Drug Contamination of Mortars and Pestles

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Abstract □ Evidence is presented suggesting that potent water-insoluble antipentylenetetrazol agents triturated in porcelain mortars and pestles are not removed from this mixing device by the usual laboratory washing procedure. Moreover, amounts sufficient to contaminate the next substance triturated in this vessel can be demonstrated by the subcutaneous pentylenetetrazol seizure threshold test. The data show that a rigorous washing routine must be followed to achieve a "clean" mortar and pestle. Attention is also directed to the importance of using disposable hypodermic syringes, test tubes, *etc.*, whenever possible and of designing an internal control test to determine when implements that must be reused are "clean."

Keyphrases □ Contamination—mortars and pestles washed by usual procedures, demonstrated by pentylenetetrazol seizure threshold test □ Equipment, laboratory—contamination of mortars and pestles washed by usual procedures demonstrated by pentylenetetrazol seizure threshold test

Previously (1), lipophilic solvents were shown to increase hexobarbital sleep time in mice. This observation suggested that other factors might compromise the results obtained in pharmacological experiments. Consequently, when marked variations appeared in the evaluation of standard and candidate antiepileptic agents subjected to the subcutaneous pentylenetetrazol test, a search for the cause of such aberrant results was begun. Careful study disclosed that highly potent, insoluble, antipentylenetetrazol agents, such as clonazepan (ED_{50} *versus* pentylenetetrazol seizures in mice, 0.009 mg/kg), can be carried over on the mortar and pestle despite careful washing.

This article discusses the possibility of cross-contamination when testing highly potent water-insoluble substances and the precautions necessary to avoid such pitfalls.

EXPERIMENTAL

Male albino mice[1] (CF No. 1 strain, 18–25 g) were allowed free access to food[2] and water, except when they were removed from their cages for the experimental procedure. Pentylenetetrazol seizures were induced by the technique of Swinyard *et al.* (2). Briefly, pentylenetetrazol[3] is administered subcutaneously as a 0.85% solution in 0.9% NaCl, in a dose of 85 mg/kg, in a loose fold of skin on the back of the neck. Seizures are induced in 97+% of normal mice. The mice are observed for 30 min after administration. Absence of a 5-sec episode of clonic spasm (a threshold seizure) is defined as protection in this test.

Clonazepam (50 mg) was triturated for 30–60 sec in a 6.5-cm (o.d.) glazed porcelain mortar and pestle with 2.5 ml of 30% polyethylene glycol, and the resulting suspension was discarded. The mortar and pestle were washed with various combinations of hot water, 0.1 *N* HCl, absolute ethanol, detergent[4], and water rinse and then dried with a paper towel. An inactive control substance (10 or 25 mg of 3,3,5,5-tetramethylcyclohexanespirohydantoin) was placed in the same mortar and triturated for 30–60 sec with 2.5 ml of 30% polyethylene glycol. Then 0.01 ml/g was injected intraperitoneally into eight animals.

At the previously determined time of peak effect for clonazepam (0.5 hr), the mice were subjected to the subcutaneous pentylenetetrazol test, and the presence or absence of a seizure in each animal was recorded. This procedure was repeated with various washing routines until the mortar and pestle were "clean," as indicated by the inability of the inactive substance to protect against pentylenetetrazol-induced seizures.

[1] Charles Rivers, Wilmington, Mass.
[2] S/L Custom Lab Diet-G4.5.
[3] Metrazol, courtesy of Knoll Pharmaceutical Co.
[4] Liqui-Mox, Alconox Inc., New York, NY 10003.

