Sesquiterpene Lactones and Thymol Esters from *Vicoa pentanema*

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The aerial parts of *Vicoa pentanema* yielded four new sesquiterpene lactones, namely, 10α-hydroxy-14*H*-inuviscolide (**1**), 4α,5α-epoxy-10α,11*β*,13*H*,14*H*-1-*epi*-inuviscolide 3-*β*-D-glucoside (**2**), 2α-*O*-acetyl-3*β*-hydroxyalantolactone (**3**), and 2α,3α-dihydroxyalloalantolactone (**4**). The structure and stereochemical assignments of all sesquiterpenes were based on their ^{1}H and ^{13}C-NMR spectral data, including those derived from 2D-NMR COSY, HETCOR, FLOCK, and NOESY experiments, as well as extensive NOE-difference studies. In addition, the same plant material yielded the known sesquiterpene lactones 8*β*-hydroxyparthenolide (**5**), 8-*epi*-confertin (**6**), 4α,5α-epoxy-10α,14*H*-1-*epi*-inuviscolide (**7**), inuviscolide (**8**), lipiferolide (**9**), and carabrone (**10**), the known thymol ester 7,8-epoxy-9-(isobutyryloxy)thymolisobutyrate (**11**), and its hydrolysis product 7-hydroxy-8,9-bis(isobutyryloxy)thymol (**12**).

The genus *Vicoa* is represented in Saudi Arabia by a single species, namely, *V. pentanema* Aitch. *et* Hemsl. (syn. *Inula pentanema*), family Compositae.[1] It is an annual herb that is widely distributed throughout the southern part of the Arabian Peninsula, where it is known as *ufaynah*. Until now, this plant has not been the subject of phytochemical analysis, but the genus *Inula* has yielded a wide array of sesquiterpenes,[2,3] sesquiterpene lactones,[4−8] thymol derivatives,[9−11] and flavones,[2,4] while the aerial parts of *V. indica* (syn. *I. indica*) have yielded germacranolides,[12,13] including vicolides B−E,[14−16] the guaianolide vicolide A,[14] the monoterpene vicodiol,[17] and the 28-nortriterpenoid glycoside vicoside A.[18]

Examination of the aerial parts of *V. pentanema* has led to the isolation and characterization of four new sesquiterpene lactones, namely, the guaianolides **1** and **2** and the eudesmanolides **3** and **4** (Chart 1). In addition, the six known sesquiterpene lactones 8*β*-hydroxyparthenolide (**5**), 8-*epi*-confertin (**6**), 4α,5α-epoxy-10α,14*H*-1-*epi*-inuviscolide (**7**),[4] inuviscolide (**8**),[19,20] lipiferolide (**9**),[21,22] and carabrone (**10**),[4,23] as well as the known thymol ester **11**,[9] and its hydrolytic product **12**, have been also isolated from the same source. The isolation and structure elucidation of these compounds are the subject of this paper.

Results and Discussion

The *n*-hexane extract of *V. pentanema* was subjected to flash chromatography over Si gel to give the major sesquiterpene lactones 4α,5α-epoxy-10α,14*H*-1-*epi*-inuviscolide (**7**),[4,24] inuviscolide (**8**),[19,20] lipiferolide,[21,22] and carabrone,[4,23] identified by comparison of their physical and spectroscopic data with those previously reported. The stereochemical assignments of **7** and **8** were confirmed from extensive NOE experiments and by comparison with the NOE data of the diepoxide **13**, prepared from **7** by epoxidation. The NOE established a *cis*-relationship between H-1 (*δ*2.66) and H-8 (*δ*4.31) in **8**, while a *trans*-relationship between these two protons was observed in **7**, as H-1(*δ*2.56) and H-7 (*δ*2.98) were correlated, while no correlation was observed between H-1 and H-8. This established **7** as a derivative of 1-*epi*-inuviscolide, rather than inuviscolide (**8**) as reported previously.[4,8,24] In addition, complete ^{13}C-NMR data for **7** and **8**, not published previously, are assigned in Table 1 using COSY, HETCOR, and ^{1}H−^{13}C long-range FLOCK[25] experiments.

