ANTIFERTILITY ACTIVITIES IN THE MOUSE AND RAT OF TRIARYLALKENES AND TRIARYLALKANOLS WITH BASIC ETHER GROUPS

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Summary. The postcoital antifertility activity of a series of triarylalkenes and triarylalkanols with basic ether groups has been examined in mice and rats. The triarylalkenes were, in general, more potent than the others, and more potent orally than by injection. Apart from having a greater sensitivity than the mouse, the rat appears to give essentially the same information about relative potency and oestrogenic properties. Most compounds examined were weak to very weak oestrogens of an 'impeded' type, not antioestrogenic in conventional tests, and many had a much higher antifertility potency than could be expected from their oestrogenic activity. The general pattern of results suggests that pre-implantation rather than postimplantation stages are affected, or more readily affected. The most potent compound examined (4h) was effective in the mouse at an injected ED_{50} of $14 \mu\text{g/kg/day}$ after coitus, with an oestrogenic (vaginal smear) ED_{50} of 250 times that dose. Several other compounds were effective at a dosage around $70 \mu\text{g/kg/day}$.

INTRODUCTION

The antifertility activities in rats and mice of a number of the 1,1,2-triarylalk-1-enes and 1,1,2-triarylalkan-1-ols with basic ether groups have been reported by Harper & Walpole (1966), Emmens (1971a, b), Collins, Hobbs & Emmens (1971) and Emmens & Carr (1973). These series of compounds have some members which are very highly potent, particularly as postcoital antifertility agents, and which have some interesting properties as precoital antifertility agents. They vary in oestrogenic and antioestrogenic properties, and in the influence of isomerism on these and on their antifertility activities. Those most active as antifertility agents tend to have atypical, flat dose-response curves as oestrogens, but in general they exhibit much less oestrogenicity than could be expected to account for their antifertility potencies. Few have any marked degree of antioestrogenicity by conventional tests.

The chemical synthesis of fourteen 1,1,2-triarylalk-1-enes and ten 1,1,2-triarylalkan-1-ols was reported by Collins *et al.* (1971), with brief details of the biological activities of some of the twenty-four compounds. Further biologi-

cal data relating to these compounds and to most of the remainder are presented below with some general comments on the two series.

MATERIALS AND METHODS

Albino mice of the QS strain or Wistar-derived rats were used throughout. Females were housed five per box with proven fertile males and examined daily for vaginal plugs. When a plug was found (Day 1), the female was isolated and dosed on Days 1 to 3 or 4 to 6, inclusive; those animals which were pregnant were then allowed to go to term. Approximate values for the ED_{50} derived from such tests are shown in the tables; the ED_{50} is defined as that dose which reduces the number of litters to 50% of control values. The dose-response lines for antifertility activity in such tests are quite steep, covering 10 to 90% in about a fourfold dose range, so although individual limits of error are not presented, as the range of activities in different compounds is so great, the limits would not be beyond 50 to 200%.

In tests of oestrogenic activity by the vaginal smear technique also briefly reported (Emmens, 1969), the low slopes and 'impeded'-type activity of many of these compounds make bioassay difficult and the ED_{50} estimates presented are sometimes quite imprecise. Thus, with the rat, *trans-4l* could only be assigned an ED_{50} within the limits 0.2 to 5.0 mg (Table 3). Nevertheless, the ED_{50} estimates for oestrogenic activity are usually so far removed from the ED_{50} estimates in antifertility tests, that little doubt exists as to the separation of activities. Tests of antioestrogenic activity were against simultaneously injected 17β -oestradiol in vaginal smear assays, using a separate injection site but otherwise conducted as by Emmens (1969).

In all tests, groups of ten were standard and several dose levels of each compound were used. Injection was in 0.05 or 0.1 ml peanut oil/dose, oral administration in 0.05 or 0.1 ml of 25 to 50% aqueous propylene glycol.

The compounds investigated are listed in Table 1. The chemistry of the compounds is discussed in Collins *et al.* (1971) and because of this, they have been given the key numbers used in that paper, together with other reference numbers used within this department or elsewhere and sometimes cited in the literature. Many of the compounds were *cis:trans* mixtures and although some of these were resolved, there were only a few cases in which sufficient material remained afterwards for biological testing on an adequate scale. Where the activity of a compound justified it, a special effort was made to prepare and identify pure isomers, not always successfully.

RESULTS

Results with mice are shown in Table 2. Some results are also included from Collins *et al.* (1971) so as to be available for comparison and discussion below. In all cases, except for a few weakly active compounds such as 3a and 4p, higher dosage was required on Days 4 to 6 than on Days 1 to 3, indicating that established implantation might not be affected, or not be as easily affected as the preimplantation stages. This was confirmed by studies made with 4i and 4j.

