

Calocedimers A, B, C, and D from the Bark of *Calocedrus macrolepis* var. *formosana*Chin-Lin Hsieh,<sup>†,‡</sup> Lung-Lin Shiu,<sup>†</sup> Mei-Huims Tseng,<sup>‡</sup> Yi-Yuan Shao,<sup>§</sup> and Yueh-Hsiung Kuo<sup>\*,†</sup>

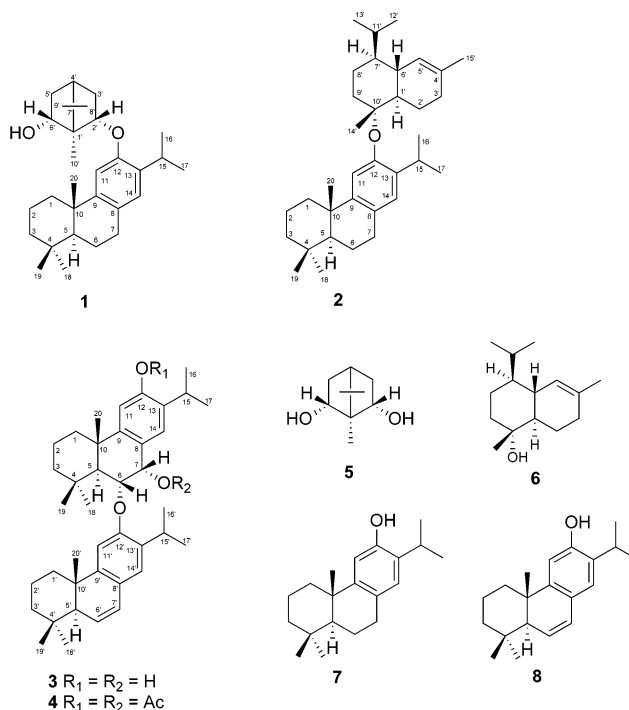
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Calocedimers A, B, C, and D, together with four known compounds, platydiol,  $\alpha$ -cadinol, ferruginol, and 6,7-dehydroferruginol, were isolated from the bark of *Calocedrus macrolepis* var. *formosana*. Calocedimers A, B, C, and D are the dimers of abietane-*O*-camphane, abietane-*O*-cadinane, abietane-*O*-abietane, and abietane-*O*-abietane, respectively. Their structures were elucidated from spectroscopic data.

*Calocedrus macrolepis* var. *formosana* (= *C. formosana*),<sup>1</sup> a member of the Cupressaceae, is an endemic plant and also an important building material in Taiwan. It grows at elevations from 300 to 2000 m in the central and northern mountains of Taiwan. Previous investigation of the heartwood<sup>2–4</sup> had shown that it contained essential oils and a large quantity of terpenoid acids, such as shonanin, thujic, and chaminic acids. The wood also contains tropolones, monoterpenes, naphthalene-type sesquiterpenes, diterpenoid phenols, and lignan components, such as hinokinin, hibactone (savinine), calocedrin, and matairesinol. The leaves<sup>5,6</sup> contain monoterpene derivatives. The bark<sup>7,8</sup> contains some phenolic diterpenes such as sugiol, ferruginol, and xanthoperol. Because the studies from nearly thirty years ago were incomplete, we continued the research on the bark of this plant. Four dimeric compounds, calocedimers A (**1**), B (**2**), C (**3**), and D (**4**), together with the known platydiol (**5**),<sup>9</sup>  $\alpha$ -cadinol (**6**),<sup>10</sup> ferruginol (**7**),<sup>11</sup> and 6,7-dehydroferruginol (**8**)<sup>12</sup> are reported in this paper. The new compounds **1**, **2**, **3**, and **4** are dimers of abietane-*O*-camphane, abietane-*O*-cadinane, abietane-*O*-abietane, and abietane-*O*-abietane, respectively. The structures of these new dimeric compounds were elucidated on the basis of spectroscopic evidence.

