Biological Synthesis of Polyketides from 6-*n*-Pentyl-α-pyrone by *Streptomyces* sp.

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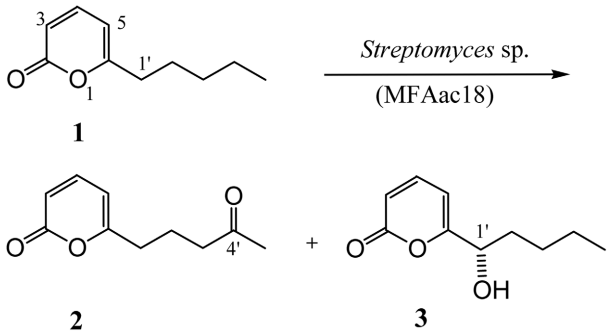
Selectivity is an essential requirement in synthetic organic chemistry. The regioselectivity of enzymes even on complex or symmetric molecules without any need of protecting groups is a fundamental strength of biocatalysis. Biocatalysis are also a powerful tool for the regioselective and enantioselective synthesis of bioactive compounds, as well as for the generation of new, active, and less toxic derivatives for the bioactive natural products.[1]

The symmetric dipyrroloquinone, terreusinone,[2] was regioselectively biotransformed to its unsymmetrical alcohol derivative, terreusinol, by the marine actinomycete *Streptomyces* sp. (MFAac18),[3] which was isolated from a sample of sea plant *Zostera marina* collected at Bijin Island, Gyeongnam, using YPG (yeast, peptone, and glucose) agar medium.

In our continuing studies of the application of biocatalysis,[3] we investigated the microbial transformation for 6-*n*-pentyl-α-pyrone (**1**), which had been isolated as the anti-tyrosinase active component from the marine isolate of the fungus *Myrothecium* sp.[4]

We wish to report the biological synthesis 6-*n*-(4-oxopentyl)-α-pyrone (**2**) and 6-*n*-[(1*S*)-hydroxypentyl]-α-pyrone (**3**) from 6-*n*-pentyl-α-pyrone (**1**) by *Streptomyces* sp. (MFAac18).



1 **2** **3**

Biotransformation was carried out in the presence of a substance, 6-*n*-pentyl-α-pyrone (**1**) (20 mg), by a two-stage fermentation protocol.[3,5] The incubation was harvested after five weeks, and the filtered broth was extracted with EtOAc to afford a crude extract (30 mg).

Chromatography of the extract (30 mg) gave the biotransformed metabolites, 6-*n*-(4-oxopentyl)-α-pyrone (**2**) (5 mg) and 6-*n*-[(1*S*)-hydroxypentyl]-α-pyrone (**3**) (3.5 mg).

6-*n*-(4-Oxopentyl)-α-pyrone (**2**) was isolated as a colorless oil. A molecular formula of $C_{10}H_{12}O_3$, which gave five degrees of unsaturation, was established by HR-EI-MS and ^{13}C NMR methods. The IR absorption spectrum of **2** showed bands characteristic of a α-pyrone (1728, 1634, 1559 cm^{-1}) and aliphatic ketone (1720 cm^{-1}) functionality.

The overall NMR data indicated the presence of an α-pyrone, which was further supported by UV spectral data [299 nm (log ε 3.8)], and a pentanone.

On the basis of 2D NMR, COSY, HMQC, and HMBC correlations, the structure of **2** was proposed as the 6-*n*-(4-oxopentyl)-α-pyrone, which had been isolated as the anti-fungal metabolite, viridepyronone, from *Trichoderma viride*.[6] In addition, compound **2** is the first example, to the best of our knowledge, from the biological synthesis.

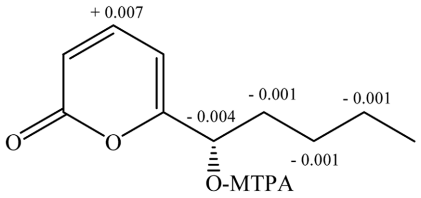
6-*n*-[1(*S*)-Hydroxypentyl]-α-pyrone (**3**) was isolated as a colorless oil. A molecular formula of $C_{10}H_{14}O_3$, which gave four degrees of unsaturation, was established by HR-EI-MS and ^{13}C NMR methods.

The ^1H and ^{13}C NMR spectra of **3** were closely similar to those of **2** except for an oxygenated aliphatic methine [δ_H 4.41 (1H, dd, H-1'); δ_C 71.0 (C-1')] newly appeared instead of an aliphatic ketone [δ_C 207.7 (C-4')].

On the basis of 2D NMR analysis of **3** in conjunction with the molecular formula, the plane structure of **3** was proposed as the known 6-*n*-(1-hydroxypentyl)-α-pyrone, which has been biotransformed from the same substrate (**1**).[7]

The unsolved stereochemistry of **3** led us to do further structural examination of this compound. The advanced Mosher's method[8] was applied to determine absolute configuration. Esterification of **3** with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPACl) by 1,3-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP) in CH_2Cl_2 yielded (*S*)-MTPA ester (**3a**) and (*R*)-MTPA ester (**3b**), respectively. The assignments of **3a** and **3b** were achieved by ^1H NMR, and the chemical shift differences between **3a** and **3b** (Figure 1) indicated an *S* configuration at C-1'. Thus, the stereostructure of **3** was established as 6-*n*-[1(*S*)-hydroxypentyl]-α-pyrone (**3**).

