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ANTIFUNGAL EUDESMANOIDS FROM *PARTHENIUM ARGENTATUM* × *P. TOMENTOSA*

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Key Word Index—*Parthenium argentatum* × *P. tomentosa*; Asteraceae; eudesmane sesquiterpenoids; argentone; carrisone; antifungal activity.

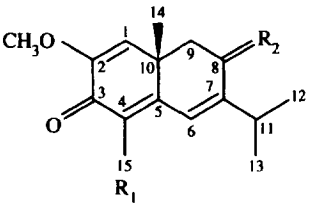
Abstract—Investigation of guayule resin for antifungal activity has led to the isolation and characterization of six eudesmane-type sesquiterpenoids, five of which are new: 2-methoxy-eudesma-1,4,6-trien-3-one (argentone); 2-methoxy-15-nor-eudesma-1,4,6-trien-3-one (15-nor-argentone); 2-methoxy-15-hydroxy-eudesma-1,4,6-trien-3-one (15-hydroxy-argentone); 2-methoxy-eudesma-1,4,6-trien-3,8-dione (8-*oxo*-argentone); 2-methoxy-15-nor-eudesma-1,4,6-trien-3,8-dione (8-*oxo*-15-nor-argentone); and the previously identified 11-hydroxy-eudesma-4-en-3-one (carissone). Their structures were elucidated by one- and two-dimensional NMR spectroscopy and mass spectrometry. After 6 weeks all inhibited the growth of *Aspergillus fumigatus* (ATCC-13073) at 1.0 mg ml^{-1} and complete inhibition of *A. niger* (UA-172-1) for 2-methoxy-eudesma-1,4,6-trien-3-one and 2-methoxy-15-nor-eudesma-1,4,6-trien-3-one. At 0.25 mg ml^{-1} 2-methoxy-eudesma-1,4,6-trien-3-one demonstrated 100% control against *A. fumigatus* and 80% control against *A. niger*.

INTRODUCTION

Parthenium argentatum Gray (guayule) is best known for its high-quality natural rubber [1]. The low-molecular-weight *cis*-polyisoprene is useful as a plasticizer or as a feedstock for depolymerized rubber [2, 3]. De-rubberized resin use has been focused on three areas to date: wood preservatives, arthropod antifeedants and plasticizers [4]. Discovering coproducts from the bagasse or de-rubberized resin continues to be an important component of guayule research [5, 6]. As a part of our search for commercially useful biocides as coproducts after guayule rubber processing, we have isolated and identified six eudesmane sesquiterpenoids, several of which display *in vitro* fungicidal activity against *Aspergillus niger* (UA-172-1) and *A. fumigatus* (ATCC-13073). The presence of eudesmane terpenoids in guayule is reported here for the first time. Isolation procedures, structural determination and antifungal assay results are reported.

RESULTS AND DISCUSSION

By analysing the fractionations obtained from the resin with an HPLC equipped with a photo diode array (PDA) detector, **1**–**5** appeared to be a family of closely related compounds since their UV spectra were very similar. The most indicative peak ranged from 305 to 326 nm for these constituents. Thus, a ketone with extended conjugation could be expected as a key chromophore for **1**–**5**.



	R_1	R_2
1	CH_3	2H
2	H	2H
3	CH_2OH	2H
4	CH_3	=O
5	H	=O

At the same time, **6** gave *m/z* 236 $[M]^+$ for $C_{15}H_{24}O_2$ with a base peak of *m/z* 59 (C_3H_7O) suggesting the presence of a hydroxy-isopropyl group. The ^1H and ^{13}C NMR (Table 1) and the optical rotation spectral data for **6** are in agreement with the literature data for 11-hydroxy-eudesma-4-en-3-one, carissone [7].

