UK-1, A NOVEL CYTOTOXIC METABOLITE FROM *Streptomyces* sp. 517-02

II. STRUCTURAL ELUCIDATION

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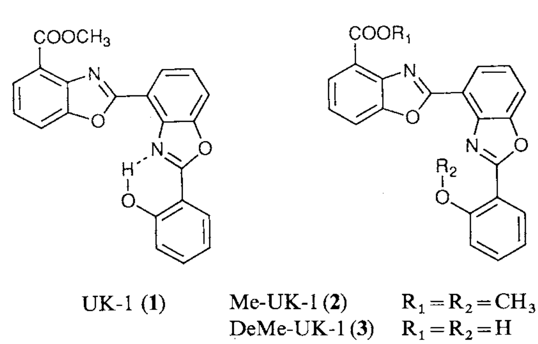
The structure of UK-1 isolated from the mycelium of *Streptomyces* sp. 517-02 was elucidated to be a novel benzoxazole dimmer derivative (**1**) on the basis of spectroscopic methods.

A novel metabolite with potent cytotoxic activity against B16, HeLa and P388 cells, UK-1, was isolated from the mycelium of *Streptomyces* sp. 517-02 as described in a previous paper[1]. The structure of UK-1 was elucidated to be a dimeric benzoxazole derivative constituted of two moles of 3-hydroxyanthranilic acid and one mole of salicylic acid on the basis of some spectroscopic methods (Fig. 1). The structure determination studies of UK-1 are described in this paper.

Results and Discussion

The IR spectrum of UK-1 (**1**) described in a previous paper showed a strong absorption based on ester group at 1725 cm^{-1} and the UV spectrum of **1** suggested the existence of conjugated system in the molecule. The molecular formula of **1** was determined as $C_{22}H_{14}N_2O_5$ from the HREI-MS (M^+: *m/z* 386.0913, Calcd for $C_{22}H_{14}N_2O_5$, 386.0903) as the base peak and ^{13}C NMR spectral data. Other fragment ions were observed at *m/z* 354.0606 (Calcd for $C_{22}H_{14}N_2O_5 \cdot CH_3OH$, 354.0572) and *m/z* 328.0888 (Calcd for $C_{22}H_{14}N_2O_5 \cdot HCOOCH_3$, 328.0928) in the HREI-MS spectrum of **1**. The absorption of a hydroxyl group could not be observed in the IR spectrum of **1** but the signal based on a strong hydrogen bonded hydroxyl group appeared at δ 11.9 ppm in the ^1H NMR spectrum in $CDCl_3$. Moreover, **1** afforded its mono-methyl ether, Me-UK-1 (**2**), by methylation with methyl iodide and anhydrous potassium carbonate in dry acetone. The IR spectrum of **2** showed the absorption based on an ester group at ν_{max} 1710 cm^{-1}. In the ^1H and ^{13}C NMR spectra of **2**, the signals of a methoxyl group appeared at δ 4.09 and δ 56.22, respectively showing to be the monomethyl derivative of **1**. Alkaline hydrolysis of **1** furnished the corresponding carboxylic acid, DeMe-UK-1 (**3**). The absorption based on a carboxyl group appeared at ν_{max} 2500～3100 and 1690 cm^{-1} in the IR spectrum of **3** and the signal of a methoxycarbonyl group disappeared in the ^1H NMR.

Fig. 1. Structures of UK-1 (**1**) and its derivatives.



The ^1H and ^{13}C NMR spectral data of **1**, **2** and **3** are shown in Table 1. The assignments of proton and carbon signals were done using ^1H-^1H COSY and ^1H-^{13}C COSY spectra (Fig. 2) and

Table 1. NMR spectral data for benzoxazoles.

