Neoclerodane Diterpenes from *Baccharis crispa*

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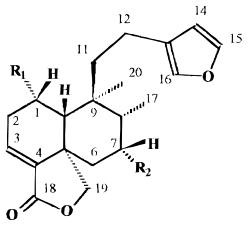
In order to confirm the previously proposed stereochemistry for bacrispine (**1**), several high-field 2D NMR (500 MHz) experiments were carried out. Complete NMR assignments, as well as the validation of the relative stereochemistry of 1-deoxybacrispine (**2**), were established. In an investigation of the aerial parts of *Baccharis crispa*, a medicinal plant from Argentina, two new neoclerodanes (**4**, and **5**) have been isolated and their structures determined by spectroscopic means.

Baccharis crispa Sprengel (Asteraceae) is well known for its medicinal value and is widely used in Argentina as an infusion with choleretic and digestive properties. This plant is indigenous to central and western regions of Argentina and is occasionally cultivated. The *Farmacopea Nacional Argentina*[1] gives the botanical description and pharmacological properties of this plant under the name "carqueja." In previous papers,[2,3] we reported the isolation and structural elucidation of two new clerodane-type diterpenes, bacrispine (**1**) and 1-deoxybacrispine (**2**), together with the known compound **3**.[4] The diterpenes **1** and **3** showed interesting antifeedant effects toward *Tenebrio molitor* L. larvae.[5]

The structures of these compounds were elucidated from spectroscopic data of several related derivatives of **1**, prepared by selective acetylation, reduction, oxidation, and dehydratation reactions. These data allowed us to assign the connectivities and arrive at some stereochemical conclusions. However, the ^{1}H-NMR study was not sufficient to establish the relative stereochemistries at all of the chiral centers of furanditerpene **1**.[6] On the other hand, the relative stereochemistry of **2** was proposed on the basis of some differences in the ^{1}H-NMR chemical shifts in the spectra run in pyridine-d_5 and $CDCl_3$ at 60 MHz.[3] In both cases, ^{13}C-NMR studies were not performed and all of the protons assignments were not established.

We report herein on the unequivocal assignments of ^{1}H- and ^{13}C-NMR spectra, the relative configurations of **1** and **2**, as well as the isolation and structural elucidation of two new minor constituents, **4** and **5**, all obtained from aerial parts of this plant collected in the flowering stage.

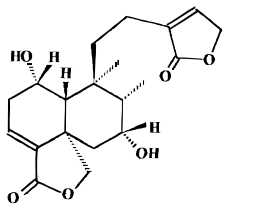
The assignments of ^{1}H-NMR (Table 1) and ^{13}C-NMR spectral data (Table 2) were made by a combination of 2D NMR (^{1}H−^{1}H-COSY, HMBC, HMQC, and HETCOR) techniques. The structure of **1** was unambiguously confirmed from analysis of HMBC, HMQC, NOESY, and NOE data. In particular, the HMBC experiment of **1** was conclusive in the assignment of the entire structure; selected correlations are shown in Table 3. The relative stereochemistry was assigned on the basis of NOE difference and NOESY data. Irradiation of the signal at δ 1.12 (H-20) caused an NOE enhancement of the signals at δ 4.70 (H-19 *pro*-S) and δ 5.37 (H-19 *pro*-*R*), but had no effect on the signals at δ 4.40 (H-1) and δ 4.01 (H-7). No cross peaks between H-19/H-1, H-19/H-7, H-20/H-1, and H-20/H-7 were displayed in a 2D NOESY experiment. The above data were in agreement with a structure where C-19 and C-20 are on the same face of the molecule while the hydroxyl groups at C-1 and C-7 are in the axial-α orientation. The *W* coupling (J = 1.5 Hz) exhibited by the H-19 *pro*-*S* diastereotopic proton with H-6β suggested the α-axial configuration of C-19.[7] All of these data are in agreement with a *trans*-fusion in the decalin moiety; therefore, the structure of **1** was confirmed as 15,16-epoxy-1α,7α-dihydroxy-neocleroda-3,13(16),14-trien-18,19-olide.



1 $R_1 = R_2 = OH$

2 $R_1 = H$ $R_2 = OH$

3 $R_1 = R_2 = H$



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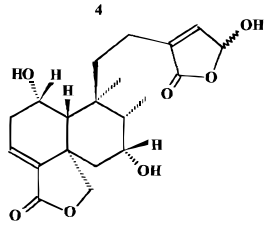

5

Our earlier report described the structure elucidation of compound **2**, which was based on the ^{1}H-NMR spectral data. In this structure determination, the sterochemistry needed further clarification.[3] Now, from the 200 MHz ^{1}H-NMR, COSY, and HETCOR spectra it was possible to assign completely the ^{1}H and ^{13}C signals (see Tables 1 and 2) in terms of structure **2**. The ^{13}C-NMR chemical shifts of C-17 and C-20 indicated the α-orientation of both methyl groups on a *trans*-clerodane skeleton.[7,8] The stereochemistry was confirmed by the NOESY spectrum, which fully supported the relative stereochemistry suggested previously. In this 2D experiment, cross peaks between H-20/H-17 and H-19-*pro*-*R*/H-20 were displayed. No cross peaks between H-19/H-7 and H-20/H-7 were observed.

