THE STRUCTURE OF A NOVEL SUGAR COMPONENT OF POLYENE MACROLIDE ANTIBIOTICS: 2,6-DIDEOXY-L-RIBOHEXOPYRANOSE

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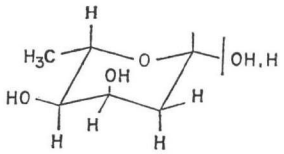
A novel carbohydrate has been isolated after acidic hydrolysis of nystatin A_3, candidinin and polyfungin B and its structure established as 2,6-dideoxy-L-ribohexopyranose.

The polyene macrolides comprise a structurally related group of antifungal antibiotics[1]. Many of these substances contain in their molecules a glycosidically linked carbohydrate moiety: 3-amino-3,6-dideoxy-D-mannose (mycosamine) or 4-amino-4,6-dideoxy-D-mannose (perosamine).

In the course of structural studies with the polyene macrolides performed in our laboratory it was found that acidic hydrolysis of nystatin A_3[2], polyfungin B[3] and candidinin[4] yields besides mycosamine, a novel carbohydrate identified as 2,6-dideoxy-L-ribohexopyranose (**I**, Fig. 1)–L-digitoxose.

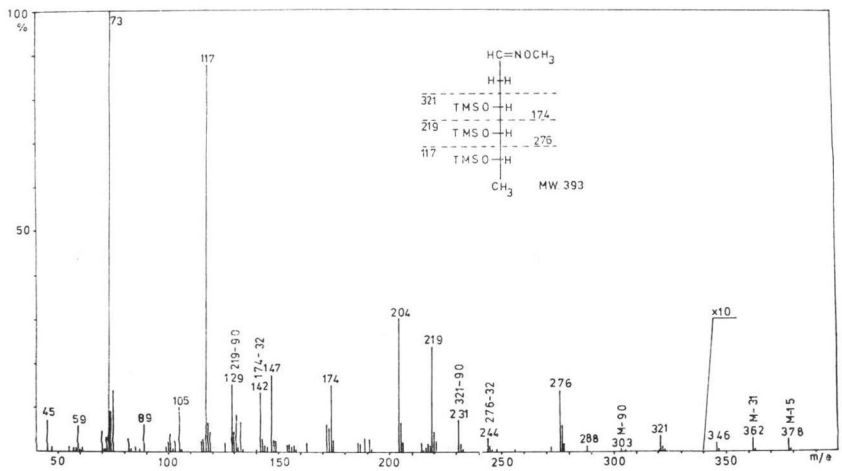
Fig. 1. The structure of L-digitoxose.



Structure Elucidation

The positions of oxygen functions in the carbohydrate moiety were revealed from the mass spectrum of its tri-O-trimethylsilyl methoxime derivative (**II**, Fig. 2). This derivative

Fig. 2. Mass spectrum of 1-methoxime-3,4,5-tri-O-trimethylsilyl-2,6-dideoxyhexose.



was obtained according to the procedure described by LAINE and SWEELEY[5].

Methanolysis of dodecahydronystatin A_3 afforded the mixture of O-methyl-glycosides (**III, IV**). These were further methylated, and the structures of these components established by means of gas chromatography-mass spectrometry as O-methyl glycosides of 3,4-di-O-methyl-2,6-dideoxyhexose in the pyranose and furanose ring forms respectively. The structure of the anomers resulted from studies of their mass spectra (Figs. 3 and 4) following the rules of KOCHETKOV[6].

Fig. 3. The mass spectrum of 1,3,4-tri-O-methyl-2,6-dideoxy-hexopyranose.

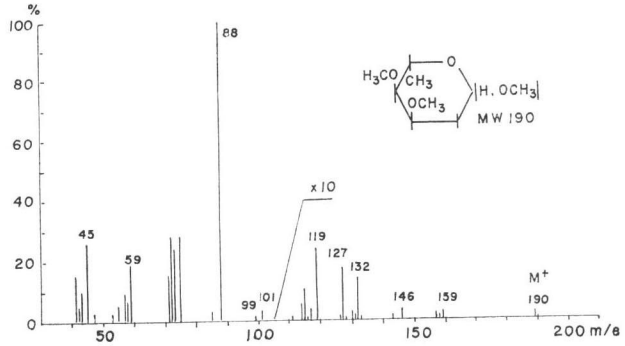
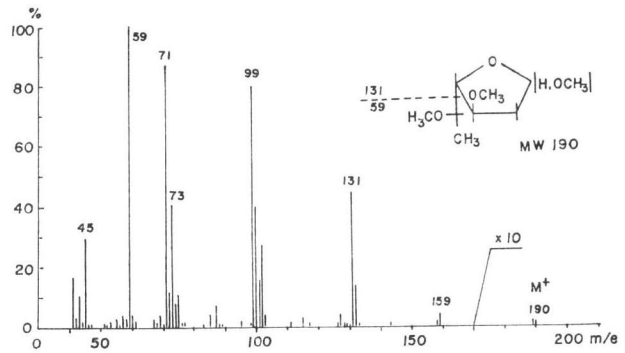


