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

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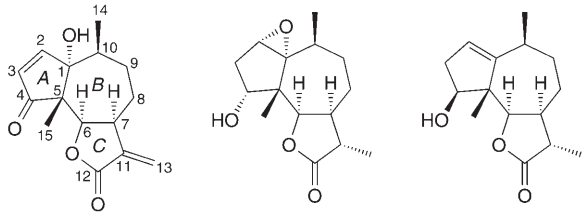
Chemical investigation on two samples of the flowers of *Parthenium hysterophorus* afforded two new pseudoguaianolides (one from each sample) together with several known constituents. The structures of the new compounds were established by extensive spectroscopic (1D- and 2D-NMR) studies. The X-ray crystallographic analysis of one of these compounds was also accomplished.

Introduction. – *Parthenium hysterophorus* L. (Compositae), an obnoxious weed, grows wild in different parts of India. It creates contact dermatitis and allergic rhinitis in animals [1]. It also exhibits allelopathic property [2–4]. The methanolic extract of the flowers showed significant antitumour activity [5]. Earlier chemical examination of the plant reported the isolation of parthenin (**1**) [6], a pseudoguaianolide, as a major constituent together with some related sesquiterpenes and flavones [7–13]. Parthenin (**1**) has been found to possess allelopathic and cytotoxic properties [2][3][14]. In continuation of our work [11–13][15] on *P. hysterophorus*, we examined the chemical constituents of two samples of its flowers and isolated the new pseudoguaianolides **2** (from the first sample) and **3** (from the second sample). Here we report the structure elucidation of these two new compounds.



1 **2** **3**

Results and Discussion. – Compound **2** was obtained as colorless crystals. Its molecular formula $C_{15}H_{22}O_4$ was assigned from elemental analysis, the ^{13}C-NMR spectrum, and ESI-MS (*m*/*z* 289, $[M+Na]^+$). The IR spectrum indicated the presence

[1]) Part 64 in the series 'Studies on Phytochemicals'.

of OH and C=O groups. The structure of the compound was derived from its ^{1}H- and ^{13}C-NMR spectral data (*Tables 1* and *2*), which were compared to those of parthenin (**1**). The H- and C-atoms were clearly assigned with the help of 2D-NMR (DQF-COSY, NOESY, HSQC, and HMBC; *Fig. 1*) and DEPT experiments.

Table 1. *^{1}H-NMR Data of* **1**–**3**[a]). δ in ppm, J in Hz.

	1	**2**	**3**
2	7.48 (*d*, *J* = 6.2)	3.43 (br. *s*)	5.38 (*t*, *J* = 2.0)
3	6.15 (*d*, *J* = 6.2)	2.23 (*dd*, *J* = 14.0, 6.0), 2.12–2.02 (*m*)	2.53 (*ddd*, *J* = 14.0, 8.0, 2.0), 2.20 (*ddd*, *J* = 14.0, 8.0, 2.0)
4	–	3.62 (br. *d*, *J* = 6.0)	4.10 (*t*, *J* = 8.0)
6	4.98 (*d*, *J* = 8.2)	5.08 (*d*, *J* = 8.5)	4.39 (*d*, *J* = 8.6)
7	3.51–3.41 (*m*)	2.66–2.58 (*m*)	2.87–2.68 (*m*)
8	2.37–2.18 (*m*)	2.12–2.02 (*m*), 1.92–1.79 (*m*)	1.66–1.60 (*m*)
9	1.88–1.80 (*m*), 1.66–1.60 (*m*)	1.92–1.79 (*m*), 1.79–1.73 (*m*)	1.74–1.66 (*m*), 1.28–1.22 (*m*)
10	2.14–2.06 (*m*)	1.92–1.79 (*m*)	2.87–2.68 (*m*)
11	–	2.49–2.39 (*m*)	2.87–2.68 (*m*)
13	6.24 (*d*, *J* = 2.0), 5.56 (*d*, *J* = 2.0)	1.28 (*d*, *J* = 7.0)	1.20 (*d*, *J* = 7.0)
14	1.24 (*s*)	1.09 (*s*)	1.21 (*s*)
15	1.12 (*d*, *J* = 7.0)	1.22 (*d*, *J* = 7.0)	1.18 (*d*, *J* = 7.0)
OH	–	–	1.83 (br. *s*)

[a]) The spectra were measured in $CDCl_3$, and the spectral data were assigned on the basis of DQF-COSY, NOESY, and HMBC experiments.

