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THE STRUCTURE OF LEVORIN A_2 AND CANDICIDIN D

J.Zieliński,H.Borowy-Borowski,J.Golik,J.Gumieniak,T.Zimiński,P.Kołodziejczyk, J.Pawlak,E.Borowski

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

Yu.Shenin, A.I.Filippova

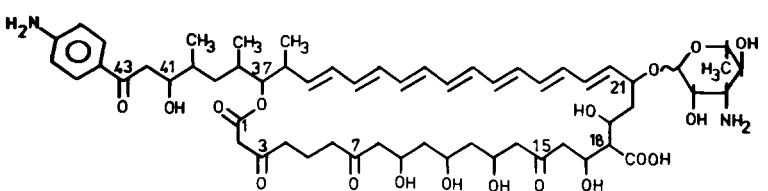
Union Research Institute of Antibiotics and Enzymes, Leningrad, USSR

Antifungal antibiotics levorin and candicidin are produced by Actinomyces levoris 26/1 [1,2] and Streptomyces griseus ATCC 3570 [3,4], respectively, in the form of complex mixtures of closely related active compounds. They all are polyene macrolides and belong to the "aromatic" subgroup of heptaenes. Levorin and candicidin are characterized by very high antifungal activity and ability to inhibit the growth of adenoma prostatae [5,6].

Differentiation of both antibiotics by chromatographic and counter current distribution (CCD) methods, pointed to similarity or even identity of levorin and candicidin [7,8], although some differences in biological properties of both antibiotic complexes were also observed.

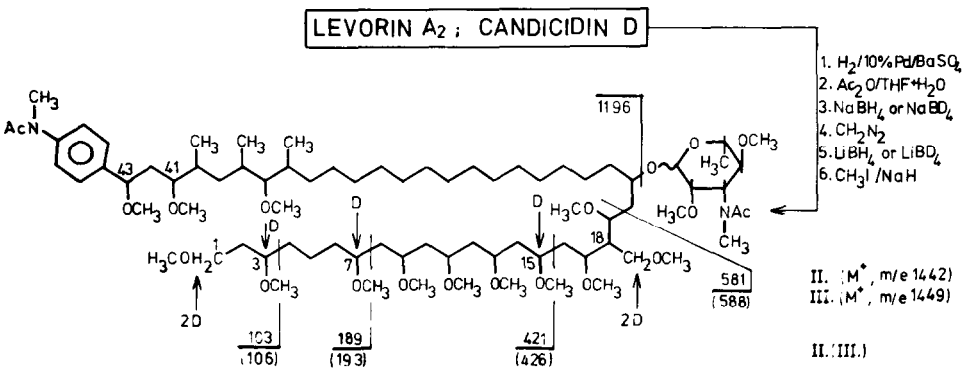
Main components of levorin and candicidin complexes, which are levorin A_2 [9] and candicidin D [10], have been preparatively isolated by CCD in a solvent system: chloroform-methanol-borate buffer pH 8,3 =(2:2:1)v/v [11]. Both compounds yield upon acidic and alkaline hydrolysis mycosamine and p-aminoacetophenone, respectively.

The complete structures of levorin A_2 and candicidin D have been established as I which unambiguously proves the identity of both compounds.



I.

The chemical structure of both antibiotics has been elucidated on the basis of mass spectrometric analysis of the products obtained in specific chemical reactions. The reduction of carbonyl functions in levorin A_2 and candicidin D with metal borohydrides or borodeuterides led to the formation of compounds II or III appropriately labelled with deuterium atoms. The hydroxyl functions in the examined antibiotics have been localized on the basis of mass spectra EI of polymethoxy derivatives II, M^+, m/e 1442 and their deuterised analogues III, M^+, m/e 1449 . Ether type fragmentations of methyl ethers, indicated in the figure, of the compounds II and III enabled to localize: carboxyl groups at C_1 and at C_{18}, ketone groups at C_3, C_7 and C_{15} and glycosidically bound mycosamine at C_{21}.