Further purification of fraction B by chromatography gave 10α-hydroxy-14*H*-inuviscolide (**1**), $C_{15}H_{22}O_4$, as off-white granules. It showed the presence of two hydroxyl groups (ν_{max} 3300 cm^{-1}) and an exocyclic methylene lactone ring (ν_{max} 1755,1660 cm^{-1}; δ_C 141.0, 169.9, 118.1; C-11, C-12, and C-13, respectively). The ^{1}H- and ^{13}C-NMR spectral data (Tables 1 and 2) of **1** were remarkably similar to those of the guaianolide inuviscolide (**8**)[19,20] but lacked the signals associated with the C-10(14)-exocyclic methylene group. Instead, **1**, like **14**,[5] was concluded to have a C-10 hydroxyl group geminal with a methyl group, as suggested by its NMR spectral data (δ_{C-10} 71.7, δ_{C-14} 24.8; δ_{H-14} 1.13, 3H, s; *vide infra*). Furthermore, the ^{1}H-NMR spectra of **1** contained a signal at δ (DMSO-d_6) 4.32 (ddd, J = 1.4, 9.8, 11.2 Hz; H*β*-8) [versus δ $CDCl_3$ 3.98 (ddd, J = 2.0, 9.5, 11.0 Hz in **14**[5])], suggesting the presence of an 8*β*-H on the lactone system. A COSY experiment established the system $CH_2CH(C{=}CH_2)CH(O)CH_2-$ in **1** and was confirmed by a HETCOR experiment (Table 1). These experiments confirmed that the signals at δ 28.4, 50.2, 79.4, and 48.3 can be assigned to C6−C9, respectively. The placements of the C-4 and C-10 tertiary hydroxyl groups and C-14 and C-15 methyl groups were established by an ^{1}H−^{13}C long-range FLOCK experiment, which revealed the key three-bond correlations between the signals δ 1.13 (H-14), δ_C 51.2 (C-1) and 48.3 (C-9), and δ 1.03 (H-15), δ_C 40.7 (C-3) and 49.3 (C-5). Also, the FLOCK experiment exhibited the key two bond

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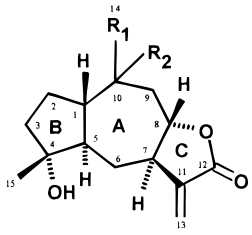
Chart 1

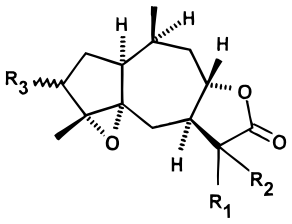


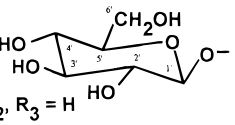
1 R_1 = β-Me , R_2 = α-OH

8 $R_1 + R_2$ = exocyclic CH_2

14 R_1 = β-Me , R_2 = α-OH , double bond C_1-C_2

15 R_1 = α-Me, R_2 = β-OH

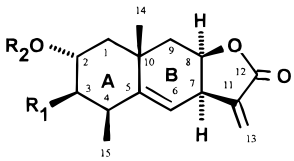




2 R_1 = α-Me, R_2 = H , R_3 =

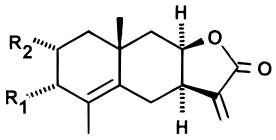
7 $R_1 + R_2$ = exocyclic CH_2, R_3 = H

13 $R_1 + R_2$ = β-11(13)-Epoxide , R_3 = H



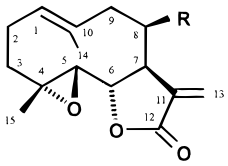
3 R_1 = OH , R_2 = Ac

16 $R_1 = R_2$ = H



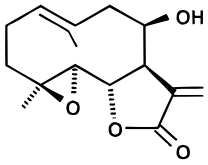
4 $R_1 = R_2$ = OH

17 $R_1 = R_2$ = H

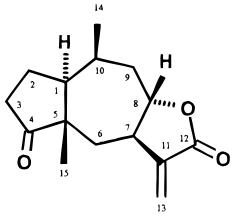


5 R = OH

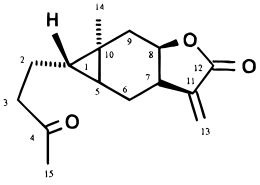
9 R = OAc



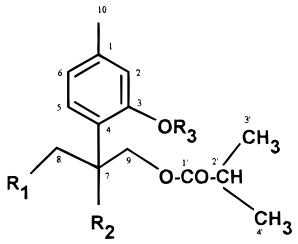
5A



6



10



11 $R_1 + R_2$ = epoxide , R_3 = isobutyryl

12 R_1 = isobutyryloxy , R_2 = OH , R_3 = H

correlations between H-14 and C-10 (δ71.7) and H-15 and C-4 (δ78.9).

The stereochemical assignment at the centers C-1, C-4, C-8, and C-10 was inferred from extensive NOE experiments, including both NOESY and NOE difference techniques. Irradiation of the H-8β (δ 4.32) signal was found to enhance the signals for H-1 (δ1.82), H-14 (δ1.13), and H-15 (δ1.03), suggesting that all of these protons were on the same face of the molecule, while there was no NOE observed between H-7 and H-8, suggesting the presence of a *trans*-fused lactone ring. In addition, irradiation of the signal at δ1.47 (H-5) enhanced the signal for H-7 (δ 2.55), indicating that these two protons were α-oriented, while no enhancement, as expected, was observed for H-1. It should be noted that compound **1** is the C-10 epimer of **15**, a guaianolide recently isolated[5] from the aerial parts of *Inula thapsoides*. The spectral features of **1** and **15** are remarkably similar, except for the chemical shift value of C-14, which is more deshielded in **15** than in **1** (δ 30.7 vs δ 24.8, respectively).

