SHORT REPORT

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Thymol Derivatives from *Eupatorium fortunei*

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Abstract: The presence of thymol derivatives in the leaves and twigs of *E. fortunei* collected in Northern Vietnam was investigated. Five thymol derivatives, including new 9-*O*-angeloxy-10-hydroxy-8-methoxythymol, 10-acetoxy-9-chloro-8,9-dehydrothymol, 9-acetoxy-8,10-epoxythymol-3-*O*-isobutyrate, 8,10-epoxy-9-hydroxy-thymol-3-*O*-tiglate, and 9-acetoxy-8,10-epoxythymol-3-*O*-angelate together with six other compounds, including taraxasteryl acetate, *o*-coumaric acid, *trans*-melilotoside, (–)-loliolide, coumarin, and *β*-sitosterol were isolated. The structures of the isolated compounds were determined on the basis of MS and NMR spectroscopic data.

Keywords: *Eupatorium fortunei*; Asteraceae; thymol derivative.

1. Plant Source

Eupatorium fortunei Turcz. (*syn. E. staechadosmum* Hance) of the family Asteraceae has been recorded as a medicinal plant under the name Man tuoi in Vietnam. The plant has been described in remedies for digestive benefits and for the treatment of fever [1].

The leaves and twigs of *E. fortunei* were collected in Gia Lam district, Hanoi, Vietnam, in December 2015. The plant material was identified by Dr. Nguyen Thi Kim Thanh, Faculty of Biology, VNU University of Science, Vietnam National University, Hanoi, Vietnam. A voucher sample (No. EF-12-15) has been deposited at the Laboratory of Chemistry of Natural Products, Faculty of Chemistry, VNU University of Science, Vietnam National University, Hanoi, Vietnam.

2. Previous Studies

Phytochemical reports showed the occurrence of monoterpenoids, sesquiterpenoids, triterpenoids, thymol derivatives, benzofuran derivatives, flavonoids, trihydroxypiperidines, pyrrolizidine alkaloids, and an acetophenone from different plant collections in Vietnam [2, 3], China [4-8], and Japan [9-12]. Concerning pharmacological studies, antiviral activity against RNA viruses [13], reduced metastatic and angiogenic potency of malignant cancer *via* suppression of MMP-9 activity and VEGF production [14] of the aqueous extract of *E. fortunei* were reported. Thymol

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derivatives have been isolated from *Eupatorium* species, particularly *E. fortunei* [3, 15], *E. glechonophyllum* [15], *E. kiirunense* [15], and *E. cannabium* [16, 17]. They have been reported to exhibit cytotoxic activity against human cancer cell lines [5, 17], inhibitory activity on LPS-induced NO production [8], anti-inflammatory activity by suppressing fMLP/CB(formyl-L-methionine-L-leucyl-L-phenylalanine/cytochalasin B)-induced elatase release [16], and antibacterial activity [18].

