Tetrahedron Letters,Vol.27,No.14,pp 1603-1606,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain

THE STRUCTURE OF A NOVEL MACROLIDE ANTIBIOTIC, NOTONESOMYCIN A

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Summary: Based on mainly NMR spectral data, the structure of notonesomycin A, a novel macrolide antibiotic has been determined as shown in Fig. 1.

Notonesomycin A is an antifungal antibiotic isolated from the mycelium of Streptomyces aminophilus subsp. notonesogenes 647-AV$_1$ and is effective in the treatment of sheath blight disease of rice plant in a green house test[1]. We wish to report herein the structural elucidation of notonesomycin A accomplished mainly by ^{1}H and ^{13}C NMR spectral analyses.

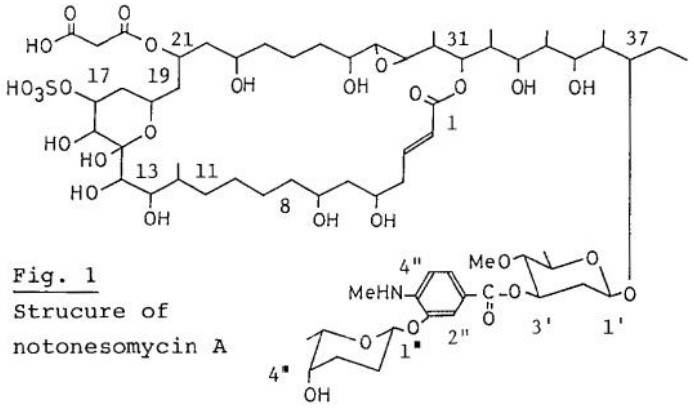


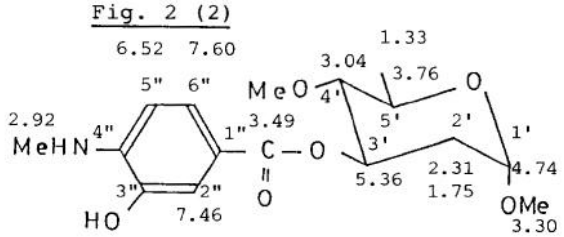
Fig. 1
Strucure of
notonesomycin A

Notonesomycin A (1) is a colorless amorphous powder possessing the following physico-chemical and spectroscopic properties: mp. 194-195° C, $[\alpha]_D^{24}$ -29.7° (c 1.0, $CHCl_3$: MeOH = 1:1), λ_{max}^{MeOH} 310 nm (ε9700), ν_{max}^{KBr} 3400, 1720, 1700, 1600, 1280 and 1060 cm^{-1}.

The molecular formula $C_{68}H_{109}NO_{30}SNa_2$ of 1 was established by elemental analysis (found C;54.21, H;7.85, N;1.33, S; 2.07%, calcd. for $C_{68}H_{109}NO_{30}SNa_2$ C;54.51, H;7.28, N;0.94, S;2.14%), mass spectral data (FAB-MS pos. m/z 1498 $[M+H]^+$, neg. m/z 1452 $[M-2Na+H]^-$) and ^{13}C NMR spectral analysis (vide infra).

A fragment ion peak (m/z 1395, $[M-SO_3Na]^+$) in FAB-MS and the strong absorptions (1280 and 1060 cm^{-1}) in the IR spectrum indicated the presence of a sulfate ester in 1.

Fig. 2 (2)



The isolated methylene protons [δ_H 3.30, s, δ_C 45.6 in DMSO-d_6] were gradually exchanged by deuterium upon addition of D_2O. This phenomenon together with the presence of no ketone carbonyl group strongly suggests that a malonate ester exists in 1.

