SYNTHESIS AND BIOLOGICAL ACTIVITIES OF CYCLOPROPENONE ANTIBIOTIC PENITRICIN AND CONGENERS

HIDETOSHI TOKUYAMA, MASAHIKO ISAKA and EIICHI NAKAMURA*

Department of Chemistry, Tokyo Institute of Technology,
Meguro, Tokyo 152, Japan

RYOICHI ANDO and YASUHIRO MORINAKA

Pharmaceuticals Laboratory, Research Center, Mitsubishi Kasei Corporation,
1000 Kamoshida-cho, Midori-ku, Yokohama 227, Japan

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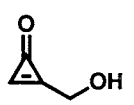
A number of derivatives of the cyclopropenone antibiotic penitricin have been synthesized by the reaction of metalated cyclopropenone acetals with electrophiles. Studies on the antimicrobial structure-activity relationships indicated that the penitricin skeleton, hydroxymethylcyclopropenone, is indispensable for antimicrobial activity. These compounds were also found to display cytotoxic activity.

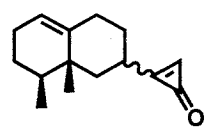
Recently three naturally occurring compounds (**1**,[1] **2**[2] and **3**[2]) containing a highly strained cyclopropenone ring have been isolated. Among them, penitricin (**1**), isolated from the culture filtrate of *Penicillium aculeatum* by OKUDA *et al.*,[1] is one of the rare classes of cyclopropenones known to have biological activity,[3] and it displays moderate activity against a broad range of Gram-positive and Gram-negative bacteria. The biosynthesis of penitricin has been investigated in some detail.[4] We recently developed the first general method for the synthesis of substituted cyclopropenones,[5~7] which is ideally suited for the synthesis of penitricin and its congeners. We report here the results of synthetic and biological studies on the penitricin class of antibiotics, focusing on the following issues: (1) the basic synthetic methodology, (2) the role of the cyclopropenone and the hydroxyl groups for the biological activity, and (3) the search for biological activity other than the antimicrobial one.

Chemistry

Only a limited range of substituted cyclopropenone derivatives have been studied for biological activity[8] due to the lack of efficient general methods for the synthesis of this interesting class of compounds.[9,10] Penitricin has been synthesized previously[3] in only *ca.* 1% overall yield, and no congeners have ever been prepared. With the aid of our general synthesis of cyclopropenones,[5,6] we have prepared penitricin and its congeners to gain the first insight into their structure-activity relationships. Representative penitricin congeners synthesized are listed in Table 1.



Penitricin (**1**)



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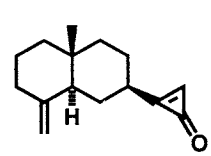

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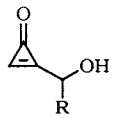
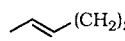
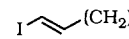
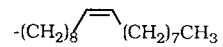
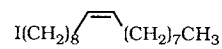
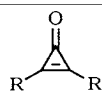
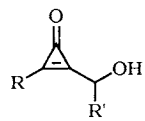
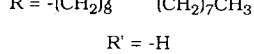
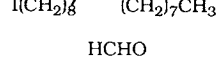
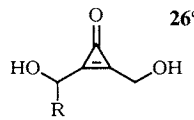
Table 1. Synthesis of penitricin and its congeners.

Cyclopropenones		R, R′	Electrophile	% yield[a]
	**1**	-H (penitricin)	HCHO	36
	12	$-C_2H_5$	C_2H_5CHO	65
	13	$-C_7H_{15}$	$C_7H_{15}CHO$	68
	14	$-(CH_2)_5-$		84
	**15**	-H	—	41
	16	$-CH_2CH(OH)CH_3$		70
	17	$-(CH_2)_3OH$	$I(CH_2)_3OTHP$	39
	18	$-(CH_2)_5OH$	$I(CH_2)_5OSiMe_3$	43
	19	$-(CH_2)CH_3$	$I(CH_2)_3CH_3$	81
	20			78
	21	-Ph	IPh	68
	22			49
	**23**	$-(CH_2)_3CH_3$	$I(CH_2)_3CH_3$	47
	**24**[b]	R = -Ph R′ = $-(CH_2)_4CH_3$	IPh $CH_3(CH_2)_4CHO$	69
	25[c]	R =  R′ = -H	 HCHO	29
	**26**[c]		HCHO 	37

