^{1}H and ^{13}C NMR of synthetic steroid sapogenins. Part II.♣ C-23 Substituted derivatives of (25*S*)-spirostanes

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Abstract
The main ^{1}H and ^{13}C NMR characteristics of synthetic (25*S*)-5β-spirostantes are described. Changes on chemical shifts due to substitution at C-23 are briefly analyzed.

Keywords: C-23 Substituted (25*S*)-spirostanes, NMR, x-ray diffraction

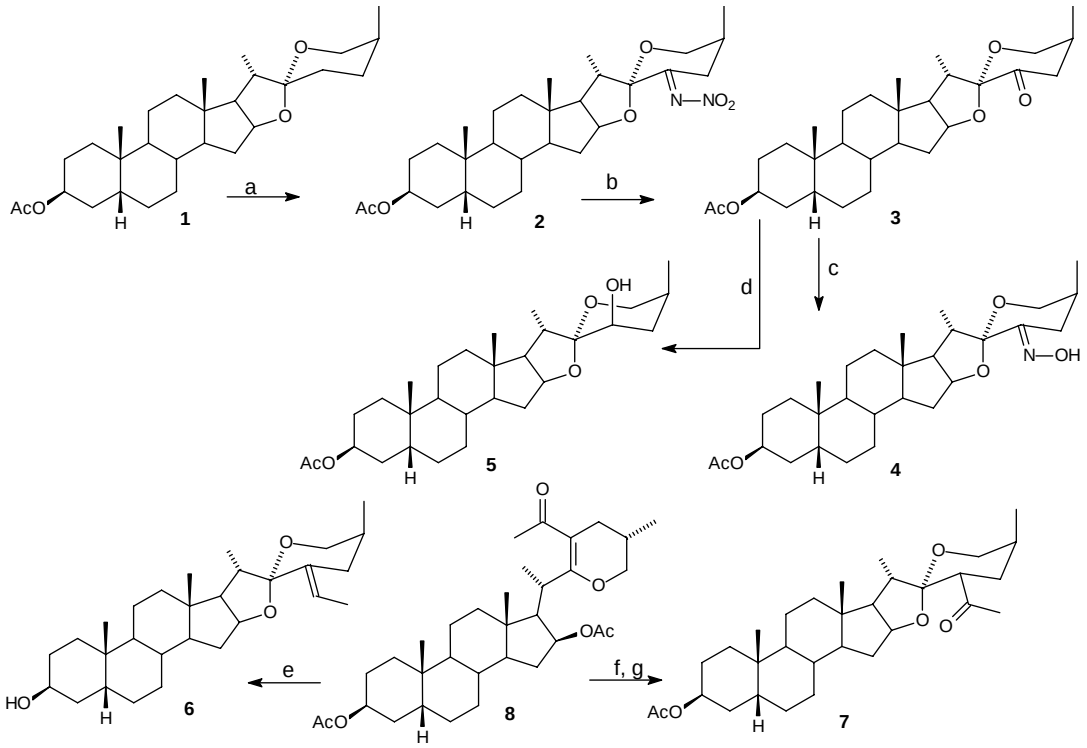
Introduction

Steroidal sapogenins are closely connected with the history of organic chemistry in Mexico.[1a,b] In general, this family of compounds are widespread in both natural and synthetic domains. During the mid part of the last century this kind of compound attracted the attention of natural product chemists due to the special reactivity of the heterocyclic side chain.[1c] Some of the members of this class have served as starting materials in the synthesis of sex hormones[2] and corticosteroids.[3] More recently, steroid sapogenin chemistry has experienced a revival; new reactions[4] and new synthetic applications[5] are being reported.

Although the NMR characteristics of the spiroketal side chain have been described and reviewed,[6] to the best of our knowledge, only one report has analyzed the changes on ^{1}H and ^{13}C chemical shifts derived from common substitution at C-23 of (25*R*)-spirostanes.[6e] We now present an analysis of the ^{1}H and ^{13}C spectra of a number of synthetic (25*S*)-spirostanes bearing oxygenated or non-common functionality at C-23, which as a part of our program on bioactive steroidal derivatives, are being examined as synthetic precursors. Main changes on the side chain chemical shifts derived from this substitution are described and rationalized.

