Tetrahedron Letters,Vol.23,No.26,pp 2667-2670,1982 0040-4039/82/262667-04$03.00/0
Printed in Great Britain

THE STRUCTURE OF A NEW ANTIBIOTIC, HYGROLIDIN

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Summary. The structure of a new antibiotic, hygrolidin has been determined as shown in Fig. 3.

During the course of our screening program for antitumor antibiotics, we found that Streptomyces hygroscopicus D-1166 produced azalomycins B[1] (elaiophylin)[2] and F_{4a}[3]. Detailed analysis of the metabolites of this organism revealed the presence of a minor component active against SV-40 transformed C-3H-2K cells[4]. We wish to report herein the structure elucidation of this antibiotic named hygrolidin.

The mycelium of St. hygroscopicus was extracted with aqueous acetone and after removal of the solvent in vacuo, the active materials were transferred to ethyl acetate. Hygrolidin (I) was isolated from this fraction by silica gel column chromatography ($CHCl_3$:MeOH = 20:1) followed by Sephadex LH-20 chromatography ($CHCl_3$). The antibiotic, I could be distinguished from azalomycins B and F_{4a} based on its chromatographic behaviors (Rf values on silica gel TLC, $CHCl_3$:MeOH = 5:1, I 0.32, Azalomycin B 0.73 and Azalomycin F_{4a} 0.13).

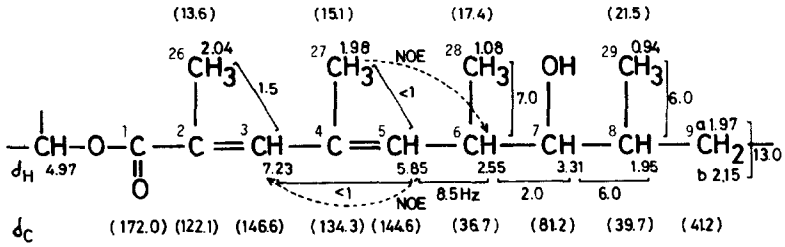
The physicochemical properties of I were as follows; white amorphous powder, mp. 105-107°, $C_{38}H_{58}O_{11}$, FD-MS M^+ (m/z) 690, anal. found: C; 66.67, H; 8.91, O; 24.41 %, calcd: C; 66.06, H; 8.46, O; 25.47 %, $[\alpha]_D^{20}$ +43.3° (c 1.30, $CHCl_3$), λ_{max}^{MeOH} 246nm (ε28200) and 277 (ε11500), $\nu_{max}^{CHCl_3}$ 3360, 1720(sh), 1710, 1670, 1645 and 1610 cm^{-1}.

The ^{13}C-nmr spectrum of I (100 MHz, in $CDCl_3$)[5] revealed the following functional groups and accounted for 54 protons, CH_3 × 9, CH_2 × 3, CH × 5, CH_3O × 1, CH-O × 6 (δ_C 70.3, 71.2, 73.1, 76.0, 81.2 and 82.6), O-C-O × 1 (99.3), CH= × 7, C= × 3 and -COO- × 3 (163.9, 168.3 and 172.0). These data suggested the presence of two ring structures and four oxygen-linked protons in I.

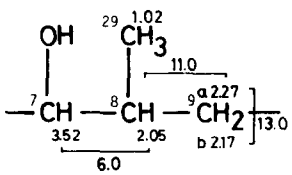
Deuterium induced upfield shift Deuterium induced upfield shift has proved to be useful to locate alcohol functions in complicated molecules[6,7]. An application of this technique proved the presence of four hydroxy or carboxylic acid functions in I. Thus, in the ^{13}C-nmr spectrum taken in $CDCl_3$ added with a 1/1 mixture of CD_3OD/CD_3OH, four carbon resonances at δ_C 70.3, 99.3, 81.2 and 168.3 showed upfield shift with the former two being broadened. Therefore, the resonances due to the carbonyl carbon at δ_C 168.3, quaternary carbon at 99.3 and two oxymethine carbons at δ_C 70.3 and 81.2 were assinged to a free carboxylic acid, a hemiketal and two alcoholic functions, respectively. It follows that the remaining two carbonyl carbons are assigned to ester groups and four oxymethines at δ_C 82.6, 76.0, 73.1 and 71.2 are involved in the formation of ester or ether functions.

Analysis of the 400 MHz ^{1}H-nmr spectrum[5] of I taken in $CDCl_3$ could be accomplished by the aid of conventional proton spin decoupling, difference spectrum[8] as well as NOE experiments to give the following partial structures.

Partial structure A



(NMR spectral data were obtained in $CDCl_3$.)

