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12-HYDROXYTOVAROL AND DERIVATIVES FROM *THAPSIA VILLOSA* VAR. *MINOR*

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Key Word Index -*Thapsia villosa*; Umbelliferae; germacrane derivatives, 12-hydroxytovarol angelate; 12-hydroxyshiromodiol angelate.

Abstract—The new germacrane esters 12-angeloyloxy- and 12-hydroxy-8-*O*-angeloyltovarol and 12-angeloyloxy-8-*O*-angeloylshiromodiol were isolated from *T. villosa* var. *minor*, and their structures established by spectral data and chemical correlations. The terpenoids geranyl acetate, δ-cadinene, γ-cadinene, γ-muurolene, β-caryophyllene and caryophyllene oxide were also isolated from the aerial parts.

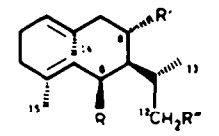
INTRODUCTION

In the course of our study on the chemical components from *Thapsia* species [1–4] we have described the isolation of some germacrane esters related to tovarol [= (6*S*,7*R*,8*S*,4*E*,9*E*)-1(10),4-germacradiene-6,8-diol] and shiromodiol (4β,5β-epoxy derivative). We would like to report here the isolation of two new 12-hydroxytovarol angelates **1** and **2** from the roots of *T. villosa* var. *minor* and a new 12-angeloyloxyshiromodiol derivative **14** as well as other known terpenoids from the umbellas of this plant.

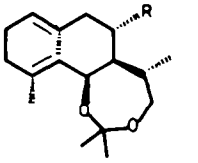
RESULTS AND DISCUSSION

The benzene extract from the roots of the plant afforded after chromatography pure **1** and **2**. Both substances showed IR spectra nearly identical and very similar to that of 8-*O*-angeloyltovarol previously isolated from the same plant [2]. The IR spectra were indicative of the presence of hydroxyl groups (3400 cm^{-1}), conjugated ester (1700, 1230 cm^{-1}) and double bonds (1640, 850 cm^{-1}). The ^1H NMR spectra of **1** and **2** showed the presence of two olefinic protons, two vinyl methyl groups as well as a secondary methyl group. Compound **1** showed also signals of two angeloxy groups but **2** displayed signals of only one angeloxy group. The alkaline hydrolysis of both **1** and **2** gave the same triol **3** which on standing at room temperature overnight with acetic anhydride–pyridine gave the triacetate **4**. The ^1H NMR spectra of **3** and **4** were quite similar to those of tovarol **5** and its diacetate **6**, respectively. However, tovarol showed signals of an isopropyl group while **1** to **4** showed only one 3H doublet and signals of a CH_2OR group. These data suggested that the third oxygenated group should be placed on C-12. Moreover, the coupling constants displayed by the acetates **4** and **6** are analogous and both showed high specific rotations with the same sign (**4**: $[\alpha]_D$ −71.0°; **6**: $[\alpha]_D$ −101.5°). This suggested that the configuration of both compounds was the same except for the new C-11 chiral centre.

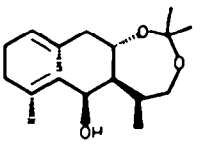
To establish the configuration at C-11 the isopropylidene derivatives of triol **3** were synthesized. As the hydroxyl groups attached to C-6 and C-8 are *trans* the 6,12 and 8,12 dioxepanes were the only expected isopropylidene derivatives. In fact only two products were obtained, **7** and **10**. Product **7** was acetylated yielding **8**. This showed the H-8 multiplet deshielded and located the free hydroxyl group at C-8. Assuming a conformation for the cyclodecane ring $_{15}D^5$, $^1D_{14}$ [5] analogous to that shown by shiromodiol [6], as suggested by the observed coupling constants, the oxepane ring may adopt the half-chair conformation **I** or **II** as the half-boat conformations are highly puckered because of the steric interactions of the isopropylidene group with the cyclodecane substituents. Although both conformations allow the same conclusions with respect to the C-11 configuration, **I** must be the more stable conformation for **7** as deduced from the deshielding of one of the C-12 protons. This deshielding could be caused by the *n* electrons of the C-6 oxygen being spatially close to the H-12 in conformation **I**. Also, the small coupling constants between the C-12 protons with H-11 (H-12β: δ4.00, *br d*, J = 12 Hz; H-12α: δ3.25, *br*



