Notes

BMY-28160†, A NEW PEPTIDE ANTIBIOTIC

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In the course of screening for new antibiotics with broad antibacterial spectrum, *Bacillus circulans* strain H913-B4 was found to produce a novel antibiotic coded as BMY-28160. Antibiotic BMY-28160 is active against Gram-positive and Gram-negative bacteria, anaerobes and fungi. BMY-28160 is closely related to permetin A in structure, differing in containing L-valine in place of L-isoleucine in permetin A. This paper reports the production, isolation, properties and structure of BMY-28160.

B. circulans strain H914-B4 was produced in shake culture at 28°C for 48 hours in a medium containing yeast extract 1%, peptone 2% and glucose 1% (pH 7.0). Five ml of the seed culture was transferred into 500-ml Erlenmeyer flasks containing 100 ml of fermentation medium composed of mashed potato 4%, corn steep liquor 2%, $CaCO_3$ 0.3% and NaCl 0.2%. The fermentation was carried out on a rotary shaker (250 rpm) at 28°C for 6~7 days and the antibiotic activity in the broth determined by bioassay using *B. subtilis* PCI-219 as the test organism.

Harvested broth was extracted with an equal volume of 1-BuOH at pH 7.0. The extract was concentrated *in vacuo* to oily residue which was taken up in MeOH and diluted with EtOAc to precipitate crude antibiotic. This solid was chromatographed on a column of Diaion HP-20 using aq MeOH followed by acidic (pH 2.0) aq MeOH as developing agents. The active fractions eluted with acidic aq MeOH were pooled and concentrated *in vacuo* to a small volume. After being acidified to pH 2.0, the solution was

Fig. 1. IR spectrum of BMY-28160 hydrochloride.