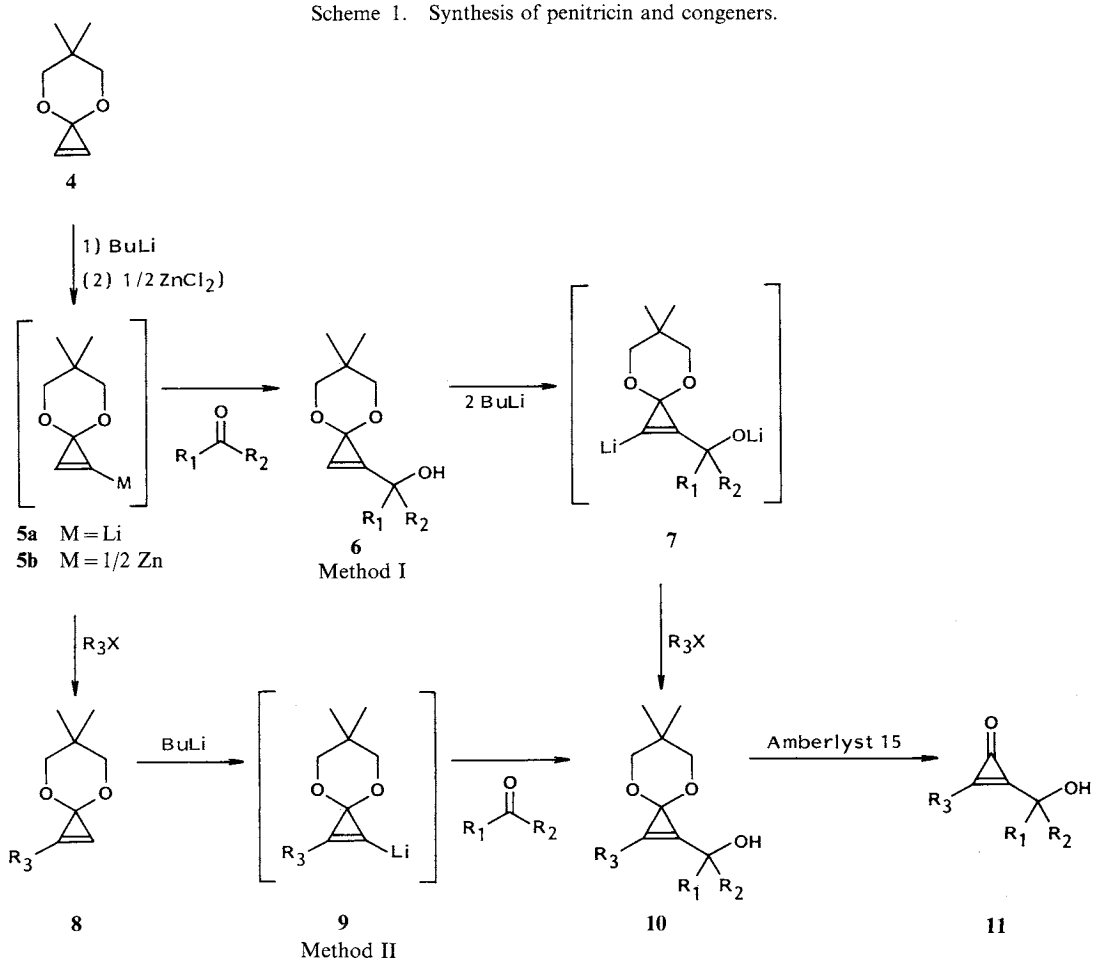
[a] Overall yield from **4**.
[b] Synthesized by Method II.
[c] Synthesized by Method I.

