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Constituents of *Chrysothamnus viscidiflorus*

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Abstract

A phytochemical investigation of the aerial parts of *Chrysothamnus viscidiflorus* var. *viscidiflorus* afforded three new [chrysothol (**1**), **2** and **4**] and seven known compounds, including five sesquiterpenes, two cinnamic acid derivatives, two ketoalcohol derivatives and one coumarin glucoside. The structures of two previously reported compounds, **1b** and **1c**, were revised on the basis of chemical reaction. Structures of the compounds were determined by extensive NMR studies, including DEPT, COSY, NOE, HMQC, HMBC and X-ray analysis. The unpublished X-ray data of the known compounds **6** and **7** are reported. Compounds chrysothol (**1**), and **8**–**10** showed anti-cancer activity against human breast cancer cells.

Keywords: *Chrysothamnus viscidiflorus* var. *viscidiflorus*; Asteraceae; Guaiane sesquiterepene; *p*-Coumaric acid derivatives; Sesquiterpenoid glucoside; Coumarin glucoside; Anti-cancer activity

1. Introduction

Green rabbitbrush (*Chrysothamnus viscidiflorus*, Asteraceae) is a common and widespread ecologically important shrub in the dry interior habitats of western North America. Previous chemical research on the genus has shown a long history of interest in using these plants, and in particular *C. nauseosus* (gray rabbitbrush) as an alternative source of rubber (Hall and Goodspeed, 1918; Hegerhorst et al., 1988). *C. nauseosus* has also been shown to exhibit antifeedant effects on the Colorado potato beetle (Rose et al., 1980). Previous investigations of *C. viscidiflorus* have focused on flavonoids (Urbatsch et al., 1975; Stevens et al., 1999), diterpene acids (Le-Van and Pham, 1980) a hydroxyacetophenone and chromanone derivatives (Le-Van and Pharm, 1981). In the present study, we wish to report on the isolation of three new compounds [chrysothol (**1**), **2**, **4**] and seven known compounds (**3**, **5**–**10**), reported for the first time in this species. Compounds chrysothol (**1**) and **8**–**10** were shown to have anti-cancer activity against human breast cancer cells.

2. Results and discussion

Compound **1** was isolated as a colorless oil, $[\alpha]_D^{25} + 13.0°$ ($CHCl_3$, $c = 0.2$) and the absorption band at 3500 cm^{-1} in the IR spectrum indicated the presence of a hydroxyl group. The molecular formula of **1**, $C_{15}H_{26}O_2$, was established by HRCI-MS (*m/z* 239.201105, $[M+H]^+$) and ^{13}C NMR data. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) in $CDCl_3$ showed signals of a guaiane sesquiterpene skeleton. The ^1H NMR spectrum of **1** displayed a broad doublet at δ 4.01 (1H, $J = 4.0$ Hz, H-6), a multiplet at δ 2.32, for two protons (H-1 and H-5), a doublet triplet at δ 2.17

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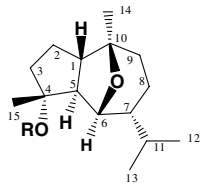

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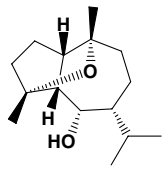
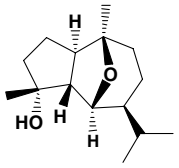
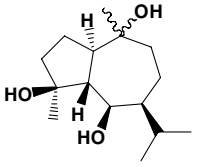
1a R = OAc



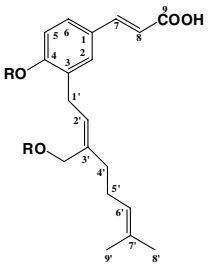
1b



1c



1d αOH, βMe
1e βOH, αMe



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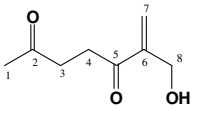
3 R =H



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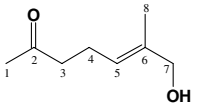

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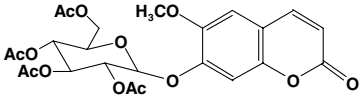

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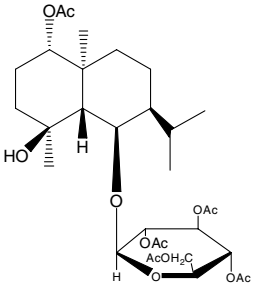

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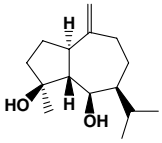

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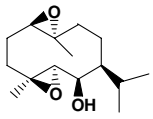

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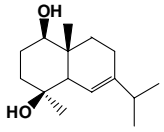

