# UK-69,753, A NOVEL MEMBER OF THE EFROTOMYCIN FAMILY OF ANTIBIOTICS

## II. STRUCTURE DETERMINATION AND BIOLOGICAL ACTIVITY

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A novel antibiotic, UK-69,753, has been isolated from a submerged fermentation of *Amycolatopsis orientalis* strain N731-15. UK-69,753 has been assigned the structure **1** using spectroscopic means, primarily by NMR analysis. UK-69,753 is a glycoside of factumycin (A40A), a previously reported member of a small group of antibiotics related to aurodox and efrotomycin. UK-69,753 was shown to have potent activity both *in vitro* and *in vivo* against the swine pathogen *Treponema hyodysenteriae*.

In the preceding paper[1], we have described the taxonomy, fermentation and isolation of UK-69,753 (**1**), a novel member of the efrotomycin[2] family of antibiotics. In this paper we describe the structure elucidation of UK-69,753 along with its antibacterial activity.

## Results and Discussion

### Structure Determination of UK-69,753

The structure of UK-69,753 (**1**) has been determined by spectroscopic means, relying heavily on the results of NMR analysis. $^{1}H$, $^{13}C$, carbon multiplicity (distortionless enhancement by polarization transfer: DEPT)[3] and 2D (homonuclear correlation: COSY[4] and heteronuclear correlation: HETCOR[5]) NMR spectra were recorded on UK-69,753 in $CD_3OD$, and the results of these experiments are summarized in Table 1. The $^{13}C$ NMR spectrum showed 57 distinct resonances with one line at 130.41 ppm accounting for two carbons, indicating that UK-69,753 contains 58 carbon atoms. Using the data available primarily from the two correlation experiments, eight distinct spin systems comprising 47 carbons were identified which correspond to the molecular fragments **2** through **9**. The olefinic proton resonances of fragments **2**, **7**, and **8** were unambiguously assigned and measurement of the vicinal coupling constants of these resonances in the $^{1}H$ NMR spectrum at 500 MHz (Fig. 1) allowed for assignment of the configuration of each disubstituted olefin in these fragments. The configuration of the two trisubstituted olefins (fragments **7** and **8**) were both assigned as drawn on the basis of nuclear Overhauser effect (NOE) experiments†. The carbonyl and quaternary olefinic carbons in fragments **7** and **8** were assigned *via* multiple bond heteronuclear correlation (MBHC) to nearby carbons bearing protons.

Consideration of the part structures **2**~**9** assembled from an analysis of NMR data lead to the hypothesis that UK-69,753 is a novel member of a small group of antibiotics characterized by compounds

† The nuclear Overhauser polarizations, observed at 500 MHz, were consistently negative.

Table 1. A summary of the $^{1}H$, $^{13}C$, DEPT, homonuclear (COSY) and heteronuclear (HETCOR) correlation data for UK-69,753 (**1**) ($CD_3OD$).

