Biosynthesis of the Polyether Antibiotic Monensin-A: Stereochemical Aspects of the Incorporation and Metabolism of Isobutyrate

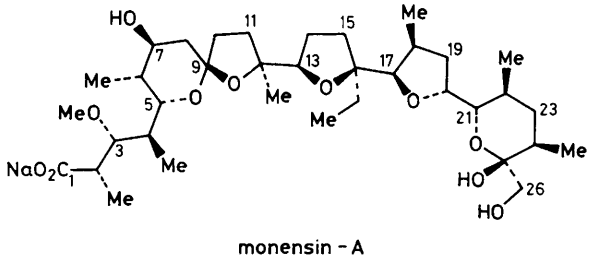
David Gani, David O'Hagan, Kevin Reynolds, and John A. Robinson*

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

The incorporation of stereospecifically labelled isotopomers of isobutyrate into monensin-A provides stereochemical and mechanistic information about the bioconversion of isobutyrate into methylmalonyl-CoA and n-butyryl-CoA in *Streptomyces cinnamonensis*; a rearrangement of isobutyryl-CoA to n-butyryl-CoA occurs without loss of the α-hydrogen, and the carbonyl carbon migrates to the *pro*-(*S*) methyl group, and is replaced by a hydrogen atom with overall retention of configuration.

The roles of L-valine and isobutyric acid in the secondary metabolism of macrolide and polyether antibiotic producing strains of Streptomyces have been described by Havranak,[1] and by Omura.[2] In particular, the Czech group[1] have shown that monensin-A is specifically enriched at C(1), C(3), C(5), C(11), C(15), C(17), C(21), and C(23) upon incorporation of either [1-^{13}C]isobutyrate or [1-^{13}C]butyrate[3] (see Figure 1). However, seven of these enrichments occur in units formally derived from propionate building blocks, and the highest enrichment was seen from labelled isobutyrate into the n-butyrate unit [*i.e.* C(15)–C(16)–C(32)–C(33)] in monensin-A. We report here the results of our studies with stereospecifically labelled isotopomers of isobutyric acid which confirm the existence of a pathway linking branched chain metabolism and straight chain metabolism in *Streptomyces cinnamonensis*, and which define stereochemical and mechanistic features of the reaction(s) linking isobutyryl-CoA and n-butyryl-CoA.

The addition of sodium isobutyrate to cultures of *S. cinnamonensis* at high concentrations (>20 mM) leads to a significant reduction in the yield of monensin-A. In two control experiments [1-^{13}C]- and [3,3′-$^{13}C_2$]-isobutyrate were administered batchwise to 60 ml cultures, to a final concentration of 17 mM. The enriched Na-monensin-A isolated in each experiment was purified by chromatography and examined by 90 MHz ^{13}C n.m.r. spectroscopy. The results shown in Table 1



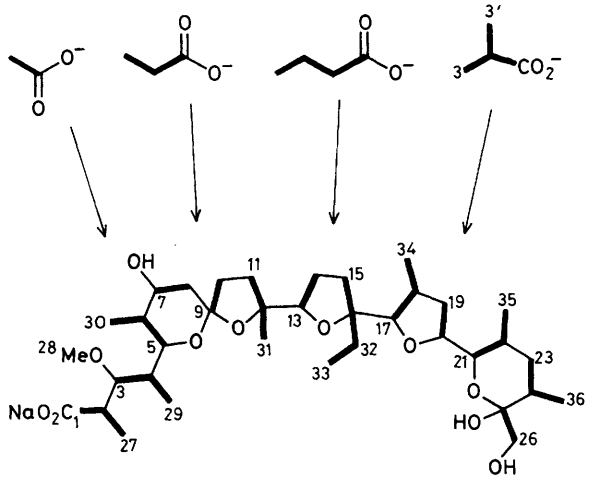
monensin - A

Figure 1

Table 1. Enrichments in monensin-A biosynthesized from [1-^{13}C]- and [3,3'-$^{13}C_2$]-isobutyrates (IBA).[a]

Carbon	[1-^{13}C]IBA	[3,3'-$^{13}C_2$]IBA
C-1	2.3	—
C-3	3.2	—
C-5	2.8	—
C-11	2.7	—
C-15	5.7	—
C-16	—	2.8 ($J_{1,3}$ 1.83 Hz)
C-17	3.5	—
C-21	3.2	—
C-23	2.6	—
C-27	—	3.1
C-29	—	2.5
C-30	—	2.2
C-31	—	2.8
C-32	—	—
C-33	—	4.0 ($J_{1,3}$ 1.83 Hz)
C-34	—	2.3
C-35	—	2.2
C-36	—	3.7

