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

III. Absolute Configuration of an Antifungal Antibiotic, UK-2A, and Consideration of Its Conformation

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Recently, the novel potent antifungal metabolites, UK-2A, B, C and D isolated from *Streptomyces* sp. 517-02, have been reported[1,2]. The structure of UK-2A contains a nine-membered dilactone skeleton constituted with *N*-(3-hydroxy-4-methoxy-picolinyl)serine and 2-benzyl-3-isobutyryloxy-4-hydroxy-pentanoic acid. Mild alkaline hydrolysis of UK-2A afforded 2-benzyl-3-isobutyryloxy-4-methyl-4-butanolide **1** and *N*-(3-hydroxy-4-methoxypicolinyl)serine **2**. Methanolysis of the UK-2 mixture gave compounds **3** and **4**. Consequently, the absolute configuration of UK-2A can be determined by elucidation of the absolute configurations of the compounds **1** and **2**. We tried asymmetric synthesis of compounds **1** and **2**.

Butanolide **1** is an analogue of blastmycinone **5** derived from alkaline hydrolysis of the nine-membered dilactone antibiotic, antimycin A_3. Preparation of the optically active butanolide **5** has been already reported[3]. So far, we have found the configuration at C-2, C-3 and C-4 of UK-2A and the key intermediate **3a** to be (2*R*, 3*R*, 4*S*) or its antipode (2*S*, 3*S*, 4*R*) based on the strong resemblance in the NMR spectra between **1** and **5**[2].

This time we attempted to prepare the diastereoisomers of butanolide **1** in which the configuration of C-4 was achieved using methyl (*S*)-(+)- and (*R*)-(−)- lactate as starting materials following an extended synthetic method of **5** by H. H. WASSERMAN *et al.*[3], as shown in Scheme 1. Both lactates were converted to (*S*)- and (*R*)-2-[(2-methoxy)methoxy]propanal by a known procedure[4]. 2-Phenylpropyl-4,5-diphenyloxazole[5] **6** prepared from a mixture of 3-phenylpropionic acid and

Fig. 1. Alkaline hydrolysis of UK-2A and methanolysis of UK-2 mixture.

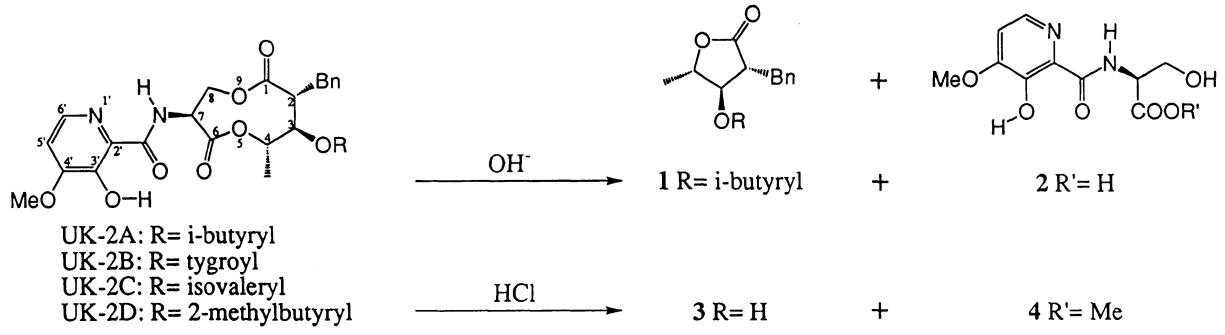
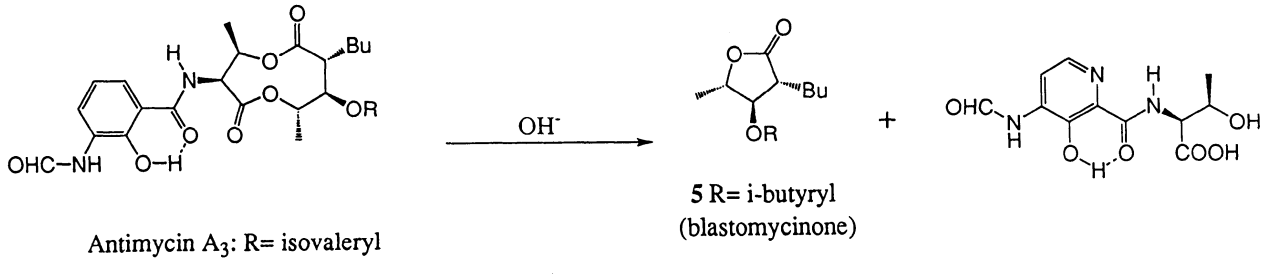
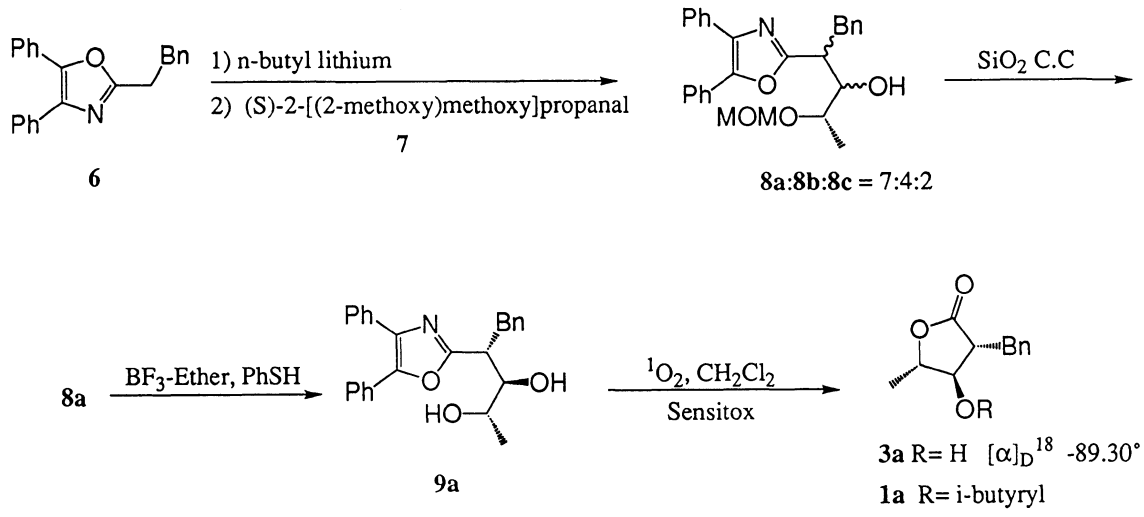


