ANSAMYCIN BIOGENESIS: INCORPORATION OF [1-^{13}C]GLUCOSE AND [1-^{13}C]GLYCERATE INTO THE CHROMOPHORE OF RIFAMYCIN S

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1. Introduction

Incorporation studies with ^{13}C-acetates and ^{13}C-propionates led to the proposal of a biogenetic scheme for rifamycin S [1], in which a single polyketide chain is initiated by a seven carbon amino unit of unknown metabolic origin (fig. 1). Such a scheme appeared applicable to ansamycin biogenesis in general and suggested that all naphthalenic ansamycins could derive from a common progenitor. Its validity has now been demonstrated by the isolation of rifamycin W [2], an intermediate in rifamycin S biosynthesis, which structurally [3] resembles the streptovaricins and thus represents the missing link in naphthalenic ansamycin biogenesis. Further evidence has come from studies on the incorporation of [1-^{13}C]propionate into streptovaricin D [4] and geldanamycin [5].

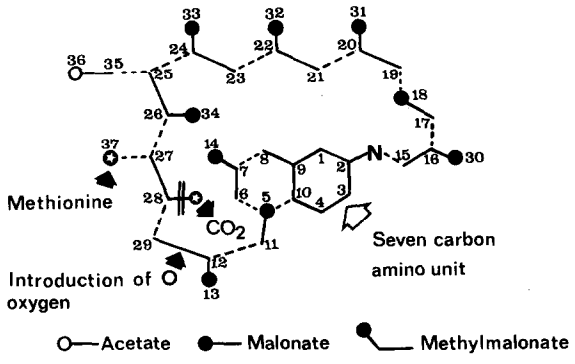

Fig. 1. Biogenetic scheme for rifamycin S (modified from ref. [6]).

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Studies on the origin of the seven carbon unit are of interest due to the unusual meta amino substitution of a benzoic acid and because of the lack of shikimate incorporation into this aromatic moiety [6]. We have reported [6] that the only precursors to specifically label the rifamycin chromophore are [3,4-^{14}C]glucose and [1-^{14}C]glycerate. In addition, the pattern of [1-^{13}C]glucose incorporation into the quinone ring and C-8 [6] recalled that obtained by Davis et al. [7] in shikimate with [1-^{14}C]glucose, suggesting an analogous origin for this moiety. However, the low level of incorporation and partial overlap of certain signals on the CMR spectrum precluded a definitive interpretation of these results.

2. Materials and methods

2.1. *Preparation and incorporation of ^{13}C enriched precursors*

Sodium [1-^{13}C]glycerate (90% enriched) was prepared from Na^{13}CN and glycolaldehyde by the method of Ashworth [8] and added to a fermentation, 48 hr after inoculation, to give a final concentration of 5 mg/ml. [1-^{13}C]glucose (15% enriched) was added to a fermentation at zero time to give a final concentration of 120 mg/ml. *Nocardia mediterranei* was cultured in a complex organic medium for a total of 168 hr and the [^{13}C]enriched rifamycin B formed was then extracted and transformed into rifamycin S as previously described [1]. ^{13}C enriched compounds were purchased from Merck Sharp and Dohme of Montreal, Canada.

2.2. *Recording of ^{13}C spectra*

Proton-decoupled Fourier transform carbon mag-

netic resonance (CMR) spectra were recorded at 25.2 MHz on a Varian XL-100 spectrometer in dimethyl sulphoxide-d_6 (DMSO-d_6) solution with dissolved tetramethylsilane (TMS) as reference and using internal deuterium lock. Spectra 2A and 2B: 46 mg samples of rifamycin S were dissolved in 0.5 ml of DMSO-d_6 in a 5 mm tube; 10 000 scans were accumulated with an acquisition time of 0.7 sec and processed by a Digilab 32K core computer. Spectra 2C and 2D: 46 mg samples of rifamycin S were dissolved in 2 ml of DMSO-d_6 in a 12 mm tube; 70 000 scans were accumulated with an acquisition time of 0.7 sec and processed with a Varian 620 L computer.