Compound **1** was identified as 2-methoxy-eudesma-1,4,6-trien-3-one, which has been named argentone. The EIMS of **1** indicated an empirical formula of $C_{16}H_{22}O_2$,

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with an unsaturation equivalence of six. This was confirmed by the high-resolution EI, mass spectrum (HREIMS) with a parent ion at *m/z* 246.1573 (calculated 246.1601). The base peak at *m/z* 203 $[M-43]^+$ provided evidence for the loss of an isopropyl group. The ^{13}C NMR data (Table 1), showed 16 carbon signals, which were discriminated by DEPT into four methyls, one methoxy, two olefinic CH, one aliphatic CH, two aliphatic CH_2 and six non-protonated carbons including one carbonyl. The olefinic resonances at 117.67, 121.72, 126.50, 149.72, 153.69 and 154.49 ppm were assigned to three double bonds at the 1, 4 and 6 positions. The downfield shifted signal at 181.40 ppm was assigned to the α–β unsaturated *oxo* group at the 3 position in the eudesmane skeleton [8]. The β- and γ-effects induced by this 3-keto group were observed at the 5 (moved downfield to 154.59 ppm) and 15 positions (moved upfield to 9.9 ppm) [9]. The IR spectrum established the presence of an α–β unsaturated carbonyl (1642 cm^{-1}). ^1H NMR (Table 1) displayed two olefinic proton singlets at 5.67 and 6.40 ppm, each integrating for one proton (H-1 and H-6, respectively) and a methoxyl signal at 3.67 ppm. The four skeletal methyl signals at 1.13 (*d*, $J = 7.5$ Hz), 1.14 (*d*, $J = 7.5$ Hz), 1.18 (*s*) and 1.98 (*s*) are consistent with those of the eudesmanes with an isopropyl group at the 7 position and a 15-methyl group attached to a double bond (the proton signal is shifted downfield, 1.98 ppm, while the carbon signal is upfield, 9.9 ppm). The assignment of **1** as 2-methoxy-eudesma-1,4,6-trien-3-one was based on ^1H–^{13}C HETCOR and by long-range correlation, through the intensive use of selective INEPT (Fig. 1) [10]. The irradiation of the methyl proton singlet at 1.18 ppm (H_3-14) resulted in an enhancement of the carbon signals at 36.53 (C-10), 121.72 (C-1) and 154.59 ppm (C-5). Irradiation of the proton singlet at 1.89 ppm (H_3-15) enhanced the carbon signals at 181.40 (C-3), 126.50 (C-4) and 154.59 ppm (C-5). Finally, irradiation of the olefinic singlet at 5.67 ppm (1H-1) gave enhancement of the carbon signals at 149.72 (C-2), 181.40 (C-3) and 154.59 ppm (C-5). These results confirm the structure of **1** as 2-methoxy-eudesma-1,4,6-trien-3-one (argentone).

Compound **2** gave *m/z* 232 $[M]^+$ for $C_{15}H_{20}O_2$ with a base peak at *m/z* 217 $[M-CH_3]^+$ and a peak at *m/z* 189 $[M-C_3H_7]^+$. These data implied that **2** had one less methylene or methyl than **1**. The ^1H and ^{13}C NMR data of **2** (Table 1) are similar to those for **1** except for the absence of the signal at 1.98 ppm in ^1H NMR and 9.9 ppm in the ^{13}C NMR, assigned to the 15 position methyl. Also, from the appearance of a new proton singlet at 6.01 ppm the presence of a proton at the 4 position was inferred. These results are in good agreement with the structure of **2** as 2-methoxy-15-nor-eudesma-1,4,6-trien-3-one (15-nor-argentone).

