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Pseudoguaianolides from the flowers of *Parthenium hysterophorus*☆

C. Ramesh[a], N. Ravindranath[a], Biswanath Das[a,*], A. Prabhakar[a], Jagadeesh Bharatam[a], K. Ravikumar[a], A. Kashinatham[b], T.C. McMorris[b]

[a]*Indian Institute of Chemical Technology, Hyderabad 500 007, India*
[b]*Department of Chemistry, University of California, San Diego, USA*

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Dedicated to Professor (Mrs) Asima Chatterjee on the occasion of her 86th birthday.

Abstract

Chemical investigation on the flowers of *Parthenium hysterophorus* has resulted in the isolation of four new pseudoguaianolides, hysterones A–D along with the known compounds, parthenin, coronopilin, 2β-hydroxycoronopilin and tetraneurin-A. The structures of the new compounds were established by interpretation of their spectral (1D and 2D NMR) data. The X-ray crystallographic analysis of hysterones A and C was also carried out.

Keywords: *Pathenium hysterophorus*; Compositae; Pseudoguaianolides; Hysterones

1. Introduction

Parthenium hysterophorus Linn, an obnoxious weed, is a somewhat unattractive member of the family Compositae. It grows wild in different parts of India. The plant is known to create contact dermatitis and allergic rhinitis in animals (Towers et al., 1966). Its allelopathic effects have also been observed (Kanchan, 1975; Patel and Hedge, 1988) Earlier investigations reported the isolation of parthenin (**1**) (Herz et al., 1962) as the major constituent of the plant along with some related pseudoguaianolides (Romo de Vivar et al., 1966; Picman et al., 1980; Sethi et al., 1987; Dela Fuenta et al., 1997). Parthenin is known to possess significant allelopathic and cytotoxic properties (Kanchan, 1975; Patel and Hedge, 1988; Kupchan et al., 1971). In continuation of our work (Das et al., 1999) on the constituents of the herb we report here the isolation (from the flowers) of four new pseudoguaianolides, hysterones A–D (**2**–**5**) along with the known compounds parthenin, (Herz et al., 1962), coronopilin (Picman et al., 1980), 2β-hydroxycoronopilin (Sethi et al., 1987) and tetraneurin A (Picman et al., 1980).

2. Results and discussion

Hysterone A (**2**) analyzed for $C_{15}H_{20}O_5$ from elemental analysis, ^{13}C NMR spectrum and ESI/MS (*m*/*z* 280, $M^{+\cdot}$). The IR spectrum indicated the presence of hydroxyl, α,β-unsaturated ketone and lactone carbonyl groups. Its molecular formula along with its ^1H and ^{13}C NMR spectra indicated that it is a dihydroparthenin derivative containing an extra hydroxyl group (the molecular formula of parthenin is $C_{15}H_{18}O_4$). The ^1H and ^{13}C NMR spectral data compared with those of parthenin (**1**) (Herz et al., 1962; Sethi et al., 1987) (Table 1) indicated that the double bond in ring A in the new compound was intact (δ 7.86, 1H, *d*, *J* = 6.1 Hz, H-2 and 6.18, 1H, *d*, *J* = 6.1 Hz, H-3) while that in ring C (that is C-12, C-13 double bond) was saturated (δ 1.33, 3H, *d*, *J* = 7.2 Hz, Me-13; 2.37, 1H, *m*, H-12). The exocyclic double bond was absent. The DQF-COSY spectrum clearly showed a correlation between Me-13 and H-12 and also showed a correlation with H-7 (δ 2.65, 1H, *m*) which was again correlated with H-6 (δ 5.08, 1H, *d*, *J* = 8.2 Hz). The two methyl groups (Me-14 and

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* Corresponding author. Tel.: +91-40-7193434; fax: +91-40-7160512.
E-mail address: biswanathdas@yahoo.com (B. Das).