Compound **1**, called calocedimer A, has been obtained as a gum. HREIMS revealed the formula C<sub>30</sub>H<sub>46</sub>O<sub>2</sub>. The IR spectrum suggested the presence of OH (3543 cm<sup>-1</sup>) and aromatic (3051, 1613, 1573, and 1497 cm<sup>-1</sup>) groups. By means of <sup>1</sup>H and <sup>13</sup>C NMR (Tables 1 and 2) analysis and 2D NMR (including HMQC, HMBC, COSY, and NOESY methods), the structure of calocedimer A (**1**) was judged as an ether-linked dimer of monoterpene and diterpene fragments rather than a triterpene. The <sup>1</sup>H NMR spectrum (Table 1) showed one set of dehydroabietane signals as follows:  $\delta$  0.92, 0.90, 1.17 (each 3H, s, Me-18, Me-19, Me-20), 1.16, 1.20 (each 3H, d,  $J = 6.8$  Hz, Me-16, Me-17), 2.15 (1H, br d,  $J = 12.4$  Hz, H $\beta$ -1, characteristic signal for dehydroabietane),<sup>11,12</sup> 3.10 (1H, sept,  $J = 6.8$  Hz, H-15), 2.86 (1H, dd,  $J = 16.7, 6.5$  Hz, H $\beta$ -7), 2.76 (1H, ddd,  $J = 16.7, 11.2, 7.1$  Hz, H $\alpha$ -7), 6.60, 6.86 (each 1H, s, H-11, H-14). The first set of signals of **1** resemble those of ferruginol.<sup>11</sup> The second set of signals resemble those of camphane-type platydiol<sup>9</sup> as follows:  $\delta$  0.92, 0.94, 1.13 (each 3H, s, Me-8', Me-9', Me-10'), 4.16 (br d, H-6'), 4.56 (br d, H-2'). The NOESY correlations of Me-8' with H-2' and Me-9' with H-6' confirmed the *exo*-orientation of H-2' and H-6'. No phenolic proton was observed, and one of the oxygenated methine protons (H-2' of platydiol moiety) was downshifted to  $\delta$  4.56 ( $\delta$  4.20 in platydiol). The <sup>13</sup>C NMR shifts of C-2' and C-12 are lower than those of the



corresponding carbons in platydiol and ferruginol, respectively, thereby suggesting the linkage between platydiol and ferruginol at C-2' and C-12 via an ether connection. Further proof of this is that the signal at  $\delta$  4.56 (H-2') had HMBC correlations to C-12. Therefore, compound **1** was elucidated as abieta-8,11,13-trien-12-yl 6 $\alpha$ -hydroxycamphan-2 $\alpha$ -yl ether.

The IR spectrum of calocedimer B (**2**) suggested the presence of an aromatic (3061, 1611, 1494 cm<sup>-1</sup>) ring. The molecular formula of C<sub>35</sub>H<sub>54</sub>O was established by HREIMS. Through the analysis of <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 1 and 2) together with 2D NMR techniques, compound **2** was proposed as an ether-linked dimer of cadinane sesquiterpene and dehydroabietane diterpene rather than a C<sub>35</sub>-terpene. The diterpene moiety is a ferruginol. The <sup>1</sup>H NMR spectrum showed three methyl singlets, an isopropyl group attached to an aromatic ring, two *para*-phenyl protons, and a typical H $\beta$ -1 proton of dehydroabietane. The <sup>1</sup>H NMR (Table 1) data of the diterpene moiety closely resemble those of compound **1**. Both the <sup>1</sup>H and <sup>13</sup>C NMR (Tables 1 and 2) data of the second moiety of **2** were similar to those of  $\alpha$ -cadinol<sup>14</sup> and were assigned as follows:  $\delta$  1.27, 1.67 (each 3H, s, Me-14', Me-15'), 0.74, 0.87 (each 3H, d,  $J = 6.9$  Hz, Me-12', Me-13'), 2.12 (1H, m, H-11'), 5.51 (br s, H-5'), 2.00 (1H, dd,  $J = 16.9, 4.8$  Hz, H $\beta$ -3'), 2.08 (1H, m, H $\alpha$ -3'). This moiety is a *trans*-fused cadinol because the olefinic proton is a

