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Oxidation of Acyclic Terpenoids by *Corynebacterium* sp.

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Squalene analogs such as lycopersene, geranylfarnesyl, digeranyl, and 2-hydroxy-2,3-dihydrosqualene and terpene alcohol derivatives such as farnesyl benzyl ether, farnesyl pivalate, geranylgeranyl pivalate, geranyl pivalate, and geranyl benzyl ether were oxidized by *Corynebacterium* sp. strain SY-79, which was isolated from soil by using squalene as a carbon source. Lycopersene and geranylfarnesyl gave no major product. Digeranyl, geranyl benzyl ether, and geranyl pivalate gave terminal oxidation products, and 2-hydroxy-2,3-dihydrosqualene, farnesyl benzyl ether, farnesyl pivalate, and geranylgeranyl pivalate were degraded to give lower molecular carboxylic acids. Strain SY-79 showed promising oxidative activities toward acyclic terpenes, although the metabolites obtained were variable, depending upon the structure of the substrate.

In search of useful microorganisms for biotransformation of acyclic terpenoids that will be applicable in organic syntheses, we have isolated *Arthrobacter* sp. strain Y-11 (9) and *Corynebacterium* sp. strain S-401 (6) from soil. In screening these bacteria, we used squalene (Fig. 1, I), an important intermediate of steroids biosynthesis, as a carbon source. Since squalene has a symmetrical structure with 8 methyl and 10 methylene groups and six double bonds which show almost identical reactivities to chemical reagents, it is a suitable model substrate for screening regioselective biotransformers. These isolates from soil showed some useful microbial transformations of hydrophobic linear compounds for organic synthesis (5, 6, 8, 10). On the other hand, the reactions involved make these experiments pertinent to studies of the degradation pathway of branched olefinic compounds in nature. Recently, we have isolated *Corynebacterium* sp. strain SY-79 from soil by using squalene as a carbon source. This bacterium used squalene as a carbon source and accumulated squalenedioic acid (Fig. 2, XI) in 26% yield in culture medium (7). In this report, we describe the application of *Corynebacterium* sp. strain SY-79 to squalene variants and acyclic terpene alcohol derivatives.

MATERIALS AND METHODS

Organism. *Corynebacterium* sp. strain SY-79, an isolate from soil, was used throughout this study.

Culture condition. The medium used for biotransformation contained the following: NH_4NO_3, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2HPO_4, 0.1%; KH_2PO_4, 0.1%; yeast extract, 0.1%, Plypepton (Wako Pure Chemical Industries Ltd., Osaka, Japan), 2%; terpenoids as substrate, 0.3 to 0.5% (vol/vol). The medium was adjusted to pH 7.2. Terpenoids were sterilized separately at 120°C under a pressure of 1.5 kg/cm^2 for 15 min and mixed with the medium aseptically before inoculation.

The bacteria were cultured in a 500-ml Sakaguchi flask containing 50 ml of the medium on a reciprocal shaker at 30°C for 5 days, except in the case of farnesyl pivalate (2 days). Seed culture was prepared in the medium containing 1% glucose in the place of the terpenoids. The inoculum size was 1%.

Isolation and purification of metabolites. The culture broth was acidified to pH 3.0 with 3 N HCl solution and extracted three times with dichloromethane. The dichloromethane extracts were combined and dried over anhydrous sodium sulfate. The metabolites in this extract were checked by silica gel thin-layer chromatography (TLC) with the solvent system of *n*-hexane–isopropanol (20:1). The solvent was evaporated under reduced pressure at 30°C, and the residue was purified by column chromatography with *n*-hexane–isopropanol (0.5 to 1.0%). After elution of the starting substrate with *n*-hexane, the metabolic products were eluted with *n*-hexane–isopropanol.

