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THE STRUCTURES OF MACBECIN I AND II, NEW ANTITUMOR ANTIBIOTICS

Masayuki Muroi*, Konomi Haibara, Mitsuko Asai and Toyokazu Kishi
Central Research Division, Takeda Chemical Industries, Ltd.,
Yodogawa-ku, Osaka 532, Japan

Summary Structures of macbecin I (1) and II (2), new antitumor antibiotics isolated from the culture broth of Nocardia sp. No. C-14919, have been elucidated as benzenoid ansamycins on the basis of chemical evidence and spectral analyses.

Macbecin I (1) and II (2) are antitumor antibiotics produced by Nocardia sp. No. C-14919, and also show antibacterial, antifungal, and antiprotozoal activities.[1,2] In this paper, we wish to report the structural elucidation of 1 and 2.

Macbecin I (1), mp 187-188°C (dec.), $[\alpha]_D$ +351° ($CHCl_3$), $C_{30}H_{42}N_2O_8$ (MS, M^+ m/e 558.2951; Calcd. for $C_{30}H_{42}N_2O_8$: 558.2941), had UV λ_{max}^{MeOH} 274 (ε 25,510) and 397 nm (ε 2,400), IR ν_{max}^{KBr} 1740 (ester C=O), 1692, 1660, 1605 (quinonoid C=O and C=C) and 1645 cm^{-1} (amide C=O). ^{1}H-NMR ($CDCl_3$, Table 1) indicated the presence of the following groups; three doublet CH_3, two vinyl CH_3, three OCH_3, four olefinic protons, two methine protons attached to oxygen functions, two quinone ring protons (δ 6.62 and 7.33, each 1H, J=2.5 Hz), one NH_2 and one amide NH proton.

Table 1. ^{1}H-NMR Chemical Shifts of 1 and 2

	>CH-CH$_3$ (3H,d)	=CH$_3$ (3H,d or bs)	-OCH$_3$ (3H,s)	-O-CH-	=CH-	ring H	-NH$_2$	phenol OH	CONH
1 ($CDCl_3$) δ ppm	0.80 1.04 1.10	1.53 2.01	3.30 3.33 3.56	4.60(dd) 5.78(d)	5.30(bd) 5.67(dd) 6.35(bt) 7.14(bd)	6.62(dd) 7.33(d)	4.61(bs)		8.87(s)
2 (d_6-DMSO) δ ppm	0.63 0.82 0.89	1.16 1.83	3.13 3.24 3.39	4.54(d) 4.64(d)	5.02(d) 5.21(d) 5.86(t) 5.98(d)	6.35(d) 6.45(d)	6.23(bs)	7.51(s) 8.64(s)	8.85(s)

Macbecin II (2), $C_{30}H_{44}N_2O_8$, mp 148°C (dec.), $[\alpha]_D$ +62° (MeOH), showed UV λ_{max}^{MeOH} 255 (ε 16,800) and 308 nm (sh.).

The functional groups in ^{1}H-NMR (d_6-DMSO) of 2 are shown in Table 1. 2 was obtained also by reduction of 1 with reducing agents such as $Na_2S_2O_4$, whereas 2 was again readily oxidized to 1 with oxidizing agents such as $FeCl_3$.

Table 2. ^{13}C-NMR Chemical Shifts of 1 and 2 (d_6-DMSO)

	C=O (s)	>C= (s)	-CH= (d)	O-CH- (d)	-OCH_3 (q)	-CH- (d)	-CH_2- (t)	-CH_3 (q)
1	186.7 182.8 170.4 155.9	145.0 141.2 133.4 132.1	140.4 131.9 129.0 128.1 123.5 113.8	82.8 81.5 79.0 77.7	59.8 57.3 55.6	35.2 34.3 33.5	30.3	17.6 15.3 14.2 13.3 12.6
2	172.8 156.0	150.5 141.0 133.0 132.1 129.8 129.2	135.5 131.1 123.4 (x2) 110.6 109.6	83.7 81.5 80.8 80.6	60.3 56.2 (x2)	34.9 34.4 34.2	26.6	18.1 17.6 14.9 13.5 11.8

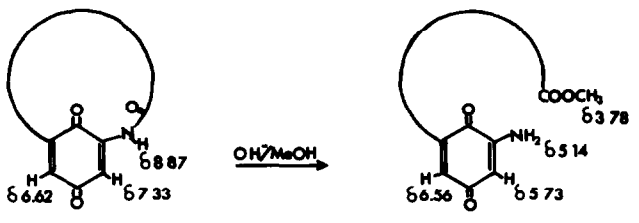
^{13}C-NMR of 1 and 2 (Table 2) confirmed 30 carbon atoms and the functional groups as shown in the table, respectively.

