NOTES

STRUCTURE OF ANTIBIOTIC Bu-2545, A NEW MEMBER OF THE CELESTICETIN-LINCOMYCIN CLASS

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Bu-2545 is a new antibiotic that has activity against various anaerobic organisms as well as aerobic Gram-positive bacteria. It was produced by a *Streptomyces* strain similar to *S. aureocirculatus*. The fermentation, isolation and properties of Bu-2545 were reported in a preceding paper[1]. The structure of Bu-2545 had been proposed[1] on the basis of microanalysis, NMR and mass spectral data in comparison with lincomycin and celesticetin. The present paper describes the structure elucidation of Bu-2545, including its stereochemistry, by ^{13}C-NMR and degradation studies.

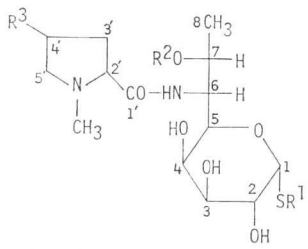
The ^{13}C-NMR spectrum of Bu-2545 (**1**) (Table 1) showed the presence of sixteen carbons including C-CH_3 (δ in ppm: 14.0), SCH_3 (14.0), NCH_3 (41.4) and OCH_3 (56.9). The carbon chemical shifts due to SCH_3 and C-1 ~ C-5 of the sugar portion were in agreement with those of lincomycin (**2**) under protonated conditions. However, the signals due to C-6 ~ C-8 and the N-acyl residue consisting of six carbons were different from those of **2**. The C-7 signal of **1** at δ 76.8 was shifted downfield by 9.4 ppm as compared with that of **2**, while the C-6 and C-8 signals were shifted upfield by 5.0 and 3.2 ppm, respectively. These three carbons along with the N-

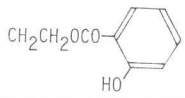
Table 1. CMR spectra of Bu-2545 and related compounds.

		Chemical shift, ppm from TMS						
	Carbon	Bu-2545 (**1**)[a]		**5**[a]		Lincomycin HCl (**2**)[b]	Celesticetin HCl (**3**)[c]	Methyl thiolincosaminide HCl (**8**)[a]
		Base	+DCl	Base	+DCl			
The sugar moiety	1	89.2(d)[d]	89.1	88.9(d)[d]	88.8	89.2	88.3	88.7
	2	69.7(d)	69.1	68.6(d)	68.2	68.8	70.3	68.3
	3	71.3(d)	71.2	71.3(d)	70.6	71.4	71.3	70.7
	4	70.0(d)	69.5	69.2(d)	69.7	69.5	69.9	69.5
	5	69.3(d)	69.8	71.7(d)	67.3	70.0	69.3	68.1
	6	49.0(d)	49.9	50.4(d)	54.8	54.9	50.8	56.9
	7	76.7(d)	76.8	77.4(d)	74.7	67.4	76.7	65.7
	8	14.0(q)	14.0	12.5(q)	14.3	17.2	14.7	17.7
	SCH_3	14.0(q)	14.0	13.9(q)	13.6	14.2	—	13.6
	OCH_3	56.7(q)	56.9	56.3(q)	57.0	—	56.9	—
The amino acid moiety	1′	177.3(s)	169.4			170.1	169.1	
	2′	68.9(d)	68.5			69.5	68.5	
	3′	30.9(t)	30.5			36.4	30.8	
	4′	23.9(t)	23.4			37.4	23.9	
	5′	56.7(t)	57.3			62.4	57.7	
	NCH_3	41.1(q)	41.4			41.8	41.9	

[a] Recorded by a Varian FT-80 spectrometer at 20 MHz in D_2O with dioxane as an internal reference.
[b] See ref. 4. The shifts of *n*-propyl on the N-acyl residue are omitted.
[c] See ref. 4. The shifts of β-salicyloxyethylthio in the 1-position of the sugar moiety are omitted.
[d] Multiplicity of resonance in single frequency off-resonance proton-decoupled spectrum.

