

Leishmanicidal Active Constituents from Nepalese Medicinal Plant Tulsi (*Ocimum sanctum* L.)

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Received September 15, 2008; accepted November 11, 2008; published online December 8, 2008

In the course of screening leishmanicidal active compounds from Asian and South American medicinal plants, a Nepalese medicinal plant, Tulsi (*Ocimum sanctum* L.), showed strong activity. We therefore studied the isolation and structural elucidation of the active constituents from *O. sanctum* L. From the ethyl acetate soluble fraction of the plant, seven new novel neolignan derivatives were isolated along with 16 known compounds. The structures of the new compounds (1–7) were elucidated as 6-allyl-3',8-dimethoxy-flavan-3,4'-diol (1), 6-allyl-3-(4-allyl-2-methoxyphenoxy)-3',8-dimethoxyflavan-4'-ol (2), 5-allyl-3-(4-allyl-2-methoxyphenoxy)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (3), 1,2-bis(4-allyl-2-methoxyphenoxy)-3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropane (4), 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-tris(4-allyl-2-methoxyphenoxy)propane (5), 1-allyl-4-(5-allyl-2-hydroxy-3-methoxyphenoxy)-3-(4-allyl-2-methoxyphenoxy)-5-methoxybenzene (6), and 3-(5-allyl-2-hydroxy-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenoxy)-prop-1-ene (7) by means of ¹H-NMR, ¹³C-NMR, and 2D-NMR spectral data. Some of these compounds showed leishmanicidal activity.

Key words *Ocimum sanctum*; Labiatae; leishmanicidal activity; neolignan; eugenol oligomer

Leishmaniasis are a group of tropical diseases caused by a number of species of protozoan parasites belonging to the genus *Leishmania* that survive and multiply in macrophages in the mammalian host and are transmitted by female flying insects of genera *Phlebotomus* and *Lutzomyia*. Currently, 12 million people in developing countries are affected by the disease annually.^{1,2} In many cases, the medicines employed for treatment are toxic, only slightly effective, given by injection, and compromised by the development of resistance.³ Since safe, effective, and affordable drugs for leishmaniasis are needed in developing countries, there have been many studies on leishmanicidal constituents from plant sources,^{4–6} but there are as yet no safe, effective, and reasonably priced drugs. In our project, sesquiterpene lactone⁷ and steroid saponins⁸ were isolated as leishmanicidal constituents. We have been testing tropical medicinal plants for developing lead compounds for leishmanicidal medicines. Of these testing samples, the Nepalese traditional crude drug, Tulsi (*Ocimum sanctum* L.), was identified as a potent leishmanicidal sample. Seven new neolignan derivatives were isolated from the sources, derived by polymerization of eugenol. This paper deals with the isolation and structural elucidation of new novel neolignan derivatives and the leishmanicidal activity of compounds isolated from *O. sanctum* L.

Results and Discussion

Methanol (MeOH) extract of *O. sanctum* L. was partitioned with ethylacetate (AcOEt) and *n*-butanol (*n*-BuOH) to give AcOEt, *n*-BuOH, and aqueous fractions. Active AcOEt fraction was separated by successive chromatographies, as described in the Experimental section, to give seven new compounds (1–7) and 16 known compounds (8–23) (Fig. 1). The structures of the known compounds were determined by data from MS and NMR spectra as follows:

eugenol (8), citrusin C (9),⁹ ferulaldehyde (10), bieugenol (11),¹⁰ dehydrodieugenol B (12),¹¹ oleanolic acid (13), ulsoric acid (14), stigmasterol (15),¹² β -sitosterol-3-*O*- β -D-glucopyranoside (16), caryophyllene oxide (17),¹³ apigenin (18),¹⁴ luteolin (19),¹⁵ crysoeriol (20),¹⁶ 4',5-dihydroxy-7,8-dimethoxyflavone (21),¹⁷ 4',5-dihydroxy-3',7,8-trimethoxyflavone (22),¹⁸ and vanillin (23).

Compound 1 was obtained as a colorless amorphous powder. HR-ESI-MS of 1 showed a pseudomolecular ion at *m/z* 365.1371 [M+Na]⁺ C₂₀H₂₂O₅Na, which accorded to the molecular formula C₂₀H₂₂O₅. The IR spectrum of 1 showed absorptions at 3433, 2361, 1495, 1272 cm⁻¹. The UV spectrum of 1 showed absorption at 238 (ϵ 10600) and 279 (ϵ 7100) nm. The ¹H-NMR spectrum of 1 showed the presence of two methoxy groups [δ_{H} 3.83 (3H, s), 3.88 (3H, s)], one allyl group [δ_{H} 3.31 (2H, br d, *J*=6.8 Hz), 5.96 (1H, ddt, *J*=6.6, 10.3, 17.1 Hz), 5.07 (1H, br d, *J*=10.3 Hz), 5.10 (1H, br d, *J*=17.1 Hz)], 3,4-dioxygenated phenyl group [δ_{H} 6.95 (1H, d, *J*=1.7 Hz), 6.91 (1H, d, *J*=8.1 Hz), 6.93 (1H, dd, *J*=8.1, 1.7 Hz)], two *m*-coupled protons [δ_{H} 6.58 (1H, d, *J*=1.5 Hz), 6.54 (1H, d, *J*=1.5 Hz)], two oxygen-bearing methine groups [δ_{H} 4.76 (1H, d, *J*=7.8 Hz), 4.11 (1H, dt, *J*=5.8, 8.1 Hz)], and a methylene group [δ_{H} 2.89 (1H, dd, *J*=8.8, 16.1 Hz), 3.06 (1H, dd, *J*=5.4, 16.1 Hz)]. The ¹³C-NMR data of 1 showed the presence of two secondary oxygen-bearing methine carbons (δ_{C} 82.0, 68.1), two methylene carbons (δ_{C} 32.7, 39.8), and 14 *sp*² carbons (Table 2). This indicated that 1 has a flavan-3-ol skeleton having two methoxy groups, an allyl group, and a hydroxyl group. The positions of functional groups were determined by heteronuclear multiple bond connectivity (HMBC) experiments of 1, as shown in Fig. 2. H-7' (δ_{H} 4.76, d, *J*=7.8 Hz) showed a correlation to C-4, 2', 6', 8', and 9' (δ_{C} 141.8, 109.5, 120.4, 68.1, 32.7); H-8' (δ_{H} 4.11, dt, *J*=5.1, 8.1 Hz) to C-5 and 1' (δ_{C} 120.7,

