rescence detector. Then norethindrone would be added as an internal standard for methyltestosterone, followed by HPLC, which permits detection of all components and quantitative measurement of methyltestosterone. This procedure was carried out using a commercial capsule dosage form and yielded results of 100.4 and 100.1% of methyltestosterone.

The two chromatograms in Fig. 4 demonstrate another use of the analytical system described in this work. The high sensitivity of the fluorescence detector in measuring dansyl estrogens allows the monitoring of impurities of one estrogen in another. The chromatograms in Fig. 4 are measurements of estradiol (at the 1% level) in estradiol valerate and $\alpha$-estradiol (1% added as an impurity) in estradiol. The USP (9) has a limit of 1% estradiol in estradiol valerate, whereas the NF (10) has a specification of 3% foreign steroids and other impurities in estradiol.

The results of the analysis of 11 different pharmaceutical dosage forms by the proposed procedure are presented in Table II. The values obtained are within 2% of each other, except for the ethinyl estradiol coated tablets. As a test of the accuracy of the method, a sesame oil solution of estradiol was prepared and assayed. Results of 99.0 and 99.6% estradiol indicated that the procedure was accurate to within 1% for this dosage form.

For comparison, analyses of all but two of the dosage forms were carried out by alternative procedures (given in the footnotes to Table II). The agreement between members in pairs of results was within an average of less than 2%, but three pairs of values differed by more than 2%. In each case, the alternative method average values were lower than the HPLC method. The greatest difference was observed for the lower dosage ethinyl estradiol (0.01 and 0.05 mg) dosage forms.

The AOAC method (footnote *h* of Table II) was collaboratively studied for antifertility tablets and gave results comparable to the HPLC method with norethindrone acetate and ethinyl estradiol tablets. With the lower dosage ethinyl estradiol dosage forms, the variation was wider. In two instances (footnote *i* in Table II), the AOAC method was modified in that chloroform was replaced by benzene and the chromogenic reaction was carried out in benzene. In both cases, somewhat higher results, closer to the HPLC results, were achieved. The alternative method (footnote *l* in Table II) consisted of extracting the estrone aqueous suspension with a chloroform solution of estradiol valerate (internal standard), followed by filtration and direct injection for HPLC (UV detection) analysis. Good agreement was observed with the HPLC procedure.

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# Synthesis and Evaluation of Sulfur-Containing Steroids against Methylmercuric Chloride Toxicity


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Received March 9, 1978, from the *Departments of *Biochemistry and ‡Biophysics, Indiana University School of Medicine,* and the §*Veterans Administration Hospital, Indianapolis, IN 46202.* Accepted for publication May 10, 1978. ¶Present address: Department of Physiological Chemistry, College of Medicine, University of Baghdad, Baghdad, Iraq.



**Abstract** □ Sulfur-containing steroids, analogs, and derivatives were synthesized for evaluation in mice suffering acute toxicity from methylmercuric chloride. Steroids were administered by intraperitoneal injection, by stomach tube feeding, or by absorption through the tail skin. Thiocholesterol and the thiocholanoic acids were effective if given prior to poisoning. The thiosteroids were significantly more effective than penicillamine or dimercaprol under these conditions.



**Keyphrases** □ Sulfur-containing steroids—synthesized, evaluated in treatment of methylmercuric chloride toxicity in mice □ Steroids, sulfur containing—synthesized, evaluated in treatment of methylmercuric chloride toxicity in mice □ Methylmercuric chloride toxicity—treatment by various sulfur-containing steroids evaluated in mice □ Chelating agents—various sulfur-containing steroids synthesized, evaluated in treatment of methylmercuric chloride toxicity in mice □ Structure–activity relationships—various sulfur-containing steroids evaluated in treatment of methylmercuric chloride toxicity in mice


Heavy metal poisoning traditionally is treated with chelating agents such as penicillamine, dimercaprol, and ethylenediamine tetraacetic acid, which are thought to combine with the metal and facilitate rapid elimination through the kidneys. Because the effectiveness of these agents frequently is limited by toxicity, novel chemotherapeutic agents of low toxicity are needed.

To minimize the burden placed on the kidney by heavy metal poisoning, such agents could include sulfhydryl-type chelators with lipophilic character that might form more nearly irreversible complexes with the heavy metal and be eliminated by other than the urinary route. Sulfhydryl analogs of bile components, specifically mercapto analogs of cholesterol and the bile acids, are such candidates.

