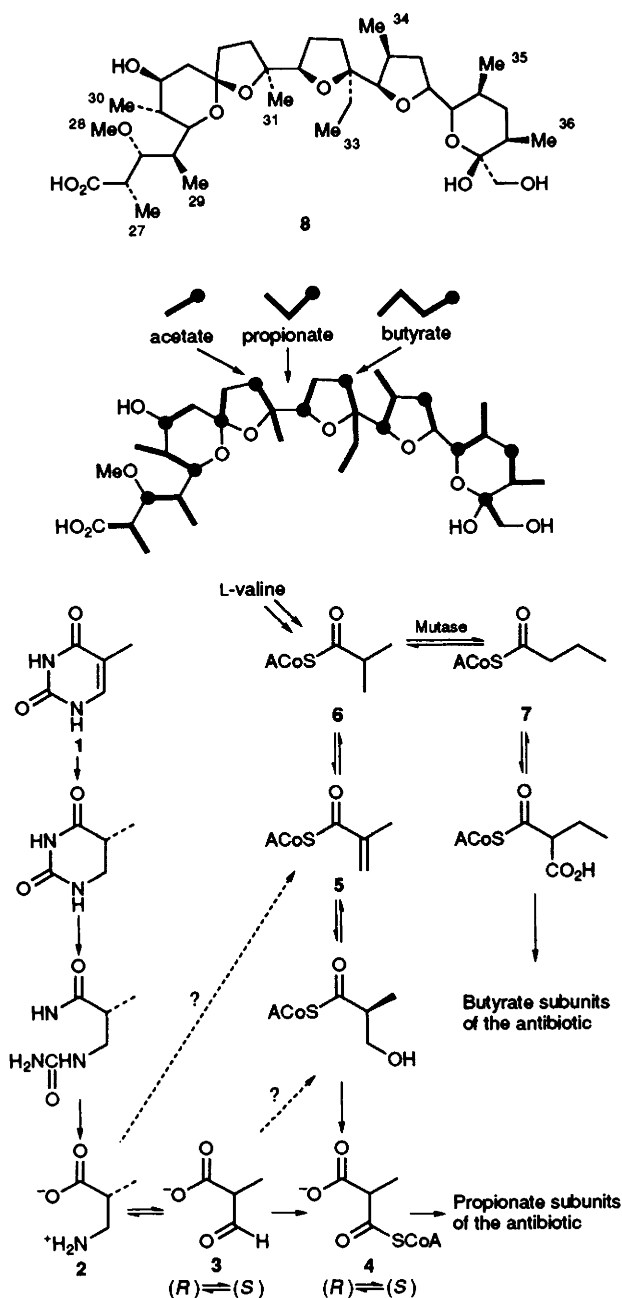
The Incorporation of Thymine and β-Aminoisobutyrate into the Polyether Antibiotic, Monensin-A

David O'Hagan,*[a] Sarah V. Rogers,[a] Kevin A. Reynolds[b] and Gordon R. Duffin[c]

[a] *Department of Chemistry, Science Laboratories, South Road, Durham, UK DH1 3LE*
[b] *Department of Biomedicinal Chemistry, School of Pharmacy, The University of Maryland at Baltimore, Baltimore, Maryland 21201, USA*
[c] *Department of Chemical Development, Sterling Winthrop Research Centre, Willowburn Avenue, Alnwick, Northumberland, UK NE66 2JH*

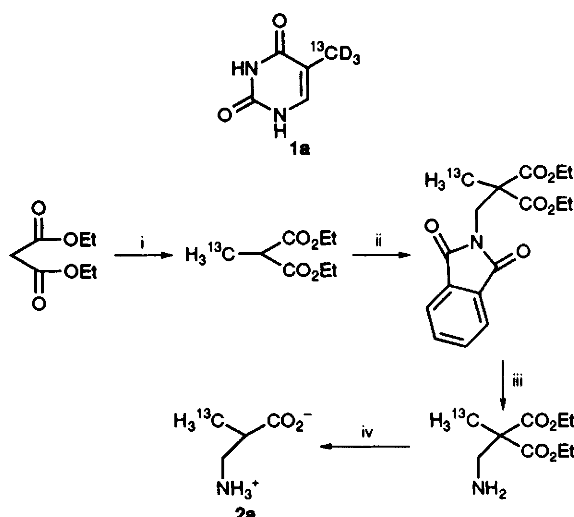
The isotopes from DL-[3-^{13}C]-β-aminoisobutyrate and [^{13}C^{2}H$_3$-*methyl*]thymine are efficiently incorporated into the seven propionate, and to a lesser extent the butyrate, derived methyl groups in monensin-A from *Streptomyces cinnamonensis*; catabolic pathways from thymine to methylmalonyl-CoA and isobutyryl-CoA are implicated in antibiotic producing Actinomycetes.