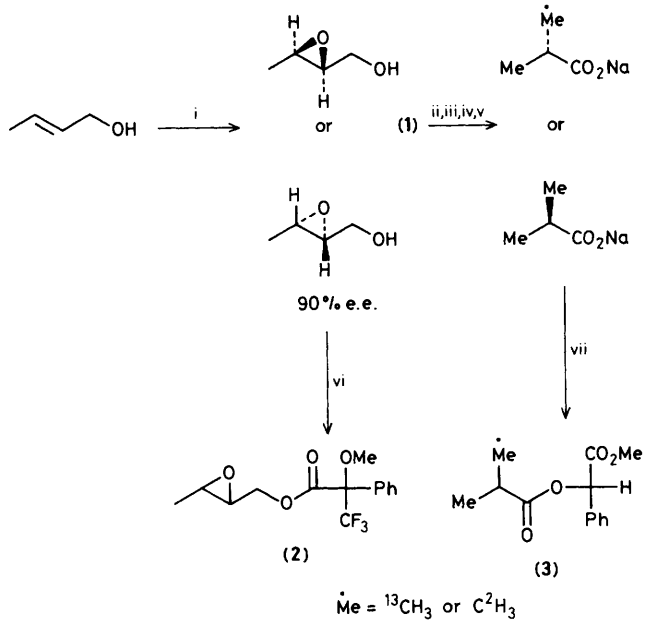
[a] Spectra were recorded at 90.5 MHz in $CDCl_3$, 64 K data points, 7 s relaxation delay, 90° pulse, exponential multiplication with 0.5 Hz line broadening. The enrichment is the factor by which the height of a signal in the $^{13}C\{^1H\}$ spectrum of the enriched monensin-A Na^+ salt is increased over the height at natural abundance.



Scheme 1

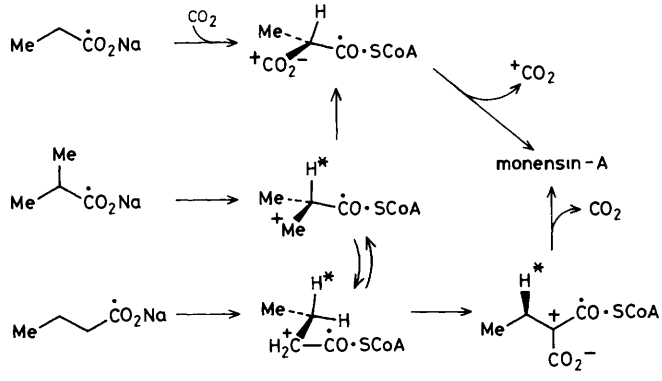
confirm the earlier findings,[1] and show also that [3,3'-$^{13}C_2$]-isobutyrate enriches both C(16) and C(33) (an intact unit: $J_{1,3}$ 1.83 Hz), centres that are formally derived from C(2) and C(4) of n-butyrate (see Scheme 1), as well as enriching each of the methyl groups formally arising from propionate.

Although the oxidation of isobutyryl-CoA to (*S*)-β-hydroxyisobutyryl-CoA is well known,[4] no other precedent exists for the conversion of isobutyryl-CoA into n-butyryl-CoA. In order to study stereochemical aspects of isobutyrate metabolism in *S. cinnamonensis*, (*S*)-[3-^{13}C]-, (*S*)-[3-$^2H_3$]-, and (*R*)-[3-$^2H_3$]-isobutyrates were synthesized by the route shown in Scheme 2, which is a modification[5] of the method developed by Aberhart.[6] The optical purities of the epoxy-alcohols (**1**) were assayed by 360 MHz ^{1}H n.m.r. spectroscopy after conversion into the Mosher derivatives[7] (**2**). The configurational purity of each labelled isobutyric acid was monitored by ^{1}H and either ^{13}C or ^{2}H n.m.r. spectroscopy after conversion into the mandelate derivatives (**3**), (90%



Me = $^{13}CH_3$ or C^2H_3

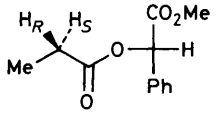
Scheme 2. *Reagents:* i, (+)- or (−)-di-isopropyl tartrate, Bu^tOOH, $Ti(OPr^i)_4$; ii, Bu^tMe_2SiCl; iii, Me_2CuLi; iv, F^-; v, $NaIO_4$, $KMnO_4$; vi, (−)-(MeO)(CF_3)CPhCOCl, *N,N*-dimethylaminopyridine, CH_2Cl_2; vii, (+)-methyl mandelate, dicyclohexylcarbodi-imide, Et_2O, *N,N*-dimethylaminopyridine.



