CARBAZOMYCINS G AND H, NOVEL CARBAZOMYCIN-CONGENERS CONTAINING A QUINOL MOIETY

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Carbazomycins G (I) and H (II), new congeners of the carbazomycin complex, have been isolated from the culture broth of *Streptoverticillium ehimense*. They have proved to contain a unique quinol moiety in the molecule. Their structures have been elucidated by mass and NMR spectrometries and X-ray crystallographic analysis. Carbazomycin G showed moderate antifungal activity against *Trichophyton* species.

In a previous paper[1], we have reported the isolation of carbazomycins C, D, E and F as the minor components of carbazomycin complex. The structures of carbazomycins C (IV) and D (V) were determined by spectroscopic and chemical means, and in the present paper are confirmed by an X-ray crystallographic analysis of IV.

In our successive search for other active substances produced by the same microorganism, we have found two new components named carbazomycins G (I) and H (II) which are shown to possess a unique quinol moiety in the molecule.

In this paper, we report the isolation, physico-chemical and antimicrobial properties, and structure elucidation of I and II by mass, ^{1}H and ^{13}C NMR spectrometries and X-ray crystallographic analysis. The confirmation of the structures of IV and V by X-ray crystallography of IV is also reported.

Results and Discussion

Isolation of Carbazomycins G and H

Streptoverticillium ehimense was cultured in the same way as reported previously[1]. The broth filtrate was extracted with ethyl acetate and the extract was fractionated by silica gel column chromatography using the solvent composed of *n*-hexane and ethyl acetate (7 : 1 ~ 1 : 1). The last fraction eluted with *n*-hexane and ethyl acetate (1 : 1) was concentrated and further purified by repeated preparative TLC on precoated Silica gel plates (Merck Art. No. 5715) developed with *n*-hexane and ethyl acetate (2 : 3). Under a UV-light (365 nm), I gave strong blue fluorescence and II gave dark yellow one, and both components gave the same brownish purple color on silica gel TLC by heating after spraying with 10% sulfuric acid.

Physico-chemical Properties and Structure Elucidation of Carbazomycins G and H

The physico-chemical properties of **I** and **II** are summarized in Table 1. The UV absorption spectra of **I** and **II** resembled that of carbazomycin B (**III**)[2], but a little differed from that of **III** suggesting a somewhat modified carbazole nucleus in **I** and **II**. The molecular formulae of **I** and **II** were established by high resolution mass spectrometry (HR-MS) as $C_{15}H_{15}NO_3$ and $C_{16}H_{17}NO_4$, respectively.

The ^1H NMR data of **I** and **II** suggested the presence of four adjacent aromatic protons, one methoxyl group, two methyl groups and two exchangeable protons as shown in Table 2. These methyl signals, however, were observed at a somewhat higher field than ordinary aromatic methyl signals such as those (δ 2.35 and δ 2.39) of carbazomycin B (**III**)[3~5] and the one at δ 2.01 could be assigned as a methyl group on a double bonded carbon and the other at δ 1.60 might be due to a tertiary methyl group on a carbon bearing one hydroxyl group.

The ^{13}C NMR data of **I** and **II** are shown in Table 3. The assignments were made by comparison with the data of carbazomycins so far obtained[1,3~5] and the reported data for cacalone[6] which was isolated from the roots of *Cacalia decomposita* and contains the same quinol system in the molecule. The surroundings described above of two methyl groups in **I** were supported by the ^{13}C NMR data

Table 1. Physico-chemical properties of carbazomycins G (**I**) and H (**II**).

	I	**II**
Appearance	Colorless prisms	Colorless prisms
MP (°C)	241~243	228~230
Molecular formula	$C_{15}H_{15}NO_3$	$C_{16}H_{17}NO_4$
HR-MS (m/z (M^+))		
Found:	257.1046	287.1150
Calcd:	257.1051	287.1157
UV λ_{max}^{MeOH} nm (ε)	214 (33,200), 253 (19,800), 272 (sh, 8,600), 278 (7,400), 340 (5,600)	214 (35,700), 253 (23,800), 261 (sh, 20,300), 292 (10,100), 310 (sh, 5,800), 340 (3,500)
IR ν_{max}^{KBr} cm^{-1}	3150, 2950, 1635 (sh), 1605, 1475, 1450, 1400, 1370, 1315, 1290, 1190, 1150, 1090, 1005, 880, 810, 750	3350, 3150, 2900, 1625 (sh), 1605, 1480, 1455, 1285, 1260, 1215, 1165, 1130, 1095, 1065, 1000, 885, 850, 800, 700
Rf† value on TLC	0.33	0.26

† Merck Silica gel plate, Art. No. 5715: Solvent; *n*-hexane - EtOAc (1 : 1).

