Kurasoins A and B, New Protein Farnesyltransferase Inhibitors Produced by *Paecilomyces* sp. FO-3684

II. Structure Elucidation and Total Synthesis

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The structures of new protein farnesyltransferase inhibitors, kurasoins A and B, were elucidated by NMR study. Kurasoins A and B are acyloin compounds having in common a 3-hydroxy-1-phenyl-2-butanone moiety, to which *p*-hydroxyphenyl and 3-indolyl moieties respectively, are connected at C-4. The structures were confirmed by total synthesis.

In the course of screening for inhibitors of protein farnesyltransferase, we have found some new compounds, kurasoins A and B (**1** and **2**, Fig. 1), from the cultured broth of *Paecilomyces* sp. FO-3684[1]. In this paper, the structure elucidation and total synthesis of **1** and **2** are described.

Structure Elucidation of Kurasoin A (1)

Chemical shifts in the ^1H and ^{13}C NMR of **1** and **2** observed in methanol-d_4 are shown in Tables 1. The HMQC experiments revealed the connectivity of each proton and carbon.

HR-FAB-MS of **1** revealed its molecular formula, $C_{16}H_{16}O_3$[1]. Compound **1** showed two methylene, one oxymethine, nine aromatic methine, one carbonyl, and three quaternary carbon signals in the DEPT spectra. Nine protons at the low field of ^1H NMR in **1** were assigned as one monosubstituted and one *p*-disubstituted benzene by the ^1H-^1H COSY (Fig. 2). The ^1H-^1H COSY also indicated that the remaining protons were assigned to be $-CH_2-CH-O-$ and isolated CH_2. The result of the HMBC experiment are shown in Fig. 2. The long-range couplings of 4-H_2 (δ 2.77, 2.97)/C-1″ (δ 129.3), 4-H_2/C-2″ (6″) (δ 131.6), and 2″ (6″)-H (δ 7.04)/C-4 (δ 40.2) revealed that C-4 connected to C-1″. The couplings of 3-H (δ 4.35)/C-2 (δ 212.3) and 4-H_2/C-2 indicated the arrangement of C-2—C-3—C-4. Also the couplings of 2′(6′)-H (δ 7.10)/C-1 (δ 46.6), 1-H_2 (δ 3.63, 3.71)/C-1′ (δ 135.5), and 1-H_2/C-2′(6′) (δ 130.9) revealed that C-1 was connected to C-1′ of monosubstituted benzene. Three oxygens were suggested to be connected to C-2, C-3 (δ 78.8), and C-4″ (δ 157.2) on the basis of their chemical shifts. The remaining atoms were two hydrogens, which were suggested to be active hydrogens as they were not observed in methanol-d_4. Therefore they were assumed to be hydroxyl protons of 3-OH and 4″-OH and remaining C-1 and C-2 were considered to be connected. This connectivity was supported by the long-range coupling between 1-H_2 and C-2. Finally the structure of **1** was elucidated as 3-hydroxy-4-(*p*-hydroxyphenyl)-1-phenyl-2-butanone by its total synthesis.

The ^1H and ^{13}C NMR signal intensities of C-1 gradually declined in methanol-d_4 solution. It may be due to the enolization of the ketone of C-2, enabling deuterium exchanges of methylene protons of 1-H_2.

Structure Elucidation of Kurasoins B (2)

The molecular formula of **2** was elucidated by HR-FAB-MS as $C_{18}H_{17}NO_2$. The ^1H and ^{13}C NMR spectra (Table 1) of **1** and **2** resembled in their high field

Fig. 1. Structures of kurasoins A and B (**1** and **2**).

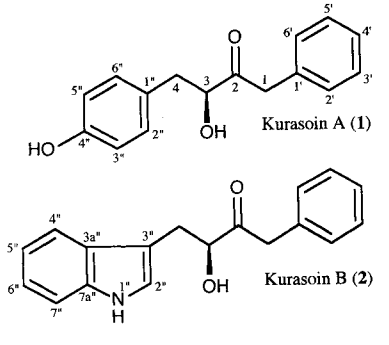
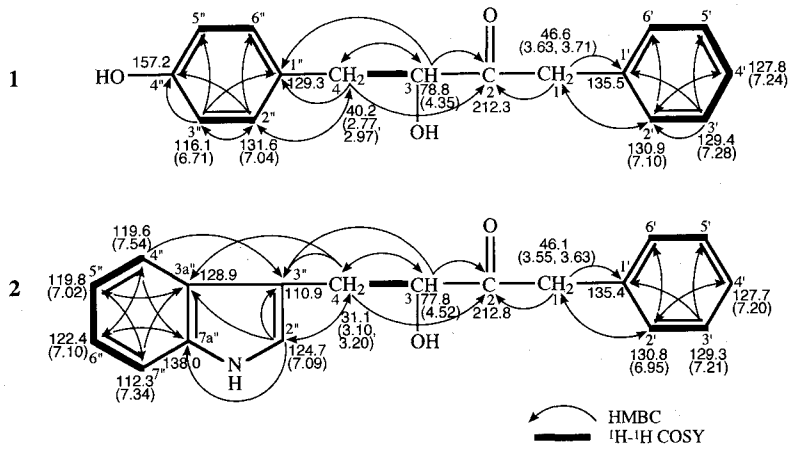


