BIOSYNTHESIS OF CARBAZOMYCIN B

II†. ORIGIN OF THE WHOLE CARBON SKELETON††

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The carbazomycins were isolated from *Streptoverticillium ehimense* H 1051 MY10 as the first antibiotics containing a carbazole nucleus and they mainly inhibit the growth of phytopathogenic fungi[3,4]. The complex consists of eight components; carbazomycins B (**1**), A, C, D, E, F, G and H, and their structures were elucidated by spectroscopic and chemical means together with X-ray crystallographic analyses[4~7] and are shown in Fig. 1. The carbazomycins are very unique carbazole derivatives having a C_{14}-skeleton and having substituents at the four adjacent carbon atoms, C-1, C-2, C-3 and C-4. Thus they are different from the carbazole alkaloids of higher plants[8,9], which have so far been isolated exclusively from *Rutaceae* plants and have structures with a C_{13}-, C_{18}- or C_{23}-skeleton†††. Very little, however, has been reported on the biosynthesis of these carbazole alkaloids and, especially, no positive experimental evidence for the origin of their tricyclic carbazole nucleus has yet been provided[8,9]. These facts prompted us to attempt biosynthetic studies of carbazomycin B (**1**), the main component of the complex.

In our previous biosynthetic studies[1] of **1** based on feeding experiments with ^{14}C- and ^{13}C-labeled compounds followed by measurement of radioactivity and ^{13}C NMR spectra, we found that all carbon atoms of the carbazole nucleus except C-1 and C-2 are derived from tryptophan, C-12 (the methoxyl carbon) comes from methionine and the C_2 unit, C-1 and C-10, from acetate. The origin of C-2 and C-11 could not be determined. During these biosynthetic studies[1], the ^{13}C NMR assignment of **1** was established.

In this paper, we describe the determination of the origin of the unsolved C_2 unit using ^{14}C- and ^{13}C-labeled precursors, which has clarified the origin of the whole carbon skeleton of **1**.

Materials and Methods

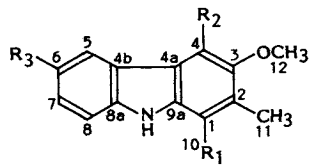
Labeled Compounds

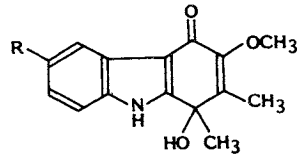
Sodium [1-^{14}C]pyruvate, sodium [2-^{14}C]pyruvate, sodium [3-^{14}C]pyruvate and L-[*U*-^{14}C]alanine were purchased from the Radiochemical Centre, Amersham and DL-[1,2,3-$^{13}C_3$]alanine was obtained from MSD Isotopes.

Fermentation

The fermentation of the producing microorganism, *S. ehimense* H 1051 MY10, was carried out by the procedure described previously[3]. For feeding experiments with ^{14}C-labeled compounds, the production medium was inoculated directly from slants. For preparation of ^{13}C-labeled carbazomycin

Fig. 1. Structures of carbazomycins.





Carbazomycin B (**1**)	$R_1=CH_3$	$R_2=OH$	$R_3=H$
Carbazomycin A	$R_1=CH_3$	$R_2=OCH_3$	$R_3=H$
Carbazomycin C	$R_1=CH_3$	$R_2=OH$	$R_3=OCH_3$
Carbazomycin D	$R_1=CH_3$	$R_2=OCH_3$	$R_3=OCH_3$
Carbazomycin E	$R_1=CHO$	$R_2=OH$	$R_3=H$
Carbazomycin F	$R_1=CHO$	$R_2=OH$	$R_3=OCH_3$

Carbazomycin G R=H
Carbazomycin H R=OCH_3

† Part I: See ref 1.

†† This study was previously presented in part (ref 2).

††† Recently, binary carbazole alkaloids have been isolated from a *Rutaceae* plant (ref 10).

B, preculture fermentation was performed as described earlier[3] and the resulting growth was used to inoculate the production medium. In all feeding experiments, labeled compounds, which were dissolved in a small amount of water, were added to the culture medium 48 hours after the inoculation of the production medium and the cultivation was continued for an additional 48 hours.

