CHEMICAL MODIFICATION OF CORIOLIN B*

YOSHIO NISHIMURA, YOSHIYUKI KOYAMA** and SUMIO UMEZAWA
Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasaki-shi, 211 Japan

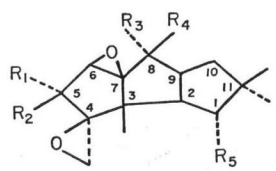
TOMIO TAKEUCHI, MASAAKI ISHIZUKA and HAMAO UMEZAWA
Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo, Japan

(Received for publication November 15,1976)

Several derivatives of 5-ketocoriolin B (**8**, **11**, **12**) chemically modified at C-8 have been synthesized. These derivatives showed antitumor and antibacterial activity of the same degree as 5-ketocoriolin B (**4**) and diketocoriolin B (**5**) which were the most active members of the known coriolin group antibiotics. These derivatives were more stable than **4** and **5** in acidic or alkaline solution.

Coriolins[1], produced by the mushroom, *Coriolus consors*, are tricyclic sesquiterpenoids as indicated in the formulas (**1**, **2**, **3**)[2,3]. Coriolin (**1**) and coriolin C (**3**) have antitumor and antibacterial activities. Coriolin B (**2**) has no activity but its oxidation products, 5-ketocoriolin B (**4**) and diketocoriolin B[4] (**5**) have antibacterial activity similar to coriolin and stronger activity than coriolin in inhibiting YOSHIDA rat sarcoma cells in tissue cultures and in prolongation of the survival periods of mice inoculated with mouse leukemia 1210 or EHRLICH asites tumor. These observations suggest that the keto group at C-5 and two epoxy groups greatly contribute to the antitumor and antibacterial activities.

Fig. 1. Coriolin (**1**): $R_1, R_2=O$, $R_3=H$, $R_4=R_5=OH$
Coriolin B(**2**): $R_1=R_3=H$, $R_2=R_4=OH$, $R_4=OCO(CH_2)_6CH_3$
Coriolin C(**3**): $R_1, R_2=O$, $R_3=H$, $R_4=OH$, $R_5=OCOCH(OH)(CH_2)_5CH_3$
5-Ketocoriolin B(**4**): $R_1, R_2=O$, $R_3=H$, $R_4=OH$, $R_5=OCO(CH_2)_6CH_3$
Diketocoriolin B(**5**): $R_1, R_2=R_3, R_4=O$, $R_5=OCO(CH_2)_6CH_3$



The mode of action of diketocoriolin B was investigated[5,6] and it has been shown that diketocoriolin B inhibits (Na^+-K^+)-ATPase of the cell membrane of tumor cells, causing cessation of growth.

* This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare. Presented in part at the 201st Meeting of the Japan Antibiotics Research Association, Tokyo, March 26, 1976

** Department of Applied Chemistry, Faculty of Engineering, Keio University, Yokohama, 223 Japan.

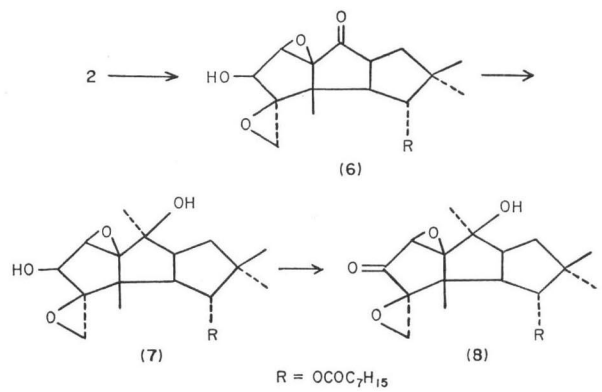
Hitherto, no further modification of the antibiotics has been reported. The presence of the epoxide groups in coriolins is thought to be essential for the biological activities but these epoxide rings are easily opened by chemical treatments. In order to study the effect of modifications at C-8 on biological activity and on the stability of coriolins in alkaline and acidic solutions, we synthesized 5-keto-8α-methylcoriolin B (**8**), 8-deoxy-5-keto-8β-methylcoriolin B (**11**) and 5-keto-8-methylenecoriolin B (**12**).

