THE STRUCTURE OF NYSTATIN A_3, A COMPONENT OF NYSTATIN COMPLEX

Sir:

Antibiotic nystatin, produced by *Streptomyces noursei*, is a complex containing three biologically active components designated as nystatins A_1, A_2 and A_3. It was established that all of them were polyene macrolides possessing a diene-tetraene chromophore and glycosidically linked mycosamine moiety[1~3]. The structure of the main component of the complex, nystatin A_1, was assigned[4,5]. Structural studies on unresolved nystatin complex were also reported[6].

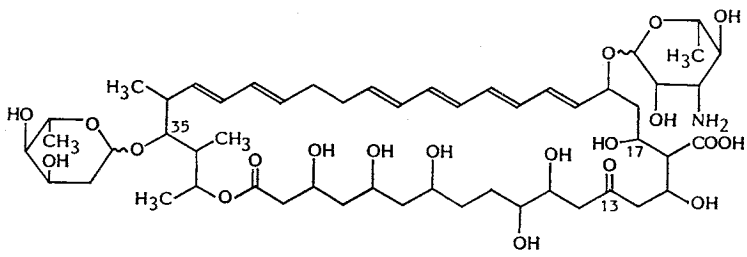
Following the preliminary studies on the structure of nystatin A_3[7] in present paper we report the data on the complete structure of this antibiotic.

The sample of nystatin A_3 was isolated in our laboratory from commercial nystatin by CCD method in two steps. As a result of the first step, nystatin A_1 (K=3.3) was separated from the complex with the solvent system chloroform - methanol - 1% aqueous NaCl solution (2:2:1) after 400 transfers. The residue was separated in the second step with the solvent system chloroform - methanol - borate buffer pH 8.2 (2:2:1) after 400 transfers into nystatins A_2 (K=2.5) and A_3 (K=6.5). All the components of nystatin complex were identified upon their CCD pattern which were identical to those reported previously[1,2]. The sample of nystatin A_3 exhibited the following physico-chemical properties: Colorless amorphous powder insoluble in water and soluble in alcohols, DMF, DMSO and pyridine; UV spectrum (MeOH) showed three maxima at 290, 304 ($E^{1\%}_{1cm}$ 650) and 318 nm; fast atom bombardment mass spectrum (FAB-MS) m/z 1,056 $(M+H)^+$.

The structure of nystatin A_3 (I) was elucidated by means of spectroscopic identification of its degradation products obtained by the procedure previously reported for nystatin A_1[4]. It was found that the only difference between structures of nystatins A_1 and A_3 was the presence in the latter compound of a moiety of L-digitoxose[8].

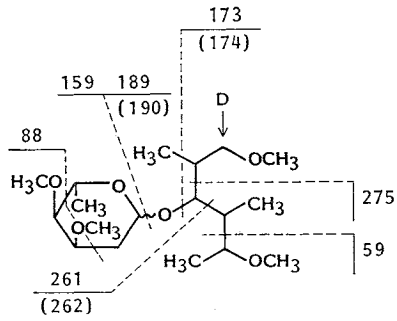
The evidence on the location of L-digitoxose moiety was derived as follows. Ozonolysis of nystatin A_3, hydrogenation of ozonides, reduction of the carbonyl groups with sodium borohydride or sodium borodeuteride and finally hydrolysis with barium hydroxide yielded 3-(*O*-digitoxosyl-1)-2,4-dimethylhexane-1,5-diol (**II**) and its 1-monodeuterio analogue (**III**). The products **II** and **III** were transformed into their tetra-*O*-methyl derivatives **IV**, and **V** and analyzed by MS. The main diagnostic ions for **IV** and **V** are shown in Fig. 1. Methanolysis of **IV** yielded 1,5-di-*O*-methyl-2,4-dimethylhexane-3-ol and tri-*O*-methyl-L-digitoxose identified upon their mass and ^{1}H NMR spectra, respectively. This pointed out the location of glycosidically bound L-digitoxose at C-35 in nystatin A_3.

