Mercapto Steroids in Protection against Mercury and Lead Poisoning

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Abstract □ When thiocholesterol is administered as liposomes, it provides significant protection against methylmercuric chloride in mice when given in three intraperitoneal injections, 0.5 hr before and 2 and 8 hr after the methylmercuric chloride. Thiositosterol, 5α-cholestane-2β,3α-dithiol, and 5β-cholane-3β,24-dithiol also are active, but 3α-mercapto-5α-pregnan-20-one, 6β-mercapto-5α-cholestane-3β,5α-diol, 3β-mercapto-5β-cholanic acid, and adamantanethiol are ineffective under these conditions. Adamantanethiol is somewhat effective when administered in soybean oil. Cholestanyl amine was treated with acetylthiosuccinic anhydride to give the half amide; cleavage with hydroxylamine liberated the thiol group. This product is active against both methylmercuric chloride and lead nitrate.

Keyphrases □ Methylmercuric chloride toxicity—protection by mercapto steroids □ Steroids—mercapto compounds, protection against mercury and lead poisoning □ Heavy metal poisoning—protection by mercapto steroids

In a study to identify lipophilic therapeutic agents for heavy metal poisoning that would minimize the burden placed on the kidney, thiocholesterol was found to be superior to dimercaprol and penicillamine in protecting mice against methylmercuric chloride toxicity (1). An investigation of how alterations in the structure of the molecule might alter activity then was desired. Compounds were synthesized containing, in addition to the thiol group, a hydroxyl, a carboxyl, or a second thiol group. A thiosemicarbazone, synthesized in connection with another problem and lacking a mercapto group, was included because it forms a precipitate with methylmercuric chloride *in vitro*. Several nonsteroidal mercaptans also were studied.

EXPERIMENTAL[1]

Thiositosterol (II)—Tosylation of 20.0 g of β-sitosterol[2] in 140 ml of pyridine[3] with 20 g of *p*-toluenesulfonyl chloride in the refrigerator overnight gave 28.7 g of crude product, mp 130°. Recrystallization from 2-butanone–acetone–water gave β-sitosteryl *p*-toluenesulfonate[4], 24.0 g (89% yield), mp 130–132°; IR: 1601 (benzene ring), 1198 and 1180 (SO_3), 950, 900, 878, 820, and 675 cm^{-1}.

Anal.—Calc. for $C_{36}H_{50}O_3S$: C, 76.00; H, 9.92; S, 5.64. Found: C, 76.24; H, 10.19; S, 5.64.

The tosylate (24.0 g) was treated with thiourea (24.0 g) in 248 ml of 95% alcohol and 25 ml of pyridine at reflux for 4.5 hr. Water precipitated the crude product, 22.8 g (82% yield), mp 233–239°. Two recrystallizations from methanol gave β-sitosteryl isothiouronium tosylate, mp 238–239°; IR: 3300–3000 (NH), 1675, 1230, 1181 (SO_3), 1139, 1050, 1025, 822, and 698 cm^{-1}.

Anal.—Calc. for $C_{37}H_{60}N_2O_3S_2$: C, 68.90; H, 9.38; N, 4.34; S, 9.94. Found: C, 68.60; H, 9.61; N, 4.14; S, 9.72.

Hydrolysis of 7.753 g of the isothiouronium salt in alcoholic sodium hydroxide (2.46 g/180 ml, 5 hr reflux) followed by precipitation with cold water and dilute hydrochloric acid gave the crude product, 4.594 g (89% yield), mp 61–65°. Two crystallizations from acetone–water gave II, mp 70–71°; IR: 1200, 968, and 802 cm^{-1}.

Anal.—Calc. for $C_{29}H_{50}S$: C, 80.86; H, 11.70; S, 7.44. Found: C, 80.59; H, 11.95; S, 7.29.

3α-Mercapto-5α-pregnan-20-one (V)—A comparable reaction of 3β-tosyloxy-5α-pregnan-20-one, mp 131.5–133° [lit. (4) mp 132–135°], with thiourea gave the corresponding isothiouronium tosylate, mp 270–272°; IR: 3275 (NH), 1701 (C=O), 1180 (SO_3), 1090, 1041, 1005, and 819 cm^{-1}.

