[Chem. Pharm. Bull. 31(2) 552—556 (1983)]

Marine Natural Products. XI.[1] An Antiinflammatory Scalarane-type Bishomosesterterpene, Foliaspongin, from the Okinawan Marine Sponge Phyllospongia foliascens (PALLAS)

HIROYUKI KIKUCHI,[a] YASUMASA TSUKITANI,[a] IWAO SHIMIZU,[a] MOTOMASA KOBAYASHI,[b] and ISAO KITAGAWA*,[b]

Tokyo Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.,[a] 3-8-3, Nukuikitamachi, Koganei, Tokyo 184, Japan and Faculty of Pharmaceutical Sciences, Osaka University,[b] 1-6, Yamada-oka, Suita, Osaka 565, Japan

(Received August 16, 1982)

On the basis of chemical and physicochemical evidence, the chemical structure of foliaspongin, an antiinflammatory active principle of the Okinawan marine sponge *Phyllospongia foliascens* (PALLAS), was elucidated as a new scalarane-type bishomosesterterpene (**3**).

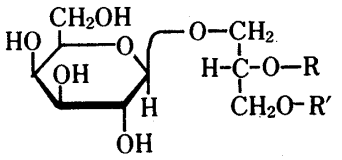
Keywords——marine sponge; *Phyllospongia foliascens;* scalarane-type bishomosesterterpene; foliaspongin; CI-MS, ^{13}C-NMR; ^{1}H-NMR; antiinflammatory activity

During the course of studies in search of bioactive marine natural products,[2] we isolated two constituents from the water-soluble portion of the Okinawan marine sponge *Phyllospongia foliascens* (PALLAS)(Spongiidae) and elucidated their chemical structures.[1] They were the galactolipid M-5 (**1**), which showed antiinflammatory activity, and the sulfonoglucolipid M-6 (**2**), which inhibited the complement-fixation reaction. Later, in the course of fractionation of the lipid-soluble constituents while monitoring the biological activities, we isolated a new antiinflammatory bishomosesterterpene named foliaspongin. This paper presents detailed evidence for a C_{27} scalarane-type structure of foliaspongin (**3**).[3]

In recent years, quite a few scalarane-type C_{25}, C_{26}, and C_{27} sesterterpenes have been isolated from various marine sponges, *Phyllospongia radiata,*[4a] *P. dendyi,*[4a] *P. foliascens,*[4a] *Dysidea herbacea,*[4b] *Spongia idia,*[4c] *S. nitens,*[4e] and *Cacospongia scalaris.*[4d] Among these species, the Australian marine sponge *P. foliascens* characteristically produced several sesterterpenes having a 4α-ethyl moiety.[4a] We investigated the bioactive principles of the same kind of marine sponge collected at Okinawa Prefecture and isolated foliaspongin (**3**), which possessed a scalarane-type structure similar to those of the compounds found in the Australian species, after repeated chromatographic purification of the ethyl acetate–soluble constituents of the sponge.

The chemical ionization (NH_3) mass spectrum [CI(NH_3)-MS] revealed the molecular composition of foliaspongin (**3**) from the ion peak at m/z 550 $(M+NH_4)^+$. The ultraviolet (UV) spectrum of foliaspongin did not show any absorption maximum above 210 nm, while the infrared (IR) spectrum exhibited absorption bands due to hydroxyl group(s) and carbonyl group(s). The presence of the carbonyl function was supported by the circular dichroism (CD) spectrum, which showed a positive maximum of $[\theta]_{286}$ + 19000 due to the carbonyl n$\rightarrow\pi^*$ transition. Ordinary acetylation of foliaspongin (**3**) furnished the fully acetylated diacetate (**3a**), whose molecular composition was also obtained by CI(NH_3)-MS.

