PENITRICIN, A NEW CLASS OF ANTIBIOTIC PRODUCED BY *PENICILLIUM ACULEATUM*

III. STRUCTURAL CONFIRMATION BY CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITY

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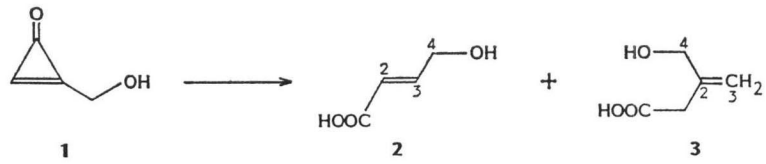
A novel antibiotic, penitricin showing anti-Gram-negative activity, has been isolated from the culture filtrate of *Penicillium aculeatum*. Chemical and physico-chemical studies determined the structure of penitricin as hydroxymethylcyclopropenone (**1**), which was confirmed by chemical synthesis from propargyl alcohol. Biological activity of penitricin and several cyclopropenones as well as two metabolites, penitricins B and C was compared.

Penitricin (**1**), a new anti-Gram-negative antibiotic was isolated and purified from the culture filtrate of *Penicillium aculeatum* NR 5165 as described in the previous paper[1,2]. We wish here to report the structure elucidation of **1** on the basis of the characteristic physico-chemical properties and degradation pattern, and chemical synthesis from propargyl alcohol.

Penitricin (**1**), a colorless oil, has the moleculer formula $C_4H_4O_2$ (EI-MS m/z 84.0203 (M^+), Calcd m/z 84.0210). The IR spectrum (KBr) revealed the presence of hydroxyl (3350 cm^{-1}), highly strained carbonyl (1830 cm^{-1}) and double bond (1590 cm^{-1}). The latter two strong absorptions were characteristic to the cyclopropenones[3]. ^1H NMR (in D_2O at 5°C) showed methylene protons adjacent to the hydroxyl group at δ 4.88 (2H, s) and a methine proton at δ 8.81 (1H, s). The latter was assigned as a ring proton of cyclopropenone since such characteristic downfield shift of the ring proton was well-known in the monosubstituted cyclopropenones[3,4]. From these data penitricin was suggested to be hydroxymethylcyclopropenone. This assumption was also supported by ^{13}C NMR. Thus in ^{13}C NMR spectrum (in D_2O at 5°C) of **1**, four signals were observed at δ 57.39 (CH_2OH), 146.46 (C-3), 158.92 (C-1) and 167.20 (C-2). The assignment of the signals were tentatively made based on the data of cyclohexylcyclopropenone reported by BOHLMANN *et al.*[3], since off-resonance spectrum of **1** could not be measured because of the instability of **1**.

Furthermore the following characteristic decomposition process of penitricin strongly supported the above conclusion. Thus, when **1** was kept at room temperature for a few days, complete decomposition of **1** into γ-hydroxycrotonic acid (**2**) and α-hydroxymethylacrylic acid (**3**) was observed from its NMR. The signals due to **2** were observed at 170.14 (s, C-1), 149.05 (d,C-3), 118.87 (d, C-2) and 60.75 (t, C-4) in ^{13}C NMR and at 7.04 (dt, J_1=16, J_2=5 Hz, C3-H), 6.01 (dt, J_1=16, J_2=2, C2-H) and 4.25 (m, C4-H) in the ^1H NMR spectrum. The signals due to **3** were observed together with the above mentioned signals at 169.56 (s, C-1), 138.97 (s, C-2), 127.14 (t, C-3) and 60.75 (t, C-4) in the ^{13}C NMR spectrum and at 6.26 (s, C3-H), 5.88 (s, C3-H) and 4.25 (m, C4-H) in the ^1H NMR spectrum. Compound **2**, mp 105~107°C, was isolated and identified with the authentic γ-hydroxycrotonic acid, synthesized according to the literature procedure[5], in ^1H and ^{13}C NMR, IR and MS. This type of the decomposition

Fig. 1. Decomposition of penitricin.



process in monosubstituted cyclopropenones has already been reported by BRESLOW[4] and BOHLMANN[8].

For further confirmation of our conclusion, penitricin was prepared as follows.

