1- AND 3-DEAMIDINO DERIVATIVES OF DIHYDROSTREPTOMYCIN AND SOME 1-*N*-ACYL DERIVATIVES

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1-Deamidino-, 3-deamidino- and 1,3-di(deamidino)dihydrostreptomycin (**1**, **2**, **3**) were prepared by treatment of dihydrostreptomycin (DHSM) with ammonia at 100°C. The 3-guanidino group of DHSM is suggested to be more important than the 1-guanidino group for the antibacterial activity of DHSM. 1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl) and 1-*N*-[(*S*)-4-guanidino-2-hydroxybutyryl] derivatives (**4**, **6**) of 1-deamidinodihydrostreptomycin were further prepared.

A dihydrostreptomycin (DHSM) derivative, dideamidinodihydrostreptomycin (**3**) in which two guanidino groups are replaced with amino groups, had already been reported[1] and found to have almost no antibacterial activity. In order to learn more clearly the role of the guanidino groups in streptomycins for their antibacterial activity, we tried to prepare monodeamidino derivatives (**1** and **2**) of dihydrostreptomycin starting from DHSM.

At first we tried to prepare **1** and **2** by basic hydrolysis of DHSM in aqueous solution, however, this attempt has not been rewarded. Monoguanidino-monoureido, diureido, monoureido-monoamino and diamino (**3**) derivatives of DHSM were formed and the formation of aimed monoguanidino-monoamino compounds (**1** and **2**), which are positive for diacetyl (a reagent for detection of a guanidino group) as well as for ninhydrin but negative for EHRLICH reagents, were only slightly detected.

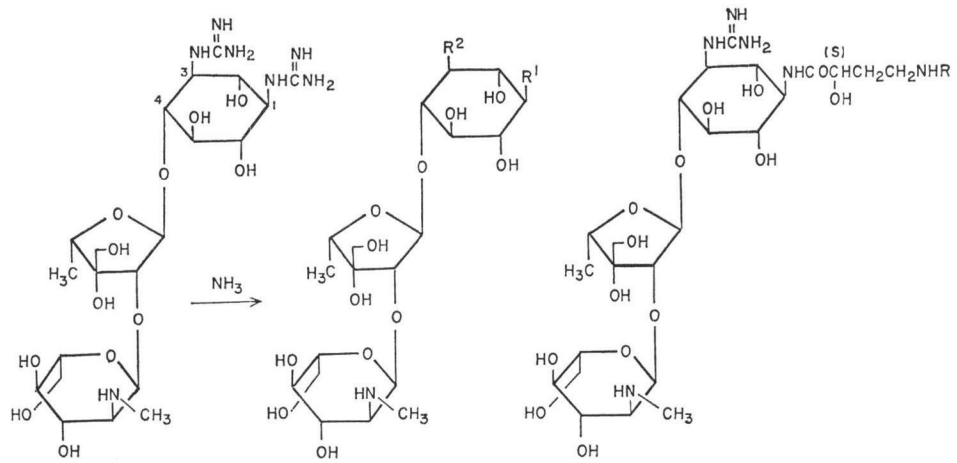
In the next place, we tried to hydrolyze DHSM in liquid ammonia, since the following reaction (1) is expected to occur without formation of any ureido compound. However, DHSM was found

$$-NHC(=NH)NH_2+NH_3 \longrightarrow -NH_2+NH_2C(=NH)NH_2 \qquad (1)$$

to be stable in liquid ammonia at $-50° \sim +25°C$ and after several days standing at room temperature in a sealed tube, DHSM was recovered unchanged. Addition of sodium amide to the ammonia solution of DHSM did not improve the situation. Therefore, an attempt was made to raise the temperature of the ammonia solution of DHSM and it was found that the optimum condition for the formation of **1** and **2** is heating the solution at 100°C for 24 hours.

Separation of **1** and **2** was carried out by column chromatography on Amberlite CG 50 developed with aqueous ammonium carbonate. The ratio of the products thus formed was estimated (before purification) to be 6 : 2 : 3 : 3 for 1-deamidino (**1**), 3-deamidino (**2**), 1,3-di(deamidino) derivatives (**3**) and unchanged DHSM. Predominant formation of **1** is noteworthy although the reason is not clear; however, we suppose that the ammonolysis may be assisted by neighbouring hydroxyl groups. 1-Guanidino group has two neighbouring hydroxyl groups at C-2 and C-6 but 3-guanidino group has only one at C-2. We have observed several similar instances in the basic de-*N*-acetylation of DHSM derivatives[2] and others* in which the presence or absence of a neighbouring hydroxyl group produced a remarkable influence on the hydrolysis of *N*-acetyl group.

