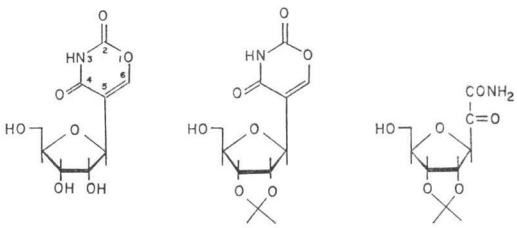
Communication to the editor

BIOSYNTHESIS OF THE C-NUCLEOSIDE, MINIMYCIN: ASYMMETRIC INCORPORATION OF GLUTAMATE AND ACETATE INTO THE OXAZINE RING

Sir:

One of the most intriguing of the C-nucleoside group of antibiotics is minimycin (**1**), which has a unique 1,3-oxazine-2,4-dione ring.[1,2] In our previous paper,[3] we proposed a C–7 sugar or C–8 branched sugar as a possible biosynthetic intermediate for both the ribose and oxazine moieties of **1**. This assumption is mainly based on the incorporation and distribution of ^{14}C in **1** from [1- or 3,4- or 6-^{14}C] glucose. Recently, we have reinvestigated the biosynthesis of this nucleoside and reached the conclusion that carbons 3, 4, and 5 of glutamate are incorporated asymmetrically into carbons 6, 5, and 4 of the oxazine ring of **1** (Scheme 1).



1 2 3

An *in vivo* feeding experiment was performed with growing *Streptomyces hygroscopicus* as described before.[3] Incorporation of [5-^{14}C; 3-^{3}H]glutamate into **1** is shown in Table 1. Both ^{14}C and ^{3}H were incorporated and the retention of ^{3}H was 23.4%. Acetonization of **1** gave the acetonide (**2**) with the same specific activity. Permanganate oxidation of **2** afforded 2′,3′-*O*-isopropylidene-β-D-ribofuranosylglyoxylamide[3,4] (**3**), which retained 85% of ^{14}C. However, ^{3}H was lost almost completely. Hydrolysis of **3** with 5 N HCl followed by decarboxylation with

Scheme 1. Proposed scheme for the biosynthesis of minimycin

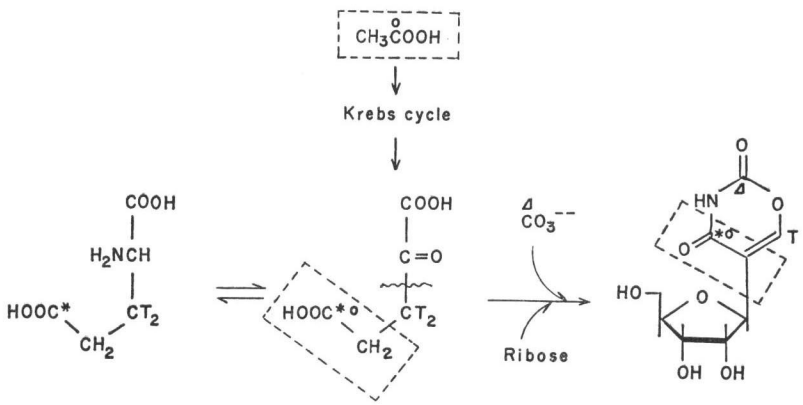


Table 1. Incorporation of labeled compounds into minimycin and its derivatives.

	DL[5-^{14}C;3-^{3}H]Glutamate			[U-^{14}C;3-^{3}H]Glutamate*			[1-^{14}C]Acetate
	Sp Act. (μCi/μmol)		$^{3}H/^{14}C$	Sp Act. (μCi/μmol)		$^{3}H/^{14}C$	Sp Act. (μCi/μmol)
	^{3}H	^{14}C		^{3}H	^{14}C		
Compound added	1.09	0.17	6.46	0.528	0.10	5.28	3.19×10^{-3}
Minimycin (**1**)	1.96×10^{-4}	1.30×10^{-4} (1300)**	1.51	1.25×10^{-4}	8.20×10^{-5} (1220)**	1.52	1.81×10^{-6} (1760)**
Acetonide (**2**)	2.06×10^{-4}	1.29×10^{-4}	1.60	1.39×10^{-4}	8.16×10^{-5}	1.71	1.50×10^{-6}
Glyoxylamide (**3**)	1.64×10^{-5}	1.09×10^{-4}	0.15	6.87×10^{-6}	4.04×10^{-5}	0.17	1.12×10^{-6}

* 25.2 μCi of L[U-^{14}C]glutamate and 266 μCi of DL[3-^{3}H]glutamate were mixed. The $^{3}H/^{14}C$ ratio was calculated on the basis of L-isomer.

