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Stereochemical analysis of the 3α- and 3β-hydroxy metabolites of tibolone through NMR and quantum-chemical investigations. An experimental test of GIAO calculations

Diego Colombo,[1] Patrizia Ferraboschi,[1]* Fiamma Ronchetti[1] and Lucio Toma[2]

[1] Dipartimento di Chimica e Biochimica Medica, Università di Milano, Via Saldini 50, 20133 Milan, Italy
[2] Dipartimento di Chimica Organica, Università di Pavia, Via Taramelli 10, 27100 Pavia, Italy

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The configuration at C-3 of the 3α- and 3β-hydroxy metabolites of tibolone was studied by extensive application of one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy combined with molecular modeling performed at the B3LYP/6–31G(d) level. Using HF and DFT GIAO methods, shielding tensors of the two molecules were computed; comparison of the calculated NMR chemical shifts with the experimental values revealed that the density functional methods produced the best results for assigning proton and carbon resonances. Although steroids are relatively large molecules, the present approach appears accurate enough to allow the determination of relative configurations by using calculated ^{13}C resonances; the chemical shift of pairs of geminal α/β hydrogen atoms can also be established by using calculated ^{1}H resonances. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; tibolone metabolites; stereochemistry; steroids; molecular modeling; HF calculations; DFT calculations

INTRODUCTION

The synthetic steroid tibolone (Org OD 14) (**1**) is widely used in hormone replacement therapy (HRT) of menopausal complaints[1] and it is metabolized mainly affording the 4-ene isomer **2** and the 3α- and the 3β-alcohols **3** and **4** obtained by reduction of the 3-keto group. The hormonal activities of these three steroids have been extensively evaluated[2] and more recently the role of tibolone and its metabolites in the protection of breast tissue in postmenopausal women with HRT has been studied.[3–7]

Considering the pharmacological significance of tibolone metabolites and the few available chemico-physical data, we decided to study the 3-hydroxy derivatives, verifying the configuration at C-3 of both epimers. They can be easily prepared from **1**, the first by reduction with lithium tri-*tert*-butoxyaluminum hydride that affords a predominant product purified by crystallization. Its 3-epimer can be obtained by inversion of the configuration at C-3 performed through a Mitsunobu reaction.[8] The 3α configuration, represented by structure **3**, might be assigned to the main product of reduction on the basis of the structural analogy of **1** with the antifertility steroid norethinodrel (**5**), of which the tibolone is the 7α-methyl analogue and which on metal hydride reduction affords as preferred product the 3α-hydroxy derivative owing to the quasi-chair conformation assumed by the A ring.[9,10] Although tibolone and norethinodrel share the same A ring, the presence of a methyl group at position 7 could, in principle, modify the A ring quasi-chair conformation and hence the stereochemical outcome of the 3-ketone reduction.

We report here a detailed NMR study of diol **3** and its epimer **4** combined with a modeling investigation through quantum-chemical calculations that allowed us to confirm the assignment of the relative configuration at C-3 and to explore the usefulness of theoretical calculations of ^{1}H and ^{13}C chemical shifts in relation to stereochemical studies of steroidal compounds.

RESULTS AND DISCUSSION

Reduction of tibolone (**1**) with lithium tri-*tert*-butoxyaluminum hydride yielded two epimeric diols (**3** and **4**) in a ratio of ca 96:4. The main product **3** was easily obtained pure by crystallization from hexane–acetone whereas its epimer **4** was prepared by treatment of **3** with benzoic acid, diisopropyl diazadicarboxylate and triphenylphosphine followed by hydrolysis of the recovered benzoate.

Complete ^{1}H and ^{13}C NMR signal assignments (Tables 1 and 2) of the spectra of **3** and **4** were achieved using a combination of 1D and 2D (COSY, HSQC and NOESY) experiments