Table 1. The compounds tested

Compound	R ₁	R ₂	R ₃	R ₄	Salt
<i>trans</i> -4l (ICI-46,474)	OCH ₂ CH ₂ N(CH ₃) ₂	H	C ₂ H ₅	H	—
<i>cis</i> -4l (ICI-47,699)	OCH ₂ CH ₂ N(CH ₃) ₂	H	C ₂ H ₅	H	HCl
4g ^a (H-1101)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₃	HCl
3l (H-1065)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	OCH ₃	OCH ₃	Citrate
4n (ICI-45,960)	OCH ₂ CH ₂ N(CH ₃) ₂	H	C ₂ H ₅	H	Citrate
4a (H-386)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	H	H	—
3a (MER-25)	OCH ₂ CH ₂ N(CH ₃) ₂	H	CH ₃	OCH ₃	Citrate
4c (H-286)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	CH ₃	H	Citrate
4d ^b (H-282)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	CH ₃	OCH ₃	Citrate
(Isomer 1) 4c ^c (H-1278)	OCH ₂ CH ₂ N(CH ₃) ₂	H	C ₂ H ₅	OCH ₃	—
(Isomer 2) 4c ^d (H-1286)	OCH ₂ CH ₂ N(CH ₃) ₂	H	C ₂ H ₅	OCH ₃	Citrate
3g (H-1274)	OCH ₂ CH ₂ N(CH ₃) ₂	H	C ₂ H ₅	OCH ₃	Citrate
4i ^e (H-774)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₃	Citrate
<i>trans</i> -4l ^f (H-1067)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₃	HCl
<i>cis</i> -4i ^g (H-1438)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₃	HCl
4h (H-1285)	OCH ₂ CH ₂ N(CH ₃) ₂	OH	C ₂ H ₅	OCH ₃	HCl
3i (H-1270)	OCH ₂ CH ₂ N(CH ₃) ₂	OH	C ₂ H ₅	OCH ₃	Citrate
4m (ICI-45,692)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	CH(CH ₃) ₂	OCH ₃	HCl
4j ^h (H-1076)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₃	Citrate
<i>trans</i> -4l (H-1075)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₃	HCl
3h (H-1089)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	CH(CH ₃) ₂	OCH ₃	HCl
4k (H-1291)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	CH(CH ₃) ₂	OCH ₃	—
4o (CN-55945-27)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	NO ₂	OCH ₃	—
3c (H-1010)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	H	OCH ₃	—
4q (H-1378)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₂ CH ₂ N(CH ₃) ₂	HCl
4p (H-1379)	OCH ₂ CH ₂ N(CH ₃) ₂	OCH ₃	C ₂ H ₅	OCH ₂ CH ₂ N(CH ₃) ₂	HCl

^a *cis:trans*, 50:50. ^b *cis:trans*, 55:45. ^{c,d} Two unidentified isomers. ^e *cis:trans*, 62:38. ^f Pure *trans*-4i. ^g 95% pure *cis*-4i. ^h *cis:trans*, 39:61.

With 4i, given in a single oral dose of 100 μ g, pregnancy was terminated in all mice dosed on any day between Days 1 and 4, inclusive, but was maintained in 4/9, 3/9 and 6/10 mice dosed on Days 5, 6 and 7, respectively, and in 6/9 controls. When given by injection, the compound was as active and pregnancy was maintained in 0/10 or 1/10 mice injected on Days 1 to 5, inclusive, and in 2/9 and 4/10 injected on Days 6 and 7, respectively. In the controls, the comparable figure was 6/10. In both cases, control values were lower than usual. With 4j at a dose of 10 μ g/day orally, single doses gave 1, 2, 1, 5, 7, 9 and 6 pregnancies in groups of 10 dosed on Days 1 to 7 as before (controls—8/10). All

Table 2. Biological activities of compounds in the mouse

Compound	Oestrogenic		Anti-oestrogenic (subcutaneous)	Antifertility (per day)			
	Subcutaneous	Oral		Days 1 to 3		Days 4 to 6	
				Subcutaneous	Oral	Subcutaneous	Oral
<i>trans</i> -4l ^a	>250	250	na ^b	2	1	5	10
<i>cis</i> -4l ^a	200	750	na	60	20	100	100
4g	>180	— ^c	—	2	—	6	—
3i	1000	1000	na	6	8	90	50
4n ^a	600	—	na	>18	—	>18	—
4a ^a	>1000	900	500	>1000	—	>1000	—
3a ^a	>1000	—	1000	1000	—	1000	—
4c ^a	1000	200	na	20	—	250	—
4d	300	300	1000	20	—	100	—
(Isomer 1) 4e	125	—	—	20	—	>80	—
(Isomer 2) 4e	10	—	—	6	<1	25	4
3g	>125	—	—	50	—	100	—
4i ^a	2000	200	na	6	<5	50	15
<i>trans</i> -4i	250	300	na	3	2	6	6
<i>cis</i> -4i	10	—	—	10	10	>80	60
4h	100	—	—	0.4	—	1.6	—
3j	200	—	—	2	—	8	—
4m ^a	<100	—	na	>18	—	>18	—
4j	250	1000	na	1.5	—	6	—
<i>trans</i> -4f	250	100	na	3	—	6	—
3h ^a	200	<100	na	50	—	200	—
4k	25	—	—	2	—	8	—
4o ^a	1000	—	na	50	—	100	—
3c	>2700	>2700	na	1000	—	1000	—
4q	1500	—	—	300	<500	300	<500
4p	1600	—	—	150	100	150	100

The biological activity of the compounds tested are given in terms of the ED₅₀ in μ g.

