UK-2A, B, C and D, Novel Antifungal Antibiotics from *Streptomyces* sp. 517-02

II. Structural Elucidation

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(Received for publication January 26, 1996)

UK-2A, B, C and D, novel antibiotics produced by *Streptomyces* sp. 517-02, exhibit strong antifungal activity. The structures were elucidated based on spectral and chemical evidence that these compounds are the derivatives of the nine-membered dilactone formed from serine and 4-hydroxypentanoic acid moiety.

Recently we have reported that a new dibenzoxazole antibiotics (UK-1) isolated from *Streptomyces* sp. 517-02, exhibited the potent cytotoxicities against B16, HeLa and P388 cells[1,2]. In the continuation of a screening program in order to discover other useful bioactive metabolites from the same microbial sources, four novel substituted nine-membered dilactone were obtained[3]. In the present paper, we report the purification and structure elucidation of UK-2A, B, C and D.

Results and Discussion

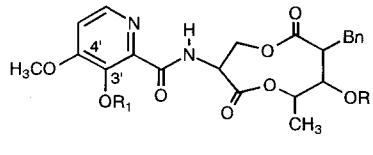
The mycelial cake was extracted with acetone and filtered. The filtrate was concentrated to give an aqueous solution, which was further extracted with chloroform. The organic layer on concentration yielded an oily material, which on purification with silica gel column gave crude UK-2. Recrystallization of this fraction from MeOH afforded colorless crystal of UK-2.

The NMR spectrum of UK-2 indicated that this compound was a mixture by observations of 4 sets of signals based on ester substituents. The purification with reverse phase HPLC gave UK-2A as the major component, UK-2B as a trace component and inseparable mixtures of UK-2C and D as minor components.

UK-2A was obtained as colorless needles, mp 207~209°C, $[\alpha]_D^{23}$ +89.11° (*c* 0.8, $CHCl_3$), whose molecular formula was established to be $C_{26}H_{30}N_2O_9$ (M^+, 514.1943, Δ −0.8 mmu) by HREI-MS and $C_{26}H_{31}N_2O_9$ ($M+H^+$, 515.2032 Δ +0.2 mmu) by HRFAB-MS. The IR spectrum of UK-2A showed the presence of an amide group at 3370, 1655 cm^{-1}, ester and lactones at 1760 cm^{-1} and aromatic groups at 1580 cm^{-1}. In the NMR spectra of UK-2A measured at 40, 27, −20 and −40°C in $CDCl_3$, and 55, 40 and 27°C in C_6D_6, both of ^1H and ^{13}C signals on the nine-membered ring were observed as broad signals that suggested the lability of the conformation in the nine-membered dilactone skeleton of UK-2A. Especially, the signals assigned to hydroxymethyl group in serine moiety, remarkably broadened. Fortunately, in the measurement at 0°C in $CDCl_3$, the NMR spectra of UK-2A were observed as relatively sharp signals. Complete assignment of ^1H and ^{13}C NMR signals could be characterized by 2D NMR experiments for UK-2A shown in Tables 1, 2 and Fig. 2. The ^{13}C NMR spectrum implied that 12 degrees of unsaturation could be attributed to two aromatic rings, three ester carbonyl groups and one amide group, and the eleven sp^3 carbons were identified as 4 methyl carbons, two methylene units (one bearing an oxygen function and one bearing a phenyl moiety) and five methine units (one bearing a nitrogen atom, two bearing oxygen functions, and two simply aliphatic). The absence of any other sp^2 carbons suggested that the remaining one degree of unsaturation was due to one additional ring system.

One of the two aromatic rings was identified as monosubstituted phenyl group based on the pattern arising from carbon signals at δ 137.72 (C-1″), 128.76 (C-2″ and

Fig. 1.



UK-2A : R = isobutyryl, R_1 = H
UK-2B : R = tigloyl, R_1 = H
UK-2C : R = isovaleryl, R_1 = H
UK-2D : R = 2-methylbutyryl, R_1 = H
Me-UK-2A: R = isobutyryl, R_1 = CH_3

Table 1. ^{1}H NMR spectral data for UK-2A and Me-UK-2A (in δ, $CDCl_3$, at 40°C).