The second guaianolide, **2**, was obtained as off-white granules and analyzed for $C_{21}H_{32}O_9$. Its CIMS revealed the base peak at *m*/*z* 249 $[C_{15}H_{21}O_3]^+$, corresponding to the sesquiterpene aglycone after elimination of the D-glucose moiety. The ^{1}H- and ^{13}C-NMR spectral data for **2** (Tables 1 and 2) were generally similar to those of the guaianolide **7**, except for the presence of signals for the glucosyl and methyl groups at C-3 and C-11, respectively. The ^{1}H-NMR spectrum of **2** contained signals at δ 4.48 (t, J = 8.0 Hz, δ_{C-3} 81.9) and 1.16 (d, J = 7.6 Hz) due to C-3 oxymethine and C-11 methyl groups, respectively. A COSY experiment established the systems −CHCH$_2$CH(O)− (ring B), −CH$_2$CH-(CHMe)CH(O)CH$_2$− (rings A and C), and −CH$_2$CH-(Me)CH− (ring A), as confirmed by a HETCOR experiment (Table 1). The placement of the C-3-glucoside and C-4(5)-epoxide groups was established by a ^{1}H−^{13}C long-

Table 1. ^{13}C-NMR Chemical Shift Values for Compounds **1**, **2**, **6**−**8**, and **13**

carbon	**1**[a]	**2**[b]	**6**	**7**	**8**	**13**
1	51.2 d	43.2 d	47.0 d	47.7 d	46.9 d	47.8 d
2	23.4 t	36.2 t	24.2 t	30.6 t	26.3 t	28.9 t
3	40.7 t	81.9 d	34.8 t	32.7 t	41.1 t	32.7 t
4	78.9 s	71.4 s	220.9 s	69.9 s	80.4 s	69.8 s
5	49.3 d	70.7 s	50.8 s	69.7 s	59.1 d	69.4 s
6	28.4 t	29.5 t	35.0 t	29.0 t	29.9 t	26.5 t
7	50.2 d	47.7 d	45.2 d	44.4 d	45.3 d	40.4 d
8	79.4 d	83.7 d	80.4 d	82.6 d	82.3 d	83.3 d
9	48.3 t	41.7 t	41.6 t	40.4 t	40.7 t	40.6 t
10	71.7 s	35.7 d	32.4 d	34.6 d	146.6 s	34.5 d
11	141.0 s	41.7 d	140.0 s	139.1 s	139.6 s	58.0 s
12	169.9 s	182.4 s	169.8 s	170.0 s	170.1 s	172.9 s
13	118.1 t	10.7 q	120.2 t	118.9 t	120.4 t	46.7 t
14	24.8 q	15.1 q	17.0 q	14.7 q	111.7 t	14.5 q
15	22.6 q	13.3 q	23.4 q	15.6 q	24.1 q	15.4 q
1′		103.2 d				
2′		75.3 d				
3′		78.2 d				
4′		71.9 d				
5′		78.3 d				
6′		63.3 t				

[a] Spectrum recorded in DMSO-d_6. [b] Spectrum recorded in CD_3OD.

range FLOCK experiment, which demonstrated the key three-bond correlation between δ 1.41(H-15), δ_{C-3} 81.9, and δ_{C-5} 70.7 and two-bond correlation between H-15 and C-4 (δ 71.4). In addition, the FLOCK experiment showed the three-bond correlation between H-13 (δ 1.16) and δ_{C-12} 182.4. The identity of the sugar moiety of **2** as β-glucose was suggested on the basis of $J_{1',2'} = 7.8$ Hz, indicating an axial α-orientation of the anomeric proton (H-1′). This was further confirmed by comparing its ^{13}C-NMR chemical shift values with those of the β-D-glucoside vicoside A;[18] the two sets were virtually identical. On the basis of the foregoing data and by comparing the ^{13}C-NMR chemical shift values with those of its derivatives **7** and **13** (Table 1), this guaianolide glucoside was formulated as **2**.

The stereochemistry at C-1, C-8, and C-11 in **2** was deduced by extensive NOE difference experiments. Since no NOE was observed between H-7 and H-8, compound **2** should contain a *trans*-fused lactone ring, as observed for **1** and **7**. Irradiation of H-8β enhanced the signals at δ 0.95 (H-14) and 1.41 (H-15), showing that these two methyl groups were also β-oriented. Conversely, irradiation of H-7 (δ 2.73) was found to enhance the signal of H-13 (δ1.16) and H-6α (δ2.10), and the latter on irradiation enhanced the signal of H-1 (δ 2.62), while no enhancement was observed for H-8β. Furthermore, irradiation of H-3 at δ 4.48 enhanced the signal of H-1′ (δ 4.23), suggesting that both two protons were α-oriented. However, since the sugar moiety can rotate freely, the stereochemistry of the glycosidic linkage remained unknown. In view of these findings, **2** was established as 4α,5α-epoxy-10α,11β,13*H*,14*H*-1-*epi*-inuviscolide 3-β-D-glucoside.