Due to the sensitive nature of the penitricin, great care was taken to avoid serious decomposition of cyclopropenone ring during the course of the reactions. Among the known procedures[3,4] for the preparation of the monosubstituted cyclopropenones, BRESLOW's procedure[4] using lithium trichloromethane[6] seemed to be the most suitable for our purpose because of the mildness of reaction condition. The choice of the protective group of primary hydroxyl group was also important, in that removal below room temperature in a short time period under non basic conditions was an essential requirement. Thus propargyl alcohol **4** was converted to the silyl derivative **5** by treatment with *tert*-butyldimethylsilyl-chloride - imidazole - DMF (80% yield) followed by protection of acetylenic proton of **5** with trimethylsilyl group (66% yield). **6** was then reacted with lithium trichloromethane, generated *in situ* at −115°C according to BRESLOW's procedure, at −96°C followed by hydrolysis of the resulting 1,1-dichlorocyclopropenone derivative with conc hydrochloric acid at −110°C to room temperature to afford the cyclopropenone **7** [*m/z* 270 (M^+), IR 1820, 1580 cm^{-1}] (2.2% yield) together with a large amount of starting material **6** after silica gel chromatography. The crude sample containing a small amount of **7** was subjected to the final deprotection reaction without further purification. Removal of the silyl protective groups in **7** was achieved by treatment with 5% solution of 46% aqueous HF in acetonitrile[7] at room temperature for 2 hours to yield the desired hydroxymethylcyclopropenone (**1**) as a pale yellow oil (nearly quantitave yield) after removal of water by freeze-drying at −25°C. This synthetic compound was completely identical with natural penitricin in ^1H and ^{13}C NMR (in D_2O at 5°C), IR and MS.

Biological activity of cyclopropenones and two other metabolites is shown in Table 1 which compares the *in vitro* antimicrobial activities of penitricin and the other synthetic cyclopropenones. Penitricin is mainly active against Gram-negative bacteria including *Pseudomonas*, *Citrobacter*, *Enterobacter* and *Serratia*. The MICs against sensitive and resistant strains were almost identical: 3.13~12.5 μg/ml, and few resistant colonies appeared at any concentration. In contrast, penitricin showed rather

Fig. 2. Chemical synthesis of penitricin.

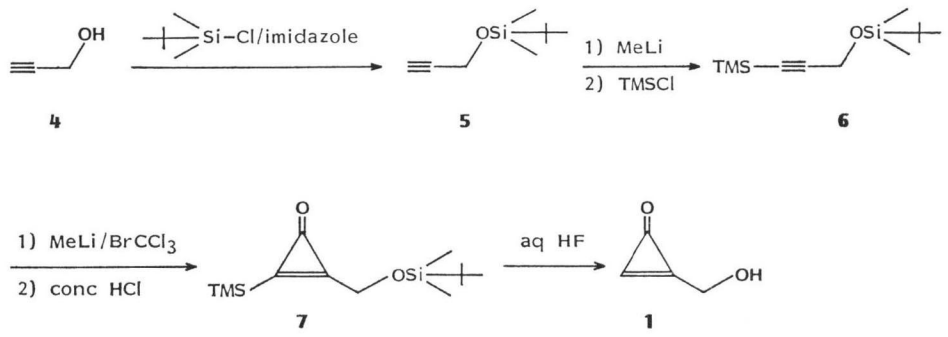
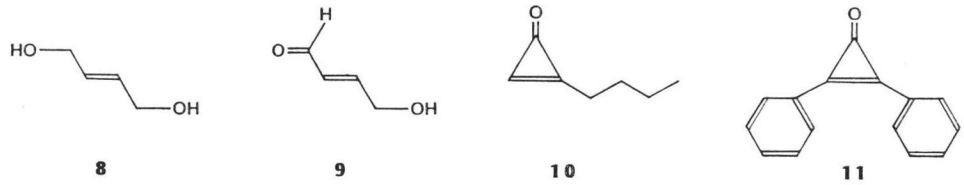


Table 1. Antimicrobial spectrum of cyclopropenones and two metabolites **8** and **9** (μg/ml).