Table 1. The ^1H and ^{13}C NMR data of **1** and **2**.

Position	**1** ^{13}C	**1** ^1H	**2** ^{13}C	**2** ^1H
1	46.6 t	3.63 d (1H, J=16.7 Hz), 3.71 d (1H, J=16.7 Hz)	46.1 t	3.55 d (1H, J=16.8 Hz), 3.63 d (1H, J=16.8 Hz)
2	212.3 s		212.8 s	
3	78.8 d	4.35 dd (1H, J=4.9, 7.6 Hz)	77.8 d	4.52 dd (1H, J=5.7, 6.8 Hz)
4	40.2 t	2.77 dd (1H, J=7.6, 14.0 Hz), 2.97 dd (1H, J=4.9, 14.0 Hz)	31.1 t	3.10 dd (1H, J=5.7, 14.6 Hz), 3.20 dd (1H, J=6.8, 14.6 Hz)
1'	135.5 s		135.4 s	
2', 6'	130.9 d	7.10 d (2H, J=6.5 Hz)	130.8 d	6.95 d (2H, J=7.8 Hz)
3', 5'	129.4 d	7.28 dd (2H, J=6.5, 7.5 Hz)	129.3 d	7.21 m (2H)
4'	127.8 d	7.24 d (1H, J=7.5 Hz)	127.7 d	7.20 m (1H)
1"	129.3 s			
2" (6")	131.6 d	7.04 d (2H, J=8.5 Hz)	124.7 d	7.09 s (1H)
3" (5")	116.1 d	6.71 d (2H, J=8.5 Hz)	110.9 s	
3a"			128.9 s	
4"	157.2 s		119.6 d	7.54 d (1H, J=7.7 Hz)
5"			119.8 d	7.02 dd (1H, J=7.6, 7.7 Hz)
6"			122.4 d	7.10 dd (1H, J=7.6, 7.7 Hz)
7"			112.3 d	7.34 d (1H, J=7.7 Hz)
7a"			138.0 s	

The MeOH-d_4 signals (3.31 ppm of ^1H and 49.0 ppm of ^{13}C) were used as references. The coupling constants (Hz) are in parentheses.

Fig. 2. Structure of **1** and **2** elucidated by NMR analysis.



regions. Common structure of kurasoins, 3-hydroxy-1-phenyl-2-butanone, and 3-substituted indole was revealed ^1H-^1H COSY and HMBC (Fig. 2). The long-range couplings of 3-H (δ 4.52)/C-3" (δ 110.9), 4-H_2 (δ 3.10, 3.20)/C-2" (δ 124.7), 4-H_2/C-3", 4-H_2/C-3a" (δ 128.9), and 2"-H (δ 7.09)/C-4 (δ 31.1) indicated the connection of C-4 and C-3". Thus the structure of **2** was elucidated as 3-hydroxy-4-(3-indolyl)-1-phenyl-2-butanone and it was confirmed by total synthesis described below.

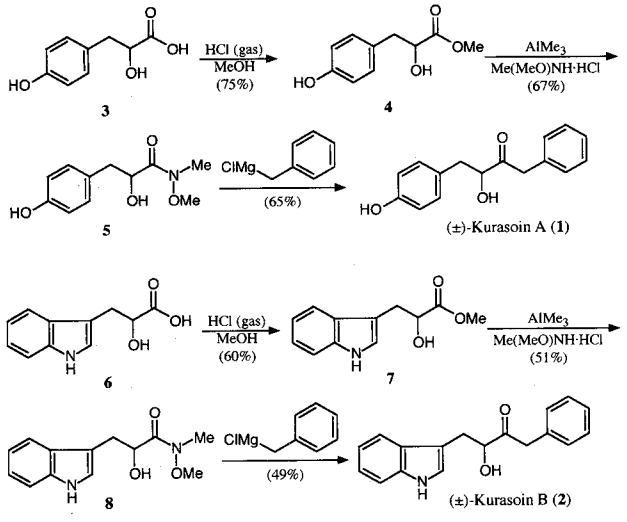
Both **1** and **2** are acyloin compounds and have a 3-hydroxy-1-phenyl-2-butanone moiety in common. The absolute configurations of **1** and **2** were shown to be both *S* by the asymmetric synthesis of (+)-**1** and (+)-**2**. The asymmetric synthesis of kurasoins will be reported elsewhere.

