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Structures of Torilolide and Oxytorilolide; Two Novel Germacranolides from *Torilis japonica* (HOUTT.) DC.[1)]

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Two novel germacranolides (torilolide and oxytorilolide) were isolated from *Torilis japonica* (HOUTT.) DC. and their structures were determined by X-ray diffraction analysis and from spectral data.

Keywords——*Torilis japonica*; sesquiterpene lactone; germacranolide; butenolide; torilolide; oxytorilolide

Introduction

Torilis japonica (HOUTT.) DC. (Japanese name "Yabujirami"; Umbelliferae) is a biennial plant distributed widely in East Asia. The fruits have been used as a crude drug "He shi" in Chinese medicine ("Kanpo"). In Japan, the fruits have been used traditionally as a substitute for a Chinese crude drug "She chuang zi" and also as an insecticide.[2)] From the methanol extract of the fruits, torilin, a guaiane-type sesquiterpene angelate, has been isolated.[3)] The essential oil of the fruits is mainly composed of certain sesquiterpene hydrocarbons; characteristically germacrene-D.[4)] In our pharmacological studies of crude drugs, the ethanol extract of the fruits was found to have antispasmodic activity.[5)] We established that the active principles are sesquiterpene derivatives,[5b)] and examination of terpenic constituents resulted in the isolation of several sesquiterpenoids which have been correlated to epoxygermacrene-D as a biogenetic intermediate.[6)] In this paper, we describe the structure determination of two novel germacrene-type sesquiterpene lactones, torilolide(I) and oxytorilolide(II), isolated from the benzene extract of the fruits.

Results and Discussion

The dried fruits of *T. japonica* collected in Komoro, Nagano Pref., Japan were extracted with benzene. Torilolide(I) and oxytorilolide(II) were isolated by silica gel column chromatography (LC) and high-performance liquid chromatography (HPLC).

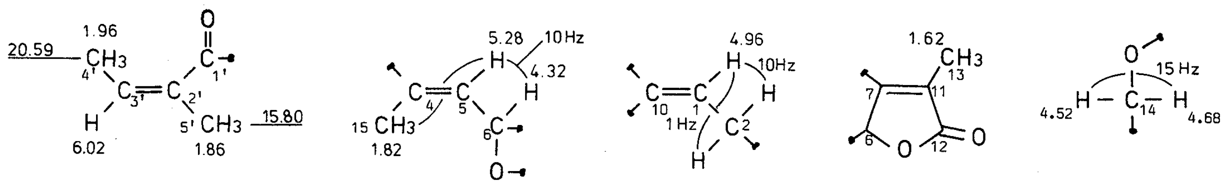
Torilolide(I), $C_{20}H_{26}O_4$ [molecular ion peak (M^+) at m/z 330 and elemental analysis] was a sesquiterpene lactone which appeared to be new, as described below.

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy with the usual decoupling procedure afforded partial structures A—E (Fig. 1), where the carbon atoms are numbered as in the final structure. Irradiation at the frequency of the quartet of quartets signal, H-3′, at δ 6.02 collapsed the signals of two methyls at δ 1.86 and 1.96, indicating the presence of an angelic ester moiety (A), and the angelic ester carbons (two methyls at δ 15.80 and 20.59 ppm) were reasonably assigned in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum.[7)]

Partial structure B was established by the fact that irradiation at the vinylic proton, H-5, at δ 5.28 collapsed not only a methyl signal, H-15, at δ 1.82 but also a proton signal under the lactone ring, H-6, at δ 4.32. The remaining vinylic proton signal, H-1, in the partial structure C appeared at δ 4.96 as a doublet of doublets ($J=10$, 1 Hz) coupled with two protons on C-2 whose signals were superimposed at δ 1.80—2.40. A singlet signal at δ 1.62 was assigned to a methyl group on the α, β-unsaturated butyrolactone moiety (D) [IR band at 1760 cm^{-1}] and two protons on C-14 appeared as a pair of doublets at δ 4.52 and 4.68 (each $J=15$ Hz) (E). These partial structures were also supported by the ^{13}C-NMR and other spectral data, and were connected as shown in structure I (Fig. 2) on the basis of biogenetical considerations.

