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Antifertility Activity and Toxicity of α-Chlorohydrin Aromatic Ketal Analogues in Male Rats

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Abstract

The antifertility activity and toxicity of α-chlorohydrin and seven aromatic ketal derivatives were investigated in male rats. At a dose of 5 mg/kg injected intraperitioneally each day for 14 days, α-chlorohydrin and the methoxy benzaldehyde derivative (compound 2) produced complete infertility. The benzaldehyde derivative (compound 1) was 89% effective and the other five compounds 71–25% effective. All compounds except the least effective antifertility agent, the methylbenzaldehyde derivative (compound 3), reduced the motility of sperm recovered from the epididymis. None of the compounds caused a decrease in body or testes weight but some increased adrenal weight.

Introduction

Since α-chlorohydrin possesses many desirable properties as a male contraceptive, but has proved too toxic for human use, numerous studies have been undertaken in an attempt to find analogues which exhibit antifertility activity without the side-effects (Jones 1983).

Fig. 1. (*a*) Basic structure necesssary for antifertility action (Jones 1978).
(*b*) α-Chlorohydrin.
(*c*) Chemical structures of the analogues of α-chlorohydrin tested:
Compound 1: R^1 = H, R^2 = phenyl;
Compound 2: R^1 = H, R^2 = *p*-methoxyphenyl;
Compound 3: R^1 = H, R^2 = *p*-tolyl;
Compound 4: R^1 = H, R^2 = *p*-chlorophenyl;
Compound 5: R^1 = H, R^2 = *p*-nitrophenyl;
Compound 6: R^1 = methyl, R^2 = phenyl.
(*d*) Compound 7: R = $-CH-CH=CH.C_6H_5$ (cinnamaldehyde derivative).

The structural requirements for antifertility activity are very specific, and thus the number of possible modifications limited. The active compound must contain three saturated carbon atoms: a primary carbon atom bearing a chlorine atom,

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adjacent to a carbon atom bearing a secondary hydroxyl group, adjacent to a primary carbon atom bearing a primary hydroxyl group. The hydroxyl groups may be free, esterified or present as ether linkages (Fig. 1) (cf. Jones 1978).

Brown-Woodman *et al.* (1979) found that compound 1 (Fig. 1*c*) was an effective antifertility agent in the male rat and apparently less toxic than α-chlorohydrin. In view of this we have synthesized ketal derivatives of α-chlorohydrin and tested their antifertility activity in male rats.

Materials and Methods

Treatment of Rats

Mature fertile Wistar rats were maintained under regulated conditions of temperature, light and humidity, and were given food and water *ad lib.*

The formulae of α-chlorohydrin and the analogues tested are show in Fig. 1. Each compound was dissolved in propyleneglycol and five male rats were injected subcutaneously daily for 15 days with each analogue, at a dose equivalent to 5 mg α-chlorohydrin per kilogram body weight. A control group of rats received propyleneglycol alone. Two female rats were caged with each male on the seventh day of injection. Male rats were killed and weighed on day 16 and the testes, epididymides, spleen, adrenal and body weights recorded. The vasa deferentia were flushed with 0·5 ml Krebs–Ringer phosphate buffer (pH 7·2) containing 0·3% (w/v) fructose, and the 0·5-ml portions from each rat were pooled. Aliquots (0·2 ml) were added to 1·0 ml formal saline for assessment of sperm concentration using a haemocytometer. Sperm motility was scored on the remainder according to the method of Emmens (1947). The female rats were killed 20 days after mating and checked for pregnancy and the litter size recorded.

Preparation of Ketal Derivatives of α-Chlorohydrin

The ketal derivatives of α-chlorohydrin were prepared from α-chlorohydrin (0·10 mol), the carbonyl compound (0·11 mol), and *p*-toluenesulfonic acid (0·005 mol) in benzene (200 ml). The mixture was refluxed for 24 h (Dean–Stark water separator being used to remove water formed in the reaction), cooled, and extracted with dilute Na_2CO_3. After evaporation of the dried solvent the residue was distilled under reduced pressure.

