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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SEVERAL STEROIDAL OXIMES

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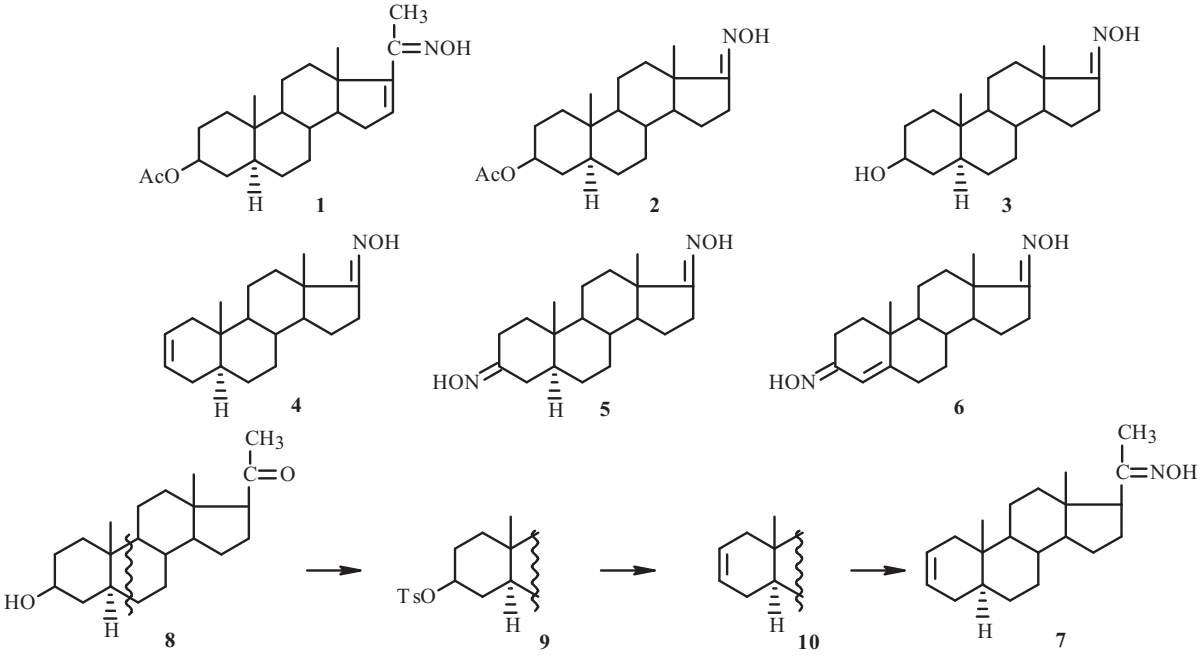
Steroidal oximes and their derivatives are known to exhibit anti-inflammatory, anticancer, and other types of physiological activity [1, 2].

In continuation of structure–activity relationship studies of 5α-steroids [3], we synthesized steroidal oximes **1**–**7** and studied their antimicrobial and anti-inflammatory activity.

The starting material for synthesizing **1**–**7** was 5α-pregnenolone acetate, which was prepared by the method developed previously by us [4].

Oximes **1**–**6** were synthesized by the known method [5] from the corresponding ketones whereas 5α-pregnen-2-one-20 (**7**) was obtained for the first time from ketone **8** using its tosylation (**9**), dehydrotosylation (**10**) [6, 7], and reaction of **10** with hydroxylamine hydrochloride in Py (Scheme 1).



Scheme 1

The structures of the products were confirmed by IR and PMR spectral data and elemental analysis.

The PMR spectrum of **10** showed resonances for angular methyls C-18 and C-19 as singlets with chemical shifts δ 0.67 and 0.85 ppm, respectively. The chemical shift of methyl C-21 was 2.05 ppm. Of the other resonances, a complicated multiplet near 5.52 ppm belonging to the C-2 and C-3 protons should be mentioned.

Introduction of the oxime (**7**) shifted resonances of all methyls to strong field (0.61, 0.74, and 1.86 ppm, respectively) and caused the appearance of a C=N–OH resonance as a broad singlet at 8.28 ppm. The C-2 and C-3 protons of the double bond resonated as a complicated multiplet at 5.56-5.58 ppm.