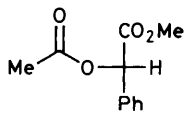
Scheme 3

enantiomer excess ≡ enantiomer ratio 95:5), and these materials were subsequently used in feeding experiments to cultures of *S. cinnamonensis*.

The incorporation of (*S*)-[3-^{13}C]isobutyrate led specifically to a single enrichment in monensin-A at C(16), the position formally derived from C(2) of n-butyrate. No other significant incorporations into propionate-derived units were detected by ^{13}C n.m.r. spectroscopy. Taken with the labelling experiments described earlier, this result indicates that the carboxy carbon of isobutyrate migrates to the *pro*-(*S*) methyl centre, to afford an n-butyrate moiety specifically enriched at C(2). Moreover, the non-appearance of carbon-13 label in the propionate units is consistent with the conversion of (*S*)-[3-^{13}C]isobutyrate into (*S*)-methylmalonyl-CoA by oxidation of the *pro*-(*S*) methyl group[4] (see Scheme 3). These conclusions were supported by the results from two additional feeding experiments using (*S*)- and (*R*)-[3-$^2H_3$][1-^{14}C]isobutyrates. The specific incorporation of ^{14}C activity in each labelled monensin-A was 8.64 and 8.34% respectively, but in the ^{2}H n.m.r. spectrum of each, run under identical conditions, only monensin-A biosynthesized



(4)



(5)

from the (*R*) isomer showed strongly enriched signals† at δ 1.5, 1.25, and 0.9, which can be assigned unambiguously to overlapping signals from all of the C-methyl resonances.

Finally, in two separate experiments [α-^{3}H,1-^{14}C]- (^{3}H/^{14}C ratio 0.84) and [α-^{2}H]-isobutyrates were administered to cultures of *S. cinnamonensis*. The first experiment afforded monensin-A with a ^{3}H/^{14}C ratio of 1.31, and the second experiment gave monensin-A whose ^{2}H n.m.r. spectrum showed only a single broad absorption at δ 1.6, which is consistent[8] with the location of deuterium label at C(32). To confirm this, the monensin-A (2.0 g) was degraded[9] by Kuhn–Roth oxidation, and the acetic and propionic acids formed were collected by steam distillation and converted into the (+)-mandelate ester derivatives (**4**) and (**5**), which were then cleanly separated by chromatography. The (+)-mandelate ester of the propionate (**4**) was characterized by ^{1}H and ^{2}H n.m.r. spectroscopy under conditions where the diastereotopic proton resonances at C(2) are clearly resolved.[10] The ^{2}H n.m.r. spectrum at 55.3 MHz in benzene revealed two strongly enriched signals at δ 2.45 and 2.32 in the ratio 85 : 15. The major signal is assigned[10] to the *pro*-(2*R*) hydrogen in propionate, and it follows that the bulk‡ of the ^{2}H enriched propionate has the (2*R*) absolute configuration.

Integrating these results reveals stereochemical details of two metabolic pathways utilizing isobutyrate, one leading to (*S*)-methylmalonyl-CoA, and the other affording an n-butyrate unit by a reaction (or reactions) not requiring the loss of the α-hydrogen of isobutyrate, but involving the migration of the carboxy carbon atom, presumably activated as a coenzyme-A thioester, to the *pro*-(*S*) methyl group, with its replacement by a hydrogen atom occurring with overall retention of configuration (see Scheme 3). These pathways presumably operate in all Streptomycetes and may also provide an important link between straight-chain fatty acid metabolism, and the branched-chain building blocks needed for antibiotic biosynthesis.[12] Carbon skeleton rearrangements are rare in intermediary metabolism and attempts to discover more details of this process, particularly at the enzymic level in cell-free systems, are currently underway.

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† The monensin-A derived from (*S*)-[3-^{2}H$_3$; 1-^{14}C]isobutyrate also showed very weakly enriched ^{2}H n.m.r. signals at δ 1.5, 1.25, and 0.9, which are almost certainly due to the presence of approximately 5% of the (*R*)-isotopomer in the synthetically derived (*S*)-isobutyrate.

‡ The (*S*)-(+)-methyl mandelate used for derivative preparation had $[\alpha]_D$ +118° (*c* 0.98, H_2O) (lit.,[11] $[\alpha]_D$ +134°) and for the derivative (**4**) $[\alpha]_D$ +91° (*c* 0.96, $CHCl_3$) (lit.[11] $[\alpha]_D^{20}$ 135.5°). The appearance of the minor ^{2}H n.m.r. signal at δ 2.32 is therefore accounted for by a small amount of epimerization that occurred at C(2) in mandelate during formation of the derivatives.

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