Test organism	**1**	**9**	**8**	**7**	**10**	**11**
Alcaligenes faecalis IFO 13111	12.5	>100	>100	50	100	>100
Citrobacter freundii IFO 12681	12.5	>100	>100	>100	100	>100
C. freundii 5D60-1	12.5	>100	>100	>100	100	>100
Comamonas terrigena IFO 12685	6.7	100				
Enterobacter aerogenes 5I497-0	12.5	>100	>100	>100	100	>100
E. cloacae 6D63-2	12.5	>100	>100	>100	100	>100
Escherichia coli NIHJ JC-2	12.5	>100	>100	>100	>100	>100
E. coli CF41	12.5	>100	>100	>100	100	>100
Klebsiella pneumoniae PCI 602	12.5	>100	>100	>100	>100	>100
K. pneumoniae 1X165	12.5	>100	>100	>100	>100	>100
Proteus rettgeri ATCC 14505	12.5	>100	>100	>100	100	>100
P. vulgaris OX19 ATCC 6898	6.25	>100	>100	100	50	100
P. vulgaris GN 7919	12.5	>100	>100	100	25	>100
Pseudomonas aeruginosa A3	6.25	>100	>100	100	100	>100
P. aeruginosa 5E81-1	12.5	>100	>100	>100	>100	>100
P. stutzeri IFO 12695	3.13	>100	>100	50		>100
P. maltophilia IFO 12690	26.8	>100				
Serratia marcescens IFO 12648	12.5	>100	>100	>100	100	>100
S. marcescens 5A405-1	12.5	>100	>100	>100	100	>100
Salmonella typhimurium IFO 12529	12.5	>100	>100	>100		>100
Bacillus subtilis PCI 219	25	>100	>100	25	>100	>100
Micrococcus luteus ATCC 9341	6.25	>100	>100	100	100	100
Staphylococcus aureus 209P JC-1	50	>100	>100	100	100	100
S. aureus MS9261	50	>100	>100	100	>100	>100

Fig. 3.

8: Penitricin C (*trans*-2-butene-1,4-diol), **9**: Penitricin B (4-hydroxycrotonaldehyde), **10** and **11**: see text.



8 **9** **10** **11**

weak activity against Gram-positive bacteria and no activity against fungi or yeasts. On the other hand, more stable mono- or di-substituted cyclopropenones, **7**, 1-butylcyclopropenone (**10**) and diphenylcyclopropenone (**11**), possessed less activity than unstable penitricin. Moreover, two other metabolites, penitricin B (4-hydroxycrotonaldehyde, **9**) and penitricin C (*trans*-2-butene-1,4-diol, **8**) were practically inactive.

There is no data regarding biological activity of monosubstituted cyclopropenones. However disubstituted cyclopropenones prepared by TOBEY[5] exhibited antibacterial, antifungal or insecticidal activity. According to the patent[5], bis(2,2-dichlorovinyl)cyclopropenone, bis(2,2-dibromovinyl)-cyclopropenone or bis(2-methylpropenyl)cyclopropenone, for instance, are active against *Staphylococci*, *Mycobacterium*, *Bacillus*, *Trichophyton* and *Aspergillus*; that is, against Gram-positive bacteria and fungi.

Experimental

General

^1H and ^{13}C NMR spectra were recorded with a Jeol FX-100 NMR spectrometer in $CDCl_3$ unless otherwise stated and were reported in δ from tetramethylsilane. IR spectra were recorded with a Hitachi 260-10 spectrometer. Low and high resolution mass spectra were obtained with a Jeol DX-300 system. Melting points were uncorrected. Thin-layer chromatography was carried out on 0.25 mm Merck precoated silica gel plates (Kieselgel 60 F_{254}) with 0.5% potassium permanganate in 1 N sodium hydroxide as a detection agent.

1-*t*-Butyldimethylsilyloxyprop-2-yne (**5**)

To a stirred solution of propargyl alcohol (5.0 ml, 85.9 mM) and *t*-butyldimethylsilyl chloride (12.95 g, 85.9 mM) in dry DMF (50 ml), imidazole (11.69 g, 171.8 mM) was added at 0°C. The mixture was stirred at 0°C for 10 minutes and then kept at room temperature overnight. The reaction mixture was diluted with ether (200 ml), washed with water (200 ml×3) and dried over anhydrous magnessium sulfate. After removel of ether by distillation, the residue was distilled under reduced pressure (bp 57~64°C/20 mmHg) to give 11.83 g (yield 81%) of pure silyl ether **5** as a colorless oil: MS *m/z* (%) 170 (M^+, 9), 155 (9), 114 (50), 113 (100), 83 (75); IR ($CHCl_3$) 3300 (H–C≡C), 1250 (Si-Me_3), 1080, 830; ^1H NMR ($CDCl_3$) δ 0.14 (6H, s, Si-Me_2), 0.97 (9H, s, Si-^{t}Bu), 2.40 (1H, t, J=2.0, H-C≡), 4.34 (2H, d, J=2.0, ≡C–CH_2–O–).

