



Modhephene derivatives from *Pluchea sericea*

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Abstract

The roots of *Pluchea sericea* (Compositae) afforded the known compounds boehmerol acetate and β -sitosterol together with two new sesquiterpenes 14-hydroxymodhephene and its acetate. Their structures were determined by ¹H and ¹³C NMR spectroscopy including the use of a lanthanide shift reagent and trichloroacetyl isocyanate. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The genus *Pluchea* comprises 80 species (Mahmoud, 1997) and characteristic constituents of this genus are sesquiterpenes with an eudesmane skeleton such as cuahtemone (Nakanishi et al., 1974), arguticin (Ahmad & Fizza, 1988) and pluchecinin (Ahmad, Fizza, & Sultana, 1989). *Pluchea sericea* (Nutt.) Caville, commonly known as ‘cachanilla’ is an evergreen wild shrub growing in sandy or saline soils of the Baja California, Sonora and Chihuahua deserts in Mexico (Martínez, 1987). Previous chemical studies on the aerial parts of this plant revealed the presence of flavonoids, triterpenes and sesquiterpenes with eremophilane skeleton (Romo De Vivar, Reyes, Delgado, & Schlemper, 1982). As a result of the present work, boehmerol acetate (Chawla, Kaith, Honda, Kulshreshtha, & Srimal, 1990), β -sitosterol and two new sesquiterpene derivatives 14-hydroxymodhephene (**2**) and 14-acetoxy-modhephene (**3**) have been isolated from the hexane extracts of the roots of *Pluchea sericea*. This type of sesquiterpene with an unusual [3.3.3]-propellane carbon skeleton, has been found in only a few genera such as *Isocoma* (Zalkow, Harris, & Van Derveer, 1978), *Liabum* (Bohlmann, Zdero, Bohlmann, King, & Robinson, 1980), *Pulicaria* (San Feliciano, Medarde, Gordaliza, Del Olmo, & Miguel del Corral, 1988), *Silphium* (Bohlmann & Jakupovic, 1980), *Berkheya* (Bohlmann et al., 1979) and

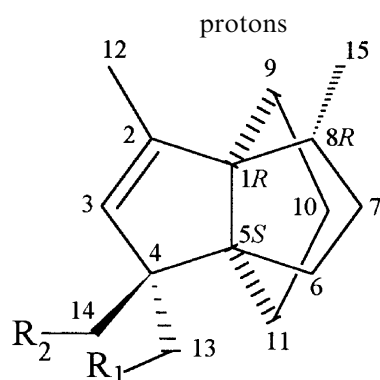
now in *Pluchea*. The structures of **2** and **3** were elucidated by means of NMR measurements including the use of lanthanide-induced shifts (LIS) and the preparation of the 3,5-dinitrobenzoate and trichloroacetyl isocyanate (TAI) derivatives.

2. Results and discussion

The hexane extracts of the roots of *Pluchea sericea* were subjected to column chromatography to afford 14-acetoxy-modhephene (**3**) and 14-hydroxymodhephene (**2**). Additionally, boehmerol acetate (Chawla et al., 1990) and β -sitosterol were identified by comparison of spectral data with those reported.

14-Acetoxy-modhephene (**3**) is a colorless oil that showed a molecular peak [M]⁺ at *m/z* 262 in its mass spectrum, in agreement with the molecular formula C₁₇H₂₆O₂. This was confirmed by its ¹³C NMR and DEPT spectra (Table 1), which indicated the existence of four methyl groups, six methylenes, two methines and five non-protonated carbon atoms. The low-field signals revealed the presence of an acetoxy group (s, δ 171.2) and a trisubstituted double bond (s, δ 144.7; d, 129.6). These three signals support the whole functionality of the molecule and as deduced from the molecular formula, compound **3** must possess a tricyclic skeleton. The ¹H NMR spectrum of **3** (Table 2) showed an acetyl methyl singlet δ 2.04, a methyl singlet at δ 1.03, a vinyl methyl doublet (*J* = 1.4 Hz) at δ 1.63 and a secondary methyl doublet (*J* = 6.5 Hz) at δ 1.00. The remaining aliphatic