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**Table 1.** <sup>1</sup>H NMR Data (CDCl<sub>3</sub>, 500 MHz) of Compounds **1–4** (δ in ppm, *J* in Hz)

no.	<b>1</b>	no.	<b>2</b>	no.	<b>3</b>	<b>4</b>
1 <sub>α</sub>	1.39 td (12.4, 3.3)	1 <sub>α</sub>	1.34 m	1 <sub>α</sub>	1.62 m	1.66 m
1 <sub>β</sub>	2.15 br d (12.4)	1 <sub>β</sub>	2.12 br d (12.7)	1 <sub>β</sub>	2.29 br d (13.9)	2.04 br d (12.8)
2	1.75m, 1.60 m	2	1.71m, 1.56 m	2	1.68 m, 1.72 m	1.64 m, 1.72 m
3	1.45 m, 1.20 m	3	1.44 m, 1.28 m	3	1.28 m, 1.46 m	1.33 m, 1.46 m
5	1.30 <sup>a</sup>	5	1.32 <sup>a</sup>	5	1.70 d (9.4)	1.81 d (9.6)
6	1.88 m, 1.68 m	6	1.81 m, 1.67 m	6	4.95 d (9.4)	5.03 dd (2.8, 9.6)
7 <sub>α</sub>	2.76 ddd (16.7, 11.2, 7.1)	7 <sub>α</sub>	2.77 ddd (16.8, 11.2, 7.1)	7	4.67 s	5.99 d (2.8)
7 <sub>β</sub>	2.86 dd (16.7, 6.5)	7 <sub>β</sub>	2.84 dd (16.8, 6.7)	11	6.68 s	6.88 s
11	6.60 s	11	6.81 s	14	7.02 s	7.22 s
14	6.86 s	14	6.82 s	15	3.11 sep (6.9)	2.90 sep (6.8)
15	3.10 sep (6.8)	15	3.29 sep (6.9)	16	1.18 d (6.9)	1.11 d (6.8)
16	1.16 d (6.8)	16	1.11 d (6.9)	17	1.20 d (6.9)	1.10 d (6.8)
17	1.20 d (6.8)	17	1.16 d (6.9)	18	0.98 s	0.99 s
18	0.92 s	18	0.89 s	19	1.15 s	1.14 s
19	0.90 s	19	0.91 s	20	1.43 s	1.43 s
20	1.17 s	20	1.15 s	1 <sub>β</sub> '	1.64 m	1.57 m
2'	4.56 br d (9.9)	1'	1.62 <sup>a</sup>	1 <sub>α</sub> '	2.22 br d (12.9)	2.16 br d (12.7)
3 <sub>α</sub> '	1.45 m	2 <sub>α</sub> '	2.35 dd (12.4, 5.8)	2'	1.68 m, 1.72 m	1.68 m, 1.76 m
3 <sub>β</sub> '	2.55 m	2 <sub>β</sub> '	1.35 m	3'	1.28 m, 1.53 m	1.24 m, 1.50 m
5 <sub>α</sub> '	1.30 m	3 <sub>α</sub> '	2.08 m	5'	2.13 dd (2.6, 3.0)	2.11 t (2.8)
5 <sub>β</sub> '	2.49 m	3 <sub>β</sub> '	2.00 dd (16.9, 4.8)	6'	5.88 dd (2.6, 9.6)	5.87 dd (2.8, 9.6)
6'	4.16 br d (9.9)	5'	5.51 br s	7'	6.50 dd (3.0, 9.6)	6.48 dd (2.8, 9.6)
8'	0.92 s	6'	1.81 m	11'	7.10 s	6.73 s
9'	0.94 s	7'	0.97 m	14'	6.93 s	6.91 s
10'	1.13 s	8'	1.56 m, 1.17 m	15'	3.23 sep (7.0)	3.21 sep (6.8)
		9 <sub>α</sub> '	1.41 m	16'	1.10 d (7.0)	1.10 d (6.8)
		9 <sub>β</sub> '	1.88 dt (12.2, 3.2)	17'	1.08 d (7.0)	1.06 d (6.8)
		11'	2.12 m	18'	0.96 s	0.95 s
		12'	0.74 d (6.9)	19'	1.04 s	1.02 s
		13'	0.87 d (6.9)	20'	1.07 s	1.04 s
		14'	1.27 s	OH-12	4.86 br s	
		15'	1.67 s	OH-7	3.13 br s	
				CH <sub>3</sub> COO-12		2.30 s
				CH <sub>3</sub> COO-7		1.83 s

