NEW ANTIBIOTICS, CARBAZOMYCINS A AND B

II. STRUCTURAL ELUCIDATION

KATSU-ICHI SAKANO and SHOSHIRO NAKAMURA

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi, Minami-ku, Hiroshima 734, Japan

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The structures of carbazomycins A and B are elucidated to be 3,4-dimethoxy-1,2-dimethylcarbazole and 4-hydroxy-3-methoxy-1,2-dimethylcarbazole, respectively. Further, ^{13}C-chemical shifts of carbazomycins and their derivatives are assigned simultaneously.

Carbazomycins were isolated from the cultured mycelia of an unidentified *Streptomyces*, tentatively designated as strain H 1051-MY 10. Carbazomycins inhibited the growth of phytophathogenic fungi and further showed weak antibacterial and antiyeast activities[1]. Carbazomycin B (I), $C_{15}H_{15}NO_2$, was suggested to have one phenolic hydroxyl, one methoxyl and two methyl groups on a carbazole nucleus. While, carbazomycin A (**II**), $C_{16}H_{17}NO_2$, was proved to be O-methylcarbazomycin B[1]. The structures of carbazomycins A and B are elucidated by studying the ^1H- and ^{13}C-NMR spectra of both antibiotics and their derivatives and described in this paper.

Structures

^1H- and ^{13}C-chemical shifts of carbazomycins and their derivatives are shown in Tables 1 and 2, respectively. Signals for H-4 and H-5 in the ^1H-NMR spectra of carbazole derivatives are reported to be observed usually at lower field than the other aromatic protons of a carbazole nucleus because those two protons are mutually deshielded[2~5]. Thus, one double doublet (1H, J=7.0, 2.0 Hz) at δ 8.31 in the ^1H-NMR spectrum of carbazomycin B (**I**) can be assigned for H-5 of a carbazole nucleus as shown in Fig. 1 and Table 1. The double doublet at δ 8.31 suggests the existence of three or four adjacent aromatic protons and the absence of H-4 in **I**. Further, chemical shifts due to four aromatic tertiary carbons in the ^{13}C-NMR spectrum of **I** are strikingly related to those of carbazole as seen in Table 2. Thus, the four aromatic protons of **I** are presumed to be located adjacently on the same ring of a carbazole nucleus.

The appropriate strong band due to four adjacent aromatic protons is also observed near 750 cm^{-1} in the infrared absorption spectra of **I** and carbazomycin A (**II**).

The four substituents of **II** are considered to be in a sterically congested environment, because the signals of two methyl carbons are observed at higher field (δ 12.6 and 13.6) than usual values (δ 15~25) and two methoxyl carbons at lower

Fig. 1. Structures of carbazomycins and their derivatives.

Carbazomycin B (**I**) R_1=OH R_2=H

Carbazomycin A (**II**) R_1=OCH_3 R_2=H

Deoxycarbazomycin B (**III**) R_1=H R_2=H

O-Acetylcarbazomycin B (**IV**) R_1=$OCOCH_3$ R_2=H

O,6-Diacetylcarbazomycin B (**V**) R_1=$OCOCH_3$ R_2=$COCH_3$

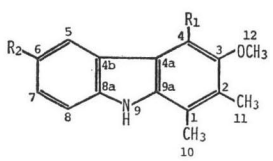


Table 1. ^{1}H-Chemical shifts of carbazomycins and their derivatives.