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Table 1. ¹H-NMR Data of Compounds 1–7 (in CDCl₃, 500 MHz)

Position	1	2	3	4	5	6	7
H-2	6.58 (1H, d, 1.5)	6.59 (1H, d, 2.0)	6.65 (1H, br s)	7.05 (1H, d, 1.7)	7.17 (1H, d, 1.7)	6.75 (1H, d, 1.7)	6.57 (1H, d, 1.5)
5				6.85 (1H, d, 8.1)	6.82 (1H, d, 8.1)	6.80 (1H, d, 8.1)	
6	6.54 (1H, d, 1.5)	6.49 (1H, d, 2.0)	6.76 (1H, br s)	6.91 (1H, dd, 8.1, 1.7)	7.01 (1H, dd, 8.1, 1.7)	6.67 (1H, dd, 8.1, 1.7)	6.61 (1H, br s)
7	3.31 (2H, br d, 6.8)	3.30 (2H, br d, 5.1)	3.33 (2H, br d, 5.4)	4.62 (1H, d, 4.9)	5.54 (1H, d, 4.6)	3.35 (2H, d, 6.6)	3.30 (2H, d, 6.6)
8	5.96 (1H, ddd, 17.1, 10.3, 6.6)	5.89–5.96 (1H, overlap)	5.95 (1H, overlap)	4.53 (1H, dt, 5.1, 4.9)	4.79 (1H, q, 4.9)	5.84–5.98 (1H, overlap)	5.94 (1H, ddt, 16.8, 10.0, 6.6)
9	5.07 (1H, br d, 10.3)	5.03–5.10 (2H, overlap)	5.07 (2H, overlap)	3.91 (1H, dd, 10.3, 4.9)	3.96 (1H, dd, 10.3, 5.4)	4.98–5.10 (2H, overlap)	5.04 (1H, br d, 10.0)
	5.10 (1H, br d, 17.1)			4.26 (1H, dd, 10.3, 5.1)	4.46 (1H, dd, 10.3, 4.4)		5.07 (1H, br d, 17.6)
2'	6.95 (1H, d, 1.7)	6.89 (1H, d, 1.7)	6.98 (1H, d, 2.2)	6.67 (1H, d, 1.7)	6.64 (1H, overlap)	6.51 (1H br s)	6.55 (1H, d, 2.7)
5'	6.91 (1H, d, 8.1)	6.83 (1H, d, 8.1)	6.86 (1H, d, 8.8)	6.71 (1H, d, 8.1)	6.75 (1H, d, 7.8)		6.81 (1H, d, 8.8)
6'	6.93 (1H, dd, 8.1, 1.7)	6.91 (1H, dd, 8.1, 1.7)	6.97 (1H, dd, 8.8, 2.2)	6.61 (1H, dd, 8.1, 1.7)	6.64 (1H, overlap)	6.30 (1H, d, 1.7)	6.49 (1H, ddt, 8.8, 2.7)
7'	4.76 (2H, d, 7.8)	5.23 (1H, d, 6.4)	5.66 (2H, br d, 6.6)	3.30 (2H, br d, 3.9)	3.29 (2H, br d, 6.1)	3.26 (2H, d, 6.6)	6.44 (1H, d, 12.0)
8'	4.11 (1H, dt, 5.8, 8.1)	4.65 (1H, q, 6.4)	3.93 (1H, m)	5.92 (1H, ddt, 16.9, 10.3, 1.8)	5.90 (1H, overlap)	5.84–5.98 (1H, overlap)	5.46 (1H, dt, 12.0, 7.3)
9'	2.88 (1H, dd, 16.1, 8.8)	3.04 (2H, d, 5.4)	4.16 (1H, t, 8.6)	5.05 (2H overlap)	5.00–5.08 (2H, overlap)	4.98–5.10 (2H, overlap)	3.33 (2H, d, 7.3)
	3.06 (1H, dd, 16.1, 5.8)		4.27 (1H, dd, 9.5, 5.9)				
2''		6.66 (1H, d, 1.7)	6.71 (1H, d, 2.0)	6.65 (1H, d, 2.0)	6.64 (1H, overlap)	6.40 (1H, d, 1.5)	
5''		6.63 (1H, d, 8.1)	6.80 (1H, d, 8.3)	6.87 (1H, d, 8.1)	7.00 (1H, d, 7.8)		
6''		6.58 (1H, dd, 8.1, 1.7)	6.68 (1H, dd, 8.3, 2.0)	6.63 (1H, dd, 8.1, 2.0)	6.64 (1H, overlap)	6.51 (1H, br s)	
7''		3.29 (2H, br d, 6.1)	3.32 (2H, br d, 6.1)	3.30 (2H, br d, 4.9)	3.30 (2H, br d, 5.1)	3.20 (2H, overlap)	
8''		5.89–5.96 (1H, overlap)	5.95 (1H, overlap)	5.93 (1H, ddt, 16.9, 10.3, 6.6)	5.90 (1H, overlap)	5.84–5.98 (1H, overlap)	
9''		5.03–5.15 (2H, overlap)	5.05–5.11 (2H, overlap)	5.05 (2H, overlap)	5.00–5.08 (2H, overlap)	4.98–5.10 (2H, overlap)	
2'''				6.64 (1H, overlap)	6.64 (1H, overlap)		
5'''				6.74 (1H, d, 8.1)	6.74 (1H, d, 8.1)		
6'''				6.52 (1H, dd, 8.1, 1.7)	6.52 (1H, dd, 8.1, 1.7)		
7'''				3.25 (2H, br d, 6.6)	3.25 (2H, br d, 6.6)		
8'''				5.90 (1H, overlap)	5.90 (1H, overlap)		
9'''				5.00–5.08 (2H, overlap)	5.00–5.08 (2H, overlap)		
3'-OMe	3.83 (3H, s)	3.89 (3H, s)	3.89 (3H, s)	3.79 (3H, s)	3.78 (3H, s)	3.74 (3H, s)	3.86 (3H, s)
3''-OMe	3.88 (3H, s)	3.77 (3H, s)	3.84 (3H, s)	3.80 (3H, s)	3.76 (3H, s)	3.82 (3H, s)	3.85 (3H, s)
3'''-OMe		3.75 (3H, s)	3.82 (3H, s)	3.76 (3H, s)	3.74 (3H, s)	3.84 (3H, s)	
3''''-OMe							
7-OMe				3.30 (3H, s)	3.73 (3H, s)		

