BIOSYNTHETIC ORIGIN OF CARBONS 3 AND 4 OF LEUCOMYCIN AGLYCONE

Sir:

Leucomycin, a 16-membered macrolide antibiotic, consists of four structural units: aglycone, mycaminose, mycarose and an acyl side chain on mycarose which arise *via* different biosynthetic routes.[1] Biosynthetic studies using ^{13}C-labeled precursors by ŌMURA *et al.*[1,2] revealed that the aglycone is derived from five acetates, one propionate, one butyrate, and a C_2 unit corresponding to carbons 3 and 4 to which a hydroxyl and a methoxy group are attached respectively. However, the biosynthetic origin of the two carbons has remained unknown. Several attempts to examine possible candidates for the origin of the C_2 unit such as [1,2-$^{13}C_2$]oxalate, [2-$^{13}C_2$]malonate, [1-^{13}C]glycine, [1,4-$^{13}C_2$]succinate and [*methoxy*-D_3]acetate were not successful. RINEHART *et al.*[3] has reported that the two C_2 units involving methoxy and hydroxyl groups in geldanamycin, an ansamycin antibiotic, originate from glycolate and glycerate. In spite of the structural resemblance of the C_2 unit of geldanamycin with that of leucomycin, the origin of C_2 unit of leucomycin was not clarified by feeding experiments with these ^{13}C-labeled compounds.[4] Recent biosynthetic studies[5~7] of polyketide and isoprenoid metabolites utilizing uniformly ^{13}C-labeled glucose have shown that it is useful as an *in vivo* precursor of intact biosynthetic units. In this communication, we wish to report that uniformly ^{13}C-labeled glucose and [2-^{13}C]glycerol are incorporated into carbons 3 and 4 of the aglycone of leucomycin.

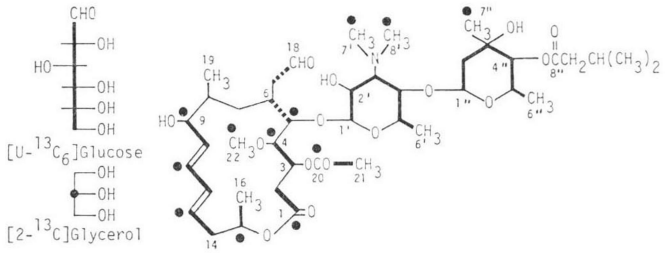
[*U*-$^{13}C_6$]glucose (90 atom%, MSD Canada, 200 mg) was administered at 24 hours to a growing culture (200 ml) of *Streptoverticillium kitasatoensis* KA-468 (68-69-1). After further 90 hours growth of the organism, the labeled antibiotic was extracted by the standard work-up.[2] Purification of the antibiotic by silica gel column chromatography followed by preparative thin-layer chromatography gave a pure sample of leucomycin A_3 (15 mg).

The 100.65 MHz ^{13}C NMR spectrum of leucomycin A_3 labeled with [*U*-$^{13}C_6$]glucose showed 21 triplet signals, as summarized in Table 1. This indicated that these carbon signals were enriched and coupled to one ^{13}C neighbor. The triplet signals corresponding to the carbons originating from six acetate units (C-1 and -2, C-9 and -10, C-11 and -12, C-13 and -14, C-15 and -16, C-20 and -21) exhibited the typical ^{13}C–^{13}C coupling pattern consisting of triplet-triplet. This is in accord with the results of a feeding experiment using doubly labeled acetate.[2] The appearance of the triplet at the signal due to C-4 suggested that this carbon atom was derived from a metabolite of glucose which retained an intact two- (or more) carbon unit. Carbon 4 at 84.9 ppm was not coupled to the signal at 71.6 ppm which had been assigned to C-3,[2] but to the signal at 69.0 ppm previously assigned to C-2′[2] with the ^{13}C–^{13}C coupling constant of 42 Hz. Therefore, the resonance assignments for C-3 and C-2′ should be reversed. In addition, the signals corresponding to four terminal carbons C-1′, -6′, -1″ and -6″ on mycaminose and mycarose moieties appeared each as a triplet, while the remaining carbons C-2′ to C-5′ and C-2″ to C-5″ appeared as multiplets, suggesting that an intact six-carbon unit from glucose was incorporated into each sugar moiety. The resonances for C-5, -6 and -18 exhibited very weakly enriched triplet signals. This observation could be rationalized by extremely low incorporation of two acetate units derived from glucose into C-5, -6, -17 and -18, which were previously suggested to originate from a butyrate unit.[2]