*Correspondence to: Patrizia Ferraboschi, Dipartimento di Chimica e Biochimica Medica, Università di Milano, Via Saldini 50, 20133 Milan, Italy. E-mail: patrizia.ferraboschi@unimi.it
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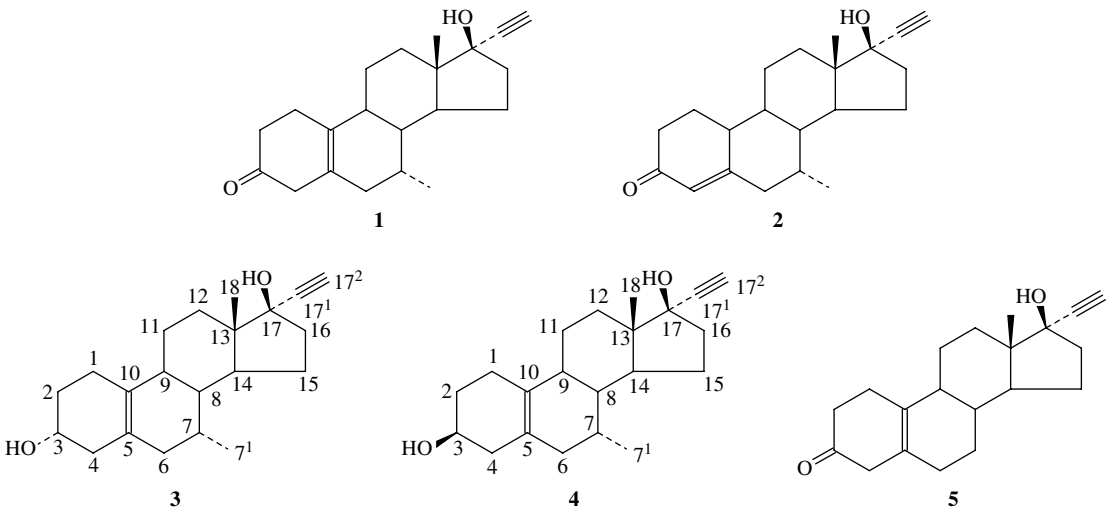

Table 1. GIAO-calculated ^{1}H NMR chemical shifts (δ, in ppm relative to TMS) for **3** and **4** based on geometries optimized at the B3LYP/6–31G(d) level in comparison with the experimental values from the spectra recorded in chloroform–pyridine (1 : 1)

Compound	H[a]	Exp.	HF/ 6–31G(d)	HF/ 6–31G(d,p)	B3LYP/ 6–31G(d)	B3LYP/ 6–31G(d,p)	B3PW91/ 6–31G(d)	B3PW91/ 6–31G(d,p)
3	1α	1.97	1.64	1.55	1.92	1.90	1.91	1.89
	1β	2.17	1.96	1.88	2.26	2.23	2.25	2.22
	2α	1.57	1.14	1.04	1.21	1.12	1.22	1.13
	2β	2.08	1.87	1.77	1.87	1.83	1.87	1.82
	3	3.94	3.59	3.44	3.79	3.72	3.81	3.74
	4α	2.05	1.33	1.24	1.50	1.43	1.51	1.44
	4β	2.33	2.10	2.00	2.26	2.21	2.26	2.22
	6α	1.62	1.33	1.21	1.56	1.47	1.57	1.48
	6β	2.23	1.98	1.90	2.22	2.19	2.21	2.18
	7	1.81	1.49	1.34	1.78	1.71	1.79	1.72
	8	1.47	1.26	1.07	1.71	1.57	1.73	1.59
	9	1.69	1.42	1.28	1.97	1.85	1.97	1.84
	11α	1.96	1.62	1.53	1.82	1.79	1.82	1.79
	11β	1.20	1.05	0.95	1.33	1.27	1.32	1.26
	12α	1.90	1.71	1.64	1.90	1.87	1.89	1.86
	12β	1.71	1.24	1.14	1.45	1.41	1.45	1.40
	14	1.90	1.51	1.35	2.13	2.03	2.13	2.02
	15α	1.66	1.48	1.41	1.66	1.64	1.65	1.63
	15β	1.34	1.25	1.17	1.46	1.42	1.44	1.40
	16α	2.40	2.24	2.18	2.30	2.29	2.31	2.30
	16β	2.13	2.05	1.95	2.12	2.09	2.11	2.08
	18	1.02	0.92	0.86	0.95	0.93	0.93	0.91
	7^1	0.81	0.85	0.79	0.91	0.87	0.88	0.85
	17^2	2.84	2.75	2.69	1.94	2.05	2.03	2.13
4	1α	1.84	1.59	1.52	1.79	1.76	1.79	1.76
	1β	2.43	1.99	1.92	2.32	2.31	2.28	2.28
	2α	1.74	1.31	1.20	1.55	1.48	1.53	1.46
	2β	1.87	1.79	1.68	1.73	1.67	1.73	1.68
	3	4.16	3.69	3.57	3.82	3.81	3.83	3.83
	4α	2.21	1.81	1.72	2.16	2.11	2.14	2.09
	4β	2.13	1.75	1.66	1.89	1.83	1.91	1.84
	6α	1.56	1.33	1.21	1.57	1.48	1.58	1.49
	6β	2.31	1.95	1.88	2.20	2.17	2.19	2.16
	7	1.81	1.50	1.35	1.79	1.73	1.81	1.74
	8	1.50	1.24	1.07	1.70	1.58	1.73	1.59

Table 1. (*Continued*)

