THE STRUCTURE OF MYCOHEPTIN, A POLYENE MACROLIDE ANTIFUNGAL ANTIBIOTIC

E. BOROWSKI, J. GOLIK, J. ZIELIŃSKI, L. FALKOWSKI,
P. KOŁODZIEJCZYK, J. PAWLAK
Department of Pharmaceutical Technology and Biochemistry,
Technical University, Gdańsk, Poland

and YU. SHENIN
Research Institute for Antibiotics, Leningrad, USSR

(Received for publication April 9, 1976)

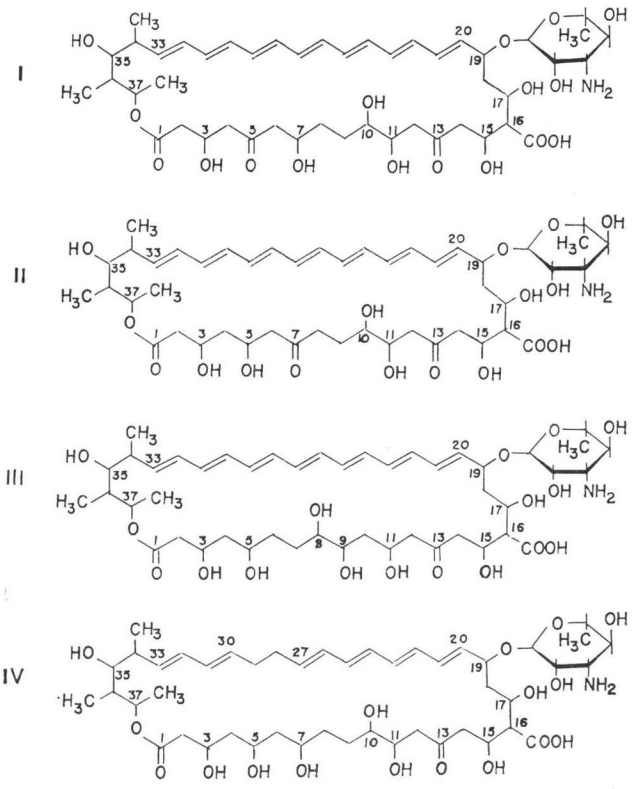
The complete structure of mycoheptin, a heptaene macrolide antifungal antibiotic, has been established as **I** by chemical degradation methods and MS and ^{1}H NMR analysis of the products.

Mycoheptin, a heptaene macrolide antifungal antibiotic produced by *Actinomyces netropsis*[1], belongs to the "nonaromatic" subgroup of the heptaenes with mycosamine as their glycosidic constituent[2].

The structure elucidation studies were performed by running selective chemical degradations followed by mass spectrometric examination of the degradation products or their suitable derivatives. The procedures applied were analogous to those reported in structural studies of amphotericin B[3], nystatin A_1,[4] and candidin[5]. The complete structure of mycoheptin (**I**) is now reported. Mycoheptin is very closely related to candidin (**II**), amphotericin B (**III**) and nystatin A_1 (**IV**). The structures of these antibiotics are shown in Fig. 1. It can be expected that mycoheptin, like other polyene macrolides with hydroxyl group in the δ position to the ketone, can form an equilibrium internal cyclic hemiketal structure[6,7].

The structure of the carbon skeleton of the heptaene chromophore region (C-19 to C-35 of **I**) was established by oxidative degradation of perhydromycoheptin with potassium permanganate

Fig. 1. The structures of mycoheptin (**I**), candidin (**II**), amphotericin B (**III**) and nystatin A_1 (**IV**).



in alkaline medium followed by chromium trioxide in acetic acid. Cleavage at the nearest oxygen functionalities on both sides of the chromophore afforded a dicarboxylic acid which was esterified with diazomethane and examined by mass spectrometry. The product was identical with an authentic sample of 2-methyl-heptadecanedioic acid dimethyl ester obtained from perhydrogenated amphotericin B[8].

