ABSOLUTE CONFIGURATION OF THE β-HYDROXYL FATTY ACID CONSTITUENT OF PERMETIN A

ASAO MURAI, YUSUKE AMINO and TOSHIHIKO ANDO

Central Research Laboratories, Ajinomoto Co., Inc. 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, Japan

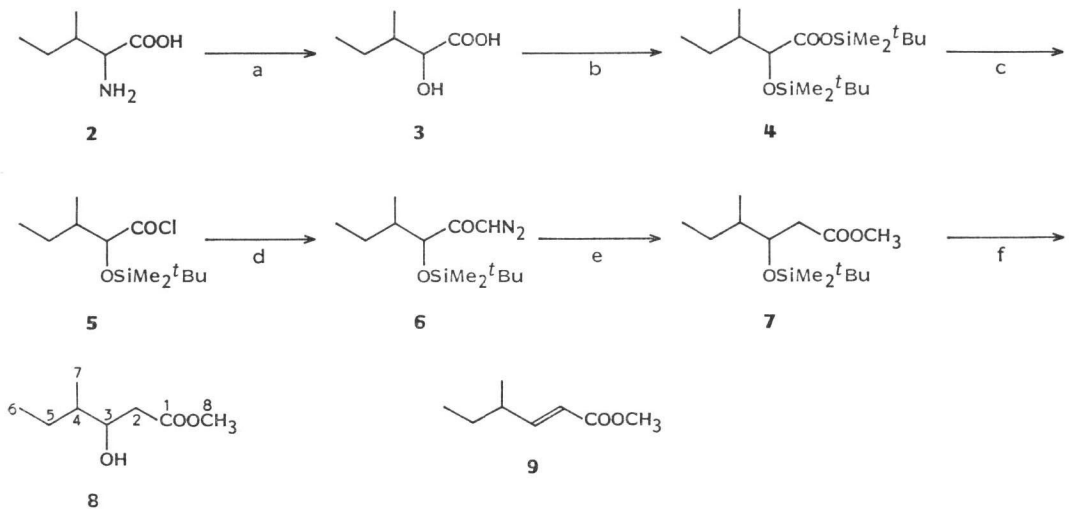
(Received for publication July 30, 1985)

The cyclic depsipeptide permetin A[1] is one member of the polypeptin-permetin family antibiotics produced by *Bacillus circulans*. The members of the family, polypeptin A[2], permetin A[3] and BMY-28160[4] have closely related structures (Fig. 1), they have the same β-hydroxyl fatty acid constituent and differ from each other only by one or two amino acids. The absolute configuration of two asymmetric centers of the β-hydroxyl fatty acid, however, has been left undetermined.

This paper reports the determination of the absolute configuration of 3-hydroxy-4-methylhexanoic acid (**1**) of permetin A as (3*S*,4*S*). This conclusion was derived from comparing $[\alpha]_D$ values and NMR spectra of methyl ester of **1** (**8**) isolated from permetin A with those of four possible stereoisomers of **8**. The stereoisomers were chemically derived from the four optical isomers of isoleucine, *i.e.* L-Ile, L-*allo*-Ile, D-Ile and D-*allo*-Ile (purchased from Sigma Chemical Co. except L-Ile from Ajinomoto Co.). The synthetic route is shown in Scheme 1.

As shown in Scheme 1, each isoleucine isomer was transformed into **8** through deamination with nitrous acid and elongation by one carbon atom through Arndt-Eistert synthesis. Both reactions are known to proceed with retention of chirality[5]. Isoleucine was treated with nitrous acid to give 2-hydroxy-3-methylpentanoic acid (**3**) which was purified by distillation (bp 120°C/4 torr, yield 50%). The secondary alcohol and carboxylic acid groups were blocked by treatment with *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of imidazole and 4-dimethylaminopyridine at 60°C for 16 hours in DMF and the TBDMS derivative (**4**) was purified by distillation (bp 120°C/4 torr, yield 90%). Treatment of **4** with oxalyl chloride in methylene chloride and catalytic quantities of DMF at 0°C for 1.5 hours, then at room temperature for 0.5 hour gave acid chloride (**5**), which was purified by distillation (bp 90°C/4 torr, yield 90%). The

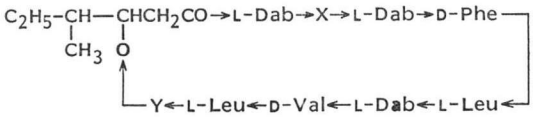
Scheme 1. Synthesis of methyl 3-hydroxy-4-methylhexanoate from isoleucine.