The spectral data described above indicated that 1 is a 2,6-disubstituted benzoquinone and 2 is its hydroquinone form. Although 1 did not give any acetates under usual conditions with Ac_2O-pyridine, 2 afforded two kinds of acetates, i.e., diacetate (3), $C_{34}H_{48}N_2O_{10}$, mp 194-195°C (dec.), $[\alpha]_D$ +90.5° ($CHCl_3$), ^1H-NMR, δ 2.20 (-OAc x 2), 7.62 (-CONH-) and triacetate (4), $C_{36}H_{50}N_2O_{11}$, mp 143-144°C (dec.), $[\alpha]_D$ +88.5° ($CHCl_3$), ^1H-NMR; δ 2.20 and 2.26 (-OAc), 2.45 (-NAc).

The presence of three double bonds in the side chains of 1 and 2 was assigned from the ^1H and ^{13}C-NMR spectra of 1 and 2, and in addition the UV absorption maximum at 255 nm (ε 16800) in 2 and the ^1H-NMR spectra of 1 and 2 suggested the presence of dienamide.[3]

Mild alkali treatment of 1 with $NaHCO_3$-MeOH afforded a reddish brown product (5), $C_{31}H_{46}N_2O_9$, mp 79-80°C (dec.), $[\alpha]_D$ +179° ($CHCl_3$). 5 had λ_{max}^{MeOH} 270 (ε 29,800) and 485 nm (ε 1950) in the UV and visible spectra, showing a typical spectrum of aminobenzoquinonoid.[4] ^1H-NMR spectrum of 5 revealed four methoxyl signals at δ 3.25, 3.35, 3.50 and 3.78, indicating that one methoxyl group increased in 5. The signal of NH_2 group was newly observed at δ 5.14 instead of disappearance of the amide NH signal in 1, and one of two benzoquinonoid ring protons at δ 7.33 (1H, d) in 1 shifted upfield to δ 5.73 (1H, d).

These changes between 1 and 5 clearly demonstrated that the amide linkage attached to benzoquinone in 1 was cleaved to give free aminobenzoquinone and the end COOH group of the other substituent of 1 was methylated. This indicated that two substituents of 5 constituted an ansa-bridge in 1.

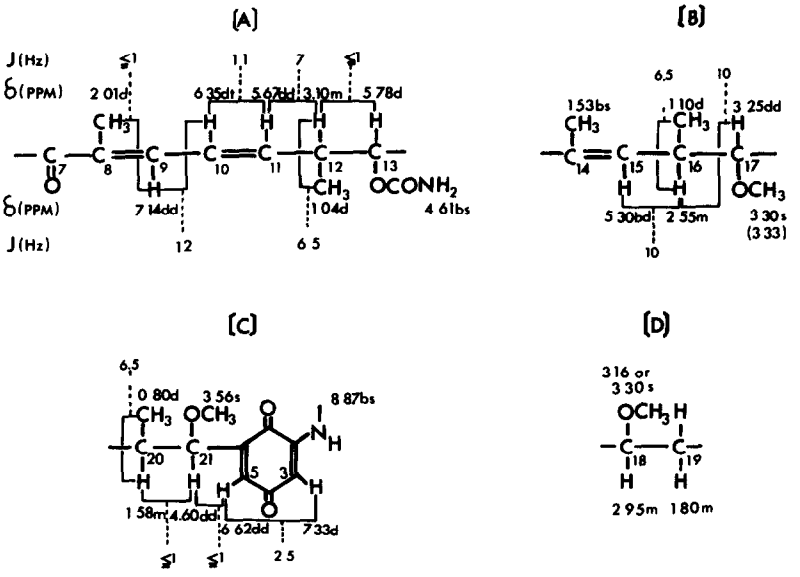


1 5

The IR absorption band of 1 at 1740 cm^{-1} (ester C=O), a significant peak of m/e 515.2905 (calcd. for $C_{29}H_{41}NO_7$· 515.2883) due to [M^+ -NHCO] fragment in the high-resolution mass spectrum and the signals (C=O) at δ 155.9 of 1 and 156.0 of 2 in the ^{13}C-NMR spectra indicated the presence of the carbamate (-$OCONH_2$) group. Acid hydrolysis of 1 with d·HCl in dioxane afforded decarbamoyl products, $C_{29}H_{41}NO_7$ (MS, M^+ m/e 515), which consisted of three isomers, 6, mp >300°C (dec.), $[\alpha]_D$ +337.7° ($CHCl_3$), 7, mp >300°C (dec.), $[\alpha]_D$ +244.5° ($CHCl_3$), and 8, mp 178-179°C (dec.), $[\alpha]_D$ +144° ($CHCl_3$), and lost the ester C=O in the IR spectra.