Determination of Incorporation Ratios of ^{14}C-Labeled Compounds

^{14}C-Labeled carbazomycin B was obtained and treated to determine incorporation ratios by the procedure previously reported[1], with the exception that a mixture of *n*-hexane and EtOAc (3:1) was used as solvent for preparative TLC in place of a mixture of benzene and acetone (30:1).

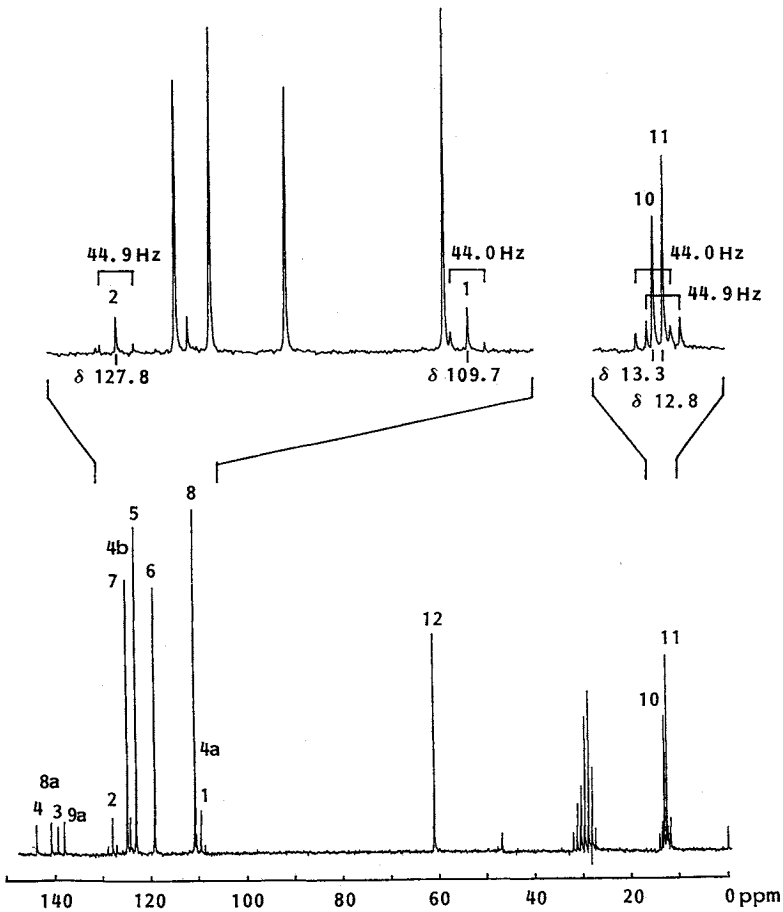
Preparation of ^{13}C-Labeled Carbazomycin B

Ten mg of DL-[1,2,3-$^{13}C_3$]alanine was added to each flask containing 100 ml of the production medium. The mycelial cake obtained from a total of 1,500 ml of the cultured broth was extracted with acetone and the acetone extract was purified by silica

Table 1. Incorporation of ^{14}C-labeled compounds into carbazomycin B (**1**).

Labeled compounds	Specific activity (mCi/mmol)	Radioactivity (dpm)		Incorporation into **1** (%)
		Fed	**1**	
Sodium [1-^{14}C]pyruvate	9.8	5.55×10^6	7.40×10^2	0.01
Sodium [2-^{14}C]pyruvate	15.8	5.55×10^6	1.07×10^4	0.19
Sodium [3-^{14}C]pyruvate	16.2	5.55×10^6	7.23×10^3	0.13
L-[*U*-^{14}C]Alanine	165	5.55×10^6	7.18×10^3	0.13

Fig. 2. ^{13}C NMR spectrum of carbazomycin B fed with DL-[1,2,3-$^{13}C_3$]alanine.



gel column chromatography using a mixture of *n*-hexane and EtOAc (5:1). Recrystallization from *n*-hexane-EtOAc afforded 35 mg of ^{13}C-labeled carbazomycin B.

