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Short communication

Characterization of related impurities in megestrol acetate

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Abstract

Three new compounds, 17α-acetoxy-2,6-dimethylpregna-1,4,6-triene-3,20-dione (**1**), 17α-acetoxy-2α,6-dimethylpregna-4,6-diene-3,20-dione (**2**), 17α-acetoxy-6α-methoxylmethylpregna-4-ene-3,20-dione (**3**), together with five known ones, 17α-acetoxy-6β-hydroxyl-6α-methylpregna-4-ene-3,20-dione (**4**), 17α-acetoxy-6α-hydroxyl-6β-methylpregna-4-ene-3,20-dione (**5**), 17α-acetoxy-pregna-4-ene-3,6,20-trione (**6**), 17α-acetoxy-pregna-4-ene-3,20-dione (**7**) and 17α-acetoxy-6-methylene-pregna-4-ene-3,20-dione (**8**), were isolated and identified from the residual mother liquor of megestrol acetate. Their structures were established by spectroscopic methods. These compounds seem to be minor impurities in production of the drug megestrol acetate.

Keywords: Megestrol acetate; Related impurities; Structural elucidation

1. Introduction

Megestrol acetate (MA), 17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione, is a semi-synthetic progestogenic hormone. It is prescribed primarily for the treatment of breast cancer and sometimes also used to treat endometrial cancer and prostate cancer [1]. Moreover, other application is described as an appetite stimulant for people experiencing loss of appetite and weight loss because of advanced cancer [2].

Five possible impurities in megestrol acetate are listed in the European Pharmacopoeia [3] (Fig. 1): medroxyprogesterone acetate (**A**), megestrol (**B**), D-homo megestrol acetate (**C**), 6-methylene hydroxyprogesterone acetate (**D**) and 6-methyl-3,20-dioxopregna-1,4,6-trien-17-yl acetate (**E**). As it is known, the impurity profile of drugs depends on the synthetic route. In our studies on impurities of MA from Shenzhou Pharm. Inc., three new compounds, 17α-acetoxy-2,6-dimethylpregna-1,4,6-triene-3,20-dione (**1**), 17α-acetoxy-2α,6-dimethylpregna-4,6-diene-3,20-dione (**2**), 17α-acetoxy-6α-methoxylmethylpregna-4-ene-3,20-dione (**3**), together with five known ones **4**–**8** were isolated and identified from the residual mother liquor of MA. Their structures were elucidated by spectroscopic methods. Here we report the isolation, structure elucidation and spectral data of impurities **1**–**8**.

2. Experimental

2.1. Chemicals and reagents

Megestrol acetate samples and its mother liquor were supplied by Shenzhou Pharm. Inc., Xianju, Zhejiang Province, China. HPLC grade acetonitrile (Merck, Germany) was used for the HPLC tests. Analytical-grade petroleum ether, ethyl acetate, cyclohexane, acetone, chloroform and methanol purchased from Shanghai Reagent Co. were used as solvents for column chromatography. Water was obtained from a Milli-Q purification system.

2.2. High-performance liquid chromatography

An Agilent 1100 series HPLC system equipped with G1311A quaternary gradient pump, G1314A variable-wavelength detector, model 7725 injector fitted with 20 μl sample loop and Agilent Chemstation were used for the analysis. The analysis was carried out on Zorbax C18 100 Å, 5 μm,

