Steroids 104 (2015) 137–144

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Crystallographic and spectroscopic study on a known orally active progestin

Patrizia Ferraboschi [a,*], Pierangela Ciuffreda [b], Samuele Ciceri [a], Paride Grisenti [c], Carlo Castellano [d], Fiorella Meneghetti [e]

[a] Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Via Saldini 50, 20133 Milano, Italy
[b] Dipartimento di Scienze Biomediche e Cliniche "Luigi Sacco", Università degli Studi di Milano, Via G.B. Grassi 74, 20157 Milano, Italy
[c] EUTICALS SpA, Via Volturno 41/43, 20089 Rozzano, MI, Italy
[d] Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133 Milano, Italy
[e] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via L. Mangiagalli 25, 20133 Milano, Italy

ARTICLE INFO

Article history:
Received 24 April 2015
Received in revised form 10 August 2015
Accepted 27 September 2015
Available online 30 September 2015

Keywords:
Progestin
Progesterone
Medrogestone
Steroidal hormone
X-ray
NMR

ABSTRACT

6,17α-Dimethyl-4,6-pregnadiene-3,20-dione (medrogestone, **2**) is for a long time known steroid endowed with progestational activity. In order to study its crystallographic and NMR spectroscopic properties with the aim to fill the literature gap, we prepared medrogestone following a traditional procedure. A careful NMR study allowed the complete assignment of the ^{1}H and ^{13}C NMR signals not only of medrogestone but also of its synthetic intermediates. The structural and stereochemical characterizations of medrogestone together with its precursor 17α-methyl-3-ethoxy-pregna-3,5-dien-20-one were described by means of X-ray analysis, allowing a deepened conformational investigation.

1. Introduction

Progesterone (4-pregnene-3,20-dione, **1**) is the natural progestational agent. Progestational agents, natural or synthetic, are compounds that, like progesterone, are able to transform an endometrium primed by estrogens into a secretory status and are named progestins [1]. All known progestins belong to the class of steroids and are structurally related to pregnane or to androstane and estrane. The pregnane related progestins can differ from progesterone only for the presence of a 17α-hydroxy group, for the stereochemistry of C-10 or for the absence of C-19 [1,2]. The research devoted to the discovery of synthetic progestins is explained by the poor bioavailability on oral administration of progesterone, due to rapid metabolism in the liver that involves the reduction of 20-keto group followed by the hydrogenation of 4,5-double bond and reduction of 3-keto group [3].

Medrogestone (6,17α-dimethyl-4,6-pregnadiene-3,20-dione, Prothil®, **2**) is a synthetic orally active progestin, known since sixties [4,5], used in the treatment of pathological deficiency of the natural hormone; in addition, its action on the enzymes involved in the estrogen biosynthesis and metabolism, in normal and cancerous breast cells, is still object of studies [6–9]. It is known, indeed, that a wide variety of progestinic analogs are potent inhibitors of enzymes involved in estrogen biosynthesis (sulfatase and 17β-hydroxysteroid dehydrogenase). These latter, at the same time, act by stimulating the sulfotransferase, the enzyme which converts estrogens into the biologically inactive sulfates. This double action leads to a significant reduction in estradiol biosynthesis which is aberrant in many breast tumors [10].

The structure of medrogestone **2** is closely related to that of progesterone **1**, two methyl groups at position 6 and 17, respectively, and a double bond at position 6 being the only differences. The presence of the two methyl group is reported as mandatory for orally activity, probably due to the block of metabolic inactivation [11]. Indeed, the additional C-17 substituent could protect the 20-keto group and the C-6 methyl group could slow down the A-ring reduction [3].

Although medrogestone **2** is known for a long time, a careful NMR and crystallographic investigation is not yet reported. This lack of data prompted us to study, in addition to medrogestone, also its precursor 17α-methyl-3-ethoxy-pregna-3,5-dien-20-one (**3**) (Fig. 1), either by NMR and X-ray analyses, and to describe the

* Corresponding author at: Università degli Studi di Milano, Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Via Saldini 50, 20133 Milano, Italy.
E-mail address: patrizia.ferraboschi@unimi.it (P. Ferraboschi).

http://dx.doi.org/10.1016/j.steroids.2015.09.007
0039-128X/

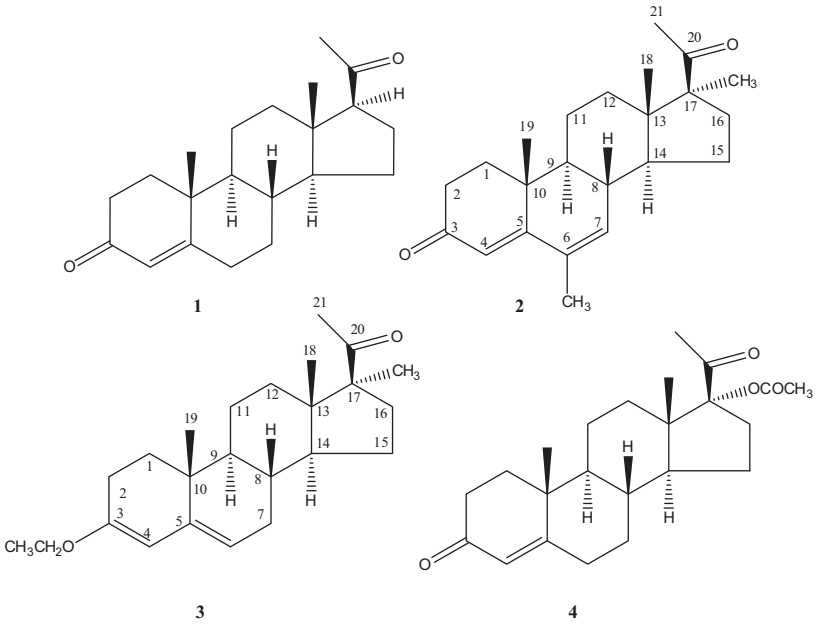


Fig. 1. Structures of progesterone **1** and related compounds **2**–**4**.

assignments of NMR spectra signals of the other intermediates involved in the synthetic route leading to **2**. In particular, the solid state structure of the drug medrogestone allowed the complete stereochemical assignment of the stereocenters, together with the deeper description of its geometrical features with the aim to relate them to its precursor **3** and to natural progestin progesterone **1**.

Commercially available **3** is usually obtained by substitution of a 17α-acetoxy group, for example on 17α-acetoxy progesterone **4**, with a methyl group, as described in Scheme 1 [12]. Starting from **3** we introduced the 6-methyl group and the 6,7-double bond in order to obtain **2** and to isolate the synthetic intermediates.

