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NEUROTROPHIC SESQUITERPENE-NEOLIGNANS FROM *MAGNOLIA OBOVATA*: STRUCTURE AND NEUROTROPHIC ACTIVITY

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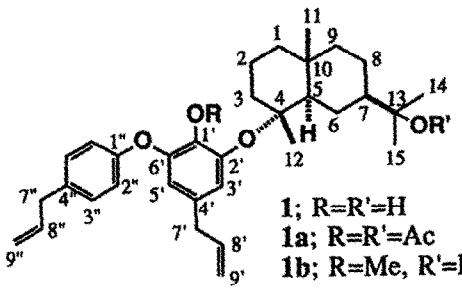
ABSTRACT: Novel sesquiterpene-neolignans, eudesobovatols A (**1**) and B (**2**), eudesmagnolol (**3**), eudeshonokiols A (**4**) and B (**5**), clovanemagnolol (**6**), and caryolanemagnolol (**7**), have been isolated from the bark of *Magnolia obovata*. Their structures were elucidated to be sesquiterpenes (eudesmol, 4,4,8-trimethyltricyclo [6.3.1.0^{2,5}] dodecane-1,9-diol, and clovanediol) combined through ether bond with neolignans such as obovatol, honokiol, and magnolol on the basis of spectral data, degradation, and/or synthesis. Compounds **1**, **6**, and **7** were found to exhibit interesting neurotrophic activity on a neuronal cell culture system derived from fetal rat hemisphere.

The bark of *Magnolia obovata* or *M. officinalis* (Magnoliaceae) has long been used as traditional medicine for neurosis and gastrointenstinal complaints in China, Korea, and Japan. The constituents of the species have been investigated intensively becaus of these pharmacological interests and various type of compounds has been isolated; i.e. neolignans[1-3] (magnolol, honokiol, and obovatol), terpenes[4,5] (α- and β-pinens, camphene, bornylacetate, α-, β-, and γ-eudesmols, humulene oxide, caryophyllene, caryophyllene oxide), monoterpene-neolignans[6] and isoquinoline alkaloides[7,8] (magnocurarine, magnoflorine) in addition to phenylpropanoids glycoside recently isolated.[9,10] While magnolol and honokiol, the major components of *M. obovata* and *M. officinalis* were claimed to be the active principle for the central depressant effect,[11] our preliminary studies suggested the presence of neurotrophic active substances in the title plant and extensive studies on the minor components led to the isolation of various sesquiterpenes linked to biphenyl- or biphenylether type neolignans, named eudesobovatols A (**1**) and B (**2**), eudeshonokiols A (**3**) and B (**4**), eudesmagnolol (**5**), clovanemagnolol (**6**), and caryolanemagnolol (**7**), some of which exhibited the activities accelerating neurite sprouting and neuronal cell network formation as well as enhancing choline acetyltransferase activity in cultured neuronal cell derived from fetal rat hemisphere.

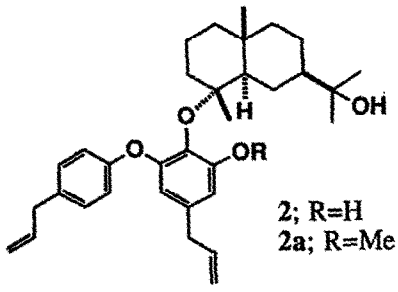
In this paper we report the full accounts of the structures and neurotrophic activities of these novel sesquiterpene-neolignans isolated from *M. obovata*.

Results and Discussions

Isolation Since the bark of *M. obovata* contains large amounts of neolignans, magnolol and honokiol, it is essential to remove these major constituents effectively for the isolation of the minor components. Thus, the ethyl acetate-soluble portion of methanolic extract was subjected to column chromatography using silica gel and



1; R=R'=H
1a; R=R'=Ac
1b; R=Me, R'=H



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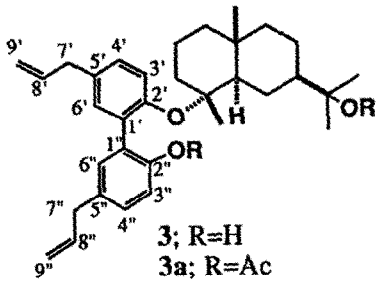

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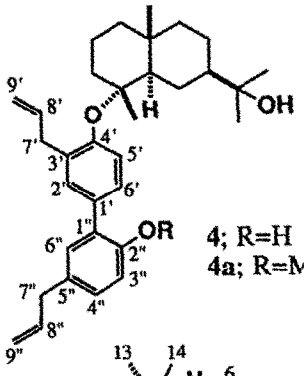

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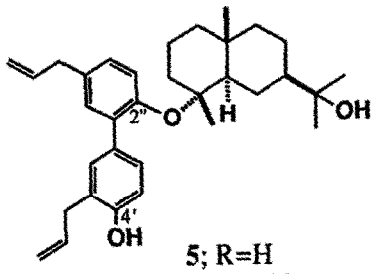

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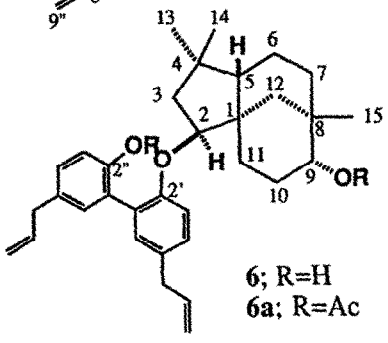
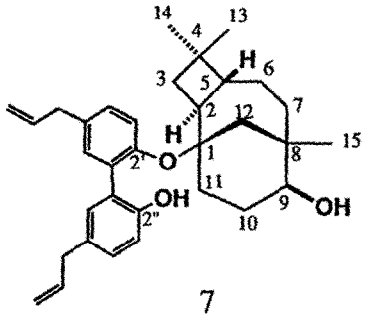
5a; R=Me



6; R=H
6a; R=Ac



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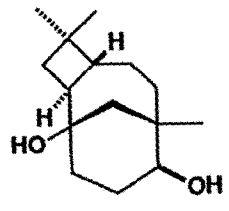

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Fig. 1

Sephadex LH-20 repeatedly to remove these neolignans. Thus obtained fractions containing sesquiterpene-neolignans were further separated by reverse-phase low-barr column chromatography (MPLC) and/or high-performance liquid chromatography (HPLC) to give seven new sesquiterpene-neolignans **1-7** (Fig. 1).

Structure of eudesobovatols A and B[12] Eudesobovatol A (**1**) was obtained as a viscous oil and showed the molecular ion peak at *m/z* 504 in the field desorption mass spectrum (FDMS) giving the molecular formula $C_{33}H_{44}O_4$ in combination with ^{13}C NMR data summarized in Table I. UV and IR spectra indicated the presence of hydroxyl groups (ν_{max} 3600, 3530 cm^{-1}) and aromatic ring [λ_{max} 208 (ε 5800), 274 (ε 7200), 281 (ε 6700) nm; ν_{max} 1600, 1500 cm^{-1}]. Acetylation of **1** afforded a diacetate **1a**, while treatment of **1** with diazomethane gave a monomethyl ether **1b** indicating the presence of two hydroxyl groups, one of which should be phenolic. ^1H NMR spectrum analyzed with the aid of 2D DQFCOSY and C/H COSY spectra disclosed the presence of two allyl groups [δ (C_5D_5N) 3.26 (2H, d, *J*=6.8 Hz), 5.03 (dd, *J*=17.2, 2.0 Hz), 5.10 (dd, *J*=10.3, 2.0 Hz), 5.91 (ddt, *J*=17.2, 10.3, 6.8 Hz) and 3.31 (2H, d, *J*=6.4 Hz), 5.04 (dd, *J*=10.3, 2.0 Hz), 5.08 (dd, *J*=17.1, 2.0 Hz), 6.01 (ddt, *J*=17.1, 10.3, 6.4 Hz)], two AB type aromatic protons [δ 7.05 (2H, d *J*=8.8 Hz) and 7.11 (2H, d, *J*=8.8 Hz)], and *meta* coupled aromatic protons [δ 6.84 (d, *J*=2.0 Hz) and 7.02 (d, *J*=2.0 Hz)] as well as four tertiary methyl groups (δ 0.93, 1.42 x 3), three of which must be located on the carbon bearing oxygen function. The ^{13}C NMR spectrum (Table I) was analyzed by using C/H and long-range C/H COSYs. Comparison of these data with those of known neolignans isolated from *M. obovata*, coupled with the substitution pattern of aromatic rings deduced from ^1H NMR spectrum, clearly revealed that **1** consists of obovatol[3] (**8**) and a bicyclic sesquiterpene linked each other *via* an ether bond. In fact, in electron inpact mass spectrum (EIMS) prominent peaks were observed at *m/z* 282 (base peak) and 222 corresponding to **8** and terpenoid part, respectively. Careful analysis of ^1H NMR spectrum of the terpenoid part gave rise to the partial structures **A-D** shown in Fig. 2. These partial structures were able to connect each other with the aid of long-range C/H COSY (shown by arrows in Fig. 2) to construct an eudesmol type structure. Namely, the ^{13}C signal at δ 84.4 (C-4) was correlated with the proton signals at δ 1.42 (H-12), 1.94 (H-5), and 2.14 (H-3), whereas the signal at δ 34.5 (C-10) with the signals at δ 0.93 (H-11), 1.83 (H-8), 1.94 (H-5), and 2.95 (H-6). The presence of these two units in **1** was confirmed by the fact that treatment of **1** with trifluoroacetic acid in dry benzene yielded (+)-γ-eudesmol (**9**) ([α]$_D$ +55.5°, *lit.*[13] 62.5°) and **8** (Scheme 1). This result also revealed that one of the hydroxyl groups on the obovatol ring must be

