THE STRUCTURE OF CARBAZOMYCIN B

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The structure of carbazomycin B was determined to be 4-hydroxy-3-methoxy-1,2-dimethylcarbazole by ^{1}H- and ^{13}C-nmr studies of carbazomycin B and its derivatives, and was unequivocally confirmed by X-ray crystallographic analysis.

Carbazomycins A and B were isolated mainly from the cultured mycelia of an unidentified Streptomyces which was also proved to produce viomycin simultaneously. Carbazomycin B was the main antibiotic active against some kinds of phytopathogenic fungi and further showed weak antibacterial and antiyeast activities, while carbazomycin A was the minor component and showed extremely weak biological activities against the above microbes.

The more polar pale yellow substance, carbazomycin B (I), mp 137.5-138.0°, had a molecular formula $C_{15}H_{15}NO_2$ and its ir spectrum showed an aromatic system with an -NH- function and a hydroxyl group. The uv spectrum of I was very similar to those of carbazole derivatives suggesting the presence of a carbazole skeleton. The ^{1}H-nmr spectrum of I revealed the presence of an -NH- function, one phenolic hydroxyl, one aromatic methoxyl and two aromatic C-methyl groups besides four aromatic protons. From these data carbazomycin B (I) was suggested to be a carbazole derivative with one hydroxyl, one methoxyl and two methyl groups on the nucleus.[1] Zinc dust distillation of I afforded carbazole confirming the

carbazole skeleton in the antibiotic.[1] The less polar pale yellow compound, carbazomycin A (II), mp 51.0-52.5°, $C_{16}H_{17}NO_2$, was similarly suggested to be a carbazole derivative with two methoxyl and two methyl groups on the nucleus by spectroscopic means, and was further identified as carbazomycin B monomethyl ether by the fact that methylation of I with diazomethane yielded II.[1]

In this communication we report results which unambiguously determine the structure of carbazomycin B (I).

In the ^{1}H-nmr spectrum of I (Table 1), the double doublet at δ 8.31 (1H, J=7.0, 2.0 Hz) can be assigned to 5-H since the aromatic proton signal due to 4-H or 5-H of carbazole derivatives usually appears at a fairly lower field than those of the other aromatic protons.[2] The existence of this double doublet at δ 8.31 and the absence of an aromatic proton singlet in the ^{1}H-nmr spectrum suggested a unique 1,2,3,4-tetrasubstituted carbazole structure. This was also inferred, though indirectly, from a strong ir band at 750 cm^{-1} which could be attributed to the 4b,8a-ortho-disubstituted benzene ring.

The location of the methoxyl group of I was proved to be on C-3 of the carbazole nucleus by the characteristic fragment ion peak at m/e 198 ($[M-CH_3-CO]^+$) in its mass spectrum as illustrated in Fig. 1.[2d]

Carbazomycin B (I) was converted to O-tosylcarbazomycin B with p-TsCl, which was subsequently reduced with Raney nickel to give deoxycarbazomycin B (III).[2b,d] A new aromatic proton singlet appeared at δ 7.45 in the ^{1}H-nmr spectrum of III as seen in Table 1 and this signal showed nuclear Overhauser effect (NOE) of ca 20 % enhancement on irradiation of the C-3 methoxyl group signal (δ 3.98), but no NOE was detected on irradiation of each methyl group signal (δ 2.47, 2.36). Therefore, this new proton singlet at δ 7.45 must be assigned to 4-H or 2-H, being ortho to 3-OMe group, but the possibility of 2-H was ruled out by the expectation that the 2-H proton signal would appear at an appreciably higher field, probably at δ 6.5-7.0 ppm region, by the ortho-substituent effects of 3-OMe and 1-Me groups.[4]

