutispina*, is capable of synthesizing at least cholest-7-enol from mevalonate via probably squalene and lanosterol etc. but not sterols other than C27-sterols. Also, it was suggested that the conversion of cholest-7-enol to cholesterol may not proceed in this starfish.

The echinoderms belonging to the class Asteroidea and Holothuroidea contain mainly \( \Delta^7 \)-sterols\(^3-^6\), while most other animals including vertebrates possess \( \Delta^9 \)-sterols. The sterols of these echinoderms are generally complex mixtures which are composed of C26-, C27-, C28-, and C29-sterols. Several workers have proved that the starfish are capable of converting cholesterol (cholest-5-en-3\( \beta \)-ol) to cholest-7-enol (5\( \alpha \)-cholest-7-en-3\( \beta \)-ol)\(^7-^10\), and \( \beta \)-sitosterol (stigmast-5-en-3\( \beta \)-ol) to 5\( \alpha \)-stigmast-7-en-3\( \beta \)-ol\(^10\), and this suggested that a variety of types of C25-, C27-, C28-, and C29-\( \Delta^7 \)-sterols occurring in them are partly derived from dietary sources of \( \Delta^9 \)-sterols. Recent investigations have also demonstrated that de novo sterol biosynthesis from acetate and mevalonate proceeds in a number of echinoderms\(^11-19\). These informations indicate that asteroids derive their sterols by both biosynthesis and modification of dietary \( \Delta^9 \)-sterols to \( \Delta^7 \)-sterols. However, it is not so clarified whether other sterols than C27-sterols are synthesized in asteroids and holothuroids or not.

Previously, the authors have shown that a starfish, *Leiaster leachii*, could synthesize cholest-7-enol from mevalonate-2-14C, and also that this starfish possesses no ability for alkylation at C-24\(^17\). In this study, hence, this subject was reinvestigated using another species of starfish, *Coscinasterias acutispina*, as part of investigations on sterol metabolism in marine invertebrates.

**Materials and Methods**

Specimens of the starfish, *C. acutispina*, were collected near Sakurajima in Kagoshima

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in October, 1974. To nine starfish, 25 μCi of DL-mevalonate-2-14C (specific activity, 5–10 mCi/m mol; Département des Radioéléments, Gif-sur-Yvette, France) was injected into the body cavity. The starfish were maintained in aquaria at 19–22°C for 12 days, and then the unsaponifiable matters were isolated from the whole bodies as described previously.17)

3β-Hydroxysterols were isolated by the digitonin method.20) Thin-layer chromatography (TLC) was carried out by using two adsorbents; Silicagel G developed with chloroform-ethyl acetate (20: 1, v/v) and a mixture of Silicagel GF254+366 and silver nitrate (5: 1, w/w) developed with hexane-benzene (5: 2, v/v) twice. The radioautography of TLC plates was performed by 3-week exposure to Sakura X-ray film.

Column chromatography on alumina (Brockmann grade III) was conducted to separate the unsaponifiable matters into methylsterols and desmethylsterols21); the elution was done with 0, 10, 20, 30, 40, 50, 70, and 90% of ether in hexane. Column chromatography on a mixture of silicic acid and silver nitrate (4: 1, w/w) was carried out by the similar method reported previously17) to separate desmethylsterols into the individual sterols according to the number and position of double bonds; the elution was done with 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50% of benzene in hexane.

Gas-liquid chromatography (GLC) on 1.5% OV-17 or 3.0% OV-17 was performed with a Shimadzu Gas-chromatographic unit, model GC-4BPF.17) In preparative GLC, the effluent was subdivided and samples were trapped in a collector at intervals of either 2 or 4 minutes at room temperature. The radioactivity of trapped samples was measured with a liquid scintillation counter, Beckman LS-230, using a toluene solution of PPO (0.6%) as a scintillator.

