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STRUCTURE OF MONAZOMYCIN, A NEW IONOPHOROUS ANTIBIOTIC

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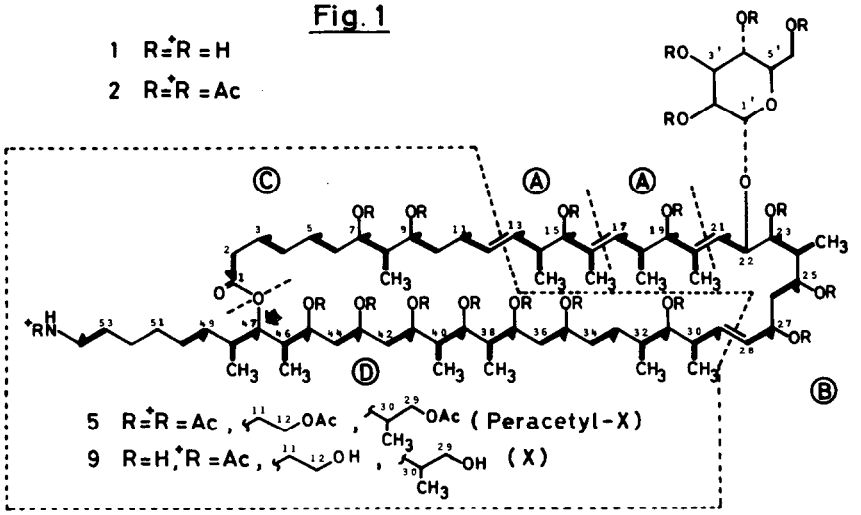
Abstract. The structure of monazomycin was determined by degradative study in combination with biosynthetic means using ^{13}C-NMR.

Monazomycin is an antibiotic isolated from the fermentation broth of Streptomyces mashiuensis and is active against Gram-positive bacteria.[1] Similar to valinomycin, it counteracts the transportation of metal cations across biological membranes induced by carboxylic acid ionophorous antibiotics such as lysocellin and lonomycin A.[2] We wish to report the structure of this biologically interesting compound.

Monazomycin (1) is a colorless amorphous powder possessing the following physicochemical and spectroscopic properties: m.p. 123-127°C; $[\alpha]_D^{22}$ +16.3° (c 1.41, MeOH); pKa' 10.5 (50% EtOH, titration equivalent 1388); λ_{max}^{MeOH} end absorption; ν_{max}^{KBr} 3200-3600 (OH, NH_2), 1710 cm^{-1} (ester carbonyl); positive ninhydrin reaction. The molecular formula $C_{72}H_{133}O_{22}N$ was established by elemental analysis, mass spectral data [FDMS m/z 1365 $(M+H)^+$, 1387 $(M+Na)^+$],[3] and spectroscopic methods as indicated below.

The 100.7 MHz ^{13}C-NMR spectrum of 1 taken in CD_3OD showed total of 66 signals, with 6 ones of double intensity. They are classified to the following groups according to the chemical shifts trends, splitting patterns in the SFORD spectrum and INEPT[4]; one ester carbonyl (δ_C^{TMS} 175.3), 8 olefinic carbons containing two quaternary ones (δ_C 146-130), one acetalic carbon (δ_C 97.8), 21 mono-oxygenated carbons including only one oxymethylene (δ_C 83-63), 10 methines and 19 methylenes (δ_C 44-26), and 12 methyls (δ_C 18-7). The ^{15}N-NMR spectrum of 1 showed the presence of only one aliphatic primary amine $\delta_N^{NH_3}$ 37.6 (t, J_{N-H}=48 Hz). The number of hydroxy functions was determined to be 18 (including one hydroxymethyl group) by making use of the

Fig. 1



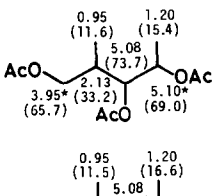
deuterium induced upfield shifts[5] of alcoholic carbons in the ^{13}C-NMR spectrum of 1 taken in CD_3OD/CD_3OH. The molecular formula thus established indicated the presence of two ring structures in 1.