Since the main chemical physical properties of synthetic intermediates **3**, **8**, **9** and final medrogestone **2** are not reported in the literature, in this paper we wish to report their description, in order to provide a complete piece of information mainly useful from a preparative point of view and for relate them to the pharmacological properties endowed by this class of compounds.

2. Experimental

2.1. General

All reagents and solvents were purchased from Sigma–Aldrich. 17α-methyl-3-ethoxy-pregna-3,5-dien-20-one (**3**) was purchased from Xi'an Reyphon Pharmaceutical CO Ltd, China.

TLC analyses were performed on silica gel 60 F_{254} precoated plates with a fluorescent indicator (Merck) with detection by a 5% phosphomolybdic acid solution in ethanol and heating at 110 °C.

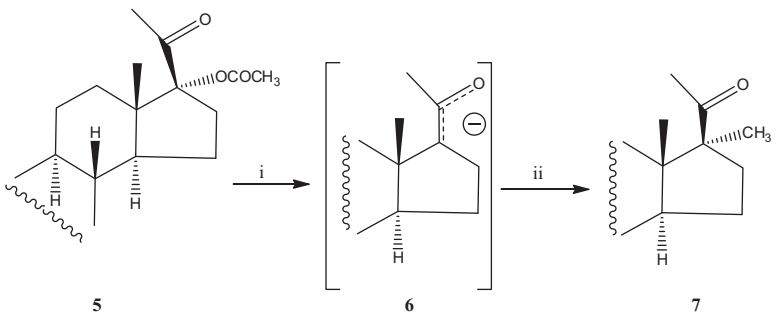
The DSC (Differential Scanning Calorimetry) were registered on a Perkin Elmer instrument (Mod. DSC7) at a heating rate of 30 °C/min from 50.0 °C to 280.0 °C.

Infrared spectra were recorded on a Perkin Elmer instrument (Mod. Spectrum One FT-IR) equipped with ATR sampling device.

Mass spectra were recorded on an Agilent 6330 Ion Trap instruments (ESI positive) using the direct inlet probe technique; the samples of compounds **2**, **8** and **9** were dissolved in a 0.1 M formic acid solution in methanol/water mixture (1:1) at a final concentration of 0.002 mg/ml and injected at an infusion rate of 0.5 ml/h. The sample of compound **3** was dissolved in methanol at a final concentration of 0.002 mg/ml and injected at an infusion rate of 0.5 ml/h.

HPLC system consisted of an Agilent 1100-series liquid chromatography, equipped with auto injector, DAD detector and a Chemostation software installed on a PC, for data collecting and processing. A Symmetry C 18 (Waters) column (250 × 4.6 mm, 5 μm) was employed. Column temperature: 25 °C. Mobile phase: A (acetonitrile/water 6:4); B (acetonitrile/water 95:5). Elution gradient from 100% A (0–33 min) to 100% B (34–45). Detection UV: wavelength 245 nm. Flow rate: 1.0 ml/min.

Optical rotation values were registered on a Perkin Elmer instrument (Mod 241) at 589 nm and 25 °C.



Scheme 1. (i) Li, NH_3; (ii) CH_3I, THF.

Table 1
Summary of crystal data and refinement of **3** and **2**.

Identification	3	2
Empirical formula	C_{24} H_{36} O_2	C_{23} H_{32} O_2
Formula weight	356.53	340.49
Temperature (K)	294(2)	294(2)
λ Mo Kα (Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	P 2_1	P 2_1
Unit cell dim. (Å, °)	a = 16.401(3)	a = 10.386(2)
	b = 7.500(1)	b = 6.527(1)
	c = 18.185(4)	c = 14.507(3)
Crystal size (mm)	0.3 × 0.20 × 0.15	0.25 × 0.20 × 0.15
Volume (Å^3)	2106(1)	979(3)
Z	4	2
Density (calc. Mg/m^3)	1.125	1.155
Refinement method	Full matrix least square on F^2	
F(000)	784	372
θ range data coll. (°)	1.19–27.18	1.41–32.35
Index ranges	$-21 \leqslant h \leqslant 21$	$-15 \leqslant h \leqslant 14$
	$-9 \leqslant k \leqslant 9$	$-9 \leqslant k \leqslant 9$
	$-23 \leqslant l \leqslant 23$	$-21 \leqslant l \leqslant 21$
Refl. coll. (Indep.)	5492/9328	3897/6320
Data/restraints/param.	9379/1/469	6320/1/226
Goodness-of-fit on F^2	1.023	0.906
Final R ind. [$I > 2\sigma(I)$]	$R1$ = 0.059	$R1$ = 0.048
	$wR2$ = 0.136	$wR2$ = 0.1312
Largest diff. peak and hole (e/Å^3)	0.197; −0.250	0.21; −0.168

2.2. Chemistry

2.2.1. 3-Ethoxy-17α-methyl-3,5-pregnadien-20-one (**3**)

Chemical–physical properties: R_f 0.67 (hexane/ethyl acetate 1:1). DSC endothermic peak at 130.00 °C. IR ν (cm^{-1}) 2972–2813, 1700, 1650, 1624, 1172. MS (*m*/*z*) 357.4 [M+1]$^+$, 379.4 [M+Na]$^+$. $[\alpha]_D^{25}$ −117.2 (c 1, CH_2Cl_2).

2.2.2. 3-Ethoxy-6-formyl-17α-methyl-3,5-pregnadien-20-one (**9**)

Title compound **9** was prepared from **3** as reported in Ref. [13]. 4.14 g, 77% (lit [13] 87.5%). R_f 0.53 (hexane/ethyl acetate 1:1). DSC endothermic peak at 83.6 °C (crystallization from methanol/trimethylamine 99:1). MS (*m*/*z*) 385.4 [M+1], 407.4 [M+Na]$^+$, 423.3 [M+K]$^+$. IR ν (cm^{-1}) 2976–2751, 1701, 1648, 1606, 1578, 1159. $[\alpha]_D^{25}$ -142.9 (c 1, CH_2Cl_2).

