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FURANEDITERPENES FROM *BACCHARIS THYMIFOLIA*

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Key Word Index—*Baccharis thymifolia*; Compositae; furanediterpenes; flavonoids.

Abstract—From the aerial part of *Baccharis thymifolia* two new furanediterpenoids have been isolated besides previously know flavonoids. The structure of thymifodioic acid and 17-acetoxymethylthymifodioic acid, were established by spectroscopic data and chemical transformations.

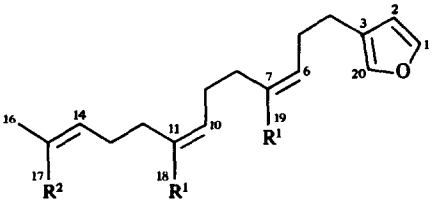
INTRODUCTION

In the course of our chemosystematic investigation of the *Baccharis* genus, we have reported the isolation and identification of diterpenoids of the clerodane type and flavonoids [1–6]. We have now studied the aerial part of *B. thymifolia*. This work has resulted in the isolation of two new furanediterpenes and three known flavonoids. This paper reports the structures (1 and 7) of the new compounds.

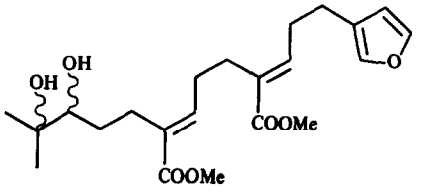
RESULTS AND DISCUSSION

Thymifodioic acid (1) had a molecular formula of $C_{20}H_{26}O_5$ which was established by high resolution mass spectrometry. The IR spectrum of 1 showed absorptions for a carbonyl group at 1690 cm^{-1} and for a hydroxy group at 3500–3200 cm^{-1}. The UV spectrum showed an absorption at 249 nm, both compatible with an α,β-unsaturated carboxyl group. By treatment with diazomethane, compound 1 gave a dimethyl ester (2). The ^{1}H NMR spectrum of 2 exhibited two signals for carbomethoxy protons at δ3.71 and 3.73 and a signal for two protons as a double doublet at δ6.76 which was ascribed to two equivalent olefinic protons on double bonds, conjugated with carboxyl groups. Furthermore, the ^{1}H NMR spectrum showed signals at δ7.30 and 7.20 for two α-protons of a β-substituted furan ring and a broad singlet at 6.20 due to the β-proton of the furan ring. Moreover, its mass spectrum showed the base peak at *m/z* 81, in addition to an intense peak at *m/z* 95. The ^{13}C NMR spectrum, with carbon multiplicities determined by APT confirmed, the presence of two carboxyl groups (δ167.4 and 167.8), six olefinic carbons (three –CH = and three $-\overset{|}{C}=$) and four aromatic protons (three –CH = and one $-\overset{|}{C}=$) which accounted for all degrees of unsaturation involved by the molecular formula. Hence a furan diterpenoid acyclic structure was inferred for 2. The irradiation of the remaining olefinic signal in the ^{1}H NMR spectrum at δ5.03, sharpened both broad singlets at 1.55 and 1.63 showing that these two methyl–vinyl groups and the olefinic proton were arranged on the same double bond and mutually underwent on allylic long-range coupling. The terminal isopentenyl group was also deduced from biogenetic considerations and from the fragment ion at *m/z* 69 of the mass spectrum.

On the other hand, the treatment of 2 with *m*-chloroperbenzoic acid produced the diol 6 probably due the opening of epoxide in the ^{1}H NMR spectrum of 6 the H-16 and H-17 methyl signals were shifted to δ1.24 and 1.28 respectively, and the H-14 vinyl-proton signal at 5.03 was replaced by a one carbinol-proton signal as a double



	R^1	R^2
1	COOH	Me
2	COOMe	Me
3	CH_2OH	Me
4	CH_2OAc	Me
5	CHO	Me
7	COOMe	CH_2OAc



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doublet at 4.02. The other signals were similar to those of 2 and thus the carboxyl groups were positioned at C-7 and C-11.