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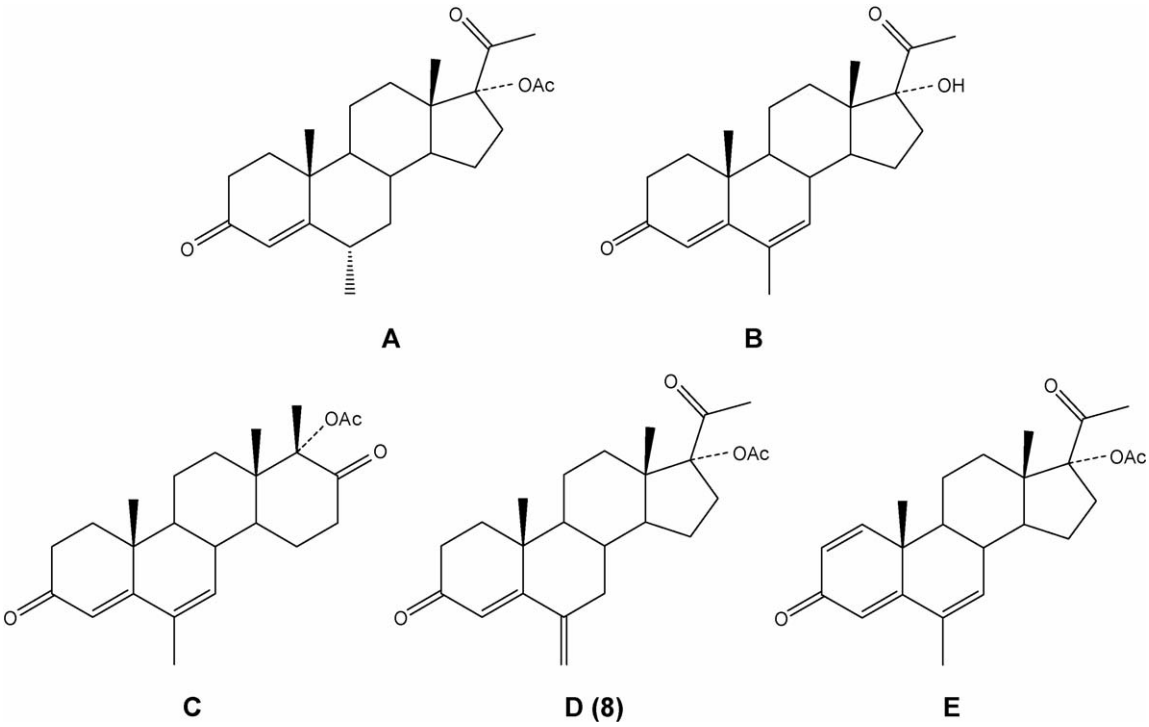

Fig. 1. List of impurities in European Pharmacopoeia.

250 mm × 4.6 mm column using a mobile phase consisting of tetrahydrofuran–acetonitrile–water (14.5:25.5:60.0, v/v/v) with UV detection at 254 nm at a flow rate of 1.5 ml/min according to the method described in the European Pharmacopoeia [3].

2.3. Column chromatography

After MA was crystallized, the residual mother liquor was evaporated to yield a powder. One hundred grams of this was subjected to column chromatography (silica gel,

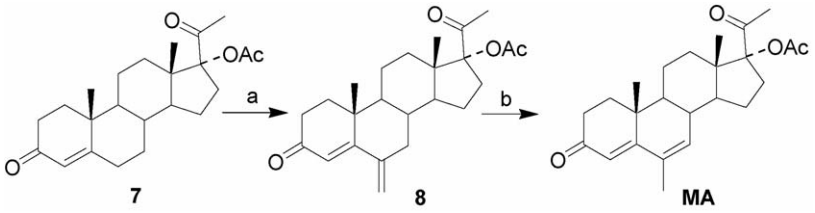

Fig. 2. Synthetic route of megestrol acetate. (a) $CH(OC_2H_5)_3$/THF/$C_6H_5N(CH_3)_2$/HCl and (b) Pd/C cyclohexene/C_2H_5OH.

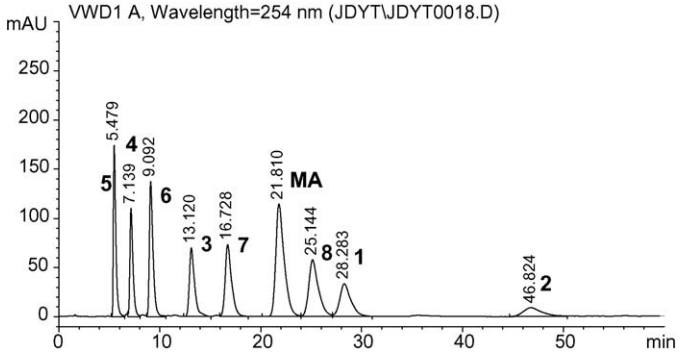

Fig. 3. HPLC chromatogram of a model mixture containing megestrol acetate and impurities **1**–**8**.