2.2.3. 17α-Methyl-6-methylene-4-pregnen-3,20-dione (**8**)

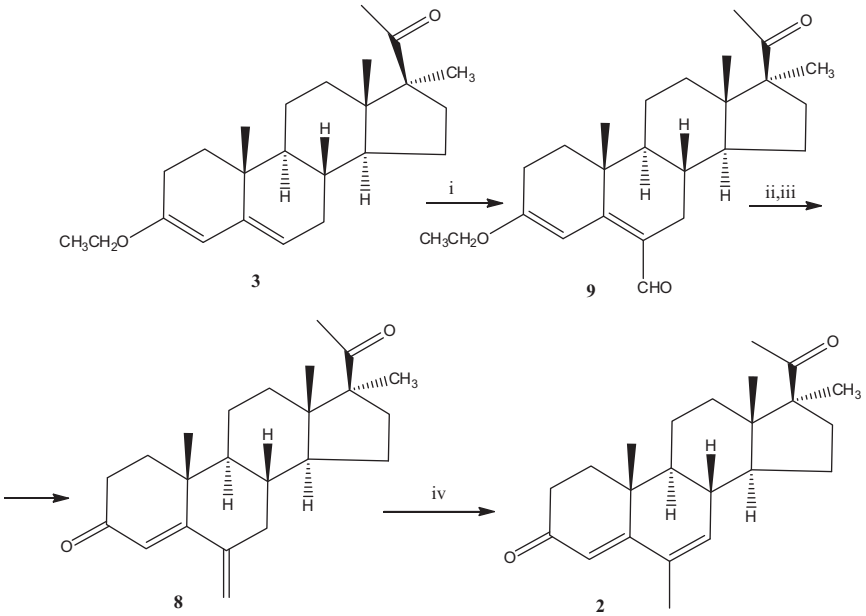
6-Methylene **8** was prepared from **9** according to Ref. [13]. 3.3 g, 94 % (lit [13] 62.7%). R_f 0.44 (toluene/ethyl acetate/triethylamine 70:30:1). DSC endothermic peak at 181.61 °C (crystallization from methanol). IR ν (cm^{-1}) 2944–2834, 1840, 1695, 1668, 1658, 918. MS (*m*/*z*) 341.4 [M+1]$^+$, 363.4 [M+Na]$^+$, 379.3 [M+K]$^+$. $[\alpha]_D^{25}$ + 264.3 (c 1, CH_2Cl_2).

2.2.4. 6,17α-Methyl-4,6-pregnadien-3,20-dione (medrogestone) (**2**)

Pure **2** was prepared from **8** in 87% yield (2.6 g) as reported [13] (lit [13] 70.8%). R_f 0.42 (toluene/ethyl acetate/triethylamine 70:30:1). DSC endothermic peak at 154.8 °C (crystallization from 2-propanol). IR ν (cm^{-1}) 2970–2858, 1691, 1663, 1631, 1593. MS (*m*/*z*) 341.4 [M+1]$^+$, 363.4 [M+Na]$^+$, 379.3 [M+K]$^+$. $[\alpha]_D^{25}$ + 79.1 (c 1, $CHCl_3$, lit [14]+79). El. Analysis ($C_{23}H_{32}O_2$): Calc. C 81.13, H 9.47, O 9.40. Found C 81.17, H 9.46, O 9.49.

2.3. NMR spectroscopy

NMR spectra were recorded on a Bruker AVANCE 500 spectrometer equipped with a 5 mm broadband inverse NMR probe with field *z*-gradient operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C, respectively. NMR spectra were recorded at 298 K for compounds **2**, **8**, **9** in $CDCl_3$ (isotopic enrichment 99.95%), for compound **3** in pyridine-d_5 (isotopic enrichment 99.95%) solution and the chemical shifts were reported on a δ (ppm) scale. The central peak of $CDCl_3$ signals (7.24 ppm for ^{1}H and 77.7 ppm for ^{13}C) and of pyridine-d_5 signals (7.22 ppm, higher field signal, for ^{1}H and 123.9 ppm, higher field signal, for ^{13}C spectra respectively) were used as an internal reference standard. The data were collected and processed by XWIN-NMR software (Bruker) running on a PC with Microsoft Windows 7. The samples (10 mg), were dissolved in appropriate solvent (0.7 ml) in a 5 mm NMR tube. Acquisition parameters for 1D were as follows: ^{1}H spectral width of 5000 Hz and 32 K data points providing a digital resolution of ca. 0.305 Hz per point, relaxation delay 2 s; ^{13}C spectral width of 29,412 Hz and 64 K data points providing a digital resolution of



Scheme 2. (i) $POCL_3$, DMF; (ii) $NaBH_4$, ethanol; (iii) $H2SO_4$; (iv) Pd/C, ethanol.

Table 2
^{1}H NMR chemical shifts (ppm)[a] and coupling constants (Hz)[b] of compounds **2**, **3**, **8**, and **9.**

