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THE COMPLETE STRUCTURE OF THE POLYENE MACROLIDE ANTIBIOTIC NYSTATIN A_1

E.Borowski, J.Zieliński, L.Falkowski, T.Zimiński, J.Golik, P.Kołodziejczyk, E.Jereczek, M.Gdulewicz

Department of Drugs Technology and Biochemistry, Technical University, Gdańsk, Poland

Yu.Shenin, T.Kotienko

Research Institute for Antibiotics, Leningrad, USSR

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Nystatin, the most commonly used clinical polyene macrolide antifungal antibiotic from Streptomyces noursei[1], and first discovered member of this group of natural products, has rather long record of chemical studies. It was generally characterised[2] and the structure of glycosidic constituent /mycosamine/ elucidated[3]. Extensive chemical studies lead to rather deep insight into the structure of the antibiotic[4,5,6]. In the most advanced studies the structure of aglycone /nystatinolide/ was postulated[6], although the place of attachement of mycosamine moiety still remained unknown. Recent finding that nystatin is not an individual compound but a mixture of two active principles /both tetraenes/, named nystatin A_1 and A_2 [7], required the verification of accumulated structural data as based on the use of unresolved antibiotic complex. In the present report the complete structure /I/ of nystatin A_1 /main component of the complex/ is postulated. The pure component was isolated from the complex according to the previously described method[7].

The reactions sequence /f/ lead to the formation of compound /IX/ which on mass spectrometry exhibited M^+ at m/e 296 and fragment ions /XVII/[x]. Thus carbon sceleton of C_{1-20} fragment of /I/ is that of 5-methyl eicosane. The degradations /c,d,e/ allowed the localisation of functional groups in C_{1-10} fragment of /I/. Decapentaol/1,3,5,7,10/ /VII/ was examined by mass spectrometry in form of pentamethyl ether derivative [xx]. Found : M^+ at m/e 292 and fragment ions /XVIII/. C_1 of /VII/ was