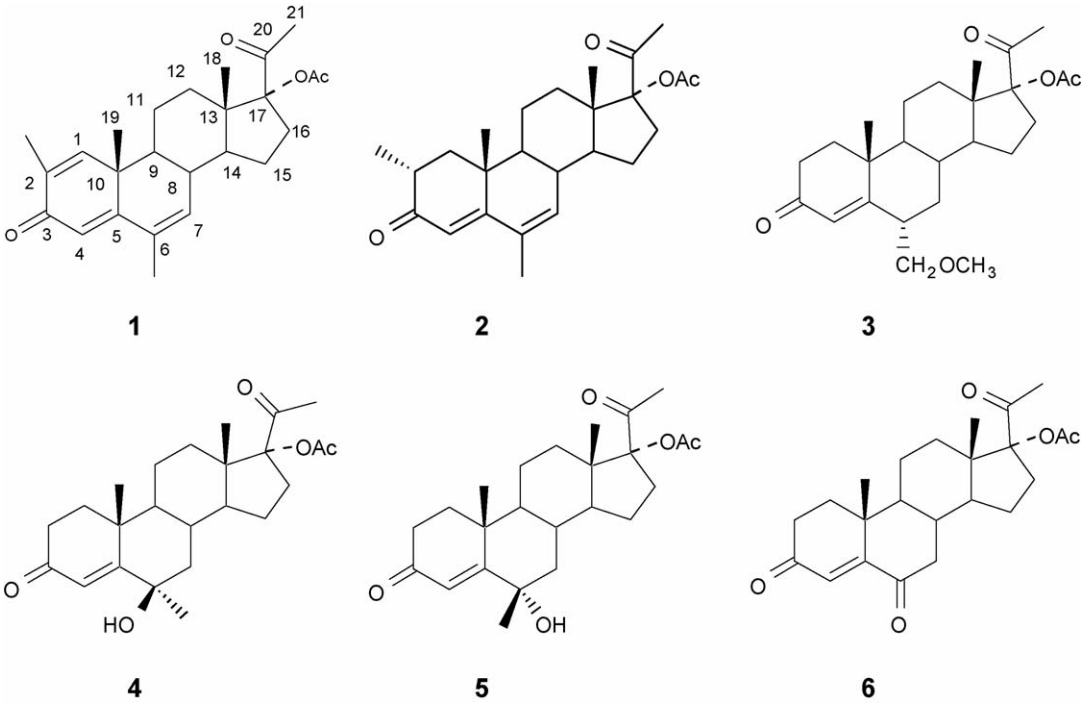

Fig. 4. Structures of impurities **1–6**.

cyclohexane–acetone 9:1 → 1:1) to get seven fractions. Each fraction was repeatedly chromatographed (silica gel, petroleum ether–ethyl acetate 4:1 → 1:1) and then was further purified by Sephadex LH-20 (chloroform–methanol 1:1) to yield impurities **1–8**.

2.4. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were run on a Brucker AM-400 instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C, using $CDCl_3$ as solvent and $SiMe_4$ as internal standard.

2.5. Mass spectrometry

MS experiments were carried out using a Finnigan MAT-95 instrument.

2.6. UV, FTIR and ORD spectroscopies

UV spectra were measured on a Varian CARY 300 Bio Spectrometer using acetonitrile as solvent. IR spectra were recorded using a Perkin-Elmer 577 IR Spectrometer with KBr disc method. Optical rotations were obtained on Perkin-Elmer 341 polarimeter (Na filter, $\lambda = 589$ nm).

3. Synthetic route of megestrol acetate

The reaction scheme used for the synthesis of megestrol acetate is shown in Fig. 2.