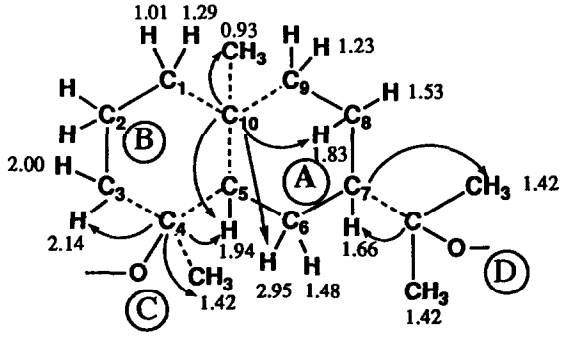
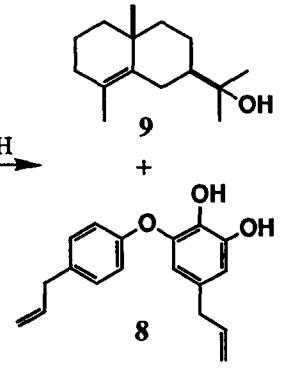


Fig. 2. Partial structures of **1** and C-H correlations observed in long-range (8 Hz) C/H COSY spectrum in C_5D_5N



Scheme 1

Table I. ^{13}C NMR data of sesquiterpene-neolignans[a)]

Carbon	1	2	3	4	5	6[b)]	7[b)]		8	10	11	12[c)]
1	40.4	40.6	40.2	40.9	40.9	45.3	84.4	(84.3)[c)]				70.4
2	19.6	20.2	19.6	20.2	20.2	89.7	39.3	(38.5)[c)]				37.8
3	38.6	38.5	37.6	39.5	38.3	44.5	36.5	(36.0)[c)]				33.8
4	84.4	87.5	84.9	83.8	84.7	37.8	34.9	(34.9)[c)]				34.6
5	51.9	53.0	51.5	53.7	52.5	50.3	44.2	(43.9)[c)]				43.4
6	22.0	21.8	21.8	22.7	22.5	21.0	20.7	(20.2)[c)]				20.1
7	49.8	49.5	49.8	50.6	50.5	33.2	35.8	(35.4)[c)]				35.1
8	22.4	22.5	22.3	22.8	23.0	34.8	40.4	(39.3)[c)]				39.1
9	44.9	44.9	44.7	45.4	45.5	74.7	71.3	(71.5)[c)]				71.6
10	34.5	35.1	34.3	35.0	35.0	26.8	28.8	(28.4)[c)]				27.8
11	18.7	19.1	18.5	19.2	19.2	27.1	29.6	(28.9)[c)]				33.0
12	19.7	21.2	20.8	20.3	21.3	35.7	40.4	(39.5)[c)]				42.1
13	70.9	72.6	70.9	71.4	71.5	25.5	20.8	(20.5)[c)]				20.6
14	27.3	26.5	27.2	27.7	27.9	31.3	30.4	(30.3)[c)]				30.2
15	27.5	27.2	27.4	28.3	27.9	28.7	26.8	(26.5)[c)]				26.5
1'	143.6	132.4	135.1	133.8	131.9	129.5	133.9	(130.6)[a)]	137.5	127.7	131.0	
2'	144.7	152.2	150.6	131.7	132.3	154.6	150.0	(151.9)[a)]	149.2	154.2	131.8	
3'	122.0	110.9	124.7	133.7	126.7	116.3	124.0	(122.8)[a)]	112.4	117.3	126.9	
4'	129.6	136.7	127.6	152.7	155.5	129.2	128.5	(127.8)[a)]	131.3	129.1	155.4	
5'	116.2	112.4	134.5	121.9	115.2	134.1	135.7	(135.7)[a)]	113.1	131.3	115.4	
6'	145.0	150.4	132.0	128.3	129.4	132.8	132.9	(132.5)[a)]	145.1	132.4	129.1	
7'	39.1	39.8	39.1	35.7	35.1	39.7	39.7	(39.7)[a)]	39.4	39.7	35.1	
8'	137.6	136.9	137.4	138.0	138.2	137.8	137.6	(138.2)[a)]	138.1	138.6	138.0	
9'	115.1	116.0	115.2	115.3	115.3	115.8	115.9	(115.7)[a)]	115.4	115.3	115.2	
1"	156.7	155.8	128.1	129.3	138.4	127.4	132.2	(128.4)[a)]	157.3	127.7	129.8	
2"	116.7	117.5	153.4	154.1	151.2	153.1	153.5	(154.4)[a)]	117.2	154.2	154.1	
3"	129.4	129.4	116.8	117.1	125.4	117.6	118.6	(117.0)[a)]	129.9	117.3	117.0	
4"	133.4	134.2	128.5	128.7	127.5	129.7	129.6	(128.9)[a)]	133.8	129.1	128.2	
5"	129.4	129.4	130.3	131.2	135.1	132.1	129.0	(133.5)[a)]	129.9	131.3	131.2	
6"	116.7	117.5	131.9	131.3	131.3	131.6	131.7	(132.7)[a)]	117.2	132.4	131.3	
7"	38.8	39.4	39.1	39.7	39.8	39.7	39.8	(39.8)[a)]	39.8	39.7	39.7	
8"	137.5	137.5	138.2	138.7	138.1	138.4	138.5	(138.8)[a)]	138.3	138.6	138.7	
9"	115.1	115.8	114.7	115.6	115.7	115.3	115.3	(115.4)[a)]	115.5	115.3	115.2	

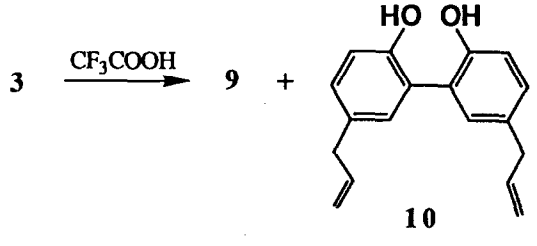
a) Chemical shifts in C_5D_5N except for **6**, **7**, and **12**. All signals were assigned with the aids of C/H COSY and HMBC technique. b) Chemical Shifts in C_6D_6. c) Chemical shifts in $CDCl_3$.

linked to the C-4 position in eudesmol framework. In order to clarify which hydroxyl group of **8** is used as ether linkage and also to determine the configuration at C-4, difference NOE experiments were investigated on methyl ether **1b**. Selective irradiation of the methyl signal at δ 1.21 (C_4-CH_3) caused NOE interaction not only for the methyl signal at δ 0.92 (C_{10}-CH_3) accounting for a 1,3-diaxial relationship of these two methyls, but also for the *meta*-coupled aromatic proton signal at δ 6.61 (H-3'), whereas no NOE was detected for any aromatic proton signal upon irradiation of the methoxy signal at δ 3.74 (Regio-isomer **2a** of **1b** showed distinct NOE between methoxy group and H-3', vide infra). These results clearly indicated that C_2'-OH of

8 was bonded to the C-4 (α-equatorial) position of eudesmol framework. The α configuration of H-5 (δ 1.94) was deduced on the basis of large coupling constant ($J_{5,6\beta}$=11.7 Hz) as well as the observation of NOE between H-5 and H-6α. Thus, the structure of eudesobovatol A was fully elucidated as **1**.

Eudesobovatol B (**2**), viscous oil, exhibited a quasi-molecular ion peak due to $[M-1]^-$ at *m/z* 503 and a base peak at *m /z* 282 in negative fast atom bombardment mass spectrum (FABMS) as in the case of **1**. It formed a monomethyl ether **2a** on treat-ment with diazomethane. The ^1H NMR spectrum was very similar to that of **1** indicating that **2** has the same structure units, obovatol and eudesmol, as eudesobovatol A (**1**). The major difference was the methyl signal at δ 1.58 (H-12) and *meta* -coupled aromatic proton signals at δ 6.61 and 6.99 . The ^{13}C NMR data of **2** (Table I) was again well corresponded to those of **1** except for the carbons adjacent to the ether linkage, i.e. carbons-4, -1', -2', -3', -4', -5', and -6'. Moreover, no NOE was observed for any aromatic protons upon irradiation of C_4-CH_3 (δ 1.58), although clear NOE was detected for the methyl signal at δ 0.92 (C_{10}-CH_3) indicating a 1,3-diaxal relationship of these two methyl groups as in **1**. These results revealed that **2** should be the regio isomer of **1** with respect to the position of ether linkage. This proposal was verified by the observation of NOE for the *meta* -coupled proton resonance at δ 6.52 upon irradiation of the methoxy signal at δ 3.81 in **2a**. Thus, the structure of eudesobovatol B was represented as the formula **2**.

