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Clerodane diterpenes from *Haplopappus deserticola*

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Abstract

From the resinous exudate of *Haplopappus deserticola* a new clerodane diterpene named deserticolic acid (**1**), the known 18-acetoxy-*cis*-cleroda-3,13*E*-dien-15-oic acid (**2**) and four flavonoids were isolated. The structure of **1** was elucidated as 19-hydroxy-*cis*-cleroda-3,13*E*-dien-15-oic acid (*ent*-5α form) by spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Haplopappus deserticola*; Asteraceae; Diterpenes; 19-Hydroxy-*cis*-cleroda-3,13*E*-dien-15-oic acid (*ent*-5α form); 18-Acetoxy-*cis*-cleroda-3,13*E*-dien-15-oic acid

1. Introduction

In continuation of our investigations of the genus *Haplopappus* (Asteraceae) (Urzúa, Tojo & Soto, 1995), we now describe the isolation and characterization of a new clerodane diterpene named deserticolic acid (**1**) from the resinous exudate of *Haplopappus deserticola* Phil. (Zdero, Bohlmann & Niemeyer, 1990). In addition, the known clerodane 18-acetoxy-*cis*-cleroda-3,13*E*-dien-15-oic acid named crotonic acid (**2**) (Bórquez et al., 1995) and four flavonoids were obtained (Harborne, 1994). The aerial parts of this plant have been used as folk medicine in Chile and a recent study (Urzúa, Torres, Muñoz & Palacios, 1995) has shown antimicrobial activities in the resinous exudate of different species of this genus.

2. Results and discussion

The methylene chloride extract of the resinous exudate of *H. deserticola* was subjected to column chromatography on silica gel using increasing amounts of ethyl acetate in hexane to afford deserticolic acid (**1**), crotonic acid (**2**), and four flavonoids.

The FAB mass spectrum of **1** exhibited the molecular ion peak at m/z 343 $[M+Na]^+$, indicating the molecular mass of 320, while in the EI mass spectrum the base peak appeared at m/z 302 corresponding to $[M-H_2O]^+$ ion. The IR spectrum showed absorption bands assignable to an α,β-unsaturated carboxylic group (1692, 1640 cm^{-1}). The ^{1}H-NMR spectrum of **1** was very similar to that of crotonic acid (**2**) (Bórquez et al., 1995). It showed secondary and tertiary methyl groups at δ 0.80 (*d*, J = 6.7 Hz) and 0.83, respectively, typical of clerodane diterpenes, and a characteristic methyleneoxy group [δ 3.39 (*d*, J = 10.9 Hz), 3.26 (*d*, J = 10.9 Hz)] tentatively assigned to C-19. Furthermore, a one-proton doublet signal at δ 5.71 (J = 0.8 Hz) and a methyl signal at δ 2.19 (*d*, J = 1.0 Hz) suggested the presence of a side chain requiring an α,β-unsaturated carboxyl group. The ^{13}C-NMR spectrum also supported structure **1**. Assignments were made on the basis of the observed multiplicities (DEPT), empirical shift rules (Breitmaier & Volter, 1978) and by comparison with reported ^{13}C-NMR spectral data of similar compounds (Atta-ur-

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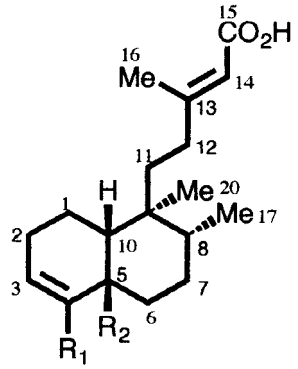
Rahman & Ahmad, 1992; Bórquez et al., 1995). High field bidimensional NMR techniques (COSY, HMBC, HMQC) confirmed structure **1** and allowed the assignment of all signals. The relative stereochemistry at the asymmetric carbons was established by NOESY experiment, which showed that H-10 was correlated with H-8 and H-19, and that 9-methyl was correlated with H-7β and H-2β. The *E*-geometry of the side chain was indicated by comparison with the ^{13}C-NMR spectral data of **2** (Bórquez et al., 1995) and confirmed by NOESY correlation between H-14 and H-12. By treatment with CH_2N_2, compound **1** was converted to its corresponding methyl ester; a new methoxy signal at δ 3.70 (3H, *s*) in the ^{1}H-NMR spectrum indicated the presence of one free carboxylic group.

