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TWO PHTHALIDES FROM *LIGUSTICUM CHUANGXIONG*

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Key Word Index—*Ligusticum chuangxiong*; Umbelliferae; rhizomes; phthalides; (*E*)-senkyunolide E; senkyunolides N, and H.

Abstract—Two novel phthalides, (*E*)-senkyunolide E and senkyunolide N, along with the 18 phthalides including senkyunolide H and senkyunolide J, were isolated from the rhizomes of *Ligusticum chuangxiong*. On the basis of spectral analyses and chemical methods the structures of (*E*)-senkyunolide E and senkyunolide N were proved to be (*E*)-(9*RS*)-3-butylidene-9-hydroxyphthalide and (3*S*,6*S*,7*S*)-3-butyl-4,5-dihydro-6,7-dihydroxyphthalide, respectively. The absolute configuration of senkyunolide J was also established by spectral analyses and chemical methods; the conformational structure of senkyunolide H was studied by X-ray diffraction analysis.

INTRODUCTION

The dried rhizome of *Ligusticum chuangxiong* (Senkyu in Japanese and Chuang-Xiong in Chinese) is one of the most important crude drugs in traditional Chinese medicine and it has been used to treat headache, anaemia, feeling of cold and irregularity of menstruation. Various phthalide derivatives have been previously isolated from this species by many workers [1–6]. We describe the structures of two novel phthalides named (*E*)-senkyunolide E and senkyunolide N, the stereochemistry of senkyunolide J [7], and the conformational structure of senkyunolide H [5, 7] in a crystal state.

RESULTS AND DISCUSSION

A methanolic extract of the rhizomes of *L. chuangxiong* gave (*E*)-senkyunolide E (**1**), senkyunolide H (**2**), senkyunolide N (**3**), and senkyunolide J (**4**) along with known phthalides, senkyunolides B (**8**), C (**9**), D (**10**), E (**5**), F (**11**), G (**12**), I (**6**), L (**13**), [5, 6, 7], (*Z*)-3-butylidene-7-hydroxyphthalide (**14**) [5], (3*S*)-3-butyl-4-hydroxyphthalide (**15**) [1, 8], ligustilide (**16**) [9], butylidenephthalide (**17**) [10], senkyunolide (**7**) [11], butylphthalide (**18**) [12], cnidilide (**19**), and neocnidilide (**20**) [13].

(*E*)-Senkyunolide E (**1**), $C_{12}H_{12}O_3$ was isolated as an oil. Its UV, IR and mass spectra were very similar to

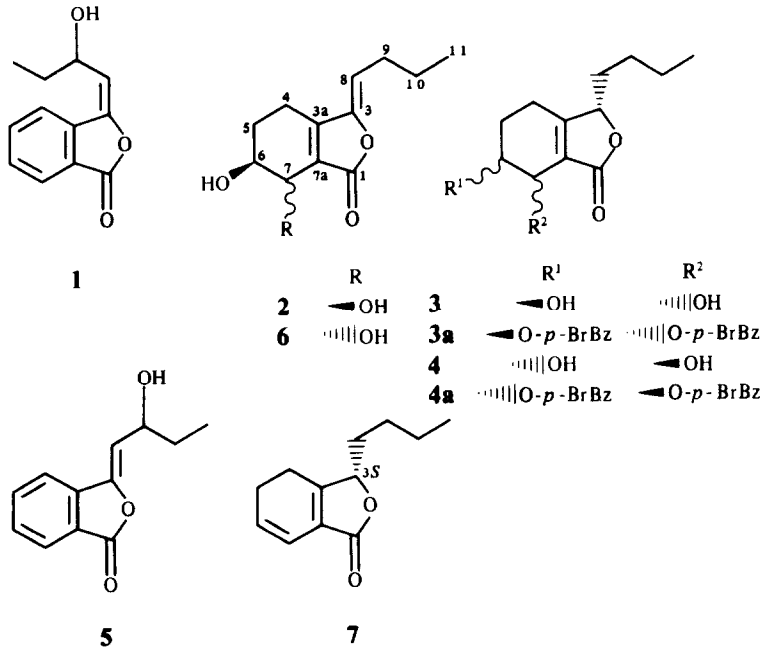

Table 1. ^{1}H NMR spectral data for compounds 1–7 (500 MHz, $CDCl_3$, TMS as int. standard)