These results can be rationalized only if C-20, C-17, C-19, and the hydroxyl group at C-7 are on the same face of the molecule. All of these data are in agreement with compound **2** being a neoclerodane skeleton similar to that of bacrispine (**1**). Thus, **2** was confirmed to be

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**, **2**, **4**, and **5** in $CDCl_3$

	compound			
position	**1** δ_H[a]	**2** δ_H[b]	**4** δ_H[b]	**5** δ_H[b]
1	4.40 m ($w_{1/2}$ = 10.0 Hz)		4.48 br s	4.48 m ($w_{1/2}$ = 13 Hz)
2H-2	2.40 m	2.40 m	2.45 m	2.50 m
3	6.58 t (3.0)	6. 73 t (3.0)	6.58 t (3.0)	6.58 t (4.0)
6α	2.35 dd (14.0, 4.0)	2.35 dd (14.0, 5.0)	2.35 dd (13.0, 4.7)	2.37 m
6*β*	1.42 ddd (14.0, 4.5, 1.5)	1.42 ddd (14.0, 4.5, 1.5)	1.40 ddd (13.0, 4.5, 1.5)	1.30 m
7	4.01 m ($w_{1/2}$ = 9.5 Hz)	4.15 m	4.08 m	4.10 m
8	1.75 m	1.78 m	1.70 m	1.78 m
10	1.81 br s		1.75 m	1.65 m
2H-11	1.65−1.83 m[c]	1.60−1.90 m[c]	1.60−1.80 m[c]	1.40−1.70 m[c]
2H-12	1.92−2.17 m[c]	2.20−2.40 m[c]	2.10−2.25 m[c]	1.90−2.20 m[c]
14	6.30 br s	6.30 br s	7.24 br s	6.90 br s
15	7.40 br s	7.40 br s	4.82 br s	6.10 br s
16	7.22 br s	7.22 br s		
17	1.08 d (6.0)	1.08 d (6.0)	1.08 d (6.5)	1.05 d (6.0)
19 *pro-S*	4.70 dd (9.0, 1.5)	3.95 dd (9.0, 1.5)	4.68 dd (9.0, 1.5)	4.68 dd (9.0, 1.5)
19 *pro-R*	5.37 d (9.0)	5.33 d (9.0)	5.35 d (9.0)	5.38 d (9.0)
20	1.12 s	0.91 s	1.15 s	1.13 s

[a] Run at 500.13 MHz. [b] Run at 200.13 MHz. [c] Overlapped signals *J* unresolved.

Table 2. ^{13}C NMR Data of Compounds **1**, **2**, **4**, and **5** in $CDCl_3$[a]

	compound			
position	**1** δ_C[b]	**2** δ_C[c]	**4** δ_C[c]	**5** δ_C[c]
1	63.3 d	18.11 t	63.0 d	63.2 d
2	35.5 t	19.16 t	35.8 t	35.4 t
3	129.9 d	135.0 d	130.1 d	129.4 d
4	137.7 s	138.3 s	137.5 s	137.7 s
5	38.7 s	38.8 s	38.7 s	38.6 s
6	41.4 t	40.3 t	41.4 t	41.1 t
7	71.8 d	72.2 d	71.7 d	71.6 d
8	49.7 d	48.1 d	50.0 d	49.6 d
9	44.3 s	44.9 s	44.3 s	44.2 s
10	41.2 d	40.3 d	41.2 d	41.0 d
11	38.2 t	38.5 t	36.5 t	36.4 t
12	18.1 t	18.1 t	18.9 t	19.1 t
13	124.6 s	124.7 s	133.7 s	136.9 s
14	110.5 d	110.7 d	144.8 d	143.7 d
15	142.6 d	142.8 d	74.8 t	97.2 d
16	138.1 d	138.9 d	174.9 s	171.1 s
17	11.3 q	11.9 q	11.3 q	11.6 q
18	171.5 s	170.3 s	171.5 s	170.6 s
19	74.8 t	72.8 t	70.5 t	74.6 t
20	19.8 q	19.2 q	19.7 q	20.0 q

[a] Multiplicities were determined by a DEPT experiment. [b] Run at 127.7 MHz. [c] Run at 50.2 MHz.