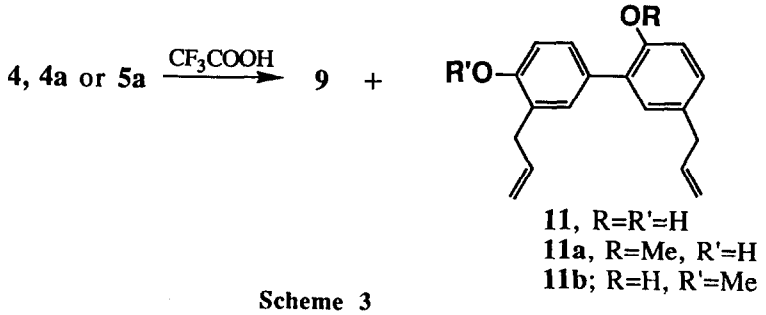
Structures of eudesmagnolol[14] The molecular formula of eudesmagnolol (**3**), viscous oil, was determined to be $C_{33}H_{44}O_3$ on the basis of FDMS (*m /z* 488 [M]+) and ^{13}C NMR data summarized in Table I The presence of hydroxyl group and aromatic ring was again indicated by IR spectrum (ν_{max} 3610, 3260, 1600, 1500 cm^{-1}). Treatment of **3** with acetic anhydride in pyridine yielded a diacetate **3a**. The ^1H NMR spectrum of **3** disclosed the presence of two 1,2,4-trisubstituted benzene rings [δ 7.13 (dd, *J*=8.3, 2.0 Hz), 7.24 (d, *J*=8.3 Hz), and 7.44 (d, *J*=2.0 Hz); 7.19 (dd, *J*=8.3, 2.0 Hz), 7.26 (d, *J*=8.3 Hz), and 7.35 (d, *J*=2.0 Hz)] and a set of allyl groups [δ 3.35 (2H, d, *J*=6.8 Hz), 5.01 (dd, *J*=10.3, 1.9 Hz), 5.10 (dd, *J*=17.1, 1.9 Hz), and 5.97 (ddt, *J*=17.1, 10.3, 6.8 Hz) ; δ 3.45 (2H, d, *J*= 6.8 Hz), 5.05 (dd, *J*= 10.3, 1.9 Hz), 5.14 (dd, *J*=17.1, 1.9 Hz), 6.01 (ddt, *J*=17.1, 10.3, 6.8 Hz) in addition to a methyl group (δ 0.81) on the quaternary carbon and three methyl groups (δ 1.22, 1.43, 1.45) on the carbon bearing oxygen function . Comparison of ^1H and ^{13}C NMR spectra to those of **1** and **2** revealed that **3** consists of eudesmol and a different type of neolignan combined through an ether linkage. Since the FDMS of **3** showed a prominent ion peak at *m /z* 266 and the ^{13}C NMR data were well corresponded (Table 1), the neolignan incorporated in **3** was determined to be magnolol[1] (**10**), one of the major component of *M. obovata.* In fact, treatment of **3** with CF_3COOH afforded (+)-**9** ($[\alpha]_D$ +55.5°) and **10** (Scheme 2). The configuration at C-4 in eudesmol part was determined to be *R* since NOE was observed between C_4-CH_3 and C_{10}-CH_3 giving the full structure of eudesmagnolol (**3**).



Scheme 2

Structure of eudeshonokiols A§,14 and B The molecular weight of eudeshonokiol A (**4**) was determined as 488 by negative- (*m/z* 487 $[M-1]^-$) and positive FABMS (*m/z* 511 $[M+Na]^+$). The IR spectrum revealed the presence of hydroxyl group (ν_{max} 3670, 3550 cm^{-1}) and aromatic ring (ν_{max} 1600, 1480 cm-1). Treatment of **4** with diazomethane afforded a monomethyl ether **4a**. ^1H and ^{13}C NMR spectra were found to be similar to those of **3**, which suggested that **4** consists of eudesmol-type sesquiterpene and biphenyl-type neolignan. However, the ^1H NMR spectrum of aromatic unit was apparently different from that of **10**, particularly appearance of benzylic methylene protons as nonequivalent AB-type pattern [δ 3.55 (dd, *J*=15.4, 6.6 Hz) and δ 3.71 (dd, *J*=15.4, 6.6 Hz)]. These facts suggest that honokiol (**11**), a component of *M. obovata.*, is incorporated in **4**. In fact, reaction of **4** with CF_3COOH afforded (+)-**9** ($[\alpha]_D$ +45.3°) and honokiol (**11**) (Scheme 3). The C_4-OH of the latter associated with an ether bond was verified by the observation of NOEs in **4a** on H-11 (δ 0.94) and H-5' (δ 7.01) upon irradiation of H-12 (δ 1.33) and on H-3" (δ 6.90) upon irradiation of the methoxy signal (δ 3.77) and was confirmed by the acid treatment of **4a** to form 2'-*O*-methylhonokiol[15] (**11a**) (Scheme 3) which has been isolated from the title plant by us and also derived from **11**.

The UV and IR spectra and the pattern of ^1H and ^{13}C NMR (see Table I) spectra of eudeshonokiol B (**5**) were very similar to those of **4**. Thus, it can be assumed readily that **5** is the regio-isomer of **4** in respective of the hydroxyl group of **11** associated with the ether bond. This assumption was supported by the observation of the following NOEs: C_4-CH_3 (δ 1.20) / one of the *ortho*-coupled aromatic protons (δ 7.24; H-3") in **5**, OCH_3 (δ 3.86) / another *ortho*-coupled aromatic protons (δ 6.87; H-5') in methoxy derivative **5a**. Finally, the structure was confirmed by the cleavage of **5a** with CF_3COOH to yield 4-*O*-methylhonokiol (**11b**) (Scheme 3), isolated from *Magnolia grandiflora*.[15]



Scheme 3

Structure of clovanemagnolol[16] The molecular formula of clovanemagnolol (**6**) was determined as $C_{33}H_{42}O_3$ by high resolution EI mass spectrum (HREIMS) (*m/z* 486.3127) and its ^1H NMR spectrum indicated the presence of an aromatic and sesquiterpene moieties. The aromatic part was readily assigned as magnolol (**10**) since the ^1H NMR revealed the presence of two allyl groups and two 1,2,4-trisubstituted aromatic rings as well as the close similarity of ^{13}C NMR data between them. However, the spectral data of the terpene part were totally different from those of above-mentioned eudesmol-type structure. The DEPT spectrum of **6** displayed the presence of fifteen carbons consisted of three methyl, six methylene, one methine,

§The name, eudeshonokiol, previously reported for **4**[14] has now been corrected to eudeshonokiol A because of the isolation of closely related compound (eudeshonokiol B) later on.

two oxygen bearing methine, and three quaternary carbons (Table I), wherease ^{1}H NMR spectrum (C_6D_6) contained three tertiary methyl signals (δ 0.64, 0.84, and 0.94) and two oxygenated methine signals (δ 3.07 and 4.11). Acetylation of **6** to diacetate **6a** caused a large down-field shift (Δδ 1.42) of the higher-field proton signal (δ 3.07), which indicated that the proton appeared at lower-field should be attached to the carbon bearing ether bond. Analysis of DQFCOSY and C/H COSY spectra revealed that these two carbinyl protons were involved in partial structures **A** and **B**, respectively, and the additional partial structures **C** and an isolated methylene group were present in **6** in addition to three tertiary methyls. These partial structures could be connected each other by the analysis of HMBC spectrum. As shown in Fig. 4, ^{1}H-signal at δ 4.11 (H-2) showed long-range (8 Hz) correlation with the ^{13}C-signals at δ 50.3 (C-5), 35.7 (C-12), and 27.1 (C-11). Thus, the partial structures **A**, **B**, **C** and the isolated methylene group could be connected through

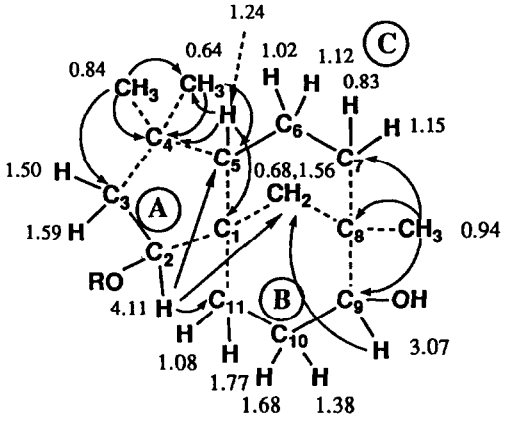
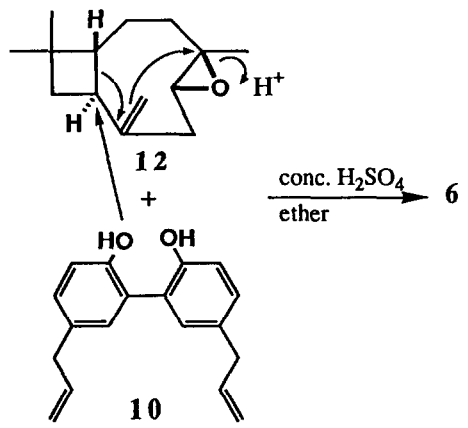


Fig. 3. Partial structures of **6** and C-H correlations observed in HMBC spectrum in C_6D_6



Scheme 4

the same quaternary carbon (C-1). Observation of cross peak between H-5 (δ 1.24) and C-1 (δ 45.3) also suported the connectivity. The correlation of C_{15}-methyl proton (δ 0.94) with C-7 (δ 33.2) and C-9 (δ 74.7) in addition to C-8 (δ 34.8) clarified the relation of the other terminals of the partial structures **B** and **C** and also the methylene group to result in the construction of bicyclo[3.3.1]nonane skeleton. Both the remaining methyl proton signals showed the correlation with C-3 (δ 44.5), C-4 (δ 37.8), and C-5 (δ 50.3). Thus, the sesquiterpene part was elucidated to be clovanediol.[17] The NOE enhancement of *ortho* -coupled aromatic resonance at δ 6.95 (H-3') upon irradiation of the oxygenated methine proton signal at δ 4.11 allowed us to connect magnolol to C-2 position of clovanediol through ether bond. The α (axial)-configuration of the secondary hydroxyl group was determined on the basis of small coupling constant between H-9 (δ 3.07) and adjacent methylene protons (J=3.2, 3.2 Hz). The proposed structure **6** is most likely to be formed by attack of magnolol as a nucleophile to the carbonium ion generated from caryophyllene oxide by successive epoxide opening, transannular cyclization followed by C-C bond migration. According to the hypothesis, one step synthesis of clovanemagnolol (**6**) was attempted. Treatment of a mixture of (-)-caryophyllene β-oxide (**12**) and **10** in anhydrous ether with one drop of conc. H_2SO_4 afforded an addition product ($[\alpha]_D$ +26.3°), whose ^{1}H and ^{13}C NMR data were superimposable with those of **6** (Scheme 4). Since the stereochemistry of this type of cyclization - rearrangement has been well established in the acid-catalyzed conversion of caryo-phyllene

