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A DITERPENE ACID FROM *BACCHARIS TUCUMANENSIS*

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Key Word Index—*Baccharis tucumanensis*; Compositae; clerodane; diterpene; structural determination.

Abstract—The new clerodane diterpene **1** was isolated from the aerial parts of *Baccharis tucumanensis* (Compositae). Its structure has been determined from spectral data combined with chemical transformations.

INTRODUCTION

In continuing our investigations on the chemical constituents of the genus *Baccharis* [1], Compositae belonging to the tribe Astereae, we nowwish to report the structural elucidation of a new clerodane diterpene (**1**), which we propose to name tucumanoic acid.

RESULTS AND DISCUSSION

The natural product has been studied as the derived methyl ester (**2**), obtained after diazomethane treatment. Methyl tucumanate (**2**) shows a UV absorption at λ_{max} 233 nm ($\log \varepsilon$ 4.15) attributable to an α,β-unsaturated ester functionality that is further evident from the IR bands at 1695 and 1645 cm^{-1}. In addition IR absorptions in the 3400–3200 cm^{-1} range are indicative of OH groups.

The elemental composition of **2** as $C_{21}H_{36}O_5$ was deduced from a combined evaluation of the ^1H and ^{13}C NMR spectra, since in the mass spectrum only the $[M-H_2O]^+$ at m/z 350 was evident. Thus, the ^{13}C NMR spectrum provides cogent evidence for the α,β-unsaturated ester due to the presence of the ester carbonyl at 166.5, a non protonated sp^2 carbon at 161.5, an sp^2 CH signal at 115.7 and the Me group at 50.7 ppm. The remaining 17 sp^3 signals appear as two OH bearing doublets at 79.5 and 69.6, an oxygen bearing singlet at 77.0, two quaternary carbons at 41.5 and 39.3, two doublets at 39.1 and 36.6, five methylene signals at 37.3, 32.9, 28.1, 27.4 and 26.9 and five Me peaks at 25.2, 22.2, 18.7, 17.8 and 16.4 ppm.

Particularly information was the 360 MHz ^1H NMR spectrum of **2**, which shows the five Me signals of a clerodane molecule [2–6] as a vinyl Me doublet (J = 1.6 Hz) at 1.89, a secondary Me (J = 6 Hz) at 0.84, two tertiary Me groups at 0.74 and 1.10 and a Me singlet on an OH bearing carbon at 1.29 ppm. The tertiary OH signal appears as a singlet at 1.25 ppm and the two secondary OH groups appear as a well defined doublet (J = 2.5 Hz) at 2.36 and a more broadened doublet (J = 3.5 Hz) at 2.06 ppm. The two secondary OH protons are coupled with CH signals at 3.61 and 4.13 ppm, respectively, as is evident on the contour plot of the two-dimensional J-correlated homonuclear ^1H NMR data depicted in Fig. 1. This plot also shows that the signals at 3.61 and 4.13 are coupled. Furthermore the signal at 4.13 is coupled with the protons of a methylene group at 1.75 and ~ 1.57 ppm. Other informative couplings that are evident, are those of the vinyl proton at 5.63 and the vinyl Me at 1.89 ppm, as well as the secondary Me at 0.84 and a CH in the 1.5 ppm region. The later is overlapped with the signals of a methylene group coupled with another two methylene protons which appear as the AB part of an ABXY system at δ_A 2.71 and δ_B 2.29 ppm with $J_{AB} = 12$, $J_{AX} = 5$, $J_{AY} = 5$, $J_{BX} = 5$ and $J_{BY} = 5$ Hz. The chemical shift and the couplings of the AB protons of the ABXY system are indicative of an allylic methylene, which combined with the chemical shift of the vinyl Me and the vinyl proton allow assignment of the C-9 side chain of **2** as a Z-olefin [7–9] rather than as an E-olefin [2, 6–11].

