Antifertility Activities of Two Diphenyl-Dihydronaphthalene Derivatives. (28070)

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(Introduced by William E. Dulin)
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Previous publications from this laboratory presented biological and chemical properties of a derivative of a 2,3-diphenylindene which possessed oral antifertility activity(1,2). In laboratory animals this compound was efficacious when administered either in small doses for the first 4 days following ovulation or in a single larger dose on any one of these 4 days. When administered later than this critical period, normal healthy litters were delivered at term. More recent investigations have led to the preparation of a series of related 1,2-diphenyl-3,4-dihydronaphthalenes(3), members of which are also orally effective antifertility agents. Pertinent biological activities of 2 members of this series are presented here.

Methods and results. All animals were fed a recommended Purina laboratory diet, maintained in an environment of 78 ± 2°F, and subjected to alternate 12-hour periods of light and dark. The dihydronaphthalenes—*U-10520A* (2-[p-(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)phenoxy]-triethylamine, hydrochloride) and *U-11100A* (1-{2-[p-(3,4-dihydro - 6 - methoxy - 2 - phenyl - 1 - naphthyl)phenoxy]ethyl}-pyrrolidine, hydrochloride)—were administered orally in sterile water containing 0.25% methylcellulose (Upjohn Sterile Vehicle #122).

Pregnancy inhibition. 1. *Rats.* Compounds were administered daily from proestrus through Day 6* of pregnancy to 200 g Sprague-Dawley rats. Females were sacrificed on Day 8 at which time uteri were examined for implantation sites and gross abnormalities. Daily doses of or above 0.25 and 0.025 mg/kg of U-10520A and U-11100A, respectively, inhibited pregnancy in all rats tested. Average number of implantation sites per pregnant rat in animals receiving sub-effective doses was 10.8 and 9.5 for each compound, respectively. Single doses of 2.5 and 0.25 mg/kg of U-10520A and U-11100A, respectively, inhibited pregnancy in all rats when administered during proestrus or within 4 days after breeding. Comparable or higher doses administered on Day 5 were ineffective. Single doses of 0.62 and 1.25 mg/kg of U-10520A were effective when administered on Day 3-4 or Day 1-4, respectively. Following sub-effective single doses, a reduction in both number of implants per pregnant rat and conception rate was observed.

2. *Rabbits.* Female New Zealand white rabbits (3.3-3.8 kg) were treated daily with U-11100A for 10 days starting immediately following mating. They were sacrificed on the 15th day following mating and their uteri examined for implantation sites. Of 8 rabbits receiving only vehicle, 7 contained an average of 8.6 implantation sites. No implantation sites were observed in remaining rabbit. Three of 4 rabbits receiving 0.014 to 0.15 mg/kg daily of U-11100A contained an average of 7.1 implantation sites. Neither implantation sites nor corpora lutea were observed in fourth rabbit. Pregnancy was completely inhibited in each of 5 additional rabbits receiving 0.5 mg/kg daily.

3. *Hamsters and guinea pigs.* Female Golden hamsters (70-110 g) were subjected to U-11100A in a manner comparable to that described for rabbits. Daily oral doses up to 7 mg/kg were ineffective in inhibiting development of implantation sites. In guinea pigs (500-800 g) similarly treated, pregnancy was completely inhibited at 0.75 mg/kg/day.

Uterotropic activity. Compounds were administered orally for 10 days to 55 g ovariectomized Sprague-Dawley rats. Uterine weights were obtained at autopsy on 11th day. Minimal uterine stimulation was observed with daily doses of 0.035 and 0.0125 mg/kg/day of U-10520A and U-11100A, re-

* Day sperm were observed in vaginal smear is considered Day 1 of pregnancy.

TABLE I. Uterine Response of Ovariectomized Immature Female Rats to 10 Oral Doses of 2 Dihydronaphthalenes.

Compound	Rats/dose	Daily dose (mg/kg)	Uterine wt (mg)
Vehicle control	5	—	26 ± .6*
	5	—	25 ± .6
Estradiol (S.C.)	5	.0008	115 ± 6.1
U-10520A	5	.035	33 ± 1.9
	5	.07	42 ± 1.9
	5	.15	63 ± 8.7
	5	.28	56 ± 1.9
	5	.56	52 ± 1.9
	5	7.0	62 ± 1.9
U-11100A	5	.0125	30 ± 1.9
	5	.025	45 ± 1.9
	5	.125	57 ± 1.9
	5	.25	54 ± 1.9
	5	.50	57 ± 1.9

* ± standard error.

spectively (Table I). Average uterine weights did not exceed 63 mg at daily doses up to 7.0 mg/kg.

Antiestrogenic activity. Administered concomitantly with estradiol for 10 days to 55 g ovariectomized Sprague-Dawley rats, both compounds inhibit uterine response to exogenous estrogen (Fig. 1). Antagonism of the uterine response to 0.04 μg of subcutaneously administered estradiol by these compounds is apparent at doses corresponding, on a mg/kg basis, to minimally effective rat antifertility doses. Maximum observed inhibition of uterine weight increase was 59% of the anticipated response to estradiol.

