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GUAIANE ESTERS FROM *THAPSIA VILLOSA*

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Key Word Index—*Thapsia villosa*; Umbelliferae; sesquiterpene esters; phenylethylcarbamate; absolute configuration.

Abstract—Three new terpenes have been isolated from *Thapsia villosa* var. *minor* [$2n=22$ ($=2x$)] and, mainly by spectroscopic methods, shown to be monoesters of the novel sesquiterpene alcohol (4*S*,5*S*,7*S*,8*S*)-1(10)-guaiene-8,11-diol. The acid moieties are *p*-coumaric acid, ferulic acid and senecioic acid, respectively. X-Ray analysis of the 8-*O*-(*S*)-1-phenylethylcarbamoyl derivative of the parent guaienediol established the relative and absolute configuration. The chemotaxonomic value of the heterogeneity of terpenes found in different specimens within the species *T. villosa* is discussed.

INTRODUCTION

Chemotaxonomic investigations of the genus *Thapsia* have revealed some interesting results concerning the distribution of sesquiterpenes as well as sesquiterpene lactones in the different species [1]. The unique polyoxygenated guaianolides especially, the thapsigargins, have attracted much attention due to their biological properties [2, 3]. Correlation of chromosome numbers with the observed pattern of secondary constituents of *T. villosa* revealed that thapsigargins could only be isolated from polyploid plants [$2n=44$ or 66 ($=4x$ or $6x$)], whereas hydroindene and other non-lactone sesquiterpenes were detected in plants with $2n=22$ ($=2x$) [4–7]. Further investigations have revealed that phytochemical differences occur within diploid plants, although all roots were collected from plants bearing ripe fruits and, thus, at the same stage of development [5].

We report the isolation and structural elucidation of three new sesquiterpenes (**1**–**3**), which are monoesters of [4*S*,5*S*,7*S*,8*S*]-1(10)-guaiene-8,11-diol (**4**), the acid moieties being *p*-coumaric acid, ferulic acid and senecioic acid. These compounds and guaiol occur only in some samples of *T. villosa* $2n=22$. The heterogeneous occurrence of guaienes within specimens of *T. villosa* $2n=22$ ($=2x$) indicates that a taxonomic revision is needed.

RESULTS AND DISCUSSION

Comparison of the NMR and mass spectra of compounds **1**–**3** indicated that they are all monoesters of the same alcohol. This conclusion was confirmed by the hydrolysis of the ester group, which in all three cases yielded the same crystalline product, **4**, analysing for $C_{15}H_{26}O_2$. The EI mass spectrum exhibited the expected $[M]^+$ at *m/z* 238. Furthermore, two prominent peaks were found at *m/z* 59 $[Me_2C{=}O]^+$ and *m/z* 147, corresponding to loss of the side chain, water and one methyl group. The NMR data of **4** showed that the alcohol is bicyclic with one fully substituted double bond. The four methyl signals in the ^1H NMR spectrum were assigned to one methyl group at a saturated carbon (δ0.89 *d*), one methyl group at a vinylic carbon (δ1.67 *s*) and the two methyl groups of a hydroxyisopropyl side chain (δ1.19 *s* and 1.25 *s*). A signal at δ3.61 (*m*) was assigned to the proton of a secondary alcohol. The structure of **4** was further confirmed by a COSY experiment (Table 1) and by decoupling experiments, which in addition enabled

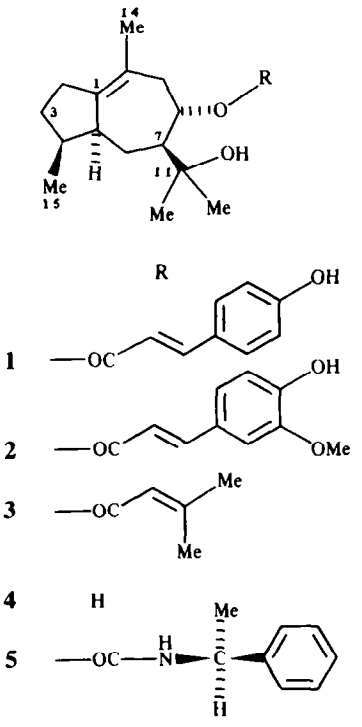

Table 1. ^{1}H NMR spectral data of compounds **1**, **4** and **5** (270 MHz and 500 MHz, $CDCl_3$, TMS as int. standard, alcoholic and phenolic protons exchanged with D_2O)