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Table 1
^{1}H NMR of **1** and **1a** (500 MHz, δ-values)

Position	**1**[a]	**1**[b]	**1a**[a,c]
1	2.32 *m*	2.25	2.35 *ddd* ($J = 14.0, 11, 5$)
2a	1.56 *m*	1.38	1.52
2b	1.54 *m*	1.38	1.54 *dt* ($J = 11.0, 9.5, 9.5$)
3a	2.17 *dt* ($J = 14.3, 9.5, 9.5$)	2.01	2.10
3b	2.80 *dd* ($J = 14.3$)	1.90	2.44
5	2.32 *m*	2.13	2.29 *dd* ($J = 14.0, 4.5$)
6	4.01 *brd* ($J = 4.0$)	3.77	4.08
7	1.39 *m*	1.61	1.53
8a	1.80 *m*	1.74	1.81
8b	1.78 *m*	1.35	1.59
9a	1.76 *m*	1.56	1.71
9b	1.74 *m*	1.35	1.39
11	1.72 *m*	1.61	1.79
12	0.97 *d* ($J = 7.0$)	0.87	0.96
13	0.97 *d* ($J = 7.0$)	0.87	0.97
14	1.19 *s*	1.05	1.17
15	1.42 *s*	1.10	1.55

[a] In $CDCl_3$.
[b] In DMSO-d_6.
[c] AcO, δ 1.98 (*s*).

Table 2
^{13}C NMR of **1**–**1$_e$** (125 MHz, δ-values)

Position	**1**[a]	**1**[b]	**1a**[a]	**1b**[c]	**1c**[a]	**1d**[a]	**1e**[a]
C-1	53.3 *s*	52.2 *s*	52.3 *s*	53.3 *s*	45.8 *s*	51.6 *s*	52.1 *s*
C-2	23.9 *t*	23.5 *t*	23.4 *t*	23.8 *t*	23.1 *t*	23.6 *t*	23.2 *t*
C-3	48.2 *d*	47.6 *d*	46.4 *d*	37.5 *d*	40.6 *d*	41.0 *d*	41.2 *d*
C-4	74.5 *s*	73.5 *s*	82.2 *s*	74.4 *s*	79.9 *s*	81.2 *s*	81.1 *s*
C-5	68.2 *d*	67.7 *d*	66.5 *d*	68.2 *d*	55.8 *d*	53.9 *d*	55.4 *d*
C-6	75.9 *d*	75.1 *d*	77.9 *d*	75.9 *d*	69.8 *d*	71.3 *d*	71.4 *d*
C-7	38.5 *d*	37.6 *d*	37.8 *d*	38.5 *d*	51.3 *d*	45.7 *d*	45.6 *d*
C-8	20.3 *d*	19.8 *d*	19.9 *d*	20.2 *d*	20.6 *d*	19.9 *d*	20.5 *d*
C-9	37.5 *d*	37.1 *d*	37.2 *d*	48.2 *d*	47.7 *d*	47.1 *d*	48.1 *d*
C-10	74.4 *s*	72.3 *s*	73.9 *s*	74.4 *s*	73.4 *s*	73.0 *s*	75.5 *s*
C-11	32.6 *s*	32.4 *d*	32.3 *d*	32.7 *s*	29.7 *s*	29.7 *s*	29.6 *s*
C-12	21.1 *q*	20.8 *q*	20.1 *q*	21.1 *q*	21.5 *q*	21.2 *q*	21.1 *q*
C-13	21.1 *q*	20.1 *q*	21.1 *q*	21.1 *q*	21.1 *q*	21.3 *q*	21.5 *q*
C-14	21.9 *q*	21.9 *q*	21.9 *q*	21.9 *q*	22.9 *q*	29.9 *q*	22.2 *q*
C-15	25.8 *q*	25.7 *q*	21.6 *q*	25.8 *q*	21.8 *q*	23.9 *q*	23.1 *q*

[a] In $CDCl_3$.
[b] In DMSO-d_6.
[c] AcO, δ 22.0 (*q*), 170.6 (*s*).

(1H, $J = 14.3$, 9.5, 9.5 Hz, H-3a). Additionally, a signal for two secondary methyl groups due to an isopropyl unit was found at δ 0.97 (6H, $J = 7.0$ Hz, H-12, 13) and two tertiary methyl signals appeared at δ 1.19, 1.42 (H-14 and H-15, respectively). The other protons were determined by ^{1}H–^{1}H COSY (Table 1). The ^{13}C NMR spectral data of **1** (Table 2) revealed 15 carbon signals that were resolved by DEPT experiments into four methyls, four methylenes, five methines and two quaternary carbons. Three oxygenated carbons were observed at δ 75.9 (*d*), 74.5 (*s*) and 74.4 (*s*) and the other 12 signals were for aliphatic carbons. The other protons and carbons were determined by HMQC and HMBC.

