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NYSTATIN. PART VI.*

CHEMISTRY AND PARTIAL STRUCTURE OF THE ANTIBIOTIC

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Nystatin, $C_{46-47}H_{73-75}O_{18}N$, the antifungal agent produced by Streptomyces noursei,[1,2] contains mycosamine, 3,6-dideoxy-3-amino-D-mannose[3,4] (I), in glycosidic linkage to the aglycone, nystatinolide,

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*Part V, ref.5. The present paper also constitutes Macrolide Antibiotics, Part XIV. (Part XIII, ref.5). This work was presented at the XIXth International Congress of Pure and Applied Chemistry, London, 1963.

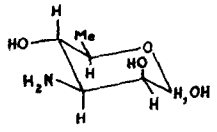
[1]E.L.Hazen and R.Brown, Proc.Soc.Exper.Biol. and Med., 1951, 76, 93

[2]J.D.Dutcher, G.Boyack and S.Fox, "Antibiotics Annual", Medical Encyclopedia Inc., New York, 1953, p.191; J.D.Dutcher, D.R.Walters and O.P.Wintersteiner, "Therapy of Fungus Diseases", Little, Brown and Co., Boston, 1955, p.168.

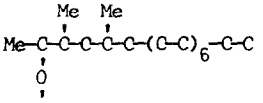
[3]D.R.Walters, J.D.Dutcher and O.P.Wintersteiner, J.Amer.Chem.Soc., 1957, 79, 5076; J.Org.Chem., 1963, 28, 995.

[4]M.H.von Saltza, J.Reid, J.D.Dutcher and O.P.Wintersteiner, J.Amer.Chem.Soc., 1961, 83, 2785; J.Org.Chem., 1963, 28, 999.

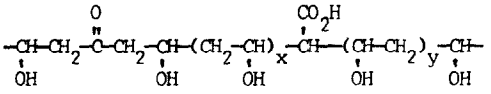
$C_{40-41}H_{62-64}O_{15}$, in which are present[5] the structural features (II) and (III) together with lactone, diene and tetraene functions.[2]



(I)

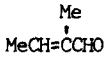


(II)

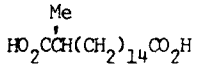


(III, x or y = 0)

Oxidation of nystatin with ozone or, more important, with lead tetra-acetate, afforded tiglic aldehyde (IV) as the only steam-volatile carbonyl compound. This aldehyde (IV) was not produced on similar oxidation of nystatin peracetate. Consequently, the skeleton (II) must carry a 1,2-diol system as in (VI), the olefinic bond of the tiglic aldehyde (IV) arising by β-elimination of an oxygen function during the steam-distillation. Hydrogenation of nystatin over platinum catalysts followed by oxidation with



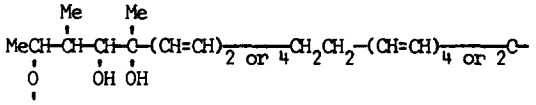
(IV)



(V)

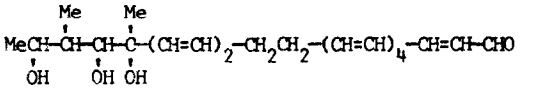
[5]A.J.Birch, C.W.Holzapfel, R.W.Rickards, C.Djerassi, M.Suzuki, J.W.Westley, J.D.Dutcher and R.Thomas, preceding paper.

nitric acid yielded a series of dibasic acids up to 2-methylheptadecane-dioic acid (V). The production of this di-acid (V) must involve hydrogenolysis of the tertiary hydroxyl of the diol system, which is therefore allylic to either the diene or the tetraene chromophore as shown in (VI). Since the highest dibasic acid obtained on nitric acid oxidation of nystatin itself is succinic acid, the chromophores must be separated by two adjacent methylene groups, enabling us further to expand the skeleton (II) to the partial structure (VI).



