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Biotransformation of Squalene to Optically Active Methylsuccinic Acid and 3-Methyladipic Acid

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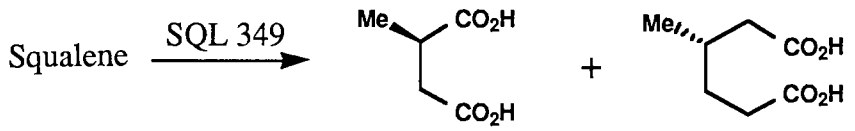
Squalene was converted to a mixture of (*R*)-(+)-methylsuccinic acid and (*S*)-(-)-3-methyladipic acid by *Saccharomycopsis lipolytica* SQL349 isolated from soil. The enantiomeric excess of each compound was determined to be 99%.

Squalene is a naturally occurring acyclic olefin which has six double bonds and eight methyl groups. It is a suitable model compound as the olefinic substrate of microbial transformation. There have been some reports on the microbial oxidation of squalene. Oxidation of the terminal carbon of the squalene molecule gave C_{30} dicarboxylic acid,[1,2] and oxidative cleavage at the central part of the squalene molecule gave two molecules of *trans*-geranylacetone.[3] But these compounds were not very useful as starting materials for organic synthesis. In order to obtain useful starting materials by microbial transformation of squalene, we screened microorganisms that could utilize squalene from soil. We isolated a *Saccharomycopsis lipolytica* SQL349 which was capable of converting squalene to a mixture of (*R*)-(+)-methylsuccinic acid (*R*)-(+)-(**1**) (99% ee) and (*S*)-(-)-3-methyladipic acid (*S*)-(-)-(**2**) (99% ee). These optically active compounds might be useful as chiral building blocks for synthesis of physiologically active substances.



(*R*)-(+)-**1** (*S*)-(-)-**2**

A strain of *Saccharomycopsis lipolytica* SQL349 which assimilates squalene was isolated as follows. The soil sample was inoculated into a test tube containing medium A composed of 1.0% (w/v) squalene, 0.25% (w/v) NH_4NO_3, 0.15% (w/v) KH_2PO_4, 0.15% (w/v) Na_2HPO_4, 0.05% (w/v) $MgSO_4 \cdot 7H_2O$, 0.001% (w/v) $FeSO_4 \cdot 7H_2O$, 0.001% (w/v) $CaCl_2 \cdot 2H_2O$, and 0.02% (w/v) yeast extract, at pH 5.0, and incubated at 30 °C for 5 days under aerobic conditions. The products were analyzed by HPLC.[4] A culture of *Saccharomycopsis lipolytica* SQL349 was inoculated into 3.0 L of medium B that was the same as medium A except for containing 2.0% (w/v) (47 mmol/L) squalene and 0.1% (w/v) glucose. The cultivation was carried out at 30 °C; agitation,

500 rpm; aeration, 0.8 vvm; for 64 h. The pH value was adjusted to 4.5 with 4 mol/L aq.NaOH once a day. The concentrations of **1** and **2** were determined to be 20 mmol/L (2.7 g/L) and 12 mmol/L (1.9 g/L), respectively, by HPLC analysis. The culture broth was centrifuged to remove cells and concentrated to 0.5 L at 60 °C *in vacuo*. The aqueous phase was acidified with 6 mol/L HCl and extracted with ethyl acetate. The ethyl acetate solution was dried over Na_2SO_4 and evaporated to give a residue which contained **1** and **2**. Each compound was purified from the residue by use of column chromatography with a silica gel and HP-2MG (Mitsubishi Kasei Co., Ltd.; Japan) to give 4.9 g of **1** [5] and 3.2 g of **2** [6] from 60 g of squalene. The recovery of **1** and **2** from the culture broth was 60% and 57%, respectively. The absolute configurations of **1** and **2** were determined to be *R* and *S*, respectively, by comparison of optical rotation with the value in the literature.[7,8] The e.e. of each compound was determined to be 99% by ^{13}C NMR analysis of their diesters formed with *l*-menthol in the presence of *p*-toluenesulfonic acid.[9,10] The mechanism of transformation of squalene to optically active compounds is under investigation. Further, we have obtained the half esters of **1** and **2**, methyl (*R*)-(+)-carboxybutyrate and methyl (*S*)-(-)-5-carboxy-3-methylpentanoate, respectively, by regioselective enzymatic hydrolysis of their dimethyl esters.

References

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2) C. Seo, Y. Yamada, N. Takada, and H. Okada, *Appl. Environ. Microbiol.*, **45**, 522 (1983).

3) Y. Yamada, H. Motoi, S. Kinoshita, N. Takada, and H. Okada, *Appl. Microbiol.*, **29**, 400 (1975).

4) HPLC analysis was carried out with a YMC AQ-312 (YMC Co., Ltd.; Japan). Mobile phase: 0.1 mol/L phosphate buffer (pH=2.8)/17 vol% MeOH; flow rate: 1.0 mL/min; detection: absorbance at 220 nm.

5) $[\alpha]_D^{27}$+15.6° (c 3.0, EtOH); IR νc=o(acid) 1698 cm^{-1}; ^1H NMR (D_2O) δ=1.22 (3H, d), 2.56 (1H, dd), 2.70 (1H, dd), 2.87-2.91 (1H, m); ^{13}C NMR (D_2O) δ=17.1, 36.7, 38.1, 177.3, 181.2; Found: C, 45.2; H, 6.2%; $(M+H)^+$, 133. Calcd for $C_5H_8O_4$: C, 45.4; H, 6.1%; M, 132.

6) $[\alpha]_D^{20}$-8.9° (c 0.76, H_2O); IR νc=o(acid) 1707 cm^{-1}; ^1H NMR (D_2O) δ=0.949 (3H, d), 1.48-1.56 (1H, m), 1.62-1.70 (1H, m), 1.89-1.97 (1H, m), 2.22 (1H, dd), 2.35-2.48 (3H, m); ^{13}C NMR (D_2O) δ=19.4, 30.5, 31.9, 32.5, 42.2, 179.3, 179.9; Found: C, 52.0; H, 7.5%; $(M+H)^+$, 161. Calcd for $C_7H_{12}O_4$: C, 52.5; H, 7.5%; M, 160.

7) E. Berner and R. Leonardsen, *Justus Liebigs Ann. Chem.*, **538**, 1 (1939).

8) J.Von Braun and F. Jostes, *Chem. Ber.*, **59**, 1091 (1926).

9) The e.e. of **1** was calculated from the ratio of the signal areas of carbon atoms at a 3-position of *l*-dimenthyl methylsuccinate; δ=37.95 ppm (*R*-form), 38.03 ppm (*S*-form).

10) The e.e. of **2** was calculated from the ratio of the signal areas of carbon atoms at a 2-position of *l*-dimenthyl 3-methyladipate; δ=41.77 ppm (*R*-form), 41.85 ppm (*S*-form).

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