Oxytetracycline Biosynthesis: Mode of Incorporation of [1-^{13}C,$^{18}O_2$]Acetate

Robert Thomas* and David J. Williams

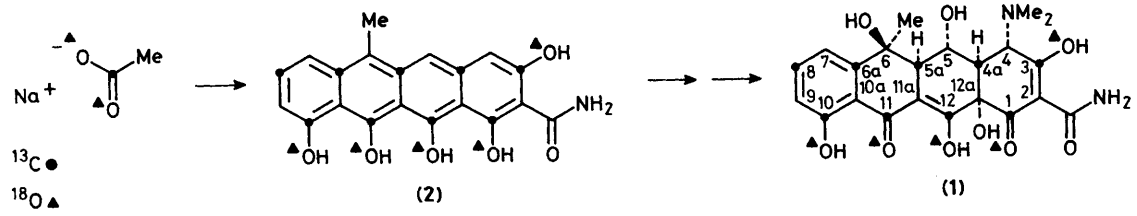
Faculty of Science, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

The regiospecific locations of [$^{18}O_2$]acetate-derived oxygen substituents of oxytetracycline (**1**) have been determined using the ^{13}C n.m.r. isotope shift technique and shown to correspond exclusively to those oxygen-bearing carbons which originate biosynthetically from the carboxy group of acetate.

N.m.r.-based studies of the incorporation of [1,2,3-$^{13}C_3$]-malonate[1] have recently established the exclusive malonate origin of the carbon skeleton of both the tetracyclic nucleus and the carboxamide substituent of oxytetracycline (**1**). The present communication describes the selective derivation of the oxygen substituents at carbons 1, 3, 10, 11, and 12 from acetate as shown in Scheme 1.

Following incorporation of [1-^{13}C,$^{18}O_2$]acetate (isotopic purity ^{13}C 90%, ^{18}O 99%) into (**1**) by *Streptomyces rimosus* under conditions described previously,[2] the sites of ^{18}O enrichment were determined by ^{13}C n.m.r. spectroscopy, based on the characteristic isotopic shifts[3] observed at ^{18}O-substituted ^{13}C atoms (Table 1).

The proton noise-decoupled ^{13}C n.m.r. spectrum of (**1**)



Scheme 1

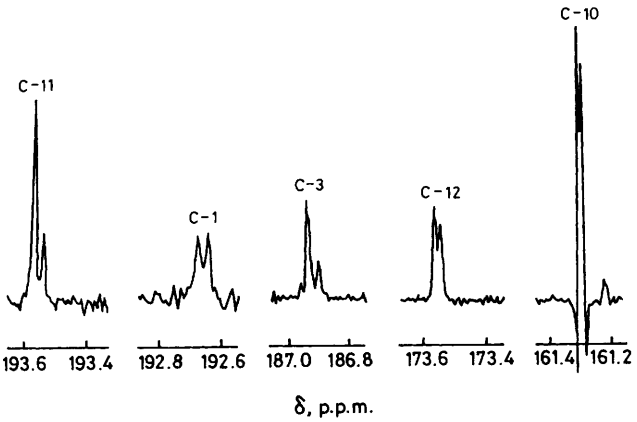


Figure 1. Expanded proton noise-decoupled ^{13}C n.m.r. spectrum of [1-^{13}C,$^{18}O_2$]acetate-derived oxytetracycline (**1**) showing ^{18}O-isotopically shifted resonances. Chemical shifts determined at 90.56 MHz relative to the midline of $(CD_3)_2SO$.

obtained at a probe temperature of 35 °C showed poor resolution of the C-1 signal; however, at an elevated temperature (80 °C), the presence of the corresponding ^{18}O-shifted satellite was readily apparent (Figure 1).

A small variation was evident in the degree of ^{18}O-retention at carbons 1, 3, 10, 11, and 12. Similar ratios of the intensities of ^{13}C signals bearing ^{18}O and ^{16}O substituents were observed for C-3, -10, and -11 (*ca.* 35 : 65) in contrast to C-1, where the corresponding ratio approximated to 57 : 43 with an intermediate value for C-12 (50 : 50).

In his early study of oxytetracycline biosynthesis, Gatenbeck[4] reported [1-^{14}C]acetate-derived labelling of the carboxamide substituent with 50% of the efficiency of the corresponding labelled carbons of the carbocyclic nucleus. He accounted for this value by suggesting an oxidative conversion of both acetate carbons into CO_2 prior to incorporation into the carboxamide. This proposal is supported by our recent report of appreciable ^{13}C-labelling of the carboxamide moiety of [1-^{13}C]acetate-derived oxytetracycline.[2,5] Using [1,2-$^{13}C_2$]-acetate we have subsequently shown that the corresponding incorporation of label is approximately doubled (2.04 : 1.00), consistent with comparable conversion efficiences for both acetate carbons.

The absence of detectable ^{18}O-labelling of the carboxamide moiety, despite significant incorporation of ^{13}C-label from [1-^{13}C,$^{18}O_2$]acetate, would be consistent with its conversion *via* acetyl coenzyme A and successive passes through the Krebs cycle to $^{13}C^{16/18}O_2$ with subsequent loss of ^{18}O by exchange with $H_2{}^{16}O$.

The remaining oxygen substituents at C-5, -6, and -12a appear to require oxidative introduction subsequent to the formation of 6-methylpretetramid (**2**) and it is proposed to investigate the specific labelling at these centres using $^{18}O_2$ and $H_2{}^{18}O$.

The authors acknowledge the award (to D.J.W.) of an S.E.R.C. (CASE) studentship and the helpful co-operation of Pfizer Limited, Sandwich, U.K. We are also particularly grateful to Dr. T. J. Simpson, Department of Chemistry, University of Edinburgh, for ^{13}C n.m.r. spectral analysis.

Received, 31st December 1984; Com. 1798

Table 1. ^{13}C N.m.r. data for ^{18}O-isotopically shifted resonances observed in oxytetracycline (**1**) derived from [1-^{13}C,$^{18}O_2$]acetate.

Carbon	Isotopic shift (Δ, p.p.m.)	Isotopic ratio[a] ^{16}O : ^{18}O
11	0.032	70 : 30
1	0.034[b]	43 : 57
3	0.033	65 : 35
12	0.017	50 : 50
10	0.010	65 : 35

[a] Isotopic ratios estimated by comparison of the relative intensities of ^{13}C–^{16}O to ^{13}C–^{18}O signals after correcting the former signal intensity for its natural abundance ^{13}C contribution. [b] ^{13}C–^{18}O Shift for carbon-1 resolved at 80 °C; data for all other carbons obtained at 35 °C.

References

1 R. Thomas and D. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1983, 677.
2 R. Thomas and D. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1983, 128.
3 J. C. Vederas, *Can. J. Chem.*, 1982, **60**, 1637; F. E. Scott, T. J. Simpson, L. A. Trimble, and J. C. Vederas, *J. Chem. Soc., Chem. Commun.*, 1984, 756.
4 S. Gatenbeck, *Biochem. Biophys. Res. Commun.*, 1961, **6**, 422.
5 D. J. Williams, Ph.D. Thesis, University of Surrey, 1983.