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GUAIANOLIDES AND OTHER TERPENOIDS FROM *ANTHEMIS AETNENSIS*

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Key Word Index—*Anthemis aetnensis*; Anthemideae; Compositae; guaianolides; germacradienolides; eudesmanolide; acetoxypectanone.

Abstract—Aerial parts of *Anthemis aetnensis* furnished the guaianolides hydruntinolide A and B and a number of new analogues, two new germacradienolides, 1-β-hydroxyarbusculin and the isofraxidin derived sesquiterpene ether 7-acetoxypectanone.

INTRODUCTION

Anthemis aetnensis Schouw is a Sicilian endemic growing in volcanic debris on the upper slopes of Mount Etna [1]. Investigation of the aerial parts has now led to the isolation of hydruntinolides A (**1a**) and B (**1d**) previously obtained [2] from *Anthemis hydruntina* Groves, an endemic of Southern Italy, the new analogues **1b**, **1c**, **2a** and **2d**, a mixture of the two germacranolides **3a**, b, 1β-hydroxyarbusculin and the isofraxidin derived sesquiterpene ether acetoxypectanone (**4**) previously isolated together with related ethers from the roots of *Anthemis cretica* [3].

RESULTS AND DISCUSSION

High resolution ^{1}H NMR spectral data of the hydruntinolide analogues are listed in Table 1; assignments were arrived at by extensive decoupling. Lactones **1d** and **2a** could not be freed of the previously unknown contaminants **1e** and **1f**, and **2b** and **2c**, respectively, as shown by the MS and the NMR spectra. In all respects, except for the change in the ester function from isobutyrate to acetate, the ^{1}H NMR spectrum of **1c** compared with that of **1d** [2]; the ^{13}C NMR spectrum of **1c** listed in the Experimental likewise established the analogy. As for **1b** the MS and the upfield shift of the H-8 resonance showed the presence of a free hydroxyl group on C-8; noteworthy also and of diagnostic value are the upfield shift of the H-7 and the downfield shift of the H-13b resonances which accompany the change from ester to hydroxyl on C-8. Comparison of the spectrum of **2a** which contains only one acetate, i.e. at C-1, but also an isobutyrate, with the spectrum of **1b** shows that the two spectra are essentially identical, hence **2a** differs from **1b** in substitution of the isobutyrate moiety for the acetate on C-9. On the other hand the NMR spectrum of monoacetate **2d** differs from that of **1b** only in the upfield shift of the H-2 signal and a smaller upfield shift of the H-1 resonance. Consequently the two hydroxyls of **2d** are on C-1 and C-8.

The mass spectrum of the mixture of **3a** and **3b** indicated the presence of two closely related substances $C_{17}H_{22}O_7$ and $C_{17}H_{22}O_6$ while the ^{1}H NMR spectrum (Table 2) which exhibited essentially only one set of signals, together with a typical hydroperoxide frequency at δ 8.04, showed that one of the components was the hydroperoxide of the other. Spin decoupling established the sequences C-1 through C-3 and C-5 through C-9 as well as the stereochemistry at the centers C-1, C-3, C-6, C-7 and C-8. The H-3 and H-5 signals were superimposed at δ 5.25; one of them (H-5) was allylically coupled to a vinyl methyl group (H-15) and vicinally coupled to H-6 which was identified by being coupled to H-7, while the second (H-3), obviously on the carbon carrying the acetoxy group, was vicinally coupled to the protons of a methylene (H-2 a,b) which in turn were coupled to a proton on a carbon carrying a hydroxyl and a hydroperoxy group, respectively. Proceeding in the other direction, H-7 was coupled to a signal (H-8) on carbon carrying a second hydroxyl which was further coupled to the protons (H-9 a,b) of a -CH_2- group one of which was allylically coupled to the protons (H-14 a,b) of

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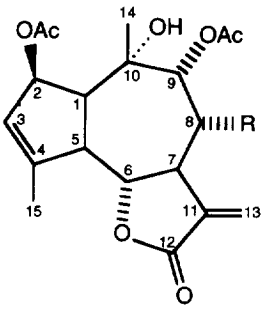

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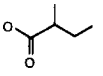
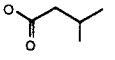
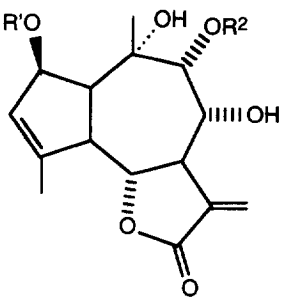
e R = 
f R = 



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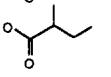
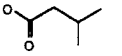
b R' = Ac, R² = 
c R' = Ac, R² = 
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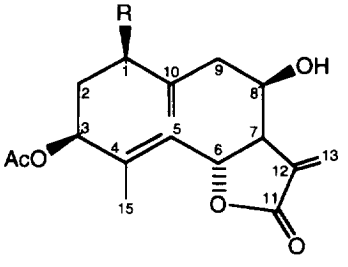

