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THE STRUCTURE OF THE ANTIBIOTIC PERIMYCIN A

P.Kołodziejczyk, J.Zieliński, J.Pawlak, J.Golik, L.Falkowski, E.Borowski
Department of Pharmaceutical Technology and Biochemistry, Technical University,
80-952 Gdańsk, Poland

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Perimycin (syn.Fungimycin,NC-1968,Aminomycin)is a polyene macrolide antifungal antibiotic produced by Streptomyces coelicolor var.aminophilus NRRL 2390 [1]. It was characterized by Borowski et al.[2] as a novel structural type member of "aromatic" subgroup of heptaene macrolides.The structure of the aminosugar moiety perosamine (4-amino-4,6-dideoxy-D-mannose) has been elucidated by Lee and Schaffner [3]. Alkaline hydrolysis of perimycin yields N-methyl-p-aminoacetophenone [4,5].

Perimycin exhibits unique biological properties.Unlike other polyene macrolides it induces specific permeability changes of the yeasts plasma membrane resulting in the loss of potassium ions from the cells ("potassiumless death")[6].

We found that perimycin is a mixture of three active components which were isolated by counter-current distribution and characterized [x].

The structure of the main component, perimycin A (I) was elucidated by mass spectrometry of chemical transformation products [xx]. The molecular weight of (I) and the number of hydroxyls and keto groups have been established by mass spectrometry of trimethylsilyl ethers of N,N'-diacetyl perimycin A (P, m/e 1900) and corresponding methoxime derivative (P, m/e 1987).

The structure of the carbon skeleton of the chromophore moiety results from the formation of 2-methylheptadecanedioic acid upon the oxidative degradation of tetradecahydroperimycin A and is the same as in other heptaenes.

The formation of (II)(P, m/e 423) or its d_2-analogue (P, m/e 425) enabled to

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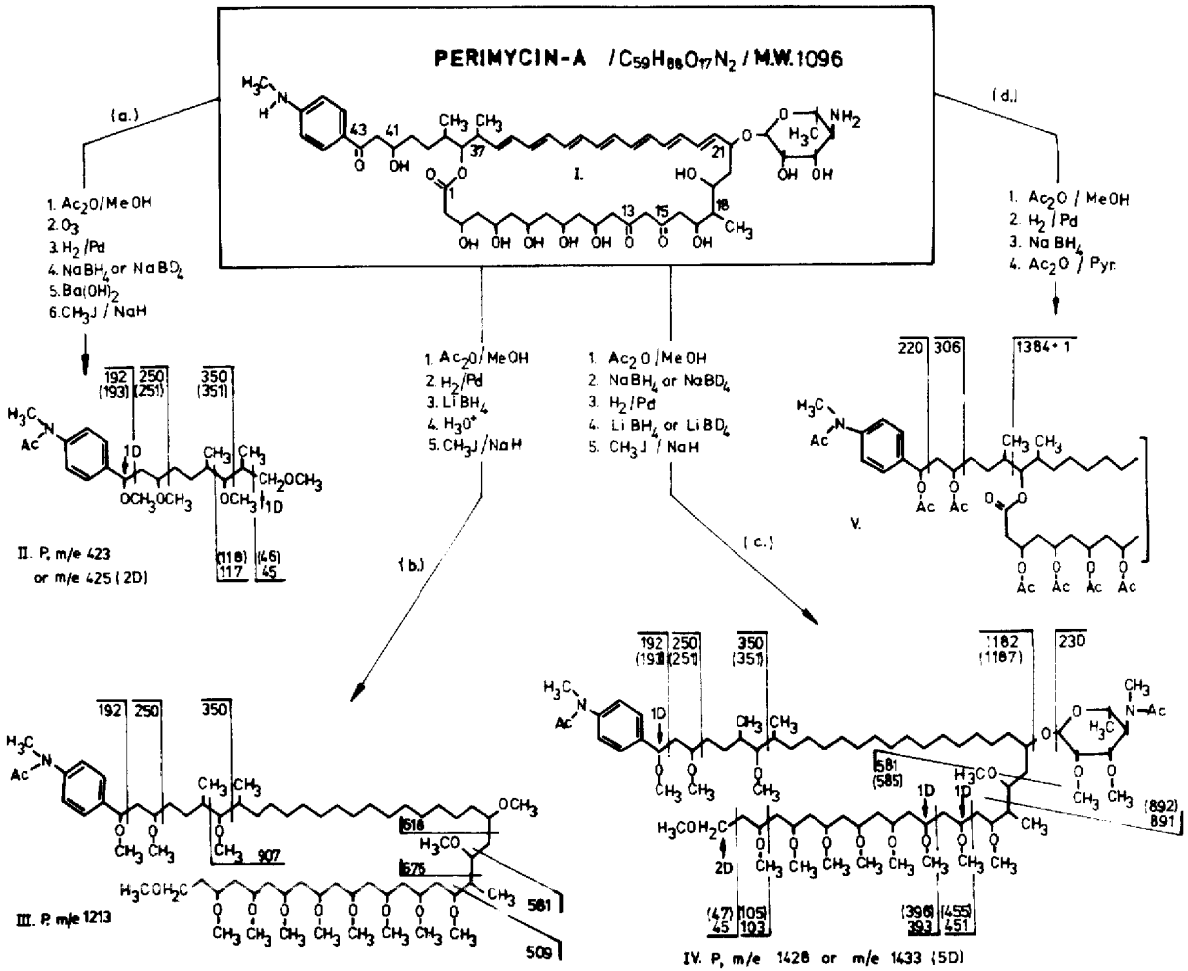
x) c.c.d in chloroform-methanol-ethylene glycol-phosphate buffer pH 7 = 4:2:1:1 v/v; Perimycin A (70-85% of total) exhibited K=0,38, perimycin B (10-15% of total) K=0,84 and perimycin C (ca. 5% of total) K=0,21.