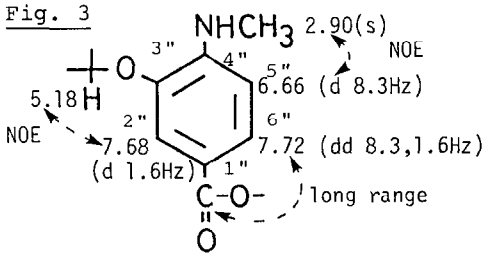
Treatment of 1 with 0.1 N HCl-MeOH followed by purification by Sephadex LH-20 column chromatography gave methyl rhodinose and a chromophore with a deoxy sugar (2) as shown in Fig. 2. Rhodinose was isolated as its 2,4-dinitrophenylhydrazone and its configuration was determined to be L by optical

rotation ($[\alpha]_D^{24}$ -11° (c 0.1, pyridine), lit. $[\alpha]_D^{25}$ -14.9°(c 0.5, pyridine))[2].

Physico-chemical properties of 2 are as follows ; $[\alpha]_D^{24}$ +7.2°(c 0.5, MeOH), $C_{16}H_{23}NO_6$, EI-HRMS m/z obsd. 325.1509, calcd. 325.1524), λ_{max}^{MeOH} 234 nm (ε 9300) and 317 nm (ε13500).

Alkaline hydrolysis of 2 with 0.5 N NaOH-MeOH followed by preparative silica gel TLC gave a methyl glycoside, which was identified as methyl 2,6-dideoxy-4-O-methyl-α-D-arabino-hexopyranoside by ^1H NMR spectral analysis and optical rotation ($[\alpha]_D^{24}$ +110°(c 0.1, $CHCl_3$), lit. $[\alpha]_D^{20}$ +142° (c 0.9, $CHCl_3$))[3]. The downfield chemical shift of H-3' (δ_H 5.36) in 2 indicated that the chromophore is connected to C-3' of the sugar by acyl linkage.

Fig. 3



The structure of the chromophore moiety was determined in the following way (Fig. 3). The coupling pattern of the aromatic protons [H-6" δ_H 7.72 (dd, J=8.3 and 1.6 Hz), H-2" 7.68 (d, J = 1.6 Hz), H-5" 6.66 (d, J = 8.3 Hz)] and the C-H long range coupling between H-6" and carbonyl carbon (δ_C 166.6) observed in 1 indicated the presence of a 1,3,4-tri-substituted benzoic acid system. The long range coupling and NOE were observed between the aromatic proton (H-5") and N-methyl protons (δ_H 2.90, s) and between the anomeric axial proton of rhodinose (δ_H 5.18, dd, J = 1.5 and 8.5 Hz) and the aromatic proton (H-2") in 1. These indicate the substitution of N-methyl at C-4" and rhodinose at C-3" with β-configuration on the aromatic system.

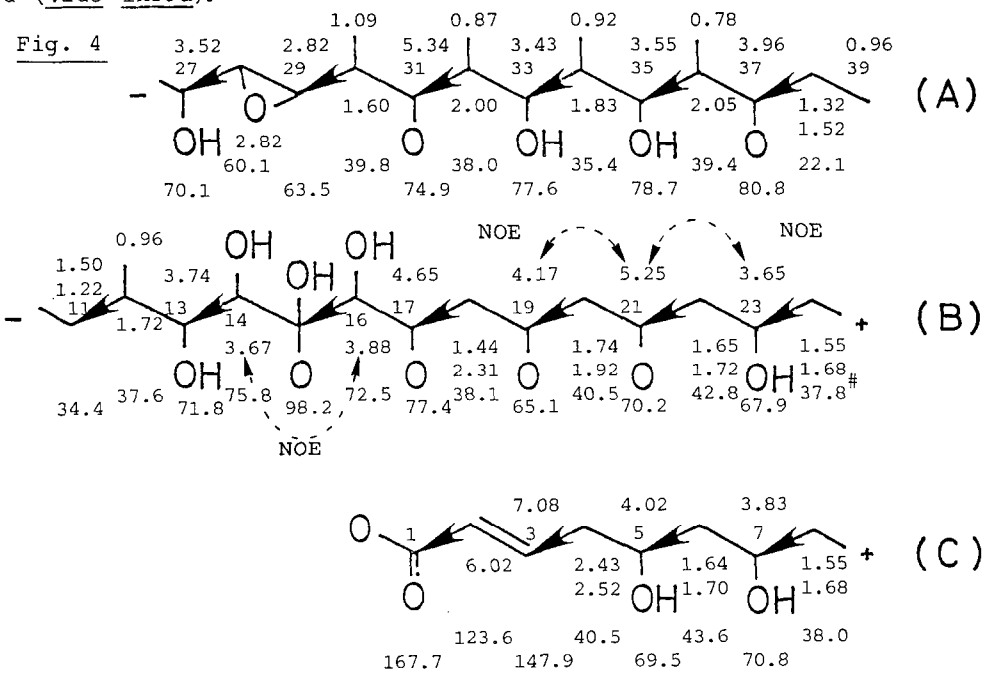
The sugar-chromophore part is glycosidically combined to C-37 of the aglycone with β-configuration by observation of NOE between the anomeric proton (δ_H4.78 (dd J = 1.8 and 9.6 Hz) and H-37 (δ_H 3.96, m). Thus the structure and the position of the sugar chain were determined as shown in Fig. 1.