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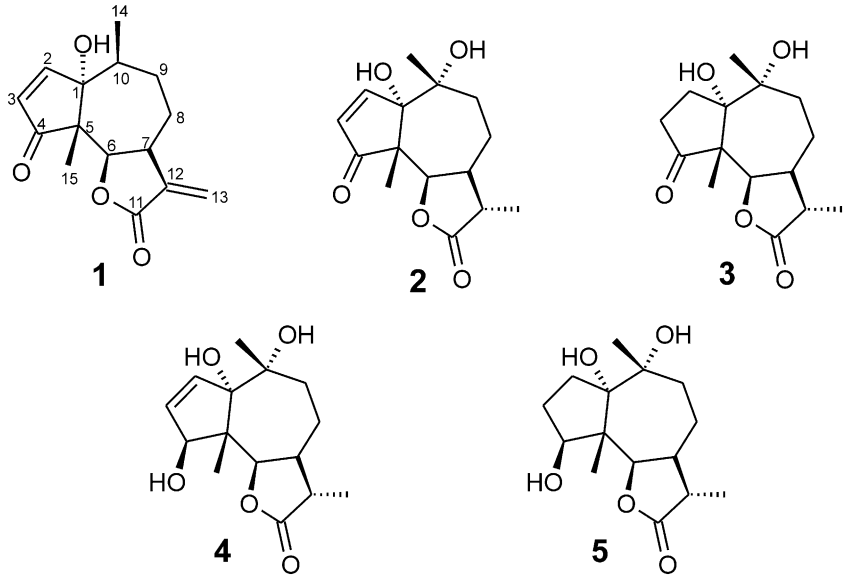

Table 1
^{1}H NMR data of compounds **1–5**[a]

Proton	**1**[b]	**2**[c]	**3**[b]	**4**[c]	**5**[b]
2	7.48, *d* (6.2)	7.86, *d* (6.1)	2.37, *m*	6.17, *dd*	2.21, *m*
			2.00, *m*	(6.0, 1.5)	1.65–1.49, *m*
3	6.15, *d* (6.2)	6.18, *d* (6.1)	2.45, *m*	5.91, *dd*	2.29, *m*
				(6.0, 2.4)	1.65–1.49, *m*
4				5.10, *dd*	4.75, *ddd*
				(2.4, 1.5)	(7.9, 7.3, 2.5)
6	4.98, *d* (8.2)	5.08, *d* (8.2)	5.01, *d* (8.2)	5.38, *d* (9.4)	5.24, *d* (9.4)
7	3.46, *m*	2.65, *m*	2.46, *m*	2.54, *m*	2.45, *m*
8	2.37–2.14, *m*	1.99, *m*	2.06, *m*	2.09, *m*	2.07, *m*
		1.91, *m*	1.77, *m*	2.00, *m*	1.65–1.49, *m*
9	1.84, *m*	1.87, *m*	1.61, *m*	1.75, *m*	2.08, *m*
	1.63, *m*				1.65–1.49, *m*
10	2.12–2.08, *m*				
12		2.37, *m*	2.27, *m*	2.38, *m*	2.29, *m*
13	6.24, *d* (2.0)				
	5.56, d (2.0)	1.33, *d* (7.2)	1.31, *d* (7.4)	1.25, *d* (7.0)	1.24, *d* (7.0)
14	1.12, *d* (7.0)	1.34, *s*	1.15, *s*	1.34, *s*	1.00, *s*
15	1.24, *s*	1.29, *s*	1.49, *s*	1.03, *s*	1.42, *s*

[a] *J* values in Hz.
[b] Spectra in $CDCl_3$.
[c] Spectra in $CDCl_3 + CD_3OD$.

Me-15) appeared as singlets while the Me-14 of parthenin was a doublet. The extra-OH group in hysterone A was thus placed at C-10. This was also supported by ^{13}C NMR and DEPT experiments. The NOESY spectra showed a correlation between two methyl groups, Me-14 and Me-15 but they were not correlated with Me-13. H-12 was also correlated with H-7 and H-6 but these two protons were related to only Me-13 among the three methyl groups. The two methyl groups, Me-14 and Me-15, were thus as β oriented while Me-13 was α.

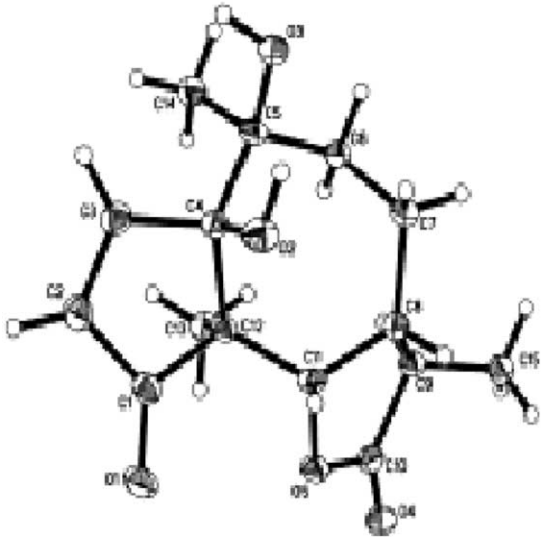

Fig. 1. ORTEP view of hysterone A(**2**).