Results and Discussion

Satisfactory single-crystals were obtained by slow evaporation for compounds **4**, **6** and **7**. Structure refinements revealed the expected geometries for the A-F steroidal nucleus, including *cis* A/B junctions. The PM3 optimized geometries are in good agreement with their corresponding X-ray structures. Bond lengths and angles for C23 unambiguously stand for sp^2 hybridized atoms in the case of **4** and **6**, while a sp^3 hybridization is observed in the case of **7**. For instance, C23-N32 and C23-C23' bond lengths in **4** and **6**, respectively, are 1.269(3) and 1.345(6) Å [1.323(6) Å for the second independent molecule], *vs*. 1.506(7) Å for the C23-C23' bond length in **7**. As a consequence, one conformation should be observed in the solid-state for groups bonded to C23 in **4** and **6**, while a free rotation for the acetyt group at C23 may be expected in the case of **7**. The observed conformations for the oxiimino and ethyliden groups in **4** and **6** are *anti*, respect to C22 bonded. In both molecules, this conformation generates close contacts between H20 and N32 or C23' atoms; corresponding observed distances for H20···N32 in **4** is 2.49 Å, and H20···C23' in **6** is 2.73 Å [2.72 Å in the second independent molecule]. The acetyl group at C23 in **7** is placed on an equatorial position of the F cycle, giving also a short contact, H20···C23' = 2.65 Å, similar to that observed in **6**. Figure 1 shows the x-Rays structures of compounds **4**, **6** and **7**.



Scheme 1. (a) $NaNO_2/BF_3/AcOH$, (b) Al_2O_3, (c) NH_2OH,HCl/AcONa/ethanol, (d) $NaBH_4$/methanol, (e) KOH/methanol, (f) $LiAlH_4$/THF, (g) Ac_2O/pyr.

