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Note

^{13}C NMR assignments and conformational evaluation of diterpenes from *Croton sarcopetalus* Muell.

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ABSTRACT: The known diterpenes junceic acid (**1**) and the stress metabolite derivatives **2**–**4** were isolated from the hexane extracts of the roots of *Croton sarcopetalus* Muell. The complete ^{13}C NMR chemical shift assignments of these compounds were achieved with the aid of DEPT and HETCOR experiments. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: ^{13}C NMR; diterpenes; clerodane, *ent*-pimarane; *ent*-beyerane; *ent*-atisane

INTRODUCTION

Croton sarcopetalus Muell. (Euphobiaceae), commonly known as 'lecherón' is a shrub that grows in northwestern and central Argentina.[1] Most of the species of this genus produce a significant variety of diterpenes, some with anti-tumoral activity,[2] including tigliane derivatives with recognized tumoral promotory activity.[3]

From the hexane extracts of the roots of *Croton sarcopetalus* Muell. we isolated the known diterpenes junceic acid (**1**)[4,5] and compounds **2**–**4**.[6] The latter compounds are interesting because they are considered to be stress metabolite derivatives.[6] Junceic acid (**1**) and its enantiomer are unique compounds with a clerodane skeleton where C-20 is oxidized to a carboxylic acid group. In addition, **2**–**4** are uncommon in nature owing to their keto group functionalization at C-3.

The ^{1}H NMR data of all these compounds have been presented in previous reports,[4–6] with the exception of the 6-CH$_2$ and 7-CH$_2$ assignments for **2**–**4**. Our assignments for these methylenes are based on the minimum energy conformation structures by means of MMX calculations. Complete ^{13}C NMR chemical shift assignments of **1**–**4** were achieved by one- and two-dimensional NMR experiments and the results are reported here.

RESULTS AND DISCUSSION

Junceic acid (**1**) was identified by agreement of their optical rotation and ^{1}H NMR data with those in the literature.[5,6] The ^{13}C NMR spectrum shows 20 signals (Table 1), one corresponding to the carboxylic acid carbonyl at 183.2 ppm and a further six signals corresponding to sp^2 carbons. Of these, according to a DEPT experiment, four appear as CH signals at 121.1, 110.9, 138.6 and 142.8 ppm, which were easily assigned to C-3, C-14, C-15 and C-16, respectively, after a HETCOR experiment, since the ^{1}H NMR assignments are known.[4,5] The remaining sp^2 carbons at 143.4 and

Table 1. ^{13}C NMR data (δ, ppm) for compounds **1**–**4**

C	1	2	3	4
1	17.9	37.1	37.1	37.5
2	27.2	34.0	34.5	34.1
3	121.1	216.1	215.7	217.2
4	143.4	47.6	47.8	47.5
5	38.9	55.0	54.4	55.3
6	37.5	20.9	23.2	20.3
7	27.4	35.7	35.2	36.3
8	37.1	49.2	137.4	39.2
9	50.0	54.2	53.2	51.9
10	48.4	36.9	38.5	37.4
11	33.9	36.1	34.5	23.1
12	20.3	211.5	212.1	44.0
13	124.7	57.3	52.1	131.8
14	110.9	58.2	127.2	135.9
15	138.6	138.9	142.6	53.5
16	142.8	136.7	115.5	74.1
17	16.6	17.1	23.5	31.5
18	17.5	26.0	25.7	26.3
19	18.1	21.7	22.2	21.9
20	183.2	13.5	14.1	15.1

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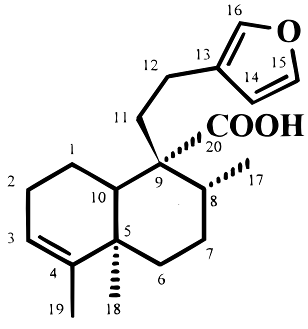

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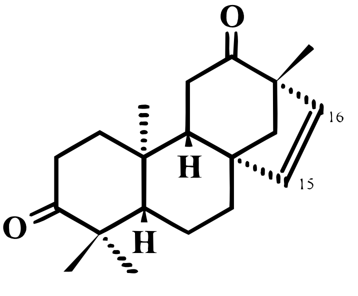