The protective action of thiocholesterol (I*a*) and 3$\beta$-mercapto-5$\beta$-cholanic acid (VII*a*) against lethal doses of methylmercuric chloride was reported recently (1). Spironolactone (III) protects against mercuric chloride toxicity in rats (2) and mice (3). The present report describes continuing efforts to test other sulfur-containing steroids and their esters and analogs as therapy for methylmercuric chloride intoxication.

## EXPERIMENTAL

**Steroids**—Thiocholesterol was converted to its acetate (I*b*) (4) and


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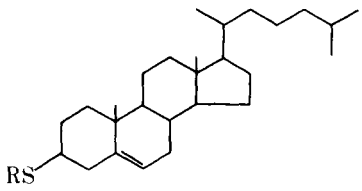
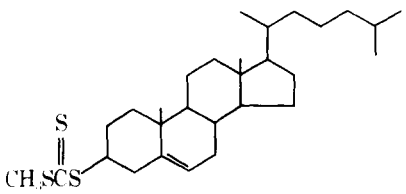
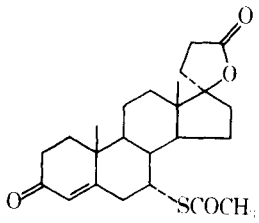


Ia: R = H
Ib: R = $CH_3CO$



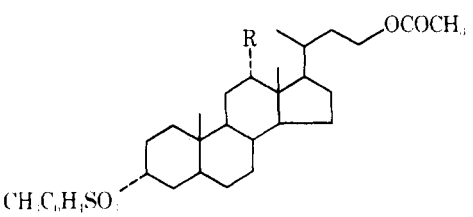
II



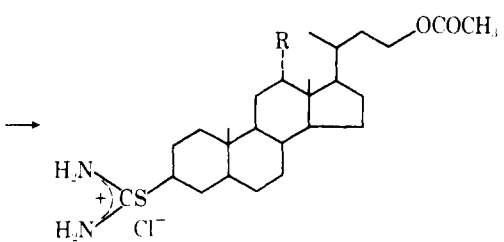
III

to the trithiocarbonate (II), the latter by treatment of the sodium salt of Ia with carbon disulfide and then methyl iodide, following the procedure for converting cholesterol to its xanthate (5). 3β-Mercapto-5β-cholan-24-oic acid (VIa) and 12α-hydroxy-3β-mercapto-5β-cholan-24-oic acid (VIb) were prepared (Scheme I) from the 3-tosylates (IVa and IVb) (6) *via* the intermediate isothiouronium salts (Va and Vb), as in the synthesis of thiocholesterol (7). Ethyl 17β-acetoxy-3-oxo-5α-androstan-2α-ylxanthate (VII, Table I) was prepared from 17β-acetoxy-2α-bromo-5α-androstan-3-one[1] (8), following the conditions for the synthesis of 2α-mercapto-5α-cholestan-3-one (9). Two additional xanthates were synthesized similarly, ethyl 11β,17α-dihydroxy-3,20-dioxo-4-pregnen-21-ylxanthate (VIII) from cortisol mesylate (10) and ethyl 17-oxo-5α-androstan-3α-ylxanthate (IX) from epiandrosterone tosylate (11).

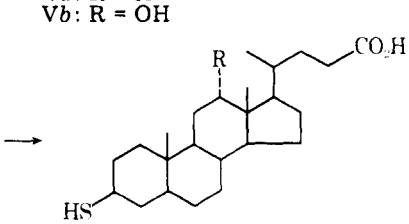
Cholesteryl thiocyanate (X) was prepared from cholesteryl tosylate



