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Total ^{1}H NMR assignment of 3β-acetoxypregna-5,16-dien-20-one

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ABSTRACT

This work describes the total and unambiguous assignment of the 750 MHz ^{1}H NMR spectrum of 3β-acetoxypregna-5,16-dien-20-one or 16-DPA (**1**), the well-known intermediate utilized in the synthesis of biological important commercial steroids. The task was accomplished by extracting the coupling constant values in the overlapped spectrum region by HSQC, and using these values in the ^{1}H iterative full spin analysis integrated in the PERCH NMR software. Comparison of the experimental vicinal coupling constants of **1** with the values calculated using Altona provides an excellent correlation. The same procedure, when applied to the published data of progesterone (**2**) and testosterone (**3**), afforded an acceptable correlation for **2** and a poor correlation for **3**. In the last case, this suggested the reassignment of all four vicinal coupling constants for the methylene signals at the C-15 and C-16 positions, demonstrating the utility of this methodology.

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1. Introduction

The total ^{1}H NMR assignment of steroids remains an important challenge in the area of organic and medicinal chemistry. The characteristics of ^{1}H NMR signals, such as shift and shape, can be used to identify steroids in the spectral fingerprints regions of biological fluids for metabolomics studies [1], in the estimation of their conformational profile association during interaction with proteins [2], and in monitoring the biocatalysis for steroid bioconversions [3].

Although the ^{13}C NMR data for a large number of relevant steroids exist in the literature [4], concerning their ^{1}H NMR data, the overlapping spin multiplets in the spectra frequently have required careful analysis using 2D techniques and most of the studies afford limited assigned hydrogen atoms and poor descriptions of coupling constant values [5].

3β-Acetoxypregna-5,16-dien-20-one or 16-DPA (**1**) is an important intermediate in the synthesis of different hormones with therapeutic value [6]. During the great worldwide impact of the steroidal industry, some 6–7 decades ago, **1** acquired economical relevance from the well-known Marker degradation route and many metric tons of the molecule were produced [7]. Since then, several NMR structural characterizations have been described, including ^{1}H and ^{13}C NMR chemical shift values [8,9]. However, the signal region between 1.54 and 1.90 ppm in the ^{1}H NMR spectrum shows a complex pattern making the description of all coupling constant values difficult. In this sense, computational tools, like spectra prediction and simulation, are useful for decoding complex ^{1}H NMR spectra.

Of relevance is also to mention that a combination of high-field NMR (at least 600 MHz) and sophisticated software, like the PERCH program used in the present study, finally provide the tools required for the complete signal assignment of steroids, a task that might be valuable for the characterization of small quantities of a given metabolite since ^{1}H NMR spectra can nowadays be obtained from microgram amounts of compounds, specially when using a cryoprobe.

In a recent paper about the complete assignment of α-cedrene, cedrol and eight related cedranolides [10], the ^{1}H iterative spin–spin analysis integrated in the PERCH NMR software [11] was efficient to determine the total set of coupling constants at 500 MHz for compounds possessing two to four methylene group signals in a narrow region. However, for the other two cedranolides which have a few extra methylene groups, like in cedrol and isocedrol, magnetic signal dispersion at 750 MHz was required for the spin–spin analysis in order to separate the overlapped signals. In this work the same procedure was attempted for **1**, but the overlap of many methylene and methine groups signals rendered this task as not possible. Consequently, the application of HSQC for extracting coupling constants in the overlapped spectra region was used in combination with the ^{1}H iterative full spin analysis to achieve the complete and unambiguous assignment of **1**. Additionally,

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experimental vicinal coupling constants were contrasted with those calculated using Altona [12], presenting a very good correlation. The same procedure when applied to the described experimental data of progesterone (**2**) [13] and to testosterone (**3**) [14] provided an acceptable correspondence for **2**, and a poor agreement for **3**. In the latter case it allowed the detection of the wrong assignment of vicinal coupling constants for the two pairs of hydrogen atoms at the C-15 and C-16 positions.