Chemicals. 2-Hydroxy-2,3-dihydrosqualene (V) was prepared from squalene by biotransformation with *Corynebacterium* sp. strain S-401 (6). Geranyl benzyl ether was synthesized from geraniol and benzyl chloride in the presence of sodium hydride. Farnesyl benzyl ether was also synthesized from farnesol (all *trans*) and benzyl chloride by the same method as was geranyl benzyl ether. Farnesyl benzyl ether (VI), NMR ($CDCl_3$) δ 7.35 (5H, s, aromatic protons), 5.42 (1H, t, OCH_2CH=), 5.15 (2H, m, CH=), 4.50 (2H, s, OCH_2Ar), 4.04 (2 H, d, OCH_2C=), 1.9 to 2.2 (8H, m, CH_2C=), 1.67 (3H, s, CH_3C=), 1.64 (3H, s, CH_3C=), 1.60 (6H, s, CH_3C=). Geranyl benzyl ether (IX), NMR ($CDCl_3$) δ 7.30 (5H, s, aromatic protons), 5.40 (1H, t, OCH_2CH=), 5.10 (1H, m, CH=), 4.45 (2H, s, OCH_2Ar), 4.01 (2H, d, OCH_2CH=), 2.0 to 2.2 (4H, m, CH_2C=), 1.70 (3H, s, CH_3C=). Farnesyl pivalate was prepared from farnesol (all *trans*) and pivaloyl chloride in chloroform in the presence of pyridine. Pivaloyl esters of geraniol, geranylgeraniol, and dimethylallyl alcohol were prepared from corresponding alcohol and pivaloyl chloride in benzene in the presence of pyridine.

For farnesyl pivalate (VII), NMR ($CDCl_3$) δ 5.25 (1H, t, OCH_2CH=), 5.10 (2H, m, HC=), 4.52 (2H, d, OCH_2), 1.9 to 2.1 (8H, m, CH_2C=), 1.65 (6H, s, CH_3), 1.56 (6H, s, CH_3C=), 1.13 (9H, s, CH_3C). Infrared (IR) spectrum (film) 1730, 1680 cm^{-1}. For geranyl pivalate (X), NMR ($CDCl_3$) δ 5.35 (1H, t, OCH_2CH=), 5.10 (1H, m, CH=), 4.55 (2H, d, OCH_2), 2.0 to 2.2 (4H, m, CH_2C=), 1.20 (9H, s, CH_3C). IR spectrum (film) 1730, 1680 cm^{-1}. For geranylgeranyl pivalate (VIII), NMR ($CDCl_3$) δ 5.32 (1H, t, OCH_2CH=), 5.10 (3H, m, CH=), 4.55 (2H, d, OCH_2), 2.2 to 2.0 (12H, m, CH_2C=), 1.70 (6H, s, CH_3C=), 1.62 (12H, s, CH_3C=), 1.21 (9H, s, CH_3C), IR spectrum 1730, 1680 cm^{-1}. For dimethylallyl pivalate, NMR ($CDCl_3$) δ 5.28 (1H, t, OCH_2CH=), 4.50 (2H,

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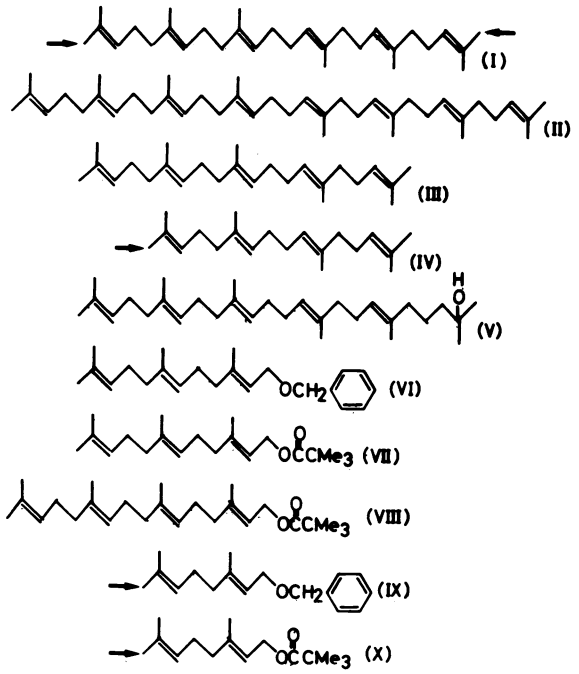

