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CHEMICAL STUDIES WITH AMPHOTERICIN B

III. THE COMPLETE STRUCTURE OF THE ANTIBIOTIC +/

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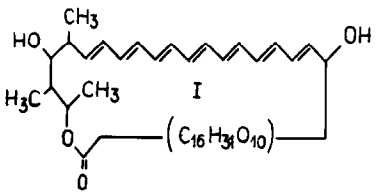
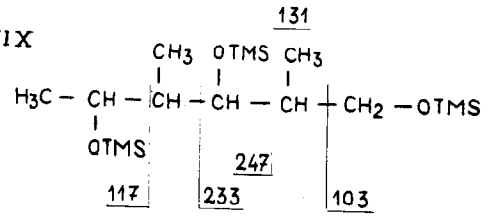
Amphotericin B, a widely used in clinical practice polyene macrolide antifungal antibiotic from Streptomyces nodosus [1], belongs to the "nonaromatic" structural subgroup of heptaenes[2]. No complete structure of any antibiotic of this subgroup has so far been reported. Amphotericin B was generally characterised[3] and the structure of glycosidic constituent /mycosamine/ [4] elucidated. The partial structure /I/ was reported by us[5] and confirmed as well as further extended by Cope et al.[6]. Our recent results permit us to postulate the comple-



XIX



te equivalent structures /II/ and /XXVII/ of amphotericin B. Our previous data supporting the partial structure /I/ are now additionally confirmed by the use of more refined techniques.

Mass spectrum of /III/ obtained in the procedure /a/ was identical with that of the synthetic product[7] /Found : M^+, m/e 342 and McLafferty ions at m/e 74 and 88/. Mass spectrometry of trimethylsilyl /TMS/ derivative of /IV/

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/reactions b,c/ gave M^++1 ion at m/e 379,TMSOH elimination ions at m/e 288 198 118 and fragment ions /XIX/.

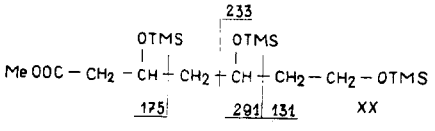
Lactone carboxyl,vic.glycol,free carboxyl,mycosamine moiety and chromophore terminus were localised in /II/ at C_1 $C_{8,9}$ C_{16} C_{19} C_{20} respectively,in reactions /d/ and /e/.Mass spectrometry of /V/ /derived from C_{1-8} of II/ gave M^+ at m/e 158 and the fragmentation pattern of methyl caprylate.Compound /VI/,derived from C_{9-20} of /II/,exhibited mass spectrum with M^+ at m/e 228,two McLafferty ions at m/e 172 and 130 and the fragmentation pattern of methyl 2-n-butyl-pelargonate.Mass spectrum of /VII/ /derived from C_{9-19} of II/ exhibited M^+ ion at m/e 214,both McLafferty ions at m/e 172 and 116 and the fragmentation pattern of methyl 2-n-propyl pelargonate. /VI/ and /VII/ differed in the length of the side chain by one carbon atom,because the partial hydrolysis of acetal remaining after periodate cleavage of mycosamine created a new /C_{19-20}/periodate oxidizable moiety.If after periodate oxidation the product was mildly treated with acid,followed by another step of periodate oxidation,only /VII/ was obtained.

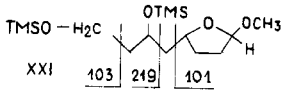
The attachement of mycosamine to the allylic C_{19} of /II/ is also in accord with the extreme ease of acid hydrolysis of glycosidic bond and with the elimination of mycosamine but not methyl mycosaminide in MeOH/HCl. Methyl mycosaminide is formed in secondary reaction proceeding with free sugar.

