Synthesis and Biological Activity of Novel Mitomycin C Analogs Derived from Mitomycin A

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A variety of mitomycin C analogs were synthesized from mitomycin A and their biological activities were studied. Mitomycin A (**1**) underwent nucleophilic displacement reactions involving intramolecular hydrogen migrations upon treatment with nitrogen nucleophiles bearing mobile hydrogens, but the mode of hydrogen migration depended on the nature of the nucleophiles. The reaction with alkoxyamines gave compounds **6** and **7** which have the 5*H*-6-alkoxyimino-4,7-dione structure in ring A of **1**. However, the reaction with hydroxylamine and benzoylhydrazine afforded compounds **11** and **13** which have the 4-hydroxy-6-hydroxyimino-7-one structure and the 4-hydroxy-6-hydrazono-7-one structure, respectively, in ring A of **1**. These products were converted into various types of mitomycin C derivatives by methylation with methyl iodide or dimethyl sulfate. The mechanistic features of these reactions are discussed. The *in vitro* and *in vivo* biological activities were tested by using P388 leukemia and Sarcoma 180 tumor cells. Several of the synthesized compounds exhibited better activity than that of mitomycin C.

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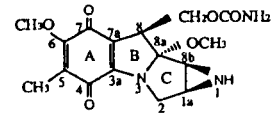
Introduction.

Mitomycins are a class of antibiotics produced by *Streptomyces caespitous* and they usually have a strong cytotoxic activity [1]. The chemistry of mitomycins has received a great deal of attention in view of their unique biological functions and also of developing clinically useful antitumor agents by chemical modification [2].

It is known that the methoxy group at C-6 of mitomycin A (**1**) is susceptible to nucleophilic substitutions and can be replaced by various nucleophiles to produce general substitution products of the type **2** [3]. However, Sawhney and Kohn have recently reported that upon treatment with acyl or alkoxycarbonyl hydrazines, **1** is converted into mitomycin C derivatives **4** [4]. This reaction apparently involves an intramolecular hydrogen migration: *i.e.*, **1** is first converted into the hydrazino compounds **3** and then isomerized to **4** by hydrogen migration. We have also found that treatment of **1** with nitrogen nucleophiles bearing mobile hydrogens, such as hydroxylamine, alkoxyamines and hydrazines, causes nucleophilic displacement reactions involving hydrogen migration, but the mode of hydrogen migration depends on the nature of the nucleophile. These reactions permitted preparation of a novel class of mitomycin C analogs.

In this paper, we report the synthesis, nmr spectral properties and biological activity of various types of mitomycin C derivatives. All compounds were synthesized from **1** and the mechanistic features of the reactions are also discussed.



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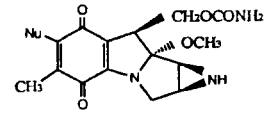

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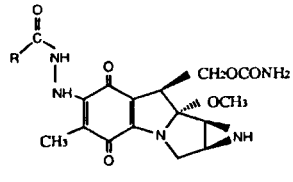

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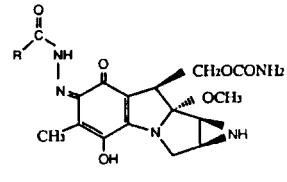

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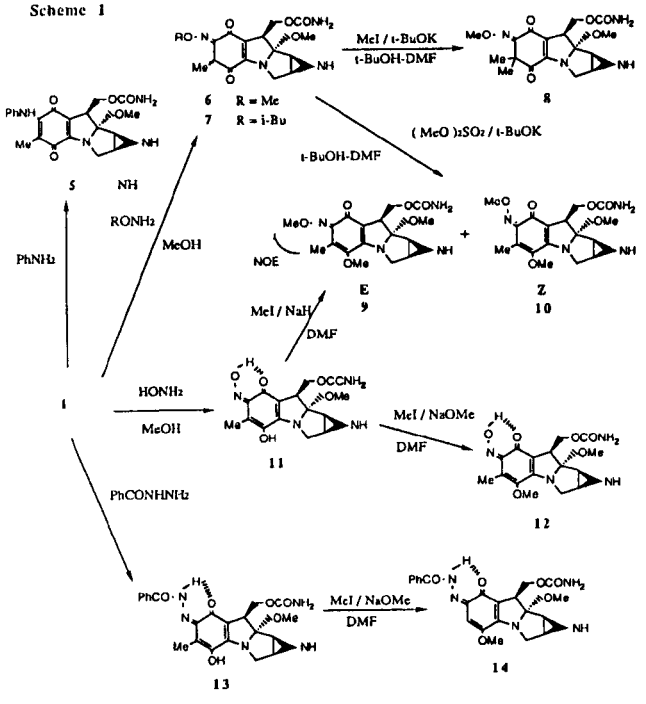
Results and Discussion.

Chemistry.

