SYNTHESIS OF THE 3-O-DEMETHYL AND 2″-N-FORMIMIDOYL DERIVATIVES OF ISTAMYCIN B

Sir:

Istamycins A and B[1], new members of fortimicin-group antibiotics are produced by *Streptomyces tenjimariensis* and exhibit good activity in inhibiting the growth of Gram-positive and Gram-negative bacteria except *Pseudomonas*. Recently, we synthesized istamycin A[2] and its 6′-N,3-O-didemethyl and 4-N,6′-N,3-O-tridemethyl derivatives[3] starting from 3′,4′-dideoxyneamine through an aziridine derivative. The former derivative was more active than istamycin A against *Pseudomonas* strains. On the other hand, it was reported that 2″-N-formimidoylfortimicin A (SF-2052)[4] produced by *Dactylosporangium matsuzakiense* showed interesting properties. In this communication, we wish to report the synthesis of the 3-O-demethyl and 2″-N-formimidoyl derivatives of istamycin B (**1**) starting from istamycin B_0[5] (deglycylistamycin B, **2**).

Ether cleavage of **2** with 48% hydrobromic acid[6] or 57% hydroiodic acid in a sealed tube at 90~93°C for 4 hours followed by column chromatography on CM-Sephadex C-25 (NH_4^+, eluted with a linear gradient of 0.15 M~0.70 M ammonia) afforded 3-O-demethylistamycin B_0 (**3**) as the dicarbonate (49%~50% yield), mp 122~126°C (decomp.), $[\alpha]_D^{25}$ +129° (*c* 1, water), MS *m/z* 319 $[(M+1)^+]$, ^1H NMR (D_2O, pD 2.0) δ 3.28 (3H s, NCH_3) and 3.36 (3H s, NCH_3).

Treatment of **3** with 3.9 equivalents of N-(benzyloxycarbonyloxy)succinimide in methanol at −10°C for 4 hours followed by chloroform extraction gave a crude powder of 1,2′,6′-tri-N-Cbz-3-O-demethylistamycin B_0 (**4**). Without purification of **4**, the powder was treated with N-hydroxysuccinimide ester of N-Cbz-glycine in dioxane in the presence of triethylamine at 55°C for 2 hours to yield 1,2′,6′,2″-tetra-N-Cbz-3-O-demethylistamycin B (**5**) which was purified by column chromatography on silica gel developed with a mixture of ethyl acetate and toluene (5: 2). The Cbz groups of **5** were removed by catalytic hydrogenation with 5% palladium on charcoal in a mixture of methanol, water and acetic acid (10: 2: 1) for

Table 1. ^{13}C chemical shifts (ppm) of istamycins B (**1**) and B_0 (**2**), 3-O-demethylistamycins B_0 (**3**) and B (**6**), 2″-N-formimidoylistamycin B (**13**) and 3-O-demethyl-2″-N-formimidoylistamycin B (**14**).

Carbon	**1** pD 5.4	**2** pD 5.5	**3** pD 2.0	**6** pD 2.0	**13** pD 6.5	**14** pD 6.5
1	47.2 d	46.6 d	47.1	47.3	47.2	47.2 d
2	29.3 t	29.4 t	32.1	33.5	29.3	33.5 t
3	71.9 d	74.8 d	65.1*	62.5	72.0	62.6 d
4	56.5 d	60.6 d	61.6*	57.4	56.5	57.5 d
5	68.3 d	62.5 d	61.9*	68.3	68.1	68.1 d
6	73.6 d	72.6 d	71.7	73.7	73.1	73.1 d
3-OCH_3	56.6 q	57.3 q			56.5	
4-NCH_3	32.0 q	31.7 q	31.7	31.9	31.9	31.9 q
1′	93.1 d	92.9 d	92.0	93.1	92.6	92.5 d
2′	49.6 d	49.2 d	49.1	49.6	49.5	49.5 d
3′	21.4 t	22.4 t	21.6	21.4	21.3	21.3 t
4′	26.7 t	26.7 t	26.3	26.7	26.7	26.7 t
5′	66.5 d	66.3 d	66.9	66.5	66.5	66.5 d
6′	53.0 t	53.0 t	52.8	52.9	53.0	53.0 t
6′-NCH_3	34.4 q	34.3 q	34.5	34.4	34.4	34.4 q
1″	168.7 s			168.9	169.1	169.3 s
2″	41.3 t			41.3	44.1	44.2 t
CH=NH					155.9	155.9 d

The ^{13}C FT NMR spectra were taken with a Varian XL-100 spectrometer in D_2O. Similar values with asterisks within each column may be interchanged. Assignments s, d, t and q show multiplicity on off-resonance experiment.

