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MACROLIDE ANTIBIOTIC STUDIES. XVI*. THE STRUCTURE OF NYSTATIN

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Macrolide antibiotics of the polyene subgroup[1] find extensive clinical application as antifungal agents[2], and may also be of value in treatment of hypercholesterolemia[3] and prostatic hypertrophy[4]. Nystatin, in 1950 the first of the polyene macrolides to be discovered[5], is the one most commonly used in antifungal therapy, and its hypothetical aglycone has been assigned the structure (I)[6]. We now present evidence which completes the structure (without regard to stereochemistry) of nystatin as (II)†.

The attachment of mycosamine, 3-amino-3,6-dideoxy-D-mannose[7], to the aglycone (I) is restricted to three possible positions, C-15, -17, and -19, by the following evidence. Nystatin reacts with lead tetraacetate by fission of the homo-allylic 35-alcohol system, subsequent β-elimination of the 37-oxygen function then yielding tiglic aldehyde (III)[6d]. Cleavage of the 10,11-diol system of nystatin with aqueous methanolic periodate, followed by alkaline hydrolysis and methylation, affords the acetal methyl ester (IV)[6b]. These products (III) and (IV) are obtained in similar yield from N-acetylnystatin, and thus their formation does not involve oxidative cleavage and solvolysis of the mycosamine moiety. Nystatin must then carry free hydroxyl functions at C-3, -5, -7, -10, -11, and -35.

The chromophore of nystatin was unchanged by treatment with manganese dioxide, indicating the absence of a free allylic hydroxyl group. Direct evidence for a glycosidic linkage at the allylic C-19 position as in (II) was obtained by hydrogenation of nystatin in methanol over platinum oxide or palladised barium sulphate to afford free mycosamine. The sugar is released by hydrogenolysis, not by solvolysis or β-elimination triggered by ketone or carboxyl functions, since no mycosamine was formed under these reaction conditions in the absence of hydrogen.

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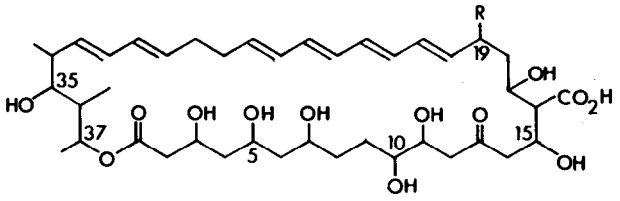
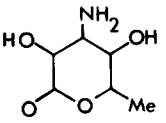
* Part XV. R.W. Rickards, R.M. Smith and B.T. Golding, J. Antibiot., submitted for publication.

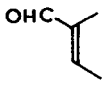
† This work was described at the Fourth National Convention of the Royal Australian Chemical Institute, Canberra, August 1970. cf. Abstracts of Symposia of the Aliphatic, Carbocyclic, and Heterocyclic Chemistry Divisions, p.73 (1970).