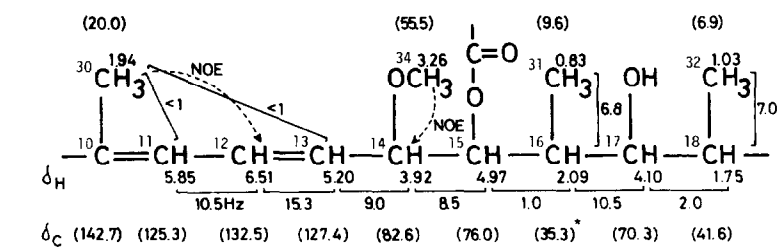


(measured in d_5-pyridine)

The linkage from C-5 to C-8 was straightforwardly revealed by the consecutive ^1H spin decoupling. Allylic couplings [H-5↔CH_3(C-4), H-5↔H-3 and H-3↔CH_3(C-2)] served to extend the sequence as far away as to C-2. Thus, simultaneous irradiation of H-3 and CH_3(C-4) collapsed H-5 to a sharp doublet. In addition, NOE enhancement was observed with H-6 upon irradiation of CH_3(C-4). In agreement with the downfield chemical shift of H-3 (δ_H 7.23), conjugation of a carbonyl carbon with the double bonds was confirmed by ^{13}C- ^1H long range selective proton decoupling (LSPD) irradiating at CH_3(C-2), whereupon the ester carbon C-1 (δ_C 172.0) collapsed to a sharp signal. The C-1 resonance further changed to a sharp doublet upon irradiation of an oxymethine proton (δ_H 4.97) which was later assigned to H-15. NOE enhancement observed between H-3 and H-5, but not between H-3 and CH_3(C-2), together with characteristic $^3J_{C_1-H_3}$ (ca. 8 Hz)[9] indicated that both the double bonds are in E configuration. High order couplings and inadequate separation of H-8 and H-9 signals in the ^1H-nmr spectrum of I taken in $CDCl_3$ prevented to establish the relationship between these protons. In the ^1H-nmr spectrum measured in d_5-pyridine, however, H-8 and H-9 were observed as well separated signals to be analyzed easily (H-7; δ_H 3.52, H-8; 2.05, H-9a; 2.27 and H-9b; 2.17, $J_{7,8}$=6.0 Hz, $J_{8,9a}$=11.0, $J_{8,9b}\simeq 0$ and $J_{9a,9b}$=13.0).

The presence of an alcohol function at C-7 (δ_C 81.2) was established by the coupling between a hydroxy proton (δ_H 4.92) and H-7 (3.16) in the ^1H-nmr spectrum taken in d_6-DMSO as well as by the deuterium induced upfield shift of the C-7 signal as explained above.

Partial structure B



* The assignments of C-16 and C-22 may be exchanged. (measured in $CDCl_3$)

The sequence H-11 to H-15 and H-17 to CH_3(C-18) could be determined unambiguously by spin decoupling. However, almost complete overlapping of H-16 and H-22 (vide infra) in the very crowded region at $\delta_H \sim 2.09$ made it very difficult to connect H-15 and H-17 via H-16. This trouble was overcome by the combined use of difference spectrum[8] and triple resonance (Fig. 1). Thus, a broad doublet at δ_H 2.09 in the difference spectrum [{CH_3(C-16)}-nondecoupled][10] obtained by the irradiation of a methyl at δ_H 0.83 (Fig. 1-B) collapsed to a sharp doublet (J=10.5 Hz) on the simultaneous irradiation of this methyl and H-15. Saturation of H-17 instead of H-15 changed the resonance

to a broad singlet (Fig. 1-D). Therefore, H-15, H-17 and the methyl at δ_H 0.83 [which must be assigned to CH_3(C-16)] are all coupled to H-16. It should be noted that the other overlapping signal at the same position, which was later shown to be coupled with the methyl at δ_H 0.81, was nulled under these experimental conditions. Allylic coupling [CH_3(C-10)↔H-11] and long range coupling through six bonds [CH_3(C-10)↔H-13, $J < 1$ Hz] enabled to connect C-10 and C-11. NOE enhancement of H-12 on the irradiation of CH_3(C-10) and the large coupling constant (15.3 Hz) between H-12 and H-13 established the E configurations of both the double bonds. The position of CH_3-O- was proved to be at C-14 by the NOE enhancement observed with H-14 on the irradiation of the methoxy signal. A hydroxy function was located at C-17, since a proton at δ_H 4.64 exchangable with D_2O was coupled to H-17 in the ^{1}H-nmr spectrum of I taken in d_6-DMSO. Again this finding is compatible with the deuterium induced upfield shift of C-17 (δ_C 70.3).