* Details will be reported in the near future.



DHSM

1 $R^1 = NH_2$ $R^2 = NHC(=NH)NH_2$

2 $R^1 = NHC(=NH)NH_2$ $R^2 = NH_2$

3 $R^1 = R^2 = NH_2$

4 R = H

6 $R = C(=NH)NH_2$

The structures of **1** and **2** were determined by $\Delta[M]_{TACu}$ method[3]. TACu can form complex only with a pair of vicinal amino and hydroxyl groups having relative special orientations of 60° dihedral angle and the $\Delta[M]_{TACu}$ shows a value of $\pm 900°$, the sign being decided by counter-clockwise (positive) or clockwise (negative) orientation in the NEWMAN projection of -(NH_2)CH-CH(OH). When the method is applied to DHSM, TACu should form complex only at $C_{(2)}NHCH_3$-$C_{(3)}OH$ *of the N*-methyl-L-glucosamine moiety and show $\Delta[M]_{TACu}+900°$. The value $\Delta[M]_{TACu}+1020°$ (lit[5] $+1020°$) of DHSM indicates that the above presumption is correct. The $\Delta[M]_{TACu}+1570°$ of **2** shows that another copper complex (positive in sign) is formed in addition to the above. This value is reasonable when considering another complexion at $C_{(2)}OH$-$C_{(3)}NH_2$ of the deamidinostreptidine moiety. The structure of **2**, therefore, was decided to be 3-deamidinodihydrostreptomycin. In the case of **1**, the value $\Delta[M]_{TACu}+830°$ indicates the presence of $C_{(1)}NH_2$ group because, in this case, copper complex can be formed between the amino group at C-1 and $C_{(2)}OH$ and between the amino group and $C_{(6)}OH$ competitively, thus resulting in cancellation of the $\Delta[M]_{TACu}$ values in these sites. The structure of **1**, therefore, was decided to be 1-deamidinodihydrostreptomycin.

Table 1. $\Delta[M]_{TACu}$ values before and after additions of ammonia.

	$[M]_{H_2O}$	$[M]_{TACu}$[1]	$\Delta[M]_{TACu}$	$[M]_{TACu-NH_3}$[2]	$\Delta[M]_{TACu-NH_3}$
1	−1190	−360	+830	−280	+910
2	−1290	+280	+1570	+420	+1710
3	−1300	−960	+340	+1230	+2530[3]
DHSM	−1250	−170	+1020	−80	+1170

1) 0.01 mmol of each sample (as trihydrochloride, ~7 mg) was dissolved in 1.0 ml of 0.16 mmol TACu solution and the rotation of the solution was measured in 0.1 dm tube at 436 nm.

2) After measuring the $[M]_{TACu}$, 0.001 ml each of 15 M ammonium hydroxide solution was added to the solution (0.55 ml) and the rotation of the solution was measured. The procedure was repeated until the rotation reached to a constant value. The final rotation for **1**, **2** and DHSM was reached after three additions, respectively.

3) The change of $\Delta[M]_{TACu}$ was as follows: 0.001 ml, +520; 0.002, +710; 0.003, +820; 0.004, +1040; 0.009, +1970; 0.014, +2340; 0.019, +2530; 0.024, +2510; 0.031, +2470.

Chart 1.

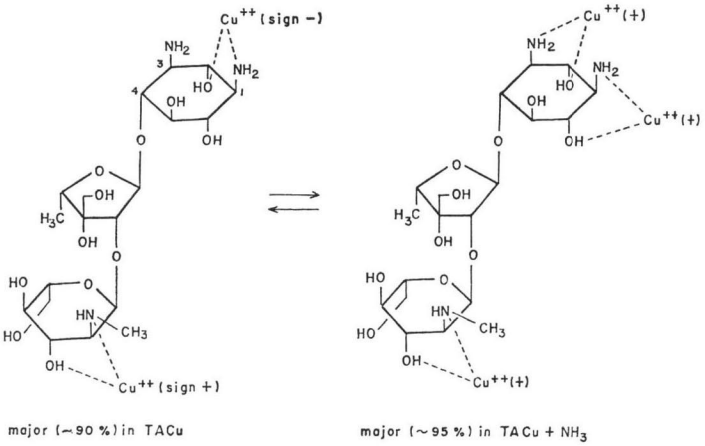


Table 2. Antibacterial spectra of **1**, **2**, **3**, **4**, **6**, DHSM and bluensomycin

