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Clerodanes from *Onoseris alata*

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Abstract

Aerial parts of *Onoseris alata* furnished five new *trans*-clerodanes, loliolide and a thiopheneacetylene.

Keywords: *Onoseris alata*; Gochnatiinae; Mutisieae; Compositae; Diterpenes; Clerodanes

1. Introduction

Onoseris (Asteraceae, Mutisieae, Gochnatiinae) is a neotropical genus of approximately 32 species ranging from Mexico to northern Argentina (Ferreyra, 1944; Bremer, 1994). Previous work on five species, mainly on the roots, resulted in isolation of 4-methylmercapto-5-methylcoumarin (Bohlmann, & Zdero, 1977, 1979; Bohlmann et al., 1980, 1985), another 5-methylcoumarin (Bohlmann et al., 1985), a sesquiterpene lactone onoseriolide (Bohlmann et al., 1980, 1985; Bittner et al., 1994) and common triterpenes. We now report isolation of the *trans*-clerodanes **1a**–**d** and **2b** from *Onoseris alata* Rusby, a species from the mountains of southeastern Bolivia and northwestern Argentina (Cabrera, 1978). Loliolide and a thiopheneacetylene **3** were also found.

2. Results and discussion

Structure **1a** was deduced by analysis of the ^{1}H NMR spectrum (Table 1). H-3, a *dd* at δ 6.89, was coupled to H-2a at δ 2.35 and H-2b at δ 2.28 each of which was in turn coupled to H-1a at δ 1.71 and H-1b at δ 1.53. Only the former was coupled significantly (J = 12 Hz) to H-1. In ring B the sequence H-6 a,b through H-8 at δ 2.78, 1.16, 4.99 and 1.64, respectively, was deduced similarly with the coupling constants $J_{6\alpha,7\alpha}$, $J_{6\beta,7\alpha}$ and $J_{7\alpha,8\beta}$ = 4, 12 and 11 Hz, thus establishing β-orientation of the acetoxy group on C-7. The nature of the lactone group in the side chain followed from the chemical shift of H-14 at δ 7.08 which was coupled vicinally to H-15 a,b at δ 4.76 and allylically to H-12 a,b at δ 2.35 and δ 2.28. The chemical shifts of H-17, H-19 and H-20 at δ 0.84, 1.33 and 0.84 showed that we were dealing with an A/B ring *trans*-fused clerodane. Substance **1a** is the 7-epimer of a neoclerodane isolated earlier from the dried pods of *Sindora sumatrana* (Leguminosae) (Heymann et al., 1994). The ^{13}C NMR spectra of **1a** and its 7-epimer tallied except for the expected changes in the frequencies of the ring B carbons.

Three other constituents **1b**–**d** were 1′-β-D-glucopyranosyl esters of **1a** as shown by the paramagnetic shift of the respective anomeric protons near δ 5.6. In **1c** the 4′-hydroxyl of the glucose moiety was benzoylated whereas in **1d** which decomposed in the NMR tube prior to analysis the esterifying group on C-4′ was 4-methoxygallate as indicated by the presence of an extra methoxy group and two equivalent aromatic protons at δ 7.28. A fifth constituent was the isomeric glucosidic ester **2b** whose ^{1}H NMR spectrum (Table 1) differed significantly from that of **1b** only in the chemical shift of H-14, now at δ 5.84 comparable to H-14 of analogous 16,15-olides (Esquivel et al., 1995; Hussein et al., 1996).

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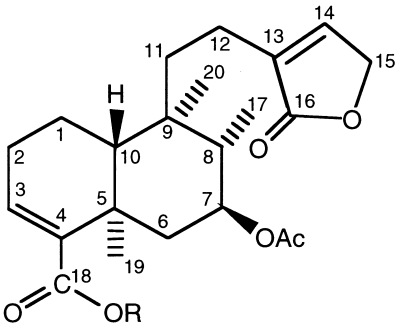
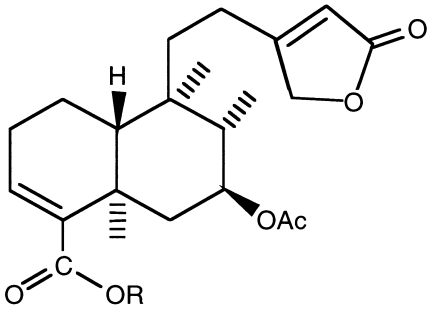


