Biosynthesis of Monensin. Evidence for a Vicinal Interchange Rearrangement linking n-Butyryl-CoA and Isobutyryl-CoA

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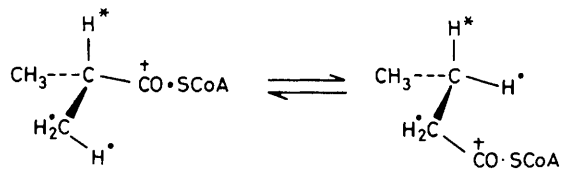
The interconversion of isobutyrate and n-butyrate in *Streptomyces cinnamonensis*, which occurs by an intramolecular carbon skeleton rearrangement, is shown by isotopic labelling experiments also to involve the 1,2-shift of a hydrogen from the *pro*-(*S*) methyl of isobutyryl-CoA to the 3-*pro*-(*S*) position in n-butyryl-CoA.

The incorporation recently[1,2] of isotopically labelled forms of isobutyrate into the polyether antibiotic monensin-A have highlighted a hitherto undisclosed intramolecular carbon skeleton rearrangement that links isobutyryl-CoA and n-butyryl-CoA. During this rearrangement the carbonyl thioester of isobutyryl-CoA undergoes a 1,2-migration to the *pro*-(*S*) methyl, and it is replaced by a hydrogen atom at C(3) of n-butyryl-CoA with overall retention.[1] We report here new results which show that during this rearrangement there is also a concomitant 1,2-hydrogen shift from the *pro*-(*S*) methyl of isobutyrate into the 3-*pro*-(*S*) position of n-butyrate. The proposed interconversion of isobutyryl-CoA and n-butyryl-CoA may therefore be viewed as a new 1,2-vicinal interchange rearrangement (see Scheme 1).

Our experiments were carried out using a whole cell system



Scheme 1

in which the rearrangement is assayed by following the incorporation of labelled isobutyrate into the single n-butyrate unit in monensin-A (C-15, -16, -32, and -33), in cultures of *Streptomyces cinnamonensis* (see Figure 1). Figure 2(b) shows the high field portion of the $^{13}C\{^{1}H,^{2}H\}$ n.m.r. spectrum of monensin-A biosynthesized from [3,3′-$^{13}C_2$, 3,3′-$^{2}H_6$]-isobutyrate. Included in the plot are the natural abundance signals assigned to C(29), C(30), and C(33), along with a multitude of isotopically shifted resonances. For comparison the spectrum in Figure 2(a) shows the same spectral region for a monensin-A sample biosynthesized from sodium [2-^{13}C,^{2}H]acetate. For each of the resonances C(29), C(30), and C(33) in Figure 2(a) there appear isotopically shifted signals which can be assigned to intact $^{13}CH_2D$, $^{13}CHD_2$, and $^{13}CD_3$ groups, as indicated. The corresponding isotopically shifted signals associated with C(29) and C(30) are also clearly evident in the spectrum in Figure 2(b), although the shifted signals for C(33) are more complex. For this carbon each of the shifted signals shows a long range ^{13}C–^{13}C coupling ($J_{1,3}$ 2 Hz) and there is no discernible signal for a $^{13}CH_2D$ group. Of more significance, however, there are additional signals upfield (by *ca.* 0.10 p.p.m.) beyond the resonances assigned to intact $^{13}CHD_2$ and $^{13}CD_3$ groups. These may arise from the presence also of deuterium at C(32) in those molecules