H	UK-2A	UK-2A[b]	UK-2A[c]	Me-UK-2A
2	2.97 (td, 9.5)[a]	2.92 (td, 10.0)[a]	2.90 (td, 9.9, 2.9)	2.94 (td, 9.5, 5.2)[a]
3	5.20 (dd, 9.5, 9.5)	5.21 (dd, 10.0, 10.0)	5.35 (dd, 9.9, 9.9)	5.18 (dd, 9.5, 9.5)
4	4.99 (dq, 9.5, 6.2)	4.95 (dq, 10.0, 6.4)	4.97 (dq, 9.9, 6.2)	4.99 (dq, 9.5, 6.4)
7	5.15 (br q, 5.5)	5.17 (ddd, 8.8, 8.8, 6.4)	5.03 (br)	5.15 (br)
8	5.33 (br)	5.31 (dd, 8.8, 10.7)	5.14 (br)	5.40 (br)
	3.68 (br)	3.63 (dd, 6.4, 10.7)	3.12 (br)	3.60 (br)
4-CH_3	1.32 (d, 6.2)	1.30 (d, 6.4)	1.10 (d, 6.2)	1.31 (d, 6.4)
$(CH_3)_2$–CH–	2.60 (septet, 7.0)	2.62 (septet, 7.1)	2.25 (septet, 7.3)	2.60 (septet, 7.3)
$(CH_3)_2$–CH–	1.23 (d, 7.0)	1.21 (d, 7.1)	0.97 (d, 7.3)	1.23 (d, 7.3)
–CONH–	8.73 (d, 6.2)	8.56 (d, 8.8)	8.72 (d, 7.7)	8.43 (d, 6.2)
5′	6.87 (d, 4.8)	6.87 (d, 5.2)	6.08 (d, 5.2)	6.90 (d, 5.2)
6′	7.99 (d, 4.8)	7.98 (d, 5.2)	7.67 (d, 5.2)	8.23 (d, 5.2)
3′-OH	11.90 (s)	11.78 (s)	12.34 (s)	
3′-OCH_3				3.934 (s)
4′-OCH_3	3.95 (s)	3.92 (s)	3.14 (s)	3.928 (s)
2″/6″	7.12 (d, 7.3)	7.15 (d, 7.3)	6.99 (d, 7.3)	7.12 (d, 7.3)
3″/5″	7.26 (t, 7.3)	7.29 (t, 7.3)	7.01 (t, 7.3)	7.26 (t, 7.3)
4″	7.19 (t, 7.3)	7.22 (t, 7.3)	7.02 (t, 7.3)	7.18 (t, 7.3)
Ph-CH_2–	2.96 (dd, 11.7, 9.5)[a]	2.95 (br d, 12.2)[a]	3.15 (dd, 13.4, 9.9)	2.99 (dd, 12.7, 9.5)[a]
	2.72 (br d, 11.7)	2.68 (br d, 10.0)	2.73 (dd, 2.9, 13.4)	2.70 (br d, 12.7)

[a] Overlapped signals.
[b] at 0°C.
[c] in C_6D_6.

C-6″), 128.61 (C-3″ and C-5″), 126.72 ppm (C-4″) in $CDCl_3$ at 0°C. The ^{1}H-^{1}H COSY experiment clearly showed the coupling correlation from benzyl methylene group bearing C-2 to methyl group bearing C-4, which suggested the existence of fragment E2 in UK-2A (Fig. 2). These data and ^{1}H-^{13}C correlation *via* long range coupling measured in C_6D_6 between the aromatic carbon signal at δ 138.52 ppm (C-1″) and benzyl proton resonance at δ 2.73 ppm and δ 129.20 ppm (C-2″) and at δ 3.15 ppm were attributed to partial structure E.

Partial structures F1 and F2 also were indicated by coupling correlation in the ^{1}H-^{13}C COSY, COLOC, HMQC and HMBC experiments in $CDCl_3$ at 40 and 0°C and in C_6D_6 at 40°C. The existence of a hydrogen-bonded phenolic hydroxyl group was indicated by the proton resonance at δ 11.78 ppm (s) and confirmed by the methylation of UK-2 with diazomethane to give the monomethyl derivative, Me-UK-2A, which gave the molecular ion at *m/z* 528.2081 ($C_{27}H_{32}N_2O_9$, Δ −2.7 mmu) in HREI-MS. In the acetylation of UK-2 with pyridine-acetic anhydride at room temperature for one day, UK-2 was recovered. Based on these results and comparison of the NMR spectral data for Me-UK-2A with known 3,4-dimethoxy-2-formylpyridine[4], the partial structures F1 and F2 were connected to the partial structure F.

