Table IV—Comparison of Calculations for Potency at a Dallas Warehouse

Calculation	Virtual Temperature	Kinetic Ratio	Number of Arithmetic Operations: Virtual Temperature	Number of Arithmetic Operations: Kinetic Ratio	Vitamin A Results
Potency after 3 years	Eqs. 1 and 3*a*	Eq. 3*b*	6	3	5914 units
Potency after 18 months (six quarters) using seasonal values	Eqs. 7*a*, 1, and 3*a*	Eqs. 7*b* and 3*b*	17	8	664 units
Potency after 18 months (six quarters) using annual values	Eqs. 1 and 3*a*	Eq. 3*b*	6	3	6570 units
Shelflife	Eq. 4*a*	Eq. 4*b*	6	4	358 weeks

warehouse location and construction, each company would have to determine its individual parameters. The inclusion of a reference temperature in these calculations is a small matter. In addition, conversion from one reference temperature to another, should this be necessary, is straightforward (Eq. 9*b*).

Sample Calculations—Constants and reference data were combined with the data from Table III for sample calculations. Twice as many arithmetic operations usually are required for calculations involving virtual temperature (Table IV). The use of reference potencies eliminates the need to calculate rate constants. The kinetic ratio reference potency and initial potency are all that are required for direct calculations. Once the reference values are calculated, they remain constant and may be used to calculate potencies at each warehouse with the appropriate α value. Virtual temperature calculations invariably require exponentiation to obtain a rate constant needed for subsequent calculations. As also can be seen from Table IV, the seasonal effect over six quarters is not significant for vitamin A in multivitamin tablets (1.5%). Shelflife calculations using the kinetic ratio are particularly easy because they only involve dividing a constant by a different α for each warehouse.

CONCLUSIONS

Although it is only a simple extension of a generalized virtual temperature definition, the kinetic ratio offers clear practical advantages. The parameter has a direct, easily understood physical relationship to potency, and commonly performed calculations are simplified greatly.

NOMENCLATURE

α = kinetic ratio (dimensionless)
b_0 = Arrhenius factor, defined by Eq. 1 (weeks^{-1})
b_1 = Arrhenius activation factor, E/R, defined by Eq. 1 (°K)
θ = time (weeks)
g = general unspecified function
k = Arrhenius kinetic constant (weeks^{-1})
P = potency or concentration variable (units)
τ = specific time (weeks)
T = temperature (°K)

Subscripts:

0 = initial value of a variable
1, 2, 3, 4 = either a specific value denoted by the number or values that relate to enumerated quarters of the year
ref = reference value of a variable or quantity enclosed in brackets
sl = value of variables corresponding to the shelflife
v = virtual value
y = cumulative annual value

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Synthesis and Skeletal Muscle Relaxant Activity of Quaternary Ammonium Salts of Dantrolene and Clodanolene

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Abstract □ A series of quaternary ammonium salts of dantrolene and clodanolene was prepared and evaluated for skeletal muscle relaxant activity. The quaternary ammonium salts exhibit greater aqueous solubility and, therefore, facilitate intravenous administration. One member of this series, although less effective orally, exhibited greater aqueous solubility than the sodium salt. When administered intravenously, it was a more potent antagonist of skeletal muscle contraction and yielded comparable therapeutic and muscle relaxant efficacy indexes.

Keyphrases □ Dantrolene—quaternary ammonium salts, synthesis, skeletal muscle relaxant activity □ Clodanolene—quaternary ammonium salts, synthesis, skeletal muscle relaxant activity □ Relaxants, skeletal muscle—dantrolene and clodanolene, quaternary ammonium salts, synthesis, activity

Several hydantoins have skeletal muscle relaxant activity (1). One member of this series, dantrolene[1], was shown subsequently to cause skeletal muscle relaxation by a unique mechanism involving direct action on the skeletal muscle (2, 3). Dantrolene sodium has been hypothesized to act by preventing the release of calcium ion (Ca^{2+}) from the sarcoplasmic reticulum (4, 5).

Dantrolene sodium recently was shown to have potential

[1] Dantrium, Morton-Norwich Products.

