BIOLOGICAL ACTIVITY OF RACEMIC D-HOMO-8-ISOESTRONE

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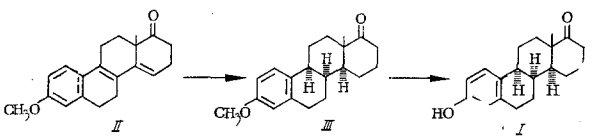
Modified estrogens are used for therapeutic purposes [1-4], and also form part of hormonal contraceptives [5, 6]. Various conditions related to the menopause or other estrogen-deficient states in women may often be ameliorated by replacement therapy with estrogens. For current methods of treatment, it is important to have estrogens with specific properties. There are literature reports of the occurrence of high vaginotropic activity in some 8-isoanalogs of steroidal estrogens, in conjunction with reduced uterotropic activity [7, 8], hypolipidemic activity with low hormonal activity [9, 10], and high antiovulatory activity [11].

It was therefore of interest to examine closely the biological activity of 8-isoanalogs of steroidal estrogens. This could lead to an improvement in our understanding of the relationship between the structures of steroids and their biological effects, thereby facilitating the discovery of drugs with directed effects.

We have studied the biological activity of the readily-accessible racemic D-homo-8-isoestrone (I), synthesized as shown below:



Catalytic hydrogenation of racemic 3-methoxy-D-homo-1,3,5(10),8,14-estrapentaene-17a-one (II) [12] with hydrogen in the presence of Raney nickel, followed by oxidation of the products with chromic anhydride in pyridine, readily afforded the methyl ether of racemic D-homo-8-isoestrone, identified by its PMR and ^{13}C NMR spectra. Acid hydrolysis of this compound gave the required steroid (I) in 64% yield, calculated on the original estrapentaene (II).

EXPERIMENTAL PHARMACOLOGY

The following methods were used to study the biological activity.

Uterotropic Effect. Experiments were carried out on 120 immature female mice of the SNR strain weighing 6-8 g, treated daily for three days with 0.1 ml oil solutions of estrone or D-homo-8-isoestrone. The control animals were treated with the same volume of the oil. The results were subjected to regression analysis. The ED_{2C} values were calculated for both compounds, having the effect of doubling the weight of the uterus in the control animals [13].

Contraceptive (Postcoital) Effect. Experiments were carried out on 80 mature female rats weighing 160-180 g. Male animals were introduced to normally-cycling rats, and from the day on which sperm were detected in the vaginal smears (day 1 of pregnancy) the test estrogens were administered subcutaneously daily for seven days, in a range of doses, or oil alone, in a volume of 0.1 ml. The contraceptive effect was assessed at the 19th-20th day following detection of sperm. The experimental results were treated as described in [14], and the ED_{50} values calculated.

Estrus-Inducing Effect ("Vaginotropic Effect"). Mature female rats were subjected to ovariectomy, and the vaginal smears were examined for 3-4 weeks thereafter (90 experiments). When stable diestrus was established, estrone or its analog, D-homo-8-isoestrone was administered subcutaneously for five days in a range of doses in 0.1 ml of oil. The vaginal smears were examined at the same time (twice daily). The vaginal smear findings were evaluated by the method described in [15]. The relationship between dose size and frequency of

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TABLE 1. Uterotropic Activity of Estrone and D-Homo-8-isosterone

Drug	Range of doses (total amounts over 3 days)		Regression coefficients*		†ED_{2c}		Relative activity, %
	μg per animal	mg per kg body weight	a	b	μg per animal	mg per kg body weight	
Estrone	0,05—0,8	0,00625—0,1	47,4	30,3	0,21	0,02625	100
D-Homo-8-iso-estrone	0,025—0,8	0,003125—0,1	24,7	8,3	1,83	0,22875	11,5

*Regression coefficients were calculated for the most curved regions of the dose—effect curves, using the equation $y = a + b \log x$.

†In calculating the ED_{2c} values, a weight of 26.88 mg was taken as twice the mass of the uterus in the control animals.

TABLE 2. Contraceptive (Postcoital) Activity of Estrone and D-Homo-8-isoestrone

Drug	Range of subcutaneous doses over 7 days, mg per kg of body weight per day	ED_{50}	Relative activity	P
Estrone	0,005—0,05	0,015	100	
D-Homo-8-isoestrone	0,02—0,1	0,043	34,5	<0.05

TABLE 3. Effects of Estrone and D-Homo-8-isoestrone on the Induction of Estrus in Castrated Female Rats

Drug	Range of subcutaneous doses over 5 days		ED_{50}		Relative activity, %	P
	μg per animal	mg per kg body weight	μg per animal	mg per kg body weight		
Estrone	3—30	0,0012—0,12	0,0036	$1,4 \cdot 10^{-5}$	100	—
D-Homo-8-isoestrone	3—30	0,0012—0,12	0,0038	$1,5 \cdot 10^{-5}$	94,8	>0.05

occurrence of reactions was, as in the preceding test, evaluated by the method described in [14] and the ED_{50} values calculated.

