Structural Elucidation of Heptaene Macrolide Antibiotics 67-121-A and 67-121-C

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Summary The total gross structures of two heptaene macrolide antibiotics of the aromatic subgroup have been determined.

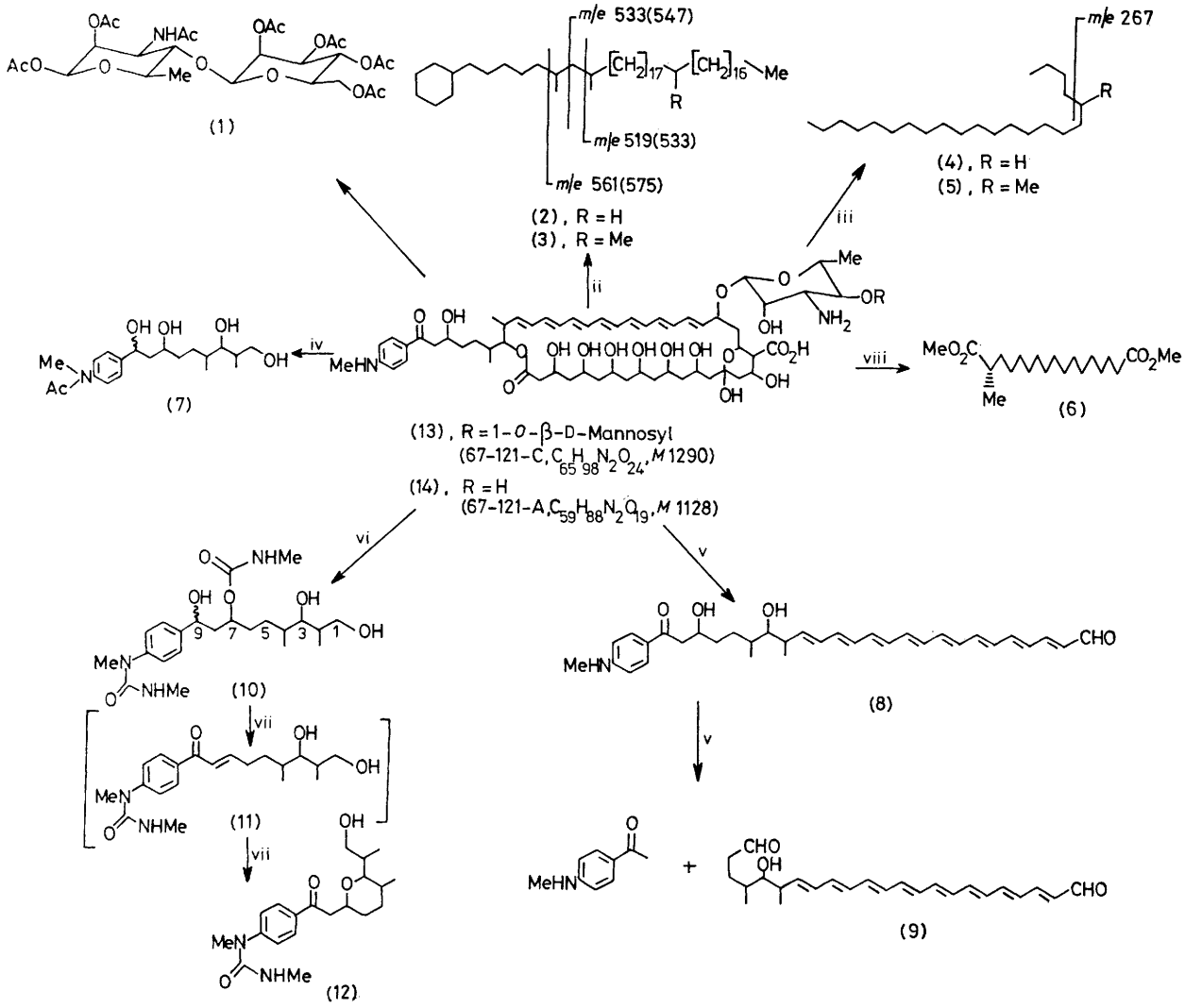
The polyene complex 67-121 (Sch 16656) is elaborated by Actinoplanes NRRL 5325 and consists of three major components, 67-121-A, -B, and -C, which are separable by

partition chromatography on silica gel.[1] The structures of components A and C have been examined in detail.[2] All three components are members of the aromatic subgroup of the heptaene macrolide family of antibiotics. Treatment with aqueous base liberates *p*-methylaminoacetophenone from components A and C. The nature of the glycoside species was in each case determined by acetolysis; 67-121-A contains D-mycosamine as do most other polyenes of this type, while 67-121-C contains a novel disaccharide, 4-*O*-β-D-mannopyranosyl-D-mycosamine (**1**), isolated as the per-*O*,*N*-acetate ($[\alpha]_D$ — 17·1°). This is the first example of a disaccharide occurring as a component of a heptaene antibiotic.

The carbon skeleton was obtained by an exhaustive hydrogenolysis sequence employing high-pressure hydrogenation.[3] Application of this sequence to 67-121-C gave the alkane (**2**) with concomitant loss of the secondary carboxylic acid group during the hydrogenation. Prior reduction of 67-121-C with $NaBH_4$ suppressed decarboxylation and led to the formation of (**3**) in which the carboxylic acid had been retained and converted into a methyl group. A highly characteristic fragmentation pattern in the electron impact mass spectrum of both compounds [*m/e* 519, 533, and 561 for (**2**) and 533, 547, and 575 for (**3**)] established the 1,3-relationship of the two other methyl groups at positions C-36 and C-38.

