Distribution of Camazepam in Rats and Mice

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Received December 1, 1977, from the **Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea, 62, 20157 Milan, Italy,* and the ‡*Laboratorio di Ricerche Simes, Milan, Italy.* Accepted for publication February 1, 1978. §Present address: Universidad Autonoma, Barcelona, Spain.

Abstract □ Camazepam, 5 mg/kg iv, was injected in rats and mice to study its distribution in the blood and brain. Peak blood levels were about 0.9 μg/ml in rats and 0.6 μg/ml in mice. Peak brain levels were about 1.5 μg/g in rats and 0.8 μg/g in mice. The apparent blood half-life of camazepam was 9 min in mice and 20 min in rats.

Keyphrases □ Camazepam—blood and brain distribution in rats and mice □ Distribution, blood and brain—camazepam in mice and rats □ Psychoactive agents—camazepam, blood and brain distribution in rats and mice

Camazepam[1], 3-*N,N*-dimethylcarbamoyloxy-7-chloro-5-phenyl-1-methyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (I), is a new psychoactive benzodiazepine derivative with a carbamic group in position C-3. Pharmacologically, camazepam displays anxiolytic properties, very weak muscle relaxant activity, no cardiodepressant effect, and very low toxicity (1–3).

During the investigation of suitable analytical parameters for the analysis of camazepam, its response to electron-capture GLC was found to be sufficiently sensitive for quantitation in the nanogram range. The electron-capture GLC assay reported here was chosen for the determination of blood and brain levels of camazepam in rats and mice given doses of 5 mg/kg iv.

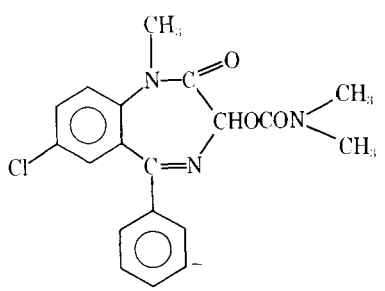
EXPERIMENTAL

Animals—Male CD-COBS Charles River rats, 220 ± 10 g, and male CD_1 mice, 25 ± 3 g, were used.

Drug Administration—Camazepam was injected at 5 mg/kg iv. It was dissolved in diethylacetamide–polysorbate 80[2] (1:10) and diluted with water to a suitable concentration so that the animals received less than 0.1 ml of polysorbate 80/kg.

At 5, 30, 60, and 180 min after drug injection, five animals were killed, blood samples were collected in heparinized test tubes, and the brain was rapidly removed and weighed.

Extraction from Blood—Aliquots of 1 ml of whole blood and 3 ml of benzene were pipetted into 10-ml test tubes with ground-glass stoppers. The tubes were stirred vigorously on a vortex mixer for 3 min and then centrifuged at 2000 rpm for 5 min. Then a 2-ml aliquot of the benzene extract was transferred to a 15-ml conical centrifuge tube, and the benzene was evaporated to dryness in the water bath of a rotary evaporator at 35–40°. The evaporation residue was dissolved in at least 100 μl of acetone in which 0.25 μg of penfluridol[3]/ml (internal standard) was dissolved. A suitable aliquot (1–2 μl) was injected into the gas chromatograph.



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[1] SB 5833, obtained from Simes S.p.A., Milan, Italy.
[2] Tween 80.

Table I—Blood and Brain Levels of Camazepam after Administration of 5 mg/kg iv in Rats

Time after Injection, min	Blood Level, μg/ml ± *SE*[a]	Brain Level, μg/g ± *SE*[a]
5	0.910 ± 0.2	1.490 ± 0.1
30	0.300 ± 0.1	0.710 ± 0.1
60	0.120 ± 0.05	0.240 ± 0.08
180	0.005 ± 0.005	0.026 ± 0.001

[a] Each value represents the means ± *SE* of five animals.