Partial structures of the aglycone were elucidated by ^1H-^1H spin decoupling experiments as shown in Fig. 4. Partial structure (A) was determined by double or triple decoupling, selective COSY[4] and relayed COSY[5] experiments. The large coupling constants observed in the two oxymethine carbons [δ_C 63.5 (J_{CH} = 170 Hz) and 60.1 (J_{CH} = 173 Hz)] indicated the presence of an epoxide ring. The oxymethine (C-27 δ_H 3.52 δ_C 70.1) located at the end of the partial structure was connected to a methylene (1.4 ppm) hidden in an envelope (1.25 ppm-2.00 ppm).

Partial structure (B) was determined by spin decoupling and NOE experiments. Due to the insufficient separation of H-20 and H-22 methylene protons, the relationship from H-19 to H-23 could not be revealed by decoupling experiments. However, the NOEs observed with H-19 and H-23 upon irradiation of H-21 (δ_H 5.25) enabled us to establish the partial structure from H-19 to H-23. These NOEs could only be observed by transient NOE experiments. The NOE between H-14 and H-16 facilitated to connect these two protons via a hemiketal carbon. The relationship between hemiketal carbon (C-15) and C-16 was proved

by the labelling pattern in the ^{13}C NMR spectrum of 1 labeled with [1,2-$^{13}C_2$] acetic acid (vide infra).

Fig. 4



The two methylenes located at the both ends of the partial structure were connected to methylene protons which could not be identified due to severe overlapping of protons at 1.2 ppm-2.00 ppm. The partial structure (C) was determined also by ^1H-^1H spin decoupling experiments.

The feeding experiments and subsequent ^{13}C-^{13}C spin decoupling experiments revealed that [1,2-$^{13}C_2$] acetic acid and [1,2-$^{13}C_2$] propionic acid were incorporated into the aglycone of 1 as summarized in Fig. 4.

The positions of free hydroxyl groups were determined by observing deuterium induced upfield shifts[6]. In the ^{13}C NMR spectrum of 1 taken in DMSO-d_6 added with one drop of a mixture of D_2O and H_2O (1 : 1), 10 oxymethine carbons (δ_C 66.2, 67.9, 69.5, 70.1, 70.8, 71.8, 72.5, 75.8, 77.6 and 78.7) appeared as doublet or broad signals. Therefore, the hydroxyl functions were located at C-5, C-7, C-13, C-14, C-15, C-16, C-23, C-27, C-33, C-35 and C-4'"as shown in Fig. 4.

The oxymethines involved in the ester bond formation were determined by measuring long range ^{13}C-^1H J modulation difference spectrum (JMD)[7]. Irradiation at the methine proton H-31 (δ_H 5.34) revealed the long range coupling with the carbonyl carbon C-1 (δ_C 167.7). The location of the malonate ester was also elucidated by irradiation at H-21 (δ_H 5.25).

