A Novel Chlorinated Biscembranoid from the Marine Soft Coral *Sarcophyton glaucum*

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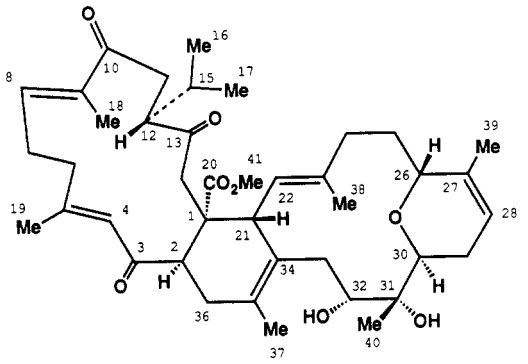
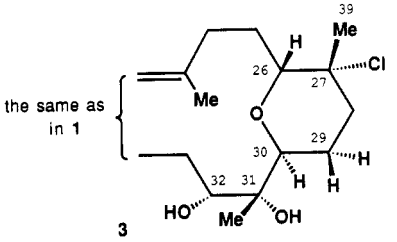
Novel tetraterpenes 1 and 3 have been isolated from an Okinawan soft coral *Sarcophyton glaucum*, and their structures have been deduced by means of spectroscopy and chemical transformation. Their carbon framework, presumably formed by Diels–Alder reaction of two cembranoids, has been found to be the same as that of methyl sartortuoate and methyl isosartortuoate.

Chemists have recently become interested in marine natural products because of their remarkable biological activities and unique structures.[1] During our search for pharmaceutically active components of marine invertebrates,[2] we isolated novel tetraterpenoids, which exhibit cytotoxic activity to KB cells, from an Okinawan soft coral *Sarcophyton glaucum*, and this report deals with their structures.

An acetone extract of freshly collected (at Henoko Bay, Okinawa, in April 1988) *S. glaucum* was chromatographed on silica gel, and two active components, methyl sarcophytoate (1) and methyl chlorosarcophytoate (3), were found.



1
2 (C-10 is CHOH)



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Methyl sarcophytoate (1), colorless oil, $[\alpha]_D$ +157° (*c* 0.34, $CHCl_3$), λ_{max} (MeOH) 232 nm (ϵ 18000), has the molecular formula $C_{41}H_{58}O_8$ [HRMS *m/e* 678.4131 (M^+)]. The presence of two hydroxy groups were deduced from the mass fragments at *m/e* 660 ($M^+ - H_2O$) and 642 ($M^+ - 2H_2O$), and the IR band at 3700–3400 cm^{-1}. The IR spectrum also suggested the ester (1733 cm^{-1}), carbonyl (1710 cm^{-1}), and conjugated carbonyl (1669 cm^{-1}) moieties, and the ^{13}C NMR spectrum indicated the existence of four carbonyl groups [δ 173.1 (ester), 203.2, 203.4, 210.4]. All of the characteristics of the ^1H NMR spectrum of 1 resembled those of cembranoids isolated from other marine soft corals. A singlet at δ 3.55 (3 H) in the ^1H NMR spectrum as well as the molecular formula inferred that 1 is a methyl ester of a bisditerpenoid, the carbon framework of which consists of 40 carbons. Analyses of 1D and 2D NMR spectra including H,H and H,C-COSY, HOHAHA and HMBC spectra[3] led to the partial structures a, b, and c (Figure 1). It was obvious that the bottom end of b (=b1) was bound to the top end of c (=c2) from the HMBC correlation peak. The spectrum revealed that structures a and b as well as a and c are bound with a carbonyl group between. The proximity of the chemical shifts of the two carbonyl carbons (δ 203.2 and 203.4), however, made it impossible to distinguish connection modes (i) cyclo-(a1-a2)-CO-(b2-b1-c2-c1)-CO and (ii) cyclo-(a2-a1)-CO-(b2-b1-c2-c1)-CO. When 1 was subjected to sodium borohydride reduction, one of the three carbonyls was selectively reduced. In the ^1H NMR spectrum of reduction product 2, the newly formed carbinyl proton at δ 4.37 was found (H,H-COSY) to be coupled with the methylene protons (δ 1.39 and 2.03) of fragment b. At the same time the signal due to H_A of fragment a (δ 6.24 in 1) shifted up to δ 5.07, while the chemical shift of H_B before (δ 6.04) and after (δ 5.89) the reduction was not appreciably different. These findings verified connection mode i, leading to the 14-membered ring of 1.

