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# Pimaricin

## I *. Determination of the Carbon Skeleton

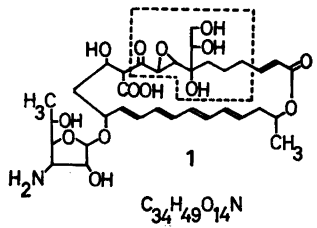
OLOF CEDER

*Department of Organic Chemistry, The Royal Institute of Technology, Stockholm 70, Sweden*

Biogenetic and chemical considerations indicated that an earlier proposed structure of the polyene antibiotic pimaricin might be incorrect. It is shown that drastic degradation of pimaricin gives 12-methylhexacosane. This finding requires a revision of the postulated formula.

Systematic investigations of antibiotics produced by *Streptomyces* strains from soil samples have in the last decade resulted in the isolation of a large number of polyene antibiotics with strong antifungal properties (for a review *cf.* Ref.[1]). For two of them, lagosin [2] and fungichromin,[3] convincing structural evidence has recently been presented. Both are nitrogen- and sugar-free macrocyclic lactones.

Pimaricin was the first polyene antibiotic chemically investigated, and in 1958 Patrick *et al.*[4] proposed structure *1*. An attempt to rationalize this carbon skeleton and oxygenation pattern in terms of an acetate biogenesis is not

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$C_{34}H_{49}O_{14}N$

successful. During structural investigations of the saturated macrolides magnamycin,[5] erythromycin,[6] methymycin,[7] and oleandomycin [8] its application

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proved very useful. The hydroxylated two carbon side chain of pimaricin is particularly remarkable. It could be furnished by a carbohydrate precursor, as proposed by the Lederle group. Grisebach [9] recently presented the first experimental proof for two biogenetic schemes operating simultaneously in the formation of magnamycin. Tracer studies showed that the four carbon fragment (C—4 to C—8) containing the aldehyde group of magnamycin is derived from glucose present in the medium and not from acetate. Pimaricin would, if the proposed structure were correct, constitute a second example of a mixed biogenesis.

The chemical evidence presented by the American authors was not entirely compelling. Thus from both chemical and biogenetic considerations a reinvestigation of the structure of pimaricin seemed justified.

Pimaricin is a crystalline compound, very stable in the solid state and without a definite melting point but decomposing gradually at approximately 200°. Its isolation and properties were first described by Hoogerheide *et al.*[10] and later by Patrick *et al.*[4] It has a very strong absorption in the ultraviolet with maxima at 279, 290, 303, and 318 mμ. The $E^{1\%}_{1cm}$ at 303 mμ is 1100. Intensity and spacing of the absorption bands are characteristic of an all-*trans*, unsubstituted tetraene system.

Patrick *et al.*[4] showed that hydrolysis of pimaricin with strong acid in methanol gives the methyl glycoside of mycosamine,[11] an aminohexose previously isolated from several other polyene antifungal antibiotics including nystatin [12] and amphotericin B.[13] Its structure was proven by Walters, Dutcher, and Wintersteiner.[14] Recently they also reported its synthesis and absolute configuration.[15] The intensity of the tetraene absorption, elemental analysis, and the presence of one atom of nitrogen in mycosamine lead to a molecular weight of about 700 for pimaricin. Its origin, general properties, and the published structural work indicate that pimaricin is a macrocyclic lactone. Conversion of the antibiotic to its parent hydrocarbon followed by determination of its molecular weight by mass spectrometry therefore seemed to be a convenient method to obtain information about the carbon skeleton.

Pimaricin was reduced catalytically and with lithium aluminum hydride; the resulting polyol was refluxed with 48 % hydroiodic acid and red phosphorus. Lithium aluminum hydride and catalytic reductions followed by chromatography on alumina produced an oil with infrared and ultraviolet spectra characteristic of a saturated hydrocarbon. High temperature gas chromatography indicated that it was probably a mixture of two very similar compounds. This was confirmed when two molecular ion peaks, one at $m/e=380$ and another at 366 were found in the mass spectrum of the reduction product. The mass numbers correspond to the molecular formulas $C_{27}H_{56}$ and $C_{26}H_{54}$. Reductions with hydroiodic acid are known to cause rearrangements and cleavage of carbon-carbon bonds. Therefore the basic skeleton of pimaricin probably contained twenty-seven carbon atoms, one of which was partly eliminated during the hydroiodic acid treatment.

