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THE STRUCTURES OF MACBECIN I AND II

NEW ANTITUMOR ANTIBIOTICS

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Abstract—Macbecin I (1), $C_{30}H_{42}N_2O_8$, and macbecin II (2), $C_{30}H_{44}N_2O_8$, were shown to be 2,6-disubstituted benzoquinone and hydroquinone derivatives by an oxidation-reduction relationship, UV, ^1H and ^{13}C NMR spectra. Alkaline methanolysis of 1 gave a 2-aminobenzoquinone derivative (5), suggesting an ansa-structure for 1, and acid hydrolysis of 1 gave decarbamoyl products 9, 10 and 11, indicative of the location of carbamoyloxy group in allylic position. Spin decoupling studies on 1, 3 and 5 clarified the partial structures [A], [B], [C] and [D]. From their mutual disposition two structures 1a and 1b, were proposed out of which 1a has been selected for the structure of 1 on the basis of the structure of oxidative degradation product 12. X-Ray analysis of the bromoacetyl derivative of 1 confirmed the above proposed structure and determined the absolute stereochemistry of 1 and 2.

Macbecin I (1) and II (2) are new antitumor antibiotics produced by *Nocardia* sp. No. C-14919, with antibacterial, antifungal and antiprotozoal activities.[1,2] Details of the isolation, characterization and antitumor activity have been reported.[2] In a preliminary communication,[3] the structures of 1 and 2 were presented. In this paper, we report the structural determination of 1 and 2 on the basis of chemical studies and X-ray analysis.

Macbecin I (1), m.p. 187–188° (dec), $[\alpha]_D + 351°$ ($CHCl_3$), gave a molecular ion peak at *m/e* 558.2951 (Calc. for $C_{30}H_{42}N_2O_8$; 558.2941) in high-resolution mass spectrum, and the molecular formula, $C_{30}H_{42}N_2O_8$, was given to 1 in conjunction with its elemental analysis. 1 showed absorption maxima at 274 nm (ϵ 25,510) and 397 nm (ϵ 2400) in the UV and visible spectra and had characteristic bands at 1740 (ester C=O), 1692, 1660 and 1605 (quinonoid C=O and C=C) and 1645 cm^{-1} (amide C=O) in the IR spectrum.

Its ^1H NMR spectrum (Table 1) indicated the presence of the following groups; three doublet Me, two vinyl Me, three OMe, two methine protons attached to O functions, four olefinic protons, two quinone ring protons (each 1H, J = 2.5 Hz), one NH_2 and one amide NH proton. The ^{13}C NMR spectrum of 1 (Table 2) revealed the following 30 C atoms; four C=O, four singlet olefinic carbons, six doublet olefinic carbons, four doublet carbons attached to O functions, three OMe carbons, three methine carbons, one methylene carbon and five Me carbons.

Macbecin II (2), $C_{30}H_{44}N_2O_8$, m.p. 148° (dec), $[\alpha]_D +$ 62° (MeOH) had λ_{max}^{MeOH} 255 nm and a shoulder at 308 nm. 2 was obtained by reduction of 1 with $Na_2S_2O_4$, whereas 2 was readily oxidized to 1 with $FeCl_3$. This reversibility suggested that 1 is a quinone and 2 is its hydroquinone form. UV absorption at 308 nm in 2 suggested that 2 is a substituted hydroquinone derivative.[4]

Like that of 1, ^1H NMR of 2 (Table 1) showed the

Table 1. ^1H 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)

Table 2. ^{13}C NMR chemical shifts of 1 and 2 (in 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 (×2) 110.6 109.6	83.7 81.5 80.8 80.6	60.3 56.2 (×2)	34.9 34.4 34.2	26.6	18.1 17.6 14.9 13.5 11.8

signals of three doublet Me, two vinyl Me, three OMe, two methine protons attached to O functions at δ 4.54 and 4.64, four olefinic protons, and two aromatic protons at δ 6.35 and 6.45 (each 1H, J = 2.5 Hz, *m*-coupling) instead of quinonoid protons of **1**. Although the ^{13}C NMR spectrum of **2** revealed similar signals to **1**, four C=O carbons in **1** decreased to two C=O carbons and two aromatic or olefinic carbons increased in **2**. The spectral data described above indicated that **1** is a 2,6-disubstituted benzoquinone and **2** is its hydroquinone form.

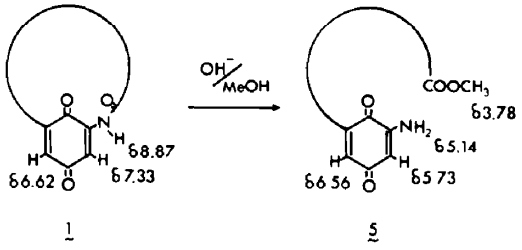
Macbecin I (**1**) did not give any acetates with Ac_2O–pyridine under usual conditions, showing that **1** does not contain any primary or secondary OH and ordinary basic amino group in spite of the NH_2 signal observed in ^1H NMR.

On the other hand, macbecin II (**2**) afforded two kinds of acetates, i.e. diacetate **3**, $C_{34}H_{48}N_2O_{10}$, m.p. 194–195° (dec), $[\alpha]_D$ +90.5° ($CHCl_3$) and triacetate **4**, $C_{36}H_{50}N_2O_{11}$, (MS, M^+ *m/e* 686), m.p. 143–144° (dec), $[\alpha]_D$ +88.5° ($CHCl_3$). Diacetate **3** showed two OAc signals at δ 2.20, while triacetate **4** exhibited an additional OAc signal at δ 2.45 besides δ 2.20 and 2.26. Furthermore, the amide NH proton at δ 7.62 in **3** disappeared in **4**, suggesting that the amide NH was also acetylated and the amide group is directly bound to the aromatic ring.

