APhA Academy of Pharmaceutical Sciences, Houston meeting, April 1972.

Abstracted in part from a dissertation submitted by D. J. Slatkin to the University of Mississippi in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

The authors thank John Naworal, Graduate School of Public Health, University of Pittsburgh, Grant No. RR00273, for mass spectra; Dr. Coy Box for help in collection of plant material; and Dr. S. J. Angyal, Dr. Takayuki Wada, and Dr. K. L. Stuart for reference samples.

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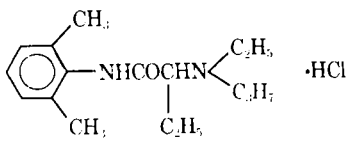
# Local Anesthetic Activity and Acute Toxicity of (±)-2-(*N*-Ethylpropylamino)-2′,6′-butyroxylidide, a New Long-Acting Agent

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

**Abstract** □ This paper is concerned with the local anesthetic activity and acute toxicity of (±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide (I). Testing in rat sciatic nerve blocks and guinea pig intradermal wheals showed that the compound has rapid onset, excellent frequency, and long durations of block. These observations were confirmed in studies of peridural anesthesia in the cat in which the durations of block were comparable to those of the long-acting agent bupivacaine. Although the compound is more irritating and more toxic than lidocaine, it is not more so than bupivacaine and tetracaine. These studies indicate that the overall pharmacological and toxicological profile of Compound I more closely resembles those of bupivacaine and tetracaine than that of lidocaine.

**Keyphrases** □ (±)-2-(*N*-Ethylpropylamino)-2′,6′-butyroxylidide—local anesthetic activity and acute toxicity □ Anesthetic activity, local—(±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide □ Toxicity, acute—(±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide

A series of α-aminobutyroxylidide derivatives was synthesized and tested for local anesthetic activity. This paper describes the local anesthetic activity and acute toxicity of one of the most interesting compounds in this series. It is chemically designated as (±)-2-(*N*-ethylpropylamino)-2′,6′-butyroxylidide[1] (I) and has the structural formula shown here.



I

In the studies reported here, Compound I was compared with lidocaine, an agent of intermediate duration, and two long-acting agents, bupivacaine[2] and tetracaine.

[1] W19053.
[2] Marcaine.

## METHODS

**Rat Sciatic Nerve Blocks**—Conduction block in a peripheral nerve trunk was studied in the female albino rat. The method was described in detail by Camougis and Takman (1). Precisely 0.2 ml. of drug solution or vehicle was injected into the midthigh region of the animal, so that it was deposited around the sciatic nerve trunk. After the injections, the animals were examined at frequent intervals for onset, depth, and duration of motor block. Frequencies of complete and of partial blocks were recorded, and overt systemic effects were noted. Mean durations and standard deviations were calculated from the durations of the complete blocks only. Groups of five rats were used at each concentration tested, and injections were made into both hind limbs.

**Guinea Pig Intradermal Wheals**—To evaluate infiltration anesthesia, the local anesthetic activity of graded concentrations of the agents was studied in the guinea pig intradermal wheal (1). Each wheal was made by injecting 0.1 ml. of drug solution or vehicle intradermally on the shaved backs of guinea pigs, and 12 wheals were made for each concentration. The presence or absence of anesthesia was determined by means of the response to pinpricks.

**Peridural Anesthesia in the Cat**—Surgical procedures and the evaluation of peridural anesthesia in cats were reported by Duce *et al.* (2). In brief, the procedure requires surgical implantation of a plastic catheter into one of the lumbar vertebrae so that local anesthetic solutions can be introduced into the peridural space. After administration of the local anesthetic solution, animals were examined at frequent intervals for onset of block. The principal endpoints recorded were: block and recovery of the animal's ability to support itself on its hind limbs, block and recovery of the flexor reflex (withdrawal of the limb when pressure is applied to the paw), and loss and complete recovery of normal motor function. Animals were also observed for effects that may result from the spread of the local anesthetic solution and from absorption into the blood.

