H_2O from the reaction was continuously removed by a Dean–Stark tube. The resulting solution, after cooling, was washed with 10 ml of H_2O, dried (Na_2SO_4), and evaporated *in vacuo*. During distillation of the residue, two fractions were collected. The first fraction, 3.5 g, bp 55° (0.15 mm), was identified by ir as the 3-methoxy-4-bromotoluene. The second fraction, which weighed 2.5 g, was a bright yellow, viscous oil, bp 132° (0.4 mm). Its ir spectrum showed the absence of an absorption peak due to C=O.

The above oil, with no further purification, was dissolved in 50 ml of Et_2O and was added dropwise to a suspension of 1.5 g of LAH in 75 ml of Et_2O, while the temperature was maintained between 0 and 5°. The mixture was refluxed for 4 hr, and H_2O and 10% NaOH were added successively to decompose excess LAH. The inorganic solids were removed by filtration, and the filtrate was evaporated *in vacuo* leaving a light yellow oil. A solution of this oil in 50 ml of Et_2O was saturated with HCl to precipitate the HCl salt. Recrystallization from MeOH–Et_2O gave 1.4 g (38% over-all, based on the recovered 3-methoxy-4-bromotoluene) of 1-(2-methoxy-4-methylphenyl)-2-aminopropane hydrochloride.

Pharmacology. Swim Maze Test.—The H-shaped swim maze,[13,14] constructed of galvanized metal sheet, has an over-all dimension of 80 × 60 × 15 cm, three swim channels 6 cm in width, and a landing strip 30 × 6 cm with a 10-cm projection at 40° angle. An 8-cm level of H_2O was maintained at 37° by several straps of heating tape placed underneath the tank.

Prior to the administration of drugs, mice were trained to swim the maze for 2 consecutive days (at intervals of 2 hr). They were placed in the H_2O at one end of the tank and the swimming time was recorded as the time from placement in the tank until exit at the landing strip. The trained animals were able to complete the two-right-turn swimming task in an average of 5 sec and with an average of less than one error. During the training period, those that did not show good performance were excluded from the study. The animals in groups of ten were injected intraperitoneally with 50 μmoles/kg of compounds in 30% aqueous propylene glycol. The control group was given only propylene glycol. The swim tests were performed at both 10 and 30 min after the injection. The results were expressed as (a) the completion time for swimming (X), and (b) number of errors (Y) during that time. With the emphasis on Y, the disruption of mouse behavior was evaluated as $X + 2Y$.

Effects on Barbiturate Sleeping Time.—Mice in groups of ten were injected intraperitoneally with 50 μmoles/kg of compounds in 30% propylene glycol. After 5 min, sodium pentobarbital (40 mg/kg) in saline was given *via* the same route. Controls were first given 30% propylene glycol, then pentobarbital in saline. The presleeping time and sleeping time (loss of the righting reflex) were recorded and treated statistically.

Acute Toxicity.—All compounds dissolved in 30% propylene glycol (10 mg/kg) were administered intraperitoneally to groups of ten male albino Yale–Swiss mice weighing 17–23 g. The LD_{50} within a 24-hr period was determined graphically according to the method of Miller and Tainter.[15]

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Pharmacologic and Metabolic Studies with Deuterated Zoxazolamine[1]

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The synthesis of deuteriozoxazolamine-4,6-d_2 is described. Nmr data on the deuterated drug and related derivatives are discussed. The deuterated drug exhibits a kinetic deuterium isotope effect in *in vitro* metabolism. No increased duration of pharmacologic action with the deuterated drug was observed.

In our continuing work on the biological consequences of deuterium substitution on drugs, we studied the effects of the muscle relaxant zoxazolamine. The results of those studies are reported herein.

We previously reported[2] that the barbiturate butethal induced a twofold increase in sleeping time of mice when D atoms were substituted on the penultimate C of the butyl side chain, which is the site of metabolic deactivation. The biological isotope effect of $k_H/k_D \cong 2$ was attributed to a slower rate of metabolism of the deuterated drug. Evidence for this conclusion was also obtained from the slower rate of metabolism observed with the deuterated species in *in vitro* studies with the liver microsomal enzymes.