Results

The starfish, C. acutispina, incorporated mevalonate-2-14C into the lipids and unsaponifiable matters. The percentage incorporations of mevalonate-2-14C into the lipids and unsaponifiable matters were 7.6% and 5.9%, respectively. When the unsaponifiable matters were analyzed by TLC on Silicagel G followed by radioautography, the radioautogram showed that the radioactivity of unsaponifiable matters was associated with squalene (Rf 0.71), dimethylsterols (Rf 0.41), monomethylsterols (Rf 0.36) and desmethylsterols (Rf 0.28).

The 3β-hydroxysterols isolated from the unsaponifiable matters were subjected to column chromatography on alumina with hexane-benzene. The radioactivity of 3β-hydroxysterols was found to be present in the desmethylsterol fraction (1,150,000 dpm, about 80% of total radioactivity) eluted with 50–90% of ether in hexane. The methylsterol fraction eluted with 20–30% of ether in hexane gave low but substantial radioactivity. This result almost agreed with that of the starfish, L. leachii, reported previously17).
One of radioactive methylsterols was identified as lanosterol (5α-lanost-8-en-3β-ol) by TLC on Silicagel G followed by radioautography.

The analytical GLC on 1.5% OV-17 showed that the isolated desmethylsterols contained cholest-7-enol (60%), cholesta-7, 22-dienol (5α-cholesta-7, 22-dien-3β-ol) (8%), 24-methylcholesta-7, 22-dienol (24-methyl-5α-cholesta-7, 22-dien-3β-ol) (13%), 24-methylcholest-7-enol (24-methyl-5α-cholest-7-en-3β-ol) (6%), 24-ethylcholest-7-enol (24-ethyl-5α-cholest-7-en-3β-ol) (4%), and other minor sterols. The details of characterization of sterol components have been described elsewhere6). For preliminary check of the radioactive distribution in the individual sterols, desmethylsterols were subjected to preparative GLC on 1.5% OV-17. As shown in Table 1, radioactivity was apparently associated with the portions corresponding to cholesta-7, 22-dienol (5%), cholest-7-enol (55%), and 24-methylcholesta-7, 22-dienol (40%).

**Table 1.** Distribution of radioactivity in the desmethylsterols isolated from the starfish, *L. leachi*, after injection of mevalonate-2-14C

<table>
<thead>
<tr>
<th>Sterols</th>
<th>% Composition</th>
<th>Radioactivity cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholestanol + cholesterol</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cholesta-7, 22-dienol</td>
<td>8</td>
<td>110</td>
</tr>
<tr>
<td>Cholest-7-enol</td>
<td>60</td>
<td>2010</td>
</tr>
<tr>
<td>24-Methylcholesta-7, 22-dienol</td>
<td>13</td>
<td>990</td>
</tr>
<tr>
<td>24-Methylcholest-7-enol</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>24-Ethylcholest-7-enol</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*1 Determined by GLC on 1.5% OV-17.

*2 An aliquot of radioactive desmethylsterols was subjected to preparative GLC on 1.5% OV-17 with sample trapping at interval of 2 min.