3. Present Study

The dried powdered leaves (3.0 kg) or dried twigs (1.4 kg) of *E. fortunei* were extracted with MeOH at room temperature (three times, each time for 7 days). The combined MeOH extract from the leaves or the twigs was concentrated under reduced pressure. The residue was successively partitioned between water and organic solvents of increasing polarities to give *n*-hexane- (120 g and 20.5 g, respectively), CH_2Cl_2- (12 g and 41.5 g), and EtOAc- (12 g and 2.4 g) soluble fractions. *Separation of the leaf soluble fractions*: Part of the *n*-hexane-soluble fraction (55 g) was separated by CC on silica gel eluted with *n*-hexane-acetone 90:1, 49:1, 29:1, 19:1, 6:1, 3:1, 1:1 to give twelve fractions. Fractions 2 (225.6 mg) and 3 (2.24 g) were washed with *n*-hexane to give **2** (586.8 mg). Fractions 7 (896 mg) and 8 (145.5 mg) were washed with acetone to give **3** (200.2 mg). Fraction 11 was separated by RP-18 CC with MeOH-H_2O 4:1, 9:1 and further purified by: 1) silica gel CC, *n*-hexane-EtOAc 29:1, 19:1, 6:1, 3:1, 1:1 and 2) preparative TLC, *n*-hexane-EtOAc 3:1 to give **1** (3 mg) and **4** (5 mg). The CH_2Cl_2-soluble fraction (12 g) was subjected to silica gel column chromatography eluted with *n*-hexane-acetone 70:1, 49:1, 29:1, 19:1, 15:1, 12:1 to give seven fractions. Fraction 2 (412 mg) was separated by silica gel CC, CH_2Cl_2-acetone 70:1, 49:1, 29:1, 19:1, 13:1, 12:1, and further purified by: 1) RP-18 CC with MeOH-H_2O 7:3, 9:1 and 2) silica gel CC with *n*-hexane-EtOAc 15:1, 12:1, 9:1, 6:1, 3:1 to give **5** (5 mg). Part of the EtOAc-soluble fraction (6 g) was separated by Sephadex LH-20 CC, MeOH, and further purified by: 1) silica gel CC, *n*-hexane-EtOAc-HCO_2H 20:5:1, 20:10:1, 20:19:1; 2) silica gel CC, 20:1:0.1, 20:5:0.1, 20:5:0.4, 20:5:1; and 3) silica gel CC, *n*-hexane-EtOAc-HCO_2H 20:5:0.1, 20:5:0.4, 20:5:0.8 to give **6** (12 mg). The water phase was concentrated under reduced pressure and the residue was subjected to RP-18 CC, MeOH-H_2O 4:1, 9:1, and further purified by: 1) silica gel CC, *n*-hexane-EtOAc-HCO_2H 10:10:1, 10:20:1, 10:40:1 and 2) silica gel CC, CH_2Cl_2-MeOH 29:1, 19:1, 15:1, 12:1, 9:1, 6:1, 3:1 to afford **7** (2.8 mg). *Separation of the twig soluble fractions*: The *n*-hexane-soluble fraction (20.5 g) was chromatographed on silica gel, *n*-hexane-acetone 90:1, 49:1, 29:1, 19:1, 9:1, 6:1, 3:1, 1:1 to give eleven fractions. Fraction 7 was purified by: 1) silica gel CC, *n*-hexane-EtOAc 99:1, 70:1, 50:1 and 2) silica gel CC, *n*-hexane-CH_2Cl_2 99:1, 70:1 to give **8** (4.5 mg) and a mixture of **9** and **10** (4.5 mg). The EtOAc-soluble fraction (2.38 g) was subjected to Sephadex LH-20 CC, MeOH. Further purification by: 1) silica gel CC, *n*-hexane-EtOAc 49:1, 29:1, 19:1, 9:1; 2) silica gel CC, *n*-hexane-EtOAc 99:1, 70:1; and 3) preparative TLC, *n*-hexane-EtOAc-HCO_2H 6:1:0.1 gave **11** (6.5 mg).

*9-O-Angeloxy-10-hydroxy-8-methoxythymol (**1**):* White amorphous powder; $[\alpha]_D^{27}$ 0.0 (*c* = 0.13, $CHCl_3$); IR ν_{max} (film): 3396, 1703, 1697, 1649, 1629, 1462, 1231, 1159, 1045 cm^{-1}; HRESIMS (positive-ion mode): *m/z* 317.13605 $[M + Na]^+$ (calcd. 317.13594 for $C_{16}H_{22}O_5Na$).

The structures of the known compounds, taraxasteryl acetate (**2**) [19], *β*-sitosterol (**3**) [20], 10-acetoxy-9-chloro-8,9-dehydrothymol (**4**) [21], (–)-loliolide (**5**) [22], *o*-coumaric acid (**6**) [23], *trans*-melilotoside (**7**) [24], 9-acetoxy-8,10-epoxythymol-3-*O*-isobutyrate (**8**) [25], 9-acetoxy-8,10-epoxythymol-3-*O*-angelate (**9**) [2], 8,10-epoxy-9-hydroxythymol-3-*O*-tiglate (**10**) [11], and coumarin (**11**) [26] (Fig. 1) were determined by comparing their spectroscopic data (MS, ^1H, and ^{13}C NMR) with reported literature values. The occurrence of **4**, **5**, and **7** in *E. fortunei* is reported for the first time in the present work.

