COMPOUNDS EXHIBITING PROLONGED ANTIOESTROGENIC AND ANTIFERTILITY ACTIVITY IN MICE AND RATS

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Summary. Various triarylalkene derivatives which are weakly oestrogenic and not antioestrogenic at any dosage in conventional tests in mice and rats cause refractoriness to oestradiol for several weeks after injection in vaginal smear tests.

The same compounds, administered in a single injection of 1 mg before mating, caused complete sterility for up to several weeks, although mating still occurred, sometimes at a slower rate than in controls. Daily injections of effective doses (5 to 50 µg) produced similar effects, but reduced mating frequencies more severely.

Oral administration was not followed by refractoriness to oestradiol. When a single dose of 1 mg was fed however, mating was normal but fertility was reduced by up to 85% compared with controls. Daily oral dosing in the vehicle used (50% aqueous propylene glycol) or even of the vehicle alone upset the animals and reduced both mating and fertility.

INTRODUCTION

Since Lerner, Holthaus & Thompson (1958) described MER-25 (1-[*p*-(2-diethylaminoethoxy)phenyl]-2-(*p*-methoxyphenyl)-1-phenylethanol), various triarylethane and triarylethylene derivatives with basic ether groups have been found to possess both antioestrogenic and antifertility activity (see Emmens, 1970, for a recent review). Antioestrogenic activity has usually been detected by administering an oestrogen and potential antagonist simultaneously in uterine weight or vaginal smear tests, and antifertility activity by administering the compounds before or shortly after coitus. For example, MRL-37 (1-[*p*-(2-diethylaminoethoxy)phenyl]-2-(*p*-methoxyphenyl)-1-phenylethane) prevents conception in rodents whether given before, or on Days 1 to 3 or 4 to 6, after coitus (Emmens, Humphrey, Martin & Owen, 1967).

Harper & Walpole (1966) showed that the *cis*- and *trans*- isomers of 1[*p*-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbut-1-ene have different properties. The *cis*-isomer, ICI-47,699, is a potent oestrogen in rats and mice, without antioestrogenic activity at any dosage level, and appears to act as a typical oestrogen in causing failure of implantation when given after coitus. The

trans-isomer, ICI-46,474, is an even more potent oestrogen in the mouse, still not antioestrogenic in that species, and highly potent as an antifertility agent, whereas, in the rat, it is a weak oestrogen, a potent antioestrogen, and also a highly potent antifertility agent after coitus.

These results were largely confirmed by Collins, Hobbs & Emmens (1971) who examined a series of similar compounds, often characterized fully with respect to their stereoisomerism. In general, such compounds do not function as antioestrogens when injected into the mouse, but are weakly oestrogenic, often with a very low slope to the dose-response line, and are highly potent as antifertility agents. Exceptions, however, were found, showing weak subcutaneous antioestrogenicity accompanied in one case by no antifertility action and in the other by fairly potent antifertility activity after coitus. For the most part, these compounds showed no convincing correlation of either oestrogenic or antioestrogenic activity with antifertility properties, when their activities were measured as described above.

It was therefore with some surprise that it was observed that in vaginal smear tests, interference with responses was occurring in animals previously dosed with triarylethylene derivatives. Particularly after subcutaneous administration, the higher doses of some compounds caused refractoriness to oestradiol and other oestrogens, even to the oestrogenic effects of the compound originally administered. This paper gives preliminary results of an investigation into the phenomenon, and into antifertility effects which were found to accompany it.

MATERIALS AND METHODS

Vaginal smear tests were conducted in QS mice or albino rats as described by Emmens (1969), with two injections or feedings in oil or 50% aqueous propylene glycol respectively on the mornings of consecutive days. Smears were taken on the evening of the 3rd day and morning of the 4th day, stained in 5% methylene blue and scored as positive or negative. The total percentage score for each group, usually of ten mice, is used in the Tables, each smear contributing one unit. When oestrogen and an experimental compound were administered simultaneously, each was given by separate injection, or one by injection and one orally.

Fertility tests were conducted by injecting or feeding a single dose of a compound to female mice 3 days before introducing males, with twenty mice per group. The mice were examined daily and segregated on the discovery of a vaginal plug. In other tests, daily injection or oral dosage was started 3 days before introducing the males and continued until mating occurred or for 10 days, whichever was the sooner.

