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STRUCTURE OF MAGNESIDIN, A NEW MAGNESIUM-CONTAINING ANTIBIOTIC FROM PSEUDOMONAS MAGNESIORUBRA

H. Kohl*, S.V. Bhat, J.R. Patell, N.M. Gandhi, J. Nazareth, P.V. Divekar and N.J. de Souza
Research Centre, Hoechst Pharmaceuticals Limited, Bombay-80, India
and
H.G. Berscheid and H.-W. Fehlhaber
Farbwerke Hoechst AG., vormals Meister Lucius & Bruening, Frankfurt, West Germany

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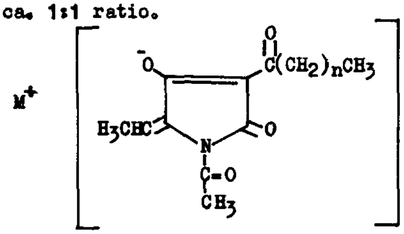
Pseudomonas magnesiorubra nov. sp. (ATCC No.21856), when grown in various nutrient media under conditions of submerged fermentation, produces a magnesium-containing antibiotic named Magnesidin showing interesting biological properties.[1,2] We have found that magnesidin is a mixture of the magnesium salts (Ia) of two new tetramic acids, 1-acetyl-3-n-hexanoyl-5-ethylidenetetramic acid (Ib, n=4) and 1-acetyl-3-n-octanoyl-5-ethylidenetetramic acid (Ib, n=6), which are present in ca. 1:1 ratio.



I. n = 4, 6 (ca. 1:1)
a. $M^+ = Mg^{++}/2$
b. $M^+ = H$

Magnesidin was isolated by extraction of the cell mass obtained by fermentation[2] and was purified by crystallization from aqueous methanol, m.p. undefined, with shrinking and softening from 123-150°, and λ_{max} (MeOH) 257 nm ($E^{1\%}_{1cm}$ 747.3). On combustion, magnesidin was found to contain carbon, hydrogen and nitrogen; it left behind a residue which analysed completely for magnesium oxide, corresponding to a content of ca. 4.1% magnesium in magnesidin. The presence of magnesium in magnesidin was confirmed by X-ray determination. The IR spectrum (KBr) of magnesidin indicated a conjugated ketone (ν_{max} 1669 cm^{-1}), a conjugated double bond (ν_{max} 1618 cm^{-1}), a trisubstituted double bond (ν_{max} 797 cm^{-1}) and two -CON< groups, one of which could be a tertiary amide (ν_{max} 1637 cm^{-1}) and the other a five-membered ring lactam

(ν_{max} 1709 cm^{-1}).[3] The NMR spectrum (60MHz, $CDCl_3$ + 1 drop $(CD_3)_2SO$) exhibited bands at δ 0.82(t, $-CH_2C\underline{H}_3$), 1.22(m, $-(C\underline{H}_2)_n$), 2.18(d, J=8Hz, $=CHC\underline{H}_3$), 2.60(s, $OC-C\underline{H}_3$), 2.83(t, $OCC\underline{H}_2CH_2$) and 7.19(q, J=8Hz, $=C\underline{H}CH_3$).

By treatment with a cation exchanger, the magnesium in magnesidin was replaced by hydrogen to give Ib, the MS[4] of which revealed two molecular ion peaks, m/e 265 and 293, with a relative abundance of ca. 1:1 corresponding to the formulae $C_{14}H_{19}NO_4$ and $C_{16}H_{23}NO_4$ respectively (high-resolution MS). The difference of C_2H_4 in the two formulae, together with the NMR data on the number of hydrogen atoms in the methylene region of the spectrum, and the observation that both Ia and Ib could be separated into two compounds on reversed phase TLC/(kieselguhr plates coated with paraffin, $AcOH-H_2O$(4:1))/ was in conformity with the conclusion that Ia and Ib were mixtures of two homologous compounds bearing alkyl chains differring from one another by two methylene units.

Ib exhibited the following physico-chemical characteristics: m.p. 80-92°, pKa 3.6(62% MeOH), λ_{max}(MeOH) 261 nm ($E^{1\%}_{1cm}$ 928.6), λ_{max}(0.1NNaOH-MeOH) 263 nm ($E^{1\%}_{1cm}$ 864.2), ν_{max}($CHCl_3$), 1745, 1668, 1634, 1600, 798 cm^{-1}, δ (60MHz, CCl_4) 14.2(enol) in addition to bands corresponding to those exhibited by magnesidin, positive ferric chloride and titanium chloride tests, and formation of a blue cupric salt, an enol methyl ether (M^+ = m/e 279, 307) and an enol acetate (M^+ = m/e 307, 335). These data, together with the major mass spectrometric fragmentation reactions of Ib (Figure 1), were compatible with the assignment of an acyl tetramic acid structure to Ib, in which the length of the acyl chain in the two components of the mixture differred by two $-CH_2$ units. Compounds with an acyl tetramic acid structure have previously been found in nature.[5]

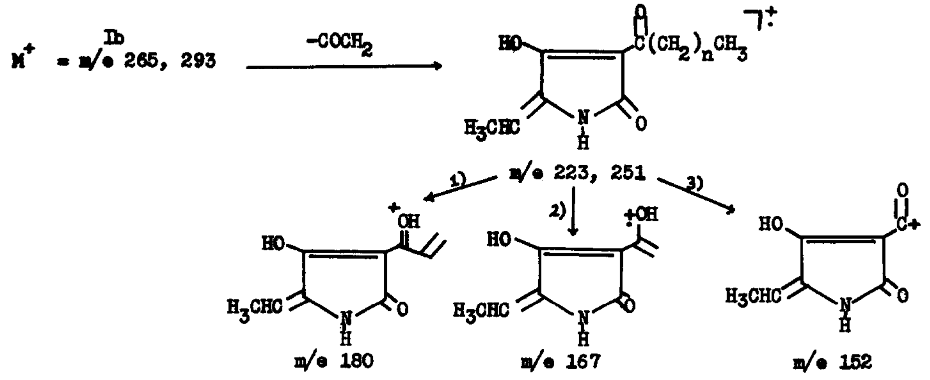


Figure 1 : Major fragments in the MS of Ib. 1) γ-cleavage; 2) McLafferty rearrangement; 3) α -cleavage.

