CARBAZOMYCINAL AND 6-METHOXYCARBAZOMYCINAL AS AERIAL MYCELIUM FORMATION-INHIBITORY SUBSTANCES OF *STREPTOVERTICILLIUM* SPECIES

Sir:

Actinomycetes, which produce a number of antibiotics, have a more complex life cycle than other microorganisms that are procaryotes. The life cycle begins at germination of the asexual arthrospores, after which filamentous vegetative mycelia (substrate mycelia) then grow from the germinated spores, and the aerial mycelia, which subsequently differentiate from the substrate mycelia, extend into the air. In most actinomycetes asexual spores are produced on this aerial mycelia.[1] Recently, this process of differentiation of aerial mycelia from substrate mycelia has attracted much attention from microbiologists and natural products chemists on account of the close correlation between this differentiation and the initiation of secondary metabolism (*e.g.* production of antibiotics) which has been shown in several actinomycetes.[2,3] We are interested in the aerial mycelium-inducing substances of actinomycetes and have continued the isolation of active substances from *Streptoverticillium* species[4] and *Streptomyces alboniger*.[5] During our chemical studies of these actinomycetes, we found that strain KCC U-0166 of *Streptoverticillium* sp. in solid agar culture produces substances that inhibit aerial mycelium formation.

These substances selectively inhibited the formation of aerial mycelia without any effect on the growth of substrate mycelia. Two active compounds were isolated and named carbazomycinal and 6-methoxycarbazomycinal. Their respective structures, elucidated as **1** and **2**, are described in this report. Carbazomycinal and 6-methoxycarbazomycinal are the first inhibitors of aerial mycelium formation to be identified chemically in actinomycetes, although an earlier publication indicated the presence of such compounds in actinomycetes.[6]

Streptoverticillium sp. was cultured on inorganic salts starch agar medium (300 liters) at 30°C for 6 days, after which the cultured mycelia and the medium were macerated in Me_2CO. The Me_2CO extract was evaporated to an aq residue that was then treated with EtOAc. The EtOAc extract was fractionated into neutral, basic and acidic fractions. The active neutral fraction was purified by repeated silica gel column chromatography, preparative TLC and recrystallization from EtOAc - *n*-hexane to give carbazomycinal (**1**) (yield 9.3 mg) and 6-methoxycarbazomycinal (**2**) (12.9 mg) (Fig. 1), which had Rf values of 0.56 and 0.45, respectively, on silica gel TLC in a solvent system of EtOAc - *n*-hexane (1 : 1).

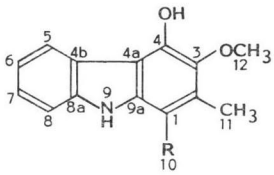
Carbazomycinal (**1**) was obtained as pale yellow crystals; mp 224°C; $C_{15}H_{13}NO_3$ (obsd *m/z* 255.0875, error −2.0).[a] ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of carbazomycinal closely resemble those of carbazomycin B (**3**) reported by NAKAMURA and co-workers.[7~9] However, these spectra and the IR spectrum[b] showed the presence of an aldehyde group (δ 10.41 (1H, s); 189.4 (d); 1660 cm^{-1}). The UV spectrum of carbazomycinal (λ_{max}^{MeOH} nm (ε) 214 (24,400), 227 (23,200), 263 (12,200), 295 (16,300), 320 (5,100), 372 (8,400)) resembles quite closely that of carbazole-1-carbaldehyde, but differs distinctly from the spectra of carbazole-2-, 3- and 4-carbaldehyde.[10] Thus, carbazomycinal was shown to be a carbazole-1-carbaldehyde derivative with one each of methyl, methoxyl and phenolic hydroxyl substituents. The ^1H NMR spectrum (Me_2CO-d_6) showed four neighboring aromatic protons of H-5 (δ 8.23 (1H, ddd, J= 7.8, 1.2, 0.7 Hz)), H-6 (δ 7.22 (1H, ddd, J=7.8, 7.3, 0.9 Hz)), H-7 (δ 7.38 (1H, ddd, J=8.0, 7.3, 1.2 Hz)) and H-8 (δ 7.72 (1H, ddd, J=8.0, 0.9, 0.7 Hz))[c] and the ^{13}C NMR spectrum similarly showed four protonated aromatic carbons (Table 2). These results indicated one unsubstituted ring and thus the four substituents must be located on the other aromatic ring at C-1 (CHO), -2, -3 and -4 positions. Of these substituents, the methyl group was shown to be at C-2 from NOE experiments; *i.e.*, irradiation of the methyl signal enhanced (9%) the aldehyde signal,