The compounds used in these tests were all triarylalkene derivatives of the compounds shown in Table 1.

RESULTS

Vaginal smear tests

The first substance investigated in some detail was H-774 (*cis*, *trans* mixture,

about 85% *trans*) and the corresponding isomers, H-1067 (*trans*) and H-1071 (*cis*). This experiment started with a vaginal smear test as described above, using the experimental compounds. At 2-week intervals thereafter, further vaginal smear tests were made on the same animals, using a total dose of 0.1 µg of oestradiol-3-17β which, on its own, would give almost 100% of positive responses. The results are shown in Table 2. Responses to the experimental compounds are irregular (Collins *et al.*, 1971), but a high proportion of positive smears indicates the weak and atypical oestrogenicity seen with these compounds in an Allen-Doisy test. Two weeks later, the response to oestradiol was abolished in mice which had received the higher doses of H-774 and H-1067 by injection ($P < 0.01$); the *cis*-compound, H-1071 had a weaker effect. No significant reduction in responses to oestradiol occurred in mice receiving these

TABLE 1
THE COMPOUNDS INVESTIGATED

Compound	R ₁	R ₂	R ₃	R ₄
ICI-46,474	H	C ₂ H ₅	H	(CH ₃) ₂ <i>trans</i>
ICI-47,699	H	C ₂ H ₅	H	(CH ₃) ₂ <i>cis</i>
ICI-45,960	H	C ₂ H ₅	H	(C ₂ H ₅) ₂ <i>cis + trans</i>
ICI-45,692	H	C ₂ H ₅	OCH ₃	(C ₂ H ₅) ₂
H-774	OCH ₃	C ₂ H ₅	OCH ₃	(C ₂ H ₅) ₂ <i>cis + trans</i>
H-1067	OCH ₃	C ₂ H ₅	OCH ₃	(C ₂ H ₅) ₂ <i>trans</i>
H-1071	OCH ₃	C ₂ H ₅	OCH ₃	(C ₂ H ₅) ₂ <i>cis</i> (impure)
H-1075	OCH ₃	C ₂ H ₅	OCH ₃	□ <i>trans</i> ?
H-1076	OCH ₃	CH(CH ₃) ₂	OCH ₃	(C ₂ H ₅) ₂ <i>trans</i> ?

compounds by mouth. The responses to oestradiol were back to normal within 6 weeks in all groups except that receiving the highest dose of H-774, which recovered 8 weeks after the first injection.

Results of a further test are shown in Table 3. In this test, ICI-46,474 and 47,699 were given together with 0.06 µg of oestradiol, all by injection, to illustrate the lack of antagonism on first injection. Subsequently, refractoriness to oestradiol developed, as with H-774. The compounds ICI-45,692 and 45,960 had not been tested before, and so were given with and without oestradiol initially, and did not significantly affect the responses to it. Refractoriness to oestradiol developed 2 weeks after injection, and disappeared by 6 weeks. All results in Table 3 are highly significant ($P < 0.01$ for inhibition by each substance during the test period).

Some of these compounds were tested by injection in spayed rats, with the results shown in Table 4. At the dose levels used, ICI-47,699 was ineffective in

the rat but, as in mice, the other compounds failed to inhibit initial responses to oestradiol, and then caused a refractoriness to it from 2 to 4 weeks after injection, which lasted up to 8 weeks. Peak activity seemed to take rather longer to develop in the rat compared with the mouse.

Two other compounds, tested in the succeeding section because of their high antifertility activity (Collins *et al.*, 1971) which have also caused refractoriness to 0.1 μg oestradiol when tested 3 weeks after injection in mice, are H-1075

TABLE 2
THE EFFECTS OF TWO CONSECUTIVE DAILY ADMINISTRATIONS OF H-774 AND
THE SEPARATED ISOMERS ON THE RESPONSE OF SPAYED MICE TO OESTRADIOL