The presence of isobutyryl group G in UK-2A, was shown by the proton resonance at δ 1.21 (d, 6H) and 2.62 ppm (septet, 1H), and the carbon signals at δ 18.99 (2C, q), 34.44 (d) and 175.06 ppm (s) in $CDCl_3$ at 0°C.

In view of the above evidence, we were led to the hypothesis that UK-2A was identified as an eight- or nine-membered dilactone system. Selective chemical degradation experiments were required to determine how these partial structures were connected.

Methanolysis of UK-2 with excess HCl-MeOH gave (3-hydroxy-4-methoxypicolinyl)-serine methyl ester (**1**) and 2-benzyl-3-hydroxy-4-methyl-4-butanolide (**2**). Mild alkaline hydrolysis of UK-2 with 5 % NaOH solution afforded (3-hydroxy-4-methoxypicolinyl)-serine (**3**), 2-benzyl-4-methyl-4-but-2-enolide (**4**) and 2-benzyl-3-isobutyryloxy-4-methyl-4-butanolide (**5**). From these results (Specially, the formation of compound (**5**) by alkaline hydrolysis), the structure of UK-2A was elucidated as the isobutyryl ester of a nine-membered dilactone derivative (Fig. 3).

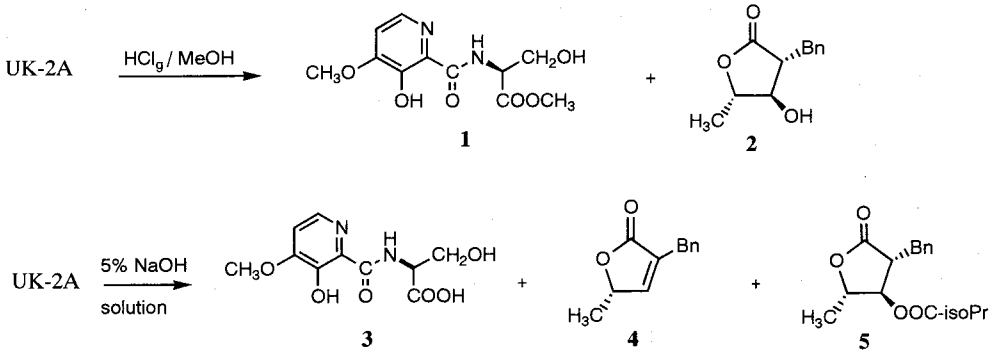
UK-2B was obtained as a minor component, $[\alpha]_D^{17}$ +87.5° (*c* 0.3, $CHCl_3$), and its molecular formula was determined to be $C_{27}H_{30}N_2O_9$ by HRFAB-MS ($M+H^+$, *m/z* 527.2030, Δ 0.0 mmu). The NMR spectral data for UK-2B (Tables 3 and 4) resembled those of UK-2A, except for the presence of tigloyl proton resonance at δ 1.82 (3H, dq, J=7.0, 1.1 Hz), 1.85 (3H, qd, J=1.1, 1.1 Hz) and 6.92 ppm (1H, qq, J=7.0, 1.1 Hz), carbon signals at δ 12.14 (q), 14.52 (q), 127.94 (s), 139.19 (d) and 166.60 ppm (s) and the absence of an isobutyryl

Table 2. ^{13}C NMR spectral data for UK-2A, and Me-UK-2A (in $CDCl_3$, at 40°C).

C	UK-2A	UK-2A[a]	UK-2A[b]	Me-UK-2A
1	171.83 s	171.76 s	171.74 s	171.88 s
2	52.04 d	52.45 d	51.85 d	52.11 d
3	75.25 d	75.53 d	75.53 d	75.34 d
4	74.86 d	74.83 d	74.72 d	74.51 d
6	169.67 s	169.98 s	169.98 s	170.43 s
7	50.25 d	50.60 d	49.79 d	50.60 d
8	64.96 t	65.31 t	65.10 t	65.89 t
3-COO-	175.61 s	175.06 s	175.06 s	175.61 s
4-CH_3	17.88 q	17.76 q	17.86 q	17.88 q
$(CH_3)_2$-*CH*-	34.18 d	34.20 d	34.44 d	34.16 d
$(CH_3)_2$-CH-	18.97 q	18.93 q	18.99 q	18.97 q
		18.90 q		
-CONH-	168.72 s	169.48 s	169.48 s	163.62 s
2′	129.56 s	130.66 s	129.74 s	142.48 s
3′	149.29 s	150.07 s	148.67 s	147.00 s
4′	156.18 s	155.91 s	155.30 s	160.75 s
5′	109.74 d	110.05 d	109.66 d	109.86 d
6′	140.23 d	140.38 d	140.75 d	145.07 d
3′-OCH_3				56.09 q
4′-OCH_3	56.29 q	55.36 q	56.16 q	61.90 q
1″	137.98 s	138.52 s	137.72 s	138.07 s
2″/6″	128.79 d	129.20 d	128.76 d	128.79 d
3″/5″	128.64 d	128.82 d	128.61 d	128.61 d
4″	126.73 d	126.92 d	126.72 d	126.67 d
Ph-CH_2-	34.69 t	34.20 t	34.10 t	34.69 t