The geometry of the Δ^6- and Δ^{10}-double bonds was assigned as *E* by the low-field chemical shift of the H-6 and H-10 olefinic protons when compared with those of the model compounds (*E*)- and (*Z*)-methyl-2-pentenoic acids [7]. In order to confirm the location of the carboxyl groups and to determine the geometries of the Δ^6- and Δ^{10}-double bonds, compound 1 was first reduced with lithium aluminium hydride in tetrahydrofuran to yielded the diol 3, compound 3 was acetylated with acetic anhydride in pyridine to give the diacetate 4. Also 3 was oxidized with pyridinium chlorochromate supported on alumina to yielded the dialdehyde 5.

The spectral data of compounds 1 –5 were nearly identical for most of the signals with the spectra in refs [8, 9]. However, some differences did appear in the ^{1}H NMR and ^{13}C NMR spectra. for centipedoic acid [9], the chemical shift of the β-hydrogen of the α,β-unsaturated carboxyl group was reported at δ6.00 but thymifodioic acid showed the signals for the protons at the anologous positions (H-6, H-10) at 6.80. On the other hand, the methyl ester of centipedoic acid showed the resonance of the β-carbon in the α,β-unsaturated carboxyl system at δ141.8 while for compound 2 the signals observed for the analogous carbons (C-6, C-10) were at δ126.0 and 123.3 respectively. This suggested in agreement with ref. [7], that centipedoic acid has the *Z*-arrangement at the Δ^6 bond while thymifodioic acid has an *E,E*-arrangement at the Δ^6- and Δ^{10}-bonds.

The *E,E*-configurations at the Δ^6- and Δ^{10}-bonds for thymifodioic acid were confirmed by the chemical shifts of H-18 and H-19 aldehyde protons in 5 which were at 9.43 and 9.53, respectively [10–14]. Conopododiol was reported [8] to have the *E,E*-configuration, although the aldehyde obtained by oxidation showed resonances at δ10.01 and 10.03, for the the aldehyde protons which is consistent with the *Z,Z*-configuration of the compound. Additionally, the assigned arrangement provides a regular isoprenoid skeleton.

The molecular formula ($C_{22}H_{28}O_7$) of the most polar furanediterpene (7) isolated indicated that this compound differed from 1 by an additional acetoxy group. The ^{1}H NMR spectrum was in part similar to that of thymifodioic acid. However, the signal of a vinylmethyl group of a terminal isopentenyl group was replaced by a broad singlet at δ4.53 (2H) and a singlet at 2.03 (3H) which are typical for the methylene protons and methyl protons, respectively, of the acetoxymethylene group. The assignment of the acetoxy group on C-17 was made on the basis of the ^{13}C NMR spectrum which showed the signal of one methyl group at δ21.1 consistent with a methyl group in position C-16 [9]. This was confirmed for the chemical shifts of the protons of the C-16 methyl group at δ1.76 which was coincident with that shown by 17,18-dihydroxygeranylnerol [11] and by 17,20-dihydroxy geranylnerol triacetate [15]. However, it differed by 0.12 ppm with that shown by 16-acetoxygeranylgeraniol acetate [16]. Consequently we propose the structure 7 for 17-acetoxythymifodioic acid.

EXPERIMENTAL

^{13}C NMR spectra were recorded in a Brucker WP-80 in $CDCl_3$; the ^{1}H NMR spectra were recorded on a Varian EM 360 A in $CDCl_3$; the mass spectra were determined utilizing a Varian Mat 112 S at 70 eV and 0.7 MA.

Plant Material. Baccharis thymifolia. H. et A. was collected in February 1985 in Villavicencio, Mendoza by J. A. Ambrosetti, F. Roig and L. A. Del Vitto (Voucher MERL 32491).

Extraction and isolation. The air-dried plant material (1.5 kg) was extracted with hot MeOH (3 l × 3). The extract was concd to 1.5 l, then H_2O was added (10, 20, and 30%) and partitioned between *n*-hexane, CCl_4, $CHCl_3$ and EtOAc respectively. The $CHCl_3$ extract was evapd and the residue (60 g) was subjected to CC on silica gel 60 and eluted successively with C_6H_6 and C_6H_6 containing increasing proportions of EtOAc.

