TOTAL CHEMICAL STRUCTURE OF STREPTOTHRICIN

Sir:

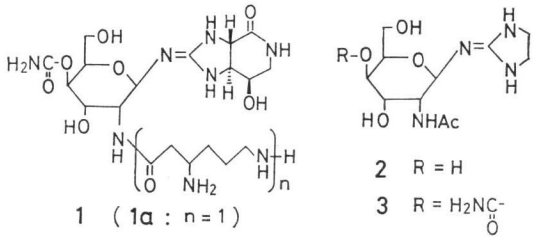
The chemical structure of streptothricin has not been fully elucidated yet in spite of its very early discovery.[1] In 1961, VAN TAMELEN *et al.* proposed its structure,[2] and the β-glycosidic linkage between gulosamine and the exocyclic guanidine nitrogen of streptolidine was unequivocally confirmed by our recent synthesis of N^g-streptolidyl gulosaminide.[3] However, the location of the carbamoyl group still remained unknown. We now clearly show that this substituent is on the 4-hydroxyl group of gulosamine moiety by comparison of ^{1}H NMR spectra of natural streptothricin F and two synthetic model compounds **2** and **3**. Thus, the structure of streptothricin is finally established as **1**.

Recently, KAWAKAMI *et al.* concluded on the basis of ^{13}C NMR data that the carbamoyl group in other streptothricin-like antibiotics, LL-AB664 and LL-AC541, are also on the same hydroxyl group.[4] Though their assignment of signals are tentative and therefore the evidence seems to be rather indirect, coincidence of the both conclusions suggests that the 4-carbamoyl structure is common in streptothricin group antibiotics.

A sample of streptothricin F (**1a**) was isolated from a natural streptothricin mixture by column chromatography on Sephadex G-25 (BuOH - AcOH - pyridine - H_2O, 15: 3: 10: 12) and converted into its trihydrochloride by passing through a column of Dowex 1X8 (Cl^- form). The ^{13}C NMR spectrum and optical rotation value ($[\alpha]_D^{20}$ −46.7° (*c* 1.0, H_2O)) of this sample were in good agreement with those reported in the literature.[5] After several unsuccessful attempts to determine the position of carbamoyl substituent chemically, we planned to analyze the high magnetic field NMR spectrum of **1a**.

In the 360 MHz ^{1}H NMR spectrum of **1a** hydrochloride, signals of all protons separated well and could be completely assigned by decoupling experiments. The chemical shifts of protons of its gulosamine moiety were then compared with those of a reference compound **2***, which contains simple β-glycosyl guanidine and *N*-acyl moieties but no carbamoyl group. As shown in Table 1, H-4 of streptothricin F (**1a**) exhibits a distinct low field shift of 0.99 ppm.

Fig. 1.



This indicates the presence of the carbamoyl group on the 4-hydroxyl group.

In order to confirm this assumption, another model compound **3** which carried the 4-*O*-carbamoyl moiety was next prepared, Allyl 6-*O*-acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-3-*O*-MEM**-α-D-gulopyranoside (**4**) (mp 80~82°C, $[\alpha]_D^{16}$ +50.7° (*c* 1.00, $CHCl_3$)) obtained from D-glucosamine[6] was employed as the starting material. After alkaline hydrolysis ($Ba(OH)_2$ in 30% EtOH under reflux for 30 minutes) of **4** followed by *N*-acetylation, the two free hydroxyl groups were benzylated (BzlBr and BaO-$Ba(OH)_2 \cdot 8H_2O$ in DMF at room temperature) to give **5** (96% from **4**, syrup).*** Removal of the MEM group in **5** ($ZnBr_2$ in dry CH_2Cl_2 at room temperature) yielded **6** (73%, syrup), which was treated with chloroacetyl isocyanate (in CH_2Cl_2 at 0°C for 40 minutes) and then with Zn-powder in methanol (at room temperature for 3 hours) to afford the 4-*O*-carbamoyl derivative (**7**) (85%, syrup, ^{13}C NMR: δ 156 and 170 ppm (carbamoyl and acetyl carbonyl carbons, respectively)). After the allyl group had been removed (10% Pd-C in EtOH - AcOH - H_2O (2: 1: 1) at 75~85°C for 6 hours), the product was treated with *p*-nitrobenzoyl chloride in pyridine - CH_2Cl_2 to give 1-β-*O*-*p*-nitrobenzoate (**8**) (mp 178~178.5°C (dec), ^{1}H NMR δ 5.95 ppm (1H d, $J_{1,2}$=9 Hz, H-1)) which was then converted into β-glycosyl isothiocyanate **9** (52%, syrup; ^{1}H NMR δ 4.98 ppm (1H d, $J_{1,2}$=9.5 Hz, H-1); IR 2040 cm^{-1} (N=C=S)) in a similar manner described previously[3] [1] dry