2. Materials and methods

2.1. General

Compound **1**, a very stable molecule, was available from a previous study that we performed over half a century ago [15]. A sample of approximately 10 mg was placed in a 5 mm tube, dissolved in 0.9 mL of $CDCl_3$ and degassed by slow bubbling of an argon stream under ultrasound during 15 min. A final volume of 0.5 mL was left, to which a small amount of TMS in $CDCl_3$ was added.

2.2. NMR spectra

All spectra were obtained on a Bruker Ascend 750 spectrometer equipped with a cryoprobe. The 750 MHz ^{1}H NMR spectrum needed for the PERCH evaluation was acquired with number of scans (NS) = 16, acquisition time (AQ) = 2.90 s, relaxation delay (RD) = 1.0 s, 90° pulse width (P1) = 7.24 ms, spectral width (SW) = 11261.26 Hz and FT size = 65,536. The ^{13}C NMR spectrum was acquired with NS = 64, AQ = 2.47 s, RD = 2.0 s, P1 = 7.12 ms, SW = 13227.5 Hz and FT size = 32,768. ^{13}C NMR (188.6 MHz, $CDCl_3$): δ = 196.93 (C-20), 170.62 (CO), 155.38 (C-17), 144.56 (C-16), 140.31 (C-5), 122.08 (C-6), 73.93 (C-3), 56.31 (C-9), 50.34 (C-14), 46.05 (C-13), 38.11 (C-4), 36.84 (C-1), 36.76 (C-10), 34.56 (C-12), 32.25 (C-15), 31.52 (C-7), 30.12 (C-8), 27.70 (C-2), 27.17 (C-21), 21.46 (C-22), 20.60 (C-11), 19.22 (C-19), 15.70 (C-18). The acquisition data for gHSQC were SW ^{1}H = 397.58 Hz with 2048 increments and ^{13}C = 2570.95 Hz with 512 increments, AQ = 2.6 s, NS = 8 and RD = 5 s. The NMR data were processed using the TOPSPIN 3.2 software.

2.3. ^{1}H NMR full spin analysis

Complete ^{1}H NMR spectra analyses of **1** was achieved using the PERCH v.2011.1 software (PERCH Solutions Ltd., Kuopio, Finland). The 750 MHz experimental data were imported into the preparation module (PAC) of the PERCH shell, and subjected to phase and baseline correction, peak picking, and integration. The most stable conformer, obtained from theoretical calculations, was imported into the molecular modeling software (MMS) and used to predict the initial calculated spectrum. As a first approximation, all known chemical shift for **1** [8,9] and the following coupling constants: ^2J: CH_2-1, -2, -7, -11, -12 -15 and ^3J: $1_{\alpha\beta}$–$2_{\alpha\beta}$; $7_{\alpha\beta}$–8; 8–9; 8–14; 9–$11_{\alpha\beta}$; $11_{\alpha\beta}$–$12_{\alpha\beta}$; 14–$15_{\alpha\beta}$ from related compounds **2** [13] and **3** [14] were incorporated in the table of the graphical spectral parameter editor (PMS). For diastereotopic hydrogen atoms described at the same chemical shift, the assignments were interchanged in several combinations until the total-line-shape fitting (T) analysis gave the lowest RMS (root mean square). This procedure was not enough to get a good congruence between experimental and calculated spectra for the crowded spectra region between 1.5 and 1.9 ppm, besides the RMS value was bigger than 0.2%. In consequence, the estimation of coupling constants was done by extraction of clean multiplicities from HSQC. With these new values, the optimization of the spectral parameters was carried out using again the total-line-shape fitting (T) analysis obtaining an excellent agreement between the experimental and the calculated spectra, and a RMS error of 0.05%. Line narrowing by means of mathematical algorithms, like the use of weighting functions for resolution enhancement, was omitted since it is critical to determine at which point a given weighted NMR spectrum starts to look unreal. In fact, our experience dictates that in favorable cases one can obtain magnetic homogeneities better than 0.2 Hz, as we have used to demonstrate that some aromatic methoxy groups appear as doublets [16].