Table 2. *^{13}C-NMR Data of* **1**–**3**[a]). δ in ppm.

	1	**2**	**3**
1	84.8	72.7	151.5
2	163.4	62.6	124.0
3	131.5	35.0	36.2
4	211.2	78.7	78.8
5	59.2	52.7	53.3
6	78.8	79.2	84.9
7	44.7	45.9	40.8
8	28.4	23.2	20.6
9	30.2	28.2	28.9
10	40.0	37.6	35.9
11	140.5	41.7	39.3
12	170.8	179.2	179.9
13	121.6	14.8	10.3
14	17.7	16.9	15.4
15	18.2	17.7	21.9

[a]) The spectra were run in $CDCl_3$, and the spectral data were assigned on the basis of DEPT, HSQC, and HMBC experiments.

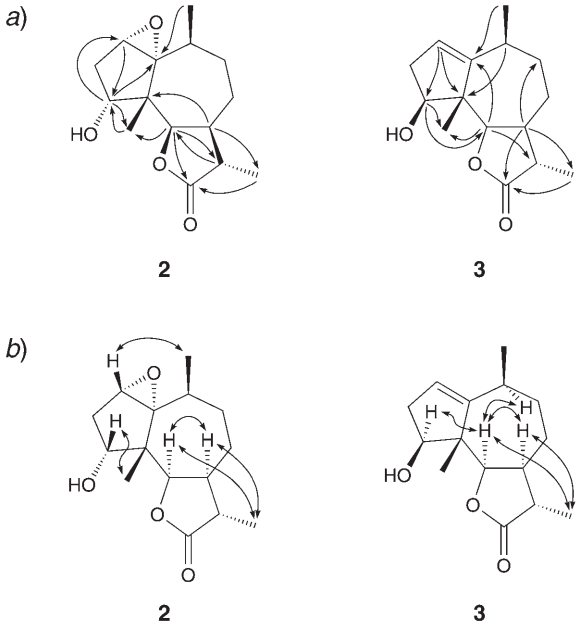


Fig. 1. *a*) *Selected HMBC* (→) *and b*) *NOESY* (↔) *correlations of* **2** *and* **3**

The spectra suggested that the structure of **2** was related to that of **1**, but the rings *A* and *C* of the two compounds were different. In **2**, the ring *A* contained no C=C bond. The H-atoms of this ring appeared at δ(H) 3.43 (br. *s*, H–C(2)), 2.23 (*dd*, *J* = 14.0, 6.0, H_a–C(3)), 2.12–2.02 (*m*, H_b–C(3)), and 3.62 (br. *s*, *J* = 6.0, H–C(4)) (*Table 1*). On the basis of these spectral data as well as DQF-COSY observations, an epoxide ring has been placed at C(1), C(2), and a OH group at C(4). In ring *C*, the exocyclic C=C bond was replaced by a Me group (δ(H) 2.49–2.39 (*m*, H–C(11)) and 1.28 (*d*, *J* = 7.0, Me(13)). The DQF-COSY experiment clearly showed the presence of the system H–C(7) to H–C(11) and Me(13). The ^{13}C-NMR spectrum and DEPT experiment also supported the above modifications of the rings *A* and *C*. The three O-substituted C-atoms of ring *A*, C(1), C(2), and C(4), appeared at δ(C) 72.7, 62.6, and 78.7, respectively, while C(11) and C(13) of ring *C* resonated at δ(C) 41.7 and 14.8 respectively (*Table 2*). The HMBC experiment (*Fig. 1*,*a*) showed that H–C(2) was related to C(4), H–C(4) to C(1), C(2), and Me(15), H–C(6) to C(11), C(12), and Me(15), H–C(7) to C(5) and Me(13), and Me(14) to C(1). In the NOESY experiment (*Fig. 1*,*b*), H–C(2) showed correlation with Me(14), H–C(4) with Me(15), and H–C(6) with H–C(7) as well as Me(13). These correlations suggested that the epoxide at C(1), C(2), the OH group at C(4), as well as Me(13) have α-configuration. The structure and configuration of **2** were thus clearly established. The X-ray crystallographic analysis of the compound (*Fig. 2*) further confirmed its structure. The molecule has been presented in a molecular-graphics programme (*Fig. 3*).

