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Chemical Studies on *Viburnum awabuki* K. KOCH

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From *Viburnum awabuki* K. KOCH (Caprifoliaceae), four new acetyl glucosides of scopoletin, 2′6′-di-*O*-acetylscopolin (**1**), 3′,6′-di-*O*-acetylscopolin (**2**), 6′-*O*-acetylscopolin (**3**) and 2′-*O*-acetylscopolin (**4**), and two lupane-type triterpenes, 6α-hydroxylup-20(29)-en-3-on-28-oic acid (**6**) and its 6β-epimer (**7**), were isolated. Their structures were elucidated on the basis of physicochemical evidence, including proton and carbon-13 nuclear magnetic resonance spectrometry.

Keywords——*Viburnum awabuki*; coumarin derivative; acetyl glucoside; scopolin; *A*-value; CD spectrum; lupane-type triterpene; ^{13}C-NMR spectrum

Viburnum awabuki K. KOCH (Caprifoliaceae) is an evergreen which is cultivated as a windbreak in the southern part of Japan.[1] Vibsanine A and B have been isolated from this plant as a piscicidal compound and a plant growth inhibitor, respectively, along with vibsanines C, D, E and F.[2]

In this paper, we describe the isolation and structural elucidation of four new acetyl derivatives of a coumarin glucoside, 2′,6′-di-*O*-acetylscopolin (**1**), 3′,6′-di-*O*-acetylscopolin (**2**), 6′-*O*-acetylscopolin (**3**) and 2′-*O*-acetylscopolin (**4**), and two lupane-type triterpenes, 6α-hydroxylup-20(29)-en-3-on-28-oic acid (**6**) and 6β-hydroxylup-20(29)-3-on-28-oic acid (**7**). Fresh leaves of the title plant were extracted with hot methanol (MeOH). The ethyl acetate (AcOEt)-soluble fraction of the extract was separated by repeated column chromatography as described in the experimental section to give four new coumarin derivatives, **1**, **2**, **3** and **4**, and two triterpenes, **6** and **7**, along with scopolin (**5**), lup-20(29)-en-3-on-28-oic acid (**8**),[3] ursolic acid, and vibsanines B and C.

Compound **1**, mp 184—186 °C, has the molecular formula, $C_{20}H_{22}O_{11}$, based on the elemental analysis and the mass spectrum (MS). The ultraviolet (UV) spectrum of **1** showed the characteristic coumarin absorption (described in the experimental section). The proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **1** (Table I) showed the presence of the coumarin skeleton [δ 6.29 (d, J= 9.0 Hz, H-3), 7.65 (d, J=9.0 Hz, H-4) and 114.8 (C-3), 143.3 (C-4)], two acetyl groups [δ 2.12 (6H, s)], a methoxyl group [δ 3.82 (3H, s) and δ 57.0 (q)] and a glucosyl moiety (Table I) having acetyl groups. The two singlet proton signals, δ 6.90 and 7.10, showed that two substituents were at C-6 and C-7 of the B-ring. From these data, **1** was supposed to be a diacetate of scopolin (7-*O*-glucosyl-6-methoxycoumarin) or iso-scopolin (6-*O*-glucosyl-7-methoxycoumarin). In a nuclear Overhauser effect (NOE) experiment, irradiation of H-4 gave 11% NOE at the δ 6.90 signal and irradiation of the methoxyl group gave 19% NOE at the same signal. These results indicated that the aglycone part was scopoletin and **1** was an acetate of scopolin, which was confirmed by methanolysis of **1** to give scopoletin and methyl α-glucopyranoside. In the ^{13}C-NMR spectra, the C-1′, C-3′ and C-5′ signals appeared at higher field (δ 100.1, 74.7 and 74.9, respectively), so the substitution pattern of the acetyl groups was considered to be 2′,6′-diacetyl. This was confirmed from the use of the exciton chirality

method of Nakanishi *et al.*[4] Compound **1** was *p*-bromobenzoylated to give di-*p*-*O*-bromobenzoate (**1a**), (confirmed by the ^{1}H-NMR spectrum). The benzoate (**1a**) showed a strong Cotton effect in the circular dichroism (CD) spectrum and the *A*-value[5] was −55.4 (Fig. 1), which accorded with the calculated *A*-value of the 3,4-di-*O*-*p*-bromobenzoate of glucose (−62). Thus, the structure of **1** was determined to be 2′,6′-di-*O*-acetylscopolin.