Table 2. ^1H NMR data of carbazomycins G (**I**) and H (**II**).

Protons	**I** R=H	**II** R=OCH_3
5-H	8.05(m)	7.66 (d, J=2.4 Hz)
6-R	7.21~7.50 (m)	3.84 (s)
7-H		6.85 (dd, J=8.8, 2.4 Hz)
8-H		7.39 (d, J=8.8 Hz)
1-CH_3	1.60 (s)	1.66 (s)
1-OH	5.99 (s)	5.45 (s)
2-CH_3	2.01 (s)	2.05 (s)
3-OCH_3	3.71 (s)	3.76 (s)
NH	12.27 (br s)	11.17 (br s)

Spectra were taken using DMSO-d_6 (δ in ppm) as solvent and TMS as an internal standard.

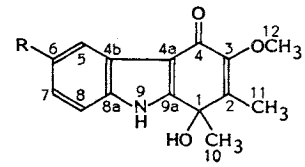
Table 3. ^{13}C NMR data of carbazomycins G (I) and H (II).

Carbon No.	Multiplicity	I	II	Δ_1[a]	Δ_2[b]
C-1	s	67.3	67.2		
C-2	s	154.3	154.4		
C-3	s	147.6	147.5		
C-4	s	177.5	177.4		
C-4a	s	108.4	108.3		
C-4b	s	123.8	124.4	+0.6	+0.4
C-5	d	121.5	102.3	−19.2	−16.9
C-6	d (s)[c]	120.5	155.0	+34.5	+34.8
C-7	d	122.9	112.4[d]	−10.5	−10.9
C-8	d	112.0	112.7[d]	+0.7	+0.5
C-8a	s	136.4	131.1	−5.3	−5.1
C-9a	s	140.8	140.6		
C-10 (1-CH_3)	q	27.9	27.9		
C-11 (2-CH_3)	q	10.1	10.1		
C-12 (3-OCH_3)	q	59.2	59.1		
C-13 (6-OCH_3)	q	—	55.2		

Spectra were taken using DMSO-d_6 as solvent and TMS as an internal standard.
[a] Δ_1=(δ of II)−(δ of I). [b] Δ_2=(δ of IV)−(δ of III). [c] Multiplicity for II. [d] Could be reversed.

of I, namely, the singlet signal at δ 10.1 could be due to a methyl group on a double bonded carbon and that at δ 27.9 could be assigned as a tertiary methyl group on a carbon bearing one hydroxyl group and the singlet signal at δ 67.3 could be attributed to a quaternary carbon bonded to one methyl group and one hydroxyl group. The carbonyl carbon signal at δ 177.5 could be assigned to a quinone or a quinol carbonyl group. Thus, it appears that I contains a quinol system similar to that of cacalone[6] whose carbonyl carbon was observed at δ 175.0. From the viewpoint of biosynthesis of carbazomycins[5], the location of two methyl groups and one methoxyl group in I could be assumed to be the same as that of other carbazomycins such as III or IV and the carbonyl group should be located at C(4). Consequently, the whole structure can be proposed as I in Fig. 1. In order to establish the structure and the stereochemistry, especially at C(1), of I, an X-ray crystallographic analysis of I was undertaken.

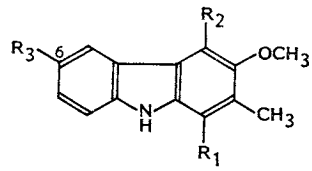
Fig. 1. Structures of carbazomycins G and H.



Carbazomycin G (I) R=H
Carbazomycin H (II) R=OCH_3

Crystals of I suitable for an X-ray analysis were grown from dimethyl sulfoxide solution and belong to the monoclinic, space group $P2_1/a$. The lattice constants and intensity data were obtained on a Syntex R3 computer-controlled four circle diffractometer using MoKα radiation monochromated by a graphite plate. The crystal data and the course of structure determination are summarized in Table 4. A total of 1305 reflections was measured by the ω scan method within a 2θ range of 3° through 50°; of these, 1076 reflections with intensities above the 1.96σ (I) level were considered to be observed, but all 1305 reflections were used for the structure determination. The crystal structure was solved by direct methods using the program MULTAN[7] and refined by a block diagonal least-squares method. All atoms in the molecule were located on the difference electron-density maps. The final R value was 0.039, assuming anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms. An ORTEP drawing[8] of the final X-ray model for I together

Fig. 2. Structures of carbazomycins.