Anal.—Calc. for $C_{29}H_{44}N_2O_4S_2$: C, 63.47; H, 8.08; N, 5.10; S, 11.67. Found: C, 63.73; H, 8.31; N, 4.90; S, 11.82.

Alkaline hydrolysis of the isothiouronium salt gave V in an 89% yield, mp 123–125° (methanol–water); IR: 1710 (C=O), 1208, 1160, and 978 cm^{-1}.

Anal.—Calc. for $C_{21}H_{34}OS$: C, 75.39; H, 10.24; S, 9.58. Found: C, 75.59; H, 10.34; S, 9.36.

***N*-(5α-Cholestan-3α-yl)mercaptosuccinic Acid Monoamide (VIII)**—A solution of 16.0 g of 5α-cholestan-3α-yl amine and 14.2 g of *S*-acetylmercaptosuccinic anhydride in acetic acid was held under nitrogen at 90–100° for 15 min. The solution was cooled to 35° and poured onto ice. The solid was filtered, the filtrate was extracted with ether, and the ethereal solution and solid were combined and evaporated. The solid residue was dissolved in 75 ml of tetrahydrofuran, 30 ml of methanol, and 10 ml of water. Hydroxylamine hydrochloride was added, and the solution was saturated with gaseous ammonia.

After standing at room temperature for 1.5 hr, the solution was made acidic with cold dilute hydrochloric acid. The precipitate was filtered, washed with water, and dried to give 20.0 g (93% yield), mp 113–118°. The analytical sample was precipitated from acidic methanol–water to yield VIII, mp 117–121°; IR: 3300 and 3170 (NH), 1710 (acid C=O), 1635 (amide C=O), 1540, 1220, and 1175 cm^{-1}.

Anal.—Calc. for $C_{31}H_{53}NO_3S$: C, 71.61; H, 10.28; N, 2.60; S, 6.16. Found: C, 71.26, 71.92; H, 10.46, 10.84; N, 2.48; S, 5.74.

3β-Hydroxy-5-androstene-16,17-dione 16-Oxime 17-Thiosemicarbazone (XI)—3β-Hydroxy-5-androsten-17-one tetrahydropyranyl ether (5, 6) (IX, 23.40 g) was added to a stirred solution of 5.89 g of potassium in 262 ml of *tert*-butanol under nitrogen. The mixture was stirred 1 hr, and 22.0 ml of isopentyl nitrite was added. The contents of the flask solidified within 2 min. After standing overnight at room temperature, the solid mass was dissolved in 1300 ml of cold water and extracted three times with ether.

Table I—Effect of Timing on Intraperitoneal Thiocholesterol Therapy for Methylmercuric Chloride Toxicity

Steroid[a]	Hours before First Injection[b]	Hours after Second Injection[b]	Hours after Third Injection[b]	Survivors	p^c
Cholesterol	24	24	48	3/20, 15%	—
Thiocholesterol	24	24	48	3/16, 19%	—
Thiocholesterol	2	24	48	9/16, 56%	<0.0093
Thiocholesterol	0.5	24	48	8/16, 50%	<0.0278
Cholesterol	0.5	2	8	1/15, 7%	—
Thiocholesterol	0.5	2	8	13/16, 81%	<0.0001
Thiocholesterol	0.25	2	4	0/16, 0%	—
Thiocholesterol	0.25	2	8	0/16, 0%	—

[a] Steroids were administered as liposomes with 400 mg of steroid/kg of body weight. [b] Before and after intraperitoneal injection of 25 mg of methylmercuric chloride/kg of body weight. [c] Normal difference test as compared to appropriate cholesterol control (13).

[1] Melting points were obtained on a Thomas-Hoover melting point apparatus and are corrected. IR spectra were taken as smears or mineral oil mulls on a Perkin-Elmer 247 spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

[2] Mann, dried by benzene azeotropy.

[3] Dried over molecular sieve.

[4] Reported without the melting point by Stoll (2) and possibly the same as the compound with a melting point of 123–124.5° reported by Rathmann and Morrow (3).