Compound	H[a]	Exp.	HF/ 6–31G(d)	HF/ 6–31G(d,p)	B3LYP/ 6–31G(d)	B3LYP/ 6–31G(d,p)	B3PW91/ 6–31G(d)	B3PW91/ 6–31G(d,p)
	9	1.73	1.48	1.34	2.02	1.90	2.02	1.90
	11α	1.99	1.69	1.61	1.89	1.88	1.89	1.88
	11β	1.21	0.99	0.90	1.28	1.24	1.27	1.22
	12α	1.91	1.74	1.66	1.93	1.90	1.92	1.89
	12β	1.70	1.25	1.16	1.46	1.42	1.46	1.42
	14	1.90	1.52	1.36	2.15	2.05	2.15	2.04
	15α	1.67	1.49	1.41	1.67	1.65	1.65	1.64
	15β	1.34	1.25	1.17	1.46	1.43	1.45	1.41
	16α	2.40	2.25	2.19	2.31	2.30	2.32	2.32
	16β	2.13	2.05	1.96	2.13	2.09	2.12	2.08
	18	0.98	0.91	0.86	0.95	0.93	0.93	0.91
	7^1	0.83	0.87	0.80	0.92	0.89	0.90	0.87
	17^2	2.83	2.76	2.70	1.95	2.06	2.04	2.14

[a] Numbered according to IUPAC–IUB Joint Commission on Biochemical Nomenclature (www.chem.qmul.ac.uk/iupac/steroid/).

Table 2. GIAO-calculated ^{13}C NMR chemical shifts (δ, in ppm relative to TMS) for **3** and **4** based on geometries optimized at the B3LYP/6–31G(d) level in comparison with the experimental values from the spectra recorded in chloroform–pyridine (1 : 1)

Compound	C	Exp.	HF/ 6–31G(d)	HF/ 6–31G(d,p)	B3LYP/ 6–31G(d)	B3LYP/ 6–31G(d,p)	B3PW91/ 6–31G(d)	B3PW91/ 6–31G(d,p)
3	1	27.5	25.0	25.4	29.7	30.2	29.6	30.1
	2	33.1	29.9	30.5	34.3	34.8	33.5	34.0
	3	67.3	59.7	60.6	66.6	67.7	66.3	67.3
	4	41.2	37.4	38.2	42.8	43.5	42.3	43.0
	5	124.6[a]	125.3	126.4	123.4	124.8	123.9	125.2
	6	38.9	34.4	35.0	39.6	40.3	39.3	39.9
	7	27.5	24.3	25.2	30.8	31.7	30.2	31.0
	8	42.0	34.8	35.9	42.9	44.1	42.3	43.4
	9	40.2	34.7	35.7	42.0	43.2	41.5	42.6
	10	128.7[a]	129.0	130.1	127.0	128.4	127.3	128.6
	11	25.7	23.7	24.2	28.1	28.6	27.6	28.0
	12	33.6	28.7	29.4	33.8	34.3	33.4	33.9
	13	47.8	40.8	42.0	50.8	52.4	50.6	52.1
	14	46.3	38.6	39.7	47.5	48.6	47.0	48.0
	15	22.3	21.0	21.3	24.4	24.8	24.4	24.7
	16	39.5	34.5	35.0	39.3	39.7	38.9	39.2
	17	79.0	69.2	70.6	79.4	81.2	79.7	81.3
	18	13.3	14.6	14.6	15.4	15.3	15.3	15.3
	7^1	13.0	13.8	13.9	14.4	14.3	14.4	14.3
	17^1	89.5	78.1	79.9	78.1	80.4	79.7	81.9
	17^2	73.2	75.3	76.1	67.7	69.0	70.4	71.6
4	1	23.2	21.3	21.8	25.1	25.6	25.0	25.4
	2	30.8	27.2	27.7	31.0	31.4	30.5	30.9
	3	65.1	58.1	58.9	64.9	65.9	64.7	65.6
	4	40.1	35.2	35.8	40.2	41.0	39.9	40.6
	5	123.4[a]	124.6	125.7	122.4	123.8	122.9	124.1
	6	39.5	34.5	35.2	39.8	40.5	39.5	40.1
	7	27.5	24.3	25.2	30.8	31.6	30.2	31.0
	8	41.9	34.9	35.9	43.0	44.2	42.4	43.5
	9	39.9	34.7	35.8	42.1	43.3	41.6	42.8
	10	128.6[a]	128.8	129.9	126.8	128.3	127.2	128.5
	11	25.6	23.7	24.3	28.1	28.6	27.6	28.0
	12	33.6	28.8	29.4	33.8	34.3	33.4	33.9

(*continued overleaf*)

Table 2. (*Continued*)