These data were identical with those published for buchariol, whose structure was reported to be **1b**, a sesquiterpene isolated from *Salvia bucharica* (Ahmad et al., 1999). Acetylation of a portion of the material for **1** (pyridine, DMAP) afforded the monoacetylated derivative **1a**. HRCI-MS of the acetylated product **1a** gave a molecular ion peak at *m/z* 281.21089 ($C_{17}H_{28}O_3$), and the ^{1}H NMR showed a new acetyl signal at δ 1.98; moreover, two carbon signals in the ^{13}C spectrum were observed at δ 22.0 (CH_3CO) and 170.6 (CH_3CO) (Table 2). However, the oxygenated proton that appeared at δ 4.01 in **1** exhibited only a minor downfield shift and appeared at δ 4.08 in **1a** (Table 1). The acetylation latter result confirmed the absence of a free hydroxyl group at C-6, and the oxygen function must be part of an ether linkage. Additionally, a clear correlation was observed in HMBC of the acetylated product **1a** between H-6 and C-10, supporting the ether linkage between C-6 and C-10, (Fig. 1). The assignments of all protons signals for **1a** and their connectivity to adjacent protons and carbons signals were established from the results of the 2D ^{1}H–^{1}H COSY, HMBC (Fig. 1) and NOE (Fig. 2). Therefore, the **1b** structure for buchariol can be revised to **1**. Recently, another compound **1c** very similar to **1** was reported from *Fagonia boveana* (Gedara et al., 2003). The NMR data for **1c** were reported in DMSO-d_6. For comparison, we recorded the ^{1}H and ^{13}C NMR of **1** in the same solvent (Tables 1 and 2). Our data were different from those of **1c**, for example the three oxygenated carbons appeared at δ 75.1, 73.5 and 72.3 in **1**, compared to 79.9, 73.4 and 69.8 in **1c** (Table 2). Careful comparison of the ^{13}C data of **1c** with those of **1** (in DMSO-d_6), **1d** and **1e** (Table 3), and the absence of H-6/C-10 or H-14/C-6 correlation in HMBC of **1c**, suggested that **1c** could be an epimer of either **1d** or **1e** (Bruno et al., 1993). The stereochemistry of **1** was established from NOE experiments of the monacetylated product, **1a** (Fig. 2). Irradiation of the signals at δ 2.35 (H-1) enhanced the signals at δ 1.55 (H-15), irradiation of the signals at δ 2.29 (H-5) enhanced the signals at δ 4.08 (H-6), the acetyl signal at δ 1.98, irradiation of the signals at δ 4.08 (H-6) enhanced the signals at δ 0.96 (H-12), 0.97 (H-13) and 1.79 (H-11). Therefore, **1** was identified to be 6β,10β-epoxy-4-α-hydroxy-guaiane, named chrysothol.

Compound **2** was assigned as a molecular formula of $C_{23}H_{29}O_6$ by HRCIMS which gave an ion peak $[M+H]^+$ at *m/z* 401.19501. The CIMS exhibited an ion peak $[M+H]^+$ at *m/z* 401, followed by a fragments at 383 $[(M+H)-H_2O]^+$, 359 $[M-CH_3CO]^+$ and 341 $[(M+H)-CH_3COOH]^+$. The ^{1}H NMR spectrum of **2** was close to **3** (Jakupovic et al., 1990), except the presence of two additional acetyl signals at δ 2.32 and 2.10. The two acetyls were placed at C-4 and C-10′ on the basis of the location of the free hydroxyl groups in **3**. Therefore, **2** was identified as 3-[10-acetoxygeranyl]-4-acetoxy-*p*-coumaric acid, a new natural product.

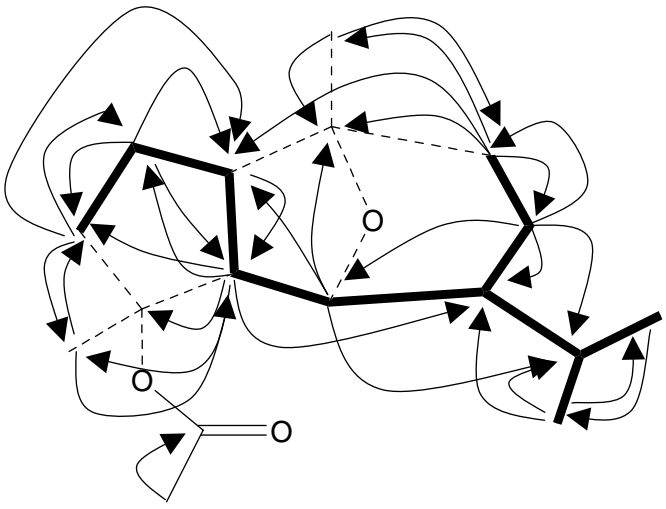


Fig. 1. ^{1}H–^{1}H correlations (bold line) and long-range ^{1}H–^{13}C correlations (arrows) of **1a**.

