Table II. Inhibitory Zone Diameters (in mm)[a] vs. Penicillin-Sensitive and -Resistant Bacterial Strains

no.	S. aureus		E. coli		Enterobacter clocae		Pseudomonas aeruginosa	
(25 μg/disk)	MB2985	MB2314	MB2482	MB2964	MB2647	MB2646	MB2835	MB3350
1	40	41	28.5	29	25.5	27	26	26
2	40	40.5	28.5	29.5	27	27.5	29	29
3	38.5	40	28	29.5	27	27	28	28

[a] In this assay a 2-mm difference in zone size corresponds approximately to a twofold difference in activity.

evaluations of in vitro antibacterial activity have shown a two- to fourfold increased potency against these species.[5] Because of its improved stability and enhanced antipseudomonal activity, *N*-formimidoylthienamycin (**2**) has been selected for clinical studies.

Acknowledgment. We thank Dr. Byron H. Arison and Mr. Herman Flynn for the ^{1}H NMR spectra and Ms. Jean S. Kahan for the antibacterial assays.

References and Notes

(1) H. Kropp, J. S. Kahan, F. M. Kahan, J. Sundelof, G. Darland, and J. Birnbaum, "Thienamycin, A New β-lactam Antibiotic. II. In Vitro and In Vivo Evaluation", presented at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., Oct. 1976.
(2) J. S. Kahan, F. M. Kahan, R. Goegelman, S. A. Currie, M. Jackson, E. O. Stapley, T. W. Miller, A. K. Miller, D. Hendlin, S. Mochales, S. Hernandez, H. B. Woodruff and J. Birnbaum, *J. Antibiot.*, **32**, 1 (1979).
(3) J. S. Kahan, F. M. Kahan, R. T. Goegelman, E. O. Stapley, and S. Hernandez, German Offen. 2652681, 1977.
(4) Reverse-phase high-performance LC analyses were performed using a Waters Associates high-pressure liquid chromatograph equipped with a 2.5 mm i.d. × 61 cm μBondapak C_{18}/Corasil column. 1, **2**, and **3** had retention times of 5, 6, and 7 min, respectively, at a flow rate of 1.0 mL/min using 10% THF–H_2O as solvent.
(5) H. Kropp, J. B. Sundelof, J. S. Kahan, F. M. Kahan, and J. Birnbaum, "MK0787 (*N*-Formimidoyl-Thienamycin): Evaluation of In Vitro and In Vivo Activity", presented at the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, Mass., Oct., 1979.

W. J. Leanza,* K. J. Wildonger, T. W. Miller B. G. Christensen

Merck Sharp & Dohme Research Laboratories Rahway, New Jersey 07065

Received July 23, 1979

Articles

Oxazepam Esters. 1. Correlation between Hydrolysis Rates and Brain Appearance of Oxazepam

Gábor Maksay,* Zsuzsanna Tegyey, Vera Kemény, István Lukovits, László Ötvös,

Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Pusztaszeri ut 59-67, H-1025 Budapest, Hungary

and Éva Pálosi

Chemical Works of G. Richter Ltd., Budapest, Hungary. *Received February 26, 1979*

Esters of the centrally acting oxazepam were investigated to find quantitative correlations between the pharmacokinetics of the parent drug and in vitro biotransformation rates and physicochemical properties of its prodrugs. The ^{14}C-labeled aliphatic and ω-phenyl-substituted esters were administered intravenously to mice. Brain levels of the esters and oxazepam were determined and the latter was fitted to a simplified exponential equation. In vitro hydrolysis rate of the esters catalyzed by the hepatic microsomal fraction was measured with a pH stat. Pharmacokinetic constants characterizing the rising part of oxazepam brain levels correlate well with the chromatographic R_M values and with in vitro maximal hydrolysis rates of the esters. The hydrolysis is capacity limited in the liver. In a closely related set of aliphatic esters, oxazepam brain penetration also correlates with the steric constant (E_S) of its esters.

Prodrugs are known to modulate the pharmacokinetic properties of the parent compound.[1,2] This approach proved to be useful in drug design.[3,4]

Only few attempts have been made at defining overall pharmacokinetic–structure relationships.[4-8] There have been, however, numerous reports wherein one pharmacokinetic property—often examined in model systems in vitro—has been correlated with physicochemical parameters.[9-21]

Quantitative aspects of the correlation between chemical structure and pharmacological activity (QSAR) have been thoroughly studied. Notari[5] proposed the inclusion of the pharmacokinetic aspect to structure–activity relationships (SPAR). This is especially justified for prodrugs because activity is directly dependent on biotransformation and pharmacokinetics, the prodrug having no activity of its own.

