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NOTE

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Revision of the Structure Assigned to the Antibiotic BU-4664L from *Micromonopora*

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Abstract The structure assigned to the antitumor antibiotic BU-4664L from *Micromonospora* sp. was revised to 5,10-dihydro-4,6,8-trihydroxy-10-(3,7,11-trimethyl-*trans*-2,*trans*-6,10-dodecatrienyl)-11*H*-dibenzo[*b*,*e*][1,4]-diazepin-11-one based on the NMR analysis.

Keywords BU-4664L, antitumor, *Micromonospora*

In the screening of antitumor compounds from rare actinomycetes, *Micromonospora* sp. TP-A0860 was found to produce BU-4664L [1] and rakicidins [2]. BU-4664L was originally isolated from *Micromonospora* sp. M990-6 as a lipoxygenase inhibitor and was shown to have potent *in vitro* and *in vivo* antitumor activity. The structure of BU-4664L is described in a patent as shown (Fig. 1). During the structural confirmation of the compounds produced by strain TP-A0860, we found that the proposed structure of BU-4664L was incorrect. We herein report the revision of the structure of BU-4664L on the basis of NMR analysis.

The producing strain was isolated from a soil sample collected in Osawano, Toyama, Japan and identified as *Micromonospora* sp. based on the taxonomic study. Seed and production fermentation was carried out in V-22 and A-3M medium, respectively, as described previously [3]. The fermentation broth (5 liters) was extracted with 1-butanol and the organic layer was concentrated *in vacuo*. The oily extract (17 g) was successively chromatographed on the column of silica gel ($CHCl_3$ - MeOH; 20 : 1～1 : 1), ODS (CH_3CN - 0.15% KH_2PO_4, pH 3.5; 20 : 80～85 : 15)

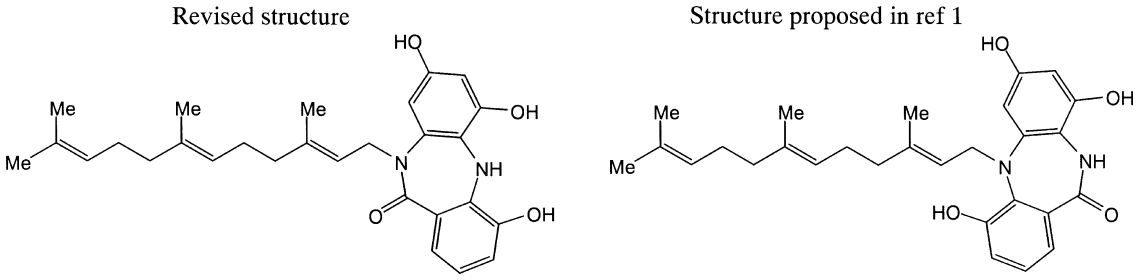

Fig. 1 Structure of BU-4664L.

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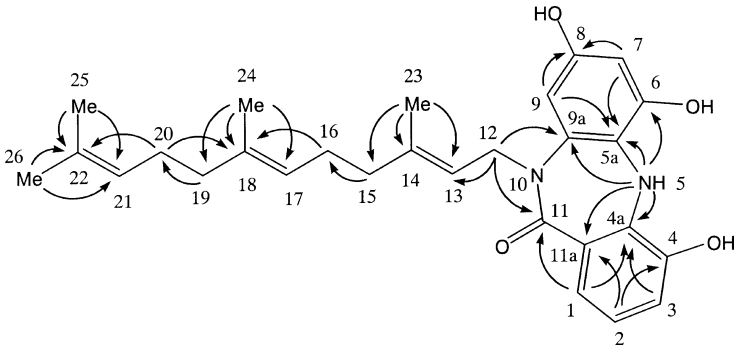

Fig. 2 Important HMBC correlations observed in BU-4664L.

and LH-20 (MeOH) to afford BU-4664L (16 mg) as light yellow needles with the melting point of 192～194°C.