Reagents: a; $NaNO_2$ - 1 N H_2SO_4 (1.6 equiv), 0°C, 2 hours, room temp, 1.5 hours, 50%. b; TBDMSCl (2.2 equiv), imidazole (4.4 equiv), DMAP (0.2 equiv), DMF, 60°C, 90%. c; $(COCl)_2$ (2.0 equiv), DMF (few drops), CH_2Cl_2, 0°C, 1.5 hours, room temp, 0.5 hour, 90%. d; CH_2N_2 (excess), Et_2O, Et_3N, room temp, 1 hour. e; $C_6H_5CO_2Ag$ (0.6 equiv), MeOH, Et_3N, −40°C to room temp, 40% from **5**. f; 47% aq $NaHCO_3$, 50%.

acid chloride (**5**) upon treatment with ethereal diazomethane in the presence of triethylamine at room temperature for 1 hour afforded diazoketone (**6**) [IR(film); 2106.3 cm^{-1} (–CH=N^+=N^-)]. Treatment of **6** with silver benzoate in MeOH in the presence of an excess of triethylamine with the temperature gradually raised from −40°C to room temperature for 15 hours gave β-silyloxy carboxylic acid methyl ester (**7**) and the α,β-unsaturated carboxylic acid methyl ester (**9**). The by-product **9** was easily removed by distillation (bp 100°C/4 torr, yield 40% from **5**). Removal of silyl group from **7** was carried out by the exposure of **7** to aqueous HF in acetonitrile at room temperature for 10 minutes, followed by neutralization with $NaHCO_3$. Purification of β-hydroxyl carboxylic

Fig. 1. Structures of polypeptin-permetin family antibiotics.



Permetin A X=L-Ile, Y=L-Ser
Polypeptin A X=L-Ile, Y=L-Thr
BMY-28160 X=L-Val, Y=L-Ser
(Dab: 2,4-Diaminobutyric acid)

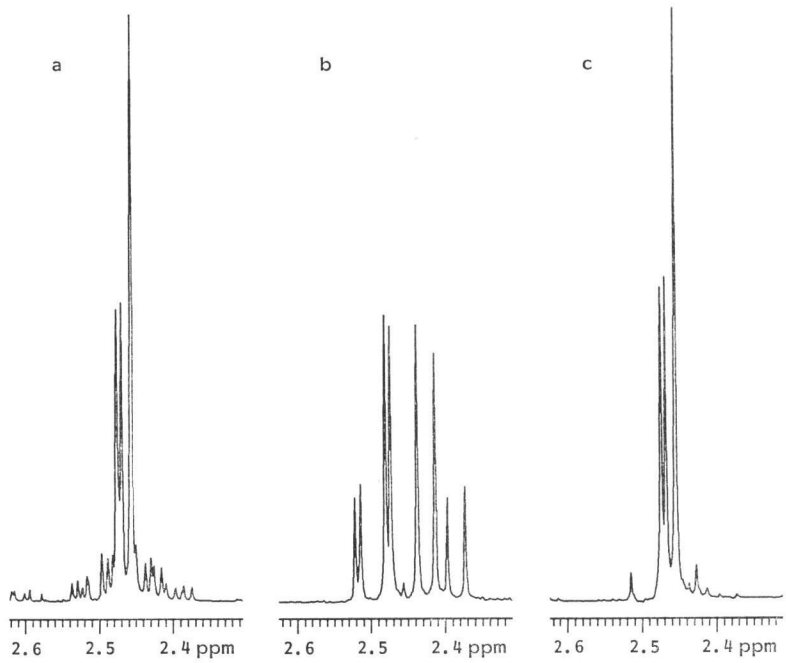
Table 1. Optical rotational values and NMR spectrum data of methyl 3-hydroxy-4-methylhexanoate (**8**).

