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

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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-methoxyphenoxymethyl)-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 ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR spectral data. Some of these compounds showed leishmanicidal activity.

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

Leishmaniases 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 *Phleobotomus* 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 leishmaniases 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 lactone[7] and steroid saponines[8] 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*-buthanol (*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**), ursoric 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 $[M+Na]^+$ $C_{20}H_{22}O_5Na$, which accorded to the molecular formula $C_{20}H_{22}O_5$. 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 ^{1}H-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 metylene group [δ_H 2.89 (1H, dd, J=8.8, 16.1 Hz), 3.06 (1H, dd, J=5.4, 16.1 Hz)]. The ^{13}C-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. ^{1}H-NMR Data of Compounds **1**—**7** (in $CDCl_3$, 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, overap)	5.94 (1H, ddt, 16.8, 10.0, 6.6)
9	5.07 (1H, br d, 10.3) 5.10 (1H, br d, 17.1)	5.03—5.10 (2H, overlap)	5.07 (2H, overlap)	3.91 (1H, dd, 10.3, 4.9) 4.26 (1H, dd, 10.3, 5.1)	3.96 (1H, dd, 10.3, 5.4) 4.46 (1H, dd, 10.3, 4.4)	4.98—5.10 (2H, overlap)	5.04 (1H, br d, 10.0) 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, dd, 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) 3.06 (1H, dd, 16.1, 5.8)	3.04 (2H, d, 5.4)	4.16 (1H, t, 8.6) 4.27 (1H, dd, 9.5, 5.9)	5.05 (2H overlap)	5.00—5.08 (2H, overlap)	4.98—5.10 (2H, overlap)	3.33 (2H, d, 7.3)
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)		
5‴					6.74 (1H, d, 8.1)		
6‴					6.52 (1H, dd, 8.1, 1.7)		
7‴					3.25 (2H, br d, 6.6)		
8‴					5.90 (1H, overlap)		
9‴					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					3.73 (3H. s)		
7-OMe				3.30 (3H, s)			

