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MACROLIDE ANTIBIOTICS XV.[1]

NYSTATIN — THE STRUCTURE OF THE AGLYCONE[2]

M. Ikeda,[3a] M. Suzuki[3b] and Carl Djerassi

Department of Chemistry, Stanford University, Stanford, California 94305

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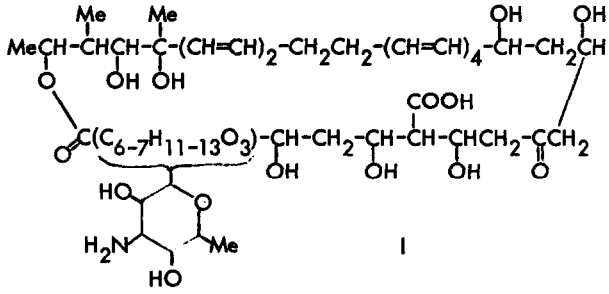
In previous papers[4,5] the partial structure I of nystatin was proposed on the basis of biosynthetic and chemical evidence. We now describe new data which establish the complete carbon skeleton and the oxygenation pattern of the aglycone, nystatinolide (XVI), of this widely used antifungal agent produced by Streptomyces noursei.



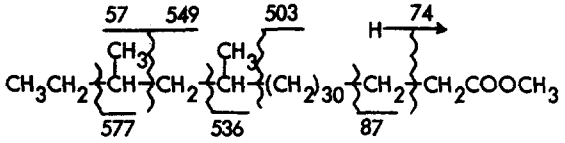
I

The high-pressure hydrogenation[6] of nystatin over 5% palladium on alumina in glacial acetic acid at 3900 psi and 280°, followed by esterification with diazomethane and repeated column chromatography resulted in the isolation of seven methyl esters (II-VIII).

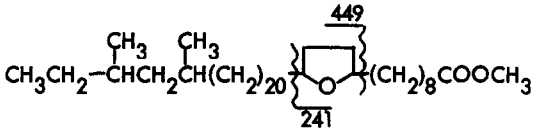
The methyl ester II (m.p. 67-68°), $C_{41}H_{82}O_2$, was identified as methyl 34,36-dimethyloctatriacontanoate by a combination of infrared, nmr and mass spectral measurements. This structure was confirmed by conversion of II, using the red phosphorus-hydriodic acid method[7] to the parent hydrocarbon IX, $C_{40}H_{82}$, whose mass spectrum was identical with that of the C_{40}-hydrocarbon obtained from amphotericin B by Cope et al.[8]

The methyl esters III (m.p. 33-34°), IV (m.p. 31-32°) and V (m.p. 40-42°) all have the same molecular formula $C_{41}H_{80}O_3$. Their mass spectra indicated the presence of a tetrahydrofuran ring for III and IV and a tetrahydropyran ring for V, as shown by the α-fission fragments marked on the structural

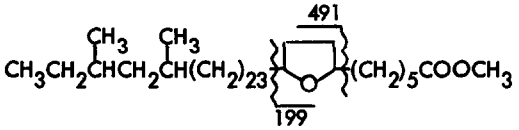
II (M^+ 606)



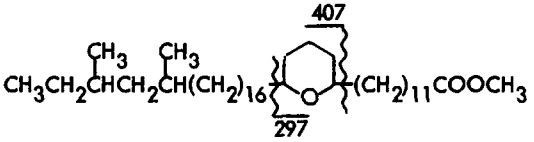
III (M^+ 620)



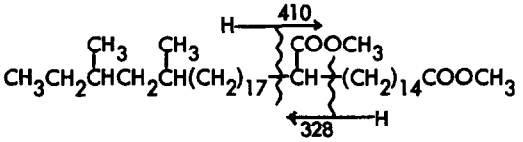
IV (M^+ 620)



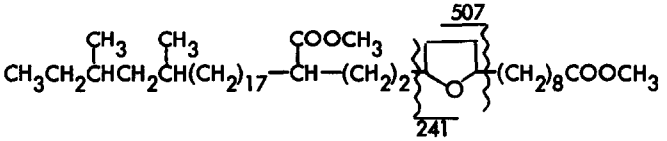
V (M^+ 620)



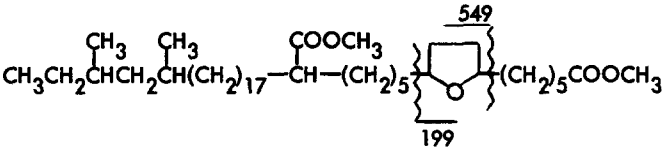
VI (M^+ 664)