No.	UK-1 (**1**)[a]		Me-UK-1 (**2**)[a]		DeMe-UK-1 (**3**)[b]		2-*o*-Methoxybenzoxazole (**5**)[a]		2-*o*-Hydroxybenzoxazole (**6**)[a]	
	^{13}C shift	^{1}H shift	^{13}C shift	^{1}H shift	^{13}C shift	^{1}H shift	^{13}C shift	^{1}H shift	^{13}C shift	^{1}H shift
1	151.41		151.86		151.83					
2	141.59		141.64		142.05					
3	122.67		122.39		122.98					
4	127.41	8.07 dd	127.11.	8.06 dd	127.82	8.39 d				
5	124.83	7.44 t	124.47	7.44 t	125.32	7.47 t				
6	114.96	7.82 dd	115.34	7.91 dd	114.71	7.85 d				
7	161.81		163.20		159.93[c]					
8	117.77		118.45		118.00		120.25	7.59 d	119.55	7.69 d
9	138.74		140.75		138.78		142.24		140.14	
10	149.98		151.47		150.28		150.53		149.23	
11	113.69	7.72 dd	113.88	7.78 dd	114.20	7.79 d	110.48	7.81 d	110.64	7.71 d
12	125.22	7.48 t	124.76	7.50 t	125.69	7.47 t	124.28	7.35 t	125.01	7.36 t
13	125.33	8.31 dd	125.79	8.43 dd	125.88	8.42 d	124.90	7.32 t	125.37	7.33 t
14	164.62		163.89		164.57		161.87		162.98	
15	110.06		116.00		110.57		116.50		110.64	
16	159.68		159.03		159.93[c]		158.62		158.85	
17	117.84	7.15 d	112.34	7.10 d	118.16	7.31 d	112.26	7.08 d	117.47	7.12 d
18	134.24	7.46 t	133.37	7.54 t	134.71	7.51 t	132.73	7.49 t	133.56	7.42 t
19	119.56	7.00 td	120.83	7.13 t	120.04	7.05 t	120.77	7.07 t	119.27	6.98 t
20	127.21	8.01 dd	131.95	8.32 d	127.65	8.04 d	131.40	8.13 d	127.15	8.01 d
C=O	166.12		165.86		167.81					
OMe	52.53	4.17 s	52.32	4.10 s						
OMe			56.22	4.09 s			56.26	4.01 s		
OH		11.90 s								11.42 s

[a] In $CDCl_3$.
[b] In pyridine-d_5.
[c] Overlapped signal.

Fig. 2. ^{1}H-^{13}C COSY spectrum of Me-UK-1 (**2**).

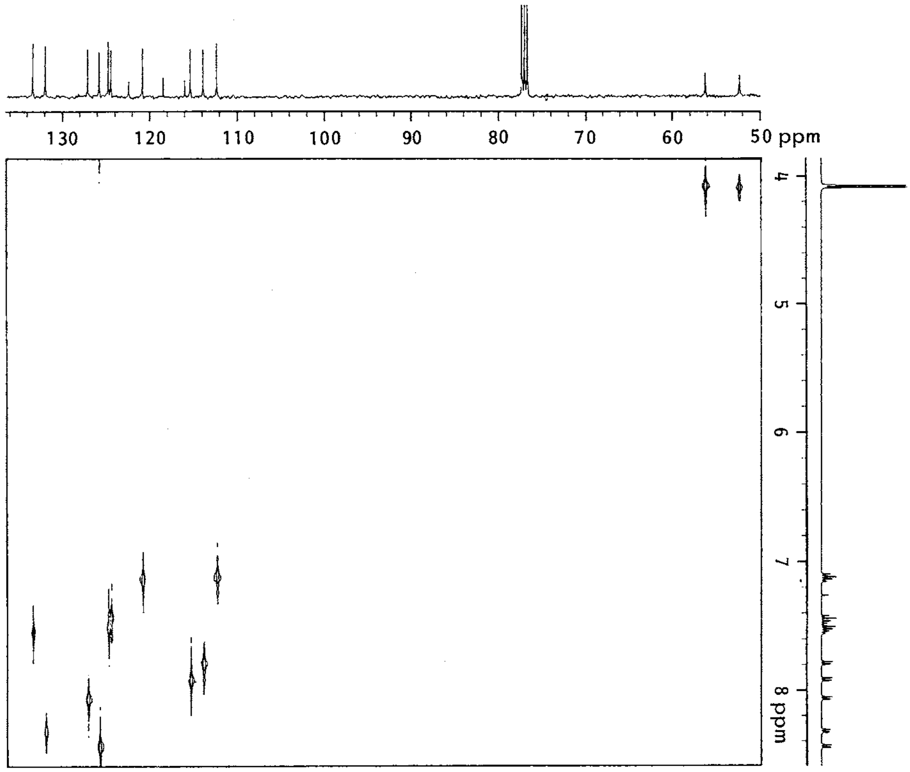
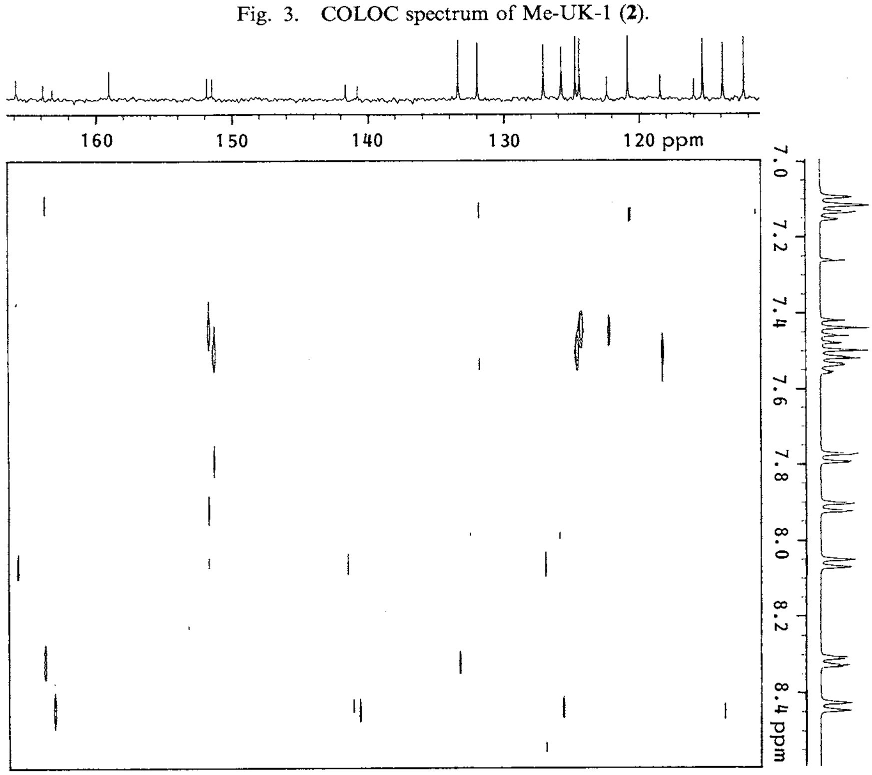