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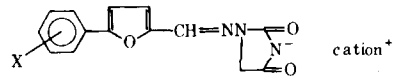
Table I—Synthesized Imidazolidinedione Compounds and Their Corresponding Cations

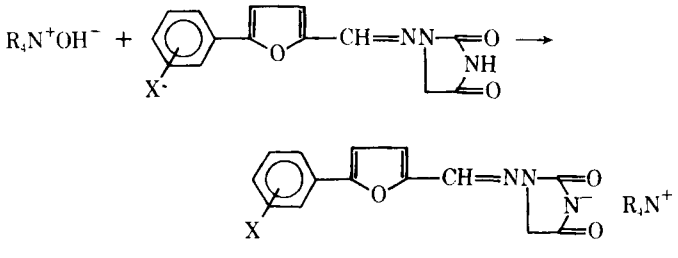


Compound	X	Cation	Water Solubility, mg/liter
I	4-NO_2	Benzyltrimethylammonium	>10
II	4-NO_2	Tetramethylammonium	—[a]
III	4-NO_2	*N*-Methylpyridinium	—[a]
IV	4-NO_2	Tetrabutylammonium	—[a]
V	3,4-Cl	2-(2,3-Dihydroxy-1-propylamino)-quinolizinium	550
VI	3,4-Cl	Safranine O	40
VII	4-NO_2	Quinolizinium	930
VIII	3,4-Cl	Quinolizinium	2000
IX	4-NO_2	2-Carbamoyl-1-methylpyridinium	90
X	4-NO_2	2,3-Dimethyl-1-phenyl-4-trimethyl-ammonium-3-pyrazolin-5-one	—[a]
XI	4-NO_2	Diethyldimethylammonium	9400
XII	4-NO_2	1,3-Dimethylimidazolium	—[a]
XIII	3,4-Cl	2,3-Dimethyl-1-phenyl-4-trimethyl-ammonium-3-pyrazolin-5-one	1200
XIV	4-NO_2	Choline	8000
XV	4-NO_2	2-(1-Hydroxy-2-methyl)propyltri-methylammonium	140
Dantrolene sodium			15
Clodanolene sodium			20

[a] The water solubility is greater than 1%.

utility for the treatment of malignant hyperthermia (6–8). The fulminating nature of this condition requires rapid institution of therapeutic measures including intravenous muscle relaxants. Because dantrolene sodium is relatively insoluble in aqueous solutions, other soluble salts have been synthesized for evaluation (Table I). This paper reports the preparation of a series of quaternary ammonium salts (9) of dantrolene and clodanolene and their pharmacological evaluation for skeletal muscle relaxant activity.

Salts of dantrolene and clodanolene, other than the sodium salts, were desired for comparative pharmacological evaluation. Since the hydantoin moiety of the two parent compounds is weakly acidic, a strong base was sought for salt formation. The broad class of quaternary ammonium hydroxides fills the requirement of a strong base and also provides versatility in selection of an organic cation. In practice, the acid–base reaction of dantrolene or clodanolene with quaternary ammonium hydroxides proceeded rapidly, and the salt products were stable to recrystallization from various solvents (Scheme I). The quaternary ammonium hydroxides were prepared from the appropriate quaternary ammonium halides by ion-exchange chromatography and then were used directly. Success of



dantrolene: X = *p*-NO_2
clodanolene: X = 3,4-Cl

Scheme I

the acid–base reaction was easily discernible upon isolation of the product. The carbonyl bands of the hydantoins at 5.65 and 5.8 μm in the IR spectrum shifted to 5.9 and 6.2 μm.

EXPERIMENTAL

Chemistry[2]—*Quaternary Halides*—The following salts were prepared by literature methods: diethyldimethylammonium iodide (10), 1,3-dimethylimidazolium iodide (11), *N*-methylpyridinium iodide (12), 1,1-dimethyl-2-hydroxyethyltrimethylammonium iodide (13), 2-carbamyl-1-methylpyridinium iodide (14), aminopyridine methiodide (15), quinolizinium bromide (16), and 2-(2,3-dihydroxypropylamino)quinolizinium bromide (17). Tetramethylammonium hydroxide, benzyltrimethylammonium hydroxide, tetramethylammonium chloride, tetrabutylammonium iodide, benzyltrimethylammonium chloride, and Safranine O were purchased[3]. Choline chloride was obtained commercially[4].

