NOTE

New Antibiotic Sch 725424 and Its Dehydration Product Sch 725428 from *Kitasatospora* sp.

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Abstract A new microbial metabolite Sch 725424 (**1**) was isolated from the culture of *Kitasatospora* sp. The structure elucidation of **1** was accomplished based on NMR spectroscopic analyses as well as extensive structure elucidation of its dehydration product Sch 725428 (**2**). Compound **1** showed inhibitory activity against *Staphylococcus aureus* with MIC values 1∼2 μg/ml, and also displayed weak antifungal activity against *Saccharomyces cerevisiae* (PM503) with an MIC 32 μg/ml.

Keywords *Kitasatospora* sp., antimicrobial agents, Sch 725424, Sch 725428

In the course of our continuing search for novel antimicrobial agents [1∼6], we have isolated a novel antibacterial polyene, Sch 725424 (**1**), from culture SPRI-0408 (*Kitasatospora* sp.). Sch 725424 was identified as a new compound based on NMR spectroscopic analyses as well as extensive structure elucidation of its dehydration product Sch 725428 (**2**). In this paper, we describe the isolation of **1** and the structure elucidation of **1** and **2**. The antimicrobial activity profile of **1** against Gram-positive and Gram-negative bacterial strains as well as fungal pathogens is also reported.

Fermentation studies of the SP culture SPRI-0408 (94-

00721) were carried out in shake flasks. Stock cultures were maintained as frozen whole broths at −80°C in a final concentration of 10% glycerol. The germination medium contained glucose (10 g/liter), trehalose (10 g/liter), Difco Tryptone (5 g/liter), soyflour (5 g/liter), and yeast extract (5 g/liter). The pH was adjusted to 7.2 and $CaCO_3$ (2 g/liter) was added. A 250 ml Erlenmeyer flask containing 70 ml of this medium was inoculated with 2 ml of the stock culture. The flasks were incubated at 28°C on a rotary shaker at 250 rpm for 96 hours. This seed culture (2.5 ml) was used to inoculate another 250 ml Erlenmeyer flask containing 70 ml of the same medium and the flask was incubated as above for 96 hours.

Five percent of this second germination was then used to inoculate the fermentation medium. The fermentation was carried out in a 500 ml Erlenmeyer flask containing 100 ml of the fermentation medium. The fermentation media used contained PD-650 Dextrin (50 g/liter), ProFlo Flour (35 g/liter), cerelose (5 g/liter), $CaCO_3$ (7 g/liter), and $CoCl_2$ (0.24 mg/liter). The flasks were incubated at 28°C on a rotary shaker at 250 rpm for 96 hours.

The completed fermentation broth (2 liters) was stirred with 100 gram of NaCl followed by 4 liters of acetonitrile (MeCN) for partition. The organic layer was separated and dried *in vacuo*. The extract was absorbed onto the polymeric resin, CG161 (∼150 ml, Tosoh Biosep LLC, Montgomeryville, PA, USA) and the NaCl was washed out with water followed by 40% MeCN (200 ml each). Then,

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other absorbed organic material was eluted with 200 ml 80% aq. MeCN, and finally bioactive material was eluted with EtOAc - MeOH (1 : 1) to yield 69 mg of dried material after removing solvent *in vacuo*. The organic material of the EtOAc - MeOH fraction was purified on an HPLC semi-preparative ODS-A column (YMC, 120 Å, S-7, 20 mm×250 mm). The column was eluted with a three-step gradient of MeCN - H$_2$O: 3~40% MeCN in 40 minutes, 40~80% gradient in 40 minutes, and then 80~100% MeCN in 30 minutes, with a flow rate of 15 ml/minutes. Fractions were collected (13 ml/fraction) by a fraction collector. Pure **1** (~4 mg) was obtained from two injections of 36 mg of crude material at retention time ~57 minutes.