IVa: R = H
IVb: R = OH

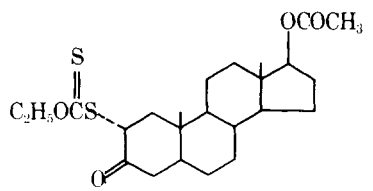


Va: R = H
Vb: R = OH

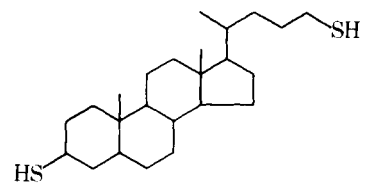


VIa: R = H
VIb: R = OH

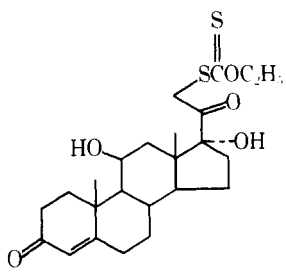
*Scheme I*



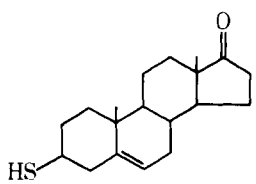
VII



XII



XIII



XVI

and potassium thiocyanate (5). 3-Cholestanone was converted to its thiosemicarbazone (XI) by standard means. Methyl lithocholate was reduced to 5α-cholane-3β,24-diol (12), the ditosylate of which was treated with thiourea; alkaline hydrolysis of the bisisothiouronium salt gave 5α-cholane-3β,24-dithiol (XII) in a sequence analogous to the preparation of VIa and VIb. Deoxycorticosterone tosylate (13) and potassium thioacetate (14) reacted to give 21-acetylthio-4-pregnene-3,20-dione (XIII), and 2α-bromo-5α-cholestan-3-one was similarly converted into 2α-acetylthio-5α-cholestan-3-one (XIV). Physical constants of all new compounds are reported in Table I. 3α-Mercapto-5α-androstan-17-one (XV) (11, 15, 16) and 3β-mercapto-5-androsten-17-one (XVI) (17–19) were prepared *via* the corresponding isothiouronium intermediates.

Toxic effects of the steroids themselves were investigated beyond 14-day survival only for thiocholesterol. Mice were fed or injected twice weekly with 400 mg of thiocholesterol/kg for 2 months without showing any obvious ill effects.

**Liposomes**—In a typical preparation, 500 mg of lecithin (L-α-phosphatidyl choline[2]) and 200 mg of the steroid were dissolved in 10 ml of acetone and 4 ml of chloroform, with warming if necessary. The solution was diluted with 10 ml of warm water, producing a milky suspension, which was concentrated[3] slowly to 2 ml. Occasionally, a second phase separated; it could be homogenized, however, by trituration. The resulting syrup was diluted to 4 ml with water and kept in the refrigerator. A 25-g mouse received 0.2 ml of the liposomal preparation containing 10 mg of steroid (400 mg/kg), unless otherwise indicated (Tables II–IV).

**Suspensions**—For the suspensions used in Experiment 2, a mixture of 200 mg of steroid, 20 mg of cholic acid, 4 drops of polysorbate 80, and 1 ml of corn oil was diluted to 4 ml with water and mixed in a tissue grinder. The suspensions used in Experiment 5 were similar, but they lacked corn oil and cholic acid.

For Experiment 8, 250 mg of steroid was dissolved in 1 ml of 1% methylcellulose solution, except with thiocholesteryl acetate because its limited solubility allowed a suspension of only 125 mg/ml. For Experiment 9, the spironolactone suspension was diluted 1:5 and the thiocholesteryl acetate suspension was diluted 2:5.

**Methylmercuric Chloride**—Crystalline methylmercuric chloride[4] was dissolved in warm water at a concentration of 1 mg/ml.

**Mice**—Male Swiss-Webster mice, 25 ± 4 g, were weighed daily. The amounts of methylmercuric chloride or steroid to be administered were calculated for each mouse from the initial weight, except for the results in Table IV, where the amount of methylmercuric chloride injected on

[1] This sample, prepared by bromination of 17β-acetoxy-5α-androstan-3-one in glacial acetic acid, had the same properties as the product prepared by bromination with *N*-bromosuccinimide and hydrofluoric acid (8).

[2] Sigma.
[3] Rotovap.
[4] R.E.L. Laboratories, Plainfield, N.J.

