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THE STRUCTURE OF CANDIDIN, A POLYENE MACROLIDE ANTIFUNGAL ANTIBIOTIC

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Candidin, the heptaene macrolide antifungal antibiotic from Streptomyces viridoflavus[1], was recognised to be a mixture of three active principles all belonging to the "nonaromatic" subgroup of heptaenes[2,3]. The name candidin was retained for the main component of the antibiotic complex while the two other components were designated candidinin and candidoin.

For pure, crystalline candidin [xx] with $E^{1\%}_{1cm}$=1640 /at 382 nm in MeOH; other absorption maxima are at 347 362 and 406 nm/ found : C 60,93; H 8,25; O 29,34; N 1,48; C-CH_3 6,80; basic neutralisation eq./$HClO_4$ in AcOH/ 870; M.w./from N contents/ 940. Calculated for $C_{47}H_{71}O_{17}N$: C 61,24; H 7,71; O 29,53; N 1,52; four C-CH_3 6,51; neutr. eq. and m.w. 921. Alcaline titration showed the presence of one free carboxyl. The molecule contains one residue of glycosidically attached mycosamine, liberated in acid hydrolysis. The free sugar was identical with an authentic sample of mycosamine in TLC and paper chromatography. N-carbobenzoxy derivative of both compounds exhibited identical IR spectrum and no depression in mixed melting point /195°/ was observed. The NMR spectrum of triacetyl mycosamine from candidin is mentioned

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[x] This paper has been presented at the VII-th IUPAC Symposium on the Chemistry of Natural Products, Riga, June 1970.

[xx] The components of the complex were separated by ccd in $CHCl_3$:MeOH:borate buffer pH 8,5 = 2:2:1 /360 transfers/. Candidin /70% of total/ exhibited K=4,16 candidinin/13% of total/ K=2,55 and candidoin/17% of total/ K=1,81.

below.

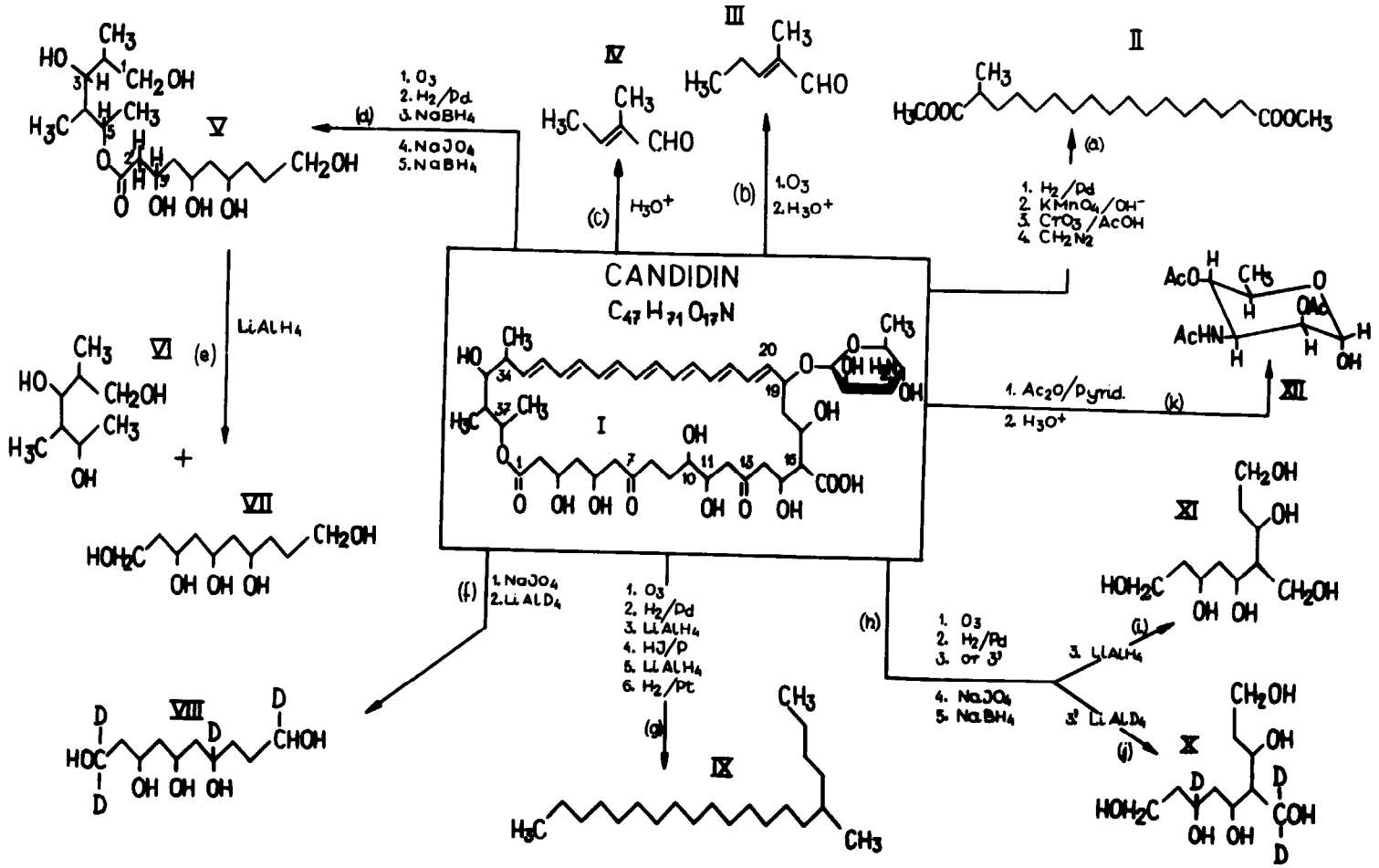
The complete structure /I/ of candidin is postulated on the basis of the following key evidence.

