New Sesquiterpenes from *Litsea verticillata*

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Seven new sesquiterpenes, named litseagermacrane (**1**), 7-*epi*-eudesm-4(15)-ene-1α,6α-diol (**2**), 5-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol (**4**), litseahumulanes A (**6**) and B (**7**), and litseachromolaevanes A (**11**) and B (**12**), as well as the known compounds 7-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol (**3**), eudesm-4(15)-ene-1*β*,6α-diol (**5**), octahydro-4-hydroxy-3α-methyl-7-methylene-α-(1-methylethyl)-1*H*-indene-1-methanol (**8**), 10-hydroxyl-15-oxo-α-cadinol (**9**), and aphanamol II (**10**), were isolated from an anti-HIV fraction of the leaves and twigs of *Litsea verticillata* Hance (Figure 1). Isolates **1**, **4**, and **12** were found to inhibit HIV-1 replication in a green fluorescent protein (GFP)-based reporter cell line (HOG.R5) with IC_{50} values of 6.5 (27.5), 17.4 (73.1), and 28.0 (119.7) *μ*g/mL (*μ*M), respectively. The structures of these isolates were determined by spectral data including 1D and 2D NMR spectra. Compound **11** was confirmed by X-ray crystallographic analysis.

Litsea verticillata Hance was the first anti-HIV plant lead identified and subjected to bioassay-directed phytochemical investigation as part of our International Cooperative Biodiversity Group (ICBG) project. The ICBG Program is a unique effort that addresses the interdependent issues of drug discovery from natural products, biodiversity conservation, and sustainable economic growth. *L. verticillata* has been found to contain a number of anti-HIV active substances.[1−3] A series of active sesquiterpenes with a new unique skeleton, which we named litseanes, has been isolated from the leaves and twigs of *L. verticillata* in our previous studies. The biological activity profiles of the litseanes were influenced by their structural features.[2,3] Further investigation of this plant material has resulted in the isolation of 12 additional compounds, belonging to seven different types of sesquiterpenes, including germacrane, eudesmane, oppositane, humulane, cadinane, isodaucane, and chromolaevane types.

Bioassay-directed fractionation of the $CHCl_3$ extract of the leaves and twigs of *L. verticillata* by repeated flash column chromatography on silica gel and RP-18 afforded two active fractions, F-17 and F-18. As previously reported,[2,3] the litseane sesquiterpenes were obtained from fraction F-17. Separation of the fraction F-18 by preparative HPLC chromatography in the current study afforded seven new sesquiterpenes, litseagermacrane (**1**), 7-*epi*-eudesm-4(15)-ene-1α,6α-diol (**2**), 5-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol (**4**), litseahumulanes A (**6**) and B (**7**), and litseachromolaevanes A (**11**) and B (**12**), as well as the known 7-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol (**3**),[4] eudesm-4(15)-ene-1*β*,6α-diol (**5**),[4] octahydro-4-hydroxy-3α-methyl-7-methylene-α-(1-methylethyl)-1*H*-indene-1-methanol (**8**),[5] 10-hydroxyl-15-oxo-α-cadinol (**9**),[6] and aphanamol II (**10**).[7] The present paper describes the isolation, identification, and biological evaluation of these isolates.

Results and Discussion

Litseagermacrane (**1**) was shown to have a molecular formula of $C_{15}H_{24}O_2$ by HRTOFMS ($[M + Na]^+$ (*m*/*z* 259.1682, calcd 259.1674), which was consistent with ^{13}C NMR and DEPT experiments. The 15 carbons were characterized by DEPT-135 and DEPT-90 spectra as three methyls, four methylenes, an olefinic methylene, two methines, two olefinic methines, an oxy-quaternary carbon, an olefinic quaternary carbon, and a quaternary carbonyl group (Table 3). One of two present double bonds was deduced to form an α,*β*-conjugated keto group with the carbonyl carbon (δ_C 206.1, C-1) based on the downfield shift of the olefinic methylene carbon (δ_C 119.7, C-14) and the upfield shift of the carbonyl carbon in the ^{13}C NMR spectra. The structure of **1** was determined by $^1H-^1H$ COSY, HMQC, and HMBC techniques using the α,*β*-conjugated keto group as a starting point for deduction. The HMQC spectrum established the olefinic methylene carbon (C-14) to be attached by two protons at δ_H 5.62 (brs) and 5.47 (d, $J = 1.4$ Hz), which were in turn shown to have long-range correlations to δ_C 28.9 (t, C-9) in the HMBC spectrum (Figure 2). In the $^1H-^1H$ COSY spectrum, the two protons of C-9 at δ_H 2.53 (brtt, $J = 13.1$, 1.9 Hz) and 2.24 (brdd, $J = 13.9$, 5.9 Hz) were observed to have correlations with the protons at δ_H 2.01 (brd, $J = 13.1$ Hz, H-8α) and 1.31 (brqd, $J = 13.1$, 2.5 Hz, H-8*β*), which subsequently correlated to H-7. A double bond [δ_C 142.9 (d, C-5), 130.3 (d, C-6) and δ_H 5.03 (ABdd, $J = 15.3$, 9.3 Hz, H-6), 4.97 (ABdd, $J = 15.1$, 8.5 Hz, H-5)] was then connected to C-7 due to the correlation of H-7 to H-6 in the $^1H-^1H$ COSY spectrum. Further analysis of the $^1H-^1H$ COSY and HMQC spectra connected the $\Delta^{5,6}$ double bond to C-4, which was bonded by C-3, and then by C-2. The fact that the proton signals of H_2-2 at δ_H 2.82 (ddd, $J = 14.0$, 12.3, 2.1 Hz) and 2.29 (ddd, $J = 14.0$, 6.1, 2.2 Hz) were coupled with the carbons of the α,*β*-conjugated keto group at δ_C 206.1 (s) and δ_C 155.4

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**5** and **8** (500 MHz, $CDCl_3$, *J* in Hz)