**Table I—Sulfur-Containing Steroids and Their Analogs**

| Compound | Yield, % | Melting Point | IR[a], $cm^{-1}$ | Formula | Analysis[b], % | Calc. | Found |
|---|---|---|---|---|---|---|---|
| II | 63 | 129–131°[c] | 1062, 958, 816 | $C_{29}H_{48}S_3$ | C | 70.66 | 70.54 |
| | | | | | H | 9.81 | 9.70 |
| | | | | | S | 19.51 | 19.72 |
| Va | 86 | 260–261°[d] | 3315 and 3100 (NH), 1739 (C=O), 1655 (C=N), 1220, 1170 ($SO_3$), 1120, 1038, 1012, 820, 678 | $C_{33}H_{52}N_2O_5S_2$ | C | 63.84 | 63.54 |
| | | | | | H | 8.44 | 8.25 |
| | | | | | N | 4.51 | 4.42 |
| | | | | | S | 10.33 | 10.27 |
| Vb | 75 | 250–251°[d] | 3580, 3555, and 3450 (OH), 3300 and 3190 (NH), 1720, 1170 ($SO_3$), 1120, 1036, 1012, 820, 680 | $C_{33}H_{52}N_2O_6S_2$ | C | 62.23 | 62.11 |
| | | | | | H | 8.23 | 8.43 |
| | | | | | N | 4.40 | 4.19 |
| | | | | | S | 10.07 | 9.72 |
| VIa | 71 | 143–149°[e] | 3400–3200 (OH), 1712 (C=O), 1275, 1100, 720 | $C_{24}H_{40}O_2S \cdot 1\frac{1}{2} CH_3OH$ | C | 69.50 | 69.75 |
| | | | | | H | 10.52 | 9.72 |
| | | | | | S | 7.27 | 7.07 |
| VIb | 67 | 99–107°[d] | 3570–3240 (OH), 1690 (C=O), 1280, 1030 | $C_{24}H_{40}O_3S \cdot H_2O$ | C | 67.88 | 68.01 |
| | | | | | H | 9.97 | 9.83 |
| | | | | | S | 7.55 | 7.59 |
| VII | 68 | 143–144°[f] | 1738 and 1718 (C=O), 1260, 1220 (C=S), 1060, 908 | $C_{24}H_{36}O_4S_2$ | C | 63.68 | 63.82 |
| | | | | | H | 8.02 | 7.94 |
| | | | | | S | 14.17 | 13.97 |
| VIII | 90 | 196–197°[c] | 3480 and 3440 (OH), 1700 (C=O), 1638, 1220 (C=S), 1110, 1054, 870 | $C_{24}H_{23}O_5S_2$ | C | 61.77 | 62.05 |
| | | | | | H | 7.34 | 7.47 |
| | | | | | S | 13.74 | 14.09 |
| IX | 37 | 145–147°[d] | 1728 (C=O), 1210, 1043, 924 | $C_{22}H_{34}O_2S_2$ | C | 66.96 | 66.67 |
| | | | | | H | 8.68 | 8.47 |
| | | | | | S | 16.25 | 16.20 |
| XI | | 204–206°[g] | 3210 and 3140 (NH), 1584, 1500, 1302, 1092, 878 | $C_{28}H_{47}N_3S$ | C | 73.46 | 73.61 |
| | | | | | H | 9.18 | 9.03 |
| | | | | | N | 10.35 | 10.17 |
| | | | | | S | 7.00 | 6.93 |
| XII | 10 | 88–92°[h] | 2600 (SH), 720 | $C_{24}H_{42}S_2$ | C | 73.02 | 73.12 |
| | | | | | H | 10.72 | 10.65 |
| | | | | | S | 16.25 | 16.19 |
| XIII | 80 | 170–172°[c] | 1708 (20 C=O), 1662 (3 C=O and acetyl C=O), 1610 (C=C), 1278, 1230, 1078, 962, 722 | $C_{23}H_{32}O_3S$ | C | 71.09 | 71.27 |
| | | | | | H | 8.30 | 8.15 |
| | | | | | S | 8.25 | 8.47 |
| XIV | 58 | 117–118°[c] | 1730 and 1698 (C=O), 1148, 1120, 964 | $C_{29}H_{48}O_2S$ | C | 75.64 | 75.37 |
| | | | | | H | 10.50 | 10.50 |
| | | | | | S | 6.96 | 6.93 |

[a] IR spectra were taken as mineral oil mulls on a Perkin-Elmer spectrophotometer, model 247. [b] Analyses by Galbraith Laboratories, Knoxville, Tenn. [c] Recrystallized from acetone. Melting points were taken on a Hoover-Thomas apparatus and are corrected. [d] Recrystallized from methanol–water. [e] Dissolved in methanolic sodium hydroxide and precipitated with hydrochloric acid. [f] Recrystallized from acetone–water. [g] Recrystallized from dimethylformamide, methanol, and water. [h] Recrystallized from methanol–acetone. The yield of dithiol was 10% from the 3,24-diol; a 6% yield of a monothiol also was obtained, tentatively identified as 5β-chol-3-ene-24-thiol, mp 64–65° (from acetone). *Anal.*—Calc. for $C_{24}H_{40}S$: C, 79.93; H, 11.18; S, 8.89. Found: C, 79.83; H, 11.15; S, 8.69.