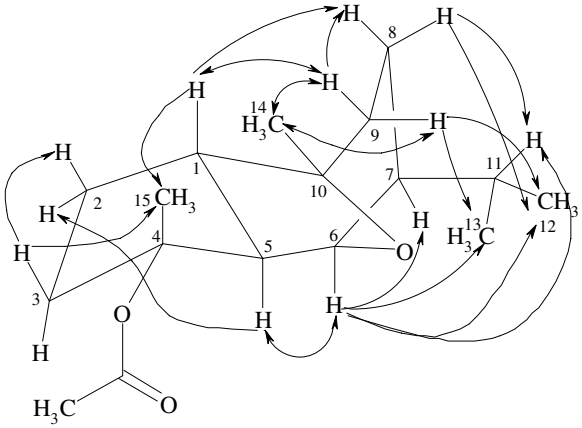


Fig. 2. NOE correlations for **1a**.

Table 3
^{13}C NMR comparison of **1**–**1e**

Carbon	1	1c	1d	1e
C-3	47.6	40.6	41.0	41.2
C-4	73.5	79.9	81.2	81.1
C-5	67.7	55.8	53.9	55.4
C-6	75.9	69.8	71.3	71.4

The structure of compound **4** could be easily deduced by comparison of its ^{1}H NMR data with those of **5**. The ^{1}H NMR spectrum of **4** exhibited two exomethylene protons at δ 6.19 (1H, *s*) and 6.03 (1H, *s*). Also, the spectrum showed a singlet at δ 4.32 (2H, *s*), two triplets at δ 3.00 (2H, *t*, $J = 6.5$ Hz) and 2.78 (2H, *t*, $J = 6.5$ Hz) and a methyl keto at δ 2.24 (*s*). The IR spectrum exhibited two absorption bands at 1730 and 1620 cm^{-1}. Therefore, **4** was identified as 6-hydroxymethyl-hepta-6-ene-2,5-dione, a new natural product. The structure of compound **5** was established by NMR analysis. The ^{1}H and ^{13}C NMR exhibited signals for an olefinic proton at δ 5.36 (*t*, $J = 7$ Hz, δ_C 124.0), hydroxyl methyl at δ3.99 (*brs*, δ_C 68.6), two methylenes at δ 2.50 (*t*, $J = 7.5$ Hz) and 2.31 (*m*, δ_C 21.9), methyl keto at δ 2.14 (*s*, δ_C 29.8), and olefinic methyl at δ 1.71 (*s*, δ_C 13.6). Compound **5** was reported as a microbial degradation product and the previously unreported ^{13}C NMR are given in Section 3 (Bock et al., 1988; Look et al., 1984; Hodgson et al., 2000).

Compounds **1**–**10** were tested for their activities as anti-cancer agents on two types of human breast cancer cells; estrogen non-responsive MDA-MB-435 human breast cancer cells and estrogen-responsive MCF-7 human breast cancer cells. Compounds **1**, **8**, **9** and **10** showed anti-cancer activities (see Section 3). Several cytotoxic sesquiterpenes were reported in the literature (Yi-Feng et al., 2005; Fatima et al., 2005).

3. Experimental

3.1. General

^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$) and the 2D spectra were recorded on Varian 500 MHz, with TMS as an internal standard. Optical rotations were determined with a JASCO-20 C automatic recording spectropolarimeter. TLC: precoated silica gel type 60 (Merck); CC: silica gel type 60 (Merck). HPLC was performed in the reverse phase on knauer pump 64 and different refractometer (column: RP-8, 250 ×;25 mm, flow = 17 mL/min, elution with MeOH–H_2O mixtures, refractive index).

3.2. Plant material

C. viscidiflorus var. *viscidiflorus* was collected during the flowering stage in September 1999, 3 miles west of Mitchell, OR, USA. A voucher specimen was deposited at the Oregon State University Herbarium (Voucher # 194231).

