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TWO PHTHALIDES FROM *LIGUSTICUM CHUANXIONG*

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Key Word Index—*Ligusticum chuanxiong*; Tousenkyu; Chuan-Xiong; Umbelliferae; phthalides; senkyunolide R; senkyunolide S.

Abstract—Two new phthalides, senkyunolides R and S, were isolated from the rhizome of *Ligusticum chuanxiong*. On the basis of spectral analyses and chemical methods, the structures of senkyunolide R and senkyunolide S were proved to be (6*RS*,7*RS*,9*SR*)-3-butylidene-4,5,6,7-tetrahydro-6,7,9-trihydroxyphthalide and (6*RS*,7*RS*,9*RS*)-3-butylidene-4,5,6,7-tetrahydro-6,7,9-trihydroxyphthalide, respectively.

INTRODUCTION

The dried rhizome of *Ligusticum chuanxiong* HORT. (Tousenkyu in Japanese and Chuan-Xiong in Chinese) is one of the most important crude drugs in traditional Chinese medicines and has been used to treat headaches, anaemia, feeling of coldness, and irregular menstrual cycles. The plant is mainly cultured in Sichuanprovince, China.

In the preceding papers, we have reported on the constituents from *L. chuanxiong* [1–3]. In a further study of this plant, we have now isolated two new phthalides which we have named senkyunolide R (**1**) and senkyunolide S (**2**) along with thymidine (**3**), uridine (**4**), adenosine (**5**) [4] and uracil (**6**) [5]. Compounds **3** and **4** were isolated from this plant for the first time. In this paper, we describe the structural determination of compounds **1** and **2**.

RESULTS AND DISCUSSION

Compounds **1** and **2** were isolated as viscous oils having the same molecular formula, $C_{12}H_{16}O_5$. Because the UV, IR, ^{1}H NMR and ^{13}C NMR spectra of **1** and **2** were very similar, they were presumed to be stereoisomers of each other. The ^{1}H NMR and ^{13}C NMR spectra of **1** and **2** were also similar to those of senkyunolide F (**7**), except for the signals neighbouring C-6 and C-7, and senkyunolide I (**8**), except for the signals neighbouring C-9 [2] (Table 1). Oxidation of **7** with *m*-chloroperbenzoic acid followed by hydrolysis [2] gave **1** and **2**. This suggested that **1** and **2** had a 6,7-*trans*-glycol moiety and a hydroxyl group at C-9, respectively, and that they only differed from each other in the configuration at C-6 and C-7. On the basis of X-ray diffraction analysis of the *p*-nitrobenzoate derivative of **1** (**1a**, Fig. 1), the structure of senkyunolide R was established as (6*RS*,7*RS*,9*SR*)-3-

Table 1. ^{1}H and ^{13}C NMR data for compounds **1**, **2**, **7** and **8** (CD_3OD, TMS as int. standard)

H	1	2	7*	8	C	1	2	7*	8
4	2.47–2.63 (*m*)	2.46–2.63 (*m*)	2.59–2.63 (*m*)	2.46–2.63 (*m*)	1	170.25	170.22	167.0	170.9
5	1.89–2.03 (*m*)	1.90–2.03 (*m*)	2.46–2.52 (*m*)	1.88–2.02 (*m*)	3	155.51	155.53	147.1	155.3
6	3.96 (*ddd*)	3.96 (*ddd*)	6.06 (*dt*)	3.95 (*ddd*)	3a	149.19	149.16	148.2	150.0
	(5.4, 3.4, 2.4)	(5.4, 3.4, 2.4)	(9.7, 4.3)	(5.4, 3.4, 2.4)	4	18.21	18.22	18.5	18.2
7	4.25 (*d*) (3.4)	4.26 (*d*) (3.4)	6.28 (*dt*)	4.25 (*d*) (3.4)	5	25.08	25.06	22.5	25.1
			(9.7, 2.1)		6	71.02	71.01	131.1	71.1
8	5.35 (*d*) (8.8)	5.34 (*d*) (8.8)	5.22 (*d*) (8.4)	5.45 (*t*) (7.8)	7	65.44	65.36	116.9	65.5
9	4.61 (*dt*)	4.61 (*dt*)	4.74 (*dt*)	2.35 (*dt*)	7a	127.81	127.80	125.2	126.6
	(8.8, 6.4)	(8.8, 6.3)	(8.4, 6.5)	(7.8, 7.3)	8	115.70	115.68	113.6	114.4
10	1.52–1.63 (*m*)	1.52–1.62 (*m*)	1.59–1.68 (*m*)	1.53 (*tq*)	9	68.69	68.76	68.3	29.1
	1.64–1.75 (*m*)	1.64–1.74 (*m*)	1.68–1.77 (*m*)	(7.3, 7.3)	10	31.16	31.19	30.2	23.3
11	0.94 (*t*) (7.3)	0.94 (*t*) (7.3)	0.97 (*t*) (7.5)	0.96 (*t*) (7.3)	11	9.96	9.96	9.6	14.1

