Table X—Ratio of Amounts in Fish at Overturn and Death

Compound	Ratio (X^*_{BO}/X^*_{BD})
I	0.590
II	0.545
III	0.585
IV	0.650
V	0.569
VI	0.273
Mean	0.535

time at that concentration. The apparent slopes do not differ much, and the intercepts are scattered about zero, indicating that all of the data may be grouped together. Linear regression of the combined data gave: $1/T_D = 0.2860(1/T_O) + 0.0001$ ($r = 0.9648$, $p < 0.01$), where T_D and T_O are the lethal and overturn times, respectively. A plot of $1/T_D$ *versus* $1/T_O$ is presented in Fig. 7.

For all data, there appears to be a linear relationship between the reciprocals of the time of response end-points at each concentration (Fig. 7). Pharmacologically, this finding suggests a simple relationship between the concentrations inducing narcosis and death. Where bulk solution concentrations are sufficiently high to make the MEC values negligible, the slope of $1/T_D$ *versus* $1/T_O$ is approximately equal to:

$$m = \frac{K_{12}V_AC^0_A}{X^*_{B_{death}}} \Big/ \frac{K_{12}V_AC^0_A}{X^*_{B_{overturn}}} \qquad \text{(Eq. 15)}$$

and:

$$m = \frac{X^*_{B_{overturn}}}{X^*_{B_{death}}} \qquad \text{(Eq. 16)}$$

Table X contains the ratio of the amount of each drug in the fish at the time of response, which may be regarded as a direct estimate of the narcosis to death ratio. The average value of the ratios is approximately 0.54. However, the value of the slope in Fig. 7, a second estimate, is only 0.29. The agreement between these two independent estimates is rough at best. The direct estimate assumes that the total fish concentration reflects the concentration in the critical CNS tissues at the moment the fish are removed from the drug solution, which may not be the case. Therefore, the pharmacological ratio would be the better estimate of the turnover to death concentration ratio. This fundamental point is worthy of more research.

REFERENCES

(1) G. Levy, K. E. Miller, and R. H. Reuning, *J. Pharm. Sci.*, **55**, 394 (1966).

(2) G. Levy and J. A. Anello, *ibid.*, **57**, 101 (1968).

(3) J. A. Anello and G. Levy, *ibid.*, **58**, 721 (1969).

(4) C. H. Nightingale, M. Tse, and E. I. Stupak, *ibid.*, **61**, 149 (1972).

(5) S. H. Yalkowsky, T. G. Slunick, and G. L. Flynn, *ibid.*, **63**, 691 (1974).

(6) S. Feldman, M. DeFrancisco, and P. J. Cascella, *ibid.*, **64**, 1713 (1975).

(7) G. Levy and S. P. Gucinski, *J. Pharmacol. Exp. Ther.*, **146**, 80 (1964).

(8) C. H. Nightingale and M. Gibaldi, *J. Pharm. Sci.*, **60**, 1360 (1971).

(9) B. J. Winer, "Statistical Principles in Experimental Design," McGraw-Hill, New York, N.Y., 1962, chap. 3.

Synthesis and Antimicrobial Evaluation of Quaternary Salts of 4-Phenyl-1,2,3,6-tetrahydropyridine and 3,6-Dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazine

NATHANIEL GRIER

Received July 21, 1978, from the *Merck Sharp & Dohme Research Laboratories, Merck & Co., Inc., Rahway, NJ 07065.* Accepted for publication September 5, 1978.

Abstract □ Fifteen predominantly alkyl bromide quaternary salts of 1-substituted 4-phenyl-1,2,3,6-tetrahydropyridine and 10 from 3,6-dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazine were synthesized. None was effective against the parasitic protozoan *Eimeria tenella* and the helminth trichostrongyle nematode. Nearly all inhibited Gram-positive and Gram-negative bacteria; maximum efficiency was obtained with nonyl through dodecyl bromide salt derivatives. Antifungal effectiveness paralleled these results. The oxazinium salt analogs were inhibitory in an *in vitro* peridontal microorganism screen. The decyl bromide derivative at 0.05% in drinking water prevented dental plaque and reduced calculus deposition in rats but not in hamsters fed cariogenic diets. A 0.01% concentration of the tetrahydropyridinium analog caused increased plaque in rats compared to nonmedicated control animals.

