Bioorganic Chemistry **27,** 207–213 (1999)
Article ID bioo.1999.1128, available online at http://www.idealibrary.com on IDEAL®

The Synthesis of Tritium-Labeled 16α,17α-Cyclohex-3′-eno-progesterone as Probe for the Study of Steroid–Receptor Binding

V. P. Shevchenko,*[,1] I. Yu. Nagaev,* N. F. Myasoedov,* A. V. Kamernitsky,† I. S. Levina,† L. E. Kulikova,† E. V. Pokrovskaya,† T. A. Shchelkunova,† and A. N. Smirnov‡

**Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Square, 123182 Moscow, Russia; †N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prosp. 47, 117917 Moscow, Russia; and ‡School of Biology, M. V. Lomonosov Moscow State University, Vorob'evy Hills 2, 119899 Moscow, Russia*

Received August 25, 1998

Tritium-labeled 16α,17α-cyclohex-3′-en-pregn-4-en-3,20-dione (16α, 17α-cyclohex-3′-eno-progesterone) with specific activity of 44 Ci/mmol, required in studying of binding with progesterone receptors, has been obtained by homogenous catalytic hydrogenation of 16α,17α-cyclohex-3′-eno-pregna-1,4-dien-3,20-dione with gaseous tritium and subsequent separation of the mixture by HPLC. Biological testing has shown it to bind to rat uterine progesterone receptor with an affinity comparable with that of progesterone itself.

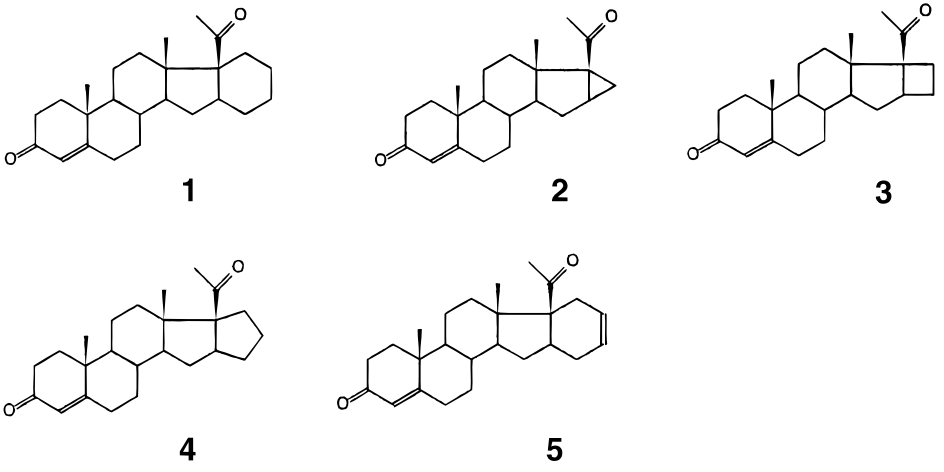
Key Words: progesterone analogs; labeled steroids; steroid-receptor binding; tritium.

INTRODUCTION

It has been found that 16α,17α-cycloalkanoprogesterones (pregna-D′-pentaranes) with an additional three- to seven-membered carbocycle, except for 16α,17α-cycloheptano-progesterone, are highly active progestins and at the same time provide selective biological action (*1*,*2*). 16α,17α-Cyclohexanoprogesterone (**1**) possess a high activity both in the proliferation of endometrium (Clauberg-McPhail test) and in the maintenance of pregnancy (Corner-Allen's test) while 16α,17α-cyclopropano-, 16α,17α-cyclobutano-, and 16α,17α-cyclopentano-progesterones (**2**, **3** and **4**, respectively), keeping the same proliferation effect, proved to be less effective in the pregnancy maintenance test (Scheme 1). On the other hand, 16α,17α-cyclohex-3′-eno-progesterone (**5**), with a weak effect on proliferation, proved to be very active in the pregnancy maintenance test.

[1] To whom correspondence should be addressed. Fax: (095) 196-0221.

0045-2068/99 $30.00



SCHEME 1

To understand reasons for such dissociation of biological functions it was necessary to study progesterone receptor binding with the pentarane series. However, a significant discrepancy between the high biological activity of the pentaranes *in vivo* and their relatively low affinity to progesterone receptor (PR) *in vitro* has been obtained in experiments on relative binding affinity, RBA (*3*). More detailed study of PR binding using tritium-labeled pentaranes **1** and **2** showed that this contradiction appears to arise from overestimation of the active concentration of the hydrophobic pentaranes due to high adsorption on tube walls (*4*). To obtain reliable data on binding of pentaranes to PR and account for their adsorption on tube walls it was necessary to use tritiated pentaranes with a relatively high specific radioactivity (*5*).

