SYNTHESIS AND *IN VITRO* ACTIVITY OF SOME METHYLENOMYCIN ANALOGS

MARCUS A. TIUS*, GREGORY M. L. PATTERSON* and DONALD P. ASTRAB

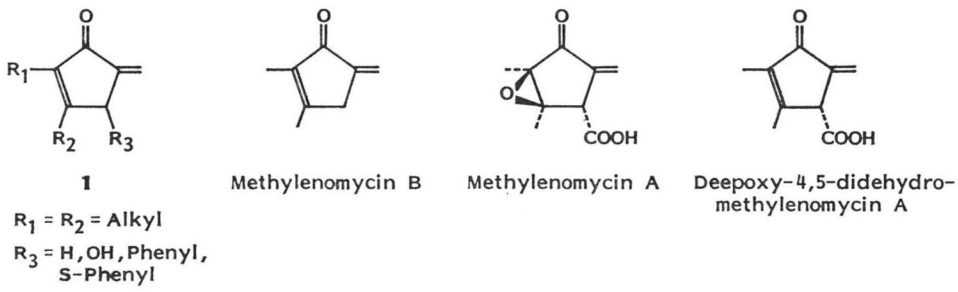
Department of Chemistry, University of Hawaii
Honolulu, Hawaii 96822, U.S.A.

(Received for publication March 25, 1985)

A cationic cyclopentannelation reaction has afforded a very simple route to a wide variety of methylenomycin analogs. The antibacterial activity of these readily accessible synthetic compounds parallels that of naturally occurring methylenomycin A. The *in vitro* antitumor activity of the synthetics assayed using human nasopharyngeal carcinoma (KB) cells is particularly promising.

The accidental discovery in our laboratory of a rapid cationic ring closure reaction has made a number of functionalized methylene cyclopentenones of general structure **1** readily available.[1] The obvious similarity between **1** and the methylenomycin antibiotics (Chart 1) suggested very strongly that antibiotic activity would be associated with the synthetic cyclopentenones which were produced during our study of the reaction. Methylenomycin B has been prepared a number of times through total synthesis,[2~12] as have methylenomycin A and deepoxy-4,5-didehydromethylenomycin A,[13~18] however, all the synthetic routes have been long and consequently impractical for the preparation of multigram quantities of material. The cationic route to compounds of general structure **1** is extremely efficient. The efficiency of the new method was demonstrated by preparing methylenomycin B in two steps (Chart 2). α-Lithio-α-methoxymethylallene[19~21] was added to 3-methyl-3-buten-2-one (**2**) in 91% yield. Cyclization of **3** with anhydrous ferric chloride in dichloromethane[22] produced methylenomycin B in 48% yield. Methylenomycin B which was used for direct comparison of biological ac-

Chart 1.

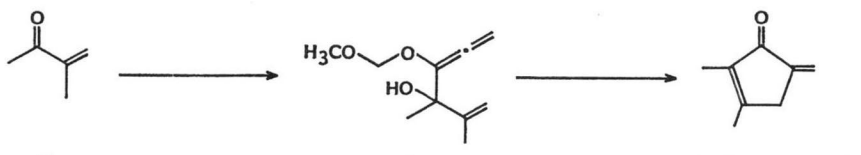


1 Methylenomycin B Methylenomycin A Deepoxy-4,5-didehydro-methylenomycin A

$R_1 = R_2 =$ Alkyl

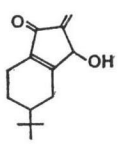
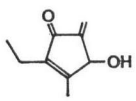
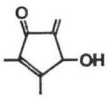
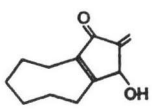
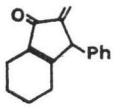
$R_3 =$ H, OH, Phenyl, S-Phenyl

Chart 2.