Thymifodioic acid (1). The C_6H_6–EtOAc (95:5) eluate, fractions 12–24, yielded a colourless oil (1.98 g), which was rechromatographed on silica gel 60 H (75 g) eluted with C_6H_6–EtOAc (95:5) and the fractions 6–15 yielded (1.81 g) of 1. HRMS calc. for $C_{20}H_{26}O_5$ M_r: 346.1780, found M_r (MS) 346.1779.; The ^{1}H NMR and ^{13}C NMR data of 1 are shown in Tables 1 and 2; MS *m/z* (rel. int.).: 346, $[M]^+$ (1), 179 (7), 167 (5), 98 (3), 81 (100), 69 (71).

Compound 2. Compound 1 (0.25 g) was dissolved in dry Et_2O and CH_2N_2 added slowly, followed by the usual work-up to give 0.23 g of 2. The ^{1}H NMR and ^{13}C NMR data of 2 are shown in Tables 1 and 2; MS *m/z* (rel. int.): 374 $[M]^+$ (3), 342 (1.5), 305 (10), 293 (35), 193 (6.7), 181 (8), 112 (3), 81 (100), 69 (78).

Compound 3. Compound 2 (0.85 g) was dissolved in 250 ml of dry THF and added slowly to a cooled soln containing 0.57 g of $LiAlH_4$ in 200 ml of dry THF. The mixture was stirred under N_2 atmosphere overnight at room temp. followed by the usual work-up to give 0.54 g of 3. The ^{1}H NMR and ^{13}C NMR data of 3 are shown in Tables 1 and 2. MS *m/z* (rel. int.): 318 $[M]^+$ (0.5), 300 (0.5), 285 (0.1), 165 (0.1), 153 (0.1), 95 (30), 81 (80), 69 (100).

Compound 4. Compound 3 40 mg was acetylated with Ac_2O–pyridine in the usual way to give 39 mg of 4. The ^{1}H NMR and ^{13}C NMR data of 4 are shown in Tables 1 and 2. MS *m/z* (rel. int.): 402 $[M]^+$ (0.1), 342 (5), 282 (6), 206 (0.2), 196 (0.2), 95 (35), 81 (70), 69 (100).

Compound 5. Compound 3 (0.25 g) was dissolved in C_6H_6 and 8 g of PCC/alumina were added slowly; the mixture was stirred for 48 hr in the dark, followed for the usual workup to give 0.12 g of 5. The ^{1}H NMR and ^{13}C NMR data of 5 are shown in Tables 1 and 2. MS *m/z* (rel. int.): 314 $[M]^+$ (0.5), 245 (0.5), 233 (1), 219 (0.5), 163 (3), 151 (1), 95 (20), 81 (100), 69 (95).

Compound 6. Compound 2 (0.40 g) was treated with 0.80 g of *m*-chloroperbenzoic acid in dry CH_2Cl_2 (20 ml) and stirred a 5° under a N_2 atmosphere for 8 hr followed by the usual workup to give 0.27 g of 6. The ^{1}H NMR and ^{13}C NMR data of 6 are shown in Tables 1 and 2. MS *m/z* (rel. int.): 390 $[M\text{-}H_2O]^+$ (0.5), 197 (2), 193 (1.8), 112 (8), 98 (7), 95 (100), 81 (14), 43 (100).

$$\alpha\,24°\ \frac{-2.59\quad -2.59\quad -2.63\quad -5.59}{589\quad 578\quad 546\quad 436}\ (CHCl_3;\ c\,1.27).$$

Flavonoid Compounds. 5-Hydroxy-6,7,8,3',4'-pentametoxyflavone (0.20 g) was eluted with C_6H_6–EtOAc(85:15); 5,4'-dihdroxy-6,7,8,3'-tetrametoxyflavone (0.24 g) with C_6H_6–EtOAc(75:25); 5,3',4'-trihydroxy-6,7,8-trimetoxyflavone (0.19 g) with C_6H_6–EtOAc (65:35).

17-Acetoxythymifodioic acid dimethyl ester (7). The crude EtOAc extract (20 g) was subjected to CC on silica gel 60 (400 g) and developed with C_6H_6 and C_6H_6 containing increasing proportions of EtOAc. Elution with C_6H_6–EtOAc (7:3) afforded a colourless oil, which after methylation with CH_2N_2 in the usual way gave 0.06 g. of 7. The ^{1}H NMR and ^{13}C NMR data of 7 are shown in Tables 1 and 2. MS *m/z* (rel. int.): 432 $[M]^+$ (0.5), 239 (5), 193 (3.4), 127 (10), 112 (4.6), 84 (5.2), 81 (100), 54 (5), 43 (100).