Upon addition of deuterium oxide the ^1H NMR signals of **2** at 1.25, 2.06 and 2.36 disappear, the signal at 3.61 collapses to a sharp doublet ($J_{2,3}$ = 4 Hz) and that at 4.13 ppm to a double doublet of doublets ($J_{2,3} = 4$, $J_{1,2} = 12$ and $J_{1',2} = 5$ Hz). These couplings are indicative of a $-CH_2-CH(OH)-CH(OH)-C-$ arrangement in which the proton vicinal to the methylene group is axial and the other methine proton is equatorial. The only alternative to placing the three OH groups on the clerodane skeleton is therefore to locate them at C-2, C-3 and C-4. This was further evident after acetylation of methyl tucumanate (**2**) which yielded **3**, **4** and **5**. In compound **3** the signal assigned to H-2 is shifted to 5.25 ppm, in **4** that due to H-3 appears at 5.03 ppm and in **5**, H-2 is at 5.26 and H-3 at 5.06 ppm. Sarrett oxidation of **3** provided the keto-ester (**7**) that shows a new carbonyl absorption at 1710 cm^{-1} in the IR spectrum and H-2 as a double doublet (J = 12.5 and J' = 7 Hz) at 5.96 ppm in the ^1H spectrum.

The stereochemistry of methyl tucumanate (**2**) was ascertained from the ^1H NMR chemical shifts of the Me groups in comparison with the data of a large body of clerodanes [2–6, 12–20]. Thus, the shifts of the secondary and tertiary Me groups at C-8 and C-9, respectively, are in agreement with the data [2–6, 12–15] of compounds having both of these substituents as *alpha* on a *trans*-clerodane skeleton. On the other hand, the C-4 and C-5 Me groups as being both also in the *alpha* configuration, is in turn consistent after evaluation of the data of other *trans*-clerodanes [2, 4, 5, 13]. Furthermore, cursory examination of the signal due to the Me group at C-5,

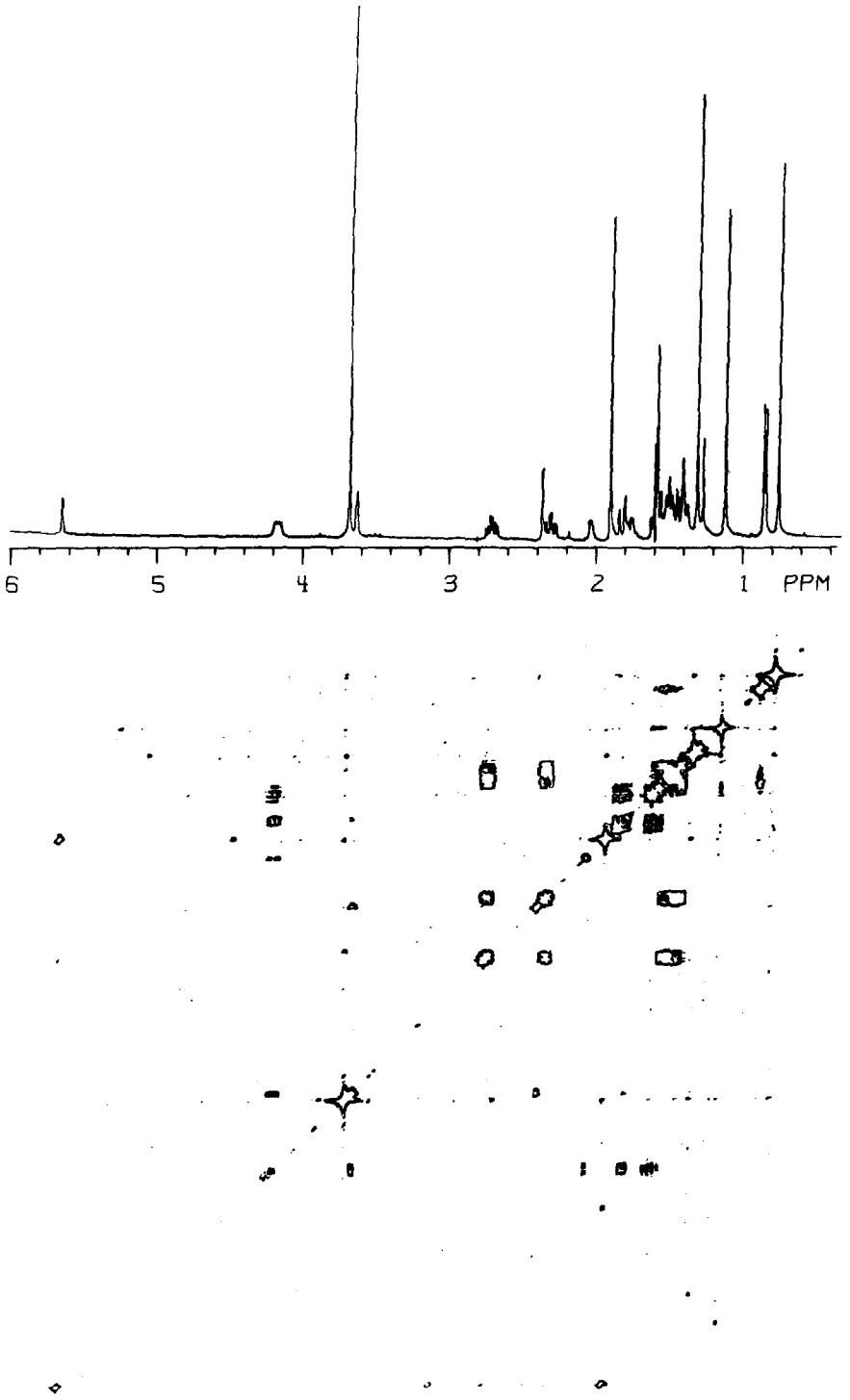
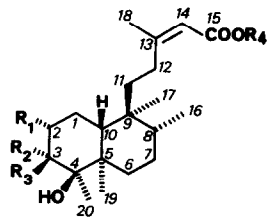