Fig. 2. The mass spectrum of 5-methyleicosane.

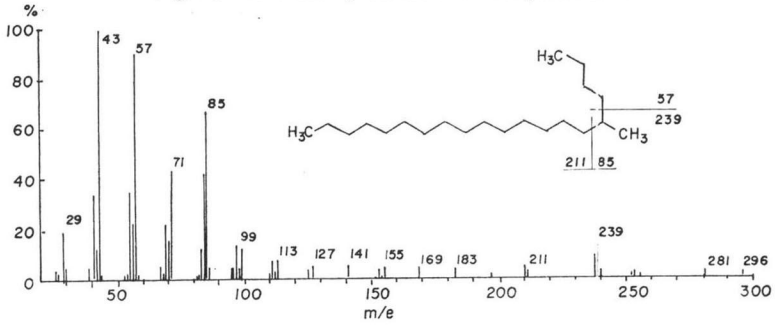
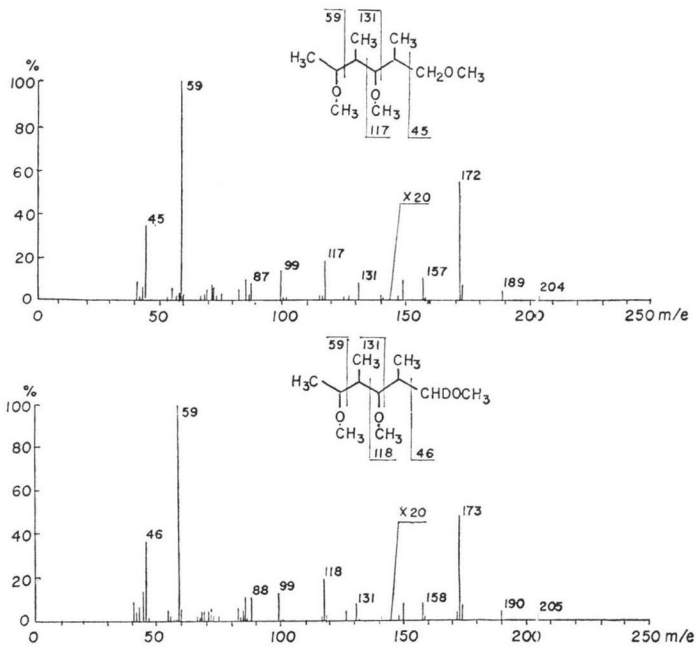


Fig. 3. The mass spectra of 2,4-dimethyl-1,3,5-trimethoxy hexane and its 1-deuterio analogue.



The carbon skeleton of the fragment C-1 to C-20 of **I** was established by the formation of 5-methyleicosane from mycoheptin by the following reaction sequence: ozonolysis, hydrogenation in the presence of palladium, reduction with lithium aluminum hydride treating with hydrogen iodide, reduction with lithium aluminum hydride and finally hydrogenation over platinum catalyst.

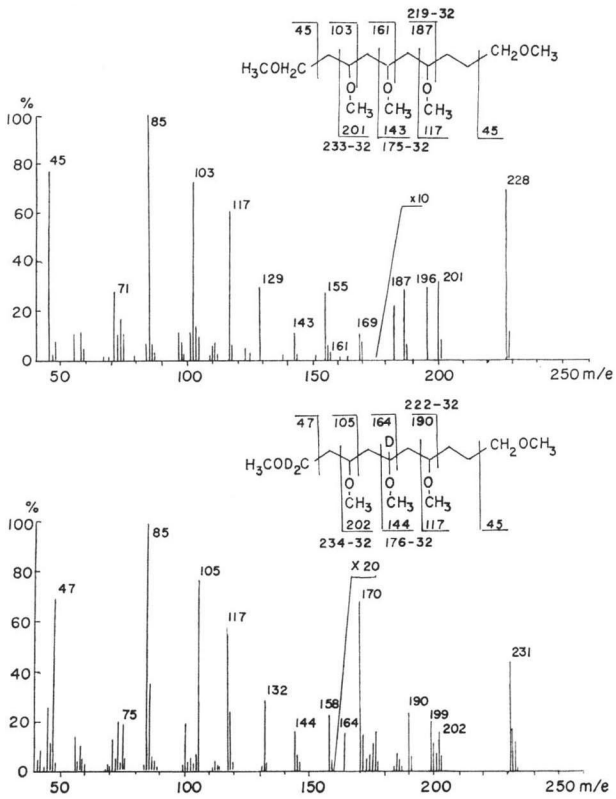
The product was identical by mass spectrometry to 5-methyleicosane obtained from candidin[5] and nystatin A_1.[4] The electron impact spectrum of 5-methyleicosane (Fig. 2) shows a molecular ion at *m/e* 296 and characteristic fragmentation peaks at *m/e* 57, 85, 211 and 239 pointing to branching of the carbon chain at C-5.