H	1	4		5*
2	2.24 *m*, 2.39[a]	2.12[c], 2.30 *m*		
3	1.41 *m*, 1.70[b]	1.30[d], 1.63[b]		
4	2.14 *m*	2.10 *m*[a]		2.1 *br*
5	2.52[c]	2.40 *br t*		2.4 *br*
6α	1.82 *br dd*	1.65[b]		1.7 *br dd*
6β	1.15[d]	0.68 *ddd*		0.9 *m*
7	2.02 *ddd*	1.65 *m*[b]		1.9 *br*
8	5.10 *m*	3.61 *m*		4.8 *br*
9α	2.55 *br dd*	2.59 *br t*		2.3–2.4 *m*
9β	2.36 *br d*	2.07 *dd*		
12 } 13 }	1.18 *s*; 1.29 *s*	1.19 *s*, 1.25 *s*		1.1 *s*, 1.2 *s*
14	1.69 *s*	1.67 *s*		1.6 *s*
15	0.86 *d*	0.89 *d*		0.8 *br d*
	O-p-Coumaroyl			1-phenylethyl carbamate
2′	6.21 *d* (16)		H-1′	*ca* 5 *br**
3′	7.58 *d* (16)		H-2′	1.4 *d*
	6.82 (H-6′, H-8′)		phenyl	7.1–7.3 *m*
	7.33 (H-5′, H-9′)			

[a–c] Signal partly overlaps H-9β, H-14, H-9α, H-12 and H-13, and H-4 signals, respectively.

* Vicinal proton coupling constants cannot be read from spectrum because of line broadening arising from chemical exchange in the amide part of the molecule [13].

J(Hz) compound **1**: 4,15 = 7; 5,6β = 6α, 6β = 12; 6β,7 = 12.6; 6α,7 = 4.2; 7,8 = 5.7; 8,9α = 8.4; 9α,9β = 14.7. Compound **4**: 4,15 = 7; 5,6β = 6α, 6β = 6β,7 = 12; 7,8 = 8.8; 8,9α = 9.5; 8,9β = 1.7; 9α,9β = 14.

determination of most of the coupling constants. The chemical shift of the methyl group at C-4 has the same value as that of the corresponding methyl group in bulnesol [8] indicating that the methyl group at C-4 and the proton at C-5 are *trans*-disposed. The β-proton at C-6 appears at an extraordinary high field (δ0.68).

Because further conclusions concerning the relative configuration were difficult to obtain from the spectroscopical data, we decided to perform an X-ray study. In order to establish the relative, as well as the absolute configuration, of the alcohol the 8-O-[(*S*)-1-phenylethylcarbamoyl] derivative **5** was prepared by reacting **4** with (*S*)-(−)-1-phenylethylisocyanate. X-Ray analysis disclosed that **5** in the crystalline state adopts two slightly different conformations, both with the hydroxy group at C-11 hydrogen bonded to the carbamate carbonyl oxygen [9]. One of the conformations is depicted in Fig. 1.

The assignment of the signals in the ^{13}C NMR spectrum of **4** is mainly based on a ^{1}H–^{13}C NMR correlation plot (Table 2). The ^{1}H NMR spectra of the natural products **1**–**3** disclosed that they were all monoesters of **4** and the chemical shift of H-8 (Table 1) showed that the alcohol group at C-8 is acylated. This acylation also caused an unexpected shift of the β proton at C-6 from δ0.68 in **4** to δ1.15 in the monoesters (Table 1). The formation of a hydrogen bond between the carbonyl group and the hydroxy group at C-11 could induce a change in conformation of the cycloheptene ring, which could explain this shift. The identity of the acyl groups in the esters **1**–**3** were based on NMR and the EI mass spectra. Ferulic and isoferulic acids were distinguished by the melting point of the acid isolated after hydrolysis of **2**.