3.3. Biological activity

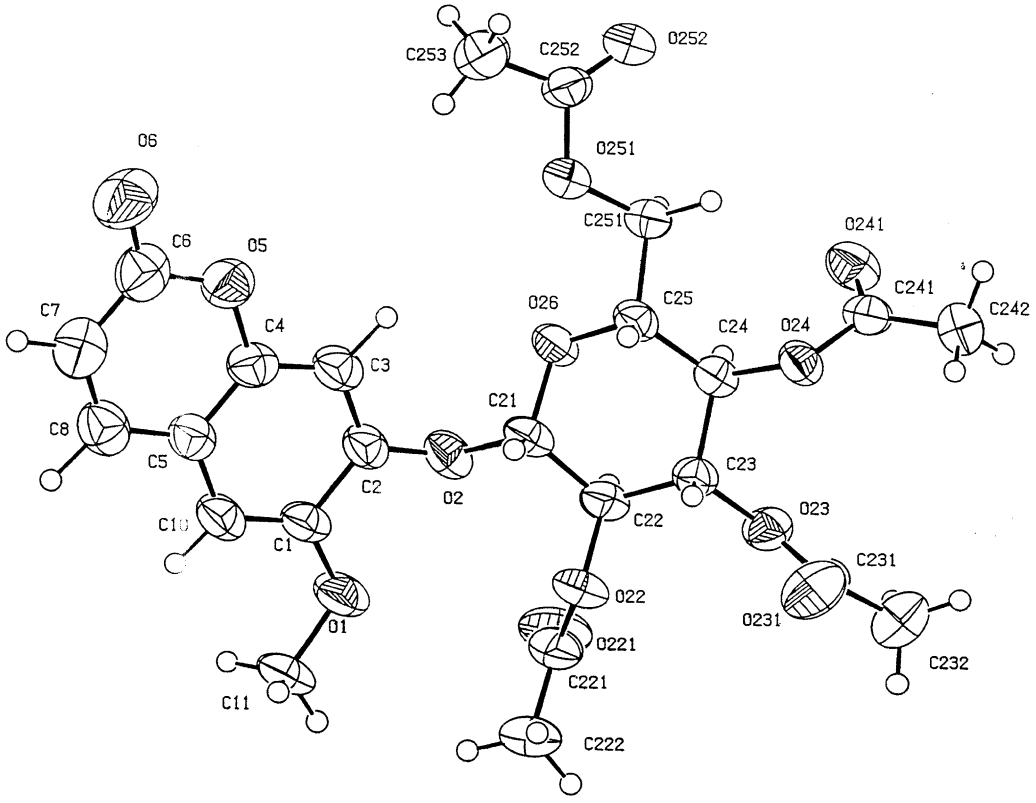
Activities of the isolated compounds were tested as anti-cancer agents on two types of human breast cancer cells; estrogen non-responsive MDA-MB-435 human breast cancer cells and estrogen-responsive MCF-7 human breast cancer cells. The two cell lines were cultured for three days with four levels. The activity of each compound against two types of human breast cancer was estimated by using apoptic protocol, which involved staining the cell nuclei with DAPI stain and determining Apoptosis by morphological criteria including condensation and DNA fragmentation (Yu et al., 1999). The most active compound was **8** against the MCF-7 cells and MDA-MB-435 cells, respectively (EC_{50}, 50 μg/mL). While compound **1** was more active against MCF-7 breast cancer cells than MDA-MB-435 cells. The less active compound was **9**, which was considered as an inactive compound against the two types of breast cancer cells. Compound **10** was toxic for MCF-7 breast cancer cells and nearly without activity against MDA-MB-435 breast cancer cells.

3.4. Extraction and isolation

Air-dried plant material (1.5 kg) was ground and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The extract was concentrated in vacuo to obtain a residue of 30 g. The residue was prefractionated by column chromatography (6 × 120 cm) on silica gel eluting with *n*-hexane (3 L) followed by a gradient of *n*-hexane–CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2–MeOH up to 15% MeOH (2 L each of the solvent mixture). The *n*-hexane–CH_2Cl_2 (3:1) fraction was subjected to a silica gel column (2 × 60 cm) eluted with *n*-hexane–CH_2Cl_2–MeOH to give two fractions. The *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5) fraction was further purified by TLC to afford compound **1** (20 mg) and *n*-hexane–CH_2Cl_2–MeOH (7:4:0.1) fraction was purified by HPLC (MeOH–H_2O, 80:20) to give compound **2** (1 mg). The *n*-hexane–CH_2Cl_2 (1:1) fraction was subjected to a silica gel column (2 × 60 cm) eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5) to afforded a residue 20 mg that was further purified by Sephadex LH-20 eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.25) yielded compound **5** (12 mg) and compound **4** (1 mg). The combined fractions *n*-hexane–CH_2Cl_2 (1:3) and 100% CH_2Cl_2 were subject to silica gel column (3 × 60 cm) eluted with *n*-hexane–CH_2Cl_2–MeOH to give two fractions. The *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5) fraction was further purified by TLC to afford **3** (6 mg), **8** (20 mg) and *n*-hexane–CH_2Cl_2–MeOH (7:4:1) fraction was further purified by TLC to give compounds **9** (15 mg) and **10** (20 mg). The CH_2Cl_2–MeOH (3:1) fraction was subject to silica gel column (2 × 60 cm) eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:2) to give 100 mg of crude fraction. Acetylation of this fraction with acetic acid anhydride in pyridine and presence of DMAP followed by purification on Sephadex LH-20 column eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5) gave compounds **6** (16 mg) (Kitajima et al., 2000) and **7** (11 mg) (Sati et al., 1989), in a crystal form (see Fig. 3).