H	1	2	3	4	5	8
H-1		4.00, dd (11.3, 4.8)	3.31, dd (11.7, 4.3)	3.92, dd (11.6, 4.8)	3.38, dd (11.6, 4.7)	3.57, dd (11.3, 4.9)
H-2a	2.82, ddd (14.0, 12.3, 2.1)	1.87, m	1.73, dtd (12.5, 5.7, 2.4)	1.86, dtd (12.5, 5.2, 2.7)	1.82, dtd (12.5, 5.1, 2.3)	1.85, m
H-2b	2.29, ddd (14.0, 6.1, 2.2)	1.53, m	1.58, m	1.58, qd (12.2, 5.7)	1.51, qd (12.7, 5.0)	1.48, tdd (13.4, 11.3, 5.4)
H-3a	2.05, brqd (13.7, 2.0)	2.24, m	2.33, ddd (14.1, 5.0, 2.3)	2.27, m	2.29, ddd (13.1, 5.0, 2.2)	2.31, m
H-3b	1.78, brddd (13.9, 6.1, 3.6)	2.24, m	2.14, tdq (13.9, 5,4, 1.3)	2.27, m	2.02, brtd (13.4, 5.2)	2.09, brdt (13.6, 5.9)
H-4	2.08, ddqd (11.7, 8.8, 6.2, 2.9)					
H-5	4.97, ABdd (15.1, 8.5)	2.18, d (11.1)	1.66, brd (1.7)	1.82, d (10.2)	1.70, brd (9.9)	1.81, brd (10.4)
H-6	5.03, ABdd (15.3, 9.3)	3.97, dd (11.0, 4.5)	4.31, brs	3.49, t (10.0)	3.68, t (9.8)	2.28, m
H-7	1.90, ddd (11.9, 9.3, 2.7)	1.71, m	0.86, dddd (12.3, 9.4, 3.7, 2.4)	1.22, tt (12.5, 2.6)	1.26, tt (12.5, 3.0)	3.21, brd (10.0)
H-8a	2.01, brd (13.1)	1.67, m	1.62, m	1.45, dq (13.4, 3.4)	1.50, m	1.89, m
H-8b	1.31, brqd (13.1, 2.5)	1.58, m	1.50, qd (13.6, 3.7)	1.26, qd (12.9, 3.2)	1.19, qd (12.8, 2.7)	1.30, m
H-9a	2.53, brtt (13.1, 1.9)	1.74, m	1.92, dt (13.0, 3.5)	2.04, dt (14.0, 3.2)	1.88, dt (12.4, 2.9)	1.73, dd (10.2, 8.2)
H-9b	2.24, brdd (13.9, 5.9)	1.28, m	1.12, td (13.1, 4.1)	1.02, td (13.7, 3.9)	1.12, td (13.1, 3.5)	1.36, brq (10.4)
H-11		1.91, m	1.61, m	2.19, se[a]d (7.1, 2.5),	2.20, sed (7.0, 2.5)	1.73, m
Me-12	1.11, s	1.08, d (6.5)	0.94, d (6.7)	0.92, d (7.1)	0.91, d (7.0)	0.97, d (6.9)
Me-13	1.07, s	0.91, d (6.7)	0.96, d (6.7)	0.82, d (7.0)	0.83, d (7.0)	0.88, d (6.8)
H-14a	5.62, brs					
H-14b	5.47, d (1.4)					
Me-14		0.87, s	0.92, s	0.84, s	0.67, s	0.64, d (0.8)
H-15a		4.97, brs	5.02, brq (1.7)	4.95, brt (2.1)	4.98, brs	4.92, brq (1.5)
H-15b		4.81, brs	4.91, brq (1.6)	4.81, brt (2.0)	4.70, brs	4.79, brq (1.4)
Me-15	0.95, d (6.2)					

[a] se represents septet.

Table 2. ^{1}H NMR Spectral Data of Compounds **6**, **7**, **11**, and **12** (500 MHz, $CDCl_3$, *J* in Hz)

H	6	6[a]	7	11	12
H-1				6.65, d (8.0)	
H_2-1					2.39, m
H-2				6.84, dd (7.9, 2.1)	
H_2−2					2.53, m
H-2a	2.89, ddd (14.7, 8.1, 2.6)	2.92, ddd (14.9, 8.4, 2.5)	2.84, brdd (15.0, 11.3)		
H-2b	2.28, ddd (14.7, 10.3, 3.0)	2.27, ddd (14.9, 10.2, 2.9)	2.25, ddd (15.1, 7.5, 1.5)		
H-3a	1.94, ddt, (14.0, 8.1, 3.0)	1.96, ddt (13.9, 8.5, 3.1)	1.96, m		
H-3b	1.85, dddd (14.0, 10.4, 7.8, 2.7)	1.80, dddd (13.9, 10.1, 7.6, 2.6)	1.66, dddd (14.7, 9.0, 4.1, 1.4)		
H-4	2.21, m	2.20, sextetd (7.1, 3.2)	1.93, m	6.81, d (1.8)	
H-5	5.09, ABm	5.23, ABdd (16.2, 7.3)	4.81, ABdd (15.7, 8.8)		5.57, brs
H-6	5.08, ABm	5.29, ABd (16.3)	4.97, ABd (15.8)	2.57, ddd (12.0, 8.8, 3.8)	
H-7	3.16, dd (4.8, 3.2)	3.49, brdd (6.8, 4.5)	3.02, dd (4.5, 2.7)		
H_2-7					2.13, t (7.7)
H-7a				2.08, m	
H-7b				1.73, dddd (13.8, 11.7, 7.9, 5.1)	
H_2-8					2.49, t (7.7)
H-8a	1.76, ddt (15.1, 12.3, 2.9)	2.03, ddt (14.8, 12.3, 2.5)	1.72, ddt (15.2, 13.3, 2.3)	2.22, ddd (16.7, 8.5, 5.6)	
H-8b	1.34, dddd (15.2, 7.5, 4.9, 2.7)	1.74, dddd (16.3,14.8, 8.6, 5.1, 2.6)	1.23, m	2.14, dd (16.6, 8.9)	
H-9a	2.67, brdd (13.4, 6.6)	2.88, brdd (13.1, 6.2)	2.81, m		
H-9b	2.15, brtd (13.3, 2.4)	2.35, td (12.6, 2.6)	2.00, m		
H-11				1.84, se[b]d (6.7, 1.9)	2.3, se (6.8)
Me-12	0.86, s	1.08, s	0.82, s	0.99, d (6.6)	1.09, dd (6.8, 0.7)
Me-13	0.96, s	1.25, s	0.96, s	0.71, d (6.7)	1.07, dd (6.8, 0.7)
H-14a	5.82, brs	5.79, brs	5.82, brs		
H-14b	5.67, dd (1.4, 0.7)	5.57, dd (1.5, 0.7)	5. 63, d (1.9)		
Me-14				2.03, s	2.08, s
Me-15	0.95, d (6.9)	0.94, d (6.9)	0.97, d (6.4)	2.23, s	1.97, s
OH		5.96, d (5.2)			

[a] Data were measured in pyridine-d_5. [b] se represents septet.