[a] Mass spectrum of carbazomycinal (**1**) (70 eV) *m/z* 255 (M^+, 62%), 240 (100), 212 (20), 142 (30).

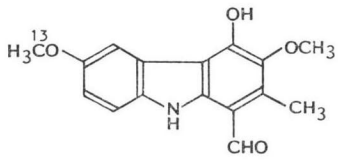
[b] IR spectrum of carbazomycinal (ν_{max}($CHCl_3$) cm^{-1}) 3520, 3450, 3000, 2950, 2860, 1660, 1590, 1575, 1485, 1455, 1345, 1300, 1265, 1160, 1010, 645, 590, 555.

[c] Other protons of carbazomycinal in Me_2CO-d_6 (200 MHz) are as follows; δ 11.12 (1H, br s, NH), 10.42 (1H, s, H-10), 9.57 (1H, br s, OH), 3.81 (3H, s, H-12), 2.77 (3H, s, H-11).

Fig. 1. Structures of carbazomycinal (**1**), 6-methoxycarbazomycinal (**2**) and carbazomycin (**3**).



1 R=CHO
3 R=CH_3



2

Table 1. ^{1}H NMR data of carbazomycinal and 6-methoxycarbazomycinal (100 MHz in $CDCl_3$).

Proton	Carbazomycinal (δ[a])	6-Methoxycarbazomycinal (δ[a])
5	8.22 (br d, J=7.6 Hz)	7.72 (br d, J=2.7 Hz)
6	7.18~7.48 (m)	
7		7.05 (dd, J=8.5, 2.7 Hz)
8		7.38 (d, J=8.5 Hz)
9 (NH)	10.60 (br s)	10.65 (br s)
10	10.41 (s)	10.51 (s)
11	2.76 (s)	2.76 (s)
12	3.87 (s)	3.86 (s)
13		3.93 (s)
OH	6.85 (br s)	6.91 (br s)

[a] Chemical shifts are in ppm relative to internal Me_4Si.

Table 2. ^{13}C NMR data of carbazomycinal and 6-methoxycarbazomycinal (50 MHz in pyridine-d_5).

Position	Carbazomycinal			6-Methoxycarbazomycinal		
	δ[a]	OFR	J_{C-H} (Hz)	δ[a]	OFR	J_{C-H} (Hz)
1	111.2	s	br d 22.0	110.3	s	br d 17.6
2	134.8	s	br q 5.0	135.0	s	br q 5.9
3	139.6	s	br s	139.0	s	br s
4	154.4	s	s	151.9	s	s
4a	111.9	s	br s	111.7	s	br s
4b	122.6	s	m	123.3	s	m
5	123.1	d	dd 161.2, 7.3	106.5	d	dd 161.2, 4.4
6	120.5	d	dd 160.5, 5.9	154.8	s	m
7	125.6	d	dd 156.8, 7.3	115.4	d	dd 158.3, 4.4
8	111.9	d	dd 161.9, 7.3	112.4	d	d 161.2
8a	140.7	s	br s	140.1	s	m
9a	139.6	s	br s	139.9	s	d 4.4
10	189.4	d	d 171.4	189.3	d	d 171.4
11	11.4	q	q 128.5	11.3	q	q 128.9
12	61.3	q	q 143.6	61.3	q	q 143.6
13				56.1	q	q 143.6