In addition, four other sesquiterpene lactones **3**−**6** were isolated in crystalline form. One of these, 2α-*O*-acetyl-3β-hydroxyalantolactone (**3**), $C_{17}H_{22}O_5$, contained a trisubstituted double bond (δ_{C-5} 145.8, δ_{C-6} 121.5) and an exocyclic methylene on a γ-lactone ring. The ^{1}H- and ^{13}C-NMR spectra of **3** (Table 3) were found to be generally similar to those reported for 2α-hydroxyalantolactone (**16**),[4,26] except for the differences associated with the presence of an acetoxy group at C-2 (ν_{max} 1750 cm^{-1}, δ_C 71.0) and a hydroxyl group at C-3 (ν_{max} 3500 cm^{-1}, δ_C 74.5). The presence of the C-7 (8)-*cis*-fused lactone was inferred from the ^{1}H-NMR data of H-8, which resonated as a doublet of triplets at δ 4.80, J = 3.3, 3.3, 6.7 Hz. These values are virtually identical to those reported for the related sesquiterpene **16**.[26] In addition, the chemical shift values for C-7 and C-8 in both **3** and **16** were very close (δ_{C-7} 39.3, δ_{C-8} 75.1 versus δ_{C-7} 39.53, δ_{C-8} 75.6 in **16**[26]).

The ^{1}H-NMR spectrum of **3** contained signals at δ 5.12 (ddd, J = 4.3, 10.2, 11.5 Hz) and 3.65 (dd, J = 6.3, 10.2 Hz) due to the two axially-disposed protons, H-2β and H-3α, respectively. The two systems $-CH_2CH$-(OAc)CH(OH)CH(Me) in ring A and $=CHCH(C=CH_2)$-$CH(O)CH_2-$ in ring B of **3** were established from the COSY spectra and were confirmed by a HETCOR experiment (Table 3). In addition, the ^{13}C-NMR spectrum revealed the deshielding of C-4 to δ_C 44.2 (versus δ_{C-4} 38.53 in **16**[26]), due to the presence of the hydroxyl group at C-3. The relative stereochemistry, depicted in **3**, was based not only on the ^{1}H- and ^{13}C-NMR spectral data but also on biogenetic correlation with other alantolactones isolated from several *Inula* species.[4−8]

The ^{1}H- and ^{13}C-NMR spectral data (Table 3) for the eudesmanolide **4**, $C_{15}H_{20}O_4$, were generally similar to those of **3** but lacked the signals associated with the C-5(6)-double bond and C-2 acetoxy group. Instead, **4** was concluded to have a C-4(5)-tetrasubstituted double bond [δ_{C-4} 129.8, δ_{C-5} 135.8, versus δ_{C-4}127.1, δ_{C-5} 137.4 in alloalantolactone (**17**)[27]] and two hydroxyl groups at C-2 and C-3 at δ_{C-2} 71.6 and δ_{C-3} 78.5. A COSY experiment established the systems $-CH_2CH$-(OH)CH(OH)− (δ 1.65, 3.78, 3.76, H-1, H-2, and H-3, respectively) and $CH_2CH(C=CH_2)CH(O)CH_2$ in rings A and B, respectively, of **4**, and this was confirmed by a HETCOR experiment and other ^{13}C-NMR spectral data (Table 3).

A series of NOE-difference experiments showed that H-7 (δ 3.18) and H-8 (δ 4.54) were *cis* and α-oriented, while no enhancement was observed for H-14 (δ 1.18), thus indicating that H-14 and H-7/H-8 were *trans*. In addition, irradiation of H-14β enhanced the signals for H-2 and H-3 (δ 3.78 and 3.76, respectively), confirming that these two protons were also β-oriented in **4**. From the foregoing data, the structure of the eudesmanolide **4** was assigned as 2α,3α-dihydroxyalloalantolactone (**4**).

The germacranolide 8β-hydroxyparthenolide (4α,5β-epoxyeupatolide, **5**) demonstrated physical and spectroscopic data that were indistinguishable from those of inulasalsolin, previously isolated from *I. salsoloides*.[6] It was originally assigned[6] structure **5A**, but the structure was recently revised[28] to **5**. Acetylation of **5** afforded the known acetate, lipiferolide (**9**), which was also isolated during this investigation, and its identity was established by comparing its physical and spectroscopic data with those previously reported.[21,22]

The last sesquiterpene lactone was found to possess physical and ^{1}H NMR data that were closely related to those reported[29,30] for the pseudoguaianolide 8-*epi*-confertin (**6**), $C_{15}H_{20}O_3$. Its hitherto unreported ^{13}C NMR assignments (Table 1) were in agreement with structure **6**.