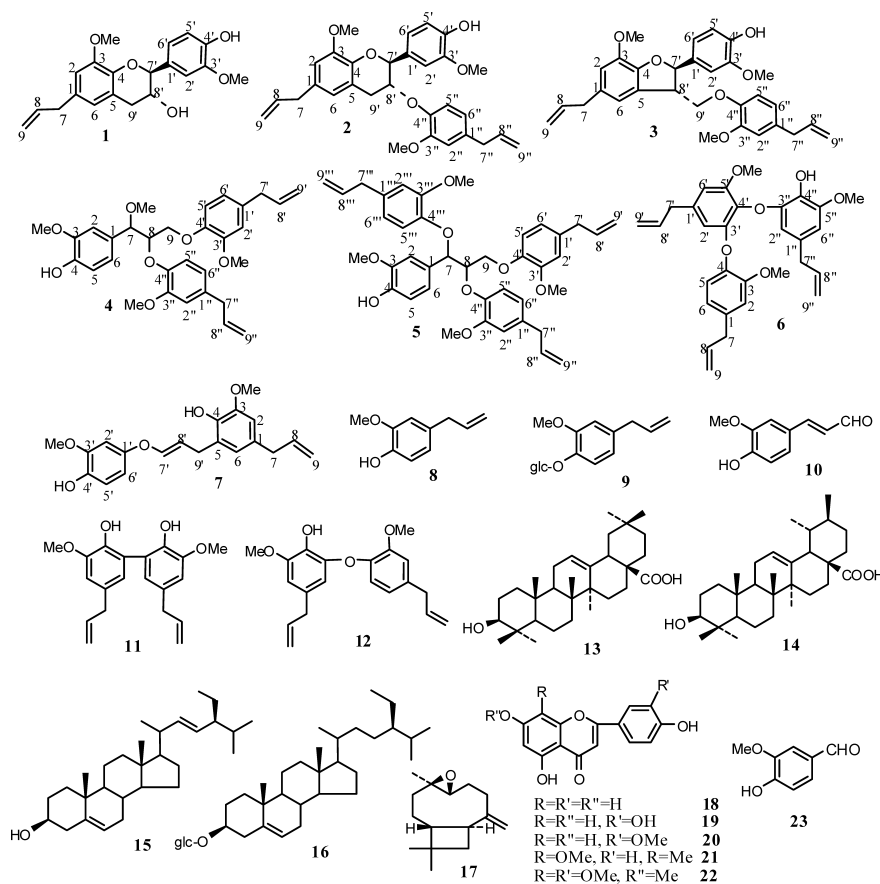


Fig. 1. Structures of Constituents Isolated from *O. sanctum*

129.7); H-6 (δ_{H} 6.54, d, $J=1.5$ Hz) to C-2, 4, 7, and 9' (δ_{C} 110.2, 141.8, 39.8, 32.7); OMe at C-3 (δ_{H} 3.83, s) to C-3 (δ_{C} 148.1); OMe at C-3' (δ_{H} 3.88, s) to C-3' (δ_{C} 146.8); H-5' (δ_{H} 6.91, d, $J=8.1$ Hz) to C-1' and 3' (δ_{C} 129.7, 146.8); H-2' (δ_{H} 6.95, $J=1.7$ Hz) and H-6' (δ_{H} 6.93, dd, $J=8.1, 1.7$ Hz) to C-4' and 7' (δ_{C} 146.0, 82.0), and so on, as shown in Fig. 2. These data indicated that **1** was 6-allyl-3',8-dimethoxy-flavan-3,4'-diol, and the compound was named tulsinol A. The relative configuration between C-7' and 8' resulted in *trans* from the coupling constant of H-7' with H-8' ($J=7.8$ Hz).¹⁹ The absolute configuration of **1** was not studied because of low optical rotation ($[\alpha]_{\text{D}} +1.5^{\circ}$) and no Cotton effect in circular dichroism (CD) spectrum of **1**.