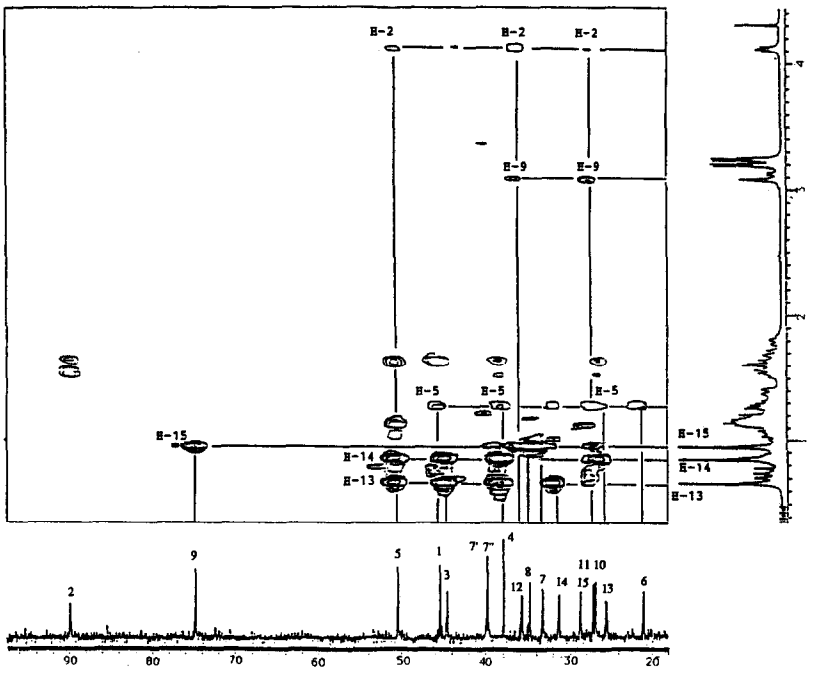


Fig. 4. HMBC spectrum of **6** in C_6D_6

oxide to clovanediol,[18] the structure of clovanemagnolol including absolute configuration was unequivocally established as the formula **6**. In accord with the stereostructure, NOEs shown in Fig. 5 were observed and β-orientation of magnolol unit on cyclopentane ring could be confirmed.

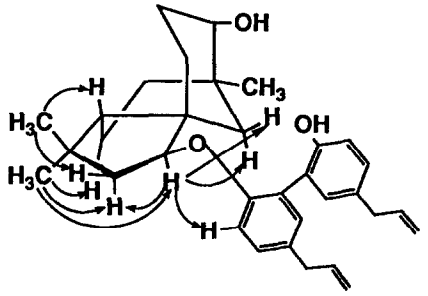


Fig. 5. NOEs observed in NOESY spectrum of **6**

Structure of caryolanemagnolol HREIMS of caryolanemagnolol (**7**), a colorless oil, gave the molecular formula as $C_{33}H_{42}O_3$ (*m/z* 486.3145). The presence of hydroxyl group and aromaic ring was again indicated by IR (ν_{max} 3600, 3300, 1600, 1480 cm^{-1}) and UV spectra [λ_{max} 211 (ε 6800), 285 (ε 8700) nm]. The aromatic part could be easily identified as **10** because the ^1H NMR spectrum revealed the presence of two 1,2,4-trisubstituted benzene rings and two allyl groups and the lower-field ^{13}C NMR signals are closely related with those of **10** (Table I) together with the observation of base ion peak at *m/z* 266 corresponding to

10 in EIMS. ^{1}H NMR spectrum showed the presence of three tertiary methyls (δ 0.71, 0.94, and 1.10) and an oxygenated methine proton (δ 3.05) and was much different from those of eudesmol or clovanediol type. On the basis of DQFCOSY and C/H COSY spectra the partial structures **A** and **B** (Fig. 6) could be identified in **7** in addition to three quaternary carbons. These partial structures were combined with the analysis of HMBC spectrum (Fig. 7). Namely, one of the tertiary methyl signals (δ 0.71) was correlated with C-7 (δ 35.8) in **B**, a quaternary carbon C-8 (δ 40.4), C-9 (δ 71.3) in **A**, and a methylene carbon (δ 40.4). The other methyl signal (δ 1.10) was correlated with the third methyl carbon (δ 20.8), C-3 (36.5) and C-5 (δ 44.2) in **B**, and C-4 (δ 34.9). Furthermore, H-3β signal (δ 2.03) had a cross peak with the oxygen-bearing

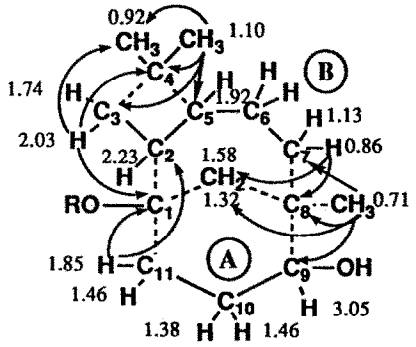
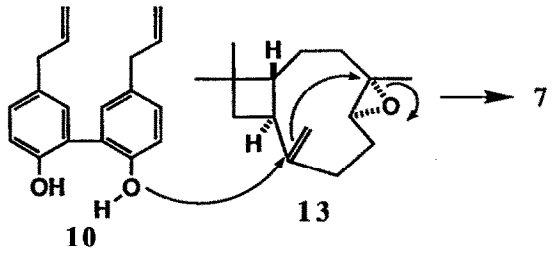


Fig. 6. Partial structures of **7** and C-H correlations observed in HMBC spectrum in C_6D_6



Scheme 5. Possible biosynthetic route of caryolanemagnolol

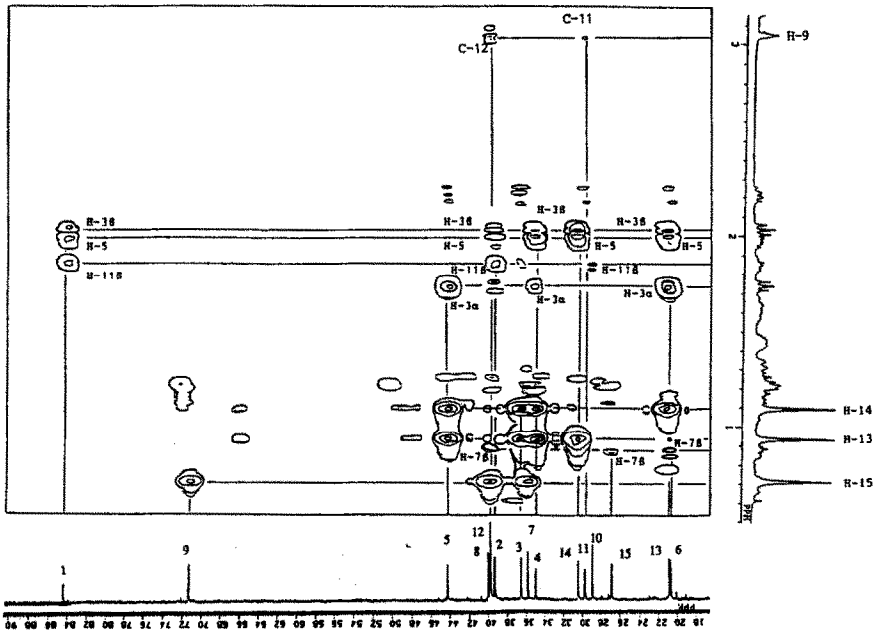


Fig. 7. HMBC spectrum of **7** in C_6D_6

carbon C-1 (δ 84.4) and C-4, while H-11β signal (δ 1.85) with C-1 and C-2 (δ 39.3). Thus obtained structure containing four-membered ring is corresponding to the glycol[17] (**12**) derived from caryophyllene oxide. In fact, 13C chemical shifts of these compounds were almost same except for the carbons around C-1 as can be seen in Table I. Large down-field shift of C-1 signal of **7** compared to **12** revealed that magnolol unit must be located at this position. Observation of clear NOEs between Me-4β (δ 1.10) and H-5b (δ 1.92) and between Me-4a (δ 0.92) and H-2α (δ 2.23) displayed trans nature of ring junction. The H-5β showed NOE interaction with one of

Table II. Effect of each compound on cell morphology and ChAT[a] actvity at 10 days in primary cell culture of fetal rat cerebral hemisphere[b]

Compound	Conc (M)	Morphological Evaluation[c]	ChAT Activity (pmol/min/dis)[d]
0.5% EtOH		-	4.1 ± 1.1
8	1x10-5	NS(-)	3.7 ± 1.6
10	1x10-5	NS(±)	5.9 ± 1.1
11	1x10-5	NS(+)	7.1 ± 1.1*
0.5% EtOH		-	23.7 ± 1.4
1	1x10-5	NS(+)	32.9 ± 1.6*
	1x10-6	NS(+)	33.6 ± 1.8*
	1x10-7	NS(+)	29.3 ± 1.4
6	1x10-5	NS(+)	41.0 ± 1.9*
	1x10-6	NS(+)	29.0 ± 0.2*
	1x10-7	NS(+)	26.2 ± 1.1
11	1x10-5	NS(+)	35.8 ± 1.1*
0.5% EtOH		-	4.0 ± 0.5
2	1x10-5	NS(-)	1.5 ± 0.6
	1x10-6	NS(±)	3.2 ± 1.1
3	1x10-5	NS(±)	2.3 ± 0 .3
0.5% EtOH		-	40.2 ± 1.0
5	1x10-5	NS(±)	80.6 ± 2.7*
	1x10-6	NS(+)	72.2 ± 3.5*
	1x10-7	NS(±)	66.5 ± 3.7*
7	1x10-5	NS(+)	94.0 ± 3.7*
	1x10-6	NS(+)	75.9 ± 1.8*
	1x10-7	NS(+)	65.6 ± 4.3*

a) Choline acetyltransferase. b)The dissociated Trypan blue negative cells were seeded at a density of 1.5 x 10^6 cells/35 mm dish containing 2.5 mL of 15% FCS-MEM. Each compound dissolved in 0.5% EtOH was added at 24 h after seeding and cell cultur was continued for 10 days. c) NS(+): Enhance neurite sprouting in comparison of neuronal cell containing 0.5% EtOH. d) The mark (*) denotes the values showing significant difference vs. 0.5% EtOH.

the methylene proton (δ 1.58) at C-12 indicating that the methylene bridge also has β-orientation. The β (axial)-configuration of secondary hydroxyl group was based on the small coupling constants (J=3.5, 3.5 Hz) observed between H-9 and adjacent protons. Caryolanemagnolol must be biosynthesized from (-)-caryophyllene α-oxide (**13**) through acid-catalyzed epoxide opening, cyclization followed by nucleophilic attack of magnolol (Scheme 5). Although any definite evidence has not been obtained so far, this biogenetic implication strongly suggests the absolute configuration of **7** as shown when one considers the co-occurence of (-)-caryophyllene in the same plant.