There is another cyclotetradecadiene ring in fragment c. The ^{13}C NMR spectra (DEPT, H,C-COSY) indicated that four carbons (p, q, r, s) possess oxygen functions, and because there are only three oxygens remaining for 1, there must be one ether linkage as well as two hydroxy moieties. To differentiate C-OH from C-O-C, a deuterium-induced shift experiment[4] was performed: When the proton-noise decoupled spectrum of 1 was measured in $CDCl_3$ in the presence of 2 equiv of CD_3OD, the carbons assignable as p and q were eminently broadened due to the partial exchange of OH to OD, whereas r and s remained sharp singlets. This indicated that carbons p and q are bound to hydroxy groups, and carbons r and s are ether-bonded through a common oxygen atom. On the basis of these facts, structure 1 (plane) was assigned to compound 1.

The 4*E*,8*E*,22*E*,34*Z* configurations of the olefinic bonds were determined by the ^{13}C NMR chemical shifts of the olefinic methyl groups (Table I) and NOEs observed by the phase-sensitive NOESY spectrum as shown in Figure

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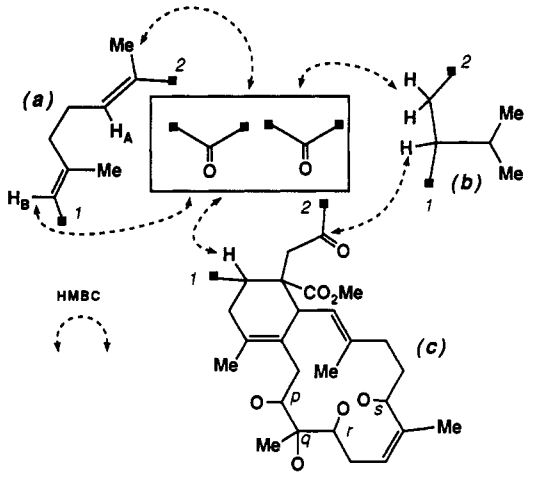
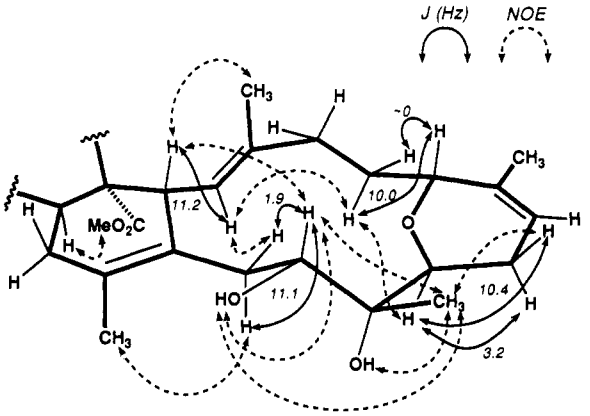


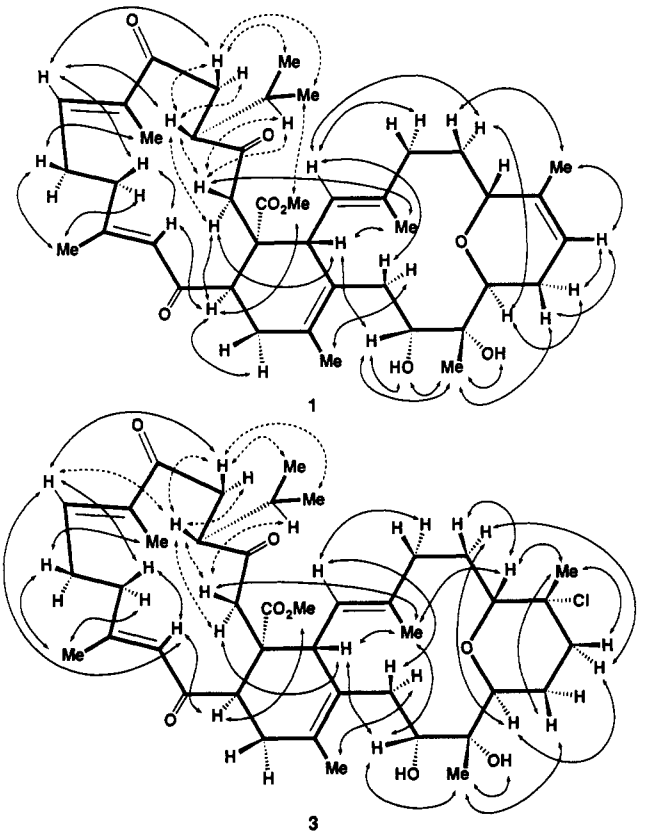
Figure 1. Partial structures deduced from NMR spectra of 1.