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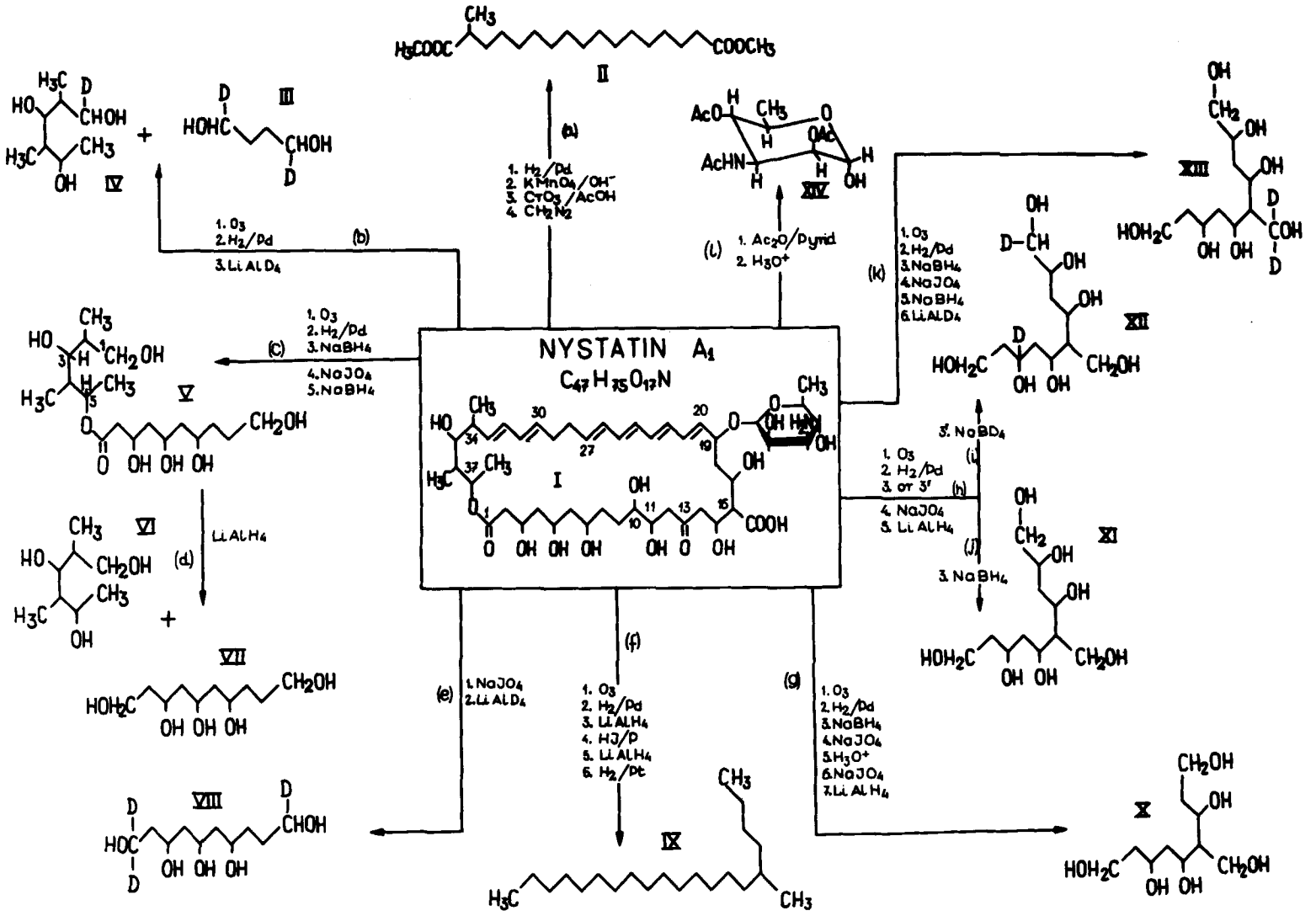
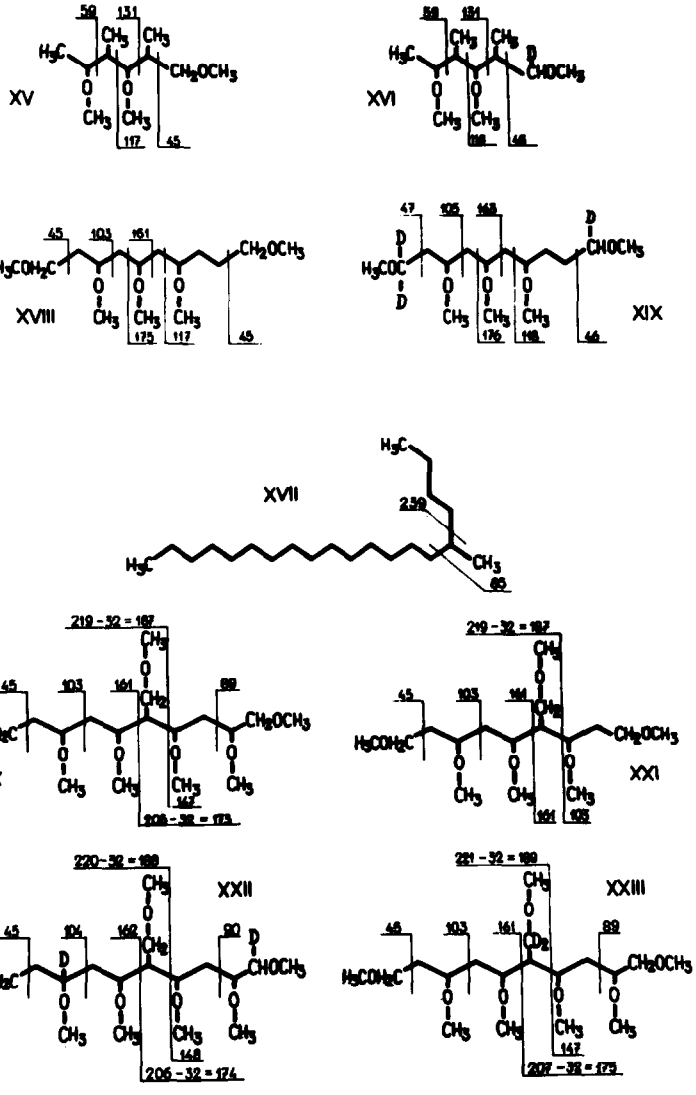
x/ all mass spectra were run on LKB instrument /Model 9000/ with E-301 column

