PHTHALIDE DIMERS FROM LIGUSTICUM CHUANGXIONG Hort.

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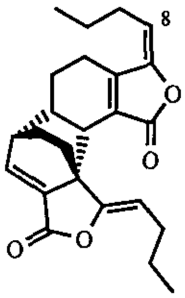
Abstract - Two new phthalide dimers, senkyunolide O (1) and senkyunolide P (2), along with three known phthalide dimers were isolated from the rhizome of Ligusticum chuangxiong Hort. On the basis of spectral analyses and chemical methods, the structures of these compounds were determined.

The dried rhizome of Ligusticum chuangxiong Hort.(Tousenkyu in Japanese and Chuang-Xiong in Chinese) is one of the most important crude drugs in traditional Chinese medicines and has been used to treat headache, anemia, feeling of cold, and irregularity of the menstruation.

In the preceding paper,[1] we reported on two new phthalides, (E)-senkyunolide E and senkyunolide N, along with eighteen phthalides from L. chuangxiong Hort. On further study of this plant, we have isolated two new phthalide dimers named senkyunolide O (1) and senkyunolide P (2), along with the known compounds levistolide A (3),[2~4] tokinolide B (4)[5] and riligustilide (5).[3,6] In this paper we describe the isolation and structural determination of these compounds from this plant.



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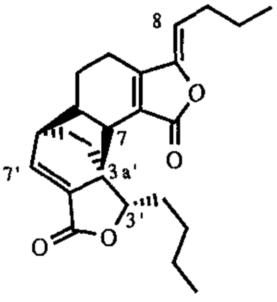

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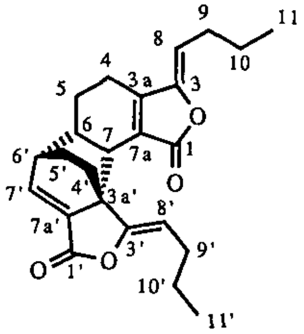

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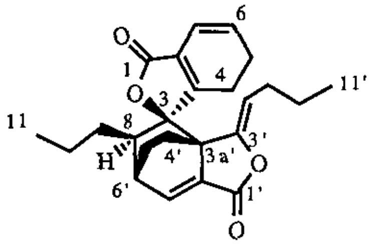

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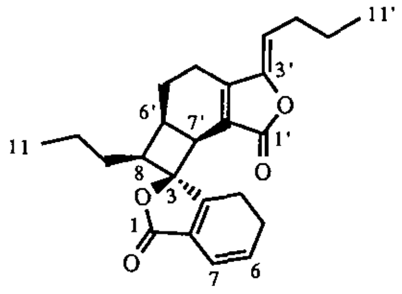

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Senkyunolide O (1), $C_{24}H_{28}O_4$, $[\alpha]_D$ 0° ($CHCl_3$), was isolated as colorless prisms, mp 173.5-174.5℃. Its uv, ir and ms spectra were very similar to those of the known compound levistolide A (3),[2~4] and also its ^{1}Hnmr and ^{13}Cnmr spectra were similar to those of 3 except for the proton and carbon signals neighboring 3,8-double bond (Tables 1 and 2). In particular, the signal assignable to H-8 (δ 5.59) shifted to the lower field in comparison with that (δ 5.07) of 3. Since the olefinic proton signal of butylidene group is known to appear at δ 5.4~5.7 if the double bond has an (E)-configuration,[5,7] the structure of 1 was presumed as the (3E)-isomer of levistolide A (3). Finally the structure and stereochemistry of senkyunolide O (1) were established as (3E, 3'Z)-(6RS, 7RS, 3a'RS, 6'RS)-6,6':7,3a'-biligustilide by X-ray diffraction analysis (Figure 1).