Fig. 1. Two-dimensional homonuclear ^1H–^1H chemical shift correlation diagram for methyl tucumanate (2) at 360 MHz. The peak near 1.6 ppm is due to moisture.

reveals that the peak is broader and therefore smaller than those signals of the other two tertiary Me groups in the molecule (2). Such a situation is due to a 4σ-bonds coupling of the Me protons and the axial hydrogen at C-6 which appears in the 1.5–1.6 ppm region as is evident in the contour plot depicted in Fig. 1. Such long range couplings of the protons of angular Me groups are well established in the steroid field and are indicative of an axial Me group on *trans*-fused decalins, since only in such situations the subtle stereochemical requirements for significative 4σ-bonds coupling are given [21]. Therefore the Me group at C-5 is α-axial and that at C-4 is α-



	R_1	R_2	R_3	R_4
1	OH	OH	H	H
2	OH	OH	H	Me
3	OAc	OH	H	Me
4	OH	OAc	H	Me
5	OAc	OAc	H	Me
6	O-C(Me)$_2$-O		H	Me
7	OAc	—O—		Me

equatorial. The coupling constants (*vide supra*) for H-2 and H-3 are thus indicative of the 2-OH group as α-equatorial and the 3-OH group as α-axial. The *cis* relationship of the alcohols at C-2 and C-3 was further confirmed by synthesis of the acetonide (**6**). The resulting antiperiplanar arrangement of the 3,4-di OH moiety seems to be responsible for the absence of the $[M]^+$ in **2** and their derived acetates (**3–5** and **7**). In fact, only in the case of the acetonide (**6**), could the $[M]^+$ be observed in the mass spectrum. The absolute configuration of methyl tucumanate as depicted in structure **2**, is consistent with the negative molecular rotation of related clerodanes [2, 13, 17].

EXPERIMENTAL

Mps are uncorr. IR spectra were obtained in KBr pellets and UV spectra from MeOH soln. ^{1}H NMR spectra were measured at 60 and 360 MHz from $CDCl_3$ solns containing int TMS. ^{13}C NMR spectra were recorded at 25.2 MHz. MS were obtained at 70 eV using a direct inlet.