	H-5	H-6	H-7	H-8	1-CH_3-	2-CH_3-	3-CH_3O-	R_1-	-NH-	6-CH_3CO-
Carbazomycin B (I) R_1=OH, R_2=H	8.31 (dd, J=7.0, 2.0)	7.14~7.38 (m)			2.36 (s)	2.28 (s)	3.80 (s)	6.21 (s)	7.71 (brs)	—
Carbazomycin A (II) R_1=OCH_3, R_2=H	8.25 (dd, J=7.0, 2.0)	7.13~7.42 (m)			2.40 (s)	2.40 (s)	3.92 (s)	4.13 (s)	7.89 (brs)	—
Deoxycarbazomycin B (III) R_1=H, R_2=H	8.08 (dd, J=7.0, 1.5)	7.16~7.52 (m)			2.47 (s)	2.36 (s)	3.98 (s)	7.45 (s)	8.00 (brs)	—
O-Acetylcarbazomycin B (IV) R_1=$OCOCH_3$, R_2=H	7.83 (dd, J=7.0, 2.0)	7.04~7.37 (m)			2.27 (s)	2.15 (s)	3.80 (s)	2.55 (s)	7.84 (brs)	—
O,6-Diacetylcarbazomycin B (V) R_1=$OCOCH_3$, R_2=$COCH_3$	8.47 (d, J=1.8)	—	7.88 (dd, J=8.5, 1.8)	7.06 (d, J=8.5)	2.25 (s)	2.07 (s)	3.83 (s)	2.64 (s)	8.32 (brs)	2.68 (s)

All spectra were run on a JEOL PS-100 spectrometer (100 MHz) using TMS as an internal standard and $CDCl_3$ as a solvent. Chemical shifts were expressed as δ in ppm.

s: singlet, d: doublet, dd: doublet of doublet, m: multiplet, brs: broad singlet.

Table 2. ^{13}C-Chemical shifts of carbazomycins and their derivatives.

Carbon No.	Multiplicity	I	II	III	IV	V	Carbazole
C-1	s	110.0[c]	114.4[d]	119.5[f]	116.6[g]	117.2[i]	110.5
C-2	s	127.0	128.7	124.2[f]	128.3	129.1	125.8
C-3	s	142.0	144.4[e]	152.6	143.4	144.0	119.4
C-4	s (d)[a]	138.5	145.9[e]	99.0	135.8[h]	135.9[j]	120.3
C-4a	s	109.3[c]	113.5[d]	118.5[f]	110.3[g]	110.3[i]	119.2
C-4b	s	123.3	122.8	120.1[f]	121.8	121.4	119.2
C-5	d	122.7	122.5	119.8	121.3	122.6	120.3
C-6	d (s)[b]	119.5	119.4	118.9	119.3	129.4	119.4
C-7	d	124.7	125.0	124.9	125.4	126.1	125.8
C-8	d	110.0	110.3	110.7	110.7	110.7	110.5
C-8a	s	139.3	139.4	139.6	139.8[h]	142.6	140.5
C-9a	s	136.8	136.4	134.2	136.4	136.6[j]	140.5
C-10	q	12.7	12.6	12.3	12.7	12.7	—
C-11	q	13.1	13.6	13.8	13.5	13.5	—
C-12	q	61.4	61.1	56.2	61.3	61.3	—
C-13	q	—	60.5	—	—	—	—

All spectra were run on a JEOL PFT-100 spectrometer (25.15 MHz) using TMS as an internal standard and $CDCl_3$ as a solvent. Chemical shifts were expressed as δ in ppm. Experimental conditions were as follows: spectral width 5 KHz, pulse width 16 μ sec, repetition time 2.0 sec. [a]: multiplicity of **III**, [b]: multiplicity of **V**. [c]~[j]: tentatively assigned. s: singlet, d: doublet, q: quartet.

field (δ 60.5 and 61.1) than usual values (δ 53~57) in the ^{13}C-NMR spectrum of **II**. Consequently, four substituents in **I** and **II** should be located on the same ring of a carbazole nucleus[6,7].