This study on oxazepam esters as prodrugs illustrates the quantitative correlation between chemical structure and brain penetration. Oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-3*H*-1,4-benzodiazepin-2-one) is a potent tranquillizer and anticonvulsant. Although substitution with bulky moieties in position 3 was stated to diminish the effect of the 1,4-benzodiazepines on the CNS,[22] oxazepam esters as bioreversible derivatives have two potential advantages. Hydrosoluble derivatives[23,24]

0022-2623/79/1822-1436$01.00/0

Table I. Pharmacokinetic Data of Oxazepam Esters and the Structural Parameters

no.	oxazepam compds	dose, mg-equiv of oxazepam/kg	a, nmol/g	k_1, min^{-1}	k_2, min^{-1}	$t_{1/2,calcd}$, min	$t_{1/2,expl}$, min	R_M,[b]	E_S,[c]
1	oxazepam	10	31.8	0.696	0.003	0.96	1.4	−0.25	
2	acetate	10	55.1	0.562	0.005	1.16	1.4	−0.15	0
3	propionate	10	38.2	0.198	0.002	3.27	4.5	−0.03	−0.07
4	*n*-butyrate	15	33.9	0.098	0.001	6.53	8.2	0.17	−0.36
5	diethylacetate	10	58.0	0.011	0.010	22.0	26	0.46	−1.98
6	isobutyrate	5	13.2	0.108	0.058	5.21	4.6	0.12	−0.47
7	succinate methyl ester	5	27.3	0.032	0.024	8.32	8.0	−0.03	−0.35[d]
8	γ-Ph-butyrate	5	21.3	0.015	0.003	29.2	26	0.65	−0.45
9	β-Ph-propionate	5	36.3	0.013	0.012	19.0	19	0.50	−0.38
10	(±)-α-Me-β-Ph-propionate	5	33.5	0.013	0.010	20.2	23	0.64	
11	(+)-α-Me-β-Ph-propionate	5	16.0	0.037	0.004	13.7	14	0.64	
12	(−)-α-Me-β-Ph-propionate	5	25.1	0.017	0.005	22.9	22	0.64	

[a] Determined graphically from the data. [b] Taken from ref 34. [c] Taken from ref 48. [d] Taken from ref 28.

Table II. Antimetrazol and Muscle Relaxant Activities of Oxazepam Esters in Mice

	30 min			60 min		
no.	antimetrazol ED_{50},[a]	rotarod ED_{50}	act. ratio[b]	antimetrazol ED_{50}	rotarod ED_{50}	act. ratio
1	0.18 (0.13-0.54)[c]	0.74 (0.02-1.40)	4.1	0.39	1.60 (1.10-2.60)	4.1
2	0.38 (0.20-0.71)	0.97 (0.69-1.35)	2.6	0.33 (0.22-0.46)	1.20 (0.96-1.72)	3.6
3	0.13	4.45	34.2	0.32 (0.28-0.51)	4.40 (2.79-6.28)	13.8
4	0.29 (0.16-0.83)	2.64 (1.96-3.51)	9.1	0.32 (0.18-0.83)	3.28 (2.10-4.84)	10.3
5	0.28	2.80 (1.03-4.32)	10.0	0.33 (0.25-0.74)	3.42 (1.77-7.05)	10.4
6	0.51 (0.34-0.92)	2.45 (1.56-3.35)	4.8	0.33 (0.13-0.76)	2.67 (1.11-3.98)	8.1
9	0.44 (0.29-0.93)	2.57 (0.91-3.85)	5.8	0.31 (0.24-0.44)	3.56 (2.11-4.88)	11.5
10	0.51	3.75 (1.64-4.71)	7.4	0.66 (0.39-1.28)	4.01 (2.25-5.19)	6.1
11	0.39 (0.20-1.24)	2.67 (1.24-4.26)	6.8	0.82 (0.63-1.28)	3.83 (2.74-5.42)	4.7
12	0.81 (0.51-1.11)	2.86 (1.72-4.09)	3.5	0.92 (0.64-1.27)	2.20 (0.91-3.29)	2.4

[a] ED_{50} values are expressed in mg-equiv of oxazepam/kg. [b] ED_{50} (rotarod)/ED_{50} (antimetrazol). [c] Figures in parentheses are 95% confidence limits.

facilitate parenteral administration, and the more lipophilic aliphatic esters[25] may modify the tissue distribution and increase the concentration of the parent compound at the site of action.

