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De-*N*-methylpamamycin-593A and B, New Pamamycin Derivatives Isolated from *Streptomyces alboniger*

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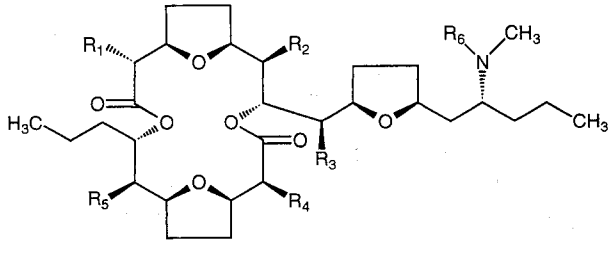
Pamamycin-607 (MW 607) was isolated from the producing strain of *Streptomyces alboniger* IFO 12738 as an aerial mycelium-inducing substance. It is a sixteen-membered macrodiolide with a dimethylamino group-bearing side chain[1,2]. We earlier isolated 14 homologues of pamamycin having different substituents at $R_1 \sim R_5$ and ranging in MW from 593 to 649, and confirmed that a change from CH_3 to CH_2CH_3 at R_3 or R_4 caused loss of aerial mycelium-inducing activity[3]. We also reported that desdimethylaminopamamycin-607 prepared by Hofmann degradation and subsequent catalytic hydrogenation entirely lost this activity[4]. We therefore investigated the structure-activity relationship of the side chain part and continued our search for pamamycin-related compounds in cultured *S. alboniger*. We here report the isolation and structures of de-*N*-methylpamamycin-593A and B (Fig. 1).

A crude pamamycin fraction[3] showed a new Dragendorff reaction-positive spot at Rf 0.30 along with spots of pamamycins (Rf 0.66) on TLC analysis (EtOAc - *n*-hexane - diisopropylamine, 3 : 7 : 0.5). This crude fraction (40 mg obtained from 14 liters of cultured material) was chromatographed in a silica gel column which first was flushed with the solvent system EtOAc - *n*-hexane - $(i\text{-Pr})_2NH$ (2 : 8 : 0.5) to wash out the pamamycins then with the same solvent system (5 : 5 : 0.5) to elute the new compounds. The fraction containing the new compounds was chromatographed by ODS-HPLC (0.2% $AcONH_4$ in 90% aq. MeOH) then by NH_2-HPLC (*n*-pentane - MeOH - *n*-$BuNH_2$, 100 : 0.5 : 0.5), giving two new compounds (2.55 and 2.38 mg).

These compounds seemed to be pamamycin-related ones, because EI-MS analysis showed an M^+ ion at *m/z* 593 and an $M^+ - 43$ ion at *m/z* 550, similar to the pattern of pamamycin-593. There was, however, a characteristic peak at *m/z* 86 rather than at *m/z* 100 the peak for pamamycins. Because the fragment ion of the pamamycins with *m/z* 100 is derived from α-cleavage of the dimethylamino group (fragment ion x in Table 1), these new compounds are thought to be de-*N*-methyl derivatives (Rx = CH_3, Ry = H, Rz = *n*-C_3H_7 in Table 1) or derivatives with the dimethylamino group and the shorter alkyl side chain (Rx = Ry = CH_3, Rz = C_2H_5) of pamamycin.

To confirm this, the incorporation of methionine-*methyl-d*$_3$ into pamamycins and the new compounds present in cultures of *S. alboniger* (1 mg/ml medium) were examined. Pamamycin-607 obtained from the feeding

Fig. 1. Structures of pamamycin-607 and de-*N*-methylpamamycin-593.



	R_1	R_2	R_3	R_4	R_5	R_6
Pamamycin-607	CH_3	CH_3	CH_3	CH_3	H	CH_3
De-*N*-methylpamamycin-593A	CH_3	CH_3	CH_3	CH_3	H	H
De-*N*-methylpamamycin-593B	CH_2CH_3	H	CH_3	CH_3	H	H

Table 1. MS fragmentation of pamamycin-607 and de-*N*-methylpamamycin-593.