Effect of sesquiterpene-neolignans on primary cell culture of fetal rat cerebral hemisphere

NGF (nerve growth factor) and FGF (fibroblast growth factor) are well known as a neurotrophic factor to control neurite sprouting and proliferation of neuroblast during develpoment of neurons. These neurotrophic factors are essentiallly related to differentiation and chemotaxis of neurons, and recently expected to be possible in medical tretment of or prevention from presbyophrenia which has increasingly caused social problems. From this point of view, we are searching for a neurotrophic substance having NGF-like property in natural products and the activity of sesquiterpene-neolignans described above were investigated using a primary neuronal cell culture derived from fetal rat hemisphere.[20] The results summarized in Table II indicated that eudesobvatol A (**1**), clovanemagnolol (**6**), and caryolanemagnolol (**7**) could accelerate neurite sprouting and also increase chloine acetyltransferase activity (ChAT)[21] at the concentration of $1x10^{-7}$ M at 10 days after seeding in comparison of control system containing 0.5 % EtOH only. Among them, caryolanemagnolol (**7**) was found to be the most active substance. In contrast to these sesquiterpene-neolignans, simple biphenyl-type neolignans, obovatol (**8**) and magnolol (**10**), have no activity even at 10^{-5} M except for honokiol (**11**). It is interesting that these exotic substances exhibited neurotrophic property similar to that of NGF in neuronal cell culture of fetal rat hemi-sphere. Detailed neurotrophic action caused by these compounds, however, must wait for further biochemical study.[22]

Experimental Section

Genaral Optical rotations were recorded in $CHCl_3$ solution on a JASCO DIP-140 polarimeter. UV spectra were measured on a Shimadzu UV-300 spectrophotometer in ethanol solution. IR spectra were recorded on a HITACHI IR 260-10 spectrometer in $CHCl_3$ solution. NMR spectra were recorded on a JEOL JNM-GX400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C nuclei. NOE and 2-dimentional experiments were performed on the same apparatus. Pyridine-d_5 was used as solvent unless otherwise stated. Chemical shifts are reported in ppm relative to tetramethylsilane as internal standard and coupling constants (J) are expressed in Hz. Mass spectra were taken on a JEOL JMS-HX100 for HRMS and a JMS-SX102 for EI-, FD-, and FABMS. Merck Kieselgel 60 (70-230 mesh, 230-400 mesh) and Wakogel C-300 were used for silica-gel chromatography. Precoated Kieselgel 60 F_{254} or RP-8 F_{254} plates were used for analytical TLC and spots were visualized by UV (254 nm) and 2% $CeSO_4$ in H_2SO_4.

Extraction and isolation. (1) Sesquiterpene-neolignans 1-7. The dried bark (10 Kg) of *Magnolia ovobata* purchased from Koshiro Co., Ltd., in Japan was powdered and immersed at room temperature with methanol (90 L) for 8 days. The methanol extract was evaporated *in vacuo* to leave the viscous residue, to which water was added. The obtained suspension was extracted three times with ethyl acetate (EtOAc). The EtOAc-soluble portion was evaporated *in vacuo* to dryness giving an EtOAc extract (1056 g), 550 g of which was divided to fr. 1 (350 g) and fr. 2 (185 g) by silica gel (Kiselgel 60; 70 - 230 mesh, 2.96 Kg) chromatography eluting with *n*-herxane-EtOAc (1:1) and CH_2Cl_2-MeOH (7:3). The fr. 1 (350 g) was chromatographed on silica-gel (Wakogel C-300, 5.2 Kg) with a stepwise gradient [*n*-hexane-EtOAc (19:1,17 L), (14:1, 15 L), (9:1, 15 L), (8.5:1.5, 15 L), (4:1, 15 L), (3:2, 17

L), EtOAc (100%, 10 L), and EtOAc-MeOH (8.5:1.5, 13 L) to give frs 3 (5 g), 4 (20 g), 6 (20 g), 7 (95 g), 8 (105 g), 9 (15 g), 10 (10 g), 11 (15 g), 12 (15 g), and 13 (40 g). The fr. 9 (15 g) was subjected to Sephadex LH-20 chromatography eluting with MeOH-CH_2Cl_2 (7 : 3) to give a fraction (11 g) containing honokiol and a fraction (3.5 g) containing sesquiterpene-neolignans. The later fraction (3.5 g) was purified by MPLC [column: Lobar RP-8, type C; solvent: MeOH-H_2O (9 : 1)] to give eudesmagnolol (3) (400 mg), caryolanemagnolol (7) (350 mg), eudeshonokiol A (4) (200 mg) and a mixture (2 g), which was subjected to HPLC [column, Cosmosil $_5C_{18}$ ϕ 10x250 mm; solvent, MeOH:CH_3CN:H_2O=62:30:8 (2.5 ml/min); det., UV (254 nm)] and the peaks appeared at retention times 19.0, 20.0, 21.5, and 24.5 min were collected to give clovanemagnolol (6) (50 mg), eudesovobatol A (1) (200 mg), eudesobovatol B (2) (150 mg), and eudesmagnolol (3) (350 mg), respectively. The fr. 11 (15 g) was chromatographed on Sephadex LH-20 (1 L) eluting with MeOH to give three fractions. The second fraction (5.2 g) was purified by BIO-BEADS SX-12 chromato-graphy (benzene) followed by neutral alumina (CH_2Cl_2) and silica-gel (CH_2Cl_2-EtOAc, 9:1) chromatographies to afford eudes-honokiol B (5) (250 mg).

(2) ***O*-Methylhonokiols 11a and 11b.** The EtOAc-soluble portion (140 g) was subjected to column chromatography on silica-gel (Kiselgel 60, 70 - 230 mesh) and eluted with *n*-hexane-EtOAc (1:1) to give frs 1 (68 g), 2 (24 g), and 3 (4.4 g). The fr. 1 (68 g) was chromatographied on silica-gel (Wakogel C-300) with a stepwise gradient [*n*-hexane-EtOAc (9:1, 14 L), (8.8:1.5, 14 L), (4:1, 6 L), (2:1, 4 L) and EtOAc-MeOH (9:1, 2 L) to give frs 4 (2.5 g), 5 (2.5 g), 6 (1.0 g), 7 (4.8 g), 8 (9.5 g), 9 (21.0 g), 10 (10.7 g), 11 (0.8 g). The fr. 7 (4.8 g) was subjected to Sephadex LH-20 chromatography and eluted with MeOH-CH_2Cl_2 (4:1) giving four fractions. The third fraction (1.3 g) was purified by silica-gel (Wakogel C-300) chromatography (*n*-hexane-EtOAc, 9:1) followed by MPLC using Lobar RP-8 (MeOH-H_2O, 9:1) to yield 2'-*O*-methylhonokiol (**11a**) (280 mg) and 4-*O*-methylhonokiol (**11b**) (120 mg).

Eudesobovatol A (1); Colorless oil. $[\alpha]_D^{24.5}$ -46.1° (*c* 2.50). UV: λ_{max} 208 (ε 58000), 274 (ε 7200), 281 (ε 6700) nm. IR: ν_{max} 3600, 3530, 1640, 1600, 1500 cm^{-1}. FDMS: *m/z* 504 ([M]$^+$), 282, 222. HRFABMS: *m/z* 527.3094; Calcd *m/z* 527.3138 for $C_{33}H_{44}O_4Na$. ^1H NMR: δ 0.93 (3H, s; H-11), 1.01 (1H, ddd, *J*=11.7, 11.7, 4.8; H-1α), 1.23 (1H, ddd, *J*=12.7, 12.7, 3.9; H-9α), 1.29 (1H, ddd, *J*=11.7, 11.7, 4.8; H-1β), 1.42 (9H, s; H-12, 14, 15), 1.4-1.5 (2H, m; H-2), 1.48 (1H, ddd, *J*=13.1, 12.2, 11.7; H-6β), 1.53 (1H, m; H-8β), 1.66 (1H, dddd, *J*=12.2, 12.2, 3.4, 3.4 ; H-7), 1.83 (1H, m; H-8α), 1.94 (1H, dd, *J*=11.7, 3.4; H-5), 2.00 (1H, m; H-3), 2.14 (1H, m; H-3), 2.95 (1H, ddd, *J*=13.1, 3.4, 3.4; H-6α), 3.26 (2H, d, *J*= 6.8; H-7"), 3.31 (2H, d, *J*=6.4; H-7'), 5.03 (1H, dd, *J*=17.2, 2.0; H-9"), 5.04 (1H, dd, *J*=10.3, 2.0; H-9'), 5.08 (1H, dd, *J*=17.1, 2.0; H-9'), 5.10 (1H, dd, *J*=10.3, 2.0 ; H-9"), 5.91 (1H, ddt, *J*=17.2, 10.3, 6.8; H-8"), 6.01 (1H, ddt, *J*=17.1, 10.3, 6.4 ; H-8'), 6.84 (1H, d, *J*=2.0; H-5'), 7.02 (1H, d, *J*=2.0; H-3'), 7.05 (2H, d, *J*=8.8; H-2", 6"), 7.11 (2H, d,*J*=8.8; H-3", 5"). ^{13}C NMR: See Table I.