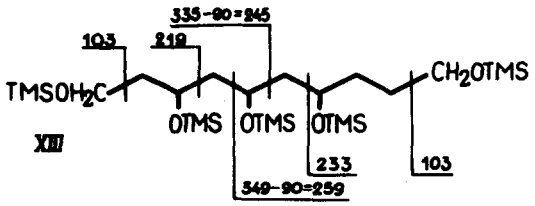
In oxidative degradation of /I/ no mono- and dicarboxylic acids higher than acetic and succinic acids were formed. Thus, the longest unsubstituted side chain can be only the methyl group, and the largest hydrocarbon fragment located between the two oxidisable functional groups is $-CH_2.CH_2-$. Oxidation of perhydrocandidin /reactions a/ permitted the elucidation of the structure of carbon skeleton of conjugated heptaene chromophore portion. Optically active /II/ obtained was identical with natural /from perhydroamphotericin B/[4] and synthetic[5] dimethyl ester of 2-methyl-heptadecanedioic acid in mass spectrometry /Found: M^+, m/e 342 and McLafferty rearrangement ions at m/e 74 and 88/.

Compound /VI/ formed in reactions /d/ and /e/ was identical in mass spectrometry of its TMS derivative with 2,4-dimethyl-hexanetriol/1,3,5/ obtained from amphotericin B[4] and nystatin A_1 [6]/Found: M^+, m/e 378, elimination ions at m/e 288 198 118 and fragment ions at m/e 103 117 131 233 247/.

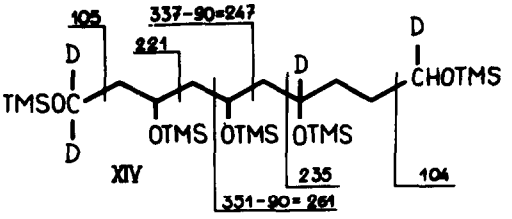
The placement of moiety /VI/ in the molecule of /I/ was established on the basis of examination of products of retroaldol and homoallylic fission of /I/. The fission in acidic conditions of /I/ as well as of /I/ reduced with $NaBH_4$ or ozonised, led to the formation of tyglic aldehyde /IV/ as the main steam volatile product. In addition to it 2-methyl-3-ethyl acrolein /III/ was also formed from ozonised /I/. None of these products were formed from perhydrocandidin or from /I/ ozonised and than reduced with $NaBH_4$. The formation of /III/ and /IV/ proved also that oxygen functions at C_{35} and C_{37} of /I/ are hydroxyls and not ketone.

The structure of carbon skeleton of macrolide ring fragment from C_1 to C_{20} of /I/ was elucidated in the procedure /g/. The hydrocarbon obtained was identified by mass spectrometry as 5-methyl-eicosane /IX/ /Found : M^+, m/e 296 and fragmentation XVII/. Functional groups in C_{1-20} fragment of /I/ were identified and localised in a series of degradations leading to the formation of corresponding polyols. Hydroxyl, ketone and carboxyl groups were distinguished in these reactions by the selective introduction of deuterium in the course of the reduction with deuterised reagent, followed by mass spectrometric analysis of TMS derivatives of

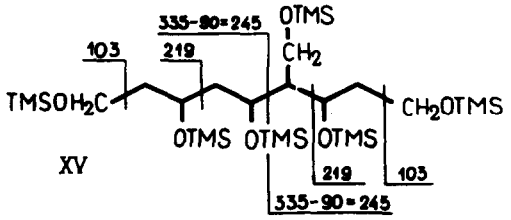

CANDIDIN
C47 H71 O17 N
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
1. O3
2. H2/Pd
3. NaBH4
4. NaIO4
5. NaBH4
1. O3
2. H3O+
H3O+
1. H2/Pd
2. KMnO4/OH-
3. CrO3/AcOH
4. CH2N2
LiAlH4
+
1. NaIO4
2. LiAlD4
1. O3
2. H2/Pd
3. LiAlH4
4. HJ/P
5. LiAlH4
6. H2/Pt
1. O3
2. H2/Pd
3. or 3'
4. NaIO4
5. NaBH4
3. LiAlH4
3' LiAlD4
1. Ac2O/Pyrid.
2. H3O+



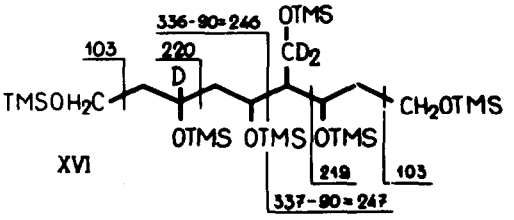
XIII



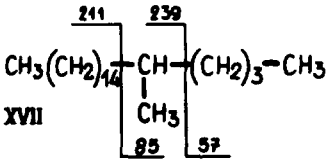
XIV



XV



XVI



XVII

corresponding polyols.

