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Crystallographic and spectroscopic study on a known orally active progestin

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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

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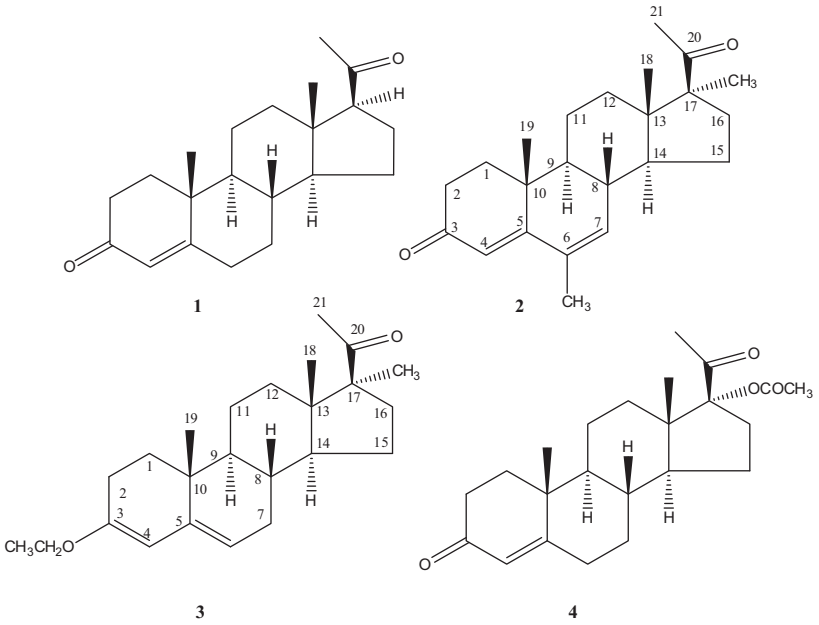


Fig. 1. Structures of progesterone **1** and related compounds **2**–**4**.

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.

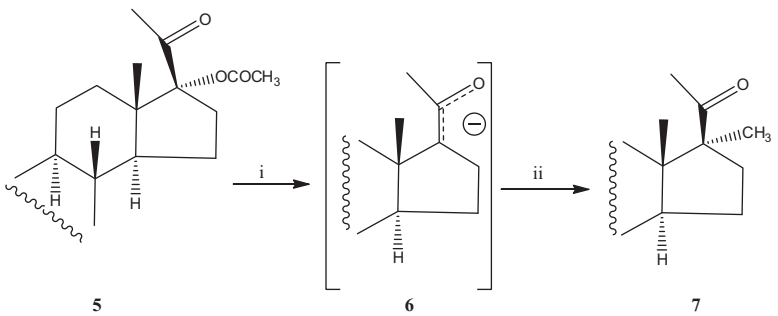
The DSC (Differential Scanning Calorimetry) were registered on a Perkin Elmer instrument (Mod. DSC7) at a heating rate of 30 °C/min from 50.0 °C to 280.0 °C.

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.