2 **3** Methylenomycin B

Table 1. Antimicrobial activities of methylenomycin analogs

	MIC (μg/ml)				
					
	4	**5**	**6**	**7**	**8**
Alcaligenes faecalis	50	12	25	25	100
Bacillus cereus	12	12	50	25	25
B. subtilis	<3	25	50	50	50
Escherichia coli K-12	>200	6	200	25	>200
E. coli ATCC 25922	>200	6	50	25	200
Klebsiella pneumoniae ATCC 13883	>200	12	25	12	200
Micrococcus roseus	50	25	100	25	25
Mycobacterium smegmatis	>200	100	>200	200	>200
Proteus vulgaris ATCC 13315	50	200	50	25	6
Pseudomonas aeruginosa ATCC 27853	100	>200	50	100	>200
P. fluorescens	50	25	50	50	>200
Salmonella typhi	50	25	25	12	50
Serratia marcescens	>200	12	25	12	50
Shigella flexneri ATCC 12022	>200	25	50	50	50
Staphylococcus aureus	25	50	50	50	25
Saccharomyces cerevisiae M-25	>200	200	>200	>200	>200
Candida albicans	>200	50	>200	>200	>200
Aspergillus flavus	>200	50	>200	>200	>200
Penicillium notatum	>200	50	>200	>200	>200
Trichophyton mentagrophytes	>200	50	>200	>200	>200

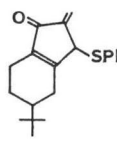
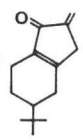
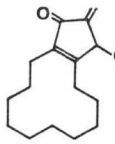
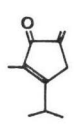
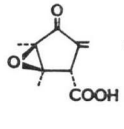
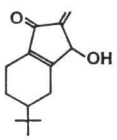
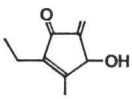
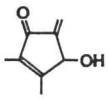
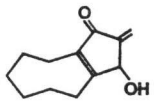
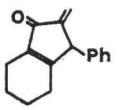
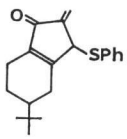
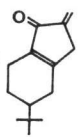
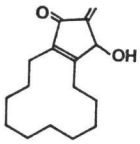
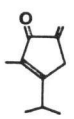
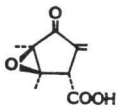
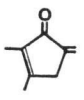
	MIC (μg/ml)					
						
	9	**10**	**11**	**12**		
Alcaligenes faecalis	200	>200	>200	>200	25	100
Bacillus cereus	3	100	50	200	100	100
B. subtilis	25	50	6	200	100	50
Escherichia coli K-12	>200	>200	>200	200	100	100
E. coli ATCC 25922	>200	>200	>200	>200	50	100
Klebsiella pneumoniae ATCC 13883	>200	>200	>200	>200	50	100
Micrococcus roseus	25	100	25	200	25	50
Mycobacterium smegmatis	>200	>200	>200	>200	100	>200
Proteus vulgaris ATCC 13315	200	100	200	100	200	200
Pseudomonas aeruginosa ATCC 27853	>200	>200	>200	>200	100	>200
P. fluorescens	>200	200	>200	>200	100	100
Salmonella typhi	50	>200	25	>200	100	100
Serratia marcescens	100	>200	>200	>200	50	100
Shigella flexneri ATCC 12022	>200	>200	100	>200	100	>200
Staphylococcus aureus	50	50	12	100	100	200
Saccharomyces cerevisiae M-25	>200	>200	>200	>200	>200	>200
Candida albicans	>200	>200	>200	>200	>200	>200
Aspergillus flavus	>200	>200	>200	>200	>200	>200
Penicillium notatum	>200	>200	>200	>200	>200	>200
Trichophyton mentagrophytes	>200	>200	>200	>200	>200	>200

Table 2. Cytotoxicity in the KB assay of methylenomycin analogs.

	 **4**	 **5**	 **6**	 **7**	 **8**
Minimum dose for >50% reduction in growth (μg/ml)	>10	0.5	10	0.1	10

	 **9**	 **10**	 **11**	 **12**		
Minimum dose for >50% reduction in growth (μg/ml)	>10	>10	>10	10	10	1

tivity of the natural products with that of the synthetic cyclopentenones, was unavailable from the natural source*, so that nearly a gram of the natural product was prepared according to Chart 2.

Biological Properties

The *in vitro* antimicrobial activities of nine synthetic products and of methylenomycins A and B toward representative bacteria, yeasts, and filamentous fungi have been listed in Table 1. Cyclopentenone **12** was the least active of the synthetics whereas **5**, **6**, and **7** were the most active. The antibacterial activity of these compounds compares favorably with that of the naturally occurring methylenomycins. The attenuation of antimicrobial activity noted for **11** may well be a result of diminished water solubility and thus reduced availability compared to the other compounds listed.