Eudesobovatol B (2); Colorless oil. $[\alpha]_D^{23}$ -26.1° (*c* 1.15). UV: λ_{max} 207 (ε 48000), 273 (ε 5000), 278 (ε 4700) nm. IR: ν_{max} 3520, 1640, 1610, 1500 cm^{-1}. Negative FABMS: *m/z* 503 ([M-H]$^-$), 282. HRFABMS: *m/z* 527.3062; Calcd *m/z* 527.3138 for $C_{33}H_{44}O_4Na$. ^1H NMR: δ 0.92 (3H, s; H-11), 1.04 (1H, ddd, *J*=11.0, 11.0, 4.8; H-1α), 1.19 (1H, ddd, *J*=12.4, 12.4, 3.6; H-9α), 1.29 (6H, s; H-14, 15), 1.29 (1H, m; H-1β), 1.38 (1H, ddd, *J*=12.4, 11.7, 9.5; H-6β), 1.50 (1H, m; H-7), 1.54 (1H, m; H-8β), 1.58 (3H, s; H-12), 1.82 (1H, m; H-8α), 2.06 (1H, dd, *J*=11.7, 2.2; H-5), 210 (1H, m; H-3α), 2.12 (1H, m; H-3β), 2.65 (1H, ddd, *J*=12.4, 2.2, 2.2; H-6α), 3.25 (2H, d, *J*=6.6; H-7'), 3.33 (2H, d, *J*=6.6; H-7"), 4.96-5.06 (4H, m; H-9', 9"), 5.94 (1H, ddt, *J*=15.4, 10.3, 6.6; H-8'), 5.98 (1H, ddt, *J*=16.8, 10.2, 6.6; H-8"), 6.61 (1H, d, *J*=1.5; H-3'), 6.99 (1H, d, *J*=1.5; H-5'), 7.10 (2H, d, *J*=8.0; H-2", 6"), 7.17 (2H, d, *J*=8.0; H-3", 5"). ^{13}C NMR: See Table I.

Eudesmagnolol (3); Colorless oil. $[\alpha]_D^{22.5}$ -74.8° (*c* 9.15). UV: λ_{max} 211 (ε 58000), 290 (ε 7400) nm. IR: ν_{max} 3610, 3260, 1650, 1500, 1400 cm^{-1}. FDMS: *m/z* 488 ([M]$^+$), 266, 223. HRFABMS: *m/z* 511.3188; Calcd *m/z* 511.3189 for $C_{33}H_{44}O_3Na$. ^1H NMR: δ 0.80 (1H, m; H-1α), 0.81 (3H, s; H-11), 1.16 (1H, m; H-1β), 1.22 (3H, s; H-12), 1.26 (1H, m; H-2β), 1.32 (1H, m; H-8β), 1.38 (1H, m; H-2α), 1.40 (1H, ddd, *J*=12.2, 12.2, 10.7; H-6β), 1.43 (3H, s; H-15), 1.44 (1H, m; H-3α),

1.45 (3H, s; H-14), 1.51 (1H, m; H-3β), 1.59 (1H, dd, *J*=12.2, 2.9; H-5), 1.66 (1H, dddd, *J*=12.2, 12.2, 3.9, 2.9; H-7), 1.82 (1H, ddd, *J*=10.7, 2.9, 2.9; H-8α), 2.56 (1H, ddd, *J*=12.2, 3.9, 2.9; H-6α), 3.35 (2H, d, *J*=6.8; H-7'), 3.45(2H, *J*=6.8; H-7"), 5.01 (1H, dd, *J*=10.3, 1.9; H-9'), 5.05 (1H, dd, *J*=10.3, 1.9; H-9"), 5.10 (1H, dd, *J*=17.1, 1.9; H-9'), 5.14 (1H, dd, *J*=17.1, 1.9; H-9"), 5.97 (1H, ddt, *J*=17.1, 10.3, 6.8 Hz; H-8'), 6.01 (1H, ddt, *J*=17.1, 10.3, 6.8; H-8"), 7.13 (1H, dd, *J*=8.3, 2.0 ; H-4'), 7.19 (1H, dd, *J*=8.3, 2.0 Hz; H-4"), 7.24 (1H, d, *J*=8.3; H-3'), 7.26 (1H, d, *J*=8.3; H-3"), 7.35 (1H, d, *J*=2.0; H-6"), 7.44 (1H, d, *J*=2.0; H-6'). ^{13}C NMR: See Table I.

Eudeshonokiol A (4); Colorless oil. $[\alpha]_D^{21}$ -48.6° (*c* 0.45). UV: λ_{max} 208 (ε 67000), 252 (ε 32000), 290 (ε 13000) nm. IR: ν_{max} 3670, 3550, 1640, 1600, 1480 cm^{-1}. FABMS: *m/z* 511 ([M+Na]$^+$), 266, 224. HRFABMS: *m/z* 511.3164; Calcd *m/z* 511.3189 for $C_{33}H_{44}O_3Na$. ^{1}H NMR: δ 0.92 (3H, s; H-11), 1.01 (1H, ddd, *J*=13.1, 13.1, 2.9; H-1α), 1.25 (1H, ddd, *J*=13.1, 2.9, 2.9; H-1β), 1.32 (3H, s; H-12), 1.43 (6H, s; H-14, 15), 1.45 (1H, ddd, *J*=11.7, 11.7, 11.7; H-6β), 1.53 (1H, m; H-8β), 1.57 (1H, m; H-3α), 1.66 (1H, dddd, *J*=11.7, 11.7, 3.6, 3.6; H-7), 1.84 (1H, dd, *J*=11.7, 2.1; H-5), 1.86 (1H, m; H-8α), 2.02 (1H, m; H-3β), 2.65 (1H, ddd, *J*=11.7, 3.6, 2.1; H-6α), 3.39 (2H, d, *J*=6.6; H-7"), 3.55 (1H, dd, *J*=15.4, 6.6; H-7'), 3.71 (1H, dd, *J*=15.4, 6.6; H-7'), 5.06 (2H, dd, *J*=10.3, 2.2; H-9', 9"), 5.14 (1H, dd, *J*=16.9, 2.2; H-9'), 5.20 (1H, dd, *J*=16.9, 2.2; H-9"), 6.06 (1H, ddt, *J*=16.9, 10.3, .6; H-8"), 6.15 (1H, dddd, *J*=16.9, 10.3, 6.6, 6.6; H-8'), 7.13 (1H, dd, *J*=8.1, 2.2; H-4"), 7.26 (1H, d, *J*=8.1; H-3"), 7.28 (1H, d, *J*=8.1; H-5'), 7.41 (1H, d, *J*=2.2; H-6"), 7.79 (1H, dd, *J*=8.1, 2.2; H-6'), 7.86 (1H, d, *J*=2.2; H-2'). ^{13}C NMR: See Table I.

Eudeshonokiol B (5) ; Colorless oil. $[\alpha]_D^{22}$ -71.7° (*c* 1.08). UV: λ_{max} 208 (ε, 56000), 258 (ε 20500) nm. IR: ν_{max} 3580, 3330, 1640, 1600,1480 cm^{-1}. FABMS: *m/z* 495 ([M+Li]$^+$), 267. HRFABMS: *m/z* 511.3193; Calcd *m/z* 511.3189 for $C_{33}H_{44}O_3Na$. ^{1}H NMR (C_6D_6): δ 0.80 (1H, m; H-1α), 0.81 (3H, s; H-11), 1.18 (1H, m; H-1β), 1.20 (3H, s; H-12), 1.37 (1H, ddd, *J*=12.2, 12.2, 12.2; H-6β), 1.42 (3H, s; H-15), 1.45 (3H, s; H-14), 1.50 (1H, ddd, *J*=12.4, 12.4, 3.6; H-3α), 1.66 (1H, dddd, *J*=12.2, 12.2, 2.5, 2.5; H-7), 1.71 (1H, dd, *J*=12.2, 2.5; H-5), 1.83 (1H, ddd, *J*=12.4, 3.6, 3.6; H-3β), 2.69 (1H, ddd, *J*=12.2, 2.5, 2.5; H-6α), 3.39 (2H, d, *J*=6.8; H-7"), 3.80 (2H, d, *J*=6.6; H-7'), 5.04 (1H, dd, *J*=17.1, 2.0; H-9"), 5.07 (1H, dd, *J*=10.0, 2.0; H-9"), 5.16 (1H, dd, *J*=10.0, 1.7; H-9'), 5.30 (1H, dd, *J*=17.1, 1.7; H-9'), 6.03 (1H, ddt, *J*=17.1, 10.0, 6.8; H-8"), 6.35 (1H, ddt, *J*=17.1, 10.0, 6.6; H-8'), 7.11 (1H, dd, *J*=8.3, 2.2; H-4"), 7.24 (1H, d, *J*=8.3; H-3"), 7.29 (1H, d, *J*=8.3; H-3'), 7.40 (1H, d, *J*=2.2; H-6"), 7.57 (1H, dd, *J*=8.3, 2.2; H-6'), 7.68 (1H, d, *J*=2.2; H-2'). ^{13}C NMR: See Table I.

