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BIOSYNTHESIS OF THE POLYETHER ANTIBIOTIC NARASIN
ORIGINS OF THE OXYGEN ATOMS AND THE MECHANISMS OF RING FORMATION

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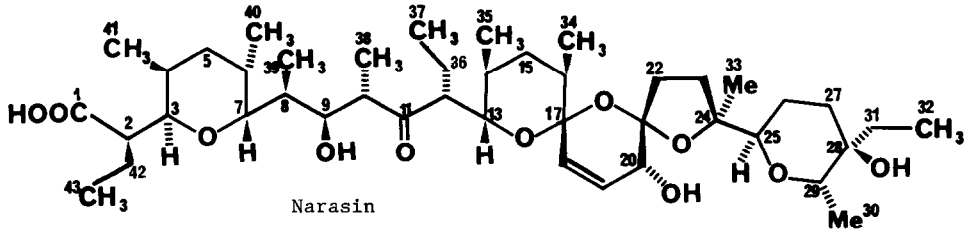
Abstract: The biosynthesis of narasin in cultures of Streptomyces aureofaciens has been studied using [1-^{13}C, $^{18}O_2$] labelled acetate and butyrate, and by incubation of cultures under an atmosphere of $^{18}O_2$. The origins of the eleven oxygen atoms in narasin have thereby been established and this provides information regarding the mechanism of heterocyclic ring and bis-spiroketal formation.

New insights into the complex biosynthetic transformations leading to the polyether antibiotics have come recently through the use of stable isotope labelling techniques designed to identify the biosynthetic origins of the carbon, oxygen and hydrogen atoms in each antibiotic. In particular, the biosyntheses of monensin-A,[1] lasalocid-A,[2] maduramicin[3] and ICI 139603[4] have been studied in these ways. An important feature of these biosynthetic pathways appears to be a requirement for a carbon chain building process capable of generating a long branched and oxygenated fatty acid backbone.[5] At first sight the large number of known, structurally unique polyethers is strongly indicative of a biosynthetic theme upon which Nature has played numerous variations to generate the diversity of observable stereochemical and regiochemical substitution patterns. On the other hand, when structural comparisons are made within this family an intriguing pattern of structural and stereochemical homologies can be perceived. This is illustrated most effectively in the recent Cane-Celmer-Westley model of polyether structure and stereochemistry.[6] The enzymic and genetic bases for these perceived similarities are, however, at present unclear.

The biosynthetic origins of all the oxygen atoms in monensin-A from primary precursors and from molecular oxygen, are now known.[1a,b] For the purposes of a detailed comparison of the pathways to monensin-A (an APPA polyether[6]) and to narasin (a PABA polyether[6]), we report here the results of initial studies with labelled precursors which define the origins of all the oxygen atoms in narasin.



Narasin

TABLE-1 Assignment of the ^{13}C n.m.r. spectrum of narasin methyl ester in deuteriochloroform relative to $CDCl_3$ (δ77.10 ppm) as internal standard. For carbon numbering see formulae above.

Peak	Carbon	δ ppm	Peak	Carbon	δ ppm	Peak	Carbon	δ ppm
43	1	175.9	25	16	40.5	19	31	30.7
27	2	49.3	39	17	99.1	1	32	6.3
36	3	77.4	41	18	121.0	14	33	25.7
15	4	28.2	42	19	132.7	8	34	15.6
21	5	35.6	30	20	67.7	9	35	17.6
16	6	29.0	40	21	106.3	11	36	18.9
33	7	73.1	23	22	36.5	4	37	12.9
22	8	35.9	18	23	30.4	6	38	13.9
31	9	69.4	38	24	87.8	2	39	7.9
26	10	47.8	34	25	74.1	5	40	13.3
44	11	213.6	12	26	22.2	10	41	17.9
29	12	56.8	17	27	29.2	13	42	23.2
37	13	78.9	32	28	70.9	3	43	12.0
20	14	33.3	35	29	77.0	28	44(CO_2Me)	52.6
24	15	39.0	7	30	14.5			

