MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2000; **38**: 1019–1022

^{1}H and ^{13}C NMR assignments and conformational analysis of some podocarpene derivatives

Manuel Arnó, Miguel A. González, M. Luisa Marín and Ramón J. Zaragozá***

Departament de Química Orgànica, Facultat de Química, Universitat de València, E-46100 Burjassot (València), Spain

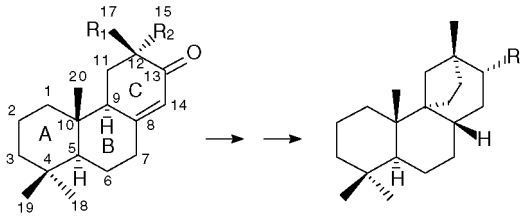
Received 11 May 2000; accepted 8 August 2000

ABSTRACT: This paper reports on the assignment of the ^{1}H and ^{13}C NMR spectra of five podocarpene derivatives. Resonance assignments were made on the basis of one- and two-dimensional NMR techniques which included ^{1}H, ^{13}C, DEPT and HMQC and also 1D NOE difference spectroscopy. The ratio of the different conformers in the six-membered C-ring of the podocarpene system was determined by molecular mechanics calculations and analysis of proton spin–spin coupling constants. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; conformational analysis; MMX calculations; podocarpene derivatives; scopadulan precursors

INTRODUCTION

In the past, several scopadulan terpenoids have been isolated from medicinal plants.[1] This new family of tetracyclic diterpenes shows interesting pharmacological properties.[2] Recently, we reported the first diastereoselective synthesis of the simplest member of this group, thyrsiflorin A methyl ester (**6**).[3] Using our synthetic route, several tricyclic podocarpene intermediates (**1**, **2**, **4** and **5**) (Scheme 1) have been prepared to achieve the synthesis of the target **6**.



	1	2	3	4	5
R_1	H	H	Me	Me	CO_2Me
R_2	H	Me	H	CO_2Me	Me

Scheme 1

In this paper, we show that the C-ring of these four podocarpene derivatives (**1**, **2**, **4** and **5**) and also of the 14β-methylpodocarpenone **3**, which was obtained from **2** by basic equilibration (see Experimental), can exist in two different half-chair conformations. Since the energies of the two conformations for podocarpenone **1** differ by only 0.9 kcal mol^{-1} (1 kcal = 4.184 kJ), the presence of substituents at C-12 (compounds **2–5**) has a great influence on the conformational equilibrium. The effect of several substituents at C-12 on the ratio of the two conformers was studied using both molecular mechanics calculations and ^{1}H NMR coupling constants.

RESULTS AND DISCUSSION

Assignments

All ^{13}C NMR signals can be separated into different classes of carbon atoms using the editing technique DEPT, and according to their chemical shifts and multiplicities most signals in this study were assigned (Table 1). Complete assignment of the remaining signals was made on the basis of their displacement, the $^{1}H–^{13}C$ shift correlation experiment (HMQC) and by comparison with similar compounds. The ^{1}H NMR spectrum was assigned using double resonance experiments, $^{1}H–^{13}C$ HMQC shift correlation experiments and some 1D NOE difference experiments (NOED). The most relevant signals and coupling constants for the conformational analysis are shown in Table 2.

The assigned stereochemistries at C-12 in **2–5** were determined from their spectroscopic data and confirmed by 1D NOE experiments. In the case of **2** and **5**, the NOE effect observed between protons H-15 (irradiated) and H-9 unambigously proved the α-orientation of the 12-methyl group. For **3** and **4**, the signals due to protons H-11α and H-11β were enhanced when proton H-17 was irradiated, confirming the β-orientation of the 12-methyl group for both compounds.

* *Correspondence to*: R. J. Zaragozá, Departament de Química Orgànica, Facultat de Química, Universitat de València, E-46100 Burjassot (València), Spain; e-mail: ramon.j.zaragoza@uv.es
** *Correspondence to*: M. Arnó, Departament de Química Orgànica, Facultat de Química, Universitat de València, E-46100 Burjassot (València), Spain.
Contract/grant sponsor: DGICYT; *Contract/grant number*: PB95-1088.