To verify this supposition a milder degradation method avoiding strongly acidic conditions was tried. Pimaricin was catalytically reduced and the resulting perhydrocompound treated with sodium borohydride. At that time, pimaricin was believed to contain one or more tertiary hydroxyl groups. Therefore

the borohydride product was treated with phosphorus oxychloride in pyridine and after isolation subjected to catalytic hydrogenation. Reduction with lithium aluminum hydride in refluxing tetrahydrofuran gave a polyol lacking carbonyl absorption in its infrared spectrum. It was treated with red phosphorus and iodine at 100°.[16] The resulting polyiodide was converted to a hydrocarbon with lithium aluminum hydride in ether. After catalytic hydrogenation and chromatography on alumina an oil with an infrared spectrum characteristic of a saturated hydrocarbon resulted. It showed no end absorption in the ultraviolet. Gas chromatography revealed only one high molecular weight component, with a retention time very close to but not identical with that of hexacosane. Mass spectrometric analysis of a collected sample showed a molec-

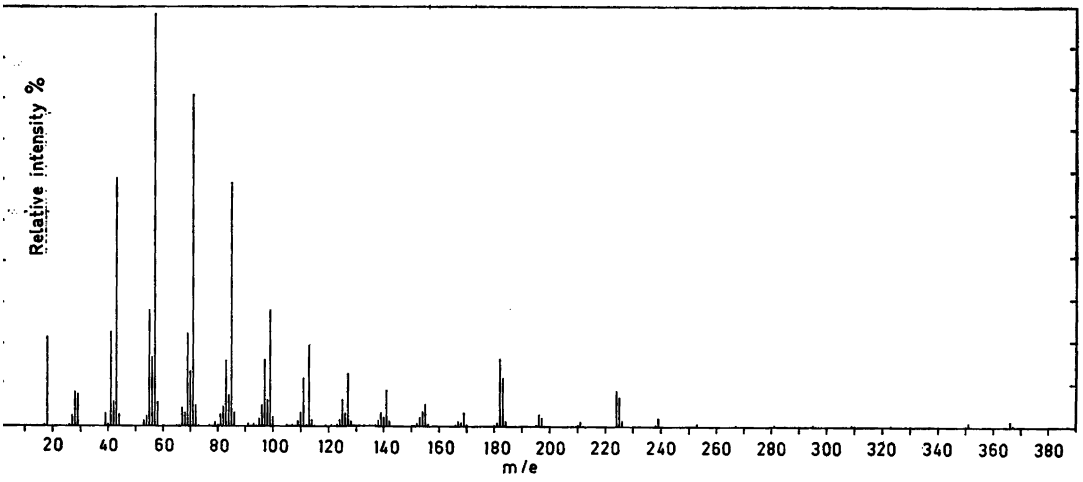
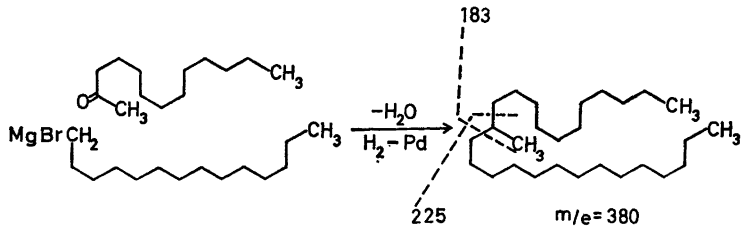



*Fig. 1.* Mass spectrum of 12-methylhexacosane.

ular weight of 380 (*cf.* Fig. 1), corresponding to a molecular formula of $C_{27}H_{56}$. The spectrum exhibited strong peaks at $m/e = 183$ and 225. Oxidation of the heptacosane with chromic anhydride in glacial acetic acid[17] gave a steam volatile acid fraction which, when analyzed as methyl esters by gas chromatography, showed a prominent peak for dodecanoic acids. The mass spectrum and the oxidation results are compatible only with 12-methylhexacosane. This

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hydrocarbon was synthesized from myristyl bromide and tridecanone-2 following standard procedures.[18] Its mass spectrum was identical with that of the hydrocarbon obtained from pimaricin.