Carbazomycin B (III)	$R_1=CH_3$	$R_2=OH$	$R_3=H$
Carbazomycin A	$R_1=CH_3$	$R_2=OCH_3$	$R_3=H$
Carbazomycin C (IV)	$R_1=CH_3$	$R_2=OH$	$R_3=OCH_3$
Carbazomycin D (V)	$R_1=CH_3$	$R_2=R_3=OCH_3$	
Carbazomycin E (carbazomycinal)	$R_1=CHO$	$R_2=OH$	$R_3=H$
Carbazomycin F (6-methoxycarbazomycinal)	$R_1=CHO$	$R_2=OH$	$R_3=OCH_3$

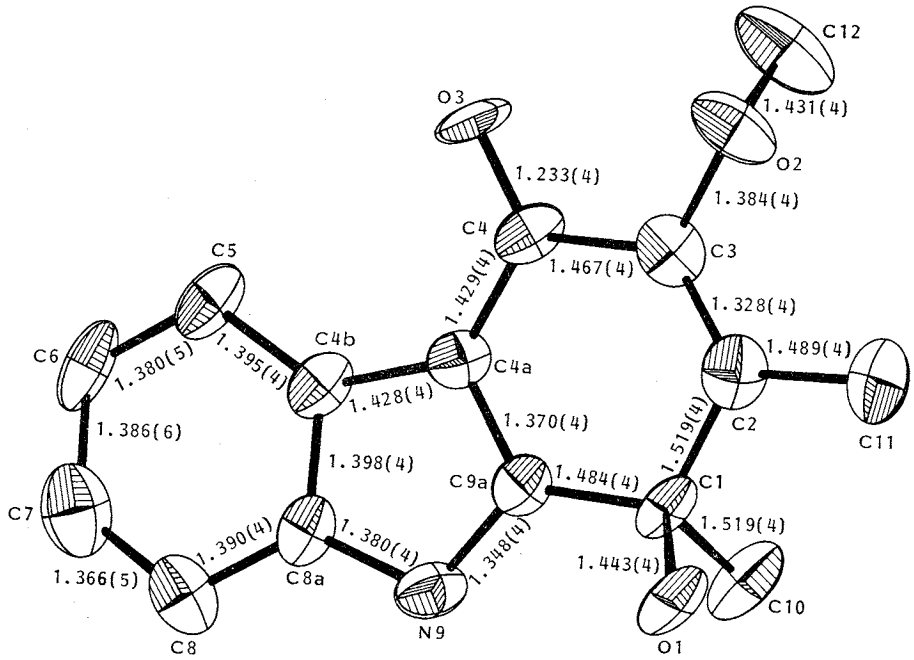
Table 4. Crystal data and the course of structure determination of carbazomycins G (I) and C (IV).

	I	IV
Chemical formula	$C_{15}H_{15}NO_3$	$C_{16}H_{17}NO_3$
MW	257.1	271.1
Crystal habit	Plate	Plate
Specimen size (mm)	$0.7\times0.5\times0.15$	$1.0\times0.7\times0.2$
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/a$	$P2_1/n$
Lattice constants		
a (Å)	9.847 (3)	6.552 (3)
b (Å)	12.571 (5)	27.72 (1)
c (Å)	11.085 (9)	15.038 (6)
β (°)	109.33 (5)	101.69 (3)
U (Å^3)	1294.8	2674.9
No. of formula unit in a cell	4	8
Calculated density (g/cm^3)	1.32	1.35
μ for MoKα (cm^{-1})	1.0	1.0
2θ range (°)	3~50	3~50
No. of possible reflections	1305	2220
No. of observed reflections	1076	1859
Method of structure determination	Direct	Direct
Method of structure refinement	Block-diagonal least-squares	Block-diagonal least-squares
No. of refined atoms		
Heavier atoms (anisotropic)	19	40
Hydrogen atoms (isotropic)	15 (all H)	32 (out of 34 in all)
Final R value	0.039	0.079

with bond lengths between the heavier atoms is shown in Fig. 3. Hydrogen atoms are omitted for clarity. Thus, the chemical structure of carbazomycin G has been determined unambiguously as that shown in Fig. 1. Carbazomycin G has an asymmetric center at C(1), but it does not show optical rotation and the crystals belong to the space group $P2_1/a$ which needs pairs of enantiomers. Therefore, carbazomycin G exists as a racemate.

