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Note

Synthesis of low-toxicity, 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives of arbekacin and its analogs, and study of structure–toxicity relationships

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Studies on structure–toxicity relationships of aminoglycoside antibiotics have rarely been reported, and, to the best of our knowledge, no studies except for ours[1,2] have been reported on the relationship between the basicity of a particular amino group in the molecule and the toxicity of the antibiotic. Lately we reported the synthesis of several 2′- (ref. 3), 3′- (refs. 4–7), 4′- (ref. 8), 6″- (ref. 9), and 5-deoxyfluoro (and 5-deoxy-5,5-difluoro) analogs[10] of kanamycins. The last group of compounds were especially found to show remarkably decreased acute toxicity (by intravenous injection in mice), with similar or sometimes improved antibacterial activity as compared to the parent antibiotics. This decrease of toxicity was considered to result[11] from the lowered basicity (induced by the strongly electronegative fluorine(s)[12] at C-5) of the amino groups at C-1 and C-3 of the deoxyfluorinated derivatives of kanamycin B analogs, relative to the parent compounds. The present study extends this research and describes the synthesis of the 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] (AHB) derivatives of the deoxyfluorinated derivatives. As was seen in amikacin[13] (1-*N*-AHB-kanamycin A) and arbekacin[14] (1-*N*-AHB-3′,4′-dideoxykanamycin B), attachment of an AHB group to the NH_2-1 group of kanamycins gave compounds of remarkably enhanced antibacterial activity, against both sensitive and resistant bacteria, relative to the parent antibiotics. However, a serious drawback in clinical use of arbekacin, for example, is its high toxicity (renal and otic toxicities, in general). Thus reduction of the toxicity of arbekacin and related aminoglycoside antibiotics is an urgent problem to be

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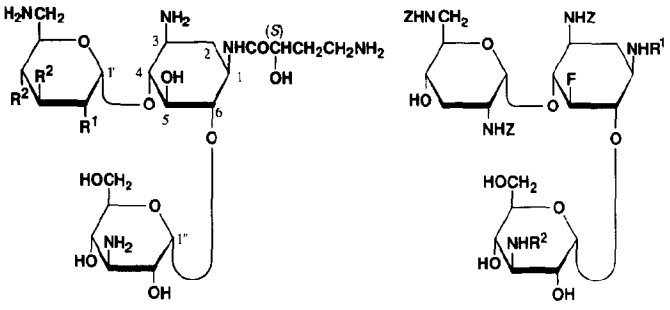
TABLE I

Minimal inhibitory concentration (μg/mL) and toxicity [a] of **4**–**8**, arbekacin (ABK), and amikacin (AMK)