None of the compounds tested had any appreciable antimycotic activity.

Cytotoxicity of the cyclopentenones was determined in cultures of human nasopharyngeal carcinoma (KB) cells (Table 2). The progressive increase in cytotoxicity of compounds **6**, **5**, and **7**, in which activity increases with an increase in the length of the carbon chain of the aliphatic groups, was suggestive of a solubility effect, possibly involving intercalation into cellular membranes. In contrast, HANEISHI *et al.*,[23] in assessing structure activity relationships in modified methylenomycins, found antifungal activity to decrease with an increase in aliphatic carbon chain length, which suggests that cytotoxicity and antifungal activity are the results of different cellular effects.

Methylenomycins A and B have little cytotoxicity and low *in vivo* toxicity, as with the related cyclopentane antibiotic sarkomycin, which has both antimicrobial and antitumor activity.[24~26] Sarkomycin, which acts by inhibition of DNase, typically requires doses in excess of 100 μg/ml to obtain observable cytological effects.[27]

* A sample of methylenomycin A was made available to us through the generosity of Dr. MAMORU ARAI of the Sankyo Company.

Experimental

^{1}H NMR spectra were recorded at 300 MHz on a Nicolet spectrometer equipped with an Oxford magnet. ^{13}C NMR spectra were recorded on the same instrument at 75 MHz. IR spectra were recorded on a Beckmann IR 10 or on a Nicolet SMX FT spectrometer. Electron impact mass spectra were recorded on a Varian MAT-311 spectrometer.

2,3-Dimethyl-3-hydroxy-4-methoxymethyl-1,4,5-hexatriene (**3**)

A solution of 4 mmol of *n*-butyllithium in 10 ml of a 1:1 mixture of ether and THF was treated at −78°C with 500 mg (5 mmol) of methoxymethyl allenyl ether. After 30 minutes a solution of 84 mg (1 mmol) of 3-methyl-3-buten-2-one in 10 ml of ether - THF, 1:1 was added *via* cannula to the rapidly stirring solution of the anion. After 5 minutes the reaction was quenched by addition of 5 ml of water. The mixture was partitioned between ether and water. The ethereal layer was washed with water and brine and was dried ($MgSO_4$). Evaporation of the solvent followed by silica gel chromatography of the crude product (33% EtOAc/hexane) produced 162 mg of **3** (88% yield) as an oil.

^{1}H NMR ($CDCl_3$) δ 1.36 (3H, d, J=0.48 Hz), 1.75 (3H, m), 2.54 (1H, d, J=0.46 Hz), 3.39 (3H, s), 4.82 (2H, d, J=0.55 Hz), 4.92 (1H, t, J=1.40 Hz), 5.16 (1H, t, J=0.59 Hz), 5.53 (2H, s). IR (neat) 3495, 2926, 1958, 1655, 1454 cm^{-1}. MS (*m/z*) 185 (M+1, weak), 184 (M, weak), 166 (M−H_2O), 123 (M−CH_3OCH_2O), 85 (C_5H_9O). ^{13}C NMR (C_6D_6) δ 18.47, 24.44, 55.34, 74.63, 90.97, 93.84, 110.07, 133.08, 148.65, 196.49.

Methylenomycin B

To a solution of 162 mg (1 mmol, 0.5 equiv) of anhydrous ferric chloride in 100 ml of dichloromethane at 0°C was added 400 mg (2 mmol, 1.0 equiv) of 2,3-dimethyl-3-hydroxy-4-methoxymethyl-1,4,5-hexatriene (**3**) in 10 ml of dichloromethane. The reaction is rapid. After 5 minutes the reaction was quenched with satd aqueous sodium bicarbonate and the mixture was allowed to warm to room temperature. The reaction mixture was partitioned between dichloromethane and water. The organic layer was washed with satd aqueous sodium bicarbonate followed by water and brine. Drying ($MgSO_4$) followed by evaporation of the solvent produced the crude product as an oil. Silica gel chromatography (25% EtOAc/hexane) produced 117 mg methylenomycin B (48% yield).