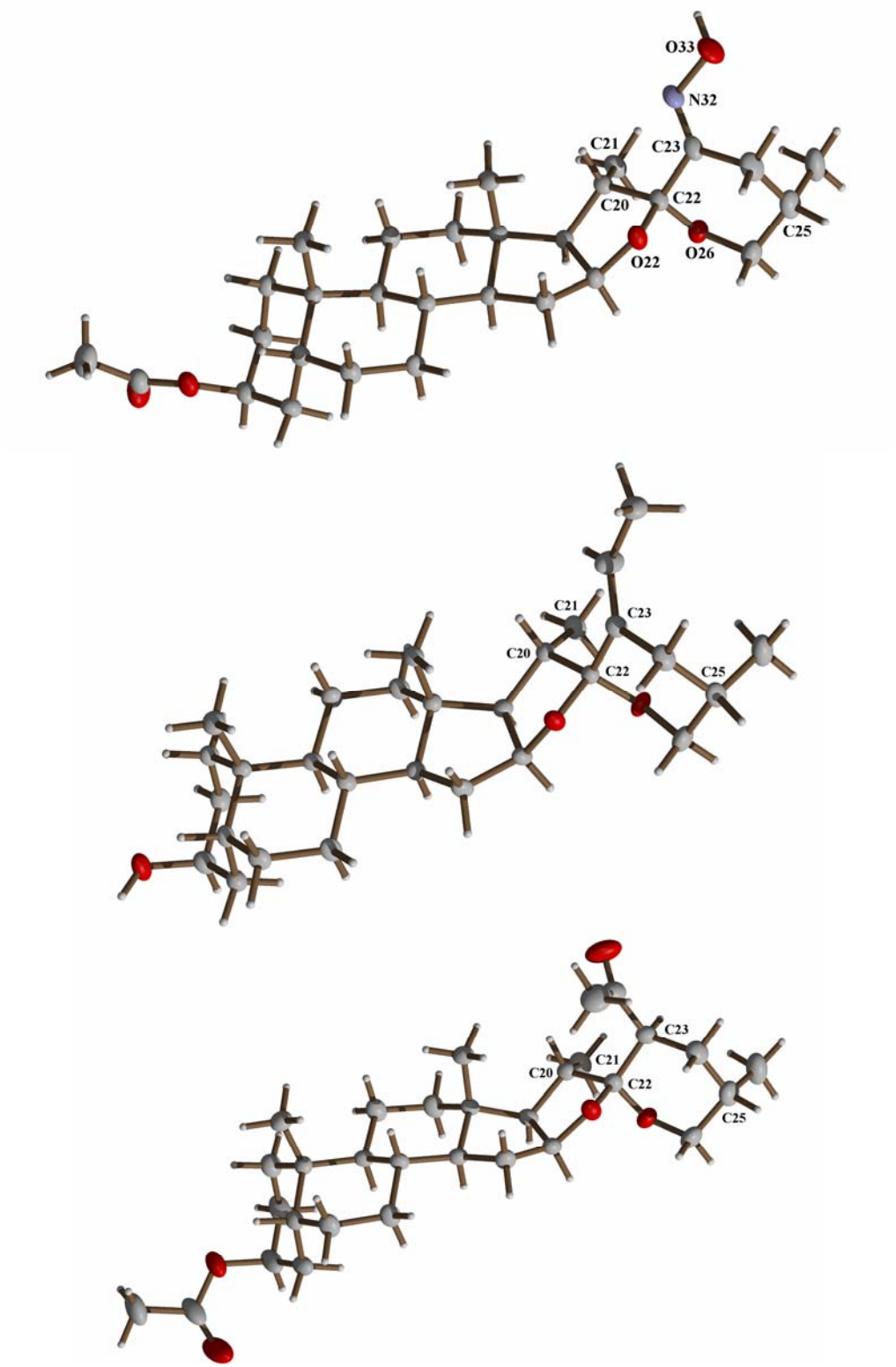


Figure 1. X-ray structures of compounds **4**, **6** and **7,** with thermal ellipsoids drawn at 30% probability level.[9]

Figure 2 shows the optimized geometries[9] of the fragments corresponding to the side chains of componds **1** to **3** and **5**. Spatial proximity between the group attached to C-23 and H-20 can be appreciated in compounds **2** and **3**.