2. The relative configurations of the substituents on the B, C, D rings of 1 and the conformations of the rings were determined to be as **1a** by considering the coupling patterns of the protons and NOEs depicted in **1a**. With respect to the isopropyl group, α-configuration was assigned to it by means of the phase-sensitive NOESY spectrum[3] of 1.



1a

Methyl chlorosarcophytoate (**3**), $[\alpha]_D$ +140° (*c* 0.38, $CHCl_3$), IR 3650–3300, 1733, 1708, 1669 cm^{-1}, λ_{max} (MeOH) 231 nm (ϵ 17 000), exhibited the molecular fragments at *m/e* 715 and 717 ($M^+ + H$), corresponding to the formula $C_{41}H_{59}O_8Cl$ in the FABMS. The fragmentation pattern [*m/e* 678 ($M^+ - HCl$), 660 ($M^+ - HCl - H_2O$), and 642 ($M^+ - HCl - 2H_2O$)] was almost identical with that of **1**. The ^1H NMR spectrum of **3**, however, lacked one olefinic proton corresponding to H-28 and exhibited a singlet at δ 1.56 assignable to H_3-39 (δ 1.63 in **1**). Because other characteristics of the NMR spectrum are extremely similar to those of **1** (Table I), and a singlet assignable to C-27 appeared at δ 73.1 in the ^{13}C NMR spectrum, we assumed that structure **3** is methyl chlorosarcophytoate. This structure was confirmed by the finding that, in the ^{13}C NMR spectrum, the carbon signal at C-27 as well as at C-26 (δ 84.7) and C-30 (δ 70.1) was unaffected by the addition of 3 equiv of CD_3OD, and instead the spectrum showed the splitting of C-31 (δ 75.7) and C-32 (δ 70.9). The β- configuration of the methyl group on C-27 was deduced by the NOE between H_3-39 and H-29β (δ 2.08), which is coupled with H-30 with J = 12.2 Hz (axial–axial coupling). The stereochemistry of other asymmetric centers of **3** was confirmed to be the same as in **1** by analyzing the 1D and 2D spectral data (Table I and Figure 2). Methyl chlorosarcophytoate (**3**) is the first example of biscembranoid possessing a chlorine atom in the molecule.

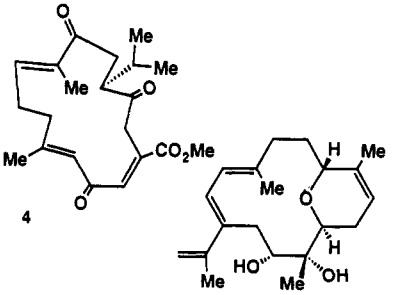


1

3

Figure 2. NOEs observed in the phase-sensitive NOESY spectra of methyl sarcophytoate (**1**) and methyl chlorosarcophytoate (**3**). For clarity NOEs which are not essential to elucidate the stereochemistry (e.g. NOEs between geminal protons) are abbreviated. Dashed arrows are those indicating the orientation of the isopropyl group. Configurations are relative ones.

The carbon skeleton of the present biscembranoids is the same as that of methyl sartortuoate[5] and methyl isosartortuoate,[6] the structures of which have been determined by X-ray analyses. As pointed out previously,[5,6] this group of tetraterpenoids would be derived by Diels–Alder coupling of two cembranes (e.g. **4** and **5**), although the corresponding "monomeric" cembranes have not been found yet. It is noteworthy that, from the number of possible combinations, only one pair of cembranes seem to have been chosen for dimerization, forming these unique tetracyclic tetraterpenoids.