Fig. 3. COLOC spectrum of Me-UK-1 (**2**).

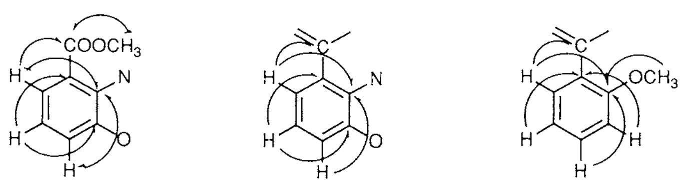


correlation spectroscopy *via* long-range couplings (COLOC) measurement (Fig. 3). These results revealed the partial structure A, B and C in **2** as shown in Fig. 4, and some possible formulae as the structure of **1** were estimated by the combination of these partial structures.

The ^{13}C NMR parameters in the benzene ring of **1** are consistent with the values calculated on the basis of the chemical shift of carbons in the benzene ring of benzoxazole (**4**)[2]. Moreover, the benzoxazole derivatives (**5** and **6**) were prepared from *o*-aminophenol and *o*-anisaldehyde or salicylaldehyde, respectively (Scheme 1)[3], and the ^{13}C chemical shifts of these derivatives were in good accordance with those of **1** and **2** (Table 1).

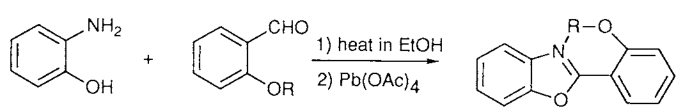
From these results, the structure of UK-1 was deduced to be formula **1**, the novel benzoxazole dimmer derivative.

Fig. 4. Partial structures of Me-UK-1 (**2**) and the correlation of ^{1}H-^{13}C long-range couplings.



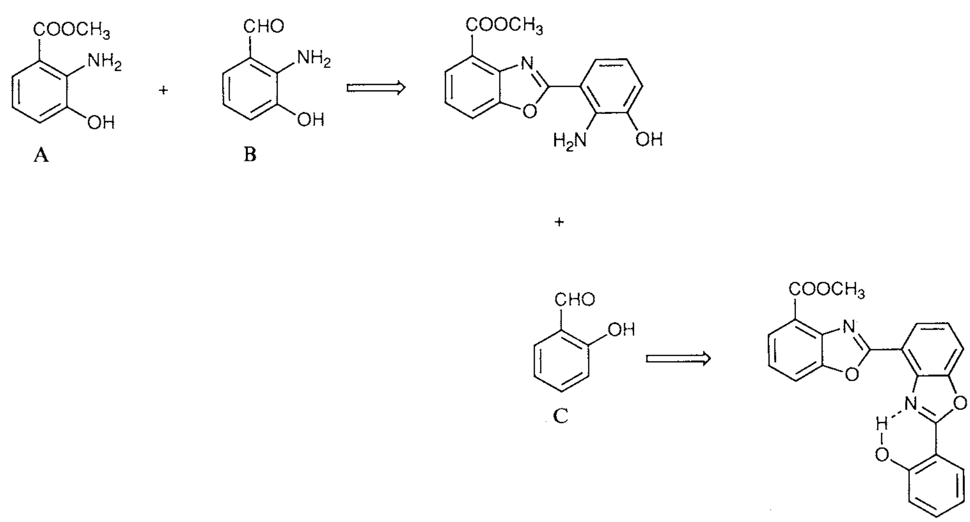
A B C

Scheme 1.