(s) in the HMBC spectrum gave strong evidence to the linking of C-1 and C-2 to form a 10-membered ring for **1** (Figure 1). A methyl group [δ_H 0.95 (d, J = 6.2 Hz) and δ_C 20.6 (q)] was positioned at C-4 due to the presence of a HMBC correlation between its proton signals and C-5. In addition, a 1-hydroxyl-1-methylethyl group was discerned

Table 3. ^{13}C NMR Spectral Data of Compounds **1**−**9**, **11**, and **12** (125 MHz, $CDCl_3$)

C	1	2	3	4	5	6	7	8	9	11	12
C-1	206.1a	68.3d	79.8d	68.1d	78.9d	203.6s	203.0s	79.0d	49.6d	115.7d	34.7t
C-2	37.7t	31.1t	30.7t	31.0t	31.8t	36.0t	36.7t	31.9t	21.3t	127.3d	31.6t
C-3	37.2t	29.7t	34.3t	29.7t	35.0t	33.3t	33.8t	34.9t	22.2t	129.8s	171.9s
C-4	41.0d	145.4s	146.8s	145.4s	146.2s	36.4d	41.2d	148.9s	142.4s	128.5d	138.7s
C-5	142.9d	55.4d	52.0d	61.6d	55.8d	134.6d	123.4d	56.4d	151.6d	129.9s	112.8d
C-6	130.3d	69.9d	68.5d	67.1d	67.0d	136.8d	137.3d	39.4d	41.4d	44.2d	152.1s
C-7	56.9d	44.0d	49.9d	49.0d	49.2d	78.5d	76.5d	82.7d	45.6d	26.7t	25.2t
C-8	32.8t	22.4t	20.3t	18.0t	18.0t	30.3t	30.1t	26.0t	22.1t	41.8t	42.7t
C-9	28.9t	29.2t	37.1t	34.3t	36.2t	31.2t	31.9t	37.3t	41.8t	210.6s	208.8s
C-10	155.4s	39.9s	39.7s	40.1s	41.6s	149.8s	150.0s	49.5s	72.1s	151.9s	208.8s
C-11	71.7s	25.0d	28.8d	26.4d	25.9d	40.2s	41.0s	31.3d	26.2d	33.0d	33.3d
C-12	27.1q	24.0q	21.1q	20.9q	21.1q	19.3q	16.5q	20.5q	21.4q	20.9q	22.2q
C-13	26.8q	22.0q	20.6q	16.2q	16.1q	26.2q	27.1q	14.7q	15.2q	21.3q	22.2q
C-14	119.7t	21.6q	13.0q	21.3q	11.5q	123.9t	123.4t	12.3q	20.5q	30.1q	25.2q
C-15	20.6q	114.4t	108.4t	114.2t	107.8t	20.6q	21.7q	107.6t	194.5d	20.7q	18.2q

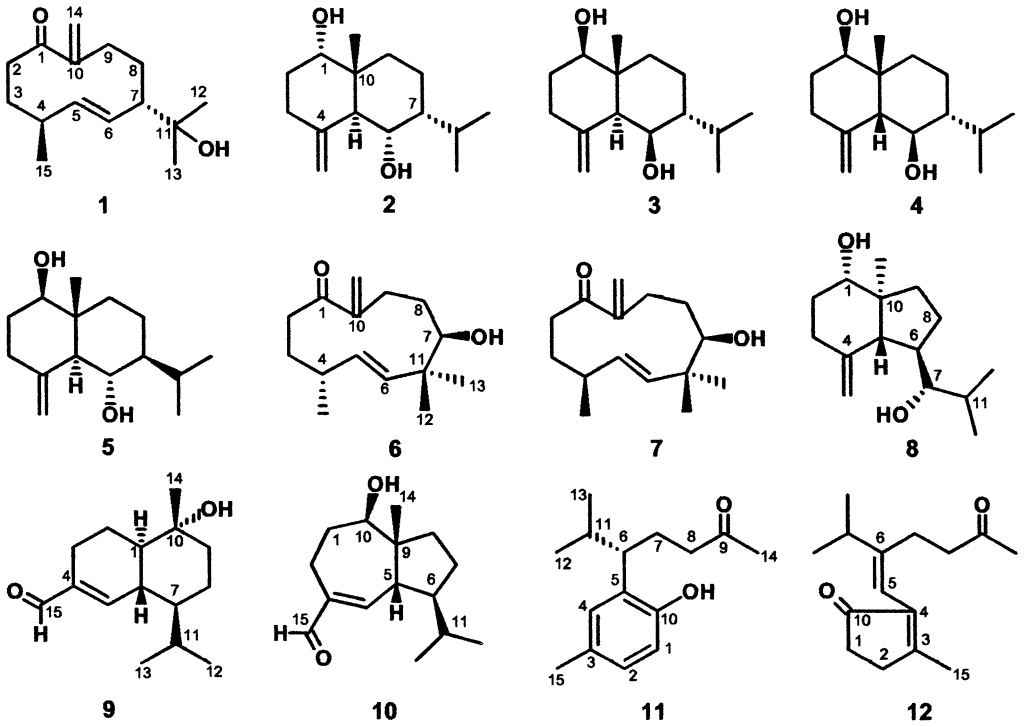

Figure 1. Structures of compounds **1**−**12**.

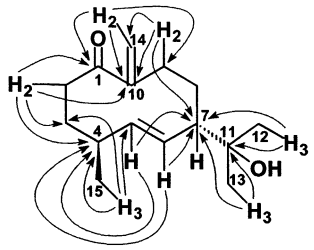

Figure 2. Selected HMBC correlations for compound **1** ($CDCl_3$).

