Biosynthesis of the Polyether Antibiotic Monensin-A. Incorporation of [2-$^2H_2$]-, (*R*)-[2-$^2H_1$]- and (*S*)-[2-$^2H_1$]-Propionate

Gulshan R. Sood, John A. Robinson,* and Abid A. Ajaz

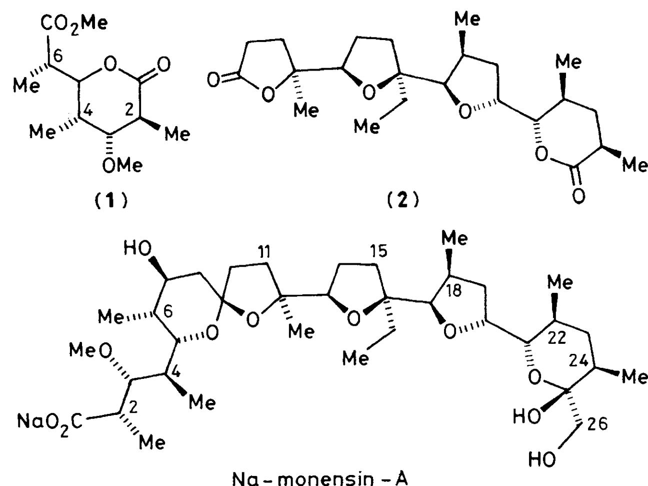
Chemistry Department, The University, Southampton SO9 5NH, U.K.

The incorporation of [2-$^2H_2$]- and (*S*)-[2-$^2H_1$]-propionate into monensin-A in cultures of *Streptomyces cinnamonensis* occurs with retention of label only at C(4) and C(6) in the antibiotic, whereas during the incorporation of (*R*)-[2-$^2H_1$]propionate the deuterium label is lost to the medium.

Carbon-13 and oxygen-18 labelling experiments have shown clearly that the carbon backbone of the polyether ionophore antibiotic monensin-A is biosynthesized from building blocks derived from acetate, propionate, and butyrate.[1] The mechanisms of activation of these precursors *in vivo* as their coenzyme-A thiol esters, and their mode of coupling, appear to resemble the processes known to occur during the biosynthesis of long-chain fatty acids. We now report the results of incorporation experiments using deuteriated isotopomers of propionic acid, which strengthen the mechanistic and stereochemical aspects of this analogy by defining the stereochemical courses of the carboxylation and decarboxylative-condensation reactions that occur during the insertion of propionyl-CoA into the carbon backbone of the antibiotic.

The aim of our experiments was to follow the incorporation of deuterium label from C(2) of propionate into the corresponding methine positions in monensin-A, *i.e.* C(2), C(4), C(6), C(18), C(22), and C(24). Initial studies using (*RS*)-[2-^3H;^{14}C]propionate ($^3H/^{14}C$ ~18.0) afforded, after addition to cultures of *Streptomyces cinnamonensis*, [^3H] and [^{14}C] labelled monensin-A of which the $^3H/^{14}C$ ratio (~0.6) indicated that a large proportion (*ca.* 95—98%) of the tritium label had been lost to the medium during the biosynthesis. In a control experiment [1-^{13}C]propionate, after incorporation, showed enhanced singlets (*ca.* ×10 enrichment) in the ^{13}C n.m.r. spectrum of the antibiotic at each of the expected[1] positions, *i.e.* C(1), C(3), C(5), C(11), C(17), C(21), and C(23).

To establish the location in monensin-A of the small amounts of deuterium label retained after incorporation of [2-$^2H_2$]propionate, ^2H n.m.r. spectroscopy was employed. Thus [2-$^2H_2$]propionate (30 g) was administered batchwise to a fermentation of *S. cinnamonensis* (6 litres), which was subsequently worked up in the usual way[2] to afford pure crystalline sodium monensin-A (3 g; m.p. 270 °C). The 55 MHz ^2H n.m.r. spectrum of this material showed an increased background level of deuterium in the antibiotic, but more importantly, also showed a strongly enhanced broad singlet at δ 2.0, a result which is consistent with the retention of deuterium label specifically at one or more methine positions. An analysis of the two-dimensional ^1H–^{13}C correlation n.m.r. spectrum[3] of unlabelled monensin indicated that the deuterium enrichment was not at C(2), although owing to the



