[Chem. Pharm. Bull. 25(3) 501—503 (1977)]

UDC 547.92.09 : 615.357.015.11.076.9

# Anti-uterotropic Activities of 16β-Ethyl-1,3,5(10)-estratrien-3,17β-diol(16β-ethylestradiol-17β) and Related Compounds

Hiroo Takikawa

*Department of Pharmaceutical Chemistry, Institute of Endocrinology, Gunma University*[1]

(Received June 3, 1976)

Uterotropic and anti-uterotropic activities of 16β-ethylestradiol-17β and their derivatives were examined in immature female rats. By subcutaneous administration, 16β-ethylestradiol-17β demonstrated a high potency in the anti-uterotropic activity but a low potency in the uterotropic activity which was approximately 0.01% of the activity of estradiol-17β in the four-day test.

**Keywords**——anti-uterotropic activity; 16β-ethyl-1,3,5-estratrien-3,17β-diol; anti-estrogens; 16β-ethylestradiol 3-methyl ether; 16β-ethylestradiol 3,17-acetate; 16β-ethylestradiol 17-acetate; 16β-ethylestradiol 3-methyl ether 17-acetate

In a previous paper,[2] it has been reported that 16-substituted estradiol-17β showed a considerable inhibition of binding of $^3$H-estradiol-17β with the estrogen receptor of human breast cancer in *in vitro* experiments. Among the 16-substituted estradiols, methyl ethers of which were synthesized by Goto *et al.*[3], 16β-ethyl-1,3,5(10)-estratrien-3,17β-diol (16β-ethylestradiol-17β) has been demonstrated to be most potent in competition with estradiol-17β in the binding assay. The present paper reports uterotropic and anti-uterotropic activities of 16β-ethylestradiol-17β and the related compounds.

## Materials ann Methods

16β-Ethylestradiol-17β, 16β-ethylestradiol 3-methyl ether, 16β-ethylestradiol 3-methyl ether 17-acetate, 16β-ethylestradiol 3,17-diacetate and 16β-ethylestradiol 17-acetate were supplied by Takeda Research Laboratories. Estradiol-17β was obtained from Mann Research Laboratories. The purity and identity of these 16β-substituted steroids were confirmed by mp and infrared (IR) spectrum and in some cases by IR, nuclear magnetic resonance (NMR) and mass spectra.[4]

Uterotropic activity was determined by the method of Lauson *et al.*[5] with a minor modification. Immature female rats, 22– to 23-day-old weighing 35—40 gm were used. The animals were injected daily for 3 days with 0.1 ml of an aqueous solution or suspension of varying doses of test compounds and killed on the fourth day. The uteri were removed from the vaginae by cutting through the cervix, separated from the surrounding tissues, and weighed fresh after pressing-out of the intra-uterine fluid.

Anti-uterotropic activity was determined by the method of Dorfman *et al.*[6] with a slight modification of using the abovementioned immature rats. A daily dose of 0.05 μg estradiol-17β on 0.1 ml of an aqueous solution was injected following administration of varying doses of test compounds once daily for 3 days and the uteri were weighed on the fourth day.

## Results and Discussion

16β-Ethylestradiol-17β and the related compounds were tested for their uterotropic activity and for their ability to inhibit estradiol-stimulated uterine growth at dosages under 300 μg.

1) Location: *3-39-15, Showa-machi, Maebashi, 371, Japan.*
2) H. Takikawa, Research on Steroids, 7, (1976) "in press".
3) G. Goto, K. Yoshioka, K. Hiraga, and T. Miki, *Chem. Pharm. Bull.* (Tokyo), **21**, 1393 (1973).
4) These analyses were performed at Takeda Research Laboratories.
5) H.D. Lauson, C.G. Heller, J.B. Golden and E.L. Severinghause, *Endocrinology*, **24**, 35 (1939).
6) R.I. Dorfman, F.A. Kincl, and H.J. Ringold, *Endocrinology*, **68**, 17 (1960).