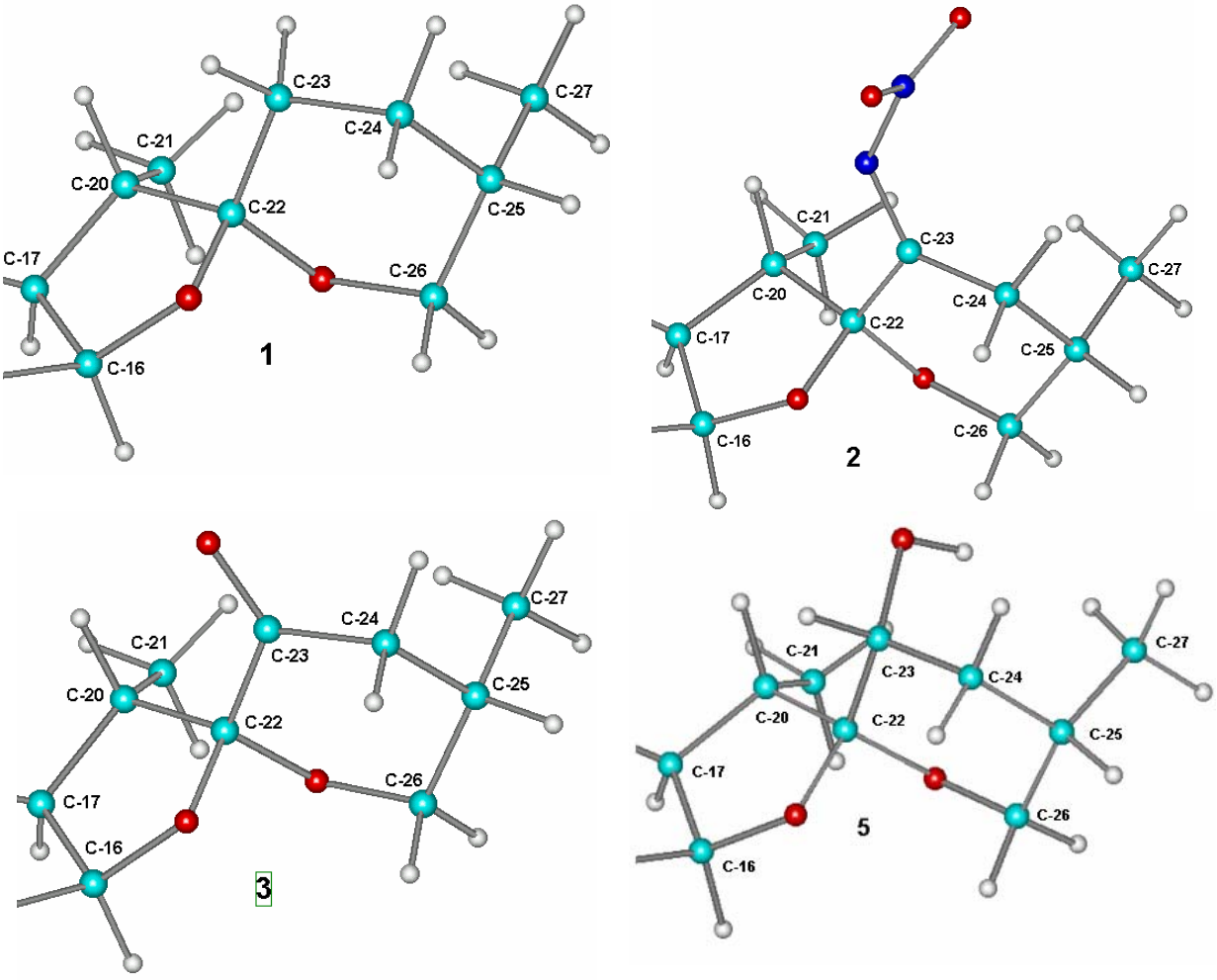


Figure 2. PM3 optimized geometries[10] of the side chains of compounds 1 to 3 and 5.

As expected, substitution at C-23 does not exert significant influence on ^{1}H and ^{13}C chemical shifts at the steroidal A, B, C and D rings, which are in good agreement with the previously reported shielding data,[6d] but significant differences for chemical shifts of H and C atoms in E and F rings were found as discussed below.

^{1}H NMR

Introduction of the new functionality at C-23 produces the expected downfield shifts in both axial and equatorial H-24 signals. Table 1 shows the main ^{1}H chemical shifts observed for compounds **1** to **7**.

Table 1. ^{1}H Chemical shifts of sarsasapogenin acetate **1** and derivatives **2 - 7** (δ ppm)

	Compound						
protons	**1**	**2**	**3**	**4**	**5**	**6**	**7**
H-18	0.76	0.80	0.77	0.75	0.79	0.80	0.64
H-19	0.98	0.99	0.98	0.95	0.98	0.98	0.97
H-21	0.99	1.04	1.07	0.97	1.14	1.01	1.00
H-27	1.07	1.07	0.95	1.00	1.24	0.99	1.08
H-3	5.06	5.06	5.05	5.03	5.06	4.10	5.06
H-16	4.40	4.54	4.61	4.45	4.49	4.39	4.41
H-20	1.81	2.82	2.87	2.76	2.30	2.40	2.50
H-24 ax	2.02	2.86	2.92	2.36	2.14	2.48	2.36
H-24 eq	1.39	2.26	2.20	2.95	1.59	2.21	1.58
H-26 ax	3.94	4.15	4.27	4.08	4.02	4.01	3.99
H-26 eq	3.29	3.41	3.41	3.32	3.38	3.29	3.33
H-23 ax	1.87	-	-	-	-	-	2.84
H-23 eq	1.39	-	-	-	3.61	-	-

Downfield shifts are observed for H-20 signals in all C-23 substituted compounds when compared with those of the C-23 non-substituted **1**. This fact could be rationalized in terms of van der Waals compression of H-20 due to the proximity of the substituent attached to C-23 in compounds **5** and **7**, and a combination of such effect with the magnetic anisotropy caused by the double bond in compounds **2 - 4** and **6**.

