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NORISOPRENOIDS FROM *VIBURNUM DILATATUM**

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Key Word Index—*Viburnum dilatatum*; Caprifoliaceae; norisoprenoids.

Abstract—A new norisoprenoid, (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one, was isolated together with seven known norisoprenoids, (−)-loliolide, (+)-isololiolide, (6*R*,7*E*)-4,7-megastigmadien-3,9-dione, (6*S*,7*E*)-6-hydroxy-4,7-megastigmadien-3,9-dione, (3*S*,7*E*)-3-hydroxy-5,7-megastigmadien-9-one, (3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmene-9-one, (3*S*,5*R*,7*E*,8*R*)-3,5-dihydroxy-6,7-megastigmadien-9-one and eight known phenolic compounds, *trans*-ferulic acid, *cis*-ferulic acid, *trans*-*p*-coumaroyl methyl ester, umbelliferone, veratric acid, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, *p*-methoxybenzoic acid, from the leaves of *Viburnum dilatatum*. Their structures were established on the basis of chemical evidence and spectral data.

INTRODUCTION

The deciduous shrub *Viburnum dilatatum* is widely distributed in Japan and China [2]. The leaves have been utilized in traditional Chinese medicine (Chinese name, jia mi) [3]. While Rice [3] reported that *V. dilatatum* exhibited allelopathic activity, this plant does harm to the neighbouring plants [4]. In our previous paper, we reported the structures of two new polyhydric alcohol glycosides isolated from the leaves of this plant [5]. In the course of further studies on its constituents, we found that the chloroform extract of the leaves showed growth and germination inhibitory effects towards the seeds of head lettuce. From this extract, we isolated a new norisoprenoid along with seven known norisoprenoids and eight known phenolic compounds. This paper deals with the structural elucidation and identification of these compounds.

RESULTS AND DISCUSSION

The fresh leaves of *V. dilatatum* were extracted successively with chloroform diethylether, ethylacetate and *n*-butanol. The chloroform extract was shown to contain growth and germination inhibitors towards the seeds of head lettuce (see Experimental). The chloroform extract was purified by silica gel column chromatography, C_{18} column chromatography and preparative HPLC to yield 16 compounds.

Compound **1** [(−)-loliolide] [6,7], **2** [(+)-isololiolide] [6], **4** ((6*R*,7*E*)-4,7-megastigmadien-3,9-dione) [8–10], **5** ((6*S*,7*E*)-6-hydroxy-4,7-megastigmadiene-3,9-dione) [11–13], **6** [(3*S*,7*E*)-3-hydroxy-5,7-megastigmadien-9-one) [11, 14, 15], **7** ((3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmene-9-one) [11, 14, 16], **8** ((3*S*,5*R*,7*E*,8*R*)-3,5-dihydroxy-6,7-megastigmadien-9-one) [17–19], **9** (*trans*-ferulic acid), **10** (*cis*-ferulic acid), **11** (*trans*-*p*-coumaroyl methyl ether), **12** (umbelliferone), **13** (veratric acid), **14** (*p*-hydroxybenzaldehyde), **15** (*p*-hydroxybenzoic acid) and **16** (*p*-methoxybenzoic acid) were identified by comparison of various diagnostic data with reported values and authentic samples.

Compound **3** was obtained as an amorphous powder. The ^1H and ^{13}C NMR spectra of **3** lacked the signals due to the cyclic ketone moiety of **4** and instead showed signals characteristic of the carbinol moiety (δ_H 4.26, δ_C 65.5). Furthermore, the ^1H NMR signals of 2-H in **3** appeared as a double doublet and shifted upfield compared with the doublet 2-H signal of **4**. From the above data, compound **3** was as shown to have a planar structure. The relative configurations of C-3 and C-6 were determined by the difference NOE experiment. As shown in Fig. 1, the difference NOE experiment indicated 3-H and 6-H to be pseudo-axial with respect to the half-chair form of the cyclohexene. The absolute configuration of **3** was determined as follows. The (+)-(*R*)-MTPA and (−)-(*S*)-MTPA ester of **3** were prepared, and a modified Mosher method was applied to these MTPA esters [20]. The positive and negative $\Delta\delta$ values were found on the right and left sides of the MTPA planes, respectively, indicating an *R*-configuration for C-3 (Fig. 2). Furthermore, the CD spectrum of **3** showed a positive Cotton effect, $\Delta\varepsilon_{239.5\ nm}$ + 11.01, suggesting C-6 to have the *R*-configuration [21]. Consequently, the structure of **3** was established as (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one. Compound **3** is a new natural product (**3** is expected as a degradation compound of carotenoids, e.g.

*Part 11 in the series 'Studies on the Constituents of *Viburnum* Species. X'. For part 10 see ref. [1].