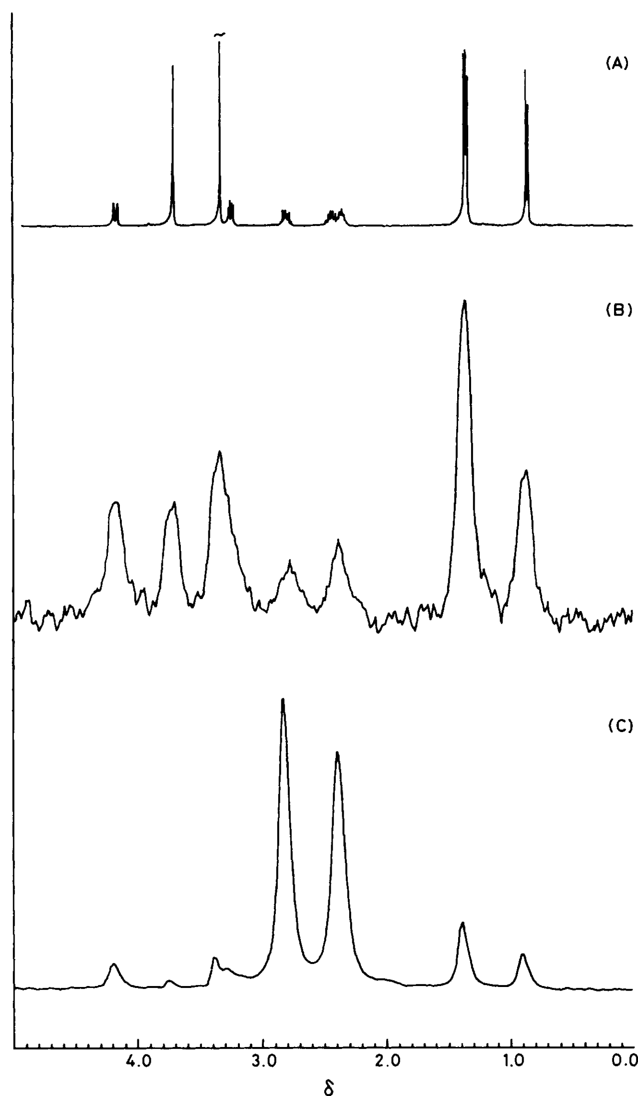


Figure 1. (A) ^1H N.m.r. spectrum of the lactone methyl ester (**1**) at 360 MHz in $CDCl_3$; (B) natural abundance ^2H n.m.r. spectrum of (**1**) at 55.3 MHz, 300 mg dissolved in 2 ml of $CHCl_3$, 2K data points, acquisition time 0.655 s, 1.0 Hz line broadening with exponential multiplication, with broad band proton decoupling; (C) ^2H n.m.r. spectrum of (**1**) derived from deuterium-enriched monensin biosynthesized from [2-$^2H_2$]propionate run under the same conditions as in (B).

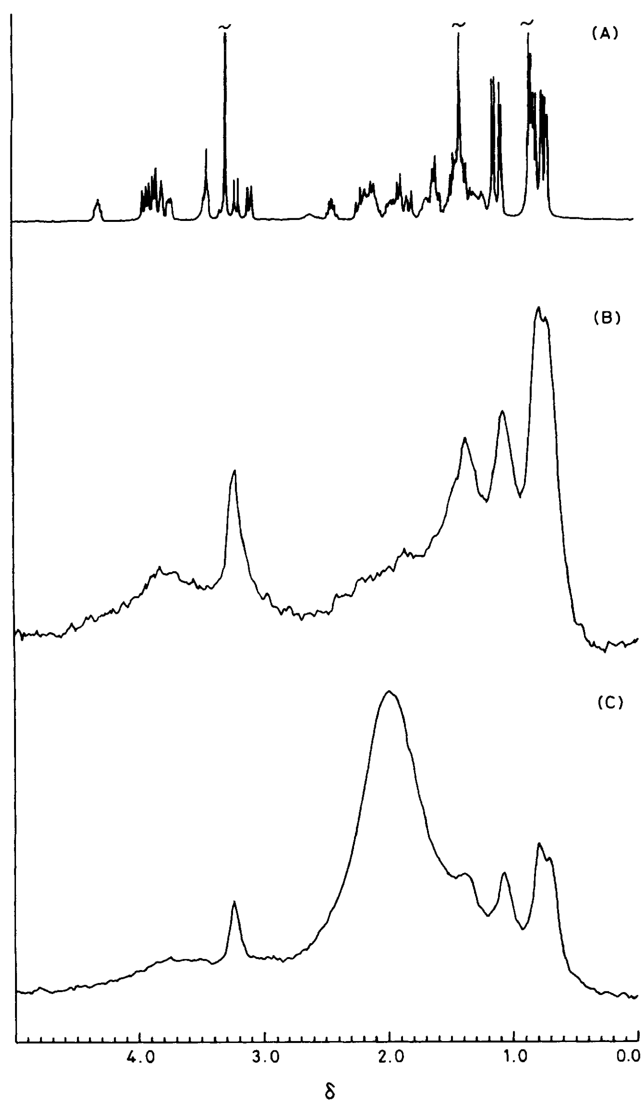
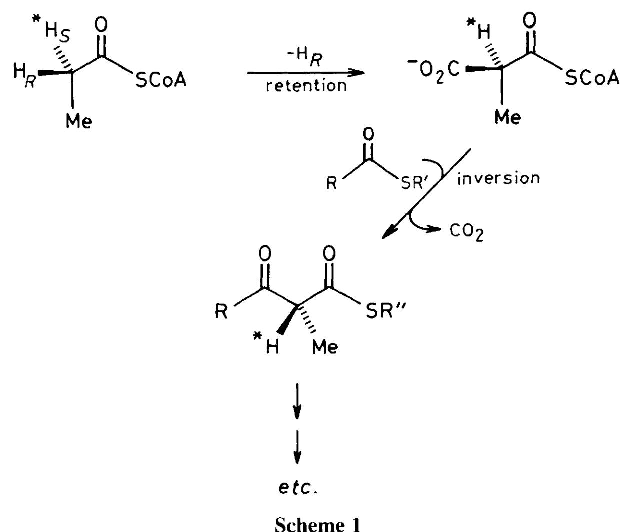