^{1}H	**2** ($CDCl_3$)	**3** (Py-d_5)	**8** ($CDCl_3$)	**9** ($CDCl_3$)
1α (ax)	1.73 (ddd) −13.7 (1β), 13.7 (2β), 5.4 (2α)	1.33 (ddd) −13.2 (1β), 13.2 (2β), 5.4 (2α)	1.77 (ddd) −13.3(1β), 13.3 (2β), 5.4 (2α)	1.46 −13.2 (1β), 13.2 (2β), 5.4 (2α)
1β (eq)	2.03 (ddd) −13.7 (1α), 5.4 (2β), 2.2 (2α)	1.79 nd	2.08 (ddd) −13.3 (1α), 5.2 (2β), 2.6 (2α)	1.99 (ddd) −13.2 (1α), 5.7 (2β), 1.6 (2α)
2α (eq)	2.45 (ddd) −17.9 (2β), 5.2 (1α), 2.2 (1β)	2.25 (ddd) −17.8 (2β), 5.4 (1α), 1.8 (1β)	2.40 (ddd) −17.3 (2β), 5.4 (1α), 2.6 (1β)	2.25 (ddd) −18.1 (2β), 5.4 (1α), 1.6 (1β)
2β (ax)	2.59 (ddd) −17.9 (2α), 13.7 (1α), 5.4 (1β)	2.41 (ddd) −17.8 (2α), 13.2 (1α), 5.4 (1β)	2.47 nd	2.41(ddd) −18.1 (2α), 13.2 (1α), 5.7 (1β)
4	5.87 (bs)	5.33 (s)	5.93 (s)	6.31 (s)
5	–	–	–	–
6	–	5.36 (bs)	–	–
6-CH_3	1.85 (s)	–	–	–
7α (ax)	5.98 (bs)	1.68 nd	1.90 nd	1.73 nd
7β (eq)		2.15 nd	2.48 nd	2.56 (dd) −17.9 (7α) 5.5 (8β)
8β (ax)	2.22 (dd) 10.6 (9α), 12.4 (14α)	1.66 nd	1.70 nd	1.63 nd
9α (ax)	1.22 (ddd) 13.4 (11β), 10.6 (8β), 3.4 (11α)	0.98 nd	1.14 nd	1.03 (ddd) 11.6 (11β), 11.6 (8β), 4.6 (11α)
11α (eq)	1.65 (dddd)* −13.2 (11β), 4.2 (12α), 2.4 (12β), 3.4 (9α)	1.61 nd	1.71 nd	1.68 nd
11β (ax)	1.44 (dddd) 13.4 (9α), −13.2 (11α), 12.5 (12α), 3.6 (12β)	1.39 nd	1.47 (dddd) 13.0 (9α), −13.4 (11α), 12.5 (12α), 4.0 (12β)	1.49 nd
12α (ax)	1.62 (ddd)* −12.5 (12β), 12.5 (11β), 4.2 (11α)	1.49 nd	1.66 nd	1.63 nd
12β (eq)	1.91 (ddd)* −12.5 (12α), 3.6 (11β), 2.4 (11α)	1.80 nd	1.91 nd	1.90 nd
14α (ax)	1.66 (ddd)* 12.4 (8β), 11.4 (15β), 7.6 (15α)	1.54 nd	1.53 (ddd) 12.4 (8β), 11.2 (15β), 7.6 (15α)	1.53 nd
15α (eq)	1.39 (dddd)* −12.1, (15β), 9.5 (16α), 7.6 (14α), 2.9 (16β)	1.15 nd	1.29 nd	1.28 nd
15β (ax)	1.89 (dddd)* −12.1, (15α), 11.4 (14α), 11.3 (16β), 6.4 (16α)	1.64 nd	1.76 (dddd) −12.1, (15α), 11.2 (14α), 11.9 (16β), 5.9 (16α)	1.87 nd
16α (ax)	1.33 (ddd)* −14.4 (16β), 9.5 (15α), 6.4 (15β)	1.24 nd	1.30 nd	1.33 nd
16β (eq)	2.70 (ddd) −14.4 (16α), 11.3 (15β), 2.9 (15α)	2.83 (ddd) −13.9 (16α), 11.3 (15β), 3.0 (15α)	2.66 (ddd) −13.7 (16α), 11.9 (15β), 3.1 (15α)	2.65 (ddd) −13.7 (16α), 11.5 (15β), 3.1 (15α)
17	–	–		
18-CH_3	0.76 (s)	0.73 (s)	0.72 (s)	0.72 (s)
19-CH_3	1.10 (s)	1.10 (s)	1.11 (s)	1.10 (s)
20	–	–	–	–
21-CH_3	2.15 (s)	2.14 (s)	2.14 (s)	2.14 (s)
17α-CH_3	1.15 (s)	1.03 (s)	1.16 (s)	1.15 (s)
CH_2CH_3	–	3.76 (q) 7.0 (CH_3)	–	3.92 (q) 7.0 (CH_3)
CH_2CH_3	–	1.25 (t) 7.0 (CH_2)	–	1.38 (t) 7.0 (CH_2)
C=CHH	–	–	5.07 (dd) −2.1 (CH), 2.1 (7)	–
C=CHH	–	–	4.95 (dd) −2.1 (CH), 2.1 (7)	–
CHO	–	–	–	10.26 (s)

nd = J(H,H) were not determined due to the overlapping; ax = axial; eq = equatorial; m = multiplet.
[a] Assignments from ^{1}H–^{1}H COSY, HSQC, HMBC and HOHAHA data.
[b] Coupling constants were obtained by direct inspection of the spectra, except those marked with an asterisk, which were obtained by HOH experiments. Experimental error in the measured ^{1}H–^{1}H coupling constants was ±0.5 Hz.

ca. 0.898 Hz per point, relaxation delay 2.5 s. The experimental error in the measured ^{1}H–^{1}H coupling constants was ±0.5 Hz.

For two-dimensional experiments, standard Bruker microprograms using gradient selection (gs) were applied. Gs-COSY-45 and phase sensitive gs-NOESY experiments were acquired with 512 t_1 increments; 2048 t_2 points; spectral width 10.0 ppm. The gs-NOESY experiments were performed with a mixing time of 0.800 s on samples degassed under a flush of argon in a screw-cap sample tube. There were not significant differences in the results obtained at different mixing times (0.5–2.0 s). The acquisition data for gs-HSQC and gs-HMBC experiments were acquired with 512 t_1 increments; 2048 t_2 points; spectral width 10.0 ppm for ^{1}H, 180 ppm for ^{13}C, gs-HSQC and 160–260 ppm for gs-HMBC experiments). Delay values were optimized for $^1J_{C,H}$ 200.0 Hz or 140 Hz, $^nJ_{C,H}$ 3.0 Hz. Zero filling in F_1 to 1 K, $\pi/2$ shifted sine-bell squared (for gs-HSQC) or sinebell (for gs-HMBC) apodization functions were used for processing.

For overlapped signals of hydrogen atoms the 1D HOHAHA technique [15] was used to obtain the chemical shifts and coupling constants.

Table 3
^{13}C NMR data of compounds **2**, **3**, **8**, and **9**.

^{13}C	**2** ($CDCl_3$)	**3** (Py-d_5)	**8** ($CDCl_3$)	**9** ($CDCl_3$)
1	34.1	35.7	35.2	32.9
2	33.6	26.3	33.8	25.3
3	200.0	154.9	200.0	161.8
4	121.1	100.2	121.7	92.3
5	164.3	141.5	168.9	159.4
6	131.2	118.5	145.9	125.5
6-CH_3	19.9	–	–	–
7	138.5	32.7	40.0	29.4
8	37.3	32.5	35.8	30.9
9	50.6	48.5	52.2	47.2
10	36.1	35.7	39.0	28.0
11	20.4	21.6	20.8	20.9
12	32.9	34.4	32.7	32.7
13	45.2	45.0	44.4	44.4
14	48.4	52.2	50.9	51.6
15	23.3	24.5	23.7	23.8
16	31.2	31.9	31.2	31.3
17	61.3	62.1	61.4	61.5
18-CH_3	15.8	16.3	15.8	15.9
19-CH_3	16.4	18.9	17.1	18.9
20	212.1	211.7	212.1	212.5
21-CH_3	27.9	28.2	27.9	27.9
17α-CH_3	21.5	22.0	21.5	21.6
CH_2CH_3	–	62.7	–	63.0
CH_2CH_3	–	15.2	–	14.5
C=CHH	–	–	114.2	–
CHO	–	–	–	190.4

2.4. X-ray crystallography

Crystals of **2** and **3** were obtained as colorless prisms from water/ethanol 1:1 and water/methanol 1:1 solutions at room temperature, respectively. Intensity data were collected at room temperature on a Bruker Apex II CCD diffractometer, using graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å). Intensity data were corrected for Lorentz-polarization effects and for absorption (*SADABS* [16]). The structures were solved by direct methods (*SIR97* [17]) and completed by iterative cycles of full-matrix least squares refinement on F_o^2 and ΔF synthesis using the *SHELXL-97* [18] program (*WinGX* suite) [19]. The positions of hydrogen atoms were introduced at calculated positions, in their described geometries and allowed to ride on the attached carbon atom with fixed isotropic thermal parameters (1.2Ueq of the parent carbon atom). A summary of crystal data and refinement is reported in Table 1.