3.4.1. Chrysothol (*1*)

Colorless oil; $[\alpha]_D^{25} + 13.0°$ ($CHCl_3$, $c = 0.2$); IR (ν_{max}^{KBr} cm^{-1}): 3500 (OH); CIMS *m/z* (rel. int): 239 $[M+H]^+$ (35), 221 $[M-H_2O]^+$ (100), 203 $[M-2H_2O]^+$ (18).



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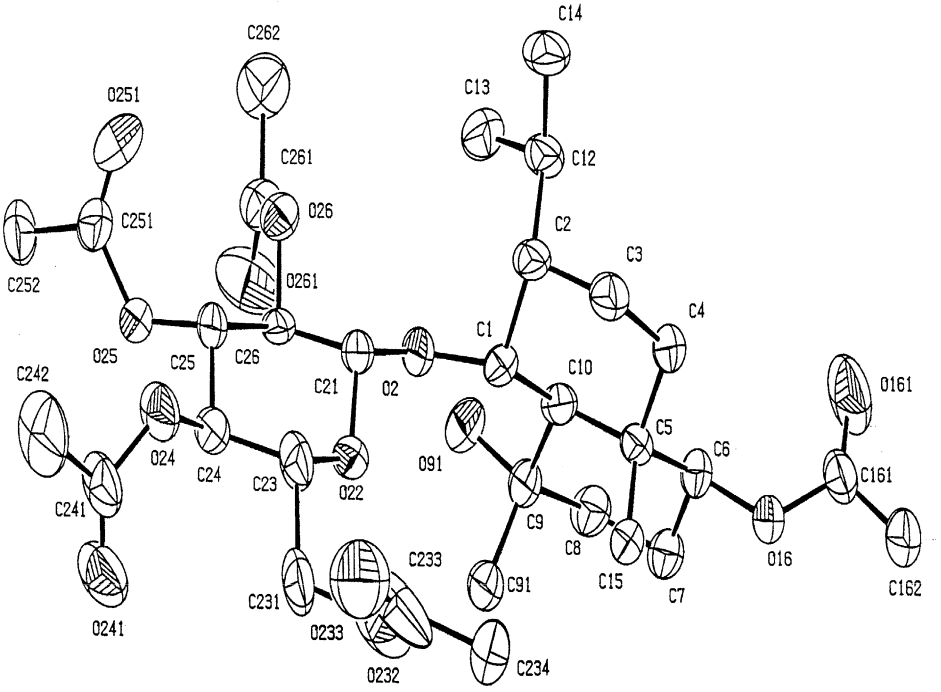

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Fig. 3. ORTEP diagram showing the atom numbering scheme and solid-state conformation of **6** and **7**.

*3.4.2. Chrysothol monoacetyl derivative (**1a**)*

CIMS *m/z* (rel. int): 281 $[M+H]^+$ (18), 221 $[M+H-CH_3COOH]^+$ (100), 203 $[221-H_2O]^+$ (78); HRCIMS 281.21089 (calc. for $C_{17}H_{28}O_3$, 281.21167).

*3.4.3. 3-[10-Acetoxygeranyl]-4-acetoxy-p-coumaric acid (**2**)*

Oil; IR (ν_{max}^{KBr} cm^{-1}): 2800, 1720, 1730, 1750; CIMS *m/z* (rel. int): 401 [M+H] (36), 383 $[(M+H)-H_2O]$ (26), 359

[M−CH_3CO] (44), 341 [(M+H)−CH_3COOH] (83), HRCIMS [M+H]$^+$ *m/z* 401.19501 (calc. for $C_{23}H_{29}O_6$, 401.19646); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.17 (1H, *d*, $J = 7.5$, H-2), 6.25 (1H, *d*, $J = 8.00$, H-5), 7.24 (1H, *brd*, $J = 16.5$, H-6), 7.71 (1H, *d*, $J = 16.0$, H-7), 6.37 (1H, *d*, $J = 15.5$, H-8), 3.34 (2H, *brd*, $J = 7.00$, H-1′), 5.36 (1H, *brd*, $J = 7$, H-2′), 2.09 (2H, *m*, H-4′), 2.15 (2H, *m*, H-5′), 5.27 (1H, *brs*, H-6′), 1.73 (3H, *s*, H-8′), 1.56 (1H, *s*, H-9′), 4.42 (1H, *brs*, H-10′), 2.31 (3H, *s*, OCOCH_3 (*q*)), 2.10 (3H, *s*, OCOCH_3 (*q*)).