Total Synthesis of Kurasoins

The deduced structures of **1** and **2** were confirmed by synthesis. The synthesis of **1** and **2** were accomplished as outlined in Scheme 1.

(±)-*p*-Hydroxyphenyllactic acid (**3**) as a starting material was converted to the methyl ester (**4**) in the treatment with hydrogen chloride gas in methanol in 75% yield. Treatment of **4** with the aluminum amide reagent derived from *N*,*O*-dimethylhydroxylamine hydrochloride and $AlMe_3$, according to the procedure of WEINREB[2], gave effectively the desired transamination

Scheme 1. Chemical synthesis of (±)-**1** and (±)-**2**.



of **4** to the *N*-methoxy-*N*-methylamide (**5**) in 67% yield. Compound **5** was treated with benzylmagnesium chloride to obtain the (±)-kurasoin A (**1**) in 65% yield.

(±)-Indollactic acid (**6**) was treated in a similar manner to that described for the preparation of (±)-**1** to obtain (±)-kurasoin B (**2**) in 15% overall yield. Synthetic (±)-**1** and (±)-**2** were identical in ^{1}H and ^{13}C NMR, IR, and mass spectra with natural **1** and **2**.

The first total synthesis of (±)-**1** and (±)-**2** confirmed the deduced structures.

Experimental

NMR spectra were obtained with JEOL JNM-EX270 and Valian Unity 400 spectrometers. Mass spectrometry was conducted on a JEOL JMS-AX505 HA spectrometer. IR spectra were recorded on a Horiba FT-210 Fourier transform infrared spectrometer.

Total Synthesis of Kurasoin A

(±)-*p*-Hydroxyphenyllactic Acid Methyl Ester (**4**)

A solution of (±)-*p*-hydroxyphenyllactic acid (**3**, 100 mg, 0.05 mmol) in MeOH (3 ml) was treated with a rapid stream of dry HCl gas until the solution boiled. The solution, saturated with HCl, was then cooled to room temperature and stirred for 1 hour.

The resulting mixture was evaporated to give a yellow solid, which was purified by preparative silica gel chromatography ($CHCl_3$-MeOH, 10:1) to obtain (±)-*p*-hydroxyphenyllactic acid methyl ester (**4**, 80.2 mg, 75%). EI-MS *m/z* 196 (M^+). HR-EI-MS calcd for $C_{10}H_{12}O_4$, 196.0735; found, 196.0729. IR (KBr) cm^{-1} 1740, 2950, 3400. ^{1}H NMR ($CDCl_3$) δ 2.83 (1H, dd, J=6.6, 14.0 Hz), 2.99 (1H, dd, J=4.3, 14.0 Hz), 3.71 (3H, s), 4.37 (1H, m), 6.64 (2H, d, J=8.5 Hz), 6.97 (2H, d, J=8.5 Hz). ^{13}C NMR ($CDCl_3$) δ 41.5, 54.4, 73.3, 117.3, 129.8, 132.5, 156.6, 176.6.

(±)-2-Hydroxy-3-(*p*-hydroxyphenyl)-*N*-methoxy-*N*-methylpropanamide (**5**)

To a suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (196 mg, 2.01 mmol) in CH_2Cl_2 (3.1 ml) was added dropwise 2.0 M trimethylaluminum in hexane (1.0 ml, 2.01 mmol) with concomitant evolution of gas. The resulting homogeneous solution was stirred for 15 minutes at room temperature.

A solution of **4** (55 mg, 0.34 mmol) in CH_2Cl_2 (2 ml) was added dropwise to the aluminum amide solution at room temperature. The resulting solution was stirred at room temperature for 4 hours. The reaction mixture was quenched by 0.5 N HCl solution (5 ml), and the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2×10 ml). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford a yellow oil, which was purified by preparative silica gel chromatography ($CHCl_3$-MeOH, 10:1) to obtain (±)-2-hydroxy-3-(*p*-hydroxyphenyl)-*N*-methoxy-*N*-methylpropanamide (**5**, 43 mg, 67%). EI-MS *m/z* 225 (M^+). HR-EI-MS calcd for $C_{11}H_{15}NO_4$, 225.1000; found, 225.1006. IR (KBr) cm^{-1} 1650, 2940, 3340. ^{1}H NMR ($CDCl_3$) δ 2.74 (1H, dd, J=6.9, 13.9 Hz), 2.93 (1H, dd, J=3.6, 13.9 Hz), 3.17 (3H, s), 3.66 (3H, s), 4.56 (1H, m), 6.59 (2H, d, J=8.6 Hz), 6.96 (2H, d, J=8.6 Hz). ^{13}C NMR ($CDCl_3$) δ 32.5, 39.9, 61.4, 69.8, 115.3, 128.4, 130.5, 154.8, 174.1.