	R	R'	R''
1	OH	OAng	OAng
2	OH	OAng	OH
3	OH	OH	OH
4	OAc	OAc	OAc
5	OH	OH	H
6	OAc	OAc	H



7 R: OH
8 R: OAc
9 R: =O

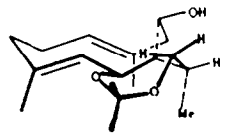


10

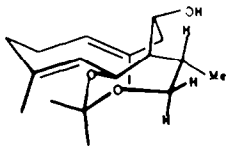
Table 1. ^{1}H NMR spectral data for 12-hydroxytovarol derivatives*

H	1	2	3	4	7	8	9	10	13	14
1	5.10 *m*	5.10 *m*	5.00 *m*	5.10 *m*	5.00 *m*	5.20 *m*	4.90 *m*	4.85 *m*	5.15 *m*	5.28 *m*
2, 3	2.12 *br s*	2.15 *br s*	2.15 *br s*	2.20 *br s*	2.12 *br s*	2.18 *br s*	2.10 *br s*	2.10 *br s*	2.15 *br s*	
5	5.10 *m*	5.10 *m*	5.00 *m*	5.10 *m*	5.00 *m*	5.20 *m*	4.90 *m*	4.85 *m*	4.90 *br d* (9)	2.82 *br d* (7)
6	5.10 *m*	5.10 *m*	4.70 *br d* (7.5)	5.40 *br d* (7)	4.60 *br d* (7)	4.60 *br d* (7)	4.55 *br d* (7.5)	4.85 *m*	4.40 *dd* (9, 6)	3.42 *br d* (7)
8	4.20 *m*	4.50 *br t* (7)	4.10 *m*	5.10 *m*	4.15 *m*	5.20 *m*		4.00 *m*	5.15 *m*	5.28 *m*
12	4.20 *m*	3.50 *m*	3.55 *m*	4.15 *dd* (10, 3)	4.15 *br d* (12)	3.92 *br d* (12)	4.25 *br d* (12)	4.00 *m*	3.80 *dd* (9, 7)	4.15 ABm
				3.85 *dd* (10, 4)	3.25 *br d* (12)	3.25 *br d* (12)	3.10 *br d* (12)	3.25 *t* (12)	3.05 *dd* (9, 7)	
13	1.15 *d* (6.5)	1.10 *d* (6.5)	0.97 *d* (6.5)	1.15 *d* (6.5)	1.10 *d* (6.5)	1.15 *d* (6.5)	1.07 *d* (6.5)	1.00 *d* (6.5)	1.10 *d* (6.5)	1.20 *d* (6.5)
14	1.62 *s*	1.68 *s*	1.60 *s*	1.70 *s*	1.60 *s*	1.65 *s*	1.50 *s*	1.60 *s*	1.67 *s*	1.80 *s*
15	1.38 *s*	1.48 *s*	1.45 *s*	1.50 *s*	1.45 *s*	1.48 *s*	1.50 *s*	1.40 *s*	1.46 *s*	1.25 *s*
Ang	6.05 *br q* (6)	6.00 *br q* (6)							5.95 *br q* (6)	6.05 *br q* (6)
	5.95 *br q* (6)									5.95 *br q* (6)
	1.96 *br d* (6)	1.96 *br d* (6)							1.96 *br d* (6)	1.95 *m*
	1.90 *br s*	1.90 *br s*							1.90 *br s*	1.90 *br s*
Ac				2.01 *s*		1.97 *s*				
				2.00 *s*						
				1.95 *s*						
gem-Me_2					1.30 *s*	1.22 *s*	1.30 *s*	1.40 *s*		
							1.19 *s*			

*Recorded at 60 MHz in $CDCl_3$ (3, 4, 10, 14) or in CCl_4. δ scale in ppm relative to TMS and J in Hz (in parentheses).



I



II

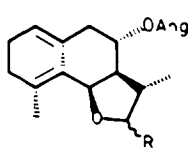
d, $J = 12$ Hz) are in agreement with the conformation shown in **I**. The Eu(fod)$_3$ induced shift on the NMR signal for Me-13 of compound **7** is small as compared with the H-8 induced shift ($\Delta\delta_{Me\text{-}13}$ 0.3 vs. $\Delta\delta_{H\text{-}8}$ 2.8) and this is against the *R*-configuration for C-11 in which the Me-13 group points in the same direction as the hydroxyl group in both conformations **I** and **II**. In consequence we propose the *S*-configuration for C-11.