The reactions studied in this investigation are summarized in Scheme 1. The reaction of **1** with aniline in methanol at room temperature gave the normal substitution product **5** in 83% yield. However, the reaction of **1** with 2.1 equivalents of methoxyamine under similar conditions gave the 5*H*-6-methoxyimino compound **6**.

Isobutoxyamine reacted with **1** in a similar fashion to yield the 5*H*-6-isobutoxyimino compound **7**. In these reactions, the hydrogens on the nitrogen of alkoxyamino groups migrated to the C-5 carbon in ring A of **1**.

Scheme 1



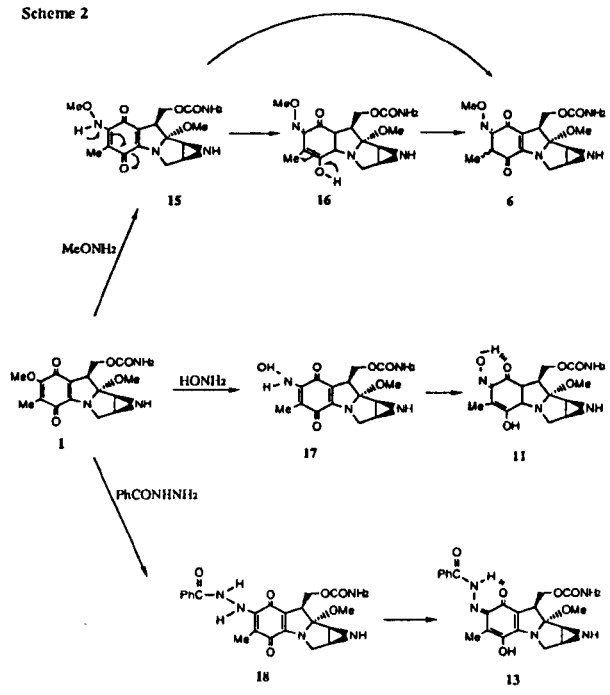
The structures of **6** and **7** were established on the basis of their spectral properties and also by chemical conversions. Table 1 shows the ^1H and ^{13}C nmr spectral data for hydrogens and carbons in ring A of the compounds synthesized. The ^{13}C nmr signals for the C-5 carbons of **6** and **7** appeared at extremely high fields (δ 44-45), compared to the ^{13}C signals for the corresponding carbons (δ 106-130) of other compounds. This indicates that the C-5 carbons of **6** and **7** have an sp^3 character [5]. Moreover, the ^{13}C signals for the C-4 carbons of these compounds appeared at relatively low fields (δ 190-192). Signals in such a downfield region are normally associated with carbonyl carbons of α,β-unsaturated ketones rather than those of quinones [6]. The ^1H nmr signals for the methyl groups attached to C-5 of **6** and **7** appeared as two doublets at higher fields (δ 1.40 and 1.41 for **6**, and δ 1.45 and 1.49 for **7**). These values also provide support for the conclusion that the methyl groups are bonded to an sp^3 carbon [7]. The appearance of the signals as two doublets suggests that the methyl groups in **6** and **7** are linked to C-5 with two different configurations; *i.e.*, both **6** and **7** are mixtures of α-methyl and β-methyl configurational isomers. In conformity with this view, the methoxy methyl protons at C-8a of **6** appeared as two singlets at δ 3.21 and 3.24 in the ^1H nmr spectrum. The intensity ratio of these signals suggested that **6** contained the α-and β-methyl isomers in a 3:2 ratio. The structure of **6** was further confirmed by chemical conversions. Treatment of **6** with methyl iodide in the presence of potassium tert-butoxide in tert-butyl alcohol-dimethylformamide gave the 5,5-dimethyl compound **8**. In this reaction, the methylation occured on the C-5 carbon. Compound **8** displayed analogous patterns of signals with those of **6** in both the ^1H and ^{13}C nmr spectra, except for the methyl groups attached to C-5. The ^{13}C nmr signals for the two methyl groups at C-5 of **8** appeared at δ 20 and 23 as two singlets and the ^1H nmr signals for these methyl groups appeared at δ 1.55 and 1.57 also as two singlets. These results confirmed that the two methyl groups are bonded to C-5 also in α and β configurations. On the other hand, treatment of **6** with dimethyl sulfate in the presence of potassium tert-butoxide in tert-butyl alcohol-dimethylformamide gave two methylated compounds **9** and **10** in a 4.5:1 ratio. In this reaction, the methylation occurred on the C-4 carbonyl oxygen. The two compounds were able to be separated by tlc on silica gel.

The configurations of the methoxyimino group at C-6 in these compounds were determined from the observation of the NOE between the methyl protons at C-5 and the methoxyimino methyl protons at C-6. The former methyl protons appeared at δ 2.18 for **9** and at δ 2.21 for **10**, and the latter methoxyiminomethyl protons at δ 4.19 for **9** and at δ 4.17 for **10**. A more important result was that a much larger Overhauser enhancement was observed for **9** than for **10**. This indicates that **9** has the *E*-configuration and **10** has the *Z*-configuration in the methoxyimino group.

