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Studies on Macrocyclic Lactone Antibiotics. III.[1] Skeletal Structure of Azalomycin F_{4a}

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The skeletal structure of azalomycin F_{4a} (1) was determined on the basis of available data about the physicochemical properties of F_{4a} and about the structures of the degradation products of this compound and of its derivatives. The structure was found to consist of a 36-membered lactone ring bearing multiple hydroxy functions, a diene and a dienoic ester group, as well as a side chain with an *N*-methylguanidine moiety as its terminal. One of the hydroxyl groups on the lactone ring forms a hemiketal ring with the keto group on a ring carbon, and another hydroxyl group forms a hemiester with a malonic acid moiety.

Keywords——azalomycin F_{4a}; skeletal structure; 36-membered $\Delta^{2,4,30,32}$-polyhydroxy lactone; *N*-methylguanidine; intramolecular hemiketal; malonic acid hemiester

In the preceding papers of this series we have reported the physicochemical properties of azalomycin F_{4a} (part I),[3] and the structures of the degradation products of this compound and of its derivatives (Part II).[1]

This paper deals with the skeletal structure of F_{4a} (1) on the basis of the data described previously.

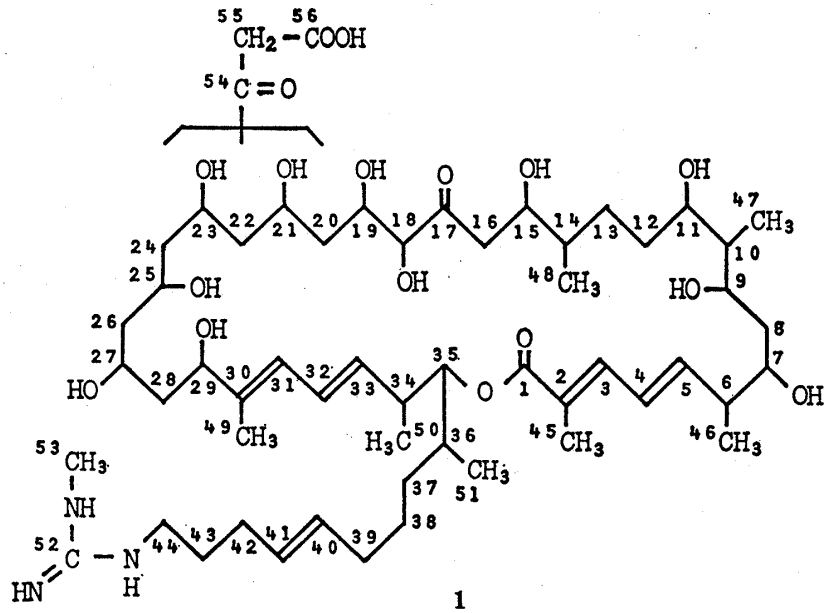

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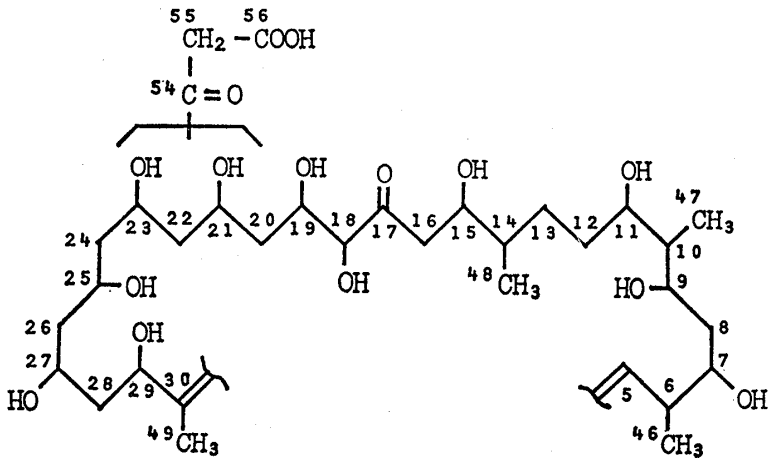
Fig. 1

Discussion

It was reported in part II[1] that ozonolysis of F_{4a} ($C_{56}H_{95}N_3O_{17}$) followed by $NaBH_4$ treatments gave rise to degradation products such as **2a** ($C_6H_{15}N_3O$), **4a** ($C_2H_6O_2$), **11a** ($C_{13}H_{26}O_5$) and **12** ($C_{33}H_{64}O_{17}$). Compounds **3a** and **5a** were obtained by hydrolysis of **11a** and **12**, respectively, and were also formed on prolonged $NaBH_4$ treatment (24 h) of the ozonide in methanol, presumably by similar hydrolysis processes.