Table II—Blood and Brain Levels of Camazepam after Administration of 5 mg/kg iv in Mice

Time after Injection, min	Blood Level, μg/ml ± *SE*[a]	Brain Level, μg/g ± *SE*[a]
5	0.640 ± 0.02	0.790 ± 0.03
30	0.060 ± 0.01	0.140 ± 0.02
60	0.010 ± 0.002	0.067 ± 0.003
180	<0.005	0.011 ± 0.001

[a] Each value represents the means ± *SE* of five determinations.

Extraction from Brain—The brains were homogenized (1:4 w/v) in 0.1 *M* glycine buffer, pH 10.2, with a polytef–glass homogenizer. Brain homogenate, 1 ml, was removed under stirring, and 3 ml of benzene was pipetted into a 10-ml test tube with a ground-glass stopper. Treatment was then identical with that for blood.

Conditions for GLC Analysis—*Column*—The column packing was a pretested preparation containing 3% OV-17 on 100–120-mesh Gas Chrom Q[4] packed into a 1-m glass column (3 mm i.d.).

Instrumental Conditions—A gas chromatograph[5] equipped with a ^{63}Ni-electron-capture detector was used. The flow rates of the carrier gas, nitrogen, and the detector purge gas were 60 and 30 ml/min, respectively. The temperature settings were: oven, 275°; injection port, 300°; and detector, 320°.

Quantitative Analysis—For quantitative camazepam analysis, the internal standard technique was used. Penfluridol [4-(4-chloro-α,α,α-trifluoro-*m*-tolyl)-1-[4,4-bis(*p*-fluorophenyl)butyl]-4-piperidinol] was chosen as an internal standard because of its suitable retention time.

Recovery studies of camazepam from the blood and brain of the two animal species were satisfactory, ranging from 80 ± 2 to 85 ± 3%. The sensitivity of the electron-capture GLC assay is 15 ng/ml of blood or g of brain. The identity of the GLC peak for camazepam was checked by mass spectrometry[6].

RESULTS AND DISCUSSION

The distribution of camazepam in rat blood and brain after administration of 5 mg/kg iv is shown in Table I. Camazepam entered the brain very rapidly from the bloodstream, giving constantly higher concentrations in the brain than in the blood from 5 min to 3 hr after administration. The highest blood and brain levels of camazepam were observed 5 min after injection; thereafter, concentration progressively decreased, reaching 5 ng/ml and 26 ng/g in the rat blood and brain, respectively, 3 hr after treatment.

Table II shows the levels of camazepam in the blood and brain of mice at different times after injection of 5 mg/kg iv. In mice, as in rats, brain

[3] Courtesy of Dr. P. Janssen, Janssen Pharmaceutica, Beerse, Belgium.
[4] Applied Science Laboratories, State College, Pa.
[5] Fractovap, model G I, Carlo Erba, Milan, Italy.
[6] LKB 9000 gas chromatograph–mass spectrometer.

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drug levels were higher than blood levels; but in both compartments, camazepam concentrations were considerably lower than those found in rats 5 min after injection. The drug declined faster in the blood of mice than in that of rats, the apparent half-lives in the two species being 9 and 20 min, respectively, as calculated according to Gibaldi (4).

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Synthesis of 1-Methyl-2-phenylcarbamoylpyrazolidines as Potential Anticonvulsant Agents

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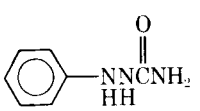
Received November 28, 1977, from the *Division of Medicinal Chemistry, College of Pharmacy, University of Kentucky, Lexington, KY 40506.* Accepted for publication January 13, 1978.

Abstract □ Lithium aluminum hydride reduction of 1,4-dimethyl-3-pyrazolidinone yielded 1,4-dimethylpyrazolidine. The latter compound and 1-methylpyrazolidine reacted with aryl isocyanates to produce 1-methyl-2-phenylcarbamoylpyrazolidines. Several of these adducts displayed significant anticonvulsant activity in the maximal electroshock seizure and pentylenetetrazol seizure threshold tests.