Synthesis

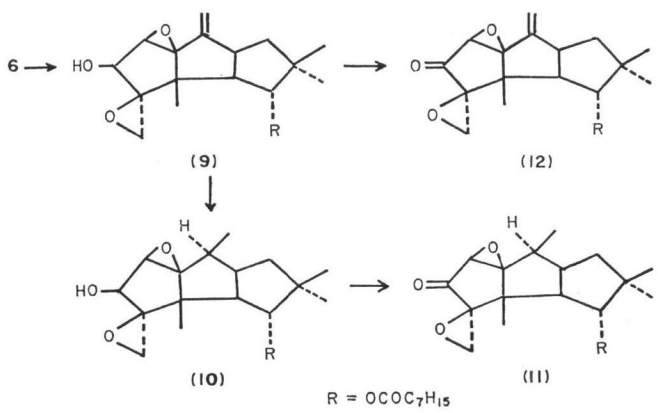
The synthetic routes to the C-8 methyl derivatives starting from coriolin B are shown in Scheme 1. The hydroxyl group at C-8 of coriolin B (**2**) was selectively oxidized with chromic anhydride-acetic acid to give 8-ketocoriolin B (**6**) in 30% yield. Treatment of **6** with methyllithium[7] in absolute ether gave 8α-methylcoriolin B (**7**) in 36% yield. The subsequent conversion of **7** into its 5-keto derivative (**8**) was performed by treatment with dimethyl sulfoxide (DMSO) in the presence of dicyclohexylcarbodiimide (DCC), pyridine (Py) and trifluoroacetic acid (TFAA)[8] in 85% yield.

On the other hand, the 8-ketocoriolin B (**6**) reacted with methylenetriphenylphosphorane[9,10] to afford 8-methylenecoriolin B (**9**), in 21% yield, which was converted to 8-deoxy-8β-methylcoriolin B (**10**) by catalytic hydrogenation in 65% yield (Scheme 2). The 5-keto derivative

Scheme 1



Scheme 2



(**11**) was obtained by oxidation of **10** with DMSO-DCC-Py-TFAA in 90% yield.

Oxidation of the 8-methylene derivative (**9**) with anhydrous chromic acid in pyridine gave 5-keto-8-methylenecoriolin B (**12**) in 57% yield.

Stereochemistry

The configurational assignments of the methyl and hydroxyl groups at C-8 of **8** were made by nuclear OVERHAUSER effect (NOE). Simultaneous irradiation of the two methyls (δ 1.18) resulted in a significant increase (H-1, δ 5.20, 30%; H-6, δ 3.52, 20%) in the integral intensity of the two proton resonances. On the other hand, NOEs of 5-ketocoriolin B (20%, 30%) were observed between H-1 and the methyl groups at C-3 and C-11, while no NOE was observed between H-6 and the methyl groups. Hence, an NOE observed between H-6 and the methyl group at C-8 of **8**, indicates that the methyl group at C-8 is proximate to H-6 and can be assigned as α.* This configuration conforms to our speculation that the 8-keto group can be attacked only from the α side by a nucleophile.

The PMR spectrum of the 8-deoxy-5-keto-8β-methylcoriolin B (**11**), which was obtained by hydrogenation of the 8-methylene derivative, showed the signal of the C-8 methyl hydrogens at δ 1.18 (doublet) and no NOE was observed between H-6 (δ 3.44) and the C-8 methyl, supporting the structure **11**.