^{1}H NMR ($CDCl_3$) δ 1.78 (3H, m, J=1 Hz), 2.04 (3H, m, J=1 Hz), 3.05 (2H, br s), 5.32 (1H, m, J=1.5 Hz), 6.02 (1H, m, J=1 Hz). IR (neat) 1690, 1660, 1625 cm^{-1}. MS (*m/z*) 122 (M), 107 (M−CH_3), 93, 86, 84, 79. Calcd for $C_8H_{10}O$ 122.0723, found 122.0732. ^{13}C NMR (C_6D_6) δ 8.28, 15.90, 36.59, 114.11, 138.18, 142.35, 162.30, 194.79.

General Procedure for the Synthesis of **4**~**12**

Nucleophilic Addition of α-Lithio-α-methoxymethylallene: A solution of a 4 mmol of *n*-butyllithium in 6 ml of a 1:1 mixture of ether and THF was treated at −78°C with 5 mmol of methoxymethylallenyl ether. Anion formation was presumed to be complete after 30~45 minutes. A solution of 1 mmol of the unsaturated ketone substrate* in 10 ml of ether/THF was added *via* cannula to the rapidly stirring lithioallene solution at −78°C. The progress of the reaction was monitored by thin-layer chromatography. After *ca.* 30 minutes the reaction was quenched by addition of 3 ml of water. Upon warming to room temperature the reaction mixture was partitioned between ether and water. The ethereal layer was washed with water and brine and was dried ($MgSO_4$) and was concentrated to produce the adducts in 65~95% yield. Although the products of this reaction were stable to chromatography on silica gel, purification prior to cyclization was unnecessary.

Cationic Cyclization: To a solution of 1 mmol of allene adduct in 10~15 ml of dichloromethane

* The preparation of *O*-trimethylsilyl hydroxymethylene ketones which were used to prepare **4**, **5**, **6**, **7** and **11** has been described.[31]

Addition of thiophenol to α-hydroxymethylene-4-*tert*-butylcyclohexanone produced the substrate for **9**. The aldol condensation product of benzaldehyde with cyclohexanone produced the substrate for **8**. The α-methylene ketones which were used for the synthesis of **10** and **12** were prepared from Eschenmoser's salt.[32,33]

at 0°C was added boron trifluoride etherate (1 μl/5 mg) dropwise with rapid stirring. The progress of the reaction was monitored by thin layer chromatography. Upon completion (<10 minutes) the reaction was quenched with 5% aqueous $NaHCO_3$ and was allowed to warm to room temperature. Partitioning between dichloromethane and water followed by drying ($MgSO_4$) and concentration produced the crude products which were purified by flash chromatography (ethyl acetate - hexane). Yields varied from 60~92%.

Spectroscopic Data of **4~12**

4 (mp 160~161°C, 76% yield): ^{1}H NMR ($CDCl_3$) δ 6.10 (1H, d, J=1.5 Hz), 5.62 (1H, br s), 4.93 (1H, br s), 0.91 (9H, s). IR (neat) 3400, 1700, 1640 cm^{-1}. MS (*m/z*) 220 (M), 202 (M−H_2O), 163 (M−*t*Bu), 136. Calcd for $C_{14}H_{20}O_2$ 220.1463, found 220.1467.

5 (66~72% yield): ^{1}H NMR ($CDCl_3$) 6.11 (1H, br s), 5.62 (1H, br s), 4.85 (1H, br s), 2.28 (2H, q, J=7.6 Hz), 2.11 (3H, s), 1.03 (3H, t, J=7.6 Hz). IR (neat) 3450, 2950, 1710, 1650 cm^{-1}. MS (*m/z*) 152 (M), 137, 134 (M−H_2O), 123, 109, 95. Calcd for $C_8H_{12}O_2$ 152.0837, found 152.0822.

6 (68% yield): ^{1}H NMR (100 MHz, $CDCl_3$) δ 6.05 (1H, br s), 5.60 (1H, br s), 4.85 (1H, br s), 3.38 (1H, br s), 2.08 (3H, s), 1.76 (3H, s). IR (neat) 3450, 2950, 1700, 1645 cm^{-1}. MS (*m/z*) 138 (M), 124, 123, 122, 120 (M−H_2O), 111, 110, 95. Calcd for $C_8H_{10}O_2$ 138.0681, found 138.0669.