Deoxycarbazomycin B (**III**) was prepared by the reduction of O-tosylcarbazomycin B over RANEY nickel to locate the hydroxyl group of **I** on a carbazole nucleus as shown in Fig. 2[3,4]. A new doublet due to the deoxylated carbon is appeared at δ 99.0 in the ^{13}C-NMR spectrum of **III**. Further, the chemical shift of the carbon substituted with a methoxyl group of **III** is shifted downfield to δ 152.6 from δ 142.0 of **I** and a methoxyl carbon of **III** shifted to δ 56.2 from δ 61.4 of **I**, while no significant shift due to two methyl carbons is observed in **I** and **III**. A new singlet for one aromatic proton is also observed at δ 7.45 in the ^{1}H-NMR spectrum of **III**. The chemical shift of an aromatic proton adjacent to a methoxyl group is reported to shift upfield by *ca.* 0.5 ppm[3,4,8,9]. Therefore, the new singlet at δ 7.45 can be assigned to H-4 in **III** as seen in Fig. 1. In the selective proton decoupling spectrum of **III** (100 MHz, in CCl_4, not degassed) irradiating at a methoxyl group (δ 3.98) and two methyl groups (δ 2.47 and 2.36), NOE of H-4 is observed by *ca.* 20% when irradiated at the methoxyl group, but not observed when irradiated at the two methyl groups.

Carbazomycin B (**I**) was acetylated with pyridine and acetic anhydride to give O-acetylcarbazomycin B (**IV**), while O,6-diacetylcarbazomycin B (**V**) was prepared by the acetylation of **I** on heating with acetic anhydride in the presence of zinc chloride as seen in Fig 2[10]. The selective proton decouping spectrum of **V** (100 MHz, in $CDCl_3$, not degassed) irradiating at the two methyl groups (δ 2.07 and 2.25) showed NOE of –NH– proton by *ca.* 15% when irradiated at one methyl group (δ 2.25).

The ultraviolet absorption spectrum of **III**; λ_{max}^{EtOH}, nm (ε); 219 (29,300), 236 (29,900), 253 (17,600), 264 (13,200), 303 (16,700), 336 (3,700) and 352 (3,500); is closely related to that of 3-methoxycarbazole[11] and 3-methoxy-2-methylcarbazole[12].

Fig. 2. Chemical transformation of carbazomycin B.

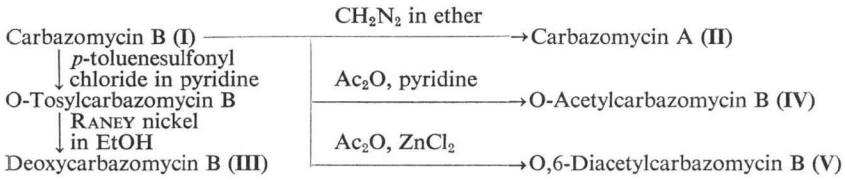
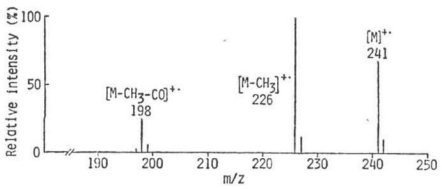


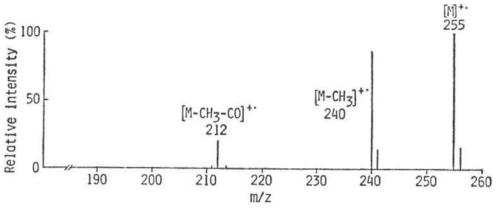
Fig. 3. GC-Mass spectra of carbazomycins.

Carbazomycin B (I)



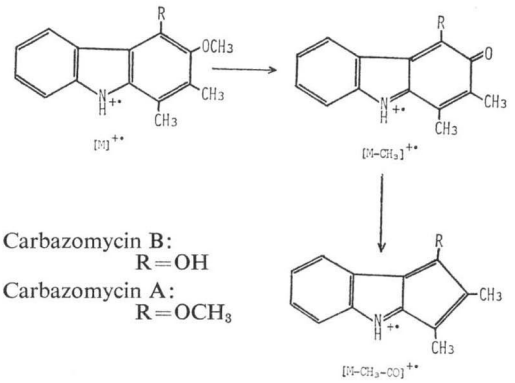
Column: glass column, 2 m, SE-30 (1 w/w%). Column temp. 220°C, Detector temp. 300°C. Carrier gas: He 28 ml/min, 1.8 kg/cm². Retention time: 4.5 min. Ion source: EI. Ionization energy: 20 eV.