Stability in Acidic and Alkaline Media

The C-8 methyl derivatives, 5-keto-8α-methyl and 8-deoxy-5-keto-8β-methylcoriolin B (**8**, **11**)

Table 1. Stabilities* of coriolin B derivatives in acidic and alkaline solutions

Compounds	Buffer solution (diethanolamine aq. HCl, pH 10)-EtOH (1:4)			H_2O-MeOH(1:4)—HCl (pH 2)	
	3 hours	20 hours	350 hours	5 hours	20 hours
5	d**	—	—	d	—
8	s***	s	d	s	s
11	s	s	d	s	s
12	s	s	d	s	s

*28°C,** decompose,*** stable

Table 2. Antibacterial activities of 5-ketocoriolin B (**4**), diketocoriolin B (**5**), 5-keto-8α-methylcoriolin B (**8**), 8-deoxy-5-keto-8β-methylcoriolin B (**11**) and 5-keto-8-methylenecoriolin B (**12**)

Test organisms	Minimal inhibitory concentration μg/ml*				
	4	**5**	**8**	**11**	**12**
Staphylococcus aureus FDA 209P	1.56	12.5	1.56	3.12	12. 5
〃 〃 Terajima	3.12	12.5	6.25	12.5	3.12
〃 〃 Smith	1.56	12.5	1.56	1.56	1.56
Bacillus subtilis NRRL B-558	1.56	25	1.56	3.12	3.12
Bacillus anthracis	0.78	12.5	0.78	0. 2	0.78
Sarcina lutea PCl 1001	3.12	12.5	3.12	1.56	3.12
Micrococcus flavus FDA 16	1.56	12.5	1.56	0.78	3.12

* Nutrient agar, 37°C, 24 hours

* Stereochemistry is denoted in the conventional manner, that is, the configuration of the methyl group under consideration is described as α when it lies behind the plane of the ring system and β when it lies in front of it.

and 5-keto-8-methylenecoriolin B (**12**) have been found more stable than diketocoriolin B (**5**) in both acidic and alkaline media, as shown in Table 1.

Antibacterial and Antitumor Activities

The antibacterial activity of the 5-keto-8α-methylcoriolin B (**8**), 8-deoxy-5-keto-8β-methylcoriolin B (**11**), 5-keto-8-methylenecoriolin B (**12**), 5-ketocoriolin B (**4**) and diketocoriolin B (**5**) were tested by agar streak method with the results shown in Table 2.

Antitumor activity was tested by the methods previously described[4] with the results shown in Table 3.

Table 3. Activity of coriolin B derivatives in prolonging survival periods of mice inoculated with L-1210

Dose mcg/mouse/day×10	T/C				
	4	**5**	**8**	**11**	**12**
100	Tox	156	163	134	Tox
50	144	144	144	134	Tox
25	132	138	138	125	163
12.5	132	138	131	125	131
6.25	125	125	125	134	131

The T/C values are the percentage ratios of the mean survival of 5 treated mice to the mean survival of the control group. 10^6 L-1210 cells were inoculated peritoneally and the treatment was started on day 1 and continued for 10 days.

Experimental

5-Ketocoriolin B (**4**). To a solution of **2** (3 g) in dry pyridine (42 ml), cooled at 0°C, a solution of anhydrous chromic acid (3 g) in dry pyridine (30 ml) was added dropwise over 30 minutes, and the mixture was stirred for 15 hours at 4°C. On silica gel tlc with benzene-acetone (5:1), the starting material (**2**, Rf 0.21) disappeared and products of Rf 0.59 and Rf 0.0 appeared. The mixture was poured into cold water (270 ml), and the water layer was extracted with four 100 ml portions of ethyl acetate. The ethyl acetate extract was washed with water, dried (Na_2SO_4), and evaporated. The resulting syrup (2.5 g) was chromatographed on a column (33×320 mm) of silica gel (Wako Gel, 150 g) with benzene-acetone (5:1) to give a colorless solid (2 g). Recrystallization from *n*-hexane gave colorless crystals of **4**, 1.8 g (60%); mp 79.5~80.5°C; $[\alpha]_D^{21}$ −45° (*c* 0.8, $CHCl_3$); ir (KBr) 3500, 3430, 2950, 2920, 2850, 1740 (ketone), 1720 (ester), 1620, 1460, 1380, 1350, 1300 (sh.), 1240 (sh.), 1220, 1190, 1160, 1130, 1090, 1060, 980, 970, 940, 930, 900, 880, 845, 810, 780, 760, 720, 660, 610 cm^{-1}; nmr (in $CDCl_3$) δ 0.96, 1.06 and 1.18 (each 3H s., CH_3), 2.02 (1H q., $J_{9,10}$ 10 Hz and $J_{10,10'}$ 13 Hz, C_{10}-H), 2.30 (2H t., J 8 Hz, -$OCOCH_2$-), 2.56 (1H q., $J_{1,2}$ 9 Hz and $J_{2,9}$ 12 Hz, C_2-H), 2.65 and 3.12 (2H ABq., J 7 Hz, an exocyclic ethylene oxide), 3.57 (1H s., C_6-H), 4.06 (1H d., $J_{8,9}$ 6 Hz, C_8-H) and 5.18 (1H d., $J_{1,2}$ 9 Hz C_1-H).