Compound **2**, mp 155—158 °C, has the same molecular formula as **1** based on elemental analysis and the MS (*m/z*: 438). The ^{1}H- and ^{13}C-NMR spectra of **2** showed almost the same signal patterns (Table I) as in the case of **1**. Only the carbon signals of the glucosyl moieties were different from each other, so **2** was concluded to be a positional isomer of the acetyl groups. In the ^{13}C-NMR spectrum of **2**, the C-2′, C-4′ and C-5′ signals appeared at higher field (δ 72.4, 69.5 and 75.4 respectively), so **2** was concluded to be 3′,6′-di-*O*-acetylscopolin.

Compound **3**, mp 229 °C, has the molecular formula, $C_{18}H_{20}O_{10}$, based on elemental

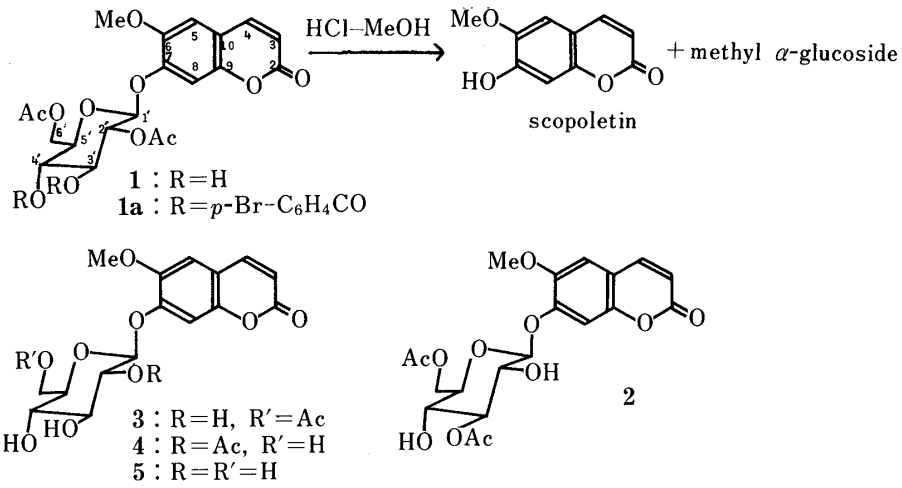


Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of **1**—**5**[a]

Carbon No.	1	2	3	4	5
C-2	161.8	160.8	160.8	160.8	160.9
C-3	114.8	114.4	114.2	114.6	114.1
C-4	143.3	143.5	143.4	143.5	143.5
C-5	110.4	110.3	110.1	111.0	110.3
C-6	147.6	147.1	147.9	147.5	147.2
C-7	150.4	151.3	151.3	151.3	151.3
C-8	106.1	104.7	104.6	105.9	104.3
C-9	149.4	150.1	150.1	149.8	150.1
C-10	114.1	113.4	113.1	113.9	113.0
C-1′	100.1	101.7	102.0	100.6	102.0
C-2′	73.4	72.4	74.4	75.9	74.7
C-3′	74.7	78.9	78.2	74.8	78.5
C-4′	70.7	69.5	71.2	71.4	71.2
C-5′	74.9	75.4	75.6	79.3	78.9
C-6′	63.5	64.0	64.4	62.1	62.4
OMe	57.0	56.3	56.3	56.8	56.5
C=O	170.6	170.6	170.6	169.9	
|	171.7				
CH_3	20.8	20.6	20.6	21.0	

a) Measured in pyridine-d_5.

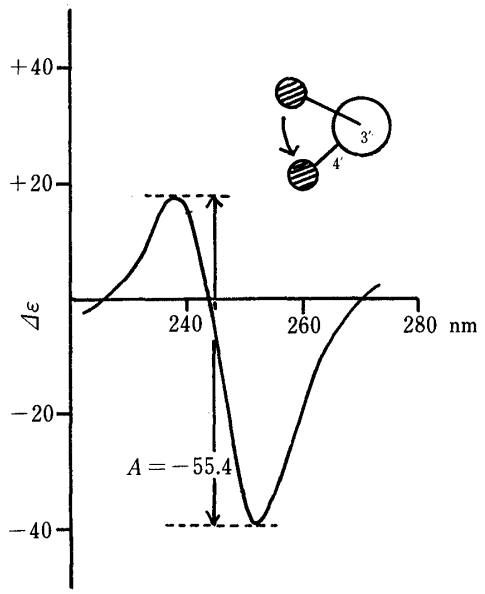
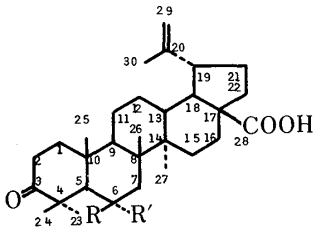
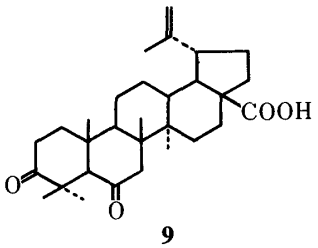