^a Some results are included for comparison from Collins *et al.* (1971).

^b Not antioestrogenic in doses up to 1 mg.

^c Not tested.

of these data indicate a drop in effectiveness at or shortly after implantation on Day 5, which does not occur with more typical synthetic oestrogens (Emmens, 1965a).

Where a comparison was made, no compound showed significantly higher subcutaneous than oral potency as an antifertility agent. This was not true of oestrogenic activity, although the same tendency was seen. Emmens (1965a) found that the daily dose of a typical oestrogen required on Days 1 to 3 or 4 to 6 in the mouse to reduce fertility to 50% of controls is approximately the oestro-

genic ED₅₀ in vaginal smear tests. It is very different with most of these compounds, which do, however, vary considerably. Thus, *cis*-4*l* and 3*h* are not much different from typical oestrogens, whereas *trans*-4*l*, 4*h* and 4*j* show oestrogenic/antifertility ED₅₀ ratios of around 250:1.

Results with the rat are shown in Table 3. They are rather sparse and were

Table 3. Biological activities of compounds in the rat

Compound	Oestrogenic		Antifertility (per day)			
			Days 1 to 3		Days 4 to 6	
	Subcutaneous	Oral	Subcutaneous	Oral	Subcutaneous	Oral
<i>trans</i> -4 <i>l</i> ^a	200 to 5000	200 to 5000	15	15	15	15
<i>cis</i> -4 <i>l</i> ^a	200	250	150	150	150	150
4 <i>d</i> ^a	—	—	10	10	50	<50
(Isomer 2) 4 <i>e</i>	—	—	3	2	>16	8
4 <i>i</i> ^a	>2700	>2700	30	10	100	30
<i>trans</i> -4 <i>i</i>	—	—	3	2	9	3
<i>trans</i> -4 <i>f</i>	—	—	3	1	9	9

The biological activity of the compounds tested are given in terms of the ED₅₀ in µg.

^a Some results included for comparison from Collins *et al.* (1971).

made mainly to check the general situation in comparison with the mouse. The most striking discrepancy is the much greater sensitivity of the rat on a weight-for-weight basis, seen to a lesser extent with potent synthetics like diethylstilboestrol (Emmens, 1965b), and not generally reported for the natural steroids. On a per kilogram basis, the daily mouse dose would be very approximately thirty-five times, and the daily rat dose would be about four times, the doses cited for individuals. Yet 4*e*, *trans*-4*i* and *trans*-4*f* were needed in about the same actual daily dose/animal in rats and mice. Otherwise, the rat shows the same tendencies as the mouse—greater oral sensitivity, a higher dosage needed on Days 4 to 6 than on 1 to 3 (except for 4*l*) and departures from parallelism of oestrogenic and antifertility potencies. It was not considered worth while to do much further work with the rat in view of these similar findings and limited amounts of material.

DISCUSSION

One of the objects of studying series of compounds like those investigated here is to see what structure–activity relationships may be discernible. Oestrogenic and antifertility compounds have proved peculiarly refractory in this regard (cf. Emmens, 1970). However, a few points emerge in the present series, in respect of postcoital antifertility potency: (i) where resolved, *trans*-compounds were more active than *cis* (4*l*, 4*i*); (ii) changes in R₃ led to great differences in potency, from virtual inactivity when R₃ = H (3*a*, 4*a*, 3*c*) to highest activity when R₃ = CH(CH₃)₂ (4*j*, 4*k*—although 4*h* was the most active compound in the series, there was no comparable substance with the CH(CH₃)₂ grouping at R₃ available); (iii) Series 3 compounds were less active than the corresponding

members of Series 4 (3i:4g, 3g:4e, 3j:4h, 3h:4f); (iv) substitution of OCH_3 for H at R_2 or R_4 made little difference; (v) substitution of $(\text{C}_2\text{H}_5)_2$ or $\triangle$ in the ether groups at R_1 for $(\text{CH}_3)_2$ may enhance potency, but there was little difference in effectiveness between the first two groups (*trans*-4i, 4k:4j); (vi) changing the site of the ether group from R_1 to R_4 caused a considerable loss of potency (4p:4i; 4g:4e). The validity of some of these comparisons could be affected by the use of unresolved mixtures of isomers. Oestrogenic activity does not follow any easily discernible patterns.

The antifertility potency of some of these compounds is perhaps up to five or ten times that of any so far reported elsewhere except for frank oestrogens. Gopalchari, Iyer, Kamboj & Kar (1970, 1971) have tested some other very potent compounds which, however, would not seem to be as active as 4h or 4j, although comparisons between results from different sources are difficult to make with any degree of confidence.

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