2.4. Theoretical calculations

Molecular models for **1**–**3** were built using the molecular modeling software Spartan′04W package (Wavefunction, Irvine, CA, USA). Monte Carlo conformational search protocols were carried out for **1**–**3** using MMFF94 calculations. Each minimum energy conformer was submitted to geometry optimization by DFT calculations at the B3LYP/DGDZVP level of theory employing the Gaussian 03W program (Gaussian Inc., Wallingford, CT, USA). The vicinal coupling constants of **1**–**3** were calculated using dihedral angle values obtained from the optimized conformers by means of Altona [12]. Although one could use the PERCH *J*-predictor tool, it was considered that an independent test for the validity of the obtained coupling constant values could be derived from DFT conformational models followed by the use of Altona which is very familiar to us [12].

3. Results and discussion

This work points towards the complete ^{1}H NMR assignment of **1**, important intermediate in the synthesis of commercial steroids, using the ^{1}H NMR iterative full spin analysis integrated in the PERCH NMR Software [11]. This methodology has been useful in the complete spectra assignment of several natural products, [17–20] and is based on the iterative minimization of the difference between the simulated and the experimental spectra, thus determining the total ^{1}H NMR data for the target molecule.

The 750 MHz free induction decay of **1** was edited in the PAC module of the PERCH shell as described in the methodology section. The molecular structure of the minimum energy conformer of **1** was imported into the MMS module of the PERCH shell, and was used to predict the preliminary ^{1}H NMR calculated spectrum. Initially, the known chemical shifts and coupling constants [8,9] were introduced in the parameter table. In this sense, two major works [8,9a] depict the ^{1}H NMR chemical shifts of **1**, nevertheless only in one of them the diastereotopic hydrogen shifts of the seven methylene groups are described [8]. In this latter work, chemical shifts at 2.29 and 1.53 ppm were assigned at CH_2-4 and CH_2-11, respectively, probably due to poor signal resolution of the individual signals at the operating proton frequency of 400 MHz. Therefore, to carry out the assignment in a 750 MHz frequency spectrum, several combinations were tested and submitted to the iterator module using the total-line shape fitting (T) analysis until the lowest root-mean-square deviation (RMS) was reached. Even though with these settings most positions of the spectrum presented a good visual congruence with the experimental (RMS ~ 0.2%), some inconsistencies were observed in the crowded region between 1.5 and 1.9 ppm. Thus, to disentangle the overlapped spin pattern, the extraction of ^{1}H multiplicities from this region was achieved from HSQC [21]. The use of slices to extract multiplicities for overlapped signals was early used during HETCOR measurements for the study of α-cedrene [21b]. By raising the increments to 2048 in the F2 axis and working in a reduced SW 397.58 Hz it was possible to obtain an improved digital resolution of 0.2 Hz/point. As a result, the extraction of traces from HSQC at