Figure 2. (A) ^{1}H N.m.r. spectrum of Na-monensin-A at 360 MHz in $CDCl_3$; (B) natural abundance ^{2}H n.m.r. spectrum of Na-monensin-A at 55.3 MHz, 1 g dissolved in 2 ml of $CHCl_3$. For acquisition parameters see Figure 1; (C) ^{2}H n.m.r. spectrum of Na-monensin-A biosynthesized from (*S*)-[1-^{14}C; 2-$^2H_1$]propionate run under the same conditions as in (B).

broadness of the peak in the ^{2}H spectrum a differentiation between C(4), C(6), C(18), C(22), and C(24) was not possible. This labelled monensin was, therefore, degraded[4] (two steps: i, CrO_3–AcOH; ii, CH_2N_2) to afford the known[4,5] lactone methyl ester (**1**) and dilactone (**2**), each of which was purified by flash chromatography on silica. The ^{2}H n.m.r. spectrum of the lactone (**1**) produced in this way, together with the natural abundance ^{2}H n.m.r. spectrum of (**1**), are shown in Figure 1. Two strongly enhanced signals at δ 2.40 and 2.85 are evident in the former spectrum and these can be assigned *via* the ^{1}H n.m.r. spectrum to specific enrichments (30—50 fold) at C(4) and C(6) in monensin. On the other hand, the ^{2}H n.m.r. spectrum of the dilactone (**2**) was virtually identical to the natural abundance spectrum. It follows, therefore, that no significant retention of deuterium at C(18), C(22), or C(24) in monensin had occurred.

Finally, in two additional experiments (*S*)- and (*R*)-[1-^{14}C; 2-$^2H_1$]propionate† were administered to separate fermentations of *S. cinnamonensis* (10 g into 5 litres). In each case the derived monensin-A Na^+ salt contained ^{14}C label corresponding to a specific incorporation of *ca.* 16%, but in the ^{2}H n.m.r. spectrum of monensin from the (*S*)-isomer an enhanced peak at δ 2.0 was again evident (shown in Figure 2), whereas from the (*R*)-isomer the ^{2}H n.m.r. spectrum showed no incorporation and was identical to that of unlabelled monensin-A.



Scheme 1

It follows from this that propionyl-CoA is converted *in vivo* into methylmalonyl-CoA with loss of the *pro*-H_R atom. This result parallels that observed using purified enzymes from other sources,[8] and indicates further that the carboxylation product is (*S*)-methylmalonyl-CoA.[9] The retention of deuterium label at C(4) and C(6) in monensin is then only consistent with a concerted decarboxylative-condensation proceeding with inversion of configuration,‡ and leading to an α-methyl-β-ketothiol ester with the (*R*) configuration at C_α (see Scheme 1). A similar mechanistic and stereochemical result has been observed during studies of the decarboxylative-condensation catalysed by fatty acid synthetases,[10] and complements related findings on the biosynthesis of the polyether antibiotic lasalocid-A.[11] The non-appearance of deuterium label at C(2), C(18), C(22), and C(24) is consistent in the case of C(2) and C(24) with the action of epimerases acting before or after the key carbon–carbon bond forming step, and additionally in the case of C(18) and C(22) with the α,β-elimination of H_2O from transiently generated α-methyl-β-hydroxythiol esters. It is relevant to note, however, that at present no clear evidence exists for the action in polyether antibiotic biosynthesis of synthetases that can directly utilize (*R*)-methylmalonyl-CoA, and this suggests that only the (*S*)-isomer may be employed for the insertion of all propionate units into the backbone, with alterations to the configuration