1 a R = H

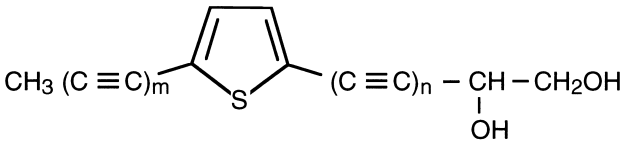
b R = β - D - Gluc

c R = 4' - Benzoyl - β - D - Gluc

d R = 4' - (*p* - MeOgallyl) β - D - Gluc



2 b



3 m+n = 3

Table 1
[1]H NMR spectra of **1a–c** and **2b**

H	**1a**	**1b**	**1c**[b]	**2b**
1a	1.71 *brdd* (12, 7, 1)	1.72	1.72	1.69 *c*
1b	1.53 *ddd* (12, 11, 5)	1.54	1.52	1.58 *c*
2a	2.35 *dddd* (20, 5, 4.5, 1)	2.33	2.37	2.37 *c*
2b	2.28 *dddd* (20, 11, 7, 3)	2.28	2.28	2.20 *c*
3	6.89 *dd* (4.5, 3)	6.82	6.90	6.81 *dd* (4, 3)
6α	2.78 *dd* (12, 4)	2.77	2.79	2.80 *dd* (12, 4)
6β	1.16 *t* (12)	1.10	1.13	1.08 *t* (12)
7α	4.99 *ddd* (12, 11, 4)	4.91	4.94	4.90 *ddd*
8β	1.64 *dq* (11, 6.5)	1.63	1.63	1.56 *c*
10β	1.42 *dd* (12, 1)	1.43	1.44	1.34 *dd* (12, 4)
11a	1.67 *brt* (13)	1.69	1.69	1.68 *c*
11b	1.51 *brt* (13)	1.52	1.52	1.58 *c*
12a	2.18 *brt* (13)	2.17	2.17	2.27 *c*
12b	2.07 *brt* (13)	2.06	2.06	2.14 *c*
14	7.08 *tt* (1.5, 1.5)	7.09	7.09	5.84 *quint* (1.5)
15a,b	4.76 *q* (1.5)	4.76	4.76	4.67 *q* (1.5)[c]
17[a]	0.84 *d* (6.5)	0.85	0.86	0.85 *d* (6.5)
19[a]	1.33 *s*	1.36	1.37	1.36 *s*
20[a]	0.84 *s*	0.84	0.85	0.87 *s*
Ac[a]	2.04 *s*	2.03	2.03	2.03 *s*
1′		5.59 *d* (8)	5.66	5.59 *d*
2′		3.67 *dd* (9.8)	3.72	3.54 *dd*
3′		3.63 *t* (9)	3.95	3.66 *t*
4′		3.54 *t* (8.5)	5.16	3.62 *t*
5′		3.49 *ddd* (8.5, 4.5, 3)	3.71	3.48 (9, 4, 2.5)
6′a		3.87 *dd* (12, 3)	3.78	3.86 *dd* (12, 2.5)
6′b		3.81 *dd* (12, 4.5)	3.63	3.81 *dd* (12, 4)

[a] Intensity three protons.
[b] H-2″,6″ 8.04 *dd* (8, 1.5), H-3″, 5″ 7.45 *t* (8), H-4″ 7.60 *tt* (8, 1.5).
[c] H-16a,b.

A non-terpenoid constituent of *Onoseris alata* isolated in only very small amounts had properties consonant with one of the two possible thiopheneacetylene structures **3** (see Experimental). A thiopheneacetylene ascribed formula **3** ($m = 1$, $n = 2$) has been reported from the roots of *Ambrosia chamissonis* (Balza et al., 1989). While the reported chemical shifts of the protons in the side chains tallied with those of our material, the chemical shifts of the two protons on the thiophene ring differed, ours being more nearly equivalent so that we are inclined to assign structure **3**, $m = 2$, $n = 1$, to our material. Other polyacetylenes have previously been reported from the roots of two *Onoseris* species (Bohlmann, & Zdero, 1979; Bohlmann et al., 1985).

Diterpenes are relatively rare in Mutisieae and have so far been isolated only from four *Gochnatia* (Bohlmann et al., 1983; García et al., 1985; Zdero, Bohlmann, & Niemeyer, 1988; Sacilotto, Vichnewski, & Herz, 1997) and one *Hyalis* species (Ybarra et al., 1997).

3. Experimental

3.1. General

For the separation of mixtures, HPLC with a differential refractometer was used. The columns were (A) a Beckman ultrasphere C-18 (10 mm i.d.×250 mm) and (B) a Beckman ultraspered C-8 (10 mm i.d.×250 mm). R_t's were measured from the solvent peak.

3.2. Plant material

Aerial parts of *Onoseris alata* Rusby were collected at the flowering stage on May 3, 1996 at Sierras de Medina, Tucumán Province, Argentina. A voucher specimen (E. Sigstad and A.S. #2) is on deposit in the herbarium of the Instituto Miguel Lillo Tucumán.

