Biosynthesis of Monensin. The Intramolecular Rearrangement of Isobutyryl-CoA to n-Butyryl-CoA

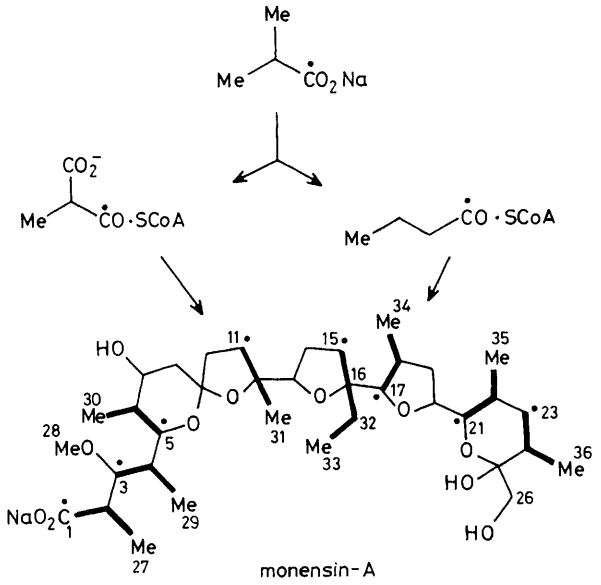
Kevin Reynolds and John A. Robinson*

Chemistry Department, The University, Southampton SO9 5NH, U.K.

The incorporation of [1,3,3′-$^{13}C_3$]isobutyrate into monensin-A in cultures of *Streptomyces cinnamonensis* reveals an intramolecular rearrangement linking isobutyryl-CoA and n-butyryl-CoA.

The incorporation recently[1] of stereospecifically labelled isotopomers of isobutyric acid into monensin-A, using shake cultures of *Streptomyces cinnamonensis* has provided strong support for the proposal, initially made by Omura and coworkers,[2] that valine metabolism in macrolide and polyether antibiotic producing strains of *Streptomyces* may involve the rearrangement of isobutyryl-CoA to n-butyryl-CoA. We report here direct evidence that this rearrangement occurs intramolecularly, involving formally a migration of the carbonyl carbon to the pro-(*S*) methyl group in isobutyryl-CoA.

The focus of attention in our labelling experiments is the ability of labelled isobutyrate to enrich efficiently the single 'n-butyrate unit,' as well as the seven 'propionate units' in monensin-A[3] (see Scheme 1). As a further probe of the mechanism of the rearrangement we administered [1,3,3′-$^{13}C_3$]isobutyrate (each site enriched 90 atom % in ^{13}C) diluted 1 : 3 with unlabelled isobutyrate, to fermentation cultures of *S. cinnamonensis*. The monensin-A generated by these cultures was isolated and purified in the usual way.[4] The most prominent feature in the ^{13}C n.m.r. spectrum of this material[5] was the presence of two strongly enriched signals assigned to C(15) and C(16). Each enrichment appears as a doublet of doublets [J 35.40 and J 1.83 Hz for C(15); J 35.40 and J 2.44 Hz for C(16)]. Moreover, in resolution enhanced ^{13}C spectra the signal assigned to C(33) is also flanked by two strongly enriched satellite signals, separated by 4.27 Hz. These enriched signals can only arise from the simultaneous presence of three ^{13}C labels at C(15), C(16), and C(33), and represent typical one bond ($J_{1,2}$ 35.4 Hz) and long range ($J_{1,3}$ 2.44 and $J_{1,4}$ 1.83 Hz) coupling constants. From this, the conclusion

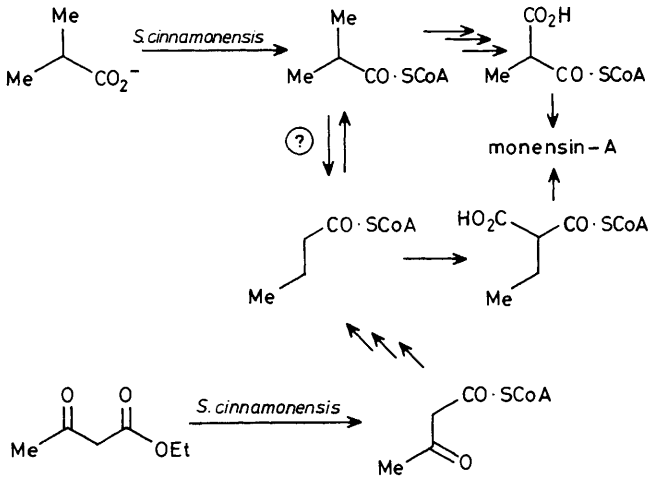


Scheme 1

follows that isobutyrate has furnished *intact* the four carbon atoms comprising the n-butyrate unit in monensin-A, and hence that an *intramolecular rearrangement* of isobutyryl-CoA to n-butyryl-CoA has occurred *in vivo*.