* Run at 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR) and measured with $CDCl_3$. Coupling constants in Hz.

†Deceased.

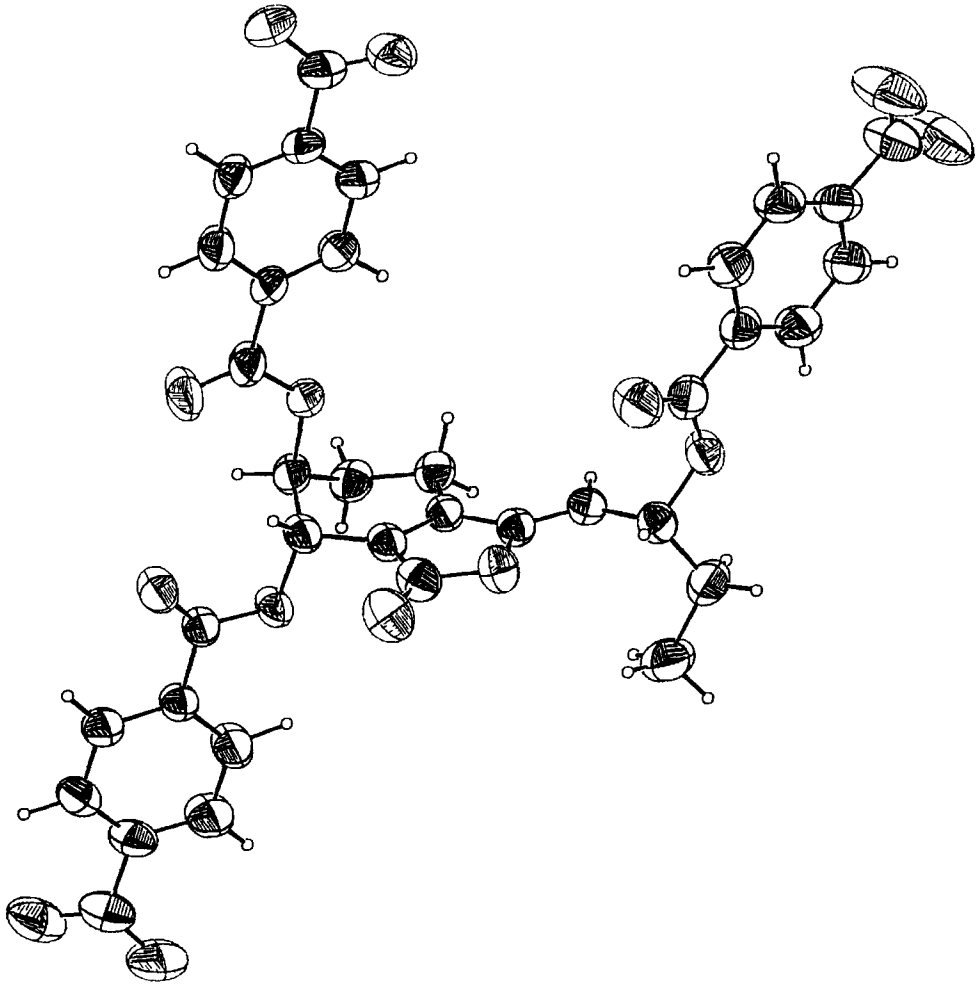


Fig. 1. ORTEP drawing of **1a**.

butylidene-4,5,6,7-tetrahydro-6,7,9-trihydroxyphthalide (**1**), and hence the structure of senkyunolide S was (6*RS*,7*RS*,9*RS*)-3-butylidene-4,5,6,7-tetrahydro-6,7,9-trihydroxyphthalide (**2**).