Fig. 1. Bu-2545 and related antibiotics.



	Antibiotic	R^1	R^2	R^3
1	Bu-2545	CH_3	CH_3	H
2	Lincomycin	CH_3	H	n-C_3H_7
3	Celesticetin		CH_3	H

acyl and O-CH_3 carbons of **1** showed a good agreement with those of celesticetin (**3**)[4]. This is consistent with the proposed structure in the previous paper[1], where the 7-hydroxy group of the methyl thiolincosaminide moiety is methylated and the 6-amino group acylated with N-methylproline (hygric acid) as in **3**.

Hydrolysis of **1** with 6 N hydrochloric acid (reflux, 3 hours) followed by purification on Amberlite IR-120 gave L-hygric acid (**4**) (45% yield) as colorless needles, mp 114~114.5°C (Ref.[5] 116°C), m/z 129 (M^+), $[\alpha]_D^{25}$ −82.5° (c 1.0, H_2O) [Ref.[5] −80.1° (c 2, H_2O)], which was identical in all respects with L-hygric acid prepared from L-proline by N-methylation with formaldehyde and sodium cyanoborohydride.

It has been reported[6] that hydrazinolysis of **2** at reflux temperature cleaved the amide bond and liberated the sugar and amino acid moieties without rearrangement or racemization. Thus, **1** was subjected to a similar hydrazinolysis (reflux for 2 days) and following chromatographic separation on a silica gel column afforded a basic substance (**5**, $C_{10}H_{21}NO_5S$) having no amide band in the IR, along with N-methylproline hydrazide (**6**) which gave **4** by 6 N HCl hydrolysis (overall yield of **4**, 35%).

Compound **5** was compared with methyl thiolincosaminide (**8**) which was prepared from **2** by a reported procedure[6]. The PMR spectrum

Fig. 2. Degradation reaction of Bu-2545.

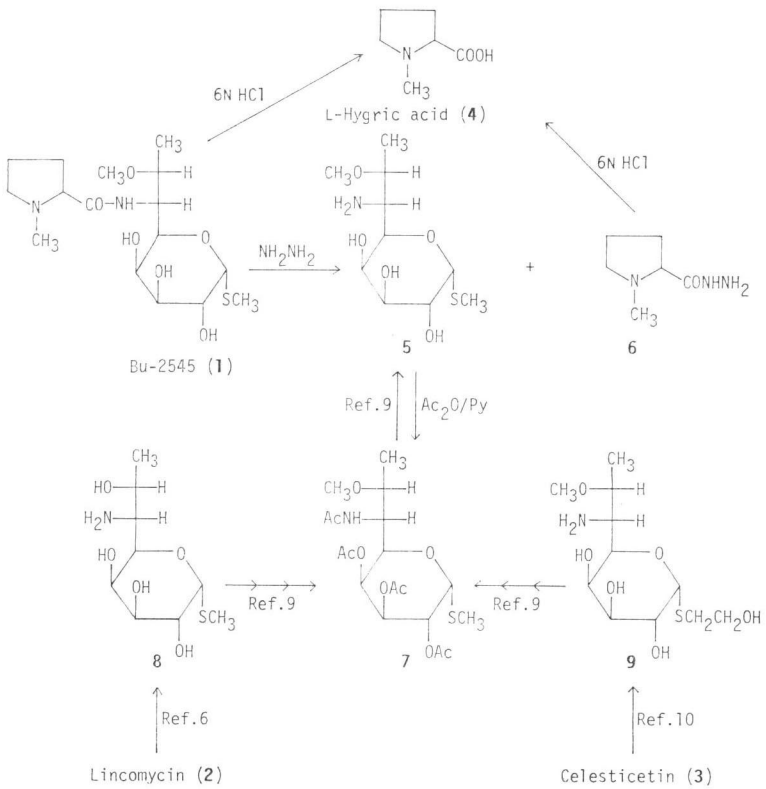


Table 2. Major signals in PMR spectra of **5** and **8** (60 MHz, D_2O).

