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NYSTATIN. PART V.*

BIOSYNTHETIC DEFINITION OF SOME STRUCTURAL FEATURES

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The isolation[1] and characterisation[2] of nystatin, the antifungal agent produced by Streptomyces noursei, havebeen described, and the structure[3] and stereochemistry[4] of mycosamine, its amino-sugar moiety,

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*Part IV, see ref.4. The present paper also constitutes Studies in Relation to Biosynthesis, Part XXXVI and Macrolide Antibiotics, Part XIII (Part XXXV and Part XII, respectively, A.J.Birch, C.Djerassi, J.D.Dutcher, J.Majer, D.Perlman, E.Pride, R.W.Rickards and P.J.Thomson, J.Chem.Soc., in the press). The work was presented in part at the 142nd Amer.chem.Soc. Meeting, Atlantic City, 1962.

[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.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.Wintersteiner, J.Amer.Chem.Soc., 1961, 83, 2785; J.Org.Chem., 1963, 28, 999.

have been elucidated. Accumulated analytical data support the molecular formula $C_{46-47}H_{73-75}O_{18}N$ for the antibiotic, rather than $C_{46}H_{77}O_{19}N$ as previously proposed.[2] The aglycone, nystatinolide, which would thus correspond to $C_{40-41}H_{62-64}O_{15}$, contains diene and tetraene chromophores in addition to lactone, carboxyl and numerous hydroxyl functions.[2] We present evidence derived from biosynthetic studies in combination with degradative chemistry, for the presence of the structural features (XII) and (XIII) in nystatin.

Shaken cultures of S.noursei were fermented in the presence of various ^{14}C-labelled substrates. In each case where incorporation into nystatin occurred, the mycosamine was inactive, in agreement with its expected direct origin in carbohydrate metabolism. The effective utilisation of sodium [1-, 2-, or 3-^{14}C]propionate (8-10%) and sodium [1-^{14}C]acetate (3-5%) showed clearly that the aglycone was derived fundamentally from these units, while negative results with [2-^{14}C]-mevalonic lactone and [methyl-^{14}C]methionine indicated the absence of introduced terpenoid or C_1 units. Degradation of the labelled nystatin samples gave fragments whose radioactivities are shown in the Table.

Oxidation of nystatin with lead tetraacetate or ozone afforded,[5] as the only steam-volatile carbonyl compound, tiglic aldehyde (I), in which the olefinic bond must arise by β-elimination of an oxygen function during the distillation. Consideration of the carbon skeleton of tiglic aldehyde (I) indicated that this portion of the aglycone originates in an acetate-propionate condensation, the acetate unit in fact representing the "primer" unit from which the chain is extended.[6] When nystatin derived from [1-, 2-, or 3-^{14}C]propionate was oxidised, the molar activity of the aldehyde (I) resulting was one-third that of the parent

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

[6] Cf. A.J.Birch, Proc.Chem.Soc., 1962, 3.

TABLE

Activities of Nystatin Fragments

(Values are relative molar activities[8] expressed as percentages of the parent nystatin activity).

	$MeCH_2{}^{14}CO_2H$	$Me^{14}CH_2CO_2H$	$^{14}MeCH_2CO_2H$	$Me^{14}CO_2H$
(I) $MeCH{=}\overset{Me}{C}CHO$	36.8	32.3	32.7	6.39
Pyrolysis $BaCO_3$				
(II) at 210°	-	-	26.8	0.75
(III) at 360°	-	-	5.3	4.68
(IV) Kuhn-Roth $MeCO_2H$				
Me	-	0.50	15.9	0.02
CO_2H	-	15.9	0.37	1.76
(V) $HO_2C\overset{Me}{C}H(CH_2)_{14}CO_2H$	67.4	35.6	-	41.3
(VI) $H_2N\overset{Me}{C}H(CH_2)_{14}NH_2$	36.0	34.2	-	36.0
(VII) $BaCO_3$ from (II)	15.5	0.70	-	3.52
(IX) MeCOMe	2.03	0.55	-	5.80
(X) MeCHO	15.3	14.9	-	2.89

[8] A.J.Birch, R.A.Massy-Westropp, R.W.Rickards and H.Smith, J.Chem.Soc., 1958, 360.

nystatin. Therefore, not only does the tiglic aldehyde contain the three carbons of one propionate unit, but also there are three propionate units in nystatin. The additional presence of sixteen acetate units in the aglycone, in good agreement with the C_{40-41} formula, was indicated by similar calculations[7] based on [1-^{14}C]acetate-derived materials.

