

**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

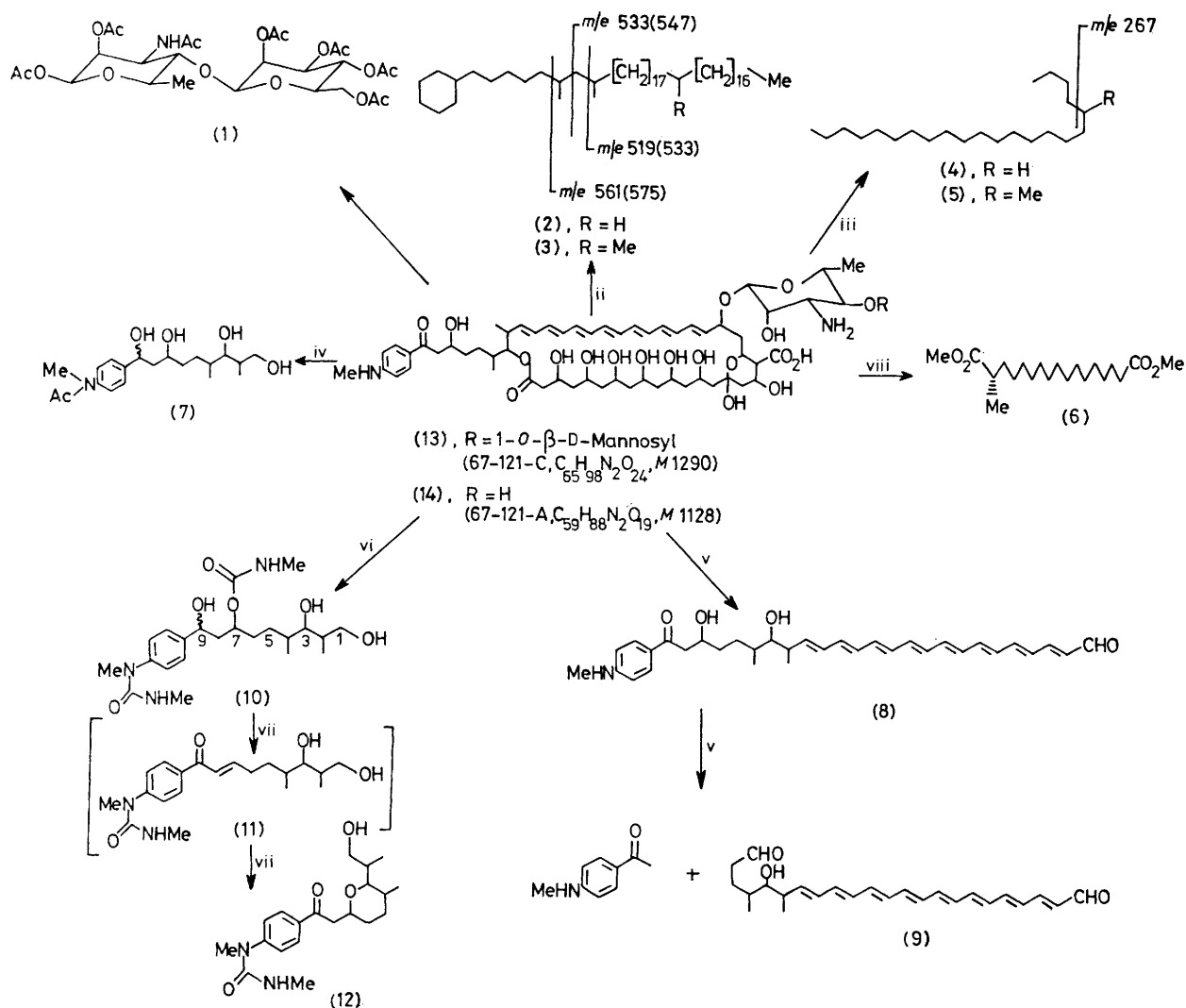
partition chromatography on silica gel.¹ The structures of components A and C have been examined in detail.² All three components are members of the aromatic subgroup of the heptaene macrolide family of antibiotics. Treatment with aqueous base liberates *p*-methylaminoacetophenone from components A and 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 ($[\alpha]_D^{25} - 17.1^\circ$). 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 NaBH₄ 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, NaBH₄ 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, Ac₂O-H₂SO₄; ii, (a) NaBH₄, (b) H₂-Pd, (c) LiAlH₄, (d) P-HI, (e) LiAlH₄, (f) H₂-Pd; iii, O₃, then as in ii; iv, (a) Ac₂O-MeOH, (b) O₃, (c) NaBH₄, (d) NaOH; v, dil. NaOH; vi, (a) MeNCO-pyridine, (b) O₃, (c) NaBH₄, (d) NaOH; vii, (a) MnO₂, (b) dil. NaOH; viii, (a) H₂-Pd, (b) HNO₃, (c) CH₂N₂.

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) ($[\alpha]_D^{26} + 17.9^\circ$) 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 NaBH₄ 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 ¹³C 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, boro-

hydride 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, NHMe), 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.

¹³C N.m.r. studies of the parent compounds and their derivatives in (CD₃)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 ¹³C 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}

We thank Mr. R. Werner for high-pressure hydrogenation experiments.

(Received, 13th June 1977; Com. 576.)

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² A preliminary account of this work has been presented at the 173rd American Chemical Society National Meeting, New Orleans, March 1977, Paper MEDI 5.

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