Extraction. Baccharis tucumanensis H. et A. (Voucher No. 19, Instituto M. Lillo, S.M. de Tucumán, Argentina) was collected near El Cadillal, Tucumán, Argentina on 10 March, 1980. Dried leaves (1960 g) were extracted with EtOAc (3 l. each, × 3) at room temp during 72 hr. The combined extracts were evapd to dryness under vac yielding a dark residue (192 g) to which H_2O was added (10%, 20 and 30) then partitioned between *n*-hexane, CCl_4 and $CHCl_3$, respectively. The $CHCl_3$ extract was evapd and the residue (56.8 g) subjected to CC on silica gel (500 g) developed successively with C_6H_6 and C_6H_6 containing increasing proportions of EtOAc, 500 ml fraction being collected. Me tucumanate (5 g) was obtained after CH_2N_2 treatment under the usual conditions.

Me tucumanate (**2**). Mp 190–191°; IR: 3400–3200, 1155 and 1040 (OH), 1695 and 1645 (α,β-unsaturated ester) and 860 cm^{-1}; UV: λ_{max} 233 nm (log ε), 4.15; MS *m/z* (rel. int.) 350.2502 $C_{21}H_{34}O_4$ require 350.2457 ($[M-H_2O]^+$, 3.6), 318.2157 $C_{20}H_{30}O_3$ require 318.2195 (350 – MeOH, 9.6), 276.2157 $C_{18}H_{28}O_2$ require 276.2089 (39.7), 275.2027 $C_{18}H_{27}O_2$ require 275.2011 (33.7), 223 ([M-side chain]$^+$, 14.4), 205 (18.0), 137 (57.8), 123 (100), 114 (36.1), 95 (43.4), 43 (51.8); $[\alpha]_D^{20°}$ −5.6, $[\alpha]_{578}$ −5.0°, $[\alpha]_{546}$ −5.8°, $[\alpha]_{436}$ −12.9°, $[\alpha]_{365}$ −25.8° (MeOH, *c*, 0.48); ^{1}H NMR (361 MHz): 5.63 (*br s*, 1H, H-14), 4.13 (*br m*, 1H, after adding D_2O: *ddd*, $J_{2,3}$ = 4, $J_{1\beta,2}$ = 12, $J_{1\alpha,2}$ = 5 Hz, H-2), 3.67 (*s*, 3H, OMe), 3.61 (*br t*, 1H, after adding D_2O: *d*, $J_{2,3}$ = 4 Hz, H-3), 2.71 and 2.29 (2*td*, 1H each, AB part of ABXY system $J_{12,12'}$ = 12, $J_{12,13}$ = 5, $J_{12,13'}$ = 5, $J_{12',13}$ = 5, $J_{12',13'}$ = 5 Hz, C-12 protons; the C-11 protons being in the 1.4–1.6 ppm region), 2.36 (*d*, 1H, *J* = 2.5 Hz, disappearing with D_2O, 3-OH), 2.06 (*br d*, 1H, *J* = 3.5, disappearing with D_2O, 2-OH), 1.89 (*d*, 3H, $J_{14,18}$ = 1.6 Hz, vinyl Me), 1.81 (*dd*, 1H, $J_{1\alpha,10}$ = 13, $J_{1\beta,10}$ = 2 Hz, H-10) the C-1 protons being at 1.75 and ~ 1.57 ppm), 1.29 (*s*, 3H, Me at C-4), 1.25 (*s*, 1H, disappearing with D_2O, 4-OH), 1.10 (*s*, 3H, Me at C-5), 0.84 (*d*, 3H, *J* = 6 Hz, Me at C-8; H-8 being at ~ 1.5 ppm) and 0.74 ppm (*s*, 3H, Me at C-9); ^{13}C NMR (pyridine-d_5): 166.5 (*s*, C-15), 161.5 (*s*, C-13), 115.7 (*d*, C-14), 79.5 (*d*, C-3), 77.0 (*s*, C-4), 69.6 (*d*, C-2), 50.7 (*q*, OMe), 41.5 (*s*, C-5), 39.3 (*s*, C-9), 39.1 (*d*, C-10), 37.3 (*t*, C-12), 36.6 (*d*, C-8), 32.9 (*t*, C-11), 28.1 (*t*, C-6), 27.4 (*t*, C-1), 26.9 (*t*, C-7), 25.2 (*q*, C-20), 22.2 (*q*, C-19), 18.7 (*q*, C-18), 17.8 (*q*, C-17) and 16.4 ppm (*q*, C-16); ^{13}C NMR (Me_2CO-d_6): 167.0 (*s*, C-15), 162.2 (*s*, C-13), 115.9 (*d*, C-14), 79.5 (*d*, C-3), 77.0 (*s*, C-4), 69.4 (*d*, C-2), 51.0 (*q*, OMe), 41.5 (*s*, C-5), 39.6 (*s*, C-9), 39.2 (*d*, C-10), 37.7 (*t*, C-12), 36.9 (*d*, C-8), 33.0 (*t*, C-11), 28.2 (*t*, C-6), 27.6 (*t*, C-1), 26.6 (*t*, C-7), 25.3 (*q*, C-20), 21.7 (*q*, C-19), 18.7 (*q*, C-18), 17.7 (*q*, C-17) and 16.4 ppm (*q*, C-16). The signals assigned to C-1 and C-6 might be reversed. The assignments were made using published clerodane data [6, 10, 11, 14, 17, 20, 22–24] and group effects.