Consequently, the structure of hysterone A was 12,13-dihydro-10-α-hydroxyparthenin (**2**). Finally the X-ray crystallographic analysis of the compound (Fig. 1) unambiguously established its structure.

Hysterone B (**3**) was assigned the molecular formula $C_{15}H_{22}O_5$ from its elemental analysis, the ^{13}C NMR spectrum and ESI/MS (*m*/*z* 282, $M^{+\cdot}$). The IR spectrum indicated the presence of hydroxyl and ketone and lactone carbonyls. The molecular formula suggested that the compound is the dihydro derivative of hysterone A (**2**). A comparison of its ^{1}H and ^{13}C NMR spectral data with those of **2** (Tables 1 and 2) showed that the compound contained no olefinic double bond but all other signals of rings B and C of these two compounds were similar i.e. the double bond in ring A of hysterone A was saturated in hysterone B. In the DQF-COSY spectrum H_2-2 (δ 2.37, 1H, *m* and 2.00, 1H, *m*) were clearly

Table 2
^{13}C NMR data of compounds **1**–**5**[a]

Carbon	**1**[b]	**2**[c]	**3**[b]	**4**[c]	**5**[b]
1	84.8 (*s*)	86.2 (*s*)	86.2 (*s*)	89.7 (*s*)	87.4 (*s*)
2	163.4 (*d*)	161.6 (*d*)	28.6 (*t*)	137.8 (*d*)	30.5 (*t*)
3	131.5 (*d*)	131.3 (*d*)	32.0 (*t*)	133.5 (*d*)	27.1 (*t*)
4	211.1 (*s*)	211.9 (*s*)	210.1 (*s*)	84.8 (*d*)	84.8 (*d*)
5	59.2 (*s*)	57.3 (*s*)	57.6 (*s*)	56.8 (*s*)	52.5 (*s*)
6	78.8 (*d*)	79.8 (*d*)	79.3 (*d*)	82.7 (*d*)	80.4 (*d*)
7	44.7 (*d*)	42.6 (*d*)	43.7 (*d*)	41.5 (*d*)	41.4 (*d*)
8	28.4 (*t*)	25.9 (*t*)	27.2 (*t*)	25.2 (*t*)	25.8 (*t*)
9	30.2 (*t*)	39.2 (*t*)	38.6 (*t*)	36.3 (*t*)	36.5 (*t*)
10	40.0 (*d*)	74.4 (*s*)	77.1 (*s*)	75.2 (*s*)	77.5 (*s*)
11	140.5 (*s*)	181.5 (*s*)	179.2 (*s*)	180.7 (*s*)	179.6 (*s*)
12	170.8 (*s*)	47.6 (*d*)	47.2 (*d*)	45.8 (*d*)	41.4 (*d*)
13	121.6 (*t*)	19.7 (*q*)	16.4 (*q*)	13.0 (*q*)	12.2 (*q*)
14	17.7 (*q*)	25.3 (*q*)	26.8 (*q*)	23.6 (*q*)	25.0 (*q*)
15	18.2 (*q*)	16.5 (*q*)	16.9 (*q*)	15.1 (*q*)	15.1 (*q*)

[a] Multiplicity derived from DEPT measurements.
[b] Spectra in $CDCl_3$.
[c] Spectra in $CDCl_3 + CD_3OD$.

related to H_2-3 (α 2.46, 2H, *m*). The methyl groups, Me-13 and Me-14 were α and β respectively, like those of hysterone A from the NOESY spectrum. The first methyl (Me-13) was correlated with H-6 and H-7 but not with Me-15 while the second methyl (Me-14) was correlated with Me-15 but not with H-6 and H-7. Thus hysterone B was 2,3,12,13-tetrahydro-10-α-hydroxyparthenin (**3**).