The presence of three double bonds in the side chains of **1** and **2** was assigned from their ^1H and ^{13}C NMR spectra and in addition the UV absorption maximum at 255 nm (ϵ 16,800) in **2** and the ^1H NMR spectra of **1** and **2** suggested the presence of dienamide group.[5]

Mild alkaline methanolysis of **1** with $NaHCO_3$–MeOH afforded two products, i.e. reddish brown compound **5**, $C_{31}H_{46}N_2O_9$ (MS, M^+ *m/e* 590; M^+ +2 *m/e* 592), m.p. 79–80° (dec), $[\alpha]_D$ +179° ($CHCl_3$) and reddish orange compound **6**, $C_{30}H_{42}N_2O_9$, m.p. 218–219° (dec), $[\alpha]_D$ +320° ($HCCl_3$).

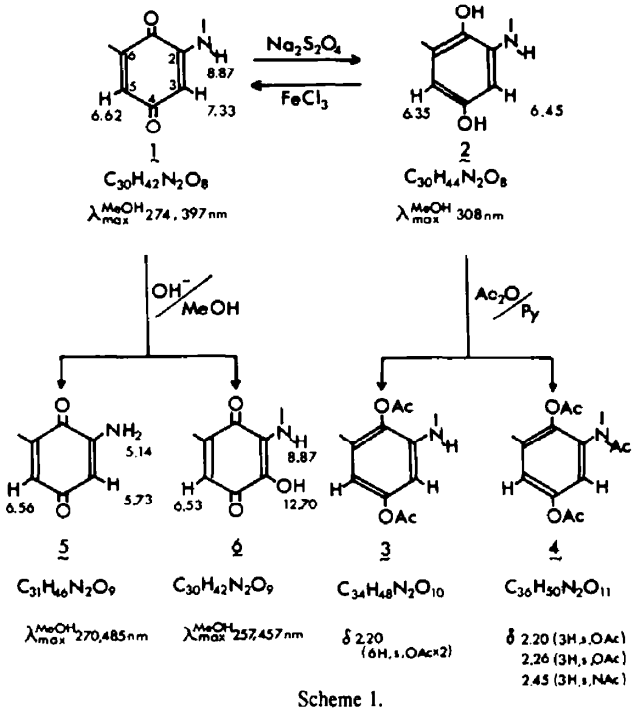
Compound **5** had λ_{max}^{MeOH} 270 nm (ϵ 29,800) and 485 nm (ϵ 1950) in the UV and visible spectra, showing a typical spectrum of aminobenzoquinone.[6] The ^1H NMR spectrum of **5** revealed four OMe signals at δ 3.25, 3.35, 3.50 and 3.78, indicating that one OMe group (δ 3.78) increased. The signal of another NH_2 group was newly observed at δ 5.14 instead of disappearance of the amide NH signal in **1** and one of the two benzoquinone ring protons at δ 7.33 (1H, d) in **1** shifted upfield to δ 5.73 (1H, d). These spectral changes between **1** and **5** clearly demonstrated that the amide linkage attached to benzoquinone nucleus in **1** was cleaved to give a free 2-aminobenzoquinone derivative and the end COOH group of the other substituent of **1** was methylated. This indicated that two substituents of **1** constituted an ansa-bridge.



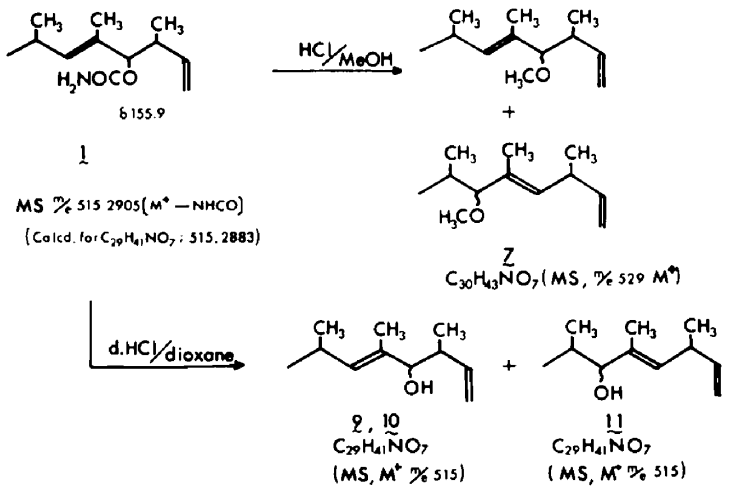
Scheme 2.

Another product **6**, which had λ_{max}^{MeOH} 257 nm (ϵ 21,900) and 457 nm (ϵ 1190) and displayed a bathochromic shift to 537 nm in alkaline conditions, showed a similar ^1H NMR spectrum to that of **1**, but remarkable differences were observed in that the quinonoid ring proton at δ 7.33 in **1** had disappeared, the multiplicity of the other ring proton at δ 6.62 in **1** changed from doublets of doublet to doublet (δ 6.53), and a new signal of chelated OH was formed at δ 12.70. Therefore, the compound **6** is concluded to be an addition product of OH to the C_3 position of *p*-benzoquinone.[7]

The IR absorption band of **1** at 1740 cm^{-1} (ester C=O), a significant peak of *m/e* 515.2905 (Calc. for $C_{29}H_{41}NO_7$: 515.2883) due to [M^+–NHCO] fragment in the high-



Scheme 1.



Scheme 3.

resolution mass spectrum and the signals of 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.

Indeed, methanolysis of **1** with HCl–MeOH afforded the decarbamoyl product (**7**), $C_{30}H_{43}NO_7$, m.p. 190–191° (dec) (MS, M^+ *m/e* 529), which had an additional OMe group in the ^1H NMR spectrum, mainly consisted of three isomers and lost the ester C=O in the IR spectrum. Likewise, macbecin II diacetate (**3**) also gave a decarbamoyl product (**8**), $C_{34}H_{49}NO_9$, m.p. 257–258° (dec), which mainly consisted of three isomers and showed a molecular ion peak at *m/e* 615. The decarbamoyl products **7** and **8** were found to be readily formed by methanolysis as mixtures of isomers through allylic rearrangement, suggesting that the carbamate group is located at the allylic position of the isolated double bond.