The proton magnetic resonance (^{1}H-NMR) spectrum[5] (in $CDCl_3$) of foliaspongin (**3**) showed the presence of a methylketone residue (δ 2.19, 3H, s), four tertiary methyl groups (δ

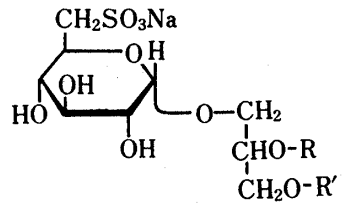


1 (**a**:**b**:**c**=1:10:10)

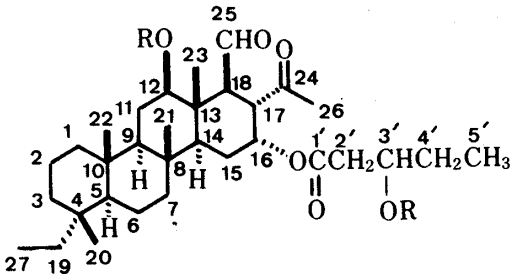
R, R′ = **a** $-C(=O)-(CH_2)_{12}CH_3$

b $-C(=O)-(CH_2)_6-CH=CH-(CH_2)_6CH_3$

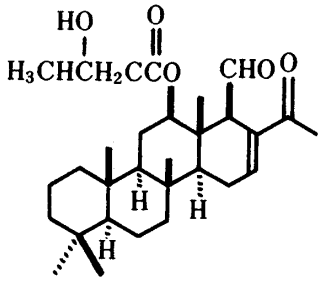
c $-C(=O)-(CH_2)_{14}CH_3$



2 (**b**:**c**=1:22)



3: R=H
3a: R=Ac



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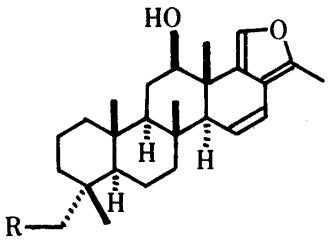
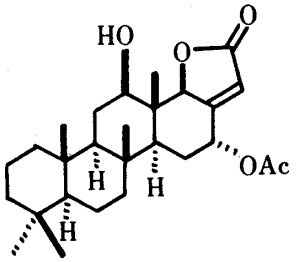


5: R=H
6: R=CH_3



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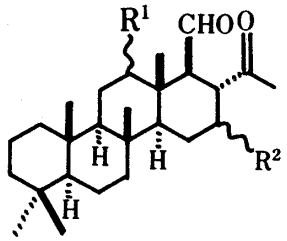

8: R^1 = α-OH, R^2 = α-OAc
9: R^1 = β-OH, R^2 = β-OAc

Chart 1

TABLE I. ^{1}H NMR Data for **3**, **3a**, **4**, **8**, and **9**

	12-H	16-H	17-H	18-H
3	3.77(dd, *J*=4, 11)	5.64(br d-like, *J*=3)	3.26(dd, *J*=3, 12)	2.93(dd, *J*=12, 1)
3a	4.89(dd, *J*=4, 11)	5.65(m)	3.24(dd, *J*=3, 12)	2.95(d, *J*=12,)
4	4.86(dd, *J*=4, 11)	—	—	—
8[4b]	—	5.62(q, *J*=*ca.* 2)	3.13(dd, *J*=10.8, 2.7)	3.45(br d, *J*=10.8)
9[4a]	3.69(dd, *J*=5, 10)	4.66(ddd, *J*=5, 11, 11)	3.21(dd, *J*=11, 11)	2.65(dd, *J*=11, 1)

0.84, 3H; 0.82, 3H; 0.80, 6H, all s), one ethyl group (δ 0.74, 3H, t, *J*=7 Hz, which changed to br s upon irradiation at *ca.* δ 1.15), and a 3-hydroxypentanoyl function (δ 2.38, H_A in ABX, J_{AB}=14 Hz, J_{AX}=8 Hz and δ 2.42, H_B in ABX, J_{AB}=14 Hz, J_{BX}=4 Hz, both ascribable to 2′-H_2; δ 3.86, 1H, m, due to 3′-H; *ca.* δ 1.50, 2H, m, due to 4′-H; δ 0.97, 3H, t, *J*=7 Hz, due to 5′-H) together with other signals as given in Table I. The presence of the 3-hydroxypentanoyl residue was further substantiated by lithium aluminum hydride reduction of foliaspongin (**3**) which yielded pentane-1,3-diol. Thus, foliaspongin (**3**) was demonstrated to be a C_{27} bishomosesterterpene alcohol esterified with 3-hydroxypentanoic acid.