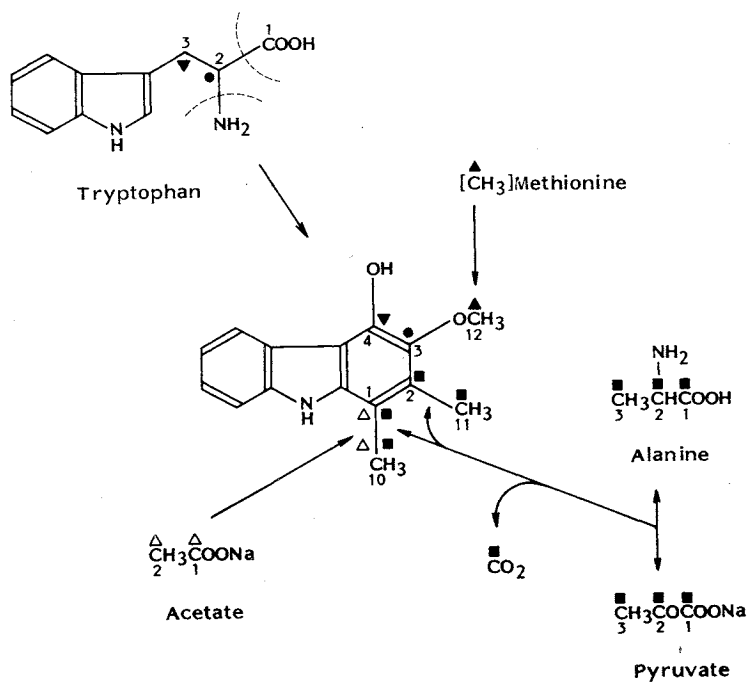
Results and Discussion

Feeding experiments with several candidates of ^{14}C-labeled precursors yielded the results shown in Table 1. Among them, sodium [2-^{14}C]pyruvate, sodium [3-^{14}C]pyruvate and L-[*U*-^{14}C]alanine afforded nearly the same total incorporation ratio as sodium [1-^{14}C]acetate (0.12%) or sodium [2-^{14}C]acetate (0.24%)[1] whereas sodium [1-^{14}C]pyruvate gave a very low ratio. This suggested that the C_2 unit of C-2 and C-3 of pyruvate could be incorporated into **1**. In order to certify this and to locate the incorporation sites, we carried out a feeding experiment with DL-[1,2,3-$^{13}C_3$]alanine, expecting the appearance of satellite signals due to ^{13}C-^{13}C coupling around C-2 and C-11 though an enhancement of each signal could not be anticipated because of the low incorporation ratio. Due to the unavailability of the ^{13}C-labeled pyruvate, we used ^{13}C-labeled alanine which is known to be a biosynthetic equivalent of pyruvate. The ^{13}C NMR spectrum of **1** after feeding with DL-[1,2,3-$^{13}C_3$]alanine was measured in acetone-d_6 on a Jeol JNM-FX100 spectrometer at 25.0 MHz and is shown in Fig. 2. As seen in the figure, the carbon signals of C-2 at δ 127.8 and C-11 (2-methyl) at δ 12.8 appeared along with satellite peaks of $J=44.9$ Hz due to ^{13}C-^{13}C coupling, which is a reasonable value for sp^3-sp^2 coupling. Furthermore, the carbon signals of C-1 at δ 109.7 and C-10 (1-methyl) at δ 13.3 were also accompanied with satellite peaks of $J=44.0$ Hz due to ^{13}C-^{13}C coupling.

These results obtained from the above feeding experiments with ^{14}C- and ^{13}C-labeled precursors indicated that the intact C_2 unit of C-2 and C-3 of pyruvate is incorporated into C-2 and C-11 of **1** and that this C_2 unit of pyruvate is also incorporated into C-1 and C-10 probably by way of acetyl-CoA. This is consistent with the previous result[1] that the C_2 unit of acetate was incorporated into C-1 and C-10 of **1**. Very low incorporation ratios of pyruvate or acetate into **1** in comparison with tryptophan or methionine[1] could be explained by a very large pool of pyruvate or acetate in living cells which would dilute isotope-labeled compounds added to the medium in the middle of a fermentation.

Hence, the origin of the all carbon atoms of carbazomycin B has been established and can be summarized as shown in Fig. 3. As far as we know, this is the first example of a natural carbazole

Fig. 3. Biosynthetic origin of carbazomycin B.



derivative whose carbon origin has been elucidated biosynthetically.

Acknowledgment

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