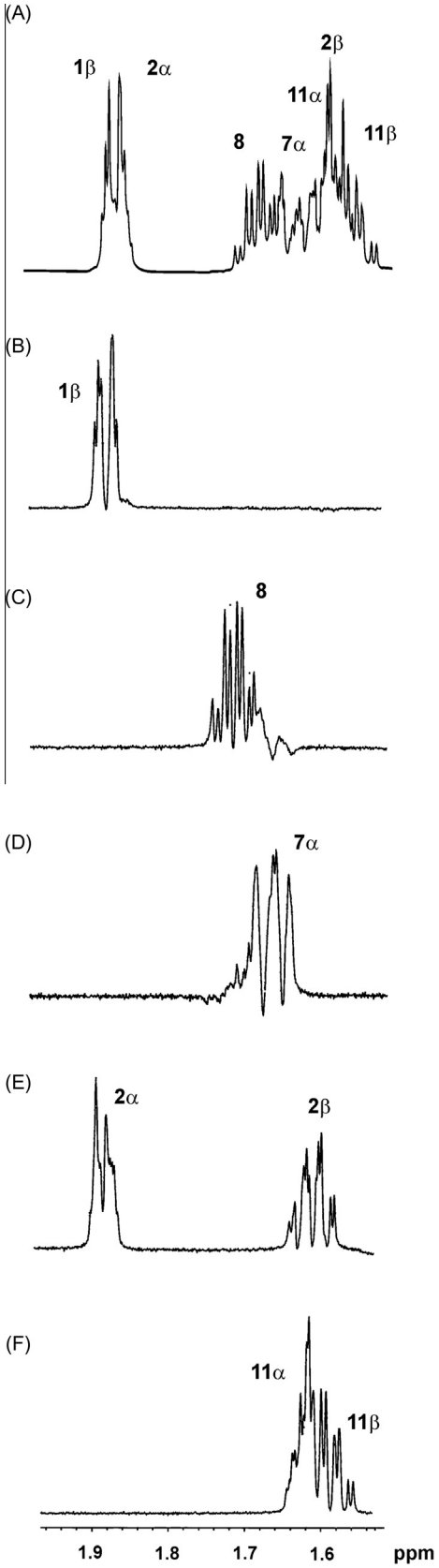


Fig. 1. (A) Partial ^{1}H NMR spectrum showing the 1.54–1.90 ppm region of **1**. Multiplets extracted at the ^{13}C chemical shifts from HSQC are: (B) C-1 at 36.9 ppm, (C) C-8 at 30.2 ppm, (D) C-7 at 31.6 ppm, (E) C-2 at 27.2 ppm, and (F) C-11 at 20.7 ppm.

the carbon frequency allows the isolation of clean proton multiplicities with high resolution and the estimation of their coupling constants in the overlapped region, Fig. 1. With these values, the new iteration process was accomplished and convergence was reached to a RMS of 0.048%.

All ^{1}H chemical shifts of **1** are shown in Table 1, while all coupling constants are shown in Table 2. By default, PERCH calculations afford chemical shift and coupling constant values with six and four decimal places, respectively. The experimental 750 MHz spectra were acquired with magnet homogeneity better than 0.9 Hz, and therefore, chemical shifts and coupling constant values with three and two digits after a decimal point, respectively, constitute a proper description, as has been done previously [17–20]. Additionally, Table 1 lists the chemical shifts of progesterone (**2**) in tetradeuteromethanol at 900 MHz [13], and of testosterone (**3**) in deuterochloroform at 600 MHz [14], which have been calculated using the PERCH software and the LAOCNCLI program, respectively (see Fig. 2 and Scheme 1).

Essentially the reported ^{1}H chemical shifts described at 400 MHz for **1** [8] and for the non acetylated analog [9a] were corroborated by iterative full spin–spin analysis. However, one inconsistency was noticed for the assignment of H-9 and H-14. The RMS value considering chemical shifts at 0.99 ppm for H-9 and at 1.38 ppm for H-14 was 0.050%, while for the inverted positions it diminishes to 0.048%. In previous works [15,18], we observed that the lowest RMS value matched to the correct assignment, however, in the case of **1**, after verifying the HMBC correlations between C-9 with CH_3-19, and C-14 with CH_3-18, and the connectivity of them in HSQC, it became evident that the described [8] chemical shifts for H-9 and for H-14 are indeed correct, even if they provide a poorer RMS value of 0.050%. Thus, for hydrogen atoms with quite similar neighbors, the assignment of the spin–spin analysis is not always the best one, and suggests the need to use a verification method.

Regarding coupling constant values, Table 2, the J_{gem} values of methylene groups at C-1, C-2, C-4, C-11 and C-12 are consistent

Table 1
Chemical shifts for **1**–**2** in $CDCl_3$ and for **3** in CD_3OD.