Antigonadotropic Effect [16]. Experiments were carried out on 100 immature male Wistar rats, which were treated subcutaneously for 5 days with various doses of oily solutions of the test estrogens in a volume of 0.1 ml. The decrease in weight of the testicles was taken as an index of antigonadotropic activity. The experimental results were treated by regression analysis, and the ED_{50} values causing a 50% reduction in the testicular mass were calculated.

Table 1 shows the results for uterotropic activity in estrone and D-homo-8-isoestrone. It will be seen from these results that D-homo-8-isoestrone shows low uterotropic activity.

Table 2 shows the contraceptive (postcoital) activity. The results of these experiments show that D-homo-8-isoestrone is 2.9 times less active than estrone in its antifertility properties.

Table 3 shows the results for vaginotropic effects, i.e., the ability of the compounds to induce estrus in castrated female rats. The results show that estrone and its analog, D-homo-8-isoestrone, have approximately the same vaginotropic activity.

The experimental results shown in Tables 1 and 3 show that the doses required to induce estrus were insufficient for the estrogenic stimulation of the uterus. These findings enabled us to regard estrus as a vaginotropic effect. The estrone analog showed a clear separation between these properties, high vaginotropic activity being accompanied by a low uterotropic effect.

TABLE 4. Antigonadotropic Activity of Estrone and D-Homo-8-isoestrone

Drug	Range of doses employed (total over 5 days), μg per animal	Regression coefficients		ED_{50}		Relative activity, %
		a	*b*	μg per animal	mg per kg body weight	
Estrone	1—5—10—20—50	179,4	—18,5	2,4	0,0275	100
D-Homo-8-iso-estrone	1—5—10—20—50	187	—109,4	7,1	0,0814	33,8

TABLE 5. Comparison of the Uterotropic (U), Contraceptive (C), Vaginotropic (V) and Antigonadotropic (A) Effects of Estrone and D-Homo-8-isoestrone

Drug	Effective dose, mg per kg body weight				Ratio of effective doses				Relative activity, %			
	ED_{50}		ED_2c									
	C	V	A	U	C/U	V/U	A/U	C/A	C	V	A	U
Estrone	0,015	$1,4\times10^{-5}$	0,0275	0,02625	0,57	0,0005	1,04	0,5	100	100	100	100
D-Homo-8-isoestrone	0,043	$1,5\times10^{-5}$	0,0814	0,22875	0,19	0,00006	9,3	0,5	34,5	94,8	33,8	11,5

The results of the study of antigonadotropic activity are shown in Table 4. It follows from these results that the estrone analog is 2.9 times less active in its inhibitory effects on hypophysis.

Table 5 presents comparative data showing the relationships between the four types of hormonal activity of estrone and D-homo-8-isoestrone. The doses compared were those giving a 50% contraceptive (postcoital) effect, inducing a vaginotropic effect in 50% of the animals, inhibiting the function of the hypophysis by 50%, and doubling the weights of the uterus in comparison with the controls.

To summarize, the data presented in Table 5 show that D-homo-8-isoestrone displays relatively high contraceptive (34.5%) and antigonadotropic (33.8%) activity, has the same vaginotropic activity as estrone, and has reduced uterotropic activity.

It is noteworthy that there is a correlation between the antifertility and antigonadotropic effects of D-homo-8-isoestrone, typical of most estrogens.

In addition, D-homo-8-isoestrone displays a marked separation of vaginotropic and uterotropic activity. Although it has high vaginotropic activity, equal to that of estrone, D-homo-8-iosestrone has only a slight effect on the uterus (11.5% of the activity of estrone).

EXPERIMENTAL CHEMISTRY

A solution of 80 g of racemic 3-methoxy-D-homo-1,3,5(10),8,14-estrapentaen-17-one (II) in 1 ml of benzene was hydrogenated over 50-60 g of Raney nickel at a temperature of 50-70°C and a hydrogen pressure of 100-120 atm. Following the usual workup procedure, the residue was dissolved in 1 liter of pyridine, and the solution was treated with cooling and stirring with Sarett's reagent, obtained from 80 g of chromium trioxide and 1 liter of pyridine. The reaction mixture was kept for 24 h at room temperature, then poured into 4 liters of water. The reaction product was extracted with benzene—ether (1:1), and the ether extract was washed with 10% hydrochloric acid followed by water until neutral, then dried over sodium sulfate. The solvent was distilled off under reduced pressure, and the residue crystallized from methanol to give 66 g of the desired product (III), mp 130-131°C (literature value, 128-128.5°C), yield 81.4%.