The structure of the C-33 to C-38 fragment of **I** and the position of its attachment to the chromophore were established by the formation of 2,4-dimethylhexane-1,3,5-triol and its deuterio analogue from **I** by the following reaction sequence: ozonolysis, hydrogenation in the presence of palladium catalyst, and reduction with lithium aluminum hydride or lithium aluminum deuteride. The structures of these compounds were established by mass-spectral analysis of their permethyl ethers. The mass spectra of the permethoxy derivatives of 2,4-dimethylhexane-1,3,5-triol and [1 – ^{2}H] 2,4-dimethylhexane-1,3,5-triol are given in Fig. 3. Diagnostic for the structures of both compounds are the molecular ions at *m/e* 204 or 205, respectively, the elimination ions at *m/e* 172 or 173 and characteristic fragmentation ions due to the cleavage of carbon-carbon bonds alpha to the oxygen functionalities. The position of

the deuterium atom in [1−^{2}H] 2,4-dimethylhexane-1,3,5-triol corresponds to C-33 of **I**, indicating that C-33 is the terminal carbon atom of the chromophore.

The remaining structural features and the orientation of the fragment C-1 to C-20 of the macrolide ring were elucidated by examination of the corresponding polyols and their deuterio analogues (labelled with deuterium selectively at the reducible positions) formed from **I** by the following reaction sequence: ozonolysis, hydrogenation in the presence of palladium, reduction with lithium aluminum hydride (or lithium aluminum deuteride), oxidation with sodium periodate, and reduction with sodium borohydride. The products were methylated with methyl iodide in the presence of sodium hydride in tetrahydrofuran and examined by mass spectrometry. The mass spectra of the permethoxy derivatives of decane-1,3,5,7,10-pentaol and its 1,1,5-trideuterio analogue, 5-hydroxymethyldecane-1,2,4,6,8,10-hexaol and its 5-dideuteriohydroxymethyl-1,8-dideuterio analogue are presented in Figs. 4 and 5. The structures of these compounds were confirmed by the fragmentation patterns shown in Figs. 4 and 5. Although the molecular ions were not observed, they can be deduced from the elimination ions at *m/e* 228 or *m/e* 231 ($M^+-2\times32$) and *m/e* 335 or *m/e* 339 (M^+-31) respectively. No methoxy groups were present originally in these compounds, since their trimethylsilyl derivatives exhibited GC retention times identical to the trimethylsilyl derivatives of the corresponding polyols obtained from nystatin A_1.[4]