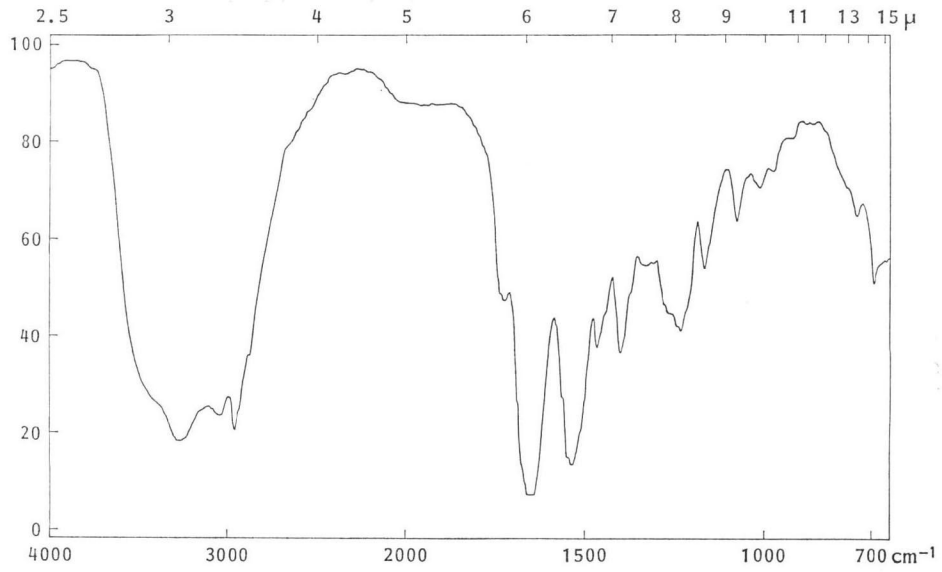

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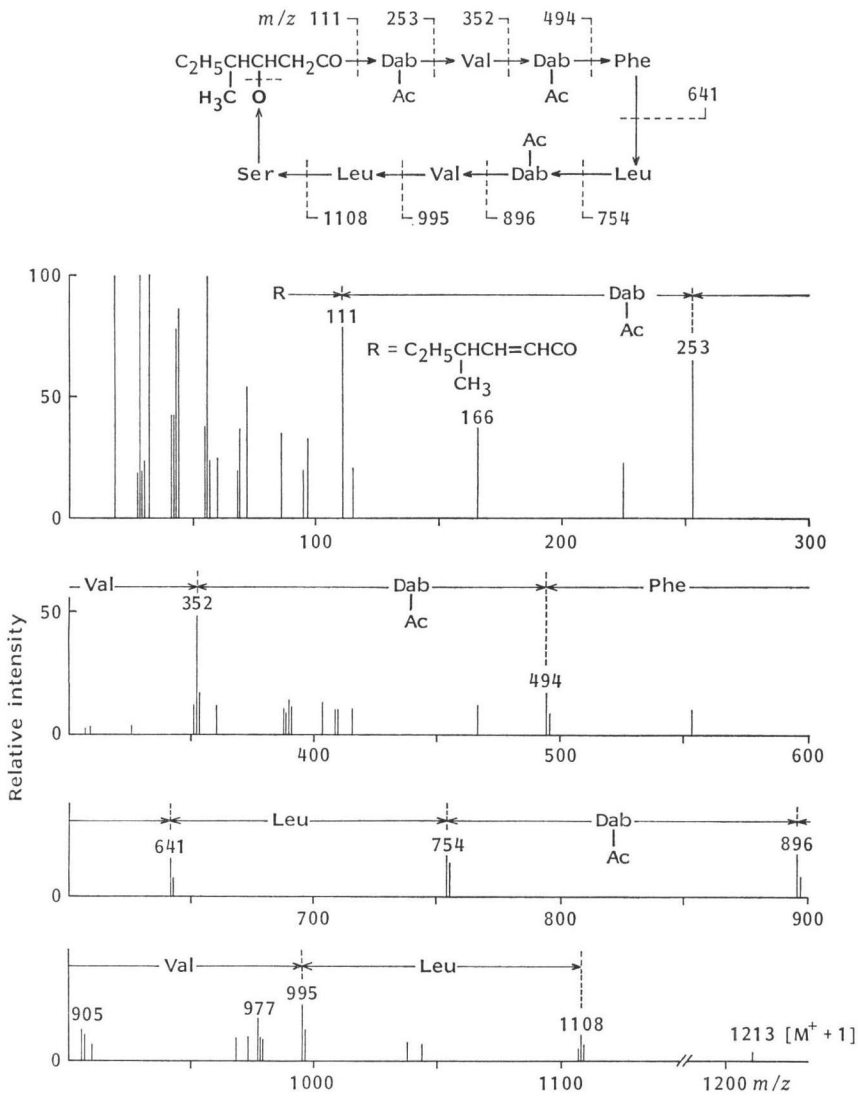
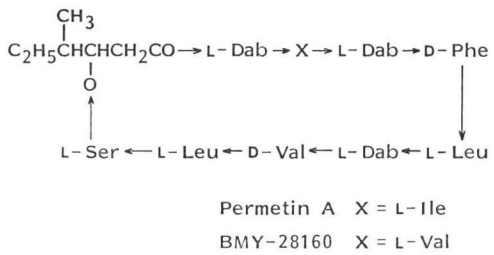
Fig. 2. In-beam EI mass spectrum of *N*-triacetyl-BMY-28160.



applied on a column of Diaion HP-20 which had been prewashed with 50% aq MeOH. Development of the column with the same solvent afforded active eluate which was concentrated and lyophilized to give a white solid of pure BMY-28160 hydrochloride.