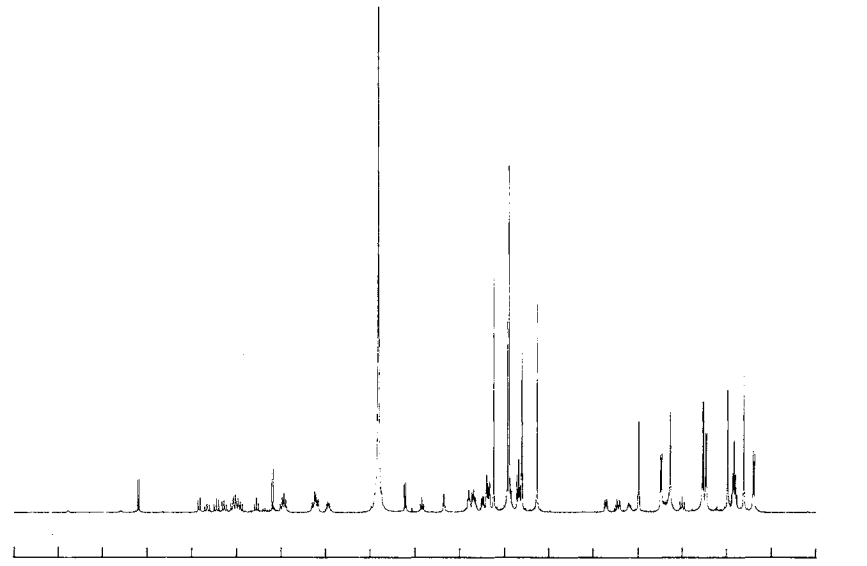
| Index | $^{13}C$ shift[a] (M)[b] | $^{1}H$ shift[a,c] ($J$) | $^{1}H$-$^{1}H$ connections[d] | Assignment[e] |
|---|---|---|---|---|
| 1 | 198.52 (0) | | | 7 |
| 2 | 176.99 (0) | | | 26 |
| 3 | 167.32 (0) | | | 5 |
| 4 | 163.72 (0) | | | 1 |
| 5 | 142.81 (1) | 7.60 d ($J$=7.6) | 23 | 3 |
| 6 | 142.17 (1) | 6.91 dd ($J$=1.4, 11.1) | 17, 55 | 9 |
| 7 | 142.04 (1) | 6.64 dd ($J$=10.8, 14.5) | 11, 17 | 11 |
| 8 | 137.88 (0) | | | 8 |
| 9 | 137.14 (0) | | | 20 |
| 10 | 136.44 (1) | 5.60 dd ($J$=6.0, 15.3) | 19, 31 | 33 |
| 11 | 134.99 (1) | 6.46 dd ($J$=10.8, 14.6) | 7, 12 | 12 |
| 12 | 133.81 (1) | 6.83 dd ($J$=11.7, 14.6) | 11, 13 | 13 |
| 13 | 132.30 (1) | 6.28 dd ($J$=11.1, 11.7) | 12, 15 | 14 |
| 14a[†] | 130.41 (1) | 5.65 m | 18, 43 | 23 |
| 14b[†] | 130.41 (1) | 5.96 dd ($J$=1.2, 11.0) | 18, 56 | 21 |
| 15 | 130.24 (1) | 5.62 m | 13, 38 | 15 |
| 16 | 130.21 (1) | 6.00 dd ($J$=10.7, 11.1) | 19, 20, 52 | 35 |
| 17 | 129.98 (1) | 6.72 dd ($J$=11.1, 14.5) | 6, 7 | 10 |
| 18 | 128.44 (1) | 6.50 dd ($J$=11.0, 15.5) | 14a, 14b, 43 | 22 |
| 19 | 128.18 (1) | 6.53 dd ($J$=11.1, 15.3) | 10, 16, 31 | 34 |
| 20 | 126.67 (1) | 5.48 dq ($J$=7.3, 10.7) | 16, 52 | 36 |
| 21 | 112.26 (0) | | | 6 |
| 22 | 104.14 (1) | 4.62 d ($J$=7.2) | 32 | 1′ |
| 23 | 101.47 (1) | 6.10 d ($J$=7.6) | 5 | 4 |
| 24 | 100.33 (1) | 4.91 br s | 26 | 1″ |
| 25 | 99.56 (0) | | | 28 |
| 26 | 89.98 (1) | 3.46 | 24, 35 | 2″* |
| 27 | 83.41 (1) | 3.67 | 45 | 30 |
| 28 | 83.22 (1) | 3.68 | 29, 33 | 3′ |
| 29 | 82.59 (1) | 3.31 | 28, 37 | 4′ |
| 30 | 82.21 (1) | 3.44 | 47 | 19* |
| 31 | 77.76 (1) | 4.18 d ($J$=6.0) | 10, 19 | 32 |
| 32 | 74.05 (1) | 3.33 | 35, 36 | 4″ |
| 33 | 73.47 (1) | 3.36 | 22, 28 | 2′ |
| 34 | 73.45 (1) | 3.86 | 38 | 17 |
| 35 | 71.98 (1) | 3.66 | 26, 32 | 3″ |
| 36 | 70.85 (1) | 3.77 | 32, 51 | 5″ |
| 37 | 69.74 (1) | 3.84 | 29, 50 | 5′ |
| 38 | 69.09 (1) | 4.43 t ($J$=8.5) | 15, 34 | 16 |
| 39 | 62.21 (3) | 3.61 s | | 3′-$OCH_3$ |
| 40 | 59.36 (3) | 3.43 s | | 2″-$OCH_3$ |
| 41 | 58.21 (1) | 2.35 dd ($J$=3.5, 9.9) | 49 | 27 |
| 42 | 56.39 (3) | 3.12 s | | 19-$OCH_3$ |
| 43 | 42.20 (2) | 3.90 | 14a, 18, NH | 24 |
| 44 | 40.30 (0) | | | 31 |
| 45 | 38.18 (2) | 1.51 t ($J$=11.7)<br>2.21 dd ($J$=4.0, 11.7) | 27 | 29 |
| 46 | 37.44 (3) | 3.46 s | | 38 |
| 47 | 37.01 (1) | 2.10 m | 30, 34, 57 | 18 |
| 48 | 23.13 (3) | 1.01 s | | 44 |
| 49 | 21.65 (2) | 1.70 m | 41, 54 | 42 |
| 50 | 18.27 (3) | 1.22 d ($J$=6.7) | 37 | 6′ |
| 51 | 18.15 (3) | 1.28 d ($J$=6.0) | 36 | 6″ |
| 52 | 13.71 (3) | 1.74 br d ($J$=7.3) | 16, 20 | 37 |
| 53 | 13.35 (3) | 0.82 s | | 45 |

