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GERMACRANOLIDES AND AN ALKYL GLUCOSIDE FROM *TANACETUM VULGARE*

AMITABH CHANDRA, LAXMI N. MISRA and RAGHUNATH S. THAKUR

Department of Phytochemistry, Central Institute of Medicinal and Aromatic Plants, Lucknow 226016, India

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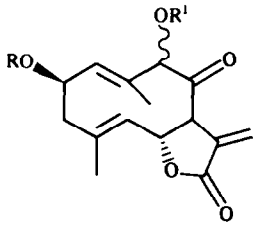
Key Word Index—*Tanacetum vulgare*; Compositae; sesquiterpene lactones; germacranolides.

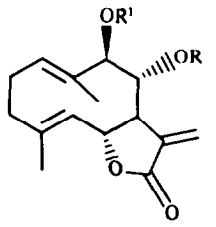
Abstract—The aerial parts of *Tanacetum vulgare* afforded two new germacranolides and an *n*-decyl glucoside. The structures have been elucidated by spectroscopic methods and chemical transformations.

INTRODUCTION

The sesquiterpene lactones of *T. vulgare*, a well known medicinal plant [1], have been the subject of several studies. The presence of various germacranolides [2–6], some eudesmanolides [7, 8] and several flavonoids [9] has been reported from different chemotypes of this plant. All the sesquiterpene lactones isolated from *T. vulgare* possess an α-methylene-γ-lactone moiety and this feature has been claimed to be the characteristic for this genus [10–12]. However, the Indian *T. vulgare* which widely occurs in the Kashmir valley of the Western Himalayas has not been chemically investigated. Our investigation on the aerial part showed the presence of germacranolides, flavonoids and alkyl glucosides.



1 R = R¹ = H
2 R = R¹ = Ac



3 R = R¹ = H
4 R = R¹ = Ac

RESULTS AND DISCUSSION

The aerial parts of *T. vulgare* afforded stearic acid, apigenin, sitosterol, stigmasterol, sitosterol-α-glucopyranoside (in its tetraacetate form), *n*-butyl-α-glucopyranoside and apigenin-trimethyl ether. The extract yielded 8-oxo-2α-9-dihydroxy-*trans*,*trans*-germacra-1(10),4-dien-*trans*-6,12-olide (**1**), 8α,9β-dihydroxy-*trans*, *trans*-germacra-1(10),4-dien-*trans*-6,12-olide (**3**) and an *n*-decyl-glucoside as new compounds.

The structure of **1** followed from its mass spectrum with the presence of an $[M]^+$ peak at *m/z* 278 accounting for $C_{15}H_{18}O_5$. The presence of strong bands at 3580–3100 and 1770–1690 cm^{-1} in its IR spectrum showed that the molecule contains hydroxy, lactone and ketonic groups. Its ^1H NMR spectrum showed two vinylic methyls at δ 2.1 and 1.68 and two doublets at 6.30 and 6.15 which established that **1** is a germacranolide. The magnitude of the couplings $J_{7,13}$ and $J_{6,7}$ indicated a *trans*-6,7-lactone if the data are compared with those of other *cis*- and *trans*-6,12-germacranolides [13–15]. The presence of the two doublets for vinylic protons at δ 5.10 and 5.00 (J = 8.0 and 10.5 Hz, respectively) showed that the C-2 and C-6 positions are substituted. The doublet of H-5 was coupled with a double doublet at δ 5.20 (J = 10.5 and 9 Hz). Spin-decoupling of H-6 collapsed the broad doublet at δ 3.90 (J = 7 Hz) into a singlet which was accounted for by H-7. Downfield shifting of H-7 and the absence of further coupling suggested the presence of a ketonic group at C-8. This was further confirmed by the presence of a singlet at δ 4.23 for H-9. The presence of another multiplet at δ 4.23 showed that C-2 was substituted by a hydroxy group. The stereochemistry at C-9 could not be established while the coupling constant of C-1 (8 Hz) was attributed to the β-hydroxy at C-2 [16]. To confirm the presence of the two hydroxy groups, the compound (**1**) was acetylated to yield **2** which showed downfield shifts of H-2 and H-9 and the appearance of two additional signals at δ 2.08 and 2.05 attributed to acetates. The interesting difference in the ^1H NMR spectrum of **2** was marked by the upfield shift of H-13′ to its usual position (δ 5.81). The unusual downfield shift of this signal in **1** could be explained by the presence of ketonic and hydroxy groups at C-8 and C-9, respectively. The unusual downfield shift of C-14 in **1** as well as **2** could have also been due to similar reasons. The Dreiding model of these molecules also confirmed the possible influence of a ketone and hydroxy at C-8 and C-9 on Me-10 and H-13. The C-8 keto-germacranolides have been isolated from *Pegolittia senegalensis* [15] but in that case the C-9 position was unsubstituted and the fusion of the 6,7-lactone was *cis*.