Acid hydrolysis of 1 with Dowex 50 (H^+, at reflux for 1hr) afforded D-mannose ($[\alpha]_D^{22}$=+16.1°, c 2.02, H_2O). The stereochemistry of the glycosidic linkage was revealed to be α by ^{13}C-NMR. The oxymethine signals due to the mannose moiety in 1 (C_3, 72.7, C_5, 74.7) can be compared with those of methyl α-D-mannopyranoside (C_3 72.1, C_5 74.5) but are markedly different from those of the β-anomer (C_3 74.2, C_5 77.5).[6]

At this point, we turned to the use of the ^{13}C-double labeling method,[7] since, in addition to the very complicated structure of 1, the aglycone of 1 with many C-methyl and hydroxy substituents seemed to be a polyketide in biosynthetic origin. In such a case, the technique is known to be a powerful tool for structural elucidation. Feeding experiments and subsequent ^{13}C-{^{13}C}{^{1}H} spin decoupling studies revealed that [1,2-^{13}C] propionic acid and [1,2-^{13}C] acetic acid were incorporated into fragments of 1 as summarized in Fig. 1. No ^{13}C-^{13}C coupling was observed with two methylene carbons (δ_C 27.3 and 30.3) as well as the mannose unit. It is very important to note that all the double bonds in 1 were formed by the condensation of two precursor units. The labeling pattern thus obtained was very useful in the following degradation studies.

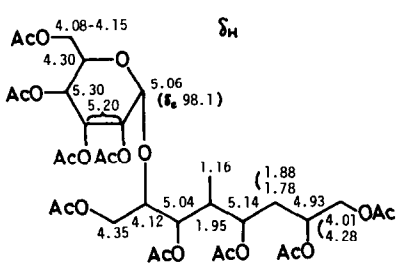
Ozonolysis of peracetyl-monazomycin (2) [$C_{110}H_{171}O_{41}N$; FDMS m/z 2185 $(M+Na)^+$][3] followed by reduction with $NaBH_4$ and acetylation with Ac_2O/pyridine gave 4 components, I, III(I')[8], II, and peracetyl-X. Compounds I and III are colorless oil [$C_{12}H_{20}O_6$; EIMS m/z 260 (M^+), 3 × CH_3COO-].

3 $\delta_H(\delta_C)$



* : Signals disappeared by reduction of $NaBD_4$.

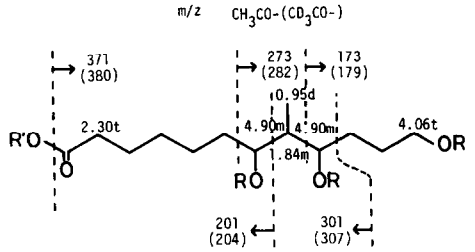
The ^{1}H- and ^{13}C-NMR spectral analysis revealed that compounds I and III are stereoisomers each other with the planar structure of 3. This implies the presence in 1 of the partial structure A in Fig. 1.[9] The yield of 3 (found 9.9%, theoretical: one unit 8.1%, two units 16.3%) indicated that the two partial structural units are contained in 1. In agreement with this, 1 has two allylic methyl groups (δ_H 1.60 and 1.71) and the other ozonolysis products do not contain any corresponding methyl groups. ^{13}C-Labeling pattern of this moiety would be represented by bold lines in A of Fig. 1. Co-occurrence of compounds I and III (I') may be ascribed to the generation of a new chiral center by $NaBH_4$ reduction and/or inherent stereostructural difference in these units.



