EPIVALIOLAMINE AND DEOXYVALIDAMINE, NEW PSEUDO-AMINOSUGARS PRODUCED BY *STREPTOMYCES HYGROSCOPICUS*

Sir:

A producer of validamycins, *Streptomyces hygroscopicus* subsp. *limoneus* (IFO 12703, ATCC 21431, FERM-P 468) was found to produce additional pseudo-aminosugars besides previously reported valiolamine, valienamine, validamine and hydroxyvalidamine[1]. In this paper, we report the isolation, structural study and α-glucosidase inhibitory effects of new minor products of this fermentation, epivaliolamine and deoxyvalidamine.

Mixtures of the pseudo-aminosugars prepared from the fermentation broth as previously reported[2], were chromatographed on a column of Amberlite IRC-50 (NH_4^+ form) using 0.2 N ammonium hydroxide as eluant to obtain four fractions, I (containing hydroxyvalidamine and validamine), II (containing validamine, valienamine and deoxyvalidamine), III (containing epivaliolamine) and IV (containing valiolamine) in order of elution. Pools of fraction II (2.2 g) containing deoxyvalidamine were subjected to column chromatography on CM-Sephadex C-25 (NH_4^+ form, 450 ml) with 0.02 N ammonium hydroxide. The deoxyvalidamine portion was desalted by chromatography on Dowex-1X2 (OH^- form, 200 ml) with water to obtain homogeneous deoxyvalidamine (120 mg). Fraction III (65 mg) containing epivaliolamine was also desalted and homogeneous epivaliolamine (50 mg) was obtained. The Rf values of these new components on silica gel TLC are given in comparison with the related pseudo-aminosugars in Table 1.

Epivaliolamine: colorless amorphous; $[\alpha]_D^{25}$ +18.2° (*c* 1.0, H_2O); *Anal* Calcd for $C_7H_{15}NO_5 \cdot H_2O$: C 39.80, H 8.11, N 6.63, Found: C 39.55, H 8.32, N 6.50. The ^{13}C NMR spectrum of epivaliolamine shows the presence of two methylenes, four methines and a quaternary carbon and was very similar to that of valiolamine (Table 2). The ^1H NMR spectrum (100 MHz, in D_2O) of epivaliolamine shows AB quartet (J=11.5 Hz) of the side-chain methylene protons as H-7 and H-7′. As shown in Table 3, the magnitude of coupling constant such as J= 6.5 Hz ($J_{2,3}$, $J_{3,4}$, $J_{5,6'}$) made it difficult to determine the relative configurations of C-2, C-3, C-4, C-5 and the doublet splitting of H-2 shows that the C-1 carbon bears no proton.

The structure of epivaliolamine was elucidated to be 1D-(1(OH),3/1,2,4,5)-5-amino-1-C-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol by the chemical synthesis from valienamine[3].

Fig. 1.

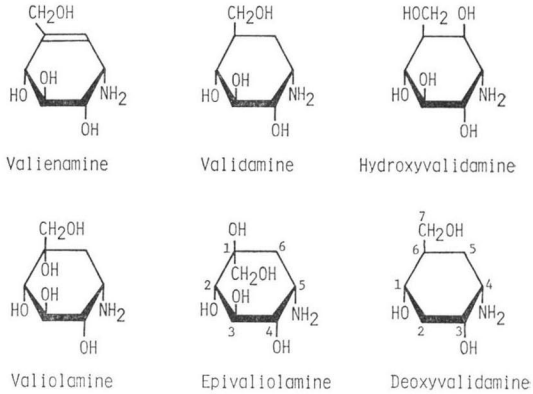


Table 1. Rf values of aminocyclitols.