Starting material	Permetin A	L-Ile (*erythro*)	D-Ile (*erythro*)	L-*allo*-Ile (*threo*)	D-*allo*-Ile (*threo*)
Absolute configuration		(3*R*,4*S*)	(3*S*,4*R*)	(3*R*,4*R*)	(3*S*,4*S*)
$[\alpha]_D^{30}$ ($CDCl_3$)	−33.50° (*c* 2.00)	+32.37° (*c* 2.00)	−31.88° (*c* 2.23)	+35.13° (*c* 2.18)	−36.10° (*c* 2.20)
^{13}C NMR (ppm) (25 MHz $CDCl_3$ TMS)					
C-6*	11.70	11.50	11.50	11.75	11.74
C-7	13.79	14.43	14.43	13.79	13.79
C-5	25.54	25.00	25.00	25.54	25.54
C-4	38.79	37.87	37.87	38.89	38.89
C-2	39.87	39.92	39.92	39.92	39.91
C-8	51.76	51.76	51.76	51.71	51.71
C-3	71.06	71.64	71.64	71.06	71.06
C-1	173.84	173.99	173.99	173.84	173.84
^1H NMR (ppm) (400 MHz $CDCl_3$ TMS)					
CH_3	0.91 (3H, d, *J*=6.8 Hz)	0.89 (3H, d, *J*=7.1 Hz)	0.89 (3H, d, *J*=7.1 Hz)	0.91 (3H, d, *J*=6.8 Hz)	0.91 (3H, d, *J*=6.8 Hz)
CH_3	0.92 (3H, t, *J*=7.3 Hz)	0.92 (3H, t, *J*=7.1 Hz)	0.92 (3H, t, *J*=7.1 Hz)	0.92 (3H, t, *J*=7.3 Hz)	0.92 (3H, t, *J*=7.3 Hz)
CH<	1.11~1.26 (1H, m)	1.11~1.23 (1H, m)	1.11~1.23 (1H, m)	1.11~1.22 (1H, m)	1.11~1.23 (1H, m)
CH_2	1.38~1.46 (1H, m) 1.46~1.69 (1H, m)	1.47~1.60 (2H, m)	1.48~1.60 (2H, m)	1.39~1.47 (1H, m) 1.47~1.59 (1H, m)	1.39~1.48 (1H, m) 1.48~1.59 (1H, m)
CH_2CO	2.46 (1H, dd, *J*=16.0, 1.8 Hz) 2.47 (1H, dd, *J*=16.0, 11.0 Hz)	2.41 (1H, dd, *J*=16.3, 10.0 Hz) 2.49 (1H, dd, *J*=16.3, 2.2 Hz)	2.41 (1H, dd, *J*=16.2, 10.1 Hz) 2.49 (1H, dd, *J*=16.2, 2.4 Hz)	2.46 (1H, dd, *J*=16.0, 1.7 Hz) 2.47 (1H, dd, *J*=16.0, 11.3 Hz)	2.46 (1H, dd, *J*=16.0, 1.6 Hz) 2.47(1H, dd, *J*=16.0, 11.3 Hz)
OH	2.78 (1H, br s)	3.01 (1H, br s)	2.99 (1H, br s)	2.99 (1H, br s)	2.97 (1H, br s)
$COOCH_3$	3.71 (3H, s)	3.71 (3H, s)	3.71 (3H, s)	3.71 (3H, s)	3.71 (3H, s)
>*CH*OH	3.93~3.99 (1H, m)	3.84~3.91 (1H, m)	3.85~3.91 (1H, m)	3.93~3.99 (1H, m)	3.93~3.99 (1H, m)

* Numbers are referred to the Scheme 1.