(±)-3-Hydroxy-4-(*p*-hydroxyphenyl)-1-phenyl-2-butanone (**1**)

To a solution of **5** (17.9 mg, 0.08 mmol) in THF (10.8 ml) at 0°C was added dropwise 2.0 M benzylmagnesium chloride in THF (200 µl, 0.40 mmol). After 4.5 hours, the reaction mixture was quenched by 5% HCl-MeOH (2 ml) and was warmed to room temperature. The mixture was poured into 10 ml each of CH_2Cl_2 and H_2O, and the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2×10 ml). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford a yellow oil, which was purified by preparative silica gel chromatography (hexane-EtOAc, 1:1) to obtain (±)-3-hydroxy-4-(p-hydroxyphenyl)-1-phenyl-2-butanone (**1**, 13.2 mg, 65%). EI-MS *m/z* 256 (M^+). HR-EI-MS calcd for $C_{16}H_{16}O_3$, 256.1099; found, 256.1072. IR (KBr) cm^{-1} 1710, 2920.

Total Synthesis of Kurasoin B

(±)-Indollactic Acid Methyl Ester (**7**)

A solution of (±)-indollactic acid (**6**, 200 mg, 0.98 mmol) in MeOH (6 ml) was treated with a rapid stream of dry HCl gas until the solution boiled for 30 minutes. The solution, saturated with HCl, was then cooled to room temperature and evaporated to give a yellow oil, which was purified by flash chromatography (hexane-

EtOAc, 3 : 1) to obtain (±)-indollactic acid methyl ester (**7**, 128.1 mg, 60%). EI-MS *m/z* 219 (M^+). HR-EI-MS calcd for $C_{12}H_{13}NO_3$, 219.0895; found, 219.0882. IR (KBr) cm^{-1} 1730, 3360, 3400. ^1H NMR ($CDCl_3$) δ 3.11 (1H, dd, J=6.1, 14.7 Hz), 3.23 (1H, dd, J=4.9, 14.7 Hz), 3.64 (3H, s), 4.45 (1H, q), 7.03~7.19 (4H, m), 7.27 (1H, d, J=7.9 Hz), 7.54 (1H, d, J=7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 30.2, 52.4, 70.8, 110.0, 111.1, 118.8, 119.5, 122.1, 123.1, 127.5, 136.1, 174.8.

(±)-2-Hydroxy-3-(3-indolyl)-*N*-methoxy-*N*-methylpropanamide (**8**)

Compound **7** (102.3 mg, 0.47 mmol) was treated with *N*,*O*-dimethylhydroxylamine hydrochloride and trimethylaluminum in a similar manner to that described for the preparation of **5** to obtain (±)-2-hydroxy-3-(3-indolyl)-*N*-methoxy-*N*-methylpropanamide (**8**, 59.4 mg, 51%). EI-MS *m/z* 248 (M^+). HR-EI-MS calcd for $C_{13}H_{16}N_2O_3$, 248.1161; found, 248.1152. IR (KBr) cm^{-1} 1650, 3350, 3400. ^1H NMR ($CDCl_3$) δ 2.99 (1H, dd, J=7.1, 14.9 Hz), 3.14 (3H, s), 3.31 (1H, dd, J=8.3, 14.9 Hz), 3.67 (3H, s), 4.61~4.62 (1H, m), 7.03~7.19 (4H, m), 7.28 (1H, d, J=6.6 Hz), 7.53 (1H, d, J=7.6 Hz). ^{13}C NMR ($CDCl_3$) δ 30.5, 32.5, 53.4, 61.4, 69.0, 111.1, 118.5, 119.3, 121.9, 123.1, 127.7, 136.0, 174.2.

(±)-3-Hydroxy-4-(3-indolyl)-1-phenyl-2-butanone (**2**)

Compound **8** (51.8 mg, 0.21 mmol) was treated with benzylmagnesium chloride in a similar manner to that described for the preparation of **1** to obtain the (±)-3-hydroxy-4-(3-indolyl)-1-phenyl-2-butanone (**2**, 28.6 mg, 49%). EI-MS *m/z* 279 (M^+). HR-EI-MS calcd for $C_{18}H_{17}NO_2$, 279.1259; found, 279.1271. IR (KBr) cm^{-1} 1710, 2920.

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