Conney, *et al.*,[3] have reported that zoxazolamine is metabolized in man by two routes. A minor pathway involves hydrolytic deamination to a pharmacologically active metabolite, 5-chloro-2-hydroxybenzoxazole. Both biologically active benzoxazole derivatives are metabolically deactivated by a ring hydroxylation pathway at C-6 to yield the respective 5-chloro-6-hydroxybenzoxazole derivatives. Since the specific site of metabolic deactivation of zoxazolamine is at the C-6 position, our goal was the preparation of the drug labeled with D at this position, and the study of its effect on the rate of metabolism.

Our inital attempt at the direct introduction of D into zoxazolamine involved acid-catalyzed exchange of the drug with D_2O at 100°. The material obtained from this reaction consisted solely of the hydrolytic deamination product, 5-chloro-2-hydroxybenzoxazole (**1**), with no evidence of incorporation of D in the aromatic ring.

Successful introduction of D atoms into the benzene ring was achieved by an acid-catalyzed exchange reaction of 2-amino-4-chlorophenol with D_2O at 100°. The intermediate deuterated phenol was condensed directly with BrCN in EtOH to yield zoxazolamine.[4] Combustion analysis indicated the incorporation of two D atoms into zoxazolamine prepared in this way. To

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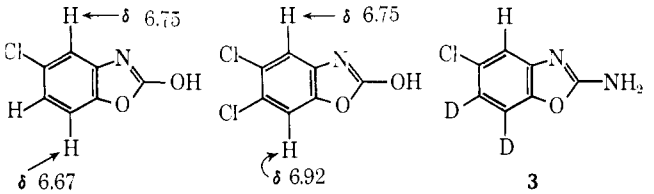
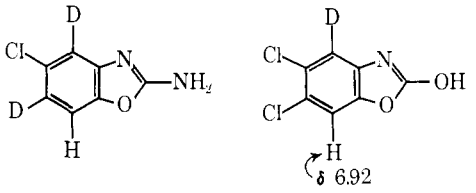
establish that a D atom was present in the crucial C-6 position of the 5-chlorobenzoxazole nucleus, the dideuteriozoxazolamine was deaminated hydrolytically at C-2 with D_2O under acid catalysis to give a dideuterated 5-chloro-2-hydroxybenzoxazole.





Chlorination of 5-chloro-2-hydroxybenzoxazole (**1**) with SO_2Cl_2 is known to produce 5,6-dichloro-2-hydroxybenzoxazole (**2**). When the dideuterated 5-chloro-2-hydroxybenzoxazole was subjected to these chlorinating conditions in deuterioacetic acid as solvent, a sample was obtained that was identical in all respects with that of the reported 5,6-dichloro-2-hydroxybenzoxazole; combustion analysis indicated the loss of exactly one D atom. This series of experiments firmly secures C-6 as the position of one of the D atoms in the benzoxazole nucleus of zoxazolamine. This leaves either structure **3** or **4** to be considered for the dideuterated derivative.

Nmr data of 5-chloro-2-hydroxybenzoxazole (**1**) were used to assign the location of the second D present in the dideuteriozoxazolamine. The aromatic protons of **1** appear as a multiplet in the δ 6.6–6.8 region of the nmr spectrum. A relatively uncomplicated singlet appears at δ 6.8 which can be assigned to the C-4 proton signal, since this proton, *ortho* to Cl, would be deshielded and absorbed at the lowest field position and be very weakly coupled to the *meta* and *para* hydrogens. The nmr spectrum of the dideuterio derivative of **1** shows the disappearance of the sharp singlet peak and only a broad peak at δ 6.79, $W/2$ = 1.5 Hz. The broadness of the band is only compatible with the replacement of the C-4 proton by D. The broadened C-7 proton signal in **4** can be attributed to coupling with the adjacent C-6 D atom: J_{D-H} values of 1–1.5 Hz have been observed.[5] This nmr evidence thus permits the choice of **4** over **3** for the dideuteriozoxazolamine.