Compound **3** was isolated as a white solid. Its molecular formula $C_{15}H_{22}O_3$ was assigned from elemental analysis, the ^{13}C-NMR spectrum, and the ESI-MS (*m*/*z* 273,

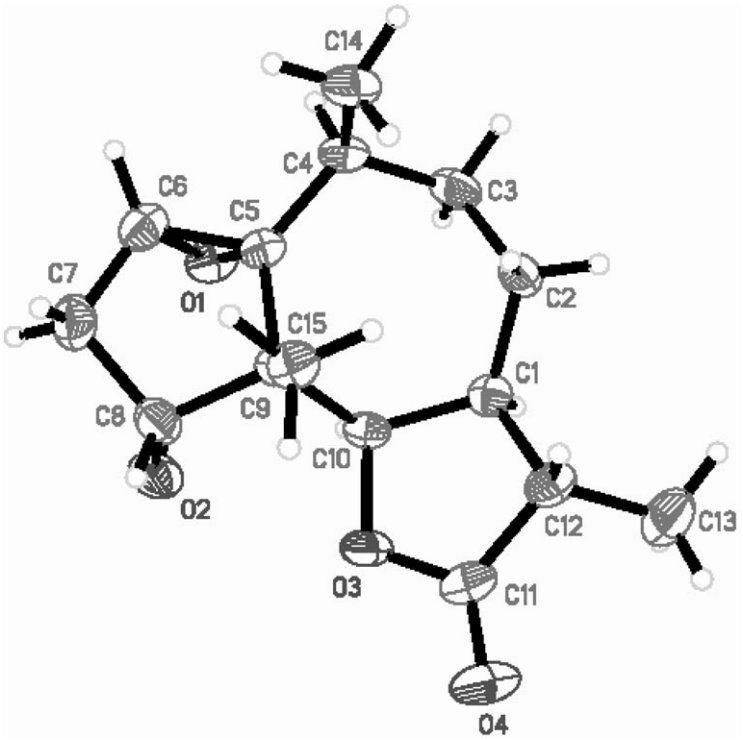


Fig. 2. *Crystal structure of compound* **2**

$[M+Na]^+$). The IR spectrum revealed the presence of OH and C=O groups. The ^{1}H- and ^{13}C-NMR spectral data of **3** (*Tables 1* and *2*) suggested that its structure was also related to that of **1**, with modifications at rings *A* and *C*. In **3**, ring *A* contained a $\Delta^{1,2}$ C=C bond, as well as a OH group at C(4). The positions of H–C(2) (δ(H) 5.38 (*t*, J = 2.0), CH_2(3) (δ(H) 2.53 (*ddd*, J = 14.0, 8.0, 2.0) and 2.20 (*ddd*, J = 14.0, 8.0, 2.0)), and H–C(4) (4.10 (*t*, J = 8.0)) in the ^{1}H-NMR spectrum, as well as the DQF-COSY experiment clearly suggested this. The $\Delta^{1,2}$ C=C bond was also observed earlier in a naturally occurring pseudoguaianolide, neoambrosin, and its derivatives [13]. Ring *C* of **3** contained a Me group at C(11) (δ(H) 1.20 (*d*, J = 7.0). The ^{13}C-NMR spectrum showed the positions of C(1), C(2), and C(4) at δ(C) 151.5, 124.0, and 78.8, respectively, while those of C(11) and C(13) were found at δ(C) 39.3 and 10.3, respectively. In the HMBC experiment (*Fig. 1*,*a*), H–C(2) showed correlations with C(4) and C(5), H–C(4) with C(6) and Me(15), H–C(6) with C(1), C(11), and Me(15), H–C(7) with C(12) and Me(13), and Me(14) with C(1) and C(9). A NOESY experiment (*Fig. 1*,*b*) indicated that H–C(6) was related to H–C(4), H–C(7), H–C(10), and Me(13), while H–C(7) was related to H–C(6) and Me(13). Consequently, the OH at C(4) was considered with β- and Me(13) with α-configuration. The structure and configuration of **3** were thus properly determined. The molecule has been presented in an energy minimized model (*Fig. 3*).