4. Results and discussion

4.1. Detection and isolation of impurities *1–8*

The details of the isolation of impurities **1–8** was described in Section 2.3. The relative retention times and HPLC chromatogram are shown in Fig. 3. Structures of these impurities are given in Figs. 4 and 2.

4.2. Structure elucidation of impurities

4.2.1. Structure elucidation of impurities *1–3*

Impurity **1** was obtained as colorless needles from methanol. Its molecular formula $C_{25}H_{32}O_4$ was determined by EI-MS and ^{13}C, DEPT NMR. In the IR spectrum, the absorption bands at 3411 cm^{-1} (OH), 1731 cm^{-1} (ester carbonyl) and 1660 cm^{-1} (C=O) were apparent. The UV data of impurity **1** (Table 1) were in agreement with those of 6-methyl-3,20-dioxopregna-1,4,6-trien-17-yl acetate (*E*) [4], which implies the presence of the

Table 1
Specific rotations, UV and IR data of impurities **1–3**

No.	Specific rotation	UV	IR
1	$[\alpha]_D^{20}$ −33.8° ($CHCl_3$, c 0.45)	225, 261.5, 299 nm	3411, 2949, 2875, 1731, 1660, 1616, 1583, 1392, 1365, 1259, 1016, 960, 613, 588 cm^{-1}
2	$[\alpha]_D^{20}$ +28.2° ($CHCl_3$, c 0.67)	283 nm	3411, 2972, 2871, 1728, 1668, 1627, 1585, 1458, 1371, 1261, 1220, 962, 883, 613 cm^{-1}
3	$[\alpha]_D^{20}$ +49.7° ($CHCl_3$, c 0.38)	238 nm	3435, 2951, 1730, 1670, 1614, 1365, 1261, 1109, 966 cm^{-1}

Table 2
^{13}C NMR data of megestrol acetate and impurities **1–8** (δ in ppm)

C	MA	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	34.3	148.9	43.1	35.6	37.5	38.1	35.4	35.6	35.3
2	33.9	133.9	36.8	33.5	33.7	33.4	33.8	33.9	34.0
3	200.1	187.0	202.0	199.5	200.8	200.3	199.2	199.4	200.0
4	121.5	121.3	120.8	121.8	123.0	122.0	125.7	123.9	121.9
5	164.3	163.1	163.3	169.9	170.7	176.0	160.3	170.7	168.9
6	138.2	131.8	131.2	39.2	71.3	71.5	201.3	30.9	145.8
7	131.7	134.0	137.3	35.7	45.2	46.4	46.4	31.8	40.0
8	37.4	37.6	36.9	35.1	30.7	30.0	34.0	35.5	35.8
9	49.2	48.1	50.2	50.9	50.8	50.6	50.1	51.0	51.5
10	36.4	40.9	36.6	38.8	38.5	38.3	39.5	38.5	39.2
11	20.6	21.6	20.1	20.7	20.6	20.4	20.4	20.6	20.8
12	30.6	31.0	30.9	30.9	30.9	30.6	30.5	32.7	31.1
13	47.8	47.0	47.4	46.7	46.8	46.6	46.7	46.7	46.9
14	50.5	49.1	48.8	53.3	52.7	52.1	51.6	53.0	51.9
15	23.6	23.2	23.1	23.7	23.7	23.7	23.5	23.8	23.9
16	31.4	30.3	30.2	30.2	30.2	30.0	30.2	30.2	30.5
17	96.7	96.2	96.3	96.6	96.7	96.4	96.2	96.7	96.8
18	14.6	14.4	14.2	14.3	14.4	14.2	14.2	14.3	14.6
19	16.6	20.6	16.7	18.1	19.9	20.3	17.5	17.3	17.3
20	204.2	204.0	203.9	204.1	204.1	203.9	203.7	204.1	204.2
21	26.6	26.4	26.3	26.3	26.4	26.1	26.4	26.3	26.6
OAc	170.9	170.6	170.6	170.7	170.1	170.6	170.5	170.7	170.9
	21.4	21.3	21.1	21.2	21.2	21.1	21.2	21.2	21.5
2-Me		16.1	15.4						
6-Me	20.1	19.3	19.8	73.8 (6 –CH_2O) 59.0 (OCH_3)	29.2	32.9			114.7 (6 =CH_2)