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	R ₁	R ₂
1	H	H
2	H	OH
3	H	OAc
4	H	3, 5-dinitrobenzoate
5	H	OCONHCOC ₁₃
6	OH	H
7	OAc	H

protons appear as complex-order overlapping multiplets between δ 1.05 and δ 2.12 corresponding to eleven protons. The mass spectrum of **3** gave a base peak ion at

Table 1
¹³C NMR data of modhephenes (75.4 MHz, CDCl₃)

C	1 ^a	2	3	4	^a
1	73.2 (s)	73.4 (s)	73.4	73.8	73.7 (s)
2	141.1 (s)	145.9 (s)	144.7	145.5	144.3 (s)
3	135.4 (d)	130.0 (d)	129.6	129.0	129.8 (d)
4	46.0 (s)	51.6 (s)	49.4	49.9	49.7 (s)
5	66.4 (s)	64.9 (s)	65.3	65.4	65.5 (s)
6	34.4 (t)	33.7 (t)	34.1	34.5	34.6 (t)
7	36.0 (t)	35.4 (t)	35.0	35.1	35.7 (t)
8	44.0 (d)	43.2 (d)	43.4	43.5	43.8 (d)
9	30.0 (t)	29.8 (t)	29.9	30.0	29.2 (t)
10	27.3 (t)	26.5 (t)	26.9	27.0	27.0 (t)
11	38.7 (t)	39.5 (t)	39.0	39.0	38.4 (t)
12	13.7 (q)	14.0 (q)	14.0	13.9	14.0 (q)
13	29.3 (q)	20.6 (q)	21.0	21.5	70.5 (t)
14	27.3 (q)	69.9 (t)	71.8	73.6	24.4 (q)
15	15.7 (q)	15.2 (q)	15.3	15.3	15.5 (q)

3: Acetate; 171.2, 21.0. **4**: 3,5-Dinitrobenzoate; 162.4 (CO), 134.3 (i), 129.3 (o), 148.7 (m), 122.5 (p).

7 Acetate; 171.3, 21.0.

^a Literature data (Bohlmann et al., 1980) reassigned by a referee.

m/z 189, suggesting that **3** possesses [3.3.3]-propellane skeleton known as modhephenes (**1**) (Bohlmann et al., 1980). This skeleton was further supported by the presence of characteristic singlets at δ 49.4, 71.8 and 73.4 (Zalkow et al., 1978) in its ¹³C NMR spectrum. The acetoxy group must be at C-14 since the ¹H NMR spectrum of **3** shows the methylene protons as an AB system at δ 3.80 and 3.97 (2H, d, J = 10.8 Hz). These resonances showed strong correlations only with the signal at δ 71.8 (t, C-14) in the HETCOR contour plot. The ¹H NMR spectral data (Table 2) of **3** were very close to those of 13-acetoxymodhephenes (**7**) isolated from *Liabum eggersii* (Bohlmann et al., 1980). On the other hand, the acetoxymethylene group is suggested to be at the *S* chiral

Table 2
¹H NMR data of modhephenes (300 MHz, CDCl₃)

H	2	3	3 (C ₆ D ₆)	Δ^*	4	5	$\Delta\delta(5-2)$
3 (q)	4.76	4.78	4.71	0.75	4.87	4.81	0.05
6 (m)	1.90	1.58	—	—	1.56	1.61	−0.29
11 (m)	2.05	2.09	2.06	4.04	2.16	2.10	0.05
11' (m)	1.07	1.56	—	—	1.66	—	—
12 (d)	1.67	1.63	1.52	0.22	1.69	1.65	−0.02
13 (s)	1.00	1.03	1.06	1.08	1.16	1.10	0.10
14 (d)	3.38	3.80	3.90	3.64	4.12	4.03	0.65
14' (d)	3.44	3.97	4.11	3.64	4.35	4.21	0.77
15 (d)	1.00	1.00	0.92	0.16	1.03	1.00	—
Ac	—	2.04	1.71	3.57	—	—	—

J (Hz); **2**, **3**, **4** and **5**; 3, 12 = 1.4; 8, 15 = 6.5; 14, 14' = 10.8. **2**: OH, δ 1.95 (br s). **4**: 3,5-Dinitrobenzoate: δ 9.15 (d) and δ 9.25 (t); J (Hz); 2', 4' = 4', 6' = 2.1. **5**: NH; δ 8.32 (br s).