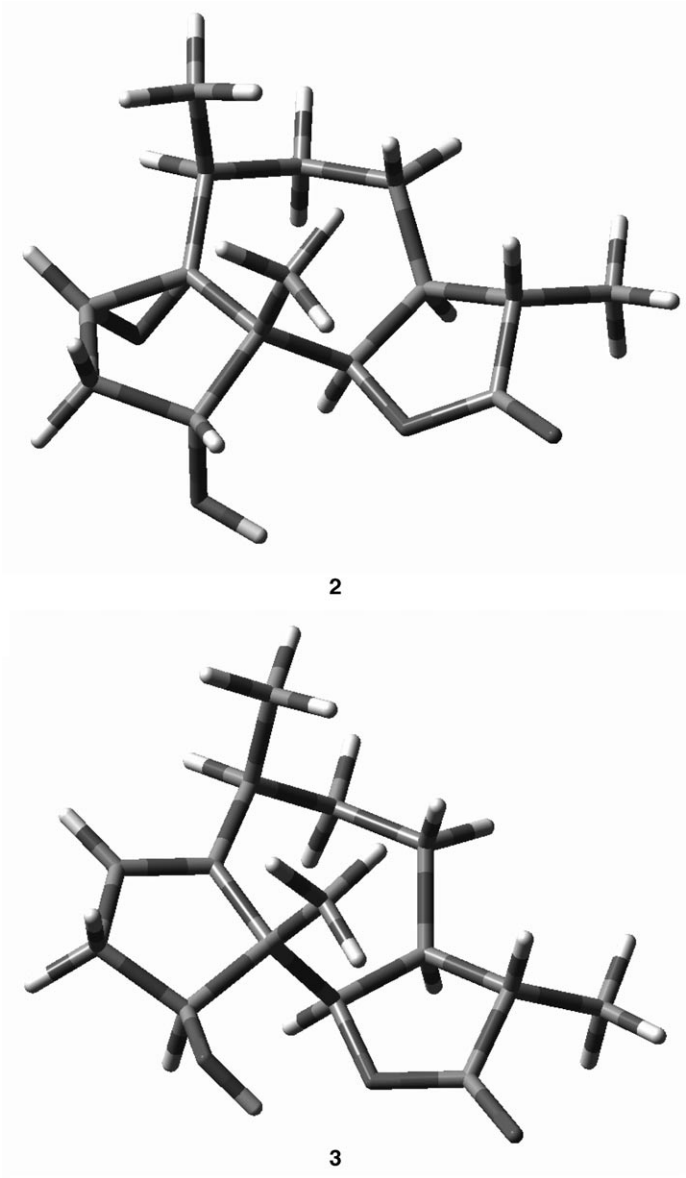


Fig. 3. *Presentation of the molecules* **2** *and* **3** *in a molecular graphics programme*

From the first sample of the flowers of *P. hysterophorus*, the known pseudoguaianolides parthenin, tetraneurin A, coronopilin, 8β-hydroxycoronopilin, hysterone D, 4β-acetoxy-11β,13-dihydroparthenin, and 3β-acetoxyneoambrosin, while from the second sample, the first four compounds along with conchasin A and deacetyl tetraneurin A were isolated. The known compounds were characterized by comparison of their

physical (m.p. and $[\alpha]_D$) and spectral (^{1}H- and ^{13}C-NMR, and MS) propererties with those of the authentic samples [11–14]. All the isolated compounds were originally present in the plant extracts as determined by TLC.