Flavonoids were identified spectroscopically by comparison with previously reported data (Harborne, 1994).



1, R_1= Me, R_2= CH_2OH
2, R_1= CH_2OCOCH_3, R_2= CH_3

3. Experimental

3.1. General

Mps were determined on a Koffler GALLENKAMP and are uncorr. Both ^{1}H-NMR and ^{13}C-NMR experiments were recorded in $CDCl_3$ on a Bruker ARX-400 spectrometer; bidimensional spectra were obtained using standard Bruker software. FAB mass spectra were recorded with a FISONS VG AUTOSPEC mass spectrometer; the samples were dissolved in a glycerol matrix with NaCl as additive. EIMS spectra were obtained with direct inlet at 70 eV. Aldrich silica gel (200–400 mesh, 60 Å) was used for column chromatography and silica gel GF-254 for TLC.

3.2. Plant material

Specimens of *H. deserticola* were collected in the IV Region of Chile (28°40′ S, 70°35′ W) in the springs of 1994 and 1996. A voucher specimen was deposited in the herbarium of the Natural History Museum in Santiago, Chile.

3.3. Extraction and isolation

The resinous exudate of *H. deserticola* Phil. was extracted by immersion of the fresh material in CH_2Cl_2 for 15–20 min at room temperature. The CH_2Cl_2 extract (60 g, 6% dry wt) was purified by CC (silica gel) using a hexane–EtOAc step gradient to afford 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone (19 mg), 5,7-dihydroxy-3,8,4′-trimethoxyflavone (39 mg), 5,7,4′-trihydroxy-3,8-dimethoxyflavone (35 mg), 5,7,4′-trihydroxy-3-methoxyflavone (15 mg), 18-acetoxy-*cis*-cleroda-3,13*Z*-dien-15-oic acid (**2**) (3 g) and 19-hydroxy-*cis*-cleroda-3,13*E*-dien-15-oic acid (**1**) (265 mg).

3.4. 19-Hydroxy-cis-cleroda-3,13E-dien-15-oic acid (ent-5α form) (*1*)

Colourless needles; mp 140–42° (EtOH); $[\alpha]_D^{20}$-54.1 ($CHCl_3$; *c* 0.19); IR ν_{max}^{KBr} cm^{-1} 1692, 1640; positive FABMS *m/z* 343 $[M+Na]^+$; EIMS *m/z* (rel. int.): 302 $[M-H_2O]^+$ (5), 289 (11), 176 (82), 149 (24), 107 (100), 95 (53), 55 (31); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.80 (3H, *d, J* = 6.7 Hz, Me-17), 0.83 (3H, *s*, Me-20), 1.26 (1H, *m*, H-7), 1.27 (1H, *m*, H-6α), 1.37 (2H, *m*, H-7, H-11), 1.42 (1H, *m*, H-8), 1.67 (1H, *m*, H-11), 1.72 (3H, *d, J* = 1.2 Hz, Me-18), 1.85 (1H, *m*, H-1α), 1.86 (1H, *m*, H-6β), 2.02 (1H, *m*, H-2β), 2.06 (3H, *m*, H-12α, H-12β, H-1β), 2.16 (1H, *m*, H-2α), 2.19 (3H, *d, J* = 1 Hz, Me-16), 3.26 (1H, *d, J* = 10.9 Hz, H-19α), 3.39 (1H, *d, J* = 10.9 Hz, H-19β), 5.60 (1H, *bs*, H-3), 5.71 (1H, *br s* , H-14); ^{13}C-RMN ($CDCl_3$, 100 MHz): δ 16.3 (*q*, C-17), 17.9 (*q*, C-20), 18.2 (*t*, C-1), 19.9 (*q*, C-16), 20.7 (*q*, C-18), 24.7 (*t*, C-2), 28.6 (*t*, C-7), 31.8 (*t*, C-6), 35.1 (*t*, C-12), 36.2 (*t*, C-11), 37.3 (*d*, C-8), 40.4 (*d*, C-10), 40.5 (*s*, C-9), 42.8 (*s*, C-5), 71.6 (*t*, C-19), 115.0 (*d*, C-14), 127.6 (*d*, C-3), 136.1 (*s*, C-4), 165.0 (*s*, C-13), 171.7 (*s*, C-15).

Acknowledgements

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