This study treats the esters of oxazepam as prodrugs, attention being focused on the brain level of oxazepam liberated. It was previously demonstrated[26] that the liver has the greatest esterase activity for oxazepam acetate; the hydrolysis in the brain is also significant and proceeds with opposite stereoselectivity. It will be demonstrated that the pharmacokinetic data characterizing the brain penetration of oxazepam can be correlated with the hepatic microsomal hydrolysis rates that are dependent on the physicochemical parameters of the esters.

Experimental Section

Substrates. Oxazepam esters were prepared from oxazepam with acyl chlorides using pyridine-catalyzed acylation as previously described.[27-29] Oxazepam phenylacetate (13) was similarly synthesized and recrystallized from methanol: yield 70%; mp 192–194 °C. Oxazepam was labeled with ^{14}C in position 2.[30] Specific activity of the esters ranged from 1.5 to 4.7 mCi/mmol and the radiochemical purities were above 99.2%.[29]

Animal Pharmacology. Oxazepam esters were administered intravenously to mice in an aqueous solution containing less than 10% of chremofor.

Antimetrazol Effect.[34] Metrazol (Fluka) was given sc at a dose of 125 mg/kg 30 or 60 min after the administration of the esters. Doses required to prevent the induction of tonic–extensor seizures in 50% of mice (ED_{50}) were determined by probit analysis.

Rotarod Test. Control mice can remain on a rod rotating with a frequency of 12 min^{-1} within the control time (2 min).[52] The ED_{50} values correspond to the dose which causes 50% of the mice to fall off the rotating rod within the control time.

Pharmacokinetics. Drug Administration. Male albino mice (18–22 g) were given 2-^{14}C-labeled esters (dose data are tabulated in Table I) intravenously in Me_2SO (35 μL of Me_2SO/20 g of body weight).

Brain Levels of Oxazepam and Its Esters. After decapitation of the mice, one hemisphere of the brain was processed as previously described.[26] The ratio of the two metabolites in the sample was determined from the ratio of the radioactivities following thin-layer chromatography. The only exception was oxazepam succinate methyl ester, where the radioactivity of a third compound, oxazepam hemisuccinate, was also taken into consideration. Total radioactivity of the other hemisphere corresponding to the sum of the levels of the metabolites was measured on a carbon–tritium analyzer (Chinoin Ltd., Hungary). Each kinetic point represents four mice. Brain level of the metabolites can be calculated from two sets of independent data. Total radioactivity of the dried liver samples was measured the same way.

Microsomal Hydrolysis of the Esters. Preparation of microsomes was carried out as described previously.[28] The liver homogenate in 0.25 M sucrose was fractionated on an ultracen-

