Oxytetracycline Biosynthesis: Mode of Incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]-Acetate

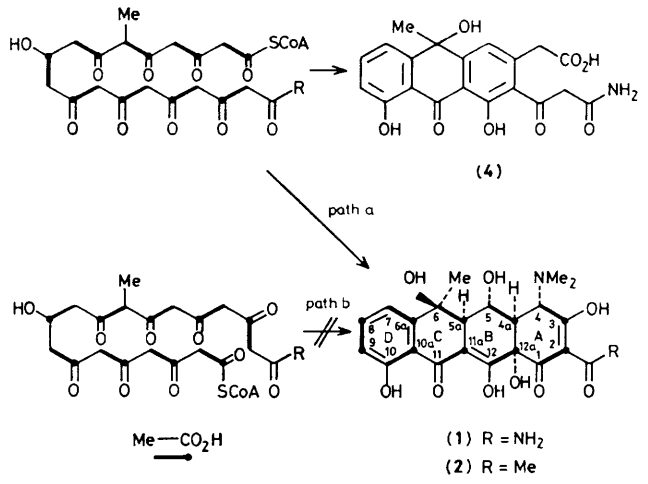
Robert Thomas* and David J. Williams

Biotechnology Unit and Chemistry Department, University of Surrey, Guildford, Surrey GU2 5SX, U.K.

Determination of the mode of incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate into oxytetracycline by *Streptomyces rimosus*, has established the exclusive polyketide origin of the tetracyclic nucleus and the direction of folding of a hypothetical linear intermediate.

Early investigations of the primary precursors of the carbon skeleton of the tetracycline antibiotics using ^{14}C-labelled acetate, methionine, glutamate, and bicarbonate,[1-4] indicated a predominant polyketide origin for the tetracyclic nucleus as originally proposed by Robinson.[5] However, the authors of a more recent labelling study,[6] based on the systematic degradation of [2-^{14}C]acetate-derived oxytetracycline (**1**), concluded that the mode of incorporation of acetate into rings A and B required further investigation. The present communication describes the labelling pattern in (**1**) derived from [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate as determined by ^{13}C n.m.r. spectroscopy. This unambiguously demonstrates the exclusive polyketide origin of the tetracyclic skeleton of (**1**) and, assuming a linear intermediate, its mode of folding; it also implies a corresponding pathway for the biosynthesis of the 2-acetyl-2-decarboxamido-analogue (**2**).

Following an autoradiographic investigation of the optimum conditions for [1-^{14}C]acetate incorporation into (**1**) by *Streptomyces rimosus* (Pfizer strain 17101) grown at 28 °C in shake culture on a glucose-based production medium (50 ml/250 ml flask), aliquots of [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate (typically 30 mg) were pulse fed following 24, 48, and 72 h