(VI)

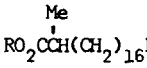
Mild treatment of nystatin with aqueous alkali gave an unsaturated hydroxy-aldehyde, $\nu_{max.}$ (in $CHCl_3$) 3420 (OH), 2700 and 1669 (CHO), and 1620 cm.$^{-1}$ (C=C). The ultraviolet absorption, $\lambda_{max.}$ 229 and 380 mμ (ε 20,000 and 45,000 respectively), of this unstable compound indicated the presence of diene and pentaenal chromophores, and the elucidation of its structure as (VII) provides full confirmation of the system (VI), and establishes the orientation of the diene and tetraene functions.



(VII)

Hydrogenation of this pentaenal over platinum afforded a diol, $C_{24}H_{50}O_2$, and a triol, $C_{24}H_{50}O_3$, separable by chromatography.

Oxidation of the diol with an excess of chromium trioxide in acetone afforded a carboxylic acid $C_{20}H_{40}O_2$. The base peak in the mass spectrum of the derived methyl ester occurred at m/e 88, which, together with the absence of an intense peak at m/e 74, indicates[6] methyl substitution in the 2-position, 2,3-cleavage with hydrogen rearrangement then yielding the ion $[MeOC(OH)=CHMe]^+$. At higher mass numbers the spectrum of this methyl ester was fully consistent[6] with the singly-branched structure (VIII).



(VIII; R = R' = Me)
(IX; R = Me, R' = CO_2Me)
(X; R = H, R' = CO_2H)
(XI; R = H, R' = Me)

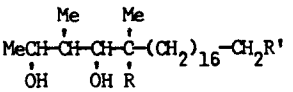
Successive reduction of the $C_{24}H_{50}O_3$ triol with hydriodic acid - red phosphorus, lithium aluminium hydride, and finally hydrogen over a platinum catalyst gave a hydrocarbon, shown to have the formula $C_{24}H_{50}$ by mass spectroscopy. Further confirmation of the triol formula followed from the mass spectrum of its O,O-isopropylidene derivative, $C_{27}H_{54}O_3$, which showed the expected ions at m/e 427 (M + 1) and m/e 411 (M − CH_3). Oxidation of the triol with an excess of chromium trioxide in acetone yielded a dicarboxylic acid, $C_{20}H_{38}O_4$. The mass spectrum of the corresponding dimethyl ester was characteristic[6] of a straight-chain system carrying a single 2-methyl branch (as in IX), the dominant peaks at m/e 74 and m/e 88 arising by 2,3-cleavage with hydrogen

[6]Cf. R.Ryhage and E.Stenhagen in "Mass Spectrometry of Organic Ions", ed. F.W.McLafferty, Academic Press, New York, 1963, p.399, and references cited therein.

rearrangement at each end of the system to give the ions $[MeOC(OH)=CH_2]^+$ and $[MeOC(OH)=CHMe]^+$ respectively.

Oxidation of the C_{24}-triol with a limited amount of chromium trioxide permitted the isolation of an acid, $C_{24}H_{46}O_4$. Infrared absorption (in CCl_4) of this acid at 3300-2500 and 1704 (CO_2H), and 1696 cm.$^{-1}$ (C=O), and of its methyl ester at 3400 (OH), 1733 (CO_2Me), and 1695 cm.$^{-1}$ (C=O), indicated the presence of hydroxyl and ketonic functions, which were shown to be in 1,3-relationship by the production of ethyl methyl ketone on treatment with hot alkali.

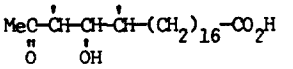
These results permit the formulation of the C_{24}-triol as (XII), which is oxidised via intermediates such as the isolated C_{24}-hydroxy-keto-acid (XV) to the C_{20}-dicarboxylic acid (X). Loss of the terminal C_4-fragment probably occurs by cleavage of a 1,3-dicarbonyl system, the presence of which in crude preparations of the acid (XV) was indicated by characteristic ultraviolet absorption ($\lambda_{max.}$ 293 mμ changing to 312 mμ in alkaline solution) and red ferric reaction. The C_{24}-diol, which must have structure (XIII), undergoes similar oxidation to the acid (XI).