The second reduction procedure is not likely to have caused rearrangements or cleavage of carbon-carbon bonds. If this assumption is correct, structure *2* represents the basic carbon skeleton of pimaricin.

A macrolide possessing the structure proposed by the Lederle group would under the same conditions give a methylethylpentacosane. They identified their antibiotic as pimaricin by comparison of infrared and ultraviolet spectra, paper chromatographic mobilities and X-ray powder diffraction patterns of the antibiotics and their N-acetyl derivatives. Their reference sample and the material used in the present investigation were obtained from the same source.* It may therefore be assumed that the present sample of pimaricin is identical with that of the American authors, and therefore their proposed structure requires revision.

## EXPERIMENTAL

The mass spectra were determined with an Atlas CH4 instrument; the heated inlet system was kept at 225°. The gas chromatographic analyses were performed on a PYE Argon chromatograph. The elemental analysis was performed by Dr. A. Bernhardt, Mülheim, Ruhr.

*Preparation of perhydropimaricin.* A solution of 5.12 g of pimaricin in 30 ml of glacial acetic acid was added to 750 ml of methanol containing 320 mg of prereduced platinum oxide. The mixture was hydrogenated at 20° for 40 min and interrupted when 1120 ml of hydrogen had been absorbed. After separation of the catalyst and evaporation of the filtrate under reduced pressure, 5.29 g of an amorphous powder resulted. It was used directly for further degradations.

*Preparation of perhydropimaricin polyol.* A solution of 5.0 g of perhydropimaricin in 300 ml of tetrahydrofuran was heated under reflux for 2 days with excess of lithium aluminum hydride. The unchanged hydride was destroyed with ethyl acetate and the mixture was evaporated to dryness. The inorganic material was dissolved in dilute sulfuric acid and the polyol was extracted with 1-butanol. The combined extracts were washed with water and the butanol was removed under reduced pressure. After drying for 24 h at 0.2 mm, 4.9 g of noncrystalline polyol was obtained.

*Reduction of perhydropimaricin polyol with hydroiodic acid.* A solution of 100 mg polyol in 2 ml of glacial acetic acid was added to a refluxing suspension of 10 ml of 48 % hydroiodic acid and 200 mg red phosphorus. After 48 h the mixture was worked up as described earlier,[3] and 13 mg of an oil was obtained. Gas chromatography at 295° using an 85 × 0.8 cm column packed with 20 % silicone grease on 60—80 mesh Chromosorb W gave only one peak. The retention time was almost identical with that of hexacosane. The mass spectrum of the oil displayed two molecular ion peaks at $m/e$ = 380 and 366, indicating the presence of $C_{27}H_{56}$ and $C_{26}H_{54}$. In the mass spectrum of this mixture an abundance of $C_{13}H_{27}^+$ ($m/e$ = 183) and $C_{16}H_{33}^+$ ($m/e$ = 225) could also be detected.

*Reduction of perhydropimaricin with sodium borohydride.* To a solution of 0.373 g of perhydropimaricin in 25 ml of methanol kept at 0°, 0.5 g of sodium borohydride was added. After 24 h at 20°, the solution was passed over an ion exchange resin (Dowex-II-X4-$Cl^-$) and the eluate evaporated under reduced pressure yielding 0.240 g of a colorless powder (I—37).

*Dehydration of I—37 with phosphorus oxychloride.* To a solution of 0.240 g of I—37 in 5 ml of pyridine 0.5 ml phosphorus oxychloride was added. The mixture was kept at 25° for 15 h and then poured on ice, acidified with dilute hydrochloric acid, and extracted

* The Royal Netherlands Fermentation Industries, Ltd., Delft, Holland.

with chloroform. The chloroform layer was filtered, washed once with water, and then evaporated yielding 0.189 g of amorphous material (I—38).

*Hydrogenation of I—38.* To 5 ml of ethanol containing 25 ml of prereduced platinum oxide 0.185 mg of I—38 was added and the mixture was reduced with hydrogen for 15 h. After separation of the catalyst and evaporation of the solvent 0.170 g of an amorphous product (I—42A) remained.

