Phytochemical Investigation of *Artemisia pallens*

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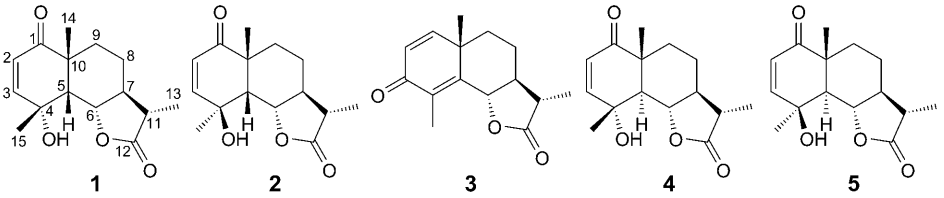
Two new eudesmanolides, pallensin (**1**) and 4-epipallensin (**2**), along with a known eudesmanolide, santonin, and a known flavone, 4-epivalgarin, have been isolated from the aerial part of *A. pallens*. Their structures were established by detailed spectral studies (1D and 2D) and by comparison of their spectroscopic data with those of known compounds.

Introduction. – *Artemisia pallens* WALLS ex DC, commonly known as *Davana*, is an aromatic herb found abundantly in humid habitats in the plains all over India. The chemical composition of the oil from *A. pallens* has been investigated by several research groups [1–3]. Further chemical investigation of the plant afforded two germacranolides [4][5]. Antidiabetic activity of the MeOH extract of the plant was reported by *Subramoniam et al.* [6].

In search for bioactive metabolites of plant origin, we have isolated two new eudesmanolides, pallensin (**1**) and 4-epipallensin (**2**), along with the known eudesmanolide santonin **3** for the first time from the aerial part of *A. pallens*.



Results and Discussion. – The aerial parts of *A. pallens* were extracted with acetone. The acetone extract (10 g) was subjected to column chromatography over silica gel (100–200 mesh) using solvents of increasing polarity from hexane to acetone. Eight fractions were collected. The fractions were pooled on the basis of similar TLC profiles, and they were further purified by repeated preparative TLC to yield two new and two known compounds.

Compound **1** was obtained as white crystalline solid. The mass spectrum displayed the molecular-ion peak at m/z 287 ($[M + Na]^+$) suggesting the molecular formula $C_{15}H_{20}O_4$. Characteristic bands at 3500, 1768, and 1678 cm^{-1} in the IR spectrum implied

the presence of OH, γ-lactone, and a conjugated CO group, respectively. The ^{1}H-NMR spectrum (*Table*) showed a *doublet* at δ(H) 1.25 ($J = 6.8$) for the Me(13) H-atoms. The configuration of the vicinal H–C(11), which resonated at δ(H) 2.35 ($dq, J = 13.2, 6.4$), was deduced from its coupling constant with H–C(7).

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2** (at 400 and 100 MHz, resp., in $CDCl_3$)

Position	δ(H)		δ(C)	
	1	**2**	**1**	**2**
1			201.66	203.10
2	5.89 ($d, J = 10.4$)	5.90 ($d, J = 10.4$)	125.64	125.32
3	6.60 ($d, J = 10.4$)	6.51 ($d, J = 10.4$)	151.64	150.20
4			70.05	68.19
5	2.42 ($d, J = 11.6$)	2.03 ($d, J = 11.2$)	54.50	51.05
6	4.15 ($t, J = 10.8$)	4.34 ($t, J = 10.8$)	79.55	79.19
7	1.63–1.74 (m)	1.63–1.74 (m)	52.44	52.32
8	1.95–2.07 (m), 1.41–1.53 (m)	1.94–2.00 (m), 1.50–1.55 (m)	22.64	22.84
9	1.95–2.07 (m), 1.52–1.58 (m)	1.94–2.00 (m), 1.52–158 (m)	34.16	32.50
10			46.19	45.93
11	2.35 ($dq, J = 13.2, 6.4$)	2.34 ($dq, J = 6.8, 5.6$)	40.64	40.83
12			178.23	178.96
13	1.25 ($d, J = 6.8$)	1.25 ($d, J = 6.8$)	12.42	12.48
14	1.20 (s)	1.33 (s)	19.71	20.59
15	1.55 (s)	1.60 (s)	23.77	31.69

Two *singlet*s appeared at δ(H) 1.20 and 1.55 for Me(14) and Me(15), respectively. Signals at δ(H) 5.89 ($d, J = 10.4$) and 6.60 ($d, J = 10.4$) were observed for the olefinic H-atoms at C(2) and C(3), respectively. A ^{13}C-NMR resonance for C(3) at δ(C) 201.66 is consistent with an α,β-enone. The downfield nature of the C(14) angular Me group (δ(H) 1.20) suggested the presence of an oxo group at C(1). The *triplet* of the lactonic H-atom at C(6), appearing at δ(H) 4.15 ($t, J = 10.8$), clearly indicated that C(5) and C(7) are not substituted with an oxygenated functional group.

