STRUCTURE AND SYNTHESIS OF NEOTHRAMYCIN

Sir:

Neothramycin is a 1,4-benzodiazepine antibiotic isolated from a culture filtrate of *Streptomyces* No. MC916-C4, and exhibits a marked therapeutic effect on mouse leukemia L-1210 and YOSHIDA rat sarcoma.[1] In this communication, the structural elucidation and total synthesis are reported.

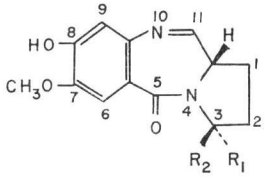
Neothramycins A and B (**1a** and **1b**) which are interconvertible in aqueous solution have been isolated.[1] Both **1a** and **1b** have the same formula $C_{13}H_{14}N_2O_4$ derived from the high-resolution mass spectrum, and give positive RYDON-SMITH, red tetrazolium, fast blue B, BRADY and ninhydrin (weak brownish yellow) reactions. The former (**1a**) shows mp 132~147°C (dec.), $[\alpha]_D^{26}$ +272° (*c* 0.52, dioxane), and UV maxima at 223, 240 (sh), 265 and 318 nm in 90% aqueous methanol. The latter (**1b**) shows mp 144~151°C (dec.), $[\alpha]_D^{26}$ +314° (*c* 0.48, dioxane), and UV maxima at 224, 240 (sh), 265 (sh) and 318 nm.

A mixture of methylneothramycins A and B (**2a** and **2b**) was easily obtained either from **1a** or **1b** by treatment with anhydrous methanol.[1] The antibiotic, **1a** or **1b**, was also converted into a mixture of butyl derivatives by treatment with anhydrous 1-butanol at 50°C for 16 hours. Butylneothramycin A (**3a**) was crystallized from benzene as colorless plates, mp 155~156°C (dec.), $[\alpha]_D^{26}$ +1025° (*c* 0.43, dioxane) and *m/e* 318 (M^+). Butylneothramycin B (**3b**) was obtained as a colorless powder, mp 52~59°C (dec.), $[\alpha]_D^{26}$ +772° (*c* 0.31, dioxane) and *m/e* 318. Catalytic hydrogenation of **3a** in dioxane with 10% palladium-charcoal at room temperature for 1.5 hours at 2.1 kg/cm^2 in a PARR apparatus gave crystalline butyldihydroneothramycin A (**4**), mp 128~130°C (dec.), $[\alpha]_D^{24}$ +150° (*c* 0.1, dioxane), *m/e* 320 (M^+).

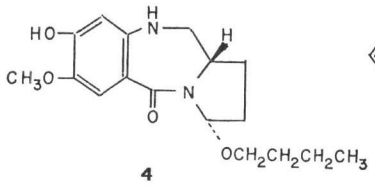
Alkaline hydrolysis of **3a** with $Ba(OH)_2$-saturated aqueous solution at 60°C for 89 hours afforded brownish crystals of 4-hydroxy-5-methoxyanthranilic acid, mp 133~134°C (dec.), UV maxima at 220, 233, 261 and 335 nm in methanol, *m/e* 183 (M^+). It was confirmed to be identical with an authentic sample which was synthesized by nitration of O-benzylvanillic acid, followed by catalytic hydrogenation in methanol with 10% palladium-charcoal.

Treatment of **3a** with phenyldiazomethane in ether at room temperature for 39 hours followed by mild hydrolysis with 0.01 N HCl - dioxane (1 : 1 in volume) at room temperature for 1 hour gave a mixture of O-benzylneothramycins A and B which was treated with $KMnO_4$ in acetone at room temperature for 1 hour to afford an oxidized compound (**5**) as a colorless powder, mp 107~113°C (dec.), $[\alpha]_D^{24}$ +214° (*c* 0.1, dioxane), *m/e* 366.1179 (calcd. for $C_{20}H_{18}N_2O_5$: *m/e* 366.1213), IR(KBr) ν_{CO} 1770, 1690 and 1610 cm^{-1}, UV maxima at 210, 243, 275 (sh) and 310 nm. Acid hydrolysis of **5** with constant boiling HCl at 105°C for 16 hours in a sealed tube gave a slightly racemized mixture of L-glutamic acid hydrochloride, $[\alpha]_D^{26}$ +26° (*c* 0.4, 6 N HCl) (authentic L-isomer: +30°).