The remaining part of nystatin A_3 structure was assigned by MS analysis of the degradation products obtained by the reaction sequence presented below. Ozonolysis of nystatin A_3, hydrogenation of ozonides, reduction of the carbonyl functions with sodium borohydride or sodium borodeuteride, oxidation with sodium periodate, reduction with sodium borohydride followed by reduction with lithium aluminium hydride or lithium aluminium deuteride and finally acidic hydrolysis afforded 2,4-dimethylhexane-1,3,5-triol (**VI**) or its 1-deuterio analogue (**VII**), decane-1,3,5,7,10-pentaol (**X**) or its 1,1-dideuterio analogue (**XI**) and 5-hydroxymethyldecane-1,2,4,6,8,10-hexaol (**XIV**) or its 1,8,1′,1′-tetradeuterio analogue (**XV**).



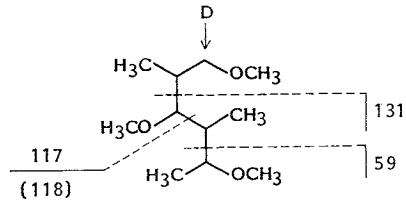
I

Fig. 1. Diagnostic ions observed in the MS of permethylated degradation products IV, V, VIII, IX, XII, XIII, XVI and XVII obtained from nystatins A_3 and A_1.



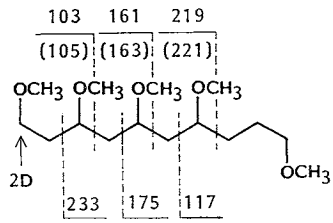
IV or **V**

m/z 316 or 317 (M^+ − MeOH)



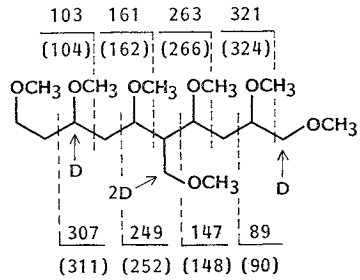
VIII or **IX**

m/z 204 or 205 (M^+)



XII or **XIII**

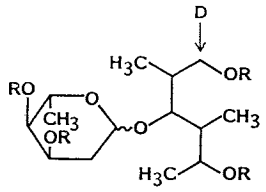
m/z 292 or 294 (M^+)



XVI or **XVII**

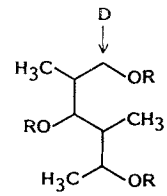
m/z 335 or 339 (M^+ − OCH_3)

Numbers in parentheses indicate the *m/z* values of the ions observed in the MS of deuterated analogues.



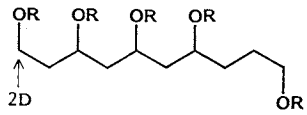
II or **III** R = H

IV or **V** R = CH_3



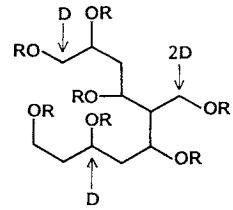
VI or **VII** R = H

VIII or **IX** R = CH_3



X or **XI** R = H

XII or **XIII** R = CH_3



XIV or **XV** R = H

XVI or **XVII** R = CH_3

Identical compounds were obtained as a result of the above sequence of reactions performed with nystatin A_1. The polyols derived from both antibiotics were transformed into their *O*-methyl derivatives **VIII**, **IX**, **XII**, **XIII**, **XVI** and **XVII** which were analyzed with LKB-9000 spectrometer combined with GC. GC conditions were as follows: Column 3 m×3 mm packed with Chromosorb W 80~100 mesh; carrier gas was helium at the flow rate 40 ml/minute. Temperatures(t) of the column and retention times (RTs) for permethylated polyols **VIII**, **XII** and **XVI** were: t=110°C, RT=3 minutes for **VIII**; t=164°C, RT=4 minutes for **XII** and t=220°C, RT=6.4 minutes for **XVI**. MS were generated at the following conditions: Ionization energy 70 eV and temperature of ionization chamber 300°C. The main diagnostic ions observed in the MS of permethylated polyols **VIII**, **IX**, **XII**, **XIII**, **XVI** and **XVII** are shown in Fig. 1.