Ib was deacetylated, hydrogenated and hydrolysed to give CO_2 (precipitated as $BaCO_3$), a mixture of hexanoic and octanoic acids (identified by GLC and MS comparison of their methyl esters with authentic specimens) and 3-aminopentan-2-one (identified by MS and NMR). On ozonolysis Ia gave acetaldehyde (identified as its 2,4-dinitrophenylhydrazone) and a mixture of N-acetyl-2-n-alkanoylacetamides II, identified by NMR and high resolution MS (Figure 2) . The data obtained from the acid hydrolysis and ozonolysis experiments conclusively established the structures Ib of the two tetramic acids.

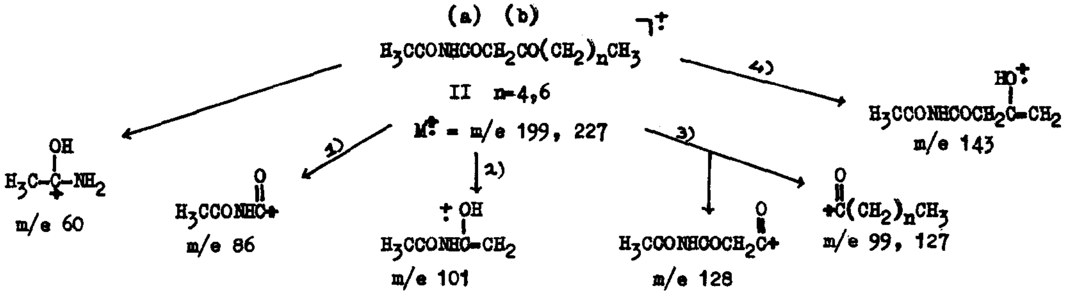


Figure 2: Major fragments in the MS of II. 1) and 3) α-cleavages of (a) and (b) respectively. 2) and 4) McLafferty rearrangements of (a) and (b) respectively.

The tetramic acids IIIa and IIIb were synthesised[6] from dl-threonine by the route depicted in Figure 3. Their magnesium salts were acetylated and mixed in a 1:1 proportion to give a product essentially identical with magnesidin on comparison of their NMR, IR, UV and biospectra.

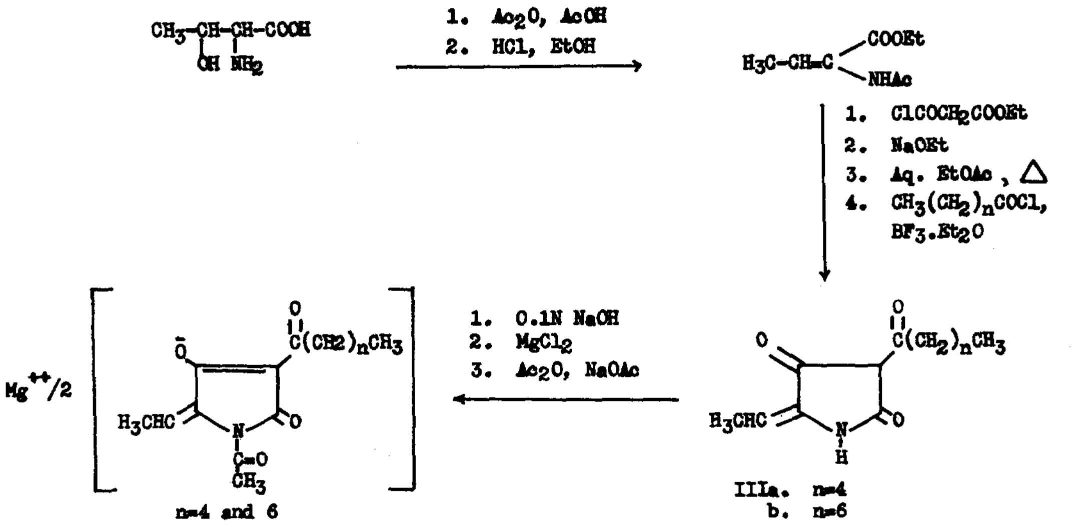


Figure 3 : Synthesis of Magnesidin

The stereochemical orientation of the 5-ethylidene group in magnesidin could not be elucidated. Double resonance experiments[7] with the methyl ether of Ib revealed no nuclear Overhauser effect to be observed between the acetyl or methoxyl group and vinylic hydrogen or vinylic methyl group indicating that the spatial distance of the different groups exceeds 3A°.

To our knowledge, magnesidin is the first magnesium-containing antibiotic to be isolated from natural sources. Preliminary biosynthetic experiments indicate an essential requirement of magnesium for optimal growth of Pseudomonas magnesiorubra. The concomitant production by the micro-organism of a second pyrrole-derived metabolite, prodigiosin and its higher homologues[8], together with magnesidin, raises interesting biosynthetic questions[9,10] which form the subject of further investigations.

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