This assignment is also in agreement with the observed chemical shift for Me-13 in the oxidation product **9**. Assuming for this compound a ring conformation like that shown in **II**, the Me-13 is placed below the carbonyl plane if the C-11 configuration is *R*, whereas if the configuration is *S* this methyl group is placed away from the carbonyl group. As the doublet of Me-13 is not significantly shielded (**7**: δ1.10; **9**: δ1.07) it is also concluded that the configuration for C-11 must be *S*.

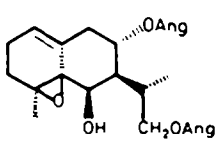
A germacradiene lactol named 'hallerin' (**11**) has been isolated recently from *Laserpitium halleri* [7]. The absolute configuration of the chiral carbon atoms C-6, C-7 and C-8 of **11** is the same that the previously proposed for our tovarol derivatives. To confirm the structure of our substances, an authentic sample of the reduction product from hallerin-lactone **12** was requested. We found that the ^{1}H NMR and IR spectra, as well as the mp and/or $[\alpha]_D$ for compounds **3** and **4** were identical to those of the triol and triacetate synthesized from natural hallerin. Consequently the configuration shown in **3** for 12-hydroxytovarol was confirmed.

The site where the angeloxy groups are attached in the natural tovarol esters **1** and **2** was deduced from their ^{1}H NMR spectra as compared with those of the derivatives **3**–**8**. The hydrolysis of diangelate **1**, caused an upfield shift for the signals of the H-12 protons ($\Delta\delta$0.6). The doublet at δ5.40 in triacetate **4** assigned to H-6 is deshielded as expected with respect to the same signal in triol **3** ($\Delta\delta$0.7 ppm) but the H-6 signal in **1**, partially overlapped near 5 ppm, is shielded after hydrolysis by 0.3 ppm only. This suggested that the non-esterified hydroxyl group is that placed on C-6 as shown in formula **1**.

Compound **2**, esterified by one angeloxy group, showed the same chemical shifts as compound **1** for H-6 and H-8, but the H-12 protons were shielded with respect to **1** ($\Delta\delta$0.7 ppm). These data are indicative that the angeloxy group in this case must be attached to C-8. This substitution pattern was also evidenced by cyclization. Treatment of **2** with TsCl gave the tetrahydrofurane **13**.



11 R = OH
12 R = =O
13 R = H



14

The benzene extract from the umbellas of the plant was also studied. The main component was identified as geranyl acetate. The known sesquiterpenoids δ- and γ-cadinene, γ-muurolene, β-cariophillene and β-cariophilene oxide were also identified, as well as falcarindiol, sitosterol, β-amyrin and glycerides (GC methyl esters: linoleate 60%, oleate 13%, linolenate 7%).

From the same extract a new germacrane ester **14** was isolated. The IR spectrum of **14** was similar to that of 8-*O*-angeloylshiromodiol [2] but the ^{1}H NMR spectrum showed in this case the presence of two angelate groups, one epoxydic proton (2.82, 1H, *d*, $J = 6$ Hz), one methyl doublet (1.20, 3H, *d*, $J = 6$ Hz) and signals for CHOAng (5.28, 1H, *m*) and CH–CH$_2$OAng (4.15, 2H, *m*). All these data led us to assign the structure shown in **14** for this new tovarol derivative.

To confirm the structure proposed for **14**, the natural tovarol (**1**) was epoxidized with one mol of *m*-CPBA to give a monoepoxide identical in all respects to compound **14**.

EXPERIMENTAL

Isolation. Plant material was collected, extracted and fractionated as previously reported [2]. Silica gel chromatography (1 kg) of the roots neutral fraction (236 g) with hexane–Et$_2$O mixtures of increasing polarity was carried out. Fractions containing **1** (12 g) were further chromatographed on silica gel with hexane–EtOAc (9:1) and on silica gel H-60 (60 g, C_6H_6–EtOAc, 97:3) yielding pure **1** (760 mg). Fractions containing **2** (19 g) yielded after chromatography on silica gel eluted with hexane–EtOAc (7:3), C_6H_6–EtOAc (8:2) and hexane–Et$_2$O (6:4), pure **2** (490 mg).