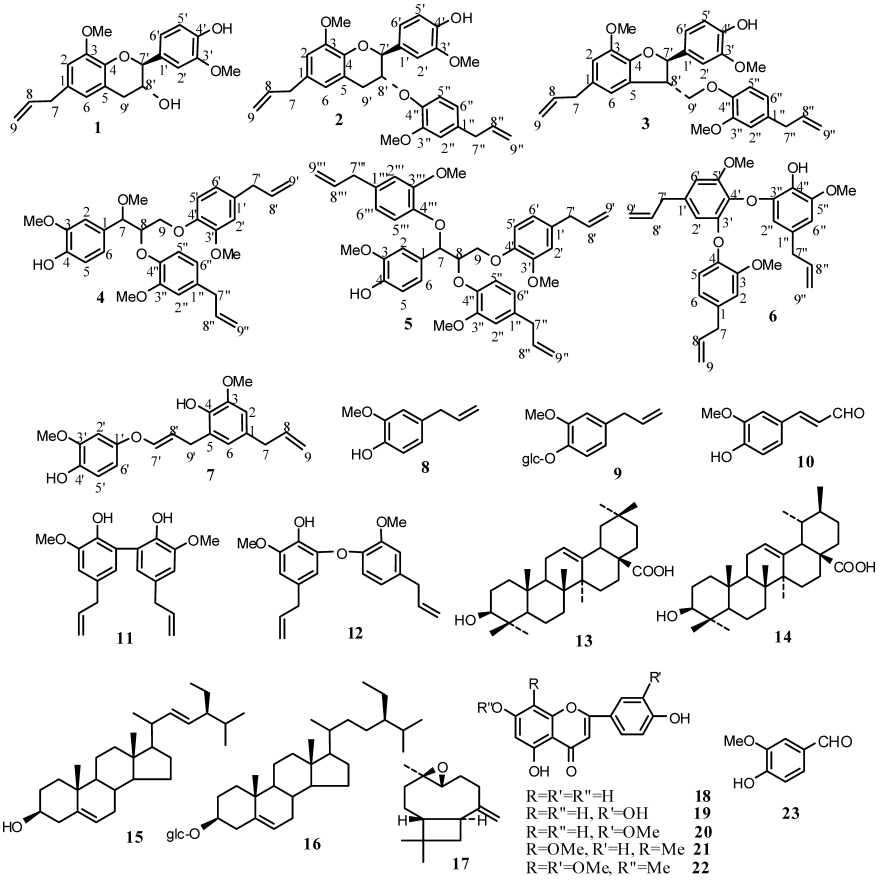

Fig. 1. Structures of Constituents Isolated from *O. sanctum*

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 ([α]$_D$ +1.5°) 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 $[M+Na]^+$ $C_{30}H_{32}O_6Na$, which accorded to the molecular formula $C_{30}H_{32}O_6$. 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 ^{1}H-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 experiment. 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 ([α]$_D$ +1.5°) 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 $[M+Na]^+$ $C_{30}H_{32}O_6Na$, which accorded to the molecular formula $C_{30}H_{32}O_6$. [α]$_D$ +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 ^{1}H-NMR spectrum of **3** showed the presence of two allyl groups [δ_H3.32 (2H, d, J=6.1 Hz), 3.33 (2H, d, J=5.4 Hz), 5.92—5.98 (2H, over lap), 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, br s), 6.76 (1H, br s)], 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 C4, 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 dihydrobenzfurane skeleton. Thus the structure of **3** was determined to be 5-allyl-3-(4-allyl-2-methoxyphenoxymethyl)-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 $[M+Na]^+$ $C_{31}H_{36}O_7Na$, which accorded to the molecular formula $C_{30}H_{32}O_6$. The IR spectrum of **4** showed absorptions at 3418, 2936, 1670, 1595, and 1270 cm^{-1}. The ^{1}H-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, 137.6×2, 39.8×2). 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-