[a] in C_6D_6.
[b] at 0°C.

Scheme 1. Hydrolysis of UK-2A.

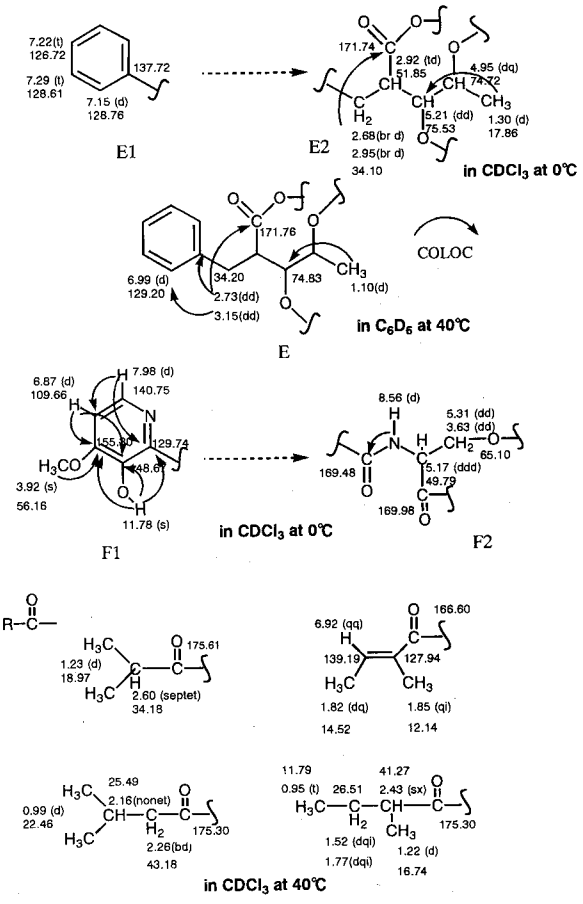


group. Based on these results, the structure of UK-2B was assigned as a tigloyl ester derivative of UK-2A (Fig. 1).

UK-2C and D were inseparable mixture in the HPLC purification and also, showed one spot in TLC. The molecular formula of both compounds were determined to be $C_{27}H_{32}N_2O_9$ based on the molecular ion in the HREI-MS (M^+, m/z 528.2114, Δ +0.6 mmu). The NMR spectral data showed the presence of isovaleryl and 2-methylbutyryl groups in a ratio of 12 : 100. It appeared that the isobutyryl ester in UK-2A replaced the isovaleryl and 2-methylbutyryl esters in UK-2C and D, respectively (Fig. 1).

Antimycin has been known as an antibiotic with a nine-membered dilactone skeleton[5], in which the absolute configuration was determined to be (2*R*,3*R*,4*S*,7*S*) by the optical selective synthesis of blastmycinolactol, 2-butyl-3-hydroxy-4-methyl-4-butanolide (**6**) and L-threonine obtained by alkaline hydrolysis of antimycin[5,6]. It was elucidated by the resemblance in the NMR spectra between compounds **2** and **6** that the configurations at C-2, 3 and 4 in UK-2A were (2*R*,3*R*,4*S*)

Fig. 2. Partial structures of UK-2A, B, C and D derived from COSY, HMQC and HMBC experiments.



or its antipode[7]. We could not obtain serine by chemical degradation and also could not determine the absolute configuration of compound **2** by modified MOSHER's method. The optical selective preparations of **1** and **5** for the determination of the absolute configuration of UK-2 is in progress.

Experimental

General Methods

All melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Spectral data were recorded on the following instruments: IR, Jasco IR-100 spectrometer in $CHCl_3$; MS and HR-MS, JEOL AX500 spectrophotometer and FAB-MS, in the positive ion mode using a glycerol matrix with the same instrument; NMR, Jeol-JNM-GX-400 and Varian Unity 500 spectrometer, ^1H NMR recording in $CDCl_3$ at 40,

Fig. 3. Partial structures of UK-2 and their connectivities.