Anal. Calcd. for $C_{23}H_{34}O_6$: C 67.95; H 8.43.
Found: C 67.93; H 8.36.

8-Ketocoriolin B (**6**). To a solution of coriolin B (1.83 g) in glacial acetic acid (91.5 ml) cooled to 0°C was added anhydrous chromic acid (450 mg) with stirring, and the reaction mixture was stirred overnight in a refrigerator. On silica gel tlc with benzene-acetone (7:1), the mixture showed 3 spots of Rf 0.75 (diketocoriolin B), 0.50 (8-ketocoriolin B) and 0.17 (the starting material). The reaction mixture was poured into ice water (700 ml), and the solution was extracted with four 300-ml portions of ethyl acetate. The extracts were washed with two 100 ml portions of 1 N aqueous sodium hydroxide solution and two 100-ml portions of water. The ethyl acetate layer was dried over anhydrous sodium sulfate, filtered and evaporated. The resulting syrup was chromatographed on a column (28×270 mm) of silica gel (Wako Gel, 90 g). The eluate of 175~275 ml containing the product of Rf 0.50 was evaporated to give a colorless solid, 650 mg. Recrystallization from *n*-hexane gave colorless crystals of 8-ketocoriolin B, 550 mg (30%): mp 78~79°C; $[\alpha]_D^{21}$ −18° (*c* 0.87, CH_3COCH_3); ir (KBr) 3400, 2950, 2930, 2870, 1750 (ketone), 1725 (ester), 1465, 1420, 1390, 1370, 1320, 1300, 1280, 1220, 1170, 1150, 1120,

1100, 1000, 950, 910, 880 cm^{-1}; nmr ($CDCl_3$) δ 1.0, 1.05 and 1.10 (each 3H s., CH_3), 3.80 (1H d., $J_{5,6}$ 2 Hz, C_6-H), 4.60 (1H d., $J_{5,6}$ 2 Hz, C_5-H), 5.20 (1H d., $J_{1,2}$ 9 Hz, C_1-H).