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5

Methyl sarcophytoate (**1**) and methyl chlorosarcophytoate (**3**) showed the following cytotoxic activities

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Table I. NMR ($CDCl_3$) Data for Methyl Sarcophytoate (1), Methyl Chlorosarcophytoate (3), and the $NaBH_4$ product (2)

	methyl sarcophytoate (1)			methyl chlorosarcophytoate (3)			2	
	δ_C	δ_H[b]	J (Hz)	δ_C	δ_H[b]	J (Hz)	δ_H[b]	J (Hz)
1	47.2 s			47.3 s				
2	46.8 d	3.97 d	7.5	46.8 d	3.95 d	7.8	3.59 d	7.5
3	203.2[a] s			203.2[a] s				
4	126.7 d	6.04 s		126.8 d	6.03 s		5.89 s	
5	159.4 s			159.2 s				
6	39.8 t	2.40 m		39.7 t	2.41[c]		2.28 m	
		2.28[c]	11.5		2.28 m	12.1	2.16 m	
7	25.5 t	2.40 m	11.5, 4.0	25.4 t	2.41 m	12.1, 4.4	2.26 m	5.9
		2.53 dt	8.8, 11.5		2.52 dt	8.4, 12.1		
8	141.4 d	6.24 dd	8.8, 4.0	141.3 d	6.23 dd	8.4, 4.4	5.07 br t	5.9
9	138.1 s			138.0 s				
10	203.4[a] s			203.3 s			4.37 dd	9.1, 4.7
11	33.3 t	3.42 dd	14.2, 6.1	33.4 t	3.41 dd	13.8, 5.7	2.03 td	14.5, 4.7
		2.01 dd	14.2, 5.7		2.03 dd	13.8, 5.2	1.39 dd	14.5, 9.1
12	56.2 d	2.57 m	6.1, 5.7	56.1 d	2.57 m	5.7, 5.2	1.82 m	14.5
13	210.4 s			210.3 s				
14	48.5 t	1.95 d	18.9	48.2 t	1.98 d	19.0	3.23 d	18.2
		3.27 d	18.9		3.23 d	19.0	2.32 d	18.2
15	30.3 d	2.14 m	7.5, 7.0	30.3 d	2.13 m	7.2	2.27 m	7.1
16	17.6 q	0.82 d	7.0	17.6 q	0.79 d	7.2	0.71 d	7.1
17	20.9 q	0.98 d	7.5	20.8 q	0.95 d	7.2	1.00 d	7.1
18	11.9 q	1.72 s		11.8 q	1.72 s		1.53 s	
19	18.9 q	2.07 s		18.8 q	2.09 s		2.02 s	
20	173.1 s			173.1 s				
21	40.7 d	3.18 d	11.2	40.4 d	3.27 d	11.2	3.49 d	11.5
22	124.1 d	4.68 d	11.2	124.7 d	4.74 d	11.2	4.70 d	11.5
23	140.8 s			139.8 s				
24	38.9 t	2.45[c]		39.0 t	2.43[c]	10.1	2.47 m	
		1.81 m			1.84 m		1.79 m	
25	33.2 t	1.67[c]	10.0	27.7 t	1.84 m	9.9	1.66 m	9.9
		1.79			1.63[c]		1.79 m	
26	79.5 d	4.00 d	10.0	84.7 d	3.96 d	9.9	4.01 d	9.9
27	134.4 s			73.1 s				
28	120.4 d	5.57 d	5.5	35.0 t	1.68[c]		5.57 d	5.9
					2.00[c]			
29	24.7 t	1.92 ddd	16.8, 5.5, 3.2	20.9 t	1.58 m	13.0, 2.0	1.94 ddd	17.7, 5.9, 4.0
		2.16 dd	16.8, 10.4		2.08 dt	13.0, 12.2	2.17 m	17.7, 10.3
30	68.3 d	3.64 dd	10.4, 3.2	70.1 d	3.63 dd	12.2, 2.0	3.66 dd	10.3, 4.0
31	75.4 s			75.7 s				
32	70.7 d	3.58 dd	11.1, 1.9	70.9 d	3.55[c]	11.2	3.61 dd	11.5, 1.6
33	31.2 t	2.47 dd	14.0, 11.1	31.5 t	2.47 dd	13.5, 11.2	2.46 dd	13.0, 11.5
		2.33 d	14.0, 1.9		2.27 d	13.5	2.31 m	13.0, 1.6
34	125.7 s			125.7 s				
35	129.2 s			129.2 s				
36	32.7 t	2.96 dd	17.7, 7.5	32.7 t	2.91 dd	18.2, 7.8	3.21 dd	19.4, 7.5
		1.84 d	17.7		1.86 d	18.2	1.85 m	19.4
37	20.2 q	1.69 s		20.1 q	1.70 d	1.2	1.68 s	
38	20.0 q	1.82 s		20.0 q	1.75 s		1.85 s	
39	20.5 q	1.63 s		30.8 q	1.56 s		1.63 s	
40	19.4 q	1.30 s		19.1 q	1.32 s		1.31 s	
41	51.4 q	3.55 s		51.4 q	3.55 s		3.55 s	
	31-OH	2.08			1.93			
	32-OH	2.67			2.36			