FIG. 1. Structures of substrates used. I, Squalene; II, lycopersene; III, geranylfarnesyl; IV, digeranyl; V, 2-hydroxy-2,3-dihydrosqualene; VI, farnesyl benzyl ether; VII, farnesyl pivalate; VIII, geranylgeranyl pivalate; IX, geranyl benzyl ether; X, geranyl pivalate.

d, OCH_2), 1.70 (3H, s, CH_3C=), 1.65 (3H, s, CH_3C=), 1.15 (9H, s, CH_3C). IR spectrum 1730, 1680 cm^{-1}.

Digeranyl and geranylfarnesyl were synthesized by the method of Biellmann and Ducep (1), starting from pure geraniol and farnesol (*cis trans* mixture). Squalene and geraniol were purchased from Tokyo Kasei Kogyo (Tokyo) Ltd. *trans*-Farnesol was a gift from Kuraray Co. Ltd. Pivaloyl chloride was purchased from Aldrich, Merck silica gel type 60 was used for TLC and Merck silica gel 60 (70 to 230 mesh) was used for column chromatography. Lycopersene was synthesized in three steps from squalenediol (10), which was derived from squalenedioic acid (XI) (7), the product of bioconversion of squalene by *Corynebacterium* sp. strain SY-79. Procedure of the synthesis is as follows. Squalene-1,24-diol(1 g, 2.26 mmol) (10) was dissolved in a mixture of 100 ml of *n*-hexane and 0.18 ml of pyridine and cooled at 5°C. Phosphorus tribromide (0.4 ml) was added dropwise to the solution, which was then stirred for 1 h. The reaction mixture was poured onto ice water, and the solvent layer was washed with water. The solvent layer was washed four times with sodium carbonate solution (5%) and again washed twice with water. The solvent was evaporated, and the residue was dissolved in 100 ml of dichloromethane and dried over anhydrous sodium sulfate. Evaporation of dichloromethane gave 0.86 g of dibromide which was used for the next reaction without purification. Mass spectrum *m/z*: 568 (M^+). NMR ($CDCl_3$) δ 5.55 (2H, t, HC=), 3.94 (4H, s, CH_2Br), 1.98 to 2.4 (20H, m, CH_2), 1.72 [6H, s, $CH_3(BrCH_2)C$=], 1.58 (12H, s, *z*-CH_3C=).

Squalene-1,24-dibromide was transformed to lycopersenedisulfone by coupling with dimethylallyl-*p*-toluenesulfinate (4). Dimethylallyl-*p*-toluenesulfinate (0.6 g, 2.7 mmol) was dissolved in tetrahydrofuran-hexamethylphosphoramide (2:1) (15 ml) and cooled at −78°C under nitrogen gas. *n*-Butyl lithium in hexane (1.12 M, 2.4 ml) was added to the solution, and it was stirred for 1.5 h. Squalene-1,2-dibromide (0.15 g, 0.898 mmol) in 5 ml of dry tetrahydrofuran was added to the solution. The reaction mixture was allowed to raise its temperature to 0°C, and then it was poured into ice water and extracted three times with ether. Ether was evaporated, and the residue was dissolved in dichloromethane and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded crude lycopersenedisulphone (2,6,10,14,19,23,27,31-octamethyldotriaconta-2,6,10,14,18,22,26,30-octaene-4,29-di-*p*-toluenesulfinate). IR spectrum (film) cm^{-1}: 1660, 1600. NMR ($CDCl_3$) δ 7.7 (4H, d, aromatic protons), 7.3 (4H, d, aromatic protons), 5.12 (6H, m, HC=), 4.88 (2H, d, HC=), 3.84 (2H, m, $CH\text{-}SO_2$), 2.80 (2H, d, CH-C=), 2.40 (6H, s, CH_3— on aromatic ring), 1.72 to 2.16 (20H, m, CH_2), 1.61 (6H, s, CH_3C=), 1.56 (6H, s, CH_3C=), 1.54 (6H, s, CH_3C=), 1.49 (6H, s, CH_3C=), 1.16 (6H, s, CH_3C=).