Compound	C	Exp.	HF/ 6–31G(d)	HF/ 6–31G(d,p)	B3LYP/ 6–31G(d)	B3LYP/ 6–31G(d,p)	B3PW91/ 6–31G(d)	B3PW91/ 6–31G(d,p)
	13	47.7	40.8	42.0	50.8	52.4	50.6	52.1
	14	46.4	38.7	39.7	47.5	48.7	47.0	48.1
	15	22.3	21.0	21.4	24.5	24.9	24.4	24.7
	16	39.4	34.6	35.0	39.4	39.8	39.0	39.3
	17	79.0	69.2	70.5	79.4	81.2	79.7	81.3
	18	13.2	14.7	14.7	15.4	15.4	15.4	15.3
	7^1	13.2	13.8	13.9	14.4	14.3	14.4	14.3
	17^1	89.6	78.1	79.9	78.1	80.4	79.7	81.9
	17^2	73.2	75.4	76.2	67.7	69.0	70.5	71.7

[a] Assigned through comparison with the calculated values.

recorded in a chloroform–pyridine (1 : 1) mixture, as this solvent gave the best spread of proton resonances of the two steroids. Starting from the characteristic resonances of the 7α-methyl group and of the H-3 proton, it was possible to assign the resonances of H-1, H-2, H-4, H-6, H-7 and H-8 of both **3** and **4** on the basis of their COSY and HSQC spectra. Also, even if many protons in the ^{1}H NMR spectrum resonated as complex multiplets in the range 1.2–2.5 ppm, some of these (Table 1) resulted in well resolved signals the coupling of which could be measured (Table 3). In particular, the assignments of some pairs of geminal protons (H-6, H-11, H-15 and H-16 of both **3** and **4** and H-2 and H-4 of **3**) were made by comparison (Table 3) of the experimental values of the vicinal coupling constants with the values calculated through the electronegativity-modified Karplus relationship (see below). NOE contacts from NOESY spectra were useful for the assignment of other geminal protons, i.e. H-12 of both **3** and **4** (NOE between H-12β and H_3-18), H-1 of **3** (NOE between H-1β and H-3) and H-4 of **4** (NOE between H-4α and H-6α), while the pairs of geminal H-1 and H-2 protons of **4** were tentatively assigned from the ^{1}H NMR chemical shifts (Table 1) calculated through the GIAO approach (see below). Finally, a cross peak between H-11α and one of the H-1 protons in the NOESY spectrum of the isomer **4** was significant for the assignment of the protons H-11 vs H-15 and, consequently, of H-9 vs H-14 and H-12 vs H-16, of the C and D rings. As this part of the molecule is identical for the two isomers, the protons of the C and D rings were assigned for **3** on the basis of the resonances already established for **4**, even though the NOESY cross peak between αH-11 and one of the H-1 protons, which is assumed from the computed distances (data not shown), was not evidenced because of resonance overlapping in the corresponding proton spectrum (Table 1).

The H-3 signal is of special interest as the four vicinal coupling constants of H-3 (Table 3) can be diagnostic for the configuration at C-3. This configurational assignment relies heavily, however, on the knowledge of the exact conformational preferences of **3** and **4** and, in particular, of the A ring. In fact, owing to the presence of the 5(10) double bond, two half-chair conformations can be envisaged (**A** and **B** type, Figure 1), the relative stability of which derives from a fine balance between steric and electronic factors. The vicinal coupling constants indicate a pseudo-axial orientation of the H-3 atom for **3** (and hence a hydroxy group equatorially oriented) and vice versa for **4**. However, these data cannot be of help until the conformational preferences of **3** and **4** have been established.

Table 3. Experimental ^{1}H NMR coupling constants (Hz) for **3** and **4** in comparison with the values calculated with the electronegativity-modified Karplus relationship

J	**3** (exp.)	**3** (calcd)	**4** (exp.)	**4** (calcd)
2α,2β	11.5			
1α,2α	5.5	5.5		5.8
1α,2β		2.5		2.5
1β,2α	11.5	11.3		11.0
1β,2β		6.1		6.4
2α,3	11.5	10.7	2.0	2.0
2β,3	3.5	3.3	6.0	5.3
4α,4β	16.8		17.0	
3,4α	9.0	9.3	3.5	4.0
3,4β	5.5	5.5	4.5	3.5
6α,6β	16.5		17.0	
6α,7	<1.0	1.4	<1.0	1.4
6β,7	7.0	6.1	6.0	6.1
7,7^1	7.0		7.0	
7,8	3.0	3.0	3.0	2.4
8,9	11.0	12.0	11.0	12.0
8,14	11.0	12.0	11.0	12.0
11α,11β	12.0		13.0	
9,11α		3.2	3.5	3.2
9,11β	12.0	12.3	13.0	12.3
11α,12α		4.0	3.5	4.0
11α,12β		2.7	3.5	2.7
11β,12α	12.0	13.1	13.0	13.1
11β,12β	3.0	4.0	3.5	4.0
15α,15β	12.0		12.5	
14,15α		6.0		6.0
14,15β	12.0	11.4	12.5	11.4
16α,16β	15.0		13.0	
15α,16α	9.0	12.1	10.0	12.1
15α,16β		3.0	4.0	3.0
15β,16α	5.5	4.7	5.5	4.8
15β,16β	12.0	12.2	12.5	12.2