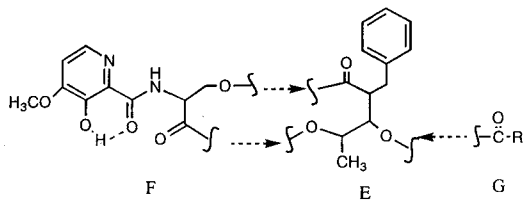


Table 3. ^1H NMR spectral data for UK-2B, UK-2C and UK-2D (in δ, $CDCl_3$, at 40°C).

H	UK-2B	UK-2C	UK-2D
2	3.01 (td, 9.5)	2.96 (td, 11.7)	2.96 (td, 11.7)
3	5.26 (dd, 9.5, 9.5)	5.22 (dd, 9.5, 9.5)	5.22 (dd, 9.5, 9.5)
4	5.01 (dq, 9.5, 6.2)	4.97 (dq, 9.5, 6.2)	4.97 (dq, 9.5, 6.2)
7	5.15 (br dt, 7.7,6.1)	5.14 (br d, 8.2)	5.14 (br d, 8.2)
8	5.33 (br)	5.32 (br)	5.32 (br)
	3.64 (br)	3.77 (br)	3.77 (br)
4-CH_3	1.31 (d, 6.2)	1.33 (d, 6.2)	1.33 (d, 6.2)
CH_3-CH=C-CH_3	1.82 (dq, 7.0, 1.1)		
CH_3-CH=C-CH_3	6.92 (qq, 7.0, 1.1)		
CH_3-CH=C-CH_3	1.85 (qd, 1.1, 1.1)		
$(CH_3)_2$-CH-CH_2-		0.99 (d, 6.9)	
$(CH_3)_2$-CH-CH_2-		2.16 (nonet, 6.9)	
$(CH_3)_2$-CH-CH_2		2.26 (b d, 7.4)	
CH_3-CH_2-CH-(CH_3)			2.43 (sextet, 7.0)
CH_3-CH_2-CH-(CH_3)			1.77 (dqi, 14.0, 7.0)
			1.52 (dqi, 14.0, 7.0)
CH_3-CH_2-CH-(CH_3)			1.22 (d, 7.0)
CH_3-CH_2-CH-(CH_3)			0.95 (t, 7.0)
-CONH-	8.58 (d, 7.7)	8.59 (d, 8.2)	8.59 (d, 8.2)
5′	6.85 (d, 5.1)	6.95 (d, 5.7)	6.95 (d, 5.7)
6′	7.97 (d, 5.1)	8.03 (b d, 5.7)	8.03 (b d, 5.7)
3′-OH	11.74 (s)	11.85 (s)	11.85 (s)
4′-OCH_3	3.92 (s)	3.99 (s)	3.99 (s)
2″/6″	7.10 (d, 7.0)	7.12 (d, 7.3)	7.12 (d, 7.3)
3″/5″	7.21 (t, 7.0)	7.25 (t, 7.3)	7.25 (t, 7.3)
4″	7.17 (t, 7.0)	7.20 (t, 7.3)	7.20 (t, 7.3)
Ph-CH_2-	2.98 (br t, 9.5)	2.92 (b d, 11.7)	2.92 (b d, 11.7)
	2.73 (br d, 9.5)	2.72 (b d, 11.7)	2.72 (b d, 11.7)

Table 4. ^{13}C NMR spectral data for UK-2B, UK-2C and UK-2D (in $CDCl_3$, at 40°C).