H	1	2	3	4	5	6	7 (400 MHz)
3	—	—	4.86 *ddd* (8.1, 3.7, 2.5)	4.86 *dd* (8.0, 3.7)	—	—	4.91*dd* (7.8, 3.7)
4	7.92 *dt* (7.6, 1.0)	2.36–2.42 (*m*) 2.62–2.69 (*m*)	2.35–2.40 (*m*)	2.31–2.38 (*m*) 2.40–2.46 (*m*)	7.67 *dt* (7.8, 1.0)	2.45–2.52 (*m*) 2.53–2.60 (*m*)	2.44–2.48 (*m*)
5	7.73 *dt* (1.0, 7.6)	1.79–1.85 (*m*) 2.10–2.17 (*m*)	1.81–1.92 (*m*) 2.07–2.12 (*m*)	1.83–1.91 (*m*) 2.06–2.11 (*m*)	7.71 *dt* (1.0, 7.8)	1.85–1.93 (*m*) 2.07–2.12 (*m*)	2.44–2.48 (*m*)
6	7.58 *dt* (1.0, 7.6)	4.06 *ddd* (7.9, 3.9, 2.5)	3.91 *ddd* (9.8, 6.1, 3.3)	3.95 *ddd* (9.1, 5.6, 3.0)	7.56 *dt* (1.0, 7.8)	3.96 *ddd* (9.2, 5.8, 3.3)	5.90 *dt* (9.8, 4.4)
7	7.92 *dt* (7.6, 1.0)	4.61 *d* (3.9)	4.41 *dddd* (6.1, 2.5, 2.1, 2.0)	4.40 *ddd* (5.6, 3.2, 1.6)	7.91 *dt* (7.8, 1.0)	4.49 *d* (5.8)	6.18 *dt* (9.8, 2.0)
8	5.85 *d* (8.9)	5.31 *t* (7.9)	1.50–1.57 (*m*) 1.81–1.92 (*m*)	1.49–1.56 (*m*) 1.83–1.91 (*m*)	5.65 *d* (8.5)	5.28 *t* (7.9)	1.48–1.57 (*m*) 1.83–1.90 (*m*)
9	4.89 *dt* (8.9, 6.5)	2.36 *dt* (7.9, 7.4)	1.30–1.45 (*m*)	1.29–1.42 (*m*)	4.86 *dt* (8.5, 6.9)	2.35 *dt* (7.9, 7.4)	1,31–1.44 (*m*)
10	1.70–1.78 (*m*) 1.80–1.89 (*m*)	1.50 *tq* (7.4, 7.4)	1.30–1.45 (*m*)	1.29–1.42 (*m*)	1.65–1.74 (*m*) 1.75–1.82 (*m*)	1.49 *tq* (7.4, 7.4)	1.31–1.44 (*m*)
11	1.03 *t* (7.5)	0.95 *t* (7.4)	0.91 *t* (7.2)	0.91 *t* (7.2)	1.00 *t* (7.5)	0.95 *t* (7.4)	0.89 *t* (7.3)

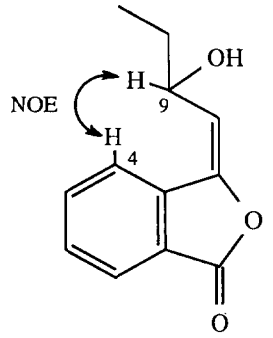

Fig 1. NOE in the NOED spectrum of compound 1.