Clovanemagnolol (6) ; Colorless oil. $[\alpha]_D^{25}$ +21.0° (*c* 1.50). UV: λ_{max} 204 (ε 46000), 208 (ε 41000), 286 (ε 5800) nm. IR: ν_{max} 3550, 3350, 1640, 1500 cm^{-1}. HREIMS: *m/z* 486.3127; Calcd *m/z* 486.3134 for $C_{33}H_{42}O_3$. ^{1}H NMR (C_6D_6): δ 0.64 (3H, s; H-13), 0.68 (1H, d, *J*=13.9; H-12α), 0.83 (1H, m; H-7β), 0.84 (3H, s; H-14), 0.94 (3H, s; H-15), 1.02 (1H, dddd, *J*=11.5, 11.5, 11.0, 6.1; H-6α), 1.08 (1H, ddd, *J*=13.6, 3.2, 3.2; H-11b), 1.12 (1H, m; H-6β), 1.15 (1H, m; H-7α), 1.24 (1H, dd, *J*=11.5, 5.6 ; H-5), 1.38 (1H, dddd, *J*=13.6, 3.2, 3.2, 3.2; H-10α), 1.50 (1H, dd, *J*=12.7, 8.5; H-3β), 1.56 (1H, d, *J*=13.9; H-12β), 1.59 (1H, dd, *J*=12.7, 5.8; H-3α), 1.68 (1H, dddd, *J*=13.6, 13.6, 3.2, 3.2; H-10β), 1.77 (1H, dddd, *J*=13.6, 13.6, 3.2, 3.2; H-11α), 3.07 (1H, dd, *J*=3.2, 3.2; H-9β), 3.19 (2H, d, *J*=6.8; H-7'), 3.24 (1H, d, *J*=6.6; H-7"), 4.11 (1H, dd, *J*=8.5, 5.8; H-2α), 4.97 (1H, dd, *J*=16.9, 1.2; H-9'), 4.99 (1H, dd, *J*=10.0, 1.2; H-9'), 5.00 (1H, dd, *J*=10.0, 1.2; H-9"), 5.02 (1H, dd, *J*=16.8, 1.2; H-9"), 5.88 (1H, ddt, *J*=16.9, 10.0, 6.8; H-8'), 5.94 (1H, ddt, *J*=16.8, 10.0, 6.6; H-8"), 6.95 (1H, d, *J*=8.3; H-3'), 7.04 (1H, dd, *J*=8.3, 2.2; H-4"), 7.06 (1H, dd, *J*=8.3, 2.4; H-4'), 7.17 (1H, d, *J*=2.4; H-6'), 7.18 (1H, d, *J*=2.2; H-6"),7.20 (1H, d, J=8.3; H-3"). ^{13}C NMR: See Table I.

Caryolanemagnolol (7) ; Colorless oil. $[\alpha]_D^{23.5}$ +11.2° (*c* 1.85). UV: λ_{max} 211 (ε 68000), 285 (ε 8700) nm. IR: ν_{max} 3600, 3300, 1670, 1640, 1600, 1480 cm^{-1}. EIMS: *m/z* 486 ([M]$^+$), 266. HREIMS: *m/z* 486.3145; Calcd *m/z* 486.3134 for $C_{33}H_{42}O_3$. ^{1}H NMR (C_6D_6): δ 0.71 (3H, s; H-15), 0.86 (1H, ddd, *J*=13.9, 4.4, 4.4; H-7β), 0.94 (3H, s; H-13), 1.10 (3H, s; H-14), 1.13 (1H, m; H-7α), 1.20-1.25 (2H, m; H-6), 1.32 (1H, d, *J*=12.9; H-12β), 1.46 (1H, dddd, *J*=11.4, 5.2, 3.6, 3.2; H-10β), 1.46 (1H, ddd, *J*=11.4, 3.6, 3.6; H-11α), 1.58 (1H, d, *J*=12.9; H-12α), 1.65 (1H, dddd, *J*=11.4, 11.4, 3.6, 3.2; H-10α), 1.74 (1H, dd, *J*=9.8, 8.1;

H-3α), 1.85 (1H, ddd, *J*=11.4, 11.4, 5.2; H-11β), 1.92 (1H, ddd, *J*=11.2, 6.8, 2.6; H-5), 2.03 (1H, dd, *J*=9.8, 9.8; H-3β), 2.23 (1H, ddd, *J*=11.2, 9.8, 8.1; H-2), 3.05 (1H, dd, *J*=3.2, 3.2; H-9α), 3.17 (2H, d, *J*=6.6; H-7'), 3.25 (2H, d, *J*=6.6; H-7"), 4.97 (1H, dd, *J*=10.0, 1.2; H-9'), 4.99 (1H, dd, *J*=17.1, 1.2; H-9'), 5.01 (1H, dd, *J*=17.1, 1.2; H-9"), 5.03 (1H, dd, *J*=10.0, 1.2; H-9"), 5.83 (1H, ddt, *J*=17.1, 10.0, 6.6; H-8'), 5.96 (1H, ddt, *J*=17.1, 10.0, 6.6; H-8"), 6.96 (1H, d, *J*=8.3; H-3'), 6.98 (1H, dd, *J*=8.3, 2.0; H-4'), 7.05 (1H, dd, *J*=8.3, 2.2; H-4"), 7.21 (1H, d, *J*=2.0; H-6'), 7.23 (1H, d, *J*=2.2; H-6"), 7.25 (1H, d, *J*=8.3; H-3"). ^{13}C NMR: See Table I.

2'-O-Methylhonokiol (11a); Colorless oil. UV: λ_{max} 207 (ε 21000), 252 (ε 9600), 283 (ε 6400) nm. IR: ν_{max} 3550, 1635, 1600 cm^{-1}. EIMS: *m/z* 280 ([M]$^+$), 224. HREIMS: *m/z* 280.1450; Calcd. *m/z* 280.1463 for $C_{19}H_{20}O_2$. ^{1}H NMR ($CDCl_3$): δ 3.41 (2H, d, *J*=6.8; H-7'), 3.50 (2H, d, *J*=6.3; H-7), 3.79 (3H, s, OCH_3), 5.06 (1H, dd, *J*=10.3, 2.0; H-9), 5.10 (1H, dd, *J*=17.1, 2.0; H-9), 5.18 (1H, dd, *J*=9.8, 2.0; H-9'), 5.22 (1H, dd, *J*=17.1, 2.0; H-9'), 6.02 (1H, ddt, *J*=17.1, 9.8, 6.8; H-8'), 6.10 (1H, ddt, *J*=17.1, 10.3, 6.3; H-8'), 6.85 (1H, d, *J*=8.3; H-5'), 6.90 (1H, d, *J*=8.3; H-3'), 7.10 (1H, dd, *J*=8.3, 2.5; H-4'), 7.11 (1H, d, *J* =2.5; H-6'), 7.28 (1H, d, *J*=2.4; H-2), 7.32 (1H, dd, *J*=8.3 , 2.4; H-6). ^{13}C NMR ($CDCl_3$): δ 35.3 (C-7), 39.4 (C-7'), 55.8 (OCH_3), 111.4 (C-3), 115.5 (C-4, 9'), 116.5 (C-9), 124.8 (C-3), 128.0 (C-6), 129.1 (C-6'), 130.4 (C-1), 131.0 (C-4'), 131.3 (C-1'), 131.5 (C-2), 132.3 (C-5'), 136.5 (C-8), 137.8 (C-8'), 153.3 (C-4), 154.9 (C-2').

4-O-Methylhonokiol (11b); Colorless oil. UV: λ_{max} 208 (ε 25000), 253 (ε 7400), 290 (ε 4700) nm. IR: ν_{max} 3580, 1640, 16905 cm^{-1}. EIMS: *m/z* 280 ([M]$^+$), 254. ^{1}H NMR ($CDCl_3$): δ 3.35 (2H, d, *J*=6.8; H-7'), 3.43 (2H, d, *J*=6.4; H-7), 3.87 (3H, s; OCH_3), 5.97 (1H, ddt, *J*=17.1, 10.3, 6.8; H-8), 6.00 (1H, ddt, *J*=17.1, 10.3, 6.4; H-8'), 6.90 (1H, d, *J*=8.3; H-3), 6.96 (1H, d, *J*= 8.3; H-3'), 7.02 (1H, d, *J*=2.4; H-6), 7.05 (1H, dd, *J*=8.3, 2.4; H-4), 7.23 (1H, d, *J*=2.4; H-2'), 7.28 (1H, dd,*J*=8.3, 2.4; H-6'). ^{13}C NMR ($CDCl_3$): δ 34.3 (C-7), 39.4 (C-7'), 55.6 (OCH_3), 111.0 (C-5), 115.5 (C-2', 9'), 115.8 (C-9), 127.9 (C-6), 128.8 (C-6'), 129.1 (C-3), 129.7 (C-1), 129.8 (C-1'), 130.2 (C-4'), 130.5 (C-2), 132.2 (C-5'), 136.5 (C-8), 137.8 (C-8'), 150.9 (C-2'), 157.1 (C-4).

Acetylation (Typical procedure). To a solution of **1** (20 mg) in pyridine (0.7 mL) was added acetic anhydride (0.7 mL) and the mixture was allowed to stand at room temperature for 48 h. The reaction mixture was diluted with cold water and exracted with ether. The extracts were washed with water, 1N HCl, sated $NaHCO_3$, and water. After drying over $MgSO_4$, solvent was evaporated *in vacuo* and the residue was chromatographed on silica-gel (*n*-hexane-EtOAc, 4:1) to give **1a** (14 mg) .

1a; Colorless oil. IR: λ_{max} 1740, 1720, 1650, 1608 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, s), 1.24 (3H, s), 1.46 (3H, s), 1.98 (3H, s), 2.19 (3H, s), 3.26 (2H, d, *J*=6.0), 3.35 (2H, d, *J*=6.0), 5.01-5.07 (4H, m), 5.79-6.01 (2H, m), 6.50 (1H, d, *J*=1.8), 6.62 (1H, d, *J*=1.8), 6.90 (2H, d, *J*=8.5), 7.11 (2H, d, *J*=8.5).