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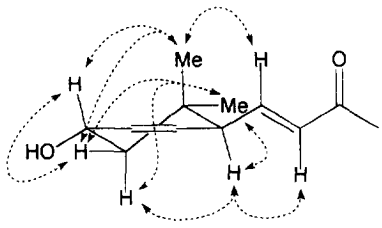


Fig. 1. Significant enhancement of signal intensity by NOE difference experiments on compound **3** in $CDCl_3$.

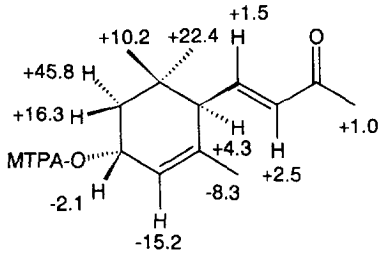
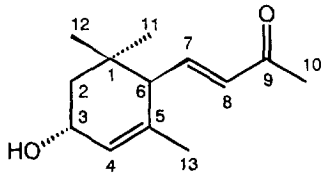


Fig. 2. $\Delta\delta$ values $\Delta\delta = \delta_S - \delta_R$ in Hz at 270 MHz obtained for the MTPA esters of compound **3**.



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flavoxanthin or lutein, containing the same cyclic end group) [22,23]; compounds **1**, **2**, **4**–**8** have now been found for the first time in the genus *Viburnum*. Furthermore, compounds **1**, **2**, **5**, **6**, **9** and **10** were shown to be growth and germination inhibitors of the lettuce, alfalfa and cress seeds [13,15,24–27]. The inhibitory activity of **3**, **4**, **7**, **8**, **11**–**16** towards the growth and germination of the seeds of head lettuce could not be examined, because of the limited samples.

EXPERIMENTAL

^1H and ^{13}C NMR: 270 and 67.8 MHz, respectively, $CDCl_3$, TMS as int. standard. EI-MS: Jeol LMS-DX 303 mass spectrometer; CC: Kieselgel 60 (Merck; 230–400 mesh) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Inc.); Prep. HPLC: Tosoh HPLC system using Cosmosil 5C_{18}-AR column (Nacalai Tesque, Inc., 10 mm i.d. × 25 cm) and TSK gel OH-120 column (Tosoh Co., 7.8 mm i.d. × 30 cm) with UV detector.

Plant material. The leaves of *V. dilatatum* Thunb were collected and identified by one of the authors (M.K) from Sendai city, Miyagi, Japan, July 1988. A voucher specimen (No. 4) is deposited in his laboratory.

Extraction and isolation. Fresh leaves of *V. dilatatum* (1.8 kg) were extracted with MeOH at room temp. The MeOH extract was concd. and the resultant aq. suspension was extracted with $CHCl_3$, Et_2O, EtOAc and *n*-BuOH, successively. The $CHCl_3$-soluble fraction was concd under red. pres. to produce a residue (35.0 g). This residue was chromatographed on a silica gel column using hexane–Me_2CO (3:1) and the eluate was sepd into three frs (frs 1–3). Fr. 2 (MeOH–H_2O, 3:1 soluble material) was rechromatographed on a C_{18} open column using MeOH–H_2O (3:1) and the eluate was separated into three frs (frs 2–1 to 2–3). Fr. 2–1 was subjected to prep. HPLC (5C_{18}–AR column; MeOH–H_2O (1:1), OH-120 column; hexane–Me_2CO (3:1 or 6:1)) to give **1** (25 mg), **2** (10 mg), **3** (10 mg), **4** (3 mg), **5** (5 mg), **6** (4 mg), **7** (4 mg), **8** (5 mg), **9** (3 mg), **10** (2 mg), **11** (3 mg), **12** (2 mg), **13** (2 mg), **14** (2 mg), **15** (3 mg) and **16** (2 mg).

Bioassay with the head lettuce seeds. For growth and germination inhibition tests, the methods reported in [15] and [27] were adapted. The $CHCl_3$ extract (concentration, 0.9 mg ml^{-1}) was shown to contain growth and germination inhibitory activities towards the head lettuce seeds (% growth of hypocotyl against control 40; % germination against control 25).

(−)-*Loliolide* (**1**). Amorphous powder, $[\alpha]_D$ − 87.5° (MeOH, *c* 0.4). EI-MS *m/z* 196 $[M]^+$. CD (MeOH) $\Delta\varepsilon$ − 9.99 (219.0 nm). The spectral data were identical with those of reported data [6,7].

(+)-*Isololiolide* (**2**). Amorphous powder, $[\alpha]_D$ + 106.9 (MeOH, *c* 0.4). EI-MS *m/z* 196 $[M]^+$. CD (MeOH) $\Delta\varepsilon$ + 10.39 (212.0 nm). The spectral data were identical with those of reported data [6].