During the isolation of the sesquiterpenes, the thymol derivatives **11** and **12** were also isolated as pure entities. Compound **11** was isolated previously from *Helenium mexicanum*,[9] while **12** was reported as the product of

Table 2. ^{1}H-NMR Chemical Shift Values and Coupling Constants (in Hz, in Parentheses) for Compounds **1**, **2**, **8**, and **13**

proton	**1**[a]	**2**[b]	**8**[c]	**13**
1α		2.62 brd (7.9)		2.55 d (7.5)
1*β*	1.82 m		2.66 brd (10.5)	
2	1.67 m	1.75 m[d]	1.96 m	1.75 m[e]
	1.47 m[f]		1.65 m	1.25 m
3	1.42 m	4.48 t (8.0)	1.85 m	1.91 m
			1.65 m	1.71 m
5α	1.47 m[f]		1.71 m	
6α	2.18 m	2.10 dd (7.0, 17.5)	2.28 td (3.7, 13.3)	1.84 m
6*β*	1.06 m	1.77 m	1.24 m	1.40 br d (17.5)
7α	2.55 m	2.73 m[g]	2.66 m	2.77 ddd (1.9, 10.1, 12.0)
8*β*	4.32 ddd (1.4, 9.8, 11.2)	4.10 ddd (4.0, 10.4, 11.5)	4.31 ddd (1.6, 9.3, 10.6)	4.43 ddd (4.0, 10.1, 12.0)
9α	1.92 dd (11.2, 14.3)	1.75 m[d]	2.55 dd (10.6, 15.5)	1.75 m[e]
9*β*	2.25 br dd (1.7, 14.3)	2.29 td (4.0, 12.7)	3.19 ddd (3.5, 6.0, 15.5)	2.36 td (4.0, 12.8)
10α		2.0 m		2.10 m
11		2.73 m[g]		
13a	5.95 d (3.3)	1.16 d (7.6)	6.22 d (3.5)	3.36 d (5.0)
13b	5.54 d (3.3)		5.54 d (3.5)	2.96 d (5.0)
14*β*	1.13 s	0.95 d (7.5)	5.09 s	0.96 d (7.4)
			4.97 s	
15*β*	1.03 s	1.41 s	1.20 s	1.33 s
1′		4.23 d (7.8)		
2′		3.18 t (7.8)		
3′				
4′		3.26−3.43 m		
5′				
6′		3.98 br d (12.0)		
		3.66 dd (5.3, 12.0)		
OH	4.50 br s	1.98 br s		

[a] Spectrum run in DMSO-d_6. [b] Spectrum in CD_3OD. [c] Data for **8** was published previously;[20] also reported here to aid the assignment of **1**. [d−g] Signals superimposed on each other, *J* unresolved.

Table 3. ^{1}H- and ^{13}C-NMR Chemical Shift Values for Compounds[a] **3** and **4**

	3		**4**		
proton/carbon	^{1}H	^{13}C	^{1}H	NOE	^{13}C
1	2.0 dd (4.3, 15.5)	44.2 t	1.65 m		44.5 t
	1.19 m				
2	5.12 ddd	71.0 d	3.78 br dd (4.5, 6.0)[b]	H-3*β*, H-14*β*	71.6 d
	(4.3, 10.2, 11.5)				
3	3.65 dd (6.3, 10.2)	74.5 d	3.76 br d[b]	H-2*β*, H-14*β*	78.5 d
4	2.80 brq (7.4)	44.2 d			129.8 s
5		145.8 s			135.8 s
6	5.30 d (4.0)	121.5 d	1.94 br t (12.5) 2.88 dd (7.5, 13.7)		28.8 t
7	3.60 m	39.3 d	3.18 m	H-8α	41.8 d
8	4.80 dt (3.3, 3.3, 6.7)	75.1 d	4.54 dd (7.6, 13.9)	H-7α	77.5 d
9	1.52 dd (3.3, 14.8)	41.9 t	1.70 m[c]		44.0 t
9′	2.21 dd (2.8, 14.8)		1.74 m[c]		
10		33.4 s			36.9 s
11		138.9 s			141.8 s
12		171.4 s			172.6 s
13	6.23 d (2.0)	122.4 t	6.18 d (2.7)		122.5 t
	5.66 d (2.0)		5.74 d (2.7)		
14	1.29 s	29.5 q	1.18 s	H-2*β*, H-3*β*	27.4 q
15	1.14 d (7.7)	16.2 q	1.77 s		15.0 q
OAc	2.09 s	21.2 q			
		169.8 s			

[a] Values in parentheses are coupling constants, in Hz. [b,c] Signals superimposed on each other, *J* (for H-3, H-9, and H-9′ of **4**) unresolved.

acid hydrolysis of **11**,[9] but has not been previously isolated from a natural source. Complete ^{13}C-NMR data for **11** and **12**, not reported previously, are assigned in Table 4 using COSY, HETCOR, and FLOCK experiments. Furthermore, the UV, IR, and MS data for 7-hydroxy-8,9-diisobutyryloxythymol (**12**) are being reported for the first time. The ^{13}C-NMR data for carabrone (**10**), not previously reported, are presented in the Experimental Section.