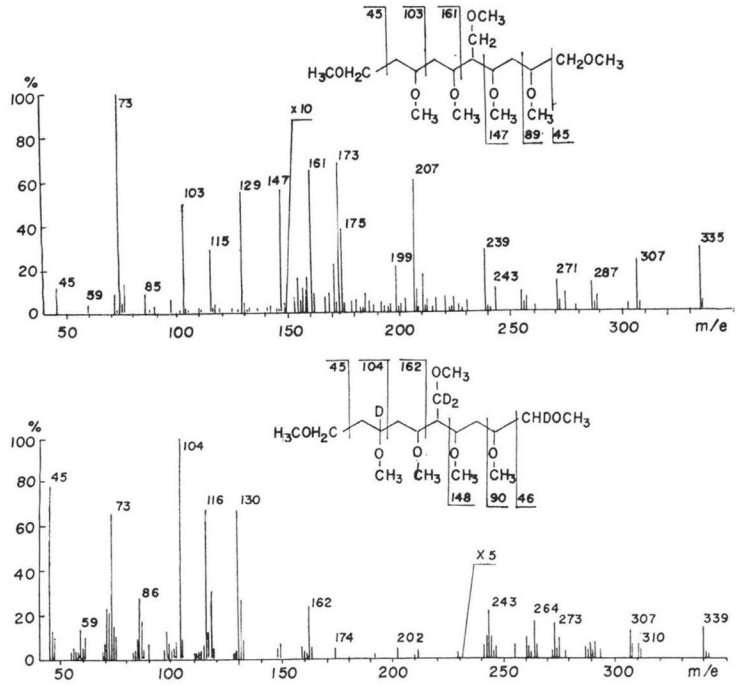
Fig. 4. The mass spectra of 1,3,5,7,10-pentamethoxydecane and its 1,1,5-trideuterio analogue.



The use of lithium aluminum deuteride led to the formation of trideuterio and tetradeuterio analogues of decane-1,3,5,7,10-pentaol and 5-hydroxymethyldecane-1,2,4,6,8,10-hexaol, respectively. The positions of deuterium labeling and the number of deuterium atoms attached to the individual carbon atoms in deuterio analogues indicate that the ketone functions are present at C-5 and C-13 in the C-1 to C-20 fragment of **I**, while C-1 and C-16′ are carboxyl carbon atoms.

The presence of only one deuterium atom at the terminal carbon atom in [1,8,5′,5′−^{2}H$_4$] 5-hydroxymethyldecane-1,2,4,6,8,10-hexaol indicates the presence of a double bond (chromophore terminus) at C-20, which was cleaved by ozone, and the aldehyde formed was reduced by lithium aluminum deuteride. Thus, the orientation of the C-1 to C-20 fragment of mycoheptin was determined. The lack of incorporation of deuterium atoms in other positions proves that the remaining oxygen functionalities in

Fig. 5. The mass spectra of 5-methoxymethyl-1,2,4,6,8,10-hexamethoxydecane and its 5-methoxydideuteriomethyl-1,8-dideuterio analogue.



C-1 to C-20 of **I**, established by the corresponding polyols, are hydroxyls. The presence of a vicinal glycol in 5-hydroxymethyldecane-1,2,4,6,8,10-hexaol which survives the sodium metaperiodate treatment indicates that the corresponding position (C-19) in **I** is blocked. This is one of the indications for the attachment of the glycosidically bound mycosamine at C-19 of **I**. Periodate treatment of the antibiotic followed by reduction with sodium borohydride transformed the mycosamine moiety to an acetal which was further cleaved during the acidification of the reaction mixture, yielding the free polyol. Further evidence for the attachment of mycosamine at the allylic position (C-19) of **I** is the extreme ease of formation of 2,3,4-triacetylmycosamine from peracetylmycoheptin by methanolysis. The elimination of the acetylated aminosugar, rather than a methyl mycosaminide derivative, is characteristic for the allylically bound carbohydrate[4]. The structure of 2,3,4-triacetylmycosamine, including the pyranose ring and the C-1 conformation, was established by ^{1}H NMR analysis of this compound. The data obtained were identical to those found for the same product from amphotericin B[8].