Antigonadotropin activity. Intact Sprague-Dawley male rats, 26-28 days of age were treated daily for 10 days. Approximately 24 hours after last dose, animals were autopsied and testes, seminal vesicle, levator ani muscle, ventral prostate, and adrenal weights were obtained. U-11100A was compared directly with estradiol subcutaneously in one assay and was tested orally in an additional assay (Table II). Antigonadotropic activity of estradiol was illustrated by dose dependent decreases in testes and accessory organ weights. A diminution in body weight gain and increased adrenal weights were also observed in estradiol-treated rats. No dose-related decrease in testes weights or increases in adrenal weights were observed with either

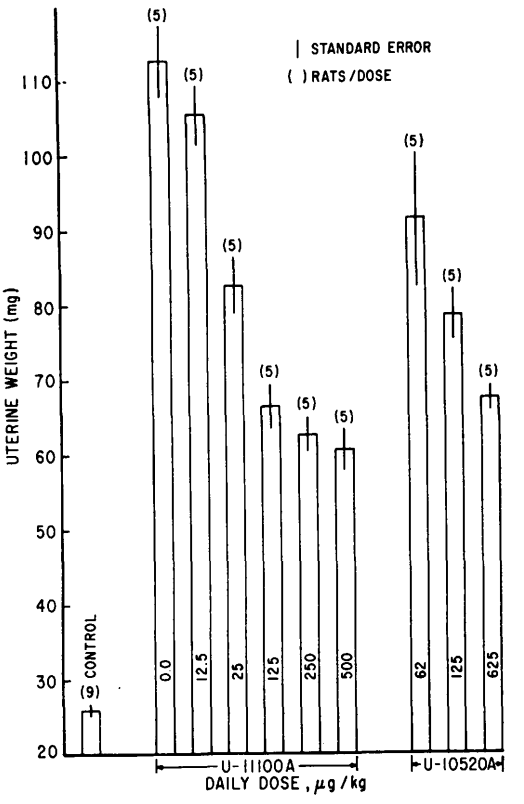

FIG. 1. Inhibition of uterine response to estradiol following concomitant oral dihydronaphthalene administration to ovariectomized immature rats. All rats, except controls, received .04 μg of estradiol, SC, daily.

parenteral or oral U-11100A at doses up to 1.6 mg/kg/day. Suppression of accessory organ weights occurred at the higher doses administered by each route.

Acute toxicity. Toxicity induced by a single oral dose of compound was evaluated in Upjohn Sprague-Dawley male rats weighing 86-184 g; effects of a single intraperitoneal administration were evaluated in Rockland Farm male mice weighing 20-30 g. Animals were observed at intervals for 7 days during which period incidence of mortality was recorded. Doses inducing 50% mortality‡ (LD_{50}) by oral and parenteral routes were, respectively, 547 (463-646) and 195 (178-214) for U-10520A, 302 (247-368) and 143 (130-157) for U-11100A, 547 (459-654) and

‡ Calculated by Spearman-Karber method(4); presented as mg/kg with 95% confidence interval.

TABLE II. Antigonadotropin Activity of U-11100A and Estradiol in Immature Male Rats (5 Rats/Dose).

Compound	Dose (mg/kg/day)	Testes wt (g)	Lev ani wt (mg)	Sem ves wt (mg)	Ventral prostate (mg)	Adrenal wt (mg)	Body wt gain (g)
Vehicle control	—	1.62	47	32	158	26	63
Estradiol (S.C.)	.0005	1.49	44	22	133	25	61
	.001	1.44	37	16	115	28	57
	.002	1.36	39	18	105	28	59
	.004	1.11	36	14	99	28	54
	.008	.92	27	15	89	29	49
	.016	.94	29	18	84	30	49
U-11100A (S.C.)	.01	1.35	39	23	121	24	61
	.02	1.40	40	24	126	24	53
	.04	1.50	39	23	144	24	58
	.08	1.52	43	26	134	24	58
	.16	1.50	35	26	124	26	48
	.32	1.48	36	20	127	26	53
Vehicle control	—	1.34	26	18	96	27	44
U-11100A (oral)	.025	1.43	33	24	123	26	52
	.10	1.31	31	21	115	25	51
	.40	1.29	28	20	121	28	49
	.80	1.40	27	18	109	26	43
	1.60	1.29	23	15	100	26	42