Table 1. ^1H and ^{13}C NMR spectroscopic data of compounds **1–6***

	1		**2**		**3**		**4**		**5**		**6**	
No.	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	5.67(*s*)	121.72	5.68(*s*)	121.78	5.72(*s*)	122.83	5.79(*s*)	121.70	6.32(*s*)	126.00	†	37.11
2	—	149.72	—	150.77	—	149.70	—	150.10	—	153.22	†	33.58
3	—	181.40	—	181.80	—	182.39	—	180.81	—	185.40	—	199.08
4	—	126.50	6.01(*s*)	122.04	—	128.80	—	133.09	6.34(*s*)	112.84	—	128.51
5	—	154.59	—	162.11	—	157.53	—	150.10	—	156.16	—	163.10
6	6.40(*s*)	117.67	6.11(*s*)	120.06	6.54(*s*)	116.57	7.36(*s*)	133.52	6.48(*s*)	145.65	†	28.60
7	—	153.69	—	155.38	—	156.37	—	147.89	—	144.30	†	49.47
8α	2.32(*m*)	23.56	2.31(*m*)	24.09	2.38(*m*)	23.65	—	196.20	—	191.13	†	22.25
8β	2.21(*m*)		2.20(*m*)		2.30(*m*)		—	—	—	—	†	—
9α	1.60(*m*)	33.56	1.65(*m*)	33.59	1.64(*m*)	33.33	2.46(*d*) (15)	49.79	2.57(*d*) (15)	48.33	†	41.77
9β	1.83(*m*)		1.86(*m*)		1.87(*m*)		2.67(*d*) (15)		2.74(*d*) (15)		†	—
10	—	36.53	—	37.69	—	37.05	—	40.58	—	40.38	—	35.69
11	2.45(*m*)	35.36	2.42(*m*)	35.16	2.46(*m*)	35.54	3.06(*m*)	28.95	3.04(*m*)	28.78	†	72.08
12‡	1.13(*d*) (7.5)	20.94	1.11(*d*) (7.5)	20.88	1.12(*d*) (7.5)	20.91	1.15(*d*) (7.5)	21.35	1.08(*d*) (7.5)	21.55	1.25(*s*)	26.36
13‡	1.14(*d*) (7.5)	21.15	1.11(*d*) (7.5)	21.18	1.13(*d*) (7.5)	21.06	1.18(*d*) (7.5)	21.70	1.11(*d*) (7.5)	21.61	1.26(*s*)	27.29
14	1.18(*s*)	25.39	1.26(*s*)	26.48	1.23(*s*)	26.45	1.34(*s*)	26.77	1.37(*s*)	25.95	1.21(*s*)	22.42
15	1.98(*s*)	9.90	—	—	4.56(*dd*) (12, 12)	55.88	2.13(*s*)	10.98	—	—	1.78(*s*)	10.71
O-CH_3	3.67(*s*)	54.36	3.68(*s*)	54.68	3.68(*s*)	54.57	3.72(*s*)	54.83	3.84(*s*)	55.88	—	—

*At 250 MHz (^1H NMR) and 62.5 MHz (^{13}C NMR), in $CDCl_3$ as a solvent, TMS as internal standard, chemical shifts (δ) in ppm, and the coupling constant J in Hz (in parentheses), *d* = doublet, *dd* = double doublet, *m* = multiplet, and *s* = singlet.

†Signal not assigned due to overlapping.

‡Assignments may be interchangeable.

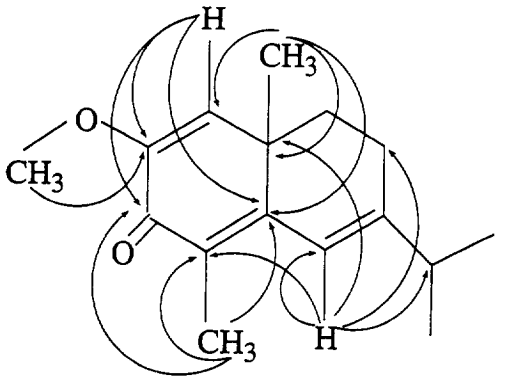

Fig. 1. Results of selective INEPT experiment for compound **1**.