xx) Mass spectra were obtained by use of Varian MAT-711 instrument.


PERIMYCIN-A /C59H88O17N2 / M.W.1096
I.
(a.)
1. Ac2O/MeOH
2. O3
3. H2/Pd
4. NaBH4 or NaBD4
5. Ba(OH)2
6. CH3J / NaH
II. P, m/e 423
or m/e 425 (2D)
(b.)
1. Ac2O/MeOH
2. H2/Pd
3. LiBH4
4. H3O+
5. CH3J/NaH
III. P, m/e 1213
(c.)
1. Ac2O/MeOH
2. NaBH4 or NaBD4
3. H2/Pd
4. LiBH4 or LiBD4
5. CH3J / NaH
IV. P, m/e 1428 or m/e 1433 (5D)
(d.)
1. Ac2O / MeOH
2. H2 / Pd
3. NaBH4
4. Ac2O / Pyr.
V.

establish the structure of C_{21}-C_{43} fragment and to localize the terminal double bond of the chromophore at C_{35} and the keto group at C_{43}.

The structure of the carbon skeleton of the aglycone and the localization of the remaining oxygen functions were based on the mass spectrometry data of polymethoxy derivative (III) obtained in the reactions sequence (b).In mass spectrum of this compound appeared ions at m/e 192, 250 and 350, identical with those found for (II). The fragmentation characteristics for beta-methoxy systems enabled to arrange the hydroxyl functions in C_1-C_{21} fragment. The presence of methyl group at C_{18} results from fragmentation at branched carbon atom (ions at m/e 509 and m/e 676) and from the sequence of elimination ions.

Further evidence for structure of perimycin A follows from mass spectra of polymethoxy derivative (IV) formed in the procedure (c). The glycosidic bonding of perosamine was indicated by ions at m/e 230 and m/e 1182 (P-246), whereas fragmentation pattern of the aminosugar moiety was characteristic for a pyranose ring. The placement of the glycosidic bond at C_{21} revealed from the presence of ions at m/e 350 and m/e 581 in mass spectra of (III) and (IV) as well as the shift of ions at m/e 907 and m/e 676 for (III) to the ions at m/e 1122 and m/e 891 for (IV) respectively.

The use of deuterium labelled reagents in the reactions (c) permitted to identify and localize reducible oxygen functions in (IV). Appearance of 2 a.m.u. shifted ions for d_5-derivative of (IV) instead ions at m/e 45,103,161 etc. for (IV) identified the lactone carbonyl at C_1. The presence of ions at m/e 393 and m/e 451 for (IV) with the appearance of corresponding ions at m/e 396 and m/e 455 for its d_5-derivative localized the two keto groups at C_{13} and C_{15}. The fifth deuterium atom was localized at C_{43} which confirms the position of the third keto group.

The analysis of mass spectra of OTMS and peracetyl derivatives of N,N'-diacetyl-eicosahydroperimycin A (V), obtained in the procedure (d), enabled to establish the position of lactone bond between C_1 and C_{37}. Ion at m/e 1385 and corresponding elimination ions are formed as a result of breaking the bond between C_{37} and C_{38} with simultaneous transfer of proton.

Perimycin A is the first member of "aromatic" subgroup of heptaene macrolides which complete structure has been elucidated. Striking similarities exist in the structure of the macrolide rings of perimycin A, amphotericin B [7,8], nystatin A_1 [9], candidin [10] and other aminosugar containing polyene macrolides. That points to the common route of biogenesis of these compounds. The presence of methyl group at C_{18} in perimycin A corroborates the suggested biogenesis of carboxyl group found in the corresponding position in other polyene macrolides. It means that the carboxyl group is formed as a result of oxidation of a methyl derived from the propionate unit.

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