Compound **2** was obtained as a pale yellow viscous oil. HR-ESI-MS of **2** showed a pseudomolecular ion at m/z 511.2113 $[\text{M}+\text{Na}]^+$ $\text{C}_{30}\text{H}_{32}\text{O}_6\text{Na}$, which accorded to the molecular formula $\text{C}_{30}\text{H}_{32}\text{O}_6$. The IR spectrum of **2** showed absorptions at 3444, 2935, 1594, and 1269 cm^{-1} . The UV spectrum of **2** showed absorption at 234 (ϵ 13300) and 279 (ϵ 6100) nm. The ^1H -NMR spectrum of **2** showed a similar signal pattern to that of **1** along with a further eugenol unit [δ_{H} 3.75 (3H, s), 3.29 (2H, br d, $J=6.1$ Hz), 5.03–5.10 (2H, m), 5.89–5.96 (1H, m), 6.58 (1H, dd, $J=8.1, 1.7$ Hz), 6.63 (1H, d, $J=8.1$ Hz), and 6.66 (1H, d, $J=1.7$ Hz)]. The ^{13}C -NMR spectrum of **2** also showed the signals of **1** and an eugenol unit (δ_{C} 39.8, 55.9, 112.9, 115.7, 119.7, 120.6, 135.1, 137.7, 144.9, 151.1). These data indicated that **2** was a derivative of **1** with one more eugenol unit. The position of the extra eugenol unit in **2** was determined from the HMBC experi-

ment. H-8' (δ_{H} 4.65, 1H, q, 6.4 Hz) showed a correlation to C-4'', C-1'', and C-5 (δ_{C} 144.9, 130.8, 120.4). The relative configuration at C-7' and 8' was determined to be *trans* from the coupling constant ($J=6.4$ Hz) between H-7' and H-8'. Optical rotation of **2** gave a low value ($[\alpha]_{\text{D}} +1.5^{\circ}$) and the CD spectrum of **2** showed no Cotton effect, indicating the possibility of low optical purity in **2**; therefore, the absolute configuration of **2** was not studied. The structure was determined to be 6-allyl-3-(4-allyl-2-methoxyphenoxy)-3',8-dimethoxyflavan-4'-ol, and the compound was named tulsinol B.

Compound **3** was obtained as a pale yellow viscous oil. HR-ESI-MS of **3** showed a pseudomolecular ion at m/z 511.2119 $[\text{M}+\text{Na}]^+$ $\text{C}_{30}\text{H}_{32}\text{O}_6\text{Na}$, which accorded to the molecular formula $\text{C}_{30}\text{H}_{32}\text{O}_6$. $[\alpha]_{\text{D}} +4.3$ ($c=0.03$, MeOH). The IR spectrum of **3** showed absorptions at 3427, 2936, 1604, and 1267 cm^{-1} . The UV spectrum of **3** showed absorption at 215 (ϵ 10300), 229 (ϵ 9100), and 281 (ϵ 3500) nm. The ^1H -NMR spectrum of **3** showed the presence of two allyl groups [δ_{H} 3.32 (2H, d, $J=6.1$ Hz), 3.33 (2H, d, $J=5.4$ Hz), 5.92–5.98 (2H, overlap), 5.05–5.11 (4H, overlap)], two 3,4-dioxygenated phenyl groups [δ_{H} 6.75 (1H, d, $J=7.8$ Hz), 7.00 (1H, d, $J=7.8$ Hz), 6.62–6.66 (4H, overlap)], two *m*-coupled H [δ_{H} 6.65 (1H, brs), 6.76 (1H, brs)], a methine group [δ_{H} 3.93 (1H, m)], an oxygen-bearing methine group [δ_{H} 5.65 (1H, d, $J=6.6$ Hz)], and an oxygen-bearing methylene group [δ_{H} 4.16 (1H, t, $J=8.6$ Hz), 4.27 (1H, dd, $J=9.5, 5.9$ Hz)]. The ^{13}C -NMR spectrum of **3** showed 30 carbon signals, which showed that **3** was a trimer of eugenol. The

Table 2. ^{13}C -NMR Data of Compounds 1–7 (in CDCl_3 , 125 MHz)

Position	1	2	3	4	5	6	7
C-1	132.6	132.2	133.8	130.4	130.0	136.7	131.1
2	110.2	110.3	112.6	110.2	110.0	113.2	121.6
3	148.1	148.0	144.1	146.5	146.4	150.8	146.2
4	141.8	141.6	146.5	145.2	145.1	143.1	141.6
5	120.7	120.4	127.7	113.8	113.9	120.4	126.1
6	121.3	121.2	116.9	120.6	120.0	120.8	109.0
7	39.8	39.8	40.1	82.6	80.6	39.9	40.0
8	137.6	137.4	137.8	82.7	82.7	137.3	137.8
9	115.6	115.5	115.7	68.1	68.4	115.9	115.4
1'	129.7	130.8	133.3	134.2	133.1	130.4	151.0
2'	109.5	109.6	108.6	112.7	112.6	106.8	101.3
3'	146.8	146.2	146.4	149.6	150.7	147.6	146.8
4'	146.0	145.3	145.3	146.8	146.8	133.0	141.1
5'	114.5	114.1	114.1	114.3	114.1	146.1	114.2
6'	120.4	119.9	119.2	120.5	120.3	110.7	108.5
7'	82.0	79.2	88.3	39.8	39.7	40.0	143.8
8'	68.1	76.2	51.4	137.6	137.6	136.8	110.2
9'	32.7	30.0	71.7	115.5	115.1	115.4	27.3
1''		135.1	133.4	133.3	134.3	137.4	
2''		119.7	112.7	112.5	112.6	106.8	
3''		151.1	149.8	150.6	150.7	153.2	
4''		144.9	146.5	146.6	146.8	134.4	
5''		112.9	114.3	118.2	118.9	151.1	
6''		120.6	120.5	120.5	120.5	106.9	
7''		39.8	39.8	39.8	39.8	40.1	
8''		137.7	137.5	137.6	137.6	137.7	
9''		115.7	115.6	115.6	115.5	116.1	
1'''					133.4		
2'''					112.7		
3'''					150.0		
4'''					146.1		
5'''					116.5		
6'''					120.6		
7'''					39.9		
8'''					137.7		
9'''					115.5		
3-OMe	55.9	55.8	56.0	55.7	55.7	55.9	55.9
3'-OMe	55.9	55.8	55.8	55.8	55.8	56.2	56.0
3''-OMe		55.9	55.9	55.9	55.8	56.2	
3'''-OMe					55.9		
7-OMe				57.2			