Compound	Total dose (mg)	Route	% Positive response to 0.1 μg injected oestradiol after			
			2 weeks	4 weeks	6 weeks	8 weeks
H-774 (<i>cis</i> + <i>trans</i>)	0.1	s.c.	100	100	—	—
	0.3	s.c.	20	75	78	100
	0.9	s.c.	0	25	78	78
	2.7	s.c.	5	0	11	78
H-1067 (<i>trans</i>)	0.1	s.c.	85	—	—	—
	0.3	s.c.	5	60	85	—
	0.9	s.c.	10	30	85	—
	2.7	s.c.	0	65	89	—
H-1071 (<i>cis</i>)	0.1	s.c.	100	—	—	—
	0.3	s.c.	80	—	—	—
	0.9	s.c.	95	—	—	—
	2.7	s.c.	25	95	80	—
H-774	0.1	oral	100	95	—	—
	0.3	oral	95	100	—	—
	0.9	oral	85	95	—	—
	2.7	oral	75	85	—	—
H-1067	0.1	oral	85	—	—	—
	0.3	oral	95	—	—	—
	0.9	oral	90	—	—	—
	2.7	oral	65	55	90	—
H-1071	0.1	oral	95	—	—	—
	0.3	oral	80	—	—	—
	0.9	oral	95	—	—	—
	2.7	oral	95	—	—	—
Nil	vehicle	s.c.	85	90	95	—

The oestradiol was given in a vaginal smear test commencing 2, 4, 6 or 8 weeks after the first administration of the compounds. Initially, each group consisted of ten mice.

(responses to oestradiol after 0.1, 0.3, 0.9 and 2.7 mg were 85%, 85%, 100% and 30%, respectively) and H-1076 (responses to the same dose levels were 100%, 85%, 65% and 15%). Neither compound, at the same dose levels, caused refractoriness when given orally.

Fertility tests

Those compounds which have so far been tested as postcoital antifertility agents in the mouse or rat in this laboratory (all but ICI-45,692 and ICI-45,960) are very potent, causing 50% inhibition of pregnancy when given on Days 1 to 3 or 4 to 6 after mating in daily doses of from 1 to 5 μg . It is not surprising,

TABLE 3

THE EFFECTS OF TWO CONSECUTIVE DAILY INJECTIONS OF VARIOUS ICI COMPOUNDS ON THE RESPONSE OF SPAYED MICE TO INJECTED OESTRADIOL

Compound	Total dose (mg)	Dose of oestradiol (μ g)	% Response	% Positive response to 0.1 μ g oestradiol after		
				2 weeks	4 weeks	6 weeks
ICI-46,474	0.3	0.06	65	75	95	95
	0.9	0.06	75	10	65	95
	2.7	0.06	65	5	0	40
ICI-47,699	0.3	0.06	95	72	69	100
	0.9	0.06	100	30	75	80
	2.7	0.06	100	20	20	85
ICI-45,692	0.1	—	60	50	100	95
	0.3	—	67	28	67	100
	0.9	—	80	15	50	78
ICI-45,692	0.1	0.06	78	78	100	100
	0.3	0.06	75	10	65	85
	0.9	0.06	50	10	25	80
ICI-45,960	0.1	—	30	65	100	95
	0.3	—	39	25	63	81
	0.9	—	72	22	31	93
ICI-45,960	0.1	0.06	95	33	61	75
	0.3	0.06	89	39	95	95
	0.9	0.06	65	11	39	95
—	—	0.06	78	93	100	100

The oestradiol was given simultaneously with the ICI compounds or 2, 4, or 6 weeks later as in Table 2. Initially, each group consisted of ten animals.

TABLE 4

THE EFFECTS OF TWO CONSECUTIVE DAILY INJECTIONS OF VARIOUS COMPOUNDS ON THE RESPONSE OF SPAYED RATS TO OESTRADIOL

Compound	Total dose (mg)	Dose of oestradiol (μ g)	% Response	% Positive response to 0.3 μ g oestradiol after			
				2 weeks	4 weeks	6 weeks	8 weeks
H-774	1	—	65	25	0	15	80
	3	—	85	35	0	15	80
	9	—	75	50	0	5	55
H-774	1	0.3	70	15	10	20	75
	3	0.3	75	60	5	50	60
	9	0.3	75	25	0	5	65
ICI-46,474	1	0.3	90	100	70	80	93
	3	0.3	85	55	15	50	83
	9	0.3	95	90	0	20	50
ICI-47,699	0.1	0.3	95	50	100	75	80
	0.5	0.3	100	85	95	100	90
	2.5	0.3	100	85	100	90	80
—	—	0.3	85	—*	100	65	95

The oestradiol was given 2, 4, 6 or 8 weeks after the first administration of the compounds. Initially, each group consisted of ten animals.