Compound **3** gave *m/z* 262 $[M]^+$ for $C_{16}H_{22}O_3$, 16 mass units greater than **1**. ^{13}C NMR spectrum of **3** was similar to **1** except for the absence of the methyl signal at 9.9 ppm, which was replaced with a new signal at 55.88 ppm, assigned to a hydroxyl methyl group at the 15 position. This was confirmed by a new doublet of doublets at 4.56 ppm (J = 12,12 Hz) for the C-15 protons in the ^1H NMR [11]. These results are consistent with the structure of **3** as 2-methoxy-15-hydroxy-eudesma-1,4,6-trien-3-one (15-hydroxy-argentone).

Compound **4** gave *m/z* 260 $[M]^+$ for $C_{16}H_{20}O_3$ and differs from **1** by 14 additional mass units. In the ^{13}C NMR data (Table 1) a new downfield shifted signal at 196.20 ppm was assigned to an α,β-unsaturated ketone at the C-8 position. Further support for this assignment was provided by the β- and γ-effects of this *oxo*-group. The ^{13}C NMR values for C-9, C-10, and C-6 shifted downfield 17, 3, and 16 ppm, respectively, compared with **1**. The two doublets in the ^1H NMR at 2.46 and 2.67 ppm were assigned to the two protons at the 9 position. These results support the structure of **4** as 2-methoxy-eudesma-1,4,6-trien-3,8-dione (8-*oxo*-argentone).

Compound **5** gave *m/z* 246 $[M]^+$ for $C_{15}H_{18}O_3$ which is 14 mass units fewer than **4**. The methyl signal at the 15 position in the ^1H and ^{13}C NMR spectra was absent and a new olefinic proton singlet at 6.34 ppm (H-4) appeared. These results and comparing **1** and **2** helped to establish the structure for **5** as 2-methoxy-15-nor-eudesma-1,4,6-trien-3,8-dione (8-oxo-15-nor-argentone).

These compounds appear to be derivatives of carissone, therefore, we have assigned the same stereochemistry for **1–5**. Since the possibility exists that these compounds are artifacts, a fresh sample of AZ 101 was collected on 20 March 1995 by Dr Dennis Ray at the Maricopa Agricultural Experiment Station in Arizona. This material was solvent extracted at room temperature with acetone and sub-fractionated by HPLC. The presence of these eudesmanoids was confirmed by co-chromatography and UV matching with the HPLC-PDA. Interestingly, when the plant was separated into leaves, stems and roots, **1** was only found in the root material. Therefore, we assume that **1–6** are true constituents of this hybrid and not artifacts of the processing and distillation of the resin.

The fungicidal activity for **1–6** are summarized in Table 2. The antifungal results indicated that an additional hydrophilic group at the 4 position decreases activity, since oxidation to the corresponding alcohol, **3**, resulted in reduction of fungicidal activity. However, the 15-nor derivative, **2**, exhibited sustained activity similar to **1**. Oxidation of the 8 position to the corresponding ketone as in **4** and **5** reduced the activity with *A. niger*. The *oxo*-group at the 3 position with the α–β unsaturation between C_4 and C_5 appears to be important for activity when compared to the known antibiotic panellon [12]. Panellon is a 14-nor-eudesmane derivative, previously isolated from *Panellus violaceofulvus* and *Resupinatus leightonii*. Panellon exhibits antimicrobial, cytotoxic, and phytotoxic activities [12]. These results indicate that further study of the structure–activity relationships may lead to more potent and versatile fungicidal agents.

Table 2. Fungicial bioassay results (% inhibition after 42 days)

	Dose (mg ml^{-1})					
	Aspergillus niger			*Aspergillus fumigatus*		
Compound	0.25	0.5	1.0	0.25	0.5	1.0
1	80	100	100	100	100	100
2	30	100	100	100	100	100
3	0	30	65	55	100	100
4	0	55	70	100	100	100
5	0	65	75	100	100	100
6	0	30	60	30	80	100
Agar control		0			0	
Solvent (0.05 ml EtOAc–EtOH/1:1)		0			0	
Amphotericin B (32 μg ml^{-1})*		46%			46%	

*Usual *in vitro* test done is 3.2 μg ml^{-1} for 2 weeks.