Now there remain two ether oxymethines. The lower chemical shift of the oxymethine proton H-17(δ_H 4.65, δ_C 77.4) indicated the substitution by a sulfate ester at C-17. By elimination, the remaining oxymethine C-19 (δ_H 4.17, δ_C 65.1) must be connected to the hemiketal carbon C-15 (δ_C 98.2) forming a 6-membered ring system. The alternate combination, i.e. linkage of C-19 to the

sulfate group and of C-15 to C-17 to form an oxetane ring can be excluded by the chemical shift of the hemiketal carbon. In addition, the coupling constants between H-16 and H-17 (9.3 Hz) and H-18 and H-19 (8.95 and 4.4 Hz) supported the formation of a pyranose ring with chair conformation.

Finally there remain 4 methylenes which were not contained in the above partial structures. They were shown to form two $-CH_2-CH_2-$ units from the results of feeding experiment of [1,2-$^{13}C_2$] acetic acid and subsequent ^{13}C-^{13}C spin decoupling studies [δ_C 26.3-27.2 (J_{C-C} = 34.1 Hz), 22.1-33.6 (J_{C-C} = 33.5 Hz)].

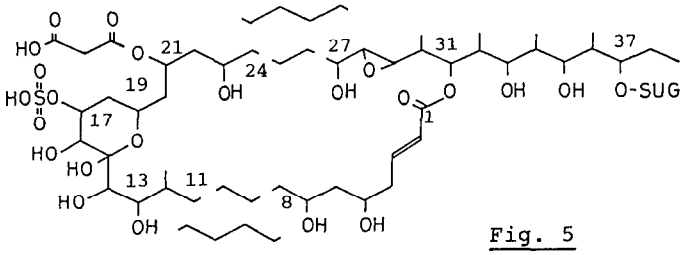


Fig. 5

Thus there exist three possibilities to connect the above partial structures by accommodating no or one $-CH_2-CH_2-$ unit, or two consecutive $-CH_2-CH_2-$ units between C-8 and C-11, and C-24 and C-27 as shown in Fig. 5.

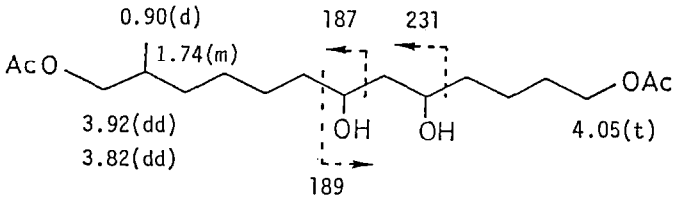


Fig. 6 (3)

This problem could not be solved by NMR experiments due to the almost identical chemical shifts of the relevant methylene protons and therefore, chemical degradation was carried out to obtain a fragment from C-1 to C-13.

Hydrogenation of 1 over PtO_2 followed by oxidation with $NaIO_4$, reduction with $NaBH_4$, acetylation with AC_2O-pyridine and preparative TLC gave a degradation product (3). Its molecular formula was established to be $C_{18}H_{34}O_6$ by FD-MS (m/z 347 $[M+H]^+$) and ^{13}C NMR spectral analyses. The structure of 3 was determined as shown in Fig. 6 by ^1H and ^{13}C NMR spectral analyses and by the fragmentation pattern of EI-MS. The existence of two hydroxymethyls in 3 indicated that the reductive cleavage had occurred at the lactone carbonyl of 1 with $NaBH_4$. The structure of 3, which contained a characteristic $-CH_2-CH(CH_3)-$ unit, suggested that it originated from C-1 to C-13 and accordingly, proved that one $-CH_2-CH_2-$ unit must be inserted between C-8 and C-11 of 1.

Thus, the unique structure of notonesomycin A with a sulfate group is established to be as shown in Fig. 1.

Acknowlegment. This work was supported in part by a Grant-in-Aid for Developmental Scientific Research from the Ministry of Education, Science and Culture, Japan (No.59860012).

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(Received in Japan 28 January 1986)