3. Results and discussion

3.1. Chemistry

Six stereogenic centers are present in the molecule of medrogestone **2**, namely the C-8, C-9, C-10, C-13, C-14 and C-17, with configuration reported in Fig. 1. The stereochemistry of the first five centers is determined considering the starting material of the synthesis, the 17α-acetoxy progesterone **4**, that is submitted to a 6 and a 17α-methylation. The 6-methyl group is usually introduced on a 3-ethylenol ether of a 3,5-diene derivative by means of a Vilsmeier reaction (DMF, $POCl_3$) [20] that leads to a 6-formyl derivative. Reduction of carbonyl function, followed by dehydration by acidic treatment, affords the 6-methylene intermediate that is isomerized to the desired 6-methyl-4,6-diene. An alternative method [21] provides the reaction of a 5α,6α-epoxide with bromomethyl magnesium in order to obtain the 5α-hydroxy, 6β-methyl derivative that by treatment with sulfuric acid affords the 3-keto-Δ^4-derivative; dehydrogenation by means of chloranil furnishes the 6,7-double bond. According to a 1965 United States patent [22], the suitable intermediate is, in this case, a 5α,6α-diol. Oxidation of 6-hydroxy group affords the carbonyl function that, by treatment with methyl magnesium bromide and dehydration is transformed into the final 6-methyl-4,6-diene.

The 17α-methyl group is introduced by the known reductive alkylation [12] of the suitable acetoxy derivative **5**. Lithium in liquid ammonia causes the formation of enolate **6** that by treatment with methyl iodide furnishes the desired 17α-methyl derivative **7**. In the course of this alkylation, the sixth stereocenter (C-17) is introduced, the methyl group attack occurring from the α-side of the molecule, that is the same side where the leaving group is bonded (Scheme 1).

We decided to prepare medrogestone **2**, necessary for crystallographic and spectroscopic studies, starting from the commercially available 17α-methyl-3-ethoxy-pregna-3,5-dien-20-one **3**. According to the above cited 1964 method [20] and following the recent revisitation and application of this synthetic route to the 17α-methyl derivative, proposed in a 2006 Solvay patent [13], the 6-methylene intermediate **8** was prepared by introduction of a 6-formyl group (compound **9**) followed by reduction to the corresponding alcohol. Desired medrogestone **2** was obtained after dehydration and isomerization of obtained 6-methylene **8** (Scheme 2). The isomerization of 6-exo double bond to the 6,7 one can be realized by acidic treatment [23], or according to a

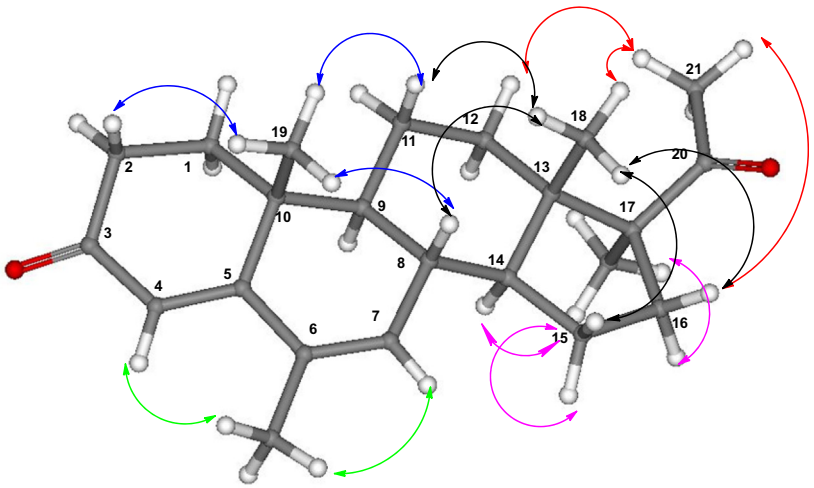


Fig. 2. Key NOE correlations for compound **2**.