Pyrolytic decarboxylation of the free carboxyl group of nystatin occurred at 210° (II), whilst at 360° a second mole of carbon dioxide (III) was released from the lactone function. Pyrolysis of nystatin labelled by [3-^{14}C]propionate and [1-^{14}C]acetate showed clearly, despite some overlap of the two decarboxylation reactions, that the carboxyl group arises by biological oxidation of the methyl group of one propionate unit, while the lactone carbon was originally an acetate carboxyl.

Kuhn-Roth oxidation of nystatin prepared from [2-^{14}C] and [3-^{14}C]-propionate gave acetic acid samples (IV) carrying one-sixth of the nystatin activity; Schmidt degradation showed this activity to be in the carboxyl and methyl groups respectively. Acetic acid arising from oxidation of propionate-derived C-methyl groups, of which there can be at most two, is being isotopically diluted in a 1:1 ratio with acid from other inactive sources. In conjunction with normal analysis, which indicates the minimum C-methyl content (Found: 3.4 - 3.8), these data unequivocally establish the presence of four C-methyl groups in nystatin.

Reduction of nystatin over a platinum catalyst, followed by oxidation with nitric acid, afforded 2-methylheptadecanedioic acid (V) as the largest dibasic acid.[5] This diacid (V) contained only one propionate methylene carbon (necessarily associated with the C-methyl group), two carbons from propionate carboxyl, and seven[7] from acetate carboxyl. Schmidt degradation of the diacid gave the corresponding C_{16}-diamine (VI) and two moles of barium carbonate (VII) which were derived from

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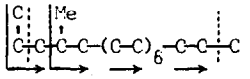
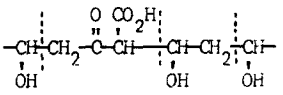
[7]Allowance is made here for the extent to which the propionate units are labelled by acetate tracer; the conversion of propionate to acetate is negligible.

carboxyl carbons of an acetate and a propionate unit. This labelling pattern is compatible only with the biogenetic arrangement (VIII) of acetate and propionate units in this section of the aglycone. Consequently, the aldehyde (I) and the diacid (V) must contain <u>different</u> propionate-derived C-methyl groups (since the biogenetic environments of these groups are different), and all four C-methyl groups of nystatin are then accounted for in degradation products.

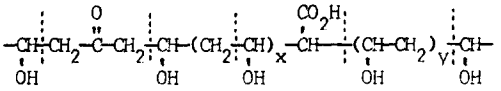


(VIII)

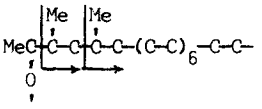
Lead tetraacetate oxidation of [3-^{14}C]propionate-labelled nystatin afforded carbon dioxide which was, however, effectively inactive, excluding oxygenation α to the carboxyl group. Labelled carbon dioxide was released from both [3-^{14}C]propionate-derived nystatin and its hydrogenation product on pyrolysis or on warming with mineral acid. Prior reduction with sodium borohydride prevented this decarboxylation, and also the retroaldolisation of nystatin in hot alkali to yield acetone and acetaldehyde. Whilst the acetone (IX) arose from acetate carbon only, the acetaldehyde (X) was derived equally from acetate and from the 1- and 2-positions of a propionate unit. The β-keto-acid system (XI), which explains the observed fission products but not their labelling patterns, must thus be rejected in favour of the δ or ζ-keto-acid structure (XII). Decarboxylation of such a system would proceed through the βγ-unsaturated acid.



(XI)



(XII, x or y = 0)



(XIII)

This environment for the carboxyl function necessitates that the propionate unit cleaved as in (VIII) during formation of the diacid (V) is that of tiglic aldehyde, and thus defines the skeletal section (XIII) in nystatin. The following communication provides degradative confirmation of this carbon skeleton (XIII), and in conjunction with other evidence leads to a tentative structure for nystatin.

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