Table 4. Relative energy (kcal mol^{-1}) and population percentages at 298K of the B3LYP/6–31G(d) optimized conformations of **3** and **4**

Conformation	E_{rel}	%
3A	0.00	88.0
3B	1.18	12.0
4A	0.00[a]	88.7
4B	1.22	11.3

[a] $E_{rel} = 0.03$ kcal mol^{-1} with respect to **3A** (1 kcal = 4.184 kJ).

The relative stability of conformers **A** and **B** was determined within the DFT framework using a hybrid exchange-correlation functional, B3LYP,[11] at the 6–31G(d) level as implemented in Gaussian 98.[12] The relative energies of these conformers are reported in Table 4 together with the population percentages, calculated through the Boltzmann equation, and their 3D representations are reported in Fig. 1. It can be seen that both compounds prefer by about 90% a conformation of type **A** that makes the OH group equatorial in **3** and axial in **4**. For each conformer the ^{1}H vicinal coupling constants were calculated with the electronegativity-modified Karplus relationship[13] and were weighted averaged on the basis of the population percentages. The values obtained are reported in Table 3 in comparison with the experimental constants for **3** and **4**. The close agreement of the experimental and calculated values confirms that the configuration at C-3 of diol **3** is α and similarly the configuration of diol **4** is β. A number of NOE contacts (e.g. between H-4β and H-3 in **3** and between H-4α and H-3 in **4**) further confirm the assigned structures. These contacts correspond to distances of <3 Å as measured on the computed **3A** and **4A** conformations of **3** and **4**, respectively.

Ab initio computation of NMR chemical shifts is becoming a convenient alternative tool for facilitating spectral assignments and rationalizing experimental chemical shifts, but has been infrequently applied to steroidal compounds. For these calculations, the gauge-including atomic orbital (GIAO)[14] method is the more widely used; Cheeseman *et al*.[15] recommended the following procedure to give a reliable estimate of shielding constants: after an optimization at the B3LYP/6–31G(d) level, the optimized geometries should be used to compute the NMR properties at the HF/6–31G(d) level, predicting the isotropic chemical shifts for carbon and hydrogen atoms with respect to tetramethylsilane (TMS). However, other workers suggested the use of different models and/or basis sets also in relation to the nuclei which are to be predicted.[16] Hence, in this work two DFT functionals,[11,17] B3LYP and B3PW91, together with the traditional Hartree–Fock method were used for GIAO calculations combined with the 6–31G(d) and 6–31G(d,p) basis sets.

We computed both the ^{1}H and ^{13}C chemical shifts for each pair of conformations of **3** and **4** and weighted averaged them on the basis of the population percentages; the results are reported in Tables 1 and 2. The shifts for the carbon atoms computed with the HF approach appear prevalently at higher fields than those measured experimentally with an error that can become higher than 10 ppm. This disagreement does not depend on the fact that GIAO calculations do not explicitly consider the solvent, in our case a 1 : 1 mixture of pyridine and chloroform, as ^{13}C chemical shifts are not sensitive to the solvent, as can be seen from the data in Table 5, where the ^{13}C resonances in chloroform and pyridine are reported; the solvent effect is in general limited to less than 1 ppm with the only exception of the quaternary acetylenic atom. The change of the basis set from 6–31G(d) to 6–31G(d,p) slightly improved the results that, however, remain unsatisfactory. An improvement could be observed by turning to the density functional methods which presented fairly good agreement of the calculated and experimental values; however, the prediction of the acetylenic carbon atoms still remains unsatisfactory.

To allow an easier comparison of methods and basis sets, we determined the root mean square (r.m.s.) errors between calculated and experimental ^{13}C resonances (Table 6). The values were calculated by inclusion or exclusion of the data for the two acetylenic carbon atoms. It appears that the use of a density functional method is largely to be preferred