C	UK-2B	UK-2C	UK-2D
1	171.96 s	171.80 s	171.80 s
2	52.15 d	52.00 d	52.00 d
3	75.09 d	75.01 d	75.01 d
4	75.73 d	74.79 d	74.79 d
6	169.81 s	169.69 s	169.69 s
7	50.20 d	50.06 d	50.06 d
8	65.33 t	64.99 t	64.99 t
3-COO–	166.60 s	175.30 s	175.30 s
4-CH_3	17.91 q	17.92 q	17.92 q
CH_3–CH=*C*–CH_3	127.94 s		
CH_3–*CH*=C–CH_3	139.19 d		
CH_3–CH=C–CH_3	14.52 q		
CH_3–CH=C–*CH_3*	12.14 q		
$(CH_3)_2$–CH–CH_2–		22.46 q	
$(CH_3)_2$–*CH*–CH–		25.49 d	
$(CH_3)_2$–CH–*CH_2*		43.18 t	
CH_3–CH_2–*CH*–CH_3			41.27 d
CH_3–*CH_2*–CH–CH_3			26.51 t
CH_3–CH_2–CH–*CH_3*			16.74 q
CH_3–CH_2–CH–CH_3			11.79 q
–CONH–	169.10 s	169.69 s	169.69 s
2′	130.22 s	129.51 s	129.51 s
3′	149.06 s	149.06 s	149.06 s
4′	155.60 s	155.89 s	155.89 s
5′	109.86 d	109.64 d	109.64 d
6′	140.69 d	140.35 d	140.35 d
3′-OCH_3			
4′-OCH_3	56.14 q	56.24 q	56.24 q
1″	138.16 s	137.88 s	137.88 s
2″/6″	128.79 d	128.76 d	128.76 d
3″/5″	128.53 d	128.62 d	128.62 d
4″	126.63 d	126.76 d	126.76 d
Ph-*CH_2*–	34.81 t	34.70 t	34.70 t

27, 0, −20 and −40°C and in C_6D_6 at 55, 40 and 27°C with TMS as an internal standard at δ 0 ppm and in CD_3OD at 40°C with solvent signal at δ 3.30 ppm for CD_3OD and in ^{13}C NMR, using the solvent signals at δ 77.03, 128.00 and 49.00 ppm as internal references, respectively; and optical rotations, Jasco DIP-370 Digital Polarimeter. The TLC was carried out on Merck silica gel GF 254 precoated plates. Column chromatography was carried out on Fuji Silysia, silica gel BW 820. For HPLC, a Waters U6K with a Soma S310A UV detector was used; Columns; Wakosil 5C18-200.

Purification of UK-2

UK-2 was purified by reverse phase HPLC (double columns, 10×250 mm and 6×25 mm, UV detection at 254 nm, eluent 7:3 acetonitrile-water, flow rate 3 ml/minute) to give UK-2A, UK-2B and mixture of UK-2C and D.

UK-2A; Colorless needles: mp 207~209°C, $[\alpha]_D^{23}$ +89.11° (*c* 0.8, $CHCl_3$), IR ($CHCl_3$): 3370, 3020~2950, 1760, 1655, 1580, 1530, 1140, 720 cm^{-1}. HRFAB-MS: $C_{26}H_{31}N_2O_9$ (Found: 515.2032, Δ +0.2 mmu), FAB-MS: *m/z* 515 ($M+H^+$), 239, 211, 185. ^1H and ^{13}C NMR data: given in Tables 1 and 2, respectively.

UK-2B; Colorless needles: mp 86~90°C, $[\alpha]_D^{17}$ +87.5° (*c* 0.3, $CHCl_3$), IR ν_{max} ($CHCl_3$): 3370, 3030~2950, 1760, 1720, 1655, 1580, 1530, 1210, 1130, 710 cm^{-1}, HRFAB-MS: $C_{27}H_{31}N_2O_9$ (Found: 527.2030, Δ +0.0 mmu), FAB-MS: 527 ($M+H^+$), 431, 239, 186. ^1H and ^{13}C NMR data: given in Tables 3 and 4, respectively.

Mixture of UK-2C and D; Colorless needles: mp 175~178°C, $[\alpha]_D^{23}$ +80.65° (*c* 0.11, $CHCl_3$), IR ν_{max} ($CHCl_3$): 3370, 3030~2950, 1760, 1655, 1580, 1530, 1140, 720 cm^{-1}. HREI-MS: $C_{27}H_{32}N_2O_9$ (Found: 528.2114, Δ +0.6 mmu), EI-MS: *m/z* 528 (M^+), 427, 383, 239, 208, 188, 144 (base peak). ^1H and ^{13}C NMR data: given in Tables 3 and 4, respectively.

Methylation of UK-2

Ethereal diazomethane was added to a solution of UK-2 in CH_2Cl_2, and the mixture was stirred for 9 hours at room temperature. The solvent was evaporated and the residue was purified by CC silica gel (CH_2Cl_2-CH_3OH) to give Me-UK-2A in small quantities and mixtures of Me-UK-2C and D in trace amounts.