Lycopersenedisulphone (200 mg, 0.23 mmol) was placed in a four-necked flask (100 ml) and cooled at −78°C under argon gas. Ethylamine (20 ml) was introduced into the flask, and the metal lithium was added portionwise until the solution became blue and its color was maintained for 30 s. The reaction mixture was quenched with ammonium chloride and ethylamine was evaporated at 20°C. Ice water was added to the residue, which was then extracted with ether. After drying over sodium sulfate, evaporation of the solvent gave 102 mg of crude product. The crude lycopersene was purified by preparative TLC (2 mm thick) with *n*-hexane. The yield was 60 mg (48%). Mass spectrum *m/z*: 546 (M^+). NMR ($CDCl_3$) δ 5.15 (8H, m, HC=), 1.8 to 2.3 (28H, m, CH_2C=), 1.68 (6H, s, *E*CH_3C=), 1.60 (24H, s, *Z*-CH_3C=).

Analytical methods. Infrared spectra were recorded on a Hitachi model 215 Grating IR spectrometer (Japan). Mass spectra and NMR spectra were obtained with a Hitachi MRU-6E spectrometer and a Hitachi R-24B (60 MHz) or a

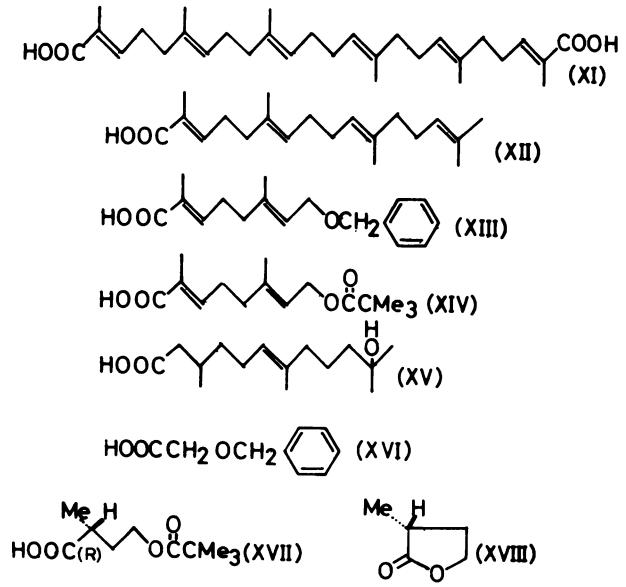

FIG. 2. Structures of major metabolic products detected. XI, Squalenedioic acid; XII, 2,6,11,15-tetramethylhexadeca-2,6,10,14-tetraenoic acid; XIII, 8-benzyloxy-2,6-dimethylocta-2,6-dienoic acid; XIV, 8-trimethylacetoxy-2,6-dimethylocta-2,6-dienoic acid; XV, 3,7,11-tetramethyl-11-hydroxy-6-dodecenoic acid; XVI, benzyloxyacetic acid; XVII, 2-methyl-4-trimethylacetoxybutyric acid; XVIII, 2-L-methylbutyrolactone.

JEOL model PS 100 (100 MHz; Japan) spectrometer, respectively. Chemical shifts are recorded in parts per million against tetramethylsilane as internal standard. Optical rotation was measured with a JASCO DIP-181 Digital Polarimeter (Japan).