3. Results and discussion

We now report a considerably improved incorporation of [1-^{13}C]glucose into rifamycin S than obtained earlier [6] and, by recording the CMR spectrum in DMSO-d_6 instead of $CDCl_3$, have obtained a better separation of the signals concerned. Assignment of the resonances was performed using the techniques previously described [9]. Fig. 2 shows the low-field region CMR spectrum of rifamycin S at natural abundance (A) and of rifamycin S biosynthetically enriched with [1-^{13}C]glucose (B). Of the seven relevant carbons only two are enriched: C-1 and C-10; the relative enrichment factors for these carbons, calculated from the ratio of intensities on the enriched and natural abundance spectra, are 3.4 and 1.9, respectively. Further evidence on the metabolic origin of this unit has come from studying the incorporation of [1-^{13}C]-glycerate. The CMR spectrum of rifamycin S biosynthetically enriched with this precursor is shown in fig. 2D (since instrumental conditions were different for

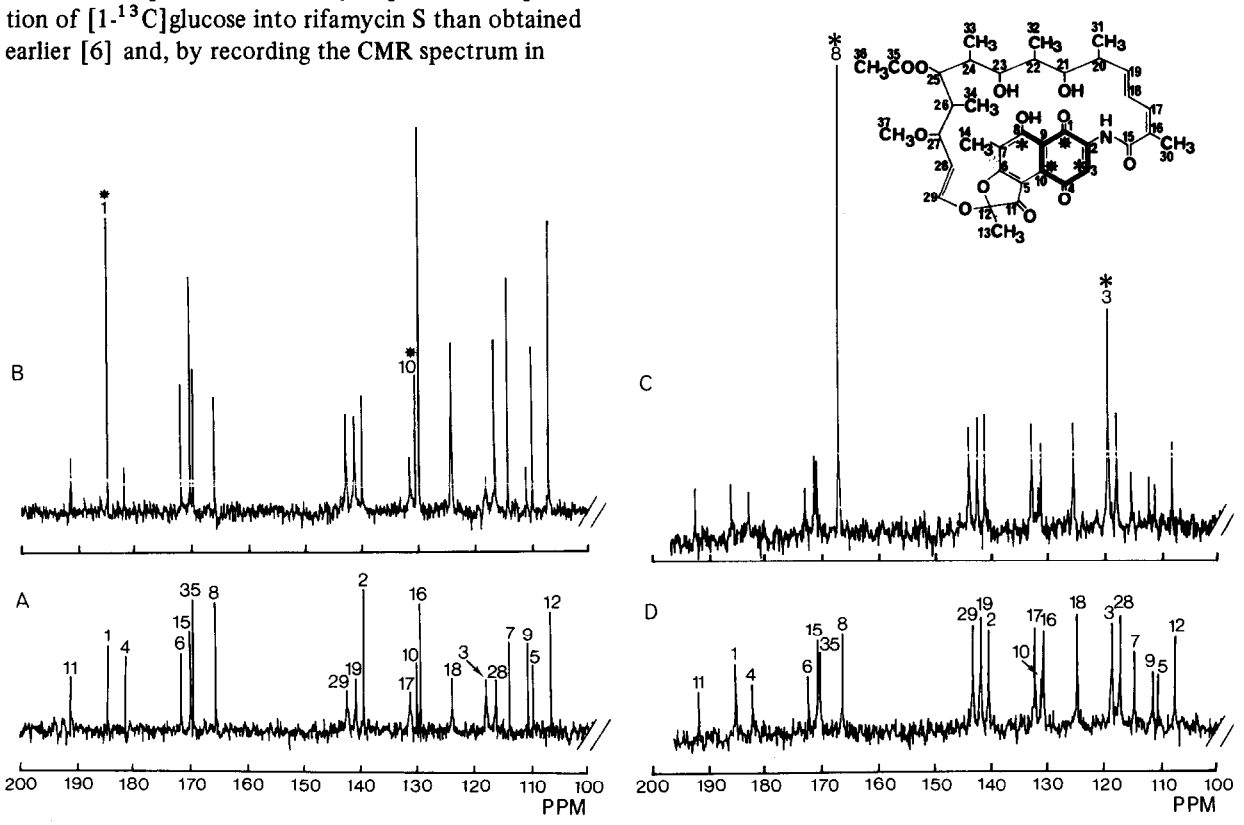

Fig. 2. Downfield region of the proton-decoupled FT CMR spectra of rifamycin S recorded at 25.2 MHz in DMSO-d_6. A and C, natural abundance; B, biosynthetically enriched with [1-^{13}C]glucose; D, biosynthetically enriched with [1-^{13}C]glycerate. Chemical shifts are in ppm from internal TMS. Enriched carbons on spectra B and D are numbered; NB. Only signals belonging to the seven carbon unit (heavy line on the formula of rifamycin S) have been considered.

