Primary Local Anesthetic Testing and Acute Toxicity of *N,N'*-Disubstituted 2,3-Diamino-2',6'-propionoxylidides

H. JACK ADAMS▲, GEORGE H. KRONBERG, and BERTIL H. TAKMAN

Abstract □ Eight *N,N'*-disubstituted 2,3-diamino-2',6'-propionoxylidides and 1-*tert*-butyl-2-aziridinecarboxy-2',6'-xylidide were prepared and tested for local anesthetic activity in the rat sciatic nerve block and in the guinea pig wheal. The durations in the rat sciatic blocks fell between those for lidocaine and tetracaine, but none of them possessed the long duration of tetracaine in the guinea pig wheal. All compounds were more toxic than tetracaine intraperitoneally in the mouse but, except for the aziridine compound, were less irritating than tetracaine in the rabbit intradermal wheal.

Keyphrases □ 2,3-Diamino-2',6'-propionoxylidides, *N,N'*-disubstituted—local anesthetic activity and acute toxicity, compared to lidocaine and tetracaine □ 1-*tert*-Butyl-2-aziridinecarboxy-2',6'-xylidide—local anesthetic activity and acute toxicity, compared to lidocaine and tetracaine □ Anesthetic activity, local—*N,N'*-disubstituted 2,3-diamino-2',6'-propionoxylidides and 1-*tert*-butyl-2-aziridinecarboxy-2',6'-xylidide □ 2,6-Xylidine derivatives—*N,N'*-disubstituted 2,3-diamino-2',6'-propionoxylidides and 1-*tert*-butyl-2-aziridinecarboxy-2',6'-xylidide, local anesthetic activity and acute toxicity

2,3-Bis(mono- and dialkyl) aminopropionanilides have been studied as local anesthetics (1–3). Except for 2,3-bis(diethylamino)-2',6'-propionoxylidide (IX), no other derivative of 2,6-xylidine has been reported previously (1). Because of the rather long duration of anesthesia observed for this substance, several related compounds (Table I) were synthesized and investigated pharmacologically.

EXPERIMENTAL

Chemistry[1]—2,3-Dibromopropionyl chloride (4, 5) was allowed to react with 2,6-xylidine to give 2,3-dibromo-2',6'-propionoxylidide (X) (1). When X was refluxed with secondary amines, the bistertiary amines were obtained. In several cases where X was allowed to react with propylamine or *tert*-butylamine under the same conditions, the expected bis(monoalkyl) aminopropionoxylidides were not formed.

Only the reaction with *tert*-butylamine was studied in some detail. The main portion of the basic products obtained was found to be 1-*tert*-butyl-2-aziridinecarboxy-2',6'-xylidide (XI). The fact that no similar compound was obtained from the reaction of propylamine and X indicates a stabilizing effect on the aziridine system by the presence of the bulky *tert*-butyl group.

The formation of aziridine derivatives from amines and 2,3-halogenopropionyl derivatives was reported previously (6, 7) but under exceedingly milder temperature conditions (liquid ammonia). Some aziridinecarboxanilides also were prepared previously, although by quite different routes (8).

Intermediates—2,3-Dibromo-2',6'-propionoxylidide (X) (1) was obtained from 2,3-dibromopropionyl chloride (5) and 2,6-xylidine in an acetate buffer system (1).

[1] Melting points were taken on the Thomas–Hoover capillary melting-point apparatus and are uncorrected. Analyses were performed by Alfred Bernhardt Microanalytical Laboratory, Elbach über Engelskirchen, West Germany, and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer; NMR spectra were recorded on a Varian A60 with tetramethylsilane as internal standard. The IR spectra were as expected.

