Notes

Sch 36605, STRUCTURE OF A NOVEL NUCLEOSIDE

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A novel nucleoside antibiotic, Sch 36605, has been isolated from the culture filtrate of a *Streptomycete* sp.[1]. All the physico-chemical and spectroscopic data presented herein indicate this compound is a new blasticidin compound. A second closely related component, Sch 36606, was isolated[1] together with Sch 36605. This compound has been characterized and identified as leucylblasticidin S[2].

Sch 36605 (**1**) is a water soluble basic compound: MP >230°C (dec); $[\alpha]_D^{26}$ +58.2° (*c* 0.1, H_2O); IR (KBr) cm^{-1} 3300 (br, NH and OH), 1650 (CONH), 1000~1150; UV λ_{max} (H_2O, pH 7) nm (ε) 272 (8,350); λ_{max} (0.01 N HCl) nm (ε) 279 (11,240).

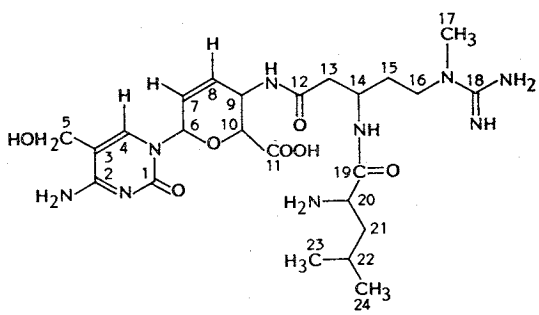
The molecular weight of Sch 36605 ($C_{24}H_{39}N_9O_7$) was established as 565 on the basis of fast atom bombardment (FAB) mass spectral data. A protonated molecular ion $M+H^+$, *m/z* 566 was observed. High resolution data is as follows: Mass measured, 566.2970; mass calculated 566.3040, for a composition of $C_{24}H_{40}N_9O_7$. The monosodiated ion at *m/z* 588 and disodiated ion at *m/z* 610 were observed. The most intense fragment ion *m/z* 425 results from the cleavage of 5-hydroxymethylcytosine ($M+H^+-141$, *m/z* 425). Other fragmentation pathways occur due to cleavage of leucine and blastidic acid from the molecule.

On acidic hydrolysis (2 N HCl, reflux, 2 hours), **1** gave 5-hydroxymethylcytosine and leucine, that were identified chromatographically by comparison with authentic samples. Dansylation of **1** followed by acid hydrolysis (6 N HCl, 17 hours, 105°C) gave dansylated leucine identified by co-chromatography with an authentic sample†. Thus in **1**, leucine is linked through the *C*-carboxy terminus.

The ^1H NMR in D_2O of Sch 36605 is consistent with the proposed structure. Characteristic ^1H NMR values are as follows: δ 0.80 (6H, br d, $2\times CH_3$), 2.92 (3H, s, NCH_3), 4.34 (2H, s, CH_2OH), 4.57 (1H, m, OC*H*COOH), 5.75 and 6.03 (each 1H, br, *J*=9 Hz, CH=CH), 6.4 (1H, br s, OCHN), 7.55 (1H, s, C=CH).

The ^{13}C NMR spectrum of **1** shows the presence of 24 carbon atoms. Fully decoupled and insensitive nuclear enhanced polarization transfer (INEPT) spectra led to the assignments of the carbon signals. The data for **1**, **2** and blasticidin S (**3**) are presented in Table 1. The assignments are in good agreement with the reported values for **3**[3] and the closely related compound mildiomycin[4].

The molecular weight of Sch 36606 (**2**) is established as 535 on the basis of FAB mass spectral data. A protonated molecular ion, $M+H^+$, *m/z* 536 was observed. The major



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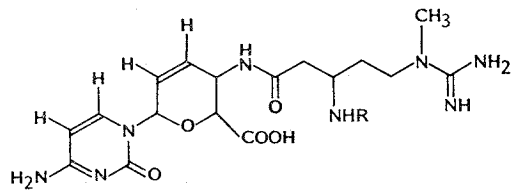


2 R = Leucine

3 R = H

† TLC performed on polyamide sheets developing with 1.5% aq formic acid.

Table 1. ^{13}C NMR data obtained on a Varian XL-400 instrument; spectra taken in D_2O (δ ppm relative to dioxane 67.4 ppm).

Carbon No.[a]	Sch 36605	Sch 36606	Blasticidin S
C-1	166.1	167.1	167.1
C-2	157.4	157.3	157.3
C-3	107.8	126.5	129.6
C-4	142.5	143.6	143.7
C-5	58.5	—	—
C-6	80.3	80.2	80.5
C-7	126.5	126.5	127.1
C-8	133.9	133.5	133.8
C-9	45.6	45.9	46.4
C-10	78.5	78.2	78.5
C-11	175.6	175.6	175.8
C-12	172.9	172.2	171.7
C-13, 15	41.3, 31.3	41.5, 31.1	38.0, 30.0
C-16, 21	48.0, 43.8	47.9, 46.0	47.4
C-14	46.2	46.2	47.4
C-17	36.5	36.4	36.6
C-18	158.3	158.3	158.3
C-19	177.2	172.1	—
C-20	53.7	52.8	—
C-22	25.0	24.6	—
C-23, 24	22.0, 22.1	21.7, 22.6	—

[a] Refers to numbering in **1**.

fragment ion *m/z* 435 represents the cleavage of cytosine from **2**, and thus follows the same fragmentation pattern observed for **1** and other related nucleoside antibiotics[5]. The UV data for **2** are as follows: λ_{max} (H_2O, pH 7) nm (ε) 269 (8,000) and (0.01 N HCl) nm (ε) 276 (11,000). Acidic hydrolysis of **2** (2 N HCl, reflux 2 hours) gave cytosine and leucine that were identified with the authentic samples.