Carbazomycin A (II)



Column: glass column, 2 m, SE-30 (1 w/w%). Column temp. 200°C, Detector temp. 300°C. Carrier gas: He 28 ml/min, 1.5 kg/cm². Retention time: 7.4 min. Ion source: EI. Ionization energy: 20 eV.

Fig. 4. Fragmentation of carbazomycins.



Carbazomycin B: R=OH

Carbazomycin A: R=OCH_3

The GC-mass spectra of **I** and **II** are shown in Fig. 3 and the location of the methoxyl group is also suggested to be on C-3 of a carbazole nucleus by the fragment ion peak[4] at *m/e* $[M-CH_3-CO]^+$ as illustrated in Fig. 4.

Thus, none of results above obtained conflicts with the structures for **I** and **II**, 4-hydroxy-3-methoxy-1,2-dimethylcarbazole (**I**) and 3,4-dimethoxy-1,2-dimethylcarbazole (**II**), respectively.

Assignments of ^{13}C-Chemical Shifts

Aromatic methyl carbons: Chemical shifts of aromatic methyl carbons of carbazomycins and their derivatives are assigned by the selective proton decoupling technique. The signals at δ 12.7 and 13.5 can be assigned to 1-CH_3 carbon (C-10) and 2-CH_3 carbon (C-11) respectively by the selective proton decoupling spectrum of O-acetylcarbazomycin B (**IV**) irradiating at 1-CH_3 protons (δ 2.27) and 2-CH_3 protons (δ 2.15). The signal which appeared in higher field in the region of δ 12~14 can be attributed to C-10 and the other to C-11 in the ^{13}C-NMR spectra of **I**~**V**.

Aromatic methoxyl carbons: The signal at δ 61.1~61.4 due to 3-CH_3O carbon (C-12) is observed in the ^{13}C-NMR spectra of **I**, **II**, **IV** and **V** and the signals at δ 61.1 and 60.5 in the ^{13}C-NMR spectrum of **II** are considered to relate to C-12 and 4-CH_3O carbon (C-13), respectively.

Aromatic tertiary carbons: The signals for C-1 and C-8 appear at higher field and that for C-2 and C-7 appear at lower field in the ^{13}C-NMR spectra of carbazole derivatives[13]. Therefore, two

doublets centered at *ca.* δ 110 and 125 can be assigned to C-8 and C-7 of **I**~**V**, respectively. Selective proton decoupling by irradiating at H-5 (δ 8.31) of **I** causes the doublet centered at δ 122.7 to become a sharp singlet. Thus, the doublet centered at *ca.* δ 120~123 can be assigned to C-5 of **I**~**V**. Consequently, the other doublet centered at *ca.* δ 119 is postulated to relate to C-6 of **I**~**IV**, while the singlet due to C-6 is shifted to δ 129.4 in **V**.

Aromatic quaternary carbons: Two singlets at δ 120~123 and δ 139~143 in the ^{13}C-NMR spectra of **I**~**V** can be assigned to C-4b and C-8a respectively by the comparison of chemical shifts of **I**~**V** with that of carbazole and its derivatives[13]. The signal for an aromatic quaternary carbon substituted with a methoxyl, hydroxyl or acetoxyl group appears at lower field. Particularly, a carbon substituted with a methoxyl group shifts more downfield than a carbon substituted with a hydroxyl or acetoxyl group[14~16]. Therefore, the singlet more downfield than δ 140 should be attributed to C-3 of **I**~**V**. Further, the singlet at δ 138.5 of **I** can be assigned to C-4 substituted with a hydroxyl group and the singlet at *ca.* δ 136 of **IV** and **V** is explained by C-4 substituted with an acetoxyl group. The signal for C-9a is considered to appear at slightly higher field than that for C-8a through the influences of *ortho*-methyl and *para*-methoxyl groups. Thus, the singlet in the region δ 134~137 is attributable to C-9a of **I**~**V**. Similarly, two signals C-4a and C-1 are expected to appear at higher field than that for C-4b and C-8 through the influences of substituents at the *ortho*- and *para*-positions. Therefore, two singlets in the region δ 109~119 are assigned to C-4a and C-1 of **I**, **II**, **IV** and **V**. Finally, the residual singlet at *ca.* δ 127~129 is presumed to relate to C-2 of **I**, **II**, **IV** and **V**.