Keyphrases □ 4-Phenyl-1,2,3,6-tetrahydropyridine quaternary salts—synthesis and antimicrobial evaluation □ 3,6-Dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazine quaternary salts—synthesis and antimicrobial evaluation □ Antimicrobial activity—quaternary salts of 4-phenyl-1,2,3,6-tetrahydropyridine and 3,6-dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazine

Two heterocyclic nitrogen bases, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 3,6-dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazine, are readily accessible from the reaction of α-methylstyrene, formaldehyde, and methylamine salts (1) among other processes. Both compound types have served as moieties of synthetic drugs, *e.g.*, analgesics (2) and antineoplastic agents (3). Quaternary *N*-alkyl salt derivatives of the two bases appeared attractive as potential antimicrobials; in addition to the microbial cell wall degradative capability of the widely used cationic quaternary ammonium compounds (4), such novel products could have a possible mechanism for formaldehyde release initiated by Hofmann elimination. The inhibitory action of some preservatives, disinfectants, and antiseptics such as 1,3,5-trisubstituted hexahydro-*s*-triazines and methenamine compounds is considered attributable in part to a latent source of formaldehyde (5).

RESULTS AND DISCUSSION[1]

Thirteen quaternary salts of the tetrahydropyridine (Table I) were synthesized by alkylation with C-1–C-18 alkyl halides, decamethylene

[1] Tests for biological activities were carried out by divisions of Merck & Co., Rahway, N.J.

0022-3549/79/0400-0407$01.00/0

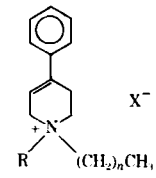

Table I—1-Substituted 1-Alkyl-4-phenyl-1,2,3,6-tetrahydropyridinium Salt Derivatives