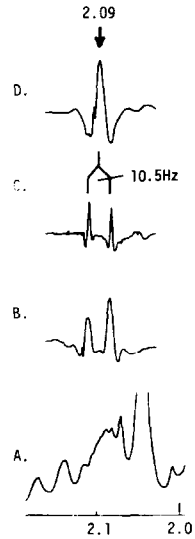
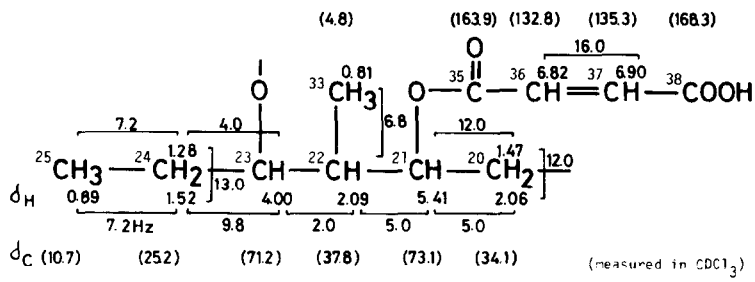


Fig.1 Difference spectra

Partial structure B thus established could be connected to partial structure A as follows. In the difference ^{1}H-nmr spectrum [{CH_3(C-10)} -nondecoupled] taken in $CDCl_3$, H-11 (δ_H 5.85) showed a small splitting (J=1.5 Hz) in addition to the coupling to H-12. This long range coupling was removed upon additional irradiation of H-9 (δ_H 2.15). Thus, C-9 and C-11 must be linked through C-10. Since the long range ^{13}C-^{1}H coupling between H-15 and C-1 had been confirmed (vide supra), C-15 must be linked to C-1 through an ester oxygen resulting in the formation of a 16-membered ring.

Partial structure C



Sequential proton decoupling resulted in this partial structure. An AB quartet (H-36; δ_H 6.82 and H-37; 6.90) with a large coupling constant (J=16 Hz) was ascribed to a fumaric acid half ester residue. The ^{1}H- and ^{13}C-nmr spectral data of this unit can be compared with literature values[11,12]. Use of LSPD also supported this conclusion; simultaneous irradiation of H-36 and H-37 collapsed two carbonyl signals at δ_C 163.9 (C-35) and 168.3 (C-38) to a doublet and a singlet, respectively. The former signal proved to be further coupled with H-21 (δ_H 5.41) and the position of the half ester was thus determined. The presence of a free carboxylic acid function at C-38 has been indicated by deuterium induced upfield shift (vide supra).

The sequence H-20 to H-25 was shown by spin decoupling experiments. The trouble caused by the coincidental chemical shifts of H-22 and H-16 has been solved by the technique as explained above.

At this point, only one hemiketal carbon (δ_C 99.3) remained to be connected. Since no more spin coupling were observed for both H-18 and H-20, these two units are linked via this

quaternary carbon, which in turn must be linked to the oxygen at C-23 forming a six-membered ring. The long range coupling between one of the methylene protons at C-20 and a hydroxy proton of the hemiketal proved the latter proton to be axially oriented[13]. Therefore, the relative stereochemistry of the six membered ring can be illustrated as shown in Fig. 2. It is biosynthetically interesting that the partial structure from C-9 to C-25, in particular, C-15 to C-23 of I is very similar to the corresponding part of azalomycin B.

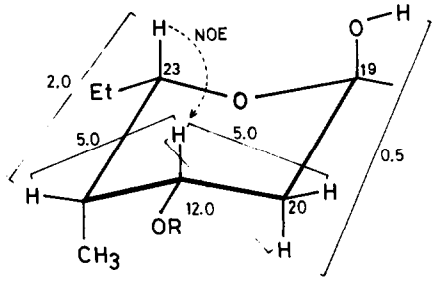


Fig.2 Relative stereochemistry of the six membered ring.

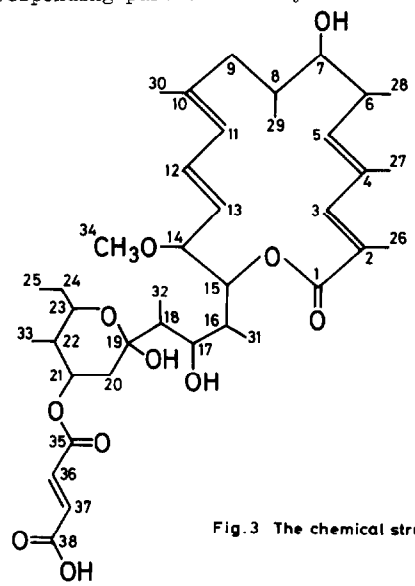


Fig.3 The chemical structure of hygrolidin

ACKNOWLEDGEMENT

The authors thank Kaken Chemical Co., Ltd. for fermentation and JEOL Ltd. for measurement of FD-MS. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

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4) Isolation procedure and biological properties of hygrolidin will be published in detail elsewhere.

5) ^{13}C and ^{1}H NMR spectra were obtained on a JEOL FX-400 spectrometer operating at 100MHz and 400MHz, respectively. Chemical shifts were given in δ values using TMS as internal standard.

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(Received in Japan 17 March 1982)