A total of seven animals received 2% lidocaine and 0.5 and 1.0% Compound I; five animals received 0.5% bupivacaine.

**Irritation Studies**—The irritation liabilities of Compound I, lidocaine, bupivacaine, and tetracaine were evaluated by means of intradermal wheals in rabbits (1). The animals' backs were shaved and a series of wheals was made by injecting 0.1 ml. of solution or vehicle intradermally at each site. Twenty-four hours later, each wheal was examined and graded for the degree of redness, degree of edema, and presence or absence of a central zone of discolor (indicative of necrosis). Grading was done on an arbitrary scale from 0 to 12, and a mean score was obtained for all wheals made at

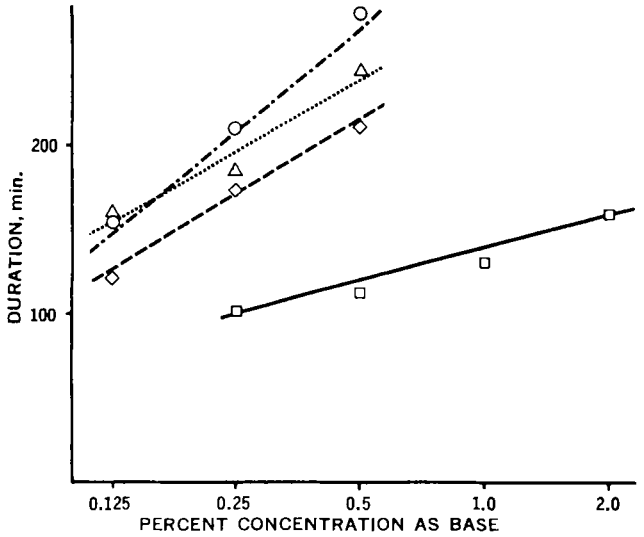



**Figure 1**—*Rat sciatic nerve blocks. Key:* O-·-O, *Compound I;* □——□, *lidocaine;* ◇---◇, *bupivacaine; and* △----------△, *tetracaine.*

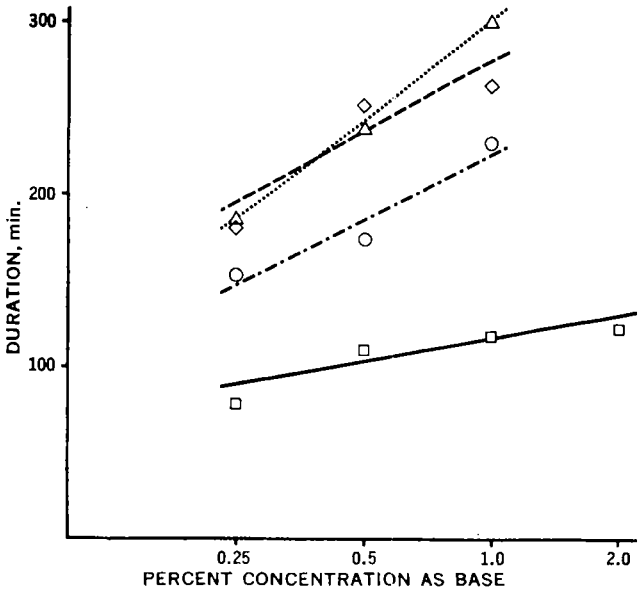



**Figure 2**—*Guinea pig intradermal wheals. Key:* O-·-O, *Compound I;* □——□, *lidocaine;* ◇---◇, *bupivacaine; and* △----------△, *tetracaine.*

each concentration of the test compound. All four compounds were tested at 0.5, 1.0, and 2.0%.

**Acute Toxicity**—Acute toxicity was evaluated by determining intravenous and subcutaneous $LD_{50}$'s in female albino mice weighing between 20 and 25 g. There were at least four doses for each $LD_{50}$ determination and 10 animals per dose level. Animals were observed for several hours immediately following drug administration. Overt effects and fatalities were recorded. The survivors were then housed as groups according to drug and dose and were checked once daily for 7 days to determine whether or not additional fatalities occurred. The $LD_{50}$'s and 95% Fieller confidence limits were calculated by the minimum logit chi-square method of Berkson (3).