HMBC spectral data showed the following correlations; H-7' (δ_{H} 5.66, d, $J=6.6$ Hz) to C-4, C-5, C-2', C-6', and C-9' (δ_{C} 146.5, 127.7, 108.6, 119.2, 71.7); H-8' (δ_{H} 3.93, 1H, m) to C-4, C-6, and C-1' (δ_{C} 146.5, 116.9, 133.3); H-9' (δ_{H} 4.16, 1H, t, $J=8.6$ Hz, 4.27, 1H, dd, $J=9.5$, 5.9 Hz) to C-5, C-4'', and C-7' (δ_{C} 127.7, 146.5, 88.3) and so on, as shown in Fig. 2. These data showed that **3** has a dihydrobenzofuran skeleton. Thus the structure of **3** was determined to be 5-allyl-3-(4-allyl-2-methoxyphenoxy-methyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran. Stereochemistry at C-7' and 8' was determined as *trans* from observation of the NOE between H-7' and CH_2 -9', and the compound was named tulsinol C.

Compound **4** was obtained as a pale yellow viscous oil. HR-ESI-MS of **4** showed a pseudomolecular ion at m/z 543.2339 $[\text{M}+\text{Na}]^+$ $\text{C}_{31}\text{H}_{36}\text{O}_7\text{Na}$, which accorded to the molecular formula $\text{C}_{30}\text{H}_{32}\text{O}_6$. The IR spectrum of **4** showed absorptions at 3418, 2936, 1670, 1595, and 1270 cm^{-1} . The ^1H -NMR spectrum of **3** showed the presence of three aromatic methoxy groups [δ_{H} 3.76 (3H, s), 3.79 (3H, s), and 3.80 (3H, s)], an aliphatic methoxy group (δ_{H} 3.30, 3H, s), two allyl moieties [δ_{H} 3.30 (4H, br d, $J=6.9$ Hz), 5.92 (1H, ddt,

$J=16.9$, 10.3, 6.8 Hz), 5.93 (1H, ddt, $J=16.9$, 10.3, 6.6 Hz), 5.05 (4H, over lap)], three 3,4-dioxygenated phenyl groups [δ_{H} 7.05 (1H, d, $J=1.7$ Hz), 6.67 (1H, d, $J=1.7$ Hz), 6.65 (1H, d, $J=2.0$ Hz), 6.85 (1H, d, $J=8.1$ Hz), 6.71 (1H, d, $J=8.1$ Hz), 6.87 (1H, d, $J=8.1$ Hz), 5.05 (3H, over lap)], two oxygen-bearing methine groups [δ_{H} 4.62 (1H, d, $J=4.9$ Hz), 4.53 (1H, dt, $J=5.1$, 4.9 Hz)], and an oxygen-bearing methylene group [δ_{H} 3.91 (1H, dd, $J=10.3$, 4.9 Hz), 4.26 (1H, dd, $J=10.3$, 5.1 Hz)]. The ^{13}C -NMR spectrum of **4** showed four methoxy carbons (δ_{C} 55.7, 55.8, 55.9, 57.2), two oxygen-bearing methine carbons (δ_{C} 82.6, 82.7), an oxygen-bearing methylene carbon (δ_{C} 68.1), 18 aromatic carbons, and two allyl groups (δ_{C} 115.5, 115.6, 137.6 \times 2, 39.8 \times 2). These data indicate that **4** is a trimer of eugenol units, as shown in Fig. 1. The structure and position of functional groups in **4** were determined by HMBC experiment as follows. The alcoholic methoxy H (δ_{H} 3.30, 3H, s) showed a correlation to C-7 (δ_{C} 82.6); H-7 (δ_{H} 4.62, 1H, d, $J=4.9$ Hz) to C-1, 6, and 9 (δ_{C} 110.2, 120.6, 68.1); H-8 (δ_{H} 4.53, 1H, q, $J=4.9$ Hz) to C-1 and 4'' (δ_{C} 130.4, 146.6); H-9 (δ_{H} 3.91, 1H, dt, $J=4.9$, 10.3, 4.26, 1H, dd, $J=5.1$, 10.3 Hz) to C-4' and 7 (δ_{C} 146.8, 82.6) and so on, as shown in Fig. 2. Thus the structure of **4** was de-

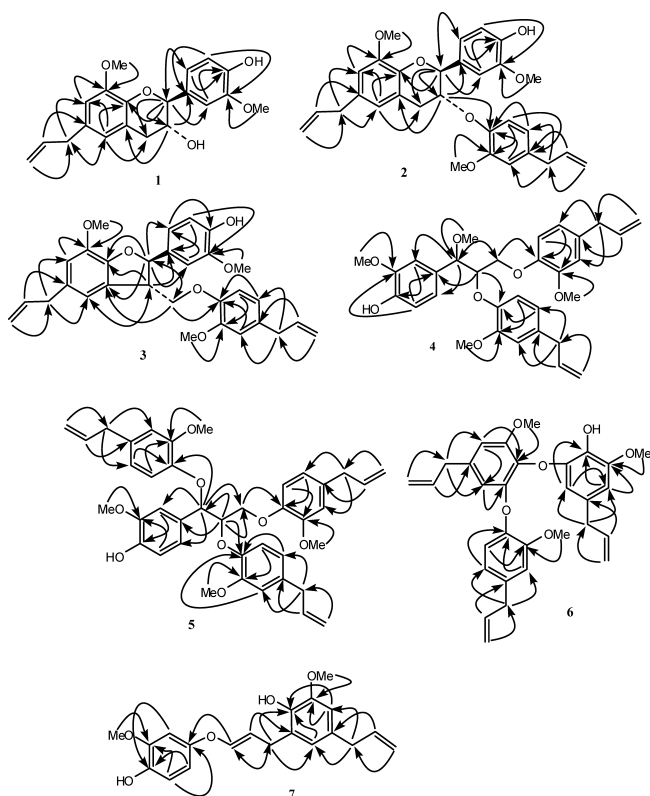


Fig. 2. Key HMBC Correlations of 1–7

terminated to be 1,2-bis(4-allyl-2-methoxyphenoxy)-3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropane, and the compound was named tulsinol D. Stereochemistry at C-7 and 8 was not determined.