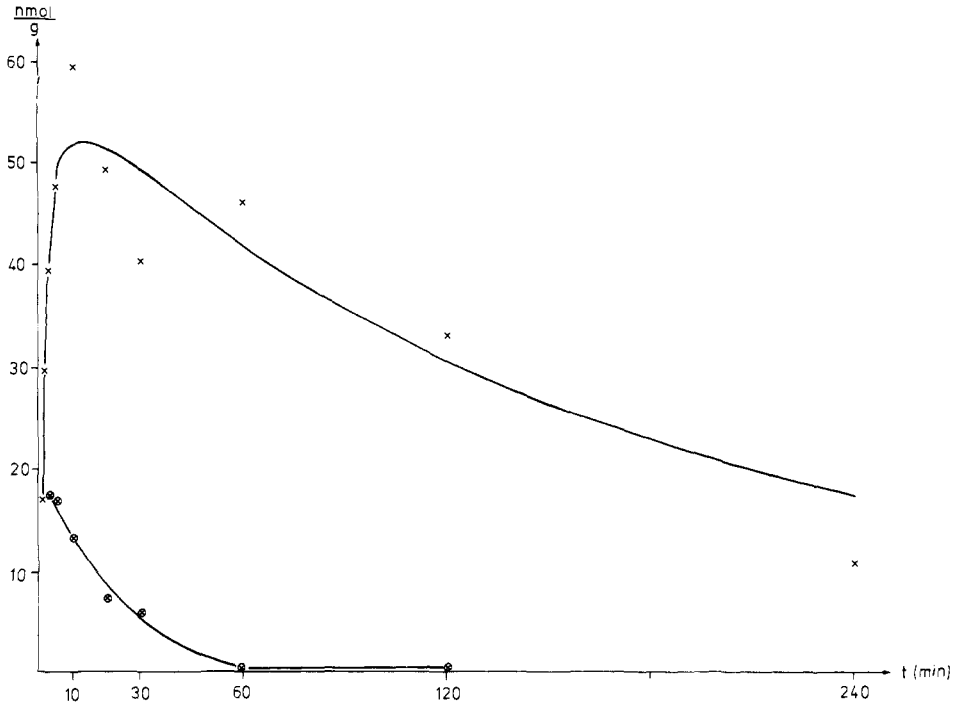


Figure 1. Brain levels of compounds 1 (x) and 2 (⊗) following iv administration of compound 2 to mice: dose, 11.5 mg/kg. The points represent four animals. The curve for 1 was fitted by computer according to eq 1.

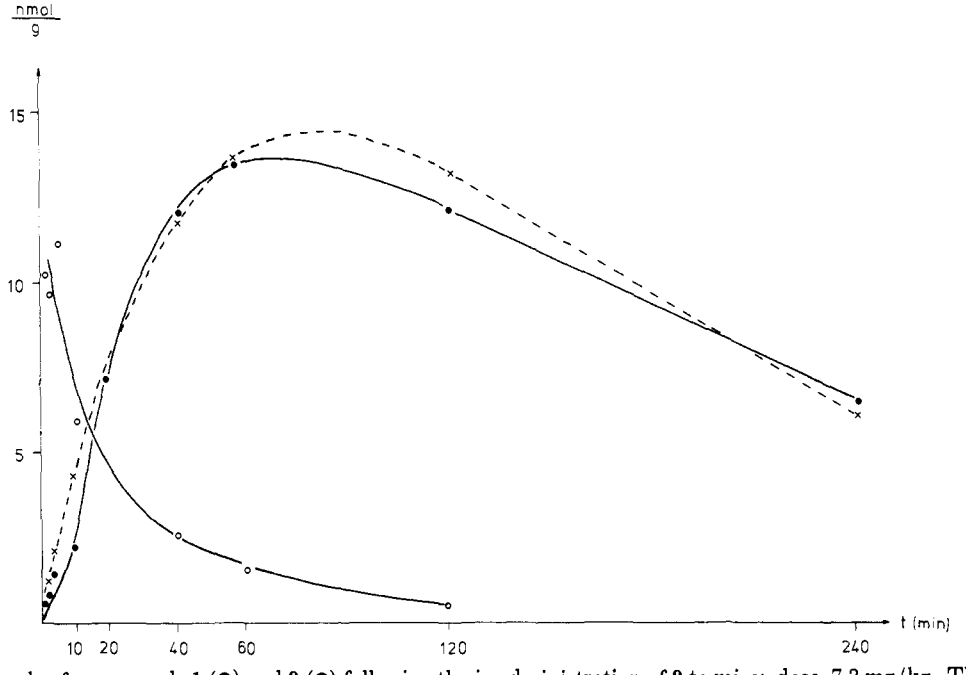


Figure 2. Brain levels of compounds 1 (●) and 9 (○) following the iv administration of 9 to mice: dose, 7.3 mg/kg. The points represent four animals. The dotted line fits to the calculated points (x) according to eq 1.

trifuge (Janetzky, Type VAC-601). The 13000*g* (for 15-min) supernatant was centrifuged at 120000*g* for 60 min.

The in vitro hydrolysis rate of the oxazepam esters was determined titrimetrically by pH stat (Radiometer, Copenhagen). Medium: 20–60 mL of 0.45% NaCl solution containing 1–50 mg of microsomes. Initial concentration of the substrates varied between 10^{-5} and 4×10^{-4} M. Initial rate of hydrolysis was determined at 37 °C, at pH 7.5. Maximal rates of hydrolysis (V_{max}) and apparent Michaelis constants (K_m) were calculated from the Lineweaver–Burk plot of the data.

The protein content of the microsomes was determined by biuret reaction[33] calibrated on human serum albumin. Reverse-phase chromatographic R_M values were taken from ref 34. R_f values were determined on silica gel plates impregnated with paraffin and developed in water containing 65% methanol.[34] Fitting of pharmacokinetic data and regression analysis were accomplished by a digital computer (CDC 3300).

Results

Animal Pharmacology. Antimetrazol and muscle relaxant activities of the compounds were measured at 30 and 60 min (Table II). Muscle relaxant activity, a side effect of the 1,4-benzodiazepine day-time tranquillants, was characterized by the rotarod test. The esters are significantly less active than the parent compound; thus, their activity pattern seems to be more advantageous. The different activity ratios can be accounted for by different tissue distribution or by the activity of the esters themselves. The study of the pharmacokinetics of the compounds might contribute to the answer.