Anal. Calcd. for $C_{23}H_{34}O_6$: C 67.95; H 8.43
Found: C 67.99; H 8.30

8α-Methylcoriolin B (**7**). To an ether solution (20 ml) of **6** (1 g, 2.46 mmol) cooled to $-70°C$, an ether solution (10 ml, 1 M) of methyllithium was added under nitrogen and the mixture was stirred for 15 minutes. The reaction temperature was then gradually raised to 0°C during 1 hour. Saturated aqueous ammonium chloride solution (30 ml) was added and the ether layer was separated. The aqueous layer was extracted with three 50-ml portions of ether. The ether extract was washed with saturated aqueous sodium chloride solution, dried over anhydrous sodium sulfate and evaporated to give a brown syrup (850 mg). On tlc with benzene-acetone (7:1), the syrup showed 5 spots of Rf 0.55, 0.35 (the starting material), 0.26, 0.22 and 0.11. The syrup was chromatographed on a column (16×390 mm) of silica gel (Wako Gel, 40 g), and the fraction of 195~280 ml containing the product of Rf 0.26 was evaporated to give syrupy **7**, 350 mg (36%): $[\alpha]_D^{20}$ +27° (*c* 1.0, CH_3COCH_3); ir (KBr) 3460, 2960, 2935, 2880, 1730 (ester), 1460, 1375, 1250, 1220, 1175, 1095, 1005, 945, 890, 860 cm^{-1}; nmr ($CDCl_3$) δ 0.97, 1.05, 1.07 and 1.17 (each 3H s., CH_3), 6.41 (1H d., $J_{5,6}$ 2 Hz, C_6-H), 5.55 (1H d., $J_{5,6}$ 2 Hz, C_5-H), 4.74 (1H d., $J_{1,2}$ 8 Hz, C_1-H).

Anal. Calcd. for $C_{24}H_{38}O_6$: C 68.22; H 9.07
Found: C 67.73; H 8.78

5-Keto-8α-methylcoriolin B (**8**). To a solution of **7** (260 mg) in a mixture of anhydrous dimethyl sulfoxide (0.34 ml), anhydrous benzene (3.4 ml) containing pyridine (0.03 ml) and trifluoroacetic acid (0.017 ml) was added dicyclohexylcarbodiimide (260 mg), and the reaction mixture was stirred at room temperature for 2 hours. On tlc with benzene-acetone (10:1), the mixture showed 4 spots of Rf 0.60 (major), 0.41 (minor), 0.16 (the starting material, minor) and 0.02 (minor). A large amount of ether was added to the reaction mixture, and the colorless precipitate was filtered off. The filtrate was evaporated to give a syrup, which was triturated with ether. The ether-soluble portion was evaporated to give a colorless syrup, 310 mg. The syrup was chromatographed on a column (17×210 mm) of silica gel (Wako Gel, 20 g) with benzene-acetone (10:1), and the fraction of 35~75 ml containing the product of Rf 0.60 was evaporated to give a colorless solid, 250 mg. Recrystallization from *n*-hexane gave colorless needles of **8**, 220 mg (85%): mp 111~112°C; $[\alpha]_D^{20}$ −35.7° (*c* 1.0, CH_3COCH_3); ir (KBr) 3425, 2950, 2925, 2870, 1760 (ketone), 1720 (ester), 1460, 1380, 1350, 1320, 1300, 1280, 1265, 1220, 1170, 1135, 1100, 1000 cm^{-1}; nmr ($CDCl_3$) δ 0.95, 1.06 (each 3H s., CH_3), 1.18 (6H s., CH_3) 2.70 and 3.13 (2H ABq., J 7 Hz, an exocyclic ethylene oxide), 3.52 (1H s., C_6-H), 5.20 (1H d., $J_{1,2}$ 9 Hz, C_1-H).

Anal. Calcd. for $C_{24}H_{36}O_6$: C 68.54; H 8.63
Found: C 68.81; H 8.61

8-Methylenecoriolin B (**9**). To a suspension of methyltriphenylphosphonium bromide (2.7 g) in anhydrous ether (30 ml) was added 20% *n*-butyllithium in *n*-hexane (3.25 ml) under nitrogen, whereupon the deep yellow color of the methylenetriphenylphosphorane was produced. To the solution 8-ketocoriolin B (**6**) (1 g) dissolved in anhydrous ether (10 ml) was added. After stirring at 25°C for 17 hours, the reaction mixture was poured into water (50 ml), and the ether layer was separated. The aqueous layer was extracted with 5 portions of ether (100 ml). The extract was washed with 2 portions of saturated sodium chloride solution (30 ml), dried with anhydrous sodium sulfate, filtered, and the filtrate was evaporated. The brown syrup (850 mg) was shaken with five 100-ml portions of pentane, dried (Na_2SO_4), and evaporated to give a brown syrup (430 mg). On tlc with benzene-acetone (7:1), the starting material of Rf 0.49 disappeared and the product of Rf 0.65 appeared. The syrup was chromatographed on a column (20×180 mm) of silica gel (25 g) with benzene-acetone (7:1) and the fraction of 29~45 ml containing the product was evaporated to give a colorless solid (230 mg). Recrystal-