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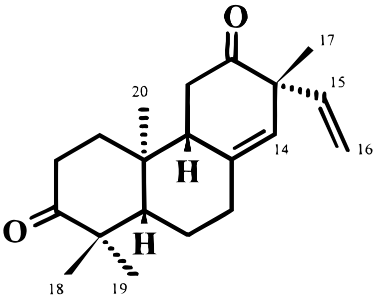

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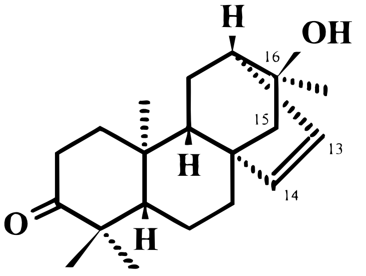

4

124.7 ppm correspond to C-4 and C-13, respectively, whose assignments were made by comparison with related compounds.[7] The aliphatic carbon atoms appear between 50.0 and 16.6 ppm, corresponding to three CH_3, six CH_2, two CH and two quaternary carbons. The protonated carbon assignments, except those corresponding to 11-CH_2 and 12-CH_2, are based on the HETCOR experiment and the ^1H NMR data.[4,5] The signals at 33.9 and 20.3 ppm were ascribed to 11-CH_2 and 12-CH_2, respectively, on the basis of the characteristic chemical shift of C-12 in related compounds.[7]

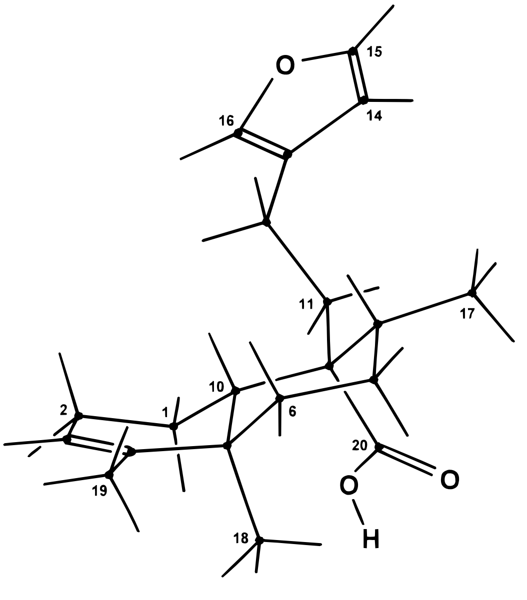

Figure 1. Minimum energy conformation of junceic acid (**1**).

The quaternary C-5 and C-9 assignments were made based on their chemical shifts and comparison with analogue compounds.[7]

In order to determine the conformation of **1**, we calculated the minimum energy conformation of its structure by means of MMX molecular modeling.[8] The calculated dihedral angles between H-1β and their vicinal protons were H1β—H2β = 46°, H1β—H2α = 71° and H1β—H10β = 74°. These angles allowed us to estimate in each case the expected coupling constant by using generalized Karplus-type equations,[9,10] which gave 5.7, 1.3 and 1.6 Hz, respectively. The H-1β signal at 2.35 ppm appears as a broadened double-doublet, $J_{gem} = 13.0$ Hz and $J(1\beta,2\beta) = 5.9$ Hz. The latter value is in agreement with the calculated coupling constant. Taking into account the rigidity of the C2—C3=C4—C5 fragment, we conclude from the above data that the A-ring conformation is that shown in Fig. 1. The same methodology as employed above was used to calculate all coupling constants involved in the C6—C7—C8 fragment. The calculated and observed coupling constants for this fragment are compared in Table 2, and the results led us to conclude that the B-ring has a chair conformation as shown in Fig. 1.