The following four methods were used for preparation of quaternary salts of dantrolene and clodanolene, with dantrolene salts given as examples (Table II).

Dantrolene [1-[[5-(p-Nitrophenyl)furfurylidene]amino]hydantoin] Quinolizinium Salt Hemihydrate (VII) (Method A)—Resin[5] (65 ml) was activated by elution with 10% NaOH, and the column was washed with water to bring to pH 7–8. Quinolizinium bromide (4.7 g, 0.021 mole) (7) was passed through the basic anion-exchange resin with water (75 ml) at ~1 drop/sec until the eluent (pH 10–11) returned to pH 8–9.

Dantrolene (5.1 g, 0.016 mole) was added slowly to the quaternary hydroxide solution at room temperature to bring to pH 8–9. The resultant yellow solid was collected and washed with cold water (3 × 5 ml) and 2-propanol (2 × 5 ml) and then was allowed to dry to give 5.1 g (70% yield), mp 184–196° dec.; IR (mineral oil): 5.9 and 6.2–6.3 μm.

Dantrolene Diethyldimethylammonium Salt (XI) (Method B)—A resin[5] column (115 ml) was prepared as in Method A. Methanol (400 ml) was eluted slowly through the column followed by a solution of diethyldimethylammonium iodide in methanol (50 ml). The column was eluted to neutrality with methanol. The methanolic solution was added to dantrolene (15.7 g, 0.050 mole) in methanol (100 ml), and the mixture was stirred for 1.0 hr.

The mixture was filtered, and the filtrate was concentrated under reduced pressure to a red solid. Recrystallization from 2-propanol (150 ml) with activated charcoal[6] yielded 16.5 g (72% yield) of product, mp 198–200°. A sample again was recrystallized from 2-propanol to analytical purity, mp 201–202°; IR (mineral oil): 5.9 and 6.2–6.3 μm.

Dantrolene Tetramethylammonium Salt (II) (Method C)—To a stirred mixture of dantrolene (21 g, 0.066 mole) in 1.0 liter of methanol was added tetramethylammonium hydroxide (50 ml of 20% solution in methanol, 0.10 mole). The mixture was stirred for 1.0 hr and then heated while methanol (400 ml) was added to complete dissolution. Filtration and cooling allowed isolation of 15 g (59% yield) of a red product. Recrystallization from methanol gave analytical material, mp 218–220°; NMR: δ 3.23 (s, 12H, methyl CH), 3.80 (s, 2H, hydantoin CH), 6.87 (d, 1H, furan CH, J = 3.5 Hz), 7.46 (d, 1H, furan CH, J = 3.5 Hz), 7.58 (s, 1H, azomethine CH), 8.00 (d, 2H, phenyl CH, J = 8 Hz), and 8.32 (d, 2H, phenyl CH, J = 8 Hz); IR (mineral oil): 5.9 and 6.2 (broad) μm.

Dantrolene Benzyltrimethylammonium Salt (I) (Method D)—To a refluxing stirred solution of dantrolene (16 g, 0.050 mole) in methanol (1.0 liter) was added rapidly benzyltrimethylammonium hydroxide (25 ml of 40% solution in methanol, 0.060 mole). The solution was filtered and concentrated under reduced pressure to ~250 ml. Ether (750 ml) was added to the cooled solution, and the resulting red solid was collected and rinsed with ether (100 ml) to give 22 g (95% yield) of product, mp 150–160° dec.; IR (mineral oil): 5.9 and 6.25 μm.

Pharmacology—Initially selected quaternary ammonium salts were evaluated intravenously in rats by gross observation for skeletal muscle relaxant activity (cholinergic and/or toxic signs). Direct effects on skeletal

[2] Melting points were determined on a Mel-Temp apparatus and are uncorrected. A Varian Associates A-60A instrument was used for NMR spectra. All spectra were run in dimethyl sulfoxide-d_6 with tetramethylsilane as the internal standard. A Perkin-Elmer model 137B recording spectrophotometer was used for IR spectra. Solubilities were determined by agitation of a sample in water at room temperature, filtration of the saturated aqueous solution, and UV comparison with a standard sample (Table I).

[3] Aldrich Chemical Co.

[4] Merck Laboratory Chemicals.

[5] Amberlite IRA-410.

[6] Darco.

