

# Microbial Transformation of Antifertility Agents, Norethisterone and 17 $\alpha$ -Ethinylestradiol

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The microbial transformation of oral contraceptive norethisterone (**1**) by *Cephalosporium aphidicola* afforded an oxidized metabolite, 17 $\alpha$ -ethinylestradiol (**2**), while the microbial transformation of **2** by *Cunninghamella elegans* yielded several metabolites, 19-nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yne-3,4,17 $\beta$ -triol (**3**), 19-nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yne-3,7 $\alpha$ ,17 $\beta$ -triol (**4**), 19-nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yne-3,11 $\alpha$ ,17 $\beta$ -triol (**5**), 19-nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yne-3,6 $\beta$ ,17 $\beta$ -triol (**6**) and 19-nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yne-3,17 $\beta$ -diol-6 $\beta$ -methoxy (**7**). Metabolite **7** was found to be a new compound. These metabolites were structurally characterized on the basis of spectroscopic techniques.

**Key words:** Norethisterone, 17 $\alpha$ -Ethinylestradiol, Microbial Transformation, *Cephalosporium aphidicola*, *Cunninghamella elegans*

## Introduction

Norethisterone (17 $\alpha$ -ethynyl-19-nortestosterone) (**1**) is a potent progestogen, widely used in oral contraceptive pills, as an antifertility agent [1]. It has a wide variety of uses including the delay in menstruation and the treatment of other menstrual disorders such as endometriosis. Among the female contraceptives, norethisterone is considered to be the safest. In continuation of our microbial transformation studies on bioactive chemical compounds [2–11], we studied the metabolism of norethisterone by *Cephalosporium aphidicola*. Metabolic studies of **1** have already been reported by various fungi and bacteria which afforded its hydroxylated metabolites at C-1 $\alpha$ , C-1 $\beta$ , C-6 $\beta$ , C-10 $\beta$  and C-11 $\beta$  positions [12, 13]. In the present study *Cephalosporium aphidicola* was used to transform compound **1** into a less polar metabolite **2** (17 $\alpha$ -ethinylestradiol). Compound **2**, is a well known estrogen which is also used as oral contraceptive [14]. In this paper we also report the microbial transformation of 17 $\alpha$ -ethinylestradiol (**2**) by *Cunninghamella elegans*, resulting in the formation of known compounds **3–6** and a new metabolite **7**. Compound **6** was already reported as metabolite of **2** by *Penicillium chrysogenum* [15].

## Results and Discussion

Screening scale experiments have shown that the *Cephalosporium aphidicola* (IMI 68689) was capable of converting norethisterone (**1**) C<sub>20</sub>H<sub>26</sub>O<sub>2</sub> into product **2**. Scale up of this reaction afforded a single metabolite **2**.

Compound **2** was obtained as a white solid. HREI MS of **2** displayed the M<sup>+</sup> at *m/z* 296.1614 (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub> calcd. 296.1616) which was 2 a.m.u. lesser than the substrate **1**. The IR spectrum of **2** did not show any ketonic absorption, but two additional bands at 1603 and 1517 cm<sup>-1</sup> (aromatic C=C) were observed. The UV spectrum exhibited an absorption at 204 nm, indicating the lack of conjugated chromophore. The <sup>1</sup>H NMR spectrum of compound **2** was remarkably different from substrate. It exhibited additional olefin protons signals at  $\delta$  7.12 (d, *J* = 8.4 Hz) and 6.60 (dd, *J* = 8.4 Hz, *J* = 2.7 Hz), while the C-4 proton appeared as a doublet at  $\delta$  6.54 (*J* = 2.6 Hz), indicating the aromatization of ring A. H-4 showed COSY 45° interactions with H-2 ( $\delta$  6.60), while H-2 also showed interaction with H-1 ( $\delta$  7.12). These assignment were further supported by the HMBC spectrum of compound **2**. The *ortho* and *meta*-coupling constant values for H-1/H-2 and H-2/H-4 signals, further indicated

the presence of an aromatic ring in the molecule. The aromatization of ring A through  $\Delta^{1,2}$  dehydrogenation in steroids, is a known microbial reaction, achieved by a number of fungi and bacteria [16]. The structure of compound **2** was further confirmed by comparison with the published data [17], and comparative TLC with commercially available  $17\alpha$ -ethynylestradiol.

In another subsequent experiment, compound **2** was subjected to screening experiments and it was found that *Cunninghamella elegans* (NRRL 1392) was able to convert compound **2** into several polar metabolites. Scale-up of this microbial transformation reaction afforded five polar metabolites **3–7**.