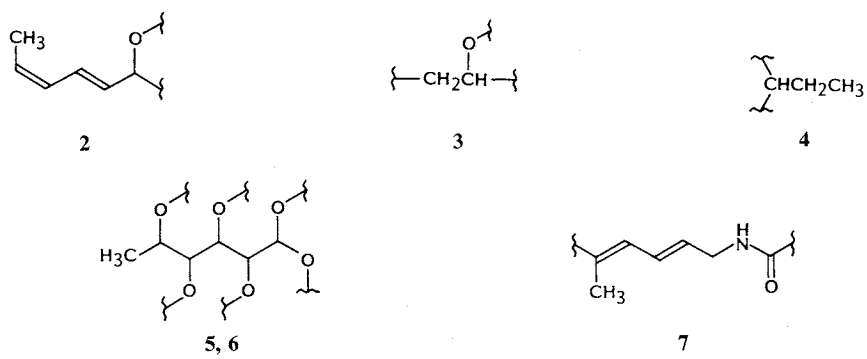
Table 1. (Continued)

| Index | $^{13}C$ shift[a] (M)[b] | $^{1}H$ shift[a,c] ($J$) | $^{1}H$-$^{1}H$ connections[d] | Assignment[e] |
|---|---|---|---|---|
| 54 | 12.47 (3) | 0.92 t ($J$=7.0) | 49 | 43 |
| 55 | 11.73 (3) | 1.98 d ($J$=1.4) | 6 | 39 |
| 56 | 11.18 (3) | 1.64 d ($J$=1.2) | 14b | 41 |
| 57 | 9.70 (3) | 0.72 d ($J$=5.9) | 47 | 40 |

[a] Chemical shifts reported in ppm.
[b] M; the number of protons attached to the carbon line.
[c] The chemical shift of the protons attached to the carbon.
[d] $^{1}H$-$^{1}H$ connections are listed by index.
[e] Assignments refer to the number system in structure **1**.
* These assignments are interchangeable.
† These carbon lines are coincidentally degenerate.
$J$: Coupling constant in Hz.

Fig. 1. $^{1}H$ NMR spectrum of UK-69,753 (**1**) (500 MHz, $CD_3OD$).

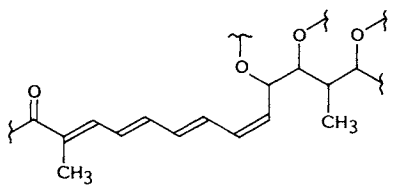



**2** **3** **4**

**5, 6** **7**

such as aurodox[6], efrotomycin[2] and factumycin[7]. The UV spectrum (Table 3) of UK-69,753 in methanol shows absorbance maxima at 232 and 358 nm, and is consistent with this hypothesis. The longer wavelength absorbance is characteristic of the tetraenone-4-hydroxy-2-pyridone chromophore which is part of the



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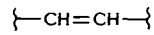

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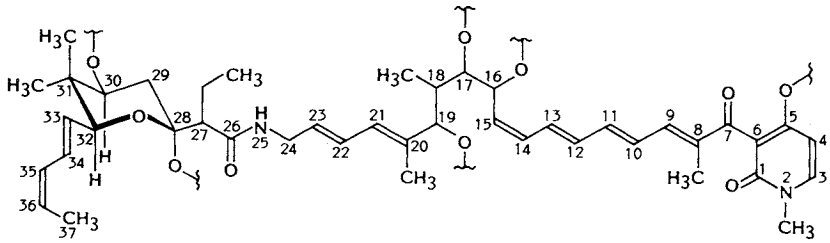