$R=H$ **5** $R=H$
$R=CH_3$ **6** $R=CH_3$

Scheme 2. Estimation of biosynthesis for UK-1.



UK-1 (**1**)

Both of the fragments A and B of **1** are related to 3-hydroxyanthranilic acid, one of the catabolic products *via* kynurenine and the fragment C is a reduced product of salicylic acid. The benzoxazoles **5** and **6** were easily prepared by oxidation of SCHIFF's bases derived from *o*-aminophenol and corresponding aldehydes. It seems that UK-1 was biosynthesized by oxidation of the SCHIFF's base prepared from methyl 3-hydroxyanthranilate and 3-hydroxyanthranilaldehyde produced in the decomposition pathway of L-tryptophan, followed to the preparation of SCHIFF's base with salicylaldehyde and the oxidative ring closure reaction of SCHIFF's base (Scheme 2). Studies on the biosynthesis of **1** are now in progress.

Experimental

MS and NMR

EI-MS and HREI-EI-MS spectra were obtained with a JEOL-JMS-AX 500 mass spectrometer. All NMR spectra were recorded on a JEOL-JNM-GX-400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Tetramethylsilane was used as an internal reference for ^{1}H NMR in the $CDCl_3$ solution. For the ^{13}C chemical shift reference, the ^{13}C peak at δ 77.03 ppm of $CDCl_3$ was used. The ^{1}H peak at δ 7.0 ppm and ^{13}C peak at δ 122.4 ppm of pyridine-d_5 were used as the internal references for NMR measurement in pyridine-d_5.

Methylation of 1

Anhydrous potassium carbonate (2 g) was suspended in a solution of 50 mg of **1** and 0.5 ml of methyl iodide in 20 ml of dry acetone, and the mixture was refluxed for 5 hours. After filtration, acetone was evaporated and the residual mixture was dissolved in CH_2Cl_2, followed to chromatographycal purification on a silica gel column. By recrystallization with methanol, **2** was obtained as colorless needles in quantitative yield. **2**; MP 145~147°C, IR (nujol) 1710, 1600, 1590, 1580, 1540 cm^{-1}, HREI-MS (M^+)=m/z 400.1067 (Calcd for $C_{23}H_{18}N_2O_5$, 400.1075), NMR, see Table 1.

Alkaline Hydrolysis of 1

Alkaline hydrolysis of **1** with aqueous NaOH in a pyridine solution at room temperature afforded **3**. **3**; MP>300°C, IR (nujol) 2500~3100, 1690, 1600, 1560 cm^{-1}, NMR, see Table 1.

Preparation of 2-Phenylbenzoxazoles

2-Phenylbenzoxazoles were prepared according to the procedure of STEPHENS and BOWER[3]. Namely, *o*-aminophenol dissolved in ethanol was mixed with the corresponding aldehyde, boiled for 10 minutes and cooled. The product obtained by filtration and recrystallization from ethanol gave a red SCHIFF's base. The treatment of the SCHIFF's base with lead tetraacetate in glacial acetic acid afforded the 2-phenylbenzoxazole, **4** or **5**.

4; The yield of SCHIFF's base from *o*-aminophenol (1.1 g) and *o*-anisaldehyde (1.4 g) was 1.04 g (70%). The dehydrogenation of SCHIFF's base (140 mg) with lead tetraacetate (211 mg) in acetic acid (3.5 ml) afforded 2-*o*-methoxyphenylbenzoxazole (38 mg). ^{1}H and ^{13}C NMR, see Table 1.

5; The yield of SCHIFF's base from *o*-aminophenol (1.1 g) and salicylaldehyde (1.2 g) was 2.24 g (100%). The dehydrogenation of SCHIFF's base (1.23 g) with lead tetraacetate (2.1 g) in acetic acid (20 ml) afforded 2-*o*-hydroxyphenylbenzoxazole. ^{1}H and ^{13}C NMR, see Table 1.

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References

1) UEKI, M.; K. UENO, S. MIYADOH, K. ABE, K. SHIBATA, M. TANIGUCHI & S. OI: UK-1, a novel cytotoxic metabolite

from *Streptomyces* sp. 517-02. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties. J. Antibiotics 46: 1089 ~ 1094, 1993
2) KALINOWSKI, H.; S. BERGER & S. BRAUN (*Eds.*): Carbon-13 NMR Spectroscopy. pp. 313 ~ 315 and 394, John Wiley & Sons Ltd., 1988
3) STEPHENS, F. F. & J. D. BOWER: The preparation of benziminazoles and benzoxazoles from SCHIFF's bases. Part I. J. Chem. Soc. 1949: 2971 ~ 2972, 1949