The formation of compound 3,5-dihydroxy-1,2,4-trimethylpentyl-3,5,7,10-tetrahydroxydecanoate from **I** by the reaction sequence: ozonolysis, hydrogenation in the presence of palladium, reduction with sodium borohydride, oxidation with sodium periodate, and reduction with sodium borohydride located the position of the lactone linkage in the mycoheptin macrolide ring. The structure of this compound was established on the basis of its ^{1}H NMR spectrum and mass spectrometric analysis of degradation products. Treatment of this compound with lithium aluminum hydride, followed by methylation of the reaction products, yielded methyl ethers of 2,4-dimethylhexane-1,3,5-triol and decane-1,3,5,7,10-pentaol, identified by mass spectrometry (Figs. 3 and 4). The ^{1}H NMR analysis of 3,5-dihydroxy-

1,2,4-trimethylpentyl-3,5,7,10-tetrahydroxydecanoate permitted the identification of the position of the ester bond. A multiplet signal centered at 5.3 ppm (d_5-pyridine: $CDCl_3 = 1:5$) was assigned to the most deshielded proton at the carbon atom with the acyloxy group. Irradiation at its resonance frequency caused the transformation of the 1.05 ppm doublet (corresponding to the most deshielded CH_3 at this atom) to a singlet. An identical compound was obtained also from nystatin A_1[4] and candidin[5] by the same degradation scheme. The ^1H NMR spectra of this compound derived from all three antibiotics coincide. A more detailed discussion of the ^1H NMR spectrum was presented earlier[4,5].

Experimental

Mycoheptin (prepared in the Institute of Antibiotics, Leningrad, USSR) exhibited $E^{1\%}_{1cm}$ 1,200 at 380 nm in methanol. Mass spectra were recorded on an LKB-9000 instrument combined with GC. For all separations a 3-m × 0.3-cm column with 3% SE-30 on Chromosorb W, 80~100 mesh, and helium flow rate 40 ml/minute were used. The ^1H NMR spectra were determined on an 80-MHz "Tesla" BS-487 instrument.

General procedures.

(a) Ozonolysis: Mycoheptin, suspended in MeOH, was ozonized at −78°C until the heptaene chromophore disappeared. The excess of ozone was removed by a flow of nitrogen and the ozonide was hydrogenated over palladium on asbestos. The catalyst was separated and the solvent was evaporated to dryness. The crude residue was reduced directly with the respective metal hydrides.

(b) Reduction with sodium borohydride or sodium borodeuteride: The substance to be reduced was dissolved in H_2O (or D_2O) and stirred, and a solution of $NaBH_4$ ($NaBD_4$) in H_2O (D_2O) was added at room temperature until an excess of the reducing agent was present. The reaction mixture was neutralized over Dowex 50W X8 (H^+).

(c) Reduction with lithium aluminum hydride or lithium aluminum deuteride: The substance to be reduced was dispersed in THF, poured into a stirred suspension of $LiAlH_4$ or $LiAlD_4$ (50% molar excess) and heated under reflux for 2 hours. The reaction mixture was cooled to 0°C and the excess of reducing agent was decomposed by addition of AcOEt, followed by MeOH - H_2O (1: 1, v/v). The suspension was acidified with 5% H_2SO_4 (pH 1), and then neutralized with K_2CO_3. The precipitate was discarded and the filtrate was evaporated to dryness, the residue was extracted with MeOH and the filtrate was again evaporated to dryness.

(d) Methylation: The respective polyols were dissolved in THF, and excess of NaH was added to the stirred solution, followed by CH_3I. The mixture was shaken for 16 hours at room temperature, diluted with chloroform (5-fold volume), and centrifuged. The supernatant was washed with H_2O, dried over $MgSO_4$ and evaporated to dryness. The residue dissolved in methanol (1 mg/ml) was directly analyzed by means of g.c.-m.s.

Dimethyl-2-methylheptadecanedioate.

This compound was obtained from perhydromycoheptin and analyzed following the procedure described previously[8]. Tetradecahydromycoheptin (1 g) yielded 165 mg of 2-methylheptadecanedioic acid. It was compared with an acid isolated from oxidative degradation products of perhydroamphotericin B and showed identical Rf (0.4) in hexane - ethyl ether - acetic acid (70: 30: 1, v/v) as well as no depression in a mixture melting point (88~89°C). The 2-methylheptadecanedioic acid obtained from perhydromycoheptin was further esterified with diazomethane and was analyzed by means of g.c.-m.s. The retention time (18 minutes at 220°C) and the analyzed compound was identical with the respective methyl ester revealed from perhydroamphotericin B.