4 (Compound II)

Compound II is a colorless oil [$C_{33}H_{48}O_{20}$; FDMS m/z 765 $(M+H)^+$, 7 × CH_3COO-] and contains D-mannose (δ_C 98.1) in it Spin decoupling experiments (400 MHz ^{1}H-NMR) determined the structure of the aglycone part of compound II as indicated in 4. The mannose-linked position was determined by the chemical shift of the proton at δ_H 4.12; the other protons being observed at much lower field (δ_H 4.93-5.12). Since no hydroxymethyl carbon exists in the aglycone of 1, 4 must be formed from the partial structure B in Fig. 1.

Peracetyl-X (5) [$C_{69}H_{115}O_{25}N$; FDMS m/z 1359 $(M+H)^+$, 11 × CH_3COO- and 1 × CH_3CON-][3] is a colorless oil and was also prepared by acetylation of compound X (9) [isolated as a N-acetyl

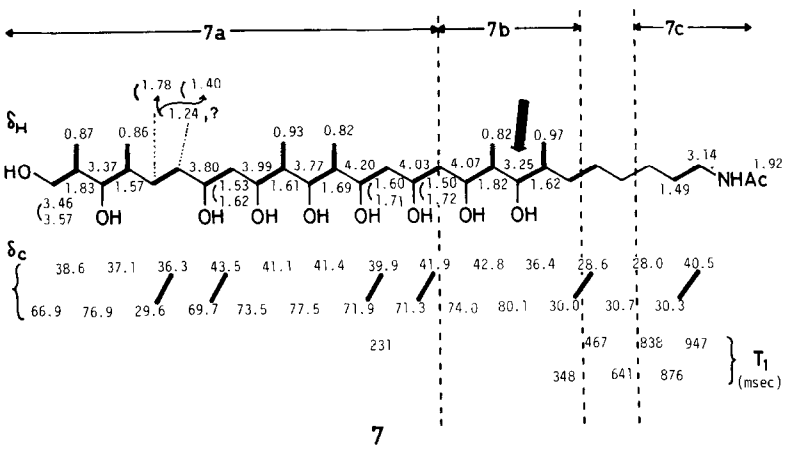


6 R=R'=H

8 R=Ac,R'=CH_3

derivative; $C_{47}H_{93}O_{14}N$, FDMS m/z 918 $(M+Na)^+$] which was obtained from 1 by ozonolysis, $NaBH_4$ reduction, followed by N-acetylation with Ac_2O/MeOH. The molecular formula of 5 accounts for all the remaining part of 1, and therefore, it contains an ester function originally present in 1.

Alkaline hydrolysis of 5 afforded a carboxylic acid (6) and a polyol (7). Treatment of 6 with Ac_2O/pyridine, then with CH_2N_2 gave a triacetyl methyl ester of 6 (8) [$C_{20}H_{30}O_8$; EIMS m/z 371 (M^+-OCH_3)]. Exhaustive ^{1}H-NMR spin decoupling experiments and biosynthetic consideration suggested the structure of 6 to be 7, 9, 12-trihydroxy-8-methyldodecanoic acid. This conclusion was supported by mass spectral analysis of 8 and its corresponding deutero-acetyl derivative. The compound 8 is derived from the partial structure C shown in Fig. 1.



7

The ^{13}C-NMR spectrum of the other compound (7) [obtained as a N-acetyl derivative; $C_{34}H_{69}O_{10}N$, FDMS m/z 674 $(M+Na)^+$] showed 32 carbon signals except for the N-acetyl substituent. ^{1}H-Decoupling difference techniques[10] were exploited to reveal the three partial structures 7a, 7b, and 7c. Due to the extensive overlapping of methylene proton signals, further combination of these fragments was accomplished in the following way.

A pair of -CHOH-CH_2- (δ_C 71.3-41.9) was located at the end of partial structure 7a by selective ^{13}C{^{1}H} spin decoupling of 7 and ^{13}C-{^{13}C}{^{1}H} spin decoupling of 7 derived from 1 labeled with [1,2-^{13}C] acetic acid. The chemical shift of this methylene carbon can be reasonably explained by its combination to another methine carbon, i.e. an oxymethine at δ_C 74.0 in 7b. This is in agreement with the expected biosynthetic labeling pattern.