Keyphrases □ Pyrazolidines, various substituted—synthesized, evaluated for anticonvulsant activity in mice □ Anticonvulsant activity—various substituted pyrazolidines evaluated in mice □ Structure–activity relationships—various substituted pyrazolidines evaluated for anticonvulsant activity in mice

Antiepileptic agents containing the semicarbazide functionality have been investigated only rarely (1–3). 1-Phenylsemicarbazide (I) is devoid of protective activity in the maximal electroshock test at 300 mg/kg (4). However, several tricyclic semicarbazides (II), which possess good potency and a favorable therapeutic ratio, have been described (5).



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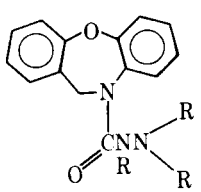
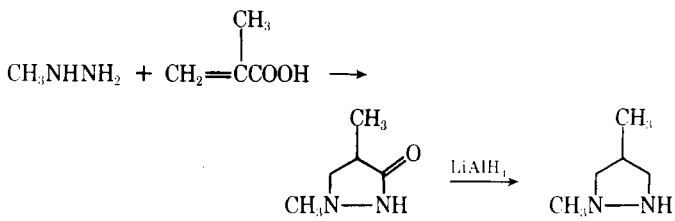


II

Interest in new anticonvulsant agents (6–8) as well as in pyrazolidine analogs of medicinals (9) prompted the synthesis and evaluation of a series of 1-methyl-2-phenylcarbamoylpyrazolidines (IV*a*–IV*w*).

DISCUSSION

Chemistry—The synthesis of the title compounds necessitated the preparation of the two pyrazolidine bases III*a* and III*b*. 1-Methylpyrazolidine (III*a*) was obtained by previously described methods (10). 1,4-Dimethylpyrazolidine (III*b*), a new base, resulted from the lithium aluminum hydride reduction of 1,4-dimethyl-3-pyrazolidinone. The latter precursor was prepared by condensing methylhydrazine with methacrylic acid (11) (Scheme I).



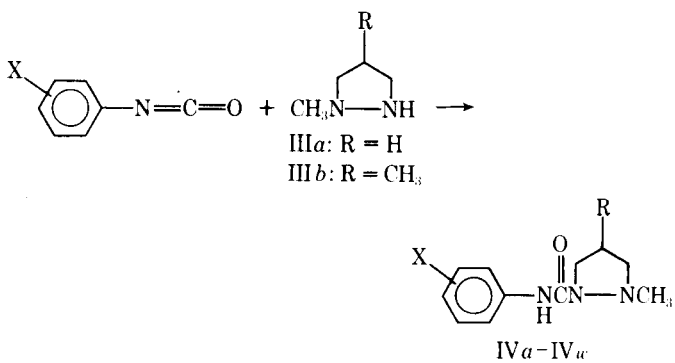
Scheme I

Addition of these pyrazolidines to aryl-substituted isocyanates occurred smoothly to give IV*a*–IV*w* (Scheme II). The physical properties of these adducts are given in Table I.

Biological Activity—All phenylcarbamoylpyrazolidines were tested for anticonvulsant activity and neurotoxicity by the methods described under *Experimental* (Table II). Of the 22 compounds tested, 19 exhibited some anticonvulsant activity. The *p*-bromo derivatives IV*b* and IV*p* were uniformly inactive.

Compounds IV*m*, IV*n*, and IV*v* showed the best activity against maximal electroshock. They all possess a 2,6-substitution pattern in the aromatic ring reminiscent of the local anesthetic–antiarrhythmic drug lidocaine, which can temporarily arrest *grand mal* as well as certain other epileptic seizures (12). However, the short time of peak effect (0.5 hr) for IV*m*, IV*n*, and IV*v* probably indicates a short duration of action and may limit their usefulness (5).

Compounds IV*e*, IV*i*, and IV*m* showed significant activity in the pentylenetetrazol test at 0.5 hr but were devoid of activity at 4 hr.



Scheme II

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