The COSY spectrum specified correlations between H–C(2) (δ(H) 5.89 ($d, J = 10.4$)) and H–C(3) (δ(H) 6.60 ($d, J = 10.4$)), H–C(5), H–C(6), H–C(7) (δ(H) 2.42 ($d, J = 11.60$), 4.15 ($t, J = 10.8$), and 1.70 (m), resp.). COSY Correlations clearly indicated the presence of the system H–C(7)/H–C(11)/Me(13).

A HMBC experiment revealed the following correlations H–C(2) → C(15), C(5), and C(6); H–C(3) → C(15), C(10), and C(4); H–C(6) → C(11), C(10), C(5), and C(4); H–C(5) → C(14), C(15), C(9), C(10), C(11), C(10), C(5), and C(4); H–C(5) → C(14), C(15), C(9), C(10), C(7), C(4), and C(6), and H–C(11) → C(13), C(7), and C(8) (*Fig. 1*).

In the NOESY experiment, H–C(6) showed correlations with H–C(5), Me(14), and Me(15), but not with H–C(7) and Me(13). These correlations suggest that Me(14), Me(15), and H–C(5) are β-oriented, and H–C(7) and Me(13) are all α-oriented. The above spectroscopic data confirm the structure and configuration of compound **1** (*Fig. 2*).

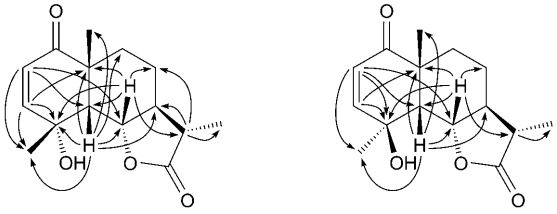


Fig. 1. *HMBCs of compounds* **1** *and* **2**

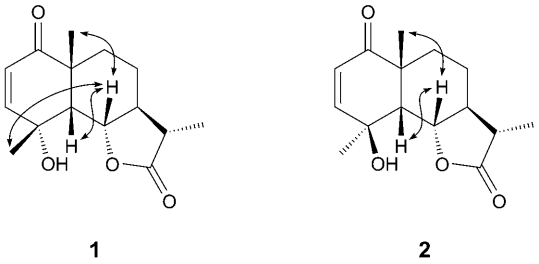


Fig. 2. *NOESY Correlations of compounds* **1** *and* **2**

Compound **2** was obtained as a viscous liquid. The mass spectrum showed the molecular-ion peak at *m/z* 287 ($[M+\mathrm{Na}]^+$) suggesting the molecular formula $C_{15}H_{20}O_4$.

The structure of **2** was found to be similar to that of compound **1**. The IR spectrum indicated the presence of OH and CO groups in the molecule. The ^{1}H- and ^{13}C-NMR data of **2** (*Table*) suggest that its structure is similar to that of compound **1**. The presence of a C=C bond between C(2) and C(3) and a OH group at C(4) in ring *A*, and a Me group at C(11) in ring *C* was evidenced.

The COSY spectrum indicated correlations between H–C(2) (δ(H) 5.90 (*d*, $J =$ 10.4)) and H–C(3) (δ(H) 6.51 (*d*, $J = 10.4$)), H–C(5), H–C(6), H–C(7) (δ(H) 2.03 (*d*, $J = 11.2$, 4.34 (*t*, $J = 10.8$), and 1.68 (*m*), resp.). COSY Correlations clearly revealed the presence of the system H–C(7)/H–C(11)/Me(13).

A HMBC experiment disclosed following correlations H–C(2) → C(15), C(5), C(4), and C(6); H–C(3) → C(10) and C(4); H–C(6) → C(8), C(12), C(10), C(5), and C(4); H–C(5) → C(14), C(15), C(10), C(7), and C(6); and H–C(11) → C(13) (*Fig. 1*).