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The biological activity of the steroidal oximes was predicted by the PASS (Prediction of Activity Spectra Substances) computer program.

Antimicrobial, antifungal, anti-inflammatory, and antiatherosclerotic activity was most probable for all oximes synthesized by us (**1**–**7**).

An *in vitro* experiment with 3,17-dihydroxyiminoandrost-4-ene (**6**) showed activity against several fungus strains (*Sporobolom salmonicolor*, *Candida glabrata*, *Penicillium notatum*, *Aspergillus terreus*, *A. niger*, *A. fumigatus*) that was comparable with that of amphotericin and nystatin.

Also, the predicted anti-inflammatory activity of 3β-hydroxy-17-hydroximino-5α-androstane (**3**) was consistent with a biological experiment because **3** acted as a selective blocker of the enzyme cyclooxygenase-2. The other oximes (**1**, **2**, **4**, **5**, **7**) exhibited moderate or low activity.

A trial was performed at the Hans Knoll Institute of Natural Compounds and Infectious Biology (Germany). It was found that a double bond, even moreso conjugated (**6**), enhanced the antimicrobial activity whereas an OH group on C-3 (**3**) increased the anti-inflammatory activity of the steroidal oximes.

IR spectra were recorded in KBr disks on a Thermo Nicolet Avatar-370 spectrometer. PMR spectra were measured in $CDCl_3$ on a Bruker AM-400 (400 MHz) spectrometer; melting points, on a Boetius heating stage.

The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates using benzene:acetone, 6:1. Elemental analyses of all compounds agreed with those calculated.

3β-(4-Methylphenylsulfonyloxy)-5α-pregnan-20-one (9). A solution of ketone **8** (1 g, 3.1 mmol) in anhydrous Py (3 mL) at 0–5°C was treated over 1 h with *p*-toluenesulfonylchloride (2 g, 10.1 mmol). The reaction mixture was stirred for 3 h at 20–25°C, treated with conc. HCl (3 mL), and held at 0–5°C for 5 h. The resulting precipitate was filtered off, washed with water, and dried. Crystallization from benzene produced 1.2 g (90%) of **9**, mp 95–98°C. IR spectrum (ν, cm^{-1}): 1740, 1680 (C=O), 1590 (C=C, Ar). PMR spectrum (δ, ppm, J/Hz): 062 (3H, s, 18-CH_3), 0.84 (3H, s, 19-CH_3), 2.12 (3H, s, 21-CH_3), 2.45 (3H, s, CH_3-Ar), 7.35 (2H, d, J = 8, H-Ar), 8.24 (2H, d, J = 8, H-Ar).

5α-Pregn-2-en-20-one (10). A solution of tosylate **9** (1.4 g, 2.9 mmol) in freshly distilled DMF (15 mL) was treated with LiBr (1.2 g, 13.6 mmol) over 2 h, refluxed for 2 h, cooled to 20°C, and diluted with water (300 mL). The resulting crystals were filtered off and washed with water to afford 0.7 g of crude product. Crystallization from MeOH afforded **10** (0.4 g, 57%), mp 125–128°C. IR spectrum (ν, cm^{-1}): 1710 (C=O), 1640 (C=C). PMR spectrum (δ, ppm): 0.67 (3H, s, 18-CH_3), 0.85 (3H, s, 19-CH_3), 2.05 (3H, s, 21-CH_3), 5.52 (2H, complicated m, H-2, H-3).

20-Hydroximino-5α-pregn-2-ene (7). A mixture of **10** (0.4 g, 1.3 mmol) and $NH_2OH{\cdot}HCl$ (0.1 g, 1.47 mmol) in Py (5 mL) was heated at 65–70°C for 3 h, cooled to 20°C, and poured into icewater (50 mL). The precipitate was filtered off, washed with water, and dried to afford **7** (0.38 g, 90%), mp 113–115°C (MeOH). IR spectrum (ν, cm^{-1}): 1680 (C=N), 1640 (C=C). PMR spectrum (δ, ppm): 0.61 (3H, s, 18-CH_3), 0.74 (3H, s, 19-CH_3), 1.86 (3H, s, 21-CH_3), 5.56 (2H, complicated m, H-2, H-3), 8.28 (br.s, CN–OH).

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