[a] Chemical shifts are in ppm relative to internal Me_4Si.
OFR: Off resonance.

whereas irradiation of the methoxyl or hydroxyl did not enhance the aldehyde signal. The free hydroxyl of carbazomycinal was methylated with dimethyl sulfate to give a dimethoxy-derivative; in its ^{1}H NMR spectrum (Me_2CO-d_6) a new methoxyl signal appeared at δ 4.25 in addition to the original one at δ 3.81. The chemical shift of the new methoxyl being lower than that of the original indicates that the former is located at the C-4 position and is deshielded by the anisotropic effect of another aromatic ring. In this way, the 3-methoxyl and 4-hydroxyl sub-

stituents were established, and the structure of carbazomycinal was determined to be **1**. Carbazomycinal was the oxidized metabolite of carbazomycin B (**3**), which was also actually isolated from the cultured broth of this fungus.

6-Methoxycarbazomycinal (**2**) has the following physico-chemical properties; pale yellow crystals, mp 221°C, $C_{16}H_{15}NO_4$ (obsd *m/z* 285.1017, error 1.6),[d] UV λ_{max}^{MeOH} nm (ε) 215 (27,000), 227 (24,500), 245 (13,000), 268 (13,000), 310 (17,000), 382 (8,500), IR ν_{max} ($CHCl_3$) cm^{-1} 3500, 3440, 3000, 2950, 1655, 1625, 1590, 1575, 1485, 1460, 1340, 1320, 1225, 1135, 1003, 560. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) were very similar to those of carbazomycinal, except that one extra methoxyl signal appeared at δ 3.93 (3H, s) and three, not four, aromatic protons at δ 7.05 (1H, dd, J=8.5, 2.7 Hz), 7.38 (1H, d, J=8.5 Hz) and 7.72 (1H, br d, J=2.7 Hz).[e] The last *meta*-coupled doublet was assigned to H-5 because it was the most deshielded due to the anisotropy of another aromatic ring. The C-6 methoxyl group was confirmed by a long range selective proton decoupling experiment (LSPD), in which irradiation of H-3 collapsed the signals of both C-4b and C-6.[f] Similarly, irradiation of H-5 and of H-7 respectively resulted in the collapse of the C-7 and C-8a signals and of the C-5 and C-8a ones.[f] On this basis, the structure of 6-methoxycarbazomycinal can be assigned as **2**.

Both carbazomycinal and 6-methoxycarbazomycinal inhibited the formation of aerial mycelia from substrate mycelia of *Streptoverticillium* sp. KCC U-0166 at the respective concentrations of 1 μg/ml and 0.5 μg/ml, but did not affect the growth of substrate mycelia even at 100 μg/ml. At concentrations of greater than 100 μg/ml neither compound had antibiotic activity against fungi or bacteria in our bioassays. Further studies of the activities of these compounds against other actinomycetes species are in progress.

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[d] Mass spectrum of 6-methoxycarbazomycinal (**2**) (70 eV) *m/z* 285 (M^+, 62%), 270 (100), 242 (9), 198 (12), 144 (9).

[e] ^1H NMR data of 6-methoxycarbazomycinal (200 MHz, Me_2CO-d_6) are as follows; δ 11.10 (1H, br s, NH), 10.40 (1H, s, H-10), 9.56 (1H, br s, OH), 7.75 (1H, d, J=2.4 Hz, H-5), 7.60 (1H, d, J=8.8 Hz, H-8), 7.02 (1H, dd, J=8.8, 2.4 Hz, H-7), 3.88 (3H, s, H-13), 3.81 (3H, s, H-12), 2.76 (3H, s, H-11).

[f] This experiment was carried out in Me_2CO-d_6 with the 200 MHz ^1H NMR spectrometry.

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