In addition, acid hydrolysis of **1** with d. HCl in dioxane afforded decarbamoyl products, $C_{29}H_{41}NO_7$ (MS, M^+ *m/e* 515), from which three isomers, **9**, **10** and **11** were isolated. The decarbamoyl compound **9**, m.p. >300° (dec), $[\alpha]_D$ +337.7° ($CHCl_3$) showed a similar ^1H NMR spectrum to **1** except for the disappearance of NH_2 signal of carbamate and an upfield shift of a signal attached to the O function. The compound **9** was considered to be a normal decarbamoyl product in which the carbamate group was simply hydrolysed without rearrangement, whereas the second decarbamoyl product, **10**, m.p. >300° (dec), $[\alpha]_D$ +244.5° ($CHCl_3$) was assigned as the stereoisomer of **9** by ^1H NMR spin decoupling study.

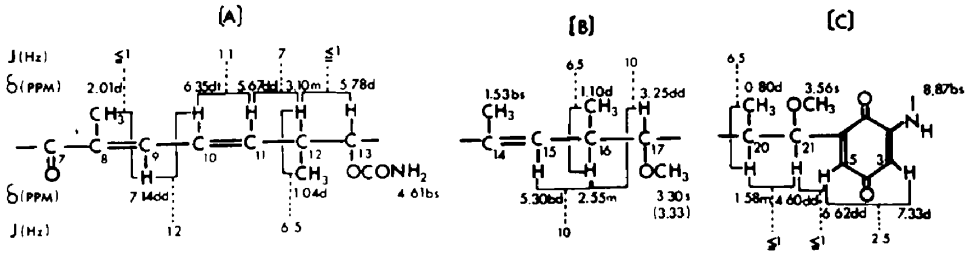
The third decarbamoyl compound (**11**), m.p. 178–179° (dec), $[\alpha]_D$ +144° ($CHCl_3$), was considered to be a positional isomer. All of these product exhibited very similar IR spectra which lost carbamate C=O. It is reasonable to speculate that they were formed through allylic rearrangement as shown in Scheme 3.

These results indicated that the carbamate group is located at the allylic position of the isolated double bond.

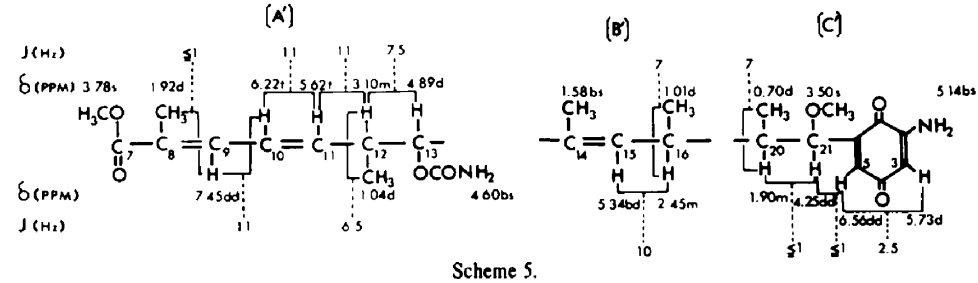
Detailed spin decoupling studies clarified the following structural units. Firstly, the partial structures [**A**], [**B**] and [**C**] in macbecin I (**1**) were disclosed as follows (Scheme 4).

The partial structures, [**A'**], [**B'**] and [**C'**] were also assigned to the amide cleavage compound (**5**) by the spin decoupling studies (Scheme 5).

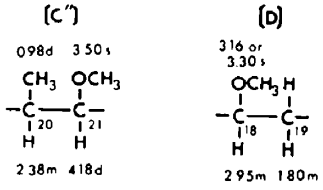
In both compounds, one of the quinonoid protons was coupled to the other quinonoid ring proton and O-benzylic proton. The remaining unit [**D**] ($-CH_2-\underset{|}{C}H-OCH_3$)



Scheme 4.



Scheme 5.



Scheme 6.

together with unit [C''] was confirmed by spin decoupling of macbecin II diacetate (**3**) (Scheme 6).

The total arrangement of these structural units [**A**], [**B**], [**C**] and [**D**] was deduced from the following facts.

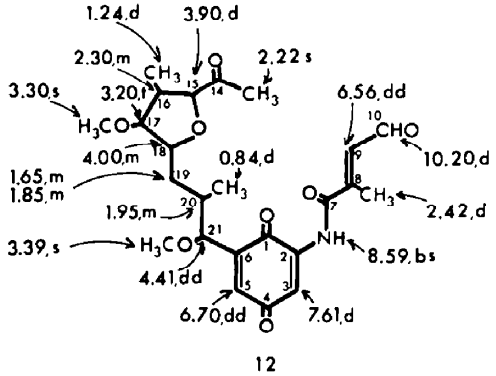
The methine proton of $-\underset{|}{C}H-OCONH_2$ was only coupled to one methine proton as shown above and acid hydrolysis of **1** and methanolysis of **1** and **3** readily yielded decarbamoyl products which consisted of three isomers through allylic rearrangement. Therefore, it is evident that the 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} carbon.

Conversion of **1** to the aminobenzoquinone **5** by alkaline methanolysis demonstrated that the amino group of the 2-amino-*p*-benzoquinone is 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**) (Fig. 1).

In order to decide the position of the remaining OMe, the normal decarbamoyl product **9** was epoxidized with *m*-chloroperbenzoic acid[8] and then oxidized with HIO_4 afford a product **12**, $C_{24}H_{31}NO_8$, $[\alpha]_D$ +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 **12** was clarified by the spin decoupling as shown in the Scheme 7. As for C_{17} to C_{19}, the methine proton of C_{18} appeared at δ 4.00 and was coupled to the C_{17} methine proton (δ 3.20, t) linked to the OMe oxygen and the C_{19} methylene protons at δ 1.65 and 1.85. The OMe group at C_{18} was cyclized with C_{15} to form a tetrahydrofuran ring. Similar cyclizations were found to occur also in rifamycin[9] and tolypomycin[10] under acidic conditions.