Compound **5** was obtained as a pale yellow viscous oil. HR-ESI-MS of **5** showed a pseudomolecular ion at m/z 675.2963 $[M+Na]^+$ $C_{40}H_{44}O_8Na$, which accorded to the molecular formula $C_{40}H_{44}O_8$. The 1H -NMR spectrum of **5** showed the presence of three eugenol units [δ_H 3.73 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 3.25 (2H, br d, $J=6.6$ Hz), 3.29 (2H, d, $J=6.1$ Hz), 3.30 (2H, d, $J=5.1$ Hz), 5.85–5.97 (3H, overlap), 5.00–5.08 (6H, overlap), 6.74 (1H, d, $J=8.1$ Hz), 6.52 (1H, dd, $J=8.1, 1.7$ Hz), 6.75 (1H, d, $J=8.1$ Hz), 7.00 (1H, d, $J=7.8$ Hz), 6.62–6.66 (5H, overlap)], a phenolic methoxy group [δ_H 3.78 (3H, s)], 3,4-dioxyphenyl group [δ_H 6.82 (1H, d, $J=8.1$ Hz), 7.01 (1H, dd, $J=8.1, 1.7$ Hz), 7.17 (1H, d, $J=1.7$ Hz)], two oxygen-bearing methine groups [δ_H 5.54 (1H, d, $J=4.6$ Hz), 4.79 (1H, q, $J=4.9$ Hz)] and an oxygen-bearing methylene group [δ_H 3.96 (1H, dd, $J=10.3, 5.4$ Hz), 4.46 (1H, dd, $J=10.3, 4.4$ Hz)]. These data showed the replacement of the methyl group in **4** to the eugenol moiety in **5**. The ^{13}C -NMR data (in Table 2) also supported the structure of **5**, in which one more eugenol moiety was substituted at C-7. The structure of **5** was confirmed by the HMBC spectrum. The HMBC of **5** showed correlation of H-7 (δ_H 5.54, 1H, d, $J=4.6$ Hz) to C-2, 6, 9, and 4'' (δ_C 110.0, 120.0, 68.4, 146.1); H-8 (δ_H 4.79, 1H, q, $J=4.9$ Hz) to C-1, 7, 9, and 4'' (δ_C 130.0, 80.6, 68.4, 146.8); H-9 (δ_H 3.96, 1H, dd, $J=10.3, 4.5$ Hz, 4.46, 1H, dd, $J=10.3, 4.4$ Hz) to C-4' and 7 (δ_C 146.8, 80.6), and so on, as shown in Fig. 2. Thus the structure of **5** was determined to be 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-tris(4-allyl-2-methoxyphenoxy)propane,

and the compound named tulsinol E. Stereochemistry was not determined.

Compound **6** was obtained as a pale yellow viscous oil. HR-ESI-MS of **6** showed a pseudomolecular ion at m/z 489.2300 $[M+H]^+$ $C_{30}H_{33}O_6$ in HR-ESI-MS, which accorded to the molecular formula $C_{30}H_{33}O_6$. The 1H -NMR spectrum of **6** showed the presence of three allyl moieties, three methoxy groups [δ_H 3.74 (3H, s), 3.82 (3H, s), 3.84 (3H, s)], five *m*-coupled protons [δ_H 6.30 (1H, d, $J=1.7$ Hz), 6.40 (1H, d, $J=1.5$ Hz), 6.51 (2H, br s), 6.75 (1H, d, $J=1.7$ Hz)], an *o*-coupled proton [δ_H 6.80 (1H, d, $J=8.1$ Hz)], and an *o, m*-coupled proton [δ_H 6.67 (1H, dd, $J=8.1, 1.7$ Hz)]. The ^{13}C -NMR spectrum of **6** showed the presence of three methoxy groups (δ_C 55.9, 56.2 \times 2), three allyl moieties (δ_C 115.4, 115.9, 116.1, 136.8, 137.3, 137.7, 39.9, 40.9, 40.1), and 18 aromatic carbons (δ_C 106.8 \times 2, 106.9, 110.7, 113.2, 120.4, 120.8, 130.4, 133.0, 134.4, 136.7, 137.4, 143.1, 146.1, 147.6, 150.8, 151.1, 153.2). These data indicate that **6** is an eugenol trimer. The three eugenol units of **6** were confirmed as two 1,2-dioxy-3-methoxy-5-allylbenzene and a 1-methoxy-2-oxy-5-allylbenzene structures from HMBC experiments, as shown in Fig. 2. Compound **6** should be derived by the phenol oxidation reaction between C-5 and oxygen at C-4 of eugenol. Thus, the structure of **6** was inevitably determined to be 1-allyl-4-(5-allyl-2-hydroxy-3-methoxyphenoxy)-3-(4-allyl-2-methoxyphenoxy)-5-methoxybenzene, and the compound was named tulsinol F.