Table I—Cross-Contamination of an Inactive Substance with Clonazepam as Determined by the Subcutaneous Pentylenetetrazol Test in Mice

Prewash Procedure[a]	Washing Procedure[b]	Postwash[c] Procedure	Dose	Subcutaneous Pentylenetetrazol Test Result[d]
Disposable test tube	—	Control	30% polyethylene glycol, 0.01 ml/g	0/8
Uncontaminated mortar and pestle	—	25 mg inactive substance triturated mortar and pestle	100 mg/kg ip	0/6
Clonazepam, 50 mg, triturated mortar and pestle	1, 7, 8	10 mg inactive substance triturated mortar and pestle	40 mg/kg ip in polyethylene glycol, 0.01 ml/g	8/8
	1, 2, 7, 8	10 mg inactive substance triturated mortar and pestle	40 mg/kg ip in polyethylene glycol, 0.01 ml/g	8/8
	1, 4, 7, 8	10 mg inactive substance triturated mortar and pestle	40 mg/kg ip in polyethylene glycol, 0.01 ml/g	2/8
	1, 3, 6–8	10 mg inactive substance triturated mortar and pestle	40 mg/kg ip in polyethylene glycol, 0.1 ml/g	2/8
	1–8	10 mg inactive substance triturated mortar and pestle	40 mg/kg ip in polyethylene glycol, 0.01 ml/g	0/8

[a] Clonazepam, 50 mg, triturated with 2.5 ml of 30% polyethylene glycol in 6.5-cm o.d. porcelain mortar and pestle.
[b] 1 = wash mortar and pestle thoroughly with hot tap water, using fingers to remove any residue of suspension.
2 = rinse mortar and pestle with 0.1 *N* HCl, using trituration motion.
3 = rinse mortar and pestle with water.
4 = rinse mortar and pestle with absolute ethyl alcohol, using trituration motion.
5 = rinse mortar and pestle with water.
6 = wash mortar and pestle with detergent, using scrubbing brush.
7 = rinse mortar and pestle with water.
8 = wipe dry with paper towel.
[c] Either 10 or 25 mg inactive substance triturated with 2.5 ml of 30% polyethylene glycol in washed mortar and pestle. [d] Number of mice protected/number of mice tested.

RESULTS AND DISCUSSION

The vehicle employed (30% polyethylene glycol) and the inactive control substance are devoid of antipentylenetetrazol activity in mice (Table I). Preliminary tests indicated that the control substance was inactive in intraperitoneal doses of 1000 mg/kg when tested from 0.5 to 6 hr after administration. Table I also shows that a mortar and pestle previously used to prepare a polyethylene glycol suspension of clona-

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zepam could not be thoroughly cleaned by washing with hot tap water and rinsing with water and/or rinsing with 0.1 *N* HCl followed by a water rinse since all animals tested were protected from pentylenetetrazol seizures when these procedures were employed. Although rinsing the mortar and pestle with absolute ethanol or washing with detergent reduced the amount of clonazepam remaining (two animals protected out of eight tested), neither procedure alone was adequate. Indeed, a clean mortar and pestle were obtained only when all of these procedures were employed in sequence (none protected out of eight tested).

The benzodiazepines in general (3) and clonazepam in particular are the most potent antipentylenetetrazol agents tested in this laboratory. The ED_{50} (and its 95% confidence interval) by the subcutaneous pentylenetetrazol test is 0.009 (0.0046–0.0165) mg/kg. Thus, a dose of only 180 ng is sufficient to protect 50% of the mice (20 g). Since the ED_{99} for clonazepam is approximately 0.013 mg/kg, at least 3.25 μg apparently was carried over on the mortar and pestle and subsequently incorporated in the polyethylene glycol suspension of the inactive substance. Chemical analysis[5] of 2.5 ml of 30% polyethylene glycol triturated in a contaminated mortar and pestle revealed that 66.25 μg (26.5 μg/ml) was, in fact, carried over in the solvent. This quantity is approximately 20 times the ED_{99} for clonazepam.

[5] Performed by Mr. Tom Jennison, Center for Human Toxicology, University of Utah.

The results presented reveal only a small fraction of the total problem. Many laboratories are working with highly potent, water-insoluble substances. Various methods such as a mortar and pestle, a sonicator, a homogenizer, a tissue grinder, and a micromixer are used to achieve a suitable solution or suspension. Subsequent use of this equipment to solubilize or suspend other chemicals could result in significant contamination unless appropriate precautions are taken. These precautions include the use of disposable laboratory utensils such as syringes, needles, test tubes, and spatulas and internal controls to determine when reused utensils are clean. One cannot help but wonder to what extent this problem has been unrecognized, ignored, or explained away on the basis of "biological variability."