Table II—Analysis of Synthesized Imidazolidinedione Compounds

Compound	Recrystallization Solvent[a]	Synthetic Method	Yield, %	Melting Point	Empirical Formula	Analysis, %	Calc.	Found
I	E	D	95	150–160° dec.	$C_{24}H_{25}N_5O_5$	C	62.19	61.79
						H	5.44	5.80
						N	15.11	14.96
II	F	C	59	218–220°	$C_{18}H_{21}N_5O_5$	C	55.81	55.82
						H	5.46	5.45
						N	18.08	18.14
III	F	B	83	173–175°	$C_{20}H_{17}N_5O_5 \cdot 0.75\ H_2O$	C	57.07	56.98
						H	4.43	4.75
						N	16.64	16.64
IV	G	B	58	187–189°	$C_{30}H_{45}N_5O_5$	C	64.84	65.04
						H	8.16	8.14
						N	12.60	12.43
V	H	A	33	132–135°	$C_{26}H_{23}Cl_2N_5O_5 \cdot 0.5\ H_2O$	C	55.23	54.92
						H	4.28	4.52
						N	12.39	12.16
VI	H	A	38	194–197°	$C_{34}H_{27}Cl_2N_2O_3 \cdot 1.5\ H_2O$	C	60.09	60.00
						H	4.45	4.64
						N	14.43	14.23
VII	E	A	70	184–196° dec.	$C_{23}H_{17}N_5O_5 \cdot 0.5\ H_2O$	C	61.06	60.86
						H	4.01	3.79
						N	15.48	15.80
VIII	F[b]	A	62	160–162° dec.	$C_{23}H_{16}Cl_2N_4O_3 \cdot 0.167\ C_3H_8O$	C	59.12	58.94
						H	3.67	3.65
						N	11.74	11.66
IX	J	B	59	203–204°	$C_{21}H_{18}N_6O_6$	C	55.60	55.85
						H	4.03	4.02
						N	18.66	18.81
X	K	B	74	150–152°	$C_{28}H_{29}N_7O_6$	C	60.10	59.91
						H	5.22	5.47
						N	17.52	17.47
XI	L	B	72	198–200°	$C_{20}H_{25}N_5O_5$	C	57.82	57.64
						H	6.07	6.15
						N	16.86	17.02
XII	F	B	53	172–175°	$C_{19}H_{18}N_6O_5$	C	55.60	55.36
						H	4.42	4.42
						N	20.48	20.43
XIII	F	B	62	190–215° dec.	$C_{28}H_{28}Cl_2N_6O_4$	C	57.64	57.51
						H	4.84	5.01
						N	14.41	14.33
XIV	F	B	48	196–198° dec.	$C_{19}H_{23}N_5O_6$	C	54.67	54.31
						H	5.55	5.54
						N	16.78	16.78
XV	J	B	54	179–181°	$C_{21}H_{27}N_5O_6$	C	56.72	56.21
						H	6.11	6.10
						N	15.72	15.64

[a] The recrystallization solvents were methanol (F), acetone (G), ethanol (H), nitromethane (J), acetonitrile (K), and 2-propanol (L), or no recrystallization solvent was used (E). [b] Sample was rinsed with 2-propanol after recrystallization, and the NMR spectrum indicated the presence of 0.167 mole of 2-propanol.

Table III—Acute Lethality of Quaternary Salts Used to Prepare Dantrolene and Clodanolene Salts

Quaternary Salt	Gastrocnemius Muscle Twitch Response, maximum % change at 25 mg/kg	Acute Oral Lethality in Mice
Benzyltrimethylammonium chloride	+10.1 ± 1.5	100% at 1600 mg/kg
Tetramethylammonium chloride	+8 ± 0.9	100% at 125 mg/kg
N-Methylpyridinium iodide	+3 ± 3.0	30% at 1600 mg/kg
Tetrabutylammonium iodide	+14.2 ± 6.5	60% at 1600 mg/kg
2-(2,3-Dihydroxy-1-propylamino)quinolizinium bromide	−5.6 ± 5.6	10% at 1600 mg/kg
Safranine O	+5 ± 1.7	10% at 1600 mg/kg
Quinolizinium bromide	+18.7 ± 6.3	100% at 1600 mg/kg
2-Carbamoyl-1-methylpyridinium iodide	+7 ± 1.4	>1600 mg/kg
2,3-Dimethyl-1-phenyl-4-trimethylammonium-3-pyrazolin-5-one iodide	−1 ± 2.1	>1600 mg/kg
Diethyldimethylammonium iodide	+7 ± 4.5	LD_{50} = 712 mg/kg (584–853)
1,3-Dimethylimidazolium iodide	+3 ± 0.1	10% at 1600 mg/kg
Choline chloride	+7.5 ± 0.5	>1600 mg/kg
2-(1-Hydroxy-2-methyl)propyltrimethylammonium iodide	+20 ± 2.7	>1600 mg/kg