Compound	R	n	X	Melting Point	Yield, %	Recrystallization Solvent	Empirical Formula	Analysis, %	Calc.	Found
Ia	H	9	Br	197–198.5°	36	Acetone	$C_{21}H_{35}BrN$	C	66.12	66.39
								H	9.25	9.62
								Br	20.71	20.95
								N	3.62	3.63
Ib	Methyl	0	I	266–268°	55	Ethanol	$C_{13}H_{18}IN$	C	49.54	49.67
								H	5.75	6.04
								N	4.44	4.45
Ic	Methyl	7	Br	199–201°	65	Acetone	$C_{20}H_{32}BrN$	C	65.55	65.08
								H	8.80	8.83
								Br	21.81	21.50
								N	3.82	3.80
Id	Methyl	8	Br	199–201°	51	Acetone	$C_{21}H_{34}BrN$	C	66.28	66.08
								H	9.01	8.93
								Br	20.99	20.49
								N	3.68	3.61
Ie[a]	Methyl	9	Br	200–202°	89	Acetone	$C_{22}H_{36}BrN$	C	66.97	66.91
								H	9.20	9.46
								N	3.55	3.61
If	Methyl	9	Dodecyl sulfate	152–156°	31	—[b]	$C_{34}H_{61}NO_4S$	C	70.42	69.96
								H	10.60	10.64
								N	2.42	2.30
Ig	Methyl	9	2-(Dodecyloxy)-ethyl sulfate	156–162°	51	—[b]	$C_{36}H_{65}NO_5S$	C	69.29	69.65
								H	10.50	10.57
								N	2.24	2.28
								S	5.14	5.13
Ih	Methyl	9	o-Sulfobenz-imide	190–190.5°	95	Acetonitrile–ether	$C_{29}H_{40}N_2O_3S$	C	70.13	69.66
								H	8.12	8.13
								N	5.64	5.55
								S	6.46	6.20
Ii	Methyl	10	Br	199–201°	37	Acetone	$C_{23}H_{38}BrN$	C	67.63	67.20
								H	9.38	9.57
								N	3.43	3.23
Ij	Methyl	11	Br	202–205°	59	Acetone	$C_{24}H_{40}BrN$	C	68.24	68.67
								H	9.54	9.67
								Br	18.91	19.20
								N	3.30	3.35
Ik	Methyl	13	Br	204–207°	49	Acetone	$C_{26}H_{44}BrN$	C	69.31	69.43
								H	9.84	9.86
								Br	17.74	18.09
								N	3.11	3.13
Il	Methyl	15	Br	211–214°	49	Acetone	$C_{28}H_{48}BrN$	C	70.27	70.43
								H	10.11	10.31
								Br	16.69	16.95
								N	2.93	2.86
Im	Methyl	17	Br	218–222°	39	Acetone	$C_{30}H_{52}BrN$	C	71.12	71.27
								H	10.35	10.34
								Br	15.77	16.12
								N	2.77	2.61
In	Ethyl	9	Br	168.5–169.5°	38	Acetone	$C_{23}H_{38}BrN$	C	67.63	67.47
								H	9.38	9.58
								Br	19.56	19.57
								N	3.48	3.41
Io	Decamethylene-bis	0	Br	259–260.5°	84	Ethanol	$C_{34}H_{50}Br_2N_2$	C	63.17	63.47
								H	7.79	8.11
								Br	24.73	24.74
								N	4.33	4.48
Ip	Phenylmethyl	0	Cl	204–206.5°	76	2-Propanol–ether	$C_{19}H_{22}ClN \cdot \frac{1}{2}H_2O$	C	73.86	73.80
								H	7.50	7.59
								Cl	11.48	11.57
								N	4.53	4.50

[a] Prepared by F. Waksmunski, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065. [b] Not recrystallized.

dibromide, or phenylmethyl chloride in refluxing acetone. The normethyl and 1-ethyl base homologs (1) were reacted with decyl bromide to provide nonquaternary 1-decyl-4-phenyl-1,2,3,6-tetrahydropyridinium hydrobromide (Ia) and 1-decyl-1-ethyl-4-phenyl-1,2,3,6-tetrahydropyridinium bromide (In). Ten oxazinium salts (Table II) were similarly prepared; the properties of several of these compounds were reported previously (6).

All compounds were evaluated for antimicrobial and antiparasitic potencies. None was active in the parasitology screen, which included *in vitro* and *in vivo* helminthiasis assays with trichostrongyle nematode, ova and larvae, and coccidiosis (*Eimeria tenella*) in chickens. The methods for these tests and the *in vitro* antibacterial and antifungal studies (Tables III and IV) were described previously (7).

The eight C-8–C-18 alkyl oxazinium bromide salts (IIb–IId and IIf–IIj) were additionally tested in an *in vitro* microbial periodontal disease screen (Table V). The decyl derivative, IId, was further studied in an *in vivo* experiment (rat and hamster) for the prevention of dental calculus and plaque.

An *in vitro* chemotherapy assay (Table III) utilized Gram-positive and Gram-negative bacteria; fungi associated with industrial and agricultural

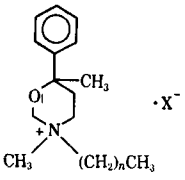

Table II—3-Alkyl-3,6-dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazinium Salt Derivatives