Downfield shifts of axial H-26 signals of 23-nitroimino **2** and 23-keto **3** may be rationalized in terms of a combination ef electric field and magnetic anisotropy of the nitroimino and carbonyl functions. Additionally, the presence of a 23-axial hydroxyl group in compound **5** results on δ-*syn* interaction which produce a downfield shift of the signal corresponding to 27-methyl group.

^{13}C NMR

Besides the expected downfield shifts on C-23 and C-24 associated to the introduction of the new functionality, the presence of a subsistent attached to C-23 exerts different effects at carbon nuclei of the side chain. Table 2 shows the assignments of chemical of the ^{13}C chemical shifts observed for compounds **1** to **7**.

Table 2. ^{13}C Chemical shifts of sarsasapogenin acetate **1** and derivatives **2 - 7** (δ ppm)

	Compound						
Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C-1	30.60	30.63	30.62	30.61	30.78	31.66	30.60
C-2	25.96	25.04	25.01	25.01	25.02	27.88	24.99
C-3	70.65	70.61	70.58	70.70	70.64	67.03	70.59
C-4	31.73	31.61	31.72	31.61	31.95	33.60	31.52
C-5	37.28	37.27	37.26	37.27	37.29	36.56	37.26
C-6	26.43	26.42	26.41	26.42	26.43	26.63	26.42
C-7	26.43	26.47	26.41	26.42	26.43	26.63	26.48
C-8	35.28	35.28	35.25	35.24	35.42	35.21	35.12
C-9	39.96	39.98	39.98	39.96	39.98	39.91	39.90
C-10	35.01	35.04	35.09	35.03	35.03	35.33	35.03
C-11	20.92	20.88	20.84	20.92	20.85	21.03	20.88
C-12	40.21	40.04	40.01	40.55	39.90	40.57	40.16
C-13	40.64	41.25	41.16	41.04	41.13	40.87	40.96
C-14	56.30	56.39	56.48	56.35	56.42	56.38	56.20
C-15	30.76	30.76	30.75	30.76	30.62	29.79	31.52
C-16	80.94	82.81	83.45	81.72	81.42	80.57	81.25
C-17	62.00	61.38	61.70	61.10	63.95	61.58	61.18
C-18	16.55	16.47	16.27	16.55	16.37	16.73	16.35
C-19	23.89	23.93	23.85	23.91	23.91	23.99	23.85
C-20	40.10	36.15	35.09	36.41	40.22	38.35	38.80
C-21	14.41	14.23	14.28	14.33	16.78	14.67	14.08
C-22	109.54	108.32	110.50	109.02	109.14	111.40	108.26
C-23	25.79	170.06	201.99	153.54	71.11	133.36	49.49
C-24	24.99	33.81	43.89	27.00	33.56	30.00	29.39
C-25	27.08	32.32	35.08	29.68	26.09	30.34	27.04
C-26	65.05	64.34	64.49	64.98	64.95	65.07	64.34
C-27	16.11	17.14	17.77	17.42	20.53	17.45	16.41
C-23[1]	-	-	-	-	-	119.14	210.19
C-23[2]	-	-	-	-	-	12.29	28.67
C=O, 3Ac	170.51	170.51	170.37	170.61	170.48	-	170.42
Me, 3Ac	21.65	21.64	21.55	21.60	21.61	-	21.58

The most salient feature is the shielding of C-20 in compounds **2** - **4**, **6** and **7**, which is in consonance with the van der Waals compression of H-20 due the *γ-gauche* interaction between C-20 and X-23. See Figures 1 and 2.

