Pseudoguaianolides from the Flowers of *Parthenium hysterophorus*[1])

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Two new pseudoguaianolide-type sesquiterpene lactones, named deacetyltetraneurin A (**3**) and hysterone E (**4**), were isolated from flowers of the plant *Parthenium hysterophorus* L., along with the seven known compounds parthenin (**1**), coronopilin, tetraneurin A, 8-β-hydroxycoronopilin, scopoletin, hysterone D, and conchasin A (**2**). The structures of all the compounds were deduced by means of elemental analysis and extensive spectroscopic (1D and 2D NMR) studies, and confirmed for **3** and **4** by X-ray crystallographic analysis.

1. Introduction. – *Parthenium hysterophorus* L. (Compositae) is an obnoxious weed accidentally introduced by mankind through seeds imported along with PL 480 wheat grain from the USA. The plant is commonly called 'Congress grass', 'Chatak chandni', 'White top', 'Gajar grass', or Parthenium. The plant is known to cause contact dermatitis and allergic rhinitis in animals [1], and to possess significant allelopathic properties [2]. Earlier investigators have reported the isolation of several pseudoguaianolide-type sesquiterpene lactones [3–8] and flavones [9] from this plant. These sesquiterpene lactones generally contain an α-methylene-γ-lactone moiety which plays a vital role in their bioactivity. The interesting cytotoxic activities of the extracts and constituents of the plant have been explored by several researchers [10–12]. For example, the MeOH extract of *P. hysterophorus* flowers has been reported to have antitumor effects in host mice bearing transplantable lymphocytic leukemia [13].

In continuation of our work on the constituents of different parts of *P. hysterophorus* [14][15], we describe here the isolation and structure determination of two new pseudoguaianolide-type sesquiterpene lactones along with seven known compounds from flowers of the plant.

2. Results and Discussion. – Extraction of the flowers of *P. hysterophorus* with CH_2Cl_2/MeOH 1:1 at room temperature yielded a mixture that was subjected to column chromatography (silica gel, hexane/AcOEt/acetone (gradient)). Thus the known compounds parthenin (**1**) [3], coronopilin [5], tetraneurin A [5], 8-β-hydroxycoronopilin [7], scopoletin [16], hysterone D [15], and conchasin A (**2**) [17], and the new compounds deacetyltetraneurin A (**3**) and hysterone E (**4**) were isolated (*Fig. 1*).

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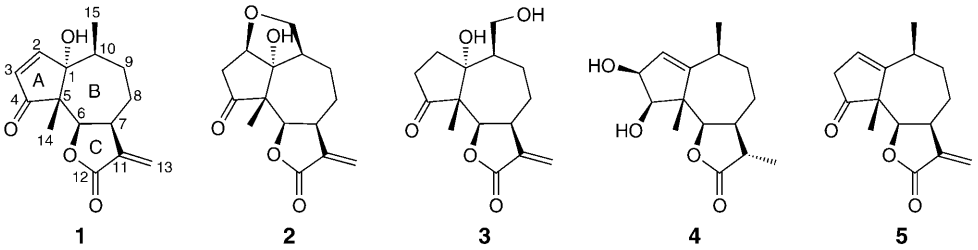

Fig. 1. *Structures of compounds* **1**–**5**[2])

Compound **3** was obtained as optically active colorless crystals. The molecular formula $C_{15}H_{20}O_5$ was determined from its elemental analysis and confirmed in the EI-MS with a molecular-ion peak at *m/z* 280 ($M^{+\bullet}$). The IR absorption at 3425 cm^{-1} indicated the presence of OH groups, and a band at 1721 cm^{-1} that of C=O groups of an α-methylene-γ-lactone and cyclopentanone moiety. The proposed structure was further corroborated by X-ray crystallography (*Fig. 2*, *a*). Although compound **3** has been prepared before as a semisynthetic derivative of tetraneurin A [18], our study represents the first report of its isolation from natural sources.