The assignment is taken from a 2D-autocorrelation experiment using narasin methyl ester (1g) in deuteriochloroform (3ml). The observed chemical shifts were followed through stepwise dilutions down to 1 mg/ml to ensure that the order of the resonances was unchanged. Most shifts varied by less than 0.05ppm and only peaks 11, 29 and 37 moved more than 0.1ppm (each moved downfield ca 0.3ppm). The 2D ^{13}C autocorrelation spectrum was acquired in 36hr with 128 increments of 160μs in the double quantum evolution period and 512 signals averaged for each step, giving an effective width in each dimension of 6250Hz (83ppm). The 4096 real points sampled from each signal were transformed with a Lorrentzian line broadening of 4Hz. The data in the second dimension was zero filled to 256 complex points and transformed without weighting before taking absolute values.[8]

The biosynthesis of narasin has been studied previously by the Lilly group,[7] who showed that the carbon backbone of the antibiotic is assembled from five acetate, seven propionate and three butyrate units. In our work the incorporation of [1-^{13}C, $^{18}O_2$] labelled acetate and butyrate, as well as $^{18}O_2$, into narasin in cultures of Streptomyces aureofaciens was studied. After each experiment the antibiotic was isolated, converted into its methyl ester by the action of diazomethane, and then examined by high field ^{13}C n.m.r. spectroscopy. Narasin methyl ester shows 44 ^{13}C resonances, whose chemical shift order was invariant over a wide range of concentrations in deuteriochloroform, unlike those of narasin free acid.[7] The ^{13}C n.m.r. spectrum of narasin methyl ester at natural abundance was assigned unambiguously by two-dimensional autocorrelation spectroscopy,[8] and this assignment is shown in Table-1.

Upon incorporation of sodium [1-^{13}C, $^{18}O_2$] acetate into narasin, the presence of intact ^{13}C-^{18}O bonds only at C(17) and C(21) were detected through the well established[9] isotope induced shift in the relevant ^{13}C n.m.r. resonances. These carbons, as well as those at C(19), C(25) and C(29), are formally regarded as originating from C(1) of acetate.

In a similar way upon incorporation of sodium [1-^{13}C, $^{18}O_2$] butyrate into narasin, intact ^{13}C-^{18}O bonds were detected atC(1) and C(11) (carbons formally derived from C(1) of butyrate), as well as at C(7), C(9) and C(13), carbons formally derived from C(1) of propionate.[14] The in-vivo interconversion of n-butyryl-CoA and methylmalonyl-CoA in other Streptomycetes is known[10] to proceed efficiently without loss of the oxygen at C(1), thereby accounting for the incorporation of label into the propionate units during this experiment.[14]