lization from *n*-hexane gave colorless crystals of **9**, 210 mg (21%): mp 78~78.5°C; $[\alpha]_D^{21}$ +25.7° (*c* 0.35, CH_3OH); ir (KBr) 3400, 2900, 2850, 2720, 1730 (ester), 1460, 1390, 1370, 1250, 1220, 1190, 1170, 1100, 1070, 1000, 940, 890, 755, 720 cm^{-1}; nmr ($CDCl_3$) δ 0.86 (3H s., CH_3), 1.03 (6H s., CH_3), 3.48 (1H d., $J_{5,6}$ 2 Hz, C_6-H), 4.42 (1H d., $J_{5,6}$ 2 Hz, C_5-H), 4.84 and 5.0 (2H ABq., J 3 Hz, C_8=CH_2), 3.05 (1H d., $J_{1,2}$ 9 Hz, C_1-H).

Anal. Calcd. for $C_{24}H_{36}O_5$: C 71.25; H 8.97
Found: C 71.42; H 8.90

8-Deoxy-8β-methylcoriolin B (**10**). 8-Methylenecoriolin B (**9**) (400 mg) was dissolved in dioxane (7 ml) and to the solution was introduced hydrogen gas for 5 minutes. Hydrogenation was performed with RANEY Ni (T-4) and hydrogen under 4 kg/cm^2 at room temperature for 40 minutes. On tlc with benzene-acetone (7:1), the starting material of Rf 0.81 disappeared and the main product of Rf 0.63 and minor products of Rf 0.29 and 0.17 appeared. The reaction mixture was filtered and the residue was washed with dioxane. The filtrate and washings were combined and evaporated to give a colorless syrup, 386 mg. The syrup was chromatographed on a column (15×230 mm) of silica gel (Wako Gel, 20 g) with benzene-acetone (7:1), and the fraction of 34~58 ml containing the product was evaporated to give a colorless solid of **10**, 289 mg. Recrystallization from *n*-hexane gave colorless needles of **10**, 271 mg (64%): mp 76~76.5°C; $[\alpha]_D^{21}$ +30° (*c* 0.7, CH_3OH); ir (KBr) 3450, 2930 (sh), 2860, 1725 (ester), 1465, 1385, 1370, 1315, 1285, 1265, 1250, 1210, 1195, 1190, 1185, 1115, 1090, 1015, 1005, 970, 945, 920, 890, 860, 830 cm^{-1}; nmr ($CDCl_3$) δ 0.97, 1.0 and 1.05 (each 3H s., CH_3), 1.10 (3H d., J 8 Hz, C_8-CH_3), 3.41 (1H d., $J_{5,6}$ 2 Hz, C_6-H), 4.36 (1H q., J 2 Hz and 9 Hz, C_5-H), 5.19 (1H d., $J_{1,2}$ 8 Hz, C_1-H).