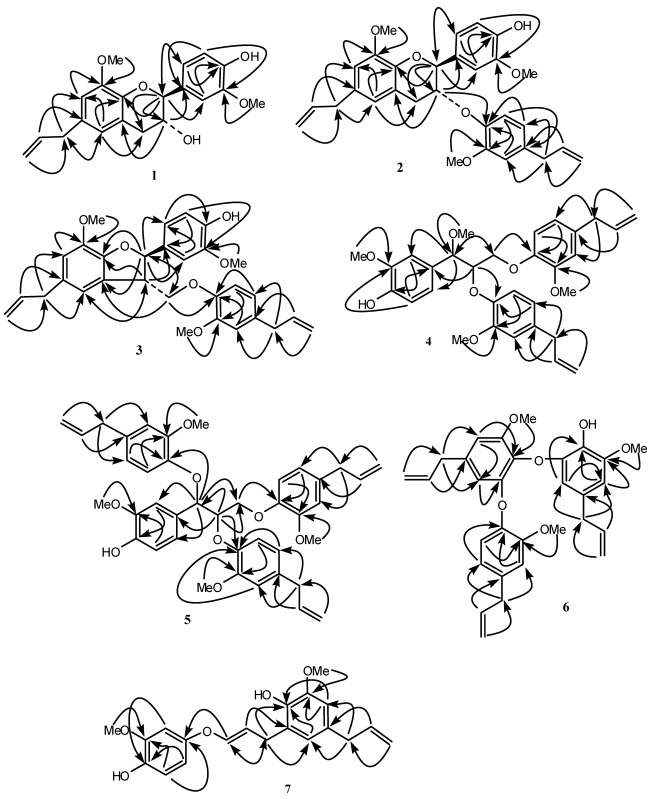

Fig. 2. Key HMBC Correlations of **1**—**7**

termined 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 ^{1}H-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 ^{1}H-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, 56.2×2), 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 106.8×2, 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-methoxyphnoxy)-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 ^{1}H-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), 3.30 (2H, d, J=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 (IC_{50} 0.9 and 2.2 μg/ml, respectively). Of the new compounds, **3** showed strong activity (IC_{50} 9.1 μg/ml). Eugenol dimers **11** and **12** also showed activity (IC_{50} 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 CH_3CN–H_2O solvent system. Silica gel 60N (Kantho Chemical Co., Ltd.) was used for column chromatography. Analytical and preparative TLC were carried out on precoated Kieselgel $60F_{254}$ (Merck) and spots were visualized by spraying the plates with 50% H_2SO_4 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 ($CHCl_3$)–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. $[\alpha]_D$ +1.5° (c=0.04, MeOH), HR-ESI-MS m/z: 365.1371 $[M+Na]^+$ (Calcd for $C_{20}H_{22}O_5Na$, 365.1365), IR ν_{max} cm^{-1} (KBr): 3433, 2361, 2343, 1495, 1272, 1223. UV λ_{max} nm (ε): 238 (10600), 279 (7100) (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **2**: Pale yellow viscous oil. $[\alpha]_D$ +1.5°(c=0.02, MeOH), HR-ESI-MS m/z: 511.2113 $[M+Na]^+$ (Calcd for $C_{30}H_{32}O_6Na$, 511.2097), IR ν_{max} cm^{-1} (KBr): 3444, 2935, 1594, 1511, 1495, 1269, 1222. UV λ_{max} nm (ε): 222sh (11700), 234 (13300), 279 (6100) (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **3**: Pale yellow viscous oil. HR-ESI-MS m/z: 511.2119 $[M+Na]^+$ (Calcd for $C_{30}H_{32}O_6Na$; 511.2097). $[\alpha]$ +4.3° (c=0.03, MeOH). IR ν_{max} cm^{-1}: 3427, 2936, 1604, 1514, 1267, 1140, 1031. UV λ_{max} nm (ε): 215 (10300), 229 (9100), 281 (3500), (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **4**: Pale yellow viscous oil. ESI-MS m/z: 543.2339 $[M+Na]^+$ (Calcd for $C_{31}H_{36}O_7Na$; 543.2359). IR ν_{max} cm^{-1}: 3418, 2936, 1670, 1595, 1512, 1270, 1139, 1032. ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **5**: Pale yellow viscous oil. $[\alpha]_D$ +8.0° (c=0.01, MeOH), HR-EI-MS m/z: 511.2113 $[M+Na]^+$ (Calcd for $C_{30}H_{32}O_6Na$, 511.2097), IR ν_{max} cm^{-1} (KBr): 3409, 2936, 1595, 1510, 1268, 1222, 1138. UV λ_{max} nm (ε): 222sh (11700), 234 (13300), 279 (6100) (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **6**: Pale yellow viscous oil. HR-ESI-MS m/z: 489.2300 $[M+H]^+$ (Calcd for $C_{30}H_{33}O_6$; 489.2277). IR ν_{max} cm^{-1} (KBr): 3422, 2940, 1673, 1591, 1508, 1211, 1131, 1090. UV λ_{max} nm (ε): 222 (11300), 275 (2400), (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-NMR is shown in Table 2.

Compound **7**: Colorless viscous oil. HR-ESI-MS m/z: 365.1401 $[M+Na]^+$ (Calcd for $C_{20}H_{22}O_5Na$; 365.1365). UV λ_{max} nm (ε): 217 (7200), 233 (6800), 284 (2300), (MeOH). ^{1}H-NMR is shown in Table 1 and ^{13}C-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 method[20] was employed except that Tetra Color One {the mixture of WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-disulfophenyl)-2*H*-tetrazorium, monosodium salt] and 1-methoxy PMS (1-methoxy-5-methylphenazinium methosukfate) (SeikagakuKogyou Co., Ltd.)} were used instead of MTT. Cultured promastigotes were centrifuged at 600 ***g*** at 4 °C for 5 min. The parasites were resuspended in each culture and diluted to a density of 1×10^5/ml. *L. major* promastigotes were seeded at 0.5×10^4/50 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% CO_2 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 (IC_{50}).

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