[δ_H 1.07, 1.11 (each 3H, s); δ_C 71.7 (s), 27.1 (q), 26.8 (q)] and was shown to be connected to C-7 through the observation of the HMBC correlations between C-11 and H-7 and between its two methyl carbons and H-7. The relative stereochemistry of **1** was established through an analysis of coupling constants and a ROESY experiment (Figure 2). The H-2 proton at δ_H 2.82 (ddd, J = 14.0, 12.3, 2.1 Hz) was determined to be in an α-orientation due to its being ROE correlated to H-14b. H-5 was also determined to be facing down to the $\Delta^{10,14}$ exocyclic double bond on the basis of its ROE correlations to H_2-14. An *E*-configuration for the $\Delta^{5,6}$ double bond was determined by the large coupling constant between H-5 and H-6 (J = 15.2 Hz). This in turn led to the assignment of H-6 as facing up to the C-1 carbonyl group. Being ROE correlated to H-6, the H-9 proton at δ_H 2.53 (brtt, J = 13.1, 1.9 Hz) was thus assigned as *β*-oriented. The configurations of H_2-3 and H-4 were subsequently determined by their coupling constants to H-2α, and those of H_2-8 and H-7 to H-9*β*. Thus, the determination of H-4α placed the C-15 methyl group in the *β*-orientation, and the assignment of H-7*β* established the 1-hydroxyl-1-methylethyl group at C-7 as being in the α-orientation. Further evidence for the determination of the orientations of the methyl group at C-4 and the 1-hydroxyl-1-methylethyl group at C-7 was confirmed by observation of the ROE correlations between H-4 and H-5 and between H-7 and H-6. Accordingly, **1** was determined to be 4*β*-methyl-7α-(1-hydroxyl-1-methylethyl)-10-methyl-enecyclodec-5*E*-enone and was given the trivial name litseagermacrane.

7-*Epi*-eudesm-4(15)-ene-1α,6α-diol (**2**), 7-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol (**3**), 5-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol (**4**), and eudesm-4(15)-ene-1*β*,6α-diol (**5**) all showed the same molecular formula of $C_{15}H_{26}O_2$ by the analysis of their HRTOFMS and ^{13}C NMR and DEPT spectra. Analysis of

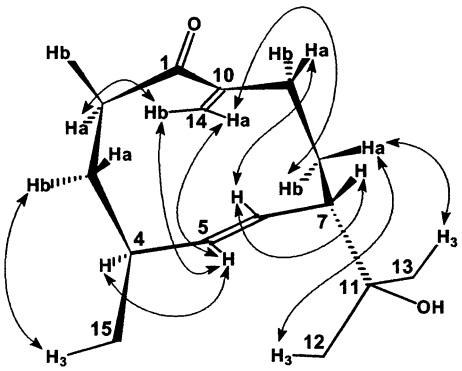

Figure 3. Selected ROESY correlations for compound **1** ($CDCl_3$).

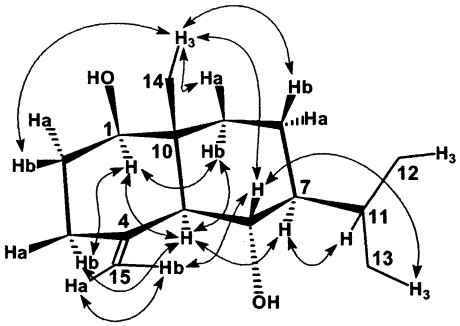

Figure 4. Selected ROESY correlations for compound **5** ($CDCl_3$).

the ^1H and ^{13}C NMR spectra (Tables 1 and 3) revealed that these four compounds are isomers sharing many structural features including an exocyclic double bond and an isopropyl, a methyl, and two hydroxyl methine groups (Tables 1 and 3). Compounds **3** and **5** were determined to be known eudesmane sesquiterpenes by comparison with literature spectral data.[4] Although the stereochemistries of these two compounds were correctly described in the literature, the chiral centers at C-5, -7, and -10 have not been confirmed either by NOE or by X-ray data. Our successful ROESY experiments consolidated the stereochemical determination of compounds **3** and **5**. The existence of ROE correlations of H-1 to H-5α and to H-9α confirmed the configurations of the hydroxyl group at C-1, the H-5, and the methyl group of C-14 as being *β*-, α-, and *β*-oreinted, respectively, while the presence of ROE correlations of H-5 to H-6 and to H-15a verified the *β*-configuration of the C-6 hydroxyl group in **3**. The ROESY correlations of compound **5** are portrayed in Figure 4. Ring B of compounds **3** and **5** exists in different conformations, with **3** being in a boat form, and the C-7 isopropyl group in an equatorial position. On the other hand, the B-ring in **5** is in a chair conformation, but its C-7 isopropyl group is in an equatorial position. Compound **2** differs from **3** in the configurations of the hydroxyl groups located at C-1 and C-6. The hydroxyl group at C-1 in **2** was assigned as α-oriented according to its dramatic downfield shift of the ^{13}C NMR signal at C-14 ($\Delta\delta_C$ 8.6 ppm) in comparison to **3**. In the case of **3**, the 1-hydroxy group is *β*-oriented and generated a *γ-gauche* shielding effect over the 14-methyl protons, which in turn resulted in an upfield chemical shift of C-14. The reason for assigning the hydroxyl group of C-6 in **2** as α-oriented is that the coupling pattern of H-6 changed from the broad singlet in **3** (δ_H 4.31) to a doublet of doublets in **2** (δ_H 3.97, dd, J = 11.0, 4.5 Hz). The broad singlet signals of H-6 in **3** determined the dihedral angles between H-6 and H-5 and between H-6 and H-7 as being approximately 90°, respectively. The existence of **2** as an epimer of **3** at C-6 could also be deduced from

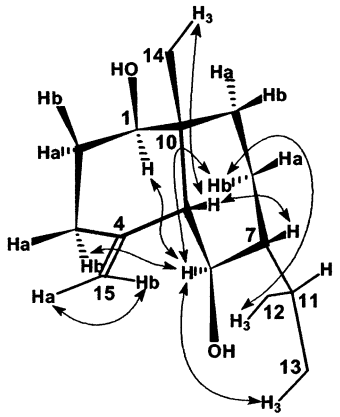

Figure 5. Selected ROESY correlations for compound **4** ($CDCl_3$).