[a] Assignments are interchangeable within the column. [b] For the methylene protons the upper ones correspond to α-protons and the lower ones to β-protons. [c] Precise multiplicities were not determined because of overlapping with other signals.

against KB cells: **1**, 7.5 μg/mL; **3**, 12.0 μg/mL.

Experimental Section

General Instrumentation. The IR spectra were recorded on a JEOL FX-60 spectrophotometer. ^1H and ^{13}C NMR spectra were taken on a Bruker AM-500 spectrometer. FABMS spectra were measured on a JEOL JMS-DX-303 instrument. Optical rotations were recorded on a JASCO DIP-181 polarimeter using a 10-cm microcell. UV spectra were obtained on a Hitachi 340 spectrophotometer.

Materials. *S. glaucum* (2 kg) was collected in April 1988, at the Henoko beach in Okinawa Island. A voucher specimen is preserved at the Natural Product Laboratory of the University of Tsukuba.

Separation of Methyl Sarcophytoate (1) and Methyl Chlorosarcophytoate (3). The soft coral was soaked in acetone immediately after collection, and the mixture was allowed to stand for 1 week. The acetone extract was concentrated, and the residue was successively washed with hexane (800 mL × 3), CH_2Cl_2 (800 mL × 3), and ethyl acetate (800 mL × 3). Concentration of the hexane and CH_2Cl_2 solutions gave a dark brown residue (40.0 g) and a pale yellow oil (70.7 g), respectively. A 7.4-g portion of the latter oil was fractionated on vacuum liquid chromatography (VLC-1) (Merck, Kieselgel 60; CH_2Cl_2–MeOH). The fraction (376 mg) eluted by CH_2Cl_2–MeOH (9:1) was further separated by VLC (VLC-2) [CH_2Cl_2–acetone] to give 14 fractions. The 10th and 11th fractions were combined, and the material (40 mg) was further separated by preparative TLC [Merck, Kieselgel GF_{254}; hexane–EtOAc (3:1); five-times development], yielding pure (TLC and NMR) **1** (2 mg). By repeating the same procedures [except that HPLC (Inertsil ODS; MeOH–H_2O (17:3) was applied in the final purification step instead of preparative TLC] for the fraction (VLC-1) which was eluted next to the one mentioned above, 18 mg of pure **1** was obtained.

During the above fractionation procedure, methyl chlorosar-

cophytonate (3; 5 mg) was obtained from the CH_2Cl_2 extract (7.4 g) as a substance slightly more polar than 1. R_f values in TLC [Merck Kieselgel 60 GF_{254}; CH_2Cl_2–MeOH (24:1)] of 1 and 3 are as follows: 1, 0.46; 3, 0.40.

$NaBH_4$ Reduction of 1. Methyl sarcophytoate (1; 4.9 mg) was treated with $NaBH_4$ (24.5 mg) in MeOH (1 mL) at room temperature for 1 h. After workup the crude product was subjected to HPLC [Inertsil ODS; MeOH–H_2O (17:3)] to give 2 (1.8 mg).

Registry No. 1, 129239-13-0; 3, 129239-14-1.

Supplementary Material Available: NMR data of 1–3, NOESY spectra of 1 and 2, and NMR spectra of 1 and 3 (14 pages). Ordering information is given on any current masthead page.