Fig. 4. The mass spectrum of 1,3,4-tri-O-methyl-2,6-dideoxy-hexofuranose.



1-β-O-Methyl-ribohexopyranoside (**III**) is the main sugar component of the reaction mixture. This glycoside was initially isolated using chromatography on silica gel and further acetylated yielding 1-β-O-methyl-3,4-di-O-acetyl-L-ribohexopyranose (**V**). The structures of these compounds were established on the basis of nmr spectroscopic data. The spectra were measured with increasing amount of Eu/dpm/3, which allowed the identification of all the carbohydrate protons on the basis of the spin-spin decoupling. The determination of the coupling constants permitted us to establish the relative configuration of the carbohydrate mole-

Table 1. ^{1}H NMR data of 1-β-O-methyl-D-digitoxide, 1-β-O-methyl-L-digitoxide and 1-β-O-methyl-3,4-di-O-acetyl-L-ribohexopyranoside

Proton	1-β-O-methyl-D-digitoxide		1-β-O-methyl-L-digitoxide		1-β-O-methyl-3,4-di-O-acetyl-L-ribohexo-pyranoside	
	δ	J	δ	J	δ	J
H_1	4.72	$J_{1,2a}$ =9.2 Hz $J_{1,2e}$ =2.3 Hz	5.14	$J_{1,2a}$ =9.2 Hz $J_{1,2e}$ =2.2 Hz	4.68	$J_{1,2a}$ =9 Hz $J_{1,2e}$ =3 Hz
H_{2a}	1.70	$J_{2a,3}$ =3.3 Hz	2.1	$J_{2a,3}$ =3.2 Hz	~2	$J_{2a,3}$ =3.2 Hz
H_{2e}	2.12	$J_{2e,3}$ =4.0 Hz $J_{2e,2a}$ =14 Hz	2.5	$J_{2e,3}$ =4 Hz $J_{2e,2a}$ =14 Hz		$J_{2e,3}$ =4 Hz $J_{2e,2a}$ =14 Hz
H_3	4.12	$J_{3,4}$ ———	4.52	$J_{3,4}$ =3 Hz	5.41	$J_{3,4}$ =3 Hz
H_4	3.31	$J_{4,5}$ =9.5 Hz	3.7	$J_{4,5}$ =9 Hz	4.55	$J_{4,5}$ =9.5 Hz
H_5	3.75	$J_{5,6}$ ———	4.17	$J_{5,6}$ =6 Hz	3.99	$J_{5,6}$ =7 Hz
H_6	1.32		1.73		1.85	

cule (Table 1).

As exemplary the spin coupling constants in **V** are discussed: high value for $H_{4,5}$ ($J=9.5$ Hz) points to the coupling H_a–H_a, whereas the value $H_{4,3}$ ($J=3$ Hz) for arrangement H_a–H_e or H_e–H_e. Since H_4 was found in the axial position, the later possibility should be eliminated. H_1 proton should be in axial position in consideration of $H_{1,2}$ value ($J=9$ Hz). In summary, the positions of protons were established as following: H_5, H_4, H_1 axial and H_3 equatorial.

The specific rotation of the isolated sugar determined in water and in methanol was opposite to the specific rotation of 2,6-dideoxy-D-ribohexopyranose–digitoxose.[7]

	$[\alpha]_D^{20}$	
	Novel sugar	2,6-Dideoxy-D-ribohexopyranose
1. Water	$-47°$ $c=1$	$+46.4°$
2. Methanol	$-38°$ $c=1$	$+37°$

Above described results permitted us to establish the structure of the carbohydrate moiety isolated from nystatin A_3, polyfungin B and candidinin as 2,6-dideoxy-L-ribohexopyranose.