7 (80% yield): ^{1}H NMR (100 MHz, $CDCl_3$) δ 5.95 (1H, br s), 5.52 (1H, br s), 4.84 (1H, br s), 3.56 (1H, br s), 1.39 (8H, m). IR (neat) 3450, 2900, 1710, 1645 cm^{-1}. MS (*m/z*) 192 (M), 177, 175, 164, 149, 135. Calcd for $C_{12}H_{16}O_2$ 192.1150, found 192.1142.

8 (75~78% yield): ^{1}H NMR ($CDCl_3$) δ 7.28 (5H, m), 6.05 (1H, d, J=0.5 Hz), 5.09 (1H, br s), 4.20 (1H, br s), 1.68 (4H, m). IR (neat) 2960, 1710, 1645 cm^{-1}. MS (*m/z*) 224 (M), 196 (M−CO), 187, 181, 167, 147 (M−Ph), 135, 77. Calcd for $C_{16}H_{16}O$ 224.1201, found 224.1198.

9 (82% yield): ^{1}H NMR ($CDCl_3$) δ 7.24 (5H, m), 6.14 and 6.12 (1H, d, J=1.6 Hz), 5.59 (1H, br s), 5.54 (1H, br s), 4.43 (1H, br s), 0.90 and 0.88 (9H, s). IR (neat) 2980, 1710, 1590 cm^{-1}. MS (*m/z*) 312 (M), 255 (M−*t*Bu), 204, 203 (M−SPh), 202, 176, 137. Calcd for $C_{20}H_{24}OS$ 312.1548, found 312.1559.

10 (56% yield): ^{1}H NMR (100 MHz, $CDCl_3$) δ 6.03 (1H, d, J=1.4 Hz), 5.32 (1H, d, J=1.6 Hz), 3.08 (2H, br s), 0.90 (9H, s). IR (neat) 2950, 1710, 1645 cm^{-1}. MS (*m/z*) 204 (M), 176 (M−CO), 148, 147 (M−*t*Bu), 117. Calcd for $C_{14}H_{20}O$ 204.1514, found 204.1507.

11 (82% yield): ^{1}H NMR (100 MHz, $CDCl_3$) δ 6.10 (1H, br s), 5.60 (1H, br s), 5.05 (1H, br s), 2.4~1.0 (20H, m). IR (neat) 3455, 2950, 1710, 1648 cm^{-1}. MS (*m/z*) 249 (M+1), 248 (M), 230 (M−H_2O), 205, 191, 177. Calcd for $C_{16}H_{24}O_2$ 248.1776, found 248.1769.

12 (48% yield): ^{1}H NMR ($CDCl_3$) δ 6.04 (1H, dd, J_1=1.3 Hz, J_2<1 Hz), 5.33 (1H, dd, J_1=1.2 Hz, J_2<1 Hz), 3.05 (2H, br s), 3.02 (1H, m), 1.76 (3H, t, J=2.1 Hz), 1.1 (6H, d, J=7.0 Hz). IR (neat) 1690, 1662, 1628 cm^{-1}. MS (*m/z*) 151 (M+1), 150 (M), 136, 135 (M−CH_3), 122 (M−CO), 107, 105. Calcd for $C_{10}H_{14}O$ 150.1048, found 150.1045.

Test Organisms

Saccharomyces cerevisiae, strain M-25, was provided by Dr. B. ADAMS, Department of Microbiology, University of Hawaii. All other microorganisms were obtained from the teaching collections maintained by the departments of microbiology and botany. The KB cell line was provided by Dr. E. FURUSAWA, of the J. A. Burns School of Medicine of the University of Hawaii.

Antimicrobial Activity

Minimum inhibitory concentration (MIC) values were determined by the broth dilution technique.[28] MIC's were determined visually after incubation at 37°C for 24 hours. The medium used for bacteria was heart infusion broth (Difco), pH 7.4, except in the case of *Mycobacterium*, which was tested using an identical medium supplemented with 6% glycerol. Yeasts were assayed using yeast nitrogen base (Difco), supplemented with 5% dextrose (pH 7). The susceptibility of filamentous fungi was determined by the broth dilution method of SHADOMY & ESPINEL-INGROFF,[29] using potato dextrose broth (Difco) adjusted to pH 7.