EXPERIMENTAL

Instrumentation. Mp are uncorr. ^{1}H NMR and ^{13}C NMR spectral data were measured on a Bruker WM 250 NMR Spectrometer at 250 MHz and 62.5 MHz, respectively, with $CDCl_3$ as the solvent and TMS as the int. st. The chemical shifts are expressed in δ ppm. DEPT, COSY, HETCOR and selective INEPT (J = 9 Hz irradiation) [10] were measured on a Bruker WM 300 NMR Spectrometer at 300 MHz. EIMS and GC–EIMS were conducted on a Hewlett Packard 5988A at 70 eV equipped with a Hewlett Packard RTE-6/VM data system and a Hewlett Packard 5890 GC using a 25 m HP-5 CC, 0.2 mm i.d., film thickness 0.33 μm, cross-linked 5% phenyl-methyl silicone, helium with head pressure of 18 psi (124.2 kPa), 1 μl injection, split ratio 1:50; injector 200°, detector 300°, temperature programme was 70°, hold for 1 min, 20° min^{-1} to 300°, hold for 6 min. HREIMS were recorded on a Varian MAT-311A. IR analysis was conducted on a Beckman Acculab I IR spectrometer UV data was obtained from a Hitachi L-4500 photo-diode-array HPLC. ORDs were measured on an Autopole III Automatic Polarimeter (Rudolph Scientific).

Bioassay. The routine screen of resin frs and compounds from the purification process was accomplished using a modification of an agar-streak assay [13]. Several generations of *A. niger* were grown under standard conditions until we isolated a fast growing culture; *A. fumigatus* was used as received. Instead of streaking the inoculum across the agar, we inoculated a 2 cm circle in the centre of the dish. This facilitated the quantitative analysis of fungal growth by measuring the increase in colony dia. Frs from the various isolation procedures were dissolved in 0.05 ml of a 1:1 mixture of EtOAc–EtOH. Final concentration in the agar varied from 0.01 mg ml^{-1} (1 ml plates) to 1 mg ml^{-1}. Controls consisted of solvent in agar and agar only, as well as the fungicidal agent, Amphotericin B at 32 μg ml^{-1}. The plates were monitored daily and colony diameter was recorded. Percent activity was calculated as 100 × (1 − (diameter of test growth/diameter of control growth)).

Resin treatment and chromatography. Guayule resin, extracted from whole plants, was obtained from the Firestone pilot scale plant at Sacaton, Arizona [14]. Plants used for extraction were grown in field plots of the Gila River Indian Tribe using guayule genetic strain AZ-101, an interspecific hybrid obtained by crossing *P. argentatum* with *P. tomemtosa* (Dennis Ray, Professor of Plant Sciences, plant geneticist, pers. commun.). Seeds are maintained by the guayule processing programme at the University of Arizona.

A major problem in dealing with this resin was the presence of large amounts of mineral oil as a result of processing equipment failure. After de-rubberization of this resin by repeated acetone pptn (15 kg resin, 48 l acetone), the resulting liquid resin was distilled using a 2″ Pope Scientific Wiped Thin Film Still. Operating cond. were: 70° feed flask, 150° evaporator, 40° internal finger condenser, − 40° external still condenser, and a − 80° trap prior to the vacuum pump. Derubberized resin was processed in 1 kg batches with the system vacuum between 0.8 and 1.5 mmHg at the start of each run; the typical resin feed rate was 250 g hr^{-1}. Four frs were generated: bottoms, distillate, overheads, and trap oils. The bottoms fr. was the residue from the distillation process, the distillate was the material that condensed on the internal condenser, the overheads condensed on the still condenser and the trap oils condensed in the vacuum pump pre-trap.