The strategy of our synthesis is summarized in Scheme 1. The synthesis basically relies on metalation of cyclopropenone acetal **4** or **8**, [7] and subsequent reaction with electrophiles. Hydrolysis of the alkylated acetals gave rise to a variety of cyclopropenones. The average overall yield of the substituted cyclopropenone **11** is about 30~80%.

The cyclopropenone acetal **4**, available in two steps from 1,3-dichloroacetone in 82% yield,[7] was converted to the corresponding lithium salt **5a** by treatment with *n*-BuLi in THF at −78°C in the presence of one or two equivalents of hexamethylphosphoric triamide or tetramethylethylenediamine. The reaction of **5a** with electrophiles such as alkyl halides, carbonyl compounds and epoxides afforded the monosubstituted acetals. Alternatively, treatment of the lithium salt **5a** with $ZnCl_2$ followed by the palladium-catalyzed cross-coupling reaction[11] of the acetal zinc salt **5b** with vinyl triflates, vinyl iodides and aryl iodides gave vinyl or aryl acetals.

Disubstituted acetals were synthesized *via* two different routes. In the first approach (Method I,

Scheme 1. Synthesis of penitricin and congeners.



"dianion method"), the 1-hydroxyalkyl group was introduced first by the reaction of the lithium salt **5a** with a carbonyl compound, and the adduct **6** was deprotonated with two equivalents of BuLi. Alkylation of the dianion **7** selectively proceeded at the vinyl position to give **10**. In the second approach (Method II), an alkyl or aryl group was introduced first, and the hydroxyalkyl side chain group was introduced next. The overall efficiency of the two methods was roughly the same.

Hydrolysis of the acetal moiety in acetone or in aqueous THF proceeded readily with an acidic ion exchange catalyst, Amberlyst 15, to afford the corresponding cyclopropenones in nearly quantitative yield. Penitricin (**1**) was prepared as a white solid in 40~50% overall yield. This compound is soluble in water, wherein it decomposes slowly. Introduction of an alkyl side chain did not significantly improve the life time of the compounds in aqueous medium. Thus, half-life times of penitricin (**1**) and its congener **13** in 10% aqueous DMSO are less than half a day.

Antibacterial Activity

Some representative data are summarized in Table 2. Monosubstituted α-hydroxycyclopropenones, **12**~**14**, showed an antibacterial activity comparable to that of penitricin. However, cyclopropenones lacking the α-hydroxyl group (**15**, **19**~**21** and **23**) or those having its hydroxy group at the β-, γ- or

Table 2. Antimicrobial activity of cyclopropenones.

Test organism	MIC (μg/ml)									
	Cyclopropenone									
	1	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**22**	**26**
Enterobacter cloacae IFO 963	100	100	50	100	>300	300	>300	300	>300	>100
Escherichia coli NIHJ JC-2	100	100	50	100	>300	300	>300	300	>300	>100
Klebsiella pneumoniae PCI 602	33	33	25	50	>300	300	>300	300	>300	>100
Pseudomonas aeruginosa IFO 3445	33	33	>100	>100	300	100	>300	>300	>300	>100
P. aeruginosa PAO1	33	33	>100	100	>300	100	>300	300	33	>100
Proteus morganii IFO 3848	33	33	12.5	50	300	300	>300	300	100	>100
P. rettgeri IFO 3850	33	33	25	50	>300	300	>300	300	>300	>100
P. vulgaris OX-19	33	33	25	50	300	100	>300	300	100	>100
Salmonella enteritidis G14	33	33	50	50	300	300	>300	300	>300	>100
Bacillus subtilis ATCC 6633	100	100	50	100	100	300	>300	>300	100	100
Micrococcus luteus ATCC 9341	33	33	50	100	300	n.d.	n.d.	n.d.	n.d.	11
Staphylococcus aureus FDA 209P JC-1	>100	100	100	>100	100	300	>300	>300	33	11
S. aureus MS353	>100	100	100	>100	100	300	>300	>300	33	3.7