muscle also were evaluated in the gastrocnemius muscle preparation by the method to be described later. Only compounds (Table III) devoid of skeletal muscle relaxant activity were used for the preparation of the imidazolidinedione compounds.

Gross Observation—Groups of unfasted male mice (TAC:SWfBr), 20–27 g, were used. The drugs were administered orally as 2% concentrations suspended in 0.5% methylcellulose 4000 cps. Gross observations for pharmacological effects were made for 2 hr, utilizing a rating of drug effect signs similar to that described by Irwin (18). Muscle relaxation was rated on a scale from 0 to 4 with 0 = no effect, 1 = slight, 2 = moderate, 3 = marked, and 4 = extreme.

Pithed Rat Gastrocnemius Muscle Preparation—A literature method was used for direct stimulation of the gastrocnemius muscle in rats (TAC:SD/NfBr) (19). The test drugs were administered intravenously at logarithmically spaced doses in tetrahydrofurfuryl alcohol, and ED_{50} values for inhibition of skeletal muscle contractions were estimated by the method of Litchfield and Wilcoxon (20).

Rotarod Testing in Mice—A method similar to that described by Dunham and Miya (21) was used to test the effect of drugs on motor coordination. Male mice (TAC:SWfBr) were trained to walk a revolving rod (20 rpm) for over 1 min. The test drugs were administered orally at logarithmically spaced doses in methylcellulose[7] to the previously trained mice, and performance trials were conducted 30 min following drug administration. The inability of an animal to stay on the rotarod for more than 30 sec was considered a positive drug effect. The ED_{50} values (the drug dose that caused 50% of the animals to fall off the revolving rod within the 30 sec) were calculated using the method of Litchfield and Wilcoxon (20).

[7] Methocel, Dow.

Table IV—Oral Testing for Skeletal Muscle Relaxant Properties of Quaternary Ammonium Salts of Dantrolene and Clodanolene

Compound	Minimum Dose (mg/kg) for Maximum Activity[a]	ED_{50}, Straub Tail[b]	ED_{50}, Rotarod[c]	LD_{50}, mg/kg	Muscle Relaxant Efficacy Index, ED_{50} (Rotarod)/ ED_{50} (Straub Tail)	Therapeutic Index, LD_{50}/ED_{50} (Straub Tail)
I	800/0	—[d]	—	—	—	—
II	800/0	—[d]	—	—	—	—
III	800/4	70 (35–140)	13 (5–36)	>2300	0.2	>33
IV	800/0	—[d]	—	—	—	—
V	800/3	>180	—[e]	—	—	—
VI	400/3	70 (46–106)	120 (56–252)	>2300	1.7	>33
VII	800/3	>180	—[e]	—	—	—
VIII	200/4	>180	—[e]	—	—	—
IX	200/3	>180	—[e]	—	—	—
X	400/2	90 (63–128)	45 (25–81)	>2300	0.5	>26
XI	400/3	>300	—[e]	—	—	—
XII	200/2	62 (43–89)	60 (20–183)	>2300	1.0	>37
XIII	400/3	75 (52–108)	54 (27–108)	>2300	0.7	>31
XIV	400/2	>180	—[e]	—	—	—
XV	400/2	86 (54–138)	34 (13–88)	>2300	0.4	>27
Dantrolene sodium[f]	400/4	20 (12–34)	25 (16–36)	1110	1.3	54
Clodanolene sodium[f]	400/4	50 (30–82)	39 (26–54)	1335	0.8	21

[a] See *Experimental* for key to rating of drug-induced skeletal muscle relaxation. [b] Dose of test drug (milligrams per kilogram) causing 50% reduction in the morphine-induced Straub tail. [c] Dose of test drug (milligrams per kilogram) causing 50% of the animals to fall from the revolving rod prior to 1 min. [d] Inactive in gross observation; no further oral testing. [e] Inactive in Straub tail; no further oral testing. [f] Expressed as parent compounds on a milligram per kilogram basis.