Fig. 1. CD Spectrum of the Di-*O*-*p*-bromobenzoate (**1a**) of **1**



6: R=H, R'=OH
7: R=OH, R'=H
8: R=R'=H



9

Chart 2

analysis and the MS (*m/z*: 396). The ^{1}H- and ^{13}C-NMR spectra of **3** indicated that **3** is a monoacetate of scopolin. In the ^{13}C-NMR spectrum the C-6′ signal appeared at lower field (δ 64.4) and the C-5′ signal appeared at higher field (δ 75.6), while the other carbon signals of the glucosyl moiety appeared at ordinary field (Table I). Thus, **3** was concluded to be 6′-*O*-acetylscopolin.

Compound **4**, mp 208—210 °C, has the molecular formula, $C_{18}H_{20}O_{10}$, based on elemental analysis and the MS spectrum. The ^{1}H- and ^{13}C-NMR spectra indicated that **4** was also a monoacetate of scopolin, like **3**. In the ^{13}C-NMR spectrum, the C-2′ signal appeared at lower field (δ 75.9), the C-3′ signal at higher field (δ 74.8) and the other signals at ordinary field. Thus, **4** was concluded to be 2′-*O*-acetylscopolin.

The molecular formula of compound **6**, mp 276—279 °C, was suggested to be $C_{30}H_{46}O_4$, from the MS. The ^{1}H- and ^{13}C-NMR spectra of **6** showed the presence of characteristic signals of lupane-type triterpenes, such as those of five tertiary methyl groups (δ 0.73, 0.99, 1.03, 1.29 and 1.32), an isopropenyl group [δ 1.69, (3H, s, vinyl methyl), 4.60 and 4.71 (each H, br s, exomethylene), and δ 109.7 (t) and 151.5 (s)], a carbonyl group (δ 221.1), a carboxyl group (δ 179.2) and a hydroxyl group [δ 3.87 (H, dt, J=4.8 and 10.9 Hz) and δ 67.5]. These results indicated that **6** is lupane-type triterpene, having a 3-keto group. A comparison of the ^{13}C-NMR spectrum of **6** with that of lupenone[6] indicated that **6** is 6-hydroxylup-20(29)-en-3-on-28-oic acid. Compound **7**, mp 274—276 °C, has the same molecular formula, $C_{30}H_{46}O_4$, as **6** based on elemental analysis and the MS. The ^{1}H- and ^{13}C-NMR spectra of **7** showed signals

TABLE II. ^{13}C-NMR Chemical Shifts of **6**—**9**

Carbon No.	**6**[a]	**7**[b]	**8**[b]	**9**[b]
C-1	39.7	40.1	39.7	41.2
C-2	33.2	34.5	34.1	33.9
C-3	217.0	216.3	216.0	214.4
C-4	42.5	42.2	47.3	47.0[c]
C-5	58.6	56.5	54.8	65.5
C-6	67.5	69.8	19.7	212.0
C-7	44.2	41.9	33.7	52.0
C-8	38.4	37.5	40.7	47.7[c]
C-9	49.2[c]	50.9[c]	49.9[c]	50.7[d]
C-10	32.2	34.5	37.0	43.8
C-11	21.9	21.3	21.5	21.7
C-12	25.6	25.2	25.6	25.2
C-13	38.0	37.5	38.6	38.3
C-14	41.4	42.9	42.5	43.1
C-15	29.8	29.9	29.8	29.8
C-16	32.9	32.2	32.2	32.1
C-17	56.2	56.9	56.5	56.3
C-18	49.0[c]	49.5[c]	49.4[c]	49.5[d]
C-19	47.4	47.0	46.9	47.0
C-20	150.5	150.3	150.2	150.1
C-21	30.6	30.7	30.7	30.7
C-22	37.1	37.0	36.8	37.0
C-23	25.6	25.7	26.7	24.3
C-24	19.4	21.3	21.0	21.6
C-25	16.4[d]	17.3[d]	15.9	16.2[e]
C-26	17.7[d]	17.1[d]	15.9	16.5[e]
C-27	14.6	15.0	14.6	15.0
C-28	179.2	181.9	182.2	185.2
C-29	109.7	109.8	109.7	110.0
C-30	19.4	19.5	19.4	19.5