Beside the thapsigargins, some guaianolides having an asymmetric C-5 with a β-disposed proton have been isolated from *T. villosa* [2*n* = 66 (= 6x)] [10]. The observation that H-5 in **4** is α-disposed indicates that the

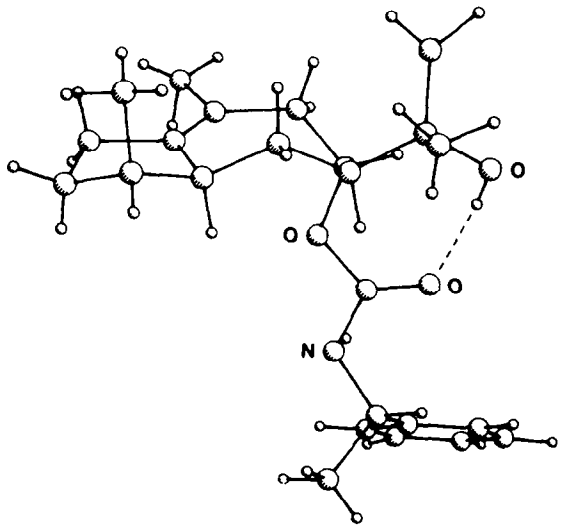

Fig. 1. Perspective drawing of compound **5**. One of the conformations in the X-ray structure is shown. Dashed line indicates intramolecular hydrogen bond.

Table 2. ^{13}C NMR spectral data of compounds **1–5** (67.9 MHz and *125 MHz, $CDCl_3$, TMS as internal standard)

C	1	2	3	4	5*
1	141.56	140.76	140.67	144.04	148.3
2	29.24[a]	29.43[a]	29.35[a]	29.74	29.1[a]
3	32.25	32.15	32.04	32.86	31.9
4	38.79[b]	38.66[b]	38.55[b]	38.50	37.9
5	45.76	45.94	45.97	45.00	46.1
6	28.69	28.80	28.82	28.01	29.4[a]
7	55.82	55.76	55.74	57.86	55.8
8	75.41	75.26	74.41	71.93	72.9
9	39.27[b]	38.89[b]	38.88[b]	45.01	39.0
10	121.99	123.24	121.92	123.23	121.7
11	74.53	73.27	73.16	75.63	75.6
12	24.55	25.04	24.79	23.75	24.7
13	29.46[a]	29.43[a]	29.50[a]	30.33	29.6
14	21.91	22.08	22.08	21.80	22.4
15	14.60	14.48	14.43	15.11	14.3
1′	166.70	167.33	166.26	—	50.4
2′	114.72	115.67	116.43	—	29.6[a]
3′	145.51	146.77	157.20	—	phenyl:
4′	126.09	126.84	20.13	-	126.0
5′	130.01	109.26[c]	27.40	—	127.3
6′	115.89	148.12	—	-	128.9
7′	158.87	145.23	—	—	129.0
8′	115.89	114.65[c]	—	—	—
9′	130.01	121.84	—	-	—

[a–c] These assignments may be interchanged in the same column.

cyclization of the lactones proceeds by a mechanism different from that of the cyclization of the guaienes, even though it proceeds in the same plant species. Some TLC investigations have revealed that the guaienes **1–3** and guaiol can only be detected in some specimens of *T. villosa* [$2n = 22$ ($= 2x$)]. This heterogeneity together with other observations will form the basis for a taxonomic revision of the genus. The results of these studies will be reported elsewhere.

EXPERIMENTAL

Extraction and purification. Extraction of plant material and the first fractionation has been described previously [4]. Further purification was carried out on silica gel with toluene–EtOAc (7:3), to which MeOH (1–4%) was added. Prep. HPLC on LiChrosorb RP-18, 5 μm, 8 × 250 mm, with MeOH–H_2O (17:3) was used alternatively. TLC examination: silica gel, petrol–Me_2CO (4:1) and visualization with UV-light or 5% phosphomolybdic acid in EtOH.

