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Constituents of *Torilis japonica* D.C. I. Isolation and Optical Purity of Germacra-4(15),5(*E*),10(14)-trien-1*β*-ol

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Humulene (I), (−)-germacrene-D (II), and (−)-germacra-4(15),5(*E*),10(14)-trien-1*β*-ol (III) were isolated from the fruits of *Torilis japonica*. The latter two compounds (II and III) were determined to have optical purities of about 40 and 30%, respectively.

Keywords——*Torilis japonica*; humulene; germacrene-D; germacra-4(15),5(*E*),10-(14)-trien-1*β*-ol; optical purity

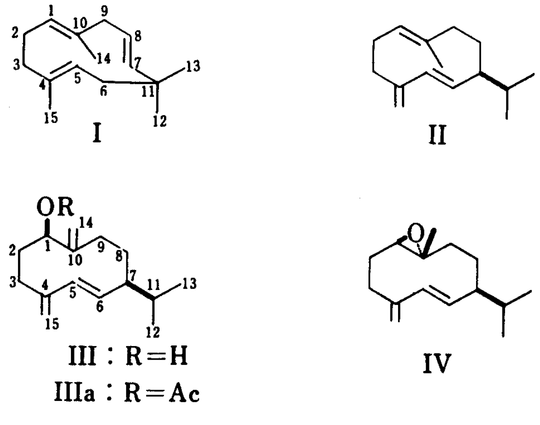
The fruits of *Torilis japonica* have been used as a substitute in Japan for a Chinese crude drug She Huangzi (蛇床子).[1] Cadinene and torilin have been isolated as constituents of the fruits.[2] In our pharmacological studies of crude drugs, the ethanol extract of the fruits was found to have strong inhibitory activity against contraction of the ileum of guinea pigs caused by histamine or barium chloride. Since we have already found that the active principles are certain sesquiterpene derivatives,[3] re-examination of the terpenic constituents was undertaken. In this paper, we report the isolation of three sesquiterpenes and discuss their optical purity.

The fresh fruits of *T. japonica* were extracted with methanol, and the *n*-hexane-soluble fraction was chromatographed on silica gel to afford three compounds, I, II, and III. By comparison of the spectral data of I and II with those of authentic specimens, these compounds were identified as humulene and (−)-germacrene-D (optical purity: approximately 40%),[4] respectively.

Compound III was shown to possess the same conjugated diene system as germacrene-D from the ultraviolet(UV) absorption and proton nuclear magnetic resonance(^{1}H-NMR) spectra. In addition, a ^{1}H-NMR decoupling experiment indicated the presence of an allylic alcohol system. Thus III can be assigned as germacra-4 (15),5(*E*),10(14)-trien-1*β*-ol. Compound III was converted to the acetate (IIIa), which was shown to be identical with an authentic acetate from Takahashi[5] by direct comparison by gas chromatography.

Fattorusso *et al.*[6] have already isolated IIIa from a brown alga, *Dilophus fasciola*, and Bohlmann *et al.*[7] have obtained III from compositae plants, *Athanasia dregeana*, *Inula cuspidata*, and *Mikania pohlii*. However, the optical rotation of III or IIIa varies widely according to its source as shown in Table I.

In order to determine the optical rotation of III in an optically pure state, germacrene-D ([α]$^{26}_{D}$ −111°, optical purity about 35%) isolated from *Solidago altissima* was converted to III in the manner described in the literature.[5] The product (III) showed an optical rotation of [α]$^{24}_{D}$ −110°. Therefore, the optical rotation of the optically pure compound illustrated by the structural formula III was estimated to be [α]$_D$ −300°. The optical purity of the samples isolated by Fattorusso *et al.*, Bohlmann *et al.*, and us can be calculated to be approximately



I

II

III : R=H
IIIa : R=Ac

IV

Chart 1

TABLE I. Optical Rotations of III and IIIa from Various Plants

Origin	III	IIIa
Dilophus fasciola	−180.3°	−106.5°
Athanasis dregeana	—	−38.0°
Torilis japonica	−84.5°	−41.1°

as 60, 30, and 30%, respectively.

It is well known that (−)- and (+)-germacrene-D co-occur in different ratios in various plants.[4] It is clear that compound III also appears as a mixture of enantiomers in various ratios in nature. It is possible the enzymatic sequence from germacrene-D to III may not be strictly stereoselective.