All oxygen and nitrogen atoms except the methoxyl oxygen at C(3) are involved in intermolecular hydrogen bonds. The hydroxyl group O(1)H at C(1) donates hydrogen to the carbonyl oxygen O(3),

Fig. 3. ORTEP drawing of the molecule of carbazomycin G with bond lengths (Å) between the heavier atoms.



and the N(9)H donates hydrogen to the hydroxyl oxygen O(1) [O(1) — O(3)^{i}, 2.739(3) Å; O(1)H — O(3)^{i}, 1.88(4) Å], [N(9) — O(1)^{ii}, 2.842(3) Å; N(9)H — O(1)^{ii}, 1.87(3) Å] where i is at $-1/2+x$, $1/2-y$, z and ii is at $-x$, $1-y$, $1-z$, and others are at x, y, z. By these intermolecular hydrogen bonds, the molecules are held together in the crystal. No intramolecular hydrogen bonds are observed.

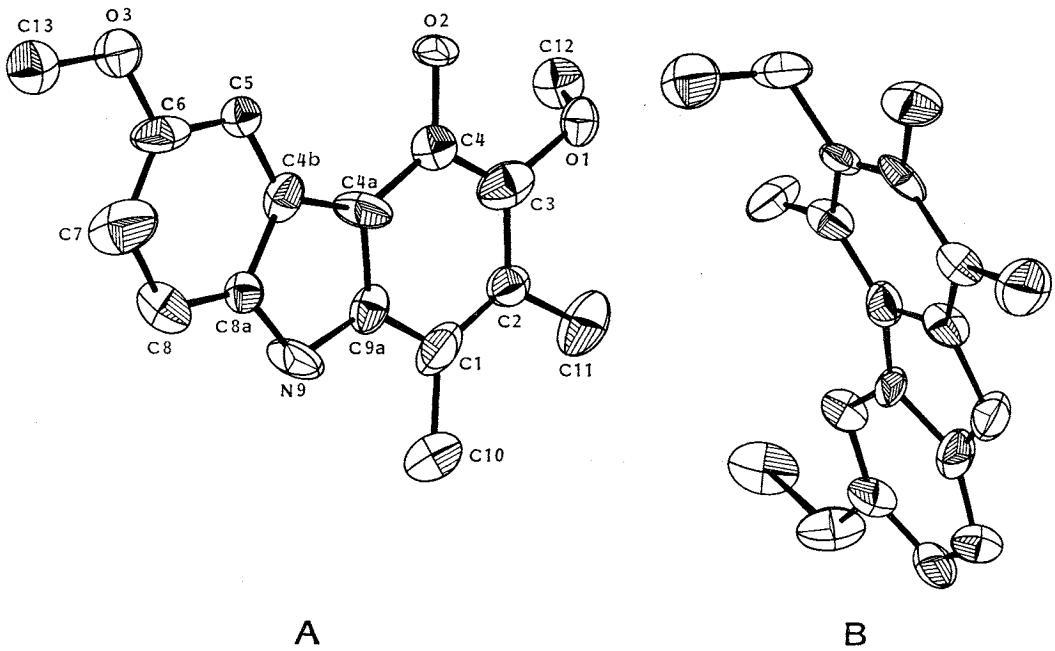
The modified carbazole skeleton of carbazomycin G formed by 13 atoms, C(1) through C(9a) and N(9), approaches planarity with an average deviation of ±0.053 Å. The hydroxyl oxygen and the methyl carbon at C(1) lie on opposite sides of the above least-squares plane with distances of 1.346 Å and 1.023 Å, respectively.

Comparison of the molecular formulae and the ^{1}H and ^{13}C NMR data of **I** and **II** suggests that one aromatic hydrogen atom in **I** is replaced by a methoxyl group on going from **I** to **II** with the remainder of the structure being identical. Furthermore, the methoxyl group should be bonded to C(6) as in **IV**[1] because the chemical shifts and the coupling system of the three aromatic protons of **II** were the same as those of **IV** as shown in Table 2. The carbon chemical shift changes on going from **I** to **II** caused by introduction of a methoxyl group at C(6) were almost identical with those observed in going from **III** to **IV**[1] as shown in Table 3. Based on these arguments, the structure of carbazomycin H is established as **II** in Fig. 1.