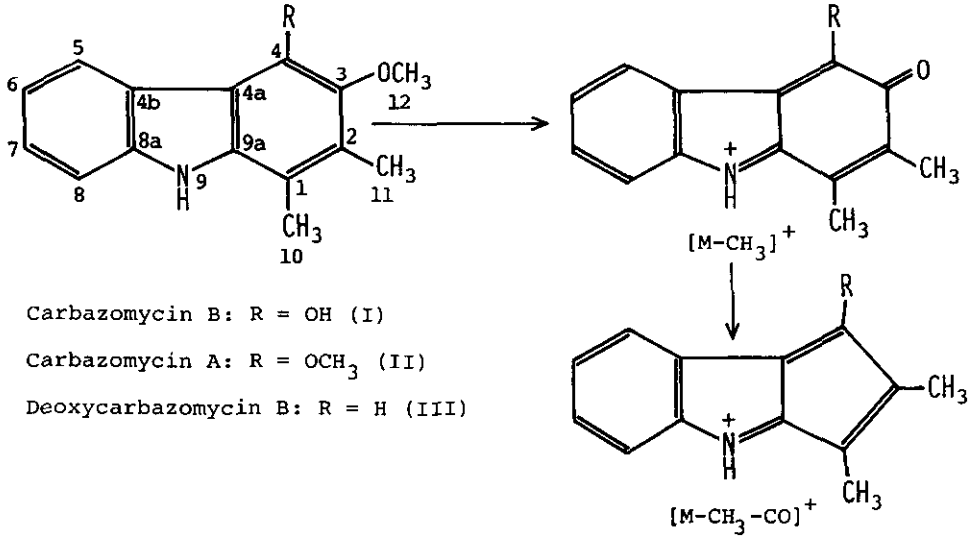
Further supports for the structure assignment of carbazomycin B (I) were obtained from the ^{13}C-nmr spectra of I-III shown in Table 2. When the spectra of I and III were compared, the C-3 signal was shifted upfield by 10.6 ppm on going from III to I by the introduction of an ortho-related OH group.[5]

Table 1. ^{1}H-Chemical Shifts of Carbazomycins[3]

Protons	Carbazomycin B (I) R=OH	Carbazomycin A (II) R=OMe	Deoxycarbazomycin B (III) R=H
4-H	-	-	7.45 s
5-H	8.31 dd(J=7.0, 2.0)	8.25 dd(J=7.0, 2.0)	8.08 dd(J=7.0, 1.5)
6,7,8-H	7.14-7.38 m	7.13-7.42 m	7.16-7.52 m
1-Me	2.36 s	2.40 s	2.47 s
2-Me	2.28 s	2.40 s	2.36 s
3-OMe	3.80 s	3.92 s	3.98 s
4-OH	6.21 s	-	-
4-OMe	-	4.13 s	-
NH	7.71 br.s	7.89 br.s	8.00 br.s

Spectra were recorded at 100 MHz in $CDCl_3$; chemical shifts are δ in ppm from internal TMS; coupling constants (J) are in Hz; signals are designated as follows: s, singlet; dd, double doublet; m, multiplet; br.s, broad singlet.

Fig. 1. Carbazomycins and Their Fragmentations by Mass Spectroscopy



Carbazomycin B: R = OH (I)

Carbazomycin A: R = OCH_3 (II)

Deoxycarbazomycin B: R = H (III)

Table 2. ^{13}C-Chemical Shifts of Carbazomycins[3]

Carbon No.	Multiplicity	I	II	III	Carbon No.	Multiplicity	I	II	III
C-1	s	110.0*	114.4*	119.5*	C-7	d	124.7	125.0	124.9
C-2	s	127.0	128.7	124.2*	C-8	d	110.0	110.3	110.7
C-3	s	142.0	144.4†	152.6	C-8a	s	139.3	139.4	139.6
C-4	s	138.5	145.9†	99.0[a]	C-9a	s	136.8	136.4	134.2
C-4a	s	109.3*	113.5*	118.5*	C-10	q	12.7	12.6	12.3
C-4b	s	123.3	122.8	120.1*	C-11	q	13.1	13.6	13.8
C-5	d	122.7	122.5	119.8	C-12	q	61.4	61.1	56.2
C-6	d	119.5	119.4	118.9	C-13	q	-	60.5	-

All values are δ ppm from internal TMS recorded at 25.15 MHz in $CDCl_3$. s, singlet; d, doublet; q, quartet. a) doublet in this case
*,†) Assignments bearing the same superscript in any one spectrum may be interchanged.