Δ^* : Changes in chemical shifts ($\Delta\delta$) upon addition of Eu(fod)₃ to a benzene solution of **3**.

center of C-4 (δ 71.8, δ 21.0). This is supported on the basis that 13- (7) (Bohlmann et al., 1980) and 14-acetoxymodhephene (3) showed similar ^{13}C NMR shift differences than those of suitable model compounds containing a chiral center having hydroxymethylene and methyl groups in either absolute configuration, as is the case of pterisin L (δ 67.1, δ 19.4) and *epi*-pterisin L (δ 66.0, δ 16.6) (Kuraishi et al., 1985; Satake, Murakami, Saiki, Chen, & Gómez, 1984).

The methyl group at C-8 in 3 remains *pseudo*-equatorial (δ 1.00, 3H, d, $J=6.4$ Hz; δ 15.3, s), this being consistent with the observed chemical shifts of the methyl group of modhephene (1) (δ 0.99, 3H, d, $J=6.0$ Hz; δ 15.7, s) (Zalkow et al., 1978) and in contrast with its C-8 epimer *epi*-modhephene (δ 0.88, 3H, d, $J=7.0$ Hz; δ 16.4, s) (Karpf & Dreiding, 1981). By comparing the lanthanide-induced shifts (LIS) of 3 and 7 (Bohlmann et al., 1980) in the presence of the $\text{Eu}(\text{fod})_3$, it becomes evident that the two methylene protons at C-14 in 3 experience small induced shifts of similar magnitude, while in 7 (Bohlmann et al., 1980) the C-13 protons experience quite different induced shifts. Furthermore, H-11 and H-12 are shifted more in 3 than in 7, in agreement with different complexation geometries with $\text{Eu}(\text{fod})_3$. All the above comparisons confirm that the new substance isolated from *Pluchea sericea* is 14-acetoxymodhephene (3).

14-Hydroxymodhephene (2) is a colorless oil whose mass spectrum gives m/z 203 [M-17] and m/z 189 [M-31], in agreement with the molecular formula $\text{C}_{15}\text{H}_{24}\text{O}$. The ^1H NMR spectrum of 2 was very similar to that of 3, but its methylene protons were observed as an AB system at δ 3.44 and 3.38 (2H, d, $J=10.8$ Hz) and its acetoxy group was replaced by one hydroxyl group at δ 1.95. The 3,5-dinitrobenzoate derivative (4) prepared from 2, m.p. 80–81°C allows a melting point differentiation with the 3,5-dinitrobenzoate derived from 13-hydroxymodhephene (6), m.p. 89°C (Bohlmann et al., 1980).

The relative stereochemistry of 2 was confirmed when its ^1H NMR data were compared with those of 5 (Table 2), obtained by addition of a drop of trichloroacetyl isocyanate (TAI) to a CDCl_3 solution. It is known (Samek & Budesínský, 1979) that when a TAI derivative is formed, the signals attributable to methylene protons vicinal to hydroxyl groups experience large low field shifts ($\Delta\delta$ 0.5–0.9 ppm), as in the case for the H-14 and H-14' protons in 5 Table 2, which shifted $\Delta\delta$ 0.65 and 0.77 ppm, respectively. In addition, the ^1H NMR spectrum of 5 showed a significant diamagnetic shift assignable to H-6 with a $\Delta\delta$ –0.29 (Table 2). This diamagnetic acylation shift is interpreted on the basis of the van der Waals effect (Samek & Budesínský, 1979). For the hydroxyl group the value of this effect is estimated in the 0.6 to 0.2 ppm range (Samek & Budesínský, 1979). This result is as expected, since acetyl groups delocalize the non-bonding electrons on the hydroxymethylene group. Similar results have

been reported for the van der Waals shifts of the C-methyl signals of steroids (Yoshioka, Mabry, Irwin, Geissman, & Samek, 1971). Alkaline hydrolysis of 3 afforded 2.

The ^{13}C NMR data interpretations 2–4 are given in Table 1, these being assigned using literature data (reassigned by a referee) in combination with COSY and HETCOR measurements.