ε-position of the side chain (**16**~**18**) showed very weak or no activity at all. Notably, the disubstituted cyclopropenones such as **24**, bearing an α-hydroxy group on the side chain, are also inactive. Interestingly, highly lipophilic compounds bearing a long aliphatic side chains **22** and **26** exhibit sizable activity against Gram-positive bacteria. For all series of compounds, the corresponding cyclopropenone acetals did not show any antibacterial activity. In conclusion, the monosubstituted α-hydroxymethyl-cyclopropenone structure in penitricin has been found to be necessary for the antibiotic activity.

Table 3. Cytotoxicity (HeLa S3) of cyclopropenones and their acetals.

Cyclopropenones	ED_{50} (μg/ml)[a]
24	2.00 (n.d.)
13	2.3 (n.d.)
16	2.32 (14.5)
18	2.44 (7.18)
14	2.7 (n.d.)
25	3.4 (12.1)
15	5.75 (20.4)
17	5.88 (8.73)[b]
26	21.3 (20.2)
22	62.8 (24.8)
21	81.6 (40.2)
23	223.2 (n.d.)

[a] Data for the corresponding acetals in brackets.
[b] Hydroxyl group was protected as THP ether.

Cytotoxicity

Most of the above cyclopropenones were also examined for their cytotoxicity against the HeLa S3 cell line. The representative effective dose values (ED_{50}) are listed in Table 3. The structure-activity relationship is quite different from that of the antibacterial activity. The α-hydroxy group which proved so important for the antibacterial activity was found to be irrelevant for the cytotoxicity. Some cyclopropenone acetals also exhibited cytotoxicity. The activity largely depends on the number of substituents on the cyclopropenone ring. The parent cyclopropenone and monosubstituted derivatives showed moderate cytotoxicity and disubstituted compounds showed much weaker activity.

In summary, we have synthesized a number of cyclopropenones and examined their biological activity to find that the α-hydroxymethylcyclopropenone structure is necessary for the antibacterial activity. The structure-activity profile of the cytotoxicity is very different from the antibacterial spectrum, suggesting that the mechanism of action is different.

Experimental

Antibacterial Activity

MICs were determined by the 2- or 3-fold agar dilution method using Sensitive Test agar (Nissui, Japan) after incubation at 37°C for 18 hours with incolumn size of 10^6 cfu/ml.

Cytotoxicity

HeLa S3 cells were grown at 37°C as suspensions in DMEM medium containing 10% fetal calf serum. Exponentially growing HeLa S3 cells were seeded in tubes at 10^5 cells/ml and the compounds were added at different concentrations at day 0. After cells were allowed to grow for 3 days at 37°C, final cell numbers were measured by the MTA method.[12] The ED_{50} were estimated by regression analysis concentration-response data.

Chemistry: General

^1H NMR (200, 270 and 500 MHz) and ^{13}C NMR (50, 67.5 and 125 MHz) spectra were measured for a $CDCl_3$ solution of samples of Jeol FX-200, GSX-270 and GSX-500 instruments. ^1H NMR spectra are reported in parts per million from internal tetramethylsilane, and ^{13}C NMR spectra from $CDCl_3$ (77.0 ppm). IR spectra were recorded on a Hitachi 260-10 instrument or a Jasco IR-800; absorptions are reported in cm^{-1}. All reactions using air and moisture sensitive compounds were carried out in a dry vessel under nitrogen. Ethereal solvents were distilled from sodium benzophenone ketyl immediately before use. Hexamethylphosphoric triamide (HMPA) was distilled from calcium hydride under nitrogen and stored over molecular sieves. Tetramethylethylenediamine (TMEDA) was distilled from calcium hydride and stored under nitrogen. Routine chromatography was performed on silica gel using a mixture of ethyl acetate and hexane as eluant.