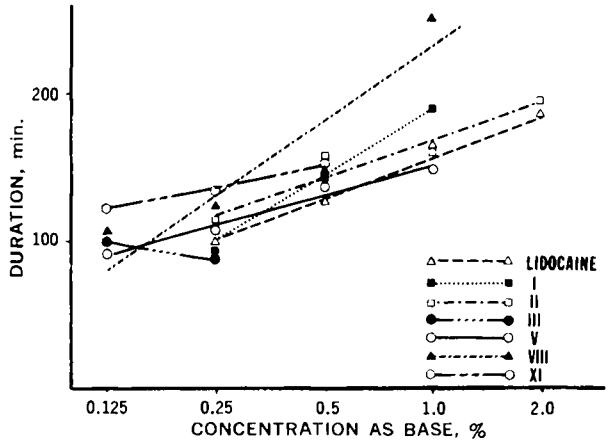

Figure 1—*Rat sciatic nerve blocks.*

Compounds of Table I—To a cool stirred solution of the secondary amine (4.5 moles) in dry benzene was added X (1 mole) in portions. After brief stirring at room temperature, the mixture was allowed to reflux for 5–6 hr. When cool, the reaction mixture was filtered, the volatiles were stripped off under reduced pressure at 50–60°, and the residue was taken up in dilute hydrochloric acid. The aqueous acid solution was washed several times with ether and then alkalinized (pH 10–11) and extracted with ether to obtain the base. The product was further purified either by direct recrystallization or through salt formation and subsequent recrystallization (*cf.*, Tables I and II).

(±)-1-tert-*Butyl-2-aziridinecarboxy-2',6'-xylidide* (*XI*)—In a glass-lined pressure vessel, X (43 g., 0.128 mole), *tert*-butylamine (73.1 g., 1.00 mole), and benzene (400 ml.) were heated for 16 hr. at 100°. After cooling, a white precipitate of *tert*-butylammonium bromide (91% yield) was filtered off. The amber filtrate was evaporated, and the waxy residue was dissolved in 200 ml. 1 *M* HCl. The solution was washed with ether, made alkaline under cooling with 7 *M* NaOH, and immediately extracted exhaustively with ether. After

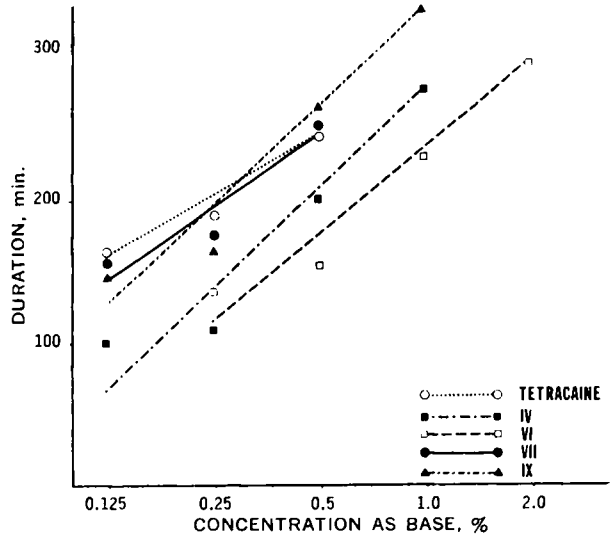

Figure 2—*Rat sciatic nerve blocks.*

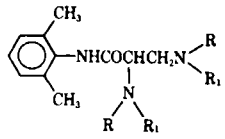

Table I Physical and Chemical Data

Number	R	R_1	Compound Form	Yield, %	Melting Point	Recrystallization Solvent
I	CH_3	CH_3	Base	55	150.5–151.5°	Cyclohexane
II	CH_3	C_2H_5	Base	55	113.5–114.5°	—[a]
III	CH_3	n-C_3H_7	Base	58	57.5–59.5°	—[b]
IIIa			Monooxalate		126.5°	Acetonitrile
IV	CH_3	iso-C_3H_7	Base	57	97–98°	Cyclohexane; n-hexane
V	CH_3	CH_2=$CHCH_2$	Base	48	88–89.5°	Aqueous ethanol
VI	CH_3	CH≡CCH_2	Base	52	101–103°	95% ethanol
VII	CH_3	$CH_3OCH_2CH_2$	Diperchlorate	40	124–125°	95% ethanol
VIII	NRR_1 = pyrrolidino		Base	43	163.5–165.5°	Benzene–cyclohexane