Aminocyclitol	I*	II	III
Validamine	0.35	0.49	0.61
Valienamine	0.41	0.49	0.61
Hydroxyvalidamine	0.41	0.39	0.56
Valiolamine	0.27	0.39	0.41
Epivaliolamine	0.40	0.43	0.56
Deoxyvalidamine	0.45	0.68	0.81

* Solvent system I: 1-PrOH - AcOH - H_2O, (4: 1: 1).
II: 1-BuOH - MeOH - $CHCl_3$ - concd NH_4OH, (4: 5: 2: 5).
III: $CHCl_3$ - MeOH - concd NH_4OH, (1: 3: 2).
Silica gel TLC: Kieselgel 60 F_{254} (Merck).

Table 2. ^{13}C NMR spectral data of epivaliolamine and deoxyvalidamine in D_2O.

Carbon	Chemical shifts δ (ppm)*	
	Epivaliolamine	Deoxyvalidamine
C-1	77.8 (s)	71.6 (d)
C-2	77.0 (d)	37.8 (t)
C-3	74.2 (d)	71.3 (d)
C-4	75.4 (d)	51.8 (d)
C-5	48.7 (d)	32.0 (t)
C-6	37.1 (t)	41.5 (d)
C-7	67.9 (t)	65.5 (t)

* δ (ppm) from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

Table 3. ^1H NMR spectral data of epivaliolamine in D_2O (100 MHz).

Proton	Chemical shift δ (ppm)*	Multiplicity and coupling constant (J in Hz)
H-2	3.58	1H, d, J=6.5
H-3	4.00	1H, t, J=6.5
H-4	3.76	1H, dd, J=4.0, J=6.5
H-5	3.42	1H, m
H-6	1.72	1H, dd, J=4.5, J=14.2
H-6′	1.96	1H, dd, J=6.5, J=14.2
H-7	3.53	Each 1H, ABq, J=11.5
H-7′	3.68	

* δ (ppm) from internal DSS.

Table 4. ^1H NMR spectral data of deoxyvalidamine N,O-tetraacetate in $CDCl_3$ (100 MHz).

Proton	Chemical shift δ (ppm)*	Multiplicity and coupling constant (J in Hz)
H-1	4.76	1H, ddd, J=4.6, J=9.7, J=9.7
H-3	4.91	1H, dt, J=4.3, J=11.4
H-4	4.20	1H, m
H-7	3.96	1H, dd, J=4.0, J=11.4
H-7′	4.12	1H, dd, J=4.8, J=11.4
–CONH–	5.71	1H, br d, J=7.3
$-COCH_3$	2.03	3H, s
	2.04	3H, s
	2.06	6H, s

* δ (ppm) from internal TMS.

** H-2, H-2′, H-5, H-5′, H-6 and Ac are overlapped each other.

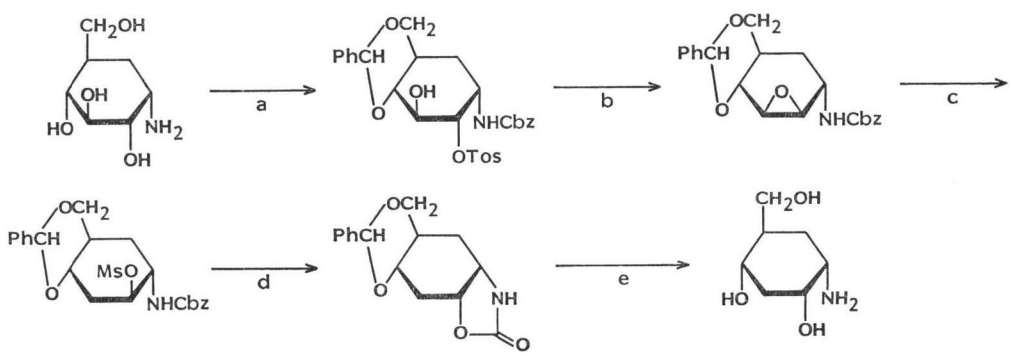
Table 5. Molar concentration required to give 50% inhibition against porcine intestinal α-glucosidases.

Aminocyclitol	Maltase	Sucrase
Valiolamine	2.2×10^{-6}	4.9×10^{-8}
Validamine	1.1×10^{-4}	7.5×10^{-6}
Epivaliolamine	1.0×10^{-3}	5.0×10^{-5}
Deoxyvalidamine	1.0×10^{-3}	2.8×10^{-4}

Substrate: 50 mM maltose, 50 mM sucrose.