3.3. Extraction and isolation

Flowers and leaves (500 g) were extracted with $CHCl_3$ (3×6 l) at room temp. for 3 days to give 34.6 g (6.9%) of crude extract which was suspended in EtOH (310 ml) at 55°, diluted with H_2O (230 ml) and extracted successively with *n*-hexane (3×450 ml) and $CHCl_3$ (3×540 ml). The $CHCl_3$ extract on evapn at red. pres. furnished 13 g of residue a portion of which (6 g) was subjected to VLC (silica gel 60) using $CHCl_3$–MeOH mixtures (250 ml) of increasing polarities (0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 50 and 70%).

Frs eluted with 5 and 7.5% MeOH (852 mg) were combined and chromatographed over Sephadex LH20 using $CHCl_3$–MeOH (100:3) to eliminate chlorophyll, four frs being collected. Fr. 3 (488 mg) was chromatographed over silica gel using $CHCl_3$ containing increasing amounts of EtOAc, 55 frs being collected. Frs 10–12 (20.3 mg), 13–16 (25.2 mg), 17–34 (47.5 mg), 35–42 (17.1 mg) and 43–45 (40.1 mg) were combined as indicated and each processed by RP-HPLC (column A, MeOH–H_2O 3:2, 2 ml min^{-1}) to give 1.1 mg of loliolide (R_t, 4.3 min), 0.9 mg of **1a** (R_t, 33.3 min), 3.4 mg of **1c** (R_t, 38 min), 0.6 mg of **3** (R_t, 40 min) and mixtures. The latter were reprocessed by HPLC (column b, MeOH–H_2O 3:2, 2 ml min^{-1}) to give 11.2 mg of **1a** (R_t, 28 and 31.1 min) and mixtures. Frs 46–50 (40 mg) and 51–55 (71.9 mg) were combined as indicated and each processed by HPLC (column B, MeOH–H_2O 3:2, 2 ml min^{-1}) to give 3.7 mg of **1b** (R_t, 10.3 min) and 0.4 mg of **1d** (R_t, 12.9 min), respectively, 14.0 mg of **1b** (R_t, 10.3 and 11.9 min^{-1}).

A portion (150 mg) of the fraction eluted with 10% MeOH from the mother column was processed by HPLC (column A, MeOH 3:2, 2 ml min^{-1}) to give 0.5 mg of **2b** (R_t, 8.3 min) and 0.5 mg of **1b** (R_t, 12.6 min), all other fractions decomposing during the chromatogram. The fraction (40 mg) eluted with 20% MeOH from the mother column on HPLC (column A, MeOH–H_2O 11:9, 2.5 ml min^{-1}) gave 16 mg of **1b** (R_t, 21 min) and a mixture (12 mg) which eventually on HPLC (column B, MeOH–H_2O 11.9, 2.5 ml min^{-1}) gave 2 mg of **2b** and mixtures. Similarly a portion (50 mg) of the fraction eluted with 50% MeOH from the mother column on HPLC (column B, MeOH–H_2O 14:11, 2.5 ml min^{-1}) and subsequently on column B (MeOH–H_2O 1:1, 2.5 ml min^{-1}) gave 3 mg of **1b** (R_t, 26.4 min) and mixtures.

3.4. (5R*,7S*,8S*,9S*,10R*)-7α-Acetoxycleroda-3,13-dien-15,16-olide-18-oic acid (**1a**)

Gum; MS PCI (isobutane) 391 (19.5 [$M^+ + H$], 373 (12.5), 331 (18), 313 (100); IR λ_{max} cm^{-1} 3400, 3030, 2950, 2875, 1750, 1730, 1450, 1425, 1380, 1250, 1075, 1055; ^1H NMR spectrum in Table 1; ^{13}C NMR spectrum in Table 2.

3.5. (5R*,7S*,8S*,9S*,10R*)-1′-β-D-Glucopyranosyl-7α-acetoxycleroda-3,13-dien-15,16-olide-18-oate (**1b**)

Gum; FAB-MS (positive mode) *m/z* 575 (100 [$M + Na^+$]; IR λ_{max} cm^{-1} 3400, 3025, 2975, 2925, 2875, 1750, 1725, 1710, 1640, 1450, 1380, 1355, 1245, 1075, 1025; ^1H NMR spectrum in Table 1; ^{13}C NMR spectrum in Table 2.

3.6. (5R*,7S*,8S*,9S*,10R*)-4′-Benzoyl-1′-β-D-glucopyranosyl-7α-acetoxycleroda-3,13-dien-15,16-olide-18-oate (**1c**)

Gum; FAB-MS (positive mode) *m/z* 679 (100 [$M + Na^+$]; IR λ_{max} cm^{-1} 3400, 3025, 2975, 2925, 2875, 1750, 1730, 1450, 1425, 1380, 1260, 1070, 1025; ^1H NMR spectrum in Table 1.