The difference in the reactivity between methyl iodide and dimethyl sulfate on the methylation of **6** may be accounted for in terms of the HSAB principle (the principle of hard and soft acids and bases) [8]. Since **6** has an enolizable hydrogen at C-5, this compound can act as an ambident nucleophile in basic media. As a result, the soft electrophile, methyl iodide, preferentially attacks the C-5 carbon that is the soft nucleophilic site, whereas the hard electrophile, dimethyl sulfate, preferentially attacks the carbonyl oxygen that is the hard nucleophilic site. Compound **1** also reacted efficiently with hydroxylamine and benzoylhydrazine in methanol at room temperature. The former reaction afforded the 4-hydroxy-6-hydroximino compound **11** and the latter reaction gave the 4-hydroxy-6-hydrazono compound **13**, both in high yields. In these reactions, the hydrogens on the nitrogens of nucleophiles migrated to the carbonyl oxygen at C-4. The structures of these compounds were also established from their spectral data and chemical conversions. For **11** and **13**, the ^{13}C nmr signals for the C-4 carbons shifted significantly upfield (δ 159-160), whereas those for the C-5 carbons shifted to downfield (δ 121), compared to the

corresponding signals for **6-8**. The ^1H nmr spectra revealed the existence of two acidic protons. The resonances at δ 19.2 for **11** and at δ 17.0 for **13** can be ascribed to the protons that are hydrogen-bonded with the carbonyl oxygens at C-7. The resonances at δ 10.2 for **11** and at δ 8.96 for **13** can be ascribed to the enolic protons at C-4. The assigned structures were also supported by chemical conversions. The reaction of **11** with methyl iodide in the presence of sodium hydride in dimethylformamide gave **9** in 31% yield. This product was identical in every respect with the compound obtained by the methylation of **6** with dimethyl sulfate. On the other hand, treatment of **11** with methyl iodide in the presence of a strong base, sodium methoxide, in dimethylformamide at room temperature gave the 4-methoxy compound **12**. The ^{13}C nmr signals for **12** almost coincided in position and pattern with those of **11**. But, the significant change was observed in the ^1H nmr spectra. The upfield proton signal (δ 10.2) due to the hydroxy proton at C-4 of **11** disappeared in **12**, although the downfield signal (δ 19.2) due to the hydrogen-bonded hydroxyimino proton in **11** remained in **12** at δ 19.0. Compound **13** was converted into the 4-methoxy compound **14** upon treatment with methyl iodide in the presence of sodium methoxide in dimethylformamide. The proton signal arising from the hydroxy group at C-4 of **13** also disappeared in **14**. Thus, all the experimental data were consistent with the assigned structures of the synthesized compounds shown in Scheme 1.

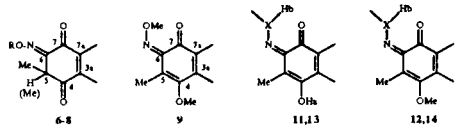
Scheme 2



Proposed mechanisms for the formation of **6**, **11**, and **13** are shown in Scheme 2. The nucleophilic displacement of the 6-methoxy group of **1** with methoxyamine affords **15**. This intermediate is converted into **6** by a 1,3-hydrogen migration or *via* the enol intermediate **16**. The primary products of the reactions with hydroxylamine and benzoylhydrazine would be **17** and **18**. These compounds are converted to **11** and **13** by a 1,5-hydrogen migration from the amino hydrazine nitrogen to the carbonyl oxygen at C-4. In these cases, the formation of the six-membered hydrogen-bonded structure plays an important role in determining the mode of hydrogen migration.

Table 1

^1H and ^{13}C NMR Data for the Ring A of Mitomycin C Derivatives



Compound	^{13}C NMR (δ/ppm)							^1H NMR (δ/ppm)		
	3a	4	5	6	7	7a	5-CH_3	Ha	Hb	5-CH_3
5	154	180	106	144	176	111	12	-	-	2.15(s)
6	152	191	44	153	177	126	18	-	-	1.40(d),1.41(d)
7	152	190	45	153	177	126	18	-	-	1.45(d),1.49(d)
8	153	192	45	154	176	124	20,23	-	-	1.55(s),1.57(s)
9	145	158	130	148	171	112	11	-	-	2.18(s)
11	139	160	121	148	169	108	10	10.17(s)	19.17(s)	2.08(s)
12	142	160	125	148	168	110	12	-	19.01(s)	2.30(s)
13	141	159	121	142	174	110	10	8.96(s)	17.04(s)	2.19(s)
14	142	159	125	144	174	109	11	-	17.08(s)	2.28(s)

Biological Activity.

The *in vitro* and *in vivo* biological activities of the compounds synthesized in this investigation were studied and compared with those of mitomycin C (Nu = NH_2, in **2**). These data are listed in Table 2. The *in vitro* tests were carried out against P388 leukemia cells. The cells were exposed to the test compounds of varing concentrations for 1 hour and then cultured at 37° for 72 hours. The IC_{50} values were estimated as the concentrations of the test compounds at which the growth of the cells was 50% inhibited. The LD_{50} values were measured by using male ddy mice and calculated by the method of Behrens and Korber [9] .