Preparation of a Protected (2*S*,3*S*)-β-Hydroxyaspartic Acid Suitable for Solid-Phase Peptide Synthesis

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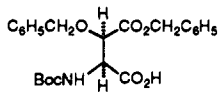
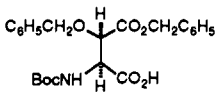
(2*S*,3*S*)-*N*-Boc-3-(benzyloxy)aspartic acid β-benzyl ester (2), a β-hydroxyaspartic acid derivative suitably protected for incorporation into peptides by solid-phase synthesis, was synthesized from *N*-Boc-(*R*)-serine via the intermediate, [4*R*-(*R**,*R**)]-4-[hydroxy[2-(trimethylsilyl)ethynyl]methyl]-2,2,-dimethyl-3-oxazolidinecarboxylic acid 1,1-dimethylethyl ester (5). Key transformations involved the ruthenium tetraoxide mediated oxidation of the ethynyl moiety to form the β-carboxylic acid and, after esterification and oxazolidine ring hydrolysis, dichromate oxidation of the resulting primary alcohol to the α-carboxylic acid. The method is suitable for the preparation of gram quantities of 2.

We recently required access to suitably protected β-hydroxyaspartic acids for incorporation into peptide analogues using solid-phase synthesis. Previously, (2*S*,3*R*)-*N*-Boc-β-(benzyloxy)aspartic acid β-benzyl ester (1) was prepared bearing appropriate protecting groups for peptide synthesis.[1,2] In the present report, we describe a complimentary route leading to (2*S*,3*S*)-*N*-Boc-β-(benzyloxy)aspartic acid β-benzyl ester (2) starting from (*R*)-serine.



1

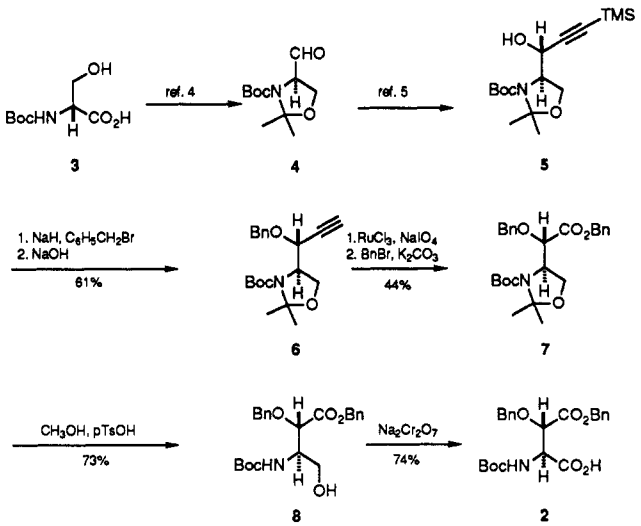


2

The three-step conversion of *N*-Boc-(*R*)-serine to the configurationally stable aldehyde 4[3] and the copper-mediated, diastereoselective addition of (trimethylsilyl)-acetylide to provide the acetylenic alcohol 5 proceeded without the incident as described.[4] Conversion of the alcohol 5 to the corresponding benzyl ether was effected by treatment with sodium hydride and benzyl bromide in DMF at room temperature for 2 h. When the reaction was quenched with water and then stirred for 45 min, complete desilylation occurred to give 6 directly. Oxidation of the acetylenic moiety of 6 in the presence of ruthenium tetraoxide and sodium periodate[5] provided an intermediate carboxylic acid as an oil, which was immediately treated with potassium carbonate and benzyl bromide in DMF to afford the benzyl ester 7 in 44% yield for the two steps.

Attempted selective hydrolysis of the acetonide contained in 7 with methanol in the presence of Amberlyst 15 as generally described by Herold[4] gave the desired alcohol 8 in low yield accompanied by large amounts of very polar products which were not characterized. After considerable experimentation, it was found that a procedure

Scheme I



3 4 5 6 7 8 2

reported by Beaulieu and Schiller was preferable.[6] Thus the acetonide 7 was treated with a catalytic amount of *p*-toluenesulfonic acid in refluxing wet methanol to provided the primary alcohol 8 as a 1:1.5 mixture with recovered starting material. Recycling afforded an overall 73% yield of 8. Finally, oxidation of 8 to the required product 2 was accomplished efficiently using chromic acid in an ether–water two-phase system.[7]

While analysis of the NMR spectra of the intermediates possessing oxazolidine rings was complicated by the

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