Therefore, the stereochemistry of **1a** or **1b** at C-11a was concluded to be the *S*-configuration. As shown in Table 1, the coupling constants ($J_{11a,1}$) indicate that the pyrrolidine ring in **2a** and **2b** exists in two different twist conformations, and the splitting patterns of the protons [(3-H)-



1a: R_1 = OH, R_2 = H
1b: R_1 = H, R_2 = OH
2a: R_1 = OMe, R_2 = H
2b: R_1 = H, R_2 = OMe
3a: R_1 = OBu, R_2 = H
3b: R_1 = H, R_2 = OBu



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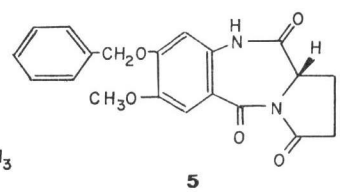

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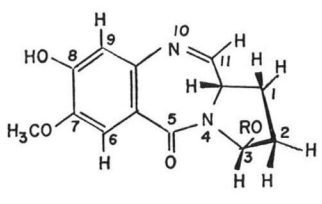
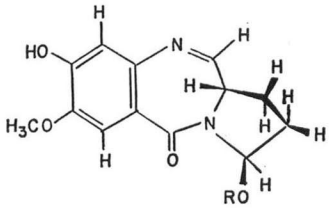
Table 1. PMR spectra of neothramycins and methylneothramycins

Proton	1a	2a	1b	2b
	δ ppm (J Hz)	δ ppm (J Hz)	δ ppm (J Hz)	δ ppm (J Hz)
1–H_2, 2–H_2	1.7~2.5 m	1.8~2.6 m	1.7~2.5 m	1.8~2.3 m
3–H	5.69 dd ($J_{3,2}$ 5.0)	5.56 d ($J_{3,2}$ 4.2)	5.78 m	5.35 dd ($J_{3,2}$ 3.0, 2.0)
3–OH or 3–OCH_3	5.00 d ($J_{3,OH}$ 3.0)	3.28 s	5.10 d ($J_{3,OH}$ 2.6)	3.44 s
6–H	7.43 s	7.48 s	7.40 s	7.36 s
7–OCH_3	3.90 s	3.90 s	3.88 s	3.88 s
8–OH	8.00 s	8.04 s	7.98 s	7.94 s
9–H	6.70 s	6.75 s	6.69 s	6.64 s
11–H	7.62 d ($J_{11,11a}$ 4.4)	7.73 d ($J_{11,11a}$ 4.5)	7.70 d ($J_{11,11a}$ 4.2)	7.54 d ($J_{11,11a}$ 4.4)
11a–H	3.80 m	3.72 m ($J_{11a,1}$ 9.0, 8.5)	3.78 m	3.80 dd ($J_{11a,1}$ 7.5)

PMR spectra were measured in deuterodioxane using TMS as the internal reference.



1a: R = H
2a: R = CH_3



1b: R = H
2b: R = CH_3

(2-H)-(1-H)-(11a-H)] were carefully analyzed. Thus, the configuration at C-3 can reasonably be assumed to be the *S*-configuration in **2a** or **1a**, and *R*-configuration in **2b** or **1b** based on consideration of the PMR spectra. From the foregoing results, the absolute structures of neothramycins A and B (**1a** and **1b**) can be proposed to be (3*S*, 11a*S*)- and (3*R*, 11a*S*)-2,3,5,11a-tetrahydro-3,8-dihydroxy-7-methoxy-5-oxo-1*H*-pyrrolo [2,1-*c*] [1,4] benzodiazepine, respectively. These structures and the structures of their derivatives can be shown by **1**~**5**.

Based on a PMR experiment, it is confirmed that the azomethine function in **1a** or **1b** is easily hydrated to form the carbinolamine in an aqueous solution.

As shown in Table 2, all carbon atoms in the structures of **2a** and **2b** can be identified by carbon-13 FOURIER-transform NMR spectra.

We attempted the total synthesis of neothramycin through a new route different from that reported for anthramycin.[2] Starting from vanillic acid (**6**), neothramycin synthesis has been accomplished by 7-step procedure* (Fig. 1), in which the key stage involves the ring formation of the 1,4-benzodiazepine.