Table 2
In vitro and *in vivo* Biological Activities of Mitomycin C Derivatives

	LD_{50} (mg Kg^{-1})	IC_{50} (g ml^{-1})	C.I. (to MMC)	P388 (ILSmax %)
6	38	0.012	0.64	110
7	19	0.003	1.9	90
8	> 100	–	–	–
9	75	0.094	0.89	>158
10	-	0.72	–	–
11	53	1.135	0.72	114
12	75	0.015	2.0	>158
13	45	0.027	-	85
14	49	0.007	-	-
MMC [a]	5.5	0.009	1	110

[a] MMC: Mitomycin C.

The *in vivo* tests were carried out by using Sarcoma 180 tumor cells inoculated into ddy mice and also by using P388 leukemia cells inoculated into CD2F1 mice. The chemotherapeutic indexes (C.I. values) were calculated as the ratios of LD_{50}/ED_{50} in which the ED_{50} value is defined as a dose capable of reducing the tumor cell volume to one-half the corresponding volume in the untreated, control animals. The ILS max values were evaluated as the % values for the maximum increase in life span for P388 leukemia-inoculated mice. As can be seen from the LD_{50} values of Table 2, all the compounds synthesized showed a lower acute toxicity than mitomycin C. All the compounds, except **8**, exhibited antitumer activity, in the *in vitro* tests. Particularly, the chemotherapeutic indexes for **7** and **12** were higher than that of mitomycin C. In the *in vitro* tests, **7** and **14** displayed the highest cytotoxic activity (low IC_{50} value). In connection with the mechanism of cytotoxity, it may be of interest to note that the methylated compounds **12** and **14** exhibited much higher cytotoxic activity than the unmethylated compounds **11** and **13**. Compounds **9** and **12** displayed the highest efficiency in the % ILS max values for P388 leukemia-inoculated mice. The results of the above biological tests suggest that **12** is the best candidate for the antitumor agent among the compounds tested. Further details of biological activities are currently under investigation and will be reported elsewhere.

EXPERIMENTAL

General.

Melting points were determined with a Buchi 510 capillary melting point apparatus and are uncorrected. The ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a JEOL JNM GX270 FT NMR spectrometer using TMS as the internal standard. Infrared spectra were obtained on a Shimadzu IR-4000 instrument. Mass spectra were recorded on a JEOL JMS-D300 Mass Spectrometer. Column chromatography was performed on silica gel (Silica gel 60, Merck). The purity of compounds was checked by tlc on silica gel plates (Silica gel, F 254, Merck). Elementary analyses were taken on a Yanagimoto CHN Corder MT-3. Mitomycin A was obtained from Kyowa Hakko Kogyo Co. Ltd.

Preparation of Mitomycin C Derivatives.

[1aS-(1aα,8β,8aα,8bβ)]-8{[(Aminocarbonyl)oxy]methyl}-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methyl-6-phenylaminoazilidino[2',3':3,4]pyrrolo[1,2-*a*]indole-4,7-dione (**5**).

Aniline (0.80 g, 8.60 mmoles) and potassium carbonate (0.1 g, 0.72 mmole) were added to a stirred solution of mitomycin A (**1**) (1.00 g, 2.87 mmoles) in methanol (80 ml). After stirring at room temperature for 3 hours, the mixture was concentrated. The residue was chromatographed on silica gel with chloroform-methanol (9:1) to give 1.02 g (83%) of **5**, mp 175-180°; ir (potassium bromide): 3450, 3290, 1715, 1635, 1560, 1505, 1450, 1320, 1065 cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.35 (3H, s), 1.77 (1H, br), 2.76 (1H, bs), 2.84 (1H, bs), 3.18 (3H, s), 3.42 (1H, d), 3.48 (1H, dd), 3.99 (1H, d), 4.19 (1H, t), 4.62 (1H, dd), 6.49 (2H, bs), 7.04 (2H, d), 7.08 (1H, t), 7.31 (2H, t); ^{13}C nmr (DMSO-d_6): δ 12.3, 31.8, 35.5, 43.4, 49.3, 49.7, 61.0, 105.9, 108.7, 110.3, 123.0, 123.8, 128.3, 131.9, 143.5, 154.0, 156.8, 178.4, 179.9; ms: (m/z) 410 (M^+).
Anal. Calcd. for $C_{21}H_{22}N_4O_5$: C, 61.46; H, 5.37; N, 13.66. Found: C, 61.21; H, 5.32; N, 13.78.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-8a-methoxy-6-(methoxy)imino-5-methyl-1,1a,2,5,8,8a,8b-heptahydroazilidino[2',3':3,4]pyrrolo[1,2-*a*]indole-4,7-dione (**6**).