Such ozonolysis products should, of course, be derived from cleavages of the five double bonds present in F_{4a}.[3] Ethylene glycol (**4a**) was expected from both conjugated dienoic ester and conjugated diene moieties.

Since the sum of the numbers of carbon atoms, methyl groups, acyl carbonyl groups and carbons of the methylguanidine group represented by these products (**2a**, **4a**×2, **11a** and **12**) coincides with that of F_{4a}, elucidation of the skeletal structure of azalomycin F_{4a} should be possible by determining the connection modes of these fragments.



partial structure I

Fig. 2

Partial Structure I

In part II[1] it was shown that compound **12** gave, on alkaline hydrolysis, compound **5a** and malonic acid, and that the periodate oxidation of **5a** gave rise to **15a** and **16a** (after acetylation) with consumption of 3 mol equivalents of the reagent. In the latter reaction the element corresponding to one methyl and two CH-OH units contained in the structure of **5a** could not be isolated, and the structure of compound **5a** has not previously been discussed.

On elucidation of the partial structure I, several questions arose about the locations of the units which were lost in the periodate oxidation of **5a**, and about the connection mode of the fragments **15a** and **16a**, and of malonic acid in the parent molecule.

It was observed (as described in part II)[1] that compound **5a**, on periodate oxidation followed by $NaBD_4$ reduction, gave **15b** and **16b** (after acetylation), of which the former has a deuterium atom on both terminal carbons (at C-19 and C-29), and the latter has a deuterium atom on one end of the carbon chain (at C-17).

Likewise, periodate oxidation of the deuterated compound **5b** followed by $NaBH_4$ reduction gave **15a** and **16c** (after acetylation), of which the former retained no deuterium, wherease the latter was found to retain a deuterium atom on both terminal carbons (at C-5 and C-17).

These results clearly indicated that one of the terminal carbons (C-5) of compound **5a**

(consequently of **10a**) constituted a terminal carbon of **16a**, and that the other terminal moiety, $CH(CH_3)$-OH, was lost in this oxidation process yielding the fragment **15a**.

On the other hand, it was shown that one of the terminal moieties, $CH(CH_3)$-OAc, of compound **10a** (consequently a terminal moiety, $CH(CH_3)$-OH, of compound **5a**) was retained in compound **23** whose structure evidently consisted of this terminal moiety and the carbon skeleton of **15a**.[1] These facts suggested that the remaining CH-OH unit which could not be trapped in the periodate oxidation of **5a** (and of **5b**) should be located at the position between the fragments **15a** and **16a** forming the C-18 unit in compound **5a** (consequently in azalomycin F_{4a}). The structure of **5a** was thus estabilished to be as shown in the preceding paper (part II).[1]

Hemiester formation of malonic acid with a hydroxy group in the partial structure **5a** was indicated by the fact that both F_{4a} and compound **12** liberated malonic acid on alkaline hydrolysis, as well as by nuclear magnetic resonance (NMR) spectroscopic evidence of an additional ester likage present in F_{4a} other than the lactone linkage between C-1 and C-35 (see Tables I and II in part II).[1] The position of the ester group could not, however, be determined with compound **12** because of the instability of this ester linkage under the purification conditions used, and hence, its position will be discussed later.

^{13}C-NMR of F_{4a} indicated the presence of a carbonyl function forming a hemiketal (signal at δ 99.78, see part I),[3] and the position (C-17) of the functional group was elucidated by deuterium incorporation at C-17 of the compound **5b**, which was determined from the deuteration position in compound **16c**.

The partial structure I was thus formulated as shown in Fig. 2.

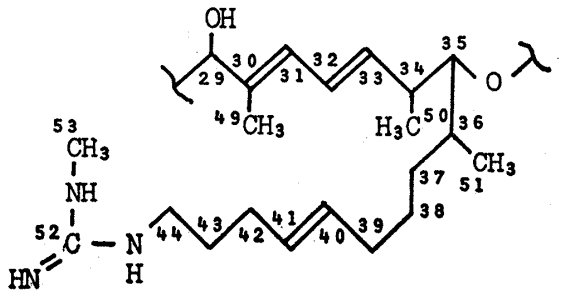
Partial Structure II

As was discussed in the preceding paper (ref. 1), the structure of compound **18** suggested that the fragments **2a** and **3a** should be connected directly through a carbon–carbon double bond (between C-40 and C-41), and formation of the homologues, **18**, **19** and **20**, at the same time could be explained by ozonolysis of a mixture of olefin isomers (Δ^{30}, Δ^{31} and Δ^{32}) formed by a non-selective partial hydrogenation of a diene system present in F_{4a} (C-30—C-33). The presence of such a diene system had already been indicated by inspection of the ultraviolet (UV) and ^{1}H-NMR spectra of F_{4a} and the partial structure was formulated as B in Fig. 2 in ref. 3.