The carbon nuclear magnetic resonance (^{13}C-NMR) spectrum of foliaspongin (**3**) showed the presence of a methylketone residue, an aldehydic residue, an ester group, and three carbinyl carbons (Table II).

In order to clarify the carbon skeleton, foliaspongin (**3**) was subjected to pyrolysis, since an allied scalarane-type derivative (**4**), isolated from the Australian sponge *P. foliascens,* affords a furano derivative (**5**) upon pyrolysis.[4a] The furano compound (**6**) thus obtained showed negative specific rotation similar to that of **5**.[6] In addition, the UV spectrum of **6** [λ_{max}^{MeOH} nm(ϵ): 223 (8500), 230 (9900), 240 (11000)] was very similar to that of **5**.[4a,6] However, the electron-impact mass spectrum (EI-MS) of **6** showed a clear distinction between the two compounds. The EI-MS of **6** gave the molecular ion peak, an ion peak due to dehydration of the molecular ion, and an ion peak at m/z 205 derivable from the A/B ring[7], all of which

TABLE II. ^{13}C-NMR Data for **3**, **3a**, and **7**[a,b]

Carbon	3	3a	7[4c]
1	40.2(t)	40.1(t)	40.0
2	18.3(t)[c]	18.2(t)[c]	18.2[c]
3	24.5(t)	24.5(t)	42.0
4	36.1(s)	36.1(s)	33.3
5	50.5(d)	50.7(d)	56.6
6	18.0(t)	18.2(t)[c]	18.6[c]
7	41.7(t)	41.7(t)	42.0
8	37.5(s)[d]	37.0(s)[d]	37.3[d]
9	58.2(d)[e]	57.0(d)	58.1
10	36.9(s)[d]	37.6(s)[d]	37.6[d]
11	28.9(t)	26.9(t)	25.3
12	79.7(d)	82.1(d)	80.7
13	45.5(s)	43.8(s)	47.5
14	51.5(d)	52.5(d)	47.8
15	27.5(t)	23.3(t)	26.9
16	68.9(d)	69.0(d)	65.9
17	58.5(d)[e]	58.3(d)[e]	162.8
18	58.7(d)[e]	58.6(d)[e]	89.5
19	36.6(t)	36.6(t)	33.3
20	25.1(q)	25.1(q)	21.3
21	17.0(q)[f]	17.1(q)	17.1
22	17.1(q)[f]	17.1(q)	16.4
23	8.6(q)	8.6(q)	6.5
24	208.6(s)	206.1(s)	116.8
25	204.1(d)	202.1(s)	
26	28.5(q)	28.5(q)	169.2
27	9.9(q)[g]	9.4(q)	
1′	171.9(s)	170.2(s)	
2′	42.0(t)	40.1(t)	
3′	69.7(d)	71.4(d)	
4′	29.7(t)	28.1(t)	
5′	10.1(q)[g]	10.9(q)	

a) Abbreviations given in parentheses denote the signal patterns observed in off-resonance experiments: d=doublet, q=quartet, s=singlet, t=triplet.
b) *c*—*g* The assignments for these signals within the same column may be interchanged.

counted 14 mass units larger than the corresponding ion peaks observed in the EI-MS of **5**.[4a] In addition, both EI-MS gave common fragment ion peaks at m/z 175, 172, 159, 148, 147 (base peak), and 146.[4a]

Furthermore, the ^{1}H-NMR spectrum of **6** confirmed a close structural resemblance of **5** and **6**. Signals ascribable to the conjugated α-methylfuran moiety were observed at δ 2.23 (3H, s, 24-CH_3), δ 5.67 (1H, dd, J=10, 2 Hz, 16-H), δ 6.38 (1H, dd, J=10, 2.5 Hz, 15-H), and δ 7.24

(1H, s, 25-H),[8] and other signals due to 12α-H (geminal to 12β-OH; δ 3.84, 1H, dd, J=11, 4 Hz) four tertiary methyl groups (δ 0.77, 0.86, 0.98, and 1.01, 3H each, all s), and one primary methyl group (δ 0.72, 3H, t, J=7 Hz) were also seen. These spectral features were quite similar to those of **5**, except that signals of five tertiary methyl groups were observed in the case of **5**.[4a,9] Therefore, **6** was presumed to be a homo-analog of **5** having an ethyl group at the *A* or *B* ring of the scalarane skeleton.