Me-UK-2A; Colorless amorphous: IR ν_{max} ($CHCl_3$): 3350, 1750, 1670, 1130 cm^{-1}. HREI-MS: $C_{27}H_{32}N_2O_9$ (Found: 528.2081, Δ −2.7 mmu), EI-MS: 528 (M^+), 397, 252, 222 (base peak), 203, 188, 143, 124, 91. ^1H and ^{13}C NMR data: given in Tables 1 and 2, respectively.

Me-UK-2C and D; Trace amounts: HREI-MS: $C_{28}H_{34}N_2O_9$ (Found: 542.2330, Δ −6.6 mmu)

Methanolysis of UK-2

HCl gas was bubbled through a solution of UK-2 in dry methanol for 30 minutes at 0°C and the reaction mixture was stirred for 2 hours at room temperature. The solvent was removed under reduced pressure, and the residue was purified by CC silica gel (CH_2Cl_2-CH_3OH) to give compounds **1** and **2**.

(**1**) (3-Hydroxy-4-methoxypicolinyl)-serine methyl ester; Colorless needles: mp 145~146°C, $[\alpha]_D^{27}$ −11.03° (*c* 0.6; $CHCl_3$), IR ν_{max} ($CHCl_3$): 3400, 1750, 1650, 1260 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.91 (s, OH), 6.86 (d, *J*=5.2 Hz, 5-H), 8.00 (d, *J*=5.2 Hz, 6-H), 3.94 (s, OCH_3), 8.75 (d, *J*=8.6 Hz, CONH), 4.83 (1H, br dt, *J*=8.6, 3.9 Hz), 3.83 (s, $COOCH_3$), 4.04 (1H, dd, *J*=11.3, 3.9 Hz), 4.13 ppm (1H, dd, *J*=11.3, 4.3 Hz). ^{13}C NMR: δ 130.48 (C-2, s), 149.00 (C-3, s), 155.60 (C-4, s), 109.74 (C-5, d), 140.69 (C-6, d), 169.42 (CONH, s), 52.85 (CH, d), 54.39 (COO*CH_3*, q), 170.30 (*C*OOCH$_3$), 63.22 ppm (CH_2OH, t). HREI-MS: $C_{11}H_{14}N_2O_6$ (Found: 270.0847, Δ −0.5 mmu), EI-MS: *m/z* 270 (M^+), 239, 211, 193, 152, 124.

(**2**) 2-Benzyl-3-hydroxy-4-methyl-4-butanolide; Colorless needles: mp 47~49°C, $[\alpha]_D^{25}$ −89.30° (*c* 0.13; $CHCl_3$), IR ν_{max} ($CHCl_3$): 3400, 1770, 1600, 1170, 1050 cm^{-1}. HREI-MS: $C_{12}H_{14}O_3$ (Found: 206.0918, Δ −2.5 mmu), EI-MS: *m/z* 206 (M^+), 188, 149 (base peak), 131, 91, 105. ^1H NMR (C_6D_6): δ 2.37 (ddd, *J*=8.2, 7.6, 5.5 Hz, 2-H), 3.26 (br t, *J*=8.2 Hz, 3-H), 3.67 (qd, *J*=6.4,

6.4 Hz, 4-H), 0.92 (d, J=6.4 Hz, 5-CH_3), 3.01 (dd, J=14.0, 5.5 Hz, 6-H), 2.65 (dd, J=14.0, 7.6 Hz, 6-H), 7.00~7.08 ppm (5H, m). ^{13}C NMR (C_6D_6): δ 174.84 (C-1, s), 50.24 (C-2, d), 77.44 (C-3, d), 79.63 (C-4, d), 17.76 (C-5, q), 33.80 (C-6, t), 138.35 (C-1′, s), 129.62 (C-2′/6′, d), 128.85 (C-3′/5′, d), 126.91 ppm (C-4′, d).

Alkaline Hydrolysis of UK-2A

UK-2A was treated with 5% NaOH for 10 minutes, and then the pH was adjusted to 2 with 5% HCl following extraction with ether. After drying and evaporation of the organic layer, purification with silica gel chromatography afforded compounds **4** and **5**. The water layer was taken to dryness under reduced pressure to obtain a residue. The residue was treated with ethanol to take up the soluble parts in ethanol. Ethanol-soluble part gave compound **3**.