A NOESY experiment indicated correlations between H–C(6) (δ(H) 4.34 (*t*, $J =$ 10.8)), H–C(5) (δ(H) 2.03 (*d*, $J = 11.2$)), and Me(14) (δ(H) 1.33 (*s*)). This confirmed the β-configuration of OH group at C(4). Consequently, the structure of compound **2** was established as the *C(4)*-epimer of **1** (*Fig. 2*).

Similarity in ^{1}H- and ^{13}C-NMR data of both pallensin (**1**) and 4-epipallensin (**2**) was observed to those of valgarin (**4**) and 4-epivalgarin (**5**) [7], except for the H-atom at C(5). The NOESY experiments unambiguously established the β-configuration of the H-atom at C(5) of **1** and **2**. Although the coupling constants of H–C(5) of **1** and **2** were

11.6 and 11.0 Hz, respectively, indicating a *trans*-relation to H–C(6), a NOE experiment showed clearly a strong correlation between H–C(5) and H–C(6). A similar large coupling ($J = 9.0$) for the α-oriented H-atoms at C(6) and C(7) were observed before in pseudoguaianolides [8].

A known compound, santonin (**3**), was also isolated and purified from the aerial part of *A. pallens*. The structure of compound **3** was assigned by detailed spectral data, and comparison with those reported in the literature.

Conclusions. – On the basis of detailed 1D- and 2D-spectral studies and comparison with compounds valgarin (**4**) and 4-epivalgarin (**5**), we have deduced the structure and configuration of the novel compounds pallensin (**1**) and 4-epipallensin (**2**). This is the first time that these compounds have been isolated from *A. pallens*.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 100–200 mesh). TLC: glass silica gel-precoated (*60 F_{254}*; *Merck*) plates; spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH with vanillin, followed by heating at 100°; hexane/acetone 50:50 and benzene/acetone 90:10 were used as developing solvents. M.p.: *Büchi B-545* apparatus. IR Spectra: *Perkin-Elmer FTIR RXI* spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: *Bruker 400* MHz, in $CDCl_3$. MS: *Perkin-Elmer SCIEX*, *API 3000* LC/MS/MS.

Plant Material. The plant *A. pallens* was collected from Jejuri, Maharashtra, India, in December 2006. It was authenticated at the Botanical Survey of India, Pune (No. BSI/WC/Tech/2008/1059).

Extraction and Isolation. The aerial parts of *A. pallens* were shade-dried and milled to coarse powder. Extraction of the powdered material (1.0 kg) was carried out with acetone (4 l × 4 h) at r.t. with continuous stirring. The combined filtered extracts were concentrated under reduced pressure at 40° to afford a brown gummy residue (25.5 g). The residue (10 g) was subjected to CC (SiO_2 (100–200 mesh); solvents of increasing polarity from hexane to acetone). Eight fractions, *A–H*, were eluted with a hexane/acetone gradient: 100% hexane, 90:10, 80:20, 60:40, 50:50, 40:60, 20:80, and 100% acetone, resp. *Frs. D* and *E* were combined (1.0 g) and subjected to CC with solvents of increasing polarity from hexane to acetone. Four subfractions were collected, *1–4*. *Subfr. 4* (110 mg) was recrystallized using a mixed solvent system (hexane/acetone). This was further purified by prep. TLC to yield a new compound **1** (55 mg). *Subfr. 2* (120 mg) was further purified by prep. TLC to yield another new compound **2** (8 mg). *Subfr. 1* (270 mg) was further crystallized from hexane/acetone to yield a known compound **3** (240 mg).

*(3a*S*,5a*R*,9*R*,9a*R*,9b*S*)-3a,5,5a,9,9a,9b-Hexahydro-9-hydroxy-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2,6(3*H*,4*H*)-dione* (**1**). White crystalline solid. M.p. 176–177°. $[\alpha]_D^{25} = +56.8$ ($c = 0.1$, $CHCl_3$). IR (KBr): 3500, 2930, 1767, 1677, 1455, 1173, 993. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table*. LC/MS: 287 ($[M + Na]^+$).

*(3a*S*,5a*R*,9*S*,9a*R*,9b*S*)-3a,5,5a,9,9a,9b-Hexahydro-9-hydroxy-3,5a,9-trimethylnaphtho[1,2-*b*]furan-2,6(3*H*,4*H*)-dione* (**2**). Colorless viscous mass. $[\alpha]_D^{25} = +77.2$ ($c = 0.1$, $CHCl_3$). IR: (KBr): 3517, 2931, 1771, 1676, 1460, 1108, 1018. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table*. LC/MS: 287 ($[M + Na]^+$).

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