The defatted umbellas extract (31.9 g) was chromatographed (silica gel, 500 g) with hexane–Et$_2$O mixtures of increasing polarity. The less polar fractions (1.2 g) were further chromatographed on 10% $AgNO_3$–silica gel yielding δ-cadinene (40 mg), γ-muurolene (60 mg), γ-cadinene (20 mg) and β-caryophyllene (200 mg). The next fractions contained geranyl acetate (10.3 g), caryophyllene oxide (98 mg) and an oily product (290 mg) which after chromatography on silica gel H-60 (30 g) with hexane–Et$_2$O (8:2) yielded pure **14** (166 mg).

12-*Angeloyloxy*-8-O-*angeloyltovarol* (**1**). Oily, $[\alpha]_D$ −132.0° ($CHCl_3$; *c* 7.3). IR ν_{max}^{film} cm^{-1}: 3400, 1700, 1640, 1380, 1220, 1150, 1070, 1030, 1000, 850. EIMS (probe) 70 eV, *m/z* (rel. int.): 218 [M − 2AngOH]$^+$ (10), 203 [M − 2AngOH − Me]$^+$ (3), 200 [M − 2AngOH − H$_2$O]$^+$ (10), 203 [M − AngOH − Me]$^+$ (3), 200 [M − 2AngOH − H$_2$O]$^+$ (4), 169 [M − AngOH − *iso* − PrOAng − H$_2$O]$^+$ (15), 134 (25), 121 (10), 119 (10), 107 (40), 105 (20), 93 (60), 83 (100), 57 (50), 55 (50), 43 (20), 41 (20).

12-*Hydroxy*-8-O-*angeloyltovarol* (**2**). Oily. IR ν_{max}^{film} cm^{-1}: 3400, 1710, 1650, 1450, 1390, 1240, 1160, 1080, 1050, 1000, 850.

12-*Hydroxytovarol* (**3**). Compounds **1** or **2** hydrolysed overnight with 2 N NaOH–MeOH yielded crystalline **3**. Mp 164–165° (Et$_2$O). $[\alpha]_D$ −88.2° (EtOH; *c* 1.9) IR ν_{max}^{nujol} cm^{-1}: 3300, 2950, 1670, 1380, 1260, 1060, 1020, 980, 860, 840, 760, 680. EIMS (probe) 70 eV, *m/z* (rel. int.): 236 [M]$^+$ (4), 221 [M − 15]$^+$ (16), 218 [M − 18]$^+$ (10), 177 (15), 159 (40), 121 (40), 107 (60), 93 (95), 81 (100), 69 (70), 55 (50), 41 (40).

Triacetate **4**. Acetylation of **3** (170 mg) in Ac$_2$O–pyridine yielded **4** (190 mg), which was purified by CC on silica gel with hexane–Et$_2$O (8:2) (110 mg). Oily, $[\alpha]_D$ −78.0° ($CHCl_3$; *c* 4.3).

IR ν_{max}^{film} cm^{-1}: 2950, 1740, 1660, 1450, 1380, 1250, 1130, 840. EIMS (probe) 70 eV, *m/z* (rel. int.): 260 [M − 2AcOH]$^+$ (4), 218 [M − C_3H_2O]$^+$ (2), 200 [M − 3AcOH]$^+$ (10), 185 (85), 159 [M − 2AcOH − *iso*-PrOAc]$^+$ (100), 93 (85), 43 (90).

6,12-*Isopropylidene derivative* **7**. Reaction of **3** (95 mg) in 4 ml Me_2CO with 2,2-dimethoxypropane (1 ml) and a trace of *p*-TsOH afforded after work up a crude oil which was chromatographed on silica gel (10 g; hexane–Et_2O, 8:2), to yield **7** (63 mg) and **10** (21 mg). Compound **7**: Oily. $[\alpha]_D$ −118.1° ($CHCl_3$; *c* 1.6). IR ν_{max}^{film} cm^{-1}: 3000, 2950, 1670, 1460, 1390, 1230, 1170, 1060, 1000, 950, 900, 870, 850, 800, 710. EIMS (probe) 70 eV, *m/z* (rel. int.): 264 [M − 2Me]$^+$ (4), 236 [M − C_3H_6O]$^+$ (10), 227 [M − C_3H_6O − Me]$^+$ (1), 218 [M − C_3H_6O − H_2O]$^+$ (1), 206 (10), 187 (10), 159 (25), 152 (25), 43 (100).