the fact that the existing ROE correlation between H-15 and H-6 in **3** was not observed in the ROESY spectra of **2**. The epimerization of the two hydroxyl groups from the *β* in **3** to the α orientation in **2** generated other significant changes of ^{13}C NMR chemical shifts at C-1 ($\Delta\delta_C$ −11.5 ppm), C-3 ($\Delta\delta_C$ −4.6 ppm), C-5 ($\Delta\delta_C$ +3.4 ppm), C-7 ($\Delta\delta_C$ −5.9 ppm), C-9 ($\Delta\delta_C$ −7.9 ppm), C-11 ($\Delta\delta_C$ −3.8 ppm), C-12 ($\Delta\delta_C$ +2.9 ppm), and C-15 ($\Delta\delta_C$ +6.0 ppm) (Table 3). Compound **4** showed NMR data very similar to those of **5**. If one compares only the ^1H and ^{13}C NMR data, **4** might be mistakenly deduced as being an epimer of **5** at C-1, mirroring the relationship between **2** and **3**. In that case, the C-1 hydroxyl group in **4** would simply be assigned to the α-orientation due to the changes in the ^{13}C NMR chemical shifts between **4** and **5** being very similar to those of **2** and **3** at C-1, C-3, and C-14 (Table 3). If this is the case, the ROE correlations of H-5 to H-3α and H-7α would exist in the ROESY spectrum of **4**. However, such ROE cross-peaks were not found in **4**. Instead, the ROE correlations of H-6 to H-3 and to H-1 were easily observed (Figure 5), which suggested an opposite direction of H-5 to H-1, H-3, and H-6. The orientation of H-5 was assigned as *β*, facing the14*β*-methyl group due to their ROE correlations, which in turn, established both the H-1 and the H-6 as α-oriented. The stereochemistry of C-7 in **4** is also different from that of **5**. Its isopropyl group was assigned to the α-orientation according to the presence of the ROE correlation between H-7 and H-5. A search for literature revealed the existence of a *cis*-fused eudesmane (10-epijunenol) that does not have a C-1 hydroxy group. Compound **4** showed a significant upfield ^{13}C NMR chemical shift of C-14 (δ_C 21.3) as compared to that of 10-*epi*-junenol (δ_C 28.2),[8] which was induced by *γ-gauche* shielding effects between the C-1 hydroxyl group and 14-methyl protons in **4**. Thus, **4** and **5** are stereoisomers. Accordingly, compounds **2** and **4** were determined to be 7-*epi*-eudesm-4(15)-ene-1α,6α-diol and 5-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol, respectively. Compounds **2**, **3**, and **5** belong to the *trans*-eudesmane sesquiterpenes, whereas **4** is a rare natural *cis*-eudesmane. The full assignments of NMR data of these eudesmanes (**2**−**5**) are given in Tables 1 and 3 through analysis of the 2D NMR spectra including $^1H-^1H$ COSY, HMQC, HMBC, and ROESY experiments.

Litseahumulane A (**6**) has a molecular formula of $C_{15}H_{24}O_2$ as shown by its HRTOFMS ($[M + H]^+$ (m/z 237.1864, calcd 237.1855). The NMR spectra (Tables 2 and 3) disclosed the presence of an α,*β*-conjugated keto group, a second double bond, two tertiary methyls, a secondary methyl, a methine, an oxy-methine, four methylenes, and a quaternary carbon. Analysis of the $^1H-^1H$ COSY, HMQC, and HMBC spectra (see Figure 6 for HMBC data) in the

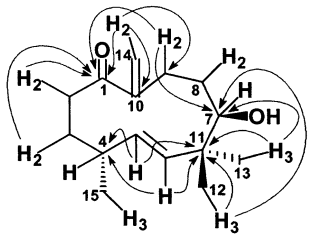

Figure 6. Selected HMBC correlations for compound **6** ($CDCl_3$).

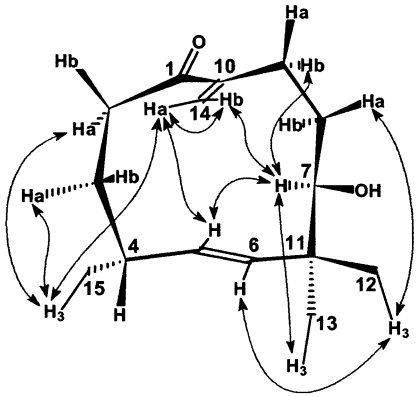

Figure 7. Selected ROESY correlations for compound **6** (pyridine-d_5).

same manner employed for the structure determination of **1** led to the elucidation of the structural relationship of these groups. The planar structure of **6** was found to be a humulane sesquiterpene with a hydroxyl group at C-7. One of the two double bonds in **6** was determined to be an exo-methylene group configured in the form of an α,*β*-conjugated keto group with the C-1 carbonyl carbon. The second double bond was located at $\Delta^{5,6}$, which due to a large coupling constant (J = 16.2 Hz) between its two protons was determined as *E*-configured. Analysis of the coupling constants and ROESY data established the conformation of **6** as shown in Figure 7. The methyl group at C-4 was assigned to an α-orientation due to the presence of the ROE correlation between H_3-15 and H-14a. By its ROE correlation to H-14a, H-5 was deemed to face down to the $\Delta^{10,14}$ double bond. The orientation of the hydroxyl group at C-7 was determined to be *β* on the basis of the ROE correlations of H-7 to H-5 and H-14b. Accordingly, the structure of **6** was elucidated to be 7*β*-hydroxyl-4α,11,11-trimethyl-10-methylenecycloundeca-5*E*-enone and was given the trivial name litseahumulane A.

Litseahumulane B (**7**), having the same molecular formula of $C_{15}H_{24}O_2$ as that of **6** (HRTOFMS ($[M + Na]^+$ *m*/*z* 237.1859, calcd 237.1855), was shown to be an isomer of **6** by a comparison of their NMR data (Figures 2 and 3). Compound **7** differs from **6** only by the configuration of the methyl group at C-4, which in **7** was positioned in the *β*-orientation due to the presence of the ROE correlation between H-4 and H-5. The epimerization of the methyl group from α in **6** to *β* in **7** resulted in a downfield chemical shift of C-4 ($\Delta\delta_C$ 4.8 ppm) and an upfield chemical shift of C-5 ($\Delta\delta_C$ 11.2 ppm). The structure of **7** was thus elucidated as 7*β*-hydroxyl-4*β*,11,11-trimethyl-10-methylenecycloundeca-5*E*-enone and was given the trivial name litseahumulane B.