(3*R*,6*R*,7*E*)-3-*Hydroxy*-4,7-*megastigmadien*-9-*one* (**3**). Amorphous powder, $[\alpha]_D$ + 84.6° (MeOH; *c* 0.3). EI-MS *m/z*: 208 $[M]^+$. UV λ_{max}^{MeOH} nm (log ε): 224.0 (4.03). ^1H NMR (270 MHz, $CDCl_3$): δ 0.89 (3H, *s*, 12-CH_3), 1.03 (3H, *s*, 11-CH_3), 1.39 (1H, *dd*, *J* = 13.5, 6.9 Hz, 2-Hα), 1.62 (3H, *s*, 13-CH_3), 1.84 (1H, *dd*, *J* = 13.5, 5.9 Hz, 2-H$_\beta$), 2.26 (3H, *s*, 10-CH_3), 2.50 (1H, *d*, *J* = 9.9 Hz, 6-H), 4.26 (1H, *br s*, 3-H), 5.63 (1H, *br t*, *J* = 1.3 Hz, 4-H), 6.10 (1H, *d*, *J* = 15.8 Hz, 8-H), 6.54 (1H, *dd*, *J* = 15.8,9.9 Hz, 7-H). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 22.7 (C-13), 24.7 (C-12), 27.2 (C-10), 29.3 (C-11), 33.9 (C-1), 43.8 (C-2), 54.3 (C-6), 65.5 (C-3), 125.9 (C-4), 133.6 (C-8), 135.5 (C-5), 147.1 (C-7), 198.0 (C-9). CD (MeOH) $\Delta\varepsilon$ + 11.01 (239.5 nm).

Preparation of (*R*)-(+)-*MTPA ester of* **3**. To a soln of **3** (1.5 mg, 7.2 μmol) in pyridine (30 μl) was added (*R*)-(+)-MTPA chloride (2.9 μl, 15 μmol), and the soln was allowed to stand at room temp. for 24 hr. 3-[(Dimethylamino)propyl]amine (1.7 μl, 15 μmol) was added, and after 30 min, the solvent was removed, and the residue diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was evapd *in vacuo* and the residue was purified by prep. HPLC (silica-60 column (Tosoh Co.), 7.8 mm i.d × 30 cm, hexane–Me_2CO, 10:3) to yield the (*R*)-(+)-MTPA ester, **3a** (2.0 mg, 65%). ^1H NMR (270 MHz, $CDCl_3$): δ 0.877 (3H, *s*, 11-CH_3), 0.884 (3H, *s*, 12-CH_3), 1.49 (1H, *dd*, *J* = 14.5, 3.6 Hz, 2-Hα), 1.67 (3H, *s*, 13-CH_3), 1.88 (1H, *dd*, *J* = 14.5, 5.9 Hz, 2-Hβ), 2.26 (3H, *s*, 10-CH_3), 2.46 (1H, *d*, *J* = 9.6 Hz, 6-H), 3.56 (3H, *s*,

OCH_3), 5.57 (1H, *m*, 3-H), 5.65 (1H, *br s*, 4-H), 6.07 (1H, *d*, $J = 15.8$ Hz, 8-H), 6.52 (1H, *dd*, $J = 15.8$, 9.6 Hz, 7-H), 7.40 (3H, *m*), 7.53 (2H, *m*). EI-MS *m/z*: 424 $[M]^+$.

Preparation of (S)-(−)-MTPA ester of **3**. Compound **3** (1.5 mg, 7.2 μmol) was treated with (*S*)-(−)-MTPA chloride as described for **3** above, to provide the (*S*)-(−)-MTPA ester (**3b**) (1.9 mg, 62%). ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.92 (3H, *s*, 12-CH_3), 0.96 (3H, *s*, 11-CH_3), 1.62 (1H, *dd*, $J = 14.3$, 4.3 Hz,2-Hα), 1.64 (3H, *s*, 13-CH_3), 1.94 (1H, *dd*, $J = 14.3$, 5.9 Hz,2-Hβ), 2.26 (3H, *s*, 10-CH_3), 2.47 (1H,*d*, $J = 9.9$ Hz, 6-H), 3.54 (3H, *s*, OCH_3), 5.57 (1H, *m*, 3-H), 5.59 (1H, *br s*, 4-H), 6.08 (1H, *d*, $J = 15.8$ Hz, 8-H), 6.53 (1H, *dd*, $J = 15.8$, 9.9 Hz, 7-H), 7.41 (3H, *m*), 7.52 (2H, *m*). EI-MS *m/z*: 424 $[M]^+$.

(6*R*,7*E*)-4,7-*Megastigmadien*-3,9-*dione* (**4**). Amorphous powder, $[\alpha]_D$ + 165.0° (MeOH, *c* 0.2). EI-MS *m/z* 206 $[M]^+$. CD (MeOH) Δε − 1.60 (320.0 nm), + 25.8 (244.0 nm), − 23.5 (210.0 nm). The spectral data were identical to those of reported data [8–10].