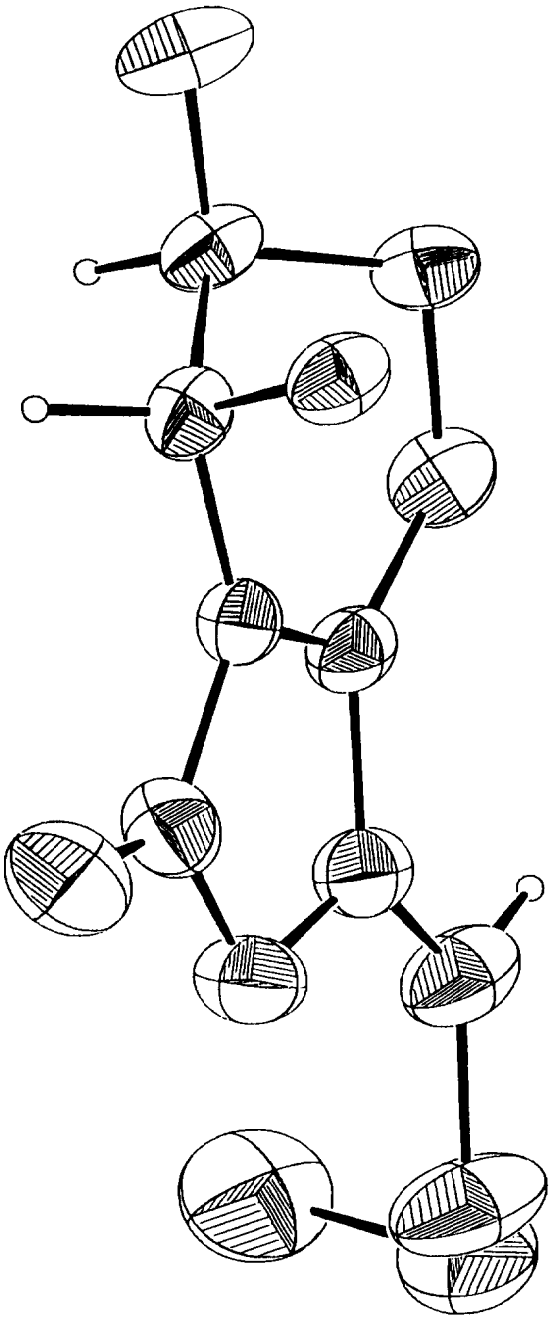

Fig. 2. ORTEP drawing of compound 2.

those of senkyunolide E (**5**) [7] and also its ^1H and ^{13}C NMR spectra were similar to those of **5**, except for the signals at C-4 and C-8 (Tables 1 and 2). From these data, the structure of **1** was presumed to be a geometrical isomer of senkyunolide E (**5**) which has the 3,8-*cis* configuration. In the NOE difference spectrum, the NOE for H-9 was observed, but not for H-8 upon irradiation at H-4. This indicated that **1** has the 3,8-*trans* configuration (Fig. 1). Thus, the structure of **1** was elucidated as (*E*)-(9*RS*)-3-butylidene-9-hydroxyphthalide.

Senkyunolide H (**2**), $C_{12}H_{16}O_4$, was obtained as prisms, its stereochemistry has been reported by Kobayashi *et al.* [7] and Wang *et al.* [5]. Because **2** was isolated in crystal line form for the first time, X-ray diffraction analysis was carried out. This analysis showed that **2** adopts a conformation in which two hydroxyl groups in the cyclohexene ring have axial (C-7) and equatorial (C-6) orientation, respectively, in the crystal state (Fig. 2).