Experimental

Deoxycarbazomycin B (III)

Carbazomycin B (77 mg) was refluxed with dry pyridine (5 ml) and *p*-toluenesulfonyl chloride (500 mg) for 2 hours. The reaction mixture was purified on a slica gel column (41×2.2 cm, diam.), eluted first with 100 ml of *n*-hexane - AcOEt (9: 1) followed by *n*-hexane - AcOEt (4: 1) and the eluate was collected in 19-ml fractions each. Fractions No. 9~21 were combined, concentrated *in vacuo* and recrystallized from *n*-hexane and AcOEt to afford O-tosylcarbazomycin B (70 mg), colorless needles, m.p. 232.0~235.5°C. Mass; *m/e* 395 (M^+). IR in KBr; 3380 cm^{-1} (–NH–), 1370 cm^{-1} ($-SO_3-$) and 1185 cm^{-1} ($-SO_3-$). ^{1}H-NMR in $CDCl_3$ (100 MHz); a broad singlet for –NH– proton (1H, δ 7.94), multiplets for eight aromatic protons (3H, δ 7.97~8.14: 5H, δ 7.04~7.44), a singlet for a methoxyl group (3H, δ 3.43) and three singlets for three methyl groups (3H×3, δ 2.46, 2.30 and 2.26).

O-Tosylcarbazomycin B (66 mg) dissolved in dry EtOH was refluxed for 10 hours with RANEY nickel (400 mg). The resulting product was chromatographed on a sillica gel column (41×2.2 cm, diam.), eluted with *n*-hexane - AcOEt (19: 1) and the eluate was collected in 17-ml fractions. Fractions No. 5~11 were combined, concentrated *in vacuo* and recrystallized from *n*-hexane and AcOEt to afford deoxycarbazomycin B (8 mg), colorless needles, m.p. 129.0~130.0°C. Mass; *m/e* 225 (M^+). IR in KBr; 3340 cm^{-1} (–NH–).

O-Acetylcarbazomycin B (IV)

Carbazomycin B (72 mg) dissolved in a mixture of pyridine (1 ml) and Ac_2O (2 ml) was kept at room temperature for 24 hours. The resulting product was suspended in H_2O and extracted with AcOEt. The extract was concentrated, applied to a silica gel column (54×2.2 cm, diam.), eluted with benzene - AcOEt (9: 1) and the eluate was collected in 10-ml fractions. Fractions No. 16~21 were combined, concentrated *in vacuo* and finally recrystallized from *n*-hexane and AcOEt to afford O-acetylcarbazomycin B (46 mg), colorless needles, m.p. 192.0~195.0°C. Mass; *m/e* 283 (M^+). IR in KBr; 3340 cm^{-1} (–NH–) and 1745 cm^{-1} (CH_3COO-). UV; λ_{max}^{EtOH}, nm (ε); 240 (52,600), 261 (22,600),

294 (22,200), 328 (4,500) and 342 (4,500).

O,6-Diacetylcarbazomycin B (V)

Carbazomycin B (72 mg) was heated with Ac_2O (2 ml) and $ZnCl_2$ (100 mg) at 70°C for 30 hours. The reaction mixture was suspended in H_2O and extracted with AcOEt. The extract was concentrated, applied to a silica gel column (53×2.2 cm, diam.), eluted with *n*-hexane - AcOEt (7: 3) and the eluate was collected in 15-ml fractions. Fractions No. 19~23 were combined, concentrated *in vacuo* and recrystallized from *n*-hexane and AcOEt to afford O,6-diacetylcarbazomycin B (57 mg), colorless crystalline powder, m.p. 242.0~242.5°C. Mass; *m/e* 325 (M^+). IR in KBr; 3300 cm^{-1} (–NH–), 1735 cm^{-1} (CH_3COO–) and 1655 cm^{-1} (Ar–$COCH_3$).

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