2-Hydroxymethyl-2-cyclopropen-1-one (1)

To a solution of the cyclopropenone acetal **4** (2.5 ml, 18 mmol) and TMEDA (4.1 ml, 27 mmol) in THF (45 ml) at −70°C was added BuLi (11.9 ml of a 1.59 M solution in hexane, 18.9 mmol) over 5 minutes, and the mixture was stirred for 20 minutes. The reaction mixture was warmed to −40°C, and formaldehyde gas (thermolysis of 3.0 g of paraformaldehyde at 160°C), was bubbled into the solution over 30 minutes (carrier gas: nitrogen). The reaction was terminated by addition of a pH 7.4 phosphate buffer - THF solution (1 - 5, 10 ml). The mixture was filtered through a short column of Celite-silica gel (elution with Et_2O). The filtrate was concentrated to obtain an oily crude product (4.1 g). Column chromatography on silica gel (20 to 70% ethyl acetate in hexane) afforded the penitricin acetal (**6**: $R_1=R_2=H$, 1.56 g, 51%). Further purification was achieved by recrystallization from Et_2O - hexane.

The penitricin acetal (124 mg, 0.73 mmol) was dissolved in THF (0.7 ml) and water (66 μl). Amberlyst 15 (21 mg) was added and the suspension was stirred for 20 minutes. The resin was removed to obtain a yellow oil (94% yield by ^1H NMR analysis). Column chromatography on silica gel (Et_2O, then with ethyl acetate) gave penitricin (**1**) as a yellow oil (43.5 mg, 71%). Further purification by silica gel column chromatography (ethyl acetate) afforded a pure sample as a white solid, which could be stored in a −20°C freezer for more than three month without loss of purity. The spectral properties fully coincided with the reported values:[3] IR (neat) cm^{-1} 3350, 1940, 1835, 1585, 1065, 640; ^1H NMR (200 MHz, $CDCl_3$) δ 3.41 (1H, br s), 4.79 (2H, br s), 8.56 (1H, t, $J=1.3$ Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 58.8, 146.7, 156.3, 169.5.

Anal Calcd for $C_4H_4O_2$: C 54.14, H 4.80.
Found: C 53.92, H 4.94.

General Procedure for the Preparation of 2-Substituted Cyclopropenones *via* 5a

2-(1-Hydroxyoctyl)-2-cyclopropen-1-one (13)

To a solution of cyclopropenone acetal **4** (0.93 ml, 6.6 mmol) and TMEDA (2.72 ml, 18 mmol) in THF (10 ml) at −70°C was added 3.77 ml of BuLi (6.6 mmol in hexane) over 5 minutes. After stirring for 0.5 hour, octanal (0.94 ml, 6 mmol) was added dropwise, and stirred for 1 hour at that temperature. The reaction was terminated by addition of a pH 7.4 phosphate buffer (1/15 M) in THF (1 : 5, 3.5 ml). Purification of the crude product (2.2 g) on silica gel gave the acetal of **13** (1.34 g, 85%).

To a solution of the acetal (1.07 g, 4 mmol) in THF (6 ml) was added Amberlyst 15 (100 mg). The mixture was stirred for 2 hours at room temperature, filtered and concentrated to afford a crude oily product. Purification on silica gel gave **13** (0.58 g, 81%): IR (neat) cm^{-1} 3400, 2930, 2860, 1830, 1590, 1465, 1080; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, d, J=6.35 Hz), 1.19~1.42 (8H, m), 1.42~1.58 (2H, m), 1.73~1.90 (2H, m), 2.63 (1H, d, J=5.37 Hz), 4.82 (1H, dt, J=5.37 and 5.86 Hz), 8.51 (1H, d, J=1.98 Hz).

Anal Calcd for $C_{11}H_{18}O_2$: C 72.49, H 9.95.
Found: C 72.30, H 9.98.