3. Experimental

3.1. General

M.p.'s: uncorr. ^1H (Table 2) and ^{13}C NMR spectra (Table 1) were measured at 300 and 75.4 MHz, respectively, from CDCl_3 solutions with TMS as the int. standard. CC were performed on silica gel 60 (70–230 mesh). LIS measurements of 3 were obtained by adding 5 mg increments of $\text{Eu}(\text{fod})_3$ to the NMR tube containing the sesquiterpene.

3.2. Plant material

The roots of *Pluchea sericea* were collected at Mexicali Valley, Baja California, Mexico in December 1995. A voucher specimen (BRT122295-1) is deposited at the Herbario de Plantas Útiles Efraím Hernández X., Departamento de Fitotecnia, Universidad Autónoma Chapingo, Mexico.

3.3. Extraction and isolation

The air dried roots of *P. sericea* (2.8 kg) were extracted exhaustively ($\times 3$) with hexane (10 l) under reflux for 4 h each time. Filtration and evapn of the extract afforded a dark yellow oil (11.2 g) which was CC on silica gel (220 g). Elution with hexane, hexane–EtOAc mixtures and MeOH afforded 93 frs of 20 ml each. Frs 31–37 (hexane–EtOAc, 9:1) were further CC on silica gel to isolate boehmerol acetate (45 mg) (Chawla et al., 1990), β -sitosterol, 2 (150 mg) and 3 (75 mg).

3.4. 14-Hydroxymodhephene (2)

Colorless oil $[\alpha]_{589} -13^\circ$, $[\alpha]_{578} -13^\circ$, $[\alpha]_{546} -15^\circ$, $[\alpha]_{436} -27^\circ$, $[\alpha]_{365} -44^\circ$ (CHCl_3 ; c 1.37); EIMS 20 eV, m/z (rel. int.): 203 [M-17] (1), 189 [M-31] (100) 161 (22), 43 (8); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 3515 (hydroxyl).

3.5. 14-Acetoxymodhephene (3)

Colorless oil $[\alpha]_{589} +15^\circ$, $[\alpha]_{578} +16^\circ$, $[\alpha]_{546} +19^\circ$, $[\alpha]_{436} +31^\circ$, $[\alpha]_{365} +47^\circ$ (CHCl_3 ; c 2.02); EIMS 20 eV, m/z (rel. int.): 262 [M] $^+$ (0.6), 203 (1.7), 189 (100), 43 (7.8); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1725 (acetate).

3.6. 3,5-Dinitrobenzoate (**4**)

A soln of **2** (51 mg) and freshly prepared and recrystallized 3,5-dinitrobenzoyl chloride (83 mg) in dry benzene (3 ml) and pyridine (1 ml) was stirred under reflux for 30 min, poured over ice-H₂O and extracted with EtOAc. The organic layer was washed with dil. HCl, H₂O, aq. NaHCO₃ and H₂O, dried over Na₂SO₄, filtered and evapd under vacuum to give a yellow oily residue (80 mg), which was chromatographed on silica gel. Elution with hexane/EtOAc (1:1) afforded ester **4** as a colorless solid. Crystallization from MeOH yielded the pure compound as colorless needles (51 mg, 50%), m.p. 80–81°C; [α]₅₈₉ +3°, [α]₅₇₈ +4°, [α]₅₄₆ +4°, [α]₄₃₆ +5°, (CHCl₃; *c* 1.67); EIMS 20 eV, *m/z* (rel. int.): 399 (0.2), 203 (0.4), 202 (0.8), 190 (17), 189 (100), 161 (13), 119 (8); UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 203 (4.84), 230 (4.64); IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 1730 (3,5-dinitrobenzoate), 1545 and 1275 (NO₂).

3.7. Hydrolysis of **3**

A soln of **3** (70 mg) in EtOH (20 ml) was treated with 2 N NaOH in 96% EtOH (5 ml) under reflux for 1.5 h, neutralized with aq. HCl (10%) and extracted with EtOAc. The organic layer was washed with H₂O, aq. NaHCO₃ and H₂O, dried over Na₂SO₄, filtered and evapd under vacuum. The yellow oily residue was chromatographed on silica gel. Elution with hexane/CH₂Cl₂ (4:1) afforded **2** (55 mg, 93%) identical to the natural product by direct comparison.

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