Acetate **8**. Acetylation of **7** (70 mg) under the usual conditions (Ac_2O–pyridine, room temp.) yielded **8** (72 mg). Oily. $[\alpha]_D$ −104.6° ($CHCl_3$; *c* 3.6). IR ν_{max}^{film} cm^{-1}: 1740, 1660, 1370, 1240, 1210, 1050, 1020, 1000, 860, 840. EIMS (probe) 70 eV *m/z* (rel. int.): 278 [M − C_3H_6O]$^+$ (20), 276 [M − AcOH]$^+$ (20), 218 [M − AcOH − C_3H_6O]$^+$ (20), 159 [M − AcOH − C_3H_6O − H_2O − *iso*-Pr]$^+$ (40), 126 (50), 107 (60), 93 (100), 67 (50), 55 (50), 43 (80).

Ketone **9**. Compound **7** (70 mg) was oxidized overnight in 5 ml CH_2Cl_2 with PDC (70 mg), yielding a product which was chromatographed on silica gel (10 g; hexane–Et_2O, 8:2) to afford crystalline **9** (30 mg). Mp 127–129° (hexane). $[\alpha]_D$ −38.9° ($CHCl_3$; *c* 2.3). IR ν_{max}^{nujol} cm^{-1}: 2950, 1705, 1460, 1380, 1290, 1230, 1170, 1110, 1070, 1010, 980, 800, 700. EIMS (probe) 70 eV *m/z* (rel. int.): 234 [M − C_3H_6O]$^+$ (4), 216 [M − C_3H_6O − H_2O]$^+$ (2), 201 [M − C_3H_6O − Me]$^+$ (2), 150 (4), 135 (10), 122 (10), 106 (10), 93 (25), 83 (100), 67 (10), 55 (20).

8,12-*Isopropylidene derivative* **10**. Mp 103–105° (hexane). $[\alpha]_D$ −87.5° ($CHCl_3$; *c* 1.08). IR ν_{max}^{nujol} cm^{-1}: 3400, 1670, 1390, 1280, 1260, 1220, 1100, 1080, 1020, 960, 890, 850, 830. EIMS (probe) 70 eV *m/z* (rel. int.): 236 [M − C_3H_6O]$^+$ (5), 218 [M − C_3H_6O − H_2O]$^+$ (10), 159 (15), 122 (25), 107 (35), 93 (100), 84 (80), 69 (40), 55 (40).

Dehydration of **2** *to yield furane* **13**. Product **2** (312 mg) was reacted at room temp. with TsCl (200 mg) in pyridine (6 ml). After 5 hr and usual work up the oily residue was chromatographed on silica gel (20 g) with hexane–Et_2O (8:2) yielding **13** (46 mg). Oily, $[\alpha]_D$ −36.6° ($CHCl_3$; *c* 3.5). IR ν_{max}^{film} cm^{-1}: 1720, 1650, 1470, 1400, 1240, 1150, 1040, 980, 850. EIMS (probe) 70 eV *m/z* (rel. int.): 318 [M]$^+$ (1), 218 [M − AngOH]$^+$ (5), 203 [M − AngOH − Me]$^+$ (3), 110 (30), 93 (30), 91 (30), 85 (60), 83 (100), 55 (60), 43 (20).

12-*Angeloxy*-8-O-*angeloylshiromodiol* (**14**). Oily. $[\alpha]_D$ −38.0° ($CHCl_3$; *c* 0.9). IR ν_{max}^{film} cm^{-1}: 3500, 1720, 1660, 1460, 1400, 1360, 1250, 1170, 1100, 1050, 1000, 840. EIMS (probe) 70 eV *m/z* (rel. int.): 234 (1), 203 (7), 170 (60), 150 (40), 141 (70), 77 (100), 58 (80), 51 (30), 43 (70), 41 (50), 39 (30), 29 (10).

To a soln of **1** (35 mg) in $CHCl_3$ (1 ml) with a small amount of K_2CO_3, portions of *m*-CPB acid were added and the reaction followed by TLC. After usual work up, the crude product was purified by chromatography (silica gel, 10 g, hexane–Et_2O, 7:3) to give a monoepoxide (25 mg) identical in all respects to **14**.

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