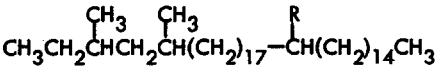
VII (M^+ 678)



VIII (M^+ 678)



IX R=H
X R=CH_3

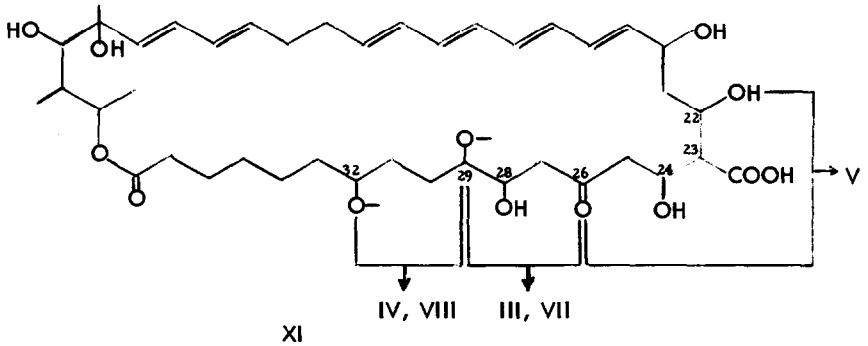


formulas. The nmr spectra of III and IV in which two protons attached to the oxygen-bearing carbon atoms appeared as a broad signal around 4δ, supported the cyclic ether structures. The conversion (phosphorus-hydriodic acid) of III and IV to the C_{40}-hydrocarbon (IX)[8] provided confirmation for the carbon skeleton of both esters.

The most important compound was the oily dicarboxylic acid ester (VI), $C_{43}H_{84}O_4$ (by high-resolution mass spectrometry). It was also converted to a hydrocarbon (X), $C_{41}H_{84}$, whose mass spectrum proved to be identical to that of the C_{41}-hydrocarbon obtained[8] from amphotericin B. In the mass spectrum of the ester VI, the base peak at m/e 410 and an accompanying peak at m/e 378 (assumed to arise from McLafferty rearrangement and subsequent loss of methanol) require the location of the secondary ester group at position 23 (see numbering system in XI).

The structures of two isomeric esters VII* and VIII were deduced from their mass spectra, in which the anticipated prominent bond cleavage occurred at the α-positions of the tetrahydrofuran ring.

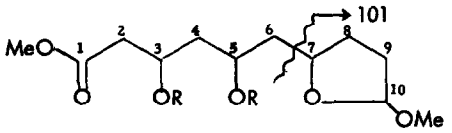
The formation of the esters II-V clearly involves the loss of the secondary carboxyl group under the stringent hydrogenation reaction conditions,[6] but the isolation of the esters VI-VIII accounts for all of the carbon atoms of nystatinolide. The structures of III, IV, V, VII and VIII necessitate the presence of oxygen functions at positions 22, 26, 29 and 32, as shown in the partial structure XI. As described in our previous papers,[4,5] labelling experiments and other data demonstrate that the keto group is present at a δ - or ζ-position with respect to the carboxyl group. The isolation[5] of acetone by alkaline treatment of nystatin requires the presence of the system -CHOH-CH_2CO-CH_2CHOH-. Hence the keto group is located at C-26 and two hydroxyl groups must be attached at C-24 and C-28 (see XI).



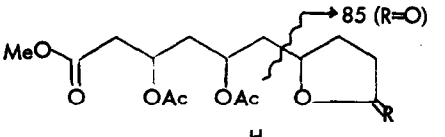
XI

* We have observed weak (M^+ + 14) peaks in the spectra of all esters (II-VIII), which presumably come from a higher homolog of nystatin. In the ester VII this peak was of abnormally high intensity.

