Oxytetracycline Biosynthesis: Mode of Incorporation of [1-^{13}C,$^{2}H_3$] Acetate

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Deuterium labelling of oxytetracycline derived biosynthetically from [1–^{13}C,$^{2}H_3$] acetate has been shown to occur exclusively at carbons 7 and 9.

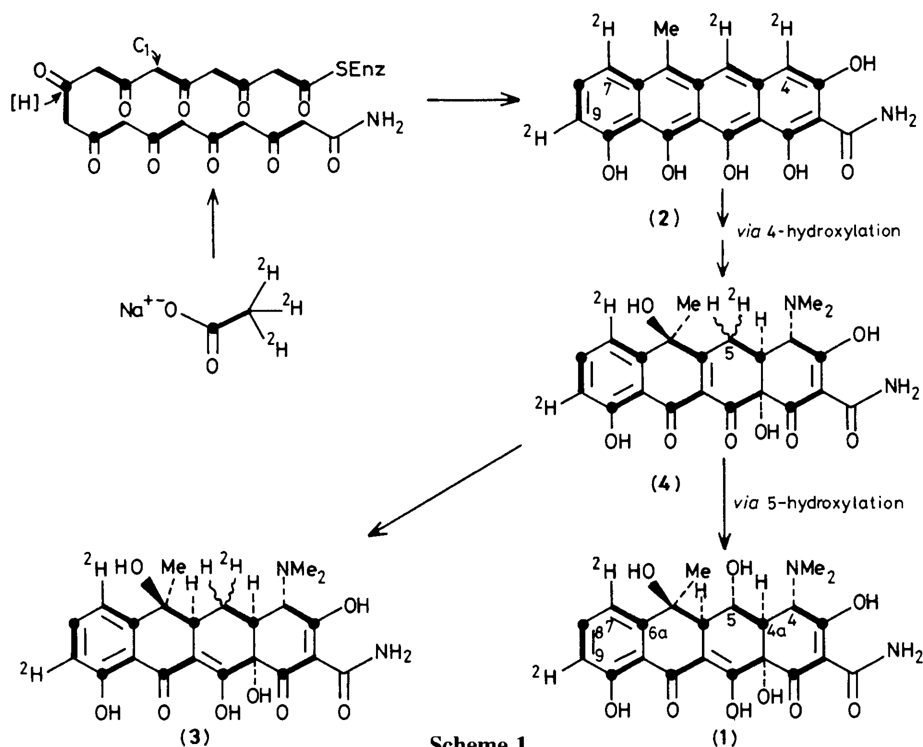
Recent ^{13}C n.m.r. studies of the labelling of oxytetracycline (**1**) derived from ^{13}C-acetate[1] and ^{13}C-malonate[2] have established the biosynthetic origin of the carbon skeleton including the carboxamide substituent, and in the present communication we report an investigation of the regiospecificity of incorporation of deuterium-labelled acetate.

The characteristic ^{13}C n.m.r. β-^{2}H isotope shift exhibited by ^{13}C nuclei carrying β-^{2}H substituents, as in [1-^{13}C,$^{2}H_3$]acetate, permits the determination of the sites of incorporation of deuterium in polyketides derived from this isotopically labelled precursor.[3,4]

Sodium [1-^{13}C,$^{2}H_3$]acetate was administered by pulsed feeding to growing cultures of *Streptomyces rimosus* as previously described for the incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]acetate,[1] and the resulting labelled oxytetracycline recovered as its crystalline hydrochloride. In the proton noise decoupled ^{13}C n.m.r. spectrum (Figure 1), the presence of ^{2}H at C-7 was apparent from the upfield signal due to a β-^{2}H isotope shift (−0.068 p.p.m.), which accompanied the characteristic resonance of C-6a (148.9 p.p.m.). The only other ^{2}H-label was detected at C-9 which gave rise to a corresponding upfield signal (−0.098 p.p.m.) relative to the C-8 resonance (136.5 p.p.m.). These data are consistent with the expected labelling pattern,[1] given the polyketide nature of (**1**).

