Bull.Chem.Soc.Ethiop., **1992**,6(2) 105-108

UTEROTROPHIC ACTIVITIES OF 6-OXA-STEROIDS, ANALOGUES OF MIROESTROL

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ABSTRACT. Six 6-oxa-steroids, miroestrol analogues,(**1-6**) have been synthesized and their estrogenic activity determined from their uterotrophic activity on immature rats using the procedure of Rubin *et al* with some modifications. Compounds **2-5** exhibited moderate estrogenic activities with compound **4** being the most potent of the series tested, but less active than 17ß-estradiol. On the other hand, compounds **1** and **6** showed no activity.

INTRODUCTION

Interest in steroid chemistry arises from significant rating that these compounds have been accorded in medicine. Commercial research on steroid is focused on finding more potent, long acting synthetic drug analogues, possessing essential pharmacological properties.

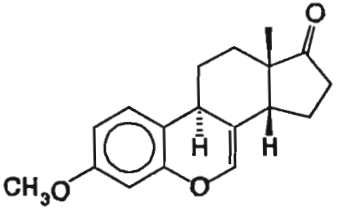
Our interest in this research arises from the report that miroestrol (**7**) isolated from the tubers of *Pueraria mirifica* proved to be the most potent phytoestrogen discovered [1,2,3]. The estrogenic activity of miroestrol was noted to be comparable to that of the animal estrogen, estradiol, and was found to be 0.25 times as active as estradiol in the rat vaginal carnification when administered subcutaneously. However, when applied *per os*, its estrogenic activity was found to be 60 to 70 times that of the estradiol [4].

Whenever a new, highly active, estrogen becomes known, it is of interest to determine the relationship between structure and activity. Following the isolation of the potent estrogen, miroestrol, several attempts have been made to synthesize it but its total synthesis has proved to be a formidable task [5,6]. To date no successful synthesis of miroestrol has been reported. We have therefore considered it of interest to synthesize miroestrol analogues and determine their estrogenic activity. The synthesis of these compounds has been previously reported [7]. Our interest in this study is based on the assumption that the high estrogenic activity of miroestrol is due to its unique structural features, particularly the novel 6-oxa-7-ene feature in ring B.

MATERIALS AND METHODS

The six synthetic analogues of miroestrol were purified by preparative TLC and were isolated as racemates. It was not possible to separate these compounds into their pure enantiomeric forms. Samples of these compounds were sent to Ayerst Research Laboratories, Montreal, Canada for estrogenic activity evaluation. Immature 21-day old

Sprague-Dawley rats weighing 30-20 g were obtained from Canadian Breeding laboratories at St. Constant, Quebec. The animals were housed in an air-conditioned room maintained at 24 to 25°C, with 45 to 50% humidity. The animals were fed on Purina Chow and water *ad libitum*.



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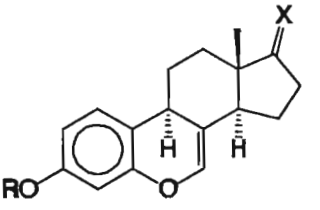

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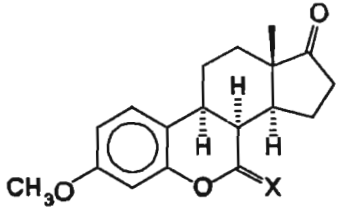
3 R = CH_3, X = α-OH, β-H
4 R = OH, X = α-OH, β-H



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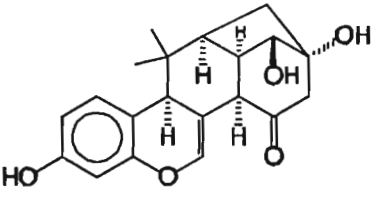

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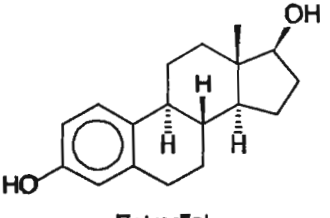

Estradiol

Uterotrophic activity of the test compounds was assessed on female rats by the uterine weight bioassay procedure [8]. The test samples were administered subcutaneously to the animals in 0.2 ml sesame oil and control animals received vehicle oil only.

The work was divided into four experiments each with a control group and three groups receiving fixed amounts of 17ß-estradiol as the standard estrogen. Each of the ten rats per group received three daily injections and were sacrificed by cervical dislocation 24 hours after the last injection. The uteri were excised free of fat and mesentery, blotted and weighed to the nearest milligram on a torsion balance. The results are given in Table 1 and each value represents the mean ± S.E.M. of 10 animals per determination.

Table 1. Uterotrophic activity[a] of miroestrol analogues (**1-6**).

Compounds	Dosage (µg/rat)				
	0.01	0.1	1.0	10.0	Control
1	0.5	0.5	0.5	0.6	0.5
2	0.6	0.7	0.8	2.2	0.6
3	0.7	0.7	1.0	2.3	0.6
4	0.7	1.2	2.0	2.4	0.6
5	0.6	0.6	0.6	1.0	0.6
6	0.6	0.6	0.6	0.6	0.6
17β-estradiol[b]	0.8	2.0	2.5	----[c]	0.6

[a]Results are expressed as uterine wt./body wt (mg/g)
[b]17ß-estrdiol used as the standard
[c]No experiment at this dosage, 17ß-estradiol is more active at lower levels.

RESULTS AND DISCUSSION

The observed estrogenic activity of miroestrol analogues was compared with that of 17ß-estradiol. The results showed that active analogues have strong activity at a dose of 10 µg. Whereas estradiol showed strong activity at a lower dose of 1 µg.

Compounds **2**,**3** and **5** showed only weak activity, while compound **4** showed significant activity, but less active than the 17ß-estradiol at lower doses. The relatively strong activity of compound **4** is not unexpected, since compound **4** is very similar to 17ß-estradiol except for the presence of the vinyl ether functionality in its B ring. Therefore the higher activity of compound **4** must be attributed to the fact that, like 17ß-estradiol and miroestrol, it has a 3,17-diol. The results also reveal that the formation of the vinyl ether functionality in B ring results in reduced activity. The reduced activity may also be attributed to the fact that the test compounds were racemates. Contrary to our observation, Herr *et al.* [9] reported that in their model experiments, the 7-ene estrogens are more active than those with saturated B ring. Compounds **1** and **6** were considered to be inactive because no significant responses

were observed at doses of up to 10 µg. The inactivity of compound **1** compared to the activity of compound **2**, may be attributed to the epimerization at C-14 which results in a *cis* C/D ring stereochemistry. This is the only difference between the two compounds. The results also show that the protection of the hydroxyl group in compound **3** at C-3 with a methyl group results in reduced activity.

The activities of the tested miroestrol analogues should be interpreted with the understanding that the compounds were tested as racemic mixtures. It is therefore likely that the isomers in each racemic mixture compete in their physiological action. It can also be deduced from the results that the unusually high estrogenic potency of miroestrol is not necessarily due to the vinyl ether moiety in its B ring but to some other structural features of the molecule.

ACKNOWLEDGEMENT

Financial support from the National Research Council of Canada is gratefully acknowledged.

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