The HREI MS of compound **3** showed the  $M^+$  at  $m/z$  312.1719, corresponding to  $C_{20}H_{24}O_3$  (calcd. 312.1725), indicated that metabolite **3** contained one additional oxygen atom. In the  $^1H$ -NMR spectrum, the C-2 proton appeared at  $\delta$  6.75, with a simplified splitting pattern as an *ortho* coupled doublet ( $J = 8.3$  Hz) with H-1 ( $\delta$  6.68). This suggested the presence of a hydroxyl group at C-4. The  $^{13}C$  NMR (broad-band decoupled and DEPT) spectra showed a new quaternary carbon signal at  $\delta$  141.2 and disappearance of C-4 methine carbon atom as compared to compound **2**. New carbon signal at  $\delta$  141.2, (C-4) showed  $^3J$  heteronuclear interactions with H-2 ( $\delta$  6.75) which further indicated the presence of catechol moiety in compound **3**.

The HREI MS of compound **4** showed the  $M^+$  at  $m/z$  312.1774 ( $C_{20}H_{24}O_3$  calcd. 312.1725) was found to be 16 a.m.u. higher than that of compound **2**. The  $^1H$ -NMR spectrum of **4** was found to be closely related to compound **2**, with an additional signal at  $\delta$  4.09 (t,  $J = 2.7$  Hz), which could be assigned to H-7 on the basis of its homonuclear coupling with 2H-6 ( $\delta$  3.01 and 2.84) in COSY  $45^\circ$  spectrum. The configuration of newly introduced hydroxyl group at C-7 was assigned to be  $\alpha$  on the basis of NOESY interaction between H-7 $\beta$  ( $\delta$  4.09) and H-8 $\beta$  ( $\delta$  1.21).

The HREI MS of compound **5** showed the  $M^+$  at  $m/z$  312.1738, corresponding to the molecular formula  $C_{20}H_{24}O_3$  (calcd. 312.1725), and indicated the presence of an additional oxygen atom. The  $^1H$  NMR spectrum of **5** was different from compound **2** in two aspects. First the C-1 proton exhibited a downfield shift at  $\delta$  7.78 (which appeared at  $\delta$  7.12 in **2**), and second the appearance of a downfield signal at  $\delta$  4.18 (1H, ddd,  $J_{11ax,9ax} = 15.2$  Hz,  $J_{11ax,12ax} = 10.2$  Hz,  $J_{11ax,12eq} = 4.2$  Hz), which indicated the introduction of a hydroxyl group at C-11. The large coupling con-

stants of H-11 signal indicated an  $\alpha$  (equatorial) configuration of geminal hydroxy group.

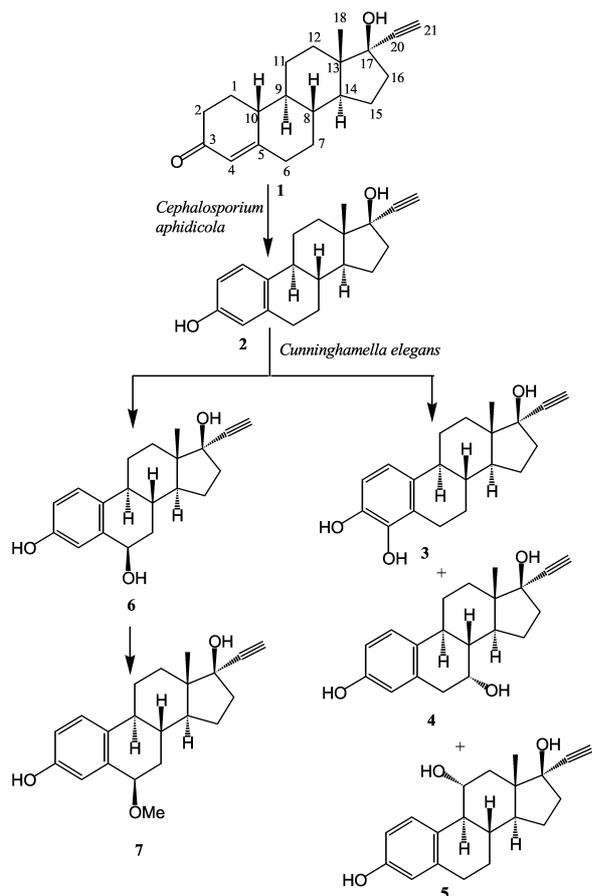
The HREI MS of compound **6** showed the  $M^+$  at  $m/z$  312.1729, corresponding to their molecular formula  $C_{20}H_{24}O_3$  (calcd. 312.1725). The  $^1H$  NMR spectrum of compound **6** was very similar to compound **2**, but with an additional methine proton triplet at  $\delta$  4.52 ( $J = 3.6$  Hz). The H-4 exhibited a downfield shift at  $\delta$  6.77, which indicated the presence of a hydroxyl group at C-6. This assignment was further supported by homoallylic coupling of H-4 with H-6 ( $\delta$  4.52) in the COSY  $45^\circ$  spectrum. H-6 also exhibited  $^2J$  and  $^3J$  heteronuclear interactions with C-5 ( $\delta$  140.1) and C-4 ( $\delta$  116.0), respectively. The stereochemistry of hydroxyl group at C-6 was deduced to be  $\beta$  on the basis of NOESY correlation between H-6 $\alpha$  ( $\delta$  4.52) and H-9 $\alpha$  ( $\delta$  1.95).