From the data discussed in this section, the partial structure II was built up (Fig. 3).

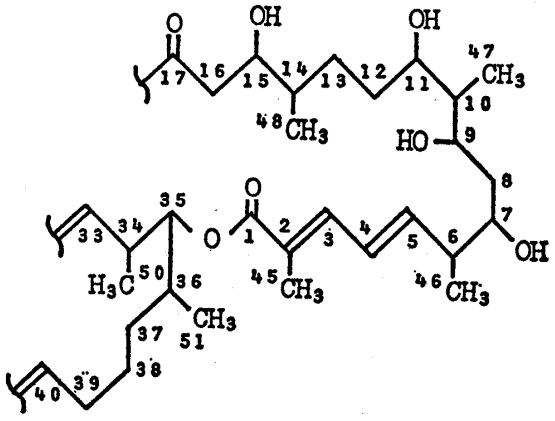
Partial Structure III

The deuteration position in compound **11b**[1] obtained by ozonolysis of F_{4a} followed by $NaBD_4$ reduction of the ozonide indicated that the carbons C-2, C-33 and C-40 form olefinic



partial structure II

Fig. 3



partial structure III

Fig. 4

linkages in F_{4a}. This result taken together with the skeletal structure of compound **22**, as well as with the partial structure A in ref. 3 suggests the partial structure III.

A terminal moiety (at C-17) of this partial structure should be formulated as a keto group, because the terminal carbon of **22** bearing a primary hydroxy group has been proved to be common to one terminal carbon of fragment **16a** (at C-17) (consequently to the C-17 position of the partial structure I), and because the presence of a carbonyl function at this position in F_{4a} was pointed out in the previous discussion on the partial structure I.

Skeletal Structure of Azalomycin F_{4a}

From inspection of the partial structure I, II and III elucidated in this paper together with the molecular formula and functional groups of the compound F_{4a} (see ref. 3), it is now clear that the skeletal structure of this compound should be depicted as shown in Fig. 1.

The C-17 keto group shown in the structure **1** forms a hemiketal in F_{4a} with a hydroxy group in this molecule, although the position of the hemiketal forming hydroxy group remained unknown.

The location of the malonyl hemiester group was deduced to be probably either at C-21 or at C-23 on the basis of the following argument:

As summarized in Table II of part I,[3] extensive studies of the ^{1}H-NMR spectra of F_{4a} revealed that the signal due to the proton on the carbon bearing the malonic acid hemiester appears at δ 5.22, and that the signals of H-7, H-18, H-27 and H-29 appear at δ 3.76, 3.34, 4.02 and 4.16, respectively. The signal of H-19 was shown to appear at δ 3.85 (overlapping with the signals of three other protons) by decoupling of the H-18 signal at δ 3.34. These positions (at C-7, C-18, C-19, C-27 and C-29) were, therefore, excluded as possible positions of the hemiester-forming hydroxy group.

The signals at δ 1.77 and 1.68 were proved to be coupled with the signal at δ 5.22 (a proton on the carbon attached to the hemiester linkage), indicating that the protons which resonate at δ 1.77 and at δ 1.68 are located vicinal to the proton giving the δ 5.22 signal, and no coupling was observed between these proton signals (at δ 1.77 and 1.68) and the proton signals of CH_3-47, CH_3-48 and H-27.

This result demonstrates that H-10, H-14 and H-26 (as well as H-28) are not located on the carbon next to the hemiester-bearing carbon. The hydroxy groups at C-9, C-11, C-15 and C-25 were, hence, also excluded as possible hemiester-forming hydroxy groups.

Consequently the position of the malonyl hemiester linkage was elucidated to be either the C-21 or the C-23 position; the exact position cannot yet be determined.

In conclusion, it has been shown that azalomycin F_{4a}, a main component of azalomycin F complex produced by *Streptomyces hygroscopicus* var. *azalomyceticus*, is a 36-membered macrocyclic lactone antibiotic, possessing *N*-methylguanidine as a terminal moiety of its side chain, an intramolecular hemiketal ring involving the keto group at C-17 and a hydroxy group in the molecule, and a malonyl hemiester group at either C-21 or C-23.

The structure of a macrocyclic lactone antibiotic, primycin, possessing a guanidino group in its side chain has been reported,[4] and F_{4a} represents the second example of an antibiotic with a guanidino group.[5]

In the course of our study on the structures of the macrocyclic lactone antibiotics, azalomycins F_3 and F_5, and copiamycin were also found to possess a guanidino group at the terminals of their side chains, and a malonyl hemiester group in their lactone rings.[6] The structures of these compounds will be published shortly.

References and Notes

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