Table 1. ^{13}C NMR data for compounds **1–5**[a]

Carbon	1	2	3	4	5
1	39.3	39.4	39.2	38.9	39.1
2	18.7	18.8	18.7	18.6	18.6
3	41.7	41.8	41.7	41.6	41.6
4	33.4	33.4	33.4	33.3	33.4
5	53.8	54.4	53.6	53.5	53.8
6	21.9	22.5	21.7	21.9	21.7
7	35.6	35.8	35.1	35.1	35.0
8	165.8	164.5	164.5	165.2	164.7
9	51.6	48.4	52.4	49.6	48.4
10	38.9	39.6	38.4	38.7	38.7
11	20.5	27.6	29.3	32.0	30.1
12	36.8	39.6	40.3	—[b]	—[b]
13	200.0	203.3	202.1	196.1	198.0
14	125.8	123.9	125.5	124.8	123.3
15		16.7		173.1	19.6
17			14.7	21.2	173.9
18	33.6	33.7	33.6	33.5	33.5
19	22.0	22.0	22.1	22.0	22.0
20	15.3	15.4	15.1	14.7	15.1
CO_2Me				52.4	52.2

[a] Spectra measured in $CDCl_3$ at 298 K and referenced relative to TMS.
[b] Signal was hidden.

Conformational analysis

The conformational study was carried out using molecular mechanics calculations by means of the PCMODEL program. For every compound (**1–5**) a rotational analysis incrementing the dihedral angle C-9—C-11—C-12—C-13 by 5° steps from −85° to 85° using the option D-DRV was performed. In all cases, two minimun energy conformations, half-chair-1 (dihedral angle about −55°) and half-chair-2 (dihedral angle about 40°), were found, the rotational barrier between them being low (1–5 kcal mol^{-1}) (see Fig. 1).

The two conformers of each compound were independently minimized. For **4** and **5** containing a CO_2Me group which can occur in different non-equivalent spatial orientations, an independent rotational study for this group was carried out in order to find the most stable conformation.

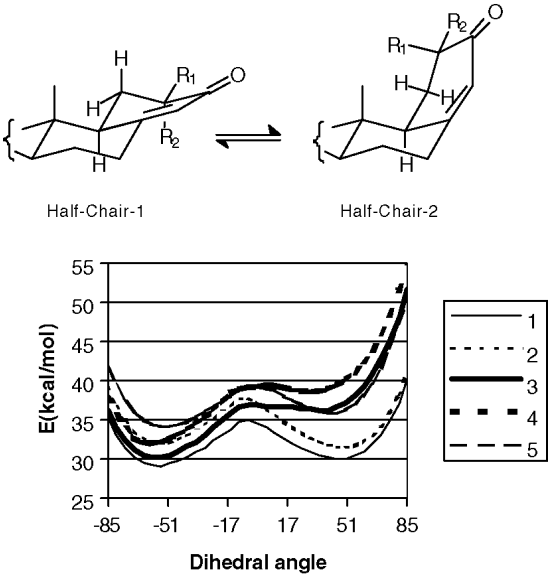


Figure 1. Rotational analysis for compounds **1–5**.

Table 2. Selected ^1H NMR chemical shifts (ppm) and coupling constants (Hz) of compounds **1–5**[a,b]

	1	2	3	4	5
H-7α	2.28 (ddddd, 15.6, 12.8, 6.6, 2.2, 1.5)	2.27 (ddddd, 14.5, 12.8, 6.4, 1.8, 1.5)	2.27 (ddddd, 15.9, 13.0, 6.7, 2.2, 1.5)	2.27 (ddddd, 15.7, 12.8, 6.5, 1.8, 1.5)	2.27 (ddddd, 15.8, 12.8, 6.7, 2.2, 1.3)
H-7β	2.54 (ddd, 15.6, 4.9, 2.0)	2.51 (ddd, 14.5, 4.6, 1.8)	2.53 (ddd, 15.9, 4.9, 1.9)	2.52 (ddd, 15.7, 5.3, 1.9)	2.56 (ddd, 15.8, 5.0, 1.8)
H-9	2.07 (dddd, 9.5, 5.3, 2.2, 1.5)	2.11 (dddd, 7.8, 6.0, 2.1, 1.5)	2.14 (dddd, 10.8, 5.1, 2.2, 1.5)	2.29 (dddd, 10.4, 5.3, 2.1, 1.5)	2.11 (dddd, 11.0, 5.0, 2.2, 1.3)
H-11α	2.00 (dddd, 13.3, 5.3, 5.3, 4.2)	1.73 (ddd, 13.9, 6.0, 6.0)	1.96 (ddd, 13.3, 5.1, 5.1)	2.42 (dd, 13.8, 5.3)	1.84 (dd, 13.4, 5.0)
H-11β	1.71 (dddd, 13.7, 13.3, 9.5, 4.8)	1.96 (ddd, 13.9, 7.8, 5.2)	1.40 (ddd, 13.5, 13.3, 10.8)	1.53 (dd, 13.8, 10.4)	2.33 (dd, 13.4, 11.0)
H-12α	2.21 (ddd, 15.9, 13.7, 5.3)		2.24 (dd[c], 13.5, 5.1)		
H-12β	2.40 (ddd, 15.9, 4.8, 4.2)	2.45 (dd[c], 6.0, 5.2)			
H-14	5.88 (dd, 2.2, 2.2)	5.80 (dd, 2.1, 1.8)	5.86 (dd, 2.2, 2.2)	5.92 (dd, 2.1, 1.8)	5.81 (dd, 2.2, 2.2)
H-15		1.07 (d, 6.8)			1.32 (s)
H-17			1.10 (d, 6.8)	1.36 (s)	
H-18	0.93 (s)	0.93 (s)	0.93 (s)	0.93 (s)	0.94 (s)
H-19	0.88 (s)	0.88 (s)	0.88 (s)	0.88 (s)	0.89 (s)
H-20	0.81 (s)	0.85 (s)	0.77 (s)	0.76 (s)	0.85 (s)
CO_2CH_3				3.66 (s)	3.74 (s)