Day 3 was calculated from the mouse weight on that day. Mice receiving the steroid by stomach tube were first deprived of food and water for 1 hr; otherwise, they received standard mouse feed and water *ad libitum*.

Male Swiss-Webster albino mice[5] were not constant in their response to methylmercuric chloride poisoning during this investigation (24 months). Therefore, controls were included with each animal study. Dose–response studies for methylmercuric chloride were carried out during June 1976, May 1977, and October 1977 with approximately 100 animals each time. Determinations of the $LD_{50}$ for the three dates were 11.3 ± 0.7, 15.1 ± 1.3, and 17.5 ± 1.8 mg/kg, respectively; the $LD_{95}$ values were 16.3 ± 2.0, 33.1 ± 3.5, and 29.4 ± 2.5, respectively. All observations in any experiment were collected within a 30-day interval. Comparisons from one experiment to another are not necessarily valid since the response of the control group may have changed.

**Experiment 1**—This experiment compared sulfur-containing steroids with dimercaprol and penicillamine (Table II).

**Experiment 2**—Six sulfur-containing steroids were assayed by oral administration *via* a stomach tube. All mice received 16 mg of methylmercuric chloride/kg ip. Steroids were administered as suspensions in corn oil, water, and polysorbate 80. In addition, thiocholesterol and its control contained diethylaminoethylcellulose. Animals were fed 400 mg of steroid/kg on each of 2 days before and 1 day following the methylmercuric chloride injection.

The compounds, survivors, number of test animals, and $p$ values (where calculated) were: Ia, 11, 20, <0.059; control, 6, 20; Ib, 18, 24, <0.089; II, 9, 20; X, 11, 24; XI, 11, 24; VIb, 5, 24; and control, 10, 20.

**Experiment 3**—In the first attempt to devise a screening assay that would utilize only a few mice, sulfur-containing steroids (400 mg/kg) were incorporated into lipsomes and administered by stomach tube on each of 2 days before and 1 day following 16 mg of methylmercuric chloride/kg ip. Neither XII nor XV performed (six mice each) significantly differently from the control group (one survivor out of nine).

**Experiment 4**—This experiment tested the feasibility of administering the steroids by absorption through skin (Table III).

**Experiment 5**—Mice were fed aqueous suspensions of steroids (400 mg/kg) every 12 hr following 16 mg of methylmercuric chloride/kg ip. The control group was fed cholesterol to balance any possible nutrient effect of the steroids. The compounds, survivors, and numbers of test animals were: XIII, 2, 11; XIV, 4, 11; and cholesterol, 3, 11.

**Experiment 6**—In a second attempt to devise a screening assay, ste-

**Table II—Effects of Chemotherapy on Methylmercuric Chloride Toxicity[a]**

| Chemotherapy[b] | Method[c] | Time[d] | Survivors | Percent | $p$[e] |
|---|---|---|---|---|---|
| None | | | 7/53 | 13 | |
| Dimercaprol | A | C | 15/63 | 24 | 0.1336 |
| Dimercaprol | B | C | 23/61 | 38 | 0.0027 |
| Dimercaprol | B | D | 5/20 | 25 | 0.2301 |
| Penicillamine | A | C | 4/12 | 33 | 0.1000 |
| Penicillamine | B | D | 13/46 | 28 | 0.0719 |
| Ia | A | C | 16/20 | 80 | 0.0001 |
| VIa | A | C | 12/15 | 80 | 0.0001 |
| Ia–VIa (10:1) | A | C | 55/73 | 75 | 0.0001 |
| Ia–VIa (2:1) | A | C | 30/32 | 94 | 0.0001 |

[a] Survivors were gaining weight 14 days after a 16-mg/kg ip dose of methylmercuric chloride in water, pH 7. [b] Total chemotherapeutic dose: steroid, 3 × 400 mg/kg; dimercaprol, 3 × 120 mg/kg; and penicillamine, 3 × 160 mg/kg. [c] A, intraperitoneal injection of liposomes; and B, intraperitoneal injection of the thiol dissolved in acetate buffer, pH 6. [d] C, administered 48 hr before, 24 hr before, and 24 hr after methylmercuric chloride injection; and D, administered 3 hr before, 3 hr after, and 24 hr after methylmercuric chloride injection. [e] Normal difference test (20) as compared to the control group.