Table I. Uterotropic Response by 16β-Ethylestradiol-17β and Related Compounds

| Steroid | Number of Rats | Total dose (μg) | Mean uterine weight (mg) ±S.E. |
|---|---|---|---|
| estradiol-17β | 5 | 3.0 | 135.8±1.2[a] |
| | 4 | 1.5 | 106.7±7.0[a] |
| | 5 | 0.9 | 92.6±4.6[a] |
| | 5 | 0.3 | 91.4±2.0[a] |
| | 5 | 0.15 | 86.8±2.0[a] |
| | 5 | 0.075 | 58.2±1.9[a] |
| | 5 | 0.015 | 50.4±1.5[a] |
| | 5 | 0.0075 | 43.6±2.0 |
| | 5 | 0.0015 | 36.6±1.4 |
| | 5 | 0.00075 | 36.0±0.7 |
| 16β-Ethylestradiol | 5 | 300.0 | 51.4±1.2[a] |
| | 5 | 150.0 | 40.6±2.5 |
| | 5 | 3.0 | 38.4±2.8 |
| | 5 | 1.5 | 38.2±3.6 |
| | 5 | 0.9 | 39.8±2.6 |
| | 5 | 0.3 | 40.4±1.6 |
| 16β-Ethylestradiol 3-methyl ether | 5 | 300.0 | 39.4±1.4 |
| | 5 | 30.0 | 38.2±1.3 |
| | 5 | 3.0 | 40.2±3.5 |
| | 5 | 1.5 | 38.0±1.7 |
| | 5 | 0.9 | 38.6±3.5 |
| | 4 | 0.3 | 40.4±1.6 |
| 16β-Ethylestradiol 3-methyl ether 17-acetate | 5 | 300.0 | 40.0±1.2 |
| | 5 | 30.0 | 39.6±0.4 |
| 16β-Ethylestradiol 3,17-diacetate | 5 | 300.0 | 41.4±0.2 |
| | 5 | 30.0 | 44.2±0.2 |
| 16β-Ethylestradiol 17-acetate | 5 | 300.0 | 38.2±2.6 |
| | 5 | 30.0 | 38.2±2.6 |
| Control | 5 | 0 | 34.4±1.8 |

a) Statistically significant against the controls ($p > 0.01$)

As shown in Table I, 16β-ethylestradiol-17β had no uterotropic activity in immature rats at dosages under 150 μg, but caused a slight increase of uterine weight at a higher dosage of 300 μg. The minimum effective dose of estradiol-17β in immature rats appeared to be 0.015 μg. In a comparison of the two compounds, uterotropic activity of 300 μg 16β-ethylestradiol-17β was found to be almost equivalent to that of 0.015 μg estradiol-17β. Therefore, 16β-ethylestradiol is estimated to have approximately 0.01% of uterotropic activity of estradiol-17β in immature rats. Other derivatives of 16β-ethylestradiol-17β had no uterotropic activity at dosages under 300 μg.

As shown in Table II, the uterotropic response to 0.15 μg estradiol in immature rats was inhibited significantly by simultaneous administration of 16β-ethylestradiol-17β at dosages over 3.0 μg. The maximal inhibition caused by a dosage of 30 μg was approximately 60%.

It must also be added that on the basis of the histological observation estradiol-17β induced a stimulation of the epiterial element of the endometrium as well as an enlargement of the uterine wall. 16β-Ethylestradiol-17β administration at the high dose of 300 μg resulted in some stimulation but no significant alteration at doses under 150 μg. Other compounds exhibited no significant changes at dosages under 300 μg.

Miyake and Tanaka[7] reported that when epithioandrostanol (2α,3α-epithio-5α-androstan-17β-ol) was administered simultaneously with 0.09 μg estradiol-17β, it inhibited uterine growth

7) T. Miyake and A. Tanaka, *Annu. Rep. Shionogi Res. Lab.*, **19**, 20 (1969).