Compound **7** exhibited the  $M^+$  at  $m/z$  326, which was 30 a.m.u. greater than the substrate **2** and 14 a.m.u. greater than the metabolite **6**. In addition, a significant ion was observed at  $m/z$  294 [ $M^+ - CH_3OH$ ]. The HREI MS of compound **7** showed the  $M^+$  at  $m/z$  326.1952, corresponding to molecular formula  $C_{21}H_{26}O_3$  (calcd. 326.1882). The  $^1H$  NMR of compound **7** was different from **6**, especially in two aspects. First, the appearance of an additional three-proton singlet at  $\delta$  3.43, secondly, an upfield shift of H-6 signal at  $\delta$  4.18 (which appeared at  $\delta$  4.52 in **6**), which indicated the methylation of C-6 hydroxyl group. The  $^{13}C$  NMR spectra of **7** (Broad-band decoupled and DEPT) exhibited resonances for 21 carbons including two methyls, five methylenes, eight methines and six quaternary carbons (Table 1). An upfield oxygen-bearing C-6 methine signal resonated at  $\delta$  76.4 ( $\Delta\delta + 8.2$  ppm vs **6**), along with a methyl signal at  $\delta$  56.7, further supporting the proposed structure **7**. Stereochemistry at C-6 was assigned to be  $\alpha$  on the basis of NOESY interaction between H-6 $\alpha$  ( $\delta$  4.18) and H-9 $\alpha$  ( $\delta$  2.19). Heteronuclear interaction of oxygen-bearing methyl protons ( $\delta$  3.43) with C-6 ( $\delta$  76.4) in the HMBC spectrum, further supported the biological methylation at C-6 hydroxyl group which is chemically less reactive than C-3 phenolic group (Scheme 1).

## Experimental Section

### General experimental procedure

IR Spectra were recorded in  $CHCl_3$  on a FT IR-8900 spectrophotometer. MPs were determined on a Buchi 535 melt-



Scheme 1. Metabolism of compounds **1** and **2** by *Cephalosporium aphidicola* and *Cunninghamella elegans*, respectively.

ing point apparatus. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. UV Spectra were recorded in  $\text{CHCl}_3$  on a Hitachi U-3200 spectrophotometer. The  $^1\text{H}$ - and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$  solutions on Bruker Avance- 400 NMR at 400 and 100 MHz, respectively. The EI and HREI MS were measured on Jeol JMS-600H mass spectrometer. TLC were performed with pre-coated plates (Silica gel 60, PF<sub>254</sub>, 0.2 mm, Merck). Compound **1** was isolated from *Primolut N*, a product of Schering AG, Berlin, Germany.

#### Organism and media

Microbial cultures were obtained from either International Mycological Institute (IMI), Institute of Fermentation (IFO) or Northern Regional Research Laboratories (NRRL). All cultures were maintained on SDA and stored at 4°C prior to use. The medium for *Cephalosporium aphidicola* (IMI 68689), was prepared by mixing the following

Table 1.  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectral data of new metabolite **7**.