Compound	*n*	X	Melting Point	Yield, %	Recrystallization Solvent	Empirical Formula	Analysis, %	Calc.	Found
II*a*	0	I	205–207°	76	Ethanol	$C_{13}H_{20}INO$	C	46.85	46.85
							H	6.05	6.36
							I	38.09	38.34
							N	4.21	4.28
II*b*	7	Br	199–200°	40	Acetone	$C_{20}H_{34}BrNO$	C	62.48	62.49
							H	8.91	8.86
							Br	20.79	20.51
							N	3.64	3.38
II*c*	8	Br	192–193.5°	34	Acetone	$C_{21}H_{36}BrNO$	C	63.30	63.64
							H	9.11	9.40
							Br	20.05	20.16
							N	3.52	3.28
II*d*	9	Br	194–195.5°	34	Acetone	$C_{22}H_{38}BrNO$	C	64.07	63.80
							H	9.29	9.33
							Br	19.37	19.13
							N	3.48	3.32
II*e*	9	*o*-Benzo-sulfimide	115–116°	64	Acetonitrile–ether	$C_{29}H_{42}N_2O_4S$	C	67.64	67.68
							H	8.23	8.52
							N	5.45	5.36
							S	6.23	6.18
II*f*	10	Br	186–187°	30	Acetone	$C_{23}H_{40}BrNO$	C	64.77	64.96
							H	9.45	9.50
							Br	18.74	18.32
							N	3.28	3.58
II*g*	11	Br	190–191.5°	32	Acetone	$C_{24}H_{42}BrNO$	C	65.48	65.29
							H	9.61	9.69
							Br	18.13	18.10
							N	3.18	3.20
II*h*	13	Br	183–184.5°	36	Acetone	$C_{26}H_{46}BrNO$	C	66.62	67.16
							H	9.90	10.05
							Br	17.05	17.41
							N	2.99	2.88
II*i*	15	Br	174–176°	67	Acetone	$C_{28}H_{50}BrNO$	C	67.62	67.99
							H	10.15	10.17
							N	2.82	2.75
II*j*	17	Br	171.5–173°	55	Acetone	$C_{30}H_{54}BrNO$	C	68.69	69.15
							H	10.38	10.60
							N	2.67	2.64

Table III—*In Vitro* Antibacterial and Antifungal Assay

Compound	Minimal Inhibitory Concentration, μg/ml							
	S. aureus[a]	*S. pyogenes*[b]	*B. bronchiseptica*[c]	*K. pneumoniae*[d]	*E. aerogenes*[e]	*E. coli*[f]	*A. pullulans*[g]	*A. niger*[h]
I*a*	60	20	—[i]	—	—	—	200	200
I*c*	60	60	200	200	100	200	—	200
I*d*	10	10	100	100	100	200	60	200
I*e*	10	10	20	60	10	60	20	10
I*h*	60	60	60	60	60	100	20	200
I*i*	1	10	10	20	60	60	—	—
I*j*	1	20	20	10	60	200	10	10
I*k*	1	1	10	10	—	—	20	60
I*l*	10	10	—	—	—	—	—	—
I*m*	10	10	—	—	—	—	—	—
I*n*	20	20	60	60	60	200	100	—
I*o*	10	10	100	—	—	200	20	—
II*b*	10	100	—	200	100	—	—	—
II*c*	10	20	60	100	60	200	200	200
II*d*	10	10	20	60	10	100	100	—
II*e*	10	10	60	60	100	—	60	200
II*f*	1	1	1	60	60	100	10	100
II*g*	20	20	20	20	20	100	10	200
II*h*	20	20	20	100	—	—	—	—
II*i*	1	10	10	20	60	60	—	—
II*j*	10	60	—	—	—	—	—	—
Cetylpyridinium bromide[j]	1	10	20	60	—	—	60	—
Benzalkonium chloride[k]	10	10	20	10	10	200	60	—

[a] Merck Bacteria (MB) 2865. [b] MB 2874. [c] MB 3551. [d] MB 3123. [e] MB 1503. [f] MB 2884. [g] MF 4341. [h] MF 442. [i] Greater than 200 μg/ml. [j] K & K Laboratories, New York, N.Y. [k] Hyamine 3500, Rohm & Haas, which contains alkyl (50% C-14, 40% C-12, 10% C-16) dimethylbenzylammonium chloride as an 80% solution in ethanol.