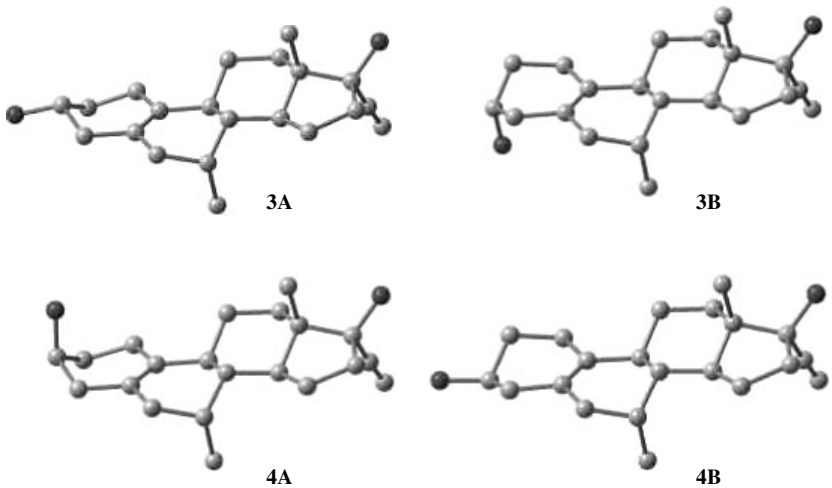

Figure 1. 3D plots of the minimum energy conformations of **3** and **4**.

Table 5. ^{13}C NMR chemical shifts (δ, in ppm relative to TMS) for **3** and **4** from the spectra recorded in chloroform and in pyridine

	Compound **3**		Compound **4**	
C	Chloroform	Pyridine	Chloroform	Pyridine
1	27.2	27.8	22.3	23.6
2	32.6	33.7	29.9	31.4
3	68.1	67.4	65.9	65.3
4	40.6	41.7	39.7	40.7
5	124.1	124.9	122.6	123.7
6	38.7	39.1	39.1	40.0
7	27.0	27.7	27.3	27.8
8	41.7	42.2	41.7	42.2
9	39.8	40.5	39.5	40.2
10	128.6	129.0	128.5	128.9
11	25.3	25.9	25.3	25.9
12	33.2	34.0	33.1	34.0
13	47.5	48.1	47.4	48.0
14	46.2	46.7	46.3	46.8
15	22.1	22.6	22.1	22.6
16	39.0	40.0	39.0	39.7
17	79.8	79.2	79.9	79.2
18	12.9	13.6	12.9	13.3
7^1	12.9	13.1	13.0	13.5
17^1	87.9	90.3	87.8	90.3
17^2	73.7	74.1	73.8	74.1

over the HF method as r.m.s. errors of 1.5 ppm are observed with the B3PW91 method if only sp^3 and sp^2 carbon atoms are considered. The use of the more extended basis set 6–31G(d,p) seems unnecessary as it gives a worsening of the errors.

We now address the question of whether the theoretical calculation of the ^{13}C resonances can be used for the assignment of the relative configuration of the diastereoisomeric pair **3**–**4**. We think that they can, in particular through minimization of the systematic errors by expressing the chemical shifts of the carbon atoms of one isomer relative to the values of the other isomer. Table 7 reports calculated and experimental $\Delta\delta(\alpha - \beta)$: obviously, these $\Delta\delta$ values are significant only for the carbon atoms of ring A, as the other rings are identical. Very close agreement between experimental and calculated $\Delta\delta$ values can be observed at all levels of calculation.

As far as the ^1H NMR resonances are concerned, also in this case the density functional methods with the 6–31G(d) basis set work better than the Hartree–Fock method as r.m.s. errors less than 0.2 ppm are observed (Table 6) if the acetylenic proton is excluded from the computation. In the case of the proton resonances, comparison of experimental and calculated values cannot be a safe tool for configurational assignments; however, it should be pointed out that in each pair of geminal α–β hydrogen atoms, the relative order in the chemical shifts is correctly predicted (Table 1). Hence this can become a method for the assignment of α- and β-hydrogens in cases in which other methods, such as vicinal coupling constant analysis or NOE contacts, fail.

CONCLUSIONS

The C-3 configuration of the diols **3** and **4**, obtained by reduction of tibolone, was assigned through a detailed modeling study combined with the analysis of the vicinal coupling constants of the ring A protons compared with the theoretical *J* values. It has been shown that B3LYP/6–31G(d) optimization followed by GIAO NMR calculations with the same method or with the other DFT approach (B3PW91) is no doubt a better way to carry out the theoretical determination of ^1H and ^{13}C resonances. These methods are accurate enough to permit the stereochemical assignment of the configuration of diastereoisomeric steroidal compounds by using ^{13}C resonance differences; the predicted ^1H resonances appear less precise but allow the assignment of the chemical shift within pairs of geminal α–β hydrogen atoms.