Atom	δ (ppm)		
	1[a]	**2**[b]	**3**[c]
1α	1.138	1.716	1.702
1β	1.869	2.047	2.034
2α	1.863	2.337	2.347
2β	1.594	2.441	2.424
3	4.606	–	–
4α	2.344	5.730	5.731
4β	2.320	–	–
6	5.388	α2.280/β2.412	α2.278/β2.391
7α	1.642	1.065	1.007
7β	2.017	1.870	1.848
8	1.690	1.570	1.579
9	1.431	0.989	0.932
11α	1.606	1.645	1.603
11β	1.571	1.461	1.436
12α	1.347	1.456	1.093
12β	2.404	2.079	1.862
14	1.041	1.181	0.978
15α	2.316	1.724	1.628
15β	2.044	1.267	1.313
16	6.716	α1.676/β 2.189	α1.466/β 2.084
17	–	2.548	3.655
Me-18	0.920	0.669	0.795
Me-19	1.060	1.196	1.198
Me-21	2.268	2.127	–
Ac-22	2.039	–	–

[a] $CDCl_3$, 750 MHz.
[b] MeOD, 900 MHz [13].
[c] $CDCl_3$, 600 MHz [14].

Table 2
Comparison of PERCH determined coupling constants for **1**–**3** with the vicinal coupling constants calculated using Altona.

J	1			2			3		
	Exp	Cal	Δ	Exp	Cal	Δ	Exp	Cal	Δ
1α,1β	−13.57	–	–	−13.40	–	–	−13.30	–	–
1α,2α	3.85	3.85	0	4.42	4.12	+0.30	4.80	4.09	+0.71
1α,2β	14.15	13.49	+0.66	14.86	13.49	+1.37	15.00	13.50	+1.50
1β,2α	3.48	3.42	+0.06	3.02	2.75	+0.27	3.30	2.78	+0.52
1β,2β	3.76	4.52	−0.76	5.20	3.58	+1.62	5.20	3.53	+1.67
1α,19	−0.65	–	–	−0.70	–	–	0.50	–	–
2α,2β	−12.52	–	–	−17.06	–	–	−17.0	–	–
2α,3	4.31	4.52	−0.21	–	–	–	–	–	–
2β,3	11.44	11.22	+0.22	–	–	–	–	–	–
2α,4α	−2.25	–	–	0.97	–	–	1.0	–	–
3,4α	5.02	4.35	+0.67	–	–	–	–	–	–
3,4β	11.65	11.22	+0.43	–	–	–	–	–	–
4α,4β	−13.04	–	–	–	–	–	–	–	–
4α,6	−0.11	–	–	–	–	–	–	–	–
4β,6	2.15	–	–	−1.90	–	–	1.90	–	–
4α,7α	–	–	–	–	–	–	–	–	–
4α,7β	−0.13	–	–	–	–	–	–	–	–
4β,7α	3.43	–	–	–	–	–	–	–	–
4β,7β	2.52	–	–	–	–	–	–	–	–
6,7α	1.99	2.94	−0.95	4.18	3.90	+0.28	4.20	3.85	+0.35
				2.39	2.69	−0.30	2.40	2.74	−0.34
6,7β	5.16	4.71	+0.45	13.96	13.12	+0.84	14.10	13.12	+0.98
	–	–	–	5.45	3.89	+1.56	5.50	3.87	+1.63
6,3	0.18	–	–	–	–	–	–	–	–
7α,7β	−17.57	–	–	−12.83	–	–	−12.90	–	–
7α,8	10.65	11.66	−1.01	11.72	12.29	−0.57	11.90	12.30	−0.40
7β,8	5.30	5.44	−0.14	3.54	3.60	−0.06	3.30	3.53	−0.23
8,9	11.49	12.00	−0.51	10.87	12.14	−1.27	10.70	12.14	−1.44
8,14	10.76	12.15	−1.39	10.69	12.14	−1.45	10.90	12.13	−1.23
9,11α	5.81	4.20	+1.61	4.08	3.49	+0.59	4.30	3.56	+0.74
9,11β	12.69	12.05	+0.64	12.37	12.29	+0.08	12.30	12.26	+0.04
9,19	−0.41	–	–	–	–	–	–	–	–
11α,11β	−14.03	–	–	−13.95	–	–	−13.30	–	–
11α,12α	4.51	4.23	+0.28	4.30	3.90	+0.40	4.30	3.99	+0.31
11α,12β	2.44	2.46	−0.02	2.85	2.82	+0.03	2.70	2.69	+0.01
11β,12α	13.24	12.86	+0.38	13.27	13.10	+0.17	13.40	13.07	+0.33
11β,12β	4.75	4.79	−0.04	4.13	4.11	+0.02	4.20	4.25	−0.05
12α,12β	−12.80	–	–	−12.49	–	–	−12.50	–	–
12α,18	−0.61	–	–	0.41	–	–	0.50	–	–
14,15α	6.33	6.36	−0.03	7.27	5.69	+1.58	7.20	6.03	+1.17
14,15β	11.98	11.74	+0.24	12.75	11.61	+1.14	12.30	11.39	+0.91
14,18	−0.29	–	–	–	–	–	–	–	–
15α,15β	−17.07	–	–	−12.37	–	–	−12.60	–	–
15α,16	3.36	4.83	−1.47	9.61	11.59	−1.98	9.50[a]	12.01	−2.51
	–	–	–	3.01	2.20	+0.81	3.50[a]	2.76	+0.74
15β,16	1.87	2.69	−0.82	6.70	5.61	+1.09	5.90[a]	4.88	+1.02
	–	–	–	11.61	11.61	0	11.90[a]	12.02	−0.12
16α,16β	–	–	–	−13.83	–	–	−13.70	–	–
16a,17	–	–	–	9.31	8.17	+1.14	8.30	8.48	−0.18
16β,17	–	–	–	9.19	9.21	−0.02	9.10	7.74	+1.36