* Omitted in error.

therefore, that a dose of 1 mg given by injection before mating should persist in effect, as shown in Table 5. It is interesting that mating was not prevented by this dose in the majority of mice, although its occurrence was delayed in some. No pregnancy followed a mating which occurred within 10 days of injection, while only 15% of matings during the next 10 days were fertile. Orally, the compounds in Table 5 were far less effective, a number of fertile matings occurring shortly after oral dosage.

TABLE 5
THE EFFECTS OF A SINGLE DOSAGE OF VARIOUS COMPOUNDS ON THE OCCURRENCE OF VAGINAL PLUGS AND RESULTING PREGNANCIES IN MICE

Compound		Plugs found and litters resulting during the following 10-day periods after injection:				Total	%
		1 to 10	11 to 20	21 to 30	31 to 40		
H-774 1 mg	Plugs	13	6	1	0	20	100
	Litters	0	2	1	0	3	15
H-1075 1 mg	Plugs	7	6	4	1	18/19	95
	Litters	0	1	3	0	4	21
H-1076 1 mg	Plugs	11	8	0	1	20	100
	Litters	0	0	0	1	1	5
Controls vehicle only	Plugs	19	1	0	0	20	100
	Litters	17	1	0	0	18	90

Compound		Plugs found and litters resulting during the following 10-day periods after oral dosage:				Total	%
		1 to 10	11 to 20	21 to 30	31 to 40		
H-774 1 mg	Plugs	14	3	1	0	18/19	95
	Litters	5	3	1	0	9	47
H-1075 1 mg	Plugs	15	3	0	0	18	90
	Litters	1	2	0	0	3	15
H-1076 1 mg	Plugs	10	8	0	0	18	90
	Litters	2	5	0	0	7	35
Controls vehicle only	Plugs	15	5	0	0	20	100
	Litters	14	5	0	0	19	95

Males were introduced on Day 3 after injection. Initially, each group consisted of twenty mice.

The results of a test, in which injections of 5 μ g or 50 μ g/day were started on Day 1 and mating was allowed from the night of Day 3 were quite different (Table 6). With 5 μ g of H-774/day, the early matings which occurred were fertile, but sterility set in after Day 6. A dose of 50 μ g/day of H-774 reduced mating frequency and all matings were sterile. With H-1075 and H-1076, only one litter was produced in any group, although the mating frequency with the latter compound at 5 μ g/day was not significantly less than in controls.

An attempt to repeat the test in Table 6 by oral administration was unsuccessful, since a daily dose of 0.1 ml of 50% aqueous propylene glycol reduced mating in controls to 30% over the test period, and litters to 15%. The mice swelled

visibly with intestinal gases and then recovered, even with continued administration, but the results of the test were clearly valueless. Further oral testing will be attempted at a later date using a different medium.

TABLE 6

THE EFFECTS OF THE INJECTION OF VARIOUS COMPOUNDS ON THE OCCURRENCE OF VAGINAL PLUGS AND RESULTING PREGNANCIES IN MICE

Compound	Dose ($\mu\text{g/day}$)	Plugs found and litters resulting during the following single days after the start of injections :									
		4	5	6	7	8	9	10	Total	%	
H-774	5	Plugs	4	1	1	9	1	2	0	18	90
		Litters	4	1	1	1	0	0	0	7	35
	50	Plugs	4	0	0	0	3	5	0	12	60
		Litters	0	0	0	0	0	0	0	0	0
H-1075	5	Plugs	4	3	1	2	1	2	0	13	65
		Litters	0	0	0	0	0	0	0	0	0
	50	Plugs	2	3	1	2	1	1	0	10	50
		Litters	0	0	0	0	0	0	0	0	0
H-1076	5	Plugs	10	1	0	3	2	0	0	16/19	84
		Litters	1	0	0	0	0	0	0	1	5
	50	Plugs	2	1	1	2	3	1	1	11	55
		Litters	0	0	0	0	0	0	0	0	0
Controls	0	Plugs	5	2	4	7	1	0	0	19	95
		Litters	5	2	4	6	1	0	0	18	90

Injections were begun on Day 1 and males were introduced on Day 3. Initially, each group consisted of twenty mice.