Thus were prepared the benzaldehyde derivative (compound 1), b.p. 103°C/1·2 mm (Baggett *et al.* 1966); (126–127°/4 mm); the *p*-methoxybenzaldehyde derivative (compound 2), b.p. 128–130°/0·2 mm (found: C, 57·8; H, 5·4; Cl, 15·6. $C_{11}H_{13}ClO_{13}$ requires C, 57·8; H, 5·7; Cl, 15·5%); the *p*-methylbenzaldehyde derivative (compound 3), b.p. 100–104°/0·04 mm (found: C, 62·3; H, 6·3; Cl, 16·9. $C_{11}H_{13}ClO_2$ requires C, 62·1; H, 6·2; Cl, 16·7%); the *p*-chlorobenzaldehyde derivative (compound 4), b.p. 106°/0·15 mm (found C, 51·2; H, 4·6; Cl, 30·8. $C_{10}H_{10}Cl_2O_2$ requires C, 51·5; H, 4·6; Cl, 30·4%); the *p*-nitrobenzaldehyde derivative (compound 5), m.p. 55–61° (found: C, 49·2; H, 4·5; Cl, 14·3. $C_{10}H_{10}ClNO_4$ requires C, 49·3; H, 4·4; Cl, 14·6%); the acetophenone derivative (compound 6), b.p. 84–86°/0·4 mm (found C, 61·9; H, 6·4; Cl, 16·7. $C_{11}H_{13}ClO_2$ requires, C, 62·1; H, 6·2; Cl, 16·7%); the cinnamaldehyde derivative (compound 7), b.p. 140–142°/0·6 mm (found: C, 62·1; H, 6·2; Cl, 16·9. $C_{11}H_{13}ClO_2$ requires C, 62·1; H, 6·2; Cl, 16·7%). All compounds tested were racemic and all of the derivatives of α-chlorohydrin were additionally mixtures of diastereoisomers which could not be separated.

Statistical Analysis

The significance of differences between treatments was examined by analysis of variance and Duncan's multiple-range test (Steel and Torrie 1960). The mean increase in body weight during the experiment for each of the analogues was compared with the controls by a paired *t*-test. The number of fetuses produced per rat was analysed by a Student's *t*-test.

Results

α-Chlorohydrin and all analogues decreased the fertility of the male rats to some extent. Of the analogues, compounds 1 and 2 were the most active (Table 1), and the latter, like α-chlorohydrin, was completely effective. Both the number

of pregnancies and the number of fetuses per litter were less than in the control group, except for compound 3 which did not decrease the litter size. All compounds except compound 3 reduced motility of the epididymal sperm (Table 2). None of the compounds caused an increase in the separation of sperm heads and tails (Table 2).

Table 1. Effect of α-chlorohydrin and α-chlorohydrin analogues on fertility of male rats (five per group) cohabited with females (10 per group)
$*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$

Compound	No. of females pregnant/rats mated	% pregnant/ rats mated	No. of normal fetuses/pregnant rat (mean ± s.d.)
Control	9/9	100	9·6±3·6
α-Chlorohydrin	0/8	0	0***
Compound 1	1/9	11	3***
Compound 2	0/6	0	0***
Compound 3	6/8	75	10·7±4·7
Compound 4	2/7	29	1·5±0·7*
Compound 5	4/10	40	2·8±2·0**
Compound 6	6/9	67	7·6±2·7
Compound 7	5/9	56	3·2±1·8**

No significant change in body weight occurred during the 15 days of injections. α-Chlorohydrin produced a significant decrease in testicular weight compared with the control; however, none of the derivatives affected testicular weight (Table 3). Spleen weight was decreased in all groups except those which received compounds 1 and 3, while adrenal gland weight was unchanged in all rats except those which received compound 7, where an increase was noted (Table 3).

Table 2. Effect of α-chlorohydrin and α-chlorohydrin analogues on epididymal spermatozoa
Values are mean ± s.d.; $n = 5$. Means with a common superscript are not significantly different at the 0·05% level