The ^1H NMR spectrum of **3** was in part similar to that of montafrusin [17]. However, the H-1 signal appeared as a triplet (J = 8 Hz) while H-2 and H-3 appeared as overlapping multiplets which suggested that C-2 and C-3 were unsubstituted. A further difference was observed owing to the absence of angelate signals. In this case the C-8 and C-9 were substituted by hydroxy groups with the

same stereochemistry as in the case of montafrusin ($J_{7,8}$ = 3 Hz and $J_{8,9}$ = 10 Hz). After acetylation compound 3 yielded 4 showing downfield shifts of H-8 and H-9 along with the appearance of two additional acetate signals (Table 1).

The IR spectrum of *n*-decyl glucoside (Experimental) showed the presence of hydroxy and ether peaks and an *n*-alkane chain. In its ^{1}H NMR spectrum (C_5D_5N) the presence of a broad signal at δ1.17 (equivalent to 14H) and a broad triplet at δ0.75 (equivalent to 3H, J = 7 Hz) along with two broad multiplets at δ3.70 and 1.50 (each equivalent to 2H) indicated that the molecule had an *O*-*n*-decyl chain. The obscured downfield signals (δ5.20 to 3.80) showed that the *n*-decyl chain was attached to a sugar moiety. Owing to its high polarity it seemed difficult to get further information about the type of sugar attached. Therefore this was acetylated to yield a tetraacetate which in its IR spectrum showed strong bands at 1750–1700 and 1220 cm^{-1}. In its ^{1}H NMR spectrum a broad triplet at δ0.90, a broad multiplet at 1.30 and two multiplets at 3.70 and 1.50 suggested the presence of a *n*-decyl chain in the molecule. The broad overlapping signals at δ2.2–2.1 (equivalent to 4 methyls of acetates) along with several downfield signals between δ5.10 and 3.40 suggested the attachment of a tetraacetate sugar moiety. The anomeric proton appeared as a doublet at δ5.10 (J = 3.0 Hz) which indicated that the alkyl attachment is of the α-type [18]. To identify the sugar, the compound was hydrolysed to obtain a compound which was comparable on paper chromatography with α-D-glucose. The ^{1}H NMR spectrum of its tetraacetate was in part similar to that of α-D-glucose pentaacetate [18]. Similarly its mass spectrum showed $[M]^+$ at m/z 488 for $C_{24}H_{40}O_{10}$. The characteristic $[M-CH_2OAc]^+$ peak for hexoses appeared at m/z 415. A relatively strong peak at m/z 330 accounted for [hexosetetraacetate – ROH]$^+$. This peak suggested that the side chain is a *n*-decyl ($C_{10}H_{22}O$) unit which was further supported by a peak at m/z 347 for $[M-R]^+$. These data showed that this material was a *n*-decyl-α-D-glucopyranoside.

Table 1. ^{1}H NMR spectral data of compounds 1–4 in $CDCl_3$ with TMS as internal standard

	1	2	3	4
H-1	5.10, *br d*	5.25*	5.40, *t*	5.30, *d*
H-2	4.23, *m*	4.7–5.4†		
H-2′	—	—	2.4–2.0†	2.50–1.90†
H-3	2.85, *t*	2.80, *t*		
H-3′	2.30, *t*	2.40, *t*		
H-5	5.00, *t*	4.7–5.4†	5.10, *d*	4.65–5.15†
H-6	5.20, *dd*	4.7–5.4†	4.95, *dd*	4.65–5.15†
H-7	3.90, *d*	4.15, *br d*	3.42, *dd*	3.40, *dd*
H-8	—	—	4.00, *dd*	4.65–5.15†
H-9	4.23, *s*	4.7–5.4†	4.20, *d*	4.65–5.15†
H-13	6.30, *d*	6.30, *d*	6.15, *d*	6.15, *d*
H-13′	6.15, *d*	5.81, *d*	5.75, *d*	5.75, *d*
H-14	2.10, *br s*	2.15, *s*		1.70, *br s*
H-15	1.68, *br s*	1.87, *s*	1.60, *br s*	1.80, *br s*
OR	2.50, *br s*	2.08, *s*	—	2.05, *br s*
	—	2.05, *s*	—	

J (Hz): **1** and **2**: 1,2 = 8; 3,3′ = 12; 5,6 = 10.5; 6,7 = 9; 7,13 = 7,13′ = 3. **3** and **4**: 1,2 = 1,2′ = 8; 5,6 = 8,9 = 10; 6,7 = 8; 7,8 = 7,13 = 7,13′ = 3.

*Signal obscured.

†Overlapping multiplet.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 80 MHz in $CDCl_3$ and C_5D_5N with TMS as internal reference. The values are given in δ units. Mass spectra were recorded at 70 eV (direct inlet).