Experimental Section

General Experimental Procedures. Melting points were recorded on an Electrothermal 9100 instrument. UV spectra were obtained in MeOH, using a Varian DMS 90 spectrophotometer, and IR were taken as KBr disks, unless otherwise specified, on a Perkin-Elmer 5808 spectrophotometer. The NMR spectra were taken on Varian VXR 300 instrument at the University of Mississippi and on a Varian XL-300 NMR spectrometer at the College of Pharmacy, University of Illinois at Chicago (UIC), operating at 299.9 MHz (^{1}H) and 75.4 MHz (^{13}C) respectively, in $CDCl_3$, unless otherwise stated, using tetramethylsilane (TMS) as internal standard. DEPT spectra were obtained from a Nicolet NT-360 spectrometer operating at 90.8 MHz (^{13}C) at the Research Resources Center (UIC) and a GE Omega-300 spectrometer at 75.0 MHz (^{13}C) at the University of Chicago. 2D NMR spectra (COSY, HETCOR, FLOCK,

Table 4. ^{1}H- and ^{13}C-NMR Chemical Shift Values for Compounds[a,b] **11** and **12**

proton/ carbon	**11**		**12**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		139.9 s		140.1 s
2	6.87 br s	122.9 d	6.69 br s	118.6 d
3		148.5 s		156.5 s
4		126.0 s		118.8 s
5	7.35 d (7.8)	128.9 d	6.90 d (8.0)	126.5 d
6	7.05 br d (7.8)	126.7 d	6.65 dd (2.0, 8.0)	120.5 d
7		56.9 s		78.7 s
8	3.03 d (5.3)	50.7 t	4.48 d (12.1)	67.3 t
	2.79 d (5.3)		4.43 d (12.1)	
9	4.57 d (12.2)	64.8 t	4.48 d (12.1)	67.3 t
	4.19 d (12.2)		4.43 d (12.1)	
10	2.35 s	21.1 q	2.27 s	21.0 q
iBu-1 1′		176.4 s		177.5 s
2′	2.52 sep (7.1)	33.8 d	2.56 sep (7.0)	33.9 d
3′	1.09 d (7.1)[c]	19.0 q	1.12 d (7.0)	18.9 q
4′	1.11 d (7.1)[c]	18.9 q	1.12 d (7.0)	18.9 q
iBu-2 1″		175.3 s		177.5 s
2″	2.85 sep (7.2)	34.2 d	2.56 sep (7.0)	33.9 d
3″	1.32 d (7.2)	18.6 q	1.12 d (7.0)	18.9 q
4″	1.32 d (7.2)	18.6 q	1.12 d (7.0)	18.9 q
OH			8.75 s	
			4.36 s	

[a] ^{1}H-NMR of **11** and **12** were published previously[9] but reported here to aid with the ^{13}C-NMR assignments, which were accomplished using 2D NMR COSY, HETCOR, and FLOCK experiments. [b] Values in parentheses are coupling constants, in Hz. [c] Interchangeable signals.

NOESY and NOE-difference experiments) were obtained using standard Varian software. Stereochemical assignments drawn for all new compounds depict relative rather than absolute stereochemistry. CIMS were recorded on a Finnegan MAT 300 mass spectrometer, using CH_4 or NH_3 as ionizing gases. Elemental analyses are within ±0.4% as determined using a Perkin-Elmer analyzer, Model 2400. Optical rotations were recorded in $CHCl_3$, unless otherwise stated, at ambient temperature using a Perkin-Elmer 241 MC polarimeter. TLC was performed on Si gel 60 F_{254}, using 15% MeOH−CCl_4, unless otherwise specified, as solvent, with visualization using 1% vanillin/H_2SO_4 spray reagent. Centrifugal preparative TLC (CPTLC, using a Chromatotron instrument obtained from Harrison Research, Inc., Model 7924) was run with either 1, 2, or 4 mm Si gel PF_{254} disks, at a flow rate of 4 mL/min.

