Biosynthesis of Simocyclinone D8 in an $^{18}O_2$-rich Atmosphere

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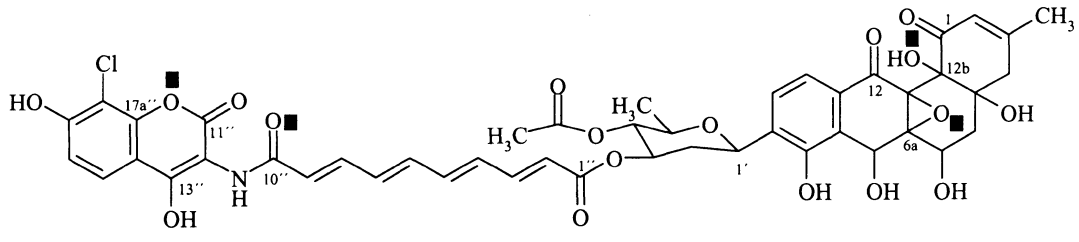
Simocyclinone D8 (**1**) is the main component of a novel class of antibiotics, which is produced by *Streptomyces antibioticus* (strain Tü 6040)[1]. **1** consists of angucyclinone, deoxysugar, octatetraene dicarboxylate and aminocoumarin structural elements (Figure 1)[2], which were biogenetically generated from different sources of primary metabolism using special pathways typical for the biosynthesis of microbial secondary metabolites[3]. Thus it has been shown that angucyclinone and the polyene dicarboxylic acid were built up from acetate/malonate being typical polyketides, while the precursor of the aminocoumarin moiety is tyrosine and of the *C*-glycoside is glucose as it has been shown earlier[2]. In addition to feeding experiments using ^{13}C-labelled precursors, we decided to perform a fermentation of strain Tü 6040 in an $^{18}O_2$-rich atmosphere in order to evaluate the origin of the oxygen atoms, which are incorporated by oxygenases during the biosynthesis of the different portions. In the case of the angucyclinones similar experiments have already been done[4], but in the case of the polyene dicarboxylic acid and aminocoumarin moieties these experiments should give further insight into the sequence of the biosynthetic steps.

Streptomyces antibioticus (strain Tü 6040) was cultivated in five 300 ml-Erlenmeyer flasks (with three baffles) each filled with 100 ml nutrient broth consisting of glycerol 2.5%, L-lysine 0.4%, NaCl 0.1%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, Amberlite® XAD-2 3.3% and 0.2 ml trace element concentrate (TEC) in deionised water (pH 7.0 prior to sterilisation). TEC (per liter): $FeSO_4 \cdot 7H_2O$ 1 g, $CuSO_4 \cdot 5H_2O$ 0.1 g, $MnSO_4 \cdot H_2O$ 0.1 g and $ZnSO_4 \cdot 7H_2O$ 0.1 g. The flasks were inoculated as described previously[2] and shaken on a rotary shaker (120 rpm) at 27°C. At the beginning of the production phase (16 hours) the flasks were flushed with nitrogen for five minutes and connected to an apparatus described previously[5]. $^{18}O_2$ (95.3% atom purity, 1 liter, Chemotrade) was fed to the culture over 20 hours. Gas circulation within the apparatus was stopped in order to minimize oxygen consumption. Isolation of simocyclinone D8 (**1**) followed the protocol given previously[2] yielding 6.8 mg of **1**.

The incorporation of ^{18}O into **1** was determined by analysis of the ^{13}C NMR spectrum (DMSO-d_6, 125.7 MHz). Signals of carbon atoms connected to oxygen originating from the $^{18}O_2$-atmosphere showed an α-isotopic shift (Table 1). In the angucyclinone portion a shift was observed for C-6a (33 ppb) and C-12b (17 ppb), respectively. The signal for C-12 was very weak and not analyzable, that for C-12a was not detectable. In the polyene dicarboxylate only the signal of C-10″ (34 ppb) showed an α-isotopic shift indicating that this carboxy group is built by oxidation of the final methyl group of the already ester bound decatetraenoic acid, as was expected from the ^{13}C labelling pattern of [^{13}C]-enriched malonate[2]. From the signals of the aminocoumarin those for C-11″ (27 ppb) and C-17a″ (23 ppb) exhibited the α-shift unambiguously. The signal for C-13″ was not detectable as described for **1** itself[2].

The ^{18}O-labelling of the aminocoumarin portion ends the discussion, which arose from molecular genetic and enzymatic investigations of the novobiocin and

Fig. 1. Structural formula of simocyclinone D8 (**1**) and proved labelling pattern of **1** by [$^{18}O_2$ ■].



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coumermycin A_1 biosynthesis. In a very early paper BUNTON *et al.*[6] reported that the heterocyclic oxygen atom of the aminocoumarin of novobiocin arises from the carboxyl group of tyrosine assuming a special oxidative cyclization. The analysis of the gene cluster of the aminocoumarin biosynthesis of novobiocin and coumermycin A_1 led to some doubt concerning this cyclisation step[7,8]. On the assumption that the results of the aminocoumarin portion in simocyclinone D8 (**1**) are applicable to similar portions of other antibiotics produced by streptomycetes, a cyclisation mechanism as proposed by WALSH *et al.*[8] seems to be most likely now. In this mechanism the 2,4-dihydroxy-β-keto-phenylalanine bound as thioester to a peptidyl carrier protein (PCP), cyclises by the attack of 2-OH on the thioester bond. The predicted flavoprotein monooxygenase for the hydroxylation step of the β-keto-tyrosyl intermediate has yet to be found (Scheme 1).

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Table 1. ^{13}C NMR signals of [^{18}O]-enriched **1** showing an α-isotopic shift.

C-Atom	δ_C (ppm)	$\Delta\delta$ (ppm)	^{16}O :^{18}O (%)*
C-6a	65.4	0.033	66.3 : 33.7
C-12b	75.1	0.017	63.4 :36.6
C-10''	165.7	0.034	72.5 : 27.5
C-11''	159.1	0.027	73.0 :27.0
C-17a''	145.9	0.023	57.5 :42.5

* ^{18}O enrichment by comparison of ^{13}C (^{16}O) : ^{13}C (^{18}O)

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Scheme 1. Proposed intermediates during the aminocoumarin biosynthesis.