Compound **7** was obtained as a colorless viscous oil. HR-ESI-MS of **7** showed a pseudomolecular ion at m/z 365.1401 $[M+Na]^+$ $C_{20}H_{22}O_5Na$ in HR-ESI-MS, which accorded to the molecular formula $C_{20}H_{22}O_5$. The 1H -NMR spectrum of **7** showed the presence of an allyl group [δ_H 5.04 (1H, br d, $J=10.0$ Hz), 5.07 (1H, br d, $J=16.8$ Hz), 5.94 (1H, ddt, $J=16.8, 10.0, 6.6$ Hz)], 1,2-dioxyphenoxy moiety [δ_H 6.49 (1H, dd, $J=8.8, 2.7$ Hz), 6.55 (1H, d, $J=2.7$ Hz), 6.81 (1H, d, $J=8.8$ Hz)], two *m*-coupled H [δ_H 6.57 (1H, d, $J=1.5$ Hz), 6.61 (1H, d, $J=1.5$ Hz)], two methoxy groups [δ_H 3.85 (3H, s), 3.86 (3H, s)], and a propene moiety [δ_H 3.33 (2H, d, $J=7.3$ Hz), 5.46 (1H, dt, $J=12.0, 7.3$ Hz), 6.44 (1H, d, $J=12.0$ Hz)]. The connecting pattern of these moieties was determined from the HMBC experiment of **7**. H-7 (δ_H 3.30, 2H, d, $J=6.6$ Hz) showed correlations to C-2, 6, and 9 (δ_C 121.6, 109.0, 115.4); H-2 (δ_H 6.57, 1H, d, $J=1.5$ Hz) to C-3, 4, 6, and 7 (δ_C 146.2, 141.6, 109.0, 40.0); H-9' (δ_H 3.33, 2H, d, $J=7.3$ Hz) to C-4, 6, and 7' (δ_C 141.6, 109.0, 143.8); H-8' (δ_H 5.46, 1H, dt, $J=12.0, 7.3$ Hz) to C-5, 7', and 9' (δ_C 126.1, 143.8, 27.3); H-7' (δ_H 6.44, 1H, d, $J=12.0$ Hz) to C-1' and 9' (δ_C 151.0, 27.3), and so on, as shown in Fig. 2. In the rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiment, H-7' showed a correlation with H-9'. Thus the C-7',8' double bond was an *E* configuration, but the coupling constant ($J=12.0$ Hz) between H-7' and H-8' was small for ordinal *E* configuration. This could be explained from substitution of the electro-negative atom oxygen at C-7'. From these data, the structure of **7** was determined to be 3-(5-allyl-2-hydroxy-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenoxy)propene, and the compound was named tulsinol G.

Compounds **1** and **2** have very interesting structures. They should be synthesized from eugenol through oligomerization, thus they belong to the neolignan group in biosynthetic

Table 3. Leishmanicidal Activity of Isolated Compounds against *L. major*

Compound	IC ₅₀ (μg/ml)
1	>25
2	43.9
3	9.1
5	47.1
6	23.8
7	89.7
8	>25
10	0.9
11	13.6
12	16.9
13	17.1
14	2.2
15	>25
17	>25
18	358.7
19	73.9
21	>25
22	>25
Amphotericin B	0.04

standpoint, but they have a flavan-3-ol skeleton and belong to the flavone group in structural standpoint.

Some isolated compounds were tested for leishmanicidal activity against promastigotes of *Leishmania major* and the results are shown in Table 3. Known compounds, ferulaldehyde and ulsoric acid, showed strong leishmanicidal activity (IC₅₀ 0.9 and 2.2 μg/ml, respectively). Of the new compounds, **3** showed strong activity (IC₅₀ 9.1 μg/ml). Eugenol dimers **11** and **12** also showed activity (IC₅₀ 13.6 and 16.9 μg/ml, respectively). Some of the new compounds showed medium activity, as shown in Table 3.

Experimental

UV and IR spectra were obtained by U-2001 (Hitachi) and FT-IR spectroscopy (Perkin Elmer). Optical rotations were measured by JASCO P-1010 polarimeter at room temperature. NMR spectra were recorded on a Unity INOVA 500 spectrometer (Varian Inc., Palo Alto, CA, U.S.A.). HR-ESI-TOF-MS spectra were obtained on a Micromass Q-ToF micro mass spectrometer (Waters Corp., Milford, MA, U.S.A.). Preparative and analytical HPLC was carried out on reverse-phase columns (Mighty sil RP-18 and 8, Kantho Chemical Co., Ltd.) with the CH₃CN–H₂O solvent system. Silica gel 60N (Kantho Chemical Co., Ltd.) was used for column chromatography. Analytical and preparative TLC were carried out on precoated Kieselgel 60F₂₅₄ (Merck) and spots were visualized by spraying the plates with 50% H₂SO₄ solution, followed by heating.

Plant Material The crude drug, Tulsi, was purchased at a market in Kathmandu, Nepal, in August 2003. The botanical identification was made by Dr. A. Takano of Showa Pharmaceutical University. A voucher specimen is deposited in the laboratory of Natural Product Chemistry of the Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Shobara, Hiroshima, Japan.

Isolation of Constituents Dried leaves of Tulsi (*Ocimum sanctum* L.) (1 kg) were extracted with methanol (MeOH) under reflux to give MeOH extract (106 g). The MeOH extract was partitioned between ethyl acetate (AcOEt) and water to give an AcOEt layer and aqueous layer. The aqueous layer was partitioned with *n*-butanol (*n*-BuOH) to give a *n*-BuOH layer. The AcOEt layer and *n*-BuOH layer were evaporated to give AcOEt extract (37.4 g) and *n*-BuOH extract (20.3 g). Both extracts showed leishmanicidal activity, but the AcOEt extract showed many constituents on TLC analysis. Thus the AcOEt extract was chromatographed on a silica gel column using a gradient chloroform (CHCl₃)–MeOH solvent system to give 11 fractions, Fr. 1–Fr. 11. Fr. 2 (6.19 g) was purified by successive column chromatography and HPLC using an ODS column to give compounds **8** (1.8 g), **10** (3 mg), **12** (118 mg) and **17** (43 mg). Fr. 3 (4.96 g) was also purified by similar procedure to give compounds **2** (51 mg), **3** (7 mg), **5** (3 mg), **6** (5 mg), and **7** (5 mg); Fr. 4 (1.62 g) gave compounds **11** (71 mg), **15** (69 mg), and **23** (47 mg); Fr. 5 (2.93 g) gave compounds **4** (6 mg) and **10** (21 mg); Fr. 6