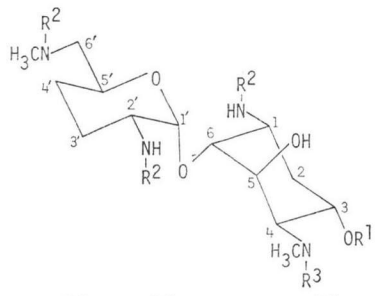
1.5 hours. The product was purified by column chromatography on Amberlite CG-50 (NH_4^+) eluted with a linear gradient of 0.2 M ~ 0.8 M ammonia to afford 3-O-demethylistamycin B (**6**) as the sesquicarbonate (23% yield from **3**), mp 182~184°C (decomp.), $[\alpha]_D^{25}$ +130° (*c* 1, water), MS *m/z* 376 [(M+1)$^+$], ^{1}H NMR (D_2O, pD 2.0) δ 3.19 (3H s, 6′-NCH_3), 3.54 (3H s, 4-NCH_3)

Table 2. Minimum inhibitory concentrations (μg/ml) of istamycin B (**1**), 3-O-demethylistamycin B (**6**), 2″-N-formimidoylistamycin B (**13**) and 3-O-demethyl-2″-N-formimidoylistamycin B (**14**).

Test organism	**1** (hemicarbonate hemihydrate)	**6** (sesqui-carbonate)	**13** (disulfate dihydrate)	**14** (disulfate trihydrate)
Staph. aureus FDA 209P	0.39	0.39	0.39	0.20
Staph. aureus Smith	<0.20	<0.20	<0.20	<0.20
Staph. aureus Ap01[a]	0.78	0.78	0.78	0.78
Staph. epidermidis 109[a]	0.78	0.78	0.78	0.78
Micrococcus flavus FDA 16	6.25	3.13	0.39	<0.20
Sarcina lutea PCI 1001	0.20	0.39	0.39	<0.20
B. anthracis	<0.20	<0.20	<0.20	<0.20
B. subtilis PCI 219	<0.20	<0.20	<0.20	<0.20
B. subtilis NRRL B-558	<0.20	<0.20	<0.20	<0.20
B. cereus ATCC 10702	1.56	1.56	1.56	1.56
Corynebact. bovis 1810	0.78	1.56	1.56	0.78
Myco. smegmatis ATCC 607	0.78	<0.20	0.39	<0.20
E. coli NIHJ	1.56	1.56	3.13	1.56
E. coli K-12	1.56	3.13	1.56	1.56
E. coli K-12 R 5[b]	3.13	6.25	3.13	3.13
E. coli K-12 R 388	1.56	1.56	1.56	1.56
E. coli K-12 J5R11-2[c]	1.56	1.56	1.56	1.56
E. coli K-12 ML 1629[c]	1.56	3.13	1.56	1.56
E. coli K-12 ML 1630	1.56	3.13	1.56	1.56
E. coli K-12 ML 1410	3.13	3.13	1.56	3.13
E. coli K-12 ML 1410 R 81[c]	1.56	3.13	1.56	1.56
E. coli K-12 LA 290 R 55[d]	3.13	6.25	1.56	1.56
E. coli K-12 LA 290 R 56	1.56	3.13	1.56	1.56
E. coli K-12 LA 290 R 64	1.56	3.13	1.56	1.56
E. coli W 677	1.56	1.56	1.56	0.78
E. coli JR 66/W 677[d,e]	3.13	6.25	1.56	3.13
E. coli K-12 C 600 R135[f]	12.5	6.25	50	12.5
E. coli JR 225[f]	0.78	1.56	1.56	0.78
Kl. pneumoniae PCI 602	1.56	3.13	1.56	1.56
Kl. pneumoniae 22 # 3038[d,e]	3.13	6.25	3.13	3.13
Sh. dysenteriae JS 11910	3.13	6.25	3.13	3.13
Sh. flexneri 4b JS 11811	3.13	6.25	3.13	3.13
Sh. sonnei JS 11746	3.13	6.25	3.13	3.13
Sal. typhi T-63	0.39	1.56	0.78	0.78
Sal. enteritidis 1891	1.56	3.13	1.56	1.56
Proteus vulgaris OX 19	0.39	0.78	0.78	0.39
Proteus rettgeri GN 311	12.5	12.5	12.5	12.5
Proteus rettgeri GN 466	6.25	6.25	6.25	3.13
Serratia marcescens	6.25	12.5	6.25	3.13
Serratia sp. SOU	100	25	>100	>100
Serratia sp. 4[d]	50	12.5	100	6.25
Providencia sp. Pv 16[g]	6.25	25	12.5	25
Providencia sp. 2991[g]	6.25	12.5	6.25	12.5
Ps. aeruginosa A 3	6.25	3.13	12.5	1.56
Ps. aeruginosa No. 12	100	12.5	100	12.5
Ps. aeruginosa H 9[e]	50	25	100	12.5
Ps. aeruginosa H 11	50	25	>100	25
Ps. aeruginosa TI-13[e]	25	12.5	100	6.25
Ps. aeruginosa GN 315[b]	50	12.5	50	6.25
Ps. aeruginosa 99[f]	>100	25	>100	100
Ps. aeruginosa B-13[e,e]	>100	50	>100	>100
Ps. aeruginosa 21-75[h]	100	50	>100	25
Ps. aeruginosa PSTI[f]	100	50	>100	25
Ps. aeruginosa ROS 134/PU 21[f]	>100	50	>100	50
Ps. aeruginosa K-Ps 102[i]	50	6.25	100	12.5
Ps. aeruginosa GN 907[i]	>100	>100	>100	>100