3.4.4. 6-Hydroxymethyl-hept-6-ene-2,5-dione (*4*)

Oil; IR (ν_{max}^{KBr} cm^{-1}): 3350, 1740, 1620; CIMS *m/z* (rel. int): 157 [M+H]$^+$ (28), 139 [M+H−H_2O], 99 [M+H−CH_2COCH_3] (18), 85 [M−$CH_2CH_2COCH_3$] (67); HRCIMS [M+H]$^+$ *m/z* 157.086442 (calc. for $C_8H_{13}O_3$,157.086469); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 6.19 (1H, *s*, H-7a), 6.03 (1H, *s*, H-7b), 4.32 (2H, *s*, H-8), 3.00 (2H, *t*, $J = 6.5$, H-4), 2.78 (2H, *t*, $J = 6.5$, H-3), 2.24 (3H, *s*, H-1).

3.4.5. 7-Hydroxy-6-methyl-hepta-5-en-2-one (*5*)

Oil; IR (ν_{max}^{KBr} cm^{-1}): 3400, 2950, 2150, 1730; CIMS *m/z* (rel. int): 125 [(M+H)−H_2O]$^+$ (100), 111 [M−CH_2] (10); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.36 (1H, *t*, $J = 7.3$, H-5), 3.99 (2H, *brs*, H-7), 2.50 (2H, *t*, $J =$ 7.5, H-3), 2.31 (2H, *m*, H-4), 2.14 (3H, *s*, H-1), 1.71 (3H, *s*, H-8). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 29.8 (C-1), 208 (C-2), 43.3 (C-3), 21.9 (C-4), 124.0 (C-5), 135.9 (C-6), 68.6 (C-7), 13.6 (C-8).

3.4.6. X-ray structure analysis of *6* and *7*

Crystal and intensity data for **6** and **7** were obtained using a Siemens P4 automated diffractometer, which utilized graphite-monochromated Mo Kα radiation. The structures were solved using direct methods and refined using full-matrix, least-squares procedures using SHELXL (1997). Positions for all hydrogen atoms were calculated based on atomic geometry. The structures were solved, refined and displayed using the program package SHELXL (1997). Crystal data and experimental details follow: scopolin tetraacetate (**6**): $C_{31}H_{48}O_{13}$, mol. wt. = 628.690, crystal size $0.50 \times 0.20 \times 0.15$ mm, orthorhombic, space group $P2_12_12_1$, $a = 7.6640$ (4), $b = 19.790$ (2), $c = 21.66650$ (19) Å, μ (Cu Kα) = 0.824 mm^{-1}, independent data, 5354 ($R_{int} = 0.0636$), θ range 13.1–25.2°, R [$I > 2\sigma(I)$] = 0.086, $wR_2 = 0.306$, $R_{all} = 0.1656$, largest peak and hole in difference map, 0.19, −0.20 e Å^{-3}. Crystallographic data of **6**, including atomic coordinates, bond lengths and angles, thermal parameters and additional experimental details, have been deposited in the Cambridge Crystallographic Data Center (CCDC 195900). Pumilaside A (**7**), $C_{24}H_{26}O_{13}$, mol. wt. = 522.450, crystal size 0.50× 0.50 × 0.10 mm, triclinic, space group $P2_1$, $a = 6.1812(3)$, $b = 9.3710$ (12), 12.0180 (9) Å, $\alpha = 71.479$ (6), $\beta = 88.135$ (7), $\gamma =$ 75.13 (11), lub 75.13 (11)°, μ (Cu Kα)= 0.962 mm^{-1}, independent data, θ range 11.6–26.6°, R [$I > 2\sigma(I)$] = 0.047, $wR_2 =$ 0.137, $R_{all} = 0.0641$, largest peak and hole in difference map, 0.19, −0.2 e Å^{-3}. Crystallographic data of **7**, including atomic coordinates, bond lengths and angles, thermal parameters and additional experimental details, have been deposited in the Cambridge Crystallographic Data Center (CCDC 195899).