Fig. 2. C-2 methylene region of ^1H NMR spectra (400 MHz) of methyl 3-hydroxy-4-methylhexanoate ($CDCl_3$, TMS).

a; Isolated from permetin A, b; (3*R*,4*S*)-isomer (*erythro*), c; (3*S*,4*S*)-isomer (*threo*).



acid methyl ester (**8**) was carried out by preparative TLC on silica gel (EtOAc - $CHCl_3$) and distillation (bp 70°C/3 torr, yield 50%). Purity of four stereoisomers of **8** was determined to be >98% by GC analysis*. NMR spectra of them showed that any *threo*- or *erythro*-isomer of **8** does not contain a detectable amounts of their diastereotopic isomer (*erythro*- or *threo*-, respectively).

β-Hydroxyl fatty acid of permetin A was obtained by hydrolysis of permetin A with 6 N HCl at 110°C for 1 hour, followed by extraction with ether. The acid was esterified with excess ethereal diazomethane and the methyl ester purified by distillation (bp 95°C/6 torr). GC analysis indicated the purity of the methyl ester to be about 90%.

Table 1 shows the values of optical rotation and ^{13}C and ^1H NMR spectrum data of four stereoisomers of **8**, synthesized from isoleucine isomers and those of the material isolated from permetin A. Fig. 2 shows partial 400 MHz ^1H NMR spectra of permetin A-derived **8** and *threo*- and *erythro*-isomers of **8** at C-2 methylene region. In Table 1, the negative value of $[\alpha]_D$ of permetin A-derived **8** proved that it has (*S*) configuration at C-3 position. However, it is difficult to assign the configuration at the C-4 position from the $[\alpha]_D$ values because of similar absolute values of the four stereoisomers. Table 1 and Fig. 2 indicate that clear differences in NMR spectra exist between *threo*- and *erythro*-isomers of **8**, and that the hydroxyl fatty acid of permetin A belongs to one of the *threo*-isomers [(3*S*,4*S*) or (3*R*,4*R*)]. From these results it is concluded that the hydroxyacyl constituent of permetin A, 3-hydroxy-4-methylhexanoic acid, has (3*S*,4*S*) configuration. This means that the asymmetric carbon at C-3 has the D-configuration and that the side-chain configuration at C-4 is the same as that of L-Ile. It is interesting to note that the here established configuration of the asymmetric centers of β-hydroxyl fatty acid component of permetin A coincides with those previously proposed for β-hydroxyacyl peptides, viscosin[6] and EM-49[7].

* Because the 4 stereoisomers could not be separated from each other under the GC analytical condition, the purity values do not mean optical purity.

Acknowledgment

The authors wish to thank Prof. S. MURAO of Kumamoto Institute of Technology for his continuous encouragement throughout this work. The authors also wish to thank Miss H. ONOE and Mr. K. HIRAYAMA for the measurement of NMR spectra and valuable discussions.

References

1) TAKAHARA, Y.; Y. TAKEUCHI, I. KOMURA, Y. HIROSE & S. MURAO: Isolation of a new peptide antibiotic, permetin A, from *Bacillus circulans*. J. Antibiotics 32: 115~120, 1979
2) SOGN, J. A.: Structure of the peptide antibiotic polypeptin. J. Med. Chem. 19: 1228~1231, 1976
3) TAKEUCHI, Y.; A. MURAI, Y. TAKAHARA & M. KAINOSHO: The structure of permetin A, a new polypeptin type antibiotic produced by *Bacillus circulans*. J. Antibiotics 32: 121~129, 1979
4) SUGAWARA, K.; M. KONISHI & H. KAWAGUCHI: BMY-28160, a new peptide antibiotic. J. Antibiotics 37: 1257~1259, 1984
5) BUSSOU, R. & H. VANDERHAEGHE: Synthesis and circular dichroism of (5*S*)-1-azabicyclo-[3.2.0]heptane-7-one. J. Org. Chem. 43: 4438~4441, 1978
6) HIRAMOTO, M.; K. OKADA & S. NAGAI: The revised structure of viscosin, a peptide antibiotic. Tetrahedron Lett. 1970: 1087~1090, 1970
7) PARKER, W. L. & M. L. RATHNUM: EM49, a new peptide antibiotic. IV. The structure of EM49. J. Antibiotics 28: 379~389, 1975