5-Methyleicosane.

Mycoheptin (1 g) was ozonized according to procedure (a) and the residue obtained was reduced with $LiAlH_4$ (procedure c). The mixture of polyols was purified on silica gel in the solvent system

AcOEt - HCOOH - H_2O (2: 1: 1, v/v). The main component (0.4 g) was dissolved in AcOH (4 ml), poured into a mixture of 57% HI (10 ml) and red phosphorus (0.2 g) and heated under reflux for 2 hours. After cooling, the reaction mixture was diluted with water (50 ml) and extracted 3 times with $CHCl_3$ (25-ml portions). The collected organic phase was washed with 5% $NaHCO_3$, 2% $Na_2S_2O_3$ and H_2O, dried over $MgSO_4$ and evaporated. The oily residue (0.42 g) dissolved in THF (4 ml) was added to a suspension of $LiAlH_4$ (0.5 g) in THF (25 ml) and heated for 2 hours under reflux. The excess of reducing agent was decomposed with AcOEt (10 ml), the precipitate was centrifuged and washed 3 times with hexane (50-ml portions), and the combined supernatants were evaporated to dryness. The residue, dissolved in hexane (10 ml) - AcOH (0.5 ml), was hydrogenated for 2 hours over Pt (0.05 g). The catalyst was centrifuged, the solvents were evaporated to dryness, and the residue was dissolved in hexane and filtered through a silica gel column. The fraction containing hydrocarbons was analyzed by g.c.-m.s. The main component (80%) was identified as 5-methyleicosane (retention time 12 minutes at 240°C). The following major peaks in the mass spectrum in order of decreasing relative intensity were found: *m/e* 43, 57, 85, 71, 99, 239, 211, 281.

2,4-Dimethyl-1,3,5-trimethoxyhexane.

2,4-Dimethylhexane-1,3,5-triol (**IV**) was isolated following the procedure of COPE, *et al.*[9] Mycoheptin (0.1 g) afforded 0.014 g of **IV**, which was methylated according to procedure (*d*) and identified by means of g.c.-m.s. (retention time 3 minutes at 110°C). The procedure and the yield for the deuterio analogue were identical.

1,3,5,7,10-Pentamethoxydecane.

Mycoheptin (0.05 g) was ozonized (procedure *a*) and reduced with $LiAlH_4$ (procedure *c*). The residue, suspended in H_2O (5 ml), was treated with $NaIO_4$ (0.05 g) in H_2O (2 ml) for 2 hours at room temperature. The excess $NaIO_4$ was destroyed with ethylene glycol, the solution was neutralized with $Ba(OH)_2$, and $BaCl_2$ was added until complete precipitation of the iodates. The mixture was filtered and the solution was evaporated to dryness. The residue obtained was dissolved in THF (2 ml) and reduced with $LiAlH_4$ following procedure (*c*). The solution (10 ml) was then extracted with 1-BuOH (3×10 ml), and the aqueous phase was evaporated to dryness. The residue purified by silica gel column chromatography in solvent system $CHCl_3$ - MeOH (5: 1), yielded 7 mg of decanepentaol which was further methylated by procedure (*d*) and characterized by g.c.-m.s. (retention time 4 minutes at 164°C). The deuterio analogue was obtained similarly.

5-Methoxymethyl-1,2,4,6,8,10-hexamethoxydecane.

Mycoheptin (0.2 g) was ozonized (procedure *a*), reduced with $LiAlH_4$ or $LiAlD_4$ (procedure *c*), further oxidized with $NaIO_4$, and worked up as described previously. The residue was dissolved in THF and reduced with $LiAlD_4$ or $LiAlH_4$ (procedure *c*). The product was purified over silica gel in the solvent system AcOEt - MeOH - H_2O (3: 1: 0.2), yielding 0.011 g of polyol, which was further methylated (procedure *d*) and analyzed by means of g.c.-m.s. (retention time 6.4 minutes at 220°C).