Table IV—Antimicrobial Evaluation of Decyl Bromide Quaternary Salts

Compound	Minimal Inhibitory Concentration[a], μg/ml										
	S. aureus[b]	*B. mycoides*[c]	*E. aerogenes*[d]	*P. aeruginosa*[e]	*A. pullulans*[f]	*A. niger*[g]	*P. luteum*[h]	*R. solani*[i]	*C. globosum*[j]	*T. viride*[k]	*V. alboatrium*[l]
Ie	10	10	50	—[m]	—	—	—	50	100	—	50
IId	10	50	50	100	100	100	100	50	50	100	100
Hexahydro-1,3,5-triethyl-*s*-triazine	100	100	100	100	100	100	100	100	100	100	100

[a] Tested at 1, 10, 50, and 100 μg/ml. [b] ATCC 6538. [c] Institute of Paper Chemistry 509. [d] Institute of Paper Chemistry 500. [e] ATCC 10145. [f] ATCC 9348. [g] ATCC 6275. [h] ATCC 10466. [i] ATCC 16115. [j] ATCC 6205. [k] ATCC 8678. [l] V3H. [m] Greater than 100 μg/ml.

Table V—Microbial Periodontal Disease Screen for 3-(C-8–C-18)-Alkyl Bromide Salts of 3-Methyl-6-phenyltetrahydro-2*H*-1,3-oxazine

Compound	Zone of Inhibition, mm diameter[a]				
	O. viscosus[b]	*Bacteroides* sp.[c]	*Strep.* SL-1[d]	*S. aureus*[e]	*E. coli*[f]
IIb	26	17	15	11	21
IIc	34	22	20	13	20
IId	32	27	21	13	20
IIf	32	23	20	10	18
IIg	25	20	17	8	13
IIh	17	12	10	0	9
IIi	10	9	0	0	8
IIj	15	0	0	0	0
Control A[g]	17	14	20	8	9
Control B[h]	28	35	24	15	23

[a] For all compounds, a concentration of 50 μg/6.35-mm diameter assay disk was used. [b] MB 2065. [c] MB 2022. [e] ATCC 9637. [f] ATCC 6538. [g] 1,1′-Hexamethylenebis[5-(2-ethylhexyl)biguanide]·2HCl, 50 μg/disk. [h] Chlortetracycline, 5 μg/disk.

Table VI—*In Vivo* Rat Dental Plaque and Calculus Inhibition

Compound	Concentration in Drinking Water, % (w/w)	Percent of Control	
		Plaque	Calculus
Ie	0.01	200	80
IId	0.01	30	65
Control A[a]	0.01	20	125
Quaternary salt[b]	0.01	15	65
IId	0.05	0	15
Quaternary salt[b]	0.05	0	30

[a] 1,1′-Hexamethylenebis[5-(2-ethylhexyl)biguanide]·2HCl. [b] Cinnamyldimethyldodecyl ammonium chloride.

economic losses were included. Method details were reported (7); concentrations of 200, 100, 60, 20, 10, 5, and 1 μg/ml of test compound in brain heart infusion agar culture medium[2] were run in duplicate as outlined.

Broad spectrum antimicrobial efficiency appeared to peak and approximate that of benzalkonium chloride in both series of compounds when the quaternary alkyl methyl bromide salts were of C-10–C-12 chain length. The higher alkyl derivatives, Il, Im, and IIj, proved effective only for Gram-positive bacteria, similar to the nonquaternary salt Ia. Failure to inhibit any microorganisms was observed with Ib, If, Ig, and Ip with *Staphylococcus aureus, Streptococcus pyogenes, Bordetella bronchiseptica, Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli, Aureobasidium pullulans,* and *Aspergillus niger.* Additionally, no inhibitory action was obtained at the maximum concentration, 200 μg/ml, for all compounds against *Pseudomonas aeruginosa* (MB 418 and MB 2245), *Proteus mirabilis* (MB 3549), *Fusarium* sp. (MF 4648), and *Cephalosporium* sp. (MF 4641).