[5] Cox, Indianapolis, Ind.

**Table III—Administration of Thiosteroids by Absorption through Tail Skin after Methylmercuric Chloride Injection [a]**

| Steroid [b] | Daily Dosage for 4 Days, mg/kg | Survivors [c] | Percent |
|---|---|---|---|
| Control (no thiosteroid) | | 6/20 | 30 |
| XVI | 400 | 3/12 | 25 |
| XII | 400 | 3/12 | 25 |
| VII | 400 | 1/11 | 9 |
| XIII | 400 | 6/11 | 55 [d] |

[a] All mice received 16 mg of methylmercuric chloride/kg ip. [b] Steroids administered at the level of 400 mg/kg, except XII, which was less soluble (see text). [c] Animals alive and gaining weight at 14 days after methylmercuric chloride injection. [d] $p < 0.0587$; normal difference test (20).

roids (400 mg/kg) were incorporated into liposomes and administered to six mice by stomach tube at 0, 12, 24, and 36 hr after 18 mg of methylmercuric chloride/kg ip. The compounds and numbers of survivors were: I*a*, 2; I*b*, 1; XII, 1; IX, 1; III, 2; VIII, 0; and cholesterol, 3.

**Experiment 7**—In a similar experiment employing 12 mice/group, steroids (400 mg/kg) were incorporated into liposomes and administered by stomach tube at 0, 12, 24, and 36 hr after 18 mg of methylmercuric chloride/kg ip. The compounds and numbers of survivors were: I*a*, 3; III, 5; cholesterol, 4; and no drug, 3.

**Experiment 8**—To determine the effect of massive doses, steroids suspended in 1% methylcellulose were administered by stomach tube at 0, 8, 24, 32, and 48 hr after 35 mg of methylmercuric chloride/kg ip. The dose of I*b* was 1250 mg/kg at each feeding; for all other steroids, the dose was 2500 mg/kg. Compounds I*b*, VIII, and III had no survivors out of 12 for each steroid, while cholesterol had three out of 16 survivors.

**Experiment 9**—Compounds I*b* and III were compared with I*a* (Table IV).

**Experiment 10**—The toxicity of 1:1 complexes formed between methylmercuric chloride and penicillamine, dimercaprol, or thiocholesterol was evaluated (Table V).

## RESULTS AND DISCUSSION

Both penicillamine and dimercaprol provided, as expected, some protection against methylmercuric chloride poisoning (Table II). All results were obtained with the therapeutic agent given a few hours to 1 day before and after the methylmercuric chloride. Penicillamine and dimercaprol were equally effective at either time period. Compounds I*a* and VI*a*, alone or in combination, were considerably more effective in protecting against acute methylmercuric chloride poisoning than were penicillamine or dimercaprol.

In the search for orally active compounds, the steroids in Experiment 2 and some analogs were administered as aqueous suspensions *via* stomach tube. It was assumed that esters like the thioacetate (I*b*), the trithiocarbonate (II), and (less likely) the thiocyanate (X) might hydrolyze to thiocholesterol. The thiosemicarbazone (XII) was tested because of its structural resemblance to diphenylthiocarbazone [$C_6H_5NHNHC(=S)N=NC_6H_5$], which binds mercuric and methylmercuric ions. Although both I*a* and I*b* had more survivors than their respective controls, the results were not significant at the 95% confidence level. Compound X gave poorer results than controls (not tabulated).

Compounds XII and XV were compared in a screening procedure designed to detect promising compounds with few mice (six for each compound) (see Experiment 3). Neither compound was active.

It was of interest to find new ways of administering the test steroid.

**Table IV—Comparison of Spironolactone and Cholesteryl Thioacetate with Thiocholesterol [a]**

| Steroid [b] | Route [c] | Survivors [d] | Percent |
|---|---|---|---|
| Control (no thiosteroid) | | 4/14 | 29 |
| I*a* | A | 9/12 | 75 [e] |
| I*a* | B | 3/12 | 25 |
| I*b* | C | 2/13 | 15 |
| III | C | 2/12 | 17 |

[a] All mice received 22 mg of methylmercuric chloride/kg ip. [b] Steroids were administered at the level of 400 mg/kg. [c] A, liposomes injected intraperitoneally each of 2 days before and 1 day after methylmercuric chloride; B, liposomes administered orally at 0, 8, 24, and 32 hr after methylmercuric chloride; and C, suspension in 1% methylcellulose administered each of 2 days before and 1 day after methylmercuric chloride. [d] Animals alive and gaining weight 14 days after methylmercuric chloride. [e] $p < 0.0278$; normal difference test (20).