Octahydro-4-hydroxy-3α-methyl-7-methylene-α-(1-methylethyl)-1*H*-indene-1-methanol (**8**), 10-hydroxyl-15-oxo-α-cadinol (**9**), and aphanamol II (**10**) are known sesquiterpenes. Compound **8** is an oppositane sesquiterpene, having been originally isolated as a reaction product of epoxygermacrene-D,[5] and was recently reported to be present in *Dysoxylum variabile*.[9] Compound **9** belongs to the cadinane type of sesquiterpenes. It was reported previously as a saponification product of the natural sesquiterpene 10-*O*-acetyl-15-oxo-α-cadinol, as well as an oxidative product of α-cadinol.[6] Since the ^{13}C NMR spectral data of **8** and **9** have not yet been reported in the literature, they are therefore being presented in the current paper. Compound **10**, an isodaucane sesquiterpenoid, was first isolated as a minor toxic component from the fruit peels of the timber tree *Aphanamixis grandifolia*.[7] Its absolute structure was established by Hansson et al. through a synthesis method.[10]

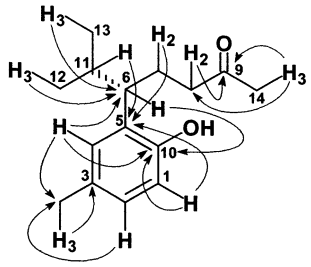

Figure 8. Selected HMBC correlations for compound **11** ($CDCl_3$).

Litseachromolaevane A (**11**), $C_{15}H_{22}O_2$ (HRTOFMS ($[M + Na]^+$ *m*/*z* 257.1521, calcd 257.1517), was shown to possess an isopropyl group, an acetyl group, and a 1,2,4-trisubstituted aromatic group by the ^1H, ^{13}C, and DEPT NMR data (Tables 2 and 3). Further, an additional tertiary methyl group, two methylene groups, and an additional methine group were also discerned from the NMR spectra. A substructural unit of 6-methylheptan-2-one was established for **11** by the linkage of the acetyl, the two methylene, the methine, and the isopropyl groups through analysis of the $^1H-^1H$ COSY, HMQC, and HMBC spectral data (see Figure 8 for HMBC data). The presence of the HMBC correlation between the NMR signals of δ_H 6.81 (d, J = 1.8 Hz) and δ_C 44.2 (d) further connected the 6-methylheptan-2-one group to the 1,2,4-trisubstituted aromatic group. Other substituents on the 1,2,4-trisubstituted aromatic group were assigned to a hydroxy group being ortho-postioned and a methyl group [δ_H 2.23 (s)] being meta-positioned to the 6-methylheptan-2-one group on the basis of HMBC analysis (Figure 8). The structure of **11** was hence elucidated as 6-methyl-5-(2-hydroxy-5-methylphenyl)heptan-2-one and was given the trivial name litseachromolaevane A. The proposed structure of chromolaevane **11** was further confirmed by a single-crystal X-ray analysis (Figure 9).

Litseachromolaevane B (**12**) was shown to have a molecular formula of $C_{15}H_{24}O_2$ on the basis of the HREIMS ($[M]^+$ (*m*/*z* 234.1617, calcd 234.1620) as well as ^{13}C NMR and DEPT spectral data. The NMR spectra (Tables 2 and 3) disclosed the presence of an isopropyl group, an acetyl group, two olefinic double bonds, a methyl group, four methylene groups, and an additional carbonyl carbon. Analysis of the $^1H-^1H$ COSY, ROESY, HMQC, and HMBC spectral data (see Figure 10 for HMBC data) of **12** showed the presence of two substructural units: 6-methylheptan-2-one and 3-methylcyclopent-2-enone. The 6-methylheptan-2-one group is the same as that found in **11**. However, this group in **12** was connected to other structural units through a double bond [δ_C 152.1 (s) and δ_C 112.8 (d)] instead of a single bond as in the case of **11**. In the HMBC spectrum, the olefinic methine group [δ_C 112.8 (d) and δ_H 5.57 (brs)] of the double bond ($\Delta^{5,6}$) was shown to have long-range correlations to the NMR signals at δ_C 171.9 (s), δ_H 2.3 (septet, J = 6.8 Hz) and 2.13 (d, J = 7.7 Hz), respectively.

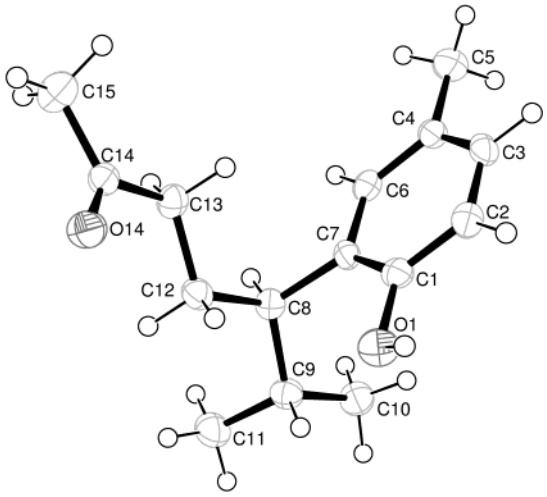

Figure 9. ORTEP drawing of one molecule of compound **11**.

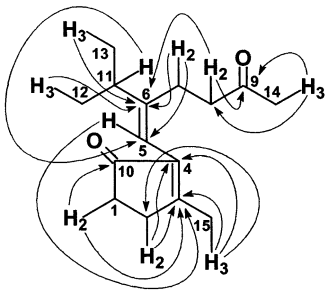

Figure 10. Selected HMBC correlations for compound **12** ($CDCl_3$).

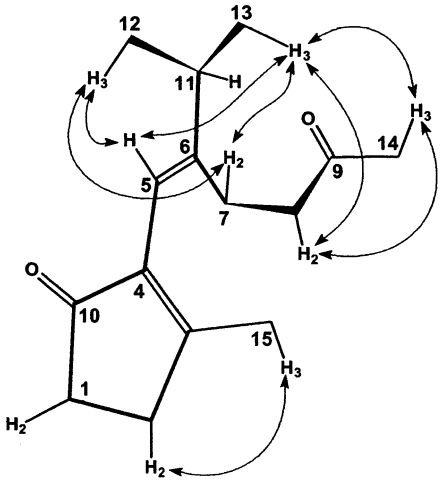

Figure 11. Selected ROESY correlations for compound **12** ($CDCl_3$).