O-Benzylvanillic acid (**7**, mp 170.5~171.5°C, lit.[3] mp 171~172°C) prepared from **6** by treatment with benzyl chloride in a mixture of 2 N NaOH and acetone at 50°C for 16 hours, was nitrated with fuming nitric acid (90%) at −60°~−20°C for 1 hour, affording yellowish needles of 5-methoxy-2-nitro-4-*p*-nitrobenzyloxybenzoic acid (**8**, mp 210~212°C, 66% yield from **7**). Treatment of **8** with thionyl chloride in an oil bath at 100°C for 3 hours followed by coupling with γ-methyl L-glutamate hydrochloride in dichloromethane in the presence of triethylamine at room temperature for 2.5 hours afforded a

* Elemental analysis and spectroscopy gave satisfactory data on all compounds cited in the synthetic study.

Fig. 1. Total synthesis of neothramycin

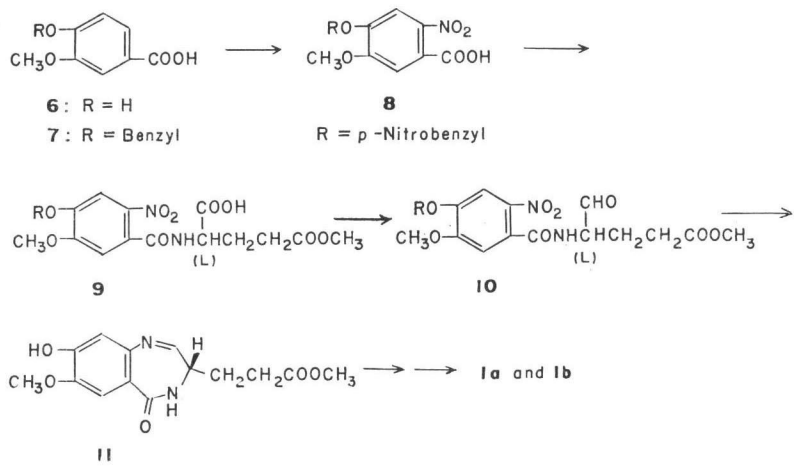


Table 2. Carbon-13 spectra of methylneothramycins

Carbon	2a		2b
	Chemical shift (δ)	Multiplicity on off-resonance	Chemical shift (δ)
1	27.3*	t	27.6*
2	31.9*	t	31.8*
3	87.6	d	88.5
5	166.0	s	165.4
5a	118.7	s	120.6
6	113.9**	d	113.2**
7	146.4***	s	146.3***
8	150.0	s	149.6
9	112.5**	d	112.5**
9a	142.8***	s	141.3***
11	164.8	d	164.2
11a	54.3	d	54.0
7-OCH_3	56.0	q	56.1
3-OCH_3	56.0	q	57.6

δ: ppm from TMS (internal) in deuterodioxane. *, **, ***: Assignments within any vertical column may be reversed.

yellowish crystalline powder of an acylglutamate (**9**), mp 107~111°C (dec.), $[\alpha]_D^{22}$ +26° (*c* 1.42, dioxane) in 74% yield. According to the method of STAAB,[4] **9** was treated with N,N′-carbonyldiimidazole and $LiAlH_4$ in tetrahydrofuran to give a yellowish crystalline aldehyde (**10**, mp 133~136°C (dec.), 33% yield). Catalytic hydrogenation of **10** in methanol with 10% palladium-charcoal at room temperature for 30 minutes under atmospheric pressure gave a colorless powder of (*S*)-4,5-dihydro-8-hydroxy-7-methoxy-3-methoxycarbonylethyl-3*H*-1,4-benzodiazepin-5-one (**11**), mp 72~84°C (dec.), $[\alpha]_D^{22}$ +71° (*c* 0.17, dioxane) in 45% yield. Hydrolysis of the ester in **11** with NaOH in an aqueous dioxane followed by acidification with HCl to pH 4.0, and lyophilization gave a powder containing the free acid. The powder dissolved in anhydrous tetrahydrofuran or dioxane was treated with N,N′-carbonyldiimidazole at 50°C and thereafter reduced with $LiAlH_4$ at −60°C or $NaBH_4$ at 0°C to afford a mixture of synthetic **1a** and **1b** (3~10% yield from **11**), which was separated by preparative thin-layer chromatography developed with chloroform - methanol (10: 1 in volume).

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