The biological activities will be described elsewhere.

Experimental

Spectral data were obtained on the following instruments: optical rotation, on a JASCO DIP-4 in $CHCl_3$ solution (all samples were freed of solvent *in vacuo* for several hours); infrared (IR), on a JASCO A-302 in CCl_4 solution; ultraviolet (UV), on a Hitachi 557; ^{1}H-NMR, on a Varian EM 390; ^{13}C-NMR, on a JEOL FX-100 in $CDCl_3$ solution; mass spectrum (MS), on a Hitachi M-80. High-performance liquid chromatography (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 Compounds I, II, and III——The fresh fruits (2.7 kg) of *Torilis japonica* collected in Hachioji, Tokyo, in July 1981 were extracted three times with methanol at room temperature. The methanol extracts were diluted with water and shaken with *n*-hexane. The *n*-hexane layer was concentrated to give a green oil (89.8 g). A half of the *n*-hexane extract was chromatographed on silica gel with *n*-hexane and ethyl acetate, and the eluates were divided into 16 fractions. The first fraction was chromatographed again on silica gel (eluent: *n*-hexane) and $AgNO_3$-coated silica gel[8] (eluent: benzene) to afford I (58 mg) and II (86 mg). Repeated chromatography of the eighth fraction (HPLC, benzene: ethyl acetate = 9: 1; $AgNO_3$-HPLC, *n*-hexane: ethyl acetate = 65: 35) also afforded III (1.72 g).

Compound I (humulene): Colorless oil. IR cm^{-1}: 2970, 2930, 1660, 1450, 1380, 1360, 970. ^{1}H-NMR (CCl_4) δ: 1.01 (6H, s), 1.43 (3H, s), 1.64 (3H, s), 1.91 (2H, d, $J=7$ Hz), 2.08 (4H, br s), 2.30 (1H, br s), 2.51 (2H, d, $J=8$ Hz), 4.98 (2H, m), 5.54 (1H, d, $J=16$ Hz), 5.60 (1H, dt, $J=7$, 16 Hz). MS *m/z*: 204 (M$^+$, 39), 161 (19), 157 (42), 147 (45), 121 (36), 93 (100), 80 (40).

Compound II (Germacrene-D): Colorless oil, $[\alpha]_D^{26}$ −124° ($c=0.38$). IR cm^{-1}: 3090, 2950, 2930, 2860, 1625, 1600, 1455, 1385, 980, 885. UV (*n*-hexane) nm (ε): 259 (3300). MS *m/z*: 204 (M$^+$, 7), 161 (47), 133 (21), 131 (13), 119 (32), 105 (60), 91 (100), 77 (57), 41 (79). ^{1}H-NMR (CCl_4) δ: 0.79 (3H, d, $J=7$ Hz), 0.84 (3H, d, $J=7$ Hz), 1.48 (3H, s), 4.68 (1H, br s), 4.71 (1H, br s), 5.06 (1H, br d, $J=10$ Hz), 5.17 (1H, dd, $J=9$, 16 Hz), 5.73 (1H, d, $J=16$ Hz).

Compound III (Germacra-4(15),5(*E*),10(14)-trien-1β-ol): Colorless oil, $[\alpha]_D^{17}$ −85° ($c=0.83$). IR cm^{-1}: 3625, 3090, 2965, 2885, 1650, 1640, 1610, 1460, 1445, 1385, 1370, 985, 895. UV (EtOH) nm (ε) : 239 (14700). MS *m/z*: 220 (M$^+$, 8, Calcd for $C_{15}H_{24}O$, 220.1825; Found 220.1806), 205 (17), 202 (27), 177 (28), 159 (50), 109 (100). ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, d, $J=6$ Hz), 0.87 (3H, d, $J=7$ Hz), 3.76 (1H, dd, $J=5$, 10 Hz), 4.82 (1H, br s), 4.88 (1H, br s), 4.97 (1H, br s), 5.26 (1H, br s), 5.41 (1H, dd, $J=9$, 16 Hz), 5.98 (1H, d, $J=16$ Hz). ^{13}C-NMR δ: 20.5 (q), 20.7 (q), 29.9 (t), 31.8 (t), 34.5 (d), 36.1 (t), 36.4 (t), 52.5 (d), 75.7 (d), 110.4 (t), 112.7 (t), 129.5 (d), 137.7 (d), 146.6 (s), 153.2 (s).