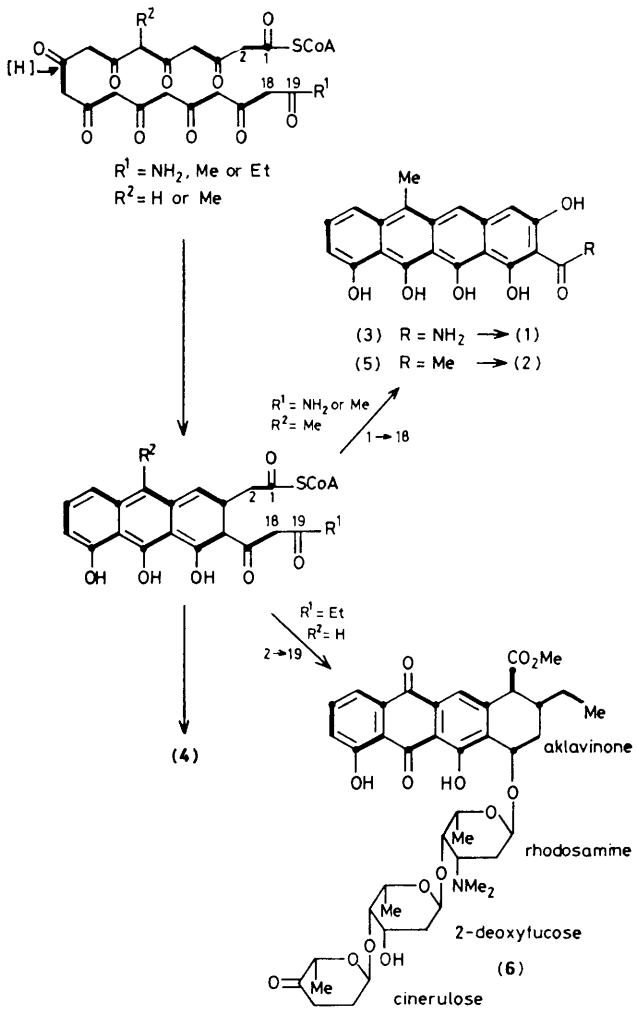


Scheme 1

Table 1. 90.52 MHz ^{13}C n.m.r. data for oxytetracycline (**1**), in $(CD_3)_2SO$, derived from [1-^{13}C]acetate.[a]

Carbon	δ, p.p.m.
Me	24.75
NMe_2	41.8
4a	42.4[b]
5a	49.9[b]
5	64.5
4	65.0
6	69.1
12a	72.7
2	95.6
11a	105.5
10a	114.6
9	114.9
7	117.1
8	136.6[b]
6a	148.9[b]
10	161.3[b]
$CONH_2$	172.2[b]
12	173.8[b]
3	187.3[c]
1	193.1[b]
11	193.8[b]

[a] ^{13}C Data (shifts relative to ext. Me_4Si) by courtesy of Dr. P. Regan, Shell Research Centre, Sittingbourne, Kent. [b] Denotes enrichment in excess of 100% relative to normal abundance (average enrichment *ca.* 400%). [c] Broadening of signal at C-3 precluded significant comparison of relative intensities.



Scheme 2. Possible biosynthetic interrelationship of tetracycline and anthracycline antibiotics.

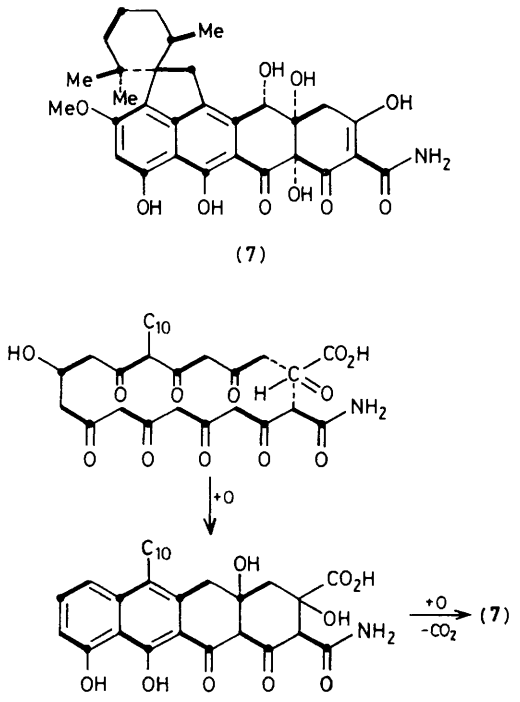
Table 2. 62.9 MHz ^{13}C n.m.r. data for oxytetracycline (**1**) derived from [1,2-$^{13}C_2$]acetate.[a]

Coupled carbons	J_{CC}/Hz
1, 2	60.5, 62.6
3, 4	41.5, 42.9
4a, 5	33.9, 34.2
5a, 6	38.1, 38.0
6a, 7	61.0, 58.6
8, 9	57.5, 56.1
10, 10a	63.2, 63.0
11, 11a	56.0, 56.2
12, 12a	52.4, 52.5
2, $CONH_2$	62.6, 64.6[b]