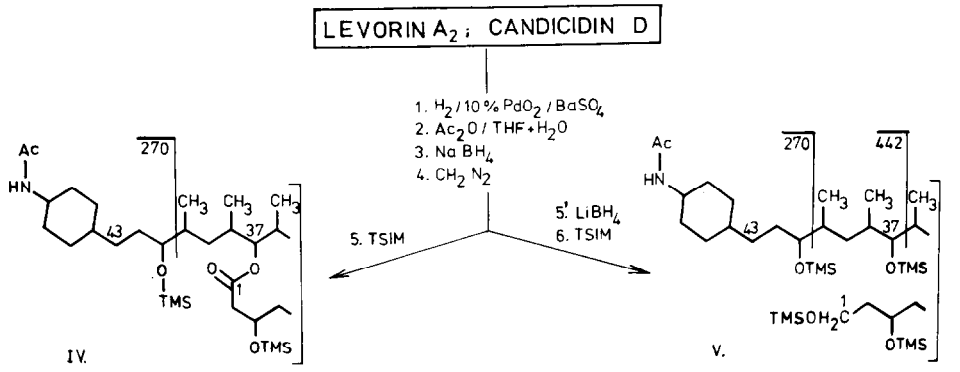
The products of reactions 1-6 indicated in the above scheme, were examined by mass spectrometry FD mode and the following molecular ions were detected:

	M^+	$(M+H)^+$	$(M+Na)^+$
N,N′-diacetyllevorin A_2 methyl ester; reactions: 2,4	-	-	1229
N,N′-diacetylhexadecahydrolevorin A_2 methyl ester; reactions: 1,2,4	-	-	1245
N,N′-diacetyldocosahydrolevorin A_2 methyl ester; reactions: 1,2,3,4	-	1229[12]	-
Product of reactions: 1,2,3,4,5	-	1205	1227
Product of reactions: 1,2,3,4,5,6	1442	-	-

Hydrolysis of glycosidic bond in polyols, obtained in reaction 5, yielded corresponding aglycons. The ether type fragmentation of methoxy derivatives of obtained aglycons confirmed and supplemented the established localization of oxygen functions.

Hydrogenation of both antibiotics in the presence of 10% $Pd/BaSO_4$ in THF-H_2O(3:1)v/v, results in saturation of seven double bonds and reduction of C_{43} carbonyl group to the hydroxyl. On the other hand, when 10% $PdO_2/BaSO_4$ is used as a catalyst under the same conditions, the reduction of C_{43} carbonyl group to methylene moiety and the hydrogenation of benzene ring to cyclohexane occurs in addition to hydrogenation of the chromophore.

The lactone bond between C_1-C_{37} has been localized on the basis of mass spectra of OTMS derivatives of IV and V.



Comparison of the fragment ions of cyclic and linear compounds IV and V, respectively, enables unambiguous localization of lactone bond between C_1-C_{37} in the examined antibiotics. The problem of localization of lactone bond has been solved on the basis of derivatives IV and V containing in their molecules cyclohexane instead of benzene ring because these compounds exhibit mass spectra simpler and easier for interpretation than the corresponding aromatic compounds.

The molecular weight of levorin A_2 and candicidin D of 1108 mass units, corresponding to the formula $C_{59}H_{89}O_{18}N_2$, has been established on the basis of ions $(M+Na)^+$ of methyl esters of N,N'-diacetyl levorin A_2 and candicidin D using a FD method.

Levorin A_2 and candicidin D, similarly to other polyene macrolides, most probably form an equilibrium hemiketal six member internal ring structure. The formation of hemiketal could be expected between C_{15} and C_{19}.

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11. Candicidin complex originated from Calbiochem, Switzerland; levorin complex was own fermentation product.
12. Ions $(M+Na)^+$ were not observed as after reduction with $NaBH_4$ ions Na^+ were removed with Dowex 50 Wx8, (H^+) form.

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