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A CMR STUDY OF THE BIOSYNTHESIS OF CHLORAMPHENICOL[1]

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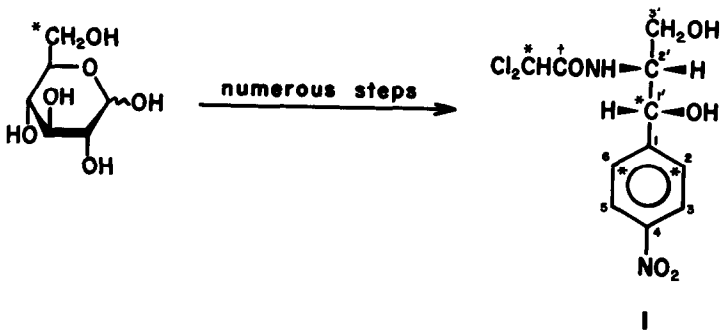
The biosynthesis of the medically important antibiotic chloramphenicol (I, Figure 1) has been a subject of considerable interest during the past 20 years. Early experiments[2] suggested the operation of a shikimate pathway[3] and this was supported by the low incorporation, with high dilution, of shikimate into chloramphenicol.[4] Our recent study[1b] based on a specific degradation scheme for chloramphenicol provided much support for the shikimate hypothesis, but the degradative scheme devised was not able to distinguish between C-1 and C-2/C-6 or between C-2' and C-3' (Figure 1). We report here a biosynthetic study of chloramphenicol employing [6-^{13}C]D-glucose which definitively locates all labeled carbons by carbon magnetic resonance (cmr) spectroscopy and more clearly implicates the shikimate pathway.



I

Figure 1. Labeling of chloramphenicol by [6-^{13}C]glucose: *heavily enriched (ca. 7X) carbons; †lightly enriched (ca. 2X) carbon.

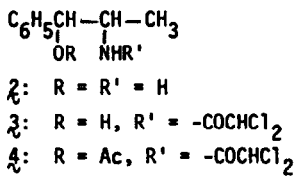
The natural abundance cmr spectrum of chloramphenicol diacetate (Table I) displays 13 resonances (C-2/C-6, and C-3/C-5 being degenerate due to the plane of symmetry in the molecule). From chemical shift considerations alone,[5] the resonances at 20.5, 20.6, 169.5 and 170.0 ppm could be assigned directly to the acetate methyl and carbonyl carbons, that at 164.0 ppm to the amide carbonyl, and the signal at 52.0 ppm to C-2'. The resonance at 62.3 ppm could be assigned from its unique multiplicity in the off-resonance spectrum (triplet) to C-3'. Specific proton decoupling at 6.35, 6.00, 8.20 and 7.60 ppm from TMS assigned the resonances at 66.3, 72.8, 123.5, and 127.9 to the $-CHCl_2$ group, C-1', C-3/C-5, and C-2/C-6, respectively.

Table I. Carbon Magnetic Resonance Data for Chloramphenicol and Its Acetate

	δ, ppm[a]					
	Chloramphenicol	Chloramphenicol diacetate	Δ[b]	Multiplicity[c]	Spd[d]	Enrichment[e,f]
C-1	151.3	144.8	-6.5	s	-	1.13
C-2	127.3	127.9	+0.6	d	7.60	7.50
C-3	122.9	123.5	+0.6	d	8.20	1.36
C-4	146.5	147.3	+0.8	s	-	1.01
C-5	122.9	123.5	+0.6	d	8.20	1.36
C-6	127.3	127.9	+0.6	d	7.60	7.50
C-1'	69.0	72.8	+3.8	d	6.00	6.61
C-2'	56.8	52.0	-4.8	d	-	1.30
C-3'	60.3	62.3	+2.0	t	-	1.22
-NH-C(=O)-	163.4	164.0	+0.6	s	-	2.17
$-CHCl_2$	66.5	66.3	-0.2	d	6.35	7.05
$-CH_3$	-	20.5, 20.6	-	s	-	-
-O-C(=O)-	-	169.5, 170.0	-	s,s	-	1.00, 1.00

[a]Ppm from TMS, determined using a Varian XLFT-100 spectrometer and Digilab computer, DMSO-d_6 (solvent) as internal lock, and TMS as internal standard. [b]Change in carbon chemical shift on acetylation. [c]Chloramphenicol diacetate, off-resonance decoupled, s=singlet, d=doublet, t=triplet. [d]Specific proton decoupling on chloramphenicol diacetate: resonance of proton decoupled, in ppm relative to TMS. [e]Calculated by taking the ratio of the intensity of a peak in the enriched spectrum relative to that in the natural abundance spectrum, with the acetate carbonyl carbons assigned the value 1.00. [f]Enrichments less than 1.50 are not considered significant.