Table 3. HMBC Correlations on Compound **1**[a]

proton	correlated carbons	proton	correlated carbons
1	C-5	15	C-13, C-16
2	C-1, C-3, C-4	16	C-15
3	C-1, C-2, C-4, C-5, C-18, C-19	17	C-7, C-9, C-10
6α	C-4, C-5, C-7, C-8, C-10	19 *pro-R*	C-4, C-5, C-6, C-18
6*β*	C-5, C-19	19 *pro-S*	C-5, C-6
8	C-17, C-20	20	C-8, C-9, C-10
14	C-13, C-15, C16		

[a] 500 MHz, in $CDCl_3$.

15,16-epoxy-7α-hydroxyneocleroda-3,13(16),14-trien-18,19-olide.

The HREIMS of compound **4** displayed a molecular ion at *m*/*z* 362.172 93 that established the elemental composition $C_{20}H_{26}O_6$ (calcd 362.172 98). The IR spectrum exhibited bands for the hydroxyl groups (3450−3465 cm^{-1}) and for an α,*β*-unsaturated-*γ*-lactone (1730, 1680 cm^{-1}). The ^{1}H-NMR spectrum in $CDCl_3$ (Table 1) showed a methyl group singlet (H-20) at *δ* 1.15 and a three-proton doublet (*J* = 6.5 Hz) at *δ* 1.08 assigned to H-17. Except for the absence of the typical *β*-substituted furan signals, the ^{1}H-NMR spectra of **4** closely resembled that of **1**. A broad singlet at *δ* 7.24 clearly coupled from the COSY spectra with a two-proton broad singlet at *δ* 4.82 replaced the furan pattern observed in **1**. The first of these signals was characteristic of a proton on the *β*-carbon of a α-substituted butenolide ring.[9] The ^{13}C-NMR signals (Table 2) assigned with the aid of HETCOR spectra revealed that only four carbons (C-13, C-14, C-15 and C-16) showed significant chemical shift differences with those observed in **1**. The above data were in agreement with the presence of an α-ethylbutenolide on C-9 of the decalin moiety, and the connectivities in the last portion should be identical with those observed in the compound **1**. The stereochemistry of **4** is proposed on the basis of NOESY cross peaks observed between H-20/H17, H6*β*/H-10, H-8/H10, and H-19 (two protons)/H-20, and these data clearly indicated that the relative stereochemistry of **4** is identical to that observed in compounds **1** and **2**.

Finally, the substitution of the furan ring by the butenolide moiety in the side chain at C-9 could be confirmed from the EIMS. Hence, the EIMS of compound **1** and **2** showed typical *β*-substituted furan fragments at *m*/*z* 95 and 81 as prominent peaks.[10] These signals were not present in the EIMS of compound **4**. The peaks at *m*/*z* at 134, 105, 91, 77, and 65 may be proposed as a consequence of the retro-Diels−Alder fragmentation with aromatization of the ring A from the *m*/*z* 326 $[M - 2H_2O]^+$ ion. The ions at *m*/*z* 105 and 91 have been observed as the more important fragments in the EIMS of secoclerodanes with a diene system that can assist the aromatization at the ring A.[9] The ion at *m*/*z* 97 could arise by the allylic cleavage of the C-11/C-12 bond. Finally, the signals at *m*/*z* 69, 53, and 39 can be attributed to the cyclopropenyl fragments[11] derived from the *m*/*z* 97 ion. Thus, **4** is assigned the structure 1α,7α-dihydroxyneocleroda-3,13-dien-16,15:18,19-diolide.

The minor constituent **5** was isolated as an inseparable mixture of epimeric cyclic hemiacetals that no gave molecular ion peak in its EIMS. The molecular formula $C_{20}H_{26}O_7$ was assigned from the combination of ^{1}H- and ^{13}C-NMR (HETCOR and DEPT) spectral data. The ^{13}C-NMR spectrum revealed the presence of a hemiacetal carbon (*δ* 97.2)[9] instead of the methylene

signal at δ 74.8, observed in the butenolide **4**; the remaining signals were very close to those observed for this diterpene. The ^{1}H-NMR (Table 1) spectrum of **5** showed the typical pattern observed for the decalin moiety of the neoclerodane described above, except for the signals corresponding to H-14, which showed a broad singlet resonance at δ 6.90 clearly coupled with H-15 (δ 6.10) from the COSY spectrum. This NMR experiment showed the coupling between H-19 *pro-S* with H-6β similar to that previously noted for compound **1**. Comparison of the chemical shifts of H-14 for compounds **4** and **5** showed that, in the latter, this proton appears 0.34 ppm upfield; this observation could be attributed to the effect of the vicinal hemiacetal hydroxyl group.