Catabolism of the DNA base thymine **1**, in mammalian systems generates (2R)-β-aminoisobutyrate **2**[1,2] *via* (5*R*)-dihydrothymine and β-ureidoisobutyric acid as illustrated in Scheme 1. (2*R*)-β-Aminoisobutyrate **2** is converted to methylmalonic acid presumably *via* methylmalonate semialdehyde **3**. With this background we were interested in assessing the metabolism of thymine in the monensin-A producer, *Streptomyces cinnamonensis* to determine if a similar metabolism can contribute to the methylmalonyl-CoA **4** pool, which is important for antibiotic biosynthesis. We were encouraged by a series of initial experiments with [^{14}C-*methyl*]thymine which indicated a 1–2% incorporation into monensin-A (data not shown). In this communication we report the regiospecific incorporations of DL-[3-^{13}C]β-aminoisobutyrate **2a** and [^{13}C, ^{2}H$_3$-*methyl*]thymine **1a** into monensin-A **8**. (2*S*)-Methylmalonyl-CoA is the direct precursor to the propionate derived subunits in the polyether and macrolide antibiotics[3] and therefore identification by ^{13}C NMR of the location of the isotopes in monensin-A, after feeding experiments employing the isotopically labelled compounds **1a** and **2a**, have allowed us to evaluate indirectly the regiospecific labelling into methylmalonyl-CoA.[4]



Scheme 1

DL-[3-^{13}C]-β-Aminoisobutyrate **2a** was synthesised by the route outlined in Scheme 2[5] and [^{13}C^{2}H$_3$-*methyl*]thymine **1a** was prepared by a modification of a previously reported procedure[6,7] for the preparation of radiolabelled thymine, where [^{13}C^{2}H$_3$]methyl iodide was used as the source of the isotopes.† In the event, stepwise supplementation (on days 3, 3.5 and 4) of a culture of *S. cinnamonensis* with DL-[3-^{13}C]-β-aminoisobutyrate **2a** corresponding to a final concentration of 5 mmol dm^{-3} in the medium, gave rise to monensin-A **8** (on day 8) which, by ^{13}C NMR analysis,‡ was highly enriched (10–20-fold) at C-27, C-29, C-30, C-31, C-34, C-35 and C-36, the seven propionate derived methyl groups. Interestingly there



Scheme 2 *Reagents and conditions*: i, ^{13}CH$_3$I, NaH, THF (93.5%); ii, *N*-(bromomethyl)phthalimide, NaH, THF (87%); iii, H$_2$NNH$_2$, MeOH, 20 °C, 12 h, (50.4%); iv, 10% HCl, reflux, 12 h, then neutralise to pH 7 with dil. NaOH (88.6%)

was a smaller, but significant (2.5-fold) incorporation into C-33, the methyl group derived from butyrate. In the second experiment, monensin-A was isolated on day 8 after incorporation of [$^{13}C^2H_3$-*methyl*]thymine, introduced in a single batch on day 3 to a final concentration of 5 mmol dm^{-3}, and was analysed by $^{13}C\{^1H,^2H\}$ NMR. Although the incorporations were lower (1–2-fold) overall, the analysis clearly demonstrated that the seven propionate and to a lesser extent (0.2-fold) the butyrate, derived methyl groups were labelled. Deuterium induced α-shifts were apparant at all eight methyl groups, and for the seven propionate derived methyl carbons there were CD_3, CHD_2 and CH_2D components averaging a ratio of 3:2:1. Interestingly, the C-33 methyl group appeared to consist predominantly of CD_3 indicating the absence of any significant exchange prior to incorporation into the butyrate subunit. It is possible that the interconversion of the methylmalonyl-CoA pool with succinyl-CoA, mediated by methylmalonyl-CoA mutase, is leading to deuterium loss at the propionate derived methyl groups.

It is widely appreciated[8,9] that the amino acids L-valine and L-isoleucine contribute to the methylmalonyl-CoA pool. L-Valine is an efficient precursor of isobutyryl-CoA **6** which is then converted to methylmalonyl-CoA **4** as shown in Scheme 1 (the thioester carbon of **6** becomes the thioester carbon of **4**).[10] Isobutyryl-CoA is also interconverted directly with butyryl-CoA **7** by the action of isobutyryl-CoA mutase.[11,12] This mutase links C_3 and C_4 metabolism in such antibiotic producing Actinomycetes. The enrichments observed from the labelled precursors into C-33, the butyrate derived methyl group indicate that β-aminoisobutyrate **2** is metabolised to isobutyryl-CoA, prior to rearrangement to butyryl-CoA **7**. This may happen directly by the action of a deaminase on β-aminoisobutyrate followed by activation to the coenzyme-A ester of methacrylate, although such a deaminase has not been described. The observation that deuterium from [$^{13}C^2H_3$-*methyl*]thymine is less efficiently washed out in the butyrate unit, compared with the propionate units, is consistent with this scenario, as the pathways would diverge at methacrylyl-CoA **5** with exchange occurring at the interconversion of methylmalonyl-CoA and succinyl-CoA as discussed above. Alternatively, the results are also consistent with a reductive pathway from methylmalonate semialdehyde **3** to isobutyryl-CoA **6** as illustrated in Scheme 1. It is noteworthy that β-aminoisobutyrate has been identified[13] as a metabolite of L-valine in mammals. This is consistent with the present observations with either of the proposed pathways operating in reverse.