Table V—Intravenous Testing for Skeletal Muscle Relaxant Properties of Quaternary Ammonium Salts of Dantrolene and Clodanolene

Compound	Gastrocnemius Muscle Twitch Tension[a], mean maximum % change ± *SE*	ED_{50}[b] mg/kg (95% confidence limits)	ED_{50}[b] μM/kg	Gross Effects[c]	LD_{50} in Rats, mg/kg	Therapeutic Index, LD_{50}/ED_{50} (Gastrocnemius Muscle Twitch Tension)	ED_{50} (Straub Tail)[d], mg/kg	LD_{50} in Mice, mg/kg	Therapeutic Index, LD_{50}/ED_{50} (Straub Tail)
I	−72 ± 3.6	3.0 (1.2–4.2)	5.3	Cholinergic	—[e]	—	—	—	—
II	−70 ± 3.7	6.0 (3.8–9.9)	21	Cholinergic	—[e]	—	—	—	—
III	−81 ± 1.8	3.0	8.5	Skeletal muscle relaxation	110 (71–172)	36.7	43 (33–57)	122 (73–205)	2.8
IV	−77 ± 1.4	4.1 (3.2–5.1)	7.4	Cholinergic	—[e]	—	—	—	—
V	−75 ± 3.2	5.0 (3.8–6.8)	9.0	Cholinergic	—[e]	—	—	—	—
VI	−72 ± 6.0	6.0 (3.7–8.6)	8.2	Skeletal muscle relaxation	—[f]	—	—	—	—
VII	−78 ± 3.4	4.0 (2.4–4.9)	7.7	—[f]	—[f]	—	—	—	—
VIII	−76 ± 2.5	7.0 (2.6–9.7)	10.7	Cholinergic	—[e]	—	—	—	—
IX[g]	—	—	—	—[f]	—[f]	—	—	—	—
X	−74 ± 0.1	4.0 (2.7–4.1)	5.9	Skeletal muscle relaxation	115 (85–155)	28.8	24 (19–31)	115 (68–196)	4.8
XI	−77 ± 1.8	3.0 (2.0–4.5)	7.5	Cholinergic	—[e]	—	—	—	—
XII	−71 ± 2.2	3.0 (0.7–4.0)	5.1	Skeletal muscle relaxation	145 (105–200)	48.3	21 (13–33)	>180–<200	>8–<8.9
XIII	−72 ± 3.0	6.0 (4.2–7.9)	9.8	Skeletal muscle relaxation	88 (69–113)	14.7	16 (10–26)	115 (91–146)	7.2
XIV	−76 ± 3.1	8.0 (4.5–11.0)	19.6	Skeletal muscle relaxation	45 (30–68)	5.6	18 (13–24)	66 (48–75)	3.7
XV	−74 ± 3.9	4.0 (2.8–5.8)	9.2	Skeletal muscle relaxation	102 (78–133)	25.5	22 (17–28)	111 (93–133)	5.0
Dantrolene sodium	−80 ± 1.0	2.6 (2.1–3.4)	7.7	Skeletal muscle relaxation	—[f]	—	—	—	—
Clodanolene sodium	−78 ± 3.7	3.7 (3.2–4.1)	10.2	Skeletal muscle relaxation	—[f]	—	—	—	—

[a] $n = 4$ for each compound: drugs were administered in tetrahydrofurfuryl alcohol at a maximum cumulative dose of 36.6 mg/kg. [b] Drug dose causing 50% inhibition of gastrocnemius muscle twitch tension. [c] Gross effects in rats following a 30-mg/kg dose in tetrahydrofurfuryl alcohol. [d] Drug dose causing 50% inhibition of morphine-induced Straub tail in mice. [e] Not tested further because of undesirable side effects. [f] Not tested further because of solubility. [g] Insoluble in tetrahydrofurfuryl alcohol or dimethyl sulfoxide.