Plant Material. The aerial parts of *V. pentanema* were collected in Abha, Saudi Arabia, in May 1993. A voucher specimen (no. 13063) is deposited at the herbarium of MAPPRC, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Extraction and Isolation. The dried ground aerial parts (1 kg) were percolated successively with *n*-hexane (8 L) followed by EtOH (8 L) at room temperature, and the extracts were dried *in vacuo* to leave 35 and 55 g of residue, respectively. The *n*-hexane extract (33 g) was subjected to column chromatography over Si gel (mesh 70−230, 1 kg) and eluted with CCl_4 followed by increasing concentrations of EtOAc (between 1% and 15%) in CCl_4 to give seven pure isolates **3**, **6**, **7**, and **9**−**12** and two fractions (A and B). Subsequent separation by centrifugal preparative TLC (using Si gel PF_{254} 4 mm disk, *n*-hexane:EtOAc 7.5:2.5) of fraction A led to the isolation of **5** and **8**. Similar treatment of fraction B ($CHCl_3$:Me_2CO 2.5:0.5) gave compounds **1** and **4**.

Successive percolation of the dried EtOH extract (50 g) prepared from aerial parts with EtOAc, followed by MeOH, afforded 30 and 16 g of solid residue, respectively. A portion of the MeOH fraction (13 g) was subjected to column chromatography over Si gel (400 g) and eluted with CH_2Cl_2−EtOAc (1:1) to yield fraction C (0.5 g), which was subsequently purified by additional chromatography (CPTLC, Si gel PF_{254} 2 mm disk, $CHCl_3$:Me_2CO Me_2CO 55:45) to give compound **2**.

10α-Hydroxy-14*H*-inuviscolide (1): off-white granules (80 mg) from Me_2CO/$CHCl_3$; mp 112−114 °C; $[\alpha]_D$ −58° (*c* 0.056, EtOH); UV (MeOH) λ_{max} 220 (log ϵ 3.96) nm; IR (KBr) ν_{max} 3300 (OH), 1755 (lactone), 1660 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Tables 2 and 1, respectively; CIMS *m*/*z* [M·NH_4]$^+$ 284 ([$C_{15}H_{22}O_4$·NH_4]$^+$, 100), 266 ([M·NH_4-NH_4]$^+$, 10), 249 (20).

4α,5α-Epoxy-10α,11*β*,13*H*,14*H*-1-*epi*-inuviscolide 3-*β*-D-glucoside (2): off-white granules (120 g) from Me_2CO/$CHCl_3$; mp 80−82 °C; $[\alpha]_D$, −26.7° (*c* 0.09, MeOH); UV (MeOH) λ_{max} 220 (log ϵ 4.38) nm; IR (KBr) ν_{max} 3500 (OH), 1750 (lactone) cm^{-1}; ^{1}H- and ^{13}C-NMR, see Tables 2 and 1, respectively; CIMS *m*/*z* [MH]$^+$ 429 ([$C_{21}H_{32}O_9$·H]$^+$, 10), 249 ([M − $C_6H_{11}O_6$]$^+$, 100), 231 (65).

2α-*O*-Acetyl-3*β*-hydroxyalantolactone (3): colorless needles (200 mg) from *n*-hexane/EtOAc: mp 182−184 °C; $[\alpha]_D$ +120° (*c* 0.084, $CHCl_3$); UV (MeOH) λ_{max} 220 (log ϵ 3.98) nm; IR (KBr) ν_{max} 3500 (OH), 1750 (br, OAc and lactone), 1660 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 3; CIMS *m*/*z* [M·NH_4]$^+$ 324 ([$C_{17}H_{22}O_5$·NH_4]$^+$, 100), [M]$^+$ 306 (15), 288 ([M]$^+$ − H_2O, 15), 246 ([M]$^+$ − 60, 15).

2α,3α-Dihydroxyalloalantolactone (4): colorless needles (60 mg) from Me_2CO/$CHCl_3$: mp 81−83 °C; $[\alpha]_D$ +25°(*c* 0.09, MeOH); UV (MeOH) λ_{max} 220 (log ϵ 3.98) nm; IR (KBr) ν_{max} 3500 (OH), 1745 (lactone), 1650 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 3; CIMS *m*/*z* [MH]$^+$ 265 ([$C_{15}H_{20}O_4$·H]$^+$, 10), 247 ([MH]$^+$ − H_2O, 75), 229 (*m*/*z* 247 − H_2O, 100), 201 (25).

8*β*-Hydroxyparthenolide (4α,5*β*-Epoxyeupatolide, 5): colorless needles (300 mg) from *n*-hexane/EtOAc; mp 144−146 °C; $[\alpha]_D$ −178° (*c* 0.811, $CHCl_3$) and corresponding literature[6] values for **5A**: mp 136−138 °C; $[\alpha]_D$ −186.3° (MeOH); spectroscopic (^{1}H- , ^{13}C-NMR, and MS) data were indistinguishable from those reported previously[28] for **5**.

8-*epi*-Confertin (6): colorless plates (100 mg) from *n*-hexane/EtOAc: mp 150−151 °C; $[\alpha]_D$ +178°(*c* 0.05 $CHCl_3$) and corresponding literature[29] values for **6**: mp 135−136 °C; $[\alpha]_D$ +147° (MeOH); ^{13}C-NMR, see Table 1; CIMS *m*/*z* [MH]$^+$ 249 ([$C_{15}H_{20}O_3$·H]$^+$, 100), 231 (95).