Senkyunolides N (**3**) and J (**4**) were isolated as oils having the same molecular formula, $C_{12}H_{18}O_4$. The UV, IR, ^1H and ^{13}CNMR spectra of **3** and **4** were very similar. From this data, **3** and **4** were presumed to be stereoisomers of each other. The ^1H and ^{13}CNMR spectra of **3** and **4** were also similar to those of senkyunolide (**7**) [11] except for the signals due to the glycol groups at C-6 and C-7 (Tables 1 and 2). This suggested that **3**, **4** and **7** possess a close structural relationship. Oxidation of **7** with *m*-chloroperbenzoic acid followed by hydrolysis gave **3** and **4**, indicating that the configurations at C-3 in **3** and **4** are the same *S* as **7**. The *J* value between H-6 and H-7 in senkyunolide I (**6**) having a 6,7-*trans* glycol was 5.8 Hz, while the $J_{6,7}$ value in senkyunolide H (**2**) having a 6,7-*cis* glycol was 3.9 Hz. The *J* values of the vicinal coupling between H-6 and H-7 was 6.1 Hz in **3** and 5.6 Hz in **4**. These values indicated that the configurations of the two hydroxyl groups in **3** and **4** are *trans*. The absolute configurations at C-6 and C-7 in **3** and **4** were determined by CD. The CD spectrum of the *p*-bromobenzoate of **3** (**3a**) showed a positive Cotton effect at 251 nm, indicating that **3a** has the 6*S* and 7*S* configuration [14]. On the other hand, the *p*-bromobenzoate of **4** (**4a**) showed a negative Cotton effect at 251 nm, indicating that **4a** has the 6*R* and 7*R* configuration. Thus, the structures of senkyunolides N (**3**) and J (**4**) were elucidated as (3*S*,6*S*,7*S*)-3-butyl-4,5-dihydro-6,7-dihydroxyphthalide (**3**) and (3*S*,6*R*,7*R*)-3-butyl-4,5-dihydro-6,7-dihydroxyphthalide (**4**), respectively.

EXPERIMENTAL

Mps: uncorr. $[\alpha]_D$: $CHCl_3$. UV and CD: EtOH. ^1H NMR: 400 and 500 MHz, $CDCl_3$, TMS as int. standard. ^{13}C NMR: 100 and 125 MHz. FAB-MS: *m*-nitrobenzylalcohol matrix. CC: Celite 545 and Merck Kieselgel 60 (70–230 and 230–400 mesh). HPLC: YMC D-ODS-10 column (Yamamura) and C.I.G. column system (silica gel, Kusano).

Plant material. Rhizomes of *L. chuangxiong* Hort. produced in China were bought from Shibata Co., Ltd.

Extraction and isolation. Dried rhizomes (55.8 kg) were extracted with MeOH at 62° (234 l × 2). The MeOH extract was partitioned with $CHCl_3$–MeOH–H_2O (3:2:1, 84 l), and then the lower layer (3.33 kg) partitioned with *n*-hexane–MeOH–H_2O (10:5:1, 16 l). The lower layer (1.35 kg) was mixed with Celite (6 kg) followed by loading onto a column and successively eluted with *n*-hexane (48 l), C_6H_6 (45 l) and MeOH (28 l). Eluates were concd to give *n*-hexane (905 g), C_6H_6 (414 g) and MeOH (128 g) frs. A portion of the C_6H_6 eluate (300.6 g) was chromatographed on silica gel (11.5 cm i.d. × 36 cm, 1.68 kg) using $CHCl_3$–MeOH to give 16 frs. Frs 3 and 4 were repeatedly chromatographed on silica gel with various solvent systems [*n*-hexane–EtOAc (3:1), *n*-hexane–Me_2CO (6:1), *n*-hexane–Et_2O (3:2), $CHCl_3$–*n*-hexane–Et_2O (30:10:1), C_6H_6–EtOAc (10:1), C_6H_6–Me_2CO (30:1), $CHCl_3$, etc.] to give **8** (0.11 g), **9** (0.24 g), **10** (0.32 g), **11** (0.83 g), **12** (0.54 g), **13** (0.27 g), **15** (0.31 g), and mixt of **1** and **5**. Purification of **1** and **5** was achieved by HPLC (H_2O–MeOH, 1:1, 5 ml min^{-1}) to afford **1** (17 mg) and **5** (0.12 g). Fr. 7 was repeatedly chromatographed on silica gel using $CHCl_3$–MeOH (20:1) and $CHCl_3$–Me_2CO (30:1) to afford **2** (0.99 g). Fr. 10 was repeatedly chromatographed on silica gel with various solvent systems ($CHCl_3$–Et_2O, 1:1, $CHCl_3$–MeOH, 1:1, *n*-hexane–Me_2CO, 3:1, etc.) to give **6** (1.06 g) and a mixt of **3** and **4**. Purification of **3** and **4** was achieved by HPLC (H_2O–THF, 9:1, 5 ml min^{-1}) to afford **3** (0.35 g) and **4** (0.37 g). A portion of the *n*-hexane eluate (99.7 g) was repeatedly chromatographed on silica gel using various solvent systems (*n*-hexane–Me_2CO, 50:1, *n*-hexane–EtOAc, 40:1, 10:1, C_6H_6, C_6H_6–EtOAc 100:1) to give **7** (1.84 g), **14** (0.12 g), **16** (0.74 g), **17** (0.37 g), **18** (0.50 g), **19** (73 mg) and **20** (0.41 g).