Test organisms*	Minimal inhibitory concentration (mcg/ml)						
	1	**2**	**3**	**4**	**6**	Bluenso-mycin	DHSM
Staphylococcus aureus FDA 209P	>100	>100	>100	>100	>100	100	6.25
" " SM, STF	>100	>100	>100	>100	>100	>100	>100
Bacillus subtilis PCI 219	25	100	>100	50	12.5	6.25	<0.2
" *agri*	>100	>100	>100	>100	>100	>100	>100
Escherichia coli K-12	50	>100	>100	50	25	25	0.78
" " ML 1629	>100	>100	>100	>100	>100	>100	>100
" " ML 1410	50	>100	>100	50	100	50	1.56
" W 677	25	100	>100	50	25	25	1.56
" JR66/W677	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa A3	>100	>100	>100	>100	>100	100	>100
Mycobacterium smegmatis ATCC 607**	6.25	>100	>100	25	12.5	12.5	0.78

* Agar dilution streak method (nutrient agar, 37°C ,18 hours)
** 48 hours.

The value $\Delta[M]_{TACu}+340°$ (lit[4] $+300°$) of **3** suggests the presence of competition in copper complexion in **3**. Trial addition of a slight amount of ammonia to the TACu solution of **3** caused a great increase in $\Delta[M]_{TACu}$ value and the value was reached to an end by further additions of ammonia (Table 1). The value (+2530) shows that incomplete three copper complexes (all positive in sign) occurred in **3**. This phenomenon is explained if the sites of copper complex are changed in equilibrium according to the ammonia content as depicted in Chart 1.

Antibacterial activity of **1**, **2**, **3** and DHSM are shown in Table 2. The result shows that the two guanidino groups in DHSM are necessary to exhibit the activity, however, the guanidino group at C-3 is more essential than the other, because **1** still retained weak activity in a level similar to bluensomycin[5] which has carbamoyloxy group instead of the guanidino group at C-1, whereas **2** and **3** had almost no antibacterial activity.

Butirosins[6] and amikacin[7] represent a new type of aminoglycoside antibiotics which have a unique side chain, (*S*)-4-amino-2-hydroxybutyryl residue. We, therefore introduced the acyl residue to

the C-1 amino group of **1**, and obtained 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-1-deamidinodihydrostreptomycin (**4**). Furthermore, (*S*)-4-guanidino-2-hydroxybutyric acid (**5**) was prepared from the above acid by reaction with *S*-methylisothiourea, and coupled to **1** to give 1-deamidino-1-*N*-[(*S*)-4-guanidino-2-hydroxybutyryl] dihydrostreptomycin (**6**).

Antibacterial activity of the 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] and 1-*N*-[(*S*)-4-guanidino-2-hydroxybutyryl] derivatives (**4**, **6**) are shown in Table 2. The result shows that introduction of these groups gave no change substantially in the activity of **1** for both common and resistant bacteria. This shows that the successful modification of kanamycins and ribostamycin series by the N-1 acylation is not available for streptomycin series.

Experimental

Microcrystalline cellulose powder "Avicel" was purchased from Funakoshi Co., Tokyo, Japan.

1-Deamidino-, 3-deamidino- and 1,3-di(deamidino)dihydrostreptomycin (**1**, **2**, **3**)

In a 500-ml stainless-steel bomb, DHSM sesquisulfate monohydrate (9.2 g) was placed and, after substitution of the air in the vessel with dry ammonia, the whole content was cooled to $-50°C$. To it, liquid ammonia ($\sim$300 ml) stored in a cold ($-50°C$) graduated 500 ml glass pressure bottle containing sodium metal ($\sim$1 ml) was introduced by raising the temperature gradually. The vessel, after stoppered tightly, was heated at 100°C for 24 hours. The vessel was cooled to $-50°C$, opened, and the ammonia was gently removed by raising the temperature ($-50° \rightarrow +30°C$; aspirator was finally used). The residue was dissolved in water (30 ml) and the solution was chromatographed on a column of Dowex 1×2 (OH form, 200$\sim$400 mesh, 270 ml) with water as developer and the eluates were detected by 1) TLC with "Avicel" developed with 1-BuOH - EtOH - $CHCl_3$ - 17% NH_3 (4 : 7 : 2 : 7) and colorized by diacetyl (for detection of a guanidine group) and by 2) TLC with silica gel developed with $CHCl_3$ - MeOH - 17% NH_3 (2 : 2 : 1) and colorized by sulfuric acid. Guanidine (95$\sim$205 ml, Rf (Avicel) 0.44), recovered dihydrostreptomycin (135$\sim$215 ml, Rf (Avicel) 0.17, Rf (silica gel) 0), a mixture of **1**+**2** (190$\sim$285 ml, Rf (Avicel) 0.33, Rf (silica gel) 0.05) and **3** (400$\sim$640 ml, Rf (silica gel) 0.56; negative for diacetyl) were eluted. From 190$\sim$285 ml fractions, a mixture of **1** and **2** contaminated slightly by guanidine and dihydrostreptomycin was isolated (4.4 g) and from 400$\sim$640 ml fractions, chromatographically homogeneous solid of **3** was obtained, 1.5 g (22%), $[\alpha]_D^{18}$ $-117°$ (*c* 0.4, H_2O) (lit[1] $-121°$).