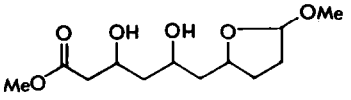
I ; R = OH

II ; R =

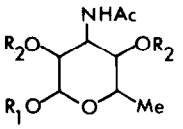




III

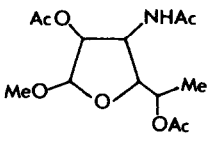


IV

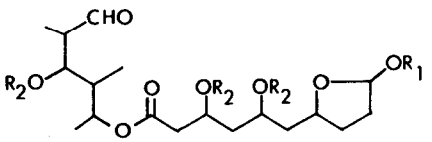


V ; R_1 = R_2 = Me

VI ; R_1 = Me, R_2 = Ac

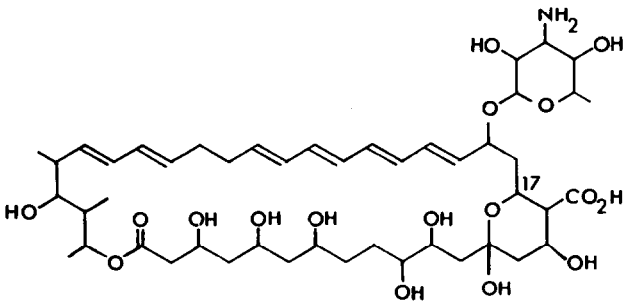


VII



VIII ; R_1 = Me, R_2 = H

IX ; R_1 = Me, R_2 = Ac



X

The pyranose nature of the mycosamine ring in nystatin (II) was established by N-acetylation of the antibiotic, reduction of the ketonic function with sodium borohydride to prevent subsequent base-catalysed β-elimination of the sugar, and permethylation of the resulting N-acetyl-dihydronystatin with methyl iodide and sodium methylsulphinylmethide. Acidic methanolysis then yielded α-methyl-N-acetyl-2,4-dimethylmycosaminide (V), m.p. 127-129°, $\nu_{max}^{CHCl_3}$ 3435 and 1670 cm^{-1} (-NHCOMe). The α-methyl-pyranoside structure (V) of this product follows from its synthesis by methylation of authentic α-methyl-N-acetylmycosaminide[7a], which has itself been shown to be pyranoid by degradation[7a]. Confirmation of these pyranoid forms was provided by acetylation of α-methyl-N-acetylmycosaminide. The product was α-methyl-2,4,N-triacetyl-mycosaminide (VI) as reported[7a], since double irradiation established that the methyl doublet (J 6.0 Hz) at τ 8.79 in its PMR spectrum (in $CDCl_3$) is coupled to a proton (H_5) appearing as a doublet of quartets (J 6.0, 9.0 Hz) centred at τ 6.10. H_5 in the alternative furanoid methyl-2,5,N-triacetylmycosaminide (VII) would be expected[8] to resonate below τ 5.5.

In the earlier work[6c] on nystatin the lactone ring was closed to C-37 by analogy with the smaller macrolides of the hydroxylated polyene type then known. Proof of this feature follows from successive oxidation of nystatin (II) with ozone and then sodium periodate in aqueous methanol. The product, expected to be the triol (VIII), on acetylation gave the triacetate (IX) and the corresponding αβ-unsaturated aldehyde arising by β-elimination. This last compound showed λ_{max}^{EtOH} 226 nm (log ε 3.90), $\nu_{max}^{CCl_4}$ 1735 cm^{-1} (broad), significant PMR resonances (in $CDCl_3$) at τ 0.56 (s, -CHO), 3.64 (d of m, -MeCHCH=CMeCHO), 4.60-5.20 (m, -CH(OMe)OR and 3 -CHOCOR-), 5.93 (m, -CHOR-), 6.68 (s, OCH_3), 7.40 (m, $-OCOCH_2-$), and 7.95-7.98 (m, 2 $-OCOCH_3$ and -CH=CMeCHO), and mass spectral ions (verified by mass measurement) at m/e 456 ($C_{23}H_{36}O_9$, M^+), 425 ($C_{22}H_{33}O_8$, M^+ - OMe), 315 ($C_{15}H_{23}O_7$, acylium ion from ester fission) and 101 ($C_5H_9O_2$, cyclic ether oxonium ion). In conjunction with the known chemistry of nystatin[6], notably the presence of a free 35-hydroxyl function, these data define the structures of the αβ-unsaturated aldehyde derived from (IX), the triacetate (IX) itself (m/e 515, $C_{25}H_{39}O_{11}$, M^+ - H), and hence the parent triol (VIII). The lactone is thus closed to C-37 in nystatin, completing the structure (II).

By analogy with the recently published X-ray crystal structure of the N-iodoacetyl derivative of the related macrolide amphotericin B[9], crystalline nystatin probably exists in the hemiketal form (X). Evidence that this form also predominates in solution will be presented elsewhere.

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