a) Measured in $CDCl_3+CD_3OD$. *b*) Measured in $CDCl_3$. *c, d, e*) Assignments may be interchanged in each column.

similar to those of **6**, except for the C-5, -6 and -7 signals (Table II). Thus, it was considered that **6** and **7** are C-6 epimers. This was confirmed by obtaining the same diketo derivative (**9**) from both **6** and **7** by oxidation. In the ^{1}H-NMR spectrum, the signal of the proton at C-6 of **6** appeared as atriplet-doublet ($J=10.9$ and 4.8 Hz) and that of the proton at C-6 of **7** appeared as broad doublet ($W_{1/2}$, $J=7.5$ Hz). These results indicate that the 6-hydroxyl group of **6** is equatorial and the 6-hydroxy group of **7** is axial. Compounds **6** and **7** were concluded to be 6α-hydroxylup-20(29)-en-3-on-28-oic acid and 6β-hydroxylup-20(29)-en-3-on-28-oic acid, respectively. Ageta and Kobayashi[7] isolated the 6α-isomer from the same source. Compound **6** was shown to be identical with an authentic sample of the 6α-isomer (as a methyl ester).

Experimental

All melting points were measured on a Yanagimoto micro melting point apparatus. Infrared (IR) spectra were recorded on a JASCO A-202 grating infrared spectrometer and UV spectra on a Shimadzu UV-210 spectrometer. Optical rotations were determined on a JASCO DIP-140 digital polarimeter. CD spectra were recorded on a JASCO J-20A automatic recording spectropolarimeter. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX 90Q FT (90 MHz) NMR spectrometer with tetramethylsilane (TMS) as an internal standard (δ value). ^{13}C-NMR spectra were recorded on a JEOL JNM-FX 90Q FT (22.5 MHz) NMR spectrometer (δ value). MS were recorded on JEOL JMS D-100 and JMS 01SG-2 mass spectrometers. Thin-layer chromatography (TLC) was carried out on

precoated Silica gel $60F_{254}$ plates (Merck). Column chromatography was carried out on Silica gel Type 60 (Merck).

Extraction and Isolation of Constituents——Fresh leaves of *V. awabuki* (9 kg), were extracted with hot MeOH. The MeOH extract was extracted with AcOEt to give the AcOEt extract (260 g). The aqueous solution was extracted with *n*-butanol to give the *n*-butanol extract (160 g). The AcOEt extract was placed on a top of a silica gel column and eluted with *n*-hexane, chloroform ($CHCl_3$), AcOEt, acetone and MeOH successively to give five eluates. The $CHCl_3$ eluate (38 g) was chromatographed on a silica gel column (benzene–acetone = 100 : 3) to give 6α-hydroxylup-20(29)-en-3-on-28-oic acid (**6**) (350 mg) and the 6β-isomer (**7**) (50 mg). The AcOEt eluate (89 g) gave ursolic acid (6.3 g), vibsanine B (1.5 g), vibsanine C (500 mg), 2′,6′-di-*O*-acetylscopolin (**1**) (90 mg), 3′,6′-di-*O*-acetyl scopolin (**2**) (30 mg) and 6′-*O*-acetylscopolin (**3**) (20 mg) on repeated silica gel column chromatography ($CHCl_3$–MeOH gradient solvent system). The acetone eluate (60 g) gave ursolic acid (2 g), **1** (120 mg), **2** (25 mg) and **3** (30 mg) repeated silica gel column chromatography ($CHCl_3$–MeOH gradient solvent system). The *n*-butanol fraction (160 g) was subjected to Mitsubishi Diaion HP-20 column chromatography. The MeOH–water (50 : 50) eluate was chromatographed on a silica gel column ($CHCl_3$–MeOH gradient solvent system) to give **1** (400 mg), **2** (20 mg), **3** (20 mg), 3′-*O*-acetylscopolin (**4**) (60 mg) and scopolin (**5**) (40 mg).