Their correlations suggested that the 6-methylheptan-2-one and 3-methylcyclopent-2-enone groups are connected through the olefinic methine carbon. Compound **12** was thus determined as another chromolaevane sesquiterpene. The configuration of $\Delta^{5,6}$ was assigned as *Z* due to the presence of ROE correlations of H-5 to H_3-12 and H_3-13 (Figure 11). Accordingly, **12** was deduced as 3-methyl-2-[(*Z*)-2-isopropy-5-oxo-hex-1-enyl)cyclopent-2-enone and was given the trivial name litseachromolaevane B.

All compounds (**1**−**12**) were isolated from an anti-HIV fraction (F-18), which exhibited 39% inhibition of in vitro HIV-1 replication in HOG.R5 cells at 10 μg/mL in the complete absence of cytotoxicity. Of these compounds, three (**1**, **4**, **12**) have been found to possess moderate to weak anti-HIV activities. Compound **1** was the most active, with an IC_{50} value of 6.5 μg/mL (27.5 μM), but it was also shown to be toxic to HOG.R5 cells at a CC_{50} value of 15.9 μg/mL (63.4 μM). Isolates **4** and **12** exhibited IC_{50} values of 17.4 (73.1 μM) and 28.0 μg/mL (119.7 μM), respectively, without any evidence of cytotoxicity up to a concentration of 20 μg/mL. Nevertheless, low selectivity index (SI) values are expected for isolates **1**, **4**, and **12**. This would warrant further evaluation of these compounds in the more pathologically relevant H9 T lymphocyte cell line that supports efficient in vitro HIV-1 replication. H9 cells have also been known to be less susceptible to toxicity. This study will be initiated upon the isolation of greater amounts of the compounds of interest.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. IR spectra were run on a Jasco FT/IR-410 spectrometer as a film on a KBr plate. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 MHz spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. All NMR data were obtained by using standard pulse sequences supplied by the vendor. Column chromatography was carried out on silical gel (200−400 mesh, Natland International Corporation). Reversed-phase flash chromatography was accomplished with RP-18 silica gel (40−63 μm, EM Science), and reversed-phase HPLC was carried out on a Waters 600E delivery system pump, equipped with a Waters 996 photodiode detector and a Watrex GROM-Saphir 110 C18 column (120 Å,12 μm, 300 × 40 mm) or a Phenomenex LUNA phenyl-hexyl column (120 Å,15 μm, 250 × 50 mm). Thin-layer chromatography was performed on Whatman glass-backed plates coated with 0.25 mm layers of silica gel 60. HRTOFMS and MS/MS spectra were recorded on a Micromass QTOF-2 spectrometer or a VG 7070-HF spectrometer. X-ray diffraction data collection was carried out on a Bruker APEX CCD area detector equipped with a Mo sealed-tube X-ray source. The direct methods SIR-92 package was used for structure solution,[11] and the WinGX package[12] was used for completing the structure determination. ORTEP[13] was used to generate Figure 9.

Plant Material. The initial collection of leaf, twig, and flowerbud sample (SVA-0001) of *Litsea verticillata* Hance (Lauraceae) was made at the Cuc Phuong National Park (CPNP) on November 22, 1998, and was documented by voucher specimens *Soejarto et al. 10352*. A large amount of the plant sample (SVA-0001, 4.5 kg, voucher specimens *Soejarto et al. 11003*) was subsequently re-collected at the same site at CPNP on November 17, 1999, for complete isolation work. Duplicate voucher specimens of both collections have been deposited at the herbaria of CPNP, Institute of Ecology and Biological Resources (IEBR at National Center for Science and Technology, Hanoi), and the John G. Searle Herbarium of the Field Museum of Natural History (Chicago, IL).

Bioassay. Anti-HIV and cytotoxicity assays were performed in parallel utilizing the green fluorescent protein (GFP)-based HOG·R5 reporter cell line that was constructed and developed specifically for quantitating HIV-1 infectivity. The system was validated and adapted as a moderately high-throughput procedure for screening natural products for anti-HIV activity in our laboratory.[1] Briefly, cultures in microtiter wells were infected with HIV-1_{IIIB} (2.5 ng/mL p24) in the presence of plant extracts after which the fluorescence output was measured at the end of 4 days. Virus was omitted from parallel cultures treated with identical concentrations of plant extracts in order to monitor changes in cellular viability by a combination of microscopic and fluorometric measurements.

Extraction and Isolation. The dried and milled leaves and twigs (4.5 kg) were extracted with MeOH, the extract was subsequently defatted with *n*-hexane and partitioned with $CHCl_3$, and resulting extract (93.0 g) was processed as previously described[3] to afford the anti-HIV fractions F-4 and F-5. The two fractions were pooled (3.83 g) and subjected to flash

column chromatography on a C-18 reversed-phase (RP-18, 107 g) column. Subsequent elution with MeOH/H_2O (1:1, 1 L) yielded an active oily fraction, F-18 (0.92 g). F-18 was subjected to preparative HPLC separation on a GROM-Saphir 110 C18 column (solvent system: MeOH/H_2O, 7:3) to afford 15 fractions (F44−F58). Fractions F48 (59.6 mg), F-52 (51.6 mg), F53 (51.6 mg), F54 (37.2 mg), F56 (181.0 mg), and F57 (97.1 mg) were subjected to further preparative HPLC separation, respectively, on the GROM-Saphir 110 C18 column. Fraction F48 afforded 5-*epi*-eudesm-4(15)-ene-1β,6β-diol (**4**, 4.85 mg), eudesm-4(15)-ene-1β,6α-diol (**5**, 22.02 mg), and litseachromolaevane B (**12**, 0.82 mg) by elution with MeCN/H_2O, 4:6. Fraction F52 afforded litseagermacrane (**1**, 3.76 mg), octahydro-4-hydroxy-3α-methyl-7-methylene-α-(1-methylethyl)-1*H*-indene-1-methanol (**8**, 5.33 mg), and litseahumulanes A (**6**, 1.32 mg) and B (**7**, 0.94 mg) by eluting with MeCN/H_2O, 5:5. Fractions F53 and F54 afforded 7-*epi*-eudesm-4(15)-ene-1α,6α-diol (**2**, 6.67 mg) and 7-*epi*-eudesm-4(15)-ene-1β,6β-diol (**3**, 3.82 mg), respectively, when eluted with MeCN/H_2O, 6:4. Fraction F56 afforded 10-hydroxyl-15-oxo-α-cadinol (**9**, 3.02 mg), aphanamol II (**10**, 4.05 mg), and litseachromolaevane A (**11**, 5.26 mg) by eluting with MeCN/H_2O, 5:5.