The connection of 7a and 7b in turn resulted in the establishment of the structure 7 and the partial structure D in Fig. 1; the longer T_1 values and ^{13}C-NMR chemical shifts of methylene carbons in the vicinity of the terminal nitrogen being compatible with this structure.

The remaining problem is to connect the partial structures A-D. Firstly, the ester linkage was located by the use of acylation shift. Comparison of ^{1}H-NMR of 6, 7, and 9 showed that the resonance at δ_H 3.25 in 7 indicated by an arrow shifts to δ_H 4.76 in 9. Hence,

the structure of 9 (composed of partial structures C and D) was established. Next, the connection of partial structures A × 2 and B was proved by the aid of 400 MHz ^{1}H-NMR spin decoupling of 1. Observation of the long-range coupling between δ_H 1.60 (CH_3 at C-16) and 5.26 (H-17), and 1.71 (CH_3 at C-20) and 5.38 (H-21)[11] proved the sequence -A-A-B-. The two trisubstituted double bonds were deduced to have E configurations based on the ^{13}C-chemical shifts of the two allylic methyl carbons (δ_C 17.2 and 17.2).[12] Lastly, these two fragments (-C-D-, -A-A-B-) were connected by spin decoupling experiments ($J_{12,13}$=$J_{28,29}$= 15.5 Hz, both in E configurations). Thus, the structure of monazomycin was established as shown in 1 which is the first natural product having a 48-membered lactone ring. To the best of our knowledge, this is the largest among the macrolide antibiotics reported so far.

With regard to biosynthetic labeling pattern, it should be noted that an acetic acid unit was incorporated in reverse direction to C-53 and C-54 and that C-51 and C-52 were labeled by [2-^{13}C] acetic acid. This labeling pattern may be compatible with the incorporation of acetic acid to these carbons via glutamic acid or its biological equivalent.

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References and Notes.

(1) K. Akasaki, K. Karasawa, H. Yonehara, and H. Umezawa, J. Antibiotics, 16, 127 (1963).

(2) M. Mitani, M. Koenuma, and N. Ōtake, J. Antibiotics, 30, 829 (1977); M. Mitani, and N. Ōtake, J. Antibiotics, 31, 750 (1978).

(3) The observed m/z values were rounded off to the nearest integrals.

(4) D. M. Doddrell and D. T. Pegg, J. Am. Chem. Soc., 102, 6388 (1980).

(5) P. E. Pfeffer, K. M. Valentine, and F. W. Parrish, J. Am. Chem. Soc., 101, 1265 (1979).

(6) H. Seto, T. Sato, S. Urano, J. Uzawa, and H. Yonehara, Tetrahedron Letters, 1976, 4367.

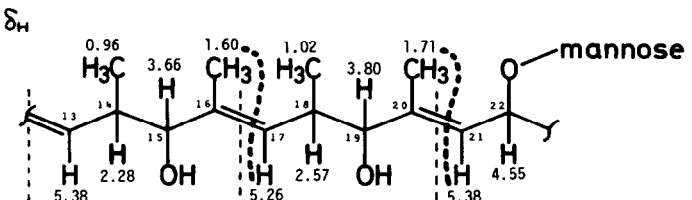
(7) J. B. Stothers; "Carbon-13 NMR Spectroscopy", p. 462, Academic Press, New York (1972).

(8) Compound III is one of the stereoisomers of I and also called I'.

(9) The positions of the double bonds broken by ozonolysis were proved by the reduction with $NaBD_4$ instead of $NaBH_4$.

(10) L. D. Hall and J. K. M. Sanders, J. Am. Chem. Soc., 102, 5703 (1980)

(11) The ^{1}H-NMR resonances relevant to the double bonds on 1 are shown below.



(12) P. A. Couperus, A. D. H. Clague, and J. P. C. M. van Dongen, Org. Magn. Reson., 8, 426 (1976).

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