[a] Reassigned in this work.

with strain-free six member rings, with values between −12.58 and −14.03 Hz. However, for CH_2-7 and CH_2-15 the values are some 3 Hz larger −17.57 and −17.07 Hz, respectively. These methylene groups are adjacent to the double bonds at C-6 and C-16, and as has been described [22], the torsion angle of the bond between the carbon atom bearing the two hydrogen atoms and the sp^2 carbon can be correlated with the value of geminal coupling constants. Thus, the φ torsional angles between C6 and C7, and between C15 and C16 are less than 20°, which correlate well with a coupling constant of approximately −17 Hz for the CH_2-7 and CH_2-15 signals, while, the torsional angle between C4–C5 is approximately 52°, corresponding to a coupling constant of −13 Hz. These angle values were calculated from the geometry optimization of **1** using the DFT methodology.

The vicinal coupling constants show characteristic magnitudes for a chair conformation in rings A and C, and a half chair conformation for ring B [23]. The *axial–equatorial* coupling constants corresponding to $J_{1\alpha,2\alpha}$, $J_{1\beta,2\beta}$, $J_{2\alpha,3}$, $J_{4\alpha,3}$, $J_{7\beta,8}$, $J_{9,11\alpha}$, $J_{11\alpha,12\alpha}$ and $J_{11\beta,12\beta}$ are in the 3.76–5.81 Hz range, the *axial–axial* coupling constants corresponding to $J_{1\alpha,2\beta}$, $J_{2\beta,3}$, $J_{3,4\beta}$, $J_{7\alpha,8}$, $J_{8,9}$, $J_{8,14}$, $J_{9,11\beta}$ and $J_{11\beta,12\alpha}$ are in the 10.65–14.15 Hz range, and the *equatorial–equatorial* coupling constants for $J_{1\beta,2\alpha}$ and $J_{11\alpha,12\beta}$; are 3.48 and 2.44 Hz, respectively. The remaining vicinal coupling constants, the *axial-pseudo*-equatorial $J_{14,15\alpha}$, *axial-pseudo-axial* $J_{14,15\beta}$ in ring D, and the vicinal coupling constants $J_{7\alpha\beta,6}$ and $J_{15\alpha\beta,16}$ followed a Karplus type relationship [23].