The plant material was collected in September 1984 from the Kashmir valley. The voucher specimen is deposited in the herbarium of our institute. The air dried aerial part (1.8 kg) was extracted with a mixture of petrol (40–60°)–Et_2O–MeOH (1:1:1) and subsequently concd. The fatty portion was precipitated by keeping the extract in an adequate amount of MeOH at 0° for 2 h. The MeOH soluble portion was column chromatographed over silica gel to give the following fractions: fraction 1 petrol; fraction 2 petrol–Et_2O (9:1); fraction 3 petrol–Et_2O (4:1); fraction 4 petrol–Et_2O (1:1); fractions 5, 6 and 7 Et_2O, fraction 8 Et_2O–MeOH (9:1) and fraction 9 Et_2O–MeOH (4:1). Fractions 2, 3 and 4 after further purification yielded stearic acid (110 mg) while fraction 5 gave sitosterol (15 mg) and stigmasterol (10 mg) together with stearic acid (30 mg). Fraction 6 on TLC separations afforded apigenin-trimethyl ether (20 mg). Fraction 7 after further CC gave 7 fractions of which the 4th on TLC (Et_2O) afforded **3** (6 mg, R_f 0.7) and the 5th after exhaustive TLC (Et_2O–MeOH, 97:3) afforded **1** (12 mg, R_f 0.5) and the 7th after TLC (Et_2O–MeOH, 97:3) yielded apigenin (8 mg). Fraction 8 on further CC yielded two complex mixtures. The first on repeated TLC (EtOAc–MeOH, 19:1) afforded *n*-decyl glucoside (25 mg, R_f 0.45) while the second after acetylation and further separation afforded sitosteryl glucoside (20 mg) and *n*-butyl glucoside (25 mg).

8-Oxo-2α-9-dihydroxy-trans,trans-*germacra*-1(10),4-*dien*-trans-6,12-*olide* (**1**). Colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580–3100 (OH), 1760 (γ-lactone), 1720 (CO), 1260, 1140, 1010, 900; MS m/z (rel. int.): 278 [M^+, $C_{15}H_{18}O_5$] (35), 260 $[M-H_2O]^+$ (5), 242 $[260-H_2O]^+$ (4), 228 $[242-CH_2]^+$ (60), 214 $[242-CO]^+$ (40), 199 $[214-Me]^+$ (45), 149 (75), 95 (40), 55 (65), 43 (82), 41 (100).

Acetylation of compound **1**. Compound **1** (8 mg) in pyridine was heated with Ac_2O over a waterbath for 2 hr. After usual work up the reaction product yielded 7 mg of **2** (for ^{1}H NMR see Table 1), $[\alpha]_D^{23°}$ −0.66° ($CHCl_3$, *c* 0.59).

8α,9β-Dihydroxy-trans,trans-*germacra*-1(10),4-*dien*-trans-6,12-*olide* (**3**). Colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3200 (OH), 1770 (γ-lactone), 1260, 1120; MS m/z (rel. int.): 264 [M^+, $C_{15}H_{20}O_4$] (10), 228 $[M-2H_2O]^+$ (8), 214 $[228-CH_2]^+$ (30), 108 (100), 96 (70); $[\alpha]_D^{25°}$ −3.43° ($CHCl_3$, *c* 0.35).

Acetylation of compound **3**. Compound **3** (5 mg) in pyridine was heated with Ac_2O over a water bath for 2 hr. After usual work up 5 mg of **4** was obtained (for ^{1}H NMR see Table 1).

n-Decyl-α-D-glucopyranoside. Colourless crystals, mp 137°, IR ν_{max}^{KBr} cm^{-1}: 3500–3100 (OH), 2900–2840 (CH), 1440 and 722 (CH_2), 1125 (C–O–C); ^{1}H NMR (C_5D_5N): 0.75 (*t*, J = 7 Hz, $-O(CH_2)_9\underline{CH_3}$); 1.17 (*br m*, $-O(CH_2)_2(\underline{CH_2})_7CH_3$); 1.50 (*m* $OCH_2\underline{CH_2}(CH_2)_7CH_3$); 3.70 (*m*, $-O\underline{CH_2}(CH_2)_8CH_3$); 5.20–3.80 (overlapping multiplets, sugar protons). After acetylation, as described above this yielded a tetraacetate: viscous liquid, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900–2840 (CH), 1750–1700 (CO), 1490, 1360, ($COCH_3$), 1440, 722 (CH_2), 1125 (C–O–C), 1220 (RCO–O–R); ^{1}H NMR ($CDCl_3$): 0.90 (*t*, J = 7 Hz, $-O(CH_2)_9\underline{CH_3}$); 1.30 (*br m*, $-O(CH_2)_2(\underline{CH_2})_7CH_3$); 1.50 (*m*, $-OCH_2\underline{CH_2}(CH_2)_7CH_3$); 3.70 (*m*, $O\underline{CH_2}(CH_2)_8CH_3$), 2.20–2.10 (overlapping singlets, $-OCOCH_3$); 5.10 (*d*, J = 3.0 Hz, anomeric proton); 4.80–3.40 (overlapping multiplets, sugar protons). MS m/z (rel. int.): 488

$[M^+, C_{24}H_{40}O_{10}]$ (2), 415 $[M-CH_2OAc]^+$(20), 347 $[M-C_{10}H_{21}]^+$ (5), 330 $[M-C_{10}H_{22}O]^+$ (22), 281 (25), 262 (20), 159 (40), 145 (38), 109 (60), 83 (100), 60 (83). Methanolic acid hydrolysis (5% HCl) of *n*-decyl glucoside (10 mg) in usual way gave α-D-glucose (7 mg).

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