To provide a more detailed framework for a discussion of the mechanism of this rearrangement, some additional experiments were carried out. Firstly, it is of value to know that the terminal methyl group, *i.e.* C(33), is derived intact from isobutyrate. This was established by feeding [3,3′-$^{13}C_2$, 3,3′-$^{2}H_6$]isobutyrate to *S. cinnamonensis*. The $^{13}C\{^1H,^2H\}$ n.m.r. spectrum of monensin-A produced in this way showed isotopically shifted resonances[6] assigned to intact-$^{13}CD_3$ groups for each of the positions C(27), C(29), C(30), C(31), C(33), C(34), C(35), and C(36). Thus the pro-(*R*) methyl of isobutyrate is incorporated intact, not only into n-butyryl-CoA, but also *via* oxidation of isobutyryl-CoA into (*S*)-methylmalonyl-CoA,[1] the precursor of the propionate units.

Secondly, it is of interest to ask whether the rearrangement is reversible. Unfortunately we cannot assay directly the metabolism of n-butyryl-CoA into isobutyryl-CoA in this way, but it is possible to observe the incorporation of labelled n-butyrate into the propionate units in monensin-A. Although the experiment is not strictly comparable, we also examined the incorporation of ethyl [1,3-$^{13}C_2$]acetoacetate (each site 90 atom % enriched; again diluted 1 : 3 with unlabelled material) into monensin-A, and were gratified to find enriched doublets (with *J* values between 33 and 51 Hz) centred about each of the natural abundance singlets assigned to C(1), C(2), C(3), C(4), C(5), C(6), C(11), C(12), C(17), C(18), C(21), C(22), C(23), and C(24), *i.e.* each of the positions derived from carbons-1 and -2 of propionate. Confirmation that these doublets have



Scheme 2

arisen specifically from the direct coupling of adjacent ^{13}C enriched sites came from a 2D ^{13}C-COSYX experiment. It is clear, therefore, that the metabolism of acetoacetyl-CoA *in vivo* has involved at one stage an intramolecular rearrangement that does not involve prior cleavage to acetyl-CoA, and affords eventually [1,2-$^{13}C_2$]methylmalonyl-CoA. Since carbon skeleton rearrangements of C_4 fatty acids are unprecedented, it is tempting and reasonable to assume that acetoacetyl-CoA is metabolized here by the route shown in Scheme 2.

Although final confirmation of the reversibility of the isobutyryl-CoA–n-butyryl-CoA rearrangement must await further studies, the labelling experiments described above together with those reported in an earlier communication,[1] place limitations on a conceivable mechanism. Indeed, it is relevant to note that a mechanistic rationale becomes available if the process represents a new coenzyme-B_{12} dependent rearrangement.

We thank Dr. David Gani for valuable discussions, and the S.E.R.C. for financial support.

Received, 9th September 1985; Com. 1320

References

1 D. Gani, D. O'Hagan, K. Reynolds, and J. A. Robinson, *J. Chem. Soc., Chem. Commun.*, 1985, 1002.
2 S. Omura, K. Tsuzuki, Y. Tanaka, H. Sakakibara, M. Aizawa, and G. Lukacs, *J. Antibiot.*, 1983, **36**, 614.
3 See also S. Pospisil, P. Sedmera, M. Havranek, V. Krumphanzl, and Z. Vanek, *J. Antibiot.*, 1983, **36**, 617.
4 J. A. Robinson, D. L. Turner, and A. A. Ajaz, *J. Chem. Soc., Perkin Trans. 1*, 1985, submitted for publication.
5 J. A. Robinson and D. L. Turner, *J. Chem. Soc., Chem. Commun.*, 1982, 148; corrected on page 568.
6 M. J. Garson and J. Staunton, *Chem. Soc. Rev.*, 1979, 539.

Electronically typeset and printed by Heffers Printers Ltd, Cambridge, England