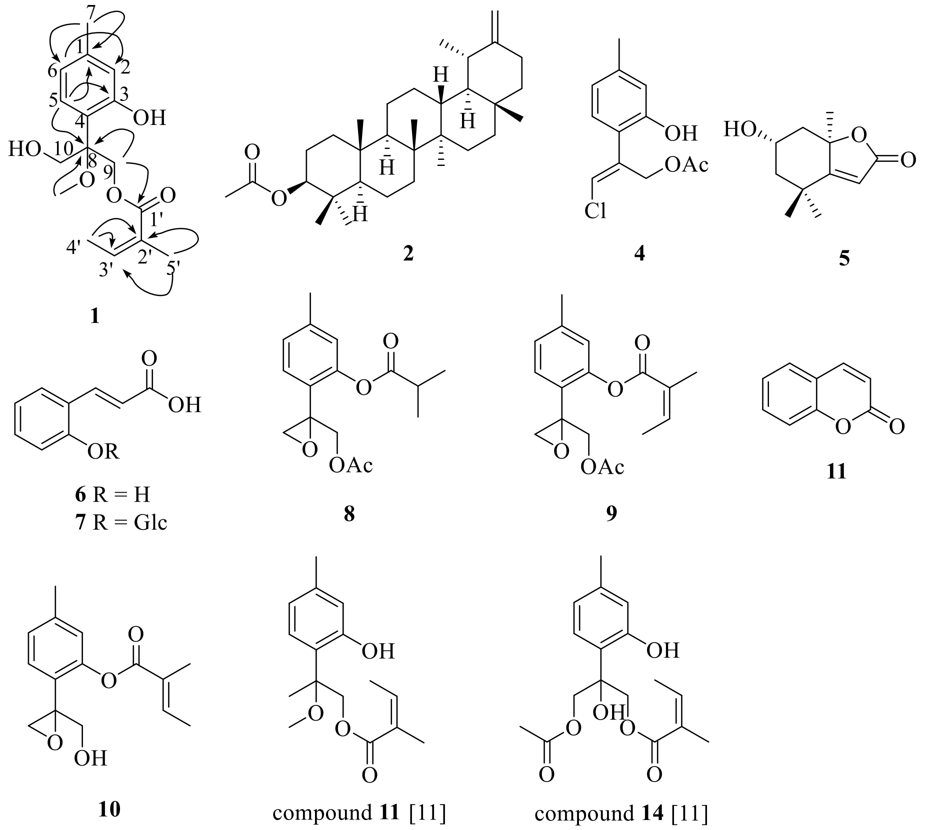

Figure 1. Chemical structures of compounds **1**, **2**, **4-11** and HMBC correlations of **1**

Compound **1** was isolated as a white amorphous powder, $[\alpha]_D^{27}$ 0.0 (*c* 0.13, $CHCl_3$). The molecular formula of **1** was determined to be $C_{16}H_{22}O_5Na$ by positive-ion HRESIMS (*m/z* 317.13605 $[M + Na]^+$). The IR spectrum showed absorption bands for a hydroxy group at ν_{max} 3396 cm^{-1}, an ester carbonyl group at ν_{max} 1703 and 1231 cm^{-1}, and an aromatic ring at ν_{max} 1697, 1462, and 1159 cm^{-1}. The ^1H and ^{13}C NMR spectra of **1** showed signals for a ten-carbon thymol skeleton, a methoxy group [δ_H 3.37 (3H, s); δ_C 51.4], and an angeloyloxy moiety [δ_H 1.88 (3H, s), 1.97 (3H, d, *J* = 7.0 Hz), 6.12 (1H, q, *J* = 6.0 Hz); δ_C 15.8, 20.5, 127.1, 139.8, and 168.0] [8]. Three oxygenated carbons were observed. The signal at δ_C 82.7 was attributed to a tertiary oxygenated carbon, and the signals at δ_C 63.3 [δ_H 3.92 (d, *J* = 12.0 Hz), 3.97 (d, *J* = 12.0 Hz)] and 62.9 [δ_H 4.62 (d, *J* = 12.0 Hz), 4.70 (d, *J* = 12.0 Hz)] were attributed to a hydroxymethyl group and an angeloyloxymethyl group, respectively. The presence of an aromatic methyl group at δ_H 2.29 (3H, s); δ_C 21.0 indicated the locations of the tertiary oxygenated carbon and two oxymethyl groups at C-8, and C-9 and C-10, respectively. Their locations were confirmed by ^1H-^{13}C long-range correlations in the HMBC spectrum (Fig. 1) between H-5 (δ_H 6.90) and C-8 (δ_C 82.7), between H_2-9 (δ_H 4.62/4.70) and C-8, between H_2-9 and C-1′ (δ_C 168.0), and between 8-OCH_3 (δ_H 3.37) and C-8. Full assignment of the ^1H and ^{13}C NMR signals was established on the basis of ^1H-^1H COSY, HSQC, and HMBC correlations. It has been reported that thymol derivatives possessing a chiral center at C-8 from *E. fortunei* may exist as racemic mixtures [11]. Having a chiral center at C-8, compound **1** showed no optical rotation and could be present in its racemic form.