Test organism [b]	**4**	**5**	**6**	**7**	**8**	ABK	AMK
St. a. FDA 209P	0.39	0.39	0.39	0.39	0.39	< 0.2	0.78
St. a. Smith	< 0.2	0.39	< 0.2	< 0.2	< 0.2	< 0.2	0.39
St. Ap01 [c]	0.78	6.25	0.78	3.12	3.12	0.39	3.12
St. ep. 109 [c]	6.25	6.25	0.78	3.12	0.39	0.2	3.12
Micr. l. FDA16	0.78	0.39	0.78	0.39	0.39	0.78	6.25
Micr. l. PCI1001	0.78	0.78	0.78	1.56	0.78	1.56	3.12
B. sub. NRRL B-558	< 0.2	0.39	< 0.2	0.39	< 0.2	< 0.2	0.39
Coryn. bovis 1810	< 0.2	< 0.2	< 0.2	0.39	0.39	0.39	0.78
E. coli NIHJ	< 0.2	< 0.2	0.2	< 0.2	< 0.2	< 0.2	0.78
E. coli K-12 (*Ec*K.)	< 0.2	< 0.2	0.2	0.2	0.39	0.39	0.39
*Ec*K. R5 [d]	3.12	12.5	50	25	25	100	25
*Ec*K. J5R11-2 [e]	< 0.2	0.39	0.2	< 0.2	< 0.2	< 0.2	0.39
*Ec*K. ML1629 [e]	0.78	0.78	0.39	1.56	1.56	1.56	1.56
*Ec*K. ML1410	0.78	0.39	0.78	0.39	0.39	1.56	3.12
*Ec*K. ML1410 R81 [e]	0.78	0.78	0.78	0.78	0.78	0.78	3.12
*Ec*K. LA290 R55 [f]	0.78	0.78	0.78	< 0.2	< 0.2	0.78	3.12
E. coli W677	0.39	< 0.2	0.2	< 0.2	< 0.2	< 0.2	0.39
E. coli JR66/W677 [f,g]	0.78	0.78	0.78	0.2	0.2	0.78	3.12
*Ec*K. C600 R135 [h]	0.78	< 0.2	0.78	0.39	0.39	0.39	0.78
E. coli JR255 [h]	0.39	< 0.2	0.2	< 0.2	< 0.2	< 0.2	1.56
Mycob. s. ATCC 607	0.78	12.5	0.78	0.78	1.56	0.39	1.56
Kl. p. PCI602	0.39	0.78	0.39	0.78	0.78	0.78	1.56
Kl. p. 22#3038 [f,g]	1.56	3.12	0.78	1.56	1.56	1.56	3.12
Sh. dys. JS11910	1.56	0.78	0.78	1.56	1.56	1.56	3.12
Sh. sonnei JS11746	0.78	0.78	0.78	0.78	0.78	0.78	3.12
Sal. typhi T-63	0.78	0.78	0.39	1.56	1.56	0.39	0.78
Sal. ent. 1891	1.56	1.56	0.78	3.12	3.12	1.56	1.56
Pr. vulgaris OX19	0.78	0.78	0.78	0.78	0.78	0.78	1.56
Pr. rettgeri GN311	< 0.2	< 0.2	< 0.2	0.39	0.39	0.78	1.56
Serr. marcescens	1.56	3.12	1.56	6.25	6.25	6.25	3.12
Serr. sp. 4 [f]	1.56	3.12	1.56	1.56	1.56	6.25	3.12
Prov. sp. Pv16 [i]	0.78	0.78	0.78	1.56	1.56	1.56	1.56
Prov. sp. 2991 [i]	0.39	0.78	0.78	3.12	3.12	6.25	1.56
Ps. aerug. A3	< 0.2	0.39	0.2	0.2	< 0.2	0.78	0.78
Ps. aerug. No. 12	1.56	1.56	1.56	3.12	6.25	1.56	3.12
Ps. aerug. H9 [g]	12.5	3.12	0.78	3.12	3.12	1.56	3.12
Ps. aerug. H11	12.5	12.5	6.25	25	25	6.25	6.25
Ps. aerug. TI-13 [e]	1.56	3.12	1.56	3.12	3.12	0.78	3.12
Ps. aerug. GN315 [d]	6.25	6.25	6.25	6.25	12.5	3.12	25
Ps. aerug. 99 [h]	3.12	6.25	3.12	6.25	12.5	3.12	6.25
Ps. aerug. 21-75 [j]	50	25	50	100	100	50	6.25
Ps. aerug. PSTI [h]	12.5	6.25	6.25	12.5	25	3.12	6.25
Ps. aerug. ROS134/ Pu21 [h]	12.5	25	25	25	50	50	50
Ps. aerug. K-Ps102 [k]	6.25	3.12	3.12	12.5	12.5	3.12	3.12
LD_{50} (mg kg^{-1}) (95% confidence limit)	140 ± 10		120 ± 9	289 ± 22	212 ± 11	62 ± 5	219 ± 13



amikacin (AMK) $R^1 = R^2 = OH$

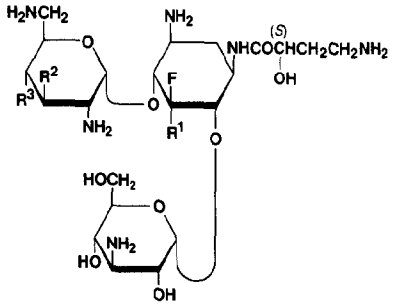
arbekacin (ABK) $R^1 = NH_2$, $R^2 = H$

1 $R^1 = R^2 = H$

2 $R^1 = H$, $R^2 = COCF_3$

3 $R^1 = (S)\text{-}COCHCH_2CH_2NHZ$, $R^2 = COCF_3$

Z: $CO_2CH_2C_6H_5$



	R^1	R^2	R^3
4	H	H	OH
5	H	OH	OH
6	H	H	H
7	F	H	OH
8	F	H	H

solved. Toward this objective, the 5-deoxy-5-fluoro and 5-deoxy-5,5-difluoro derivatives of arbekacin and its analogs have been prepared.