Finally, the absence of the peaks at *m/z* 95 and 81 in the EIMS, both observed in the spectra of compound **1** and **2**, confirmed the replacement of the furan ring in the side chain at C-9 by the γ-hydroxybutenolide function. Accordingly, compound **5** is assigned as 1α,7α,15-trihydroxyneocleroda-3,13-dien-16,15:18,19-diolide.

Experimental Section

General Experimental Procedures. Melting points were taken on a Leitz hot plate microscope and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter. The ^{1}H-NMR spectra were recorded in $CDCl_3$ at 200.13 and 500.13 MHz on a Bruker AC-200 and a Bruker AMX-500 spectrometer, respectively. The ^{13}C-NMR spectra were obtained with the same instruments at 50.23 and 125.7 MHz. COSY, HMQC, HMBC, NOESY, HETCOR, and COLOC experiments were obtained using standard Bruker software. IR spectra were recorded on a Bruker IFS-25 spectrometer. EIMS were collected at 70 eV on a VG Trio-2 instrument, and HR-EIMS were obtained with a VG-ZAB-BEQQ spectrometer at LANAIS-EMAR-CONICET, University of Buenos Aires. Column chromatography was performed on Si gel G 70−230 mesh and Kieselgel 60 H; TLC was carried out on Si gel 60 F_{254} (0.2 mm-thick plates) using C_6H_6−dioxane−AcOH (30:5:1) as solvent.

Plant Material. Aerial parts of *B. crispa* Sprengel were collected during November 1994 in El Volcán, Departamento La Capital, San Luis, Argentina, and a voucher specimen is deposited at the herbarium of INTA-Villa Mercedes (No. 2604).

Extraction and Isolation. The dry aerial parts (3.0 kg) were chopped and macerated twice for 10 day periods each time with MeOH. The solvent was evaporated under reduced pressure, and the residue (550 g) was taken up in MeOH, which after the addition of H_2O (10, 20 and 30%) was partitioned between *n*-hexane, CCl_4, and $CHCl_3$, respectively. The $CHCl_3$ solution was evaporated and the residue chromatographed on Si gel using *n*-hexane:EtOAc gradient. After several purifications, compounds **1** (1.5 g), **2** (896 mg), **4** (150 mg), and **5** (20 mg) were obtained.

Compound 1: white solid from EtOH; mp 210−212 °C; $[\alpha]^{25}_D$ −56.8° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 228.6 (3.59) nm; IR (KBr) ν_{max} 3580, 3400, 1039, 1025 (OH); 1500, 1460, 880, 788 (furan ring); 1750, 1668, 1220, 1200 (α,β-unsaturated-γ-lactone) cm^{-1}; ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2; *anal.* EIMS (70 eV) *m/z* 346 $[M]^+$ (3), 249 (19), 119 (42), 117 (43), 95 (36), 81 (43), 43 (100); C 69.36%, H 7.51%, calcd for $C_{20}H_{26}O_5$, C 69.10%, H 7.55%.

Compound 2: crystallized from MeOH: *n*-hexane; mp 198−202 °C; $[\alpha]^{25}_D$ −49.1 (*c* 0.72, MeOH); UV (MeOH) λ_{max} (log ϵ) 228.0 (3.14) nm; IR (KBr) ν_{max} 3450, 1030 (OH); 1500, 1450, 875, 788 (furan ring); 1750, 1667, 1210, 1195, 850 (α,β-unsaturated-γ-lactone) cm^{-1}. ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2; EIMS (70 eV) *m/z* 330 $[M]^+$ (40), 312 (2), 300 (10), 234 (2), 233 (82), 218 (16), 217 (10), 205 (31), 187 (6), 149 (15), 135 (21), 96 (16), 95 (57), 94 (14), 82 (53), 81 (100); *anal.* C 72.70%, H 7.93%, calcd for $C_{20}H_{26}O_4$, C 72.78%, H 8.24%.

Compound 4: white solid from MeOH:*n*-hexane; mp 247−248 °C; $[\alpha]^{25}_D$ −55.9° (*c* 1.07, MeOH); IR (KBr) ν_{max} 3460, 2940, 1040 (OH), 1730, 1680 (C=O), 1446, 1212 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2; HREIMS *m/z* 362.172 93 $[M]^+$ ($C_{20}H_{26}O_6$) calcd 362.172 98. EIMS (70 eV) *m/z* 362 $[M]^+$ (5), 326 (1), 221 (14), 134 (4), 107 (18), 105 (22), 97 (8), 91 (43), 77 (32), 69 (25), 65 (21), 53 (37), 43 (100).

Compound 5 (epimeric mixture): IR (KBr) νmax 3410, 1030, 1020 (OH); 1745, 1730 (C=O), 1205, 1120, 950 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR see Tables 1 and 2; EIMS (70 eV) *m/z* 279 (9), 167 (16), 149 (72), 84 (100), 66 (96), 57 (54), 46 (65), 43 (69).

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