Table 1. NMR spectroscopic data of **1** and reference compounds [11] (δ in ppm, J in Hz)

Position	**1** ($CDCl_3$)		Compound **11** ($CDCl_3$) [11]		Compound **14** ($CDCl_3$) [11]	
	δ_C (125 MHz)	δ_H (500 MHz)	δ_C (100 MHz)	δ_H (400 MHz)	δ_C (100 MHz)	δ_H (400 MHz)
1	140.5		140.1		140.1	
2	118.2	6.74 *br s*	117.8	6.70 *br s*	118.6	6.70 *d* (1.1)
3	156.1		156.1		156.6	
4	119.9		121.0		118.9	
5	127.4	6.90 *d* (7.5)	127.5	6.93 *d* (7.7)	126.3	6.92 *d* (8.1)
6	120.9	6.68 *br d* (7.5)	120.7	6.67 *br d* (7.7)	120.5	6.65 *dd* (8.1, 1.1)
7	21.0	2.29 *s*	21.0	2.29 *s*	20.9	2.27 *s*
8	82.7		81.2		78.5	
9	62.9	4.62 *d* (12.0)	68.2	4.28 *d* (11.7)	67.3	4.47 *s*
		4.70 *d* (12.0)		4.43 *d* (11.7)		4.47 *s*
10	63.3	3.92 *d* (12.0)	20.6	1.67 *s*	67.6	4.51 *d* (12.1)
		3.97 *d* (12.0)				4.54 *d* (12.1)
1'	168.0		167.4		168.1	
2'	127.1		127.5		126.9	
3'	139.8	6.12 *q* (7.0)	138.6	6.07 *qq* (7.3, 1.5)	140.2	6.13 *qq* (5.9, 1.5)
4'	15.8	1.97 *d* (7.0)	15.7	1.95 *dq* (7.3, 1.5)	15.8	1.92 *dq* (7.3, 1.5)
5'	20.5	1.88 *s*	20.6	1.88 *dq* (1.5, 1.5)	20.4	1.85 *dq* (1.5, 1.5)
OCH_3	51.4	3.37 *s*	50.8	3.29 *s*		
$CH_3\underline{C}O$					171.2	
$\underline{C}H_3CO$					20.7	2.07 *s*

Compounds **2**, **5-7** were tested in an agar diffusion assay [27] for their antifungal and antibacterial properties toward *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25923), *Bacillus subtillis* (ATCC 11774), *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Aspergillus niger* (439), *Fusarium oxysporum* (M42), *Candida albicans* (ATCC 7754) and *Saccharomyces cerevisiae* (SH 20). No activity was observed at concentration of 50 μg/mL (**5**) or 100 μg/mL (**2**, **6**, and **7**).

More than 300 compounds were reported from the genus *Eupatorium* including flavonoids; monoterpenoids; guaiane, germacrane, and cadinane sesquiterpenoids; *ent*-labdane and *ent*-kaurane diterpenoids; taraxasterane, dammarane, epifriedelane, ursane triterpenoids; pyrrolizidine alkaloids; phenylpropanoids; and quinonoids [15]. These diverse compounds form the chemical profile of *Eupatorium* species. Compounds isolated from the collection in Vietnam are in accordance with the *Eupatorium* chemical profile. The nor-terpene hydroxylactone (–)-loliolide (**5**) occurs in many plants and marine organism but has not been reported from any *Eupatorium* species. Chen *et al.* reported eudesmane sesquiterpenes and menthane monoterpenes as chemotaxonomic important compounds for *E. fortunei* [7]. However, eudesmanes were also isolated from some *Eupatorium* species such as *E. quadrangulare* [28] and *E. cannabium* [29]. Thymol derivatives predominantly occur in *E. fortunei*, some of which may be considered as species-specific substances for the identification of *E. fortunei* among *Eupatorium* species judging from their plant-dependent characteristic oxygenated patterns. (–)-Loliolide (**5**) may also be considered as a useful marker to distinguish *E. fortunei* from the other *Eupatorium* species.

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Supporting Information

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