The importance of thymine relative to amino acids and citric acid cycle intermediates as a nutrient source for antibiotic production remains to be evaluated. It is generally appreciated[14] however that most secondary metabolites, generated in batch culture, are produced during the second growth period—the trophophase—after cell growth and nucleic acid synthesis have ceased. Indeed for the production of methylenomycin from *S. coelicolor*[15] the maximum concentration of the antibiotic is reached at the point DNA synthesis ceases and for the production of candicidin from *S. griseus*[16] it has been shown that the total DNA concentration, which has steadily increased during cell growth—the idiophase—diminishes during the tropophase and presumably contributes to the nutrient source for antibiotic production.

We thank Dr John Parkinson of the University of Edinburgh for high field NMR spectra, Dr John A. Robinson, University of Zurich, for providing a strain of *S. cinnamonensis*, the SERC for a studentship (S. V. R.) and NATO for additional financial support.

Received, 8th April 1994; Com. 4/02109F

Footnotes

† *Selected analytical data* for **1a** and **2a**, **1a** δ_H $(CD_3)_2SO$ 7.21 (1H, d, $^3J_{13C-H}$ 3.6 Hz); δ_C 11.1 (septet, J_{13C-2H} 19.8 Hz), (In the [$^{13}C^2H_3$-*methyl*]thymine the unlabelled carbons were not observed), ν_{max} (KBr)/cm^{-1} 3160, 3045, 2814, 1734, 1673; *m/z* (EI) 130 (99.28%); (Found: M^+ 130.0663. $C_4{}^{13}CH_3D_3O_2N_2$ requires M^+ 130.0651). **2a**, δ_H (D_2O) 3.03 (2H, m, CH_2), 2.77 (1H, m, CH), 1.14 (3H, dd, $^{13}CH_3$, *J* 6.6 Hz, $^1J_{13C-H}$ 129 Hz); δ_C 180.3 (C), 44.0 (CH_2), 39.8 (d, CH, $^1J_{13C-13C}$ 32.7 Hz), 16.7 (CH_3).

‡ The ^{13}C NMR of monensin-A were recorded as solutions in $CDCl_3$ at 150 MHz. The ^{13}C NMR spectrum of monensin-A has been unambiguously assigned previously. See A. A. Ajaz, J. A. Robinson and D. L. Turner, *J. Chem. Soc., Perkin Trans. 1*, 1982, 27; D. E. Cane, T. C. Liang and H. Hasler, *J. Am. Chem. Soc.*, 1982, **104**, 7274.

References

1 D. Gani, P. B. Hitchcock and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1363.
2 O. W. Griffith, *Ann. Rev. Biochem.*, 1986, **55**, 855.
3 G. R. Sood, D. M. Ashworth, A. A. Ajaz and J. A. Robinson, *J. Chem. Soc. Perkin Trans. 1.*, 1988, 3183.
4 D. E. Cane, T. C. Liang and H. Hasler, *J. Am. Chem. Soc.*, 1982, **104**, 7274.
5 H. Bohme, R. Broese and F. Eiden, *Chem. Ber.*, 1959, **92**, 1258.
6 C.-Y. Shiue, A. P. Wolf and D. N. Slatkin, *J. Labelled Cpds. Radiopharm.*, 1980, **17**, 177.
7 L. Pichat, J. Deschamps, B. Masse and P. Dufay, *Bull. Soc. Chim. Fr.*, 1971, **6**, 2110.
8 S. Omura, K. Tsuzuki, Y. Tanaka, H. Sakakibara, M. Aizawa and G. Lukacs, *J. Antibiotics*, 1983, **36**, 614.
9 D. O'Hagan, *The Polyketide Metabolites*, pp. 122–123, Ellis Horwood, Chichester, 1991.
10 D. Gani, D. O'Hagan, K. A. Reynolds and J. A. Robinson, *J. Chem. Soc. Chem. Commun.*, 1985, 1002.
11 K. A. Reynolds, D. O'Hagan, D. Gani and J. A. Robinson, *J. Chem. Soc. Perkin Trans. 1*, 1988, 3194.
12 G. Brendelberger, J. Retey, D. M. Ashworth, K. A. Reynolds, F. Willenbrock and J. A. Robinson, *Angew Chem. Int. Ed. Engl.*, 1988, **27**, 1089.
13 R. Floyd, P. Kupiecki and M. J. Coon, *J. Biol. Chem.*, 1957, **229**, 743.
14 J. F. Martin and A. L. Demain, *Microbiol. Revs.*, 1980, **44**, 230.
15 G. Hobbs, A. I. C. Obanye, J. Petty, J. C. Mason, E. Barratt, D. C. J. Gardner, F. Flett, C. P. Smith, P. Broda and S. G. Oliver, *J. Bacteriol.*, 1992, **174**, 1487.
16 J. F. Martin and L. E. McDaniel, *Biotechnol. Bioeng.*, 1975, **17**, 92.