Antitumor Activity *In Vitro*

Human epidermoid carcinoma (KB) cells were cultured in the presence of various concentrations

of antibiotic in Eagle's minimal essential medium supplemented with 10% calf serum and 50 μg/ml gentamicin sulfate. Cells were added to tubes to give an initial density of 2.5×10^4 cells/ml.[30] Cultures were incubated at 37°C in an atmosphere of 5% carbon dioxide in air. Cell cultures were observed daily for 3 days following inoculation. MIC values were determined by the criteria of morphology and density of cells relative to control cultures.

Acknowledgment

Our acknowledgment is made to the National Science Foundation, CHE-84-03495, and to the National Institutes of Health, GM-30390-3, for their generous support. We thank Dr. MAMORU ARAI of the Sankyo Co., Ltd., for a generous sample of methylenomycin A.

References

1) TIUS, M. A. & D. P. ASTRAB: A cationic cyclopentannelation. Tetrahedron Lett. 25: 1539~1542, 1984
2) JERNOW, J.; W. TAUTZ, P. ROSEN & T. H. WILLIAMS: Methylenomycin B: Revised structure and total synthesis. J. Org. Chem. 44: 4212~4213, 1979
3) NEWTON, R. F.; D. P. REYNOLDS & T. EYRE: Total synthesis of methylenomycin B. Synth. Commun. 11: 527~532, 1981
4) TAKAHASHI, Y.; H. KOSUGI & H. UDA: A total synthesis of methylenomycin B. J. Chem. Soc. Chem. Commun. 1982: 496~497, 1982
5) BILLINGTON, D. C. & P. L. PAUSON: Methylenomycin B: An efficient synthesis from (2-butyne)-hexacarbonyldicobalt. Organometallics 1: 1560~1561, 1982
6) STRUNZ, G. M. & G. S. LAL: Fittig bislactones in cyclopentenone synthesis: Short synthesis of methylenomycin B. Can. J. Chem. 60: 2528~2530, 1982
7) SIWAPINYOYOS, T. & Y. THEBTARANONTH: Novel route to α-methylene cyclopentenones. High-yield synthesis of methylenomycin B. J. Org. Chem. 47: 598~599, 1982
8) DOUTHEAU, A.; J. SARTORETTI, & J. GORE: Synthese et cyclisation oxydante de triene-2,3,5 ols-1 en hydroxyalkyl-5 cyclopentene-2 ones-1. Tetrahedron 39: 3059~3065, 1983
9) NEGISHI, E. & J. A. MILLER: Novel syntheses of α,β-unsaturated cyclopentenones *via* allylzincation of alkynes. J. Am. Chem. Soc. 105: 6761~6763, 1983
10) MIKOLAJCZYK, M. & P. BALCZEWSKI: A new, total synthesis of methylenomycin B using organic sulfur and phosphorus reagents. Synthesis 1984: 691~694, 1984
11) POHMAKOTR, M. & S. CHANCHARUNEE: Intramolecular acylation of α-sulfinyl carbanion. A new general route to 5-methylene-2-cyclopentenones. Tetrahedron Lett. 25: 4141~4144, 1984
12) CAMERON, A. G.; A. T. HEWSON & M. I. OSAMMOR: Synthesis of cyclopentanoid natural products *via* vinyl phosphonium salts. Tetrahedron Lett. 25: 2267~2270, 1984
13) SCARBOROUGH, R. M. & A. B. SMITH: A stereospecific total synthesis of (±)-methylenomycin A. A novel antibiotic possessing an α-methylene ketone functionality. J. Am. Chem. Soc. 99: 7085~7087, 1977
14) SCARBOROUGH, R. M.; B. H. TODER & A. B. SMITH: A stereospecific total synthesis of (±)-methylenomycin A and its epimer, (±)-epimethylenomycin A. J. Am. Chem. Soc. 102: 3904~3913, 1980
15) JERNOW, J.; W. TAUTZ, P. ROSEN & J. F. BLOUNT: Stereospecific total synthesis and absolute configuration of (+)-methylenomycin A. J. Org. Chem. 44: 4210~4212, 1979
16) TAKAHASHI, Y.; K. ISOBE, H. HAGIWARA, H. KOSUGI & H. UDA: A total synthesis of (±)-methylenomycin A. J. Chem. Soc. Chem. Commun. 1981: 714~715, 1981
17) KOREEDA, M. & Y. P. L. CHEN: Synthesis of (±)-desepoxy-4,5-didehydromethylenomycin A. Tetrahedron Lett. 22: 15~18, 1981
18) BOSCHELLI, D.; R. M. SCARBOROUGH & A. B. SMITH: Total synthesis of (±)-desepoxy-4,5-didehydromethylenomycin A. Tetrahedron Lett. 22: 19~22, 1981
19) HOFF, S.; L. BRANDSMA & J. F. ARENS: Preparation, metallation and alkylation of allenyl ethers. Recl. Trav. Chim. Pays-Bas 87: 916~924, 1968
20) GANGE, D. & P. MAGNUS: An unusual new allene cyclization reaction. Synthesis of dihydrofuran-3(2H)-ones. J. Am. Chem. Soc. 100: 7746~7747, 1978
21) REICH, H. J. & M. J. KELLY: Silyl ketone chemistry. Synthesis and reactions of olefinic and acetylenic silyl ketones. J. Am. Chem. Soc. 104: 1119~1120, 1982
22) DENMARK, S. E. & T. K. JONES: Silicon-directed Nazarov cyclization. J. Am. Chem. Soc. 104: 2642~2645, 1982