Copies of the data of **6** and **7** can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0) 1223 336 033 or e-mail:deposite@ccdc.cam.ac.uk].

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References

Ahmad, V.U., Zahid, M., Ali, M.S., Jassbi, A.R., Abbas, M., Ali, Z., Iqbal, M.Z., 1999. Buchariosides and buchariol from *Salvia bucharica*. Phytochemistry 52, 1319–1322.

Bock, G., Benda, I., Schrier, P., 1988. Microbial transformation of geranioll and nerol by *Botrytis cinerea*. Appl. Microbiol. Biotechnol. 27, 351–357.

Bruno, M., De la Torrea, M.C., Rodrígueza, B., Omar, A.A., 1993. Guaiane sesquiterpenes from *Teucrium leucocladum*. Phytochemistry 34, 245–247.

Fatima, H., Otto, S., Jeorg, H., 2005. New cytotoxic sesquiterpenes lactones from *Warionia saharae*. Planta Med. 69, 462–464.

Gedara, S.R., Abdel-Halim, O.B., El-Sharkawy, S.H., Salama, O.M., Shier, T.W., Halim, A.F., 2003. New erythroxane-type diterpenes from *Fagonia boveana* (Hadidi) Hadidi & Graf. Z. Naturforschung C 58, 23–32.

Hall, H.M., Goodspeed, T.H., 1918. An emergency supply of rubber. Science 47, 452–454.

Hegerhorst, D.F., Weber, D.J., Bhat, R.B., Davis, T.D., Sanderson, S.C., McArthur, E.D., 1988. Seasonal changes in rubber and resin contents in *Chrysothamnus nauseosus* ssp. Hololeucus and ssp. Turbinatus. Biomass 15, 133–142.

Hodgson, D.M., Bailey, J.M., Villalonga-Barber, C., Drew, M.G.B., Harrison, T., 2000. Selectivity in the cycloadditions of carbonyl ylides with glyoxylates: an approach to the zaragozic acids-squalestatins. Perkin 1 (20), 3432–3443.

Jakupovic, J., Granz, M., Miski, M., Bohlmann, F., Boldt, P.E., 1990. Geranyl derivatives of *p*-coumaric acid from *Chrysothammus pulchellus*. Phytochemistry 29, 617–620.

Kitajima, J., Kimizuka, K., Tanaka, Y., 2000. Three new sesquiterpenoid glucosides of *Ficus pumila* fruit. Chem. Pharm. Bull. 48, 77–80.

Le-Van, N., Pham, T.V.C., 1980. Viscidic acid A and B, two *ent*-labdone derivatives from *Chrysothammus visicidifflorus*. Phytochemistry 19, 1971–1974.

Le-Van, N., Pharm, T.V.C., 1981. An unusual *m*-hydroxyacetophenone and three new chromanone derivatives from *Chrysothamnus viscidiflorus*. Phytochemistry 20, 485–487.

Look, S.A., Buchholz, K., Fenical, W., 1984. 12-Hydroxy-*E*-γ-bisabolene, a new sesquiterpene alcohol from a Caribbean sea whip of the genus *Pseudoptergorgia* (gorgonacea, cnidaria). Experientia 40, 931–933.

Rose, A.F., Butt, B.A., Jermy, T., 1980. Polyacetylenes from the rabbitbrush, *Chrysothamnus nauseosus*. Phytochemistry 19, 563–566.

Sati, S.P., Chaukiyal, D.C., Sati, O.P., Yamada, F., Ono, M., 1989. 2D NMR structure elucidation of a new coumarin glycoside from *Xeromphis spinosa*. J. Nat. Prod. 52, 376–379.

Sheldrick, G.M., 1997. SHELXS-97 and SHELXL-97. Release 7-2. University of Göttingen, Germany.

Stevens, J.F., Wollenweber, E., Ivancic, M., Hsu, V.L., Sundberg, S., Deinzer, M.L., 1999. Leaf surface flovonoid of *Chrysothamnus*. Phytochemistry 51, 771–780.

Urbatsch, L.E., Bacon, J.D., Mabry, T.J., 1975. Flavonol methyl ethers from *Chrysothamnus viscidiflorus*. Phytochemistry 14, 2279–2282.

Yi-Feng, H., Qi, Z., Kun, G., Zhong-Jian, J., 2005. New sesquiterpenes from *Sonchus Transcaspicus*. Planta Med. 71, 543–547.

Yu, W., Israel, K., Liao, Q.Y., Aldaz, C.M., Sanders, B.G., Kline, K., 1999. Vitamen E succinate (VES) induces fast sensitivity in human breast cancer cells. Cancer Res. 59, 953–961.