RESULTS

2-Hydroxy-2,3-dihydrosqualene. Strain SY-79 was cultured in the medium (100 ml) with monohydrated squalene (V) (0.40 g) at 30°C for 5 days. Dichloromethane extraction of the broth gave 0.187 g of oil which showed a main spot of the product (R_f = 0.17) and the recovered hydrated squalene (V) (R_f = 0.55) on TLC(*n*-hexane–ether [1:1]). The crude oil was dissolved in 20 ml of *n*-hexane and was extracted twice with 10 ml of methanol-H_2O (8:1). From the *n*-hexane layer, 50 mg of 2-hydroxy-2,3-dihydrosqualene was recovered. The methanol-H_2O layer gave 130 mg of oil which was purified on column chromatography with 10 g of silica gel. After elution with *n*-hexane, a trace amount of minor product (ca. 10 mg) was eluted with *n*-hexane–isopropanol (3%). After the elution of the minor product, the main product (40 mg) was eluted. The main product showed the following spectra. IR spectrum (film) 3,400, 3,100 to 2,700 (broad), 1,705 cm^{-1}. Mass spectrum *m/z*: 238(M^+-H_2O), 182, 122, 95, 69, 59($Me_2C{=}O^+H$). NMR ($CDCl_3$) δ 5.1 (1H, t, HC=), 2.4 to 1.8 (7H, m, CH_2CO, CH_2C=, —CHMe—), 1.55 (3H, s, CH_3C=), 1.5 to 1.2 (6H, m, CH_2), 1.16 (6H, s, Me_2C—O), 0.96 (3H, d, CH_3CH—). Irradiation of peaks at 1.95 ppm transformed the doublet signal at 0.96 to a singlet one. For confirmation of the molecular weight of the metabolite, it was transformed to methyl ester by treatment with diazomethane in ether solution. The mass spectrum of the methyl ester showed M^+ peak 270 and 252(M^+-H_2O), 238, 237. As the IR spectrum of the metabolite did not show absorption bands of an α,β-unsaturated carboxyl group and on the basis of the NMR spectrum, we assigned the structure of this product as 3,7,11-tetramethyl-11-hydroxy-6-dodecenoic acid (XV).

Geranyl benzyl ether. Cultivation of strain SY-79 in 300 ml of the medium with 0.96 g of geranyl benzyl ether gave 0.49 g of crude oil. After column chromatography as described above, 0.22 g of recovered geranyl ether and 154 mg of the metabolite were obtained. The yield of the product was 14%. Mass spectrum *m/z*: 274(M^+), 256, 244, 183(M^+-91). IR spectrum (film) 1690, 1640 cm^{-1}. NMR ($CDCl_3$) δ 9.74 (1H, broad, HOOC), 7.35 (5H, s, aromatic protons), 6.90 (1H, t, HC=), 5.44 (1H, t, HC=), 4.58 (2H, s, OCH_2 phenyl), 4.04 (2H, d, OCH_2C=), 2.1 to 2.5 (4H, m, CH_2C=), 1.84 [3H, s, CH_3(HOOC)C=], 1.64 (3H, s, CH_3C=).

The IR absorption bands indicate that the metabolite is an α,β-unsaturated carboxylic acid. The position of the carboxyl group was determined from the NMR signal of olefinic methyl protons (δ 1.84). The geometric isomerism of the carboxyl group was determined as *E* from the NMR signals of one of the olefinic protons (δ 6.90) and olefinic methyl protons (δ 1.84), since in an alkyl CH=C(COOH) alkyl system, the olefinic proton signal *Z* to carboxyl group appears at about δ 6.9 whereas that of *E* appears at δ 6.2 (2). From these spectrometric data, the structure of the metabolite was assigned as 8-benzyloxy-2,6-dimethylocta-2,6-dienoic acid (XIII).