[a] Spectra measured in $CDCl_3$ at 298 K and referenced relative to TMS.
[b] Some coupling constants were determined by analysis of the multiplet in an NOE difference spectrum and double resonance experiments.
[c] When H-15 (for **2**) or H-17 (for **3**) were irradiated.

Results obtained from these calculations are shown in Table 3, together with the conformer population (n_{HC1}, n_{HC2}) expressed as a molar fraction and calculated using the relationship between the Gibbs free energy (ΔG) and the equilibrium constant (K):[4]

$$\Delta G_{HC1-HC2} = -RT \ln(K_{HC1-HC2})$$

where $R = 1.988 \times 10^{-3}$ kcal K^{-1} mol^{-1} and $T = 298$ K. Considering $K_{HC1-HC2} = n_{HC2}/n_{HC1}$, $n_{HC1} + n_{HC2} = 1$ and $\Delta S \approx 0$, so that $\Delta G \approx \Delta H \approx \Delta E_{MMX}$, then

$$n_{HC2} = 1/\{\exp[(E_{HC2} - E_{HC1})/RT] + 1\} \quad (1)$$

and

$$n_{HC1} = 1 - n_{HC2} \quad (2)$$

From the above results, it can be seen that in the podocarpenone **1** the half-chair-1 conformation (82% of the population) is 0.9 kcal mol^{-1} more stable than the half-chair-2 conformation (18% of the population). Also, owing to the small barrier between them, this compound exists at room temperature in a rapid conformational equilibrium between the two half-chair conformations. When a methyl group is present at R_2 (**2**), the 1,3-diaxial interaction between H-9 and the methyl group makes the half-chair-1 conformation slightly less stable than before. Hence the energy difference between the two half-chair conformations is reversed, the half-chair-2 conformation being more stable (71% of the population).

In the case of **3–5**, in which a substituent different from H is present at R_1, the strong steric interaction between H-20 and the R_1 group makes the half-chair-1 conformation clearly more stable. When R_1 is a methyl group (see **3** and **4**), the energy difference is about 6 kcal mol^{-1}, in this case the half-chair-1 conformation being the preferred form (100% of the population). If R_1 is a $-CO_2Me$ group (**5**), the sp^2 hybridization of the carbonyl group exerts a smaller steric interaction between this group and protons H-20. Consequently, there is a decrease in the energy difference to 1.6 kcal mol^{-1}, thereby increasing the population of molecules in the half-chair-2 conformation until it reaches 6%.

Finally, it is worth noting that the calculated 1.25 kcal mol^{-1} energy difference between the more stable conformers of the epimeric compounds at C-12, **2** and **3**, means that in an equilibration process between the two isomers, at room temperarure, there would exist an 11% population of **2** versus 89% of **3**. This theoretical calculation is in good accordance with the 20 : 80 ratio obtained experimentally (see Experimental).