Two representatives of the more active compounds, Ie and 1-decyl-1,6-dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazinium bromide (IId), were compared *in vitro* (Table IV) with a commercial antimicrobial, hexahydro-1,3,5-triethyl-*s*-triazine, using microorganisms relevant to biodeterioration and the noted procedure (7). Compound IId was at least equipotent to the triazine; Ie had a significantly narrower antifungal spectrum than both but was markedly superior against Gram-positive bacteria.

The suspected periodontal disease organisms, *Odontomyces viscosus, Bacteroides* sp., and the cariogenic *Streptococcus* SLI strain, along with a general Gram-positive type, *S. aureus,* and a Gram-negative bacteria, *E. coli,* were studied for sensitivity to the oxazinium salts (Table V). Brain heart infusion agar, enriched with 5% sucrose for the *Streptococcus,* was used as the culture medium. A paper disk, medicated by wetting with 0.025 ml of compound stock solution, was placed on the surface. Five milligrams of compound was dissolved in 0.1 ml of dimethylformamide and diluted to 2.5 ml with distilled water to provide 50 μg/0.025 ml. All inoculum cultures were 16–24 hr old, and 5 ml was added to 200 ml of medium (10 ml/200 ml of medium for *O. viscosus*); 10 ml of each mixture was used per plate.

Incubation was overnight at 37° in anaerobe jars under nitrogen–carbon dioxide, and diameters of inhibition zones were measured. Antibacterial efficiencies appeared maximal with the nonyl, decyl, and undecyl bromides (IIc–IIe) and were much higher than with the bisbiguanide (Table V) control across the spectrum of microorganisms. The bacterial efficacy and structural requirements for *in vitro* dental antiplaque activity of this compound and 15 related guanide and biguanide derivatives were studied previously (8). The second control, chlortetracycline, at one-tenth the dosage was appreciably more effective against *Bacteroides* sp. and approximately matched the best compounds for the other four bacteria.

Compounds Ie and IId were further evaluated as inhibitors *in vivo* of dental plaque and calculus in rats (Table VI) and hamsters. Ten male weanling rats, Holtzmann strain, in each group were given a high sucrose diet, NIH 2000 (9), *ad libitum* and drinking water medicated with each compound at 0.01% concentration. After 14 days and then at weekly intervals, the upper six molar teeth were scored for plaque and calculus. Control animals on the same diet were given nondosed distilled drinking water.

Data were averaged over the 5–10-week test period and compared with the bisbiguanide (Control A) and cinnamyldimethyldodecyl ammonium chloride. Compound Ie-treated animals appeared to have twice the surface deposition of plaque as compared to those without medication. In similar studies, antimicrobials such as subtilin and nalidixic acid caused increased caries over untreated controls; one mechanism postulated is the selective removal of competing noncariogenic microflora (10).

The cinnamyl quaternary salt derivative and IId at 0.05% concentration in drinking water prevented plaque completely and reduced calculus to less than one-third of the control. Corresponding tests in hamsters indicated both compounds to be ineffective. Differences in dental plaque properties and treatment responses between the two species (11) and methods for caries prevention assay in hamsters (9) were reported previously.

EXPERIMENTAL[3]

Table I compounds were prepared as halide salts according to the method described for Ic; the normethyl and 1-ethyl base homologs were intermediates for Ia and In, respectively. Other salts were synthesized from the bromide as outlined for Ih. The same procedures were employed for Table II compounds, and 3,6-dimethyl-6-phenyltetrahydro-2*H*-1,3-oxazine (1) served as the base for all. No effort was made to improve yields or to separate isomers.