Experimental

1. Materials and methods

The antibiotics used were nystatin A_3—supplied by the Institute of Antibiotics, Leningrad, USSR. Polyfungin B—supplied by the Institute of Pharmaceutical Industry in Warsaw, Poland. Candidin complex originated from Tarchomin Pharmaceutical Works "Polfa", Poland. Candidinin was isolated from the complex of candidin in our Laboratory by means of counter current distribution in solvent system: chloroform - methanol - borate buffer (2: 2: 1, v/v)

The nmr spectra were obtained with a Tesla 80 MHz BS 487 instrument. The mass spectra were taken with a LKB 9000 instrument. The thin-layer chromatographic identification had been done on DC—Alufolien Kieselgel (Merck).

2. Dodecahydronystatin A_3

One g of nystatin A_3 was dissolved in 60 ml tetrahydrofuran - water (3: 1 v/v) mixture and hydrogenated 16 hours over 0.6 g of 10% Pd/$BaSO_4$. The catalyst was discarded, 15 ml of *n*-butanol was added to the supernatant, and the solution was concentrated under reduced pressure. Dodecahydronystatin A_3 was precipitated with ethyl ether, centrifuged, washed and dried. The yield was 920 mg.

3. 2,6-Dideoxy-L-ribohexopyranose (I)

Dodecahydronystatin A_3 (500 mg) was hydrolyzed in 8 ml of 0.1 N sulphuric acid for one hour at 40°C. The solution was then neutralized on Dowex 1×8 (OH^-), filtered and evaporated to dryness under reduced pressure. The residue was purified on silica gel (column bed 1.5×10 cm) with the solvent system: chloroform - methanol (5: 1, v/v) yielding 57 mg of 2,6-dideoxy-L-ribohexopyranose. $[\alpha]_D^{20}$ $-47°$ (*c* 1, H_2O), $[\alpha]_D^{20}$ $-38°$ (*c* 1, MeOH).

Rf ~ 0.3 in the solvent system: chloroform - methanol (5: 1, v/v).

4. 3,4,5-Tri-O-TMS-methoxime of 2,6-dideoxy-L-ribohexopyranose (II)

Three mg of **I** dissolved in 0.2 ml of pyridine was treated with 3 mg of methoximine hydrochloride for 8 hours at room temperature and afterwards with 50 mcl of trimethylsilylimidazole for 3 hours. The reaction mixture was evaporated to dryness at 10^{-3} mmHg and at 40°C. The residue was dissolved in 0.5 ml of *n*-heptane and analyzed by means of gas chromatography - mass spectrometry. Gas chromatography was performed on a column ($3\,m\times3$ mm) filled with 3% OV-17 Chromosorb W (80/100 mesh) at a temperature of 150°C and with the gas, helium, at a flow rate of 30 ml/min. A single compound was detected and its retention time was 0.74 relative to MO-tetra-O-TMS-rhamnose.

5. 1-β-O-Methyl-2,6-dideoxy-L-ribohexopyranoside (III)

Dodecahydronystatin A_3 (500 mg) was dissolved in 40 ml of 0.1 N sulphuric acid in methanol for 10 hours at room temperature. The reaction mixture was neutralized using Dowex 1×8 (OH^-)

form, filtered and evaporated to dryness at reduced pressure. The residue was purified on silica gel (column bed 1×7 cm) with the solvent system, chloroform - acetone - methanol (50: 3: 3, v/v). The components were visualized after spraying with vanilline in ethanol—1% sulphuric acid mixture and heating up to 120°C. The main component (42 mg) exhibited Rf~0.27.

6. 1,3,4-Tri-O-methyl-2,6-dideoxy-ribohexopyranose

Five mg of **III** was dissolved in 2 ml of tetrahydrofuran, stirred with 5 mg sodium hydride and 50 mcl of methyl iodide at room temperature. After 16 hours the mixture was diluted with 25 ml of hexane, centrifuged and the supernatant was evaporated to dryness. The remained substance was dissolved in 0.1 ml of *n*-hexane and was analyzed by means of gas chromatography—mass spectrometry.

7. 1-*β*-O-Methyl-3,4-di-O-acetyl-2,6-dideoxy-L-ribohexopyranoside (**V**)

Forty mg of **III** dissolved in 0.5 ml of pyridine was treated with 1 ml pyridine - acetic anhydride (1: 1, v/v) for 6 hours at room temperature. The solution was poured on ice and extracted with chloroform (2×10 ml). The extract was dried over magnesium sulphate, filtered and evaporated to dryness. The residue was purified on silica gel (column bed 1×6 cm) with the solvent system: heptane - ethyl ether (3: 2, v/v) yielding 38 mg of **V**.

Acknowledgments

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