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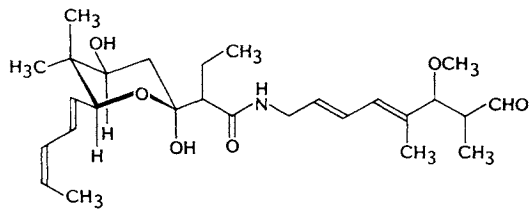

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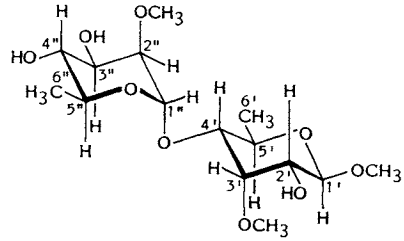


12

structure of only two members of this class, factumycin (A40A) and its stereoisomer A73A[8]. The presence of sugar residues in the structure of UK-69,753, as evidenced by **5** and **6**, distinguishes it from the two aglycones A40A and A73A.

Using the structure of factumycin as a model, the part structures **2**~**4** and **7**~**9** can be combined to give **10**, the backbone of the aglycone portion of the structure of UK-69,753. All of the structural elements of **10** not defined in the analysis above can be assigned from the COSY, HETCOR and MBHC spectra, including the N-2 methyl (37.44; 3.46 ppm), carbons C-1 (163.72), C-5 (167.32) and C-6 (112.26) of the pyridone ring, the hemiacetal C-28 (99.56) and the quaternary carbon C-31 (40.30) bearing geminal methyls (23.13; 1.01 and 13.35; 0.82). A comparison of the coupling constants observed for 32-H ($J$=6.0 Hz), 30-H ($J$=4.0 and 11.7 Hz), 29-$H_{eq}$ ($J$=4.0 and 11.7 Hz) and 29-$H_{ax}$ ($J$=11.7 and 11.7 Hz) in **10** with those of the corresponding protons in **11**, a degradation product isolated from the sodium metaperiodate oxidation of kirrothricin[9], allows for the assignment of the relative stereochemistry of the cyclic hemiacetal moiety (C-28 through C-32) as drawn in **10**. Attempts to confirm these stereochemical assignments by direct observation of NOE's were inconclusive.

The structure of the carbohydrate portion of UK-69,753 was determined by degradation and X-ray crystallography. UK-69,753 was treated with anhydrous HCl in methanol at room temperature for 5 hours, resulting in the formation of **12**, the *O*-methylglycoside of a disaccharide with a molecular formula of $C_{15}H_{28}O_9$. Careful recrystallization of **12** from 2-propanol gave crystals suitable for single crystal X-ray analysis which provided the structure for **12** shown below. A perspective diagram of **12** is shown in Fig.

2, and a summary of the NMR data for **12** is found in Table 2.

In order to complete the structure of UK-69,753, the location of the disaccharide and the remaining methoxyl group on the aglycone **10** needed to be determined. An isotope shift experiment in $CDCl_3$ indicated that the substituents on carbons C-5, C-16, C-17, and C-28 are hydroxyl groups, so that the disaccharide and methoxyl substituents are attached at either C-19 or C-30. The substituent at C-19 was assigned as a methoxyl group *via* multiple bond HETCOR, thus requiring that the disaccharide substituent be attached at C-30 and leading to the final assignment of the structure of UK-69,753 as **1**. The molecular formula of UK-69,753 is thus determined to be $C_{58}H_{86}N_2O_{18}$ with a MW of 1,098. This formula was directly

Fig. 2. Single crystal X-ray structure of **12**.