The synthesis was accomplished by the general procedure developed in our laboratory[15]; the synthesis of the 1-*N*-AHB derivative of 5,3′-dideoxy-5-fluoro-

Notes to Table I:

[a] An aliquot of each compound (the weight is based on the free base) was dissolved in saline (0.5 mL, pH 7) and intravenously injected, at 0.1 mL s^{-1}, into an ICR-JCL mouse ($n = 6$, 4 weeks old, 20 ± 0.7 g weight) and the values of LD_{50} were measured after 7 days. [b] Agar dilution streak method (Mueller–Hinton agar, 17 h, 37°C). Abbreviations are: *St.*, *Staphylococcus*; *a.*, *aureus*; *ep.*, *epidermidis*; *Micr. l.*, *Micrococcus luteus*; *B. sub.*, *Bacillus subtilis*; *Coryn.*, *Corynebacterium*; *E.*, *Escherichia*; *Ec*K., *Escherichia coli* K-12; *Mycob. s.*, *Mycobacterium smegmatis*; *Kl. p.*, *Klebsiella pneumoniae*; *Sh. dys.*, *Shigella dysenteriae*; *Sal.*, *Salmonella*; *ent.*, *enteritidis*; *Pr.*, *Proteus*; *Serr.*, *Serratia*; *Prov.*, *Providencia*; *Ps. aerug.*, *Pseudomonas aeruginosa*. [c] Resistant strain producing ANT(4′), [d] AAC(6′), [e] APH(3′)-I, [f] ANT(2″), [g] APH(3′)-II, [h] AAC(3), [i] AAC(2′), [j]APH(3′)-III. [k] Resistance by permeability.

kanamycin B is described as a typical example (see Experimental). Antibacterial activities of **4–8**, arbekacin, and amikacin are shown in Table I. The results indicate that **4–8** exhibit similar activity to that of arbekacin in most of the strains. However, acute toxicities (LD_{50}, Table I) were quite different, showing a remarkable decrease of the toxicity in comparison with that of arbekacin. This indicates that 5-deoxy-5-fluorination and 5-deoxy-5,5-difluorination lowered the toxicity, and suggests that this was attributable to the decrease in basicity at NH_2-3 of the fluorinated kanamycin B derivatives *. At the same time it was concluded, by comparison of the toxicities † of the 5-fluorinated kanamycin B derivatives[10] and those of the corresponding AHB-derivatives (reported in this paper), that attachment of the 1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl] group had no appreciable effect on toxicity.

In conclusion, this study strongly suggests that the toxicity of the aminoglycoside antibiotics may be largely influenced by the basicity of specific amino groups in their molecules, rather than by the number of amino groups, although the latter has been generally considered to be a rough indication of their toxicity.

EXPERIMENTAL

General methods.—Optical rotations were determined with a Perkin–Elmer 241 polarimeter. ^1H NMR (at 250 MHz) and ^{19}F NMR (at 235.3 MHz) spectra were recorded with Bruker WM 250 and AC 250P spectrometers, and the chemical shifts (δ) for ^1H and ^{19}F were measured downfield from internal Me_4Si and external Freon 11 ($CFCl_3$), respectively. Column chromatography was performed on Wakogel C-200.

1-N-[(S)-4-Amino-2-hydroxybutanoyl]-5,3′-dideoxy-5-fluorokanamycin B (**4**).—A mixture of 5,3′-dideoxy-5-fluorokanamycin B sesquihydrate[10] (31 mg, 0.062 mmol) and zinc acetate dihydrate (67 mg, 0.31 mmol) in dry Me_2SO (0.31 mL) was stirred for 1.5 h at 80°C. To the resulting clear solution, after cooling to room temperature, was added *N*-(benzyloxycarbonyloxy)succinimide (49 mg, 0.20 mmol) and the mixture was stirred for 1 h. Addition of diethyl ether gave a precipitate, which was treated as previously reported[15] to give 5,3′-dideoxy-5-fluoro-3,2′,6′-tris(*N*-benzyloxycarbonyl)kanamycin B (**1**), 48 mg. Treatment of **1** (47 mg, 0.054 mmol) with ethyl trifluoroacetate (8.4 μL, 0.070 mmol) in dry Me_2SO (0.24 mL) as reported[15] gave the 3″-*N*-(trifluoroacetyl) derivative **2** (52 mg). Acylation of **2** (52 mg) with *N*-[(*S*)-4-(benzyloxycarbonylamino)-2-hydroxybutanoyloxy]succinimide (24 mg, 0.069 mmol) in the presence of Na_2CO_3 (4.5 mg) in 2:1 oxolane–water (2.4 mL)