Structure Confirmation of Carbazomycins C and D by X-Ray Analysis

Crystals of carbazomycin C (**IV**) suitable for an X-ray analysis were grown from *n*-hexane and ethyl acetate solution and belong to the monoclinic, space group $P2_1/n$. The crystal data and the course of structure determination are also summarized in Table 4. The asymmetric unit contains two independent molecules A and B, as was found also in the crystal of carbazomycin B[4].

Fig. 4. ORTEP drawing of the molecules A and B of carbazomycin C.



A total of 2220 reflections was measured within a 2θ range of $3\sim50°$; of this total, 1859 reflections with intensities above 1.96σ (I) level were considered to be observed and were used for the structure determination.

The crystal structure was solved by direct methods using the program MULTAN[7] and refined by a block diagonal least-squares method. The atomic species were assigned on the basis of the Fourier map and the temperature factors with the help of chemical considerations. All non-hydrogen atoms and 32 out of 34 hydrogen atoms in the asymmetric unit were located on the difference electron-density maps. The final R value was 0.079, assuming anisotropic temperature factors for 32 heavier atoms and isotropic ones for 32 hydrogen atoms. An ORTEP drawing[8] of the molecules A and B of carbazomycin C in the asymmetric unit is shown in Fig. 4. Hydrogen atoms are omitted for clarity. The bond lengths agree well with the chemical structure.

The hydroxyl groups O(2)H at C(4) of each molecule form intramolecular hydrogen bonds to the methoxyl oxygen O(1) [A-O(2) — A-O(1)†, 2.760(9) Å; B-O(2) — B-O(1), 2.780(9) Å]. Several intermolecular hydrogen bonds summarized in Table 5 hold the molecules A and B in the crystal to form a three-dimensional network.

The carbazole skeleton of carbazomycin C formed by 13 atoms, C(1) through C(9a) and N(9), is quite planar with an average deviation of ±0.026 Å in the case of the molecule A. All other non-hydrogen atoms except for the methoxyl carbon C(12) are almost coplanar with this skeleton plane, while C(12) deviates by 1.138 Å from this least-squares plane. Thus, the chemical structure of carbazomycin C has been established unambiguously. As carbazomycin D had been shown to be carbazomycin C monomethyl ether[1], its structure has also been confirmed. The final atomic parameters for carbazomycins G and C have been sent to the Cambridge Crystallographic Data Center.

† A-O(1) designates O(1) in the molecule A.

Table 5. Intermolecular hydrogen bonds (Å) involved in the crystal of carbazomycin C.

A-HO(2) — A-O(3)[i]	2.876 (8)
A-O(1) — B-O(2)	2.845 (8)
A-N(9) — B-O(3)[ii]	2.890 (9)
B-N(9) — B-O(1)[iii]	2.963 (8)

Symmetry codes: i at $-x, -y, -z$. ii at $2-x, -y, 1-z$. iii at $1/2+x, -1/2-y, 1/2+z$. Others at x, y, z.

Antimicrobial Properties of Carbazomycins G and H

Carbazomycin G showed inhibitory activity against some kinds of fungi, particularly *Trichophyton mentagrophytes* 833 and *Trichophyton asteroides* 429, as shown in Table 6, but carbazomycin H showed no significant activity against the microorganisms examined below the concentration of 100 μg/ml.

Table 6. Antimicrobial spectrum of carbazomycin G (I).

Test organisms	MIC (μg/ml)
Aspergillus niger F-16	>100
Colletotrichum gloeosporioides	100
C. lagenarium	100
Gloeosporium laeticolor	100
Glomerella cingulata	100
G. cingulata No. 3	100
Trichophyton asteroides 429	25
T. mentagrophytes 833	6.25

Agar dilution method on potato - sucrose agar. Incubated at 26°C for 48 hours.

Experimental

MP's were measured on a Yanaco hot plate apparatus and are uncorrected. UV spectra were measured on a Hitachi model 124 spectrophotometer. IR spectra were recorded on a Shimadzu IR 408 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured with a Jeol JNM-FX 100 spectrometer at 99.55 MHz and 25.00 MHz, respectively. Mass spectrometry was carried out using a Shimadzu 7000S and a Jeol JMS-DX 300 (JMA 3100 data system) mass spectrometer by the direct inlet method. For analytical and preparative TLC, Merck precoated Silica gel plates, Art. No. 5715, were used. For column chromatography, Merck Silica gel 60, Art. No. 7734, was used.

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