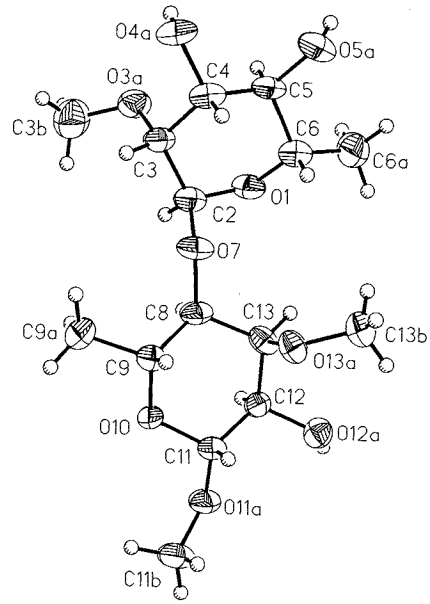


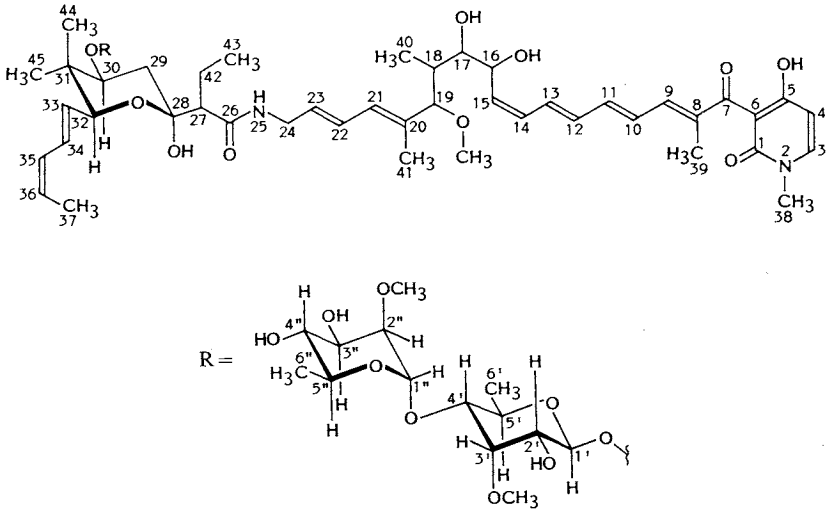

Table 2. A summary of the $^1H$, $^{13}C$, DEPT, homonuclear (COSY) and heteronuclear (HETCOR) correlation data for compound **12** ($CDCl_3$).

| Index | $^{13}C$ shift[a] (M)[b] | $^1H$ shift[a,c] (*J*) | $^1H$-$^1H$ connections[d] | Assignment |
|---|---|---|---|---|
| 1 | 101.87 (1) | 4.45 d (*J*=7.5) | 7 | 1′ |
| 2 | 98.23 (1) | 4.92 br s | 4 | 1″ |
| 3 | 80.88 (1) | 3.40 | 10, 5 | 4′ |
| 4 | 80.23 (1) | 3.48 | 2, 8 | 2″ |
| 5 | 80.18 (1) | 3.76 | 3, 7 | 3′ |
| 6 | 73.70 (1) | 3.38 | 8, 9 | 4″ |
| 7 | 72.08 (1) | 3.46 | 1, 5 | 2′ |
| 8 | 71.38 (1) | 3.70 | 4, 6 | 3″ |
| 9 | 68.94 (1) | 3.77 | 6, 15 | 5″ |
| 10 | 68.82 (1) | 3.85 dq (*J*=6.5, 9.7) | 3, 14 | 5′ |
| 11 | 61.42 (3) | 3.61 s | | $OCH_3$ |
| 12 | 58.89 (3) | 3.45 s | | $OCH_3$ |
| 13 | 57.00 (3) | 3.51 s | | $OCH_3$ |
| 14 | 17.78 (3) | 1.27 d (*J*=6.5) | 10 | 6′ |
| 15 | 17.60 (3) | 1.32 d (*J*=6.5) | 9 | 6″ |

[a] Chemical shifts reported in ppm.
[b] M; the number of protons attached to the carbon line.
[c] The chemical shift of the protons attached to the carbon.
[d] $^1H$-$^1H$ connections are listed by index.
*J*: Coupling constant in Hz.