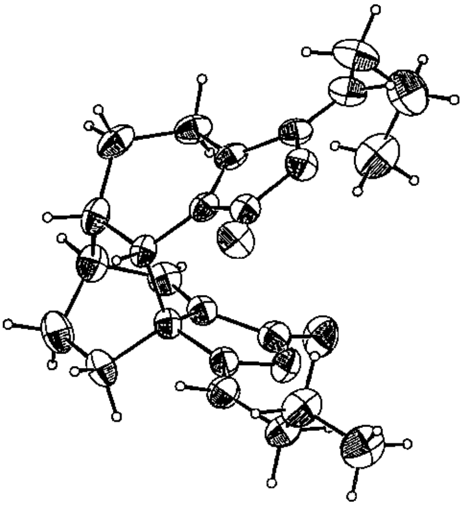
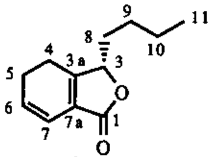


Figure 1. ORTEP drawing of 1

Senkyunolide P (2), $C_{24}H_{30}O_4$, $[\alpha]_D$ +206.2° ($CHCl_3$), was isolated as colorless oil. Its ms spectrum showed the molecular ion peak at m/z 382 and the other fragment ions of 2 were very similar to those of senkyunolide (6) and ligustilide (7).[1,8,9] The ^{1}Hnmr spectrum of 2 showed the signals of two olefinic protons[δ 5.17(1H, t, J=7.9 Hz), H-8 ; 7.30(1H, d, J=6.5 Hz), H-7'] and the signal at δ 5.17 was assigned to the olefinic proton of butylidene group, and its ^{13}Cnmr spectrum showed a quaternary sp^3 carbon[δ 48.33 (s), C-3a'] (Tables 1 and 2). From those data, the planar structure of 2 was presumed to be one of 2a~2d which were formed by Diels-Alder reaction between 6 and 7.



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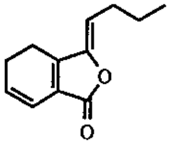

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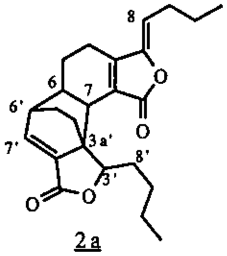
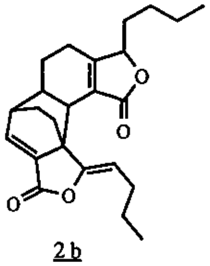


2a



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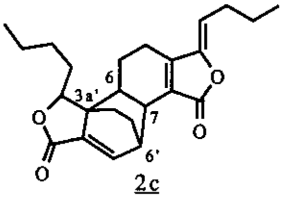

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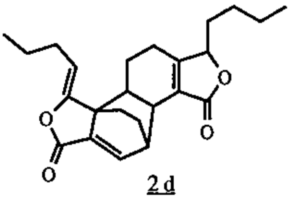

2d

In the ^{1}Hnmr spectrum of 2, the signal assignable to H-7 was observed as doublet[δ 3.02(1H, br d, J=8.8 Hz)](Table 2), and in its COLOC spectrum, the correlated peak was observed between the C-8 olefinic proton signal and the C-3a carbon signal(Figure 2). From these data, 2 was presumed as 3',8'-dihydro-6,6':7,3a'-biligustilide (2a).

In the NOESY spectrum of 2, the NOEs were observed between H-4α and H-8, H-4β and H-7', H-7 and H-3', H-7 and H-4'β, respectively(Figure 3). This indicated that relative configuration of 2 is (3Z)-(6RS, 7RS, 3'SR, 3a'SR, 6'RS). Retro-Diels-Alder reaction of 2 gave the mixture of senkyunolide (6) and ligustilide (7) in the ratio of 1.3 : 1.0. The $[\alpha]_D$ value($[\alpha]_D$ -65.0°) of the mixture closed to the half value of senkyunolide (6) ($[\alpha]_D$ -128.3°),[1,8] indicating that configuration at C-3' in 2 is the same S as 6. Thus, the structure and stereochemistry of senkyunolide P (2) were elucidated as (3Z)-(6R,7R,3'S,3a'S,6'R)-3',8'-dihydro-6,6':7,3a'-biligustilide.