4α,5α-Epoxy-10α,14*H*-1-*epi*-inuviscolide (7): colorless plates (750 mg) from *n*-hexane/EtOAc; mp 69−71 °C; $[\alpha]_D$ +89° (*c* 0.084, $CHCl_3$) and corresponding literature[4] value for **7**: a gum (mp not reported); $[\alpha]_D$ +84.4° ($CHCl_3$); spectroscopic (UV, IR, MS, and ^{1}H-NMR) data were indistinguishable from those reported previously;[4] ^{13}C-NMR, see Table 1.

Inuviscolide (8): gum (80 mg); physical/spectroscopic ($[\alpha]_D$, UV, IR) and ^{1}H-NMR data were indistinguishable from those reported previously;[19] ^{13}C-NMR, see Table 1; CIMS *m*/*z* [MH]$^+$ 249 ([$C_{15}H_{20}O_3$·H]$^+$, 40), 231 (100), 185 (35).

Lipiferolide (9): gum (200 mg); $[\alpha]_D$ −114° (*c* 0.05, $CHCl_3$) [literature[21] $[\alpha]_D$ −125° (MeOH)]; spectoscopic data (UV, IR, ^{1}H- and ^{13}C-NMR) were indistinguishable from those reported previously.[21,22]

Carabrone (10): off-white needles (500 mg) from *n*-hexane/EtOAc; mp, $[\alpha]_D$, IR, ^{1}H-NMR, and MS data were indistinguishable from those reported previously;[4,23] ^{13}C-NMR δ 17.1 (s, C-10), 18.2 (q, C-14), 22.9 (d, C-5), 23.3 (t, C-2), 30.0 (q, C-15), 30.7 (t, C-6), 34.2 (d, C-1), 37.2 (t, C-9), 37.7 (d, C-7), 43.5 (t, C-3), 75.5 (d, C-8), 122.4 (t, C-13), 139.0 (s, C-11), 170.3 (s, C-12), 208.4 (s, C-4).

7,8-Epoxy-9-(isobutyryloxy)thymolisobutyrate (11): oil (75 mg); physical and spectroscopic data ($[\alpha]_D$, UV, IR, and ^{1}H-NMR) were indistinguishable from those previously reported;[9] ^{13}C-NMR, see Table 4; CIMS *m/z* $[MH]^+$ 339 ($[C_{18}H_{26}O_6{\cdot}H]^+$, 5), 321 ($[MH]^+ - H_2O$, 100), 145 (*m/z* 321 − 2 × iBuOH, 35).

7-Hydroxy-8,9-bis(isobutyryloxy)thymol (12): oil (60 mg); UV (MeOH) λ_{max} 220 (log ϵ 3.80), 2.55 (log ϵ 2.3) nm; IR (neat) ν_{max} 3300 (OH), 1760 (iBuCOO), 1600 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Table 4; CIMS *m/z* $[MH]^+$ 321 ($[C_{18}H_{24}O_5{\cdot}H]^+$, 70), 145 ($[MH]^+ - 2 \times$ iBuOH, 100).

Epoxidation of 4α,5α-Epoxy-10α,14*H*-1-*epi*-inuviscolide (7) to 13. Compound **7** (100 mg) in CH_2Cl_2 was treated with *m*-chloroperbenzoic acid (125 mg) at rt and stirred for 12 h. Regular workup[31] yielded crude compound **13** (90 mg), purified by chromatography (CPTLC, 1 mm silica gel PF_{254} disk, solvent: 10% EtOAc−*n*-hexane) to provide 4α(5α),11α(13)-diepoxy-10α,11*β*,14*H*-1-*epi*-inuvicolide (**13**) (80 mg, R_f 0.45, solvent: 15% MeOH−CCl_4): $[\alpha]_D$ +69.5° (*c* 0.05,$CHCl_3$); IR (KBr) ν_{max} 1735 (lactone) 1235, 1155 cm^{-1}; ^{1}H- and ^{13}C-NMR, see Tables 2 and 1, respectively; CIMS *m/z* $[MH]^+$ 265 ($[C_{15}H_{20}O_4{\cdot}H]^+$, 100).

Acetylation of 8*β*-Hydroxyparthenolide (5) to 9. Compound **5** (75 mg) was dissolved in pyridine (1 mL) and treated with Ac_2O (0.5 mL) at rt for 24 h. Regular workup gave **9** (70 mg) as a gum, $[\alpha]_D$ −110° (*c* 0.05, $CHCl_3$). The physical and spectral data (IR, ^{1}H- and ^{13}C-NMR) of the acetate **9** were indistinguishable from those previously reported for lipiferolide (**9**).[21,22]

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