Furthermore, the 3-OMe (C-12) signal of III appeared at 56.2 ppm, while the signals of 3-OMe (C-12) of I and 3-OMe (C-12) and 4-OMe (C-13) of II, being ortho-disubstituted, were shifted downfield and resonated at 61.4, 61.1 and 60.5 ppm, respectively.[6] Because of this congested environment, on the contrary, the two methyl carbon signals (C-10 and C-11) appeared at higher field, such as 12.7 (C-10) or 13.1 (C-11) ppm in I, than ordinary methyl signals (15-25 ppm),[7] and remained almost unshifted in the spectra of I, II and III.

From the above results, the structure of carbazomycin B (I) was deduced to be 4-hydroxy-3-methoxy-1,2-dimethylcarbazole, and in order to establish this molecular structure, X-ray crystallographic analysis of carbazomycin B was carried out.

Crystal data: $C_{15}H_{15}NO_2$, M = 241.3, monoclinic, space group $P2_1/a$, a = 9.681(6), b = 22.237(11), c = 12.390(5) Å, β = 107.53(4)°, Z = 8, U = 2543.5 Å^3, Dx = 1.260 g·cm^{-3}.

The intensity data were measured on a Rigaku AFC-5 automatic four-circle diffractometer, equipped with a rotating anode tube (50 kV, 170 mA), by the θ-2θ scan method using graphite-monochromated Cu$K\alpha$ radiation (λ = 1.54184 Å). Out of the total of 5126 independent reflections within a 2θ value of 145°, 4032 reflections having intensities above $2\sigma(I)$ level were used for the structure determination and refinement. The intensities were corrected for Lorentz-

polarization factors and were placed on an absolute scale by Wilson's method, but no absorption correction was made. The size of the crystal was about 0.1 x 0.2 x 0.4 mm.

The crystal structure was solved by the direct method using the MULTAN[8] program. An E map calculated for the most probable set revealed 30 plausible non-hydrogen atoms. The remaining six atoms were located by successive use of difference Fourier and least-squares methods. After block-diagonal least-squares refinement all 30 hydrogen positions were located on the difference electron density maps. The final R factor was 0.085 including anisotropic thermal parameters for the non-hydrogen atoms and isotropic ones for the hydrogen atoms.

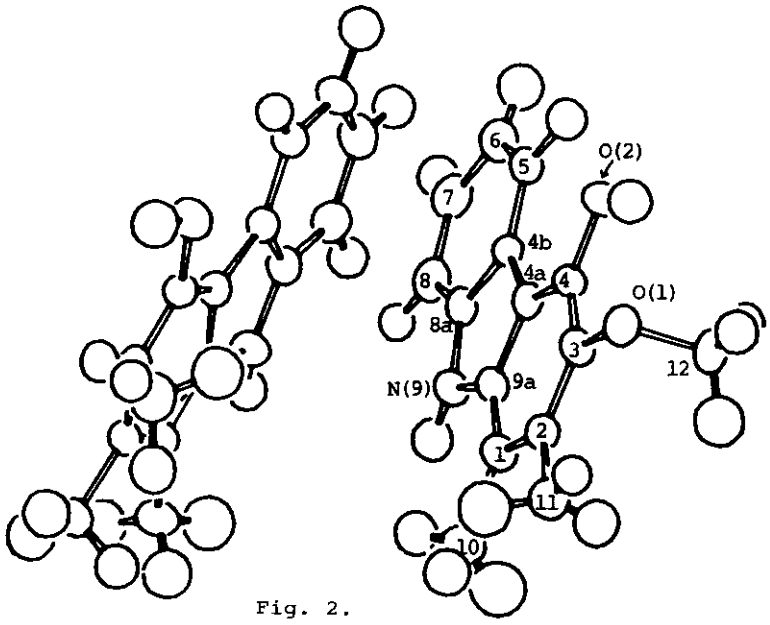


Fig. 2.

A perspective drawing[9] of the independent two molecules of carbazomycin B in the asymmetric unit is shown in Fig. 2, together with the atomic labeling which is in accordance with that of Fig. 1.

Thus, the structure of carbazomycin B has unambiguously been established.

Carbazomycins A and B are the first antibiotics having a carbazole skeleton and their congested and one-sided substitution pattern is interesting from a viewpoint of biosynthesis, and biosynthetic studies of them are now being performed in our laboratory.

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