(6*S*,7*E*)-6-*Hydroxy*-4,7-*megastigmadien*-3,9-*dione* (**5**). Amorphous powder, $[\alpha]_D$ + 142.0° (MeOH, *c* 0.2). EI-MS *m/z* 222 $[M]^+$. CD (MeOH) Δε − 1.95 (318.0 nm), + 27.6 (243.0 nm), − 25.5 (209.5 nm). The spectral data were identical to reported data [11–13].

(3*S*,7*E*)-3-*Hydroxy*-5,7-*megastigmadien*-9-*one* (**6**). Amorphous powder, $[\alpha]_D$ − 76.9° (MeOH, *c* 0.1). EI-MS *m/z* 208 $[M]^+$. The spectral data were identical to reported data [11, 14, 15].

(3*S*,5*R*,6*S*,7*E*)-5,6-*Epoxy*-3-*hydroxy*-7-*megastigmene*-9-*one* (**7**). Amorphous powder, $[\alpha]_D$ − 144.3° (MeOH, *c* 0.2). EI-MS *m/z* 224 $[M]^+$. CD (MeOH) Δε − 10.5 (232.0 nm). The spectral data were identical to reported data [11, 14, 16].

(3*S*,5*R*,7*E*,8*R*)-3,5-*Dihydroxy*-6,7-*megastigmadien*-9-*one* (**8**). Amorphous powder, $[\alpha]_D$ − 48.0° (MeOH, *c* 0.2). EI-MS *m/z* 224 $[M]^+$. CD (MeOH) Δε − 8.50 (252.0 nm), + 9.80 (228.5 nm). The spectral data were identical with those of reported data [17–19].

Trans-ferulic acid (**9**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.79 (3H, *s*), 6.30 (1H, *d*, $J =$ 15.8 Hz), 6.80–7.42 (3H, *m*), 7.64 (1H, *d*, $J = 15.8$ Hz). EI-MS *m/z* 194 $[M]^+$. The spectral data were identical with those of an authentic sample.

Cis-ferulic acid (**10**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.73 (3H, *s*), 5.83 (1H, *d*, $J =$ 12.5 Hz), 6.80–7.60 (3H, *m*), 6.80 (1H, *d*, $J = 12.5$ Hz). EI-MS *m/z* 194 $[M]^+$. The spectral data were identical with those of an authentic sample.

Trans-p-coumaroyl methyl ester (**11**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.38 (3H, *s*), 4.07 (2H, *br d*, $J = 6.0$ Hz), 6.14 (1H, *dt*, $J = 16.2$, 6.0 Hz), 6.54 (1H, *d*, $J = 16.2$ Hz), 6.78 (2H, *d*, $J = 8.9$ Hz), 7.28 (2H, *d*, $J =$ 8.9 Hz). EI-MS *m/z* 164 $[M]^+$. The spectral data were identical to those of an authentic sample.

Umbelliferone (**12**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 6.26 (1H, *d*, $J = 9.6$ Hz), 6.79 (1H, *dd*, $J = 8.3$, 2.3 Hz), 6.82 (1H, *d*, $J = 2.3$ Hz), 7.36 (1H, *d*, $J = 8.3$ Hz), 7.64 (1H, *d*, $J = 9.6$ Hz). EI-MS *m/z* 162 $[M]^+$. The spectral data were identical to those of an authentic sample.

Veratric acid (**13**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.89 (3H, *s*), 3.95 (3H, *s*), 6.94 (1H, *d*, $J = 8.2$ Hz), 7.55 (1H, *d*, $J = 2.0$ Hz), 7.64 (1H, *dd*, $J =$ 8.2, 2.0 Hz). EI-MS *m/z* 182 $[M]^+$. The spectral data were identical to those of an authentic sample.

p-Hydroxybenzaldehyde (**14**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 6.96 (2H, *d*, $J = 8.6$ Hz), 7.81 (2H, *d*, $J = 8.6$ Hz), 9.88 (1H, *s*). EI-MS *m/z* 122 $[M]^+$. The spectral data were identical with those of an authentic sample.

p-Hydroxybenzoic acid (**15**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 6.91 (2H, $J = 9.2$ Hz), 8.18 (2H, *d*, $J = 9.2$ Hz). EI-MS *m/z* 138 $[M]^+$. The spectral data were identical with those of an authentic sample.

p-Methoxybenzoic acid (**16**). Amorphous powder, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.88 (3H, *s*), 6.85 (2H, *d*, $J = 8.9$ Hz), 7.96 (2H, *d*, $J = 8.9$ Hz). EI-MS *m/z* 152 $[M]^+$. The spectral data were identical to those of an authentic sample.

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