Downfield shifts of C-27 signals of compounds **2** - **4** and **6** (sp_2 C-23) may be attributed to the lost of a shielding γ-gauche interaction present in compound **1**, (sp_3 C-23). Deshielding of C-27 in compound **5** may be interpreted on term of 1,3 repulsive diaxial interaction with the axial hydroxyl group, which compress C-27. This respulsive interaction also produces a deformation of F-ring which results on weaker γ-shielding effects over C-17. Additionally, spatial proximity of the axial 23-OH to C-21 in compound **5** (See figure 2) produces a δ-deshielding effect on C-21 signal. Table 3 shows the effects of the substitution at C-23 on the ^{13}C shifts of the (25*S*) spirostanic side chain.

Table 3. Effects (Δδ ppm) of substituents at C-23 in compounds **2** - **7**, referred to **1**

	Compound						
Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C-17	62.00	-0.62	-0.30	-0.90	+1.95	-0.42	-0.82
C-20	40.10	-3.95	-5.01	-3.69	+0.12	-1.75	-1.30
C-21	14.41	-0.18	-0.13	-0.08	+2.37	+0.26	-0.33
C-22	109.54	-1.22	+0.96	-0.52	-0.40	+1.86	-1.28
C-23	25.79	+144.27	+176.2	+127.75	+45.32	+107.57	+23.70
C-24	24.99	+8.82	+18.90	+2.01	+8.57	+5.01	+4.40
C-25	27.08	+5.24	+8.00	+2.60	-0.99	+3.26	-0.04
C-26	65.05	-0.71	-0.56	-0.07	-0.10	+0.02	-0.71
C-27	16.11	+1.03	+1.66	+1.31	+4.42	+1.34	+0.30

Experimental Section

Spectroscopy. NMR spectra were measured in $CDCl_3$ on a Varian Mercury spectrometer at 400 MHz for ^1H or 100 MHz for ^{13}C. Chemical shift are expressed on ppm downfield from TMS and were assigned with the aid of DEPT, HETCOR and COSY spectra.[7] Single-crystal X-ray analyses were carried out on a Bruker P4 diffractometer, at room-temperature, using standard procedures for data collection as for structure solutions and refinements. In the case of **4** and **6**, H atoms for the hydroxyl groups (including a water molecule for **6**) were found on difference maps. Non-H atoms were refined anisotropically in final least-squares cycles, with a riding model for all H atoms. It should be mentioned that for **6**, the asymmetric unit contains two independent molecules with very similar geometries.

Chemistry. (25*S*)-5β-Spirostan-3β-ol, named sarsasapogenin, a well known steroidal sapogenin first isolated from the Mexican plant *Smilax aristolochiaefolia,*[8] known as *sarsaparrilla*, was

converted into the acetate **1** using the standard Ac_2O/pyridine procedure. Treatment of **1** with $NaNO_2/BF_3$ in glacial acetic acid as previously described[5b,f,i] afforded the 23-nitroimino **2** which on treatment with neutral Al_2O_3 led to the 23-ketone **3**. Treatment of **3** with hydroxylamine/sodium acetate in refluxing ethanol afforded the 23-oxime **4**, meanwhile $NaBH_4$ reduction of **3** produced the axial alcohol **5**. 23-ethylidene sarsasapogenin **6** was obtained by $LiAlH_4$ reduction of the previously described 22,26-epoxycholest-22-ene **8**;[4f] basic treatment of **8** followed by acetylation led to 23-acetyl sarsasapogenin acetate **(7)**. See Scheme 1.