The MS of compounds **VIII**, **IX**, **XII**, **XIII**, **XVI** and **XVII** exhibited identical features to those reported for nystatin A_1[4].

All the results described above allowed us to postulate the structure of nystatin A_3 as **I**.

It might be expected that hemiketal bond can be formed involving C-13 and C-17 of nystatin A_3, analogically to other polyene macrolides belonging to the same diene-tetraene group[5,9].

Acknowledgments

The authors acknowledge financial support of these studies by The Institute of Organic Chemistry, Polish Acad. of Sci., Warsaw (Project CPBP-01.13).

J. ZIELINSKI
J. GOLIK
J. PAWLAK
E. BOROWSKI

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdansk, 80-952 Gdansk, Poland

L. FALKOWSKI

Institute of Oceanology, Polish Academy of Sciences, 81-967 Sopot, Poland

(Received June 15, 1987)

References

1) SHENIN, Y. D.; T. V. KOTIENKO & O. N. EKSEMPLAROV: Komponentnyi sostav nystatina. Antibiotiki 13: 387~392, 1968
2) POROWSKA, N.; L. HALSKA, Z. PLOCIENNIK, D. KOTIUSZKO, H. MORAWSKA, Z. KOWSZYK-GINDIFER & H. BOJARSKA-DAHLIG: Composition of polifungin, a new antifungal agent. Recl. Trav. Chim. 91: 780~784, 1972
3) BOROWSKI, E.; L. FALKOWSKI, J. ZIELINSKI, P. KOLODZIEJCZYK, J. GOLIK, B. CYBULSKA, T. ZIMINSKI, E. JERECZEK, J. PAWLAK, E. JAKOBS, Y. D. SHENIN & J. M. TERESHIN: The structure, modification and biological properties of antibiotics from group of polyene macrolides. Khim. Farm. Zh. 11: 57~61, 1977
4) BOROWSKI, E.; J. ZIELIŃSKI, L. FALKOWSKI, T. ZIMIŃSKI, J. GOLIK, P. KOŁODZIEJCZYK, E. JERECZEK, M. GDULEWICZ, Y. SHENIN & T. KOTIENKO: The complete structure of the polyene macrolide antibiotic nystatin A_1. Tetrahedron Lett. 1971: 685~690, 1971
5) PANDEY, R. C. & K. L. RINEHART, Jr.: Polyene antibiotics. VII. Carbon-13 nuclear magnetic resonance evidence for cyclic hemiketals in the polyene antibiotics amphotericin B, nystatin A_1, tetrin A, tetrin B, lucensomycin, and pimaricin. J. Antibiotics 29: 1035~1042, 1976
6) CHONG, C. N. & R. W. RICKARDS: Macrolide antibiotic studies. XVI. The structure of nystatin. Tetrahedron Lett. 1970: 5145~5148, 1970
7) BOROWSKI, E.; J. ZIELINSKI, M. GDULEWICZ, J. GOLIK, E. JERECZEK, J. PAWLAK, T. ZIMINSKI, P. KOLODZIEJCZYK, L. FALKOWSKI, N. POROWSKA, D. KOTIUSZKO & Z. PLOCIENNIK: Structural elucidation studies with tetraene antifungal antibiotic nystatin A_3. Abstracts of 8th International, Symposium on the Chemistry of Natural Products, p. 437, New Delhi, February, 1972
8) ZIELINSKI, J.; E. JERECZEK, P. SOWINSKI, L. FALKOWSKI, A. RUDOWSKI & E. BOROWSKI: The structure of a novel sugar component of polyene macrolide antibiotics: 2,6-Dideoxy-L-ribohexopyranose. J. Antibiotics 32: 565~568, 1979
9) SOWIŃSKI, P.; J. K. PAWLAK, E. BOROWSKI & T. IWASHITA: The structure of amphotericin A. II. The complete structure of the antibiotic. J. Antibiotics 38: 175~180, 1985