The distillate fraction demonstrated antifungal activity and still contained the contaminating mineral oil. A three-funnel partition utilizing C_6H_{14}:10% aqueous MeOH, 1.5 l of each saturated phase per 200 g sample, efficiently separated the mineral oil into the upper phase and the antifungal compounds into the lower phase. The lower phase was taken to dryness by rotoevaporation (17.2 g) and dissolved in Et_2O (1 l). Partition of the ethereal solution with 5% Na_2CO_3 (w/v) afforded a neutral Et_2O phase which was washed with water to neutrality and air dried (11.4 g, 5×). 48 g of the neutral fr. was subjected to flash CC (air pressure *ca* 10 psi (≈ 69 kPa)) using a silica gel 60 (60–230 μ, 2.6 kg, 10 cm i.d. × 58 cm) column. The eluant was C_6H_{14} followed by step gradient elution with *iso*-PrOH collected in 500 ml fractions. The elution profile was 5.3 l C_6H_{14}, 0.6% *iso*-PrOH/C_6H_{14} (2 l), 1.25% (3 l), 2% (6.7 l), 50% (3 l), and 100% (3 l). Frs were combined based on TLC results (pre-coated aluminium backed silica gel 60 F_{254}; eluant C_6H_{14}–EtOAc, 80:20). The 100% *iso*-PrOH wash contained the antifungal material in two frs designated A and B.

Fr. A (3.4 g) was subjected to flash CC using silica gel 60 (40–60 μm, 300 g, 3.5 × 45 cm column). The elution profile was C_6H_{14}–CH_2Cl_2 (1:1), C_6H_{14}–CH_2Cl_2 (1:1), with 1% Me_2CO, C_6H_{14}–CH_2Cl_2 (25:75), with 1% Me_2CO, CH_2Cl_2–Me_2CO 99:1, 97:3, 95:5, 92.5:7.5, 90:10, 87.5:12.5 and 85:15 (400 ml each). Three main fractions were generated; frs 10–11, 12–14, and 15–17.

Frs 10–11 were eluted with CH_2Cl_2–Me_2CO 99:1, yielding a brownish yellow oil (680 mg). On TLC (C_6H_{14}–Me_2CO 80:20), two major uv quenching spots were seen at R_f 0.4 and 0.3. The upper spot gave a yellow colour with vanillin/sulphuric acid spray reagent, turning greyish brown on heating. The lower spot gave a brown colour after vanillin/sulphuric acid spray reagent and heating. Two successive prep. TLCs using 1-mm-thick silica gel plates, eluting with C_6H_{14}–Me_2CO (75:25) and CH_2Cl_2–MeOH (98:2) with 1% HCO_2H for the second run, recovered using CH_2Cl_2–MeOH (1:1), yielded a yellow oil (330 mg) designated as Band 10A. The second band gave a yellow oil (135 mg) designated as Band 10B. MPLC of Band 10A gave **1** (300 mg) (135 g silica gel 40, 15–25 μ, 2.6 cm × 46.0 cm column, 40 ml min^{-1}). Elution began with 0.5 l of C_6H_{14}–EtOAc (95:5) followed by 1 l each of 90:10; 95:15, 80:20 and 70:30. The major antifungal material was recovered in the 80:20 eluant. Compound **1** was found to be 98% pure (area ratio) by GC (retention time of 10.02 min, 10 m SGE BP-1, 0.25 μm film thickness, 0.25 mm i.d.; temperature program was 130° for 6 min, 20°/min to 280°, hold for 6 min; 2.0 ml min^{-1} He carrier gas; injector 180°, FID detector

300°; 1:50 split ratio). Band 10B from frs 10–11 was resolved into two compounds after prep. TLC on silica gel plates, 1 mm thickness, using C_6H_{14}–Me_2CO (80:20) and multiple developments (9×), recovered using CH_2Cl_2–MeOH (1:1). The two compounds were designated as **4** and **5**, with yields of 46 and 40 mg, respectively; both were yellow oils. With vanillin/sulphuric acid spray, compound **4** gave a dark brown colour while compound **5** gave a reddish brown colour. Crystallization from acetone gave needles of **4** and **5**.