Assignments for the remaining two resonances (those for C-1 and C-4) were based on shift data for the relevant resonances in 1-phenyl-2-dichloroacetamido-1-propanol (3, prepared from 1-phenyl-2-amino-1-propanol, 2)[6] and its acetate (4) as well as those in benzyl alcohol and benzyl acetate.[6] On acetylation, C-4 of benzyl alcohol moves downfield from 127.2 to 128.1 ppm



(Δ = +0.9), and C-4 of 3 moves downfield from 126.9 to 127.9 ppm (Δ = +1.0). On the other hand, C-1 of benzyl alcohol moves upfield on acetylation, from 140.8 to 136.2 ppm (Δ = -4.6), and C-1 of 3 moves upfield from 142.9 to 137.4 (Δ = -5.5). For chloramphenicol this is consistent only with assigning C-1 to the peak at 151.3 ppm and C-4 to that at 146.5 ppm (Table I); for chloramphenicol diacetate C-1 is at 144.8 ppm and C-4 at 147.3 ppm.

Streptomyces venezuelae was grown in twelve 500-ml Erlenmeyer flasks, each containing 100 ml of medium which was a modification of the glycerol-lactate type[7] in which glycerol was replaced by glucose. Only 84% of the required sugar, the unlabeled glucose, was added initially. At 27 hr, when the cultures were actively growing, a total of 2.0 g (the remaining 16% of the sugar) of [6-^{13}C]D-glucose (64 atom %)[8] was divided equally among the twelve flasks. These flasks were incubated for a total of 126 hr at 26° in a reciprocal shaker (94 strokes per min, 2.5-in strokes). Chloramphenicol was isolated along with other neutral compounds from the filtered medium by ethyl acetate extraction and purified by conversion to the diacetate, which was chromatographed on silica gel and crystallized.

The cmr spectrum of the labeled chloramphenicol diacetate (27 mg, Table I) revealed that C-2/C-6, C-1' and the -$CHCl_2$ carbons were about equally labeled (6-7 times natural abundance). The amide carbonyl was labeled to a lesser extent (2 times natural abundance). This pattern is entirely in accord with that expected for the shikimate pathway (Figure 2) and extends our

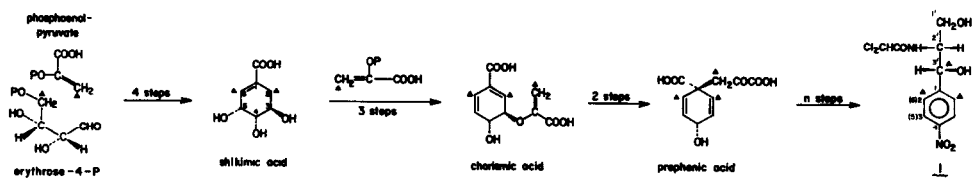


Figure 2. The shikimate pathway to chloramphenicol. Label (▲) from C-6 of glucose is converted in several steps[1b] to label at C-3 of phosphoenolpyruvate and at C-4 of erythrose-4-phosphate, and ultimately to C-2, C-6 and C-3' of chloramphenicol.

earlier work[1b] to locate the label from [6-^{13}C]glucose specifically at C-2/C-6 (rather than at C-1 or C-2/C-6, as established earlier) and to define the labeling pattern in the side chain and dichloroacetyl group. Labeling of the $CHCl_2$ carbon undoubtedly proceeds through [3-^{13}C]phosphoenolpyruvate, while the (lower) labeling of the amide carbonyl presumably arises *via* operation of the tricarboxylic acid cycle. Greater labeling of the dichloromethyl carbon *vs.* the amide carbon (7:1) has also been observed from [3-^{14}C]phenylalanine.[7]

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