Table 2
^{13}C NMR spectra of compounds **1a**, **b** ($CDCl_3$, 67.89 MHz)

C	1a	1b
1	17.6 *t*	17.6 *t*
2	27.3 *t*	27.3 *t*
3	140.8 *d*	140.6 *d*
4	140.0 *s*	140.0 *s*
5	39.8 *s*[a]	39.8 *s*[a]
6	41.3 *t*	41.3 *t*
7	72.6 *d*	72.7 *d*
8	41.4 *d*	41.2 *d*
9	38.2 *s*[a]	38.3 *s*[a]
10	46.4 *d*	46.4 *d*
11	36.3 *t*	36.3 *t*
12	17.6 *t*	19.1 *t*
13	134.6 *s*	134.4 *s*
14	143.6 *d*	143.6 *d*
15	70.1 *t*	70.1 *t*
16	174.1 *s*	174.4 *s*
17	10.9 *q*	10.9 *q*
18	169.5 *s*	164.6 *s*
19	21.6 *q*	21.6 *q*
20	19.2 *q*	19.3 *q*
Ac	21.2 *q*	21.3 *q*
	170.5 *s*	171.1 *s*
1′		94.0 *d*
2′		73.7 *d*
3′		77.2 *d*
4′		69.8 *d*
5′		76.4 *d*
6′		61.7 *d*

[a] Signals in same column may be interchanged.

3.7. (5R,7S*,8S*,9S*,10R*)-4′-(3,5-Dihydroxy-4-methoxybenzoyl)-1′-β-D-glucopyranosyl-7α-acetoxycleroda-3,13-dien-15,16-olide-18-oate (***1d***)*

Gum; the substance decomposed in the NMR tube prior to decoupling and mass spectral analysis. The following peaks were clearly visible in the NMR spectrum ($CDCl_3$, 500 MHz) at δ 7.28 (2H, *s*, H-2″,6″), 7.06 (*tt*, J = 1.5 Hz, H-14), 6.77 (*dd*, J = 4.5, 3 Hz, H-3), 6.15 (*broad*, -OH), 5.93 (*d*, J = 8 Hz, H-1′), 5.08 (*t*, J = 9 Hz, H-4′), 4.86 (*ddd*, J = 11, 11, 4 Hz, H-7), 4.75 (2H, *d*, J = 1.5, H-15), 3.92 (3H, *s*, -OMe), 3.92 (*dd*, J = 12, 3 Hz, H-6′ a), 3.86 (*dd*, J = 12, 4 Hz, H-6′ b), 3.85 and 3.80 (both *t*, J = 9 Hz, H-2′, H-3′), 3.55 (*ddd*, J = 4, 3 Hz, H-5′), 2.76 (*dd*, 12, 4 Hz, H-6α), 2.28 (*br ddd*, 20, 5,5 Hz, H-2a), 2.15 (*c*, H-2b), 2.12 (*brt*, 13, H-12a), 2.01 (3H, *s*, Ac), 1.96 (*brt*, 13, H-12b), 1.66-1.41 (*c*, 6-7p), 1.28 (3H, *s*, H-19), 0.81 (*d*, J = 6.5 Hz, H-17), 0.80 (3H, *s*, H-20).

3.8. (5R,7S*,8S*,9S*,10R*)-1′-β-D-Glucopyranosyl-7α-acetoxycleroda-3,13-dien-16,15-olide-18-oate (***2b***)*

Gum; FAB-MS (positive mode) *m/z* 575 (100 [M + Na^+; ^1H NMR spectrum in Table 1.

3.9. Thiophene **3**

Gum; MS PCI 231 (8.5 [M^+ + H]), 213 (100); ^1H NMR spectrum ($CDCl_3$, 500 HMz) δ 7.08 (*d*, J = 3.5) and 7.02 *d* (J = 3.5 Hz, H-3 and H-4), 4.66 (*d*, J = 6.5, 4 Hz, H-1′), 3.81 (*dd*, J = 11, 4 Hz, H-2′a), 3.75 (*dd*, J = 11, 6.5 Hz, H-2′b), 2.02 (3H, *s*, Ac).

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Note added in proof

Thiophene **3**, m = 2, n = 1, has been reported from *Echinops sphaerocephala* (Bohlmann et al., 1965) and synthesized (Bohlmann, Blasjkiewicz, & Bresinsky, 1968). The synthetic material exhibited ^1H NMR signals at δ4.62 (*m*, H-3′), 367 (*d*, j = 5.3 Hz, H-4′), at 2.03 (3p, vinyl methyl).