**Table V—Toxicity of Complexes of Methylmercuric Chloride with Penicillamine, Dimercaprol, or Thiocholesterol [a]**

| Complex of Methylmercuric Ion | Dose: Complex, $\mu M$ | Dose: Methylmercuric Chloride [b], mg/kg | Survivors |
|---|---|---|---|
| Penicillamine | 32 | 320 | 4/6 |
| Penicillamine | 40 | 400 | 1/7 |
| Dimercaprol | 32 | 320 | 4/6 |
| Dimercaprol | 40 | 400 | 2/7 |
| Thiocholesterol | 32 | 320 | 5/5 |
| Thiocholesterol | 40 | 400 | 6/6 |
| Thiocholesterol | 80 | 800 | 6/6 |

[a] Complexes were incorporated into liposomes and injected intraperitoneally. [b] In the other experiments, survivals of 11–50% in control groups were observed with dosages of 16–35 mg of methylmercuric chloride.

Compounds VII*b*, XII, XIII, and XVI (Table III) were dissolved in dimethyl sulfoxide, and the tail of each mouse was soaked in the solution until it was absorbed (15 min). Each animal was treated at 0, 24, 48, and 72 hr after the methylmercuric chloride injection. The soluble compounds were administered at 400 mg/kg; XII, however, slowly crystallized out, and the amount actually absorbed is unknown. Only the xanthate (IX) had more survivors than the control group; the difference was barely significant.

Because of the possible activity in I*b* (Experiment 2) and XIII, other thioesters were examined (Experiment 5). In this case, the control group was fed cholesterol to balance the possible nutritional value of the thioesters, XIII and XIV. No significant activity was observed. The same result was obtained from screening the compounds listed in Experiment 6, made up as liposomes and administered by stomach tube following the methylmercuric chloride injection.

In previous studies (3), massive doses (2500 mg/kg) of spironolactone (III), given orally, protected rats and mice following a lethal dose of mercuric chloride. In the present study, spironolactone had no protective effects against methylmercuric chloride (Experiment 8). However, the protective effects against mercuric chloride observed by Eyble *et al.* (3) could be due to the strong diuretic action of spironolactone and are, therefore, not as appropriate against methylmercuric chloride. It was recommended (21) that spironolactone be used with other diuretics such as organic mercurials to obtain the additive effects of more than one diuretic. Such therapeutic doses are at the 1-mg/kg level, however.

Spironolactone was assayed and compared with thiocholesterol and its acetate (Table IV). Only the mice receiving thiocholesterol before and after methylmercuric chloride injection exhibited increased survival.

Since the dissociation of a molecule into ionized parts should be favored by a polar solvent and restricted by a nonpolar solvent, it might be expected that the methylmercuric–thiocholesterol complex, being more lipid soluble than the corresponding complex of penicillamine or dimercaprol, would dissociate less to release free methylmercury. To test this idea, each complex was synthesized by mixing equimolar amounts of methylmercuric chloride with thiocholesterol, penicillamine, or dimercaprol in absolute ethanol. Each complex was incorporated into liposomes and injected in mice intraperitoneally. Results (Table V) strongly imply that the methylmercuric–thiocholesterol complex is indeed of very low toxicity, indicating a very stable complex. To depress the toxic effects of methylmercury, it may be almost as good to form an irreversible complex as to promote the rapid excretion.

Almost no information about the metabolism of thiocholesterol and the thio bile acids is available. Thus far, only the first of the possible metabolic products of thiocholesterol, the disulfide, has been tested, and it was devoid of any possible effects.

No ill effects from thiocholesterol, administered orally or intraperitoneally for several weeks, were observed. On the other hand, 6 days of penicillamine or dimercaprol produced fatalities. The combination of thiocholesterol with either penicillamine or dimercaprol produced even more fatalities. This result is under further investigation.

In conclusion, thiocholesterol and mercaptocholanic acid are more effective orally or intraperitoneally than either penicillamine or dimercaprol in protecting against acute doses of methylmercuric chloride. The assumption is that the thiosteroids form irreversible complexes with methylmercuric ion that enhance excretion through the bile, thereby protecting the kidneys.