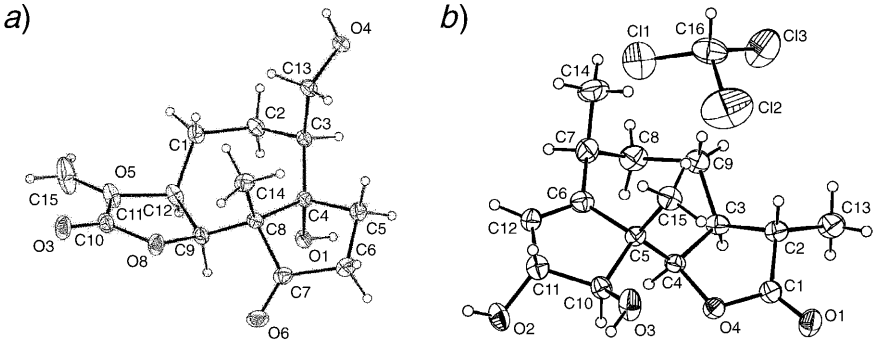

Fig. 2. *Thermal ellipsoid plot of the X-ray structure of compounds* **3** (*a*) *and* **4** (*b*)[2])

The ^{1}H-NMR spectrum of compound **3** (*Table*), when compared with that of the known compounds tetraneurin A [5] and conchasin A (**2**) [17], indicated that these compounds are structurally closely related. The diagnostic exocyclic-methylene signals at δ 6.18 (*d*, *J* = 2.0 Hz) and 5.63 (*d*, *J* = 2.0 Hz), a lactone-proton signal at δ 4.82 (*d*, *J* = 8.4 Hz) and the absence of signals due to an α,β-unsaturated ketone moiety indicated the presence of a saturated ring A and an α-methylene-γ-lactone moiety in **3**. The ^{1}H-NMR spectrum of **3**, in comparison with that of **2**, showed the presence of an oxygenated CH_2(15)[2]) moiety at δ 3.71 (*dd*, *J* = 10.0, 1.2 Hz, 1 H) and 3.62 (*t*, *J* = 10.0 Hz, 1 H), but the absence of the H–C(2) signal typical of an oxygenated C(2) suggested that C(15) of **3** bears an OH group and not an ether function. On the other hand, the ^{1}H-NMR spectrum of **3**, when compared with that of tetraneurin A [5], showed similar spectral patterns but no acetyl signal at δ 2.04, indicating that **3** is deacetylated tetraneurin A. Due to the paucity of the sample, the ^{13}C-NMR spectrum of compound **3** could not be recorded.

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[2]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Table. *[1]H- and [13]C-NMR Data of Compounds* **2**–**4**[2]). At 300 MHz for ^{1}H and 75 MHz for ^{13}C; δ in ppm, J in Hz.