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
1	126.5, d	7.17, d (8.5)
2	116.5, d	6.72, dd (8.4, 2.8)
3	153.5, s	—
4	115.2, d	6.78, d (2.7)
5	137.3, s	—
6	76.4, d	4.18, dd (2.3, 3.7)
7	32.7, t	1.87, m, 1.72, m
8	33.8, d	1.92, m
9	43.7, d	2.19, m
10	131.8, s	—
11	25.9, t	1.51, m, 1.22, m
12	31.1, t	2.14, m, 1.47, m
13	47.3, s	—
14	49.0, d	1.8, m
15	22.6, t	1.74, m, 1.23, m
16	38.9, t	2.32, m, 2.04, m
17	79.7, s	—
18	12.7, q	0.85, s
20	87.4, s	—
21	74.0, d	2.58, s
OCH <sub>3</sub>	56.7, q	3.43, s

Carbon multiplicities were determined by DEPT experiments; s = quaternary, d = methine, t = methylene, q = methyl carbons.

ingredients into distilled water (3.0 l); glucose (150 g),  $\text{KH}_2\text{PO}_4$  (3 g), KCl (3 g),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (6 g), glycine (6 g), and *Gibberella* trace element solution (6 ml). The *Gibberella* trace element solution was prepared by dissolving  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  (0.01 g),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (0.1 g),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.1 g),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.161 g),  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  (0.01 g) and  $\text{NH}_4$  molybdate (0.01 g) in to distilled water (100 ml). The medium for *Cunninghamella elegans* (NRRL 1392) was prepared by mixing the following ingredients into distilled  $\text{H}_2\text{O}$  (3.0 l); glucose (30.0 g), peptone (15.0 g), yeast extract (15.0 g),  $\text{KH}_2\text{PO}_4$  (15.0 g), and NaCl (15.0 g).

#### General fermentation and extraction protocol

The fermentation media thus obtained was distributed among 30 flasks of 250 ml capacity (100 ml in each) and autoclaved. The fermentation was carried out according to a standard two-stage protocol [18]. Substrates were dissolved in DMSO and were evenly divided into 30 flasks (20 mg/0.5 ml in each flask), containing 24-h-old stage II cultures and fermentation continued for further additional time on a rotatory shaker (200 rpm) at 29°C. During the fermentation period, aliquots from flasks were taken daily and analyzed by TLC in order to determine the degree of transformation of substrate. In all experiments, one control flask without biomass (for checking substrate stability) and one flask without exogenous substrate (for checking endogenous metabolite) were used. The culture media and mycelium were separated by filtration. The mycelium were washed with  $\text{CH}_2\text{Cl}_2$  (1 l) and the filtrate was extracted with  $\text{CH}_2\text{Cl}_2$  (3 × 1.5 l).

The combined organic extract was washed with brine and dried over anhydrous  $\text{Na}_2\text{SO}_4$ , evaporated under reduced pressure, and analyzed by thin layer chromatography. Control flasks were also harvested and compared by TLC, to detect the bio-transformed products.

*Fermentation of norethisterone (1) with Cephalosporium aphidicola (IMI 68689)*

Compound **1** (600 mg), dissolved in 15 ml DMSO, was evenly distributed among 30 flasks containing stage II cultures. Fermentation was stopped after 8 days, together with the control flasks. The organic metabolites were extracted from the medium and evaporated to afford a brown gum (3.1 gm). The crude residue was subjected to column chromatography. Elution with gradient of petroleum ether and EtOAc yielded compound **2** (164 mg, petroleum ether-EtOAc 81:19). The spectral data of compound **2** was already reported [17].

19-Nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yne-3,4,17 $\beta$ -diol (**2**) was obtained as a white solid. – M.p. 181–182°C. –  $[\alpha]_D^{25}$ :  $-131^\circ$  ( $c = 0.1$ , MeOH). – IR (CHCl<sub>3</sub>):  $\nu_{\text{max}} = 3294, 2927, 2866, 1603, 1517 \text{ cm}^{-1}$ . – <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta = 7.12$  (1H, d,  $J_{\text{ortho}} = 8.4$  Hz, H-1), 6.60 (1H, dd,  $J_{\text{ortho}} = 8.4$ ,  $J_{\text{ortho}} = 2.7$  Hz, H-2), 6.54 (1H, d,  $J_{\text{meta}} = 2.6$  Hz, H-4), 2.58 (1H, s, H-21), 0.85, (3H, s, Me-18). – <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta = 126.3$  (C-1), 112.6 (C-2), 154.0 (C-3), 115.2 (C-4), 137.9 (C-5), 131.7 (C-10). – MS (EI, 70 eV):  $m/z$  (%) = 298 [M<sup>+</sup>] (57), 252 (2), 228 (16), 213 (100), 160 (41), 134 (26), 107 (26), 55 (32). – MS (HREI):  $m/z = 296.1614$  (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>, 296.1616).