(E)-*Senkyunolide E*, (E)-(9RS)-3-*butylidene*-9-*hydroxyphthalide* (**1**). Oil. $[\alpha]_D$ 0° ($CHCl_3$; *c* 1.54). UV λ_{max}^{EtOH} nm (log ε): 211 (4.19), 216 (4.16), 239 (4.08), 261 (4.14), 270 (4.03), 307 (3.67). IR ν_{max}^{neat} cm^{-1}: 3420, 1782, 1674, 1612, 1474, 1462, 1282, 1076, 1004, 766. ^1H and ^{13}C NMR: see Tables 1 and 2. MS *m/z* (rel. int.): 186 ($[M-18]^+$, 47), 175 (6), 158 (30), 129 (46), 115 (51), 104 (43), 76 (53), 43 (100); HRFAB-MS *m/z*: 205.0850 ($[M+H]^+$) (calc. for $C_{12}H_{13}O_3$: 205.0850).

Table 2. ^{13}C NMR spectral data for compounds **1**–**7** (125 MHz, $CDCl_3$, TMS as int. standard)

C	1	2	3	4	5	6	7 (100 MHz)
1	166.7	169.4	173.0	172.9	166.6	169.1	171.2
3	147.6	153.4	82.9	82.8	145.8	152.9	82.5
3a	137.5	148.2	166.7	166.5	139.3	148.1	161.4
4	124.0	18.5	21.3	21.1	120.2	19.1	20.8
5	134.6	25.6	26.5	26.3	134.5	26.6	22.4
6	125.7	67.5	71.5	71.3	125.5	71.8	128.3
7	130.4	63.3	67.4	67.4	130.2	67.9	116.9
7a	126.4	125.5	126.3	126.1	124.6	126.0	124.5
8	115.4	114.5	31.9	31.8	110.6	114.3	31.9
9	68.1	28.1	26.8	26.6	68.2	28.1	26.8
10	31.1	22.3	22.4	22.4	30.3	22.3	22.3
11	9.7	13.8	13.8	13.8	9.6	13.8	13.9

Senkyunolide H, (E)-(6RS,7SR)-3-*butylidene*-4,5-*dihydro* 6,7-*dihydroxyphthalide* (**2**). Prisms (from Et_2O), mp 92–93°. $[\alpha]_D$ 0° ($CHCl_3$; *c* 1.07). UV λ_{max}^{EtOH} nm (log ε): 274 (4.29). IR ν_{max}^{KBr} cm^{-1}: 3360, 1768, 1680, 1640, 998, 796. ^1H NMR and ^{13}C NMR: see Tables 1 and 2. MS *m/z* (rel. int.): 224 ($[M]^+$, 33), 206 (4), 180 (100), 165 (20), 151 (49), 138 (16), 123 (21), 95 (36), 55 (63).