Hysterone C (**4**) analyzed for $C_{15}H_{22}O_5$ from the ^{13}C NMR spectrum and ESI/MS (*m*/*z* 282, $M^{+\cdot}$). The IR spectrum showed the presence of hydroxyl and lactone carbonyl groups. The molecular formula of the compound was found to be similar to that of hysterone B (**2**) indicating that it might be a tetrahydro derivative of parthenin containing an extra hydroxyl group. The ^{1}H and ^{13}C NMR spectral values of the compound (Tables 1 and 2) clearly showed that only the A-ring differed from that of hysterones A (**2**) and B (**3**) while rings B and C were identical, In ring A C-2, C-3 double bond being present and the carbonyl group at C-4 being replaced by a hydroxyl (δ 6.17, *dd*, 1H, J = 6.0, 1.5 Hz, H-2; 5.91, *dd*, 1H, *J* = 6.0, 2.4 Hz, H-3; 5.10, *dd*, J = 2.4, 1.5H, H-4). The DQF-COSY spectrum clearly showed a correlation between H-2 and H-3 and H-3 and H-4. In the NOESY spectrum H-4 was correlated with H-6 but not with Me-15 indicating that the hydroxyl group at C-4 is β. Me-14, Me-15 established connectivity and had the same β-configuration. The other methyl group, Me-13, was not related to these two methyl groups but it was related to H-6 and H-7 indicating its configuration as α. Hence hysterone C was 12,13-dihydro-4-deoxy-4α, 10β-dihydroxyparthenin (**4**). X-ray crystallographic analysis of the compound (Fig. 2) confirmed the structure.

Hysterone D (**5**) was assigned the molecular formula $C_{15}H_{24}O_5$ from its elemental analysis, ^{13}C NMR spectrum

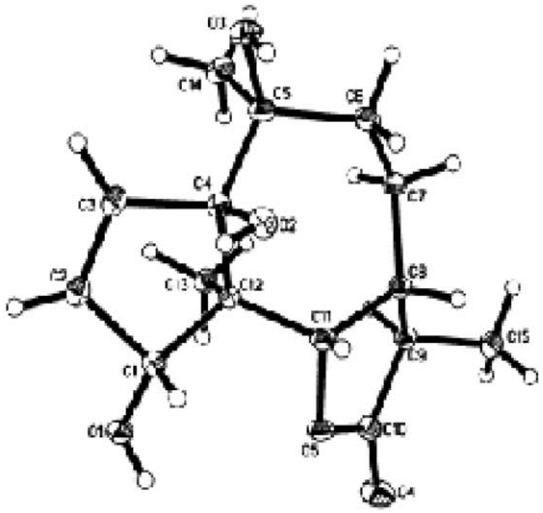

Fig. 2. ORTEP view of hysterone C(**4**).

and ESI/MS (*m*/*z* 284, $M^{+\cdot}$). The IR spectrum indicated the presence of hydroxyl and lactone carbonyl groups. The molecular formula suggested that it was a dihydro derivative of hysterone C (**4**). Comparison of the ^{1}H and ^{13}C NMR spectra with those of hysterone C (**4**) (Tables 1 and 2) indicated that the double bond in ring A of the latter was saturated in hysterone D, indicating in a change of the coupling of H-4 (δ 4.75, 1H, *dd*, *J* = 7.9, 7.3 Hz) with H_2-3. However, all other signals were exactly idendical. The NOESY spectrum showed the correlation between H-4/H-6, H-6/H-7, H-7/Me-13, and Me-14/Me-15. This suggested that HO-4 and Me-14 were β while Me-13 was α. Hence hysterone D was 2,3,12,13-tetrahydro-4-deoxy-4α,10β-dihydroxyparthenin (**5**).

Along with hysterone A-D the known pseudoguaianolides, parthenin, coronopilin, 2 α-hydroxycoronopilin and tetraneurin A were isolated and characterized by comparison of their physical (mp, $[\alpha]_D$) and spectral (IR, NMR and MS) properties with those reported in the literature. To our knowledge, this is the first report of isolation of C-4 and C-10 hydroxylated pseudoguaianolides from a natural source.

