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# Antifertility Effects of Chlorine-Substituted Dioxolanes, Dithiolanes, and Dithianes in Male Rats

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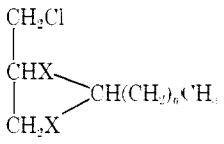
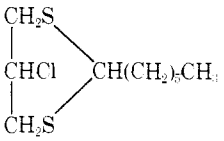
Interest in male antifertility agents was renewed by the disclosure that 1-chloro-2,3-propanediol (1) exerted a posttesticular antifertility effect in male rats.[1,2] Subsequent findings indicated similar effectiveness in monkeys,[3] guinea pigs,[4] rams,[5] and swine.[6] Recently, Banik, *et al.*,[7] have shown that 4-(chloromethyl)-2-methyl-2-pentyl-1,3-dioxolane induced sterility in adult male rats with no interference with mating nor any apparent irreversible effects. This report describes the synthesis and biological activity of two acetals **2** and **3** of 1-chloro-2,3-propanediol and two thioacetals **4** and **5** of 1-chloro- and 2-chlorodimercaptopropane.

The compounds were prepared by reaction of the glycol or dithiol,[8] *p*-TsOH, and the corresponding aldehyde in benzene. We were able to isolate 2-pentyl-2-nonenal, presumably formed *via* aldol condensation of heptanal, during the preparation of **5**.



**2**, $n = 5$; X = O
**3**, $n = 16$; X = O
**4**, $n = 5$; X = S



**5**

**Biological Activity.** The antifertility activity was evaluated in adult male Wistar and Sprague–Dawley rats (250–300 g). The compounds were dissolved or suspended in propylene glycol and administered either orally or subcutaneously for 14 consecutive days with controls receiving an equal quantity of the vehicle only. On the last day of treatment, each male was individually cohabited with a proestrus female. Vaginal washings were checked the following morning for evidence of positive mating and those males failing to mate were given another opportunity the following night. After the mating, all males were autopsied for examination of the testes, epididymides, and accessory sex organs. All females were autopsied and examined for pregnancy (implantation sites) 14 days after cohabitation.

Compounds **1** and **2** were both effective antifertility agents when given orally or subcutaneously to male rats (Table I). However, **2** appeared to have some therapeutic advantage since its minimum effective oral antifertility dose was similar to that for **1** and no apparent toxic side effects were observed at doses as high as 500 mg/kg whereas compound **1** caused extensive weight loss and deaths at 100 mg/kg. The $LD_{50}$ values (mouse, ip, 48 hr) for compounds **1** and **2** were 73 and >1000 mg/kg, respectively. The longer chain acetal **3** was active orally and subcutaneously but at higher dose levels. Thioacetals **4** and **5** did not exhibit any antifertility activity at the doses tested.

Epididymal cysts and antispermatogenic effects were observed in a few of the rats which received the higher doses of compounds **1** and **2**. This has been previously reported[2,4,7] and appears to occur only in rats. No epididymal cysts or abnormal effects on the testes were observed in males receiving compounds **3**, **4**, and **5**. The sex accessory organs were normal in all animals which is consistent with other reports[2,7] that compounds in this series do not cause androgenic or antiandrogenic effects.

Our findings substantiate the previously reported male antifertility activity of compound **1** and 2-substituted-4-(chloromethyl)-1,3-dioxolanes. We have also found that dithiolane **4** and dithiane **5** are devoid of biological activity at doses comparable to their oxygen analogs.

## Experimental Section

Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Analytical results are within ±0.4% of the calculated values. Ir spectra were recorded on a Beckman IR-8 as neat samples and nmr spectra were determined on a Varian A-60 spectrometer using $CDCl_3$ as solvent with trimethylsilane as internal standard. The ir and nmr data of all compounds were consistent with the proposed structures.

**2-Heptadecyl-4-chloromethyl-1,3-dioxolane (3).** A solution of 5.5 g (0.05 mol) of 1-chloro-2,3-propanediol, 13.9 g (0.05 mol) of octadecyl aldehyde (prepared from the bisulfite),[9] and 100 ml of benzene was refluxed in a Dean Stark apparatus for 15 min. Then 200 mg of *p*-TsOH was added and the solution azeotroped for an additional 4 hr. The solution was washed with 10% aqueous $Na_2CO_3$ and $H_2O$, dried ($MgSO_4$), and concentrated. Column chromatography of the residue on Silic AR, CC-7, eluting with ethyl acetate–hexane (1:3) and distillation of the crude product gave **3** (9.8 g, 54%): bp 144–150° (0.001 mm). *Anal.* ($C_{21}H_{41}ClO_2$) C, H, Cl.

**2-Hexyl-4-chloromethyl-1,3-dioxolane (2).** This compound was prepared in the same manner as **3** and afforded **2**: bp 82° (0.4 mm) [lit.[10] 123° (14 mm)].

**2-Hexyl-4-chloromethyl-1,3-dithiolane (4).** To an azeotropically distilled mixture of 14.4 g (0.1 mol) of heptanal, 100 ml of benzene, and a catalytic amount of *p*-TsOH was added 14.5 ml (0.1 mol) of 1-chloro-2,3-dimercaptopropane.[11] When the theoretical amount of water was collected the solution was washed with saturated aqueous $K_2CO_3$ and $H_2O$, dried ($K_2CO_3$), and concentrated. The residue was purified by an initial distillation (bp 118–121°, 0.1 mm), followed by column chromatography through Silic AR, CC-7, using ethyl acetate–hexane (5:95) as the eluent and a final distillation through a short-path distillation apparatus to afford **4**: 3.0 g (12%). *Anal.* ($C_{10}H_{19}ClS_2$) C, H, Cl, S.