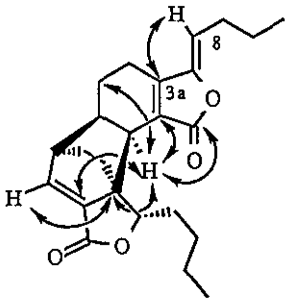

Figure 2. Correlated peaks in the COLOC spectrum of 2

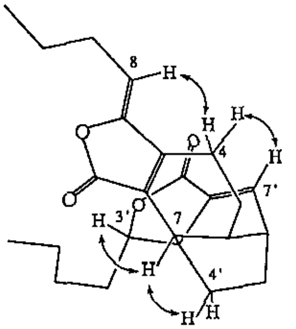

Figure 3. NOEs in the NOESY spectrum of 2

Tokinolide B (4), $C_{24}H_{28}O_4$, $[\alpha]_D$ 0° ($CHCl_3$), was isolated as colorless plates, mp 120.5-122℃. Tokinolide B (4) already has been isolated from Angelica acutiloba[5] and L. chuangxiong Hort.,[10] and its structure has been elucidated on the basis of its ^{1}Hnmr spectrum.[5] Because 4 could be isolated as crystal for the first time, the X-ray diffraction analysis was carried out(Figure 4) and the previously reported[5] stereostructure, (3'Z)-(3RS, 8SR, 3a'RS, 6'SR)-3,3a':8,6'-biligustilide, was confirmed.

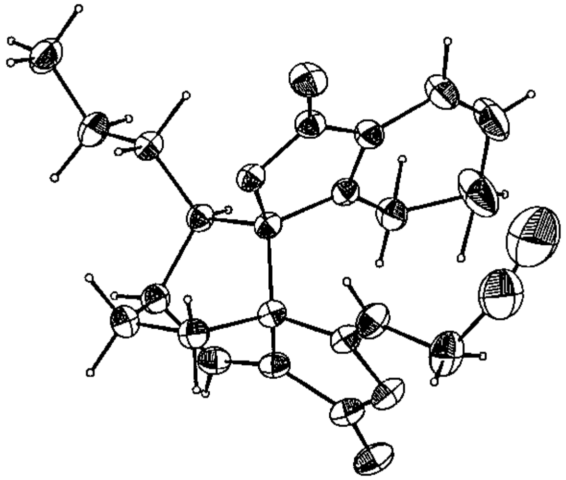

Figure 4. ORTEP drawing of 4

Table 1. ^{13}Cnmr data for phthalide dimers (125 MHz, $CDCl_3$)

C	1	3	2	4	5
1	168.13	168.45	170.53	170.40	170.34
3	153.46	154.98	156.09	89.64	91.99
3a	147.59[a]	148.06[b]	148.31	161.73	160.21
4	23.00	19.75	19.78	21.11	20.07
5	29.13	28.97	28.79	22.65	22.58
6	38.26	38.36	39.06	129.35	128.77
7	41.17	41.50	39.86	116.65	116.98
7a	129.76	126.58	126.07	125.60	122.31[c]
8	115.68	112.08	113.15	42.76	44.01
9	27.84	28.00	28.06	28.41	26.26
10	23.14	22.33	22.31	21.17	20.67
11	13.94	13.95	13.84	14.12	14.12
1'	164.80	164.88	167.62	164.22	168.54
3'	150.34[a]	150.50[b]	84.30	148.52	154.66
3a'	47.78	47.62	48.33	50.14	149.19
4'	31.18	31.08	25.85	28.17	19.61
5'	25.72	25.78	25.88	17.00	20.99
6'	41.49	41.60	40.67	36.73	32.36
7'	142.02	141.99	141.77	144.85	34.95
7a'	134.36	134.27	136.01	133.06	123.47[c]
8'	108.79	108.63	32.01	107.51	112.15
9'	27.47	27.48	28.72	27.04	27.97
10'	22.27	22.27	22.55	22.54	22.39
11'	13.60	13.82	13.98	13.54	13.85

Assignments were confirmed by $^{1}H-^{1}H$ COSY and $^{1}H-^{13}C$ COSY.

[a-c] Assignment of signals may be interchangeable.