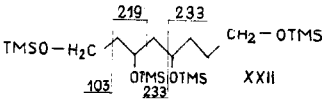
Selective deuterisation in the procedure /d/ and /f/ followed by the mass spectrometric examination of the reaction products confirmed the above findings and also enabled the localisation of ketone in /II/ at C_{13}.Substance /V/ was unlabelled.For /VIII/ M^+ was found at m/e 230,McLafferty ions at m/e 173 and 131 and the fragmentation pattern of 5,5'-dideutero methyl 2-n-butyl pelargonate.Compound /IX/ exhibited M^+ at m/e 215,McLafferty ions at m/e 116 and 173 and the fragmentation of 5-deutero methyl 2-n-propyl pelargonate.The rate of deuterisation at C_5 of /VIII/ was about 25% of that at $C_{4'}$,as calculated from the relative intensities of ions at m/e 230 and 229 and also at m/e 173 and 172.Ions at m/e 229 and 172 are derived from the $C_{4'}$-monodeutero analogue of /VIII/.Low rate of labelling at C_5 of /VIII/ will be discussed later.

The remaining oxygen functions in C_{1-19} fragment of /II/ are hydroxyls,lo-

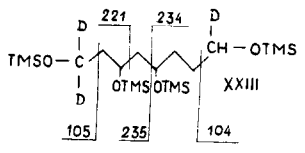
cated at C_3 C_5 C_{11} C_{15} C_{17} on the basis of the following key evidence. Methyl 3,5,8-trihydroxy-caprylate /X/ and its 1,5-lactone analogue /XI/ were obtained in the reactions /b/ and /c/. TMS derivative of /X/ gave on mass spectrometry M^+-15 at m/e 407 and the fragmentation /XX/, and of /XI/ M^+-15 at 303. In the reactions /g/ two isomeric /alpha and beta, distinguishable on GLC as TMS derivatives/ five membered ring cyclic methyl acetals /XII/ were obtained. Acid catalysed methylation occured in the course of the reaction due to the use of periodic acid. Methoxyl appeared in ^{1}H-NMR /$CDCl_3$/ as singlet at 5,2 ppm. TMS de-

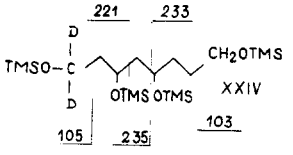


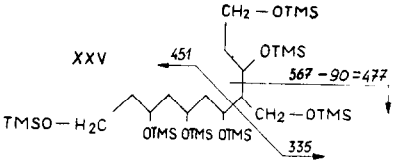




rivatives of both isomers exhibited identical mass spectrum with M^+ at m/e 334 and the fragmentation /XXI/. In the reactions /h/ octatetra-1,3,5,8-ol /XIII/ was obtained. Mass spectrometry of its TMS derivative gave M^+-90 at m/e 376 /at 70 eV/ or M^+-15 at m/e 451 /at 20 eV/ and the fragmentation /XXII/. The way of attachement of C_{1-8} fragment of /II/ to the rest of the molecule was finally established by the selective deuterisation in the reactions /i/ and /j/ leading to the formation of poliols /XIV/ and /XV/ respectively, in which two deuterium atoms were introduced at the lactone carboxyl carbon atom and one at the aldehyde formed in periodate vic.glycol cleavage. Mass spectrum of TMS derivative of /XIV/ showed M^+-90 at m/e 379 as well as the fragmentation /XXI/, and of /XV/ M^+-90 at m/e 378 with fragmentation /XXII/. In the reactions /h/ 8-hydroxymethyl-