The IR spectrum indicated the presence of an amide (1620 cm^{-1}) and hydroxyl (3250 cm^{-1}) groups. The UV-vis spectrum showed the absorption maxima λ_{max} (ε) at 211 (45,600) and 292 (sh) in MeOH or 0.01 N HCl - MeOH, and at 206 (30,800), 252 (sh), 282 (sh), 344 (8,500), 550 (2,200) in 0.01 N NaOH - MeOH. These properties closely resemble to the data described for BU-4664L in ref 1. The high resolution EI-MS indicated a molecular formula $C_{28}H_{34}N_2O_4$ [*m/z* 462.2518 (M^+); 462.2519 calcd. for $C_{28}H_{34}N_2O_4$]. ^1H and ^{13}C NMR spectra in DMSO-d_6 are also in good accordance with the ones in ref 1. The ^{13}C NMR spectrum showed 28 carbons assignable to four methyl, five methylene, eighteen sp^2 and one carbonyl carbon (Table 1). Of these, fifteen carbons (four methyl, five methylene and six sp^2 carbons) were assigned to a farnesyl residue based on the HMQC and HMBC analysis (Fig. 2). The remaining carbons were assigned to the aromatic chromophore by the HMBC analysis. The NH proton at 6.72 ppm showed HMBC correlations to aromatic carbons at C-4a, C-5a, C-6, C-9a and C-11a. On the other hand, HMBC correlations were observed from the meta-coupled protons H-7 and H-9 to C-5a and C-8. In addition, DQF-COSY revealed the contiguous coupling of three aromatic protons H-1, H-2 and H-3 and HMBC correlations were detected from H-1 and H-3 to C-4a and H-2 to C-4 and C-11a. Substitution of the farnesyl residue on the amido nitrogen at position 10 was unambiguously determined by the HMBC correlations from the C-12 methylene (δ_H 4.38; δ_C 47.8) to C-9a and the carbonyl carbon C-11. Although the direct comparison with the authentic compound of BU-4664L was not possible, our spectral data strongly indicate that the compound produced by strain TP-A0860 is identical with BU-4664L. We thus propose that the structure assigned for BU-4664L in ref. 1 should be revised to 5,10-dihydro-4,6,8-trihydroxy-10-

Table 1 NMR assignment for BU-4664L (DMSO-d_6)

Position	$\delta^{13}C$	$\delta^{1}H$
1	122.3	7.06 (1H, d, 7.8 Hz)
2	120.4	6.70 (1H, t, 7.6 Hz)
3	116.5	6.82 (1H, d, 7.8 Hz)
4	145.5	
4a	141.1	
5a	124.8	
6	147.6	
7	100.4	6.15 (1H, d, 2.2 Hz)
8	153.0	
9	99.4	6.17 (1H, d, 2.2 Hz)
9a	134.9	
11	167.6	
11a	124.8	
12	47.8	4.38 (2H, d, 6.1 Hz)
13	121.6	5.24 (2H, t, 5.8 Hz)
14	136.9	
15	39.0	1.98 (2H)
16	25.9	2.00 (2H)
17	123.7	5.05 (2H, t, 6.1 Hz)
18	134.6	
19	39.2	1.89 (1H), 1.98 (1H)
20	26.2	1.90 (1H), 1.98 (1H)
21	124.2	5.03 (2H, t, 7.1 Hz)
22	130.6	
23	16.2	1.64 (3H, s)
24	15.8	1.54 (3H, s)
25	17.5	1.51 (3H, s)
26	25.5	1.60 (3H, s)
5-NH		6.72 (1H, s)
4-OH		9.04* (1H, br.s)
6-OH		9.97* (1H, br.s)
8-OH		10.05* (1H, br.s)

Spectra were recorded in DMSO-d_6 at 400 MHz for ^1H and 100 MHz for ^{13}C; solvent peaks were used as a reference (δ_H 2.50; δ_C 39.5).

* interchangeable

(3,7,11-trimethyl-*trans*-2,*trans*-6,10-dodecatrienyl)-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one.

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