The well-known W arrangement [24] of CH_3-18 with H-12α and H-14, and of CH_3-19 with H-1α and H-9 showed values smaller than 1 Hz [24,25]. Further, H-2α and H-4α, which are positioned in an 1,3-diequatorial distribution, also showed a coupling through 4σ-bonds of −2.25 Hz [25].

Another type of $^4J_{H-H}$ coupling is the allylic coupling between CH_2-4 and H-6, which follows in a very good way the empirical correlation of the torsional angle between the plane of the double

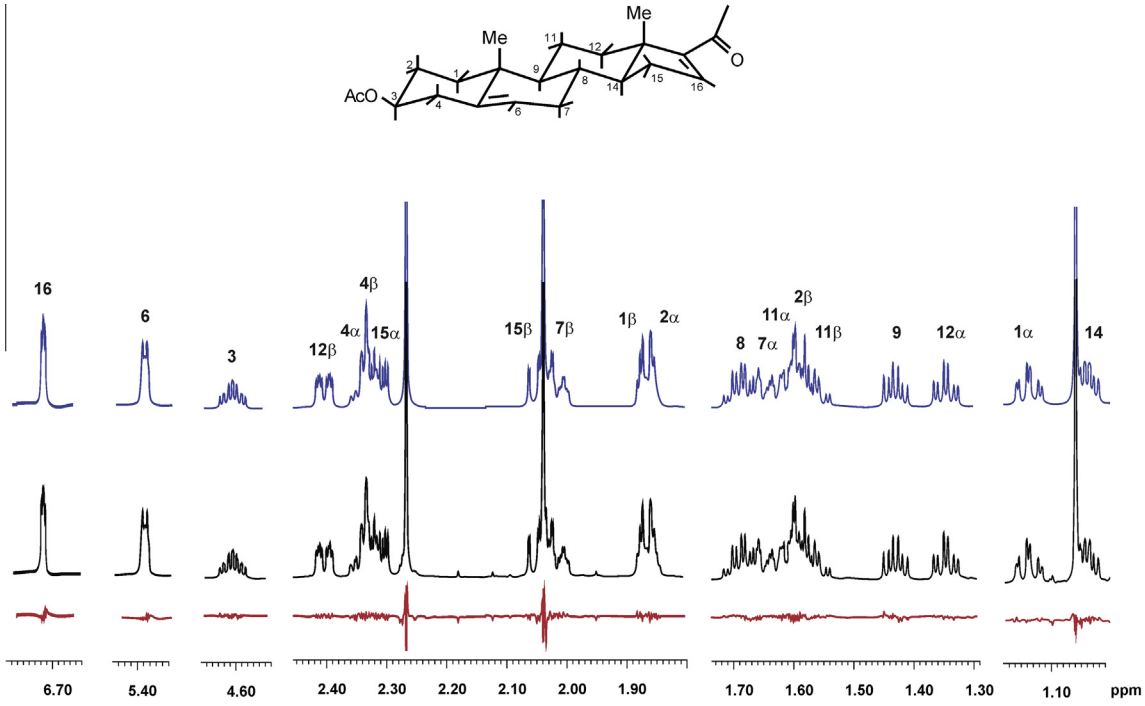
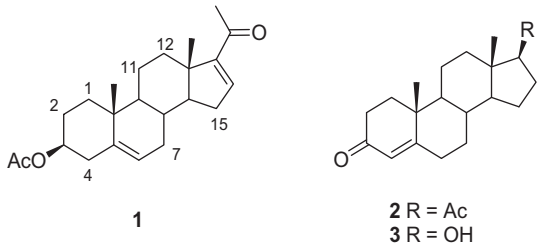


Fig. 2. Comparison of the PERCH calculated (top) and the experimental (center) ^{1}H NMR spectra of **1** in $CDCl_3$ at 750 MHz. Residuals are shown at the bottom.