<sup>a</sup> Overlapping with other signals.

broad singlet.<sup>10,15</sup> H-6' has NOESY correlations with Me-14' and Me-12'; this evidence confirmed the α-cadinol derivative. For compound **2**, no phenolic proton was observed, and C-10' and C-12 resonated at lower field than the corresponding carbons in α-cadinol and ferruginol. Only one O atom existed in the formula C<sub>33</sub>H<sub>54</sub>O; therefore, the structure of **2** can be defined as abieta-8,11,13-trien-12-yl α-cadina-10-yl ether.

Calocedimer C (**3**), on the basis of exact mass (HREIMS) at *m/z* 584.4231, had the molecular formula C<sub>40</sub>H<sub>56</sub>O<sub>3</sub>, suggesting the presence of 13 degrees of unsaturation. It showed hydroxyl (3365 cm<sup>-1</sup>) and aromatic (3051, 1614, 1584, 1496 cm<sup>-1</sup>) absorptions in its IR spectrum. From <sup>1</sup>H, <sup>13</sup>C, and 2D-NMR analysis, compound **3** was determined to be an ether-linked dimer of two dehydroabietanes rather than a tetraterpene. One moiety of **3** is a 6,7-dioxygenated ferruginol, which was revealed from its <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 1 and 2). The 6,7-dioxygenated ferruginol moiety showed three methyl singlets, an exchangeable phenolic proton, an isopropyl group attached to an aromatic ring, two *para*-phenyl protons, a typical H<sub>β</sub>-1 proton of dehydroabietane, a carbinol proton (δ<sub>H</sub> 4.67, s) located at C-7 (δ<sub>C</sub> 75.1), and another oxygenated proton (δ<sub>H</sub> 4.95, d, *J* = 9.4 Hz) located at C-6 (δ<sub>C</sub> 79.2), which is connected with an ether functional group. The coupling constant between H-6 (d, *J* = 9.4 Hz) and H-5 defined the β-axial orientation of H-6. A broad singlet signal of H-7 established the β-equatorial orientation of H-7. The other moiety showed signals of three methyl singlets, a typical H<sub>β</sub>-1 proton of dehydroabietane, an isopropyl group attached to an aromatic ring, and two *para*-phenyl protons. An AMX pattern among three protons was assigned as follows: two olefinic protons (δ<sub>H</sub> 6.50, dd, *J* = 9.6, 3.0 Hz, H-7'; δ<sub>H</sub> 5.88, dd, *J* = 9.6, 2.6 Hz, H-6') and H-5' (δ<sub>H</sub> 2.13, dd, *J* = 3.0, 2.6 Hz). These data resemble those of 6,7-dehydroferruginol.<sup>16</sup> Only one phenolic proton (δ 4.86, s, exchangeable) and an HMBC correlation between H-6 and C-12' were observed. These results suggested that