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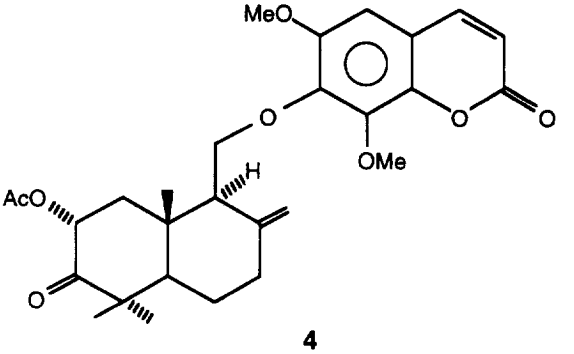

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Table 1. ^{1}H NMR spectra of compounds **1**b, **1**c, **2**a and **2**d (500 MHz, 67.89 MHz)

H	1b	1c	2a*	2d
1	2.55*dd*	2.61*dd*	2.59*dd*	2.30*dd*
2	5.43*dd*	5.46*dd*	5.43*dd*	4.61*dd*
3	5.77*ddq*	5.82*ddq*	5.79*ddq*	5.73*ddq*
5	2.84*ddd*	2.86*ddd*	2.85*ddd*	2.79*ddd*
6	4.23*dd*	4.27*dd*	4.20*dd*	4.29*dd*
7	3.47*dddd*	3.73*dddd*	3.48*dddd*	3.48*dddd*
8	4.11*dd*	5.59*dd*	4.18*dd*	4.14*dd*
9	5.62*d*	5.83*d*	5.56*d*	5.77*d*
13a	6.23*d*	6.20*d*	6.25*d*	6.22*d*
13b	5.97*d*	5.46*d*	5.99*d*	5.98*d*
14‡	1.29*s*	1.25*s*	1.33*s*	1.43*s*
15‡	1.97*brs*	1.99*brs*	1.99*brs*	1.99*brs*
Ac‡	2.14*s*, 2.40*s*	2.14*s*, 2.14*s* 2.14*s*	2.15*s*	2.17*s*

*Major signals; minor signals superimposed or closely adjacent. H-2′ 2.59*m*, H-3′ 1.11*d* (7)‡, H-4′ 1.08*d* (7),‡. Weaker signals of 2-methylbutyrate, H-2′ 2.2*m*, H-3′ a,b 1.6*m*, 1.5*m*, H-4′ 0.85*t* (7)‡ H-5′ 1.18*d* (7)‡; isovalerate H-2′ 2.2*m*, H-3′ obsc., H-4′, H-5′ 0.93*d* (7)‡, 0.90*d* (7)‡.

‡Intensity three protons.

J(Hz): 1,2 = 5.5; 1,5 = 7; 2,3 = 2.5; 3,5 = 3,15 = 1.5; 5,6 = 11; 6,7 = 10; 7,8 = 9; 7, 13a = 3.5; 7, 13b = 3; 8,9 = 5.

Table 2. ^{1}H NMR spectrum of **3a**, b (500 MHz, $CDCl_3$)

H		H	
1	4.19*dd*	9b	2.32*dd*
2a	2.57*dd*	13a	6.37*dd*
2b	2.33*dd*	13b	6.19*dd*
3	5.25*dd*	14a	5.31*d*
5	5.25*dd*	14b	5.29*brs*
6	4.26*t*	15*	1.73*d*
7	2.76*dddd*	Ac*	2.06*s*
8	3.98*ddd*	-OOH	8.04*brs*
9a	2.98*brd*		

*Intensity three protons.

J(Hz): 1,2a = 8, 9b = 11; 1,2b = 5; 2a,2b = 13; 2a,3 = 7,8 = 6; 2b,3 = 9; 5,6 = 6,7 = 10; 5,15 = 1.5; 7,13a = 8,9a = 3; 7,13b = 2.5; 9a,9b = 14; 9a,14a = 2; 9a,14b = 13a,13b = 1.

a methylene. The stereochemistry of H-8 could be deduced from the coupling constants and the chemical shifts of H-7 and H-13 a,b.

EXPERIMENTAL

Plant material. Above ground parts of *Anthemis aetnensis* Schouw were collected on the slopes of Mount Etna in June 1995. A voucher specimen PA 113/94 is on deposit in the herbarium of the Botanical Garden, Palermo, Sicily.