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Experimental Part

General. TLC: SiO_2 *GF 254*. Column chromatography (CC): SiO_2 (*BDH*, 100–200 mesh). M.p.: *Büchi-510* instrument, uncorrected. Optical rotations: *Jasco DIP-360* digital polarimeter. IR Spectra: *Perkin-Elmer RX1* FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: *Inova 400* (400 MHz). ESI-MS: *Agilent 1100* series *LC-MSD Trap SL*.

Plant Material. The first sample of the flowers of *P. hysterophorus* was collected from Medak district, Andhra Pradesh in August, 2005, while the second sample was collected from Adilabad district, Andhra Pradesh in September, 2005. Both samples were botanically identified. The voucher specimens (No.s IICT-5505 and 5507) were preserved in the herbarium of our institute.

Extraction and Isolation. First Sample. The air-dried and powdered plant material (2 kg) was extracted with $CHCl_3$ and MeOH (1:1, 4 l) at r.t. for 72 h. The extract was concentrated under reduced pressure to afford a brown gummy residue (34 g). This was subjected to CC over SiO_2 with solvents of increasing polarity from hexane to AcOEt. The following compounds were isolated according to the increasing order of polarity: coronopilin (120 mg), parthenin (5.97 g), tetraneurin A (42 mg), 8β-hydroxycoronopilin (61 mg), hysterone D (36 mg), 4β-acetoxy-11β,13-dihydroparthenin (28 mg), 3β-acetoxyneoambrosin (12 mg), and compound **2** (9 mg).

Second Sample. The shade dried and powdered flowers (2 kg) of the second sample were extracted for 72 h with $CHCl_3$ and MeOH (1:1, 5 l), and the extract (32 g) was subjected to CC over SiO_2 following the same procedure as that mentioned above. The following compounds were isolated according to the increasing order of polarity: coronopilin (98 mg), parthenin (7.24 g), tetraneurin A (35 mg), conchasin A (23 mg), compound **3** (11 mg), 8β-hydroxycoronopilin (49 mg), and deacetyltetraneurin A (26 mg).

*(3a*R*,3b*S*,4*R*,5a*S*,6a*R*,7*S*,9a*S*)-Octahydro-4-hydroxy-1,3b,7-trimethyl-4*H*-oxireno[1,8a]azuleno[4,5-*b*]furan-2(1*H*)-one* (**2**). Colorless crystals. M.p. 235–236°. $[\alpha]_D^{25} = -54.1$ ($c = 0.002$, $CHCl_3$). IR: 3450, 1716, 1464, 1182. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 289 ($[M+Na]^+$). Anal. calc. for $C_{15}H_{22}O_4$: C 67.67, H 8.27; found: C 67.34, H 8.35.

*(3a*S*,6*S*,9*S*,9a*S*,9b*R*)-3a,4,5,6,8,9,9a,9b-Octahydro-9-hydroxy-3,6,9a-trimethylazuleno[4,5-*b*]furan-2(3*H*)-one* (**3**). White solid. M.p. 155–156°. $[\alpha]_D^{25} = +23.6$ ($c = 0.003$, $CHCl_3$). IR: 3421, 1766, 1601, 1382, 1168. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 273 ($[M+Na]^+$). Anal. calc. for $C_{15}H_{22}O_3$: C 72.00, H 8.80; found: C 72.38, H 8.69.

X-Ray Crystallographic Analysis of **2**[2]). Compound **2** was crystallized from $CHCl_3$. The crystal system was orthorhombic and the space group was $P2_12_12_1$. The unit cell dimensions were as follows: $a =$ 6.9221(6), $b = 12.0503(10)$, $c = 16.5059$ (14) Å, and $\alpha = \beta = \gamma = 90°$. The reflections collected were 9959, and the independent reflections were 1354. The refinement method was full-matrix least squares on F^2.

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[2]) The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-687053. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk)).

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