O-Methylhydroxylamine hydrochloride (150 mg, 1.80 mmoles) and sodium methoxide (97 mg, 1.80 mmoles) were added to a stirred solution of **1** (300 mg, 0.86 mmole) in methanol (30 ml). After stirring at room temperature for 24 hours, the mixture was concentrated. The residue was chromatographed on silica gel with chloroform-methanol (15:1) to give 170 mg (54%) of **6** as an orange colored powder, mp 185° dec: ir (potassium bromide): 3450, 1705, 1640, 1565, 1450, 1340, 1070 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ1.40 and 1.41 (3H, d), 2.84 (1H, m), 2.93 (1H, m), 3.21 and 3.24 (3H, s), 3.45 (1H, d), 3.73 and 3.77 (1H, dd), 3.88 and 4.24 (1H, d), 4.09 (3H, s), 4.16 (1H, m), 4.59 and 4.65 (1H, dd), 4.75 and 4.79

(1H, dd), 4.89 (2H, br); ^{13}C nmr (deuteriochloroform): δ 17.5, 18.1, 32.4, 32.6, 36.6, 43.3, 43.9, 44.0, 49.1, 49.2, 49.9, 61.8, 62.2, 63.6, 105.7, 105.8, 125.8, 126.0, 152.2, 152.7, 152.9, 154.3, 156.7, 156.8, 176.4, 176.8, 190.7, 191.9; ms: (m/z) 365 (M^+).

Anal. Calcd. for $C_{16}H_{20}N_4O_6$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.62; H, 5.72; N, 15.25.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-6-(isobutyloxy)imino-8a-methoxy-5-methyl-1,1a,2,5,8,8a,8b-heptahydroazilidino[2',3';3,4]pyrrolo[1,2-*a*]indole-4,7-dione (**7**).

O-Isobutyl hydroxylamine hydrochloride (240 mg, 1.91 mmoles) and triethylamine (0.5 ml) were added to a stirred solution of **1** (300 mg, 0.86 mmole) in methanol (30 ml). After stirring at room temperature for 18 hours, the mixture was concentrated. The residue was chromatographed on silica gel with chloroform-methanol (20:1) to give 110 mg (30%) of **7**, mp 160° dec; ir (potassium bromide): 3400, 1710, 1640, 1570, 1460, 1340, 1070 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.01 and 1.03 (6H, d) , 1.45 and 1.49 (3H, d) , 2.21 (1H, m) , 2.95 (1H, m), 3.06 (1H, m), 3.30 and 3.33 (3H, s), 3.74 (1H, m), 3.77 and 3.85 (1H, dd), 3.98 and 4.29 (1H, d), 4.08 (1H, m), 4.12 and 4.14 (2H, d), 4.70 (1H, m), 4.80 (1H, m); ^{13}C nmr (deuteriochloroform): δ 16.3, 17.7, 18.2, 27.2, 27.5, 32.4, 32.7, 37.1, 43.1, 43.6, 44.8, 45.2, 48.8, 49.0, 62.0, 62.9, 63.3, 105.7, 105.9, 125.9, 126.0, 152.1, 152.6, 152.9, 153.7, 158.3, 177.2, 177.4, 190.1, 190.5; ms: (m/z) 407 (M^+).

Anal. Calcd. for $C_{19}H_{26}N_4O_6$: C, 56.02; H, 6.39; N, 13.76. Found: C, 56.33; H, 6.45; N, 13.98.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-5,5-dimethyl-8a-methoxy-6-(methoxy)imino-1,1a,2,5,8,8a,8b-heptahydroazilidino[2',3':3,4]pyrrolo[1,2-*a*]indole-4,7-dione (**8**).

To a solution of **6** (313 mg, 0.86 mmole) in dimethylformamide (10 ml) and *tert*-butyl alcohol (10 ml) was added potassium *tert*-butoxide (120 mg, 1.07 mmoles), and the mixture was stirred for 10 minutes at room temperature. Methyl iodide (107 μl, 1.72 mmoles) was then added and the resulting mixture was further stirred for 3.5 hours. After quenching excess potassium *tert*-butoxide by adding a small amount of dry-ice, the reaction mixture was concentrated, and extracted with chloroform. The extract was dried over sodium sulfate and concentrated. Column chromatography of the residue on silica gel with chloroform-methanol (15:1) gave 166 mg (51%) of **8**, mp >200°; ir (potassium bromide): 3450, 2940, 1718, 1638, 1588, 1458, 1339, 1245, 1063, 1016 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.55 (3H, s), 1.57 (3H, s), 2.86-2.82 (1H, m), 2.95-2.90 (1H, m), 3.23 (3H, s), 3.45 (1H, dd), 3.77 (1H, dd), 4.10 (1H, d), 4.12 (3H, s), 4.64 (1H, t), 4.75 (1H, dd), 4.82 (2H, bs); ^{13}C nmr (deuteriochloroform): δ 20.1, 22.5, 32.2, 35.8, 43.6, 44.7, 49.2, 49.8, 61.3, 63.8, 107.7, 124.4, 153.0, 153.8, 156.8, 176.1, 192.3; ms: (m/z) 379 (M^+).