Next, the ^{13}C-NMR spectra of **3** and **3a** were examined in detail in comparison with the reported ^{13}C-NMR data for scalarane-type sesterterpenes[4e,10] and the signals of all carbons were assigned as shown in Table II. The characteristic signals in the spectra of **3** and **3a** as compared to **7**[4e] were those due to the 4α-ethyl carbons (δ_c 9.9, q; 36.6, t). In comparison with data for **7**,[4e] the signals assignable to 4-C was observed at lower field while the signals due to 3-C and 5-C were observed at higher field, presumably due to enhanced steric compression caused by the 4α-ethyl group. All other signals due to the *A*/*B* ring carbons of **3** and **3a** were observed with chemical shifts similar to those in the case of **7**, and thus the structure of foliaspongin was assigned as **3**. Foliaspongin (**3**) is a bishomosesterterpene having a 4α-ethyl residue and possesses the same scalarane-type carbon skeleton as sesterterpenes previously isolated from the Australian marine sponge of the same species.[4a]

In regard to the location of the acyl residue and to the configurations at C-12, -16, -17, and -18 in **3**, detailed ^{1}H-NMR decoupling experiments carried out for **3** and **3a** and comparison of ^{1}H-NMR data for **3** and **3a** with those for **4**,[4a] **8**,[4b] and **9**[4a] (Table I) confirmed the assignment as depicted in **3**.

M-5 (**1**) exhibited 22.3% inhibition of superoxide radical production at the concentration of 22 μg/ml in the lactase dehydrogenase-catalyzed oxidation of nicotinamide adenine dinucleotide (NADH) test,[11] while M-6 (**2**) exhibited 100% inhibition of hemolysis at the concentration of 4 μg/ml in the anti-complement-fixation test.[12] On the other hand, foliaspongin (**3**) exhibited 18.1% ($p<0.01$) inhibition at the concentration of 10 μg/disk in the antiinflammatory test utilizing chorio-allantoic membrane of chick embryo.[13]

Experimental[14]

Isolation of Foliaspongin (3)——The fresh sponge (*Phyllospongia foliascens*, 28 kg) was extracted successively with MeOH (60 l) and acetone (60 l) at room temperature and then with hot MeOH (60 l). After removal of the solvent from the combined extract under reduced pressure, the residue was partitioned into AcOEt–H_2O. The AcOEt-soluble portion (160 g) was purified successively by repeated silica gel column chromatography (benzene–AcOEt=5:1→3:1, $CHCl_3$–MeOH=10:1 as eluants) and by Sephadex LH-20 column chromatography (benzene–AcOEt=5:1), with monitoring of the antiinflammatory activity of each fraction, to furnish furospongin-1[15] (*ca.* 105 g) and crude foliaspongin (200 mg) from the active fraction. Repeated recrystallization from MeOH gave a pure sample of foliaspongin (**3**) as colorless fine crystals of mp 186—189°C, $[\alpha]_D^{15}$ +44° (c=0.17, $CHCl_3$). CI (NH_3)-MS: m/z 550 [25%, $(M+NH_4)^+$]. *Anal.* Calcd for $C_{32}H_{52}O_6 \cdot H_2O$: C, 69.78; H, 9.88. Found: C, 69.94; H, 9.65. UV (MeOH): transparent above 210 nm. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1710 (CO). CD ($c=2.64\times10^{-1}$, MeOH): $[\theta]_{330}$ 0, $[\theta]_{286}$ +19000 (pos. max.), $[\theta]_{232}$ +500 (pos. min.), $[\theta]_{206}$ +7000! ^{1}H-NMR ($CDCl_3$): as given in Table I and as described in the text. ^{1}H-NMR (d_6-benzene: δ 10.14 (1H, s, 25-H), 5.47 (br s, 16-H), 3.08 (2H, s, 17-H and 18-H), 3.25 (1H, dd, J=4, 11 Hz, 12-H, changed to br s, upon irradiation at *ca.* δ 1.1), 0.82, 0.73, 0.69, 0.58 (each 3H, s, *tert.* $CH_3\times4$), 2.27 (H_A in ABX, J_{AB}=14, J_{AX}=0 Hz), 2.29 (H_B in ABX, J_{AB}=14, J_{BX}=12 Hz) (2′-H_2), 3.89 (1H, m, 3′-H). ^{13}C-NMR ($CDCl_3$) as given in Table II.