Frs 12–14 eluted with Me_2CO–CH_2Cl_2 (3:97), yielded a dark brown oil (740 mg). TLC with silica gel GF_{254}, C_6H_{14}–Me_2CO (80:20) gave the same picture as frs 10–11, in addition to a lower spot at R_f 0.28. This lower spot gave a yellowish brown colour after spraying with vanillin/sulphuric acid and gently heating with a heat gun for 10–20 s. TLC in CH_2Cl_2–MeOH (97:3 with 1% HCO_2H), gave an R_f of 0.5. Prep. TLC on 1 mm thick silica gel G plates in the last solvent gave a yellow oil (40 mg) designated as compound **2**.

Frs 15–17 eluted with C_6H_{14}–Me_2CO (95:5) and (92.5:7.5) gave a dark brown oil (750 mg). TLC on silica gel GF_{254}, C_6H_{14}–Me_2CO (80:20), displayed one major spot at R_f 0.36; using CH_2Cl_2–MeOH (97:3 with 1% HCO_2H) displayed one major spot at R_f 0.58, giving a dark yellow colour after spraying with vanillin/sulphuric acid spray reagent. Successive prep. TLC (1 mm thickness silica gel) using the latter two solvent systems, yielded a yellow oil (250 mg) designated as **6**. Crystallization from acetone gave needles.

Fraction B (1.8 g) was subjected to flash chromatography, (silica gel 60; 40–60 μm, 80 g, 2 cm × 45 cm) column. Elution was with C_6H_{14}–MeOH (95:5, 90:10, 80:20, 70:30) and 100% Me_2CO, 500 ml for each step. The 80:20 eluant gave one major TLC spot with an R_f of 0.19 in CH_2Cl_2–MeOH (95:5 with 1% HCO_2H). Vanillin/sulphuric acid spray reagent gave a yellow colour that changed to dark brown upon heating. Prep. TLC, 1 mm thick silica gel, in the aforementioned solvent yielded a yellow oil (60 mg) of **3**.

The yields for **1**–**6** are presented as a percentage of the dry weight of the entire plant (roots, stems and leaves).

Compound **1**. 2-methoxy-eudesma-1,4,6-trien-3-one. Yellow oil (3.1×10^{-4}%), $[\alpha]_D^{20}$ − 4.5° ($CHCl_3$; *c* 1.50); UV λ_{max} nm (227.6, 285.8, 323.5); $IR^{KBr}\nu_{max}$ cm^{-1}: 2990, 1642, 1615, 1460, 1260, 1200, 1070; EIMS *m/z* (rel. int.): 246 $[M]^+$ (40), 231 $[M\text{-}CH_3]^+$ (58), 218 $[M\text{-}CO]^+$ (45); 216 $[M\text{-}2CH_3]^+$ (8), 215 $[M\text{-}O\text{-}CH_3]^+$ (10), 203 $[M\text{-}C_3H_7]^+$ (100), and 201 $[M\text{-}3CH_3]^+$ (8). HREIMS gave *m/z* 246.1573 for $C_{16}H_{22}O_2$ (calcd 246.1601).

Compound **2**. 2-*methoxy*-15-*nor*-*eudesma*-1,4,6-*trien*-3-*one*. Yellow oil (4.2×10^{-5}%), $[\alpha]_D^{20}$ − 0.33° ($CHCl_3$; *c* 0.40); UV λ_{max} nm (223.4, 238.8, 315.2); $IR^{KBr}\nu_{max}$ cm^{-1}: 1980, 1650, 1615, 1210, 1175, 1010; EIMS *m/z* (rel. int.): 232 $[M]^+$ (26), 217 $[M\text{-}CH_3]^+$ (100), 204 $[M\text{-}CO]^+$ (12), 202 $[M\text{-}2CH_3]^+$ (5), 215 $[M\text{-}O\text{-}CH_3]$ (2), and 189 $[M\text{-}C_3H_7]^+$ (84), HREIMS gave *m/z* 232.1449 for $C_{15}H_{20}O_2$ (calcd 232.1464).