Digeranyl. Digeranyl (0.30 g) was incubated in the medium (50 ml) with strain SY-79. Dichloromethane extraction gave 0.22 g of crude oil. After column chromatography and preparative TLC (Silica Gel 60 F 254, 2 mm thick, with solvent system of *n*-hexane–isopropanol–isopropyl ether [17:1:2]), the metabolite (0.02 g) and digeranyl (0.04 g) were obtained. The yield of the metabolite was 6%. Mass spectrum *m/z*: 304 (M^+). IR spectrum (film) 1690, 1640 cm^{-1}. NMR ($CDCl_3$) δ 6.88 (1H, t, HC=), 5.14 (3H, t, HC=), 1.9 to 2.4 (12 H, m, CH_2C=), 1.82 [3H, s, CH_3(HOOC)C=], 1.66 (3H, s, *E*-CH_3C=), 1.58 (6H, s, *Z*-CH_3C=). The IR spectrum indicated that this product is an α,β-unsaturated carboxylic acid. The carboxyl group was located on the terminal of the digeranyl skeleton on the basis of the chemical shift of the olefinic methyl protons (δ 1.82). The geometric isomerism of the carboxyl group was determined as *E* from the lower field shift of the NMR signals of one of the olefinic protons (δ 6.88) and olefinic methyl protons (δ 1.82) (2). From these spectrometric data, the structure of the metabolite was determined as 2,6,11,15-tetramethylhexadeca-2,6,10,14-tetraenoic acid (XII).

Farnesyl benzyl ether. SY-79 was cultured on the medium (700 ml) with 2.44 g of farnesyl benzyl ether. Dichloromethane extraction and purification by column chromatography afforded 0.98 g of the metabolite. Farnesyl benzyl ether was not recovered. The mole yield of the metabolite was 75%. Mass spectrum *m/z*: 166 (M^+). IR spectrum (film) 1730, 1640 cm^{-1}. NMR ($CDCl_3$) δ 10.50 (1H, s, HOOC), 7.35 (5H, s, aromatic protons), 4.62 (2H, s, OCH_2 phenyl), 4.11 (2H, s, OCH_2COOH).

From these data, the structure of the metabolite was assigned as benzyloxyacetic acid (XVI).

Farnesyl pivalate. Farnesyl pivalate (VII) (2.06 g) was incubated in 700 ml of the medium with SY-79 for 2 days. After dichloromethane extraction and purification by column chromatography, pure metabolite (0.41 g) was obtained. The yield of the metabolite was 30%. Mass spectrum *m/z*: 202 (M^+). IR spectrum (film) 1725, 1715 cm^{-1}. NMR ($CDCl_3$) δ 10.7 (1 H, broad, HOOC), 3.89 (2H, t, CH_2OCO), 2.2 to 2.6 (1H, m, CHCOOH), 1.4 to 2.0 (2H, m, CH_2), 1.05 (3H, d, CH_3—CH—), 0.95 (9H, s, Me_3C). Optical rotation $[\alpha]_D^-$ = −3.11° (c = 4.47 in ethanol). Analysis found: C, 58.95; H, 8.90%. Calculated for $C_{10}H_{18}O_4$: C, 59.38; H, 8.97%. The IR spectrum suggested that the product is an ester acid. From NMR and mass spectra, we assigned the metabolite as 2-methyl-4-trimethylacetoxybutyric acid (XVII). As the ester acid (XVII) is optically active, we converted it to 2-methylbutyrolactone (XVIII) to determine its absolute configuration. The ester acid (VII) (0.23 g) was dissolved in methanol (3 ml) and water (0.5 ml) with KOH (0.5 g), and the solution was kept at room temperature for 2 h. After the hydrolysis, methanol was evaporated and pH was adjusted to 2.0 with diluted HCl solution. The acidified solution was stirred for 1 h at room temperature to complete lactonization, and it was extracted with dichloromethane. The extract was passed through an alumina column (5 g, Merck aluminum oxide 90). Pure lactone (XVIII) (26 mg) was obtained. IR spectrum (film) 1760. NMR ($CDCl_3$) δ 4.0 to 4.5 (2H, m, CH_2O), 1.28 (3H, d, J = 4 Hz, CH_3). Optical rotation $[\alpha]_D^-$ = +2.69° (c = 1.3 in ethanol). Since the specific rotation of pure 2-*R*-methylbutyrolactone is $[\alpha]_D^-$ = −21.5° (c = 5.5 in ethanol) (3), the optical purity of the lactone (XVIII) is about 12.5% and major enantiomer of the product (XVII) is *R*-configuration.