Anal. Calcd. for $C_{24}H_{38}O_5$: C 70.90; H 9.42
Found: C 71.08; H 9.36

8-Deoxy-5-keto-8β-methylcoriolin B (**11**). To a solution of 8-deoxy-8β-methylcoriolin B (**10**) (271 mg) in anhydrous dimethyl sulfoxide (0.34 ml), benzene (3.4 ml) containing pyridine (0.03 ml) and trifluoroacetic acid (0.017 ml), dicyclohexylcarbodiimide (271 mg) was added and the reaction mixture was stirred at room temperature for 3 hours. On tlc with benzene-acetone (7:1), the starting material of Rf 0.63 disappeared and the product of Rf 0.87 appeared. A large amount of ether was added to the reaction mixture, and a precipitate was filtered off. The filtrate was evaporated and the resulting syrup was triturated with ether. The ether-soluble portion was evaporated to give a colorless syrup, 250 mg, which was chromatographed on a column (8×150 mm) of silica gel (Wako Gel, 12 g) with benzene-acetone (10:1). The fraction of 18~78 ml containing the product was evaporated to give a colorless syrup of **11**, 226 mg (90%): $[\alpha]_D^{21}$ −35.2° (*c* 0.93, $CHCl_3$); ir (KBr) 3400, 2900, 2860, 2720, 1760 (ketone), 1735 (ester), 1640, 1470, 1380, 1350, 1310, 1230 (sh), 1190, 1170 (sh), 1100 (sh), 1090, 1010, 980, 935, 905, 880, 840, 770, 720, 660, 610 cm^{-1}; nmr ($CDCl_3$) δ 0.97, 1.06 and 1.11 (each 3H s., CH_3), 1.18 (3H d., J 8 Hz, C_8-CH_3), 2.58 and 3.08 (2H ABq., J 7 Hz, exocyclic ethylene oxide), 3.44 (1H s., C_6-H), 5.11 (1H d., $J_{1,2}$ 8 Hz, C_1-H).

Anal. Calcd. for $C_{24}H_{36}O_5$: C 71.25; H 8.97
Found: C 70.89; H 8.83

5-Keto-8-methylenecoriolin B (**12**). To a solution of **9** (0.21 g) in dry pyridine (9 ml) cooled at 0°C, a solution of anhydrous chromic acid (0.12 g) in dry pyridine (1 ml) was added and the mixture was stirred for 20 hours at room temperature. Anhydrous chromic acid (0.1 g) was added and the reaction was continued for another 5 hours. On tlc with benzene-acetone (7:1), the starting material of Rf 0.62 disappeared and the products of Rf 0.80 and Rf 0.0 appeared. The mixture was poured into cold water (100 ml), and the aqueous layer was extracted with four 50-ml portions of ethyl acetate. The ethyl acetate extract was washed with water, dried (Na_2SO_4) and evaporated. The resulting syrup (0.2 g) was chromatographed on a column (10×200 mm) of silica gel (Wako Gel, 10 g) with benzene-acetone (7:1) to give a syrup of **12**, 0.12 g

(57%): $[\alpha]_D^{20}$ −61° (*c* 0.89, $CHCl_3$); ir (KBr) 2950, 2925, 2860, 1755 (ketone), 1735 (ester), 1460, 1380, 1300, 1245, 1225, 1160, 1120, 1095, 1045, 1010, 980, 935, 910 (methylene), 850, 770, 710, 650, 630 cm^{-1}; nmr ($CDCl_3$) δ 0.98 (3H s., CH_3), 1.03 (6H s., CH_3), 2.03 (1H q., $J_{9,10}$ 8 Hz and $J_{10,10'}$ 12 Hz, C_{10}-H), 2.31 (2H t., J 8 Hz, -OCO-CH_2-), 2.59 (1H q., $J_{1,2}$ 7.5 and $J_{2,9}$ 12 Hz, C_2-H), 2.60 and 3.10 (2H ABq., J 7 Hz, an exocyclic ethylene oxide), 3.36 (1H sixtet, $J_{2,9}$ and $J_{9,10'}$ 12 Hz, $J_{9,10}$ 8 Hz, C_9-H), 3.51 (1H s., C_6-H), 4.98 and 5.16 (2H ABq., J 3 Hz, C=CH_2), 5.12 (1H d., $J_{1,2}$ 7.5 Hz, C_1-H).

Anal. Calcd. for $C_{24}H_{34}O_5$: C 71.61; H 8.51
Found: C 71.59; H 8.42

Stability of **5**, **8**, **11**, **12**.