Except for the acute toxicity and irritation studies, all testing was done with solutions containing epinephrine at a concentration of 1:100,000.

## RESULTS

**Rat Sciatic Nerve Blocks**—The results are shown in Fig. 1. Frequency of block was 80% or greater at the lowest concentrations tested and 100% at all higher concentrations. Compound I, bupivacaine, and tetracaine are similar in their durations, and all three are markedly different from lidocaine.

**Guinea Pig Intradermal Wheals**—As in the rat sciatic nerve, Compound I, bupivacaine, and tetracaine produced blocks that are markedly longer in duration than those produced by lidocaine. The dose-duration curves for the four compounds are shown in Fig. 2.

**Peridural Anesthesia in the Cat**—The results are summarized in Table I. In this preparation, Compound I exhibits short latency, good frequency, and long durations. Durations with 0.5%, for example, are about twice as long as those observed with 2.0% lidocaine. Unpaired *t* tests show that the duration of block of support of weight with 0.5% Compound I is not different from the duration of 0.5% bupivacaine; the duration of block of the flexor reflex, however, is significantly longer ($0.05 < p < 0.02$) than with bupivacaine. Durations with 1.0% Compound I are about three times longer than with 2.0% lidocaine.

**Irritation Studies**—The totals for the mean scores of the three concentrations tested are: lidocaine, 2; Compound I, 14; bupivacaine, 17; and tetracaine, 18. With the three long-acting agents, there was some moderate redness and edema and, especially at the higher concentrations, occasional central zones of discolor, indicative of necrotic tissue, in the wheals. The results show that Compound I, although more irritating to tissue than lidocaine, is not more so than bupivacaine and tetracaine.

**Acute Toxicity**—The results are summarized in Table II.

Intravenously and subcutaneously, lidocaine is the least toxic and tetracaine is the most toxic of the agents tested. Compound I and bupivacaine are equitoxic intravenously in the mouse; subcutaneously, however, Compound I is only about one-half as toxic as bupivacaine.

The overt effects, other than deaths, most frequently noted were, depending upon dose level, ataxia, depression, and clonic convulsions. Death apparently was due to respiratory failure, since thoracotomy immediately upon cessation of respiration revealed that the hearts were still beating. All fatalities occurred within the observation period immediately following drug administration. The overt effects and the types of death observed with Compound I were qualitatively identical to those seen with the other local anesthetic agents.

## DISCUSSION

The results of these studies show that Compound I is a potent local anesthetic agent with a profile of activity and acute toxicity that more closely resembles those of bupivacaine and tetracaine than that of lidocaine. The results obtained with rat sciatic nerve blocks show that the compound exhibits short latency and excellent frequency of block. In addition, durations are quite long com-

**Table I**—Peridural Anesthesia in the Cat[a]

| Compound and Concentration | Block of Weight Support | | Block of Flexor Reflex | | |
|---|---|---|---|---|---|
| | Onset | Duration | Frequency, % | Onset | Duration |
| 2.0% Lidocaine[b] | <1 | 96 ± 6 | 85 | 5 | 53 ± 14 |
| 0.5% Compound I | <1 | 209 ± 23 | 80 | 8 | 101 ± 33 |
| 1.0% Compound I | <1 | 308 ± 21 | 90 | 7 | 171 ± 54 |
| 0.5% Bupivacaine | <1 | 182 ± 21 | 60 | 10 | 67 ± 25 |

[a] Onset times and durations are in minutes. All solutions contained 1:100,000 epinephrine. [b] Xylocaine.