3,5-Dihydroxy-1,2,4-trimethyl-3,5,7,10-tetrahydroxydecanoate.

Mycoheptin (1 g) was ozonized (procedure *a*), reduced with $NaBH_4$ (procedure *b*), oxidized with $NaIO_4$ and again reduced with $NaBH_4$. The residue was purified over silica gel in the solvent system $CHCl_3$ - MeOH (5: 0.6, v/v), yielding 0.091 g of the ester.

2,3,4-Triacetylmycosamine.

Mycoheptin (1 g), dissolved in pyridine (10 ml), was treated with pyridine - acetic anhydride (4: 1, 4 ml) for 24 hours at -5°C. The mixture was poured into ice and extracted with $CHCl_3$. The organic phase was washed with water, dried over $MgSO_4$, concentrated to 2 ml, and the peracetylated antibiotic precipitated by the addition of hexane (100 ml). The precipitate was centrifuged, washed 3 times with 50-ml aliquots of hexane, and dried in vacuum to afford 1.1 g of decaacetylmycoheptin. The product was dissolved in methanol, treated with 1% methanolic HCl and allowed to stand for 12 hours at room temperature. The reaction mixture was then neutralized with $NaHCO_3$ and filtered, and the solution was evaporated to dryness. The sugar component was purified by silica gel column chromatography in the solvent system chloroform - acetone (5: 1, v/v) to yield 0.13 g of triacetyl-

mycosamine.

Acknowledgements

The authors wish to thank the Institute of Organic Chemistry, Polish Acad. of Sci. and Polish United Pharmac. Ind., "Polfa", for financial support of these studies.

References

1) Tsyganov, V. A.; Yu. E. Koniev & W. P. Namestnikova: The characterization of Actinomycete 44-B-1, producing mycoheptin a new antifungal antibiotic. Antibiotiki 7: 599~602, 1965
2) Borowski, E.; M. Malyshkina, T. Kotienko & S. Soloviev: Mycoheptin, a new member of non-aromatic group of heptaene macrolide antifungal antibiotics. Chemotherapia 9: 359~369, 1964
3) Borowski, E.; J. Zieliński, T. Ziminski, L. Falkowski, P. Kozłodziejczyk, J. Golik, E. Jereczek & H. Adlercreutz: Chemical studies with amphotericin B. III. The complete structure of the antibiotic. Tetrahed. Lett. 1970: 3909~3914, 1970
4) Borowski, E.; J. Zieliński, L. Falkowski, T. Zimiński, J. Golik, P. Kozłodziejczyk, E. Jereczek, M. Gdulewicz, Yu. Shenin & T. Kotienko: The complete structure of the polyene macrolide antibiotic nystatin A_1. Tetrahed. Lett. 1971: 685~690, 1971
5) Borowski, E.; L. Falkowski, J. Golik, J. Zieliński, T. Zimiński, W. Mechliński, E. Jereczek, P. Kozłodziejczyk, H. Adlercreutz, C. P. Schaffner & S. Neelakantan: The structure of candidin, a polyene macrolide antifungal antibiotic. Tetrahed. Lett. 1971: 1987~1992, 1971
6) Mechlinski, W.; C. P. Schaffner, P. Ganis & G. Avitabile: Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. Tetrahed. Lett. 1970: 3873~3876, 1970
7) 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
8) Borowski, E.; W. Mechlinski, L. Falkowski, T. Ziminski & J. D. Dutcher: Oxidative degradation studies with perhydroamphotericin B. Roczniki Chem. 39: 607~611, 1965
9) Cope, A. C.; U. Axen, E. P. Burrows & J. Weinlich: Amphotericin B. I. Carbon skeleton, ring size and partial structure. J. Amr. Chem. Soc. 88: 4228~4235, 1966