Table 2. Calculated and observed vicinal coupling constants and dihedral angles for the B-ring of **1**

	J_{obs} (Hz)	J_{calcd} (Hz)	ϕ (°)
6β,7α	12.7	13.2	171
6β,7β	4.2	3.8	56
6α,7α	3.3	3.7	56
6α,7β	3.3	3.5	58
7α,8β	12.8	12.3	177
7β,8β	3.3	2.8	61

In **2**, the ^{1}H NMR signals at 1.47 and 1.68 ppm were assigned to 6-CH$_2$ and those at 1.49 and 1.93 ppm to 7-CH$_2$ from HETCOR and COSY experiments and with the aid of MMX calculations.[8] The signals at 1.47 and 1.49 ppm along with that of H-5 at 1.37 ppm are observed as a complex system of signals. However, the signal at 1.93 ppm appears as a broadened double-triplet with $J_{gem} = 13.2$ Hz and two vicinal coupling constants of 3.2 Hz.

The calculated dihedral angles for H-7α were obtained from the minimum energy conformation using MMX molecular modeling,[8] which gave H6α—H7α = 56° and H6β—H7α = 59°. With these data we calculated, as above, the vicinal coupling constant values for H-7α as $J(6\alpha,7\alpha) = 3.78$ Hz and $J(6\beta,7\alpha) = 3.12$ Hz. These results are in agreement with the signal at 1.93 ppm corresponding to H-7α and that the B-ring has a chair conformation (see Fig. 2).

The ^{13}C NMR decoupled spectrum of **2** showed 20 lines (Table 1). DEPT analysis indicated two sp^2 methine carbons at 138.9 and 136.7 ppm and two saturated methines at 55.0 and 54.2 ppm. In addition, six methylene and four methyl carbons were observed. All these signals were assigned with the aid of a HETCOR experiment, taking into account the described proton assignments.[6] The remaining signals at 216.1, 211.5, 57.3, 49.2, 47.6 and 36.9 ppm are due to the non-hydrogen-bearing carbons. Those at 216.1 and 211.5 ppm, corresponding to keto carbonyl groups, were ascribed to C-3 and C-12, respectively, by comparison with related compounds.[11] The remaining quaternary carbon signals were assigned on the basis of their chemical shifts and by comparison with analogous compounds.[11]

In **3**, the ^{1}H NMR signals at 1.60 and 1.66 ppm were assigned to H-6α and H-6β, respectively, and those at 2.12 and 2.51 ppm to H-7β and H-7α, respectively, from HETCOR and COSY experiments and with the aid of MMX calculations.[8] The H-7α signal appears as a broadened *ddd* with $J_{gem} = 14.3$ Hz, $J(6\alpha,7\alpha) = 4.2$ Hz and $J(6\alpha,7\alpha) = 2.9$ Hz. The calculated dihedral angles for H-7α gave H6α—H7α = 54° and H6β—H7α = 60°. The calculated vicinal coupling constants using generalized Karplus-type equations[9,10] for these angles were 4.17 and 2.94 Hz. These data correspond to a chair conformation for the B-ring.

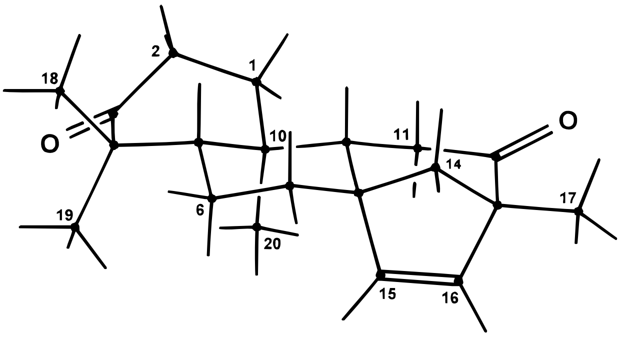

Figure 2. Minimum energy conformation of **2**.

The ^{13}C NMR spectrum of **3** showed significant changes only in the C—C double bond region when compared with the spectrum of **2** (Table 1). In this region four signals appear at 142.6, 137.4, 127.2 and 115.5 ppm; a DEPT experiment showed that the signals at 115.5 and 137.4 ppm correspond to a methylene carbon and a quaternary carbon, respectively, while the remaining two signals at 127.2 and 142.6 ppm correspond to methine carbons, which were ascribed to C-14 and C-15, respectively, as follows from a HETCOR experiment.