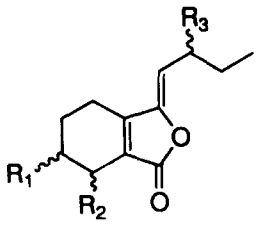
EXPERIMENTAL

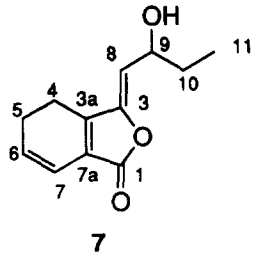
Mps: uncorr. $[\alpha]_D$: MeOH; UV: EtOH; ^{1}H NMR: 400 MHz, CD_3OD, TMS as int. standard; ^{13}C NMR: 100 MHz; FABMS: *m*-nitrobenzylalcohol matrix; CC: Merck Kieselgel 60 (230–400 mesh), Diaion HP-20 and MCI GEL CHP20P (75–150 μm); HPLC: YMC D-ODS-5 column (Yamamura Scientific) and C.I.G. column system (silica gel, Kusano Scientific). Compound **3** was bought from Tokyo Kasei Kogyo.

Plant material. Rhizomes of *L. chuanxiong* HORT. produced in China were bought from Shibata and were identified by one of the authors (M.O.).

Extraction and isolation. The dried rhizomes of *L. chuanxiong* (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), then the upper layer was partitioned with BuOH–H_2O (1:1, 72 l). The BuOH layer (373 g) was subjected to CC on Diaion HP-20 (2 l) and successively eluted with H_2O (18 l, fr. 1), 50% aq. MeOH (18 l, fr. 2) and MeOH (12 l, fr. 3). Fr. 1 (54.1 g) was subjected to CC on MCI GEL CHP20P (950 ml) using H_2O and MeOH to give 6 frs (fr. 1–1 to fr. 1–6). Fr. 1–2 (16.5 g) was repeatedly chromatographed on silica gel using various solvent systems [$CHCl_3$–MeOH (10:1), $CHCl_3$–MeOH–H_2O (100:30:3)] to give **4** (0.12 g) and **6** (12 mg). Fr. 1–6 (23.6 g) was purified by silica gel chromatography using various solvent systems [C_6H_6–Me_2CO (1:1), $CHCl_3$–MeOH (5:1), C_6H_6–Me_2CO (2:1)] to give **3** (0.15 g), **5** (0.26 g) and a mixt. of **1** and **2**. Purification of **1** and **2** was achieved by HPLC [H_2O–THF, 20:1, 3 ml min^{-1}] to afford **1** (108 mg) and **2** (69 mg). Compound **3** was identified as thymidine by direct comparison with an authentic sample (IR, MS, ^{1}H and ^{13}C NMR). Compound **4** was identified as uridine [6, 7].

Senkyunolide R, (6RS,7RS,9SR)-3-*butylidene*-4,5,6,7-*tetrahydro*-6,7,9-*trihydroxyphthalide* (**1**). Oil, $[\alpha]_D$ 0° (MeOH; *c* 0.97). UV λ_{max}^{EtOH} nm (log ε): 272 (4.15);





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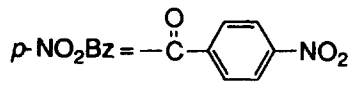
	R_1	R_2	R_3
1	—OH	····OH	····OH
1a	—O-*p*-NO_2Bz	····O-*p*-NO_2Bz	····O-*p*-NO_2Bz
2	····OH	—OH	····OH
8	—OH	····OH	H



IR ν_{max}^{neat} cm^{-1}: 3392, 1758, 1704, 1680, 1640, 1302, 1090, 1028, 966, 800; ^1H and ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 240 $[M]^+$ (3), 222 (10), 211 (100), 193 (81), 165 (60), 148 (61), 139 (62), 71 (74), 57 (87); HRMS *m/z*: 240.1003 $[M]^+$ (calc. for $C_{12}H_{16}O_5$: 240.0998).