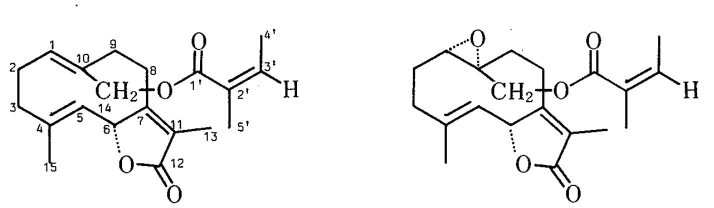
The molecular formula of oxytorilolide(II) was indicated to be $C_{20}H_{26}O_5$ from the mass spectra (MS) [346, M$^+$] and elemental analysis. Spectral data of II were similar to those of compound I. An angelic acid ester moiety was present [C3′ proton at δ 6.08 and two methyls at δ 1.84 and 2.00] and a butenolide moiety was indicated [IR band at 1760 cm^{-1}]. Another oxygen atom of II was assigned as an epoxy oxygen on the C10–C1 double bond of torilolide(I) by ^{13}C-NMR [δ 60.91 (s) and 66.52 (d)]. The oxidation of I with *meta*-chloroperbenzoic acid (*m*-CPBA) afforded II. Thus, the structure of compound II was assigned as shown in Fig. 2.

The whole structure of II, including the stereochemical configurations, was solved by X-ray diffraction analysis by the direct method. A stereoscopic drawing of II is shown in Fig. 3,



A B C D E

Fig. 1



torilolide (I) oxytorilolide (II)

Fig. 2

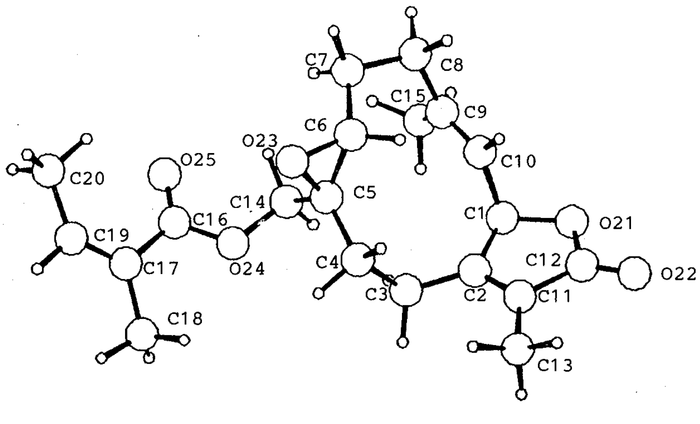


Fig. 3

Numbering of carbon and oxygen atoms used in Tables I, II and III.

and the structures of I and II are illustrated in Fig. 2.

Sesquiterpene lactones are distributed in many plants, including the Umbelliferae family. However, sesquiterpene lactones previously obtained from this family have been classified into eudesmane or guaiane types.[8] This is the first example of the isolation of sesquiterpene lactones from *Torilis* species, and of germacranolides from the Umbelliferae family.

Experimental

Spectral data were obtained on the following instruments: optical rotation, on a JASCO DIP-4 in $CHCl_3$ solution; infrared (IR) on a JASCO A-302 in CCl_4 solution; ultraviolet (UV) on a Hitachi 557 in ethanol solution; NMR on a JEOL FX-200 in $CDCl_3$ solution with tetramethylsilane as an internal standard; MS on a Hitachi M-80. HPLC was carried out on a CIG column system (Kusano Scientific Co., Tokyo) with WAKO GEL (50 μ silica gel) as the stationary phase.

Isolation of Torilolide(I) and Oxytorilolide(II)——The dried fruits (4.5 kg) of *T. Japonica* collected in July, 1982 in Komoro, Nagano, were extracted three times with benzene at room temperature. A green oil (305 g) was obtained by evaporation of the solvents and a part of it (200 g) was chromatographed on silica gel with *n*-hexane and ethyl acetate, yielding 13 fractions. Compound I (1778 mg) was obtained from the 10th fraction by HPLC (eluents, benzene : ethyl acetate = 2 : 1, *n*-hexane : ethyl acetate = 7 : 3, *n*-hexane : ethyl acetate : acetonitrile = 7 : 2 : 1), and II (225 mg) was obtained from the 11th fraction by HPLC (eluents, benzene : ethyl acetate = 2 : 1, benzene : ethyl acetate : acetonitrile = 10 : 2 : 1, *n*-hexane : ethyl acetate = 7 : 3, *n*-hexane : ethyl acetate : acetonitrile = 7 : 2 : 1).