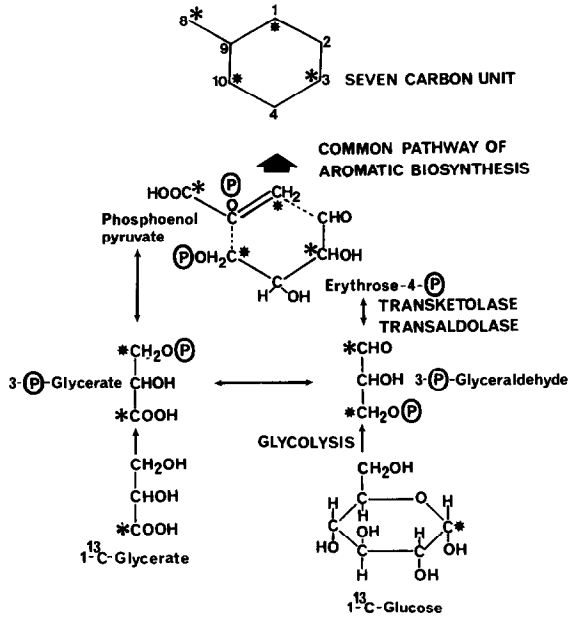

Fig. 3. Suggested metabolic scheme explaining the pattern of labelling obtained in the seven carbon unit with [1-^{13}C]-glycerate and [1-^{13}C]glucose as precursors.

the two enriched samples a second natural abundance spectrum, 2C, is included as control for 2D). Also in this case there are only two carbons enriched: C-3 and C-8, with relative enrichment factors of 2.0 and 4.4, respectively. We interpret these data as evidence for a shikimate-type origin of this moiety. However, experiments with ^{14}C-shikimate [6] have shown a very poor, non-specific incorporation of this precursor into rifamycin S. This apparent contradiction would be accounted for if the seven carbon unit derived from an intermediate of aromatic biosynthesis before the level of shikimate, i.e. 5-dehydroquinate or 5-dehydroshikimate. A metabolic scheme explaining the observed pattern of labelling is proposed in fig. 3. In analogy with the established incorporation of [1-^{14}C]-glucose into shikimate [7], the higher enrichment at C-1 of rifamycin S with [1-^{13}C]glucose is taken to indicate that it derives from the methylene carbon of phosphoenol pyruvate rather than C-4 of tetrose phosphate. Similarly, the higher enrichment at C-8 with [1-^{13}C]glycerate suggests that it derives from the carboxyl of phosphoenol pyruvate. This establishes the orientation of the seven carbon unit placing the double

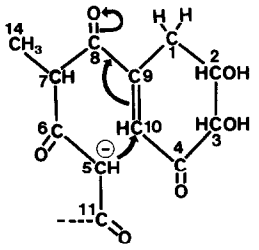

Fig. 4. Proposed mechanism of ring closure to give the naphthalenic chromophore.

bond of 5-dehydroshikimate and shikimate between C-9 and C-10. This in turn would favour ring closure between C-5 and C-10 by Michael addition giving rise to the naphthoquinone chromophore in a manner analogous to the menaquinones [10] (see fig. 4), with the C-8 carboxyl having initiated polyketide chain growth. Classical studies on the origin of the carbon skeleton of shikimate [7] have shown that [3,4-^{14}C]-glucose labels C-3 and C-4 (equivalent to C-2 and C-3 of rifamycin) via the intermediate formation of [1,2-^{14}C]erythrose-4-phosphate. Assuming a shikimate type origin, the absence of label at C-3 of rifamycin indicates that no [3,4-^{13}C] glucose-6-phosphate was formed by reverse glycolysis from [1-^{13}C]glycerate. However, label from this precursor must arrive at 3-phosphoglyceraldehyde to form [2-^{13}C]erythrose-4-phosphate, thus selectively enriching C-3 of rifamycin. Similar seven carbon meta amino moieties are evident in the other ansamycins [2,3] and also in mitomycin C, and a common biosynthetic origin has been suggested [11]. Data on the biosynthetic origin of this unit in mitomycin C are insufficient to allow definite conclusions, but it is difficult to reconcile the reported [12] specific incorporation of [1-^{14}C]glucose into the methyl carbon on the benzoquinone ring with a shikimate-type origin, as this position should derive from C-3 and C-4 of the labelled hexose.

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