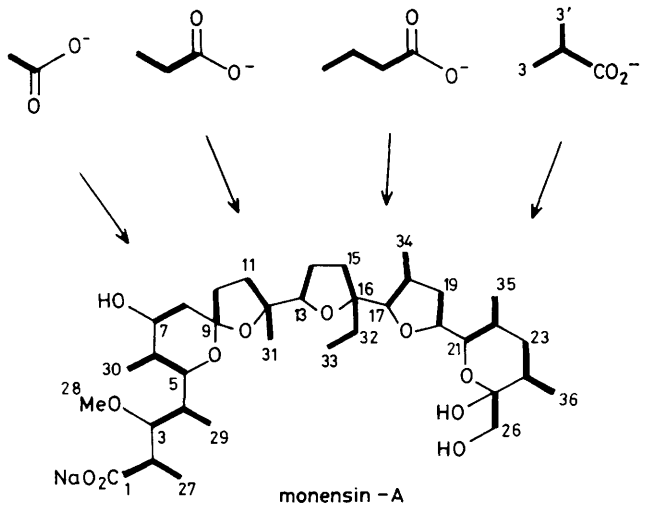


Figure 1

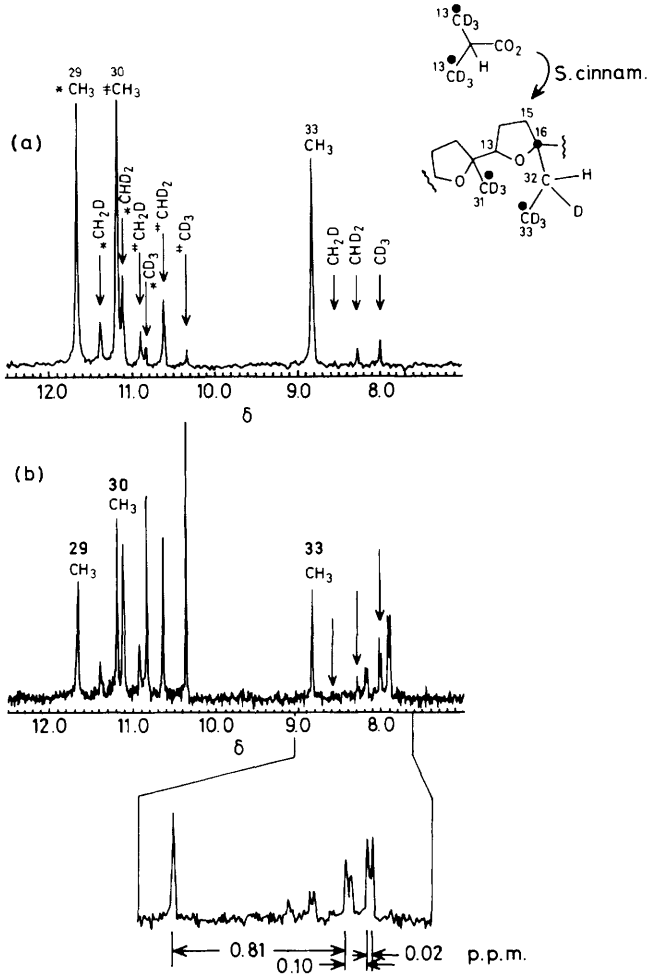
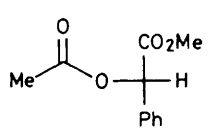
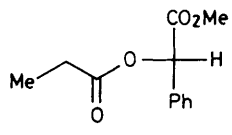


Figure 2. High field portion of the $^{13}C\{^{1}H,^{2}H\}$ n.m.r. spectra of monensin-A. (a) Biosynthesized from Na[2-^{13}C,2-$^{2}H_3$]acetate; (b) biosynthesized from Na[3,3′-$^{13}C_2$, 3,3′-$^{2}H_6$]isobutyrate. The arrows here indicate isotopically shifted signals assigned to C(29), C(30), and C(33). For each spectrum 64K data points, 1.638 s acquisition time, transformed with Gaussian line broadening of 0.4 Hz, broad band proton (1 W) and deuterium (3 W) decoupling, operating frequency 90.5 MHz.