The usage of tritium labeled pentaranes for steroid–receptor study gave an opportunity for investigating both the competitive activity of pentaranes and the kinetics of the formation and dissociation of steroid–receptor complexes and to evaluate adsorption of pentaranes on the tube wall. The binding of steroid molecule to PR proved to proceed very quickly and in most cases finished after 1 h incubation time. Besides, two types of steroid–receptor complexes were revealed based on their dissociation rate. The proportion of the slow-dissociating complexes increases with increasing preincubation time and correlates with the steroid hydrophobicy (*4*,*6*).

The aim of the current work was to investigate the steroid–receptor binding using tritium-labeled **5** (synthesized according to the Scheme 2). Labeled pentaranes **1** and **2** were obtained by the selective catalytic tritiation of double bonds in appropriate pentarane precursors (*6*). Selective introduction of tritium into steroids accompanied by preservation of isolated double bonds and hydrogenation of conjugated ones seems to be a complicated task (*7*).

The 16α,17α-cyclohex-3′-eno-pregna-1,4-dien-3,20-dione (**6**) was chosen as the precursor that could be easily synthesized starting from **5** according to the method (*5*). The next steps were to find an optimal hydrogenation condition and isolate the target labeled product [^{3}H]**5** from the reaction mixture with a radiochemical purity of no less than 95–97%.

EXPERIMENTAL

Materials and Methods

Solvents and other reaction materials were prepared and purified according to standard procedures. Lindlar catalyst and tris-triphenylphosphine (I) rhodium chloride were purchased from Fluka. Standard steroids were synthesized as described earlier (*5*). Analysis and purification of preparations was performed by HPLC and TLC (Table 1).

Radioactivity distribution along TLC plates was determined by a Berthold Model LB2832 TLC automatic linear analyzer. Radioactivity was measured by a liquid scintillation spectrometry. HPLC was performed using a Gilson gradient system equipped with a UV spectrophotometer and a Model LB506 Berthold radioactivity monitor with a solid scintillation cell.

The optimal reaction conditions were worked out using 0.1% tritium according to the procedure described in (*8*).

Synthesis of 16α,17α-Cyclohex-3′-eno-[^{3}H]progesterone

A solution of 9.8 mg of **6** and 20 mg of tris-triphenylphosphine (I) rhodium chloride in 0.5 ml of ethyl acetate was placed in an ampoule, frozen in liquid nitrogen, and the ampoule filled with carrier-free tritium gas (10 Ci) to a pressure of 400 hPa. The reaction was stirred for 40 min at room temperature. The contents of the ampoule were frozen again and the system evacuated. Labile radioactivity was removed by threefold evaporation of the reaction mass with ethyl acetate–methanol (1:5) mixture. The reaction products were applied to a silica gel plate and developed three times in system IV. Three zones of interest in the R_f range of 0.25–0.65 were clearly visualized under UV illumination at 254 nm and were extracted by ethyl acetate (5 × 10 ml) and combined. About 6.9 mg of labeled steroid mixture was recovered from TLC plate with an overall radioactivity of 2.3 Ci.

TABLE 1

HPLC and TLC Systems Used for Purification and Analysis of Steroid Compounds

	Retention time (min) or R_f value for compounds				
System No.	**6**	**5**	**7**	**1**	**8**
I (HPLC)	9.95	11.29	11.29	13.94	17.80
II (HPLC)	5.95	9.15	9.15	12.11	15.21
III (HPLC)	—	9.94	13.30	—	—
IV (TLC)	—	0.51	0.39	—	—
V (TLC)	—	0.64	0.53	—	—

Note. System I (used for preparative HPLC). Silasorb 13 C_{18} 10 × 250-mm column. Eluent: methanol–water, 95:5, 2 ml/min. System II (used for analysis). Column: Separon SGX C_{18} 3.3 × 150 mm. Eluent: methanol–water, 4:1, 0.5 ml/min. System III (used both for analytical and preparative separations). Column: Separon SIX 3.3 × 150 mm. Eluent: hexane–*i*-propanol, 95:5, 0.5 ml/min. System IV (used for purification). Sorbfil UV 254 (Russia) silica gel plates. Eluent: hexane–diethyl ether, 1:1, three passes. System V (used for analysis). Sorbfil UV 254. Eluent: hexane–*i*-propanol, 95:5.