[4S,5S,7S,8S]-8-p-*Coumaroyloxy*-1(10)-*guaien*-11-*ol* (**1**). Crystalline compound, mp 178–179° (petrol–Me_2CO). $[\alpha]_D^{20}$ +65.0°, $[\alpha]_{436}^{20}$ +169.5° ($CHCl_3$; *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1715 (C=O), 1630 (C=C), 1610, 1590, 1520 (Ar), 980 (C=C, *trans*). CIMS (*iso*-butane, probe) *m/z* (rel. int.): 385 $[MH]^+$ (10), 367 $[MH-H_2O]^+$ (38), 221 $[MH-p$-coumaric acid$]^+$ (100), 203 $[221-H_2O]^+$ (42), 165 [*p*-coumaric acid H]$^+$ (42). EIMS (probe) *m/z* (rel. int.): 366 $[M-H_2O]^+$ (0.5), 220 $[M-p$-coumaric acid$]^+$ (25), 202 $[220-H_2O]^+$ (15), 164 [*p*-coumaric acid]$^+$ (8), 162 $[M-p$-coumaric acid $-Me_2C{=}O]^+$ (100), 147 $[162-Me]^+$ and $[R-Ar{-}CH{=}CH{-}C{\equiv}O]^+$ (90), 59 $[Me_2C{=}OH]^+$ (60).

[4S,5S,7S,8S]-8-*Feruloyloxy*-1(10)-*guaien*-11-*ol* (**2**). Crystalline compound, mp 102–103° (CCl_4–Me_2CO). $[\alpha]_D^{20}$ +58.0°, $[\alpha]_{436}^{20}$ +158.8° ($CHCl_3$; *c* 0.6). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1680 (C=O), 1640 (C=C), 1610, 1600, 1520 (Ar), 980 (C=C, *trans*). CIMS (*iso*-butane, probe) *m/z* (rel. int.): 415 $[MH]^+$ (10), 397 $[MH-H_2O]^+$ (28), 221 $[MH-$ferulic acid$]^+$ (100), 203 $[221-H_2O]^+$ (40), 195 [ferulic acid H]$^+$ (49). EIMS (probe) *m/z* (rel. int.): 396 $[M-H_2O]^+$ (0.2), 220 $[M-$ferulic acid$]^+$ (10), 195 [ferulic acid H]$^+$ (10), 194 [ferulic acid]$^+$ (18), 187 $[202-H_2O]^+$ (10), 177 $[R-Ar-CH{=}CH-C{\equiv}O]^+$ (50), 162 $[M-$ferulic acid $-Me_2C{=}O]^+$ (100), 59 $[Me_2C{=}OH]^+$ (45). ^1H NMR (270 MHz, $CDCl_3$); signals from the alcoholic part of **2** were identical to those of **1**; *O*-feroyl: δ 6.25 (1H, *d*, $J_{2',3'} = 16$ Hz, H-2′), 7.58 (1H, *d*, $J_{2',3'} = 16$ Hz, H-3′), 7.05 (1H, H-5′), 6.90 (1H, H-8′), 7.00 (1H, H-9′), 3.90 (3H, –OMe).

[4S,5S,7S,8S]-8-*Senecioyloxy*-1(10)-*guaien*-11-*ol* (**3**). Amorphous compound, $[\alpha]_D^{20}$ +34.0°, $[\alpha]_{436}^{20}$ +80.3° ($CHCl_3$; *c* 0.5). CIMS (*iso*-butane, probe) *m/z* (rel. int.): 321 $[MH]^+$ (1), 303 $[MH-H_2O]^+$ (10), 221 $[MH-$senecioic acid$]^+$ (26), 203 $[221-H_2O]^+$ (33), 201 (100), 101 [senecioic acid H]$^+$ (42). EIMS (probe) *m/z* (rel. int.): 220 $[M-$senecioic acid$]^+$ (5), 162 $[M-$senecioic acid $-Me_2C{=}O]^+$ (30), 83 $[Me_2C{=}CH{-}C{\equiv}O]^+$ (100), 59 $[Me_2C{=}OH]^+$ (33). ^1H NMR (270 MHz, $CDCl_3$); signals from the alcoholic part of **3** were almost identical to those of **1** except that the 9α and 9β proton signals collapsed; *O*-senecioyl: δ 5.62 (1H, *m*, H-3′), 1.90 and 2.15 (3H each, *br s*, H-4′ and H-5′). Compound **3** is rather unstable. After storage in the refrigerator for some days the pure and colourless compound turns yellow and smells of senecioic acid.