PMR spectrum, δ, ppm: 1.11 (3H, s, CH_3 at C_{13}), 2.4-2.8 (2H, m. protons at C-6 and C-9), 3.74 (3H, s, CH_3O-), 6.6-6.8 (2H, m, protons at C-2 and C-4), 7.02 (1H, d.d., $J_{1,2}$ 8 Hz, $J_{2,4}$ 2 Hz, proton at C-1).

^{13}C NMR spectrum: 130.02 (C-1); 133.77 (C-10); 28.15 (C-11); 41.06 (C-8 and C-9), and 18.90 (C-18). Found, %: C 80.56; H 8.90. $C_{20}H_{26}O_2$. Calculated, %: C 80.49; H 8.78.

Acid hydrolysis of (III) in the usual way gave D-homo-8-isoestrone (I) in 86% yield, mp 272-273°C (literature value, 262-263°C [12]). The compound obtained gave no depression of melting point on admixture with a known, authentic sample, and the IR spectra were identical. Found, %: C 79.90; H 8.60. $C_{19}H_{24}O_2$. Calculated, %: C 80.24; H 8.51.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF N,N'-SULFURYLDIBENZOTRIAZOLE

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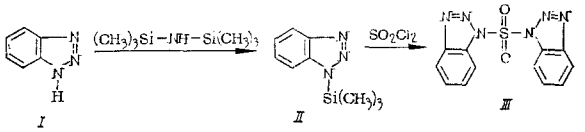
Diazolides of carboxylic and thiocarboxylic acids have been used extensively in the synthesis of biologically active compounds [1, 2]. The use of N,N'-carbonyldiimidazole and N,N'-thiocarbonyldiimidazole has been particularly successful in the preparation of O-aminoacyl derivatives of nucleotides and nucleoside polyphosphates [3, 4]. In addition, it has previously been shown that compounds of this type possess mutagenic properties [5].

In further developing work along these lines, we have synthesized N,N'-sulfuryldibenzotriazole (III), and tested for the first time the mutagenic activity of this diazolide. Compound (III) was synthesized as follows:



Reaction of 1,2,3-benzotriazole (I) with hexamethyldisilazane afforded N-trimethylsilylbenzotriazole (II), which on condensation with sulfuryl chloride in dry CCl_4 afforded (III) in 96% yield. The composition and structure of (III) were established by its elemental analysis and IR spectrum, which contained bands at 1120-1390 cm^{-1} (SO_2).

EXPERIMENTAL CHEMISTRY

IR spectra were obtained on a Spectromom-2000 instrument (Hungary) in vaseline oil.

N-Trimethylsilylbenzotriazole (II). This compound was obtained as described in [6].

N,N'-Sulfuryldibenzotriazole (III). To a solution of 5.5 g (0.029 mole) of (II) in 5 ml of dry toluene, cooled to −10°C, was added dropwise with stirring 1.9 g (0.014 mole) of sulfuryl chloride. The synthesis was carried out over 2 h at −10°C. The bright yellow solid which separated was filtered off and dried over P_2O_5, to give 4.08 g (96%) of (III), mp 130°C. Found, %: C 48.00; H 2.66; N 28.00; S 10.60. $C_{12}H_8N_6O_2S$. Calculated, %: C 48.22; H 2.60; N 27.50; S 9.55.

EXPERIMENTAL BIOLOGY

The test subject selected for the study of the mutagenic activity of the compound obtained (III) and the starting material (I) was *Drosophila melanogaster* of the wild strain Oregon-R.

Males and females of this strain in a ratio of 1:1 numbering 30 individuals were placed in a test tube containing nutrient medium. The composition of the nutrient medium was as follows: yeast 40 g, raisins 40 g, semolina 40 g, sugar 40 g, agar 5 g, and water 1 liter. A series of experiments was carried out by adding a saturated alcoholic solution of (I) or (III) to the nutrient medium, followed by dilution by a factor of 2, 4, or 8. Testing for recessive lethal mutations in the X-chromosome was carried out by the standard Meller-5 method [7]. In all, 162 chromosomes were tested for the presence of recessive lethal mutations induced by (I), and 858 chromosomes for (III). The experimental results are shown in Tables 1 and 2.

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