[a] The base was obtained from an oxalate, m.p. 130.5–131°, which was not analyzed. [b] Not recrystallized; obtained from IIIa.

drying (sodium sulfate) and evaporation, a light-brown powder (29.9 g.) was obtained. Repeated recrystallizations from petroleum ether (b.p. 60–110°) gave colorless crystals (12.5 g., 0.0508 mole, 40%), m.p. 112.5–113°. Approximately another 2.5 g. of XI could be isolated from the mother liquors with the help of chromatographic purification of a benzene solution on aluminum oxide. The total yield of XI was thus approximately 48%; NMR ($CDCl_3$): 1.08 [s, 9H, $(CH_3)_3C$], 1.84–2.04 (m, 2H, CH_2), 2.20 (s, 6H, 2 CH_3—C_6H_5), 2.36–2.52 (m, 1H, CH), 7.07 (s, 3H, C_6H_5), and 8.18 (s, 1H, NH).

Anal.—Calc. for $C_{15}H_{22}N_2O$: C, 73.1; H, 9.00; N, 11.37. Found: C, 73.0; H, 8.97; N, 11.50.

Pharmacology and Toxicology—*Rat Sciatic Nerve Blocks*—This technique was described by Camougis and Takman (9). Each compound was tested at three to five concentrations. For this primary evaluation, all solutions contained 1:100,000 epinephrine. Precisely 0.2 ml. drug solution was injected into the midthigh region so that it was deposited around the sciatic nerve trunk. Five animals were used at each concentration, and both thighs were injected on each animal. After being injected, animals were examined at frequent intervals for onset, depth, and duration of motor block. Linear regression lines were fitted to the dose–duration data[2] for the rat sciatic nerve blocks and the guinea pig wheals.

Guinea Pig Intradermal Wheals—A modification of the method described by Bülbring and Wajda (10) was used. Each wheal was made by injecting 0.1 ml. of drug solution or vehicle intradermally on the shaved back. Six wheals were made per animal, and eight to 12 wheals were made at each concentration. All solutions contained 1:100,000 epinephrine. Presence or absence of anesthesia was determined at frequent intervals by means of pinpricks.

Irritation Studies—This method was described by Camougis and Takman (9). The backs of rabbits were shaved, and a series of eight wheals per animal was made by injecting 0.1 ml. of drug solution or vehicle intradermally at each site. Twenty-four hours later the wheals were graded for degree of erythema, degree of edema, and presence or absence of necrosis. Grading was done on a numerical scale, and mean scores were obtained for all wheals at each concentration of test compound.

Acute Toxicity—The LD_{50}'s were determined in female CRCD mice, weighing about 20–25 g. Test compounds were dissolved in isotonic saline or distilled water and administered intraperitoneally. Three to five dose levels were used for each LD_{50}, and there were 10 animals at each dose level. Control groups received vehicle at a dose volume comparable to the highest dose volume of test compound. The LD_{50}'s and 95% confidence limits were calculated by the minimum logit chi-square method (11).

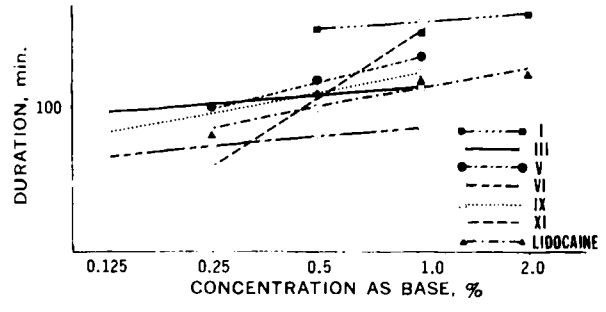

Figure 3—*Guinea pig intradermal wheals.*

[2] Using a Hewlett-Packard 9100B calculator, a 9125B plotter, and a linear regression program No. Stat-Pac X-7.

