The Utilization of Oxygen Atoms from Molecular Oxygen during the Biosynthesis of Monensin-A

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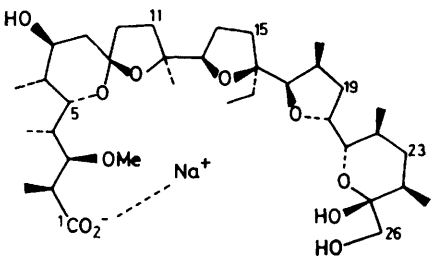
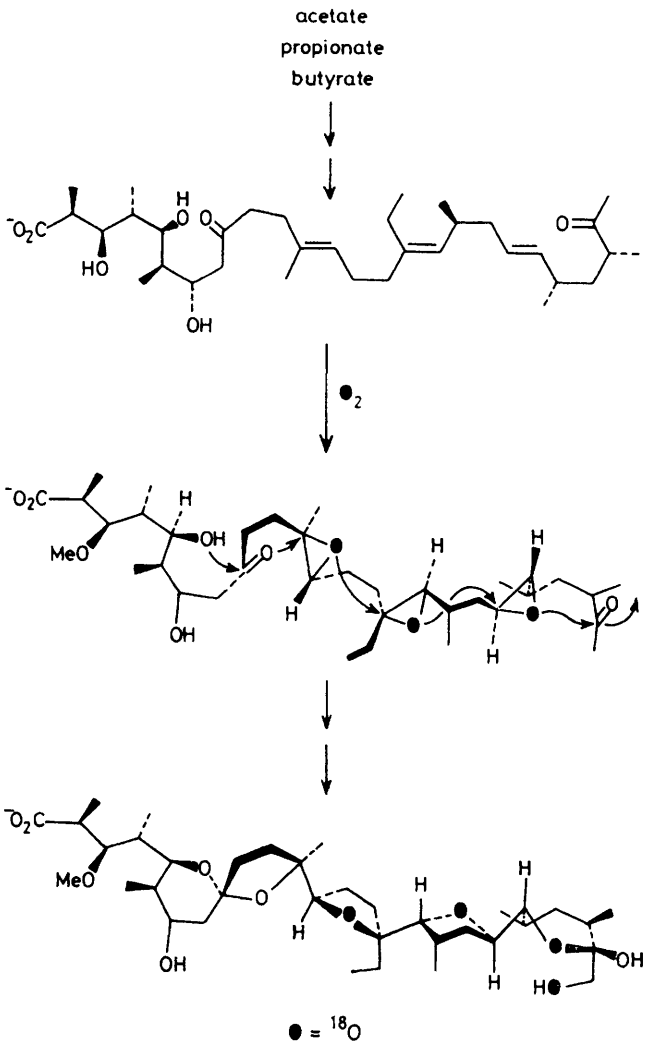
The biosynthesis of monensin-A in cultures of *Streptomyces cinnamonensis* is shown to occur with the incorporation of four oxygen atoms from molecular oxygen.

Monensin-A is a polyether ionophore antibiotic possessing, in common with most other antibiotics of this class, a polyoxygenated and branched carbon backbone.[1] The ^{13}C n.m.r. spectrum of sodium monensin-A has been assigned unambiguously by three groups,[2,3] and recently the biosynthetic origin of the carbon atoms of this antibiotic from acetate, propionate, and butyrate was established in feeding experiments[4,5] with ^{13}C labelled precursors to cultures of *Streptomyces cinnamonensis*. The derivation of the oxygen atoms in monensin-A from $^{13}C/^{18}O_2$ labelled acetate, propionate, and butyrate,[4] as well as molecular $^{18}O_2$,[3] has also been studied by Cane and co-workers, and from their results an attractive mode of



Monensin-A sodium salt.



Scheme 1

biosynthesis of the antibiotic was suggested (Scheme 1). Central to this biosynthetic scheme is the origin of at least three oxygen atoms in monensin-A from molecular O_2. We have developed a technique for detecting the incorporation of oxygen atoms from O_2 into monensin-A, which has shown clearly the origin of four of the backbone oxygen atoms from this source.

The success of a shake flask fermentation of *S. cinnamonensis* depends critically on maintaining the highest possible aeration of the culture within the constraints imposed by the need to use a closed fermentation vessel containing $^{18}O_2$. A 60 ml shake flask culture of *S. cinnamonensis* A3823.5 was grown for 24 h at 32 °C in a continuous stream of sterile air, and then for a further 50 h under an atmosphere of 99 atom% $^{18}O_2$ and N_2 (50:50). The rate of oxygen consumption remained steady at approximately 27 ml/h, and this was replenished regularly at four-hourly intervals. The antibiotic was subsequently isolated in the usual way[6] and purified rigorously to afford 45 mg of sodium monensin-A, m.p. 169 °C. An analysis of this material by electron impact mass spectroscopy revealed that the major fragment ion occurring at *m/z* 617 in unlabelled sodium monensin-A[7] (M^+−OMe−CO_2)† was now displaced

† The fragment ion at *m/z* 617 arises from loss of C(1) as CO_2 and •OMe [attached to C(3)] from the sodium monensin-A complex. These oxygen atoms are derived from propionate and/or water (ref. 3).

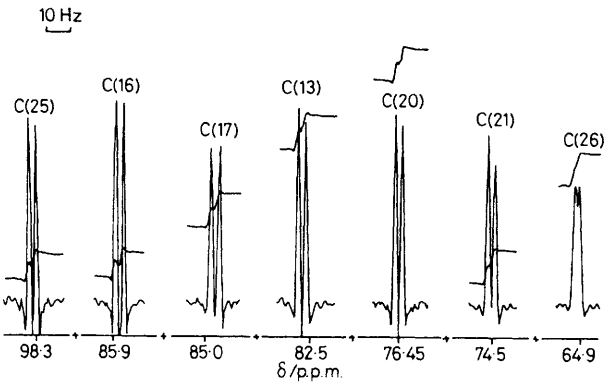


Figure 1. Section of the 100 MHz $^{13}C\{^1H\}$ n.m.r. spectrum of $^{18}O_4$-(sodium monensin-A) (20 mg) + unlabelled sodium monensin-A (20 mg) in $CDCl_3$. The spectrum is resolution enhanced: scan width 25000 Hz, 65° pulse, 6 s pulse delay, 6794 transients, 64 K data points.

clearly to *m/z* 625, indicating that the sample was *ca.* 85 atom% $^{18}O_4$ labelled. A portion of this material was diluted with an equal weight of unlabelled sodium monensin-A and the $^{13}C\{^1H\}$ n.m.r. spectrum was then recorded at 100 MHz. The spectrum showed sharp singlets for all the peaks except those assigned to C(13), C(16), C(17), C(20), C(21), C(25), and C(26). These peaks appeared as 'doublets' (see Figure 1) owing to the presence of ^{13}C–^{18}O resonances upfield[8] by 3.1, 3.4, 3.5, 2.9, 2.9, 2.9, and 2.0 Hz, respectively, from the normal ^{13}C–^{16}O signals.

These data complement the results of Cane and co-workers. They showed that the three oxygen atoms attached to C(13), C(17), and C(21) are derived from molecular oxygen, in accord with the pathway depicted in Scheme 1. The timing of the fourth oxygen atom insertion at C(26) is at present unclear. Whereas the other three inserted oxygens are required for the key cyclisation processes, the hydroxy groups at C(25) and C(26) appear[9] to form two strong hydrogen bonds with the carboxy oxygens thereby tightening and stabilizing the metal ion complex.

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