Extraction and isolation. The dried aerial parts (750 g) were extracted with Me_2CO (3 × 5 l) for 1 week each. The crude gum (23 g) was adsorbed on silica gel (50 g, Merck no. 7734 deactivated with 15% H_2O) and chromatographed over 400 g of the same adsorbent, 500 ml frs being collected as follows: Frs 1–19 (petrol), frs 20–22 (petrol–EtOAc, 1 : 1), 23–26 (petrol–EtOAc, 3 : 2), 27–30 (petrol–EtOAc, 1 : 4), 31–36 (EtOAc), 37–40 (EtOAc–MeOH, 9 : 1). Frs 20–22 were rechromatographed over silica gel and then by radial chromatography (petrol–Et_2O, 4 : 1, 7 : 3, 1 : 1) to give, in order of polarity, 20 mg of **1d** [1] contaminated with **1e** and **1f** as shown by the ^{1}H HMR spectrum which exhibited minor signals of the 2-methylbutyrate and isovalerate moieties and by the CI-MS, *m/z* (rel. int.): 465 $[M+H]^+$ of **1e** and **1f** (57.3), 451 $[M+H]^+$ of **1d** (40.1), 447 (31.7), 433 (44.1), 363 (44.1), 345 (100), 10 mg of **1a** [1], 10 mg of **1b**, 10 mg of a monoacetate $C_{17}H_{22}O_5$, MS PCI *m/z* (rel. int.): 307 (100), 247 (22.8), 229 (24.5) which exhibited only very broad signals in $CDCl_3$ and in C_6D_6 at rt or at 70°, 6 mg of the **3a**, b mixture and 20 mg of **4**. Frs 23–26 were rechromatographed over silica gel and then by radial chromatography (petrol–Et_2O, 7 : 3, 1 : 1, 2 : 3) to give, in order of polarity, 5 mg of the $C_{17}H_{22}O_5$ compound, 4 mg of **1a**, 30 mg of impure **1d** and 10 mg of **2a**. Frs 27–30 were rechromatographed over silica gel and then by radial chromatography (silica gel petrol–Et_2O, 1 : 4, and then Et_2O) to give, in order of polarity, 20 mg of **1c**, 5 mg of **2a** contaminated by **2b** and **2c** and 30 mg of **1b**. Frs 31–36 were rechromatographed over silica gel and then by radial chromatography (silica gel, EtOAc) to give, in order of polarity, 30 mg of **1b**, 10 mg of **2d** and 10 mg of 1β-hydroxyarbusculin. Known compounds were identified by MS and ^{1}H NMR spectrometry.

(1S*, 2R* 5R*, 6R*, 7S*, 8R*, 9R*, 10S*)-2,9-*Diacetoxy*-8,10-*dihydroxyguaia*-3,11(13)-*diene*-6,12-*olide* (**1b**). Gum; MS PCI *m/z* (rel. int.): 381 $[M+H]^+$ (14.3), 363 (10.6), 321 (98.3), 303 (100), 243 (73.4), 121 (41.5), 103 (26.6); ^{1}H NMR: Table 1; ^{13}C NMR ($CDCl_3$, 67.89 MHz): δ 170.5*s* (C-12), 169.2*s* (two Ac-C-O), 151.5*s* (C-4), 138.6*s* (C-11), 126.1*d* (C-3), 121.8*t* (C-13), 80.6*d* (C-6), 78.1*d* (C-2), 77.8*s* (C-10), 73.9*d*, 72.7*d* (C-8, C-9), 55.8*d* (C-1), 55.0*d* (C-5), 26.7*q* (C-14), 21.2*q* and 20.7*q* (Ac-Me), 18.1*q* (C-15).

(1S*, 2R*, 5R*, 6R*, 7S*, 8R*, 9R*, 10S*)-10-*Hydroxy*-2,8,9-*triacetoxyguaia*-3,11(13)-*diene*-6,12-*olide* (**1c**). Solid, mp not taken; MS PCI *m/z* (rel. int.): 423 $[M+H]^-$ (52.3), 405 (22.5), 363 (46.5), 345 (100); ^{1}H NMR: Table 1.

(1S*, 2R*, 5R*, 6R*, 7S*, 8R*, 9R*, 10S*)-2-*Acetoxy*-8,10-*dihydroxy*-9-(2-*methylpropanoyloxy*)-*guaia*-3,11(13)-*diene*-6,12-*olide* (**2a**). Gum; MS PCI *m/z* (rel. int.): 391 $[M+H^- -H_2O,]^+$ (100); ^{1}H NMR: Table 1 which also lists peaks due to the two contaminants giving rise to a minor peak in the MS at *m/z* 405 $[M+H-H_2O]^+$, (67.5).

(1S*, 5R*, 6R*, 7S*, 8R*, 9R*, 10S*)-9-*Acetoxy*-2,8,10-*trihydroxyguia*-3,11(13)-*diene*-6,12-*olide* (**2d**). Gum; MS PCI *m/z* (rel. int.): 339 $[M+H]^+$, (69.0), 321 (65.1), 303 (100), 261 (26.3); ^{1}H NMR: Table 1.

Mixture of (1R*, 3S*, 6S*, 7R*, 8R*)-1-*Hydroxy*- *and* 1-*hydroperoxy*-3-*acetoxy*-8-*hydroxygermacra*-4*Z*, 10(14), 11(13)-*triene*-6,12-*olide* (**3a** and **3b**). Gum: MS PCI *m/z* (rel. int.): 339 $[M+H]^+$ of **3b** (100), 323 $[M+H]^+$ of **3a** (93.1), 321 (88.5), 305 (13.6), 279 (3.2), 263 (16.1), 261 (21.0), 245 (33.8); ^{1}NMR: Table 2.

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