**Table I.** Fertility of Male Rats Treated for 14 Days with Various Chlorine-Substituted Dioxolanes, Dithianes, and Dithiolanes

| Compd | Dose, mg/kg | No. males co-habited | No. mated | No. females pregnant | Total implants | No. implants per pregnancy[a] |
|---|---|---|---|---|---|---|
| Propylene glycol | | 25[b,c] | 23 | 21 | 265 | 12.6 ± 0.6 |
| **1** | 5 po | 10[b] | 9 | 1 | 2 | 2.0 |
| | 5 sc | 5[b] | 4 | 0 | 0 | 0.0 |
| | 15 po | 13[b] | 10 | 2 | 2 | 1.0 ± 0.0 |
| | 15 sc | 5[b] | 4 | 0 | 0 | 0.0 |
| **2** | 5 po | 5[b] | 2 | 1 | 11 | 11.0 |
| | 15 po | 5[b] | 4 | 0 | 0 | 0.0 |
| | 15 sc | 5[b] | 4 | 2 | 32 | 16.0 ± 2.0 |
| | 50 po | 5[b] | 4 | 1 | 1 | 1.0 |
| | 50 sc | 5[b] | 4 | 0 | 0 | 0.0 |
| **3** | 80 po | 5[c] | 5 | 1 | 2 | 2.0 |
| | 100 sc | 4[b] | 3 | 3 | 11 | 3.7 ± 1.5 |
| **4** | 80 po | 5[c] | 5 | 5 | 51 | 10.2 ± 4.1 |
| **5** | 80 po | 5[c] | 5 | 5 | 60 | 12.0 ± 4.6 |

[a] Mean ± standard error. [b] Wistar. [c] Sprague-Dawley.

**5-Chloro-2-hexyl-1,3-dithiane (5).** The same procedure was followed as with 4 except an excess of heptanal (0.18 mol as compared to 0.12 mol of 2-chloro-1,3-dimercaptopropane[11]) was employed. Purification was achieved by column chromatography on Silic AR, CC-7, using ethyl acetate–hexane (1:99) as eluent affording first compound 5 and then 4.6 g of 2-pentyl-2-nonenal. The chromatographed compound 5 was further purified by distillation affording 5 (2.45 g, 9%): bp 89.5° (0.1 mm). *Anal.* ($C_{10}H_{19}ClS_2$) C, H, Cl, S.

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# Triphenylmethane Dyes as Inhibitors of Reverse Transcriptase, Ribonucleic Acid Polymerase, and Protein Synthesis. Structure–Activity Relationships

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The discovery of RNA-directed DNA polymerase (reverse transcriptase) activity in RNA tumor viruses[1,2] stimulated an intensive search for inhibitors of this enzyme. It was hoped that such inhibitors might lead to synthesis of drugs that would be useful in the chemotherapy of viral disease and cancer.[3] The anionic triphenylmethane dyes described in this paper represent a class of compounds whose activity against reverse transcriptase has not previously been reported. The structure–activity relationships presented represent a potential starting point for development of new chemotherapeutic agents.

The prototype inhibitor in the triphenylmethane series is aurintricarboxylic acid (ATA, 1). This dye blocks initiation of protein synthesis in cell-free extracts prepared from bacteria or animal cells[4-6] and the related compound, gallin (5), inhibits activity of *Escherichia coli* RNA polymerase.[7] In this paper, we show that these dyes and their analogs are potent inhibitors of a reverse transcriptase prepared from Rauscher leukemia virus. The same compounds were also tested as inhibitors of RNA polymerase activity and of protein synthesis and for their capacity to prevent formation of a DNA–RNA polymerase complex. The essential structure–activity relationships in each of these experimental systems are similar. We also report that Congo Red, ethidium bromide, and 2,6-dimethyl-4-benzyl-4-demethylrifampicin (AF/ABDP)† inhibit protein synthesis in lysates prepared from rabbit reticulocytes.

Structures of dyes used in these experiments are indicated in Figure 1 and Table I. Compounds 1–9 inhibit activity of both polymerases, block formation of the complex between DNA and RNA polymerase, and prevent synthesis of globin (Table II). The most active inhibitors in the triphenylmethane series (1–8) inhibit reverse transcriptase activity by 50% at concentrations of 1–2 $\mu M$. This concentration is equal or lower than that previously reported for inhibition of this enzyme by compounds 16–18.[8-10] ATA (1) has also been shown to inhibit a highly purified preparation of avian myeloblastosis reverse transcriptase, primed by partially degraded thymus DNA or avian myeloblastosis RNA.[11]

Among triphenylmethane dyes tested, 9 is generally less inhibitory than 1–8. Aurin (12) retained some activity as an inhibitor of globin synthesis; otherwise, 10–13 were essentially inactive as inhibitors in all four assays. Compounds 14–17. whose inhibitory activity against reverse

† The nomenclature used is that of Gruppo LePetit.