Table 3. Molecular geometry and distribution of the different conformations of compounds **1–5**

	Half-chair-1			Half-chair-2		
Compound	E_{HC1}[a]	n_{HC1}[b]	Dihedra angle[c]	E_{HC2}[a]	n_{HC2}[b]	Dihedra angle[c]
1	29.24	0.82	−57	30.13	0.18	46
2	32.14	0.29	−55	31.62	0.71	49
3	30.37	1.00	−57	36.31	0.00	35
4	32.11	1.00	−59	38.66	0.00	32
5	34.19	0.94	−53	35.79	0.06	41

[a] Steric energy in kcal mol^{-1} obtained from MMX calculations.
[b] Molar fraction calculated from Eqns (1) and (2).
[c] Dihedral angle C-9—C-11—C-12—C-13.

^{1}H NMR and coupling constants analysis

There are some signals in the ^{1}H NMR of **1–5** that are useful for studying the favoured conformations adopted by those compounds, specifically the coupling constants between protons H-9 and H-11α/H-11β. As can be seen in Fig. 1, the dihedral angle between proton H-9 and the two protons H-11 is different enough in the two conformations to permit not only a qualitative but also a quantitative study.

Coupling constant data [^3J(HH)] between H-9 and H-11α/H-11β for **1–5** are shown in Table 4. The ^3J(HH) ($J_{half-chair-1}$, $J_{half-chair-2}$) values were obtained using the PMR option of the PCMODEL program for each calculated conformation (Table 3). This option makes use of a generalization of the Karplus equation.[5] The average ^3J(HH) was calculated by including the mole fractions of both conformations from the formula $n_{HC1}J_{half-chair-1}$ +

Table 4. Coupling constants ^3J(HH) (Hz) of H-9 protons of **1–5**

Compound	Vicinal protons	$J_{half-chair-1}$[a]	$J_{half-chair-2}$[a]	Average[b]	Expt.	Diff.[c]
1	9–11α	4.6	6.8	5.0	5.3	0.3
	9–11β	12.1	1.2	10.0	9.5	−0.5
2	9–11α	4.8	7.0	6.4	6.0	−0.4
	9–11β	11.9	1.1	4.0	7.8	3.8
3	9–11α	4.8	8.9	4.8	5.1	0.3
	9–11β	12.0	1.0	12.0	10.8	−1.2
4	9–11α	4.6	9.5	4.6	5.3	0.7
	9–11β	12.0	1.2	12.0	10.4	1.6
5	9–11α	4.5	8.2	4.7	5.0	0.3
	9–11β	12.0	1.0	11.3	11.0	−0.3

[a] Coupling constants for the half-chair-1, and half-chair-2 conformations calculated from the option PMR of the PCMODEL program based on Karplus' generalization equation.[7]
[b] Calculated from the formula $n_{HC1}J_{half-chair-1} + n_{HC2}J_{half-chair-2}$.[8] (for n_{HC1} and n_{HC2}, see Table 3).
[c] Difference = experimental − average.

$n_{HC2}J_{half-chair-2}$,[6] where n_{HC1} and n_{HC2}, are calculated from Eqns (1) and (2).

Reasonable agreement can be seen between the experimental values and the calculated averages. Most of the differences (last column in Table 4) can be explained by the fact that the calculations were performed using only the most stable conformations, without considering the small vibrational variations and the possibility of similar energy rotamers when the —CO_2Me group is present. In particular, in the case of **2**, the experimental values could accommodate a higher population for half-chair-1 (about 50–60%). It can be concluded that the mole fractions calculated using molecular mechanics are very similar to the real values, and the study of the ^{1}H coupling constants in general confirms the results of the previous MM-based conformational analysis.

CONCLUSIONS

Podocarpene intermediates in the synthesis of scopadulan diterpenes have a six-membered C-ring that can exist in two different half-chair conformations. From a detailed study of the ^{1}H NMR data together with molecular mechanics calculations, the ratio of the two conformers (half-chair-1 and half-chair-2) of those systems was calculated. It can be concluded that when no substituents different from H are present at C-12β (**1** and **2**), both conformations have a similar energy. Hence these compounds exist at room temperature in a rapid equilibrium between the half-chair-1 and half-chair-2 conformations. However, when bulkier substituents (such as Me or —CO_2Me) at C-12β are present (**3–5**), the population in the half-chair-1 conformation increases to the detriment of half-chair-2, owing to the strong steric interaction between protons H-20 and the substituent. When the substituent at C-12β is a methyl group (**3** and **4**), the energy difference increases to approximately 6 kcal mol^{-1}, in these cases the half-chair-1 conformation being the favoured form.

EXPERIMENTAL

Calculations

Molecular mechanics calculations[7] were performed using the MMX force field, which is a derived version of the MM2 program, developed by Allinger and implemented in the PCMODEL (4.0) program (Serena Software, Bloomington, In, USA).