Torilolide(I)——Colorless plates. mp 63.5—64.5 °C. $[\alpha]_D + 3.5$ ($c = 0.58$). MS *m/z* (%): 330 (M^+, 1), 230 (50), 215 (18), 202 (17), 201 (14), 160 (7), 105 (15), 83 (100). UV λ nm (log ε): 221 (4.35). IR ν cm^{-1}: 2950, 2870, 1760, 1720, 1675, 1650, 1445, 1380, 1360, 1250, 1230, 1150, 1000. ^1H-NMR δ: 1.64 (3H, s), 1.82 (3H, br s), 1.86 (3H, br s), 1.96 (3H, d, $J = 6$ Hz), 2.76 (1H, d, $J = 16$ Hz), 4.32 (1H, d, $J = 10$ Hz), 4.52 (1H, d, $J = 15$ Hz), 4.68 (1H, d, $J = 15$ Hz), 4.96 (1H, dd, $J = 10$, 1 Hz), 5.28 (1H, br d, $J = 10$ Hz), 6.02 (1H, br q, $J = 6$ Hz). ^{13}C-NMR δ: 8.76 (q), 15.80 (q), 16.53 (q), 20.59 (q), 25.02 (t), 26.19(t), 35.36 (t), 37.99 (t), 60.18 (t), 80.76 (d), 125.06 (d), 125.88 (s), 127.51 (s), 133.65 (d), 134.02 (s), 138.32 (s), 138.64 (d), 163.55 (s), 167.84 (s), 174.61 (s). *Anal.* Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.60; H, 7.92.

Oxytorilolide(II)——Colorless plates. mp 124.5—125.5 °C. $[\alpha]_D + 19.8$ ($c = 1.10$). MS *m/z* (%): 346 (M^+, 2.5), 264 (8), 228 (14), 203 (6), 149 (8), 110 (15), 83 (100). UV λ nm (log ε): 218 (4.36). IR ν cm^{-1}: 2950, 2860, 1760, 1720, 1675, 1650, 1445, 1390, 1355, 1255, 1230, 1150, 1080, 1000. ^1H-NMR δ: 1.80 (3H, s), 1.84 (3H, s), 1.92 (3H, s), 2.00 (3H, d, $J = 8$ Hz), 3.79 (1H, d, $J = 12$ Hz), 4.56 (1H, d, $J = 12$ Hz), 4.73 (1H, d, $J = 10$ Hz), 5.34 (1H, dd, $J = 10$, 1.5 Hz), 6.08 (1H, q, $J = 8$ Hz). ^{13}C-NMR δ: 8.64 (q), 15.88 (q), 16.88 (q), 20.50 (q), 21.90 (t), 23.53 (t), 32.85 (t), 36.21 (t), 60.91 (s), 63.27 (t), 66.52 (d), 79.98 (d), 122.78 (d), 125.38 (s), 127.10 (s), 139.57 (d), 139.63 (s), 162.67 (s), 167.52 (s), 174.35 (s). *Anal.* Calcd for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.47; H, 7.56.

Epoxidation of I——A methylene chloride solution of I (157 mg in 7 ml) was added dropwise to a CH_2Cl_2 solution of *m*-CPBA (70 mg in 5 ml) and the solution was stirred for 3 h at room temperature. After the reaction, ether

TABLE I. Atomic Parameters of Oxytorilolide(II) ($\times 10^4$)

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
C1	7729 (4)	−398 (2)	10492 (6)	C14	8716 (5)	−2156 (2)	12142 (7)
C2	6543 (5)	−651 (2)	11402 (5)	C15	10517 (5)	−996 (2)	10120 (8)
C3	6666 (5)	−1151 (2)	12535 (6)	C16	8821 (5)	−3031 (2)	13592 (7)
C4	6359 (4)	−1737 (2)	11753 (6)	C17	8000 (4)	−3444 (2)	14571 (6)
C5	7570 (4)	−2027 (2)	10963 (6)	C18	6971 (5)	−3219 (2)	15809 (7)
C6	7757 (4)	−2017 (2)	9103 (6)	C19	8159 (5)	−3983 (2)	14311 (7)
C7	9111 (5)	−2048 (2)	8190 (7)	C20	9116 (6)	−4270 (2)	13154 (9)
C8	9477 (5)	−1463 (2)	7512 (7)	O21	7181 (3)	117 (1)	9762 (4)
C9	9355 (4)	−1035 (2)	8914 (6)	O22	5143 (4)	497 (1)	9445 (5)
C10	8200 (4)	−757 (2)	9049 (6)	O23	7230 (3)	−2510 (1)	9928 (5)
C11	5451 (5)	−351 (2)	11093 (6)	O24	8182 (3)	−2558 (1)	13371 (5)
C12	5827 (5)	132 (2)	10026 (6)	O25	9904 (4)	−3109 (2)	13002 (9)
C13	4035 (5)	−420 (2)	11681 (8)				