Scheme 1. Molecular formulas.

bond and the adjacent methylene. Thus, for $J_{4\alpha,6}$ with an angle smaller than 20° the coupling constant is close to zero (0.18 Hz), and for $J_{4\beta,6}$ with the angle between 100° and 110° the coupling is −2.15 Hz [24].

The homoallylic coupling constants $J_{4\alpha7\alpha}$, $J_{4\alpha7\beta}$, $J_{4\beta7\alpha}$, $J_{4\beta7\beta}$ and $J_{3,6}$ show different values, and like in the case of the allylic constants, the magnitude is dependent on the angles between the plane of the double bond and the C–H bonds of each coupled hydrogen [24]. Thus, the coupling between H-3 and H-6, and between $H_{4\alpha}$ and H-$7_{\alpha,\beta}$ are close to zero, while those between H-4_{β} and H-$7_{\alpha,\beta}$ are larger than 2 Hz.

In order to validate the coupling constant values obtained by PERCH analysis of **1**, the $^{3}J_{H-H}$ values were compared with those calculated using Altona, in the DFT optimized geometry of **1**. Thus, the molecular model of **1** was built and the conformational space explored with the Monte Carlo protocol using the Merck Molecular Force Field (MMFF) in the Spartan′04 software package. The low energy conformer was subjected to single-point DFT energy calculations at the B3LYP/6-31G(d) level of theory, followed by geometry optimization at the B3LYP/DGDZVP level employing the Gaussian 03W program. Additionally the same procedure was followed for steroids **2** [13] and **3** [14], and the comparison was realized using the reported vicinal coupling constant values given in Table 2.

Comparison of the observed vicinal coupling constants with the values calculated by Altona provides differences among the three steroids. The *R*-squared (R^2) value derived from linear regression of experimental and calculated values were 0.97, 0.95 and 0.55 for **1**, **2** and **3**, respectively, indicating the best correlation is for **1**, followed by **2** and a very poor value for **3**. This can be explained by the wrong assignment of $^{3}J_{HH}$ values between the CH_2-15 and CH_2-16 signals. The reported values of $J_{15\alpha,16\alpha}$, $J_{15\alpha,16\beta}$; $J_{15\beta,16\alpha}$ and $J_{15\beta,16\beta}$ for **3**, are 3.50, 9.50, 11.90 and 5.90 Hz, respectively [14], however by redistributing the values as: 9.50, 3.50, 5.90 and 11.90 Hz, respectively, significantly improved the correlation to R^2 = 0.95, a similar value than for **2**. This fact reveals the importance of a second methodology for data verification of values that are obtained for spectral simulations.

The experimental data of testosterone (**3**), given in Table 2, correspond to literature values [14] of excellent quality, and therefore they were considered fully useful. Furthermore, from the point of view of data comparison of experimental and calculated spectra, the agreement would be highly improved by simple interchange of the two C-16 hydrogen atoms. However this is an invalid approach since the individual hydrogen atoms assignment was based [14] on nuclear Overhauser enhancement effects.

4. Conclusions

The complete ^{1}H NMR assignment of 3β-acetoxypregna-5,16-dien-20-one (**1**) was achieved applying HSQC to measure coupling constants in the overlapped spectrum region combined with the ^{1}H iterative full spin analysis integrated in the PERCH NMR software. The comparison of experimental vicinal coupling constants with values calculated using Altona revealed good agreement for **1**, while the same procedure applied for described values of progesterone (**2**) and testosterone (**3**), showed a good correlation for **2** and a very poor correlation for **3**. This allowed us to identify inconsistencies in the assignment of the four vicinal coupling constants of the hydrogen atoms at C-15 and C-16. This fact also evidences

the convenience of a second methodology for validation of the iterative spin analysis results.

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