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Quaternary Acetate and Propionate Esters of 3-Hydroxyquinolizidine

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Received February 16, 1977, from the **Department of Medicinal Chemistry and the* ‡*Department of Pharmacology, School of Pharmacy, Southwestern Oklahoma State University, Weatherford, OK 73096.* Accepted for publication April 18, 1978.

Abstract □ The preparation of the quaternary acetate and propionate esters of 3-hydroxyquinolizidine is described. Tentative structures are assigned on the basis of NMR data. Results of preliminary pharmacological screening of the methiodide and hydrochloride salts are given.

Keyphrases □ 3-Hydroxyquinolizidine esters, various—synthesized, structures assigned, cholinergic activity evaluated *in vitro* □ Cholinergic activity—various 3-hydroxyquinolizidine esters evaluated *in vitro* □ Structure–activity relationships—various 3-hydroxyquinolizidine esters evaluated for cholinergic activity *in vitro*

The quinolizidine ring system has been used to prepare semirigid analogs of several classes of biologically active compounds (1–4). The reported reversal of configuration of the *trans*-fused rings of quinolizidine to the *cis*-configuration upon quaternization of the acetate and propionate esters of axial and equatorial 1-hydroxyquinolizidine (1) led to the present investigation of the analogous esters at the 3-position.

This report describes the preparation of the acetate and propionate esters of axial and equatorial 3-hydroxyquinolizidine and their behavior upon quaternization with methyl iodide. These isomers were screened for cholinergic activity since they are structurally related to acetylcholine.

DISCUSSION

The synthesis of the desired compounds began with the preparation of 3-oxoquinolizidine (I) by the method of Counsell and Soine (5). Then, I was reduced according to the method of Aaron *et al.* (6). The structures of II and III (Scheme I) were assigned (6, 7) primarily on the basis of IR spectral data. The intermediate esters, IV and V (Scheme I), were obtained by reaction of the appropriate alcohol with either acetic or propionic anhydride and pyridine as a catalyst. The esters so prepared were assigned the same configuration as the respective parent alcohols since the conditions used for ester formation would not be expected to result in epimerization. The assignments appear valid since all esters exhibited Bohlmann bands (Table I) in their IR spectra, which is usually taken as empirical evidence for *trans*-ring fusion (6–8).

The desired quaternary salts were obtained by treating the purified esters with excess methyl iodide in refluxing acetone. The NMR spectra of each quaternized ester exhibited only one *N*-methyl signal, which appeared as a three-proton singlet (Table I). The appearance of only one *N*-methyl signal, along with the small differences in the chemical shifts of the signals, suggested that the quaternized esters were configurationally pure and probably all of the same configuration. These conclusions seem reasonable in view of the work of Williamson *et al.* (9), who showed a 12-Hz difference between the *N*-methyl groups of *trans*- (171 Hz) and *cis*- (183 Hz) fused *N*-methylquinolizidinium iodide. Also, since the *N*-methyl signals of the quaternary salts prepared have chemical shifts very close to the *N*-methyl signals of the analogous *cis*-fused quaternary salts substituted at the 1-position (see introduction and Ref. 1), the *cis*-configuration was tentatively assigned to the quaternary salts prepared.

Examination of Drieding models of the *cis*-fused products revealed that difficultly interconvertible conformers were probably in equilibrium as depicted in Scheme II. It was reasonable to assign a conformational preference to the products, and this assignment was made on the basis that less steric interaction would occur if the ester function was in the equatorial position in the *cis*-fused system. Therefore, XII and XVI (Table I) were tentatively assigned Structure VI, where R is methyl and ethyl, respectively. Likewise, XIII and XVII (Table I) were assigned Structure IX. Support for this conclusion was obtained by the observation

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