3. Experimental

3.1. General

Melting points were measured in a Buchi-510 instrument and are uncorrected. Spectra were recorded with the following instruments: IR: Perkin Elmer spectrophotometer, ^{1}H and ^{13}C NMR: Varian Gemini 200 MHz, ESI/MS: VG Micromass 7070 H (70 eV). Optical rotations were determined with a Jasco DIP 360 digital polarimeter. Column chromatography was performed on silica gel (BDH 100–200 mesh) and TLC with silica

gel GF254. The spots were detected in an iodine chamber and under an UV lamp. The spots were also visualized by spraying the plates with 10% methanolic H_2SO_4 and subsequently heating on a hot plate.

3.2. Plant materials

The flowers of *P. hysterophorus* were collected from Venkatapur village in the Adilabad district (Andhra Pradesh) in the month of August 2001 and were botanically identified by Professor T. Rajugopal, Department of Botany, Osmania University. A voucher specimen (No. IIC-5002) was preserved in the herbarium of our institute.

3.3. Extraction and isolation

The air-dried and powdered plant material (2 kg) was extracted with CH_2Cl_2–MeOH (1:1, 5 l) at room temperature for 120 h. The extract was concentrated under reduced pressure to afford a residue (30 g). This was subjected to column chromatography over silica gel (1.5 kg) using solvents of increasing polarity from *n*-hexane through EtOAc. The following compounds were obtained according to increasing order of polarity: coronopilin (26 mg), parthenin (12.4 g), tetraneurin-A (31 mg), 2-β hydroxycoronopilin (12 mg), hysterone B (11 mg), hysterone A (14 mg), hysterone D (9 mg) and hysterone C (34 mg).

3.4. Hysterone A (2)

Colourless crystals; mp 198–201 °C; $[\alpha]_D^{25}$ −14.84° ($c = 1.0$ MeOH); IR: ν_{mas} (KBr) cm^{-1} 3425, 1721, 1678, 1026; ESI/MS: *m/z* 280 [M^+·]. 280 (Found: C, 64.20; H, 7.21. $C_{15}H_{20}O_5$ require: C, 64.26; H, 7.19%).

3.5. Hysterone B (3)

Colourless viscous mass. $[\alpha]_D^{25}$ + 3.40° ($c = 1.25$, MeOH); IR: ν_{mas} (KBr) cm^{-1} 3500, 1756, 1456, 1180; ESI/MS: *m/z* 282 [M^+·]. 282 (Found: C, 63.96; H, 7.92. $C_{15}H_{22}O_5$ require: C, 63.89; H, 7.85%).

3.6. Hysterone C (4)

Colourless crystals. mp 186–188 °C $[\alpha]_D^{25}$ + 30.54° ($c = 1.25$, MeOH); IR: ν_{mas} (KBr) cm^{-1} 3428, 1765, 1649, 1406; ESI/MS: *m/z* 282 [M^+·] 282 (Found: C, 63.90, H, 7.92. $C_{15}H_{22}O_5$ require: C, 63.89, H, 7.85%).

3.7. Hysterone D (5)

Colourless viscous mass. $[\alpha]_D^{25}$ −12.04° ($c = 1.25$, MeOH); IR: ν_{mas} (KBr) cm^{-1} 3495, 1747, 1631, 1373, 1229; ESI/MS: *m/z* 284 [M^+·] 284 (Found: C, 63.39; H, 8.61. $C_{15}H_{22}O_5$ require: C, 63.36; H, 8.51%).

3.8. X-ray crystallographic analysis of hysterones A and C

Hysterone A crystallized from MeOH. The crystal system was orthorhombic and the space group was P2 (1) 2 (1) 2 (1). The unit cell dimensions were as follows: $a = 6.7952$ (4) Å, $\alpha = 90°$; $b = 13.1589$ (8) Å, $\beta = 90°$ and $c = 15.2721$ (9) Å, $\gamma = 90°$. The crystal size was $0.60 \times 0.55 \times 0.50$ mm^3. The reflections collected were 11444 and the independent reflections were 3064. The refinement method was full-matrix least-squares on F^2.

Hysterone B crystallized from MeOH. The crystal system was monoclinic and space group was P 2(1). The unit cell dimensions are as follows: $a = 8.3662$ (5) Å, $\alpha = 90°$; $b = 7.7438$ (5) Å, $\beta = 101\times$; $c = 10.7258$ (7) Å, $\gamma = 90°$. The crystal size was $0.50 \times 0.50 \times 0.40$ mm^3. The reflections collected were 5698 and the independent reflections were 2876. The refinement method was full-matrix least squares on F^2.

Acknowledgements

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