2′,6′-Di-*O*-acetylscopolin (1)——mp 184—186 °C ($CHCl_3$–MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500, 1720, 1260, 1080. UV λ_{max}^{MeOH} nm (log ε): 205.3 (4.4), 227.5 (4.2), 250 (3.7), 257.5 (3.6), 287 (3.8), 339 (4.0). MS *m/z*: 438 (M^+) ($C_{20}H_{22}O_{11}$), 192. *Anal.* Calcd for $C_{20}H_{22}O_{11} \cdot H_2O$: C, 52.63; H, 5.30. Found: C, 52.77; H, 4.97. ^{1}H-NMR ($CDCl_3$–CD_3OD): 2.08 (3H, s, Ac), 2.12 (3H, s, Ac), 3.82 (3H, s, OMe), 6.29 (H, d, J=9.0 Hz, H-3), 6.90 (H, s, H-5), 7.10 (H, s, H-8), 7.65 (H, d, J=9.0 Hz, H-4). ^{13}C-NMR: Table I.

Methanolysis of 1——2′,6′-Di-*O*-acetylscopolin (**1**) (30 mg) was dissolved in 5% HCl–MeOH (5 ml) and refluxed for 1 h. The filtrate was evaporated to give the residue, which was separated by TLC to give scopoletin and methyl α-glucoside. Scopoletin was identified by comparison with an authentic sample. Methyl α-glucoside was *p*-bromobenzoylated to give the per-*p*-bromobenzoate, which was shown to be identical with an authentic sample by high performance liquid chromatography.

3′,6′-Di-*O*-Acetylscopolin (2)——mp 155—158 °C ($CHCl_3$–MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500, 1740, 1280, 1080. UV λ_{max}^{MeOH} nm (log ε): 204 (4.5), 226 (4.2), 287 (3.7), 340 (4.9). MS *m/z*: 438 (M^+) ($C_{29}H_{22}O_{11}$). *Anal.* Calcd for $C_{20}H_{22}O_{11} \cdot 1/2H_2O$: C, 53.71; H, 5.18. Found: C, 53.14; H, 4.91. ^{1}H-NMR (pyridine-d_5): 1.82 (3H, s, Ac), 1.89 (3H, s, Ac), 3.46 (3H, s, OMe), 6.10 (H, d, J=9.0 Hz, H-3), 6.81 (1H, s, H-5), 7.28 (H, s, H-8), 7.45 (H, d, J=9.0 Hz, H-4). ^{13}C-NMR: Table I.

6′-*O*-Acetylscopolin (3)——mp 229 °C ($CHCl_3$–MeOH). IR ν_{max}^{KBr} cm^{-1}: 3450, 1730, 1280, 1080. UV λ_{max}^{MeOH} nm (log ε): 205 (4.2), 227 (3.8), 287 (3.2), 338 (3.5). MS *m/z*: 396 (M^+) ($C_{18}H_{20}O_{10}$). *Anal.* Calcd for $C_{18}H_{20}O_{10}$: C, 54.54; H, 5.09. Found: C, 54.10; H, 5.02. ^{1}H-NMR (pyridine-d_5): 2.18 (3H, s, Ac), 3.98 (3H, s, OMe), 6.36 (H, d, J=9.0 Hz, H-3), 7.10 (H, s, H-5), 7.56 (H, s, H-8), 7.80 (H, d, J=9.0 Hz, H-4). ^{13}C-NMR: Table I.

2′-*O*-Acetylscopolin (4)——mp 208—210 °C ($CHCl_3$–MeOH). IR ν_{max}^{KBr} cm^{-1}: 3510, 1725, 1255, 1060. UV λ_{max}^{MeOH} nm (log ε): 204 (4.4), 224 (4.1), 287 (3.6), 342 (3.5). MS *m/z*: 396 (M^+) ($C_{18}H_{20}O_{10}$), 192. *Anal.* Calcd for $C_{18}H_{20}O_{10} \cdot 3/5H_2O$: C, 53.09; H, 5.24. Found: C, 52.90; H, 5.00. ^{1}H-NMR (pyridine-d_5): 1.89 (3H, s, Ac), 3.54 (3H, s, OMe), 6.15 (H, d, J=9.0 Hz, H-3), 6.85 (H, s, H-5), 7.26 (H, s, H-8), 7.47 (H, d, J=9.0 Hz, H-4). ^{13}C-NMR: Table I.