To elucidate the radioactive sterols more rigorously, the acetates (Ac) of desmethylsterols were chromatographed on a silver nitrate-impregnated silicic acid with hexane-benzene. In this chromatography, the presence of at least two radioactive metabolites (metabolites A and B) was demonstrated as shown in Fig. 1. However, the fractions containing cholesteryl (5α-cholestan-3β-yl) Ac, cholesteryl Ac, cholesta-7, 22-dienyl Ac, 24-norcholesta-7, 22-dienyl Ac, 24-methylcholesta-7, 22-dienyl Ac, and 24-methylenecholest-7-enyl (24-methylene-5α-cholest-7-en-3β-yl) Ac gave no significant radioactivity. The radioautography after TLC also revealed two spots corresponding to the metabolites A (RF 0.69) and B (RF 0.47) but not cholesta-5, 7-dienyl (cholesta-5, 7-dien-3β-yl) Ac (RF 0.20). The metabolite A obtained from argentation column chromatography was found by GLC on 1.5% OV-17 to contain cholest-7-enyl Ac, 24-methylcholest-7-enyl Ac, and 24-ethylcholest-7-enyl Ac. Hence, the metabolite A was further subjected to preparative GLC on 3.0% OV-17 followed by radioactive determination of the trapped samples. In this GLC, radioactivity was associated with the portion corresponding to cholest-7-
enyl Ac but not 24-methylcholest-7-enyl Ac and 24-ethylcholest-7-enyl Ac. The metabo-
lite B was more polar than \( \Delta^{7,22-}\text{C}_{27}\)-sterol Ac and less polar than \( \Delta^{7,24(28)}-\text{C}_{28}\)-sterol Ac in
the argentation column chromatography; the mobility suggested that the metabolite B
may be \( \Delta^{7,24-}\text{C}_{27}\)-sterol Ac. The metabolite B obtained from column chromatography
contained a number of steryl Ac which had RRT to cholesteryl Ac of 0.90, 1.15, 1.34,
1.52, 1.80, 1.90 and 2.00 in analytical GLC on 1.5\% OV-17. However, the preparative
GLC analysis indicated that major radioactivity was associated with the portion cor-
responding to the steryl Ac with RRT of 1.34. Recently, SMITH et al. have isolated
cholesta-7, 24-dienol (5\( \alpha \)-cholesta-7, 24-dien-3\( \beta \)-ol) from the tissues of starfish, Asterias
rubens, and this sterol has been shown to be formed from mevalonate-2-\( ^{14}\)C in this star-
fish.\(^{19}\) Considering these data, the metabolite B was assumed to be probably cholesta-7,
24-dienol.

The results obtained in the present study concluded that the starfish, \( C. \) acutispina, is
capable of synthesizing at least cholest-7-enol from mevalonate but not cholestanol,
cholesterol, \( \text{C}_{3\alpha^*} \), \( \text{C}_{25^*} \), and \( \text{C}_{27^*} \)-sterols.

**Discussion**

Recently the sterol metabolism in echinoderms has been actively carried out by tracer
techniques. Data available up to the present indicated that desmethylsterols are formed from acetate or mevalonate in most echinoderms including the asteroids, holothuroids, echinoids, and ophiuroids. In addition, a number of possible intermediates in the biosynthesis of cholest-7-enol have been isolated from the tissues of starfish, *A. rubens*.[4] SMITH and GOAD[9] have proposed the possible biosynthetic routes for cholest-7-enol on the basis of the isolation of intermediates from the tissues and the incorporation of mevalonate-2-14C into the intermediates. On the other hand, there are a few reports showing that some echinoderms are incapable of synthesizing their sterols from lower units[22,23]; their conclusion was based on the decrease of specific activity (dpm/mg) of isolated sterol mixtures during repeated crystallizations. However, now such criteria for the sterol-synthesizing ability in marine invertebrates which contain complex sterol mixtures appears to give not always the reliable evidence of no ability for sterol synthesis[9,16,19]. Considering these informations, it is likely that most echinoderms are capable of synthesizing their sterols from mevalonate or acetate. However, the sterol-synthesizing ability seems to vary according to physiological conditions such as maturity of gonads[19].

In the present study, it was shown that the starfish, *C. acutispina*, possesses the ability for sterol synthesis from mevalonate via probably squalene, lanosterol etc. as well as other species of the asteroids. Furthermore, the detailed investigation of radioactive distribution in desmethylsterols demonstrated that this starfish is capable of cholest-7-enol but not cholestanol, cholesterol, C_{25}-, C_{26}-, and C_{27}-sterols. This result is in agreement with those of other asteroids, *A. rubens*, *Henrica saguinolenta*, and *L. leachii*. Although the echinoids seemed to be apparently capable of synthesizing both cholesterol and cholest-7-enol from lower units[18,24], the starfish, *C. acutispina*, belonging to the class Asteroidea was suggested to be incapable of synthesizing cholesterol from mevalonate on the basis of the failure in detection of radioactive cholesterol and possible intermediates, cholestanol[10] and cholesta-5, 7-dienol[25].

Since the asteroids are generally regarded to possess the ability for conversion of Δ^2- sterols to Δ^7-sterols, all sterols other than C_{27}-sterols occurring the tissues of starfish are conceived to be of dietary origin.

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References