The ^{13}C NMR data for **2** are given in Table 1. The data are consistent with the structure of **2** and establish the identity as leucylblasticidin S.

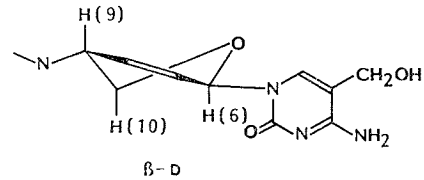
The configurations of amino acids and of the pyran-3-ene ring were determined as follows. Acid hydrolysis of **1** and **2** (6 N HCl, 105°C, 17 hours) and blasticidin S (**3**)† yields blastidic acid[6] and leucine, together with other hydrolysis products. The amino acids were separated and purified by passing the acid hydrolysate through Amberlite IRA-401S (OH^-). Elution of the resin with H_2O gave blastidic acid, followed by elution with 0.5 N HCl to give leucine. The specific rotations for each sample of blastidic acid were measured and within experimental error are in good agreement. The results are shown in Table 2. Previous reports show that blastidic acid obtained from blasticidin S was assigned the L-configuration by comparative ORD studies[6]. On the basis of our rotation measurements of blastidic acids obtained from **1**, **2** and **3**, these acids are identical and are assigned the L-configuration in **1** and **2**. The configuration of leucine, derived from **1** and **2**, as the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) derivative[7] was determined by HPLC using peak enhancement with an authentic sample and shown to be in the L-form.

† A sample of blasticidin S was kindly supplied by Kaken Pharmaceutical Co., Ltd., Japan.

Table 2. Specific rotations of blastidic acid.

Compound	$[\alpha]_D^{26}$ (c 0.5, H_2O)
Blastidic acid (Sch 36605)	+6.3°
Blastidic acid (blasticidin S)	+9.1°

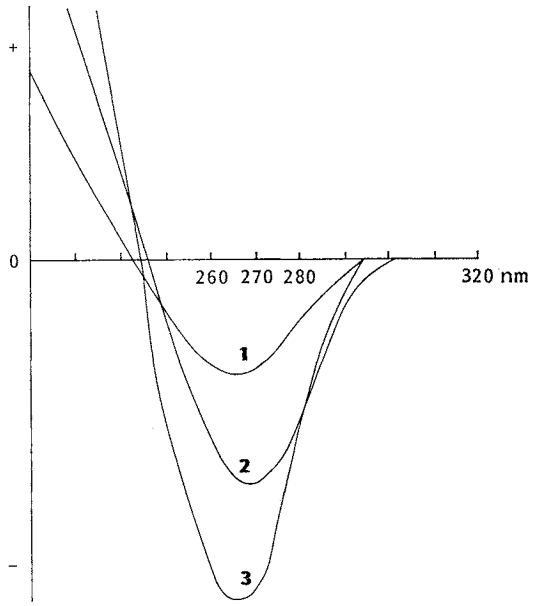
Fig. 1. Stereochemical form of the pyran-3-ene ring.



We examined the absolute configuration of the pyran-3-ene ring indicated in Fig. 1. The stereochemistry of the 9-H and 10-H protons should be diaxial on the basis of the coupling constant $J_{9,10}$=10 Hz in the ^1H NMR spectrum of **1**. Also the stereochemistry of the 6-H proton was assigned axial from $J_{6,7}$=10 Hz and $J_{6,8}$=2 Hz. The three bulky groups in the pyran-3-ene ring should reasonably be all equatorial. Only two sterically stable stereostructures of β-D or α-L could be permitted among all the possible isomers of the pyran-3-ene as shown in Fig. 1.

CD spectra for **1**, **2** and **3** are shown in Fig. 2. They all show a negative cotton effect with the λ_{max} close to 270 nm. The absolute configuration of blasticidin S was previously reported as possessing a pyran-3-ene ring in the β-D form[6]. Since the signs of the cotton effect are identical for all three compounds (and the amino acids have the same L-configuration) it is reasonable to assume the β-D form for the pyran-3-ene ring

Fig. 2. CD spectra of Sch 36605 (**1**), Sch 36606 (**2**), and blasticidin S (**3**), taken in H_2O, 0.1 mg/ml.



of **1** and **2**. Thus the structure of Sch 36605 (**1**) inclusive of stereochemistry is 4-[3-(2-amino-4-methylvaleramido)-5-(1-methylguanidino)valeramido]-1-(5-hydroxymethyl-4-amino-2-oxo-1-(2*H*)-pyrimidinyl)-1,2,3,4-tetradeoxy-D-*erythro*-hex-2-enopyranuronic acid.

Acknowledgments

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