This result led to the conclusion that macbecin I (**1**) is a benzoquinonoid ansamycin represented by **1a** and macbecin II (**2**) is its hydroquinone form (Fig. 2).

In confirmation of these proposed structures for **1** and **2** and in determination of the stereochemistry of these antibiotics, several halogen containing derivatives of **1** and **2** were synthesized. Among these derivatives, N-



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Scheme 7.

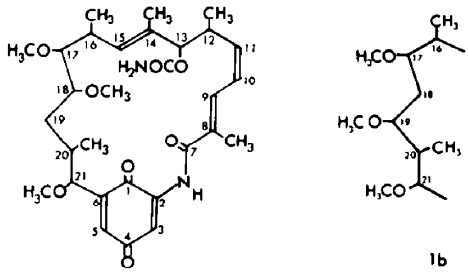

Fig. 1.

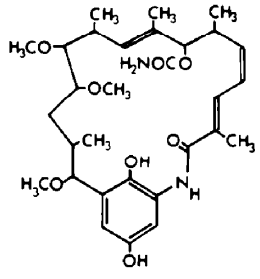

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Fig. 2.

bromoacetylmacbecin I (**13**), which was bromoacetylated at the amino group of the carbamate with bromoacetylbromide in the presence of pyridine, gave suitable crystals for X-ray analysis. In ^1H NMR spectrum of **13**, the signal of NH_2 of the carbamate disappeared and instead another amide NH proton was observed. The other signals were very similar to those of **1**.

The crystal of bromoacetylmacbecin I (**13**) crystallized from MeOH–H_2O is triclinic with P1, having cell dimensions of a = 12.99 (2), b = 16.83 (2), c = 9.648 (7) Å, α = 95.21 (8), β = 110.91 (9), γ = 107.77 (9) and U = 1828 (3) Å^3. Assuming two independent molecules in a unit-cell, the calculated density becomes a resonable value of 1.23 g cm^{-3}. Crystal data of **13** are listed in Table 3.

Based on relative positions of Br atoms obtained by Patterson synthesis, coordinates of 88 non-H atoms contained in a unit cell were determined by the heavy atom method. Atomic coordinates and isotropic temperature factors were refined by the block-diagonal least-squares method to an R-value of 0.143.[11]

Table 3. Crystal data of bromoacetyl macbecin I (**13**)

Formula	$C_{64}H_{86}N_4O_{18}Br_2$
Formula weight	1359.2
Crystal system	Triclinic
Space group	P1
Unit-cell	a = 12.99(2) Å
	b = 16.83(2) Å
	c = 9.648(7) Å
	α = 95.21°(8)
	β = 110.91°(9)
	γ = 107.77°(9)
Unit-cell volume	U=1828(3) Å^3
Number of molecules in a unit-cell	Z=2
Density	D_x = 1.23 g cm^{-3}
Wave length	λ (MoKα) = 0.7107 Å
Observed reflexions	4782 ($2\theta \leq 45°$)
	2797 ($F \geq 3\sigma(F)$)

Perspective views of two independent molecules (tentatively named as **A** and **B**) projected on each least-squares plane of 19-membered ring calculated for 19 atoms constituting each ring are shown in Figs. 3a and 3b.

Two separate structure factors with coordinates corresponding to the two possible enantiomers were calculated for the establishment of the absolute configuration. Two residual factors were 0.145 and 0.148, respectively, suggesting that the structures of Fig. 3 represent the absolute configuration of the molecule correctly. In confirmation of this result, observed and calculated structure factors of 11 Bijvoet pairs were compared (Table 4). In all cases the observed differences corresponded to expected ones, and the absolute configuration was established (Fig. 4).

The structure of macbecin I (**1**) was confirmed to be **1a** and the absolute configuration was established to be that of Fig. 4 from the present study. The configurations at seven asymmetric carbons were 12-(*S*), 13-(*R*), 16-(*S*), 17-(*R*), 18-(*S*), 20-(*S*) and 21-(*R*), respectively. The geometries of the olefinic bonds were 8*E*, 10*Z* and 14*E*.

The structure of macbecin I (**1**) is closely related to those of antibiotics geldanamycin[6] and herbimycin,[12] of which absolute configurations have not been reported up to the present. Therefore, the absolute stereochemistry of this group of antibiotics has been established for the first time in this study.

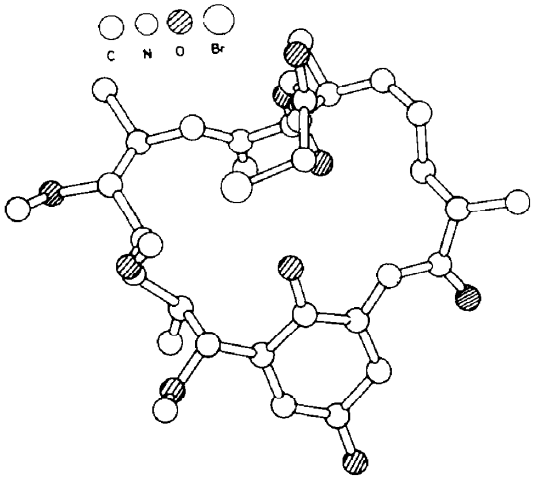

Fig. 3a. Perspective view of molecule **A**.