Anal. Calcd. for $C_{17}H_{22}N_4O_6$: C, 53.83; H, 5.80; N, 14.78. Found: C, 54.09; H, 5.63; N, 14.60.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-4,8a-dimethoxy-1,1a,2,8,8a,8b-hexahydro-6-(methoxy)imino-5-methylazilidino[2',3':3,4]pyrrolo[1,2-*a*]indol-7-one (**9**) and (**10**).

To a solution of **6** (898 mg, 2.46 mmoles) in dimethylformamide (35 ml) and *tert*-butyl alcohol (35 ml) was added potassium *tert*-butoxide (369 mg, 3.29 mmoles), and the mixture was stirred for 10 minutes at room temperature. Dimethyl sulfate (0.28 ml, 2.96 mmoles) was then added and the resulting mixture was stirred for 30 minutes at room temperature. After quenching excess potassium *tert*-butoxide by adding a small amount of dry-ice, the reaction mixture was concentrated and extracted with aqueous chloroform solution. The chloroform extract was dried over sodium sulfate and concentrated. Column chromatography of the residue on silica gel with chloroform-methanol (20:7) gave the crude product. The tlc on silica gel plate with chloroform-methanol (9:1) permitted separation of the crude product to give **9** (252 mg, 27%) and **10** (52 mg, 6%)

Compound **9** had mp 142° dec; ir (potassium bromide): 3420, 2938, 1706, 1616, 1549, 1480, 1452, 1333, 1218, 1067 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 2.18 (3H, s), 2.83-2.78 (1H, m), 2.91-2.93 (1H, m), 3.22 (3H, s), 3.49 (1H, d), 3.61 (1H, dd), 3.69 (3H, s), 4.09 (1H, d), 4.19 (3H, s), 4.53 (1H, t), 4.78 (2H, br), 4.82 (1H, dd); ms: (m/z) 379 (M^+).

Anal. Calcd. for $C_{17}H_{22}N_4O_6$: C, 53.83; H, 5.80; N, 14.78. Found: C, 53.70; H, 5.98; N, 14.95.

Compound **10** had mp 148 dec; ir (potassium bromide): 3420, 2938, 1715, 1623, 1541, 1480, 1455, 1331, 1218, 1051 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 2.21 (3H, s), 2.81 (1H, dd), 2.93 (1H, d), 3.23 (3H, s), 3.48 (1H, dd), 3.60-3.67 (1H, m), 3.69 (3H, s), 4.01 (1H, d), 4.17 (3H, s), 4.54 (1H, t), 4.72 (1H, dd), 4.91 (2H, br) ms (m/z) 379 (M^+).

Anal. Calcd. for $C_{17}H_{22}N_4O_6$; C, 53.83; H, 5.80; N, 14.78. Found: C, 54.11; H, 6.02; N,14.70.

[1aS-(1aα,8δ,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-6-(hydroxy)imino-8a-methoxy-5-methylazilidino[2',3':3,4]pyrrolo[1,2-*a*]indol-7-one (**11**).

A solution containing hydroxyamine hydrochloride (2.38 g, 34.2 mmoles) in water (10 ml) and sodium carbonate (2.18 g) was added to a solution of **1** (6.00 g, 17.2 mmoles) in methanol (400 ml) and the mixture was stirred at room temperature for 2 hours. After evaporation of solvent, the residue was chromatographed on silica gel with chloroform-methanol (9:1) to give 5.12 g (87%) of **11**, mp 173° (dec); ir (potassium bromide): 3400, 1705, 1590, 1480, 1330, 1060 cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 1.89 (H, t) 2.06 (3H, s), 2.76 (1H, dd), 2.89 (1H, d), 3.16 (3H, s), 3.45 (1H, dd), 3.53 (1H, dd), 4.09 (1H, dd), 4.17 (1H, d), 4.63 (1H, dd), 6.55 (2H, br), 10.17 (1H, s), 19.17 (1H, s); ^{13}C nmr (DMSO-d_6): δ 9.5, 31.2, 35.5, 41.6, 49.3, 49.6, 60.0, 105.9, 107.7, 120.9, 139.0, 148.2, 156.5, 160.1, 169.0; ms: (m/z) 351 (M^+).

Anal. Calcd. for $C_{15}H_{18}N_4O_6$: C, 51.43; H, 5.18; N, 16.00. Found: C, 51.22; H, 5.32; N, 15.88.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-4,8a-dimethoxy-1,1a,2,8,8a,8b-hexahydro-6-(methoxy)imino-5-methylazilidino[2',3':3,4]pyrrolo[1,2-*a*]indol-7-one (**9**).