[4S,5S,7S,8S]-1(10)-*Guaiene*-8,11-*diol* (**4**). Compound **1** (85 mg) in 1 M NaOMe (5 ml) was kept at 60° for 3 hr. After addition of H_2O (5 ml) the mixt. was heated to 60° again for

30 min. The reaction mixt. was neutralized with solid CO_2 and extracted with $CHCl_3$. The extract was purified on silica gel (20 g), using hexane to which increasing amounts of Me_2CO were added (3–12%). Crystalline **4** (50 mg) was obtained, mp 118–118.5° (hexane–Me_2CO). (Found: C, 75.47%; H, 11, 12%. $C_{15}H_{26}O_2$ requires: C, 75.58%; H, 11.0%). $[\alpha]_D^{20}$ −35.0°, $[\alpha]_{436}^{20}$ −71.5° ($CHCl_3$; *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1620 (C=C), 1370, 1380 (–CMe_2). EIMS (probe) *m/z* (rel. int.): 238 $[M]^+$ (1), 220 $[M-H_2O]^+$ (55), 162 $[M-H_2O-Me_2C{=}O]^+$ (42), 147 $[162-Me]^+$ (97), 59 $[Me_2C{=}OH]^+$ (100).

p-Coumaric acid. After hydrolysis of **1** and extraction with $CHCl_3$ the H_2O phase was acidified with HOAc and extracted twice with Et_2O. Evapn left *p*-coumaric acid (30 mg), mp 213–214° (H_2O) (lit. [1] 206°, uncorr.). The IR spectrum was identical with that of an authentic sample.

Ferulic acid. After hydrolysis of **2** (22 mg) in the same way as **1**, the alcoholic part (10 mg) was found to be identical with **4** (mp, $[\alpha]_D^{20}$, $[\alpha]_{436}^{20}$ and IR) and the acid moiety (8 mg) was identified as ferulic acid, mp 170.5–171.0° (H_2O) (lit. [12] 174°). The IR spectrum was identical with that of an authentic sample.

Hydrolysis of **3** was carried out following the same procedure as above, but due to the unstability of the compound only 1 mg of **3** was hydrolysed. The identity of the alcoholic moiety with **4** was shown by co-chromatography (TLC).

[4S,5S,7S,8S]-11-*Hydroxy*-1(10)-*guaiene*-8-*yl*-[1S]-(1-*phenyl*)-*ethylcarbamate.* Compound **4** (10 mg) in dry C_6H_6 (0.2 ml) was mixed with 4-(*N,N*-dimethyl)aminopyridine (0.2 mg) and [*S*]-(−)-1-phenylethylisocyanate (reagent from Merck, $[\alpha]_D^{20}$ −11.5 to −12.5°) [14] (50 mg). The mixt. was left at room temp. for 70 hr, after which excess reagent was removed by addition of MeOH (0.2 ml), heating briefly to 60° and leaving the mixt. at room temp. for 1 hr. TLC examination showed that **4** had disappeared. After evapn of solvent the product was purified on silica gel (10 g), using CH_2Cl_2 to which MeOH (0.5–2%) was added. Crystalline **5** (13 mg) was isolated, mp 128–129° (Et_2O–petrol). $[\alpha]_D^{20}$ +11.2°, $[\alpha]_{436}^{20}$ +21.3° ($CHCl_3$; *c* 0.6).

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