RESULTS

Rat Sciatic Nerve Blocks—Compounds I, II, III, V, and XI had dose–duration curves similar to that of lidocaine (Fig. 1). Only two points were obtained with III because 100% fatalities occurred at all concentrations above 0.25%. The dose–duration curve for VIII indicates somewhat longer durations than for the other compounds, chiefly due to the long blocks at the 1.0% concentration. Compounds IV and VI (Fig. 2) had durations greater than that of lidocaine but less than that of tetracaine; the dose–duration curves for VII and IX were comparable to that of tetracaine (Fig. 2).

Table II—Analyses of Compounds I–VIII

Number	Formula	Analysis, % Calc.	Found
I	$C_{15}H_{25}N_3O$	C 68.4	C 68.6
		H 9.59	H 9.80
		N 15.95	N 15.88
II	$C_{17}H_{29}N_3O$	C 70.1	C 70.0
		H 10.03	H 10.08
		N 14.42	N 14.32
III	$C_{19}H_{33}N_3O$	C 71.4	C 71.4
		H 10.41	H 10.19
		N 13.15	N 13.33
IIIa	$C_{21}H_{35}N_3O_5$	C 61.6	C 61.2
		H 8.62	H 8.26
IV	$C_{19}H_{33}N_3O$	C 71.4	C 71.3
		H 10.41	H 10.18
		N 13.15	N 13.20
V	$C_{19}H_{29}N_3O$	C 72.3	C 72.3
		H 9.28	H 9.51
		N 13.32	N 13.34
VI	$C_{19}H_{25}N_3O$	C 73.3	C 73.4
		H 8.09	H 8.19
		N 13.49	N 13.51
VII	$C_{19}H_{35}Cl_2N_3O_{11}$	C 41.3	C 41.4
		H 6.39	H 6.47
		O 31.9	O 31.9
VIII	$C_{19}H_{29}N_3O$	C 72.3	C 72.6
		H 9.27	H 9.11
		N 13.32	N 13.41

Table III—Acute Intraperitoneal Toxicity in Female Mice

Compound	LD_{50} and 95% Confidence Limits, mg./kg. as Base
I	44 (26–58)
II	17 (14–22)
III	16 (14–21)
IV	9 (6–13)
VII	7 (5–9)
VIII	16 (14–22)
IX	14 (11–16)
Lidocaine hydrochloride	133 (121–150)
Tetracaine hydrochloride	55 (44–69)

Frequency of block was 70% or greater at the lowest concentration tested for all compounds except VI and IX; frequency was 70% or greater at concentrations above 0.25% with these two compounds.

Guinea Pig Intradermal Wheals—The dose–duration curves for lidocaine and for Compounds I, III, V, VI, IX, and XI are shown in Fig. 3. Except for XI, these compounds exhibit flat dose–duration curves in this preparation and, in general, their curves lie close to that obtained with lidocaine. The curves for tetracaine and for Compounds II, IV, VII, and VIII are shown in Fig. 4. Although durations with these test compounds tended to be greater than those obtained with lidocaine, none of them was comparable to tetracaine in this test procedure. Because of the toxicity of VII, only two points were obtained with that compound.

In the rat sciatic nerve block experiments, Compounds VII and IX exhibited the longest durations of any test compounds; in the guinea pig wheal, VII was one of the two test compounds with the longest duration of action. However, this pattern is not consistent throughout. For example, Compound VIII was short acting in the rat sciatic but long acting in the guinea pig wheal. Such apparent inconsistencies can probably be accounted for by: (*a*) marked physiological differences between the two sites of drug application, and (*b*) differences in the physicochemical and pharmacological properties of the test compounds (12).

Irritation Studies—Because of differences in toxicity, all compounds were not tested at the same three concentrations. Lidocaine, tetracaine, and Compounds I, II, V, VI, and VIII were tested at 0.5, 1.0, and 2.0%. The others were tested at 0.25, 0.5, and 1.0%. Therefore, data are available at 0.5 and 1.0% for all compounds except VII, which was lethal at 1%. The rank order of the compounds for which irritation indexes were obtained at 0.5 and 1.0% is: least irritating = lidocaine < V < I < II < IV < III < VI < VIII < tetracaine < XI = most irritating.