Table 3. Selected physico-chemical properties of UK-69,753.

| | | |
|---|---|---|
| UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) | 232 (58,240), 358 (36,320) | |
| Combustion analysis | Calcd for $C_{58}H_{86}N_2O_{18}$: | C 63.37, H 7.82, N 2.55 |
| | Found: | C 63.34, H 7.95, N 2.19 |
| Negative ion FAB-MS | 1,097 $(M-H)^-$ | |
| Optical rotation $[\alpha]_D^{25}$ | −134.1° (*c* 0.44, MeOH) | |
| IR (KBr) $cm^{-1}$ | 3416, 2967, 2926, 1648, 1585, 1453, 1414, 1380, 1259, 1193, 1083, 1025 | |



UK-69,753 (**1**)

Table 4. *In vitro* antibacterial activity of UK-69,753.

| Organism | MIC (μg/ml) |
|---|---|
| *Staphylococcus aureus* 01A106 | >100 |
| *Escherichia coli* 51 A266 | >100 |
| *Pasteurella multocida* 59A006 | 6.25 |
| *P. haemolytica* 59B018 | 6.25 |
| *Bordetella bronchiseptica* 73A006 | >100 |
| *Clostridium perfringens* 10A006 | 6.25 |
| *C. difficile* 10J002 | 0.39 |
| *Actinomyces pyogenes* 14D008 | 25 |
| *Actinobacillus pleuropneumoniae* 54B004 | 12.5 |
| *Bacteroides fragilis* 78C024 | 100 |
| *Treponema hyodysenteriae* 94A008 | 0.78 |

Table 5. *In vivo* activity of UK-69,753 against *Treponema hyodysenteriae* 94A008.

| Dose (mg/kg/day) | Mice cleared of infection (%) | | |
|---|---|---|---|
| | Fourth day of treatment | Day-5 post-treatment | Day-12 post-treatment |
| 7.1 | 100 | 100 | 100 |
| 3.6 | 100 | 100 | 100 |
| 1.8 | 100 | 60 | 60 |
| Control[a] | 0 | 0 | 0 |

[a] 5% ethanol in $H_2O$.

verified as correct using negative ion fast atom bombardment mass spectrometry (FAB-MS) and combustion analysis (Table 3).

The spectroscopic analysis described above has served to characterize the structure of UK-69,753 as **1**, a novel glycosylated antibiotic related to efrotomycin. Previously reported glycosides in this antibiotic class include efrotomycin and the related phenelfamycin[10] and LL-E19020[11] complexes. The structure of UK-69,753 represents an interesting hybrid of structural elements found in other members of this class. The aglycone of UK-69,753 is identical in structure to factumycin (A40A) (or a stereoisomer), while the disaccharide moiety of UK-69,753 is nearly identical to the disaccharide of efrotomycin, differing only in that the C-4″ substituent is a hydroxyl in UK-69,753 as compared to a methoxyl group at C-4″ in efrotomycin.

## Biological Activity of UK-69,753

The *in vitro* antibacterial activity of UK-69,753 against a battery of veterinary pathogens was measured and the data from this study are summarized in Table 4. The spectrum of antibacterial activity observed for UK-69,753 is similar to that reported for other members of this structure class[12,13]. UK-69,753 exhibited particularly good potency against certain anaerobic bacteria such as *Clostridium difficile* and *Treponema hyodysenteriae*, a causative agent of swine dysentery. In a *in vivo* study, UK-69,753 was effective

when orally administered to mice colonized with *T. hyodysenteriae* (Table 5). No viable *T. hyodysenteriae* cells were detectable from fecal pellets of mice treated with 7.1 or 3.6 mg/kg/day of UK-69,753, even at 12 days post-treatment. Treatment with 1.8 mg/kg/day also affected colonization, although at this level only 60% of the treated mice remained free of infection by day-5 post-treatment.

## Experimental

### General Procedures

Samples of UK-69,753 used in the spectroscopic and biological studies described herein were isolated as reported in the companion paper to this report[1], and further purified by preparative HPLC using a Rainin Dynamax C18 column (21.4 mm i.d. × 25 cm, 8 $\mu$m irregular particle, 60 Angstrom pore) eluted with 35% acetonitrile - 65% 0.05 M $KH_2PO_4$ (pH 3.25) at a flow rate of 10 ml/minute. Fractions containing pure UK-69,753 were combined and the acetonitrile was removed under reduced pressure. The UK-69,753 was then extracted into ethyl acetate, washed with distilled water, dried over $Na_2SO_4$, filtered and evaporated to give a bright yellow solid.

The NMR studies leading to the structure assignment were conducted on a Brucker WM-250 spectrometer, modified to incorporate a pulse programmer and Aspect-3000 data system. All of the experiments described used standard automation programs supplied by the manufacturer. The final assignments were verified, (COSY), and extended by inclusion of long-range heteronuclear shift correlations and NOE difference spectra, on a Brucker AM-500 spectrometer. The long-range heteronuclear shift correlation experiment utilized the same standard pulse sequence employed for the one-bond correlations, with delays scaled to $^{13}C$, $^{1}H$ couplings of approximately 5 Hz.