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Table II—Activity of Compounds against Methylmercuric Chloride Toxicity [a]

	Compound [b]	Survivors	p [c]
	Cholesterol	15/78, 19%	—
I	Thiocholesterol	16/22, 73%	<0.0001
II	Thiositosterol	13/30, 43%	<0.0124
III	5α-Cholestane-2β,3α-dithiol	17/22, 77%	<0.0001
IV	6β-Mercapto-5α-cholestane-3β,5α-diol	0/10, 0%	—
V	3α-Mercapto-5α-pregnan-20-one	3/10, 30%	—
VIII	*N*-(5α-Cholestan-3α-yl)mercaptosuccinic acid monoamide	16/36, 44%	<0.0069
XI	3β-Hydroxy-5-androstene-16,17-dione 16-oxime 17-thiosemicarbazone	2/9, 22%	—
XII	3β-Mercapto-5β-cholanic acid	4/10, 40%	<0.1936
XIII	5β-Cholane-3β,24-dithiol	6/10, 60%	<0.0001
XIV	1-Adamantanethiol	1/10, 10%	—

[a] All mice received 20 mg of methylmercuric chloride/kg of body weight by intraperitoneal injection. [b] Administered as liposomes injected intraperitoneally 0.5 hr before and 2 and 8 hr after methylmercuric chloride injection at the level of 400 mg of steroid or 170 mg of adamantanethiol/kg of body weight. [c] Normal difference test (13) as compared to the control group.

The combined ethereal layer was washed with water; the water wash was added to the original alkaline layer, which then was cooled and acidified with acetic acid. Filtration of the mixture after several hours of refrigeration gave 22.36 g of crude product, mp 161–166° (88% yield). Three recrystallizations in methanol–water gave 3β-hydroxy-5-androstene-16,17-dione 16-oxime 3-tetrahydropyranyl ether (X), mp 176–191°; IR: 3245 (OH), 1735 (C=O), 1632, 1135, 1112, 1058, 1022, 974, 940, 908, and 868 cm^{-1}.

Anal.—Calc. for $C_{24}H_{35}NO_4$: C, 71.79; H, 8.79; N, 3.49. Found: C, 71.69; H, 8.78; N, 3.64.

The thiosemicarbazone was prepared in the usual way with 5 ml of concentrated hydrochloric acid, 2.00 g of the oxime (X), and 1.50 g of thiosemicarbazide in 39 ml of boiling 95% ethanol and 6 ml of water. Two recrystallizations from methanol–dimethylformamide–water gave XI[5], mp 296–298° dec.; *IR*: 3408 (OH), 3230 (OH), 3145 (NH), 1580, 1300, 1080, and 968 cm^{-1}.

Anal.—Calc. for $C_{20}H_{30}N_4O_2S$: C, 61.51; H, 7.74; N, 14.35; S, 8.21. Found: C, 61.32; H, 7.91; N, 14.37; S, 8.10.

Assays—Assays were conducted with male, Swiss–Webster mice, 22–25 g. Liposomes were prepared as described previously (1); with VIII and XI, cholesterol was added (compound to cholesterol, 4:1) to obtain stable liposomes. 1-Dodecanethiol was tested in cottonseed oil because it would not form liposomes under these conditions. Methylmercuric chloride was dissolved in warm water at a concentration of 1 mg/ml, and lead nitrate was dissolved at a concentration of 20 mg/ml. Mice were injected with test compounds in the left groin and with mercury or lead in the right groin.