Compound	10^{-5} × No. of sperm in vas deferens/mg vas: Normal	Separate heads	Separate tails	Motility score
Control	$26{\cdot}2\pm38{\cdot}2^{a}$	$0{\cdot}4\pm0{\cdot}7^{a}$	$2{\cdot}0\pm1{\cdot}5^{a}$	$5{\cdot}9\pm3{\cdot}4^{c}$
α-Chlorohydrin	$15{\cdot}7\pm11{\cdot}7^{a}$	$0{\cdot}1\pm0{\cdot}1^{a}$	$2{\cdot}1\pm1{\cdot}8^{a}$	$1{\cdot}6\pm1{\cdot}0^{ab}$
Compound 1	$65{\cdot}0\pm123{\cdot}0^{a}$	0^{a}	$1{\cdot}4\pm1{\cdot}1^{a}$	$1{\cdot}2\pm0{\cdot}8^{a}$
Compound 2	$36{\cdot}2\pm21{\cdot}4^{a}$	$0{\cdot}1\pm0{\cdot}1^{a}$	$1{\cdot}2\pm0{\cdot}7^{a}$	$2{\cdot}1\pm1{\cdot}3^{ab}$
Compound 3	$27{\cdot}2\pm28{\cdot}2^{a}$	$0{\cdot}2\pm0{\cdot}4^{a}$	$2{\cdot}1\pm2{\cdot}5^{a}$	$4{\cdot}2\pm3{\cdot}8^{bc}$
Compound 4	$18{\cdot}2\pm18{\cdot}1^{a}$	$0{\cdot}4\pm0{\cdot}9^{a}$	$1{\cdot}7\pm2{\cdot}1^{a}$	$1{\cdot}2\pm1{\cdot}1^{a}$
Compound 5	$55{\cdot}6\pm45{\cdot}6^{a}$	$0{\cdot}3\pm0{\cdot}7^{a}$	$2{\cdot}2\pm2{\cdot}3^{a}$	$2{\cdot}0\pm1{\cdot}4^{ab}$
Compound 6	$13{\cdot}2\pm8{\cdot}3^{a}$	$1{\cdot}4\pm3{\cdot}2^{a}$	$1{\cdot}7\pm1{\cdot}6^{a}$	$0{\cdot}5\pm0{\cdot}4^{a}$
Compound 7	$30{\cdot}1\pm13{\cdot}0^{a}$	$0{\cdot}3\pm0{\cdot}4^{a}$	$1{\cdot}7\pm1{\cdot}3^{a}$	$0{\cdot}6\pm0{\cdot}9^{a}$

Discussion

These experiments confirm that it is possible to mask the hydroxyl groups of α-chlorohydrin with ketal groups and still maintain at least a degree of antifertility activity. α-Chlorohydrin itself was completely effective and did not increase adrenal

Table 3. Body weight and organ weights of rats injected with α-chlorohydrin and α-chlorohydrin analogues

Values are mean ± s.d. Organ weights are expressed as mg/100 g body weight (n = 5). Means with a common superscript in any one column are not significantly different at the 0·05% level

Compound	Body weight (g)		Testes	Epididymides	Adrenal glands	Spleen
	Day 0	Day 15				
Control	403 ± 20^{a}	397 ± 18^{a}	$842 \cdot 0 \pm 111 \cdot 5^{b}$	$244 \cdot 8 \pm 25 \cdot 6^{ab}$	$10 \cdot 1 \pm 2 \cdot 8^{a}$	$256 \cdot 0 \pm 51 \cdot 5^{c}$
α-Chlorohydrin	450 ± 26^{ab}	452 ± 26^{abc}	$684 \cdot 0 \pm 102 \cdot 8^{a}$	$211 \cdot 7 \pm 37 \cdot 6^{a}$	$10 \cdot 4 \pm 1 \cdot 1^{a}$	$163 \cdot 0 \pm 26 \cdot 6^{a}$
Compound 1	450 ± 55^{ab}	461 ± 48^{bc}	$720 \cdot 3 \pm 64 \cdot 4^{ab}$	$249 \cdot 8 \pm 22 \cdot 7^{ab}$	$10 \cdot 2 \pm 1 \cdot 9^{a}$	$204 \cdot 9 \pm 35 \cdot 8^{abc}$
Compound 2	475 ± 27^{b}	473 ± 25^{bc}	$777 \cdot 9 \pm 79 \cdot 1^{ab}$	$254 \cdot 5 \pm 23 \cdot 9^{ab}$	$10 \cdot 0 \pm 1 \cdot 1^{a}$	$185 \cdot 2 \pm 44 \cdot 4^{ab}$
Compound 3	420 ± 29^{ab}	416 ± 24^{ab}	$807 \cdot 8 \pm 138 \cdot 2^{ab}$	$260 \cdot 5 \pm 64 \cdot 9^{ab}$	$11 \cdot 7 \pm 1 \cdot 8^{a}$	$239 \cdot 3 \pm 69 \cdot 6^{bc}$
Compound 4	430 ± 76^{ab}	424 ± 77^{ab}	$716 \cdot 2 \pm 77 \cdot 1^{ab}$	$254 \cdot 3 \pm 31 \cdot 9^{ab}$	$11 \cdot 5 \pm 2 \cdot 0^{a}$	$183 \cdot 9 \pm 20 \cdot 7^{ab}$
Compound 5	475 ± 26^{b}	464 ± 48^{bc}	$752 \cdot 8 \pm 142 \cdot 2^{ab}$	$247 \cdot 4 \pm 31 \cdot 4^{ab}$	$11 \cdot 3 \pm 1 \cdot 3^{ab}$	$192 \cdot 8 \pm 44 \cdot 5^{ab}$
Compound 6	479 ± 75^{b}	493 ± 62^{c}	$779 \cdot 4 \pm 98 \cdot 7^{ab}$	$253 \cdot 5 \pm 27 \cdot 9^{ab}$	$11 \cdot 6 \pm 1 \cdot 7^{a}$	$190 \cdot 9 \pm 32 \cdot 1^{ab}$
Compound 7	458 ± 36^{ab}	456 ± 33^{abc}	$781 \cdot 4 \pm 70 \cdot 1^{ab}$	$270 \cdot 3 \pm 52 \cdot 4^{b}$	$12 \cdot 3 \pm 1 \cdot 7^{b}$	$191 \cdot 8 \pm 20 \cdot 8^{ab}$

weight at the low dose in this experiment, in contrast to the increase following a single injection of 90 mg/kg (Brown-Woodman and White 1975).