*Fermentation of 17 $\alpha$ -ethynylestradiol (2) with Cunninghamella elegans (NRRL 1392)*

Compound **2** was added as a solution in DMSO (20 mg/0.5 ml in each flask), among 30 flasks containing stage II cultures. Fermentation was continued for 12 days. Culture filtrate was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The resulting organic extract was dried to afford a brown gum (2.6 g). The crude residue was subjected to column chromatography. Elution with gradient system of petroleum ether and ethyl acetate afforded metabolites **3** (17.4 mg, petroleum ether-EtOAc, 76:24), **7** (11.3 mg, petroleum ether-EtOAc, 71:29) and **4** (8.8 mg, petroleum ether-EtOAc, 61:29), while elution with petroleum ether (57%)-EtOAc (43%), afforded impure fraction containing compounds **5** and **6**, which were purified by TLC using EtOAc-petroleum ether (4:6) as mobile phase, where pure compounds **5** (21.7 mg) and **6** (31.4 mg) were obtained.

19-Nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yne-3,4,17 $\beta$ -triol (**3**) was obtained as a colorless crystalline solid. – M.p. 174–175°C. –  $[\alpha]_D^{25}$ :  $-2.1^\circ$  ( $c = 0.1$ , MeOH). – IR (CHCl<sub>3</sub>):  $\nu_{\text{max}} = 3286, 2927$  and  $2869 \text{ cm}^{-1}$ . – <sup>1</sup>H NMR (CDCl<sub>3</sub>,

400 MHz):  $\delta = 6.68$  (1H, d,  $J_{\text{ortho}} = 8.4$  Hz, H-1), 6.75 (1H, d,  $J_{\text{ortho}} = 8.3$  Hz, H-2), 2.58 (1H, s, H-20), 0.85, (3H, s, Me-18). – <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta = 117.1$  (C-1), 112.4 (C-2), 140.7 (C-3), 141.2 (C-4), 123.5 (C-5), 134.0 (C-10). – MS (EI, 70 eV):  $m/z$  (%) = 312 (53) [M<sup>+</sup>], 286 (12), 229 (100), 188 (12), 176 (42), 149 (24), 115 (21), 91 (18), 55 (34). – MS (HREI):  $m/z = 312.1719$  (C<sub>20</sub>H<sub>24</sub>O<sub>3</sub>, calcd. 312.1725).

19-Nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,7 $\alpha$ ,17 $\beta$ -triol (**4**) was obtained as a colorless crystalline solid. – M.p. 197–198°C. –  $[\alpha]_D^{25}$ :  $-2.4^\circ$  ( $c = 0.19$ , MeOH). – IR (CHCl<sub>3</sub>):  $\nu_{\text{max}} = 3441, 2974$  and  $2816 \text{ cm}^{-1}$ . – <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta = 7.18$  (1H, d,  $J_{\text{ortho}} = 8.5$  Hz, H-1), 6.64 (1H, dd,  $J_{\text{ortho}} = 8.5$ ,  $J_{\text{meta}} = 2.8$  Hz, H-2), 6.55 (1H, d,  $J_{\text{meta}} = 2.6$  Hz, H-4), 4.09 (1H, t,  $J = 2.7$  Hz, H-7 $\beta$ ), 2.59 (1H, s, H-20), 3.01 (1H, dd,  $J_1 = 15.9$  Hz,  $J_2 = 2.4$  Hz, H<sub>a</sub>-6), 2.84 (1H, d,  $J = 16.4$  Hz, H<sub>b</sub>-6), 0.85 (3H, s, Me-18). – <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta = 126.8$  (C-1), 117.2 (C-2), 156.2 (C-3), 114.3 (C-4), 137.4 (C-5), 133.9 (C-10), 67.8 (C-7), 39.1 (C-6). – MS (EI, 70 eV):  $m/z$  (%) = 312 (71) [M<sup>+</sup>], 294 (50) [M<sup>+</sup> – H<sub>2</sub>O], 261 (36), 226 (100), 211 (74), 158 (53), 145 (42), 91 (41), 55 (69). – MS (HREI):  $m/z = 312.1774$  (C<sub>20</sub>H<sub>24</sub>O<sub>3</sub>, calcd. 312.1725).