(**3**) (3-Hydroxy-4-methoxypicolinyl)-serine; IR ν_{max} (Neat): 3400, 1740, 1680, 1600, 1535, 1440, 1310, 1240, 1050, 930, 820, 780 cm^{-1}. EI-MS: m/z 256 (M^+), 238, 225, 211, 194, 181, 166, 152, 124 (base peak), 109, 95, 80. ^{1}H NMR (CD_3OD): δ 8.43 (d, J=4.3 Hz), 7.20 (d, J=4.3 Hz), 4.81 (br s), 4.29 (s, $-OCH_3$), 4.08 (dd, J=11.0, 2.4 Hz), 4.00 ppm (dd, J=11.0, 3.1 Hz), ^{13}C NMR: δ 171.56 (–COOH, s), 164.62 (–CONH–, s), 128.62 (C-2, s), 147.77 (C-3, s), 159.62 (C-4, s), 111.21 (C-5, d), 138.09 (C-6, d), 62.36 ($-CH_2OH$, t), 53.28 (–CH–, d), 56.82 ppm ($-OCH_3$, q).

(**4**) 2-Benzyl-4-methyl-4-but-2-enolide; IR ν_{max} ($CHCl_3$): 1750, 1320, 1200, 1070, 1020 cm^{-1}. HREI-MS: $C_{12}H_{12}O_4$ (Found: 188.0820, Δ −1.7 mmu), EI-MS: m/z 188 (M^+), 143 (base peak), 128, 115, 91. ^{1}H NMR ($CDCl_3$): δ 6.80 (d, J=1.8 Hz, 3-H), 4.98 (qd, J=6.7, 1.8 Hz, 4-H), 1.38 (d, J=6.7 Hz, $-CH_3$), 3.58 (2H, br d, J=1.8 Hz, 6-H), 7.23 (d, J=7.3 Hz, 2′/6′-H), 7.26 (t, J=7.3 Hz, 3′/5′-H), 7.34 ppm (t, J=7.3 Hz, 4′-H), ^{13}C NMR: δ 173.32 (C-1, s), 134.16 (C-2, s), 150.24 (C-3, d), 77.63 (C-4, d), 19.07 (C-5, q), 31.73 (C-6, t), 137.45 (C-1′, s), 129.83 (C-2′/6′, d), 128.76 (C-3′/5′, d), 126.74 ppm (C-4′, d).

(**5**) 2-Benzyl-3-isobutyryloxy-4-methyl-4-butanolide; $[\alpha]_D^{25}$ −23.20° (c 0.6; $CHCl_3$), IR ν_{max} ($CHCl_3$): 3050~2900, 1780, 1740, 1500, 1460, 1395, 1360, 1320, 1180, 1160, 1080, 720, 660 cm^{-1}. HREI-MS: $C_{16}H_{20}O_4$ (Found 276.1337, Δ −2.5 mmu), GCEI-MS: m/z 276 (M^+), 188, 143 (base peak), 129, 91. ^{1}H NMR ($CDCl_3$): δ 2.99 (dd, J=13.4, 7.9 Hz), 3.25 (dd, J=13.3, 4.3 Hz), 3.05 (ddd, J=7.9, 6.1, 4.3 Hz, 2-H), 4.95 (dd, J=6.1, 4.9 Hz, 3-H), 4.29 (qd, J=6.7, 4.9 Hz, 4-H), 1.16 (d, J=6.7 Hz, 4-CH_3), 7.22 (d, J=7.3 Hz, 2′/6′-H), 7.27 (t, J=7.3 Hz, 3′/5′-H), 7.30 (t, J=7.3 Hz, 4′-H), 2.37 (septet, J=6.7 Hz, CH), 1.01 (d, J=6.7 Hz, CH_3), 1.06 ppm (d, J=6.7 Hz, CH_3), ^{13}C NMR ($CDCl_3$): δ 175.20 (C-1, s), 48.41 (C-2, d), 77.55 (C-3, d), 79.58 (C-4, d), 18.85† (4-CH_3), 34.70 (C-6, t), 137.18 (C-1′, s), 129.28 (C-2′/6′, d), 128.79 (C-3′/5′, d), 127.09 (C-4′, d), 176.25 (C-1″, s), 33.62 (C-2″, d), 18.56† (C-3″, q), 18.66† ppm (C-4″, q).

Acknowledgments

We wish to express our sincere gratitude to Professor TAKASHI TOKOROYAMA and HIDEO IIO, Faculty of Science, Osaka City University, for their generous support of this work, and the Ministry of Education, Science and Culture (MONBUSHO) for the scholarship support for M. HANAFI. Thanks are also due to Mr. S. SHIMADA for measurements of mass spectra.

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† Assignments interchangeable within the superscript.