xx/ methylation of all polyols was performed with MeJ/NaH/diglime


NYSTATIN A1
C47 H75 O17 N
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
(a) 1. H2/Pd 2. KMnO4/OH⁻ 3. CrO3/AcOH 4. CH2N2
(b) 1. O3 2. H2/Pd 3. LiAlD4
(c) 1. O3 2. H2/Pd 3. NaBH4 4. NaJO4 5. NaBH4
(d) LiAlH4
(e) 1. NaJO4 2. LiAlD4
(f) 1. O3 2. H2/Pd 3. LiAlH4 4. HJ/P 5. LiAlH4 6. H2/Pt
(g) 1. O3 2. H2/Pd 3. NaBH4 4. NaJO4 5. H3O+ 6. NaJO4 7. LiAlH4
(h) 1. O3 2. H2/Pd 3. or 3' 4. NaJO4 5. LiAlH4
(i) 3'. NaBD4
(j) 3. NaBH4
(k) 1. O3 2. H2/Pd 3. NaBH4 4. NaJO4 5. NaBH4 6. LiAlD4
(l) 1. Ac2O/Pyrid 2. H3O+



derived from carboxyl and C_{10} from aldehyde /formed in periodate cleavage of C_{10-11} vic.glycol in /I/ /,because selective deuterisation in reactions /e/ lead to the formation of1,1,10-trideutero analogue /VIII/ of /VII/.Pentamethyl ether of /VIII/ gave on mass spectrometry M^+ at m/e 295 and fragment ions /XIX/.

Functional groups in C_{11-20} fragment of /I/ were localised in the reactions /g,h,i,j,k/.The basic compound was 6-hydroxymethyl-decahexaol/1,3,5,7,10/ /XI/formed in the reactions /h,j/.Its heptamethyl ether gave on mass spectrometry M^+-31 at m/e 335 and fragment ions /XX/.In /XI/ hydroxymethyl at C_6 was derived from

carboxyl group at C_{16} of /I/ as demonstrated by the selective introduction of two deuterium atoms in /XIII/ in the course of the reactions /k/.Heptamethyl ether of /XIII/ exhibited on mass spectrometry M^+-31 at m/e 337 and fragment ions/XXIII/. Hydroxyl groups at C_3 and C_{10} of /XI/ were derived from ketone group and chromophore terminus at C_{13} and C_{20} of /I/ respectively as again shown by the selective deuterisation in the procedures /h,j/.Heptamethyl ether of /XII/ gave on mass spectrometry M^+-31 at m/e 337 and fragment ions /XXII/.Finally C_1 hydroxymethyl of /XI/ was derived from C_{11} vic.glycol hydroxyl of /I/to be in accordance with the structure of /IX/.Oxygen function at C_{11} of /I/ is hydroxyl as in the case of ketone compound /XII/ would contain a third deuterium atom at C_1.

Mycosamine moiety is attached glycosidically at the allylic C_{19} of /I/.Compound /XI/ contains C_{9-10} vic.glycol which survived periodate treatment in reactions /h,j/ due to the protection of C_9 hydroxyl by the acetal remaining after periodate degradation of mycosamine.Mild treatment of the acetal with acid followed by another step of periodate oxidation /reactions g/ enabled the degradation of vic.glycol with the formation of 6-hydroxymethyl-nonapentaol/1,3,5,7,9/ /X/. Hexamethyl ether of /X/ exhibited on mass spectrometry M^+-31 at m/e 301 and the fragmntation /XXI/.The attachement of mycosamine to the allylic C_{19} of /I/ is also

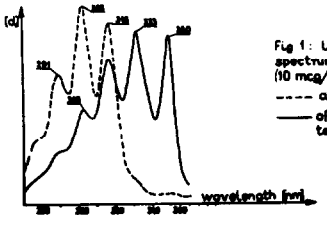


Fig 1: Light absorption spectrum of Nystatin A_1 (10 mcg/ml in 1% HCl/MeOH)
---- at 0 h.
—— after 10 h at room temperature.