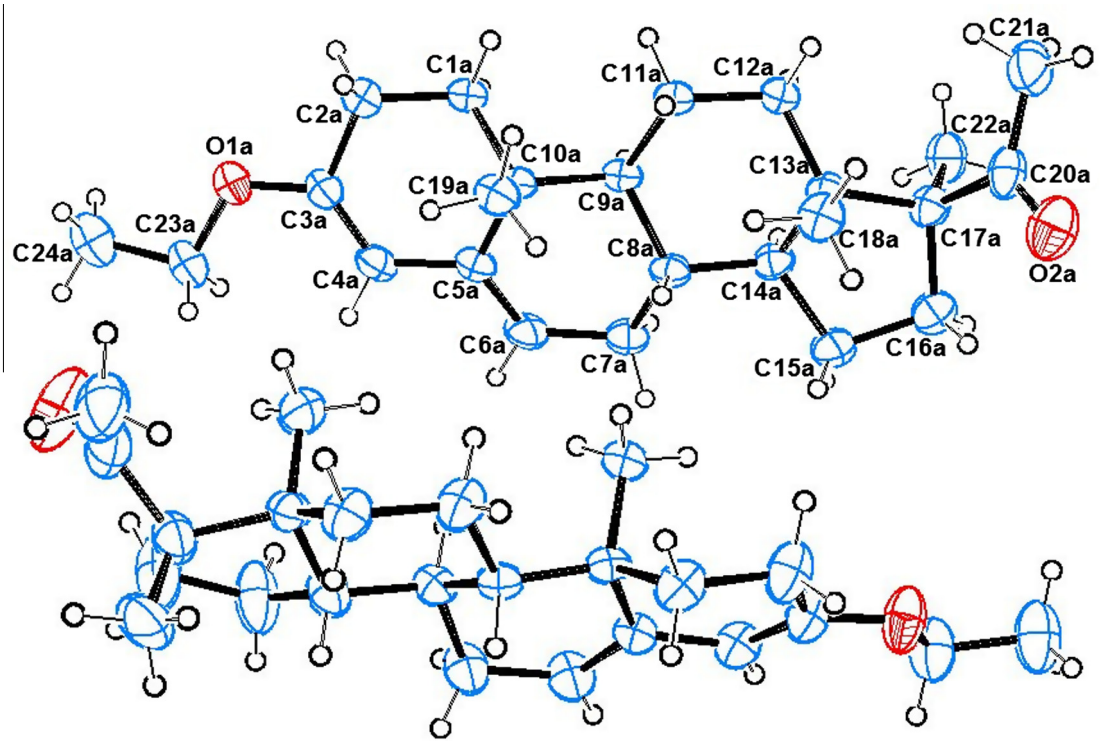


Fig. 3. ORTEP [25] drawing of **3**. For sake of clarity, only molecule "a" of the two independent molecules present in the asymmetric unit shows the labeling scheme which is followed also by molecule "b". Thermal ellipsoids are at 40% probability.

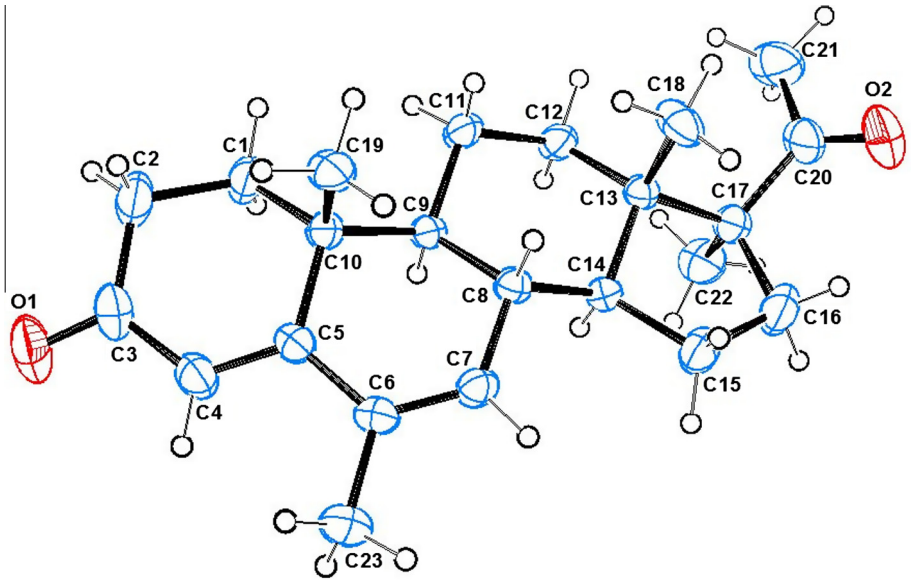


Fig. 4. ORTEP [25] of **2** showing the atom labeling scheme. Thermal ellipsoids are at 40% probability.

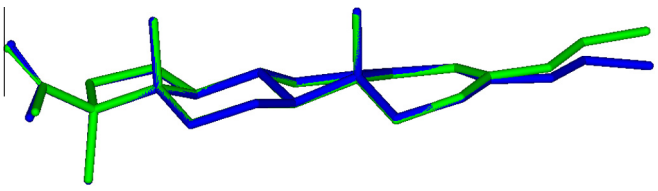


Fig. 5. Superimposition of the two independent molecules of **3** obtained through the best rms fit of the tetracyclic atoms: the "a" labeled ones is reported in green.

1965 method by reaction with palladium on charcoal, sodium acetate and cyclohexene in ethanol [24]. According to the recent modification of this procedure [13], final medrogestone **2** was obtained in 87% yields from **8** and 63% overall yields from **3**.

3.2. NMR spectroscopy

The NMR study was carried out either on medrogestone **2**, on its commercially available precursor **3** and on **8** and **9** intermediates (Scheme 2). Compounds **2**, **8**, and **9** were dissolved in $CDCl_3$, while

Table 4
Summary of the puckering [26] parameters.

Ring	**3** b			**2**		
	QT (Å)	ϑ (°)	ϕ (°)	QT (Å)	ϑ (°)	ϕ (°)
A:C1/C2/C3/C4/C5/C10	0.447(3)[0.452(3)]	52.41(4)[49.4(3)]	−150.0(5)[−147.3(4)]	0.436(2)	50.4(2)	−105.7(3)
B:C5/C6/C7/C8/C9/C10/	0.489(3)[0.486(2)]	130.6(4)[131.4(4)]	−34.6(5)[−35(5)]	0.485(2)	130.6(2)	−84.3(2)
C:C8/C9/C11/C12/C13/C14	0.555(3)[0.554(2)]	7.83(3)[8.9(2)]	176.5(2)[−116.7(1)]	0.588(2)	5.1(1)	−55(2)
D:C13/C14/C15/C16/C17	/	/	−154.8(6)[158.3(2)]	/	/	−153.1(3)

Table 5
Selected torsion angles of the tetracycle.

Torsion angle (°)	Compound		
	3 a	**3** b	2
C10–C1–C2–C3	−44	−45	−54
C5–C6–C7–C8	14	14	1
C9–C11–C12–C13	−55	−54	85
C14–C13–C17–C20	159	159	159

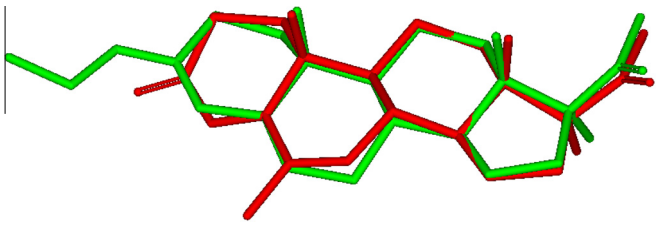


Fig. 6. Overlay between **2** (red) and **3** ("a" molecule in green) obtained through the best rms fit of C1 and C16 atoms.

compound **3** was dissolved in d_5-pyridine in order to avoid the fast degradation of the enol ether moiety, observed in chloroform. Unambiguous assignments of protons and carbons of compounds (Tables 2 and 3) were recognized by 1D NMR spectra, as well as 2D NMR homocorrelation (COSY, TOCSY and NOESY) and heterocorrelation (HSQC and HMBC) spectra were employed for complete structural assignments. For overlapped signals of hydrogen atoms in the final product **2**, the 1D HOHAHA [15] technique was used to obtain the chemical shifts and coupling constants.