**Table 2.** <sup>13</sup>C NMR Data (CDCl<sub>3</sub>, 125 MHz) of Compounds **1–4** (δ in ppm)

no.	<b>1</b>	<b>2</b>	no.	<b>3</b>	<b>4</b>	no.	<b>3</b>	<b>4</b>
1	38.8	39.0	1	39.2	41.0	16'	23.3	23.5
2	19.3	19.4	2	19.0	18.8	17'	23.3	23.0
3	41.6	41.7	3	43.1	42.8	18'	32.6	31.6
4	33.3	33.4	4	34.1	34.1	19'	22.6	22.6
5	50.4	50.4	5	55.1	54.6	20'	20.4	20.0
6	19.2	19.2	6	79.2	76.7	CH <sub>3</sub> COO-12		169.7
7	29.6	30.3	7	75.1	75.5	CH <sub>3</sub> COO-12		21.3
8	127.9	129.0	8	128.3	130.3	CH <sub>3</sub> COO-7		169.7
9	148.3	147.5	9	149.6	149.8	CH <sub>3</sub> COO-7		21.0
10	37.7	37.5	10	38.4	38.5			
11	108.1	118.2	11	110.2	117.1			
12	153.2	150.4	12	153.0	148.5			
13	133.9	139.2	13	132.2	137.8			
14	128.4	126.1	14	127.6	129.1			
15	26.5	26.4	15	26.8	27.2			
16	23.1	23.8	16	22.5	22.9			
17	22.6	23.1	17	22.6	22.5			
18	33.4	33.3	18	35.5	35.3			
19	21.6	21.6	19	22.8	22.7			
20	24.9	24.9	20	25.5	25.2			
1'	50.9	49.1	1'	36.1	39.2			
2'	86.9	23.0	2'	19.0	19.0			
3'	38.1	31.1	3'	41.1	36.1			
4'	43.4	135.1	4'	32.9	32.9			
5'	39.6	122.4	5'	51.0	50.9			
6'	78.8	39.8	6'	127.6	127.5			
7'	48.6	46.5	7'	127.2	127.2			
8'	20.3	21.8	8'	125.8	125.9			
9'	19.8	37.8	9'	147.1	146.6			
10'	12.3	82.1	10'	38.3	38.0			
11'		26.0	11'	107.1	106.1			
12'		15.1	12'	153.4	152.8			
13'		21.5	13'	134.9	135.0			
14'		19.5	14'	124.7	124.8			
15'		23.9	15'	25.8	25.4			

the C-6 position of 7 $\alpha$ -hydroxyferruginol and the C-12' position of 6,7-dehydroferruginol were connected by an ether linkage.

The IR spectrum of calocedimer D (**4**) revealed the absorptions of phenolic acetate and alcoholic acetate (1751 and 1742 cm<sup>-1</sup>) groups and aromatic (3051, 1601, 1495 cm<sup>-1</sup>) groups. The molecular formula of C<sub>44</sub>H<sub>60</sub>O<sub>5</sub> was established by HREIMS, suggesting the presence of 15 degrees of unsaturation. The <sup>1</sup>H NMR spectrum (Table 1) showed signals for six methyl singlets, two isopropyl groups attached to an aromatic ring, and two acetoxy groups. Resonances were also observed for protons on oxygenated carbons at  $\delta$  5.03 (H-6) and 5.99 (H-7), an AMX pattern at  $\delta$  5.87 (H-6'), 6.48 (H-7'), and 2.11 (H-5'), and signals of four aromatic protons at  $\delta$  6.88, 7.22, 6.73, 6.91 (H-11, H-14, H-11', H-14'). The signals at  $\delta$  2.04 (br d) and 2.16 (br d) are characteristic for H $\beta$ -1 signals of dehydroabietanes. <sup>1</sup>H NMR data of compound **4** were similar to those of **3**, the only major difference being two additional acetyl groups located on C-7-OH and C-12-OH of **3**. To prove this, acetylation of **3** with Ac<sub>2</sub>O in pyridine gave a product that was identical with compound **4**. Thus, the structure of calocedimer D (**4**) was established as 7 $\alpha$ ,12-diacetoxyabieta-8,11,13-trien-6 $\alpha$ -yl 6,7-dehydroabieta-8,11,13-trien-12-yl ether.

### Experimental Section

**General Experimental Procedures.** Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer 983 G spectrophotometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were performed on a Bruker Avance 500 NMR. EIMS, HREIMS, and specific rotations were taken on JEOL JMS-HX 300 and JEOL SX-102 mass spectrometers and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merk 70–230 mesh, 230–400 mesh, ASTM) and purified with a semipreparative normal-phase HPLC column (250  $\times$  10 mm, 7  $\mu$ m, LiChrosorb Si 60) on an LDC Analytical-III.

**Plant Materials.** The bark of *C. macrolepis* was collected in Nan-Tou, Taiwan (1998). The plant was identified by Dr. Shang-Tzen Chang, a Professor in the Department of Forestry. A voucher specimen (voucher no. 223133) has been deposited in the Herbarium of the Department of Botany of the National Taiwan University, Taipei, Taiwan.