Additional evidence for the biosynthetic pathway to C-3 and C-4 was obtained by the feeding experiment with [2-^{13}C]glycerol (90 atom%, MSD Canada) performed in the same manner as mentioned above. As shown in Table 1, the ^{13}C enrichment pattern clearly showed that the central carbon of glycerol was incorporated into C-4 of the aglycone. The enrichment of carbons 1, 5, 9, 11, 13, 15 and 20 indicated that [1-^{13}C]acetate derived from [2-^{13}C]glycerol was incorporated in these positions. The ^{13}C enrichment of carbons 22, 7′, 8′ and 7″ arising from the methyl of methionine[1] implies that the carbon 2 of glycerol was converted to a C_1 unit.

From these results, we postulate that C-3 and C-4 of leucomycin are derived from glycerol *via* divergent pathways. Namely, glycerol is metabolized to glycerate to enter the glycolytic pathway. Glycerate is further oxidized in two ways: one to pyruvate and acetate, the other one to serine and glycine. The serine metabolism is

Table 1. Incorporation of labelled precursors into leucomycin A_3.



Carbon atom	Chemical shift[a]	[U-$^{13}C_6$]-[b] glucose multiplicity (J_{CC} Hz)[e]	[2-^{13}C]-[c] glycerol relative enrichment[d]	Carbon atom	Chemical shift[a]	[U-$^{13}C_6$]-[b] glucose multiplicity (J_{CC} Hz)[e]	[2-^{13}C]-[c] glycerol relative enrichment[d]
C- 1	169.9	t 60	2.07	C- 1′	103.7	t 39	1.00
C- 2	37.0	t 60	1.08	C- 2′	71.6	m	1.09
C- 3	69.0	t 42	0.95	C- 3′	69.0	m	0.95
C- 4	84.9	t 42	1.62	C- 4′	76.0	m	[g]
C- 5	77.5	t 38	1.42	C- 5′	72.9	m	[f]
C- 6	28.8	t 38	1.00	C- 6′	18.8	t 39	0.88
C- 7	30.4	s	0.85	C- 7′	41.9	s	1.48
C- 8	33.5	s	1.36	C- 8′			
C- 9	73.1	t 50	1.96				
C-10	127.6	t 50	1.03	C- 1″	97.0	t 39	0.94
C-11	135.7	t 57	1.98	C- 2″	41.9	m	[f]
C-12	132.1	t 57	1.08	C- 3″	69.3	m	0.83
C-13	132.6	t 43	2.00	C- 4″	77.1	m	[g]
C-14	40.9	t 43	[f]	C- 5″	63.5	m	1.77
C-15	68.8	t 40	1.77	C- 6″	17.8	t 39	0.93
C-16	20.3	t 40	1.04	C- 7″	25.5	s	1.66
C-17	42.4	[f]	[f]	C- 8″	172.9	s	0.90
C-18	201.2	t 39	1.01	C- 9″	43.3	s	[f]
C-19	14.7	s	1.08	C-10″	25.5	s	[f]
C-20	170.8	t 60	1.71	C-11″	22.4	s	1.05
C-21	21.3	t 60	1.01	C-12″			
C-22	62.4	s	1.46				

[a]; Chemical shifts[a] in ppm are downfield from Me_4Si in $CDCl_3$. Spectra were recorded on a Bruker WM 400 spectrometer [b] and on a JEOL FX-100 spectrometer [c].

[d]; I(enriched)/I(unenriched) from spectra run under essentially identical instrumental conditions. Intensity of each peak was normalized based on the intensity of the unenriched carbon (C-1′) as an internal standard.

[e]; s=singlet, t=triplet, m=multiplet.

[f]; These could not be analyzed due to overlap with other signals.

[g]; The resonances of these carbons overlapped with solvent.

again divergent: one to pyruvate, the other one to glycine and a C_1 unit. Glycine itself is not only a source of a C_1 unit but also a source of glycolate, possibly through glyoxylate.[8] Another possible pathway, hydroxypyruvate to glycolate or glyoxylate by decarboxylation, is less likely because it is inconsistent with the labeling pattern, if one assumes that C-3 and C-4 of leucomycin correspond to the carboxyl and hydroxymethylene of glycolate. Consequently, it is reasonable to assume that glycolate derived from glycerol is the direct precursor of C-3 and C-4 of the aglycone. The previous results[4] that [1-^{13}C]glycine and [1-^{13}C]glycolate did not in-

corporate to C-3 of the aglycone were probably due to the impermeability of these compounds to cell membrane.

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