Most of the proton assignments were accomplished using general knowledge of chemical shift dispersion with the aid of the proton-proton coupling pattern (^{1}H NMR spectra), the gs-COSY and gs-NOESY experiments. In ambiguous cases, the gs-HSQC and gs-HMBC spectra were used as a final and unequivocal tool to make specific assignment.

In particular, in medrogestone **2** ^{1}H NMR chemical shifts of H-2α and H-2β (2.59 and 2.45 ppm) and H-16β (2.70 ppm) protons can be assigned to the influence of the anisotropy of the neighboring carbonyl group. Starting from the characteristic resonances of this three protons and the two olefinic protons H-4 (5.87 ppm) and H-7 (5.98 ppm) it was possible to assign the resonances of all the other protons.

NOE experiments were performed to confirm the configuration of the 17-CH_3. The following significant enhancements were observed when methyl protons at 1.15 ppm were irradiated (Fig. 2). The NOE correlations of 17-CH_3 (1.15 ppm) with H-15α, H-16α and H-14α indicated that all these protons were on the same face of the ring system. On the other hand, the NOE correlations of CH_3-21 (2.15 ppm) with CH_3-18, H-16β and H-12β showed that these protons were on the opposite face of the ring system demonstrating unequivocally that the 17-CH_3 was in α position.

When the CH_3-18 (0.77 ppm) was irradiated, NOE enhancement was observed on H-8β, H-11β, H-15β, H-16β, and CH_3-21; when the CH_3 in C-6 (1.85 ppm) was irradiated, NOE enhancement was observed on H-4 and H-7. Finally NOE were observed on H-2β, H-8β and on proton at 2.02 ppm, assigned to the H-1β, when the CH_3-19 (1.10 ppm) was irradiated.

The assignments of carbon atoms of CH, CH_2 and CH_3 groups were confirmed by the gs-HSQC experiment. The quaternary carbon atoms were assigned unambiguously using the information obtained from ^{1}H/^{13}C gsHMBC experiment.

^{1}H NMR and ^{13}C NMR spectra of compounds **3**, **8** and **9** showed similar chemical shifts and coupling constants to those of **2** except for C/H-6, C/H-7 and C/H-8, due to different double bond position, putting in evidence that these compounds closely resemble compound **2**. In particular, the same relative configuration of 17-CH_3 of the latter was determined also in precursor **3**, based on its NOE correlations. Indeed, when the signal at 1.03 ppm (17-CH_3) was irradiated NOE was observed on H-15α, H-16α and H-14α showing that these protons were on the same side of the D ring.

3.3. X-ray analysis

We started the X-ray analysis examining the molecular structure of the commercially available precursor **3** (see Scheme 2) in order to assess the maintenance of the proper stereochemistry at C17 during the synthetic route leading to **2**.

Compound **3** crystallized with two independent molecules in the asymmetric unit, that are shown in Fig. 3 as a ORTEP [25] drawing, while the solid state conformation of medrogestone (**2**) is shown in Fig. 4.

The crystallographic data allowed the complete stereochemical assignment of all the stereogenic carbons for both compounds and their absolute configuration was assigned according to the known *S* configurations of C9, C10 and C13, which were unaffected in the reaction pathway. Consequently, it was possible to unambiguously establish for both compounds the stereochemistry *S* at C17.

Their overall molecular structure is characterized by the common tetracyclic skeleton formed by three condensed hexatomic rings fused with a pentacyclic ring, all in *trans* configuration.

The two entities of **3** have a very similar geometry, except for a slightly different orientation of the lateral chain linked to C3 (see Fig. 5), as indicated by the torsion angles τ_1 C16–C17–C20–O2 of 6(1)[−13(1)]°, τ_2 C4–C3–O1–C23 of 5(1)[2(1)]° and of τ_3 C2–C3–O1–C23–174(1)[−179(1)]°. The values in the square brackets refer to the "b" labeled molecules.

The two molecules of the precursor and the final product have a similar rings conformation and the main deviations are related to the ϕ angles values of the six-membered rings, indicating their different puckering (Table 4) [26]. The A and B rings exhibit a "twisted envelope" conformation, but in **3** the ring A is less distorted with respect to **2**. In the precursor, atom C10 of ring A has the higher distance from the mean plane C1/C2/C3/C4/C5, being 0.618(1)[0.580(1)] Å, while in **2**, the out-of-plane atom is C1 by 0.593(1) Å. In ring B, the distances of C8 and C9 calculated from the best mean plane of the remaining ring atoms are 0.324[0.318] and 0.423[0.426] Å in **3** and they are 0.251(1) and 0.787(1) Å for **2**, respectively. The ring

C is in chair conformation, with atoms C9 and C13 out of the mean plane of C8/C14/C12/C11, with distances for C9 of 0.593(2)[0.720 (2) Å] and for C13 of −0.713(3)[−0.585(2)] Å in **3**, while they are 0.705(1) and −0.664(1) Å in **2**. The pentatomic cycle D has a similar distorted envelope conformation in both compounds, with the atom C13 out of mean plane C14/C15/C16/C17 by 0.671(3) [−0.666(3)] Å in **3** and by 0.665(1) Å in **2**. The different rings conformation between the two molecules is also put in evidence in Table 5 where at least one significant torsion angle for every ring is reported. In addition, their structural differences could be best showed in the superimposition reported in Fig. 6.

In conclusion, this solid state investigation allowed the complete stereochemical assignment of the asymmetric carbons together with the deeper description of its geometrical features with the aim to relate them to the pharmacological properties endowed by this class of compounds. Referring to the previous structure–activity-relationship (SAR) [27,28], we can confirm for medrogestone the quite rigidity of the molecular tetracycle, in particular of rings B, C and D. Though the presence of a double bond in ring B, medrogestone maintains a similar value of the distance between the carbonyl oxygens O1···O2 with respect to progesterone, being 11.7(1) Å. As the orientation of the acetyl side chain is able to influence the progestinic activity, we found in the solid state of **2** a value of τ_1 of −9(1)°, that is in agreement with that determined in other compounds [29]. In this respect, NOESY spectra demonstrated that the chain can assume different orientations, leading to a populated class of conformers.