** Dilution: Sp Act. of compound added/Sp Act. of **1** isolated.

Table 2. Distribution of ^{14}C on the amide carbon of compound **3** (carbon 4 of **1**)

Compound fed	% ^{14}C in CO_2 (C–4)*
DL[5-^{14}C;3-^{3}H]Glutamate	104.9
[U-^{14}C;3-^{3}H]Glutamate	62.1
[1-^{14}C]Acetate	103.3

* (Total disintegration per minute of CO_2 trapped in hyamine/Total disintegration per minute of **3** used) × 100.

ceric sulfate in 2 N H_2SO_4[4,5] resulted in quantitative recovery of ^{14}C as CO_2 (Table 2). This result clearly indicates that carbons 3, 4, and 5 of glutamate are the origins of carbons 6, 5, and 4 of the oxazine ring of **1** as illustrated in Scheme 1. Partial loss of ^{3}H from carbon 3 of **1** (theoretical ^{3}H retention is 50%) may be reasonable, since it would become labile in the equilibrium between enol and keto forms of α-ketoglutarate.

The [U-^{14}C; 3-^{3}H]glutamate experiment also supports this scheme. In this case, **3** retained 50% of ^{14}C (Table 1). On decarboxylation of **3**, 62% of ^{14}C was evolved as CO_2 (Table 2). The rest of ^{14}C should be located on carbon 5. ^{14}C from [1-^{14}C]acetate* was distributed predominantly on carbon 5 as in the case of the [5-^{14}C]glutamate experiment (Table 2).

Incorporation of an acetate unit into carbons 4 and 5 of **1** was further confirmed by the feeding of [1,2-^{13}C]acetate. ^{13}C-NMR analysis of **1** isolated has shown a satellite coupling (J_{cc} = 64.5 Hz) between carbon 4 (114.9 ppm)** and carbon 5 (163.9 ppm). This is an independent and unambiguous proof for the incorporation of an intact acetate unit.

The reported data[3] on incorporation and distribution of [1-^{14}C]glucose, [3,4-^{14}C]glucose and [1-^{14}C]ribose into **1** would not conflict with the present scheme. The low incorporation of ^{14}C into the oxazine ring from [6-^{14}C]glucose needs explanation. It may be that the hexose monophosphate oxidative pathway is predominant over the glycolytic pathway in this organism, resulting in almost exclusive incorporation of ^{14}C into C–5′ of the ribose moiety. In contrast, the contribution of the glycolytic pathway becomes significant in the [1-^{14}C]glucose experiment, because the operation of the hexose monophosphate oxidative pathway results only in the formation of unlabeled pentose phosphate. Thus, the present data together with the data reported earlier[3] support strongly the pathway for the biosynthesis of **1** as shown in Scheme 1.

It should be especially worth noting that all the C-nucleoside antibiotics whose biosyntheses have been studied, *i.e.*, showdomycin,[4] formycin,[6] and minimycin, have the common biosynthetic precursor, glutamate for their nucleobases.

* Very low incorporation of ^{14}C from [1-^{14}C]-acetate previously misdirected us to our former conclusion (ref. 3).

** Solvent: D_2O - H_2O (1 : 1). δ_c was calculated from internal dioxane (67.39 ppm).

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KIYOSHI ISONO*

The Institute of Physical and Chemical Research
Wako-shi, Saitama 351, Japan

ROBERT J. SUHADOLNIK

Department of Biochemistry
Temple University School of Medicine
Philadelphia, Pennsylvania 19140
U.S.A.

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* Correspondence should be addressed to this author.