Geranylgeranyl pivalate. Geranylgeranyl pivalate (VIII) (0.80 g) was incubated in 200 ml of the medium with SY-79. The dichloromethane extract gave 220 mg of crude oil. It was purified on column chromatography, and 60 mg of recovered starting material and 37 mg (10%) of the metabolite were obtained. IR, NMR, and mass spectra coincided with those of the metabolite obtained with farnesyl pivalate.

Geranyl pivalate. Geranyl pivalate (X) (1.6 g) was incubated in 400 ml of medium with SY-79. The dichloromethane extract gave 1.09 g of crude oil after evaporation of solvent. It was purified on column chromatography, and 0.53 g of recovered geranyl pivalate and 120 mg of the product were obtained. IR spectrum 3,400 to 2,500 (broad), 1,730, 1,700, 1,650 cm^{-1}. NMR ($CDCl_3$) δ 9.75 (1H, broad, HOOC), 6.86 (1H, t, HC=), 5.35 (1H, t, HC=), 4.55 (2H, d, OCH_2C=), 2.5 to 2.0 (4H, m, CH_2C=), 1.82 [3H, s, $CH_3(HOOC)C$=], 1.70 (3H, s, CH_3C=), 1.15 (12H, s, Me_3C). Mass spectrum *m/z*: 250 (M^+-H_2O), 166, 121, 100, 57.

The IR spectrum of this metabolite showed one ester carbonyl band (1,700 cm^{-1}) and α,β-unsaturated carboxylic acid absorption bands (3,500 to 2,500, 1,700, and 1,650 cm^{-1}). The carboxyl group was located at the terminal position on the basis of the lower field shift of one olefinic methyl proton (δ 1.84) which appeared at δ 1.5 to 1.6 ppm in the NMR spectrum of the starting material. The lower field shift of one olefinic proton (δ 6.90) indicated that the geometric isomerism of the carboxyl group is *E* (2). Consequently, the structure of the metabolite was determined as 8-trimethylacetoxy-2,6-dimethyl-octa-2,6-dienoic acid (XIV).

Geranylfarnesyl. Geranylfarnesyl (2.10 g) was incubated with SY-79 in 700 ml of the medium. After isolation and purification, 0.30 g of geranylfarnesyl was recovered, and no major metabolite was obtained.

Lycopersene. SY-79 was cultured on the medium (20 ml) with lycopersene (0.11 g). After extraction and purification, 66 mg of lycopersene was recovered and a trace amount of carboxylic acid (2 to 3 mg) was obtained.

DISCUSSION

Corynebacterium sp. strain SY-79, a squalene-utilizing bacterium, transformed squalene (I) into squalenedioic acid (XI), which is a useful starting material for organic synthesis of terpenoids with functional groups at each terminal position (10). Some sites of oxidation of acyclic terpenoids by this strain leading to the accumulated metabolite are shown by arrows in Fig. 1. The major metabolic products are shown in Fig. 2. Lycopersene (II), which is one isoprene unit longer than squalene at both terminals, was recovered in 60% yield from the culture broth giving no major metabolite. Geranylfarnesyl (III), which is asymmetrical and one isoprene unit shorter at one terminal than is squalene, was recovered in 14% yield without giving a major metabolite. 2-Hydroxy-2,3-dihydrosqualene (V) was degraded to hydroxy acid (XV) in 16.6% yield. The other half-moiety of this molecule was not recovered. Digeranyl (IV), a symmetrical squalene analog which is two isoprene units shorter than squalene, was oxidized at one terminal methyl group, although in low yield (6%). Geraniol and farnesol did not undergo microbial transformation but inhibited the growth of SY-79. Therefore, we used their benzyl ether and pivaloyl ester as stable derivatives to microbial hydrolysis. The *t*-butyl group of the pivaloyl moiety is also a mimic of the "head" of the isoprene unit. Isobutyric acid esters of geraniol and farnesol were also tried as substrates of biotransformation by strain SY-79, but these esters were easily hydrolyzed to the corresponding alcohols and gave no interesting metabolite. Geranyl benzyl ether (IX) and geranyl pivalate (X) were oxidized at the terminal methyl group. Farnesyl benzyl ether (VI) and farnesyl pivalate (VII) gave the highly degraded products XVI and XVII, respectively, in good yield. Both are tail parts of the farnesyl moiety. Geranylgeranyl pivalate (VIII) was also degraded to give the same product (XVII) as did farnesyl pivalate. Dimethylallyl pivalate, which has one isoprene unit, was not oxidized and was recovered without giving any metabolite. Among the pivalate derivatives, farnesyl pivalate is especially interesting with respect to organic synthesis because it was metabolized quickly and gave an optically active product.