Acetylation of Me tucumanate (**2**). A soln containing 450 mg of **2** in 9 ml of Ac_2O was treated with 1 mg of *N,N*-dimethylaminopyridine at room temp. After 72 hr the reaction mixture was extracted as usual. The resulting mixture was sepd by CC on silica gel eluting with C_6H_6–EtOAc. This yielded 361 mg (72%) of **3**, 40 mg (8%) of **4** and 83 mg (15%) of **5**.

2-Acetyl Me tucumanate (**3**). Mp 143–145°; IR: 3600–3250, 2950, 1720, 1700, 1650, 1100, 1040 and 880 cm^{-1}; MS *m/z* (rel. int.): 350 ($[M-AcOH]^+$, 8.8), 276 (32.4), 275 (25.7), 223 (14.8), 205 (21.6), 137 (28.4), 123 (100), 95 (43.2), 43 (85.1); $[\alpha]_D^{20°}$ −29.3°, $[\alpha]_{578}$ −30.5°, $[\alpha]_{546}$ −35.0°, $[\alpha]_{436}$ −63.5°, $[\alpha]_{365}$ −106.6° (*c*, 0.57, $CHCl_3$); ^{1}H NMR (60 MHz): 5.66 (*br s*, 1H, H-14), 5.25 (*ddd*, 1H, $J_{2,3}$ = 4, $J_{1,2}$ = 7, $J_{1',2}$ = 12 Hz, H-2), 3.66 (*d*, 1H, $J_{2,3}$ = 4 Hz, H-3), 3.64 (*s*, 3H, OMe), 2.08 (*s*, 3H, Ac), 1.90 (*d*, 3H, $J_{14,18}$ = 1.6 Hz, vinyl Me), 1.28 (*s*, 3H, Me at C-4), 1.10 (*s*, 3H, Me at C-5), 0.83 (*d*, 3H, *J* = 6 Hz, Me at C-8) and 0.73 ppm (*s*, 3H, Me at C-9).

3-Acetyl Me tucumanate (**4**). IR: 3580–3280, 2920, 1720, 1695, 1640, 1260, 1160 and 880 cm^{-1}; MS *m/z* (rel. int.): 350 ($[M-AcOH]^+$, 6.7), 276 (29.2), 275 (25.6), 223 (18.9), 205 (20.7), 137 (26.2), 123 (100), 95 (43.8), 43 (86.0); ^{1}H NMR (60 MHz): 5.63 (*br s*, 1H, H-14), 5.03 (*d*, 1H, $J_{2,3}$ = 4 Hz, H-3), 4.30 (*td*, 1H, $J_{2,3} \cong J_{1,2} \cong 5$, $J_{1',2}$ = 12 Hz, H-2), 3.61 (*s*, 3H, OMe), 2.13 (*s*, 3H, Ac), 1.88 (*d*, 3H, $J_{14,18}$ = 1.6 Hz, vinyl Me), 1.13 (*s*, 3H, Me at C-4), 1.05 (*s*, 3H, Me at C-5), 0.80 (*d*, 3H, *J* = 6 Hz, Me at C-8) and 0.73 ppm (*s*, 3H, Me at C-9).