Isotope shift measurements in $CDCl_3$ solution consisted of identically measuring the $^{13}C$ spectrum following successive washes with $H_2O$ - $D_2O$ (1 : 1), $H_2O$, and finally, $D_2O$. In the latter two instances, the washes were repeated several times prior to recording the $^{13}C$ spectrum.

FAB-MS were recorded on a VG Analytical 70/250-S mass spectrometer in the negative ion mode using a dithiothreitol - dithioerythritol matrix. UV spectra were measured on a Hewlett-Packard HP 8450A diode array spectrophotometer. IR spectra were recorded on a Perkin-Elmer PE 1420 ratio recording IR spectrophotometer. Optical rotations were measured using a Perkin-Elmer PE 241 MC polarimeter.

### Preparation of **12**

To a solution of 2.0 g of UK-69,753 in 80 ml of methanol was added 40 ml of anhydrous HCl in methanol which was prepared by the addition of 2.0 ml of acetyl chloride to 40 ml of anhydrous methanol. The resulting red-brown mixture was stirred at room temperature for 5 hours, at which time it was concentrated to a brown residue under reduced pressure. The residue was loaded onto a silica gel column which was eluted with chloroform - methanol (19 : 1) to give 135 mg of **12** as an off-white solid. This material was further purified by recrystallization from 2-propanol to yield 57 mg of colorless needles with mp 155~157°C, $[\alpha]_D^{25}$ −37.8° (*c* 1.07, $CHCl_3$). The NMR analysis of **12** is summarized in Table 2 and its single-crystal X-ray structure is shown in Fig. 2.

### Single Crystal X-Ray Analysis of **12**

A representative crystal was surveyed and a 1 Angstrom data set (maximum $\sin\theta/\lambda = 0.5$) was collected on a Nicolet R3m/$\mu$ diffractometer. Atomic scattering factors were taken from the International Tables for X-ray Crystallography[14]. All crystallographic calculations were facilitated by the SHELXTL[15] system. All diffractometer data were collected at room temperature.

A trial structure was obtained by direct methods. This trial structure refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were all less than 0.1 of their corresponding standard deviations. The final R-index was 0.034. A final difference Fourier revealed no missing or misplaced electron density.

The refined structure was plotted using the SHELXTL plotting package (Fig. 2). Coordinates,

anisotropic temperature factors, distances and angles are available as supplementary material.

### Antimicrobial Susceptibility Test

MICs were determined as described by DIRLAM *et al.*[16] except that all anaerobes were tested on Tryptose agar (Difco) supplemented with 5% bovine blood (TBA) and incubated 48 hours at 39°C in a Coy (Ann Arbor, Mich.) anaerobe chamber containing an $N_2$-$CO_2$-$H_2$ (80:10:10) atmosphere. MICs for aerobes were determined in an identical manner except Brain heart infusion (BHI) agar (Difco) was used and plates were incubated aerobically at 37°C for 18~20 hours.

### Mouse *Treponema hyodysenteriae* Colonization Model

Female CF-1 mice (Charles River, Wilmington, MA) were colonized with *T. hyodysenteriae* 94A008 by oral administration of cells harvested from TBA plates and diluted in BHI broth to approximately $2 \times 10^7$ cfu/ml. Three 1-ml challenge doses were administered at 0,18 and 24 hours. Mice were housed in groups of five/cage on wood chip bedding (changed twice weekly); water and pellet ration were provided *ad libitum*. Intestinal colonization was monitored by collecting two fecal pellets from individual mice, homogenizing in 4.5 ml sterile BHI broth and plating duplicate samples of the homogenate in pour plates using TBA supplemented with 400 $\mu$g/ml spectinomycin and 25 $\mu$g/ml rifampicin. Plates were incubated anaerobically (see above) at 39°C for 72 hours; cfu were identified by hemolytic zones. A sample showing $\geq$50 cfu/ml of homogenate was considered to indicate intestinal colonization.