During the NMR analysis of **1** in anhydrous CDCl$_3$, transformation of **1** to another compound (**2**) was observed. Since the transformation took place slowly, ^{1}H-, ^{13}C-, and ^{1}H-^{1}H COSY were able to be acquired for **1**. Leaving compound **1** to remain in CDCl$_3$ for two days allowed for the complete transformation, and the ^{1}H-NMR spectrum showed that transformed compound **2** was adequately pure for structure elucidation. Thus, different NMR techniques were applied for structure elucidation of **2**. Two aliphatic methylenes, two olefinic methyls, nine olefinic methines and five non-protonated sp^2 carbons were observed on the basis of analyses of ^{1}H-, ^{13}C-NMR, APT and ^{1}H-^{1}H COSY data. Based on ^{1}H-^{1}H COSY correlations from H-9 through H$_3$-12 and from H-2 through H-7 (Figure 1), the structures of Fragments A and B in Figure 1 were proposed, respectively. The coupling constant (J=14.4 Hz) between H-10 and H-11 established the *trans* configuration of Δ10, 11. Based on the same reason, the configurations of double bonds Δ2, 3, Δ4, 5, and Δ6, 7 were all determined as *trans* configuration (J=15.0 Hz). Another doublet coupling pattern of H-2, H-7, and H-9 indicated that they were connected to non-protonated carbons at the other end. The non-protonated carbon C-8, neighboring to H-7 and H-9, was determined by the following HMBC correlations: H$_3$-13 (δ 1.89) to C-7 (δ 143.1), C-8 (δ 132.7), and C-9 (δ 135.0). The correlations from H$_3$-13 to C-7, C-8, and C-9 established the location of H$_3$-13 as well as the linkage of Fragments A and B. The correlations of H-2 and H-3 to a carboxamide carbon (C-1, δ165.9) indicated the connection of amide functionality to the highly conjugated chain. The amide linkage of the conjugated chain to the rest of the unidentified moiety was further confirmed by observation of the amide proton (δ 7.65), correlated to C-1, and two non-protonated sp^2 carbons C-15 (δ 197) and C-18 (δ 174.8). Thus the polyene skeleton was established as 8-methyl-dodeca-2,4,6,8,10-pentaenoic amide.

Due to the limited material of **2**, the correlations of the rest of two methylenes (C-16 and C-17) and three non-
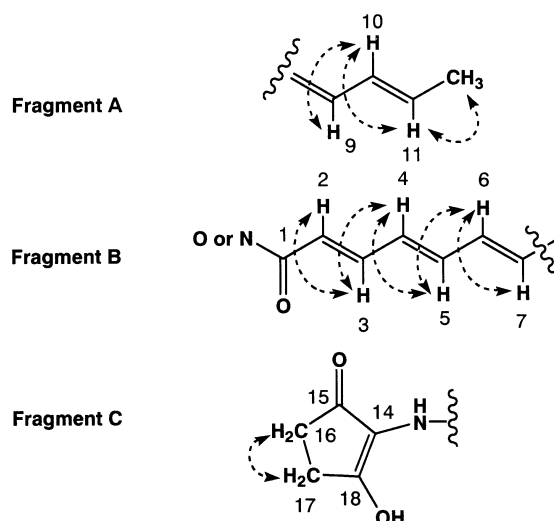

Fig. 1 Partial structures and ^{1}H-^{1}H COSY correlations of **2**.
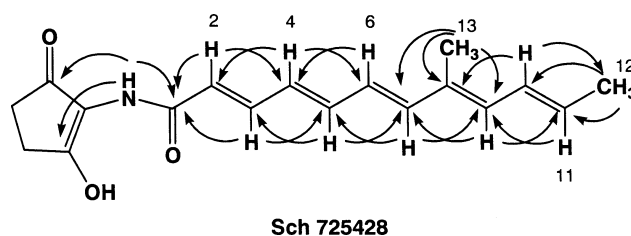

Sch 725428

Fig. 2 Key HMBC correlations of **2**.

protonated carbons (C-14, C-15, and C-18) could not be established from HMBC experiment. The 2-hydroxy-5-oxo-cyclopent-1-enyl moiety (Fragment C) was eventually determined through comparison of the NMR data to those of model compound asukamycin, shown in Figure 3 [7~9]. Thus, the structure elucidation of **2** was completed. Full assignment of proton and carbon chemical shifts was achieved based on 2D NMR data analyses including ^{1}H-^{1}H COSY, HSQC, and HMBC and shown in Table 1. The established molecular formula C$_{18}$H$_{20}$NO$_3$ is consistent with the measured exact mass m/z 300.1587 [M+H]$^+$ in high resolution ESI-MS (calcd. 300.1594).