Antibiotic BMY-28160 hydrochloride is soluble in H_2O, MeOH and aq lower alcohols, but practically insoluble in EtOAc, Me_2CO and other organic solvents. It melts at 225~229°C with decomposition and is optically active: $[\alpha]_D^{27}$ −65.5° (*c* 1.0, MeOH). Its molecular formula was established as $C_{53}H_{90}N_{12}O_{12}$ by elemental analysis (Calcd for $C_{53}H_{90}N_{12}O_{12}\cdot 3HCl\cdot 5H_2O$: C 49.46, H 8.07, N 13.06, Cl 8.27. Found: C 49.54, H 7.67, N 12.76, Cl 8.84) and from the in-beam EI mass spectral data obtained with triacetyl-BMY-28160 ($M^+ + 1$, *m/z* 1,213.) BMY-28160 hydrochloride did not exhibit absorption maximum above 220 nm. The IR spectrum (Fig. 1) indicated an ester carbonyl band at 1735 cm^{-1} and amide carbonyl bands at 1650 and 1540 cm^{-1}. The ^{13}C NMR spectrum demonstrated 10 C-CH_3 and 9 CO signals along with several methylene and methine carbon signals. Acid hydrolysis of BMY-28160 afforded serine (Ser, 1 L), valine (Val, 1 D+1 L), leucine (Leu, 2 L), phenylalanine (Phe, 1 D) and 2,4-diaminobutyric acid (Dab,

Fig. 3. Structures of BMY-28160 and permetin A.



3 L). In addition, an acidic lipophilic substance was isolated from the hydrolysate, which was identified as 3-hydroxy-4-methylhexanoic acid by the ^{1}H NMR and MS analyses of its methyl ester. These physico-chemical data indicate BMY-28160 is structurally related to polypeptin[1] and permetin A[2], yet differs from them in amino acid composition. The mass spectrum of *N*-triacetyl-BMY-28160 afforded useful information to elucidate the structure of the antibiotic. The protonated molecular ion ($M^+ + 1$, *m/z* 1,213) and diagnostic fragment ions caused by sequential loss of the amino acids from the molecule were analyzed as shown in Fig. 2. In the mass spectrum, the fatty acid moiety was observed in its dehydrated form indicating that its hydroxyl group was esterified by *C*-terminal serine[3].

Upon hydrolysis with 6 N HCl at 37°C, BMY-28160 yielded a tripeptide of Dab→Val→Leu. The chirality of valine was determined, after isolation by complete acid hydrolysis of the tripeptide, to be D-form from its optical rotational value. Thus, the structure of BMY-28160 is assigned as shown in Fig. 3.

The antibacterial activity of BMY-28160 was determined by two-fold agar dilution method and the result is shown in Table 1. BMY-28160 showed a broad antibacterial spectrum with moderate intrinsic activity against Gram-positive and Gram-negative bacteria, anaerobes and fungi. The median lethal dose (LD_{50}) of BMY-28160 in mice was 80 mg/kg by im administration.

BMY-28160 is a new cyclic depsipeptide antibiotic of the polypeptin-permetin A family. It differs from permetin A only in an amino acid between two L-diaminobutyric acid moieties, where L-isoleucine is present in permetin A and L-valine in BMY-28160. It is interesting to note that the molecular ion of a big molecule like BMY-28160 and all key fragment ions were observed by in-beam EI mass spectrometry.

Table 1. Antibacterial activity of BMY-28160.

Test organism	Test medium*	MIC (μg/ml)
Staphylococcus aureus 209P	NA	12.5
〃 〃 Smith	〃	25
Streptococcus pyogenes A20201	〃	3.1
Micrococcus flavus	〃	3.1
Bacillus subtilis PCI 219	〃	3.1
Mycobacterium phlei	〃	12.5
Escherichia coli NIHJ	〃	6.3
Klebsiella pneumoniae D11	〃	3.1
Pseudomonas aeruginosa D15	〃	25
Proteus vulgaris A9436	〃	>100
Bacteroides fragilis A22053	GAM	6.3
〃 〃 A22695	〃	6.3
Clostridium difficile A21675	〃	12.5
C. perfringens A9563	〃	25
Propionibacterium acnes A21933	〃	6.3
Candida albicans IAM-4888	SDA	50
Cryptococcus neoformans D49	〃	50
Trichophyton mentagrophytes D155	〃	12.5

* NA: Nutrient agar (Eiken).
GAM: Gifu anaerobe medium (Nissui).
SDA: Sabouraud dextrose agar.

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References

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