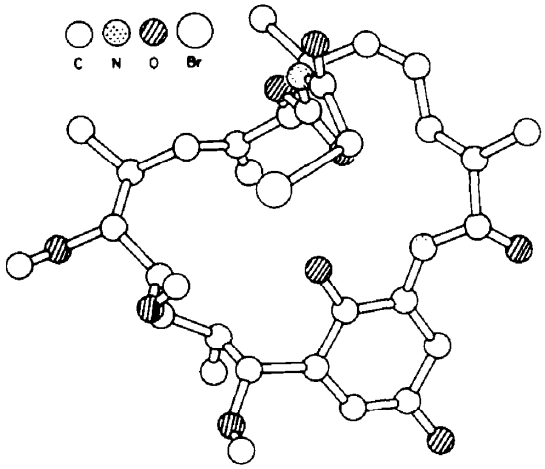

Fig. 3(b). Perspective view of molecule **B**.

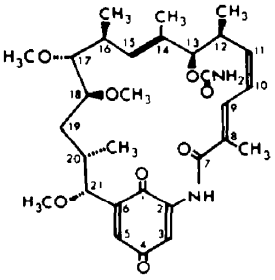

Fig. 4.

Table 4. Comparison of observed and calculated structure factors

h	k	l	Fo(h k l)	Fo($\bar{h}\bar{k}\bar{l}$)	Fc(h k l)	Fc($\bar{h}\bar{k}\bar{l}$)
0	6	2	25.6 <	30.6	23.0 <	27.7
$\bar{1}$	4	5	42.0 <	46.5	42.8 <	46.2
1	0	$\bar{5}$	47.5 >	42.1	43.8 >	40.6
1	$\bar{9}$	0	31 3 >	26.2	29.3 >	25.2
1	$\bar{3}$	4	10.1 <	15.9	9 1 <	13.1
1	$\bar{1}0$	1	25.4 >	20.3	23.9 >	21.1
2	$\bar{4}$	4	28.1 <	33.8	21.8 <	26.4
3	$\bar{9}$	0	24.2 >	19.1	22.3 >	18.3
5	$\bar{7}$	0	37 3 >	32.8	37.3 >	33.0
5	$\bar{5}$	2	25 9 >	19.7	24.0 >	20.9
$\bar{6}$	3	3	16.5 <	23.1	16.7 <	21.2

Bond lengths and angles obtained by the present analysis are given in Figs. 5 and 6, respectively.

Standard deviations range from 0.03 to 0.07 Å for distances and from 2 to 5 degrees for angles. Though these large deviations make it impossible to discuss details about bond distances and angles, the close proximity of corresponding values between two independent molecules **A** and **B** affords an irrefragable proof of molecular geometry obtained.

The low level of accuracy achieved in this analysis is mainly due to large scattering effects of bromine atoms and a glass capillary, but the former is profitable for the determination of the absolute configuration.

Now that the crystal structure of N-bromoacetyl macbecin I (**13**) has been thus elucidated, it should be mentioned whether natural macbecin I (**1**) also has the same stereochemistry as N-bromoacetyl derivative (**13**) ^{1}H NMR spectrum of **13** in $CDCl_3$ showed almost the identical chemical shifts and coupling constants with those of **1**. This fact suggests that natural macbecin I (**1**) also adopts a similar conformation to **13**.

It is interesting that among known ansamycins macbecin I and II have the similarities to maytansinoids such as maytansine[13] and ansamitocins[14] in that they exhibited significant antitumor activities against P-388 leukemia and B-16 melanoma *in vivo*,[2] consisted of 19-membered macrolactams and have the same stereochemistry at the C_{12}, C_{13} and C_{16} positions.

However, macbecins differ from maytansinoids in that the former did not show antitublinic activity unlike the latter[1] and further studies on the structure-activity relationship and on the mechanism of action are expected.

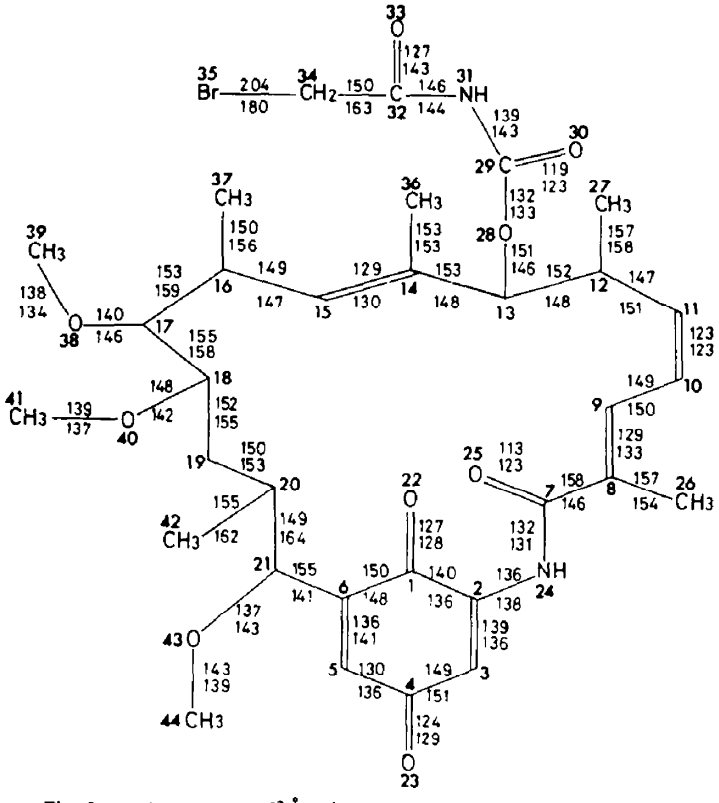

Fig. 5. Bond lengths ($\times 10^2$ Å) of molecules A (upper) and B (lower).