	2 ($(CD_3)_2CO$)		**3** ($CDCl_3/CD_3OD$)	**4** ($CDCl_3$)	
	δ(H)	δ(C)	δ(H)	δ(H)	δ(C)
C(1)		86.0			151.8
H–C(2) or CH_2(2)	4.19 (*d*, *J* = 7.2)	81.7	2.01–1.38 (*m*, 2 H)[a])	5.82 (*t*, *J* = 1.6)	129.1
CH_2(3) or H–C(3)	2.65 (*dd*, *J* = 18.0, 1.2, H_α), 2.45 (*dd*, *J* = 18.0, 3.0, H_β)	47.7	2.55–2.45 (*m*, 2 H)	4.57 (*dd*, *J* = 5.6, 1.6)	88.4
C(4) or H–C(4)		211.2		4.08 (*dd*, *J* = 5.6, 1.6)	87.9
C(5)		57.5			55.3
H–C(6)	5.21 (*d*, *J* = 8.0)	78.2	4.82 (*d*, *J* = 8.4)	4.48 (*d*, *J* = 8.1)	79.4
H–C(7)	3.59–3.51 (*m*)	42.4	3.38 (*m*)	2.32–1.60 (*m*)	40.4
CH_2(8)	2.31–1.80 (*m*)	21.7	2.55–2.45 (*m*, H_α), 2.01–1.38 (*m*, H_β)[a])	1.90–1.80 (*m*)	22.4
CH_2(9)	2.31–1.80 (*m*)	38.9	2.01–1.38 (*m*)[a])	1.61–1.49 (*m*)	29.7
H–C(10)	2.58–2.42 (*m*)	21.6	2.01–1.38 (*m*)[a])	3.02–2.83 (*m*)	35.9
C(11) or H–C(11)		138.1		2.32–2.16 (*m*)	47.2
C(12)		170.2			170.2
CH_2(13) or Me(13)	6.12 (*d*, *J* = 2.0, H_a), 5.54 (*d*, *J* = 2.0, H_b)	116.1	6.18 (*d*, *J* = 2.0, H_a), 5.63 (*d*, *J* = 2.0, H_b)	1.16 (*d*, *J* = 7.2)	14.0
Me(14)	1.21 (*s*)	12.7	0.98 (*s*)	1.12 (*s*)	15.8
CH_2(15) or Me(15)	4.28 (*dd*, *J* = 12.2, 0.8, $H_{\alpha'}$), 3.65 (*dd*, *J* = 12.2, 0.8, $H_{\beta'}$)	73.8	3.71 (*dd*, *J* = 10.0, 1.2, H_α), 3.62 (*t*, *J* = 10.0, H_β)	1.14 (*d*, *J* = 7.2)	23.5

[a]) Six protons (CH_2(2), H_β–C(8), CH_2(9), and H–C(10)) resonate in the described region as *m*.

Compound **4** was obtained as optically active colorless crystals. The molecular formula $C_{15}H_{22}O_4$ was assigned to the compound from its elemental analysis and EI-MS, which showed a significant peak at *m/z* 284 ($[M+H_2O]^+$) and was consistent with its ^{13}C-NMR spectrum. The IR spectrum showed absorption bands at 1750 and 3450 cm^{-1}, indicating the presence of a C=O and OH groups, respectively. The structure of **4** was established from its ^{1}H-NMR spectrum which was compared to that of parthenin (**1**) [3] and other earlier reported constituents of the plant. However, the compound did not resemble any of them with the exception of neoambrosin (**5**) [19], suggesting that the two compounds are structurally related.

Considering the spectral evidences, the structure of **4**, named hysterone E, was deduced as 11,13-dihydro-4-deoxo-3,4-dihydroxyneoambrosin, representing a novel pseudoguaianolide. The proposed structure was corroborated by X-ray crystallography (*Figure 2*, *b*).

In the ^{1}H-NMR spectrum of **4** (*Table*), H–C(2)[2]) resonated at δ 5.82, as the corresponding proton in neoambrosin (**5**) [19]. Further, the spectrum of **4** also showed two 1-H *dd* at δ 4.57 and 4.08 (J = 5.2, 1.6 Hz), indicating that these protons are at vicinal oxygenated C-atoms. The downfield chemical shift of the