For drug treatment, mice successfully colonized with *T. hyodysenteriae* were dosed *per os* BID for four consecutive days with 0.2 ml volumes of UK-69,753 solubilized in ethanol and diluted in sterile water to the desired concentrations; the final ethanol concentration did not exceed 5%. A fresh drug solution was prepared each day and stored at 4°C between doses. Treatment groups consisted of five mice housed and fed as described above. A control group of four mice received 5% ethanol in water. Colonization of mice was monitored as described above.

### Acknowledgments

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### References

1) PACEY, M. S.; M. R. JEFSON, L. H. HUANG, W. P. CULLEN, H. MAEDA, J. TONE, S. NISHIYAMA, K. KANEDA & M. ISHIGURO: UK-69,753, a novel member of the efrotomycin family of antibiotics. I. Taxonomy of the producing organism, fermentation and isolation. J. Antibiotics 42: 1453~1459, 1989
2) DEWEY, R. S.; B. H. ARISON, J. HANNAH, D. H. SHIH & G. ALBERS-SCHÖNBERG: The structure of efrotomycin. J. Antibiotics 38: 1691~1698, 1985
3) DODDRELL, D. M.; D. T. PEGG & M. R. BENDALL: Distortionless enhancement of NMR signals by polarization transfer. J. Magn. Reson. 48: 1490~1495, 1987
4) AUE, W. P.; E. BARTHOLDI & R. R. ERNST: Two-dimensional spectroscopy: Application to nuclear magnetic resonance. J. Chem. Phys. 64: 2229~2246, 1987
5) BAX, A. & G. MORRIS: An improved method for heteronuclear shift correlation by two-dimensional NMR. J. Magn. Reson. 42: 501~505, 1981
6) BERGER, J.; H. H. LEHR, S. TEITEL, H. MAEHR & E. GRUNBERG: A new antibiotic X-5108 of *Streptomyces* origin. I. Production, isolation and properties. J. Antibiotics 26: 15~22, 1973
7) GULLO, V. P.; S. B. ZIMMERMAN, R. S. DEWEY, O. HENSENS, P. J. CASSIDY, R. ŌIWA & S. ŌMURA: Factumycin, a new antibiotic (A40A): Fermentation, isolation and antibacterial spectrum. J. Antibiotics 35: 1705~1707, 1982
8) DEWEY, R. S.; J. E. FLOR, S. B. ZIMMERMAN, P. J. CASSIDY, S. OMURA & R. OIWA (Merck): Discovery of A73A, a new efrotomycin-like antibiotic in fermentation broth. U.S. 4,262,002, Apr. 14, 1981
9) ZEECK, A.; H.-U. HOPPE & I. HUMMEL: Die Konstitution des kirrothricins. Tetrahedron Lett. 22: 2357~2360, 1981
10) HOCHLOWSKI, J. E.; M. H. BUYTENDORP, D. N. WHITTERN, A. M. BUKO, R. H. CHEN & J. B. MCALPINE: Phenelfamycins, a novel complex of elfamycin-type antibiotics. II. Isolation and structure determination. J.

Antibiotics 41: 1300~1315, 1988

11) CARTER, G. T.; D. W. PHILLIPSON, J. J. GOODMAN, T. S. DUNNE & D. B. BORDERS: LL-E19020$\alpha$ and $\beta$, novel growth promoting agents: Isolation, characterization and structures. J. Antibiotics 41: 1511~1514, 1988

12) FROST, B. M.; M. E. VALIANT, B. WEISSBERGER & E. L. DULANEY: Antibacterial activity of efrotomycin. J. Antibiotics 29: 1083~1091, 1976

13) FROST, B. M.; M. E. VALIANT & E. L. DULANEY: Antibacterial activity of heneicomycin. J. Antibiotics 32: 626~629, 1979

14) International Tables for X-ray Crystallography. Vol. IV. *Eds.*, J. A. IBERS & W. C. HAMILTON, pp. 55, 99, 149, Kynoch Press, 1974

15) SHELDRICK, G. M.: Desktop Shelxtl Nicolet X-Ray Instrument, 1986

16) DIRLAM, J. P.; L. J. CZUBA, B. W. DOMINY, R. B. JAMES, R. M. PEZZULLO, J. E. PRESSLITZ & W. W. WINDISCH: Synthesis and antibacterial activity of 1-hydroxy-1-methyl-1,3-dihydrofuro{3,4-b}quinoxaline 4,9-dioxide and related compounds. J. Med. Chem. 22: 1118~1121, 1979