Acetylation of III——Compound III (45 mg) and acetic anhydride (0.15 ml) were dissolved in 0.1 ml of pyridine and the solution was allowed to stand overnight. The reaction mixture was worked up in the usual way and the product was chromatographed on silica gel (eluent, *n*-hexane: ethyl acetate = 8: 2) to give IIIa (27 mg).

Compound IIIa Germacra-4(15),5(*E*),10(14)-trien-1β-ol acetate): colorless oil, $[\alpha]_D^{18}$ −41° ($c=0.27$). IR cm^{-1}: 3080, 2940, 2880, 1740, 1645, 1610, 1460, 1370, 1240, 1015, 955, 890. UV (EtOH) nm (ε): 238 (13500). MS *m/z* 262 (M$^+$, 0.5), 220 (4), 202 (13), 187 (7), 174 (7), 160 (10), 159 (52), 145 (16), 131 (31), 117 (30), 105 (28), 91 (51), 79 (35), 43 (100). ^{1}H-NMR ($CDCl_3$) δ: 0.81 (3H, d, $J=6$ Hz), 0.88 (3H, d, $J=6$ Hz), 1.94 (3H, s), 4.89 (2H, br s), 4.94 (1H, dd, $J=5$, 10 Hz), 5.12 (1H, br s), 5.36 (1H, br s), 5.42 (1H, dd, $J=9$, 16 Hz), 6.09 (1H, d, $J=16$ Hz). ^{13}C-NMR: 20.5 (q), 20.7 (q), 21.4 (q), 29.0 (t), 31.8 (d), 32.9 (t), 34.4 (t), 35.9 (t), 52.5 (d), 77.4 (d), 113.2 (t), 113.8 (t), 129.4 (d), 138.1 (d), 145.9 (s), 149.1 (s), 170.4 (s).

Epoxidation of Germacrene-D[4,5]——Germacrene-D ($[\alpha]_D^{26}$ −111°, $c=0.53$: $[\alpha]_D^{18}$ −120° in MeOH $c=0.1$, 758 mg) obtained from *Solidago altissima* by the silver nitrate method[9] and *m*-CPBA were dissolved in 40 ml of ether at −10° and the solution was allowed to stand at −4° overnight. It was then washed with 10% $Na_2S_2O_3$ and 5% $NaHCO_3$, the solvent was evaporated off, and the reaction mixture was chromatographed on alumina (eluent: benzene) to afford the epoxide IV (150 mg).

Compound IV: Colorless oil, $[\alpha]_D^{16}$ −91° (c=0.98). IR cm^{-1}: 3080, 2960, 2860, 1455, 1380, 970, 880. UV (*n*-hexane) nm (ε): 234 (12700). MS m/z: 220 (M^+, 15), 205 (4), 202 (6), 187 (5), 167 (27), 159 (32), 123 (100), 107 (26), 91 (37), 81 (67). ^1H-NMR (CCl_4) δ: 0.82 (3H, d, J=7 Hz), 0.89 (3H, d, J=7 Hz), 2.58 (1H, dd, J=5, 8 Hz), 4.83 (2H, br s), 5.47 (1H, dd, J=9, 16 Hz), 6.00 (1H, d, J=16 Hz). ^{13}C-NMR: δ: 16.5 (q), 19.7 (q), 20.8 (q), 29.2 (t), 30.1 (d), 31.0 (t), 32.3 (t), 38.7 (6), 51.8 (d), 61.0 (s), 68.8 (d), 112.5 (t), 132.8 (d), 136.3 (d), 146.3 (s).

Reaction of IV with Lithium Diisopropylamide to give III[5]——An ether solution of IV (100 mg) was added to an ether solution of LDA at −74°C and the mixture was stired for 1 h at −74°C and then for 2 h at room temperature. The mixture was washed with water and concentrated, and the residue was purified by HPLC (eluent: benzene: ethyl acetate=9: 1) to afford III (17 mg), $[\alpha]_D^{24}$ −110° (c=0.17).

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