RESULTS AND DISCUSSION

Thiositosterol (II) was synthesized from sitosterol and 3α-mercapto-5α-pregnan-20-one (V) was synthesized from 3β-hydroxy-5α-pregnan-20-one *via* the corresponding tosylates and isothiouronium salts in a sequence comparable to the synthesis of thiocholesterol (7). Cholestane-2β,3α-dithiol (III) (8), 6β-mercapto-5α-cholestane-3β,5α-diol (IV) (9), and 1-adamantanethiol (XIV) (10) were synthesized by literature methods. 5α-Cholestan-3α-yl amine (VI) reacted with *S*-acetylmercaptosuccinic anhydride to give the corresponding hemiamide (VII) following a reported method (11). The product was deacylated with hydroxylamine to give the mercapto compound (VIII). The mercapto group may be on the carbon adjacent to either the carboxyl or the amide group, and, although the product exhibited a single spot by TLC, it may be a mixture of the two isomers. The tetrahydropyranyl ether (IX) of androstenolone was oximated with isopentyl nitrite and potassium *tert*-butoxide (12). When the intermediate (X) was converted to the corresponding thiosemicarbazone (XI), the tetrahydropyranyl ether group was cleaved, giving the 3-hydroxy derivative. Compounds XII and XIII were reported previously.

[5] Mixing equal volumes of 0.01 *N* mercuric acetate in 70% alcohol and 0.01 *N* XI in alcohol caused a rapid precipitation.

Table III—Activity against Methylmercuric Chloride of Compounds Tested in Vegetable Oil [a]

Compound [b]	Survivors	p [c]
Soybean oil	2/20, 10%	—
III	5/10, 50%	<0.0594
XIV	5/10, 50%	<0.0594
1-Dodecanethiol	0/13, 0%	—

[a] All mice received 20 mg of methylmercuric chloride/kg of body weight by intraperitoneal injection. [b] Administered in soybean oil solution by intraperitoneal injection 0.5 hr before and 2 and 8 hr after the methylmercuric chloride injection at the level of 400 mg (of steroid or 1-dodecanethiol) or 170 mg (of adamantanethiol)/kg of body weight. [c] Normal difference test (13) as compared to the control group.

Table IV—Activity of VIII against Lead Nitrate Toxicity [a]

Compound	Dose [b], mg/kg	Survivors	p [c]
Cholesterol	400	2/10, 20%	—
VIII	292	9/10, 90%	<0.0019

[a] All mice received 180 mg of lead nitrate/kg of body weight by intraperitoneal injection. [b] Administered as liposomes injected 0.5 hr before and 2 and 8 hr after lead acetate injection. [c] Normal difference test (13) as compared to the control group.

The desirability of having an assay in which the test compound is administered only following administration of the toxic dose of the heavy metal, thereby mimicking real life conditions, is obvious. However, in earlier work (1), thiocholesterol was an effective protective agent when given 24 and 48 hr *before* methylmercuric chloride but not when given 0, 12, 24, and 36 hr *after* methylmercuric chloride. To decrease the time between pretreatment and the toxic dose, the comparisons in Table I were carried out. Significant protection was afforded when the first of three injections of thiocholesterol was given 0.5 or 2 hr before the methylmercuric chloride but not when given 0.25 or 24 hr before. The highest survival rate was obtained with the sequence 0.5 hr before and 2 and 8 hr after the toxic dose, and this regimen was used in subsequent assays.

The results reported in Table II are composites of a series of assays and illustrate that II, III, VIII, and XIII are active in addition to thiocholesterol. 3β-Mercapto-5β-cholanoic acid (XII) was active when tested under a less stringent protocol (1). Adamantanethiol, inactive under these conditions, exhibited some activity when administered in soybean oil (Table III), but 1-dodecanethiol was totally inactive.

The hemisuccinamide (VIII) exhibited high protective activity when tested against lead nitrate (Table IV).

While there are too few examples for extensive structure–activity generalizations, it is useful to note that 3-mercapto (but not 6-mercapto) steroids were active against methylmercuric chloride. The 2,3- and 3,24-dithiols also were active. The high activity of the hemisuccinamide against lead may be the result of coordination of the lead to both carboxyl and thiol groups. These observations provide leads for the synthesis of other mercapto steroids for heavy metal therapy.

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Application of Queueing Theory to Pharmacokinetics

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Abstract □ This paper considers the steady-state plasma drug concentration in a one-compartment, open pharmacokinetic model with multiple doses and first-order kinetics using a classical deterministic technique as well as a queueing theoretical stochastic analysis. The stochastic analysis employs a new method for obtaining the steady-state probability distribution of the content of a dam with compound Poisson input and a general release rule. It is shown that if the deterministic steady-state average concentration exists, it is equal to the mean value of the steady-state concentration, the probability distribution of which is obtained using the stochastic model. Moreover, the steady-state probability distribution of the concentration and its mean always exist in the stochastic model. Ramifications of the stochastic method of analysis are discussed.