Deoxyvalidamine: colorless syrup; $[\alpha]_D^{25}$ +52.8° (c 1.0, H_2O). The ^{13}C NMR spectral data (Table 2) of deoxyvalidamine shows the presence of three methylenes and four methines. Acetylation of deoxyvalidamine with acetic anhydride in pyridine afforded an N,O-tetraacetate, colorless needles; mp 151.0~152.5°C; $[\alpha]_D^{25}$ +46.4° (c 1.0, $CHCl_3$); EI-MS m/z 329 (M^+); *Anal* Calcd for $C_{15}H_{23}NO_7$: C 54.70, H 7.04, N 4.25, Found: C 54.87, H 7.27, N 4.22. The ^1H NMR spectrum (100 MHz, in $CDCl_3$, Table 4) of deoxyvalidamine N,O-tetraacetate shows two doublets of doublets at δ 3.96 and δ 4.12 of side-chain methylene protons ($J_{6,7}$ = 4.0 Hz, $J_{6,7'}$ = 4.8 Hz, $J_{7,7'}$ = 11.4 Hz). The splitting patterns of ring methine H-3 (δ 4.91, dt, $J_{3,4}$ = 4.3 Hz, $J_{3,2ax}$ = 11.4 Hz, $J_{3,2eq}$ = 4.3 Hz) indicates that H-3 and H-4 are axial and equatorial orientation, respectively. The splittings of H-1 (δ 4.76, ddd, $J_{1,6}$ = 9.7 Hz, $J_{1,2ax}$ = 9.7, $J_{1,2eq}$ = 4.6 Hz) show *trans* diaxial orientation of H-1 and H-6. The multiplet at δ 4.20 of H-4 was changed to a doublet of triplets (J = 4.3 Hz, $J_{3,4}$, $J_{4,5ax}$, $J_{4,5eq}$)

Scheme 1.



a: i) CbzCl, $NaHCO_3$; ii) $PhCH(OCH_3)_2$, H^+; iii) $CH_3C_6H_4SO_2Cl$/pyridine
b: CH_3ONa
c: i) NaI, NH_4Cl; ii) Bu_3SnH; iii) CH_3SO_2Cl/pyridine
d: 120°C/DMF
e: i) 80% AcOH, 80°C; ii) $Ba(OH)_2$

by deuterium oxide exchange. Thus, the relative configuration of deoxyvalidamine was elucidated from the above results. The structure of deoxyvalidamine, including the absolute stereochemistry, was confirmed to be 1L-(1,3, 4/6)-4-amino-6-C-(hydroxymethyl)-1,3-cyclohexanediol by the chemical conversion of validamine into deoxyvalidamine, as shown in Scheme 1.

The inhibitory activities (Table 5) of epivaliolamine and deoxyvalidamine against porcine intestinal α-glucosidases are very weak. Based on the fact that epivaliolamine showed only 1/1,000 activity of valiolamine, the configuration of hydroxymethyl group on the C-1 is extremely important for the inhibitory activity.

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References

1) KAMEDA, Y.; N. ASANO, M. YOSHIKAWA, M. TAKEUCHI, T. YAMAGUCHI, K. MATSUI, S. HORII & H. FUKASE: Valiolamine, a new α-glucosidase inhibiting aminocyclitol produced by *Streptomyces hygroscopicus*. J. Antibiotics 37: 1301~1307, 1984
2) IWASA, T.; E. HIGASHIDE, H. YAMAMOTO & M. SHIBATA: Studies on validamycins, new antibiotics. II. Production and biological properties of validamycins A and B. J. Antibiotics 24: 107~113, 1971
3) HORII, S.; H. FUKASE & Y. KAMEDA: Stereoselective conversion of valienamine and validamine into valiolamine. Carbohydr. Res. 140: 185~200, 1985