Resistance mechanisms: a) AAD (4′), b) AAC (6′), c) APH (3′)-I, d) AAD (2″), e) APH (3′)-II, f) AAC (3), g) AAC (2′), h) APH (3′)-III, i) permeability

Fig. 1.



	R^1	R^2	R^3 (1'' 2'')
1	CH_3	H	$COCH_2NH_2$
2	CH_3	H	H
3	H	H	H
4	H	Cbz	H
5	H	Cbz	$COCH_2NHCbz$
6	H	H	$COCH_2NH_2$
7	CH_3	Boc	H
8	H	Boc	H
9	CH_3	Boc	$COCH_2NHCbz$
10	H	Boc	$COCH_2NHCbz$
11	CH_3	Boc	$COCH_2NHCH{=}NH$
12	H	Boc	$COCH_2NHCH{=}NH$
13	CH_3	H	$COCH_2NHCH{=}NH$
14	H	H	$COCH_2NHCH{=}NH$

Cbz: benzyloxycarbonyl
Boc: *tert*-butoxycarbonyl

and 4.51 (2H s, 2''-CH_2).

Two 2''-N-formimidoyl derivatives of **1** and **6** were synthesized by starting from 1,2',6'-tri-N-Boc-istamycin B_0 (**7**), mp 71~74°C (decomp.), $[\alpha]_D^{22}$ +50° (*c* 1, methanol), and 1,2',6'-tri-N-Boc-3-O-demethylistamycin B_0 (**8**), mp 119~121°C (decomp.), $[\alpha]_D^{26}$ +99° (*c* 1, methanol), respectively. Compounds **7** and **8** were prepared by partial protection of their amino and methylamino groups of **2** or **3** with 2-(Boc-oxyimino)-2-phenylacetonitrile (Boc-ON, Aldrich) followed by column chromatography on silica gel developed with a mixture of chloroform and methanol (10:1) in 61% and 67% yield, respectively. The 4-methylamino group of **7** or **8** was acylated with N-hydroxysuccinimide ester of N-Cbz-glycine in dioxane in the presence of triethylamine at 55~60°C for 2 hours to afford 2''-N-Cbz-1,2',6'-tri-N-Boc-istamycin B (**9**, 94% yield), mp 104~110°C (decomp.), $[\alpha]_D^{23}$ +44° (*c* 1, methanol), or 2''-N-Cbz-1,2',6'-tri-N-Boc-3-O-demethylistamycin B (**10**, 92% yield), mp 122~128° (decomp.), $[\alpha]_D^{24}$ +86° (*c* 1, methanol). The 2''-N-Cbz group of **9** or **10** was removed by catalytic hydrogenation with 5% palladium on charcoal in a mixture of methanol, water and acetic acid for 3 hours. The free amino group on 2''-C was converted into an amidine by treatment with benzylformimidate hydrochloride or ethylformimidate hydrochloride[7] in an aqueous methanol for 1~4 hours at pH 8.0~8.5 adjusted with 0.5 N KOH to yield 1,2',6'-tri-N-Boc-2''-N-formimidoylistamycin B (**11**, 38% yield), or 1,2',6'-tri-N-Boc-3-O-demethyl-2''-N-formimidoylistamycin B (**12**, 39% yield), which was purified by column chromatography on silica gel developed with a mixture of chloroform and methanol. Removal of the Boc