Further evidence for the placement of the second D at C-4 in the benzoxazole nucleus comes from examination of the nmr spectra of the deuterated 5,6-dichloro-2-hydroxybenzoxazole (**5**) and 5-chloro-2-hydroxybenzoxazole (**1**). The nmr of **1** shows the C-4 proton at δ 6.75 and a multiplet for the C-6 and C-7 protons centered at δ 6.67. On conversion to the dichloro derivative **2** (the C-4 proton) remains at δ 6.75, whereas the C-7 proton is shifted to δ 6.92. The nmr of the deuterio derivative of **2** shows a single peak at δ 6.92, which can be assigned to a C-7 proton and is in accord with the assignment of structure **5**.

Pharmacological Studies.—The durations of pharmacological action of zoxazolamine and the deuterated analog were compared, using male Sprague–Dawley rats weighing 215–290 g. To minimize the deviation of the mean due to individual variation among rats, the rats were separated into two groups, long sleepers and short sleepers. This procedure is based on the duration of sleep exhibited by each rat after the intraperitoneal administration of hexobarbital (100 mg/kg).[6] Both of these groups of rats were used the day after hexobarbital treatment to examine the duration of paralysis caused by zoxazolamine and deuteriozoxazolamine. As can be seen in Table I, in neither group was the duration of action of zoxazolamine significantly different from that of its deuterated analog. The data were analyzed by Students' *t* test. From these experiments *in vivo*, it would appear that there was no isotope effect on the biological hydroxylation of the aromatic ring.

TABLE I
DURATION OF PARALYSIS IN RATS AFTER ZOXAZOLAMINE INJECTION

Group	Compound[a]	Duration of paralysis, min
Long sleepers	Zoxazolamine	485 ± 162 (4)[b]
	Deuteriozoxazolamine-4,6-d_2	417 ± 78 (3)
Short sleepers	Zoxazolamine	287 ± 30 (4)
	Deuteriozoxazolamine-4,6-d_2	242 ± 38 (4)

[a] Each rat was injected intraperitoneally with 60 mg/kg of zoxazolamine or deuteriozoxazolamine-4,6-d_2, dissolved in a 1:1:1 mixture of dimethylacetamide–propylene glycol–50% aqueous glycerol. [b] Figures in parentheses refer to the number of rats in each group. Each value is a mean ± standard deviation.

In Vitro **Metabolic Studies.**—The metabolism of zoxazolamine and the deuterated analog by liver microsomes was next studied in order to compare the results *in vitro* with those obtained *in vivo*. This study serves to augment the *in vivo* sleeping time data, which indicate that zoxazolamine-4,6-d_2 is metabolized at an essentially unaltered rate compared to zoxazolamine.

Liver microsomes were prepared from male Sprague–Dawley rats which were injected intraperitoneally with 20 mg/kg of 3,4-benzpyrene in corn oil 24 hr before sacrifice. The liver was homogenized in three volumes (v/w) of 1.15% KCl and was centrifuged at 10,000*g* for 10 min. The supernatant fraction was used for incubation. The comparative metabolic data are presented in Table II.

The *in vitro* data indicated that regardless of the method of analysis, the deuteriozoxazolamine was metabolized considerably more slowly than zoxazolamine. Whether this reduced rate of metabolism of deuteriozoxazolamine is due to decrease in the affinity to the degradative enzyme or to the slower cleavage rate of C–D bond than that of C–H bond cannot be answered from these experiments.

The reason is not clear for the absence of difference in the duration of pharmcologic action between the two forms of zoxazolamine despite their difference in the *in vitro* degradation rates by the liver supernatant fraction. A loss in the potency of a drug by deutera-

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TABLE II
RELATIVE RATES OF HYDROXYLATION OF ZOXAZOLAMINE AND ZOXAZOLAMINE-4,6-d_2[a]

μmole of substrate consumed[b]			μmole of product formed[b]		
Zoxazolamine	Deuteriozoxazolamine	k_H/k_D	6-Hydroxy-zoxazolamine	Deuteria-6-hydroxy-zoxazolamine	k_H/k_D
0.163 ± 0.010	0.134 ± 0.017	1.22	0.166 ± 0.002	0.116 ± 0.002	1.43
0.143 ± 0.010	0.097 ± 0.018	1.47	0.101 ± 0.002	0.065 ± 0.005	1.54
0.129 ± 0.004	0.087 ± 0.012	1.48	0.089 ± 0.012	0.062 ± 0.005	1.43
		Av 1.39			Av 1.47