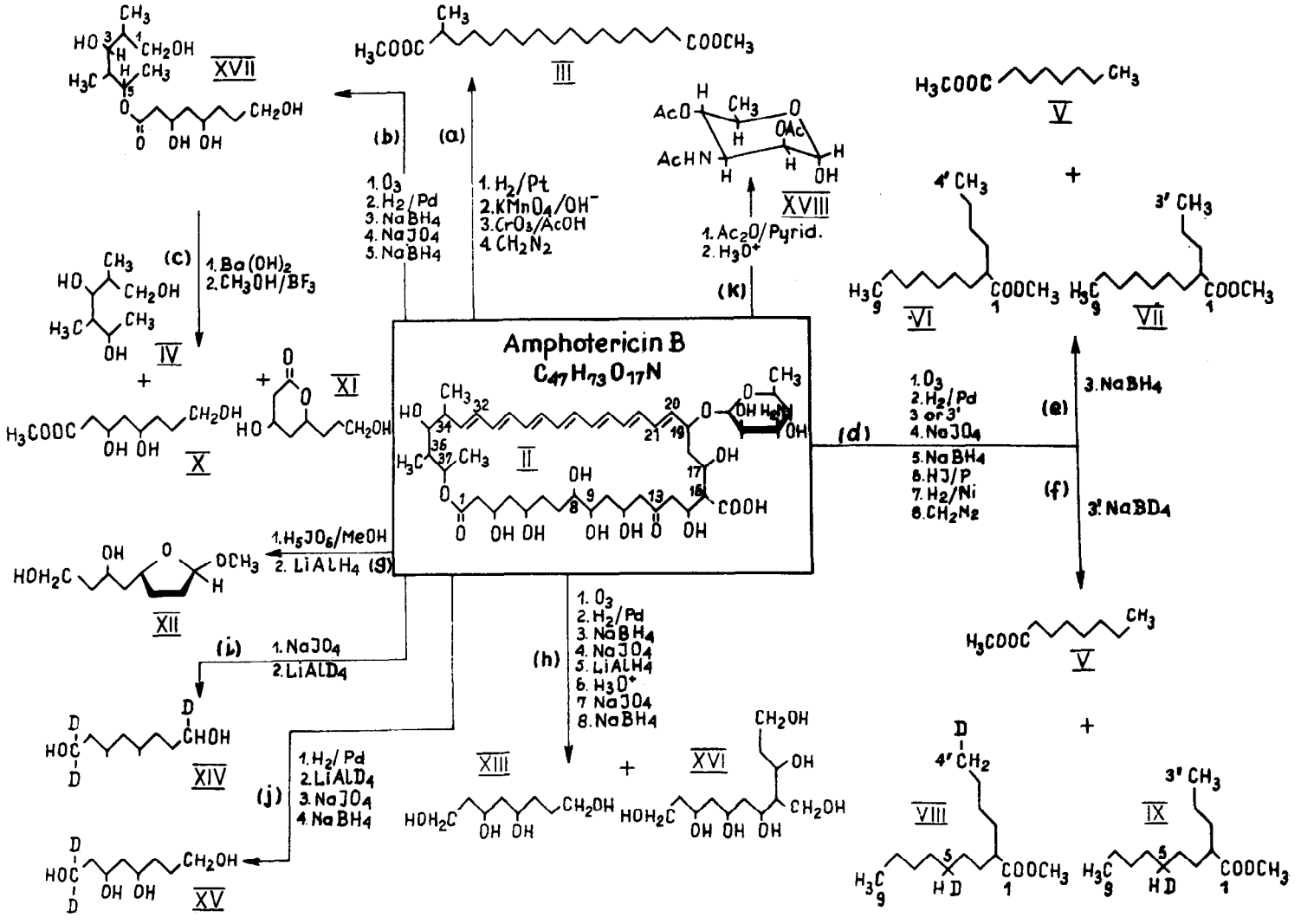






-undecanhexa-1,3,5,7,9,11-ol /XVI/ was formed. Its TMS derivative gave in mass spectrometry $M^+-/3x90/$ at m/e 516 and the fragmentation. /XXV/.