With the unambiguous NMR data and the elucidated structure for **2**, the comparison of NMR data between **1** and **2** indicated the following: in the ^{13}C NMR, chemical shifts of C-6 to C-13 and C-14 to C-18 of **1** matched well with those of **2**. However, the signals of double bond Δ2, 3 shifted to the aliphatic region, which represented a methylene (δ 42.6, t) and a hydroxyl substituted methine (δ 64.9, d) functionality. From a chemical point of view, this is a typical dehydration transformation from **1** to **2**, shown in Figure 4. The same phenomenon was observed in their ^{1}H-NMR spectra (Table 1), and again indicated that
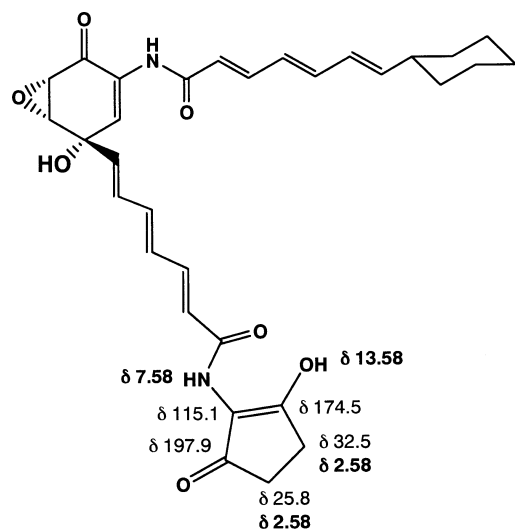
Fig. 3 Structure, partial ^{1}H (boldfaced) and ^{13}C chemical shifts of model compound asukamycin.

the only structural change from **1** to **2** occurred between C-1 and C-5. The proton signals of the paired olefin ($\varDelta$2, 3) shifted to the aliphatic region representing a methylene (δ 2.59, dd and δ 2.66, dd) and a hydroxyl substituted methine (δ 5.08, ddd). From ^{1}H-^{1}H COSY correlations of **1** (Figure 5), the location of hydroxyl group at 3 position was determined and the assignments of protons H-2 through H-5 (Table 1) were accomplished. Thus, the structure elucidation of **1** was completed.

To our best knowledge, this is the first discovery of a twelve carbon carboxamide chain having four or five conjugated double bonds with a single carbon substitution on the C-8 position. The partial structures of asukamycin and manumycin A have the same carbon units *vs.* compounds **1** and **2**, however in the former the end of the six-carbon units was cyclized to form a functionalized cyclohexyl ring [7~10]. Similar observations occurred in asukamycin analogues such as asuka-mABA and 64-pABA, derived from precursor-directed synthesis [9, 10]. All asuka-mABA, 64-pABA, and related analogues have a