similar conjugation. In the ^{13}C NMR spectrum (Table 2), 25 carbon signals were observed, constituted by six methyls, four methylenes, six methynes (including three olefinic carbons), six quaternary carbons (including three olefinic carbons) and three carbonyls. Analysis of ^{13}C NMR data of impurity **1** and comparison with those of MA showed that they possessed the same skeletons except for an additional olefinic bond (δ 148.9; δ 133.9) and a methyl (δ 16.1) attached to an olefinic bond. The above conclusion was supported by signals (δ 6.84, d, J = 1.2 Hz, H-1; δ 1.89, s, 2-Me) in the ^{1}H NMR. The positions of the additional olefinic bond and methyl group were concluded on basis of the HMBC experiments: key correlations were observed between H-1 and C-3, 5, 9, 10, 19, 2-Me; 2-Me and C-1, 3 (Fig. 5). Thus, it was concluded that the structure of impurity **1** was 17α-acetoxy-2,6-dimethylpregna-1,4,6-triene-3,20-dione. The reason for its formation is the addition of another methylene to position 2, followed by re-arrangement in the synthetic reaction.

Impurity **2**, colorless needles (methanol), had a molecular formula of $C_{25}H_{34}O_4$ determined by EI-MS (*m*/*z*, 398), ^{13}C and DEPT NMR data. The λ_{max} in its UV spectrum was at 283, which is the same as that of megestrol acetate. The IR spectrum showed the absorption bands of a hydroxyl group (3411 cm^{-1}), an ester carbonyl group (1728 cm^{-1}) and a carbonyl group (1668 cm^{-1}). The ^{1}H and ^{13}C NMR spectra were very similar to those of impurity **1** except that an olefinic bond in C1=C2 was replaced by a single bond. The position of 2-Me was justified by HMBC experiments (Fig. 5). The ^{1}H (Table 3), ^{13}C NMR (Table 2), HSQC and HMBC data led to the assignment of all H- and C-atoms. To determine the stereochemistry of 2-Me, ROESY experiment was carried out. When the signal

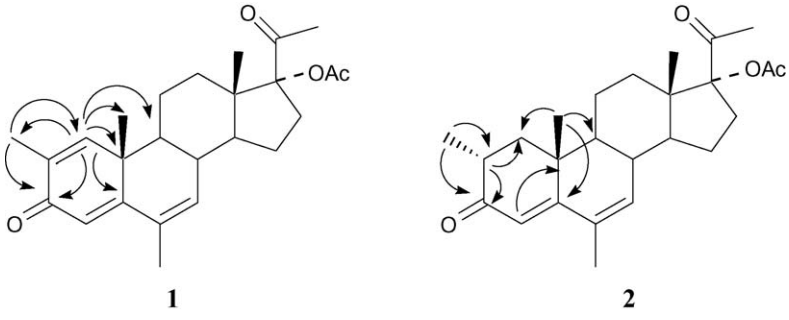

Fig. 5. Key HMBC correlations of impurities **1** and **2**.

Table 3
^{1}H NMR data of megestrol acetate and impurities **1–8** (δ in ppm, J in Hz)

H	MA	1	2	3	4	5	6	7	8
H-1		6.84, d, J = 1.2 Hz							
H-2			2.58, m						
H-4	5.85, s	6.20, s	5.84, s	5.75, d, J = 1.4 Hz	6.02, s	6.38, s	6.18, s	5.73, s	5.87, s
H-7	5.94, s	5.80, s	5.91, s						
H-16β	2.98, m	2.99, m	2.96, m		2.93, m	2.94, m	2.95, m	2.92, m	2.91, m
H-18	0.70, s	0.74, s	0.70, s	0.67, s	0.69, s	0.68, s	0.68, s	0.66, s	0.62, s
H-19	1.07, s	1.13, s	1.10, s	1.19, s	1.38, s	1.25, s	1.16, s	1.17, s	1.05, s
H-21	2.03, s	2.05, s	2.04, s	2.04, s	2.03, s	2.04, s	2.06, s	2.03, s	2.01, s
6-CH_3 or 6-CH_2	1.82, s	1.87, s	1.82, s	3.52, 3.38 each dd, J = 9.1, 5.4 Hz	1.40, s	1.41, s			5.03, 4.92, each br s, 6 = CH_2
2-CH_3		1.89, s	1.13, d, J = 6.7 Hz						
OAc	2.07, s	2.05, s	2.07, s	2.10, s	2.09, s	2.10, s	2.11, s	2.10, s	2.08, s
OMe				3.35, s					