23) HANEISHI, T.; A. TERAHARA, K. HAMANO & M. ARAI: New antibiotics, methylenomycins A and B. III. Chemical modifications of methylenomycin A and structure-activity correlations in methylenomycins. J. Antibiotics 27: 400~407, 1974
24) TAKEUCHI, T.: Bacteriological studies on actinomycetes products exhibiting anti-tumor activity. II. The bacteriostatic effect of sarkomycin and its relation to sulfhydryl group. J. Antibiotics, Ser. A 7: 37~44, 1954
25) UMEZAWA, H.; T. TAKEUCHI & K. NITTA: Sarkomycin, an anti-tumor substance produced by streptomyces. J. Antibiotics, Ser. A 6: 101, 1953
26) UMEZAWA, H.; T. YAMAMOTO, T. TAKEUCHI, T. OSATO, Y. OKAMI, S. YAMAOKA, T. OKUDA, K. NITTA, K. YAGISHITA, R. UTAHARA & S. UMEZAWA: Sarkomycin, an anti-cancer substance produced by streptomyces. Antibiot. & Chemother. 4: 514~520, 1954
27) SUNG, S.: Sarkomycin. *In* Antibiotics, Vol. I. *Eds.*, D. GOTTLIEB & P. D. SHAW, pp. 156~165, Springer Verlag, New York, 1967
28) WASHINGTON, II, J. A. & A. L. BARRY: Dilution test procedures. *In* Manual of Clinical Microbiology. 2nd Ed., *Ed.*, E. H. LENNETTE *et al.*, pp. 410~417, Am. Soc. Microbiol., Washington, D.C., 1974
29) SHADOMY, S. & A. ESPINEL-INGROFF: Susceptibility testing of antifungal agents. *In* Manual of Clinical Microbiology. 2nd Ed., *Ed.*, E. H. LENNETTE *et al.*, pp. 569~574, Am. Soc. Microbiol., Washington, D.C., 1974
30) GERAN, R. I.; N. H. GREENBERG, M. M. MACDONALD, A. M. SCHUMACHER & B. J. ABBOTT: Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother. Rep. 3: 1~103, 1972
31) TIUS, M. A. & S. ALI: Benzoannelation of ketones. J. Org. Chem. 47: 3163~3166, 1982
32) BRYSON, T. A.; G. H. BONITZ, C. J. REICHEL & R. E. DARDIS: Preformed Mannich salts: A facile preparation of dimethyl(methylene)ammonium iodide. J. Org. Chem. 45: 524~525, 1980
33) ROBERTS, J. L.; P. S. BORROMEO & C. D. POULTER: Addition of Eschenmoser's salt to ketone, ester and lactone enolates. A convenient synthesis of α-methylene carbonyls *via* Mannich intermediates. Tetrahedron Lett. 1977: 1621~1624, 1977