2,3-Diacetyl Me tucumanate (**5**). Mp 120–122°; IR: 3600–3300, 2940, 1740, 1720, 1695, 1645, 1240, 1160, 880 cm^{-1}; MS *m/z* (rel. int.): 350 (15.5), 276 (50.0), 275 (25.1), 223 (24.1), 205 (32.7), 137 (31.0), 123 (100), 95 (32.7), 43 (53.4); ^{1}H NMR (60 MHz): 5.58 (*br s*, 1H, H-14), 5.26 (*ddd*, 1H, $J_{2,3}$ = 4, $J_{1,2}$ = 7, $J_{1',2}$ = 12 Hz, H-2), 5.06 (*d*, 1H, $J_{2,3}$ = 4 Hz, H-3), 3.61 (*s*, 3H, OMe), 2.08 and 1.92 (2*s*, 3H each, 2Ac), 1.86 (*d*, 3H, $J_{14,18}$ = 1.6 Hz, vinyl Me), 1.10 (*s*, 3H, Me at C-4), 1.06 (*s*, 3H, Me at C-5), 0.83 (*d*, 3H, *J* = 6 Hz, Me at C-8) and 0.73 ppm (*s*, 3H, Me at C-9).

Oxidation of **3**. A sample of 89 mg of **3** was treated with CrO_3 (180 mg) under Sarrett conditions during 12 hr. The oily keto-ester (**7**) obtained after work-up as usual (42 mg) showed IR: 3600–3300, 2950, 1720, 1710, 1695, 1650, 1490, 1020 and 880 cm^{-1}; MS *m/z* (rel. int.) 348 ($[M-AcOH]^+$, 38.1), 316 ($[348-MeOH]^+$, 11.9), 221 ($[348-$side chain$]^+$, 47.6), 203

(16.6), 147 (41.6), 127 (20.2), 123 (73.8), 95 (65.5), 43 (100); ^{1}H NMR (60 MHz): 5.96 (*dd*, 1H, $J_{1,2}$ = 12.5, $J_{1',2}$ = 7 Hz, H-2), 5.63 (*br s*, 1H, H-14), 3.61 (*s*, 3H, OMe), 2.13 (*s*, 3H, Ac), 1.88 (*d*, 3H, $J_{14,18}$ = 1.6 Hz, vinyl Me), 1.22 (*s*, 3H, Me at C-4), 1.15 (*s*, 3H, Me at C-5), 0.85 (*d*, 3H, J = 6 Hz, Me at C-8) and 0.73 ppm (*s*, 3H, Me at C-9).

Acetonide (**6**). Treatment of **2** (80 mg) in Me_2CO (25 ml) with *p*-TsOH (270 mg) at room temp during 4 hr gave 77 mg of **6** as a gum. It showed IR: 3550–3350, 1720, 1645, 1450, 1380, 880 cm^{-1}; MS *m/z* (rel. int.) 408 ([M]$^+$, 19.5), 393 ([M − Me]$^+$, 4.2), 350 ([M − Me_2CO]$^+$, 13.5), 276 (15.2), 275 (17.8), 223 ([350 − side chain]$^+$, 6.8), 205 (18.6), 143 (100), 127 (30.5), 123 (67.8), 95 (35.6), 43 (61.8). ^{1}H NMR (60 MHz): 5.63 (*br s*, H-1, H-14), 4.30 (*ddd*, 1H, $J_{2,3}$ = 5, $J_{1,2}$ = 7, $J_{1',2}$ = 12 Hz, H-2), 3.86 (*d*, 1H, $J_{2,3}$ = 5 Hz, H-3), 3.68 (*s*, 3H, OMe), 1.86 (*d*, 3H, $J_{14,18}$ = 1.6 Hz, vinyl Me), 1.26 (*s*, 3H, Me at C-4), 1.50 and 1.28 (2*s*, 3H each, Me_2C), 1.13 (*s*, 3H, Me at C-5), 0.85 (*d*, 3H, J = 6 Hz, Me at C-8) and 0.73 ppm (*s*, 3H, Me at C-9).

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