Keyphrases □ Pharmacokinetics—steady-state plasma drug concentration, queueing theory □ Models, pharmacokinetic—steady-state plasma drug concentration, queueing theory □ Queueing theory—application to pharmacokinetics, steady-state plasma drug concentration

This paper introduces a method of analyzing drug accumulation based on a stochastic model of multiple-dosing regimens. In classical pharmacokinetic theory, transient and steady-state drug concentrations in the blood for multiple-dosing regimens are derived from knowledge of the deterministic dose sizes, intervals between doses, volumes of distribution, and kinetics of elimination and transfer between body compartments (1–4). This analysis may be clinically useful only if two major conditions are true: (*a*) the dosage intervals are known, and (*b*) the amount of drug absorbed as a consequence of each dose is known.

These conditions rarely are met in practice. As noted previously (2), nonstandard definitions of *bid*, *tid*, and *qid* may lead to varying dosage intervals. Family physicians and drug package inserts often direct that drugs should be taken near meal times, bed time, or three or four times daily without defining these terms specifically (2). Patients rarely take drugs on schedule unless they are in the hospital. Furthermore, dose size may vary because the initial dose or other doses may be different from the usual maintenance dose. The fractional absorption of oral drugs depends on several factors. For drugs administered intramuscularly, the fractional absorption partially depends on the injection site (1). Moreover, completeness of drug absorption always is clinically important (1, 5).

These observations suggest the desirability of obtaining the drug concentration in the plasma when the dose intervals, doses, or absorption fractions are subject to random fluctuations and must be considered random variables. Accounting for such random variations in these parameters leads to the consideration of a stochastic analysis of pharmacokinetic models. This paper emphasizes the new technique itself rather than its application to general multiple-dosing models. The model presented is a one-compartment, open model with instantaneous input and first-order elimination. A classical, well-known deterministic method of analysis is presented for convenience of reference, and a stochastic, queueing theoretical approach for studying the same model then is given. The latter technique is based on a new method of analyzing the model of a dam with a general release rule[1], which was studied previously in stochastic processes using other approaches.

THEORETICAL

The Model—The model treated is a one-compartment, open model with multiple dosing and first-order kinetics (1–4, 6). For clarity and to focus attention on the new technique, very rapid administration of each dose directly into and instantaneous distribution throughout the compartment is assumed. This assumption is a good approximation for intravenous bolus dosing. For outpatient oral dosing (to which the stochastic technique is more applicable), first-order absorption may be a more appropriate assumption. However, for many drugs in common clinical use, the absorption constant is appreciably larger than the elimination constant. Examples are digoxin, sublingually administered nitrites, salicylates, and phenylbutazone. For such drugs, the assumption of instantaneous absorption, although only approximate, is not entirely unreasonable.

The following notations are used in both the deterministic and stochastic analyses:

V = volume of compartment
K_e = elimination rate constant (time^{-1})
$t_{1/2e}$ = elimination half-life; $1/k_e = t_{1/2e}/\ln 2 = 1.44t_{1/2e}$
τ_n = administration time of nth dose, $n = 1, 2, 3, \ldots$; $\tau_1 = 0$
T_n = nth dosage interval; $T_n = \tau_{n+1} - \tau_n$
D_n = amount of nth dose
F_n = fraction of D_n distributed throughout V
E_n = effective increase in drug concentration at time τ_n; $E_n = F_nD_n/V$
$C(t)$ = drug concentration at time t; $t \geq 0$
C_n = drug concentration at time τ_n; $C_1 = C(0) = 0$
λ = rate of administration of doses
B = common distribution function of effective increases in concentration, E_n, independent of n; $B(0) = 0$
$r(x)$ = drug elimination rate when the concentration is x (mass times volume^{-1} times time^{-1})

Deterministic Analysis—The following well-known deterministic analysis is presented for comparison with the stochastic analysis. Vari-

[1] P. H. Brill, unpublished data.

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