Table 2. ^{1}Hnmr data for phthalide dimers (500 MHz, $CDCl_3$)

H	1	3	2	4	5
4	2.25-2.32(m) 2.47-2.51(m)	2.04-2.11(m) 2.18-2.23(m)	2.10-2.17(m) 2.20-2.25(m)	2.03-2.21(m)	2.52-2.63(m) 2.72-2.80(m)
5	1.51-1.60(m) 1.90-1.95(m)	1.39-1.57(m) 1.91-1.95(m)	1.53-1.65(m) 1.84-1.91(m)	2.26-2.32(m)	2.43-2.50(m) 2.52-2.63(m)
6	2.54-2.57(m)	2.53-2.56(m)	2.45-2.48(m)	5.92(dt) (9.6, 4.2)	5.93(dt) (9.6, 4.1)
7	3.27(br d) (9.1)	3.25(br d) (8.9)	3.02(br d) (8.8)	6.17(dt) (9.6, 2.1)	6.17(dt) (9.6, 1.9)
8	5.59(t) (8.6)	5.07(t) (7.9)	5.17(t) (7.9)	1.67(dddd) (9.2, 5.3, 1.9, 1.7)	2.94(dt) (7.8, 7.8)
9	2.23(dt) (8.6, 7.5)	2.29(dt) (7.9, 7.5)	2.30-2.35(m)	1.24-1.38(m) 1.43-1.51(m)	1.41-1.53(m)
10	1.38-1.50(m)	1.39-1.57(m)	1.35-1.52(m)	1.09-1.17(m) 1.24-1.38(m)	1.10-1.18(m)
11	0.92(t) (7.4)	0.93(t) (7.4)	0.94(t) (7.4)	0.88(t) (7.1)	0.86(t) (7.4)
3'			5.11(dd) (10.1, 2.9)		
4'	1.38-1.50(m) 2.04(ddd) (12.6, 9.8, 5.0)	1.39-1.57(m) 2.04(ddd) (12.7, 9.8, 5.0)	1.35-1.52(m) 1.73-1.80(m)	1.24-1.38(m) 2.47-2.53(m)	2.11-2.23(m) 2.52-2.63(m)
5'	1.30(ddt) (5.0, 2.8, 12.6) 1.88(dddd) (12.6, 9.8, 3.4, 2.8)	1.30(ddt) (5.0, 2.8, 12.7) 1.88(dddd) (12.7, 9.8, 3.3, 2.8)	1.27(ddt) (4.6, 2.8, 12.2) 1.84-1.91(m)	1.24-1.38(m) 2.03-2.21(m)	1.99-2.03(m) 2.11-2.23(m)
6'	2.99(ddt) (6.6, 2.0, 2.8)	2.98(ddt) (6.5, 2.0, 2.8)	2.95(ddt) (6.5, 2.2, 2.8)	3.10(dddd) (6.7, 2.9, 2.2, 1.9)	2.52-2.63(m)
7'	7.37(d) (6.6)	7.35(d) (6.5)	7.30(d) (6.5)	7.51(d) (6.7)	3.46(br d) (7.8)
8'	4.99(t) (7.7)	5.00(t) (7.7)	1.53-1.65(m) 1.73-1.80(m)	4.62(dd) (8.7, 6.8)	5.21(t) (7.9)
9'	2.13-2.18(m)	2.14-2.22(m)	1.35-1.52(m)	1.98(ddt) (14.2, 6.8, 7.6) 2.03-2.21(m)	2.29-2.38(m)
10'	1.38-1.50(m)	1.39-1.57(m)	1.35-1.52(m)	1.24-1.38(m)	1.41-1.53(m)
11'	0.92(t) (7.4)	0.92(t) (7.4)	0.93(t) (7.3)	0.82(t) (7.4)	0.95(t) (7.4)

Assignments were confirmed by ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY.

Coupling constants in Hz.

EXPERIMENTAL

Melting points were uncorrected. Optical rotations were determined by a JASCO DIP-360 digital polarimeter. Ir spectra were taken on a Hitachi 270-30 infrared spectrophotometer. Uv spectra in EtOH solution were recorded with a Hitachi U-3200 spectrophotometer. Nmr spectra were recorded with Bruker AM-500 spectrometer and the chemical shift δ (ppm) measured with TMS as int. standard. Ms were determined by JEOL JMS-DX300 ms spectrometer. Column chromatography was carried out on celite 545, Merck Kieselgel 60(70-230 and 230-400 mesh) and C.I.G. column system(silica gel, Kusano scientific Co., Ltd.).