Calcd. for $C_{19}H_{37}N_3O_{12}\cdot\frac{1}{2}H_2CO_3\cdot\frac{1}{2}H_2O$: C 43.30, H 7.29, N 7.79%.
Found: C 43.68, H 7.31, N 7.69%.

Separation of **1** and **2** was performed as follows: The crude mixture (4.4 g) described above was dissolved in 100 ml of 0.5% aqueous $(NH_4)_2CO_3$ and the solution was charged on a column (4×80 cm) of Amberlite CG50 (100$\sim$200 mesh). The column was washed in advance with 8% $(NH_4)_2CO_3$ ($\sim$4 liters) and with 0.5% $(NH_4)_2CO_3$ solution ($\sim$2 liters) in turn. The charged column was firstly washed with 0.5% $(NH_4)_2CO_3$ (2.2 liters) and then with $(NH_4)_2CO_3$ with gradual increase in concentration (2% $\rightarrow$8%). The fraction of 900$\sim$1,040 ml was concentrated *in vacuo* in a warm bath (35°C) to give a residue which was dissolved in water and the solution was concentrated. The procedure was repeated five times more to remove remaining ammonium carbonate. A solid of **2** was obtained as carbonate (0.98 g). Similar treatment of the fraction of 1,100$\sim$1,500 ml gave a solid of **1** (3.1 g) as carbonate. On TLC with "Avicel" developed with pyridine - H_2O - AcOEt - AcOH (5 : 3 : 2 : 1), **1** and **2** gave spots of Rf 0.36 and 0.41, respectively.

An aqueous solution of the above carbonate (**2**) was neutralized with hydrochloric acid* to pH 6.

* Attempt to isolate the pure free base of **1** or **2** from the corresponding carbonate by passing a column of Dowex 1×2 (OH form) gave no good yield of the base owing to long-lasting tailing of the base from the column. However, when the carbonate salt was neutralized with hydrochloric acid in advance to the column treatment, the free base was obtained in good yield without tailing, although the base obtained has a tendency to catch CO_2 from the air fairly quickly.

The solution was slowly passed through a column of Dowex 1×2 (Cl form, 100~200 mesh, 50 ml) and eluted with water. The diacetyl positive fractions were collected and concentrated. The concentrate was acidified to pH 3 by addition of hydrochloric acid and to the solution, acetone was added to cause precipitation. The precipitate was washed thoroughly with acetone and dried to give trihydrochloride of **2**, 910 mg (11%), $[\alpha]_D^{18}$ −99° (*c* 0.5, H_2O). PMR (D_2O) (benzene was used as internal reference, δ 7.53): δ 1.28 (3H d, J=6.5 Hz, CCH_3), 2.91 (3H s, NCH_3), 5.48 (1H d, J=2.7 Hz, H-1′), 5.78 (1H d, J=3.5 Hz, H-1″).

Calcd. for $C_{20}H_{39}N_5O_{12}\cdot 3HCl\cdot H_2O$: C 35.91, H 6.63, N 10.47, Cl 15.90%.
Found: C 36.01, H 6.55, N 10.03, Cl 16.02%.

Similar treatment of **1** carbonate gave the trihydrochloride, 2.96 g (36%), $[\alpha]_D^{18}$ −93° (*c* 0.5, H_2O). PMR (D_2O): δ 1.22 (3H s, J=6.5 Hz, CCH_3), 2.90 (3H s, NCH_3), 5.36 (1H d, J=1.7 Hz, H-1′), 5.61 (1H d, J=3.5 Hz, H-1″).

Calcd. for $C_{20}H_{39}N_5O_{12}\cdot 3HCl\cdot H_2O$: C 35.91, H 6.63, N 10.47, Cl 15.90%.
Found: C 36.11, H 6.88, N 10.14, Cl 16.12%.