Table 1. ^{1}H NMR spectra (60 MHz) of compounds 1–7

	1	2	3	4	5	6	7
H-1	7.30 *m*	7.34 *m*	7.34 *m*	7.37 *m*	7.34 *m*	7.35 *m*	7.33 *m*
H-2	6.20 *br s*	6.26 *br s*	6.26 *br s*	6.27 *br s*	6.27 *br s*	6.27 *m*	6.23 *br s*
H-6	6.80 *dd*	6.76 *dd*	5.43 *dd*	5.47 *dd*	6.43 *br dd*	6.96 *dd*	6.76 *dd*
H-8			2.42 *br t*				
H-10	6.80 *dd*	6.76 *dd*	5.43 *dd*	5.47 *dd*	6.43 *br d*	6.96 *dd*	6.76 *dd*
H-12			2.11 *br s*	2.10 *br s*	2.12 *m*		
H-13							
H-14	5.03 *dd*	5.10 *dd*	5.10 *dd*	5.10 *dd*	5.13 *dd*	4.02 *dd*	5.33 *dd*
H-16	1.55 *br s*	1.57 *br s*	1.59 *s*	1.58 *s*	1.53 *s*	1.24 *s*	1.76 *br s*
H-17	1.63 *br s*	1.67 *br s*	1.67 *s*	1.67 *s*	1.65 *s*	1.28 *s*	4.53 *br s*
H-18	12.5 *br s*						
			4.03 *s*	4.47 *s*	9.43 *s*		
					9.53 *s*		
H-19	12.5 *br s*						
H-20	7.20 *m*	7.21 *m*	7.21 *m*	7.22 *m*	7.21 *m*	7.24 *m*	7.21 *m*
O-Me		3.66 *s*; 3.70 *s*				3.74 *s*; 3.70 *s*	3.66 *s*; 3.73 *s*
OAc				2.03 *s*			2.03 *s*
OH			2.87 *br s*				

j(Hz) Compounds 1–7: 6,5 = 10,9 = 6.5; 6,5′ = 10,9′ = 3. Compounds 1–4,6,7: 14,13 = 5.5; 14,13′ = 2. Compound 5: 14,13 = 5; 14,13′ = 2.5.

Table 2. ^{13}C NMR spectra of compounds 1–7

	1	2	3	4	6	7
C-1	142	142.6	142.5	142.5	142.5	142.6
C-2	110.6	110.4	110.7	110.7	110.6	110.6
C-3	123.5	123.5	124.3	124.1	125.9	123.7
C-20	138.9	138.7	138.7	138.7	138.7	138.8
C-4	29.5	28.9	28.0	26.7	23.0	27.2
C-8	28.0	27.7	28.0	28.0	25.3	27.2
C-12	27.5	27.4	27.8	28.3	25.7	28.8
					27.4	
C-5	23.7	26.6	24.7	24.6	28.4	26.2
C-9	25.4	25.8	26.3	25.0	28.7	23.8
C-13	26.4	27.3	26.9	26.2		23.8
C-6	143.8	141.1	126.0	129.8	141.1	142.1
C-10	142.8	142.2	123.9	129.3	142.2	140.9
C-7	132.2	132.2	139.0	134.4	131.7	131.5
C-11	131.9	131.8	138.9	134.0	131.9	131.6
C-15	130.8	131.2	131.7	133.9	70.89	130.5
C-14	123.3	123.3	123.8	123.5	85.4	129.2
C-16	25.4	25.3	25.5	25.5	25.1	21.1
C-17	17.4	17.2	17.5	17.5	24.4	62.8
C-18	173.2	167.4	66.7	68.2	167.5	167.8
C-19	172.8	167.8	66.7	68.2	166.2	167.7
		51.4			51.4	51.4
O-Me		51.3			51.3	50.9
Me-CO.				20.8		20.6
				20.8		
Me-CO.				169.7		170.7
				170.7		

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