* Compound **2** was prepared by *N*-acetylation (Ac_2O - Et_3N in MeOH) of a derivative with free amino group described previously.[3]

** 2-Methoxyethoxymethyl

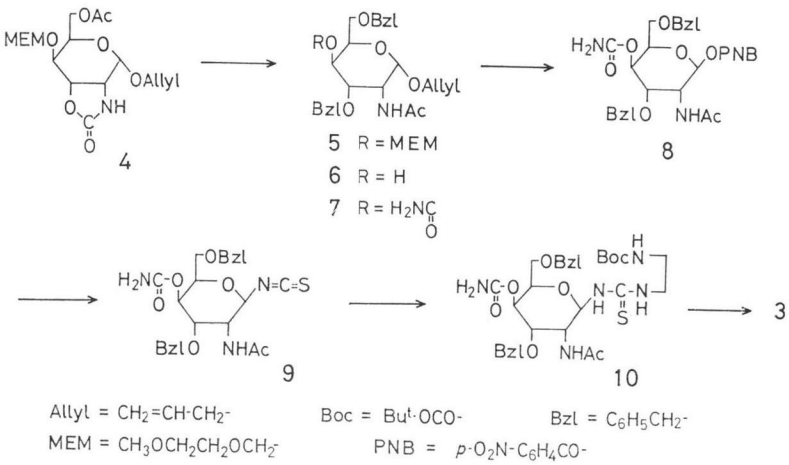
*** All syrupy compounds were purified by silica gel column or thin-layer chromatography and the structures were confirmed by spectroscopic methods.

Table 1. Chemical shifts and coupling constants of gulosamine protons.

Compound	H-1	H-2	H-3	H-4	H-5	H-6	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
1a	5.14	4.28	4.20	4.80	4.36	3.75	9.7	3.4	3.4	0	6.0
2	4.91	4.22	4.02	3.81	4.08	3.75	9.7	3.3	3.3	0	6.0
3	4.99	4.15	4.10	4.73	4.24	3.68	9.6	3.4	3.4	1.5	6.0

Spectra were recorded at 360 MHz for D_2O solutions of hydrochlorides. Chemical shifts and coupling constants are given in ppm (δ value from ext. DSS) and Hz, respectively.

Fig. 2.



HBr in CH_2Cl_2 at room temperature for 100 minutes, 2) KSCN in dry acetone at room temperature overnight]. Coupling of **9** with mono-Boc-ethylenediamine (in THF at room temperature overnight) afforded syrupy thiourea derivative **10** (61%; IR: 1720 (H_2NCO–), 1530 cm^{-1} (–NH–CS–NH–)). This compound was then subjected to the procedure for glycosyl guanidine formation [1) EtI in THF under reflux for 6 hours, 2) trifluoroacetic acid for 20 minutes, 3) Et_3N in THF].[3] Hydrogenolytic removal of the *O*-benzyl groups followed by purification by a Sephadex G-25 column (BuOH - AcOH - pyridine - H_2O, 30: 3: 10: 12) gave the desired model compound **3** acetate (24% from **10**, $[\alpha]_D^{17}$ +3.9° (*c* 0.45, H_2O)).

NMR signals of the corresponding sugar protons in streptothricin F and **3** show very similar chemical shifts and coupling patterns (Table 1), clearly indicating the 4-*O*-carbamoyl structure **1** for the natural antibiotic. This conclusion was finally confirmed by our recent total synthesis of streptothricin F (**1a**).[6]

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