* This will be discussed in detail in ref. 11; for example, the p*K*a values of NH_3^+-3 in arbekacin, **6**, and **8** in D_2O were 7.3, 6.8, and 6.5, respectively. † The values[10] of LD_{50} (intravenous mouse) for 5,3′-dideoxy-5-fluorokanamycin B, 5,3′,4′-trideoxy-5-fluorokanamycin B, 5,3′-dideoxy-5,5-difluorokanamycin B, and 5,3′,4′-trideoxy-5,5-difluorokanamycin B were 130, 130, 270, and 250 mg kg^{-1}, respectively.

gave the 1-*N*-AHB derivative **3** (59 mg). De(*N*-trifluoroacetyl)ation of the product with 2 M NH_3 in 4:3 oxolane–water followed by catalytic hydrogenolysis (Pd) of the *N*-protecting groups in 4:1:1 1,4-dioxane–water–AcOH and subsequent column chromatography of the product with CM Sephadex C-25 (7 mL, 0 → 0.5 M aq NH_3) gave **4** as the monocarbonate (21.2 mg, 55% based on the starting material); $[\alpha]_D^{24}$ +92° (*c* 2, H_2O); ^1H NMR (DCl in D_2O; pD ~1): δ 4.42 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,F} = 10$ Hz, H-4), 4.90 (dt, 1 H, $J_{4,5} = J_{5,6} = 8.5$, $J_{5,F}$ 51 Hz, H-5), 5.14 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.62 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′); ^{19}F NMR (DCl in D_2O; pD ~1): δ −190.8 (dt, 1 F). Anal. Calcd for $C_{22}H_{43}FN_6O_{10} \cdot H_2CO_3$: C, 43.66; H, 7.17; F, 3.00; N, 13.28. Found: C, 43.98; H, 6.85; F, 2.84; N, 13.46.

1-N-[(S)-*4-Amino-2-hydroxybutanoyl]-5-deoxy-5-fluorokanamycin B* (**5**).—Prepared from 5-deoxy-5-fluorokanamycin B (ref. 10, as 0.4 carbonate, 28.6 mg, 0.056 mmol), similary as just described, gave **5** as the monocarbonate, 12.4 mg (34%); $[\alpha]_D^{23}$ +91° (*c* 1, H_2O); ^1H NMR (DCl in D_2O; pD ~1): δ 4.90 (dt, 1 H, $J_{4,5} = J_{5,6} = 8.5$, $J_{5,F}$ 50 Hz, H-5), 5.14 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″), and 5.76 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1′); ^{19}F NMR (DCl in D_2O; pD ~1): δ −190.0 (dt, 1 F, $J_{4,F} = J_{6,F} =$ 10.5 Hz). Anal. Calcd for $C_{22}H_{43}FN_6O_{11} \cdot H_2CO_3$: C, 42.59; H, 6.99; F, 2.93; N, 12.96. Found: C, 42.81; H, 6.98; F, 2.96; N, 12.35.

1-N-[(S)-*4-Amino-2-hydroxybutanoyl]-5,3′,4′-trideoxy-5-fluorokanamycin B* (**6**).—Prepared likewise from 5,3′,4′-trideoxy-5-fluorokanamycin B (ref. 10, as 0.8 carbonate, 51 mg, 0.10 mmol); **6**, 38 mg; $[\alpha]_D^{25}$ +83° (*c* 1, H_2O); ^1H NMR (20% ND_3 in D_2O): δ 4.71 (dt, 1 H, $J_{4,5} = J_{5,6} = 8$, $J_{5,F}$ 51 Hz, H-5), 5.08 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), and 5.17 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′).