These data indicated that the carbamate group (-$OCONH_2$) is located at the allylic position of isolated double bond.

Detailed spin decoupling experiments on 1 (in $CDCl_3$) clarified the structural units, A, B and C.



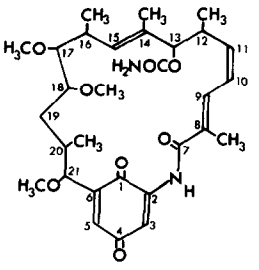
The partial structures of 5 were also assigned by spin decoupling studies. One of the quinonoid protons in 1 and 5 was coupled to the other quinonoid ring proton and O-benzylic proton. In addition to the structural units, A, B and C was confirmed the unit D (-CH_2-$\overset{1}{C}$H-OCH_3) by spin decoupling of 3.

The total arrangement of these structural units, A, B, C and D was assigned as follows. The methine proton of -$\overset{1}{C}$H-$OCONH_2$ (δ 5.78) was only coupled to the methine proton of δ 3.10, and hydrolysis of 1 readily yielded decarbamoyl products consisting of three isomers through allylic rearrangement. It is evident that the carbamate group is located at the allylic position of the isolated olefine in the unit B, indicating the connection of C_{13} with C_{14}.

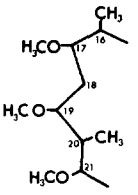
Conversion of 1 to the aminobenzoquinone 5 by alkaline methanolysis indicated that

the amino group of the 2-amino-p-benzoquinone was linked to the α,β,γ,δ-conjugated carboxyl group to form macrolactam.

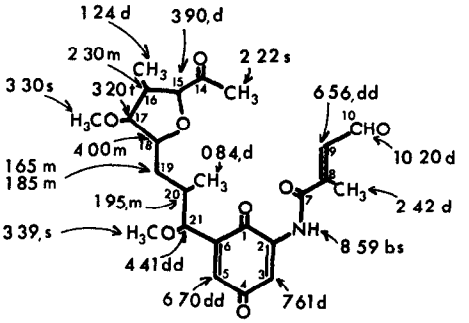
Because the junction of the unit D is not decisive, two tentative structures 1a and 1b were given to macbecin I (1).



1a

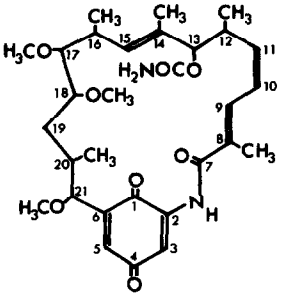


1b

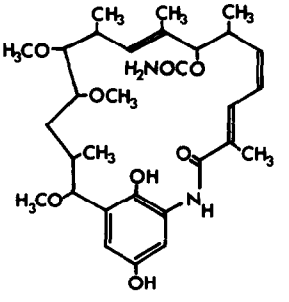


9

In order to decide the position of the remaining OCH_3, the normal decarbamoyl product (6) was epoxidized with m-chloroperbenzoic acid and then oxidized with HIO_4 to afford a product 9, $C_{24}H_{31}NO_8$, $[\alpha]_D$ +129.1° ($CHCl_3$), (MS, M^+ m/e 461) which was a cleavage compound between C_{10} and C_{11}, and between C_{13} and C_{14}. The structure of 9 was clarified by spin decoupling as shown above, and it led to the conclusion that macbecin I (1) is a benzoquinonoid ansamycin represented by 1a and macbecin II (2) is its hydroquinone form.[5]



1



2

References and Notes

1. S. Tanida, T. Hasegawa and E. Higashide, J. Antibiotics, paper in preparation.
2. M. Muroi, M. Izawa, Y. Kozai and M. Asai, J. Antibiotics, paper in preparation.
3. D. Peters, J. Chem. Soc., 1832 (1960).
4. K. Sasaki, K. L. Rinehart, Jr., G. Slomp, M. F. Grostic and E. C. Olson, J. Am. Chem. Soc., 92, 7591 (1970).
5. X-Ray analysis of the N-bromoacetyl derivative of macbecin I (1) confirmed these structures 1 and 2 and in addition determined the absolute stereochemistry of 1 and 2. The details including the X-ray analysis will be reported in the near future.

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