The NMR examination of compound /XVII/ formed in the reactions /b/ supplied the direct evidence for the position of lactone bond between C_1 and C_{37} of /II/. Multiplet signal centered at 4,5 ppm /d_5-pyridine:$CDCl_3$=1:5/ was assigned to


Amphotericin B
C47H73O17N
(a) 1. H2/Pt 2. KMnO4/OH⁻ 3. CrO3/AcOH 4. CH2N2
(b) 1. O3 2. H2/Pd 3. NaBH4 4. NaJO4 5. NaBH4
(c) 1. Ba(OH)2 2. CH3OH/BF3
(d) 1. O3 2. H2/Pd 3 or 3' 4. NaJO4 5. NaBH4 6. HJ/P 7. H2/Ni 8. CH2N2
(e) 3. NaBH4
(f) 3' NaBD4
(g) 1. H5JO6/MeOH 2. LiAlH4
(h) 1. O3 2. H2/Pd 3. NaBH4 4. NaJO4 5. LiAlH4 6. H3O⁺ 7 NaJO4 8. NaBH4
(i) 1. NaJO4 2. LiAlD4
(j) 1. H2/Pd 2. LiAlD4 3. NaJO4 4. NaBH4
(K) 1. Ac2O/Pyrid. 2. H3O⁺
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
XVII
XVIII

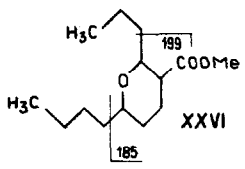
the most unshielded proton at the carbon atom with acyloxy group.This is a C_5 proton of /XVII/ because the irradiation with its resonance frequency caused the transformation of 1,1 ppm doublet /corresponding to the most unshielded CH_3 at C_5 of /XVII/ to the singlet.

The ring structure of mycosamine moiety is of pyranose type.Mild hydrolysis of peracetylamphotericin B /reactions k/ yielded 2,3,4-triacetyl mycosamine /XVIII/.The pyranose ring structure and C-1 conformation of /XVIII/ was based on the following NMR /$CDCl_3$/ evidence. δ=1,10/d,J=6 Hz/for CH_3 at C_5 ; δ=1,85 /s/for Ac-N ; δ=2,00/s/ for Ac at C_2 ; δ=2,09 /s/ for Ac at C_4 ; δ=4,15 /m/ for H at C_5 ; δ=5,05/d,J=1,5 Hz/ for H at C_1 ; δ=4,9 /m/ for H at C_2 ; δ=4,55-4,8 /m/ for H at C_3 and C_4 ; δ=6,1 /d,J=8 Hz/ for NH ; δ=4,45 /s/ for OH.

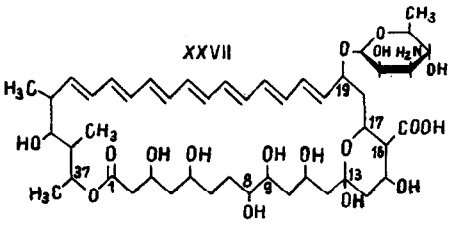
Amphotericin B being a polyhydroxy ketone has structural features adequate for the formation of equilibrium amounts of various cyclic ketal forms.The existence of a six membered ring cyclic ketal structure in the antibiotic joining carbon atoms 13 and 17 was indeed proved.Amphotericin B was degraded similarly to the procedures /d/ and /e/ except that HBr/AcOH was used instead of HJ/P and the polybromide obtained was reduced with Zn/AcOH instead of H_2/Ni. Among other products methyl ester of 2-n-propyl-6-n-butyl-pyrane-carboxylic acid-3 /XXVI/ was obtained and isolated in a pure form on silicagel column /petroleum ether:ethyl ether=10:1/.Mass spectrometry of /XXVI/ gave M^+ at m/e 242 and the characteristic fragmentation with the most important ions /XXVI/. The above evidence is based on the approach commonly used in the carbohydrate chemistry.

The existence of the ketal might be the reason for the low rate of labelling with deuterium at C_5 in compounds /VIII/ and /IX/ formed in the reactions /d/ and /f/.

The presence of ketal ring in amphotericin B is the first reported case of such a structural feature in the group of polyene macrolides.The particular conformation of the molecule resulting from the formation of ketal structure might be the reason for the exceptional insolubility and high stability of amphotericin B.The possibility of the formation by some ketone containing poly-



XXVI



XXVII

enes of various ketal ring structures of different conformation and also a free ketone structure, might be as well the explanation for the well known phenomenon of significant variations in potency of a given polyene without the noticable chemical changes during its handling or storage.

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