Table II. Effect of 16β-Ethylestradiol-17β and Related Compounds on the Estrogen-stimulated Uterine Weight of Immature Rats

| Steroid and total dose (μg) | | Number of rats | Mean uterine weight (mg) ±S.E. |
|---|---|---|---|
| Estradiol | 16β-ethylestradiol | | |
| 0 | 0 | 5 | 35.4±1.3 |
| 0.15 | 0 | 5 | 70.8±3.6 |
| 0.15 | 1.0 | 5 | 69.2±0.6 |
| 0.15 | 3.0 | 5 | 54.0±1.3[a)] |
| 0.15 | 15.0 | 5 | 50.8±1.3[a)] |
| 0.15 | 30.0 | 5 | 42.4±1.0[a)] |
| 0.15 | 150.0 | 5 | 46.4±2.2[a)] |
| 0.15 | 300.0 | 5 | 46.2±2.6[a)] |
| Estradiol | 16β-ethylestradiol 3-methyl ether | | |
| 0.15 | 30.0 | 5 | 68.8±2.8 |
| 0.15 | 300.0 | 5 | 69.2±1.2 |
| Estradiol | 16β-ethylestradiol 3-methyl ether 17-acetate | | |
| 0.15 | 30.0 | 5 | 71.0±1.5 |
| 0.15 | 300.0 | 5 | 68.2±2.8 |
| Estradiol | 16β-ethylestradiol 3,17-diacetate | | |
| 0.15 | 30.0 | 5 | 68.6±1.9 |
| 0.15 | 300.0 | 5 | 69.8±2.7 |
| Estradiol | 16β-ethylestradiol 17-acetate | | |
| 0.15 | 30.0 | 5 | 68.6±1.8 |
| 0.15 | 300.0 | 5 | 66.0±1.7 |

*a*) Statistically significant against estradiol-alone ($p<0.01$)

at dosages over 30 μg. Miyake *et al.*[8] also reported that the uterotropic response to 0.09 μg estradiol-17β in immature mice was inhibited by simultaneous oral administration of 2α,3α-epithio-5α-androstan-17β-ol 1-methoxy-cyclopentyl ether at doses over 30 μg. Our results presented above show that 16β-ethylestradiol-17β inhibited the uterotropic response to 0.15 μg estradiol-17β by simultaneous injection at dosages over 3.0 μg. It is not possible to compare exactly the anti-uterotropic activity of both compounds, since the experimental animals are different in species. Though the minimum effective dosage of estradiol-17β in both animals appeared almost equal (0.01 μg in mice, 0.015 μg in rats), it is assumed that 16β-ethylestradiol-17β is ten times more potent in the anti-estrogenic activity than epithioandrostanol.

The present data demonstrate that 16β-ethylestradiol-17β has an anti-estrogenic activity in the uterus at dosages over 3.0 μ, but shows no uterotropic activity at dosages under 150 μg in immature rats. Among the 16β-ethylestradiol derivatives tested, only 16β-ethylestradiol-17β possesses anti-uterotropic activity in immature rats.

It is also appropriate to mention that when phenolic hydroxyl group on carbon-3 and/or the alcoholic hydroxyl group on carbon-17 was substituted, the anti-uterotropic activity in immature rats was diminished. It is known that, generally, anti-estrogens compete with natural estrogens for common receptor sites.[9] These results seemed to agree rather closely with those of *in vitro* experiments on the binding of $^3$H-estradiol-17β with the estrogen receptor of human breast cancer tissues.[2] They also indicate that the *in vitro* binding experiment may be effective in a survey of the anti-estrogenic compounds.

**Acknowledgements** The author would like to thank Dr. Takuichi Miki of Takeda Research Laboratories for the synthesis of 16-substituted estradiols, and Mr. Akio Matsui, Miss Aiko Sekiguchi, and Mr. Kazuyoshi Nishikawa for their technical assistance. This work was supported by a Grant-in Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

8) T. Miyake, T. Hori, G. Kato, M. Ide, N. Uchida, and K. Yamaguchi, *Annu. Rep. Shionogi Res. Lab.*, **24**, 46 (1974).
9) M.R.N. Prasad and M.S. Sarkaran, *Acta Endocrinol.* (suppl.), **166**, 448 (1972).