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References and Notes

♣ For Part I see reference 6e

1. (a) Lehmann, P. A. *Steroids* **1992**, *57*, 403. (b) Juaristi, E.; Martinez R. Arkivoc MX-484T **2003**, 1. (c) Fieser, L.; Fieser, M. Steroids. Reinhold. San Francisco, 1959; and references therein.
2. Rosenkranz , G.; Mancera O. *J. Org. Chem.* **1956**, *2*, 520.
3. (a) Ringold, H. J.; Rosenkranz, G.; Sondheimer, F. *J. Am. Chem. Soc.* **1956**, *78*, 820. (b) Rosenkranz, G.; Pataki, J.; Djerassi, C. *J. Am. Chem. Soc.* **1951**, *73*, 4055.
4. (a) Hernandez, R.; Marrero-Tellado, J. J.; Prout, K.; Suarez, E. *J. Chem. Soc., Chem. Commun.* **1992**. 275. (b) Sandoval-Ramirez, J.; Castro-Mendez, A.; Meza-Reyes, S.; Reyes-Vazquez, S.; Santillan, R.; Farfan, N. *Tetrahedron Lett*. **1999**, *40*, 5143. (c) Morzycki, J. W.; Jastrzebska, I. *Tetrahedron Lett*. **2001**, *42*, 5989. (d) Anulewicz-Ostrowska, R.; Morzycki, J. W.; Jastrzebska, I.; Wojcik, J. *J. Org. Chem.* **2002**, *67*, 6916 (e) Betancour C.; Dorta R. L.; Freire R.; Martin A.; Prange, T.; Suarez, E. *J. Org. Chem*. **2002**, *67*, 6355. (f) Sandoval-Ramirez, J.; Meza-Reyes, S.; del Rio, R. E.; Hernandez-Linares, G; Suarez-Rojas, A.; Rincon, S.; Farfan, N.; Santillan, R. L. *Steroids* **2003**, *68*, 199.
5. (a) Iglesias-Arteaga M.A.; Perez, R.; Leliebre V.; Perez, C.S.; Coll F. *J. Chem. Research (S)* **1996**, 504. (b) Iglesias-Arteaga M.A.; Leliebre V.; Perez, C.S.; Coll F. *Quim. Nova* **1997**, 20, 361; Chem. Abstr. **1997**, *127*, 95449. (c) Iglesias-Arteaga M.A.; Perez, R.; Leliebre V.; Perez, C.S.; Rosado A.; Coll, F. *Synth. Commun.* **1998**, *28*, 1381. (d) Iglesias-Arteaga M.A.; Perez, R.; Leliebre V.; Perez, C.S.; Coll F. *Synth. Commun.* **1998**; *28*, 1779. (e) LaCour, G.T.; Tong, Z.; Fuchs, P. L. *Org. Lett*. **1999**, *1*, 1815. (f) Iglesias-Arteaga, M. A.; Perez, R.; Perez, C. S.; Coll, F. *J. Chem. Soc., Perkin Trans.* 1 **2001**, 261–266. (g) Lee, S.; Fuchs, P. L. *Org. Lett*. **2002,** *4*, 317. (h) Lee, S.; LaCour, G.T.; Lantrip, D.; Fuchs, P. L. *Org. Lett*. **2002,**

4, 313.(i) Iglesias-Arteaga, M. A.; Perez, R.; Perez, C. S.; Coll, F. *Steroids.* **2002**, *67*, 159. (j) Yu, B.; Tao, H. *J. Org. Chem*. **2002**, *6*7, 9099. (k) Betancour, C.; Freire, R.; Perez-Martin, I.; Prange, T.; Suarez, E. *Org. Lett*. **2002,** *4*, 1295**.**

6. (a) Eggert, H.; Djerassi, C. *Tetrahedron Lett.* **1975**, *42*, 3655. (b) Tori, K.; Seo, S. Yoshihiro, T.; Nishikawa, J. *Tetrahedron Lett.* **1981**, *22*, 2405. *(*c) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479. (d) Agrawal, P. K.; Bunsawansong. P.; Morris, G. A. *Magn. Reson. Chem*. **1997**, *35*, 441. (e) Iglesias-Arteaga M.A.; Perez, C.S.; Perez, R.; Coll F. *J. Chem. Research (S)* **1999**, 48.
7. A copy of all ^{1}H and ^{13}C NMR spectra may be obtained from the authors on request.
8. Marker, R. E. *J. Am.. Chem. Soc*. **1940**, *62*, 2543.
9. Crystallographic data for these structures have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 219940 (compound 4), 219941 (compound 6) and 219942 (compound 7). Copies of the data can be obtained free of charge on application to CCDC. 12 Union Road, Cambridge CB21EZ, UK (Fax: +44 1223-336-036. Email: deposit@ccdc.cam.ac.uk)
10. *Hyperchem Molecular Modeling System* v. 5.02; HyperCube, 1997.