Other terpene alcohols, such as nerolidol (C_{15}), geranylgeraniol, and isodecaprenol (C_{50}), did not inhibit the growth of SY-79 but were not metabolized and were recovered in almost 100% yield.

None of these substrates which were used in this study gave the dicarboxylic acid that was produced from squalene; the only products were one-terminal oxidized metabolites or degraded monocarboxylic acids. The low yields of products and recovered substrates suggested that the regiospecificity of this microbial oxidation is not high and that the first oxidation site directs subsequent metabolism, resulting in various products depending upon the small structural differences between substrates. However, the use of appropriate derivatives such as pivaloyl esters gives promising products for organic synthesis in tolerable yields.

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LITERATURE CITED

1. **Biellmann, J. F., and J. B. Ducep.** 1971. Synthese du squalene et d'analogues. Tetrahedron **27**:5861–5872.
2. **Gaudemer, A.** 1977. Determination of configurations by NMR spectroscopy, p. 48–50. *In* H. B. Kagan (ed.), Determination of configurations by spectrometric methods, vol. 1. Georg Thieme Publishers, Stuttgart.
3. **Kaneko, T., K. Wakabayashi, and H. Katsura.** 1962. The absolute configuration of α-methyladipic acid. Bull. Chem. Soc. Jpn. **35**:1149–1153.
4. **Sato, K., S. Inoue, A. Onishi, N. Uchida, and N. Minowa.** 1981. Stereoselective synthesis of solanesol and all-*trans*-decaprenol. J. Chem. Soc. Perkin Trans. **I**:761–769.
5. **Seo, C., Y. Yamada, and H. Okada.** 1981. Synthesis of fatty acid esters by *Corynebacterium* sp. S-401. Agric. Biol. Chem. **46**:405–409.
6. **Seo, C., Y. Yamada, N. Takada, and H. Okada.** 1981. Hydration of squalene and oleic acid by *Corynebacterium* sp. S-401. Agric. Biol. Chem. **45**:2025–2030.
7. **Seo, C. W., Y. Yamada, N. Takada, and H. Okada.** 1983. Microbial transformation of squalene: terminal methyl group oxidation by *Corynebacterium* sp. Appl. Environ. Microbiol. **45**:522–525.
8. **Yamada, Y., N. Kusuhara, and H. Okada.** 1977. Oxidation of linear terpenes and squalene variants by *Arthrobacter* sp. Appl. Environ. Microbiol. **33**:771–776.
9. **Yamada, Y., H. Motoi, S. Kinoshita, N. Takada, and H. Okada.** 1975. Oxidative degradation of squalene by *Arthrobacter* sp. Appl. Microbiol. **29**:400–404.
10. **Yamada, Y., C. Seo, and H. Okada.** 1981. Asymmetric synthesis of (−)-(S)-squalene-2,3-epoxide. Agric. Biol. Chem. **45**: 1741–1742.