X-Ray crystallographic analysis of **2**. Crystal: 0.5 × 0.3 × 0.1 mm, monoclinic, space group $P2_1/c$, a = 19.147 (2) Å, b = 12.666 (1) Å, c = 9.6317 (7) Å, β = 95.605 (5) Å, V = 2325 (2) $Å^3$, Z = 8, D_{calc} = 1.27 g cm^{-3} and μ (CuK_α) = 7.5 cm^{-1}. Reflections were measured on an Enraf-Nonius CAD-4 diffractometer, with ω/2θ scan mode and using graphite monochromated CuK_α radiation (λ = 1.54184 Å). Cell constants were determined by least-squares refinement using 25 centred reflections in the range 20° < θ < 28°. Intensities were measured for 4126 independent reflections in the range 2θ ≦ 140°, of which 3558 reflections were considered as observed [$I > 3\sigma(I)$]. The data were corrected for Lorentz and polarization effects. No absorption correction was made. The structure was solved by the direct-methods program Multan [14] and refined by full-matrix least-squares, using the Enraf-Nonius SDP program [15]. Hydrogen atoms were located on a difference Fourier synthesis map. The last difference Fourier map was essentially featureless with no peaks greater than 0.658 $eÅ^{-3}$. The final discrepancy index was $R = 0.078$.

Senkyunolide N, (3S,6S,7S)-3-*butyl*-4,5-*dihydro*-6,7-*dihydroxyphthalide* (**3**). Oil. $[\alpha]_D$ −41.7° ($CHCl_3$; *c* 1.01). UV λ_{max}^{EtOH} nm (log ε): 214 (4.0). IR ν_{max}^{neat} cm^{-1}: 3424, 1744, 1674, 1040. ^1H NMR and ^{13}C NMR: see Tables 1 and 2. MS *m/z* (rel. int.): 226 ($[M]^+$, 1), 208 (1), 182 (71), 139 (48), 126 (100); HRMS *m/z*: 226. 1200 (M^+) (calc. for $C_{12}H_{18}O_4$: 226.1205).

Senkyunolide J, (3S,6R,7R)-3-*butyl*-4,5-*dihydro*-6,7-*dihydroxyphthalide* (**4**). Oil. $[\alpha]_D$ +9.8° ($CHCl_3$; *c* 1.04). UV λ_{max}^{EtOH} nm (log ε): 214 (4.05). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3448, 1744, 1678, 1042. ^1H and ^{13}C NMR: see Tables 1 and 2. MS *m/z* (rel. int.): 226 ($[M]^+$, 1), 208 (1), 182 (72), 139 (50), 126 (100); HRMS *m/z*: 226.1205 (M^+) (calc. for $C_{12}H_{18}O_4$: 226.1205).

Glycolation of senkyunolide (**7**). To a solution of **7** (111.5 mg) in CH_2Cl_2 (2.5 ml) *m*-chloroperbezoic acid (219.3 mg) was added and the mixt. stirred at room temp. for 1 hr. 10%aq. $Na_2S_2O_3$ soln (5 ml) was then added and the reaction mixt. extd with EtOAc, washed with aq. $NaHCO_3$ soln and H_2O, followed by concn of the extract and soln in 1.5 ml of dioxane–H_2O (2:1). To this soln 0.15 ml of aq. H_2SO_4 (3M) was added and the mixt. stirred at 0° for 30 min; H_2O and aq. $NaHCO_3$ soln were then added. The reaction mixt. was extracted with EtOAc, washed with satd NaCl soln and the extracted concd. The residue was purified by HPLC (H_2O–THF, 9:1, 5 ml min^{-1}) to give **3'** (28 mg, yield 22.9%) and **4'** (38 mg, 31.1%). Compounds **3'** and **4'** were identified as senkyunolide N (**3**) and J (**4**), respectively, by direct comparison with authentic samples ($[\alpha]_D$, UV, IR, ^1H NMR and MS).