(XII; R = H, R' = OH)

(XIII; R = R' = H)

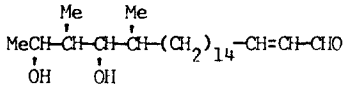
(XIV; R = R' = OH)



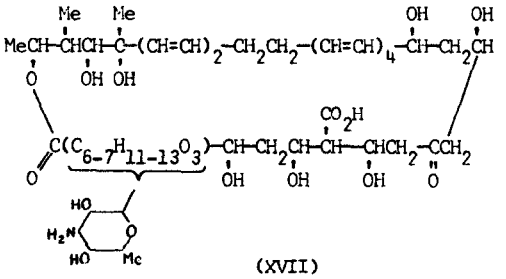
(XV)

In contrast to the pentaenal (VII) and nystatin itself, which gave tiglic aldehyde (IV) in high yield on oxidation with lead tetraacetate, similar oxidation of both the triol (XII) and nystatin which had previously been hydrogenated over platinum catalysts, afforded no volatile carbonyl compound. Reduction of the polyene system over active catalysts clearly involves hydrogenolysis of a tertiary hydroxyl group allylic to the diene chromophore. Accordingly, the pentaenal (VII) was hydrogenated over palladised charcoal, and the resulting saturated aldehyde {$\nu_{max.}$ (in CCl_4) 2670, 1722 cm.$^{-1}$ (CHO)} reduced directly with sodium borohydride. The product was shown to be the expected tetrol (XIV) by lead tetraacetate oxidation to tiglic aldehyde (IV), thus conclusively establishing the structure (VII) for the pentaenal. Reduction of this pentaenal to the C_{24}-diol (XIII) involves hydrogenolysis of both tertiary and primary allylic oxygen functions.

The base-catalysed cleavage of nystatin to acetone,[5] acetaldehyde[5] and the pentaenal (VII) is blocked by preliminary reduction with sodium borohydride, and therefore involves a reverse aldol reaction triggered by a carbonyl function. The initially-produced unconjugated aldehyde suffers β-elimination of an oxygen function to yield the pentaenal (VII). As expected, alkaline cleavage of nystatin which had been previously hydrogenated over platinum gave the dihydroxy-enal (XVI), $\nu_{max.}$ (in CCl_4) 3420 (OH), 2720 and 1692 (CHO), and 1642 cm.$^{-1}$ (C=C), $\lambda_{max.}$ 222 mμ (ε 14,000), which could be reduced to the triol (XII).



(XVI)



(XVII)

These results, in conjunction with biosynthetic evidence[5] leading notably to the structure of the ketonic section (III), are accommodated in the tentative partial structure (XVII) for nystatin. The sequence of acetate and propionate units in this structure (XVII) necessitates that x = o, y = 1 in (III), since if x = 1, y = o the acetaldehyde obtained from [1-^{14}C] propionate-labelled nystatin would not carry significant radioactivity. The mycosamine moiety (I) is probably connected through a glycosidic linkage to the undefined C_{6-7}-section (shown in brackets) of nystatinolide, since although free mycosamine would be deaminated readily by base,[7] no ammonia could be detected in mild alkaline hydrolysates of nystatin. The lactone function is positioned to close the macrolide ring by analogy with known antibiotics of this hydroxylated polyene type.[8]

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[7]Cf. F.A.Hochstein and P.P.Regna, J.Amer.Chem.Soc., 1955, 77, 3353.

[8]M.Berry, Quart.Rev., 1963, XVII, 343; W.Oroshnik and A.D.Mebane, Fortschr.Chem.org.Naturstoffe, 1963, 21, 17; cf. also O.Ceder, Angew.Chem., 1964, 76, 53.