IR spectra appeared consistent with the structures assigned. Halide salts were characterized by PMR spectra in methanol-d_4, except for Io which required trifluoroacetic acid for solubilization. The 4-phenyl group of I compounds appeared as a multiplet (5H) between 7.2 and 7.6 ppm

[2] Difco.

[3] Melting points were taken in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. IR spectra were obtained from mineral oil mulls with a Perkin-Elmer model 137 spectrophotometer, and NMR spectra were obtained with a Varian T-60 (60 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane.

[the phenylmethyl derivative, Ip, 7.1–7.8 ppm (10H)], and the vinyl proton (1H) appeared as a broad singlet at 6.0–6.3 ppm. The 6-phenyl group of II compounds was in the same range downfield, and the 2-methylene group (OCH_2N) appeared as a doublet of doublet, 4.41 and 4.58 ppm and 4.80 and 4.95 ppm, probably corresponding to the axial and equatorial protons, respectively[4].

1-Methyl-1-octyl-4-phenyl-1,2,3,6-tetrahydropyridinium Bromide (Ic)—A solution of 3.5 g (0.02 mole) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (1) and 10 g (0.05 mole) of 1-bromooctane in 50 ml of anhydrous acetone was heated on a steam bath for 12 hr. The mixture obtained upon final cooling in an ice bath was filtered. Then the crystalline solid was washed with cold acetone and diethyl ether and purified by recrystallization, 1 g/50 ml of acetone, to provide colorless needles.

1-Decyl-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium *o*-Sulfobenzimide (Ih)—One gram (0.0025 mole) of 1-decyl-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium bromide (Ie) was suspended in 100 ml of warm (35°) deionized water with stirring, and a solution of 3.0 g (0.012 mole) of sodium *o*-sulfobenzimide dihydrate in 10 ml of deionized water was dripped in (10 min). The product separated initially as a colorless oil and solidified upon mixing an additional 2 hr, followed by cooling in an ice bath. The water-washed and air-dried colorless solid was recrystallized by solution of 1 g in 2 ml of acetonitrile and addition of approximately 18 ml of ether until incipient turbidity. The colorless plates obtained were washed with cold acetonitrile–ether (1:9 v/v).

REFERENCES

(1) C. J. Schmidle and R. C. Mansfield, *J. Am. Chem. Soc.*, **78**, 425, 1702 (1956). *Ibid.*, **77**, 5698 (1955). C. J. Schmidle and R. C. Mansfield, U.S. pats. 2,847,414 (1958) and 2,750,385 (1956).

(2) M. Rajšner, E. Adlerová, and M. Protiva, *Coll. Czech. Chem. Commun.*, **28**, 1031 (1963).

(3) T. Urbański, D. Guene, S. Šlopek, H. Modarska, and M. Modarska, *Nature*, **187**, 426 (1960).

(4) A. N. Petrocci, in "Disinfection, Sterilization, and Preservation," 2nd ed., S. S. Block, Ed., Lea & Febiger, Philadelphia, Pa., 1977, p. 325.

(5) J. DeMare, H. W. Rossmore, and T. H. F. Smith, *Dev. Ind. Microbiol.*, **13**, 341 (1972).

(6) N. Grier, U.S. pat. 3,920,642 (Nov. 18, 1975).

(7) N. Grier and R. Strelitz, *J. Pharm. Sci.*, **65**, 616 (1976).

(8) J. M. Tanzer, A. M. Slee, and B. A. Kamay, *Antimicrob. Agents Chemother.*, **12**, 721 (1977).

(9) P. L. Block, C. L. Dooley, and E. E. Howe, *J. Periodont.*, **40**, 41 (1969).

(10) R. J. Fitzgerald, *Antimicrob. Agents Chemother.*, **1**, 296 (1972).

(11) H. B. Woodruff, K. H. Nollstadt, R. B. Walton, and T. H. Stoudt, *Dev. Ind. Microbiol.*, **17**, 405 (1976).