Compound /VII/ obtained in the course of reactions /d/ and /e/ and derived from C_{1-10} fragment of /I/ was identified as decapentaol/1,3,5,7,10/by mass spectrometry of its TMS derivative /Found: M^+-2x90 at m/e 402 and fragment ions XIII/. If the reducing agent was $LiAlD_4$ instead of $LiAlH_4$ /reactions f/ the corresponding polyol /VIII/ exhibited in form of its TMS derivative M^+-2x90 ion of four molecular units higher /Found:m/e at 406/,indicating the introduction of four deuterium atoms.Fragment ions /XIV/ permitted the localisation of deuterium in /VIII/.Thus in the corresponding C_{1-10} moiety of /I/ carboxyl,ketone and vicinal hydroxyl can be placed at C_1,C_7 and C_{10} respectively.Positions at C_3 and C_5 of /I/ are occupied by hydroxyls due to the lack of labelling in the corresponding positions in /VIII/.

Functional groups in C_{11-20} fragment of /I/ were localised in reaction sequence /h/ and /i/ or /j/. Using $LiAlH_4$ as the reducing agent /reactions h, i/a branched chain polyol was obtained and identified as 4-hydroxymethyl-nonapentaol/1,3 5,7,9/ /XI/ by mass spectrometry of its TMS derivative /Found: M^+-2x90, m/e 490 and fragmentation XV/. Selective deuterisation with $LiAlD_4$/reactions h, j/ led to the formation of compound /X/, the analogue of /XI/, exhibiting M^+-2x90 ion/TMS derivative/ of three molecular units higher /Found: m/e 493/, indicating the introduction of three deuterium atoms which were localised in /X/ on the basis of fragment ions /XVI/. The localisation of deuterium atoms in the polyol /X/ points to the presence of ketone and carboxyl groups at C_{13} and C_{16} and hydroxyl groups at C_{11}, C_{15} and C_{17} of /I/ respectively.

Mycosamine moiety is attached glycosidically to the allylic C_{19} /I/ of aglycone /candidinolide/. The polyol /XI/ is one carbon atom shorter than it could be expected from the structure /IX/ due to the periodate cleavage of $C_{19,20}$ vic.glycol created after $LiAlH_4$ removal of aminosugar from ozonised /I/ in the reactions /h/ and /i/. For the same reason compound /X/ does not bear deuterium atom at C_1. The deuterised C_{20} after $LiAlD_4$ reduction of ozonised /I/ is eliminated in the course of periodate oxidation in reactions /h/ and /j/. Moreover, in the conditions of methanolysis of peracetyl candidin 2,3,4-triacetyl mycosamine was obtained with extreme ease. The easy elimination of aminosugar moiety in such conditions with unsubstituted C_1, and not of methyl mycosaminide derivative, is characteristic for the allylic bound carbohydrate [6].

The ring structure of mycosamine moiety in candidin is of pyranose type. Mild hydrolysis of peracetyl derivative of /I/ /reactions k/ yielded 2,3,4-triacetyl mycosamine /XII/. The pyranose ring structure and C-1 conformation of /XII/ was based on the determination of chemical shift and spin-spin coupling constants in 80-MHz NMR /$CDCl_3$/. The data obtained were identical with those found for the same compound from amphotericin B [4].

The NMR spectrum of compound /V/ formed in the reactions /d/ 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 helpful marker for the identification on the NMR spectrum of the proton at the carbon with acyloxy group was well developped doublet signal of C_2, protons of /V/ at 1,12 ppm which decoupled after the irradiation with the frequency corresponding to 5,37 ppm. The latter frequency identifies the position on the spectrum of C_3, methine proton which is the most unshielded one among the all methine protons of /V/ at carbon atoms with hydroxyl groups. Thus the signal of the proton at the carbon atom with acyloxy group should be found at still lower field.

It was demonstrated that another ketone containing polyene antibiotic amphotericin B[4,7] can form an internal cyclic hemiketal structure. Similar type of structure is also possible in candidin. This problem is subject of our further studies.

Comparing the structure of candidin to other polyene macrolide antibiotics the great similarity exists between this antibiotic and nystatin [6] or amphotericin B [4]. The most striking resemblance is between candidin and nystatin A_1. The presence in candidin molecule of second ketone group as compared with amphotericin B may explain markedly higher solubility of the former antibiotic in organic solvents.

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