Table 4
MS and melting point data of megestrol acetate and impurities **1–8**

No.	Melting point, mp (°C)	Molecular formula	EI-MS
MA		$C_{24}H_{32}O_4$	384 (M^+), 342, 324, 299, 281 (100%), 266, 223, 187
1	222–223	$C_{25}H_{32}O_4$	397 (M^+ + 1), 337, 322, 294 (100%), 279, 200
2	180–182	$C_{25}H_{34}O_4$	398 (M^+), 358, 338, 323, 295 (100%), 201
3	185–187	$C_{25}H_{36}O_5$	416 (M^+), 374, 356, 331, 313 (100%), 281
4		$C_{24}H_{34}O_5$	402 (M^+), 384, 359, 299, 281 (100%), 241, 187
5		$C_{24}H_{34}O_5$	402 (M^+), 384, 359, 299, 281 (100%), 241, 187
6		$C_{23}H_{30}O_5$	386 (M^+), 343, 326, 301, 283 (100%), 265, 243, 189
7		$C_{23}H_{32}O_4$	372 (M^+), 330, 312, 287, 269 (100%), 229
8		$C_{24}H_{32}O_4$	384 (M^+), 369, 341, 324, 299, 281 (100%), 269, 256, 241

of H-2 was irradiated, the signal of 19-Me showed an obvious NOE enhancement. Based on the above data, the structure of impurity **2** was identified as 17α-acetoxy-2α,6-dimethylpregna-4,6-diene-3,20-dione. The appearance of impurity **2** is likely to be attributed to addition of another methylene to position 2, followed by hydrogenation in the course of the synthesis.

Impurity **3** ($C_{25}H_{36}O_5$) was obtained as colorless needles from methanol. The EI-MS displayed [M^+] at m/z 416 (Table 4). The ^{13}C NMR spectrum revealed 25 signals including four methyls, one methoxyl, eight methylene, five methines, four quaternary carbons and three carbonyls. Comparison with reported data of the known compound, 17α-acetoxy-6α-hydroxylmethylpregna-4-ene-3,20-dione [5], showed that impurity **3** is its methyl ether. The relative stereochemistry at C-6 was determined based on the characteristic $J_{H\text{-}4,\ H\text{-}6}$ long-range coupling constant: for H-6 in α form, the signal in H-4 must be a single peak, while H-6 in β form, H-4 are generally double-peak (J in ∼1.5 Hz). Therefore, structure of impurity **3** was determined to be 17α-acetoxy-6α-methoxylmethylpregna-4-ene-3,20-dione.

4.2.2. *Structure elucidation of impurities **4–8***

Comparison of spectra data with the reported values allowed us to identify other known impurities to be 17α-acetoxy-6β-hydroxyl-6α-methylpregna-4-ene-3,20-dione (**4**) [6], 17α-acetoxy-6α-hydroxyl-6β-methylpregna-4-ene-3,20-dione (**5**) [6], 17α-acetoxy-pregna-4-ene-3,6,20-trione (**6**) [7], 17α-acetoxy-pregna-4-ene-3,20-dione (**7**) [8] and 17α-acetoxy-6-methylene-pregna-4-ene-3,20-dione (**8**) [9].

5. Conclusions

Three new compounds and five known ones were isolated and identified from the residual mother liquor of megestrol acetate. Their structures were elucidated mainly by means of 1D and 2D NMR spectra supported by MS, UV and IR spectral data.

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