Table 1 NMR spectral data for compounds **1** and **2** in CDCl$_3$^{a}

C/H no.	1		2	
	^{1}H (δ)	^{13}C (δ)^b	^{1}H (δ)	^{13}C (δ)
1		172.0 s		165.9 s
2a	2.59, dd, 15.6, 3.0	42.6 t	5.98, d, 15.0	118.6 d
2b	2.66, dd, 15.6, 9.0			
3	5.08, ddd, 9.0, 9.0, 3.0	64.9 d	7.38, dd, 15.0, 11.4	144.9 d
4	5.39, dd, 10.8, 9.0	129.0 d	6.36, dd, 15.0, 11.4	128.4 d
5	6.17, dd, 11.4, 10.8	131.5 d	6.69, dd, 15.0, 11.4	143.0 d
6	6.44, dd, 15.0, 11.4	128.1 d	6.34, dd, 15.0, 11.4	126.5 d
7	6.33, d, 15.0	141.1 d	6.49, d, 15.0	143.1 d
8		132.4 s		132.7 s
9	6.12, d, 10.8	133.5 d	6.19, d, 11.4	135.0 d
10	6.41, dd, 14.4, 10.8	128.1 d	6.43, dd, 14.4, 11.4	128.3 d
11	5.82, dq, 14.4, 6.8	131.9 d	5.88, dq, 14.4, 6.8	133.0 d
12	1.85, d, 6.8	18.7 q	1.86, d, 6.8	18.8 q
13	1.89, s	12.6 q	1.89, s	12.4 q
14		116 s		116 s
15		199.0 s		197 s
16	2.53, m	32.1 t	2.61, m	32.0 t
17	2.59, m	25.5 t	2.56, m	25.7 t
18		172.0 s		174.8 s
NH	8.40, br s		7.65, br s	
O*H*	12.97, s		13.85, s	

^{a} Recorded on a Varian Unity 500 NMR instrument at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using standard Varian pulse sequence programs (VNMR Version 6.1 Software). δ in ppm; *J* in Hz.
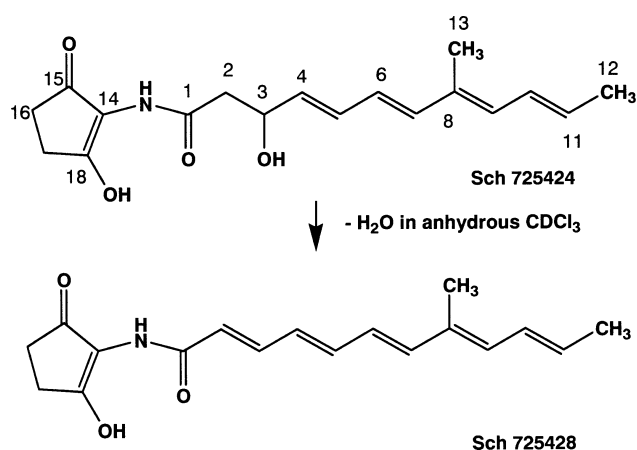^{b} The ^{13}C chemical shifts of **1** were not determined unambiguously.

Fig. 4 Dehydration conversion of Sch 725424 to Sch 725428.
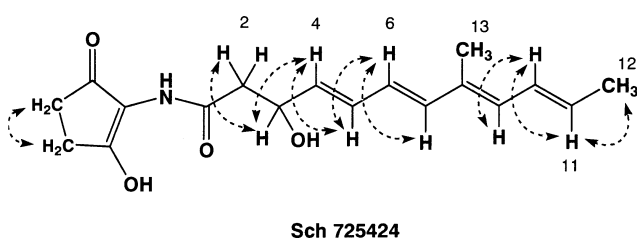

Fig. 5 ¹H-¹H COSY correlations of **1**.

cyclized phenyl ring at the end of the polyene chain, which resembles the cyclization of C-8 to C-13 of **1**. Whether compound **1** is involved in the biosynthesis or degradation of asukamycin type antibiotics is currently unknown.

Compound **1** exhibited antibacterial activity against various strains. The MIC values of **1** are listed in Table 2, in comparison with gentamicin as a reference standard. Sch 725424 (**1**) showed inhibitory activity against *Staphylococcus aureus* with MIC values 1~2 μg/ml, and also displayed weak antifungal activity against *Saccharomyces cerevisiae* (PM503) with an MIC 32 μg/ml.

Table 2 Antimicrobial activity of compound **1**

Strain[a]	MIC (μg/ml)	
	1	Gentamicin
Staphylococcus aureus sensitive strain (HS999)	1	8
S. aureus (ATCC 29213)	2	0.06
Escherichia coli (HS294)	>128	2
E. coli (ATCC 10536)	>128	0.125
Saccharomyces cerevisiae sensitive strain (PM503)	32	>64
Candida albicans (C43)	>128	>64
Aspergillus fumigatus (ND158)	>128	>64

[a] Incubation for 24 hours for bacteria, 48 hours for fungi.

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