EXPERIMENTAL

All solvents and reagents were purchased from Sigma. Tibolone (**1**) was obtained according to Ref. 18. All reactions were monitored by TLC on silica gel 60 F_{254} plates (Merck) with detection by spraying with 10% phosphomolybdic acid in ethanol solution and heating at 110 °C. Column chromatography was performed on silica gel 60 (0.063–0.200 mm) (Merck). Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC-7 instrument. GC analysis was performed on a Hewlett-Packard HP5890 instrument at 260 °C oven temperature, with an HP5 capillary

Table 6. Comparison of the different methods for prediction of ^1H and ^{13}C chemical shifts by r.m.s. errors (in ppm)

Compound		HF/ 6–31G(d)	HF/ 6–31G(d,p)	B3LYP/ 6–31G(d)	B3LYP/ 6–31G(d,p)	B3PW91/ 6–31G(d)	B3PW91/ 6–31G(d,p)
3	All ^{13}C	5.3	4.6	3.2	3.1	2.6	2.5
	sp^2 and sp^3 ^{13}C	4.9	4.2	1.7	2.3	1.5	2.0
	All ^1H	0.29	0.39	0.26	0.26	0.25	0.25
	sp^3 ^1H	0.30	0.40	0.19	0.20	0.19	0.20
4	All ^{13}C	5.3	4.6	3.2	3.1	2.6	2.5
	sp^2 and sp^3 ^{13}C	4.9	4.3	1.7	2.3	1.5	2.0
	All ^1H	0.29	0.38	0.23	0.22	0.22	0.21
	sp^3 ^1H	0.30	0.39	0.15	0.16	0.16	0.15

Table 7. Experimental and calculated ^{13}C NMR chemical shifts differences, $\Delta\delta(\alpha - \beta)$ (in ppm), between the resonances of **3** and **4**

C	Exp.	HF/ 6–31G(d)	HF/ m6–31G(d,p)	B3LYP/ 6–31G(d)	B3LYP/ 6–31G(d,p)	B3PW91/ 6–31G(d)	B3PW91/ 6–31G(d,p)
1	4.3	3.7	3.6	4.6	4.6	4.6	4.7
2	2.3	2.7	2.8	3.3	3.4	3.0	3.1
3	2.2	1.6	1.7	1.7	1.8	1.6	1.7
4	1.1	2.2	2.4	2.6	2.5	2.4	2.4
5	1.2	0.7	0.7	1.0	1.0	1.0	1.1
6	−0.6	−0.1	−0.2	−0.2	−0.2	−0.2	−0.2
7	0.0	0.0	0.0	0.0	0.1	0.0	0.0
8	0.1	−0.1	0.0	−0.1	−0.1	−0.1	−0.1
9	0.3	0.0	−0.1	−0.1	−0.1	−0.1	−0.2
10	0.1	0.2	0.2	0.2	0.1	0.1	0.1
11	0.1	0.0	−0.1	0.0	0.0	0.0	0.0
12	0.0	−0.1	0.0	0.0	0.0	0.0	0.0
13	0.1	0.0	0.0	0.0	0.0	0.0	0.0
14	−0.1	−0.1	0.0	0.0	−0.1	0.0	−0.1
15	0.0	0.0	−0.1	−0.1	−0.1	0.0	0.0
16	0.1	−0.1	0.0	−0.1	−0.1	−0.1	−0.1
17	0.0	0.0	0.1	0.0	0.0	0.0	0.0
18	0.1	−0.1	−0.1	0.0	−0.1	−0.1	0.0
7^1	−0.2	0.0	0.0	0.0	0.0	0.0	0.0
17^1	−0.1	0.0	0.0	0.0	0.0	0.0	0.0
17^2	0.0	−0.1	−0.1	0.0	0.0	−0.1	−0.1

column (25 m × 0.32 mm i.d., 0.52 μm film thickness). Optical rotations were determined on a Perkin-Elmer model 241 polarimeter in ethyl acetate solutions ($c = 1.0$) in a 1 dm cell at 25 °C. Electron ionization mass spectrometry (EI-MS) was carried out at 70 eV by LC particle beam introduction with a Hewlett-Packard HP 5988A quadrupolar mass spectrometer equipped with a PB 59980A interface and an HP 1050 low-pressure liquid chromatograph.

Compounds

*17α-Ethynyl-7α-methyl-5(10)-estren-3α,17β-diol (**3**)*

A solution of lithium tri-*tert*-butoxyaluminum hydride (1.1 M, 10.7 ml) in tetrahydrofuran was added dropwise to a solution of tibolone (**1**) (1 g, 3.2 mmol) in anhydrous tetrahydrofuran (10 ml) kept under N_2, at −70 °C. After 2 h the reaction mixture was poured into 10% aqueous acetic acid (30 ml) and disodium ethylenediaminetetraacetate (0.2 g) was added. The mixture was extracted with chloroform (5 × 25 ml). The collected organic phases were dried over sodium sulfate; evaporation of the solvents afforded a crude product: by trituration with three portions of methylene chloride (5 ml) and crystallization from acetone–hexane pure 3α-diol **3** (0.7 g, 70%) was recovered. Endothermic peak fusion (DSC) at 187 °C; $[\alpha]_D^{25}$ +67.1°; $[\alpha]_{546}^{25}$ +79.9°; EI-MS: m/z 314 (M^+, 43%), 296 (100%), 288 (100%); GC, retention time (t_R) = 12.75 min; TLC [$CHCl_3$–AcOEt (7 : 3)], $R_f = 0.49$.