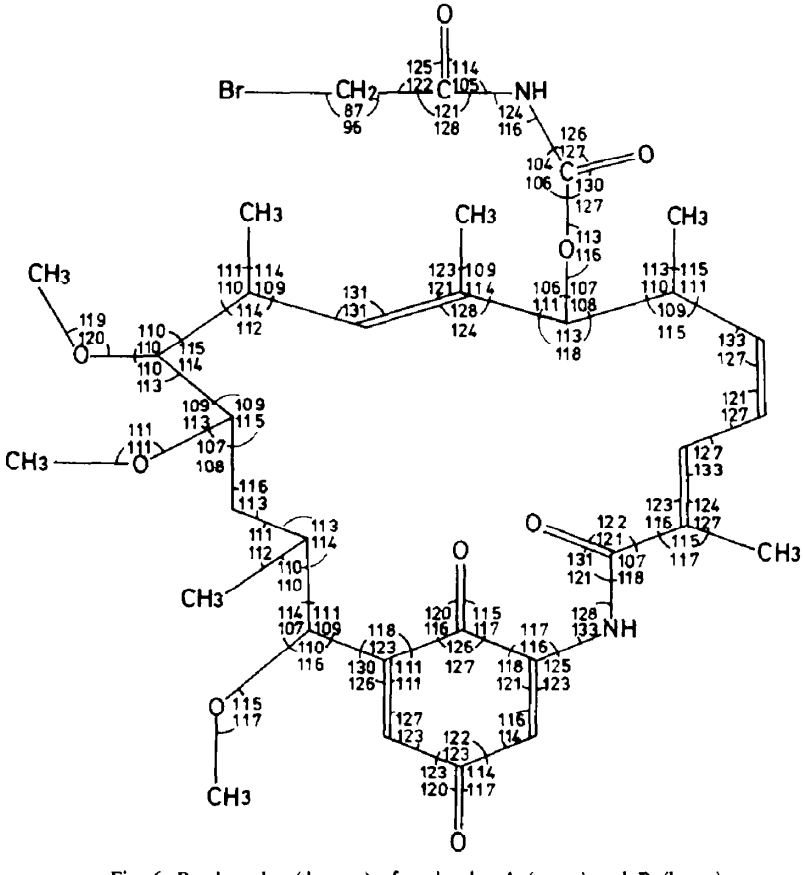

Fig. 6. Bond angles (degrees) of molecules A (upper) and B (lower).

EXPERIMENTAL

M.ps were determined with a Mettler FP5 apparatus. IR spectra were obtained with a Hitachi model 285 grating IR spectrometer. UV and visible spectra were recorded with a Hitachi model EPI-G2 spectrometer. NMR spectra were recorded on a Varian XL-100 or EM-390 with tetramethylsilane as the internal standard. Chemical shifts are reported on the δ scale. Mass spectra were obtained with a JEOL JMS-01SG mass spectrometer using a direct inlet system. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter.

Tlc was performed on silica gel spot film (Tokyo Kasei Co.) unless otherwise noted. Abbreviations are as follows; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad.

Macbecin-II diacetate (**3**) *and triacetate* (**4**). To a soln of **2** (500 mg) in dry pyridine (3.5 ml) was added Ac_2O (1.75 ml) and kept at room temp for 24 hr. The mixture was poured onto ice-water and the resulting ppt was collected by filtration to give a mixture of **3** and **4**. (387 mg). The mixture (384 mg) was purified on a column of silica gel (20 ml). After washing with hexane (50 ml), the column was developed with mixtures of hexane-EtOAc (4:1, 100 ml → 1:1, 100 ml → 1:3, 80 ml) and then EtOAc (100 ml) and 8 ml fractions were collected.

The fractions Nos. 32–33 and 36–37 were concentrated to **4** (65 mg) and **3** (106 mg), respectively.

Diacetate (**3**): m.p. 194–195°, $[\alpha]_D^{26.5}$ +90.5° (c = 0.1, $CHCl_3$). (Found: C, 61.81; H, 7.52; N, 3.99; O, 27.04. Calc. for $C_{34}H_{48}N_2O_{10}$: C, 61.61; H, 7.60; N, 4.23; O, 26.55%). IR ν_{max}^{KBr} cm^{-1}: 1765 (phenolic OAc), 1720 (carbamate C=O), 1600 (aromatic), 1165 (phenolic OAc). Triacetate (**4**): m.p. 143–144°, $[\alpha]_D^{26.5}$ +88.0° (c = 0.1, $CHCl_3$). (Found: C, 62.80; H, 7.45, N, 3.90; O, 24.49. Calc. for $C_{36}H_{50}N_2O_{11}$: C, 62.95; H, 7.34; N, 4.08; O, 25.62%). IR ν_{max}^{KBr} cm^{-1}: 1770 (phenolic OAc), 1730 (carbamate C=O), 1685 (imide C=O), 1600 (aromatic), 1165 (phenolic OAc).

Alkaline methanolysis of **1** *to give* **5** *and* **6**. To a soln of **1** (1 g) in MeOH (200 ml) was added 2% $NaHCO_3$ aq (100 ml) and kept at 0–5°. After 70 hr, the mixture was adjusted to pH 6.65 and evaporated to remove MeOH. The aqueous concentrate was extracted with EtOAc at pH 6.40 and the washed and dried extract was again concentrated to give a brown residue (fraction 1, 988 mg). The aqueous layer after extraction at pH 6.40 was further acidified to pH 5.0 and extracted with EtOAc. The washed and dried extract was concentrated to give a reddish orange mixture (fraction 2, 89 mg).

The fraction 1 (976 mg) was subjected to preparative tlc on silica gel (Merck HF_{254}) with the solvent system of $CHCl_3$–MeOH (10:1) and extraction of red bands with EtOAc gave a crude sample of **5** (155 mg), which was further purified by preparative tlc on silica gel with the solvent system of toluene–EtOAc (1:3), giving pure **5** (85 mg): m.p. 79–80° (dec), $[\alpha]_D^{27.5}$ +179° (c = 0.1, $CHCl_3$). (Found: C, 61.69; H, 8.16; N, 4.23. Calc. for $C_{31}H_{46}N_2O_9 \cdot H_2O$: C, 61.17; H, 7.95; N, 4.60%) IR ν_{max}^{KBr} cm^{-1}: 1720 (carbamate C=O), 1640, 1600 (quinonoid C=O and C=C), 1695 (α, β, γ, δ-unsaturated carboxylate).