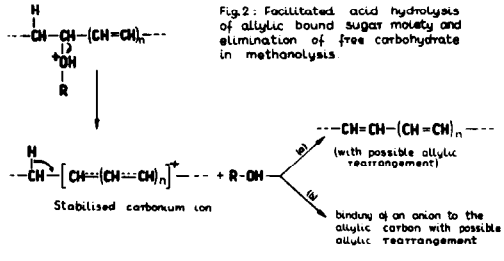


Fig.2: Facilitated acid hydrolysis of allylic bound sugar moiety and elimination of free carbohydrate in methanolysis

in accord with the extreme ease of acid hydrolysis of glycosidic bond and with the elimination of mycosamine but not methyl mycosaminide in MeOH/HCl.The suggested reaction mechanism is indicated on Fig.2.The elimination reaction,which proceeds with very good yield,leads to the formation of a new conjugated double bond.The transformation of the light absorption spectrum of /I/ from tetraene to pentaene one is shown on Fig.1.The formation of pentaene points also to the fact that the chromophore terminus at C_{20} of /I/ belongs to the tetraene and not diene conjugated system,the latter one also being present in the molecule of the antibiotic.

The ring structure of mycosamine moiety is of pyranose type. Mild hydrolysis of peracetyl derivative of /I/ /reactions 1/ yielded 2,3,4-triacetyl mycosamine /XIV/. The pyranose ring structure and C-1 conformation of /XIV/ was based on the determination of chemical shift and spin-spin coupling constants in 80-MHz NMR /$CDCl_3$/. Found: δ=1,10/d,J=6Hz/for CH_3 at C_5; δ=1,85/s/for Ac-N; δ=2,00/s/for Ac at C_2; δ=2,09/s/for Ac at C_4; δ=4,15/m/for H at C_5; δ=5,05/d,J=1,5Hz/for H at C_1; δ=4,9 /m/for H at C_2; δ=4,55-4,8/m/for H at C_3 and C_4; δ=6,1/d,J=8 Hz/for NH; δ=4,45/s/for OH.

The structure of carbon sceleton of tetraene and diene chromophores portion including C_{19-35} fragment of /I/ was established in the reactions /a/. Dimethyl ester of 2-methyl-heptadecanedioic acid obtained /II/ exhibited mass spectrum identical with that of the synthetic product [8] /Found: M^+, m/e 342 and McLafferty ions at m/e 74 and 88/. Tetraene and diene chromophores are separated by $-CH_2.CH_2-$ fragment which gives rise to 1,4-dideutero-butanediol/1,4/ /III/ formed in the reactions /b/. Trimethylsilyl /TMS/ derivative of /III/ exhibited retention time in GLC identical with that of the authentic sample. Deuterium atoms in /III/ were localised by mass spectrometry of TMS derivative. Found: M^+ at m/e 236 as well as elimination and fragment ions at m/e 221,146 and 104.

The structure of the remaining fragment of /I/ including C_{33-38} was established in the reactions /b,c,d/. Compound /VI/ obtained in the reactions /c,d/ gave in mass spectrometry of its trimethyl ether M^+ at m/e 204 and fragment ions /XV/. The attachement of this fragment to the terminus of the diene chromophore in /I/ was established by the selective deuterisation in the reactions /b/. Monodeutero analogue /IV/ of /VI/ exhibited on mass spectrometry of its trimethyl ether derivative M^+ at m/e 205 and fragment ions /XVI/. The introduction of only one deuterium atom in /IV/ also proves that the remaining oxygen functions in the corresponding moiety of /I/ are hydroxyls and not ketone.

The NMR examination of compound /V/ formed in the reactions /c/ supplied the direct evidence for the position of lactone bond between C_1 and C_{37} of /I/. Multiplet signal centered at 5,3 ppm /d_5-pyridine:$CdCl_3$=1:5/ was assigned to the most unshielded proton at the carbon atom with acyloxy group. This is a C_5 proton of /V/ because the irradiation with its resonance frequency caused the transformation of 1,05 ppm doublet /corresponding to the most unshielded CH_3 at C_5 of

/V// to the singlet.

The postulated complete structure of nystatin A_1 shows striking similarities with the heptaene macrolide antibiotic amphotericin B [9].

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