Table II—Acute Toxicity in Female Mice

| Compound | $LD_{50}$ and 95% Confidence Limits, mg./kg. as Base | |
|---|---|---|
| | Intravenous | Subcutaneous |
| Compound I | 6.7 (5.8–7.5) | 99 (85–147) |
| Lidocaine | 26 (23–33) | 211 (183–256) |
| Bupivacaine | 6.4 (5.5–7.3) | 45 (38–54) |
| Tetracaine | 4.1 (2.9–5.3) | 32 (25–42) |

pared with lidocaine, indicating that the agent should have a long duration of action in peripheral nerve blocks. The long duration of anesthesia is confirmed by the results obtained in guinea pig wheals, and testing in the surgically prepared cat shows that the compound should have a rapid onset and long duration of blocks in peridural anesthesia. In addition, its tissue irritation propensities and acute toxicity are within acceptable limits as judged by comparison with bupivacaine and tetracaine. Further studies of the pharmacology and toxicology of this compound are in progress.

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## ACKNOWLEDGMENTS AND ADDRESSES


Received May 1, 1972, from the *Research Laboratories, Astra Pharmaceutical Products Inc., Worcester, MA 01606*

Accepted for publication June 22, 1972.

Presented in part at the Joint Meeting of the American Society for Pharmacology and Experimental Therapeutics, Inc., and the Division of Medicinal Chemistry of the American Chemical Society, Burlington, Vt., August 1971.

The authors thank Mrs. Phyllis Hughes and Miss Georgette Kilpatrick for their technical assistance.



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# GLC Determination of Sorbitol and Mannitol in Aqueous Solutions


G. MANIUS, F. P. MAHN▲, V. S. VENTURELLA*, and B. Z. SENKOWSKI



**Abstract** □ A rapid and specific method is described for the determination of the concentration of sorbitol and mannitol in aqueous solutions. The procedure was also applied to sorbitol USP powder. The developed GLC procedure requires the preparation of the acetate derivative, the addition of an internal standard (dioctyl sebacate), and the use of a hydrogen flame-ionization detector. The liquid phase, ECNSS-M, 5% on Gas Chrom Q, operated at 175°, is well suited for the separation of sorbitol acetate and mannitol acetate, although it is subject to gradual depletion with time. A study was made showing how the loss of this liquid phase affects peak efficiency and resolution. The GLC method is easier, more specific, and less time consuming than the official USP procedure.

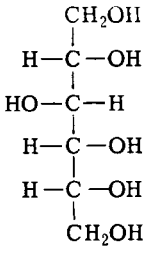


**Keyphrases** □ Sorbitol—GLC determination from aqueous solutions □ Mannitol—GLC determination from aqueous solutions □ GLC—determination of sorbitol and mannitol in aqueous solutions


A quantitative GLC method was developed for the routine control of aqueous solutions containing sorbitol and mannitol. The present USP procedure is a laborious and highly exacting two-step process. Step 1 involves column chromatography to remove interfering excipients. This process includes the sectioning-off of column material assumed to contain all of the sorbitol and then the quantitative transfer back to the column. A final water wash is expected to desorb all of the sorbitol. Step 2 involves the determination of the amount of sorbitol by titration.

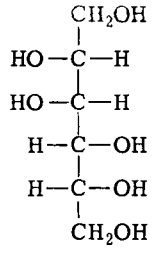
As will be shown, the GLC procedure entirely eliminates Step 1 because the sorbitol is successfully partitioned directly on the GLC column from mannitol, lower reduced sugars, and other excipients. Furthermore, GLC is well suited as a quantitative tool, particularly when an internal standard is used.

The GLC method outlined in this paper is demonstrative of a direct approach requiring neither extractions, column chromatographic purifications, nor recrystallizations. Sample preparation is restricted solely to a simple acetylation reaction, necessitating approximately 30 min. for completion. The described conditions offer an analytical alternative that greatly re-



D-sorbitol,
$C_6H_{14}O_6$

mol. wt. 182.17
anhydrous m. p. 110–112°



D-mannitol,
$C_6H_{14}O_6$

mol. wt. 182.17
m. p. 166–168°