† The (*S*)- and (*R*)-[2-$^2H_1$]propionates were prepared using the methods described by Armarego *et al.*,[6] and were assayed for configurational purity by the method described by Parker:[7] each isomer was >95% optically pure.

‡ The reasonable assumption is made that the interconversion of (*S*)- and (*R*)-methylmalonyl-CoA on the *S. cinnamonensis* methylmalonyl-CoA epimerase would effectively lead to the complete loss of deuterium from C(2). However this remains to be proven. The unlikely possibility that (*S*)- and (*R*)-methylmalonyl-CoA can be interconverted through hydrolysis followed by re-thioesterification is rendered untenable by the high retentions of ^{18}O label at C(1), C(3), and C(5) upon incorporation of [1-^{13}C;$^{18}O_2$]propionate.[1]

of chiral methine centres, where necessary, occurring after carbon–carbon bond formation.

Grateful acknowledgement is made to Dr. David Gani for valuable discussions, to Eli Lilly Ltd. for gifts of monensin-A and *S. cinnamonensis,* and to the S.E.R.C. for financial support.

Received, 27th July 1984; Com. 1101

References

1 D. E. Cane, T. C. Liang, and H. Hasler, *J. Am. Chem. Soc.*, 1982, **104**, 7274.
2 M. E. Haney and M. M. Hoehn, *Antimicrob. Agents Chemother.*, 1967, 349.
3 The ^{13}C n.m.r. spectrum of Na-monensin-A has been assigned unambiguously: J. A. Robinson and D. L. Turner, *J. Chem. Soc., Chem. Commun.*, 1982, 148; and ref. 1.
4 For details see: L. E. Day, J. W. Chamberlin, E. Z. Gordee, S. Chen, M. Gorman, R. L. Hamill, T. Ness, R. E. Weeks, and R. Stroshane, *Antimicrob. Agents Chemother.*, 1973, **4**, 410.
5 A. Agtarap and J. W. Chamberlin, *Antimicrob. Agents Chemother.*, 1967, 359; D. B. Collum, J. H. McDonald, and W. C. Still, *J. Am. Chem. Soc.*, 1980, **102**, 2117.
6 W. L. F. Armarego, B. A. Milloy, and W. Pendergast, *J. Chem. Soc., Perkin Trans. 1*, 1976, 2229.
7 D. Parker, *J. Chem. Soc., Perkin Trans. 2*, 1983, 83.
8 For propionyl-CoA carboxylase see: D. Arigoni, F. Lynen, and J. Retey, *Helv. Chim. Acta.*, 1966, **49**, 311; J. Retey and F. Lynen, *Biochem. Z.*, 1965, **342**, 256; D. J. Prescott and J. L. Rabinowitz, *J. Biol. Chem.*, 1968, **243**, 1551; for transcarboxylase see: Y. F. Cheung, C. H. Fung, and C. Walsh, *Biochemistry*, 1975, **14**, 2981.
9 See also A. R. Hunaiti and P. E. Kolattukady, *Arch. Biochem. Biophys.*, 1982, **216**, 362.
10 B. Sedgwick and J. W. Cornforth, *Eur. J. Biochem.*, 1977, **75**, 465; B. Sedgwick, J. W. Cornforth, S. J. French, R. T. Gray, E. Kelstrup, and P. Willadsen, *Eur. J. Biochem.*, 1977, **75**, 481; B. Sedgwick, C. Morris, and S. J. French, *J. Chem. Soc., Chem. Commun.*, 1978, 193; K. I. Arnstadt, G. Schindbeck, and F. Lynen, *Eur. J. Biochem.*, 1975, **55**, 561.
11 C. R. Hutchinson, M. M. Sherman, A. G. McInnes, J. A. Walter, and J. C. Vederas, *J. Amer. Chem. Soc.*, 1981, **103**, 5956; C. R. Hutchinson, *Acc. Chem. Res.*, 1983, **16**, 7.