groups in **11** or **12** with 90% trifluoroacetic acid at 0~5°C for 2 hours, followed by salt-exchange with a column of Amberlite IRA-400 (SO_4^{2-}) gave 2''-N-formimidoylistamycin B (**13**) as the disulfate dihydrate (97% yield), mp 202~210°C (decomp.), $[\alpha]_D^{23}$ +47° (*c* 1, water), ^1H NMR (D_2O) δ 3.23 (3H s, 6'-NCH_3), 3.59 (3H s, 4-NCH_3), 3.92 (3H s, 3-OCH_3), 4.89 (2H s, 2''-CH_2) and 8.46 (1H s, CH=NH), or 3-O-demethyl-2''-N-formimidoylistamycin B (**14**) as the disulfate trihydrate (95% yield), mp 210~240°C (decomp.), $[\alpha]_D^{26}$ +85° (*c* 1, water), ^1H NMR (D_2O) δ 3.22 (3H s, 6'-NCH_3), 3.62 (3H s, 4-NCH_3), 4.87 (2H s, 2''-CH_2) and 8.45 (1H s, CH=NH).

The chemical shifts of FOURIER-transform ^{13}C NMR spectra of compounds **3**, **6**, **13** and **14** were assigned by comparing with those of istamycins B (**1**) and B_0 (**2**), as shown in Table 1.

The minimum inhibitory concentrations of **6**, **13** and **14** were tested and compared with those of **1**. As shown in Table 2, all these derivatives have good activity, and compounds **6** and **14** are more active than **1** against *Pseudomonas* strains. Intravenous injection of 160 mg/kg of **6**, **13** or **14** to mice caused no deaths.

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References

1) OKAMI, Y.; K. HOTTA, M. YOSHIDA, D. IKEDA,

S. KONDO & H. UMEZAWA: New aminoglycoside antibiotics, istamycins A and B. J. Antibiotics 32: 964~966, 1979

2) IKEDA, D.; T. MIYASAKA, M. YOSHIDA, Y. HORIUCHI, S. KONDO & H. UMEZAWA: Synthesis of istamycin A. J. Antibiotics 32: 1365~1366, 1979

3) IKEDA, D.; Y. HORIUCHI, S. KONDO & H. UMEZAWA: Synthesis of demethyl derivatives of istamycin A. J. Antibiotics 33: 1281~1288, 1980

4) INOUYE, S.; K. OHBA, T. SHOMURA, M. KOJIMA, T. TSURUOKA, J. YOSHIDA, N. KATO, M. ITO, S. AMANO, S. OMOTO, N. EZAKI, T. ITO, T. NIIDA & K. WATANABE: A novel aminoglycoside antibiotic, substance SF-2052. J. Antibiotics 32: 1354~1356, 1979

5) KONDO, S.: Some chemical modifications of aminoglycoside antibiotics. Jap. J. Antibiotics 32 (Supplement): S228~S236, 1979

6) WATANABE, I.; A. IWASAKI & T. MORI: New antibiotics. Japan Kokai 55-55198, April 22, 1980

7) OHME, R. & E. SCHMITZ: A simple synthesis of alkyl formimidates. Angew. Chem. Internat. Ed. 6: 566, 1967