Scopolin (5)——mp 208—213 °C ($CHCl_3$–MeOH). IR ν_{max}^{KBr} cm^{-1}: 3460, 1700, 1280, 1080. UV λ_{max}^{MeOH} nm (log ε): 205 (4.4), 227 (4.1), 289 (3.6), 340 (3.8). *Anal.* Calcd for $C_{16}H_{18}O_9 \cdot H_2O$: C, 51.61; H, 5.41, C, 51.61; H, 5.30. ^{1}H-NMR (pyridine-d_5): 3.52 (3H, s, OMe), 6.12 (H, d, J=9.0 Hz, H-3), 6.83 (H, s, H-5), 7.19 (H, d, J=9.0 Hz, H-4), 7.28 (H, s, H-8). ^{13}C-NMR: Table I.

6α-Hydroxylup-20(29)-en-3-on-28-oic Acid (6)——mp 276—279 °C (*n*-hexane–AcOEt). MS *m/z*: 470 (M^+) ($C_{30}H_{46}O_4$). $[\alpha]_D$ +78.3° (*c*=0.10, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3370, 1739, 1700, 1240. ^{1}H-NMR ($CDCl_3$+CD_3OD): 0.73, 0.99, 1.03, 1.29, 1.32, 1.69 (3H each, s, Me × 6), 3.87 (H, dt, J=4.80, 10.69 Hz, H-6), 4.62, 4.71 (1H each, br s, H-29). ^{13}C-NMR: Table II.

6β-Hydroxylup-20(29)-en-3-on-28-oic Acid (7)——mp 274—276 °C (*n*-hexane–AcOEt). MS *m/z*: 470 (M^+) ($C_{30}H_{46}O_4$). *Anal.* Calcd for $C_{30}H_{46}O_4$: C, 76.55; H, 9.85. Found: C, 76.46; H, 10.05. IR ν_{max}^{KBr} cm^{-1}: 3520, 1697, 1460, 1379. $[\alpha]_D$ −27.1 (*c*=0.66, MeOH). ^{1}H-NMR ($CDCl_3$): 0.93, 1.14, 1.34, 1.69 (3H each, s, Me × 4), 1.41 (6H, s, Me × 2), 4.45 (1H, br s, H-6), 4.61, 4.72 (1H each, br s, H-29). ^{13}C-NMR: Table II.

Lup-20(29)-en-3-on-28-oic Acid (8)——Amorphous powder. MS *m/z*: 454 (M^+) ($C_{30}H_{46}O_3$). $[\alpha]_D$ +31.5 (*c*= 0.84, MeOH)), IR ν_{max}^{KBr} cm^{-1}: 1704, 1697, 1460, 1380. ^{1}H-NMR ($CDCl_3$): 0.97, 0.99, 1.01, 1.08, 1.44, 1.69 (each 3H, s, Me × 6), 4.57, 4.68 (each H, br s, H-29). ^{13}C-NMR: Table II.

Oxidation of 6 and 7——**6** (40 mg) was oxidized with Jones' reagent to give the diketo derivative (**9**). Colorless powder. MS *m/z*: 468 (M^+) ($C_{30}H_{44}O_4$). ^{1}H-NMR ($CDCl_3$): 1.01, 1.14, 1.48, 1.71 (each 3H, s, Me × 4), 1.09 (6H, s, Me × 2), 4.64, 4.76 (each H, br s, H-29). ^{13}C-NMR: Table II. Compound **7** was oxidized in the same way to give **9**.

***p*-Bromobenzoylation of 1**——A mixture of **1** (30 mg) and *p*-bromobenzoyl chloride (excess) in pyridine (2 ml) was stirred overnight at 100 °C. The reaction solution was poured into ice-water and extracted with $CHCl_3$ to give the residue, which was purified by preparative layer chromatography to give the di-*p*-bromobenzoate (**1a**) of **1**; the structure of **1a** was identified by ^{1}H-NMR spectrum, which showed the presence of two acetyl groups and two characteristic AA′BB′′ type signals of *p*-bromobenzoate groups. CD (*c*=0.003, MeOH): Δε −38.7 (254) (negative

maximum), +16.7 (237) (positive maximum).

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