To a solution of **11** (0.30 g, 0.85 mmole) in dimethylformamide (15 ml) was added 60% sodium hydride (0.67 g, 16.7 mmoles). The mixture was stirred at room temperature for 30 minutes, and methyl iodide (0.12 ml, 1.93 mmoles) was then added. The resulting mixture was stirred for 1 hour, and concentrated. The residue was poured into water and extracted with chloroform. The extract was washed with water, dried over sodium sulfate and concentrated. The residue was chromatographed on silica gel with chloroform-methanol (15:1) to give 0.10 g

(31%) of **9**, mp 140 (dec); ir (potassium bromide): 3450, 2940, 1705, 1620, 1540, 1480, 1450, 1330, 1210, 1050 cm^{-1}; ^1H nmr (DMSO-d_6): δ 2.14 (3H, s), 2.44 (1H, dd), 2.48 (1H, d), 3.38 (3H, s), 3.49 (1H, dd), 3.66 (3H, s), 3.88 (1H, dd), 3.95 (1H, d), 4.06 (3H, s), 4.49 (1H, dd), 4.55 (1H, dd), 6.55 (2H, br); ^{13}C nmr (DMSO-d_6): δ 10.5, 32.5, 37.3, 44.5, 49.4, 49.9, 60.1, 62.4, 65.2, 106.7, 112.2, 129.9, 145.3, 147.9, 156.4, 158.2, 171.3; ms: (m/z) 379 (M^+).

Anal. Calcd. for $C_{17}H_{22}N_4O_6$: C,53.83; H,5.80; N,14.78. Found: C,53.75; H,5.89; N, 14.45.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-4,8a-dimethoxy-1,1a,2,8,8a,8b-hexahydro-6-(hydroxy)imino-5-methylazilidino[2',3':3,4]pyrrolo[1,2-*a*]indol-7-one (**12**).

To a solution of **11** (3.00 g, 8.55 mmoles) in dimethylformamide (100 ml) was added sodium methoxide (0.42 g, 7.78 mmoles) and the mixture was stirred at room temperature for 20 minutes. Methyl iodide (0.69 ml, 11.08 mmoles) was then added. The resulting mixture was stirred at room temperature for 1.5 hours and concentrated. The oily residue was poured into water and extracted with chloroform. The chloroform layer was washed with water, dried over sodium sulfate and concentrated. The residue was chromatographed on silica gel with chloroform (15:1) to give 1.32 g (42%) of **12**, mp 148° dec: ir (potassium bromide): 3400, 1715, 1605, 1480, 1335, 1065 cm^{-1}; ^1H nmr (deuteriochloroform): δ 2.30 (3H, s), 2.87 (1H, dd), 2.98 (1H, d), 3.24 (3H, s), 3.57 (1H, dd), 3.72 (3H, s), 3.74 (1H, dd), 4.24 (1H, d), 4.51 (1H, dd), 4.84 (1H, dd), 19.01 (1H, s); ^{13}C nmr (deuteriochloroform): δ 11.9, 32.3, 37.0, 43.0, 49.6, 49.8, 60.3, 61.6, 107.3, 109.7, 124.7, 142.0, 148.0, 158.0, 160.0, 168.1; ms: (m/z) 365 (M^+).

Anal. Calcd. for $C_{16}H_{20}N_4O_6$: C, 52.60; H, 5.48; N, 15.34. Found: C, 52.80; H, 5.43; N, 15.19.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-1,1a,2,8,8a,8b-hexahydro-4-hydroxy-8a-methoxy-5-methyl-6-phenylhydrazonoazilidino[2',3':3,4]pyrrolo[1,2-*a*]indol-7-one (**13**).

Benzohydrazide (1.17 g, 8.60 mmoles) and potassium carbonate (0.10 g) were added to a solution of **1** (1.00 g, 8.87 mmoles) in methanol (80 g). After stirring at room temperature for 3 hours, the mixture was concentrated. The residue was chromatographed on silica gel with chloroform-methanol (6:1) to give 1.04 g (80%) of **13**, mp 178 dec; ir (potassium bromide): 3430, 3200, 1710, 1650, 1610, 1580, 1450, 1330, 1070 cm^{-1}; ^1H nmr (deuteriochloroform-DMSO-d_6 (3:1)): δ 2.19 (3H, s), 2.83 (1H, m), 3.00 (1H, m), 3.24 (3H, s), 3.59 (1H, d), 3.63 (1H, dd), 4.32 (1H, d), 4.39 (1H, dd), 4.83 (1H, dd), 5.61 (2H, br), 7.54 (3H, m), 8.00 (2H, dd), 8.96 (1H, s), 17.04 (1H, s); ^{13}C nmr (deuteriochloroform-DMSO-d_6 (3:1)): δ 10.3, 31.7, 36.2, 42.5, 49.3, 49.8, 60.8, 105.6, 109.3, 120.8, 127.6, 128.8, 132.6, 140.7, 141.6, 156.8, 158.9, 164.4, 173.8; ms: (m/z) 454 (M^+).

