# 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.

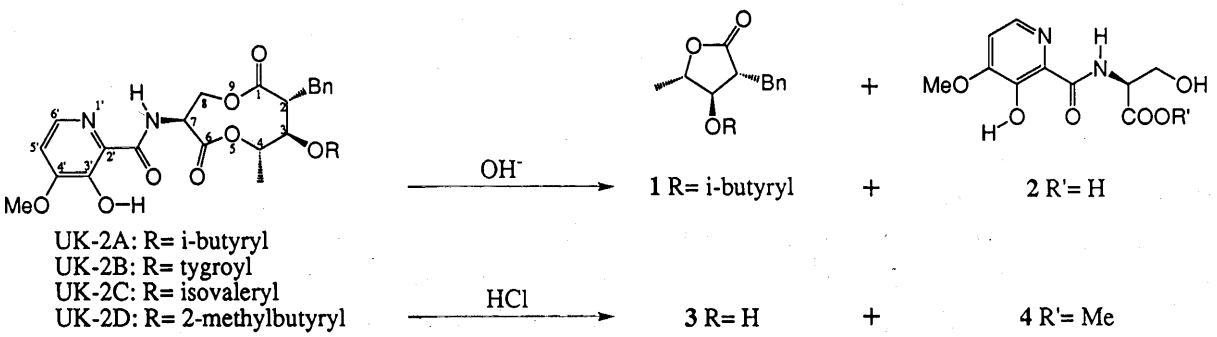
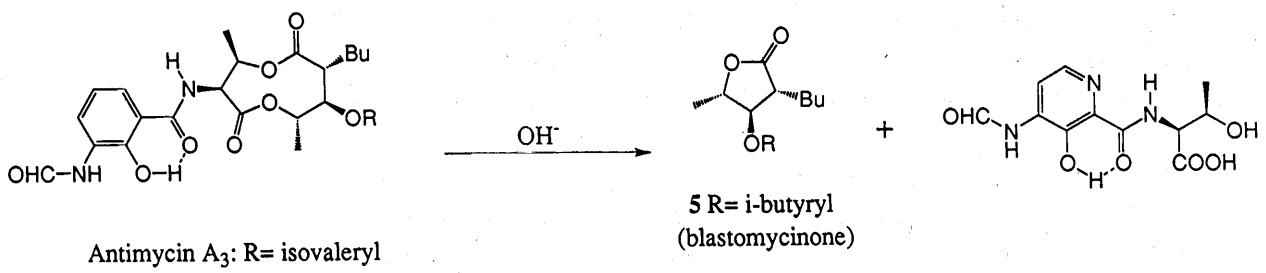
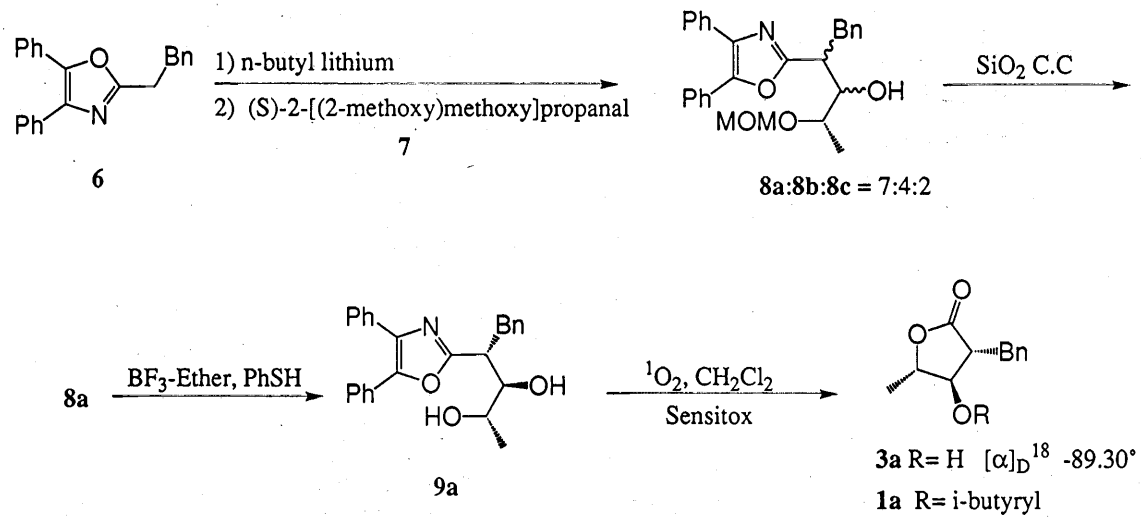


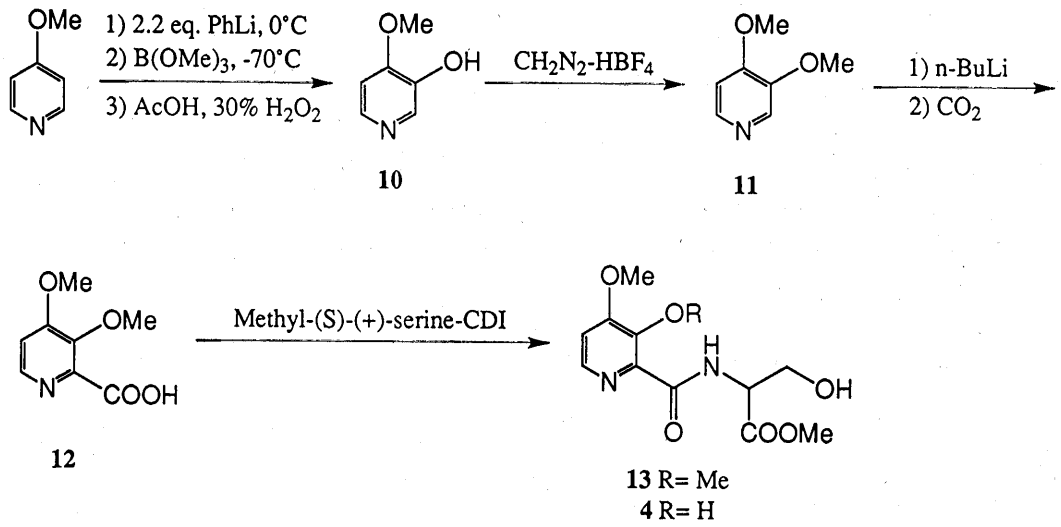

Fig. 2. Alkaline hydrolysis of antimycin $A_3$.



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 | $\delta$=2.37 (1H, H-2, ddd, $J$=7.6, 7.3, 5.5) | $\delta$=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, brt, $J$=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 | $\delta$=34.00 (t) | $\delta$=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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