Straub Tail in Mice—The method used was similar to that reported by Ellis and Carpenter (22). The drugs were administered either orally in 0.5% methylcellulose or intravenously in distilled water 20 min after the subcutaneous morphine injection to male mice (TAC:SWfBr). The drugs administered orally were evaluated 30 min after drug dosing, while the compounds administered intravenously were evaluated 5 min after drug administration. The drugs were judged effective if they caused the elevated Straub tail to lie flat on the table. The compounds were administered in logarithmically spaced doses, and ED_{50} values were obtained by the Litchfield and Wilcoxon method (20).

LD_{50} in Mice—The test compounds were administered orally or intravenously to groups of unfasted male mice (TAC:SWfBr). The drugs administered orally were suspended in saline to prevent the gel formation that occurred occasionally in methylcellulose at the higher concentrations. Drugs administered intravenously were dissolved in distilled water. The animals were held in cages for 72 hr, and deaths were recorded. The LD_{50} values were calculated using the Litchfield and Wilcoxon method (20).

Intravenous Injections in Rats—Adult male rats (TAC:SD/NfBr) were used to determine the intravenous muscle relaxant potential of each drug in unanesthetized animals. The drugs were injected intravenously at a dose of 30 mg/kg (0.25 ml) in 100% tetrahydrofurfuryl alcohol into the tail vein. This dose was more than three times the effective muscle relaxant dose and was selected to uncover undesirable cholinergic and/or toxic effects of the drug. The drugs were evaluated by gross observation for skeletal muscle relaxation, gross changes, and acute toxicity.

Intravenous LD_{50} in Rats—Groups of five male rats (TAC:SD/NfBr) were administered the drug intravenously in distilled water for acute toxicity. The animals were held in their cages for 72 hr, and all deaths were recorded. The LD_{50} values were calculated using the method of Litchfield and Wilcoxon (20).

RESULTS AND DISCUSSION

To increase the aqueous solubility of dantrolene and clodanolene, quaternary ammonium salts of these compounds were synthesized. Initially, representative quaternary ammonium halide salts were evaluated for skeletal muscle relaxant activity (cholinergic and toxic signs such as loss of the righting reflex and death), and only compounds devoid of these pharmacological activities were used to form salts (Table III).

Fifteen compounds were synthesized and evaluated, including four salts of clodanolene and 11 salts of dantrolene (Table I). The aqueous solubilities for these compounds as well as those for dantrolene sodium and clodanolene sodium are listed in Table I. The compounds were evaluated initially by gross observation in mice, and 12 of the 15 compounds were effective oral muscle relaxants (Table IV). Dantrolene sodium has been shown to relax the tail in the Straub tail mouse (22). The 12 active compounds then were tested orally in the Straub tail mouse, and six compounds were effective (Table IV). These six compounds then were evaluated for motor incoordination (rotating rod), and the activities of these compounds were compared to the parent structures (Table IV). From these data, VI and XII yielded muscle relaxant efficacy indexes equal to those of dantrolene and slightly superior to those of clodanolene.

The objective in the synthesis and pharmacological evaluation of these compounds was to increase aqueous solubility. This objective was achieved since the solubilities for the respective compounds represent increases in solubilities of 10–50 times over those of the parent sodium salts. This increased solubility facilitates the dosage flexibility, specifically for intravenous administration of a skeletal muscle contraction antagonist required for conditions such as malignant hyperthermia (6–8).

The compounds were tested intravenously for inhibition of the gastrocnemius muscle twitch response and for gross effects including cholinergic symptoms and skeletal muscle relaxation. Fourteen of the 15 compounds tested were equally effective in inhibiting the gastrocnemius muscle twitch tension (IX was insoluble in the standard solvents, tetrahydrofurfuryl alcohol and dimethyl sulfoxide) (Table V). Six compounds caused cholinergic effects and were not tested further.

The six compounds without cholinergic properties that exhibited sufficient aqueous solubility were evaluated further. The LD_{50} intravenous values in rats were calculated, and therapeutic indexes were calculated using the ED_{50} for gastrocnemius muscle twitch tension inhibition (Table V). All six compounds were effective intravenously in abolishing the Straub tail phenomenon (Table V). The LD_{50} intravenous values in mice were estimated for these active compounds, and the resulting therapeutic indexes were calculated (Table V).

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