p-*Bromobenzoylation of* **3**. To a soln of **3** (24.3 mg) in pyridine (5 ml) *p*-bromobenzoyl chloride (69.8 mg) and 4-dimethylaminopyridine (4.7 mg) as catalyst was added, and the mixt. stirred at room temp. for 1 day. The reaction mixt. was worked-up as usual and purified by silica gel chromatography to afford **3a** (45 mg, yield 70.8%). Compound **3a**; amorphous powder. $[\alpha]_D$ +167.9° ($CHCl_3$; *c* 1.01). CD (EtOH; *c* 0.0013) $[\theta]^{30}$ (nm): +88 440 (251). UV λ_{max}^{EtOH} nm (log ε): 203 (4.80), 247 (4.67), 282 (3.23). IR ν_{max}^{KBr} cm^{-1}: 1762, 1728, 1682, 1592, 1486, 1268, 1098, 1012, 846. ^1H NMR ($CDCl_3$) δ: 0.95 (3H, *t*, *J* = 7.2 Hz, Me-11), 4.97 (1H, *ddd*, *J* = 8.2, 3.2, 2.4 Hz, H-3), 5.47 (1H, *ddd*, *J* = 7.2, 4.9, 3.2 Hz, H-6), 6.10 (1H, *dddd*, *J* = 4.9, 2.4, 2.0, 1.4 Hz, H-7), 7.55 (2H, *dt*, *J* = 8.9, 2.2 Hz), 7.57 (2H, *dt*, *J* = 8.9, 2.2 Hz), 7.83 (2H, *dt*, *J* = 8.9, 2.2 Hz), 7.85 (2H, *dt*, *J* = 8.9, 2.2 Hz). FD-MS *m/z* (rel. int.): 595 ($[M+H]^+$, 78), 593 ($[M+H]^+$, 100), 591 ($[M+H]^+$, 80), 393 (58), 392 (76), 390 (67).

p-*Bromobenzoylation of* **4**. Compound **4** (25.8 mg) was treated in exactly the same manner as **3** and recrystallization from a mixt. of *n*-hexane–EtOAc gave **4a** (37.3 mg, yield 55.3%). **4a**; needles, mp 166–167°. $[\alpha]_D$ −153.7° ($CHCl_3$; *c* 1.01). CD (EtOH; *c* 0.0016) $[\theta]^{29}$ (nm): −114050 (251). UV λ_{max}^{EtOH} nm (log ε): 203 (4.79), 247 (4.67), 282 (3.25). IR ν_{max}^{KBr} cm^{-1}: 1756, 1728, 1682, 1590, 1484, 1264, 1096, 1012, 848. ^1H NMR ($CDCl_3$) δ: 0.91 (3H, *t*, *J* = 7.1 Hz, Me-11), 5.03 (1H, *dd*, *J* = 6.9, 3.8 Hz, H-3), 5.51 (1H, *ddd*, *J* = 6.1, 3.8, 2.4 Hz, H-6), 6.03 (1H, *br d*, *J* = 3.8 Hz, H-7), 7.56 (2H, *dt*, *J* = 8.9, 2.1 Hz), 7.57 (2H, *dt*, *J* = 8.9, 2.1 Hz), 7.81 (2H, *dt*, *J* = 8.9, 2.1 Hz), 7.87 (2H, *dt*, *J* = 8.9, 2.1 Hz). FDMS *m/z* (rel. int.): 595 ($[M+H]^+$, 74), 593 ($[M+H]^+$, 100), 591 ($[M+H]^+$, 69), 394 (25), 392 (61), 390 (49).

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