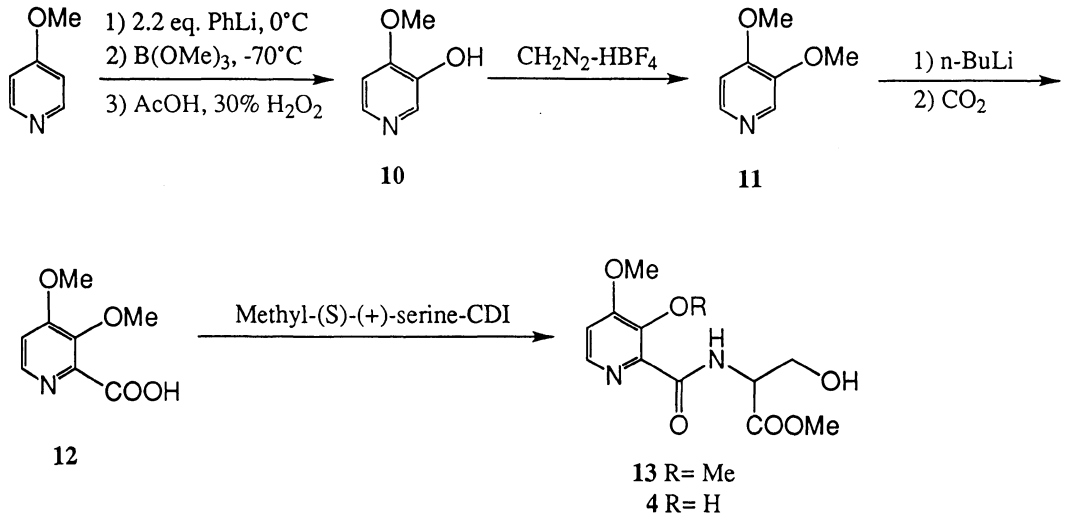
Fig. 2. Alkaline hydrolysis of antimycin A_3.



Scheme 1.



Scheme 2.



benzoin was treated with *n*-butyllithium in THF at −78°C, followed by the addition of (*S*)-2-[(2-methoxy)methoxy]propanal **7** to give a mixture of three diastereoisomers which had the (*S*)-configuration at C-4 (**8a**, **8b** and **8c** 7:4:2, 56%). The mixture (**8a**∼**c**) was separaed by a silica gel chromatography. The major hydroxyoxazole **8a** was deprotected with BF_3-Et_2O and thiophenol to afford the dihydroxyoxazole **9a** (81%). Photooxygenation of **9a** using Rose Bengal bis(triethylammonium salt) as a photosensitizer produced 2-benzyl-3-hydroxy-4-methyl-4-butanolide **3a**[2] (42%) of which $[\alpha]_D^{26}$ −88.42° (*c* 0.12, $CHCl_3$) agreed with $[\alpha]_D^{18}$ −89.30° (*c* 0.13, $CHCl_3$) of **3a** derived from UK-2A by acidic hydrolysis. By esterification of **3a** with isobutyryl chloride-DMAP, **1a** was obtained. ^1H and ^{13}C NMR spectra of the synthetic **3a** and **1a** were completely in accord with those of the derivatives from UK-2A. Consequently, the absolute configuration of **1a** was established as (2*R*, 3*R*, 4*S*).