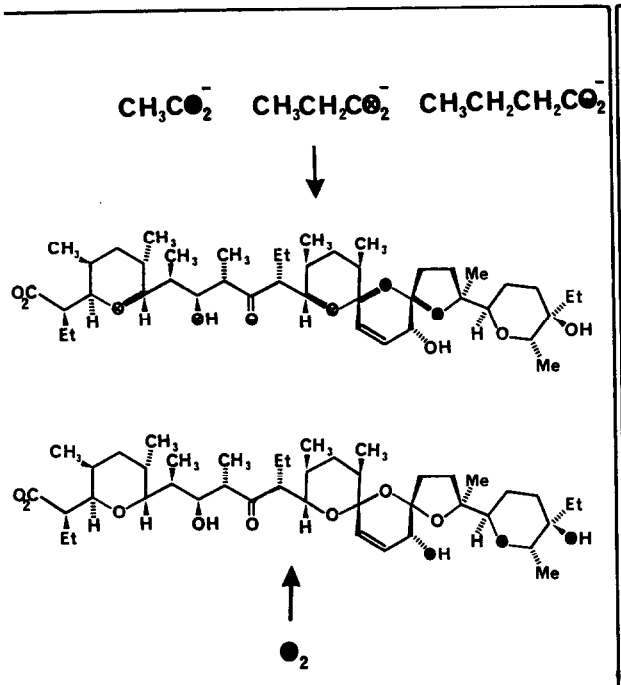


Figure-1

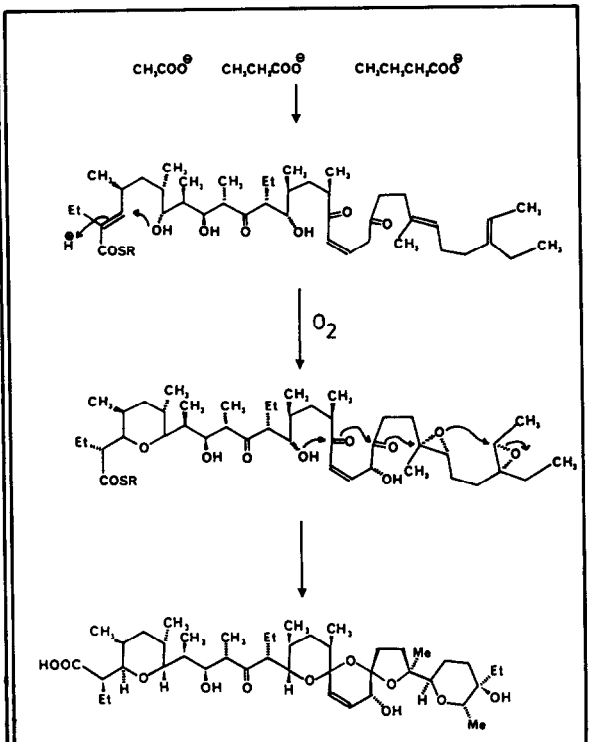


Figure-2

Finally, a fermentation culture of S.aureofaciens was grown under an atmosphere of $^{18}O_2$ and N_2 (50:50), and the narasin obtained was characterized by ^{13}C n.m.r. and mass spectroscopy. The incorporation of three oxygen-18 atoms was clearly indicated in the FAB mass spectrum ($^{m}/_{z}$ 806 ($M+Na^{+}-H$), 767; whereas for unlabelled narasin methyl ester $^{m}/_{z}$ 800 ($M+Na^{+}-H$, 761)) of this labelled narasin, and high field ^{13}C n.m.r. spectroscopy revealed the location of the label at C(20), C(25), C(28) and C(29). These results are summarised in Figure-1 and Table-2, and support the proposed biosynthetic pathway shown in Figure-2 in which the ring-A tetrahydropyran is generated by a syn-Michael addition of the C(7) hydroxyl onto an E-α,β-unsaturated thiol ester intermediate.[11] The formation of the other rings may then proceed by a cascade, as shown, in which the E-ring is closed by epoxide opening through the secondary centre. There is no evidence as yet to indicate whether the hydroxyl group at C(20), which is derived from molecular oxygen, is introduced before or after these cyclizations, although the influence of this group in biomimetic processes leading to bis-spiroketals has been noted.[12]

The enzymic processes leading to narasin and monensin now become of paramount interest, and recent advances in Streptomyces genetics[13] provides one powerful tool for the study of these secondary metabolic pathways, at the level of individual enzymes and genes.

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TABLE-2 The incorporation of sodium [1-^{13}C, $^{18}O_2$] acetate and butyrate,† as well as of $^{18}O_2$ into narasin. All $^{13}C\{^1H\}$ n.m.r. spectra were recorded at 90.5 MHz, on narasin methyl ester, in deuteriochloroform.

Carbon	$^{16}O:^{18}O$ ratios‡ with isotope shifts (Δppm) Acetate	Butyrate	$^{18}O_2$*
1	84:14	58:42 (0.03)	-
7	-	55:45 (0.023)	-
9	-	58:42 (0.019)	-
11	82:18	45:55 (0.051)	-
13	-	52:48 (0.022)	-
17	75:26	83:17 (0.028)	-
20	-	-	54:46 (0.015)
21	74:26	80:20 (0.027)	-
25	-	-	50:50 (0.028)
28	-	-	57:43 (0.034)
29	-	-	56:44 (0.028)

† Doubly labelled precursors are ca 70% $^{13}C\ ^{18}O_2$ + 14% $^{13}C\ ^{18}O_1\ ^{16}O_1$.

‡ The $^{16}O:^{18}O$ ratios are the ratios of the integrated signal intensities.

* Narasin methyl ester (18mg), isolated from the fermentation under $^{18}O_2$, was diluted with unlabelled material (18mg) before this ^{13}C n.m.r. spectrum was recorded

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10. See : D O'Hagan, J A Robinson and D L Turner, J.Chem.Soc.Chem.Comm., 1983, 1327; and Refs. 1(d) and 1(e).
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14. The retention of ^{13}C-^{18}O bonds at C(7), C(9) and C(13) was also observed upon incorporation of sodium [1-^{13}C, $^{18}O_2$] propionate.

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