	Addition of methionine-*methyl*-d_3	Substituents			Fragmentation (*m/z*)		
		R_x	R_y	R_z	M^+	x	y
Pamamycin-607	−	CH_3	CH_3	n-C_3H_7	607	100	564
	+	CD_3	CH_3	n-C_3H_7	610	103	567
		CD_3	CD_3	n-C_3H_7	613	106	570
De-*N*-methylpamamycin-593A and B	−	CH_3	H	n-C_3H_7	593	86	550
	+	CD_3	H	n-C_3H_7	596	89	553

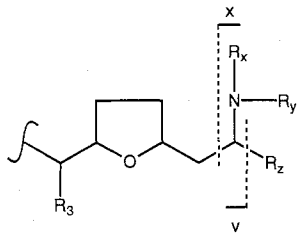
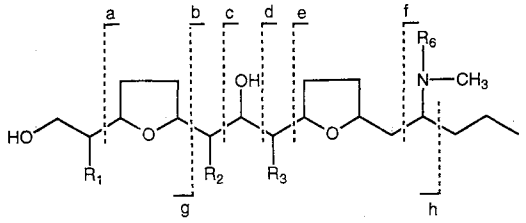


Table 2. MS fragmentation of large diol fragments obtained from pamamycin-607 and de-*N*-methylpamamycin-593A and B.

	Substituents				Fragmentions (*m/z*)								
	R_1	R_2	R_3	R_6	M^+	a	b	c	d+1	e	f	g	h
Pamamycin-607	CH_3	CH_3	CH_3	CH_3	399	340	270	242	213	184	100	129	356
De-*N*-methylpamamycin-593A	CH_3	CH_3	CH_3	H	385	326	256	228	199	170	86	129	342
De-*N*-methylpamamycin-593B	CH_2CH_3	H	CH_3	H	385	312	242	228	199	170	86	143	342



experiment had isotope peaks of 3 and 6 units larger mass at the M^+ ion as well as fragment ions x and y (Table 1). This resulted from the replacement of CH_3 at Rx and Ry with CD_3, confirmation that the two methyl groups on the dimethylamino group of pamamycin are derived from methionine. The two new compounds obtained from the feeding experiment also had isotope peaks of 3 units larger mass at the M^+ ion and fragment ions x and y, but no peaks of 6 units larger mass (such as *m/z* 599, 92 or 556) were detected. These findings confirm that the two new compounds are de-*N*-methyl derivatives of pamamycin but not derivatives with the shorter alkyl side chain. We named them de-*N*-methylpamamycin-593A and B (Fig. 1).

The alkyl substituents $R_1 \sim R_5$ of de-*N*-methylpamamycin-593A and B were identified by GC-MS analysis

of the respective diol products obtained by $LiAlH_4$ degradation of each compound[2,3]. The respective small diol fragments from de-*N*-methylpamamycin-593A and B were identical to those obtained from pamamycin-607 and 621B by GC-MS analysis. The structures of the large diol fragments were determined by comparing their fragment ions in the MS spectra with those of large fragments obtained from known pamamycins (Table 2). De-*N*-methylpamamycin-593A has the same alkyl substituent pattern as pamamycin-607 (Fig. 1).

De-*N*-methylpamamycin-593A and B showed aerial mycelium-inducing activity comparable to that of pamamycin-607. Their growth-inhibitory activity, however, was only half that of pamamycin. These findings support the idea[3], based on the structure-activity relationship of $R_1 \sim R_5$ in pamamycins, that aerial mycelium-inducing and growth-inhibitory activities are produced by different mechanisms.

We now are investigating ways to improve the yield of the de-*N*-methyl derivatives using methylation inhibitors to search for dide-*N*-methyl derivatives. Preparation of various compounds by chemical modification of these pamamycin derivatives will help to clarify the structure-activity relationship of the side chain and the mechanism of aerial mycelium-induction by pamamycin.

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