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# Molecular Connectivity and Substructure Analysis

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**Abstract** □ Antimicrobial and antiviral data sets were analyzed by molecular connectivity. Standard structure–activity relationship equations of high quality were produced in both cases. For phenyl propyl ether activity against *Staphylococcus aureus*, the three variables ${}^{1}\chi$, ${}^{3}\chi_P$, and ${}^{4}\chi^{u}_{PC}$ yielded an $r$ of 0.957, significantly better than a $\pi,\sigma$ analysis. Analysis of benzimidazole antiviral data (Lee strain, B flu virus) revealed that the one variable, ${}^{6}\chi_P$, yielded an $r$ of 0.950, also better than a reported Hansch analysis. Both data sets were further analyzed by partitioning the important regression variables into terms representing various structural features of the molecules. For the phenyl propyl ethers, the *para*-region of the phenyl ring is important for improved activity and the negative coefficient on ${}^{3}\chi_P$ corresponds to decreased activity for *vic*-dihydroxy compounds. For the alkylbenzimidazoles, substitution on the five-membered ring is highly important. No discrimination of six-membered ring positions was revealed. These structure–activity relationship observations can form the basis for synthetic decisions to improve activity.

**Keyphrases** □ Molecular connectivity—antimicrobial and antiviral data sets analyzed, structure–activity relationships produced □ Structure–activity relationships—antimicrobial and antiviral data sets analyzed by molecular connectivity method □ Antimicrobial data sets—analyzed by molecular connectivity method, structure–activity relationships produced □ Antiviral data sets—analyzed by molecular connectivity method, structure–activity relationships produced □ Topological indexes—molecular connectivity, antimicrobial and antiviral data sets analyzed, structure–activity relationships produced

Before there was any serious effort to quantify structure–activity relationships, it was popular to draw structural conclusions in terms of molecular fragments. Thus, combinations of atoms and groups were judged essential, based on studies of series of molecules with similar biological actions (1–3). This approach attempted to convey direct structural information to permit the familiar iterative process: synthesis → test → synthesis → test.

## BACKGROUND

These fragment proposals are certainly in an understandable and convenient form. However, the results of structure–activity relationship analyses frequently are stated in terms of values of physical properties, information far less valuable to the synthetic chemist. This approach has led to a greater quantification of structure–activity relationship results but also a loss of more useful information, *i.e.*, information directly related to structures.

The basic problem has been the lack of a numerical structural description that can be applied universally to all fragments and that can be translated from numbers back to structural fragments. Such a method, molecular connectivity (4–11), is now available for the numerical representation of molecular structure in a form suitable for multiple regression analysis. The molecular connectivity indexes give quantitative expression to structural variations described traditionally in such qualitative terms as branching, cyclization, and bond type, as well as number and kind of atoms. Structural information is encoded in the set of connectivity indexes, which may be calculated for any molecular structure.

The molecular connectivity indexes, referred to as $\chi$ indexes, are based on the hydrogen-suppressed graph; this graph is simply the molecular skeleton, including all atoms (except hydrogen) and the bonds between them. Information describing the molecular structure is extracted in numerical form from the connectivity relationships in the hydrogen-suppressed graph (4).

This paper discusses methods of identifying molecular fragments that the structure–activity relationship regression equation suggests are important. In a systematic fashion, the important molecular fragments or atomic arrangements may be identified. In this further development of the connectivity method, a high quality regression equation first is established with one or more $\chi$ indexes. Each $\chi$ index is a summation of subgraphs of a specific type. Then the subgraphs may be divided into chemically sensible sets. By regression analysis, the most important sets are picked out. Based on the topology of the important subgraphs, structure–activity relationship conclusions may be drawn as an aid to drug design.

**Formalism of Method**—Each ${}^{m}\chi_t$ is a sum of terms called subgraph terms:

$$ {}^{m}\chi_t = \sum {}^{m}S_j \qquad \text{(Eq. 1)} $$

Each term ${}^{m}S_j$ is defined for a subgraph—a skeletal fragment of $m$ bonds arranged in a particular fashion (type $t$)—and the summation is over all such subgraphs in the hydrogen-suppressed graph. The symbol $m$ is called the order or the index of the subgraph. The zero-order subgraph is simply a skeletal atom (graph vertex), and ${}^{0}\chi$ is a sum of the numerical

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