3 (30 mg) was similarly acetylated as described above to give diacetate **3a** (29 mg) as colorless oil. $[\alpha]_D^{18}$ -49.4° (*c* 0.91) . UV: λ_{max} 234 (ε 17500), 274 (ε 3900) nm. IR: ν_{max} 1760, 1725, 1635, 1490 cm^{-1}. EIMS: *m/z* 308, 266, 223. FDMS: *m/z* 572 ([M]$^+$), 307, 264, 205. ^{1}H NMR: δ ($CDCl_3$) 0.80 (3H, s; H-11), 1.08 (3H, broad s; H-12), 1.41 (3H, s; H-15), 1.46 (3H, s; H-14), 2.02 (3H, s; H-17), 2.34 (3H, s; H-11"), 3.35 (2H, d, *J*=6.8; H-7'), 3.41 (2H, d, *J*=6.4; H-7"), 5.04-5.10 (4H, m; H-9',9"), 5.91 (1H, ddt, *J*=17.1, 9.8, 6.4; H-8"), 6.00 (1H, ddt, *J*=16.6, 9.7, 6.8; H-8'), 7.27 (1H, d, *J*=8.3; H-3'), 7.37 (1H, d, *J*=2.4; H-6'), 7.43 (1H, dd, *J*=8.3, 2.4; H-4'), 7.44 (1H, d, *J*=7.8; H-3"), 7.52 (1H, d, *J*=7.8, 2.2; H-4"), 7.53 (1H, d, *J*=2.2; H-6"). ^{13}C NMR ($CDCl_3$): δ 19.0 (C-11), 19.9 (C-2), 21.0 (C-12, Ac), 21.5 (C-6), 22.0 (C-8), 22.6 (Ac), 23.4 (C-14), 23.8 (C-15), 34.7 (C-10), 38.1 (C-3), 39.5 (C-7'), 39.6 (C-7"), 40.5 (C-1), 44.7 (C-9), 47.1 (C-7), 51.7 (C-5), 84.4 (C-13), 85.2 (C-4), 115.7 (C-9'), 115.9 (C-9"), 122.4 (C-3"), 124.1 (C-3'), 128.1 (C-4"), 128.2 (C-4'), 131.2 (C-6'), 132.1 (C-6"), 133.2 (C-1"), 133.4 (C-1'), 134.3 (C-5"), 136.9 (C-5'), 137.4 (C-8"), 137.6 (C-8'), 146.6 (C-2"), 151.0 (C-2'), 169.4 (CO), 170.5 (CO).

6 (6 mg) was similarly acetylated as described above to give **6a** (6 mg) as colorless oil. IR: ν_{max} 1760, 1720, 1640, 1600, 1480 cm^{-1}. FABMS: *m/z* 593 ([M+Na]$^+$), 570 ([M]$^+$), 308, 266, 224, 203. ^{1}H NMR ($CDCl_3$): δ 0.81 (3H, s; H-15), 0.87 (3H, s),

0.93 (3H, s; H-14), 1.98 (3H, s; H-11"), 2.00 (3H, s; H-17), 3.33 (2H, d, *J*=6.6; H-7'), 3.37 (2H, d, *J*=6.8; H-7"), 4.12 (1H, dd, *J*=7.3, 5.6; H-2α), 4.49 (1H, m; H-9β), 5.03-5.10 (4H,m; H-9',9"), 5.94 (1H, ddt, *J*=16.9, 10.0, 6.8; H-8"), 5.96 (1H, ddt, *J*=16.8, 10.0, 6.6; H-8'), 6.87 (1H, d, *J*=8.3; H-3'), 7.00 (1H, d, *J*=2.4; H-6'), 7.02 (1H, d, *J*=8.3; H-3"), 7.08 (1H, dd, *J*=8.3, 2.4; H-4'), 7.13 (1H, dd, *J*=8.3, 2.2; H-4"), 7.19 (1H, d, *J*=2.2; H-6").

Methylation (Typical procedure). Eudesobovatol A (**1**) (10 mg) was treated with etherial solution of diazomethane at room temp. for 36 h. Ether was evaporated and the residue was chromatographed on silica-gel (CH_2Cl_2-EtOAc, 96:4) to give a mono-methyl ether **1b** (5 mg).

1b; Colorless oil. Negative FABMS: *m/z* 517 ([M-H]$^-$), 297, 282. ^{1}H NMR ($CDCl_3$): δ 0.92 (3H, s; H-11), 1.21 (3H, s; H-12), 1.23 (3H, s; H-14), 1.25 (3H, s; H-15), 3.24 (2H, d, *J*=6.4; H-7'), 3.35 (2H, d, *J*=6.3; H-7"), 3.74 (3H, s; OMe), 6.54 (1H, d, *J*=2.0; H-5'), 6.61 (1H, d,*J*=2.0; H-3'), 6.87 (2H, d,*J*=8.3; H-2",6"), 7.11 (2H, d, *J*=8.3; H-3",5").

2a; Colorles oil. Negative FABMS: *m/z* 517 ([M-1]$^-$), 503, 297, 282. ^{1}H NMR: δ ($CDCl_3$) 0.85 (3H, s; H-11), 0.95 (3H, s; H-15), 0.98 (3H, s; H-14), 1.32 (3H, s; H-12), 3.27 (2H, d, *J*=6.8; H-7'), 3.33 (2H, d, *J*=6.8; H-7"), 3.81 (3H, s; OMe), 6.41 (1H, d, *J*=2.0; H-5'), 6.52 (1H, d, *J*=2.0; H-3'), 6.80 (2H, d, *J*=8.3; H-2",6"), 7.07 (2H, d, *J*=8.3; H-3",5").

4a; Colorless oil; Negative FABMS: *m/z* 501 ([M-1]$^-$). ^{1}H NMR: δ ($CDCl_3$) 0.94 (3H, s; H-11), 1.23 (3H, s; H-14), 1.25 (3H, s; H-15), 1.33 (3H, s; H-12), 3.36 (2H, d, *J*=5.4; H-7"), 3.38 (1H, dd,*J*=15.6, 6.8; H-7'), 3.48 (1H, dd, *J*=15.6, 6.3; H-7'), 3.77 (3H, s; OMe), 4.90-5.12 (4H, m; H-9',9"), 5.96 (1H, dddd, *J*=17.1, 10.5, 6.8, 6.3; H-8'), 5.97 (1H, ddt, *J*=17.1, 10.3, 5.4; H-8"), 6.90 (1H, d, *J*=8.3; H-3"), 7.01 (1H, d, *J*=8.3; H-5"), 7.08 (1H, dd, *J*=8.3, 2.4; H-4"), 7.13 (1H, dd, *J*=8.3, 2.4; H-6'), 7.31 (1H, d, *J*=2.4; H-6"), 7.40 (1H, d, *J*=2.4; H-2').

5a; Colorless oil. Negative FABMS: *m/z* 501 ([M-1]$^-$). ^{1}H NMR: δ ($CDCl_3$) 0.81 (3H, s; H-11), 1.04 (3H, s; H-12), 1.20 (6H, s; H-14,15), 3.37 (2H, d, *J*=6.8; H-7"), 3.42 (2H, d, *J*=6.3; H-7'), 3.86 (3H, s; OMe), 5.99 (1H, ddt, *J*=17.1, 10.0, 6.8; H-8"), 6.03 (1H, ddt, *J*=17.1, 10.0, 6.3; H-8'), 6.87 (1H, d, *J*=8.3; H-5'), 6.92 (1H, d, *J*=8.3; H-3"), 7.00 (1H, dd, *J*=8.3, 2.4; H-4"), 7.09 (1H, d, *J*=2.4; H-6"), 7.28 (1H, d, *J*=2.2; H-2'), 7.31 (1H, dd, *J*=8.3, 2.2; H-6').

Acid-cleavage (Typical procedure). To a solution of eudesobovatol A (**1**) (12 mg) in dry benzene (1 mL) was added CF_3COOH (0.3 mL) and the mixture was stirred at room temp for 10 h. Solvent was evaporated *in vacuo* and the residue was chromatographed on silica-gel (*n*-haxane-EtOAc, 8:1) to yield obovatol (**8**) (7 mg) and (+)-γ-eudesmol (**9**) (2 mg), $[\alpha]_D^{22}$ +55.5° (*c* 0.08).

Eudesmagnolol (**3**) (20 mg) yielded magnolol (**10**) (12 mg) and (+)-**9** (2 mg), $[\alpha]_D^{22}$ +55.5° (*c* 0.08).

Eudeshonokiol A (**4**) (14 mg) yielded honokiol (**11**) (7 mg) and (+)-**9** (3 mg), $[\alpha]_D^{22}$ +45.3° (*c* 0.17).

Methyl ether (**4a**) (5 mg) yielded 2'-*O*-methylhonokiol (**11a**) (2 mg) and (+)-**9** (0.5 mg).

Methyl ether (**5a**) (10 mg) yielded 4-*O*-methlhonokiol (**11b**) (6 mg) and (+)-**9** (2 mg).

Synthesis of clovanemagnolol (6). To an ice-cooled solution of magnolol (**10**) (600 mg) and (-)-β-caryophyllene oxide (45 mg) in 2 mL of dry ether was added conc. H_2SO_4 (0.05 mL) under argon. After stirring at 0° C for 3 h, the reaction mixture was diluted with water and extracted with ether. The extracts were washed with brine and dried over $MgSO_4$. Solvent was evaporated in vacuo and the residue was subjected to column chromatography on Sephadex LH-20 (MeOH-CH_2Cl_2, 7:3) to remove unreacted **10**. The fraction containing **6** was then chromatographed on silica-gel (*n*-hexane-CH_2Cl_2, 1:9) to give **6** (14 mg), $[\alpha]_D^{21.5}$ +26.3° (*c* 0.5).

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