former proton was attributed to its allylic position. A deuterium-exchange experiment confirmed the presence of 2 OH groups. In addition, the ^{1}H-NMR spectrum of **4** showed a signal at δ 4.48 (*d*, $J=8.1$ Hz, 1 H), suggesting a γ-lactone unit, which is characteristic for a pseudoguaianolide [3]. The absence of an exocyclic $CH_2(13)=C(11)$ group in conjugation with the lactone C=O and the presence of an extra Me *d* at δ 1.16 ($J=7.2$ Hz, Me(13)) and a *m* at δ 2.32–2.16 (H–C(11) indicated a 11,13-dihydrolactone moiety. The *m* at δ 3.02–2.83 (1 H) and the *d* at δ 1.14 ($J=7.0$ Hz, Me) resembled the signals of Me–CH(10) of neoambrosine (**5**) [19]. The ^{13}C-NMR spectrum of **4** showed the presence of 15 non-equivalent C-atoms indicating that **4** is a sesquiterpene. Signals for the lactone C=O at δ 170.2, for two olefinic C-atoms at δ 151.8 and 129.1, for three OCH groups at δ 88.4, 87.9, and 79.4, and for three Me groups at δ 23.5, 15.8, and 14.0 were present. The important point is that the ^{13}C-NMR spectrum of **4** did not show any signal in the downfield region beyond δ 170.2, suggesting [1] the absence of a 4-keto group. Instead, it showed an extra O-bearing CH group at δ 88.4 suggesting the reduction of a keto to a hydroxy group. The ^{1}H-NMR data indicated (see above) that the O-bearing C-atoms are adjacent which was confirmed by the ^{1}H,^{1}H-COSY correlation H–C(3)/H–C(4). C(3) appeared in the ^{13}C-NMR spectrum at δ 88.4. Further, the HMBC correlations H–C(2) (δ 5.82, *t*, $J=1.6$ Hz)/C(4) (δ 87.9), H–C(3)/C(1) (δ 151.8), and H–C(10) (δ 3.02–2.83, *m*)/C(2) (δ 129.1) suggested the presence of a cyclopentene moiety (ring A) containing two vicinal OH groups and the correlations H–C(6)/C(12) (δ 170.2) and C(14) (δ 15.8) that of a lactone moiety (ring C).

The configuration of **4** was determined by analysis of the relevant ^{1}H-NMR coupling constants and NOESY correlations (*Fig. 3*). In the NOESY plot, H–C(3) at δ 4.57 (*dd*, $J=5.6$, 1.6 Hz) was related to H–C(4) at δ 4.08 (*dd*, $J=5.6$, 1.6 Hz) indicating that these protons are on the same side of the molecule. Further, H–C(6) (δ 4.48, *d*, $J=8.1$ Hz) showed a correlation with H–C(4) at δ 4.08 (*dd*, $J=5.6$, 1.6 Hz) suggesting that H–C(3) and H–C(4) are on the α-side since, in parthenin pseudoguaianolides, H–C(6) is α-positioned. H–C(3) and H–C(4) did not correlate with Me(14) and Me(15) indicating that these Me groups are positioned on the opposite β-face. Consequently the OH groups at C(3) and C(4) are β-positioned. Me(13) is on the α-side as H–C(11) (δ *ca.* 2.24, *m*) showed correlation with Me(15) which is β-positioned in parthenin pseudoguaianolides.

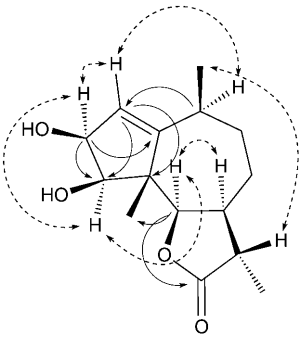

Fig. 3. *Selected HMBC* (→) *and NOESY* (←–––→) *correlations of compound* **4**

Conchasin A (**2**) was isolated as colorless viscous mass. This compound has been isolated before from *Parthenium confertum* [17] but was now isolated from *P. hysterophorus* for the first time, and its previously unknown ^{13}C-NMR data are included in the *Table*.

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Experimental Part

General. Column chromatography (CC): silica gel (*BDH* 100–200 mesh). TLC: silica gel GF_{254}; visualization under UV light, with I_2 vapors, or by spraying with 10% H_2SO_4 in MeOH and subsequently heating on a hot plate. Melting points: *Büchi 510* instrument; uncorrected. Optical rotations: *Jasco DIP-360* digital polarimeter. IR Spectra: *Perkin-Elmer* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR: *Varian Gemini-200-MHz*, *Bruker 300-MHz*, *Unity 400-MHz* and *Varian 500-MHz* spectrometer. MS: *Micromass Quattro-LC* (LSI) and *Micromass VG-7070H* (EI, 70 eV) spectrometer; *m/z* (rel.%).