19-Nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,11 $\alpha$ ,17 $\beta$ -triol (**5**) was obtained as a white crystalline solid. – M.p. 139–140°C. –  $[\alpha]_D^{25}$ :  $-93.3$  ( $c = 0.15$ , MeOH). – IR (CHCl<sub>3</sub>):  $\nu_{\text{max}} = 3288, 2925$  and  $2864 \text{ cm}^{-1}$ . – <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta = 7.78$  (1H, d,  $J_{\text{ortho}} = 8.5$  Hz, H-1), 6.62 (1H, dd,  $J_{\text{ortho}} = 8.4$  Hz,  $J_{\text{meta}} = 2.2$  Hz, H-2), 6.57 (1H, d,  $J_{\text{meta}} = 2.1$  Hz, H-4), 4.18 (1H, ddd,  $J_{11\text{ax},9\text{ax}} = 15.2$  Hz,  $J_{11\text{ax},12\text{ax}} = 10.2$  Hz,  $J_{11\text{ax},12\text{eq}} = 4.2$  Hz, H-11 $\beta$ ), 2.60 (1H, brs, H-21), 0.84, (3H, s, Me-18). – <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta = 127.6$  (C-1), 112.6 (C-2), 153.6 (C-3), 114.9 (C-4), 132.4 (C-5), 49.1 (C-9), 139.4 (C-10), 71.0 (C-11). – MS (EI, 70 eV):  $m/z$  (%) = 312 (12.1) [M<sup>+</sup>], 294 (38), 260 (22), 224 (19), 211 (61), 157 (100), 141 (82), 91 (77.6), 55 (83.3). – MS (HREI):  $m/z = 312.1738$  (C<sub>20</sub>H<sub>24</sub>O<sub>3</sub>, calcd. 312.1725).

19-Nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,6 $\beta$ ,17 $\alpha$ -triol (**6**) was obtained as a white crystalline solid. – M.p. 201–202°C. –  $[\alpha]_D^{25}$ :  $-81.4$  ( $c = 0.12$ , MeOH). – IR (CHCl<sub>3</sub>):  $\nu_{\text{max}} = 3296, 2933$  and  $2864 \text{ cm}^{-1}$ . – <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta = 7.14$  (1H, d,  $J_{\text{ortho}} = 8.5$  Hz, H-1), 6.67 (1H, dd,  $J_{\text{ortho}} = 8.4$ ,  $J_{\text{meta}} = 2.4$  Hz, H-2), 6.77 (1H, d,  $J_{\text{meta}} = 2.3$  Hz, H-4), 4.52 (1H, t,  $J = 3.6$  Hz, H-6 $\alpha$ ), 2.89 (1H, brs, H-21), 0.89 (3H, s, Me-18). – <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta = 127.1$  (C-1), 117.4 (C-2), 156.3 (C-3), 116.0 (C-4), 140.1 (C-5), 132.6 (C-10), 68.2 (C-6), 37.6 (C-7). – MS (EI, 70 eV):  $m/z$  (%) = 312 (41) [M<sup>+</sup>], 250 (9), 226 (14), 200 (31), 157 (25), 145 (70), 91 (56), 53 (100). – MS (HREI):  $m/z = 312.1729$  (C<sub>20</sub>H<sub>24</sub>O<sub>3</sub>, calcd. 312.1725).

19-Nor-17 $\alpha$ -pregna-1,3,5(10)-trien-20-yne-3,17 $\alpha$ -diol-3 $\beta$ -methoxy (**7**), was obtained as a white crystalline solid.

– M. p. 178–179 °C. –  $[\alpha]_D^{25}$ : –1.8 ( $c = 0.21$ , MeOH). – IR (CHCl<sub>3</sub>):  $\nu_{\max} = 3294, 2925$  and  $2858 \text{ cm}^{-1}$ . – UV/vis (CHCl<sub>3</sub>):  $\lambda_{\max} (\text{lg } \epsilon) = 202 \text{ nm} (3.4)$ . – <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): see Table 1. – <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): see

Table 1. – MS (EI, 70 eV):  $m/z$  (%) = 326 (71.2) [M<sup>+</sup>], 308 (4.3) [M<sup>+</sup>–H<sub>2</sub>O], 294 (45) [M<sup>+</sup>–CH<sub>2</sub>OH], 234 (4), 211 (98), 157 (100), 115 (29), 81 (39), 55 (12). – MS (HREI):  $m/z = 326.1952$  (C<sub>21</sub>H<sub>26</sub>O<sub>3</sub>, calcd. 326.1882).

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