Plant material.

The rhizome of Ligusticum chuangxiong Hort. produced in China was bought from Shibata Co., Ltd.

Extraction and isolation.

The dried rhizome of L. chuangxoing Hort.(55.8 kg) was extracted with MeOH at 62 ℃ (234 ℓ × 2). The MeOH extract was partitioned with a mixture of $CHCl_3$-MeOH-H_2O(3:2:1, 84 ℓ), and then the lower layer(3.33 kg) was partitioned with a mixture of hexane-MeOH-H_2O(10:5:1, 16 ℓ). The lower layer(1.35 kg) was mixed with celite(6.0 kg) followed by charged on a column and successively eluted with hexane(48 ℓ), C_6H_6 (45 ℓ) and MeOH(28 ℓ) to give hexane eluate(905 g), C_6H_6 eluate(414 g), and MeOH eluate(128 g).

A portion of hexane eluate(99.7 g) was repeatedly chromatographed on silica gel with various solvent systems[hexane-AcOEt, C_6H_6-AcOEt(100:1)] to give tokinolide B(4, 0.19 g).

A portion of hexane eluate(703.5 g) was repeatedly chromatographed on silica gel using various solvent systems[hexane-AcOEt, hexane-AcOEt(5:2), C_6H_6-AcOEt(30:1), hexane-AcOEt(3:1), and hexane-AcOEt(2:1)] to give levistolide A(3, 3.03 g), riligustilide(5, 0.59 g), senkyunolide O(1, 31 mg) and senkyunolide P(2, 0.25 g).

Senkyunolide O, (3E, 3'Z)-(6RS, 7RS, 3a'RS, 6'RS)-6,6':7,3a'-biligustilide (1); colorless prisms(acetone—hexane), mp 173.5-174.5℃. $[\alpha]_D$ 0° (c=1.00, $CHCl_3$). Uvλ_{max}^{EtOH} nm(logε): 203(4.08), 229(4.00), 279(4.25). Irν_{max}^{KBr} cm^{-1}: 1774, 1754, 1708, 1660, 1620, 1270, 1052, 1018, 746. ^{1}Hnmr and ^{13}Cnmr : see Tables 1 and 2. Eims m/z(rel. int.): 380(M$^+$, 9), 190(100), 161(40), 148(69), 106 (11), 105 (13). Hrms m/z: 380.2013(M$^+$) (calcd for $C_{24}H_{28}O_4$: 380.1988).

X-Ray crystallographic analysis of 1. The size of crystal was 0.3× 0.25× 0.15 mm, monoclinic, space group $P2_1/n$, a=25.794(2) Å, b=8.256(1) Å, c=9.3112(8) Å, β=95.719(8) Å, V=1973(2) Å^3, Z=4, D_{calcd}=1.28 gcm^{-3} and μ (CuK$_\alpha$)=6.5 cm^{-1}. Reflections were measured on an Enraf-Nonius CAD-4 diffractometer, with $\omega/2\theta$ scan mode and using graphite monochromated CuK$_\alpha$ radiation(λ=1.54184 Å). Cell constants were determined by least-squares refinement using 25 centered reflections in the range $14° < \theta < 23°$. Intensities were measured for 3932 independent reflections in the range $2\theta \leqq 140°$, of which 3135 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[11] and was refined by full-matrix least-squares, using the Enraf-Nonius SDP program[12]. Hydrogen atoms were located on a difference Fourier synthesis map. The last difference Fourier map was essentially featureless with no peaks greater than 0.23 eÅ^{-3}. The final discrepancy index was R=0.063.

Senkyunolide P, (3Z)-(6R,7R,3'S,3a'S,6'R)-3',8'-dihydro-6,6':7,3a'-biligustilide (2); colorless oil. $[\alpha]_D$ +206.2° (c=1.05, $CHCl_3$). Uvλ_{max}^{EtOH} nm(logε): 226(4.06), 282(4.23). Ir$\nu_{max}^{CHCl_3}$ cm^{-1}: 1746, 1666, 1630, 1058. ^{1}Hnmr and ^{13}Cnmr: see Tables 1 and 2. Eims m/z(rel. int.): 382(M$^+$, 8), 193(100), 190(95), 161(28), 107(17), 105(10), 79(9). Hrms m/z: 382.2166(M$^+$) (calcd for $C_{24}H_{30}O_4$: 382.2145).