Acetylation of Foliaspongin (3)——A solution of **3** (50 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and the total mixture was left standing at room temperature (20°C) for 2 d. After treatment with ice-water, the reaction mixture was extracted with AcOEt (15 ml×3). The AcOEt extract was washed successively with dil. aq. HCl, sat. aq. $NaHCO_3$, and sat. aq. NaCl, then dried over $MgSO_4$. The residue (60 mg), obtained after removal of the solvent, was purified by column chromatography (SiO_2 10 g, benzene–acetone=15:1) to furnish **3a** (30 mg), mp 175—176°C (colorless fine crystals from MeOH), $[\alpha]_D^{15}$ +45° (c=0.16, $CHCl_3$). CI(NH_3)-MS: m/z 634 [16%, $(M+NH_4)^+$]. *Anal.* Calcd for $C_{36}H_{56}O_8$: C, 70.09; H, 9.15. Found: C, 69.69; H, 9.39. IR ν_{max}^{KBr} cm^{-1}: 1740 (OAc), 1720 (CO). ^{1}H-NMR ($CDCl_3$): δ 9.63 (1H, br s, 25-H), 2.08 (3H, s, 24-CH_3), 2.01, 1.98 (both 3H, s, OAc×2), 0.96, 0.90 (both 3H, s), 0.82 (6H, s)(*tert.* $CH_3\times4$), 5.10 (1H,

quintet, J=6 Hz, 3'-H), 2.49 (2H, d, J=6 Hz, 2'-H), and other signals as given in Table I. ^{13}C-NMR ($CDCl_3$): δ 170.2 (s), 169.7 (s), 21.5 (q), 21.1 (q) (OAc×2), and other signals as given in Table II.

$LiAlH_4$ Reduction of Foliaspongin (3)———A solution of **3** (3 mg) in dry ether (4 ml) was treated with $LiAlH_4$ (25 mg) and the total mixture was heated under reflux for 5 h with stirring. After treatment with aqueous AcOEt (5 ml), the clear supernatant was taken and concentrated for thin-layer chromatography (TLC) ($CHCl_3$–AcOEt=1:2, Rf=0.25; benzene–acetone=2:1, Rf=0.25), gas liquid chromatography (GLC) (15% PEGS on Chromosorb WAW 3 mm×2 m, column temp. 160 °C, N_2 at a flow rate of 30 ml/min, t_R=4'12"; 15% NPGS on Chromosorb WAW 3 mm×2 m, column temp. 150 °C, N_2 at a flow rate of 30 ml/min, t_R=7'24") and mass spectrometry combined with gas chromatography (GC–MS) analyses, which showed that the product was identical with authentic pentane-1,3-diol.[16]

Hydroboration of 1-Penten-3-ol———A solution of 1-penten-3-ol (630 mg) in tetrahydrofuran (5 ml) was treated with $NaBH_4$ (250 mg) and the whole mixture was treated dropwise with a solution of BF_3-etherate (1.1 ml) in tetrahydrofuran (3 ml) over a period of 10 min. After stirring for further 30 min at room temperature, the reaction mixture was treated with water (5 ml) and made alkaline by addition of 2N aq. NaOH (7.5 ml). The total mixture was then treated with 30% aq. H_2O_2 (5 ml) and was kept stirring at *ca.* 30 °C for 2 h. After treatment with sat. aq. NaCl, the reaction mixture was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner yielded pentane-1,3-diol (700 mg). ^{1}H-NMR ($CDCl_3$): δ 0.95 (3H, t, J=7 Hz), 1.30—1.85 (4H, m), 3.50 (2H, br, OH), 3.60—3.95 (3H, m). EI-MS (m/z, %): 105 (2, M^++1), 86 (35, M^+-H_2O), 75 (85, $M^+-C_2H_5$), 57 (100, $M^+-C_2H_5-H_2O$).