(12) Y. E. Kazantsev, I. P. Boiko, Y. F. Malina, O. I. Zhuk, Y. Y. Samitov, and B. V. Unkovskii, *Zh. Org. Khim.*, **9**, 2616 (1972) (in English).

(13) H. Booth and R. U. Lemieux, *Can. J. Chem.*, **49**, 777 (1971).

ACKNOWLEDGMENTS

The author thanks Microanalytical Services for the elemental analyses, the Basic Microbiology Department for the *in vitro* antimicrobial assays, Dr. R. B. Walton for the *in vitro* periodontal disease screen, and Dr. E. E. Howe and associates for the *in vivo* dental study.

[4] Detailed NMR studies of the tetrahydro-1,3-2*H*-oxazine ring system were reported (12, 13).

Plasma Naltrexone Kinetics after Intravenous Bolus Administration in Dogs and Monkeys

R. H. REUNING *x, V. K. BATRA *§, T. M. LUDDEN *¶, M. Y. JAO *, B. E. MORRISON *, D. A. McCARTHY ‡†, S. E. HARRIGAN ‡, S. B. ASHCRAFT *, R. A. SAMS *‖, M. S. BATHALA *•, A. E. STAUBUS *, and L. MALSPEIS *

Received April 14, 1978, from the **College of Pharmacy, Ohio State University, Columbus, OH 43210,* and the ‡*Pharmaceutical Research Division, Warner-Lambert/Parke-Davis, Ann Arbor, Mich.* Accepted for publication September 6, 1978.

§Present address: Lederle Laboratories, Pearl River, NY 10965. ¶Present address: University of Texas Health Science Center, San Antonio, TX 78284. ‖Present address: Department of Veterinary Clinical Sciences, Ohio State University, Columbus, OH 43210. •Present address: A. H. Robins Research Laboratories, Richmond, VA 23220. †Deceased.

Abstract □ This investigation generated data characterizing a specific electron-capture GLC assay reported previously for naltrexone and applied the method to a determination of naltrexone pharmacokinetics. Extraction efficiencies are reported for the assay, and mass spectral evidence indicates that naltrexone forms a triester when derivatized for electron-capture GLC with pentafluoropropionic anhydride and a base catalyst. Plasma level–time data for intravenous naltrexone at two dose levels in monkeys yielded no evidence of dose-dependent kinetics. A two-compartment open pharmacokinetic model was fitted to plasma level–time data for naltrexone in two dogs and yielded a total body clearance of 51–55 ml/min/kg. Urine collected for 0–24 hr contained 36% of the dose as naltrexone conjugates with less than 1% as unchanged naltrexone. Plasma level–time data for intravenous naltrexone in six monkeys yielded an average terminal half-life of 7.8 hr and a total body clearance of 64 ml/min/kg. The total body clearance for naltrexone was greater than the hepatic plasma or blood flow in both dogs and monkeys. This finding, together with the extremely low renal excretion of naltrexone, suggests the existence of elimination mechanisms besides liver metabolism and renal excretion.

Keyphrases □ Naltrexone—plasma kinetics after intravenous bolus administration, dogs and monkeys, electron-capture GLC assay □ Pharmacokinetics—naltrexone after intravenous bolus administration, dogs and monkeys, electron-capture GLC assay □ GLC, electron capture—specific assay for naltrexone □ Narcotic antagonists—naltrexone, pharmacokinetics after intravenous bolus administration, dogs and monkeys, electron-capture GLC assay

Naltrexone, a narcotic antagonist, has been investigated for use in the treatment of opioid dependence (1). In humans, most of the typical responses to a heroin challenge (25 mg iv) are blocked by naltrexone for 48–72 hr after a 100-mg oral dose (2). Because the duration of action of naltrexone is short in relation to that considered optimal

0022-3549/79/0400-0411$01.00/0