Spectra

^{1}H and ^{13}C NMR and DEPT spectra were measured on a Varian XL-300 spectrometer (299.95 MHz for ^{1}H and 75.43 MHz for ^{13}C) operating at a probe temperature of 298 K using a dual ^{1}H/^{13}C 5 mm probe. The ^{1}H measurement conditions were spectral width 4000 Hz, 90° pulse with 18 μs, acquisition time 3.7 s, number of transients 16–64 and 0.1 Hz digital resolution.

^{1}H–^{13}C HMQC and NOED spectra were measured on a Varian 400 spectrometer (399.95 MHz for ^{1}H and 100 MHz for ^{13}C) equipped with a 5 mm indirect detection probe operating at 298 K. Sample concentrations were typically in the range 5–20 mg per 0.5 ml of $CDCl_3$. The signal of the TMS was taken as the reference. All these experiments were performed either using standard pulse sequences supplied by the spectrometer manufacturer or slightly modified pulse sequences. NOED experiments were typically acquired with 8K data points covering a spectral width of 3200 Hz and with a 1.5 s presaturation time. Spectra at each presaturation position were interleaved in groups of four scans to minimize artefacts due to instrument inconsistencies and processed with a 1 Hz exponential line broadening to reduce subtraction artefacts. The HMQC spectrum was obtained using a spectral width of 3200 Hz in the ^{1}H dimension and 16 000 Hz in the ^{13}C dimension. A total of 256 increments were collected with eight transients per increment and an acquisition time of 0.1 s.

Preparation of 12β-methyl-8(14)-podocarpen-13-one (3)

A solution of NaOMe in MeOH (2 M, 2 ml, 4 mmol) was added to a solution of 12α-methyl-8(14)-podocarpen-13-one (**2**) (50 mg, 0.19 mmol) in THF (1 ml) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for a further 30 min. The solution was poured into water and extracted with CH_2Cl_2. The organic extract was washed with brine, dried over sodium sulfate and concentrated under vacuum to give an oily residue which resulted in an 8 : 2 (β : α) mixture (determined by ^{1}H NMR) of epimers at C-12. When longer reaction times were used, lower yields of the same 8 : 2 (β : α) mixture were obtained. Chromatography of the crude with hexane–ethyl acetate (from 9 : 1 to 8 : 2) gave the 12β epimer (**3**) as an oil which solidified on standing (36 mg, 72%) and 9 mg (18%) of starting material (**2**).

Acknowledgments

This work was financially supported by DGICYT (Research Program PB95-1088). M.A.G. thanks the Conselleria d'Educació i Ciència de la Generalitat Valenciana for a research fellowship.

REFERENCES

1. (a) Hayashi T, Kishi M, Kawasaki M, Arisawa M, Shimizu M, Suzuki S, Yoshizaki M, Morita N, Tezuka Y, Kikuchi T, Berganza LH, Ferro E, Basualdo I. *Tetrahedron Lett.* 1987; **28**: 3693; (b) Hayashi T, Kishi M, Kawasaki M, Arisawa M, Morita N. *J. Nat. Prod.* 1988; **51**: 360; (c) Hayashi T, Asano S, Mizutani M, Takeguchi N, Kojima T, Okamura K, Morita N. *J. Nat. Prod.* 1991; **54**: 802; (d) Chamy MC, Piovano M, Garbarino JA, Miranda C, Gambaro V, Rodriguez ML, Ruiz-Perez C, Brito I. *Phytochemistry* 1991; **30**: 589.
2. Hayashi K, Niwayama S, Hayashi T, Nago R, Ochiai H, Morita N. *Antivir. Res.* 1988; **9**: 345.
3. Abad A, Agulló C, Arnó M, Marín ML, Zaragozá RJ. *Synlett* 1997; 574.
4. Isaacs NS. *Physical Organic Chemistry*. Longman: London, 1987.
5. Haasnoot CAG, De Leeuw FAAM, Altona C. *Tetrahedron* 1980; **36**: 2783.
6. (a) Wehrli FW, Marchand AP, Wehrli S. *Interpretation of Carbon-13 NMR Spectra*. John Wiley & Sons: New York, 1988; (b) Marshall JL. *Carbon–Carbon and Carbon–Proton NMR Couplings: Applications to Organic Stereochemistry and Conformational Analysis*. Verlag Chemie: Weinheim, 1983.
7. Allinger NL. *J. Am. Chem. Soc.* 1977; **99**: 8127.