The intensities† of the two β-^{2}H shifted signals relative to the corresponding non-isotopically shifted C-6a and C-8 resonances, were respectively 7% and 11%. Allowing for the intrinsic quantitative limitations of the present data this

† β-^{2}H Signal intensity is expressed as an isotopic labelling ratio, calculated by dividing the integral of the β-^{2}H shifted signal by the sum of the integrals of this and the non-isotopically shifted signal, after correcting for the natural abundance ^{13}C contribution: this is equivalent to an earlier analogous expression, ref. 5.



Scheme 1

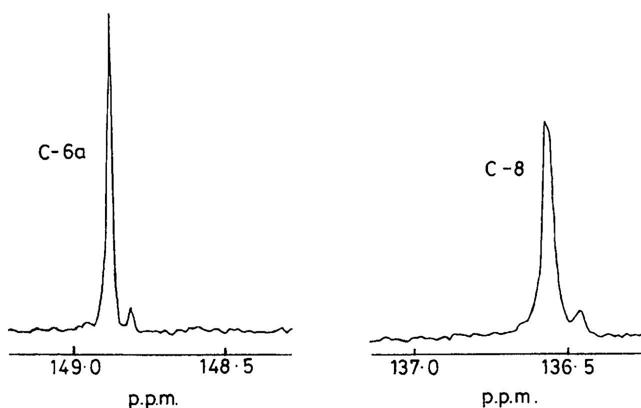


Figure 1. Expansion of proton noise decoupled ^{13}C n.m.r. spectrum of [1-^{13}C,$^{2}H_3$]acetate-derived oxytetracycline (**1**) determined at 90.56 MHz in $(CD_3)_2SO$, for the C-6a and C-8 regions showing β-^{2}H isotope shift.

indicates marginally lower ^{2}H retention at C-7 than at C-9. It is of interest to compare these results with the recently reported preferential ^{2}H-retention at olefinic centres of fungal polyketides similarly formed by dehydration of a reduced carbonyl group, *e.g.* C-3 of 6-methylsalicylic acid.[6] This contrasts with C-4 of alternariol[6] and C-7 of the aflatoxin-precursor averufin[4] where in both metabolites these respective carbons are flanked by enolic hydroxyls and hence susceptible to proton exchange.

Substantial evidence for the structure of a number of advanced intermediates involved in the conversion of 6-methylpretetramid (**2**) to (**1**) has been provided by the elegant mutant-based studies of McCormick *et al.*[7] Oxytetracycline biosynthesis is generally considered to diverge from that of its congener tetracycline (**3**) following dehydrotetracycline (**4**) formation (Scheme 1).[8] The conversion of (**4**) into (**1**) involves an oxidative step leading to the introduction of a C-5 α-hydroxyl substituent. Stereospecific hydroxylation with either retention or inversion of configuration, would necessitate the selective elimination of either the α- or the β-hydrogen atoms at this prochiral centre in (**4**), only one of which is likely to be derived from 5-H of 6-methylpretetramid (**2**) and hence acetate.

Assuming incorporation of ^{2}H-acetate at C-5 of (**4**) with comparable efficiency to that observed at carbons 7 and 9 of (**1**), it follows from the absence of a detectable β-^{2}H isotope shift at C-4a (42.2 p.p.m.) that only one of the diastereotopic hydrogen atoms at C-5 of the oxytetracycline precursor (**4**) is probably acetate-derived and that this is stereospecifically eliminated on insertion of the C-5 α-hydroxyl substituent of (**1**). Determination of the geometry of ^{2}H-acetate incorporation at C-5 of tetracycline (**3**) or 7-chlorotetracycline would consequently allow the characterisation of the stereochemistry of the C-5 hydroxylation step in oxytetracycline biosynthesis.

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