Litseagermacrane (1): colorless gum, $[\alpha]_D^{20}$ +11.1° (*c* 0.14, $CHCl_3$); IR (film) ν_{max} 3456.8 (br), 2960.2, 2925.5, 2866.7, 1708.6, 1676.8, 1450.2, 1375, 1284.4, 1218.8, 1162.9, 1088.6, 1066.4, 979.7, 920.8, 861.1, 756.0 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRTOFMS *m*/*z* 259.1682 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1674), 219.1756 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{23}O$, 219.1749).

7-*Epi*-eudesm-4(15)-ene-1α,6α-diol (2): white powder, $[\alpha]_D^{20}$ −35.3° (*c* 0.05, $CHCl_3$); IR (film) ν_{max} 3538.7 (br), 2994.9, 1708.6, 1661.4, 1458.9, 1376.9, 1266.0, 1165.8, 1044.3, 894.8 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRTOFMS *m*/*z* 261.1803 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1830).

7-*Epi*-eudesm-4(15)-ene-1β,6β-diol (3): white powder, $[\alpha]_D^{20}$ −16.0° (*c* 0.03, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 3.

5-*Epi*-eudesm-4(15)-ene-1β,6β-diol (4): white powder, $[\alpha]_D^{20}$ +36.5° (*c* 0.32, $CHCl_3$); IR (film) ν_{max} 3444.2 (br), 3072.1, 2979.5, 2915.8, 2847.4, 1701.9, 1651.7, 1458.9, 1365.4, 1266.0, 1165.8, 1041.4, 898.7 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRTOFMS *m*/*z* 221.1902 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{25}O$, 221.1905), 203.1773 $[M - 2H_2O + H]^+$ (calcd for $C_{15}H_{23}$, 203.1800).

Eudesm-4(15)-ene-1β,6α-diol (5): white powder, $[\alpha]_D^{20}$ −27.1° (*c* 1.47, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 3; HRTOFMS *m*/*z* 239.2022 $[M + H]^+$ (calcd for $C_{15}H_{27}O_2$, 239.2011).

Litseahumulanes A (6): colorless gum, $[\alpha]_D^{20}$ −34.1° (*c* 0.09, $CHCl_3$); IR (film) ν_{max} 3379.6 (br), 2960.2, 2930.3, 2879.2, 1714.4, 1664.3, 1626.7, 1452.1, 1365.4, 1284.4, 1178.3, 1028.8, 985.5, 942.1, 917.0 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRTOFMS *m*/*z* 259.1701 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1674), 237.1864 $[M + H]^+$ (calcd for $C_{15}H_{25}O_2$, 237.1855); TOFMS/MS *m*/*z* (from 237.1864) 237.1864 (100), 219 (18), 201 (32), 173 (15), 159 (19), 145 (18), 121 (13), 105 (9), 95 (7%).

Litseahumulanes B (7): colorless gum, $[\alpha]_D^{20}$ −23.8° (*c* 0.02, $CHCl_3$); IR (film) ν_{max} 3457.7 (br), 3009.4, 2904.3, 1714.4, 1671.0, 1458.9, 1372.1, 1165.8, 1041.4, 984.3 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRTOFMS *m*/*z* 237.1859 $[M + H]^+$ (calcd for $C_{15}H_{25}O_2$, 237.1855).

Octahydro-4-hydroxy-3α-methyl-7-methylene-α-(1-methylethyl)-1*H*-indene-1-methanol (8): white powder, $[\alpha]_D^{20}$ −60.8° (*c* 0.36, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 3.

10-Hydroxyl-15-oxo-α-cadinol (9): white powder, $[\alpha]_D^{20}$ +2.6° (*c* 0.19, $CHCl_3$); ^{13}C NMR data, see Table 3.

Aphanamol II (10): white powder, $[\alpha]_D^{20}$ +37.5° (*c* 0.26, $CHCl_3$).

Litseachromolaevane A (11): colorless gum, $[\alpha]_D^{20}$ +6.7° (*c* 0.23, $CHCl_3$); IR (film) ν_{max} 3380.6 (br), 2956.3, 2928.4, 2866.7, 1701.9, 1608.3, 1509.0, 1458.9, 1423.2, 1363.4, 1259.3, 1203.4, 1165.8, 1129.1, 1097.3, 1041.4, 960.4, 886.1, 813.8 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRTOFMS *m*/*z* 257.1521 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1517), 217.1589 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{21}O$, 217.1592).

Litseachromolaevane B (12): colorless gum, $[\alpha]_D^{20}$ +0° (*c* 0.05, $CHCl_3$); IR (film) ν_{max} 2959.2, 2922.6, 2866.7, 1695.1, 1614.1, 1458.9, 1440.6, 1160.0, 1001.5 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HREIMS *m*/*z* 234.1617 $[M]^+$ (calcd for $C_{15}H_{22}O_2$, 234.1620).

X-ray crystal structure of litseachromolaevane A (11): colorless crystal, 0.1 mm on edge, obtained from MeOH. Cell parameters: $a = 9.590(10)$ Å, $b = 10.140(10)$ Å, $c = 13.810(10)$ Å, $V = 1343(2)$ Å ^{3}, space group $P2_12_12_1$, $Z = 4$, $D_{calc} = 1.159$ g/cm^3, $\lambda = 0.71073$ Å, μ(Mo Kα) = 0.075 mm^{-1}, $F(000) = 512$, $T = 100(2)$ K. Data collection yielded 3905 reflections resulting in 2297 unique, averaged reflections. Full-matrix least-squares refinement on F^2 led to a final $R(4\sigma_F)$, R(all), and GOF of 0.0387, 0.0461, and 0.952. Crystallographic data, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 192170. Copies of the information can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-0-1223-336033, e-mail: deposit@ccdc.cam.ac.uk).

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Supporting Information Available: Crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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