The benzaldehyde derivative (compound 1) was again found to be highly effective (89%) in reducing the fertility of male rats although it did not completely abolish fertility as was the case in the previous trial (Brown-Woodman *et al.* 1979). As there was again no increase in adrenal weight, no loss of body weight, and no change in spleen weight, the compound is possibly non-toxic. Stress or injection of toxic substance are known to cause enlargement of the adrenals and atrophy of the spleen (Selye 1976).

Substitution of a methoxy ($-OCH_3$) group in the para position of the benzene ring of compound 1 apparently increased antifertility activity as compound 2 was 100% effective, but only six of the 10 rats mated. This may reflect reduced libido or some degree of toxicity, as although body and adrenal weights were not affected by the compound, spleen weight was significantly decreased. Addition of a methyl group to the benzene ring (compound 3) greatly reduced antifertility activity and the harmful effect on epididymal sperm motility. The compound was also apparently less toxic than the methoxy derivative (compound 2) since there was no increase in adrenal weight, and no change in spleen weight. Substitution with chloro (compound 4) and nitro (compound 5) groups in the benzene ring did not reduce antifertility activity nearly as much as methyl group substitution; compound 5, however, may be toxic as the weight of the spleen was decreased. Substitution of a methyl group in the carbon link (compound 6) of the ketal group or lengthening the carbon link (compound 7) also reduced antifertility activity and presumably increased toxicity as judged by the decreased spleen weights observed in the rats receiving both compounds, and the increased adrenal weight seen with compound 7.

We had hoped that increasing the lipophilic nature of the α-chlorohydrin derivatives might have enhanced uptake by the epididymis and this, followed by slow release into the blood stream, would reduce the toxicity of the compounds compared to α-chlorohydrin, without impairing antifertility activity. However, this does not appear to be the case.

It is not known whether these ketal derivatives exert their antifertility activity as such or after degradation to α-chlorohydrin, although the decrease in activity observed in the series reflects expected ease of hydrolysis to the starting carbonyl compounds and α-chlorohydrin and we believe that α-chlorohydrin is the active compound. Some support for this was obtained by *in situ* experiments. Thus the ketals were heated at 37°C at pH 7·4 (KH_2PO_4–Na_2HPO_4 buffer) and the mixtures were analysed by g.l.c. for the presence of the free carbonyl component and α-chlorohydrin. Compound 2 was 5% hydrolysed after 24 h, compound 1 was 1% hydrolysed after 24 h, whilst no hydrolysis products were observed under these conditions for compounds 3–7. The fact that these compounds were relatively inert under these near-physiological conditions is not surprising since ketal hydrolyses generally become rapid around pH 3; on the other hand their resistance to hydrolysis at pH 7·4 *in situ* does not necessarily indicate resistance to hydrolysis *in vivo* where enzymatic catalysis may occur.

Jones and O'Brien (1980) studied the metabolism by the rat of two ketal derivatives which had been shown previously to have antifertility activities in the rat comparable to that of α-chlorohydrin (Banik *et al.* 1972; Hirsch *et al.* 1975). Both compounds

produce β-chlorolactate, the major oxidative metabolite of α-chlorohydrin, indicating that these ketal derivatives are degraded to α-chlorohydrin. The antifertility effectiveness of the compounds tested in the present experiment may therefore, reflect their ease of degradation to α-chlorohydrin.

The reduction in epididymal sperm motility produced by α-chlorohydrin and all the analogues apart from compound 3 was not unexpected in view of the data that the primary effect of α-chlorohydrin is directed towards the sperm in the epididymis and it produces biochemical changes in the organ (Crabo and Appelgren 1972; Brown-Woodman and White, 1975, 1976; Lobl 1980; Tsang *et al.* 1981; Paz and Homonnai 1982). Low doses of α-chlorohydrin do not affect the weight or ultrastructure of the epididymis (Reijonen *et al.* 1975) and thus the fact that none of the derivatives produced change in epididymal weight was not surprising. Compounds 1 and 2 would seem to warrant further investigation as possible male antifertility agents.

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