The carbon and oxygen atoms were numbered as in Fig. 3.

TABLE II. Bond Distances of Oxytorilolide(II) (Å)

Atom 1	Atom 2	Length	Atom 1	Atom 2	Length
C1	C2	1.505 (6)	C9	C10	1.330 (6)
C1	C10	1.501 (6)	C9	C15	1.498 (7)
C1	O21	1.466 (5)	C11	C12	1.477 (6)
C2	C3	1.499 (6)	C11	C13	1.489 (7)
C2	C11	1.323 (6)	C12	O21	1.361 (6)
C3	C4	1.563 (6)	C12	O22	1.198 (6)
C4	C5	1.522 (6)	C14	O24	1.466 (6)
C5	C6	1.477 (7)	C16	C17	1.496 (7)
C5	C14	1.500 (7)	C16	O24	1.309 (6)
C5	O23	1.454 (5)	C16	O25	1.186 (7)
C6	C7	1.526 (7)	C17	C18	1.512 (7)
C6	O23	1.445 (5)	C17	C19	1.315 (7)
C7	C8	1.543 (7)	C19	C20	1.486 (8)
C8	C9	1.512 (7)			

The carbon and oxygen atoms were numbered as in Fig. 3.

TABLE III. Bond Angles of Oxytorilolide(II) (°)

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C2	C1	C10	112.0 (4)	C10	C9	C15	125.6 (4)
C2	C1	O21	103.6 (3)	C8	C9	C15	116.4 (4)
C10	C1	O21	107.5 (3)	C12	C11	C2	108.8 (4)
C3	C2	C1	122.8 (4)	C12	C11	C13	120.1 (4)
C3	C2	C11	127.6 (4)	C2	C11	C13	131.0 (4)
C1	C2	C11	109.6 (4)	O21	C12	C11	108.5 (4)
C4	C3	C2	117.8 (4)	O21	C12	O22	121.3 (4)
C5	C4	C3	114.6 (4)	C11	C12	O22	130.2 (4)
C6	C5	C4	119.9 (4)	O24	C14	C5	105.7 (4)
C6	C5	C14	121.5 (4)	C17	C16	O24	112.1 (4)
C6	C5	O23	59.1 (3)	C17	C16	O25	126.3 (5)
C4	C5	C14	116.0 (4)	O24	C16	O25	121.5 (5)
C4	C5	O23	114.1 (4)	C18	C17	C16	117.7 (4)
C14	C5	O23	111.1 (4)	C18	C17	C19	122.0 (4)
C7	C6	C5	125.3 (4)	C16	C17	C19	120.2 (4)
C7	C6	O23	119.5 (4)	C20	C19	C17	128.8 (5)
C5	C6	O23	59.7 (3)	C1	C10	C9	128.0 (4)
C8	C7	C6	109.1 (4)	C1	O21	C12	109.1 (3)
C9	C8	C7	110.1 (4)	C5	O23	C6	61.3 (3)
C10	C9	C8	117.8 (4)	C14	O24	C16	118.8 (4)

The carbon and oxygen atoms were numbered as in Fig. 3.

(20 ml) was added and the organic layer was washed with 10% $Na_2S_2O_3$ soln., sat. $NaHCO_3$ soln., water, and then brine, and dried over $MgSO_4$. The product (II) was obtained (40 mg) by HPLC (eluent, *n*-hexane : ethyl acetate = 2 : 1).

Crystal Data of II: $C_{20}H_{26}O_5$, $M_r = 346$, $P2_12_12_1$, $Z = 4$, $a = 9.9250$ Å, $b = 23.9492$ Å, $c = 7.8810$ Å, orthorhombic. A total of 1624 reflections were recorded and the final R value was 0.049.

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