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THE STRUCTURE OF THE AGLYCONE OF THE MACROLIDE ANTIBIOTIC NYSTATIN*

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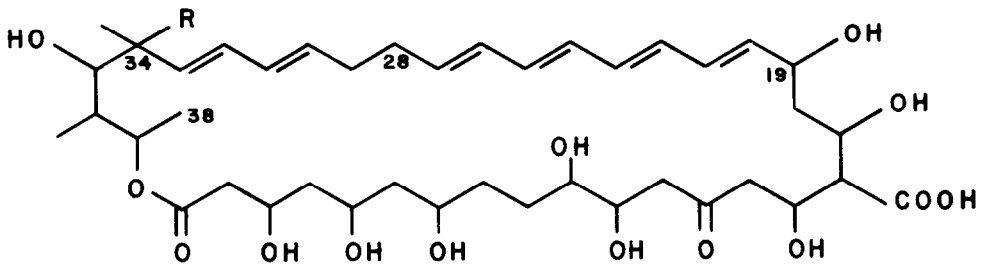
Ikeda, Suzuki and Djerassi[1] extended previous joint work[2,3] on nystatin, the chemotherapeutically-important antifungal agent[4] from Streptomyces noursei[5], and proposed constitution (I; R = OH) for nystatinolide, its hypothetical aglycone. This structure (I; R = OH) corresponds to $C_{41}H_{64}O_{15}$, and with the attachment of mycosamine[6] leads to the molecular formula $C_{47}H_{75}NO_{18}$ for nystatin itself. We present here evidence that nystatin is correctly formulated as $C_{47}H_{75}NO_{17}$, and that nystatinolide has the constitution (I; R = H).



I

Accumulated analytical data[2] indicated formulae $C_{46-47}H_{73-75}NO_{18}$ for nystatin, but were not definitive for a relatively unstable compound of such high molecular weight which also strongly occludes impurities[7]. Mass spectrometry of trimethylsilyl (TMS) derivatives** of

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** A procedure successfully applied in the determination of molecular weights of the smaller polyene macrolides lagosin, filipin, pimaricin and lucensomycin[8].

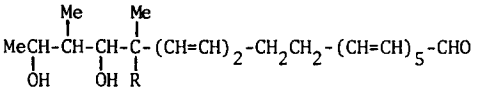
N-acetyl nystatin and N-acetyl nystatin methyl ester gave clear molecular ions at m/e 1759 and 1701 respectively[9]. Allowing for the mass difference between TMS and methyl esters, these values are in mutual agreement, but are so high as to preclude the direct determination of molecular formulae by double-focussing mass measurement. However, in conjunction with analytical and chemical data[2,3] then available[9], these molecular weights could only correspond to the TMS and methyl esters of N-acetyl-deca-TMS-ethers of a parent compound $C_{47}H_{75}NO_{17}$ or $C_{46}H_{71}NO_{18}$. This latter possibility is eliminated by the subsequent establishment[1] of the C_{47} carbon skeleton of nystatin. The remaining formula $C_{47}H_{75}NO_{17}$ necessitates deletion from the proposed nystatinolide structure[1] (I; R = OH) of one hydroxyl group, which is now shown to be that at C-34.

The primary evidence for the presence of the 34,35-diol system in nystatinolide (I; R = OH) was the reaction of nystatin with lead tetra-acetate to yield tiglic aldehyde (MeCH=CMeCHO) after subsequent β-elimination of the lactone[3]. This cleavage in fact results from fragmentation of the βγ-unsaturated 35-hydroxyl system as in the revised structure (I; R = H), a known reaction of homo-allylic alcohols[10]. In agreement, the same reaction fails on perhydronystatin prepared by hydrogenation over palladised charcoal, conditions extremely unlikely to cause hydrogenolysis of an allylic 34-hydroxyl if present. Such hydrogenolysis was suggested to occur over platinum, subsequent oxidation with nitric acid then giving 2-methylheptadecanedioic acid[3]. The acid obtained (m.p. 86-88°, $[\alpha]_D^{20}$ + 8.6° for c. 0.065% in EtOAc) corresponds, however, to the known (+)-acid[11] (m.p. 88.5°, $[\alpha]_D^{20}$ + 16 ± 2° in EtOAc), and differs from the racemate[11] (m.p. 99-100°). Hence either the suggested hydrogenolysis is stereospecific, or else nystatin carries hydrogen, not hydroxyl, at C-34.

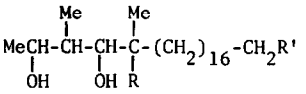
Further evidence for structure (I; R = H) for nystatinolide follows from comparison of nystatin with amphotericin B[12], which apart from an additional 28,29-double bond and possible stereochemical differences, has the same aglycone structure as (I; R = H) between C-19 and C-38, and in particular has no 34-hydroxyl. Thus amphotericin B with lead tetra-acetate gave tiglic aldehyde in similar yield to nystatin. After ozonolysis and alkaline hydrolysis the two macrolides afforded similar yields of tiglic aldehyde, propionaldehyde, and (notably) 2-methylpent-2-enal (as their 2,4-dinitrophenylhydrazones), whilst if the ozonolysis products were reduced with lithium aluminium hydride similar mixtures of polyols were formed (analysed by GLC after trimethylsilylation).

The second line of evidence for the presence of a 34-hydroxyl group in nystatin (as in I; R = OH) was the structure (II; R = OH) proposed[3] for the heptaenal formed on mild treatment of nystatin with alkali. The pmr spectrum (in $CDCl_3$) of the total material extractable into benzene directly from this reaction was that of a homogeneous compound, and showed three secondary methyl doublets centred at τ8.82, 9.01 and 9.21 (J 6.0, 6.5 and 6.5 Hz respectively), with no indication of a tertiary methyl singlet. The heptaenal is therefore correctly represented as (II; R = H), not (II; R = OH), a conclusion confirmed by mass spectrometry (Found: M^+, m/e 370.25154. $C_{24}H_{34}O_3$ requires M^+, m/e 370.25078) and in accord with the revised structure (I; R = H) for nystatinolide. The reported reactions[3] of this heptaenal can be accommodated by the constitution (II; R = H). Lead tetra-acetate causes fragmentation of

the homo-allylic alcohol system[10], as with nystatin itself, to give tiglic aldehyde. Hydrogenation over platinum is unexceptional, yielding the triol (III; R = H, R' = OH), and the diol (III; R = R' = H) by hydrogenolysis of a primary allylic hydroxyl. The tetrol (III; R = R' = OH), formed from hydrogenation over palladised charcoal followed by reduction with sodium borohydride, probably arises from a product of autoxidation* of the heptaenal at the tertiary allylic position.



II



III

Completion of the structure of nystatin itself requires the attachment of the sugar mycosamine[6] to the aglycone nystatinolide (I; R = H). The nystatin used in this and previous[1-3] structural work was provided by the Squibb Institute for Medical Research. Mass spectra of TMS derivatives of this material, and mass and pmr spectra of the freshly-prepared heptaenal (II; R = H), show no evidence of analogues bearing an additional hydroxyl group. It is important to note, however, that absolute criteria of purity for polyene macrolides are lacking, and counter-current distribution of our material shows the presence of 5-10% of a second tetraene, probably the A_2 mycosamine-carrying component recently described[13] in nystatin from other sources. From its distribution coefficient, this second tetraene is considerably less polar than nystatin itself.

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* Analytical data[7] for the heptaenal were not satisfactory, being very high in oxygen content even for the previous structure (II; R = OH). The compound was amorphous, unstable, and decomposed on chromatography.

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