Fraction 2 (85 mg) was subjected to preparative tlc on silica gel (Merck HF_{254}), containing oxalic acid with the solvent system of EtOAc containing 1% oxalic acid–toluene (1:1). The washed and dried EtOAc extract of a main band was concentrated to yield reddish orange **6** (65 mg) which was recrystallized from CH_2Cl_2–hexane or EtOAc–hexane as reddish orange needles. **6**: m.p. 218–219° (dec), $[\alpha]_D^{26.5}$ +320° (c = 0.1, $CHCl_3$). (Found: C, 62.62; H, 7.40; N, 4.76. Calc. for $C_{30}H_{42}N_2O_9$: C, 62.70; H, 7.37; N, 4.87%) IR ν_{max}^{KBr} cm^{-1}: 1745 (carbamate C=O), 1665, 1605 (quinonoid C=O and C=C). UV λ_{max}^{MeOH} nm (ϵ): 257 (21,000), 457 (1190).

Methanolysis of **1**. Macbecin I (**1**) (500 mg) dissolved in 40 ml 5% HCl–MeOH was heated under reflux for 30 min and the mixture was poured onto ice-water (200 ml). The resulting mixture was extracted with EtOAc and the extract was washed with H_2O, 2% $NaHCO_3$ and H_2O, successively. The dried extract was concentrated to a small volume and allowed to stand to give yellow crystals of **7** (225 mg), which were found to consist of three isomers from ^1H NMR spectrum. **7**: m.p. 191–192° (dec). (Found: C, 68.00; H, 8.28; N, 2.54; O, 21.77. Calc. for $C_{30}H_{43}NO_7$: C, 68.03; H, 8.18; N, 2.64; O, 21.15%) IR ν_{max}^{KBr} cm^{-1}: 1700 (quinonoid C=O), 1650 (amide C=O), 1610 (quinonoid C=C), no carbamate C=O.

Methanolysis of macbecin II diacetate (**3**). Macbecin II diacetate **3** (390 mg) was heated under reflux in 5% HCl–MeOH and work-up as in the case of **1** afforded colorless crystals of **8** (185 mg), which were also found to consist of three isomers from ^1H NMR spectrum. **8**: m.p. 257–258° (dec). IR ν_{max}^{KBr} cm^{-1}: 1760 (phenolic OAc), 1640 (amide C=O), 1600 (aromatic), 1175 (phenolic OAc), no carbamate C=O.

Acid hydrolysis of macbecin I (**1**). Macbecin I **1** (600 mg) dissolved in 30 ml dioxane and 30 ml 1N HCl was heated at 70° for 2 hr and the mixture was extracted with EtOAc after addition of 300 ml cold $NaHCO_3$ aq. The washed and dried extract was concentrated to give a mixture of decarbamoyl products (561 mg). The mixture (557 mg) was objected to preparative tlc on silica gel (Merck HF_{254}) using EtOAc–toluene (1:1) as eluant; three yellow bands were separated and extracted with EtOAc. The washed and dried extracts gave three isomeric decarbamoyl products **9** (192 mg), **10** (111 mg) and **11** (90 mg). **9** (yellow needles from EtOAc): m.p. > 300° (dec), $[\alpha]_D^{26.5}$ +337.7° (c = 0.11, $CHCl_3$). (Found: C, 67.50; H, 8.14; N, 2.66. Calc. for $C_{29}H_{41}NO_7$: C, 67.55; H, 8.01; N, 2.72%) IR ν_{max}^{KBr} cm^{-1}: 1695 (quinonoid C=O), 1645 (amide C=O), 1610 (quinonoid C=C), no carbamate C=O; ^1H NMR ($CDCl_3$) δ 0.77 (3H, d), 0.98 (3H, d), 1.07 (3H, d), 1.46 (3H, brs, 14-CH_3), 2.0 (3H, brs, 8-CH_3), 2.48 (1H, m, H-16), 3.02 (1H, m, H-12), 3.31 (3H, s, OCH_3), 3.25 (1H, brd, H-17), 3.35 (3H, s, OCH_3), 3.52 (3H, s, 21-OCH_3), 4.55 (1H, brs, H-13), 4.60 (1H, dd, H-21), 5.52 (1H, brd, H-15, J = 10.5 Hz), 5.83 (1H, dd, H-11), 6.35 (1H, dt, H-10), 6.60 (1H, dd, H-5), 7.15 (1H, brd, H-9), 7.27 (1H, d, H-3), 8.60 (1H, brs, –CONH–), no carbamoyl NH_2, MS *m/e* 515 (M^+). **10** (yellow prisms from EtOAc): m.p. >300° (dec), $[\alpha]_D^{27.5}$ +244.5° (c = 0.1, $CHCl_3$). (Found: C, 67.52; H, 8.06; N, 2.76%) IR ν_{max}^{KBr} 1695 (quinonoid C=O), 1650 (amide C=O), 1605 (quinonoid C=C), no carbamate C=O; ^1H NMR ($CDCl_3$) δ 0.78 (3H, d), 0.93 (3H, d), 1.08 (3H, d), 1.50 (3H, brs, 14-CH_3), 1.96 (3H, brs, 8-CH_3), 2.48 (1H, m, H-16), 2.95 (1H, m, H-12), 3.20 (1H, brd, H-17), 3.32 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.50 (3H, s, 21-OCH_3), 3.78 (1H, d, H-13), 4.63 (1H, brs, H-21), 5.45 (1H, brd, H-15), 5.65 (1H, dd, H-11), 6.48 (1H, t, H-10), 6.57 (1H, dd, H-5), 7.32 (1H, brd, H-9), 7.36 (1H, d, H-3), 8.78 (1H, brs, –CONH–), no carbamoyl NH_2. MS *m/e* 515 (M^+). **11** (yellow needles from EtOAc): m.p. 178–179° (dec), $[\alpha]_D^{27.5}$ +144° (c = 0.1, $CHCl_3$). (Found: C, 67.26; H, 8.09; N, 2.93%). IR (KBr) no carbamate C=O; ^1H NMR ($CDCl_3$) δ 0.83 (6H, d), 1.13 (3H, d), 1.37 (3H, brs, 14-CH_3), 1.94 (3H, brs, 8-CH_3), 3.34 (3H, s, OCH_3), 3.42 (3H, S, OCH_3), 3.48 (3H, s, OCH_3), 3.85 (1H, brd, H-13), 4.42 (1H, brs, H-21), 5.48 (1H, brd, H-15), 5.78 (1H, dd, H-11), 6.20 (1H, dt, H-10), 6.60 (1H, dd, H-5), 6.70 (1H, brd, H-9), 7.36 (1H, d, H-3), 8.25 (1H, brs, –CONH–), no carbamoyl NH_2). MS *m/e* 515 (M^+).