General Procedure for the Preparation of 2-Substituted Cyclopropenones *via* **5b**

2-(*trans*-1-Hexenyl)-2-cyclopropen-1-one (**20**)

To a solution of the cyclopropenone acetal **4** (2.95 ml, 21 mmol) and HMPA (12.2 ml, 70 mmol) in THF (30 ml) at −70°C was added BuLi (12.9 ml of a 1.63 M solution in hexane, 21 mmol) over 6 minutes and the mixture was stirred for 30 minutes. Zinc chloride (10.5 ml of a 1 M solution in THF, 10.5 mmol) was added, and the dry ice/hexane bath was removed. *trans*-1-Iodo-1-hexene (2.0 ml, 14 mmol) and $Pd(PPh_3)_4$ (0.75 g, 0.70 mmol) were added, and the mixture was stirred at room temperature for 2 hours. Triethylamine (0.7 ml) was added, and the solution was diluted with hexane and passed through silica gel (27 g, 20% Et_2O in hexane). The filtrate was concentrated to afford an orange oil (3.5 g). Column chromatography on silica gel (5% ethyl acetate in hexane) afforded the acetal of **20** as a colorless oil (2.92 g, 94%).

To a solution of the acetal compound (49.2 mg, 0.22 mmol) in acetone (2 ml) was added Amberlyst 15 (7.5 mg), and the mixture was stirred for 20 minutes. The mixture was filtered and concentrated to afford a crude oily product. Purification on silica gel afforded **20** (25.1 mg, 84%): IR (neat) cm^{-1} 3050, 1830, 1640, 1570; ^1H NMR (200 MHz, $CDCl_3$) δ 0.93 (3H, t, J=7.4 Hz), 1.25~1.60 (4H, m), 2.25~2.40 (2H, m), 6.21 (1H, ddt, J=15.6, 1.3 and 1.3 Hz), 6.96 (1H, dt, J=15.6 and 6.9 Hz), 8.09 (1H, d, J=1.3 Hz).

Anal Calcd for $C_9H_{12}O$: C 79.39, H 8.88.
Found: C 79.11, H 8.63.

General Procedure for the Preparation of 2,3-Disubstituted Cyclopropenones *via* Dianion **7**

2-Hydroxymethyl-3-oleyl-2-cyclopropen-1-one (**25**)

To a solution of penitricin acetal (**6**, R_1=R_2=H) (74.7 mg, 0.439 mmol) and HMPA (0.229 ml, 1.32 mmol) in THF (1.5 ml) at −70°C was added 0.57 ml of BuLi (0.92 mmol in hexane) over 3 minutes, and the mixtures was stirred for 0.5 hour. Oleyl iodide (170 μl, 0.66 mmol) was added and stirred for 8 hours at that temperature. A solution of a pH 7.4 phosphate buffer in THF (0.24 ml) was added to the mixture, which was then diluted with hexane. The mixture was passed through silica gel (Et_2O), and concentrated to obtain 0.32 g of an orange oil. Column chromatography on silica gel afforded the acetal of **25** (114 mg, 62%).

To a solution of the disubstituted acetal (67.3 mg, 0.16 mmol) in THF (0.3 ml) was added Amberlyst 15 and water (14 μl). The suspension was stirred for 15 minutes. Filtration and concentration afforded a crude oily product. Purification on silica gel gave the title compound (48.9 mg, 91%): IR (neat) cm^{-1} 3350, 2920, 2850, 1850, 1625, 1060; ^1H NMR (200 MHz, $CDCl_3$) δ 0.89 (3H, m), 1.2~1.4 (22H, m), 1.71 (2H, m), 2.02 (4H, m), 2.66~2.70 (3H, m), 4.76 (2H, d, J=5.7 Hz), 5.34~5.37 (2H, m).

Anal Calcd for $C_{22}H_{38}O_2$: C 78.99, H 11.45.
Found: C 79.00, H 11.48.

Acknowledgment

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