(4.05 g) gave compounds **1** (16 mg) and **22** (42 mg); Fr. 7 (2.93 g) gave compound, **13** (173 mg) and **14** (262 mg); Fr. 8 (2.82 g) gave compound **21** (15 mg); Fr. 9 (4.27 g) gave compounds **18** (72 mg) and **20** (9 mg); and Fr. 10 (1.98 g) gave compounds **9** (10 mg), **16** (104 mg), and **19** (19 mg).

Compound 1: Colorless amorphous powder. [α]_D +1.5° (*c*=0.04, MeOH), HR-ESI-MS *m/z*: 365.1371 [M+Na]⁺ (Calcd for C₂₀H₂₂O₅Na, 365.1365), IR *v*_{max} cm⁻¹ (KBr): 3433, 2361, 2343, 1495, 1272, 1223. UV λ_{max} nm (ε): 238 (10600), 279 (7100) (MeOH). ¹H-NMR is shown in Table 1 and ¹³C-NMR is shown in Table 2.

Compound 2: Pale yellow viscous oil. [α]_D +1.5° (*c*=0.02, MeOH), HR-ESI-MS *m/z*: 511.2113 [M+Na]⁺ (Calcd for C₃₀H₃₂O₆Na, 511.2097), IR *v*_{max} cm⁻¹ (KBr): 3444, 2935, 1594, 1511, 1495, 1269, 1222. UV λ_{max} nm (ε): 222sh (11700), 234 (13300), 279 (6100) (MeOH). ¹H-NMR is shown in Table 1 and ¹³C-NMR is shown in Table 2.

Compound 3: Pale yellow viscous oil. HR-ESI-MS *m/z*: 511.2119 [M+Na]⁺ (Calcd for C₃₀H₃₂O₆Na; 511.2097). [α] +4.3° (*c*=0.03, MeOH). IR *v*_{max} cm⁻¹: 3427, 2936, 1604, 1514, 1267, 1140, 1031. UV λ_{max} nm (ε): 215 (10300), 229 (9100), 281 (3500), (MeOH). ¹H-NMR is shown in Table 1 and ¹³C-NMR is shown in Table 2.

Compound 4: Pale yellow viscous oil. ESI-MS *m/z*: 543.2339 [M+Na]⁺ (Calcd for C₃₁H₃₆O₇Na; 543.2359). IR *v*_{max} cm⁻¹: 3418, 2936, 1670, 1595, 1512, 1270, 1139, 1032. ¹H-NMR is shown in Table 1 and ¹³C-NMR is shown in Table 2.

Compound 5: Pale yellow viscous oil. [α]_D +8.0° (*c*=0.01, MeOH), HR-EI-MS *m/z*: 511.2113 [M+Na]⁺ (Calcd for C₃₀H₃₂O₆Na, 511.2097), IR *v*_{max} cm⁻¹ (KBr): 3409, 2936, 1595, 1510, 1268, 1222, 1138. UV λ_{max} nm (ε): 222sh (11700), 234 (13300), 279 (6100) (MeOH). ¹H-NMR is shown in Table 1 and ¹³C-NMR is shown in Table 2.

Compound 6: Pale yellow viscous oil. HR-ESI-MS *m/z*: 489.2300 [M+H]⁺ (Calcd for C₃₀H₃₃O₆; 489.2277). IR *v*_{max} cm⁻¹ (KBr): 3422, 2940, 1673, 1591, 1508, 1211, 1131, 1090. UV λ_{max} nm (ε): 222 (11300), 275 (2400), (MeOH). ¹H-NMR is shown in Table 1 and ¹³C-NMR is shown in Table 2.

Compound 7: Colorless viscous oil. HR-ESI-MS *m/z*: 365.1401 [M+Na]⁺ (Calcd for C₂₀H₂₂O₅Na; 365.1365). UV λ_{max} nm (ε): 217 (7200), 233 (6800), 284 (2300), (MeOH). ¹H-NMR is shown in Table 1 and ¹³C-NMR is shown in Table 2.

Leishmanicidal Activity Test The leishmanicidal activities of isolated compounds were tested by an improved MTT method as follows. Basically, the reported method²⁰ was employed except that Tetra Color One {the mixture of WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-disulfophenyl)-2H-tetrazolium, monosodium salt] and 1-methoxy PMS (1-methoxy-5-methylphenazinium methosulfate) (SeikagakuKogyo Co., Ltd.)} were used instead of MTT. Cultured promastigotes were centrifuged at 600g at 4°C for 5 min. The parasites were resuspended in each culture and diluted to a density of 1×10⁵/ml. *L. major* promastigotes were seeded at 0.5×10⁴/50 μl in medium/well in a 96-well microplate, and then a further 50 μl medium/well with different concentrations of test compounds dissolved in dimethyl sulfoxide (DMSO) were added to each well. Each concentration was tested in triplicate. As positive controls, amphotericin B and pentamidine were investigated. The microplate was incubated at 27°C in 5% CO₂ for 72 h. Tetra Color One was added to each well and the plates were incubated at 27°C for 6 h. Optical density at 630 nm was measured using a microplate reader (Molecular Devices Co., Ltd.). Leishmanicidal activity was expressed as the 50% inhibitory concentration (IC₅₀).

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