Anal. Calcd. for $C_{22}H_{23}N_5O_6$: C, 58.15; H, 5.07; N, 15.42. Found: C, 58.34; H, 4.96; N, 15.58.

[1aS-(1aα,8β,8aα,8bα)]-8{[(Aminocarbonyl)oxy]methyl}-4,8a-dimethoxy-1,1a,2,8,8a,8b-hexahydro-5-methyl-6-phenylhydrazonoazilidino[2',3':3,4]pyrrolo[1,2-*a*]indol-7-one (**14**).

To a solution of **13** (1.09 g, 2.40 mmoles) in dimethylformamide (100 ml) was added sodium methoxide (0.14 g), and the mixture was stirred at room temperature for 30 minutes, and methyl iodide (0.22 ml, 3.53 mmoles) was added. The resulting mixture was stirred at room temperature for 2.5 hours, and concentrated. The residue was poured into water and extracted with chloroform. The chloroform layer was washed with water, dried over sodium sulfate and concentrated. The residue was washed chromatographed on silica gel with chloroform-acetone (1:1), and then with chloroform-acetone (1:2) giving 0.14 g (13%) of **14** as an orange yellow powder, mp 170 dec; ir (potassium bromide): 3430, 1700, 1610, 1570, 1450, 1320, 1065 cm^{-1}; ^1H nmr (deuteriochloroform): δ 2.28 (3H, s), 2.88 (1H, m), 2.99 (1H, d), 3.26 (3H, s), 3.57 (1H, d), 3.73 (3H, s), 3.75 (1H, dd), 4.22 (1H, d), 4.57 (1H, dd), 4.84 (2H, br), 4.92 (1H, dd), 7.55 (3H, m), 8.05 (2H, m), 17.08 (1H, s); ^{13}C nmr (deuteriochloroform): δ 10.7, 32.0, 36.5, 42.6, 49.7, 49.9, 60.4, 62.0, 106.8, 109.4, 125.4, 127.2, 128.8, 131.5, 134.8, 141.5, 143.5, 157.2, 159.3, 165.0, 174.2; ms: (m/z) 468 (M^+).

Anal. Calcd. for $C_{23}H_{25}N_5O_6$: C, 58.97; H, 5.34; N, 14.96. Found: C, 59.11; H, 5.20; N, 14.75.

Biological Activity.

Cytoxicity *in vitro*.

Leukemia P388 cells were grown in RPMI-1640 media supplemented with 10% fetal calf serum, 10 μ*M* 2-hydroxyethyl disulfide and kanamycin (100 μg/ml). At the beginning of culture, the cell lines were exposed for 1 hour to various concentrations of the test compounds in each culture medium in a carbon dioxide incubator. The starting cell densities were 2 x 10^4 cells/ml. After exposing for 1 hour, the cells were collected by centrifugation at 400 G for 5 minutes, resuspended in a fresh culture medium and then cultured at 37° for 72 hours in a humidified atmosphere containing 5% carbon dioxide. The number of the cell were counted with a micro cell counter. The P388 cells were counted after trypsinization in a calicum and magnesium-free Ringer buffer solution for 5 minutes at 37 °. The IC_{50} values were estimated as the concentrations of the test compounds at which the growth of the cells were 50 % inhibited.

Acute toxicity (LD_{50}).

The test compound was injected into male ddy mice (male, SLC Inc., Hamamatsu), intraperitoneally (i.p.). The death ratio of the animals treated with the test compound was observed for 14 days, from which the LD_{50} value was calculated by the method of Behrens and Korber.

Anititumor Test. Sarcoma 180 Solid.

Sarcoma 180, 5 x 10^6 cells (a generous gift of the National Cancer Institute, Tokyo) were inoculated subcutaneously (n = 5) into ddy mice (male, body weight 20 ±1 g). The tumor cells were implanted for 24 hours and then the test compound was injected into the mice i.p., and the major axis (a) and minor axis (b) of the tumor mass were measured to calculate the tumor volume ($ab^2/2$). The antitumor effect was evaluated by the ratio (T/C) of the tumor volume in the test animals (T) to the corresponding volume of the control (untreated) animals (C). The ED_{50} value, which is defined as a dose capable of reducing the tumor volume to one-half the corresponding volume of the untreated group, was estimated by a regression line plotted on the T/C values (arithmetic scale) vs. dose (logarithmic scale).

Chemotherapeutic Index (C.I.).

The ratio of the LD_{50} to ED_{50} values for the test compound was calculated and defined as C.I.

Mouse Leukemia P388 (Survival Effect).

The 1 x 10^5 cells of P388 (obtained from the Cancer Chemotherapy Center, the Japan Foundation of Cancer Research, Tokyo) were inoculated into CD2F1 mice (SLC Inc.) i.p., and the test compound was injected into the mice after the tumor cells were inoculated for 24 hours. The effect of the test compound was evaluated by the percent increase of life span (% ILS), and the maximum value (ILS max) is listed in the Table 2.

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