Components A and C, when subjected to sequential ozonolysis, $NaBH_4$ reduction, and exhaustive deoxygenation, gave two main products identified by g.l.c.–mass spectrometry as (**4**) and (**5**) from both components A and C. The position of the methyl group derived from the original carboxylic acid function was located at C-18 by a specific mass spectral fragmentation of (**5**) leading to an intense ion at *m/e* 267.

Incorporation of a periodate oxidation step in the degradative sequence leading to (**4**) and (**5**) did not affect the out-



i, Ac_2O–H_2SO_4; ii, (a) $NaBH_4$, (b) H_2–Pd, (c) $LiAlH_4$, (d) P–HI, (e) $LiAlH_4$, (f) H_2–Pd; iii, O_3, then as in ii; iv, (a) Ac_2O–MeOH, (b) O_3, (c) $NaBH_4$, (d) NaOH; v, dil. NaOH; vi, (a) MeNCO–pyridine, (b) O_3, (c) $NaBH_4$, (d) NaOH; vii, (a) MnO_2, (b) dil. NaOH; viii, (a) H_2–Pd, (b) HNO_3, (c) CH_2N_2.

come of the experiment, arguing against the presence of vicinal diol functions in the aglycone of either 67-121-A or -C.

Catalytic hydrogenation of the heptaene species followed by oxidation under carefully controlled conditions and esterification of the derived dicarboxylic acids gave the diester (**6**) as the highest molecular weight diester. The rotation of (**6**) ($[\alpha]_D^{26}$ + 17·9°) proved it to have the same absolute configuration as the material derived from amphotericin B.[4]

Ozonolysis of the di-*N*-acetate of 67-121-C followed by reduction with $NaBH_4$ gave the tetraol (**7**) which gave a tetra-acetate on acetylation in pyridine.

Treatment of either 67-121-A or 67-121-C with aqueous base under carefully controlled conditions gave the same octaenal (**8**). The characteristic octaenal absorption in the u.v. spectrum (λ_{max} 435 nm) changed to that of an octaene (λ_{max} 376, 396, and 420 nm) on borohydride reduction. Detailed off-resonance ^{13}C n.m.r. studies established the number and nature of the carbon atoms in (**8**). Mild base treatment of (**8**) induced a retro-aldol reaction to generate *p*-methylaminoacetophenone identical with an authentic sample, and an octaene dialdehyde (**9**). We have assigned the latter compound the same gross structure as the compound prepared similarly by Rinehart *et al.* from vacidin A.[5] Formation of the octaenal is considered to arise by retroaldol cleavage of the aglycone initiated by a ketone function, followed by elimination of the allylic glycoside. The ease of acid hydrolysis of the glycoside linkage is strong supporting evidence for its attachment to an allylic hydroxy group, a feature common to many polyene macrolides. The chemical shifts of the mycosaminyl anomeric carbons in 67-121-A (97·0 p.p.m.) and 67-121-C (96·8 p.p.m.) are very similar to that of the corresponding carbon in *N*-acetyl amphotericin B (97·0 p.p.m.) strongly suggestive of the same anomeric configuration of mycosamine (β) in all three compounds.

The aglycone ring size was established by a sequence involving reaction of the antibiotic with methyl isocyanate to form the percarbamate, followed by ozonolysis, borohydride reduction, and mild base hydrolysis, from which both epimers of a triol (**10**) were obtained in good yield. Location of the position of the methylcarbamate group proved difficult owing to lack of diagnostic mass spectral fragmentations in the triols themselves, their tri-*O*-acetyl derivatives, or their per-*O*,*N*-methyl derivatives. The location was unambiguously established by selective oxidation of the benzylic hydroxy group with manganese dioxide followed by treatment with cold dilute aqueous base to generate the tetrahydropyran (**12**) [*m/e* 362 (M^+); δ 0·7 (3H, d, *J* 7 Hz, CMe), 0·95 (3H, d, *J* 7 Hz, CMe), 2·7 (3H, d, *J* 5 Hz, NH*Me*), 3·28 (3H, S, NMe), 7·35 (2H, d, *J* 9 Hz, Ar), and 8·0 (2H d, *J* 9 Hz, Ar)] which is formed *in situ* from the intermediate α,β-unsaturated ketone (**11**). The hydroxy group involved in lactone formation is, therefore, that at position-3 in (**10**) rather than at the alternative position-7.

Drastic oxidation sequences gave oxalic, malonic, methylmalonic, and 2-methylglutaric acids as the only dicarboxylic acids produced. Conditions were optimized using amphotericin B in a control experiment.

^{13}C N.m.r. studies of the parent compounds and their derivatives in $(CD_3)_2SO$ revealed only one ketone carbon absorption (196·3 p.p.m. in the *N'*-acetyl derivative) and this corresponds to the carbonyl group conjugated to the aromatic ring.

An absorption at 97·1 p.p.m. in the ^{13}C n.m.r. spectrum can be assigned to a hemiacetal carbon atom and, by analogy with results obtained on related compounds,[6] this carbon atom has been assigned to position-15 of the lactone ring.

On the basis of the combined evidence, the gross structure (**14**) is proposed for antibiotic 67-121-A and structure (**13**) for antibiotic 67-121-C. These compounds are clearly closely related to other heptaene macrolides on which structural studies have been reported recently.[5,7]

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