Plant Material. The flowers of *P. hysterophorus* were collected at Balapur village near Hyderabad district (Andhra Pradesh) in August, 2003, and were botanically identified. A voucher specimen (IICT-52003) was deposited in the herbarium of the Indian Institute of Chemical Technology.

Extraction and Isolation. The shade dried plant material (2 kg) was extracted thrice with CH_2Cl_2/MeOH 1:1 at r.t. Each extraction was performed for 3 days with 4 l of solvent. The combined extract was evaporated: brown gummy residue (34 g, 1.7% based on dry weight). A part of the residue (2 g) was preserved, and the remaining material (32 g) was subjected to CC (silica gel (100–200 mesh), gradient, hexane/AcOEt/acetone of increasing polarity: *Fractions A–F. Fr. B* (15 g) was resubjected to CC (silica gel, gradient hexane/AcOEt): parthenin (**1**; 13.3 g, 0.665%) [3]. Coronopilin (120 mg, 0.006%) [5] was obtained from *Fr. C* (2 g). *Fr. D* (7 g) was further purified by CC (silica gel, gradient hexane/AcOEt): tetraneurin A (42 mg, 0.0021%) [5], 8-β-hydroxycoronopilin (61 mg, 0.0031%) [7], scopoletin (12 mg, 0.0006%) [16], and hysterone D (36 mg, 0.0018%) [15]. *Fr. E* (several spots on TLC) was subjected to CC (silica gel, gradient $CHCl_3$/MeOH; 100-ml fractions). *Fr. E.4–7* yielded conchasin A (**2**; 23 mg, 0.0012%) [17], *Fr. E.10–13* deacetyltetraneurin A (**3**; 3 mg, 0.0002%), and *Fr. E.15–19* hysterone E (**4**; 14 mg, 0.0007%). All of these constituents were found (by TLC) to be present in the crude extract of the plant material.

Conchasin A (**2**): Colorless viscous mass. $[\alpha]_D^{25} = -29.40$ ($c = 1.05$, EtOH). IR (KBr): 3495, 1747, 1631, 1373, 1229. ^1H- and ^{13}C-NMR: *Table.* LSI-MS: 279 (40, $[M+1]^+$), 296 (100, $[M+H_2O]^+$). Anal. calc. for $C_{15}H_{18}O_5$: C 63.74, H 6.52; found: C 63.66, H 6.59.

Deacetyltetraneurin A (=(*3a*S,*6*R,*6a*R,*9a*S,*9b*R)*-3,3a,4,5,6,6a,7,8,9a,9b-Decahydro-6a-hydroxy-6-(hydroxymethyl)-9a-methyl-3-methyleneazuleno[4,5*-b]*furan-2,9-dione*; **3**): Colorless crystals. M.p. 203–205°. $[\alpha]_D^{25} = -12.04$ ($c = 0.25$, MeOH). IR (KBr): 3425, 1721, 1678, 1380, 1026. ^1H-NMR: *Table.* EI-MS: 280 (18, $M^{+\cdot}$), 149 (92), 97 (28), 71 (45), 57 (100). Anal. calc. for $C_{15}H_{20}O_5$: C 64.27, H 7.19; found: C 64.19, H 7.24.

Hysterone E (=*(3*S,*3a*S,*6*S,*8*S,*9*R,*9a*S,*9b*R*)-3a,4,5,6,7,8,9,9a,9b-Octahydro-8,9-dihydroxy-3,6,9a-trimethylazuleno[4,5*-b]*furan-2(3*H*)-one*; **4**): Colorless crystals. M.p. 198–201°. $[\alpha]_D^{25} = -14.84$ ($c = 0.1$, MeOH). IR (KBr): 3450, 1750, 1300. ^1H- and ^{13}C-NMR: *Table.* EI-MS: 284 (8 $[M+H_2O]^+$), 167 (10), 149 (38), 141 (44), 69 (46), 57 (92), 43 (100). Anal. calc. for $C_{15}H_{22}O_4$: C 63.81, H 7.85; found: C 63.76, H 7.79.

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