Compounds **1** exhibited a tyrosinase inhibitory activity[9] with ED_{50} value of 0.8 μM, which are more active than kojic acid (ED_{50}, 7.7 μM). However, the biotransformed metabolites (**2**, **3**) were inactive in tyrosinase inhibitory assay. Thus, the size and functional group of *n*-pentyl side chain in **1** were supposed to be important for displaying the anti-tyrosinase activity.



3a: (*S*)-MTPA ester
3b: (*R*)-MTPA ester

Figure 1. $\Delta\delta$ values ($\delta_S - \delta_R$) for (*S*)- and (*R*)-MTPA esters of compound **3**.

Experimental Section

General. Optical rotation was determined on a Perkin Elmer model 341 polarimeter. IR spectrum was recorded on a Bruker FT-IR model IFS-88 spectrometer. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. UV/visible spectra were measured on a Hitachi U-2001 UV/Vis spectrometer.

Isolation of the bacterium *Streptomyces* species. The actinomycete species, isolate MFAac18, was isolated using YPG agar medium, composed of 0.5% yeast extract, 0.5% peptone, 1.0% glucose, 1.6% agar, 40% deionized water, and 60% seawater, containing penicillin and streptomycin (each 250 μg/mL), from the sea plant *Zostera marina* collected at the Bijin Island, Tongnyeong, Gyeongnam Province, Korea in 2000. The culture showed ash gray vegetative mycelia and demonstrated fatty acid composition (Korean Culture Center of Microorganisms, Seoul, Korea) and growth characteristics typical of actinomycete belong to the *Streptomyces* and related genera.

Biotransformatin of 1. A two-stage fermentation protocol[5] was used for preparative scale formation of the metabolite of **1**. The SWS medium contained soytone (0.1%), soluble starch (1.0%), and seawater (100%), and it was autoclaved at 121 °C for 15 min. Preparative incubation was conducted in 1 L of sterile medium held in 3 L culture flask that was incubated at 29 °C for 1 week (static). A 10% inoculum derived from one week old stage I culture was used to initiate stage II culture, which was incubated for 24 h more before receiving 20 mg of **1** in 0.75 mL of *N,N*-dimethyl formamide (DMF), and incubation was continued at 29 °C for five weeks (static). Substrate control consisted of sterile medium and substrate incubated under the same conditions but without microorganism. Also, culture control was composed of fermentation blanks in which the microorganism was grown under identical condition but without the addition of substrate. After five weeks of incubation, each control was harvested and analyzed by TLC. The culture was filtered through cheesecloth, and the filtrate was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered through sintered glass, and vacuum-concentrated to yield a crude extract (30 mg).

Isolation of the biotransformed metabolites (2 and 3). The crude extract (30 mg) was subjected to silica gel flash column chromatography using in sequence *n*-hexane-EtOAc (100:0-0:100) to give two fractions A and B, which contained substrate (**1**) and metabolites (**2**, **3**), respectively. Fractions A and B were separately purified by reversed-phase ODS-A gel (YMC Co.) flash column chromatography using MeOH, followed by HPLC (ODS-A, MeOH) to furnish the substrate (**1**) (9 mg) and the metabolites, **2** (5 mg) and **3** (3.5 mg), respectively.

6-*n*-(4-Oxopentyl)-α-pyrone (**2**) and 6-*n*-[(1*S*)-hydroxypentyl]-α-pyrone (**3**) were isolated as a colorless oil which showed spectral data virtually identical to those reported in the literatures, respectively.[6,7] The rotation recorded for **3** isolated in this study was $[\alpha]_D$ +15° (*c* 0.7, $CHCl_3$).

Preparation of MTPA esters (3a, 3b). To a CH_2Cl_2 solution (50 µL) of **3** (0.5 mg), 4-DMAP (50 µg), and DCC (50 µg) was added (*R*)-(−)-MTPACl (5 µL) and (*S*)-(+)-MTPACl (5 µL) at r.t., respectively. Each mixture was stirred for 24 hr and 3-[(dimethylamino)propyl]amine (3 µL) was added. After standing for 10 min, the solvent was evaporated. The residues were purified by preparative TLC (*n*-hexane-EtOAc, 2 : 1) to give (*S*)-MTPA ester (**3a**) (0.3 mg) and (*R*)-MTPA ester (**3b**) (0.3 mg), respectively.

(*S*)-MTPA ester (**3a**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ6.168 (1H, d, J = 9.4 Hz, H-3), 7.299 (1H, dd, J = 9.4, 6.4 Hz, H-4), 6.168 (1H, d, J = 6.4 Hz, H-5), 4.122 (1H, dd, J = 7.3, 5.1 Hz, H-1'), 1.685 (1H, m, H-2'), 1.417 (1H, m, H-2'), 1.313 (4H, m, H_2-3'/4'), 0.902 (3H, t, J = 7.3 Hz, H_3-5'), 3.492 (3H, s, OMe), 7.529 (3H, m, Ph), 7.706 (2H, m, Ph).

(*R*)-MTPA ester (**3b**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.168 (1H, d, J = 9.4 Hz, H-3), 7.292 (1H, dd, J = 9.4, 6.4 Hz, H-4), 6.168 (1H, d, J = 6.4 Hz, H-5), 4.126 (1H, dd, J = 7.3, 5.1 Hz, H-1'), 1.685 (1H, m, H-2'), 1.418 (1H, m, H-2'), 1.314 (4H, m, H_2-3'/4'), 0.902 (3H, t, J = 7.3 Hz, H_3-5'), 3.492 (3H, s, OMe), 7.529 (3H, m, Ph), 7.706 (2H, m, Ph).

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