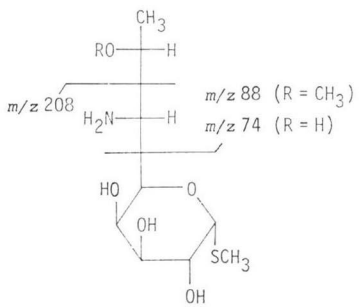
Proton	Chemical shift, δ (ppm from DSS)	
	5	**8**
$>CH-CH_3$	1.15 (d, J=6.5 Hz)	1.13 (d, J=6.5 Hz)
$S-CH_3$	2.11 (s)	2.12 (s)
$O-CH_3$	3.32 (s)	—
Anomeric H	5.28 (d, J=5.5 Hz)	5.33 (d, J=5.5 Hz)

(60 MHz, D_2O) of **5** (Table 2) showed signals assignable to $>CH-CH_3$ and $S-CH_3$ groups as well as an anomeric proton, which were also observed in the spectrum of **8**[7], and, in addition, indicated the presence of an $O-CH_3$ singlet at δ 3.32. The CMR spectrum of **5** (Table 1) which included 10 carbon signals was similar to that of **8** in the C-1 ~ C-5 signals. The C-7 signal of **5** located at 9.0 ppm lower field than that of **8** and the C-6 and C-8 signals of **5** shifted to the higher field by 2.1 ppm and 3.4 ppm, respectively, than those of **8**. These observations and an $O-CH_3$ signal at δ 57.0 in **8** indicated a methoxy group present at the C-7 position of **8**. The mass spectrum of **5** (Fig. 3) indicated the M^+ ion at m/z 267 and an intense peak at m/z 88 due to cleavage between C-5 and C-6. These peaks of **5** were higher by 14 mass units than the corresponding peaks of **8**[8], whereas the base peak at m/z 208 due to cleavage between C-6 and C-7 was common to both **5** and **8**. This also supported the location of a methoxy group at the 7-position of **8**.

The above spectral data suggested that **5** was methyl 7-O-methyl thiolincosaminide or its 7-epimer. Methyl 7-O-methyl-1-thio-α-lincosaminide has been reported[9] to be prepared from the tetra-N,O-acetate (**7**) derived from lincomycin and celesticetin *via* **8** and 2-hydroxyethyl 1-thio-α-celestosaminide (**9**)[10], respectively. The identities of **5** and its tetra-N,O-acetate (**7**) with those reported in literature were verified by melting point and specific rotation data: **5**: mp 127 ~ 128°C (Ref.[9], 126 ~ 126.5°C); $[\alpha]_D^{23}$ +250° (*c* 0.3, H_2O) [Ref.[9], $[\alpha]_D$ +263° (*c* 0.83, H_2O)]. **7**: Ac_2O/Pyridine; yield 82%; mp 211 ~ 213°C (Ref.[9], 211.5 ~ 213°C); $[\alpha]_D^{25}$ +234° (*c* 0.5, $CHCl_3$) [Ref.[9], $[\alpha]_D$ +229° (*c* 0.72, $CHCl_3$)]; M^+ m/z 435; $\nu_{c=o}$ 1755, 1660 cm^{-1}.

Thus, the sugar part of Bu-2545 was found to have the same stereochemistry as that of lincomycin and celesticetin. Accordingly, the structure of Bu-2545 was established as 7-O-methyl-4′-de-*n*-propyllincomycin or methyl 6-N-(1-methyl-L-prolyl)-1-thio-α-celestosaminide.

Fig. 3. Diagnostic peaks in mass spectrum of **5** and **8**.



5: R = CH_3 (M^+ m/z 267)

8: R = H (M^+ m/z 253)

References

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