The isolation of esters IV and VIII requires the presence of an oxygen function on C-29 and hence of an actual or potential glycol grouping at C-28 and C-29. In fact, when nystatin was subjected to successive treatment with periodic acid, alkaline hydrolysis, and esterification with diazomethane, a diol XII, $C_{12}H_{22}O_6$, νmax (liq. film); 3400 (OH), 1740 cm^{-1} (C=O), was obtained, which could be acetylated with acetic anhydride in pyridine to a diacetate XIII, $C_{16}H_{26}O_8$, $[\alpha]_D^{25}$ -35°, ν max (liq. film); 1740 cm^{-1} (C=O). The nmr spectra of XII and XIII, in combination with chemical and mass spectral data, provided proof for the structure of XII. The methyl group of the methoxycarbonyl moiety appeared at 3.71δ and 3.69δ in XII and XIII, respectively. The acetyl methyl groups in XIII gave signals at 2.05δ and 2.06δ and two (those at C-3 and C-5) of the three protons, which appeared at ca. 3.8-4.5δ in XII, shifted downfield to ca. 4.8-5.5δ by acetylation, whereas, one (C-7) remained at the same position (ca. 4.1δ). These shifts clearly show the presence of two hydroxyl groups in XII. The methylene protons on C-2 appeared as a doublet centered at 2.51δ (J=6.4c/s) and 2.63δ (J=6.3c/s) in XII and XIII, respectively. Decoupling experiments with XIII revealed that this doublet is coupled to a proton attached to an acetoxyl-bearing carbon with a signal around 5.3δ, indicating that one hydroxyl group must be present at C-3 in XII. The acetal methyl group appeared at 3.33δ in XII and XIII, and the methine proton of the acetal as a multiplet between 4.8-5.2δ. This acetal structure was confirmed by the following reaction sequence. Mild hydrolysis of the diacetate XIII with 10% sulfuric acid gave a hemiacetal (XIV), $C_{15}H_{24}O_8$, νmax (liq. film); 3450 (OH), 1740 cm^{-1} (C=O). The acetal methyl signal in the nmr spectrum of XIV disappeared and instead a hydroxyl proton appeared as a broad signal between 3.0-3.5δ. Oxidation of XIV with the chromium trioxide-pyridine complex gave a γ-lactone (XV), $C_{15}H_{22}O_8$, νmax (liq. film); 1775 (γ-lactone carbonyl), 1738 cm^{-1} (C=O).



XII R=H
XIII R=Ac



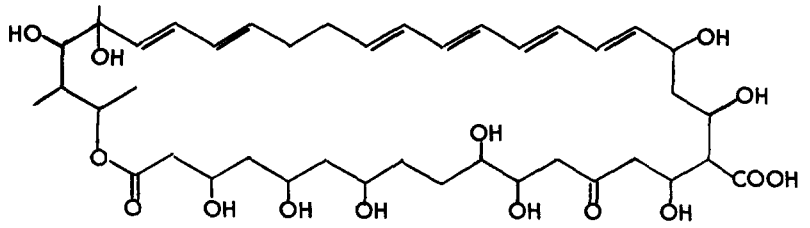
XIV R=CHOH
XV R=O

The mass spectra of XII, XIII and XV also confirmed the acetal structure by the appearance of intense peaks at m/e 101 in XII and XIII and at m/e 85 in XV, which are associated with α-fission next to the ether oxygen atom.

The possible positions of the one remaining hydroxyl group in XII are limited to C-4, C-5 and C-6 taking into account the C_{10}-straight chain skeleton required from the structure of the high-pressure

hydrogenation products. However, C-4 and C-6 are excluded as possible sites by the fact that the diol XII was produced by periodate fission and hence cannot contain a vicinal glycol. Nmr decoupling experiments on the diacetate (XIII) and the lactone (XV) revealed that a proton on C-7 couples only to methylene protons and not to a proton attached to an acetoxyl-bearing carbon, thus providing further evidence for the absence of an acetoxyl group at C-6.

The structures of the hydrogenolysis products II - VIII and the periodate oxidation product XII are uniquely accommodated by expression XVI for nystatinolide and only the site of attachment of the sugar mycosamine[9] remains for completing the structure of nystatin itself. It is pertinent to note that until now no definite empirical formula could be attributed to nystatin, although the analytical data[4] pointed toward a $C_{46-47}H_{73-75}O_{18}N$ formula. Structure XVI for nystatinolide corresponds to $C_{41}H_{64}O_{15}$, which in turn leads to $C_{47}H_{75}O_{18}N$ for nystatin.



XVI

The earlier recorded[4] labelling experiments established the biosynthetic origin of nystatin from 16 acetate and 3 propionate units. The oxygenation pattern of XVI is in accord with such a scheme, except for the glycol grouping at C-28 and C-29, which must have arisen through a rearrangement or possibly through a 29,30-epoxide precursor.

We are indebted to Dr. A. M. Duffield and Mr. R. G. Ross for the mass spectra (A.E.I. MS-9 double-focussing mass spectrometer with direct insertion probe) and to Dr. L. J. Durham for the 60 and 100 Mc NMR spectra.

References

1. For part XIV see ref. 5.

2. Financial assistance (grant No. GM 11309) from the National Institutes of Health of the U.S. Public Health Service is gratefully acknowledged. Generous supplies of nystatin were provided by Dr. J. D. Dutcher, Squibb Institute for Medical Research, New Brunswick, New Jersey.

3. Postdoctoral research fellow, (a) 1966-1967; (b) 1962-1964.

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