A) Stability in an acidic solution. A solution of a small amount (2 mg) of diketocoriolin B (**5**), 5-keto-8α-methylcoriolin B (**8**), 8-deoxy-5-keto-8β-methylcoriolin B (**11**) or 5-keto-8-methylenecoriolin B (**12**) in a mixture (0.1 ml) of methanol and water (4:1) was adjusted to pH 2.0 with a small amount of 0.1 N hydrochloric acid. After standing for 5 hours at 28°C, tlc was carried out on silica gel (Wakogel B-5) with benezen-acetone (7:1). The acidic solutions of **8**, **11** and **12** were further kept at 28°C for 15 hours and tlc was carried out in the same manner.

B) Stability in an alkaline solution. A small amount (2 mg) of **5**, **8**, **11** or **12** was dissolved in a mixture (0.1 ml) of buffer solution of diethanolamine—0.1 N hydrochloric acid (pH 10) and ethanol (1:4), and the mixture was allowed to stand at 28°C for 3 hours. Tlc was carried out in the same manner as described above. The alkaline solutions of **8**, **11** and **12** were further kept at 28°C for 17 hours, and, again for 330 hours. Tlc was carried out in the same manner.

References

1) TAKEUCHI, T.; H. IINUMA, J. IWANAGA, S. TAKAHASHI, T. TAKITA & H. UMEZAWA: Coriolin, a new basidiomycetes antibiotic. J. Antibiotics 22: 215~217, 1969
2) TAKAHASHI, S.; H. NAGANAWA, H. IINUMA, T. TAKITA, K. MAEDA & H. UMEZAWA: Revised structures and stereochemistry of coriolins. Tetrahedron Letters 1971: 1955~1958, 1971
3) NAKAMURA, T.; T. TAKITA, H. UMEZAWA, M. KUNISHIMA, Y. NAKAYAMA & Y. IITAKA: Absolute configuration of coriolin, a sesquiterpene antibiotic from *Coriolus consors*. J. Antibiotics 27: 301~302, 1974
4) TAKEUCHI, T.; S. TAKAHASHI, H. IINUMA & H. UMEZAWA: Diketocoriolin B, an active derivative of coriolin B by *Coriolus consors*. J. Antibiotics 24: 631~635, 1971
5) KUNIMOTO, T.; M. HORI & H. UMEZAWA: Mechanism of action of diketocoriolin B. Biochim. et Biophys. 298: 513~525, 1973
6) KUNIMOTO, T. & H. UMEZAWA: Kinetic studies on the inhibition of $(Na^+ + K^+)$-ATPase by diketocoriolin B. Biochim. et Biophys. 318: 78~90, 1973
7) MILJKOVIĆ, M.; M. GLIGORIJEVIĆ, T. SATOH & D. MILJKOVIĆ: Synthesis of macrolide antibiotics. I. Stereospecific addition of methyllithium and methylmagnesium iodide to methyl α-D-*xylo*-hexopyranoside-4-ulose derivatives. Determination of the configuration at the branching carbon atom by carbon-13 nuclear magnetic resonance spectroscopy. J. Org. Chem. 39: 1379~1384, 1974
8) PFITZNER, K. E.; & J. G. MOFFATT: A new and selective oxidation of alcohols. J. Am. Chem. Soc. 85: 3027~3028, 1963
9) PAQUETTE, L. A.; G. V. MEEHAN, R. P. HENZEL & R. F. EIZEMBER: The photochemistry of conjugated *cis*-bicyclo[5.1.0]octenenes, *cis*- and *trans*-bicyclo[5.2.0]non-2-en-4-ones, and their methylene analogs. J. Org. Chem. 38: 3250~3256, 1973
10) PAQUETTE, L. A.; R. P. HENZEL & R. F. EIZEMBER: Thermochemical behavior of conjugated *cis*-bicyclo[5.1.0]octenones, *cis*- and *trans*-bicyclo[5.2.0]non-2-en-4-ones, and their methylene analogs. J. Org. Chem. 38: 3257~3268, 1973