DISCUSSION

Harper & Walpole (1966) found that ICI-46,474 and ICI-47,699 have anti-fertility activities which, in the mouse, can be explained by their oestrogenic potencies. Collins *et al.* (1971) feel that neither with these compounds, nor with others of the series, can frank oestrogenicity be adequate to explain the high antifertility potencies observed, and that dissociation of these properties is seen in the series. The absence of antioestrogenic properties, as tested both by Harper & Walpole (1966) and by Collins *et al.* (1971), naturally led these authors to neglect such properties as contributing to antifertility effects in the mouse, although ICI 46,474 is strongly antioestrogenic in the rat. The recent suggestion of Emmens & Miller (1969) that at least some types of antioestrogen owe this property to their being short-acting oestrogens which displace others from the site of action and then do not continue to act themselves, would make it feasible that an antifertility compound could well show oestrogenic properties in one species and antioestrogenic properties in another, and yet act as an antifertility agent by the same mechanism in both. Terenius (1970) has now demonstrated that *meso*-butoestrol is a short-acting compound, behaving as an antioestrogen only in local tests or in tests *in vitro*, whereas U-11,100A remains at receptor sites and acts as a systemic antioestrogen.

The effects of triarylalkenes as antifertility agents when administered either immediately or shortly after coitus, and their longer-term effects when given before coitus might well be due to the same mechanism, particularly since high doses were given in the tests showing infertility for 10 or more days. Such doses might well have stayed in the body or at the site of injection for this period. However, if either oestrogenic or anti-oestrogenic activity is, in fact, directly involved, antifertility effects must be supposed to depend on first one and then the other as time goes on. The period of infertility after injection of H-774 lasted for 20 or more days (Table 5), one third or less matings resulting in litters. The period of infertility after oral dosage was quite brief. The periods of anti-oestrogenic activity after injection and oral dosage (Table 2) were similar to those of infertility. Since the route of administration affects both types of result, it seems likely that the compound stays around for longer when injected and gives rise to both effects, whether or not these are due to the same mechanism.

The first problem suggested by these observations is an investigation of the long-term effects of the triarylalkenes on the physiology and morphology of the female reproductive tract. The second is a fuller investigation of the infertility resulting from dosage before mating, with better estimates of its duration and nature. These are both under investigation.

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REFERENCES

- COLLINS, D. J., HOBBS, J. J. & EMMENS, C. W. (1971) Antiestrogenic and antifertility compounds. IV. 1,1,2-triarylalkan-1-ols and 1,1,2-triarylalk-1-enes containing basic ether groups. *J. medicinal Chem.* (in press).
- EMMENS, C. W. (1969) *Estrogens*. In: *Methods in Hormone Research*, Vol. 2A, p. 61. Ed. R. I. Dorfman. Academic Press, New York.
- EMMENS, C. W. (1970) Antifertility agents. *A. Rev. Pharmac.* **10**, 237.
- EMMENS, C. W., HUMPHREY, K., MARTIN, L. & OWEN, W. H. (1967) Antifertility properties of two non-oestrogenic steroids and MRL-37. *Steroids*, **9**, 235.
- EMMENS, C. W. & MILLER, B. G. (1969) Estrogens, proestrogens and antiestrogens. *Steroids*, **13**, 725.
- HARPER, M. J. K. & WALPOLE, A. L. (1966) Contrasting endocrine activities of *cis* and *trans* isomers in a series of substituted triphenylethylenes. *Nature, Lond.* **212**, 87.
- LERNER, L. J., HOLTHAUS, F. J. & THOMPSON, C. R. (1958) A non-steroidal oestrogen antagonist 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2-*p*-methoxyphenyl ethanol. *Endocrinology*, **63**, 295.
- TERENTUS, L. (1970) Two modes of interaction between oestrogen and anti-oestrogen. *Acta endocr., Copenh.* **64**, 47.