*Reduction of I—42A with lithium aluminum hydride.* To 5 ml of tetrahydrofuran containing 0.170 g of I—42A an excess of lithium aluminum hydride was added and the mixture was refluxed for 2 days. The unchanged hydride was destroyed with ethyl acetate and the mixture was evaporated to dryness under reduced pressure. The inorganic material was dissolved in dilute sulfuric acid at 0° and the polyol was extracted with chloroform. The combined extracts were washed with water, filtered, and the chloroform was removed under reduced pressure to give 0.150 g of an oily polyol (I—42B).

*Conversion of polyol I—42B to the "parent hydrocarbon".* Following the method of Downing, Kranz, and Murray [16] 50 mg of polyol was treated with 100 mg of iodine and 20 mg of red phosphorus, and the resulting polyiodide was reduced with lithium aluminum hydride in ether. The procedure was repeated once more. The hydrocarbon formed was reduced with hydrogen in 10 ml of distilled hexane containing 10 mg of prereduced platinum oxide. After separation of the catalyst and evaporation of the solvent, 5 mg of an oil remained. Its infrared spectrum (liquid film) was characteristic of a saturated hydrocarbon. Gas chromatography at 295° using an 85 × 0.8 cm column packed with 25 % silicon grease on 60—80 mesh Chromosorb W gave only one peak with a retention time between those of hexacosane and heptacosane. A 0.5 mg sample was collected from the column under the same conditions. The mass spectrum (*cf.* Fig. 1) showed a molecular ion peak at $m/e = 380$ and was identical with that of synthetic 12-methylhexacosane.

*Chromium trioxide oxidation of the "parent hydrocarbon".* A 2 mg sample of the hydrocarbon obtained from perhydropimaricin was oxidized with 5 mg of chromium trioxide in 0.5 ml of glacial acetic acid for 2 h at 60°. The cooled solution was neutralized with 5 % aqueous potassium hydroxide and the neutral components were extracted with ether. The alkaline solution was acidified with 5 % sulfuric acid and then extracted with ether. The ether layer was dried over sodium sulfate and then filtered. An ethereal solution of diazomethane was added and the solvent was carefully distilled through a column. Gas chromatographic analysis of the methyl esters at 205° on a 85 × 0.8 cm column packed with 20 % silicon grease on 60—80 mesh Chromosorb W gave a large peak having a retention time identical with that of methyldodecanoate.

*Preparation of 12-methylhexacosane.* To a mixture of 35 g of 48 % hydrobromic acid and 11 g of concentrated sulfuric acid 21.4 g of myristyl alcohol was added. The mixture was refluxed for 6 h, then worked up following the described procedure.[19] Distillation gave 22.1 g of myristyl bromide, b.p. 205°/30 mm.

To a solution of the Grignard reagent prepared from 2 g of magnesium and 22.1 g of myristyl bromide in 200 ml of absolute ether was added 15 g of tridecanone-2 (Fluka, *purum*). Following the procedure of Sörensen and Sörensen [18] 20 g of the tertiary alcohol was isolated. It was used without further purification.

A solution of 4.5 g of crude tertiary alcohol and 0.9 g of *p*-toluenesulfonic acid in 15 ml of benzene was heated under reflux for 12 h. The catalyst was separated by passing the mixture over 30 g of alumina (Woelm, activity I). After evaporation of the solvent 2.0 g of an oil remained. It was dissolved in 50 ml of petroleum ether (b.p. 40—60°), purified by redistillation, and hydrogenated for 12 h with 0.5 g of 5 % palladium on carbon as catalyst. The petroleum ether solution was then filtered through 10 g alumina (Woelm, activity I) and the solvent removed. After drying for 24 h at 80°/0.2 mm, 1.96 g of 12-methylhexacosane remained; $n_D^{24} = 1.4460$. (Found: C 85.21; H 14.73. Calc. for $C_{27}H_{56}$: C 85.17; H 14.83). Gas chromatography at 215° (5 % SE—30 on Celite) proved it free from homologues. The oil started to crystallize after standing for several months at room temperature.

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