247 (205-296) for U-11555A.§

Discussion. A derivative of 2,3-diphenylindene (U-11555A) was reported to be an orally effective mammalian antifertility agent which, at effective antifertility doses, does not exhibit significant progestational, antiprogestational, antigonadotropic, androgenic, or estrogenic activities(1). Present compounds, derivatives of 1,2-diphenyl-3,4-dihydronaphthalenes, possess improved physical(3) and biological characteristics along with markedly increased potency. Efficacy of this new series is also limited to the period approximating tubal transport in rats. However, single oral doses administered prior to mating are equally effective in inhibiting pregnancy. Blastocysts, once in the uterine cavity, are apparently not affected by this type of compound even when they are subjected to it for extended periods as during Provera†-induced delayed implantation(5). Likewise, development of hamster blastocysts is not inhibited at doses far in excess of effective rat, rabbit, and guinea pig doses. At minimal 100% orally effective antifertility doses (0.250 and 0.025 mg/kg/day for U-10520A and U-11100A, respectively) no antigonadotropic or frank estrogenic activity is observed. At doses more than 60 times that required for pregnancy inhibition no diminution in testes weights of immature rats occurs. Uterine stimulation observed over a wide dosage range is not comparable to frank estrogenic effects on this organ and does not exceed the equivalent of 0.4 μg/kg of subcutaneously administered estradiol-17β. This dose of estradiol, subcutaneously, is ineffective in this rat antifertility test. Both compounds are estrogen antagonists in the immature rat assay. Diminution of estradiol-induced uterine weight increases is observed at effective antifertility doses, dropping to below 50% of the anticipated response as dosages are increased. Acute toxicity data suggest that level of general systemic toxicity is not correlated with antifertility potency, U-11100A possessing an improved therapeutic ratio over both U-11555A and U-10520A. U-11100A is capable of inhibiting pregnancy in rats at 5 μg/day. Except for frank estrogenic agents, it is the most potent oral mammalian antifertility agent reported to date.

Summary. U-10520A and U-11100A, derivatives of 1,2-diphenyl-3,4-dihydronaphtha-

§ U-11555A - 2-[p-(6-methoxy-2-phenylinden-3-yl)-phenoxy]-triethylamine, hydrochloride.

† Registered Trademark of The Upjohn Co. for 17α-hydroxy-6α-methylprogesterone acetate.

lene, possess oral antifertility activity in rats, guinea pigs, and rabbits. Efficacy, following daily doses as low as 5 μg, is restricted to first 4 days of pregnancy. Antifertility activity has not been observed in hamsters. These compounds cause minimal uterotropic stimulation and also inhibit estradiol-induced uterine weight increases. Gonadotropin inhibiting activity is not observed at therapeutic doses. In contrast to many compounds reported to effectively inhibit pregnancy, both U-10520A and U-11100A possess very favorable therapeutic ratios.

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Distribution of Tritium Labeled β-3-thienyl-L-alanine in Tissues of Adult Male Rats Bearing Murphy-Sturm Lymphosarcoma.* (28071)

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β-3-thienylalanine (β3TA) is a phenylalanine analogue that has been studied in several laboratories for its ability to inhibit growth and protein synthesis in both microorganisms and mammalian tissues(1,2,3,4,5). The β3TA and a closely related compound, the β-2-thienylalanine (β2TA) were found to inhibit tumor growth in mice and rats(6,7,8, 9). In all the studies of which we are aware, the DL form of β3TA was used. Ferger and Du Vigneaud showed that L-β2TA is twice as potent an inhibitor as is the DL-β2TA (10). Experience with other amino acids further suggests that only the L-isomer of β3TA has a significant biological activity.

In view of the known oncostatic effect of β3TA, a localization study was undertaken using a new method of tritium analysis developed by Jacobson *et al.*(11).

Materials and methods. The tritium-labeled beta-3-thienyl-L-alanine was prepared from acetyl beta-3-thienyl-DL-alanine with the tritium label attached to the beta carbon of the alanine side chain. The synthesis of the radioactive acetyl-beta-3-thienyl-DL-alanine was carried out by W. G. Brown, Arthur R. Lepley and Frederick H. Greenberg who also prepared a non-radioactive sample of the same compound (Dept. of Chemistry, Univ. of Chicago). Infra red spectra of the 2 compounds were identical. The method of synthesis is to be published later. The specific activity of the radioactive compound was 170.4 mc/g. This compound was resolved into its D and L isomer, and only the L-isomer was used in this study. The method of resolution was patterned after the procedure for beta-2-thienylalanine used by Ferger and Du Vigneaud(10). The specific rotation of the brucine salt of the D-isomer in methanol was $[\alpha]_D^{25} = -37.9°$ and that of the L-isomer in pyridine was $[\alpha]_D^{25} = -61.5°$. These rotations resembled the values of −38.7 and −57.8° for the D and L brucine formyl beta-2-thienylalanine diastereoisomers reported by Ferger and Du Vigneaud(10).

After removal of brucine the resulting acetyl-L-beta-3-thienylalanine, dissolved in ethanol, gave a specific rotation of $[\alpha]_D^{25} = +39.8°$ and the acetyl-D-beta-3-thienylalanine $[\alpha]_D^{25} = -39.96°$. The free beta-3-

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