The ^{1}H NMR spectrum of **4** shows a broadened double-triplet at 1.89 ppm ($J = 13.2$ and 3.2 Hz) which, taking into account the MMX calculations made for **2**, was ascribed to H-7α. From HETCOR and COSY experiments we assigned the 6-CH$_2$ signals at 1.49–1.58 ppm (*m*) and H-7β at 1.24–1.34 ppm (*m*).

The ^{13}C NMR spectrum of **4** showed that the signals corresponding to the A-ring carbon atoms essentially appear at the same chemical shifts as those of **2** and **3**. In contrast, some significant changes are observed for the carbon chemical shifts of the remaining atoms. The assignment strategy for the protonated carbon atoms is similar to that used for the above cases, while the quaternary carbon atom assignments were made by comparison with related compounds.[12]

It is well known that in conformationally rigid molecules the chemical shifts of an equatorial methyl group, *gem* to a tertiary hydroxyl group, is around of 31 ppm and the corresponding axial methyl group appears around 25 ppm.[13] The 17-Me signal of **4** is observed at 31.5 ppm, which is indicative of its equatorial nature. Compounds **2** and **4** may probably derive from **3**. The possible biogenetic pathway are for **2** a C8—C15 bond formation and for **4** a [1,2]sigmatropic shift of the C13—C16 bond of **2**, probably through formation of a 12,13-oxide intermediate.

EXPERIMENTAL

Plant material

Roots of *C. sarcopetalus* Muell. were collected in December 1994 at San Pedro de Colalao, Tucumán Province, Argentina. A voucher specimen (Catalán No. 623) is deposited in the herbarium of the Fundación Miguel Lillo, Tucumán, Argentina.

Extraction and isolation

For the separation of mixtures, HPLC with a differential refractometric detector was used. The column employed was Beckman C$_{18}$ (5 μm, 250 × 10 mm i.d.). Retention times (t_R) were measured relative to the solvent peak.

The roots (500 g) of *Croton sarcopetalus* were extracted with petrol at room temperature for 6 days to give 12.9 g (yield 2.6%) of crude extract, which was chromatographed over silica gel. The fractions obtained were processed by HPLC, giving **1** (19 mg), m.p. 47–49 °C, t_R 7.5 min [MeOH–H_2O (95 : 5), 2.5 ml min^{-1}]; **3** (17 mg), m.p. 113–115 °C, t_R 5.7 min [MeOH–H_2O (9 : 1), 2.5 ml min^{-1}]; **2** (10 mg), m.p. 70–73 °C, t_R 9.7 min [MeOH–H_2O (85 : 15), 2.0 ml min^{-1}]; and **4** (9 mg), gum, t_R 17.5 min [MeOH–H_2O (4 : 1), 2.0 ml min^{-1}].

NMR spectra

^{1}H and ^{13}C NMR spectra were measured on a Varian XL-300GS spectrometer operating at 300 and 75.4 MHz, respectively, from $CDCl_3$ solutions with TMS as the internal standard. The probe temperature was 22 °C. For the HETCOR and COSY experiments 1024 data points were collected. The HETCOR spectra were obtained using a spectral width of 2348.5 Hz in f_1 and 14 064.7 in f_2 for **1**, 2382.7 Hz in f_1 and 11 198.2 in f_2 for **2**, 2358.5 Hz in f_1 and 11 376.6 Hz in f_2 for **3** and 2423.1 Hz in f_1 and 10 893.2 Hz in f_2 for **4**. A total of 256 time increments were collected with 256 transients per increments. The relaxation delay was 1 s. The COSY spectra were obtained using a spectral width of 2348.5 Hz for **1**, 2382.7 Hz for **2**, 2358.2 Hz for **3** and 2423.1 Hz for **4**. Other conditions were acquisition time 0.2 s, eight scans per t_1 increment and a relaxation delay of 1.75 s. DEPT experiments were acquired and processed with the software version provided by the spectrometer manufacturer.

MMX calculations

Molecular models were generated using MMX force-field calculations, which is a derivative version of the MM2 program developed by Allinger (QCPE 395), University of Georgia, as implemented in the PCMODEL program.

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