[a] The incubation mixture consisted of 1.0 ml of 0.1 *M* phosphate buffer, pH 7.4, 2 μmoles of ATP, 5 μmoles of DPN, 25 μmoles of glucose 6-phosphate, 15 μmoles of nicotinamide, 0.25 μmole of TPN, 20 μmoles of magnesium chloride, 1 μmole of substrate, and 1.0 ml of the 10,000*g* supernatant fraction in a total volume of 3.5 ml. Incubation was carried out for 20 min at 37° in a Dubnoff shaker. The reaction was approximately linear up to 30 min of incubation period. [b] The amounts of zoxazolamine remaining after incubation or of 6-hydroxyzoxazolamine formed during incubation were assayed according to the method of Conney, *et al.*[3] Each figure is a mean ± standard deviation from six separate incubation mixtures.

tion, as was reported by Elison, *et al.*,[7] for deuteriomorphine, can be ruled out in the present study. Determination of ED_{50} values and the 95% confidence limits by the method of Litchfield and Wilcoxon[8] revealed no difference between the two compounds, 58 mg/kg (55–61) for zoxazolamine and 59 mg/kg (53–66) for deuteriozoxazolamine.

Our apparent inability to detect this expected pharmacologic difference can be rationalized by the apparent insensitivity of the *in vivo* sleep time method employed to distinguish between the compounds.

The present *in vitro* data differ from those of acetanilide,[9] phenobarbital,[10] and dimethylphenol,[11] where substitution of H by D or a T atom at the site of aromatic ring hydroxylation had little effect on the rate of hydroxylation.

Experimental Section

2-Amino-4-chlorophenol-3,5-d_2.—In a 50-ml, thick-wall glass tube, 2.934 g of 2-amino-4-chlorophenol was dissolved in a solution of 25 ml of D_2O and 1.0 g of PCl_5. The tube was sealed and heated at 100–110° for 4.5 days. The reaction mixture was evaporated to dryness, and H_2O was added. This solution was neutralized with 1 *N* NaOH to pH 6, and again evaporated to dryness. The solid residue was extracted with Et_2O, and the extract was dried over Na_2SO_4. Evaporation yielded 2.802 g of product.

2-Amino-5-chlorobenzoxazole-4,6-d_2.—A mixture of 2.351 g of 2-amino-4-chlorophenol-3,5-d_2, 3.4 g of CNBr, and 225 ml of H_2O was heated on a steam bath for 15 min. The mixture was cooled to room temperature and neutralized with 15 *M* NH_4OH to pH 8 with cooling, and the product was filtered. After drying the product, it was sublimed at 160° (0.020 mm), yielding 1.926 g of **4**, mp 182–183°. *Anal.* ($C_7H_3ClD_2N_2O$) atom % excess D: calcd, 40; found, 38.10.

5-Chloro-2-hydroxybenzoxazole-4,6-d_2.—To 0.500 g of 2-amino-5-chlorobenzoxazole-4,6-d_2 in 15 ml of D_2O was added 1.0 g of PCl_5. The mixture was heated at 95–110° for 18 hr and then cooled. The product was filtered, dissolved, and evaporated from EtOH four times to exchange the acidic D on N to give 0.372 g of product.

5,6-Dichloro-2-hydroxybenzoxazole-4-d.—To 0.205 g of 5-chloro-2-hydroxybenzoxazole-4,6-d_2 dissolved in 3 ml of AcOH was added 0.178 g of SO_2Cl_2. The solution was stirred overnight and then heated on a steam bath for 1 hr and cooled, and 30 g of ice was added. The product was filtered, dissolved, and evaporated from MeOH four times to exchange labile D to give 0.201 g of **5**. A sample was sublimed at 130° (0.02 mm), mp 198.5–201°.[12] *Anal.* ($C_7H_2Cl_2DO_2N$) atom % excess D: calcd, 33.33; found, 33.20.

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