1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]-1-deamidinodihydrostreptomycin (**4**)

To a solution of **1** trihydrochloride monohydrate (167 mg, 0.25 mmol) in aqueous dioxane (1 : 5, 6 ml), triethylamine (0.3 ml, 2.5 mmols) and *N*-hydroxysuccinimide ester[7] (300 mg, 0.6 mmol) of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid were added and the solution was kept at room temperature for 16 hours. The *N*-hydroxysuccinimide ester (50 mg, 0.1 mmol) was further added and the solution was again kept at room temperature for 20 hours. On checking of the solution by "Avicel" TLC (1-BuOH - EtOH - H_2O - 28% NH_3=16 : 4 : 8 : 1 detected by diacetyl), the solution showed a spot at Rf 0.5 accompanied by minor spots of Rf 0.1 (**1**) and 0.55 (diacyl derivative). The solution was concentrated and the residue was extracted with aqueous methanol (9 : 1). The solution was chromatographed on a column of Dowex 1×2 (OH form) with water and the fractions containing **4** (detected by silica gel TLC with $CHCl_3$ - MeOH - 17% NH_3=2 : 2 : 1; Rf 0.27) was concentrated to ~2 ml. During this procedure, the *N*-benzyloxycarbonyl group of the product was partially cleaved. To the concentrate, palladium black and drops of acetic acid were added and the mixture was treated under hydrogen (50 lb/in^2). The reaction mixture was filtered and the filtrate was concentrated to give a solid (69 mg). The solid was purified by Amberlite CG50 in a manner as described for **1** to give a solid of carbonate of **4** (34 mg). The solid was then transformed to its trihydrochloride in a manner as described for **1** to give a solid, 29 mg (15%), $[\alpha]_D^{18}$ −94° (*c* 0.6, H_2O). Rf_{DHSM} 0.45 (ppc with Whatman No. 1 paper, descending, developed with 1-BuOH - pyridine - H_2O - AcOH=6 : 4 : 3 : 1). PMR (D_2O) (benzene, as internal reference): δ 1.23 (3H d, J=6.5 Hz, CCH_3), 2.1 (2H m, $COCH(OH)\underline{CH_2}$-CH_2NH_2), 2.88 (3H s, NCH_3), 5.36 (1H d, J=1.7 Hz, H-1′), 5.59 (1H d, J=3.5 Hz, H-1″).

Calcd. for $C_{24}H_{45}N_6O_{14}\cdot 3HCl\cdot H_2O$: C 37.48, H 6.55, N 10.93, Cl 13.83%.
Found: C 37.63, H 6.48, N 10.43, Cl 14.01%.

(*S*)-4-Guanidino-2-hydroxybutyric acid (**5**)

To a mixture of (*S*)-4-amino-2-hydroxybutyric acid (201 mg) and *S*-methylisothiourea (235 mg) in water (1 ml), 2 M aqueous sodium hydroxide (0.8 ml) was added and the solution was kept at room temperature for 25 hours and then in a refrigerator for 5 hours. Resulting precipitate was filtered and washed with ethanol. From the mother liquor anothre crop was obtained. Total yield 216 mg (80%), mp 246~248°C (dec), $[\alpha]_D^{21}$ −21° (*c* 0.5, H_2O).

Calcd. for $C_5H_{11}N_3O_3$: C 37.26, H 6.88, N 26.07%.
Found: C 36.86, H 6.70, N 25.73%.

1-Deamidino-1-*N*-[(*S*)-4-guanidino-2-hydroxybutyryl]dihydrostreptomycin (**6**)

Compound **5** was treated with 1 equivalent of hydrochloric acid in water and the solution was concentrated to give a thick syrup, which was dried *in vacuo* to give hydrochloride of **5**. To a solution of the hydrochloride (65 mg) in DMF (1.5 ml), *N*-hydroxysuccinimide (42 mg) and dicyclohexylcarbodiimide (78 mg) were added and the solution was kept at room temperature overnight. The dicyclohexylurea precipitated was removed by centrifuge and the precipitate was washed with DMF. To the combined DMF solution (~3 ml), **1** trihydrochloride monohydrate (34 mg) and triethylamine (0.2 ml)

were added and the solution was kept at room temperature overnight. The solution was concentrated and the residue was dissolved in water. The aqueous solution was treated successively with Dowex 1×2 (OH form), Amberlite CG50 and Dowex 1×2 (Cl form) in a manner as described for **1** to give a solid of trihydrochloride of **6**, 21 mg (51%), $[\alpha]_D^{18} -83°$ (*c* 0.6, H_2O). Rf_{DHSM} 0.7 (ppc in the same manner as described for **4**).

Calcd. for $C_{25}H_{47}N_8O_{14} \cdot 3HCl \cdot H_2O$: C 37.02, H 6.46, N 13.81, Cl 13.11%.
Found: C 36.93, H 6.41, N 13.29, Cl 12.78%.

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