[a] ^{13}C Data including confirmation of direct couplings between carbons 1 and 2, 3 and 4, 4a and 5, and 2 and $CONH_2$ using homonuclear decoupling, by courtesy of Professor D. E. Cane, Brown University, Rhode Island. We are also grateful to Professor H. Seto, Institute of Applied Microbiology, University of Tokyo, for additional ^{13}C n.m.r. measurements. [b] Low intensity doublet consistent with indirect incorporation *via* $^{13}CO_2$.

growth. The cultures were harvested at 96 h and, after vacuum concentration of the filtrate from the acidified broth (pH 2) and precipitation of protein with $Na_4Fe(CN)_6$, the free base (**1**) was precipitated at pH 4.7 prior to conversion into the yellow crystalline hydrochloride (*ca.* 50 mg flask) by treatment with methanolic HCl. Based on previously assigned chemical shifts,[7] the proton noise decoupled ^{13}C n.m.r. spectrum of (**1**) derived from [1-^{13}C]acetate, showed enrichment of the nine alternate ring carbons (Table 1) required by the Robinson hypothesis. In addition, significant enrichment of the carboxamide signal was observed, consistent with the earlier observation by Gatenbeck of the corresponding incorporation of label from [1-^{14}C]acetate into this substituent.[3]

In the spectrum of the [1,2-$^{13}C_2$]acetate-derived hydrochloride of (**1**), all 18 ring carbons appeared as doublets (Table 2) with coupling constants corresponding to the incorporation of nine intact C_2-units. These dominant doublets were accompanied by lower intensity satellite peaks due to less frequent incorporation of adjacent doubly labelled acetate units. On the basis of the observed incorporation of $^{14}CO_2$ into the C-2 carboxamide substituent, Gatenbeck proposed that malonamyl CoA provides the polyketide primer



Scheme 3. Hypothetical pathway to viridicatumtoxin *via* glyoxylate and acetate.

unit.[3] This implies the cyclisation of a linear polyketide intermediate leading to the subsequently discovered 6-methylpretetramide (**3**), or to its hypothetical acetyl analogue (**5**) where the primer unit is of acetate origin. The observed coupling pattern would consequently require a folding mode as indicated in Scheme 1 (path a).

Subsequent steps in the conversion of (**3**) into (**1**) have been elucidated in some detail by the elegant mutant-based studies of McCormick and colleagues[8] which led *inter alia* to the detection of a methyl analogue of the tricyclic metabolite protetrone (**4**), the structure of which is also consistent with this mode of cyclisation.

The carbon skeleton of the related group of tetracene-based anthracycline antibiotics, *e.g.* aclacinomycin A (**6**) is similarly formed by cyclisation of a linear polyketide chain which has an initial propionate unit.[9] While in contrast with the oxytetracycline congener protetrone, no potential tricyclic anthracycline intermediate has been observed, the currently available biosynthetic data allow a possible common derivation of both groups of tetracyclic antibiotics by alternative cyclisations of a protetrone-like precursor (Scheme 2).

The interesting recent report[10] of the biosynthesis of the tetracycline-like fungal toxin viridicatumtoxin (**7**), unexpectedly established a different labelling pattern from [1,2-$^{13}C_2$]acetate to that which we have observed for (**1**). However, the retention of oxygen at C-4a from [1-$^{13}C^{18}O$]-acetate precludes a pathway involving a fully aromatic tetracene intermediate analogous to pretetramid. In addition, the derivation of C-2 and the carboxamide carbon of (**7**) from an intact acetate unit, together with the reported non-acetate origin of C-3, further distinguishes it biosynthetically from the streptomycete tetracycline antibiotics. Conceivably, C-3 of (**7**) may be derived from C-2 of glyoxylate which could be incorporated into the polyketide moiety *via* a condensing enzyme similar to malate synthase (EC.4.1.3.2), with subsequent oxidative decarboxylation (Scheme 3).

We acknowledge the award (to D. J. W.) of an S.R.C. (CASE) studentship and the helpful co-operation of Pfizer Central Research, Sandwich, U.K.

Received, 27th September 1982; Com. 1139

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