1-N-[(S)-*4-Amino-2-hydroxybutanoyl]-5,3′-dideoxy-5,5-difluorokanamycin B* (**7**). —Prepared from 5,3′-dideoxy-5,5-difluorokanamycin B (ref. 10, as monohydrate, 335 mg, 0.66 mmol); **7** as 0.9 carbonate, 210 mg (49%); $[\alpha]_D^{23}$ +89° (*c* 3, H_2O); ^1H NMR (20% ND_3 in D_2O): δ 5.06 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″) and 5.17 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′); ^{19}F NMR (20% ND_3 in D_2O): δ −110.3 (d, 1 F, $J_{Fax,Feq}$ 247 Hz, F-5*eq*) and −129.5 (dt, 1 F, $J_{4,Fax} = J_{6,Fax} = 20$ Hz, F-5*ax*). Anal. Calcd for $C_{22}H_{42}F_2N_6O_{10} \cdot 0.9H_2CO_3$: C, 42.68; H, 6.85; F, 5.90; N, 13.04. Found: C, 42.83; H, 6.91; F, 5.63; N, 12.88.

1-N-[(S)-*4-Amino-2-hydroxybutanoyl]-5,3′,4′-trideoxy-5,5-difluorokanamycin B* (**8**).—Prepared from 5,3′,4′-trideoxy-5,5-difluorokanamycin B (ref. 10, as 0.4 carbonate, 830 mg, 1.7 mmol); **8** as 0.9 carbonate, 612 mg (58%); $[\alpha]_D^{21}$ +92° (*c* 3, H_2O); ^1H NMR (20% ND_3 in D_2O): δ 5.08 (d, 1 H, $J_{1'',2''}$ 3 Hz, H-1″) and 5.20 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′); ^{19}F NMR (20% ND_3 in D_2O): δ −110.4 (d, 1 F, $J_{Fax,Feq}$ 247 Hz, F-5*eq*) and −129.5 (dt, 1 F, $J_{4,Fax} = J_{6,Fax} = 20$ Hz, F-5*ax*). Anal. Calcd for $C_{22}H_{42}F_2N_6O_9 \cdot 0.9H_2CO_3$: C, 43.77; H, 7.03; F, 6.05; N, 13.37. Found: C, 43.84; H, 6.78; F, 5.93; N, 13.09.

REFERENCES

1 T. Tsuchiya, T. Torii, S. Umezawa, and H. Umezawa, *J. Antibiot.*, 35 (1982) 1245–1247.
2 T. Tsuchiya, T. Torii, Y. Suzuki, and S. Umezawa, *Carbohydr. Res.*, 116 (1983) 277–287.

3 T. Tsuchiya, Y. Takahashi, M. Endo, S. Umezawa, and H. Umezawa, *J. Carbohydr. Chem.*, 4 (1985) 587–611.
4 T. Tsuchiya, Y. Takahashi, Y. Kobayashi, S. Umezawa, and H. Umezawa, *J. Antibiot.*, 38 (1985) 1287–1290.
5 Y. Takahashi, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Carbohydr. Res.* 210 (1991) 221–232.
6 E. Umemura, T. Tsuchiya, Y. Kobayashi, and K. Tanaka, *Carbohydr. Res.*, 224 (1992) 141–163.
7 Y. Kobayashi, T. Tsuchiya, T. Ohgi, N. Taneichi, and Y. Koyama, *Carbohydr. Res.*, 230 (1992) 89–105.
8 Y. Takahashi, S. Tsuneda, T. Tsuchiya, Y. Koyama, and S. Umezawa, *Carbohydr. Res.*, 232 (1992) 89–105.
9 Y. Takagi, T. Tsuchiya, and S. Umezawa, *Nippon Kagaku Kaishi*, (1985) 2001–2009.
10 T. Shitara, Y. Kobayashi, T. Tsuchiya, and S. Umezawa, *Carbohydr. Res.*, 232 (1992) 273–290.
11 T. Tsuchiya, *Carbohydr. Res.*, in preparation.
12 T. Tsuchiya, *Adv. Carbohydr. Chem. Biochem.*, 48 (1990) 91–277.
13 H. Kawaguchi, T. Naito, S. Nakagawa, and K. Fujisawa, *J. Antibiot.*, 25 (1972) 695–708.
14 S. Kondo, K. Iinuma, H. Yamamoto, K. Maeda, and H. Umezawa, *J. Antibiot.*, 26 (1973) 412–415.
15 T. Tsuchiya, Y. Takagi, and S. Umezawa, *Tetrahedron Lett.*, (1979) 4951–4954.