Oxidative decomposition of the decarbamoyl product **9**. To a cold soln of **9** (1 g) in 30 ml $CHCl_3$ was added a soln of *m*-chloroperbenzoic acid (purity 85%; 1.025 g) in 20 ml $CHCl_3$ under stirring. The mixture was kept at room temp in the dark for 22 hr, then diluted with $CHCl_3$ (200 ml), and washed successively with 2% Na_2SO_3 aq, 2% $NaHCO_3$ aq and water. The dried organic soln was evaporated to give a crude product (1.138 g). To a cold soln of this crude product (1.105 g) dissolved in 60 ml dioxane was added 0.5 M HIO_4 soln (12 ml) under stirring. The mixture was allowed to stand at room temp for 24 hr and then concentrated to remove dioxane. The concentrate was extracted with EtOAc and the EtOAc extract was washed with dilute $NaHCO_3$ aq and water, and evaporated to give a yellowish orange residue (830 mg). The residue (826 mg) was chromatographed on a silica gel column (40 g, Merck kiesel gel 60); Elution was started with toluene and then successively with toluene–EtOAc (8:1, 4:1, 2:1). Fractions containing the main product were evaporated to yield a crude yellow oily product (242 mg) which was further pruified by preparative tlc with toluene–EtOAc (1:1); A main yellow band was collected and extracted with EtOAc. The washed and dried extract was evaporated to give **12** (208 mg) as a yellow gummy substance. $[\alpha]_D^{27.5}$ +129.1° (c = 0.11, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 1710–1715, 1690, 1670, 1655, 1640, 1610; MS *m/e* 463 (M^+ +2), 461 (M^+).

Monobromoacetylmacbecin I (**13**). To a cold soln of **1** (1 g) in 30 ml THF and 1.7 ml pyridine was added monobromoacetyl bromide (3 g) under stirring and after 1 hr monobromoacetyl

bromide (3 g) was again added. The mixture was further stirred for 4 hr under cooling, then poured onto ice-water and extracted with EtOAc. The extract was washed with 1% $NaHCO_3$ aq and water and the dried extract was concentrated to give a yellow residue which was chromatographed on a silica gel column (Merck, 26 × 450 mm); Elution was begun with hexane (200 ml) and then successively with hexane–EtOAc (3:1, 320 ml), hexane–EtOAc (2:1, 450 ml) and hexane–EtOAc (1:1, 400 ml). Fractions (15 ml) were collected and monitored by tlc; Fractions No. 37 to No. 41 were evaporated to yield a yellow powder (420 mg) by addition of petroleum ether. The yellow powder (10 mg) was dissolved in 4 ml of MeOH and water (5 ml) was added to give yellow crystals of **13**: m.p. 184–186°. (Found: C, 56.20; H, 6.42; N, 4.21; Br, 12.37. Calc. for $C_{32}H_{43}N_2O_9Br$: C, 56.55; H, 6.38; N, 4.12; Br, 11.75%) ^1H NMR ($CDCl_3$) δ 0.81 (3H, d), 1.06 (3H, d), 1.07 (3H, d), 1.2–2.0 (4H, m), 1.54 (3H, brs, 14-CH_3), 2.01 (3H, brs, 8-CH_3), 2.53 (1H, m), 3.2–3.5 (2H, m), 3.33 (6H, s, OCH_3 × 2), 3.52 (3H, s, OCH_3), 4.19 (1H, d, J = 13 Hz), 4.35 (1H, d, J = 13 Hz), 4.56 (1H, brs, H-21), 5.26 (1H, brd, J = 10 Hz, H-15), 5.67 (1H, dd, J = 7, 10 Hz), 5.85 (1H, brs, H-13), 6.37 (1H, dd, J = 10, 11 Hz), 6.63 (1H, dd, J = 1, 2.5 Hz), 7.09 (1H, brd, J = 11 Hz), 7.33 (1H, d, J = 2.5 Hz). The ^1H NMR spectrum of **13** was superimposable with **1** except for the signals of $BrCH_2CO$ (δ 4.19 and 4.35) and acylated carbamoyl NH. MS *m/e* 681 (M^+ +2), 680 (M^+ +1), 679 (M^+), 595, 593, 515, 500, 483, 465, 451, 433, 412.

X-Ray analysis of **13**. Recrystallization of **13** from aqueous MeOH gave yellow columnor crystals. Because the crystal was easily cracked under the atmospheric condition, the crystal used for diffraction studies was sealed in a capillary tube containing a small amount of mother liquor of crystallization. Crystal data and intensities of 4782 diffractions up to 2θ of 45° were measured using Rigaku AFC-5 4-circle diffractometer with monochromated (graphite monochrometer) M_0K_α radiation. Owing to large scattering effects of Br atoms and a glass capillary, background intensities were so high that only 2797 reflexions, which were about 60% of total reflexions measured, satisfied the criterion of $F \geq 3\sigma$ (F). Calculations of the structure analysis were performed on IBM 370/148 computer using the program system X-RAY 76 edited by J. M. Stewart.[15]

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