*17α-Ethynyl-7α-methyl -5(10)-estren-3β,17β-diol (**4**)*

A solution of 3α-diol **3** (0.44 g, 1.4 mmol) and triphenylphosphine (0.474 g, 1.81 mmol) in anhydrous diethyl ether (8 ml) was added dropwise to a solution of diisopropyl azodicarboxylate (0.37 g, 1.81 mmol) and benzoic acid (0.222 g, 1.81 mmol) in diethyl ether (0.8 ml). The reaction mixture was kept at room temperature with stirring overnight. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (silica gel, 1 : 10); elution with hexane–ethyl acetate (9 : 1) afforded the benzoate (0.38 g, 65%). The ester was treated with sodium carbonate (0.42 g) in methanol–water (9 : 1) (13 ml) at 50 °C for 4 h. The reaction mixture was poured into cool water (20 ml) and the precipitated crude product was recovered by suction. Column chromatography [silica gel, 1 : 10; hexane–ethyl acetate (7 : 3) as eluant] and crystallization (2-propanol–water) afforded pure 3β-diol **4** (0.15 g, 53%). Endothermic peak fusion (DSC) at 142 °C; $[\alpha]_D^{25}$ +15.7°; $[\alpha]_{546}^{25}$ +18.5°; EI-MS: m/z 314 (M^+, 65%), 296 (67%), 288 (100%); GC, t_R = 12.56 min; TLC [$CHCl_3$–AcOEt (7 : 3)], $R_f = 0.40$.

NMR spectroscopy

All NMR spectra were recorded at 297 K with a Bruker AM-500 spectrometer operating at 500.13 and 125.76 MHz for ^1H and ^{13}C, respectively, using a 5 mm broadband reverse probe. Chemical shifts are reported on the δ (ppm) scale and are relative to TMS as an internal reference. Compounds **3** and **4** (ca 15 mg) were dissolved in $CDCl_3$–pyridine-d_5 (1 : 1) (0.5 ml) under N_2, and their assignments were given by a combination of 1D and 2D COSY, HSQC and NOESY experiments, using standard Bruker pulse programs. The pulse widths were 7.5 μs (90°) and 9.6 μs (90°) for ^1H and ^{13}C, respectively. Typically 16K and 32K data points were collected for one-dimensional proton and carbon spectra, respectively. Spectral widths were 5747 Hz for ^1H NMR (digital resolution: 0.70 Hz per point) and 38 461 Hz for ^{13}C NMR (digital resolution: 2.34 Hz per point). 2D experiments parameters were as follows. For ^1H–^1H correlations (COSY and NOESY): relaxation delay 1.2 s, data matrix 1K × 2K (512 experiments to 1K, zero filling in F_1, 2K in F_2), 16 transients in each experiment, spectral width 5.9 ppm (2958.6 Hz). The NOESY spectra were generated with a mixing time of 1.0 s and acquired in the TPPI mode. There were no significant differences in the results obtained at different mixing times (0.5–1.5 s). For ^{13}C–^1H correlations (HSQC): relaxation delay 1.5 s, data matrix 0.5K × 2K (256 experiments to 0.5K, zero filling in F_1, 2K in F_2), 32 transients in each experiment, spectral width 5.9 ppm (2958.6 Hz) in the proton domain and 147.2 ppm (18 518.5 Hz) in the carbon domain. All 2D

spectra were weighted with sine-bell squared and shifted ($\pi/2$ in both dimensions) window functions, and processed with the Bruker software package.

Calculations

All calculations were carried out with the Gaussian 98 program.[12] Geometry optimization of the conformations of **3** and **4** was performed without constraints at the B3LYP/6–31G(d) level. The population percentages were calculated from the gas-phase electronic energies of the conformers through the Boltzmann equation at 298K; the entropic terms were neglected. Attempts to evaluate the influence of the solvent on the relative energies of the conformers were made using a continuum solvent model (C-PCM)[19] at different dielectric constant values, but the runs stopped without completion owing to the molecular size of **3** and **4**. However, solvent calculations on smaller models of **3** and **4** lacking the D ring could be performed and confirmed the preference for conformers such as **3A** and **4A**. NMR chemical shifts were calculated at the Hartree–Fock and density functional levels with the 6–31G(d) or the 6–31G(d,p) basis sets using the GIAO method. All the ^{1}H and ^{13}C chemical shifts are referenced to those of TMS. The absolute ^{1}H and ^{13}C shielding of TMS, based on the B3LYP/6–31G(d) optimized geometry, were calculated at the same level/basis set used in the calculation to which they refer.

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