1-*t*-Butyldimethylsilyloxy-3-trimethylsilylprop-2-yne (**6**)

To a stirred cold (−78°C) dry ether (100 ml) was added a solution of methyllithium in ether (1.4 M solution, 4.2 ml) under argon. The silyl ether **5** (11.83 g, 69.4 mM) was added dropwise into the above solution and the mixture was stirred at −78°C for 10 minutes and at −40°C for 25 minutes. Then trimethylsilyl chloride (8.81 ml, 69.4 mM) was added dropwise at −78°C and the mixture was allowed to warm up to room temperature. The reaction mixture was diluted with water, and ether layer was separated, washed with water and dried over anhydrous $MgSO_4$. After removal of ether, the residue was distilled under reduced pressure (bp 67~69°C/2 mmHg) to give 12.9 g (yield 87%) of pure **6** as a colorless oil: MS *m/z* (%) 185 (M^+, 55), 155 (100); IR ($CHCl_3$) 2240 (C≡C), 1240, 1080, 900; ^1H NMR ($CDCl_3$) δ 0.11 (6H, s, Si-Me_2), 0.19 (9H, s, Si-Me_3), 0.90 (9H, s, Si-^{t}Bu), 4.34 (2H, s, ≡C–CH_2–O–).

2-*t*-Butyldimethylsilyloxymethyl-3-trimethylsilylcyclopropenone (**7**)

Bromotrichloromethane (3.0 ml, 30.2 mM) was added dropwise over a 30-minute period to a stirred suspension of methyllithium (21.6 ml of a 1.4 M solution in ether, 30.2 mM) in ether (46 ml) held at −115°C (ether - frozen ether slurry) under an argon atmosphere. After 50 minutes of additional stirring, the silyl ether **6** (12.9 g, 60.4 mM) was added dropwise over a 30-minute period. The reaction mixture was then warmed to −100~−95°C (methylene chloride - frozen methylene chloride slurry) and stirred for 2 hours. Then concd HCl (3.45 ml) was added dropwise over 10 minutes at −115°C and the mixture was allowed to warm to 10°C. Cold water (50 ml) and EtOAc (100 ml) was added to the reaction mixture. The phases were separated and the organic phase was washed with cold water, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was chromatographed on silica gel (Wako C-200, 100 g). After elution of the unreacted starting material with hexane - EtOAc (10: 1), 164 mg (yield 2.2% based on methyllithium) of the desired cyclopropenone **7** was eluted with hexane - EtOAc (6: 4). Although containing a small amount of the corresponding detrimethylsilyl compound, this material was subjected to next deprotection reaction without further purification: MS *m/z* (%) 270 (M^+, 3), 185 (50), 155 (100); IR ($CHCl_3$) 1820 (C=O), 1580 (C=C), 1240, 1100, 840; ^1H NMR ($CDCl_3$) δ 4.83 (2H, s, =C–CH_2–O–), 0.93 (9H, s, Si-^{t}Bu), 0.34 (9H, s, Si-Me_3), 0.13 (6H, s, Si-Me_2).

Hydroxymethylcyclopropenone (**1**)

A solution of the silyl ether **7** (49 mg, 0.23 mM) in 5% solution of 46% aq HF in acetonitrile (2 ml) was kept at room temperature for 2 hours. The reaction mixture was concentrated at 20°C by rotary evaporator. The aq concentrate was diluted with H_2O (0.5 ml) and lyophilized at −25°C for 3 hours to give hydroxymethylcyclopropenone (**1**) (19 mg) as a colorless oil in quantitative yield: IR (KBr) 3350

(OH), 1830 (C=O), 1590 (C=C); MS *m/z* (%) 84 (M^+, 98), 67 (M−OH, 71), 57 (55), 56 (M−CO, 100), 54 (M−CH_2O, 52); ^1H NMR (D_2O at 5°C) δ 8.77 (1H, s, HC=), 4.80 (2H, s, =C-CH_2-O); ^{13}C NMR (D_2O, at 5°C) δ 168.32 (C2), 160.24 (C1), 147.65 (C3), 58.55 (C2-CH_2-O-).

1-Butylcyclopropenone (**10**)

1-Butylcyclopropenone was prepared using 1-hexyne as a starting material according to the procedure for the synthesis of 1-propylcyclopropenone described by BRESLOW *et al.*[4]: IR ($CHCl_3$) 1820 (C=O), 1580 (C=C); MS *m/z* (%) 111 (M+1, 100); ^1H NMR ($CDCl_3$) δ 8.48 (1H, s), 2.78 (2H, t, J=7.0 Hz), 2.05~1.15 (4H, m), 0.97 (3H, unresolved t).

Diphenylcyclopropenone (**11**)

Diphenylcyclopropenone was purchased from Aldrich Chemical Co.

Minimum Inhibitory Concentration

MICs of the antibiotics against various microorganisms were determined by a serial two-fold agar dilution method on Mueller-Hinton agar (Difco). The pH of the medium was adjusted to 6.0 before sterilization. Final inoculum level was 10^6 cells/ml. Determination was made after 18-hour incubation at 37°C.

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