Retro-Diels-Alder reaction of 2.
2(52.5 mg) was distilled at 220-250℃ under 3 mmHg(Argon gas) in 2 h, and gave the distillate(17.6 mg) containing senkyunolide (6) and ligustilide (7) in the ratio of 1.3 : 1.0{$[\alpha]_D$ -65.0° (c=1.56, $CHCl_3$)}. The products were identified by ^{1}Hnmr[6: 0.91(t, J=7.1 Hz, CH_3-11), 4.92(dd, J=7.6, 3.9 Hz, H-3), 5.91(dt, J=9.8, 4.2 Hz, H-6), 6.20(dt, J=9.8, 1.8 Hz, H-7) ; 7: 0.96(t, J=7.3 Hz, CH_3-11), 1.51(sext, J=7.3 Hz, CH_2-10), 5.22(t, J=8.1 Hz, H-8), 6.00(dt, J=9.8, 4.4 Hz, H-6), 6.29(dt, J=9.8, 2.0 Hz, H-7)] and Gcms[6 m/z: 192, 135, 107, 79 ; 7 m/z: 190, 161, 148, 133, 106], and its ratio was determined by ^{1}Hnmr.

Levistolide A (3); colorless prisms (acetone−hexane), mp 127.5-129℃. $[\alpha]_D$ 0° (c=1.01, $CHCl_3$). Uv λ_{max}^{EtOH} nm (log ε): 202 (4.21), 229 (4.11), 276 (4.35). Ir ν_{max}^{KBr} cm^{-1}: 1778, 1758, 1710, 1676, 1658, 1626, 1268, 1230, 1052, 1020, 744. ^{1}Hnmr and ^{13}Cnmr : see Tables 1 and 2. Eims m/z (rel. int.): 380 (M^+, 1), 190 (42), 161 (25), 148 (100), 105 (41).

Tokinolide B (4); colorless plates (C_6H_6−hexane), mp 120.5-122℃. $[\alpha]_D$ 0° (c=1.02, $CHCl_3$). Uv λ_{max}^{EtOH} nm (log ε): 201 (4.24), 218 (4.06), 280 (3.70). Ir ν_{max}^{KBr} cm^{-1}: 1786, 1756, 1700, 1662, 1606, 1238, 1022, 704. ^{1}Hnmr and ^{13}Cnmr : see Tables 1 and 2. Eims m/z (rel. int.): 380 (M^+, 3), 191 (71), 190 (100), 161 (41), 148 (89), 105 (22).

X-Ray crystallographic analysis of 4. The size of crystal was 0.6×0.5×0.2 mm, orthorhombic, space group Pbca, a=11.640(4) Å, b=27.339(4) Å, c=13.214(4) Å, V=4205(3) $Å^3$, Z=8, D_{calcd}=1.203 gcm^{-3}, μ (CuK_α)=6.1 cm^{-1} and R=0.089. Cell constants were determined using 24 centered reflections in the range $16° < \theta < 22°$. Intensities were measured for 4045 independent reflections in the range $2\theta \leq 130°$, of which 2872 reflections were considered as observed $[I > 3\sigma(I)]$. The last difference Fourier map was essentially featureless with no peaks greater than 0.32 $eÅ^{-3}$.

Riligustilide (5); colorless leaflets (acetone−hexane), mp 138-139.5℃. $[\alpha]_D$ 0° (c=1.07, $CHCl_3$). Uv λ_{max}^{EtOH} nm (log ε): 202 (4.12), 283 (4.37). Ir ν_{max}^{KBr} cm^{-1}: 1754, 1676, 1650, 1632, 1056, 720. ^{1}Hnmr and ^{13}Cnmr : see Tables 1 and 2. Eims m/z (rel. int.): 380 (M^+, 2), 191 (100), 162 (24), 149 (17), 133 (22), 105 (33).

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