Acute Toxicity—Because insufficient quantities remained after the other tests had been performed, acute toxicity determinations were not done with V, VI, and XI. The LD_{50}'s for the other test compounds and for lidocaine and tetracaine are shown in Table III. All of the compounds tested are more toxic than lidocaine, and all but I are significantly more toxic than tetracaine.

CONCLUSIONS

All of the test compounds exhibit short latency and excellent frequency of local anesthetic block. Durations are, in general, intermediate between those of lidocaine and tetracaine. All but one of the compounds are more irritating than lidocaine but less irritating than tetracaine; except for one compound, all are more toxic than tetracaine. Further synthetic efforts along these lines should obviously be aimed at retaining the excellent local anesthetic activity while attempting to reduce the toxicity.

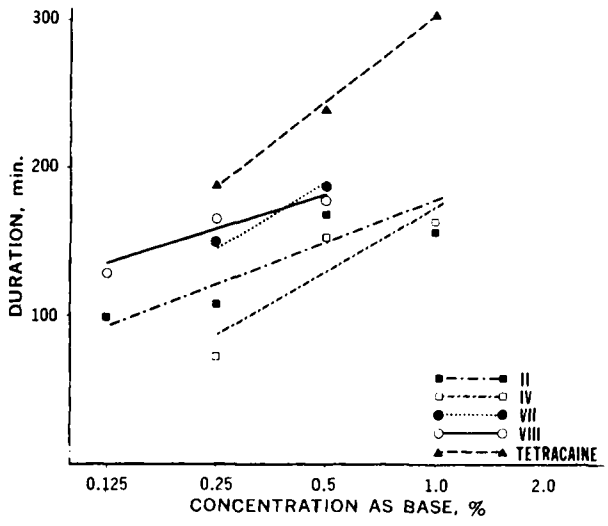

Figure 4—*Guinea pig intradermal wheals.*

REFERENCES

(1) N. Löfgren and G. Widmark, *Sv. Kem. Tidskr.*, **58**, 323 (1946).

(2) H. Weidmann and P. V. Petersen, *J. Pharmacol. Exp. Ther.*, **115**, 246(1955).

(3) H. Lundbeck and Co., Danish pat. 85,553 (1958); *Chem. Abstr.*, **53**, 13104(1959).

(4) E. Fischer, *Chem. Ber.*, **37**, 2508(1904).

(5) C. S. Marvel, J. Dec, H. G. Cooke, Jr., and J. C. Cowan, *J. Amer. Chem. Soc.*, **62**, 3495(1940).

(6) F. Hoffmann-La Roche Co., British pat. 847,205 (1960); *Chem. Abstr.*, **55**, 7433(1961).

(7) R. A. Clark, Ph.D. thesis, University of Maryland, College Park, Md., 1966; *Diss. Abstr.*, **27**, 1082-B(1966–1967).

(8) T. Wagner-Jauregg and L. Zirngibl, *Justus Liebigs Ann. Chem.*, **668**, 30(1963).

(9) G. Camougis and B. H. Takman, in "Methods in Pharmacology," A. Schwartz, Ed., Appleton-Century-Crofts, New York, N. Y., 1971, pp. 1–40.

(10) E. Bülbring and I. Wajda, *J. Pharmacol. Exp. Ther.*, **85**, 78(1945).

(11) J. Berkson, *J. Amer. Stat. Ass.*, **48**, 565(1953).

(12) F. P. Luduena, *Ann. Rev. Pharmacol.*, **9**, 503(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 8, 1973, from the *Research Laboratories, Astra Pharmaceutical Products, Inc., Worcester, MA 01606*

Accepted for publication June 6, 1973.

Presented in part at the Meeting of the Federation of American Societies of Experimental Biologists, Atlantic City, N. J., April 1970.

The authors thank William R. Millington and Sigmund M. Waraszkiewicz for their capable preparative assistance and Mrs. Marie Hogan for the statistical treatment of the data.

▲ To whom inquiries should be directed.