The availability of X-ray and NMR data of medrogestone could be a useful starting point for the study of similar molecules. Since the synthetic intermediates could be utilized also for the preparation of other steroidal compounds, the reported spectroscopic and chemical physical data have a value not limited only to the present work. Besides, this complete characterization is relevant as could help in defining the bioactive conformation of this drug.

Acknowledgments

This work was financially supported by Università degli Studi di Milano and by Euticals SpA.

S.C. acknowledges the financial support (study and research fellowship) provided by Euticals SpA.

Appendix A. Supplementary material

The crystallographic data can be obtained *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336 033; or deposit@ccdc.cam.ac.uk). CCDC-1058837 (**2**) and 1058836 (**3**) numbers contain the supplementary crystallographic data for this paper.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2015.09.007.

References

[1] J.R. Pasqualini, Progestins: present and future, J. Steroid Biochem. Mol. Biol. 59 (1996) 357–363.
[2] A.E. Schindler, C. Campagnoli, R. Druckmann, J. Huber, J.R. Pasqualini, K.W. Schweppe, et al., Classification and pharmacology of progestins, Maturitas 46S (2003) S7–S16.
[3] R. Bursi, M.B. Groen, Application of (quantitative) structure–activity relationships to progestagens: from serendipity to structure-based design, Eur. J. Med. Chem. 35 (2000) 787–796.
[4] C. Revesz, C.I.J. Chappel, Biological activity of medrogestone: a new orally active progestin, J. Reprod. Fert. B 12 (1966) 473–487.
[5] A. Carraro, R. Klinger, M. Motta, Pharmacological activity of a new synthetic progestational agent, Boll. Soc. Ital. Biol. Sperim. 43 (8) (1967) 427–431.
[6] G.S. Chetrite, C. Ebert, F. Wright, J.-C. Philippe, J.R. Pasqualini, Effect of medrogestone on 17β-hydroxysteroid dehydrogenase activity in the hormone-dependent MCF-7 and T-47D human breast cell lines, J. Steroid Biochem. Mol. Biol. 68 (1999) 51–56.
[7] G.S. Chetrite, C. Ebert, F. Wright, A.-C. Philippe, J.R. Pasqualini, Control of sulfatase and sulfotransferase activities by medrogestone in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines, J. Steroid Biochem. Mol. Biol. 70 (1999) 39–45.
[8] J.R. Pasqualini, Progestins in the menopause in healthy women and breast cancer patients, Maturitas 62 (2009) 343–348.
[9] J.R. Pasqualini, Breast cancer and steroid metabolizing enzymes: the role of progestogens, Maturitas 65S (2009) S17–S21.
[10] J.R. Pasqualini, Progestins and breast cancer, Gynecol. Endocrinol. 23 (S1) (2007) 32–41.
[11] R. Deghenghi, C. Revesz, R. Gaudry, New synthesis and structure activity relationship in the17-alkylated progesterone series, J. Med. Chem. 6 (1963) 301–304.
[12] H. Laurent, R. Wiechert, Selective introduction of alkyl and methylene groups into the steroid system, in: J. Fried, J.A. Edwards (Eds.), Organic Reactions in Steroids Chemistry, vol. II, Van Nostrand Reinhold Co., New York, 1972, pp. 97–99.
[13] J.-L. Moutou, B. Rondot, J. Lafay, P. Maillos, Method for preparing medrogestone 2006, WO 077209.
[14] R. Deghenghi, Y. Lefebvre, P. Mitchell, P.F. Morand, R. Gaudry, Synthesis of 17α- methylprogesterone derivatives. New class of orally active gestations, Tetrahedron 19 (1963) 289–298.
[15] D.G.D. Davis, A. Bax, Simplification of ^{1}H NMR spectra by selective excitation of experimental subspectra, J. Am. Chem. Soc. 107 (1985) 7197–7198.
[16] G.M. Sheldrick, SADABS Area-Detector Absorption Correction Program, Bruker AXS Inc., Madison, WI, USA, 2000.
[17] A. Altomare, M.C. Burla, M. Camalli, G.L. Cascarano, C. Giacovazzo, A. Guagliardi, et al., *SIR97*: a new tool for crystal structure determination and refinement, J. Appl. Crystall. 32 (1999) 115–119.
[18] G.M. Sheldrick, A short history of *SHELX*, Acta Crystall. A64 (2008) 112–122.
[19] L.J. Farrugia, *WinGX* suite for small-molecule single-crystal crystallography, J. Appl. Crystall. 32 (1999) 837–838.
[20] D. Burn, G. Cooley, M.T. Davies, J.W. Ducker, B. Ellis, P. Feather, et al., Modified steroid hormones-XXXIII. Steroidal 6-formyl-3-alkoxy-3,5-dienes and some of their transformations, Tetrahedron 20 (1964) 597–609.
[21] R. Deghenghi, 17α-methyl steroids, 1963, BE 622366, CA 61, 40618.
[22] P.F. Morand, R. Deghenghi, Process for preparation of 6,17α-dimethyl-4,6-pregnadiene-3,20-dione, 1965, US 3170936.
[23] J.R. Parikh, M.E. Breuer, Method for the preparation of 6-methyl-3-oxo-Δ^4,6-steroids, 1972, US 3705181.
[24] D. Burn, D.N. Kirk, V. Petrow, Modified Steroid Hormones. XXXVIII. Some transformations of steroidal 3-alkoxy-6-formyl-3,5-dienes and related compounds, Tetrahedron 21 (1965) 1619–1624.
[25] M.N. Burnett, C.K. Johnson, ORTEP-III: Oak Ridge Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, Oak Ridge National Laboratory Report, ORNL, 1996, 6895.
[26] D. Cremer, J.A. Pople, General definition of ring puckering coordinates, J. Am. Chem. Soc. 97 (6) (1975) 1354–1358.
[27] D. Colombo, P. Ferraboschi, P. Prestileo, L. Toma, A comparative molecular modelling study of dydrogesterone with other progestational agents through theoretical calculations and nuclear magnetic resonance spectroscopy, J. Steroid Biochem. Mol. Biol. 98 (2006) 56–62.
[28] D. Colombo, P. Ferraboschi, L. Legnani, P. Prestileo, L. Toma, A full conformational characterization of 13-ethylprogestogens trough theoretical calculations and nuclear magnetic resonance, J. Steroid Biochem. Mol. Biol. 103 (2007) 163–169.
[29] S. Profeta Jr., P.A. Kollman, M.E. Wolff, Conformation of the progestinic side chain: resolution of the apparent conflict between X-ray data and force-field calculations using MM2, J. Am. Chem. Soc. 104 (1982) 3745–3747.