Compound **3**. 2-*methoxy*-15-*hydroxy*-*eudesma*-1,4,6-*trien*-3-*one*. Yellow oil (6.2×10^{-5}%), $[\alpha]_D^{20}$ − 0.83° ($CHCl_3$; *c* 0.30); UV λ_{max} nm (235.1, 296.5, 322.8); $IR^{KBr}\nu_{max}$ cm^{-1}: 3420, 2975, 1635, 1605, 1450, 1250, 1190, 1070; GC-EIMS *m/z* (rel. int.): 262 $[M]^+$ (24), 247 $[M\text{-}CH_3]^+$ (38), 244 $[M\text{-}H_2O]^+$ (18), 234 $[M\text{-}CO]^+$ (6); 229 $[M\text{-}CH_3\text{-}H_2O]^+$ (32), 219 $[M\text{-}C_3H_7]^+$ (18), 217 $[M\text{-}3CH_3]^+$ (21), 201 $[M\text{-}2CH_3\text{-}OCH_3$ or $CH_2OH]^+$ (28), and 191 $[M\text{-}CH_3H_7\text{-}CO]^+$ (100). HREIMS gave *m/z* 262.1554 for $C_{16}H_{22}O_3$ (calcd 262.1570).

Compound **4**. 2-*methoxy*-*eudesma*-1,4,6-*trien*-3,8-*dione*. Needles (4.8×10^{-5}%), mp 78–80°, $[\alpha]_D^{20}$ − 1.7° ($CHCl_3$; *c* 0.75); UV λ_{max} nm (232.2, 243.9, 305.2); $IR^{KBr}\nu_{max}$ cm^{-1}, 2980, 1670, 1640, 1605, 1460, 1380, 1260, 1200, 1100, 1060, 980. EIMS *m/z* (rel. int.): 260 $[M]^+$ (34), 245 $[M\text{-}CH_3]^+$ (52), 232 $[M\text{-}CO]^+$ (64); 230 $[M\text{-}2CH_3]^+$ (4), 229 $[M\text{-}CH_3O\text{-}]^+$ (4), and 217 $[M\text{-}C_3H_7]^+$ (100). The high-resolution EIMS gave *m/z* 260.1551 for $C_{16}H_{20}O_3$ (calcd 260.1413).

Compound **5**. 2-*methoxy*-15-*nor*-*eudesma*-1,4,6-*trien*-3,8-*dione*. Needles (4.2×10^{-5}%), mp 133–135°, $[\alpha]_D^{20}$ − 0.6° ($CHCl_3$; *c* 0.475); UV λ_{max} nm (267.2, 325.7); $IR^{KBr}\nu_{max}$ cm^{-1}, 2990, 1700, 1655, 1635, 1605, 1262, 1060; EIMS *m/z* (rel. int.): 246 $[M]^+$ (20), 231 $[M\text{-}CH_3]^+$ (100), 218 $[M\text{-}CO]^+$ (4); 216 $[M\text{-}2CH_3]^+$ (3), 215 $[M\text{-}O\text{-}CH_3]^+$ (2), and 203 $[M\text{-}C_3H_7]^+$ (30). HREIMS gave *m/z* 246.1291 for $C_{15}H_{18}O_3$ (calcd 246.1256).

Compound **6**. 11-*hydroxy*-*eudesma*-4-*en*-3-*one*. Needles (2.6×10^{-4}%), mp 77–79°, $[\alpha]_D^{20}$ + 1.8°; ($CHCl_3$; *c* 1.70); $IR^{KBr}\nu_{max}$ cm^{-1}, 3415, 2950, 1645, 1600, 1440, 1350, 1315, 1200, 1000, 910, 810. EIMS *m/z* (rel. int.): 218 $[M\text{-}H_2O]^+$ (64), 203 $[M\text{-}H_2O\text{-}CH_3]^+$ (30), 190 $[M\text{-}H_2O\text{-}CO]^+$ (12); 177 $[M\text{-}C_3H_7O]^+$ (6), and 59 $[C_3H_7O]^+$ (100).

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