Pyrolysis of Foliaspongin (3)———Foliaspongin (**3**, 10 mg) was heated at 240 °C under an N_2 atmosphere for 2 min. Purification of the product by column chromatography (SiO_2 5 g, benzene–acetone=50:1) furnished **6**, mp 157—159 °C (MeOH), $[\alpha]_D^{18}$ −120° (c=0.1, MeOH). High resolution MS: Found 396.302, 378.293. Calcd for $C_{27}H_{40}O_2$= 396.302, $C_{27}H_{38}O$ (M^+-H_2O)=378.293. UV (MeOH): as given in the text. EI-MS (m/z, %): 396 (57, M^+), 378 (21), 205 (21), 175 (24), 172 (22), 159 (38), 148 (43), 147 (100), 146 (50). ^{1}H-NMR ($CDCl_3$): as given in the text.

Acknowledgement The authors are grateful to Dr. T. Hoshino, Mukaijima Marine Biological Station, Hiroshima University, for identification of the marine sponge.

References and Notes

1) Part X: H. Kikuchi, Y. Tsukitani, T. Manda, T. Fujii, H. Nakanishi, M. Kobayashi, and I. Kitagawa, *Chem. Pharm. Bull.*, **30**, 3544 (1982).
2) For examples: *a*) I. Kitagawa, T. Nishino, M. Kobayashi, T. Matsuno, H. Akutsu, and Y. Kyogoku, *Chem. Pharm. Bull.*, **29**, 1942 (1981); *b*) I. Kitagawa, M. Kobayashi, and Y. Kyogoku, *ibid.*, **30**, 2045 (1982).
3) H. Kikuchi, Y. Tsukitani, I. Shimizu, M. Kobayashi, and I. Kitagawa, *Chem. Pharm. Bull.*, **29**, 1492 (1981) (preliminary communication).
4) *a*) R. Kazlauskas, P. T. Murphy, R. J. Wells, and J. J. Daly, *Aust. J. Chem.*, **33**, 1783 (1980); *b*) Y. Kashman and M. Zviely, *Tetrahedron Lett.*, **1979**, 3879; *c*) R. P. Walker, J. E. Thompson, and D. J. Faulkner, *J. Org. Chem.*, **45**, 4976 (1980); *d*) F. Yasuda and H. Tada, *Experientia,* **37**, 110 (1981); *e*) G. Cimino, S. De Rosa, and S. De Stefano, *Experientia,* **37**, 214 (1981).
5) The assignments were based on detailed decoupling experiments.
6) **5**,[4a] $[\alpha]_D^{20}$ −124° (MeOH), UV λ_{max}^{MeOH} nm(ϵ): 223 (8312), 230 (9550), 240 (10600). 12α-OH epimer of **5**,[4a] UV λ_{max}^{MeOH} nm(ϵ): 223 (8527), 231 (10740), 244 (12690); this is distinguishable from the UV data for **5**.
7) E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, *Tetrahedron,* **28**, 5993 (1972).
8) This signal overlapped with the signal of $CHCl_3$ in $CDCl_3$.
9) **5**, ^{1}H-NMR ($CDCl_3$)[4a]: δ 7.29 (1H, s), 6.44 (1H, dd, J=10, 3 Hz), 5.70 (1H, dd, J=10, 2.5 Hz), 3.86 (1H, dd, J=11, 4.5 Hz), 2.24 (3H, s), 1.02, 1.00 (both 3H, s), 0.86 (9H, s).
10) D.S. Miranda, G.Brendolan, P.M. Imamura, M.G. Sierra, A.J. Marsaioli, and E.A. Rúveda, *J. Org. Chem.*, **46**, 4851 (1981).
11) P.C. Chan and B.H.J. Bielski, *J. Biol. Chem.*, **249**, 1317 (1974).
12) K. Nagai, *Rinsho Byori* (*The Japanese J. of Clinical Pathology*), **25**, 41 (1976).
13) P.F. D'Arcy and E.M. Howard, *Br. J. Pharmac. Chemother.*, **29**, 378 (1967).
14) The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our preceding paper.[1]
15) G. Cimino, S. De Stefano, L. Minale, and E. Fattorusso, *Tetrahedron,* **27**, 4673 (1971).
16) Due to shortage of material, the specific rotation of pentane-1,3-diol obtained from **3** was not measured.