Senkyunolide S, (6RS, 7RS, 9RS)-3-*butylidene*-4,5,6,7-*tetrahydro*-6,7,9-*trihydroxyphthalide* (**2**). Oil, $[\alpha]_D$ 0° (MeOH; *c* 1.09). UV λ_{max}^{EtOH} nm (log ε): 271 (4.09); IR ν_{max}^{neat} cm^{-1}: 3376, 1754, 1704, 1678, 1640, 1304, 1090, 1026, 970, 800; ^1H and ^{13}C NMR: Table 1; MS *m/z* (rel. int.): 240 $[M]^+$ (2), 222 (9), 211 (100), 193 (73), 165 (59), 148 (56), 139 (58), 71 (76), 57 (93); HRMS *m/z*: 240.0974 $[M]^+$ (calc. for $C_{12}H_{16}O_5$: 240.0998).

p-*Nitrobenzoylation of* **1**. To a soln of **1** (9.4 mg) in pyridine (0.5 ml), *p*-nitrobenzoyl chloride (36.7 mg) and 4-dimethylaminopyridine (4.3 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 **1a** (23.1 mg, yield 85.9%). Compound **1a** was crystallized from $CHCl_3$–MeOH to give needles. Compound **1a**: needles, mp 239.5–241°. IR ν_{max}^{KBr} cm^{-1}: 1776, 1726, 1646, 1608, 1526, 1348, 1264, 1100, 870, 830, 718; ^1H NMR ($CDCl_3$) δ: 1.09 (3H, *t*, *J* = 7.3 Hz, Me-11), 1.90–2.10 (2H, *m*, H-10), 2.33–2.37 (2H, *m*, H-5), 2.68–2.83 (2H, *m*, H-4), 5.44 (1H, *d*, *J* = 7.8 Hz, H-8), 5.57–5.61 (1H, *m*, H-6), 5.98 (1H, *dt*, *J* = 7.8, 6.4 Hz, H-9), 6.25 (1H, *d*, *J* = 4.9 Hz, H-7), 8.15–8.32 (12H, *m*); FABMS *m/z*: 688 $[M + H]^+$, 521 $[M - C_7H_4NO_4]^+$.

X-ray crystallographic analysis of **1a**. Crystal: 0.07 × 0.20 × 0.32 mm, triclinic, space group P-1, *a* = 15.318 (2) Å, *b* = 16.593 (1) Å, *c* = 7.102 (1) Å, α = 97.79 (1)°, β = 98.02 (1)°, γ = 111.21 (1)°, V = 1632.1 (4) $Å^3$, Z = 2, $D_{calc.}$ = 1.074 g cm^{-3} and μ (Cu K_α) = 5.5 cm^{-1}. Reflections were measured on an Enraf-Nonius CAD-4 diffractometer, with ω/2θ scan mode and using graphite monochromated Cu K_α radiation (λ = 1.54184 Å). Cell constants were determined by least-squares refinement using 25 centred reflections in the range 20° < θ < 30°. Intensities were measured for 6429 independent reflections in the range 2 < 2θ ≦ 140°, of which 4301 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 a direct-methods program [8] and refined by a full-matrix least-squares program [9]. H atoms were located on a difference Fourier synthesis map. The last difference Fourier map was essentially featureless with no peaks greater than 0.285 e$Å^{-3}$. The final discrepancy index was *R* = 0.096.

Glycolation of senkyunolide F (**7**). To a soln of **7** (72.2 mg) in CH_2Cl_2 (1.5 ml), *m*-chloroperbenzoic acid (190.8 mg) was added and the mixt. was stirred at room temp. for 30 min. 10% aq. $Na_2S_2O_3$ soln was then added and the reaction mixt. was extracted with EtOAc, washed with aq. $NaHCO_3$ soln and H_2O, followed by concn of the extract. The extract was dissolved in 1.6 ml dioxane–H_2O (5:2) and 0.25 ml aq. H_2SO_4 (3 M) was added. The mixt. was stirred at 0° for 1 hr, then H_2O and aq. $NaHCO_3$ soln were added. The reaction mixt. was charged on to a SEPABEADS SP207 (6.01 g, Mitsubishi) column and eluted with H_2O (350 ml) and MeOH (200 ml). The MeOH eluate was concd and the residue was purified by HPLC (H_2O–THF, 20:1, 4 ml min^{-1}) to give **1** (3.7 mg, yield 4.4%) and **2** (3.9 mg, 4.6%). Compounds **1** and **2** were identified as senkyunolide R and S, respectively, by direct comparison with authentic samples (HPLC, MS, ^1H and ^{13}C NMR).

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