(1)



(2)

containing ^{13}C labels at C(16) and C(33); the enriched signals at C(16) and C(33) then experience an additional β-shift[3] (typically *ca.* 0.03—0.1 p.p.m.) as well as any α-shifts[4] due to directly attached deuterium. This interpretation requires that during the carbon skeleton rearrangement there is also an intramolecular 1,2-hydrogen shift from the methyl of isobutyryl-CoA to the C(3) position in n-butyryl-CoA.

It was clearly desirable to corroborate this conclusion and for this reason sodium [3,3′-$^{2}H_6$]isobutyrate was also administered to cultures of *S. cinnamonensis*. The labelled monensin-A produced was then degraded by Kuhn–Roth oxidation and the mixture of acetic and propionic acids formed was isolated and converted into the mixture of derivatives (**1**) and (**2**) by reaction with (+)-methyl mandelate (dicyclohexylcarbodiimide, dimethylaminopyridine, CH_2Cl_2, −5 °C). The pure propionate derivative {$[\alpha]_D$ +125° (*c* 0.8, $CHCl_3$)} (**2**) could then be obtained by h.p.l.c. The ^{2}H n.m.r. spectrum of this material at 55.3 MHz in C_6H_6 showed three major resonances at δ 2.4, 2.3, and 1.1 p.p.m. in the ratio 0.2 : 1 : 10, which are assigned[5] to deuterium enrichments at the 2-*pro*-(*R*), 2-*pro*-(*S*), and the C(3) methyl of propionate, respectively.

Secondly, and in a similar fashion, sodium [$^{2}H_7$]isobutyrate was incorporated into monensin-A, and this monensin was degraded as above to afford the pure derivative (**2**), whose ^{2}H n.m.r. spectrum now showed three major resonances at δ 2.4, 2.3, and 1.1 p.p.m., in the ratios 1 : 1 : 8. These signals are again assigned to deuterium at the 2-*pro*-(*R*), 2-*pro*-(*S*), and 3-Me positions, respectively.

These two experiments support the conclusions drawn earlier that the α-hydrogen of isobutyrate is retained and that during the carbon skeleton rearrangement a hydrogen (or deuterium) atom undergoes a 1,2-migration from the *pro*-(*S*) methyl of isobutyrate[1] to the C(3) position in the n-butyrate (see Scheme 1). Moreover, this migrating hydrogen is subsequently located largely in the *pro*-(*S*) position, although a significant amount of deuterium label is also subsequently detected in the *pro*-(*R*) position during the experiment with [$^{2}H_6$]isobutyrate. The reasons for this last observation are presently undefined, but may reflect a partial epimerization.

The intra- *vs.* inter-molecular nature of the hydrogen shift is also of great interest. Our results indicate that this should be intramolecular, but do not rule out the possibility of an intermolecular shift if the mechanism of the rearrangement involves a hydrogen carrier, such as that known[6] to function during the vicinal interchange rearrangements catalysed by coenzyme-B_{12} dependent mutases. This question is difficult to address unambiguously using the whole cell system described here. Nevertheless, we also looked at the incorporation into monensin-A of a mixture of [2-^{13}C]- and [3,3′-$^{2}H_6$]-isobutyrates (in 1 : 3 ratio), but could see no evidence in the monensin-A produced for the direct attachment of deuterium to the ^{13}C label incorporated at C(32), which would have indicated an intermolecular hydrogen shift. This could be due, however, to an isotope effect, or to the dilution *in vivo* of labelled isobutyryl-CoA by endogenous material.

The observation of a carbon skeleton rearrangement that involves a vicinal interchange of carbon and hydrogen atoms finds precedent elsewhere only in the action of coenzyme-B_{12}

dependent mutases.[7] This lends further support to the view[2] that it is a coenzyme-B_{12} dependent mutase here that catalyses the interconversion of isobutyryl-CoA and n-butyryl-CoA.

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7 The closest analogy is to methylmalonyl-CoA mutase: See for example; J. Retey, in 'Vitamin B_{12},' vol. 2, ed. D. Dolphin, J. Wiley, New York, 1982, p. 357.