Application of the same method to **8b** and **8c** afforded **3b** and **3c**, respectivelly. In addition, the diastereoisomers were obtained and had the (*R*)-configuration at C-4, **3d** ($[\alpha]_D^{24}$ +85.24° (*c* 0.4, $CHCl_3$)), **3e** and **3f** from (*R*)-2-[(2-methoxy)methoxy]propanal using the same procedure.

On the other hand, the configuration of C-7 position of UK-2A was elucidated using methyl *N*-(3-hydroxy-4-methoxypicolinyl)serine **4** prepared from (*S*)-(+)-serine.

Table 1. Physico-chemical data of compound **3a** and **4**.

	3a	**4**
Appearance	Colorless needles	Colorless cubic
Melting point	49~50°C	127~128°C
NMR	C_6D_6, 40°C	$CDCl_3$, 40°C
^{1}H NMR	δ=2.37 (1H, H-2, ddd, J=7.6, 7.3, 5.5)	δ=3.95 (3H, 4′-OCH_3, s)
	2.65 (1H, dd, J=14.4, 7.6)	4.05 (1H, CH_2, dd, J=4.0, 11.4)
	3.02 (1H, dd, J=14.4, 5.5)	4.12 (1H, CH_2, dd, J=7.7, 11.4)
	3.26 (1H, H-3, br t, J=7.3)	4.81 (1H, CH, ddd, J=3.7, 7.3, 7.7)
	3.67 (1H, H-4, dq, J=6.4, 6.4)	6.87 (1H, H-5′, d, J=5.1)
	6.97~7.09 (5H, m)	8.00 (1H, H-6′, d, J=5.1)
		8.79 (1H, CONH, d, J=7.3)
		11.91 (1H, 3′-OH, s)
^{13}C NMR	δ=34.00 (t)	δ=52.86 (d)
	50.31 (C-2, d)	54.41 ($COOCH_3$)
	77.87 (C-3, d)	56.14 (4′-OCH_3)
	79.52 (C-3, d)	63.22 (t)
	126.95 (C-4″, d)	109.68 (C-5′, d)
	128.91 (C-3″/C-5″, d)	130.39 (C-2′, s)
	129.53 (C-2″/C-6″, d)	140.61 (C-6′, d)
	138.33 (C-1″, s)	149.03 (C-3′, s)
	174.57 (C-1, s)	155.66 (C-4′, s)
		169.36 (CONH, s)
		170.28 (s)

3-Hydroxy-4-methoxypyridine **10** was prepared from 4-methoxypyridine *N*-oxide using the method of synthesis of orelline by F. TRECOURT *et al.* (Scheme 2)[6]. The carboxylation of 3,4-dimethoxypyridine **11** afforded 3,4-dimethoxypicolinic acid **12** which gave methyl-(3,4-dimethoxypicolinyl)serine **13** with serine methyl ester and CDI by the usual amide bond preparation method. The $[\alpha]_D^{28}$ +51.34° (*c* 0.14, $CHCl_3$) of methyl-(3,4-dimethoxypicolinyl)serine **13** prepared from (*S*)-(+)-serine was consistant with the $[\alpha]_D^{28}$ +55.52° (*c* 0.05, $CHCl_3$) of a product obtained by methanolysis of UK-2A. Regioselective demethylation of **13** with BBr_3 produced methyl-(3-hydroxy-4-methoxypicolinyl)serine **4**. The synthetic **4** with $[\alpha]_D^{28}$ +13.86° (*c* 0.25, $CHCl_3$) was identical in spectral data to a degradation product of UK-2A, $[\alpha]_D^{28}$ +14.79° (*c* 0.20, $CHCl_3$)[2]. The physico-chemical data of **3a** and **4** are summerized in Table 1.

Based on the above results, the absolute configuration of UK-2A was elucidated as (+)-(2*R*, 3*R*, 4*S*, 7*S*)-configuration.

The broadening of ^{1}H and ^{13}C NMR signals on the nine-membered dilactone skeleton (especially, serine moiety) was observed in the measurements in D_6-benzene and $CDCl_3$ at ambient temperature, 40°C and 50°C. In the measurement at 0, −20 and −40°C in $CDCl_3$, the sharp spectra were observed, indicating a temperature dependence of the conformation of the nine-membered dilactone skeleton in UK-2A. MM2 calculation gives the sole stable conformation and these calculated dihedral angles supported the coupling constants observed in ^{1}H NMR at −20°C in $CDCl_3$ shown in Table 2.

Table 2. Predicted and observed *J* coupling data for UK-2A.

	Dihedral angle	Predicted *J* coupling	Observed *J* coupling
H-2~H-3	179.0°	9.2 Hz	9.8 Hz
H-3~H-4	179.7°	9.2 Hz	9.8 Hz
H-7~H-8a	−157.2°	7.8 Hz	8.5 Hz
H-7~H-8b	−36.1°	5.3 Hz	6.1 Hz

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