**Extraction and Isolation.** The dried bark of *C. macrolepis* (16 kg) was extracted with Me<sub>2</sub>CO (140 L) at room temperature (7 days  $\times$  2). After removal of Me<sub>2</sub>CO, H<sub>2</sub>O was added to bring the total volume to 1 L. This suspended phase was extracted with EtOAc (1 L  $\times$  3). Evaporation of the combined EtOAc layers afforded a black syrup (734 g), which was purified by means of silica gel chromatography and repeated HPLC (normal phase on Lichrosorb Si 60), using a hexane–EtOAc gradient solvent system. Compounds **2** (3.5 mg), **5** (15.0 mg), **8** (10.5 mg), **6** (6.8 mg), **4** (8.4 mg), **5** (3.6 mg), **1** (5.6 mg), and **3** (6.0 mg) were eluted with 5%, 5%, 5%, 10%, 10%, 10%, 30%, and 30% EtOAc in hexane solvent systems, respectively.

**Abieta-8,11,13-trien-12-yl 6 $\alpha$ -hydroxycamphan-2 $\alpha$ -yl ether (1):** gum; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +21.8 (c 0.2, MeOH); IR (KBr)  $\nu_{\max}$  3543, 3051, 1613, 1573, 1497 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 221 (3.95), 277 (3.47), 287 (3.37) nm; <sup>1</sup>H and <sup>13</sup>C NMR, Tables 1 and 2; EIMS  $m/z$  438 (M<sup>+</sup>,

11), 368 (5), 286 (85), 271 (39), 256 (69), 162 (25), 145 (67), 118 (20), 105 (14), 91 (18), 77 (32); HREIMS  $m/z$  438.3491 (calcd for 438.3500).

**Abieta-8,11,13-trien-12-yl  $\alpha$ -cadina-10-yl ether (2):** gum; [ $\alpha$ ]<sub>D</sub><sup>25</sup> -2.7 (c 0.3, MeOH); IR (KBr)  $\nu_{\max}$  3061, 1673, 1611, 1494, 822 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 217 (3.60), 225 (3.60), 281 (3.08) nm; <sup>1</sup>H and <sup>13</sup>C NMR, Tables 1 and 2; EIMS  $m/z$  490 (M<sup>+</sup>, 1), 286 (100), 204 (6), 189 (10), 161 (12), 149 (5), 121 (7), 105 (7), 91 (4), 81 (6), 69 (7); HREIMS  $m/z$  490.4199 (calcd for 490.4177).

**6,7-Dehydroabieta-8,11,13-trien-12-yl 7 $\alpha$ ,12-dihydroxyabieta-8,11,13-trien-6 $\alpha$ -yl ether (3):** gum; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +54.6 (c 0.2, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3365, 3051, 1614, 1496 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 221 (3.24), 281 (2.94), 304 (2.58) nm; <sup>1</sup>H and <sup>13</sup>C NMR, Tables 1 and 2; EIMS  $m/z$  584 (M<sup>+</sup>, 1), 566 (100), 550 (16), 495 (10), 430 (30), 328 (63), 314 (60); HREIMS  $m/z$  584.4231 (calcd for 584.4232).

**7 $\alpha$ ,12-Diacetoxyabieta-8,11,13-trien-6 $\alpha$ -yl 6,7-dehydroabieta-8,11,13-trien-12-yl ether (4):** oil; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +60.8 (c 0.3, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3051, 1751, 1742, 1601, 1495 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 221 (3.14), 281 (3.01), 304 (2.60) nm; <sup>1</sup>H and <sup>13</sup>C NMR, Tables 1 and 2; FABMS (NBA) 668 (M<sup>+</sup>, 40), 608 (18), 284 (92), 213 (100), 199 (50), 83 (77), 55 (54); HREIMS  $m/z$  668.4434 (calcd for 668.4443).

**Acetylation of 3 with Ac<sub>2</sub>O–Pyridine.** Compound **3** (3 mg) was reacted with Ac<sub>2</sub>O (0.5 mL) and pyridine (0.5 mL) at room temperature overnight. Usual workup gave compound **4** (3 mg).

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