Pharmacokinetics. Previous studies[35,36] dealing with

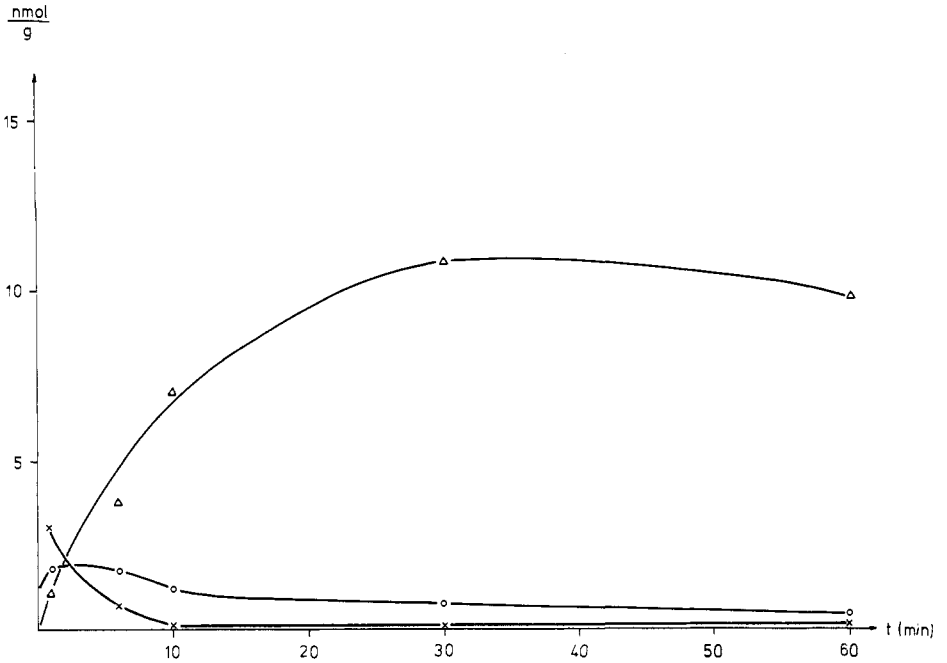


Figure 3. Brain levels of 1 (△), its hemisuccinic ester (○), and **7** (x) following iv administration of **7** to mice: dose, 7.0 mg/kg. The points represent four animals.

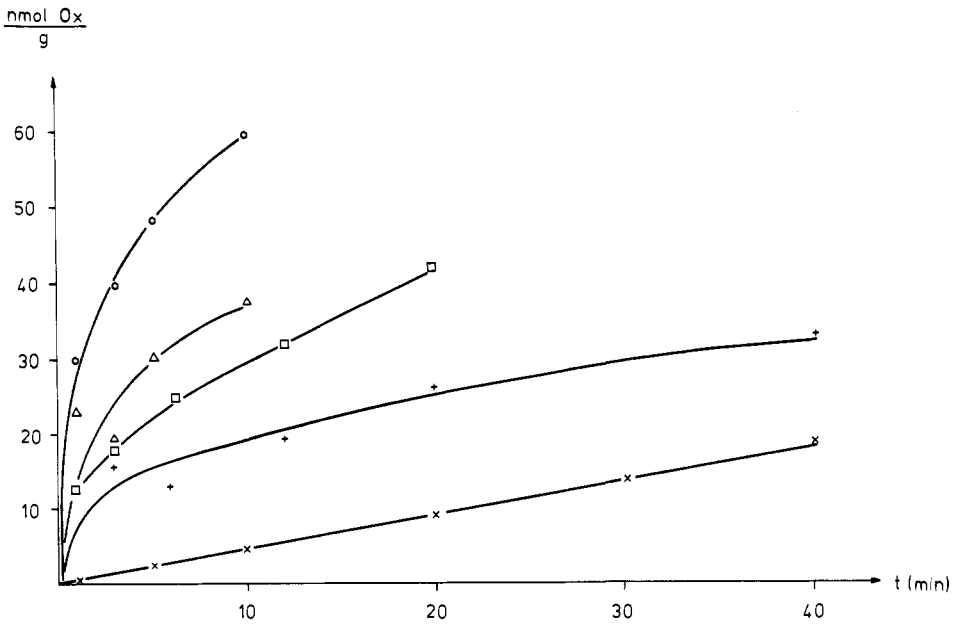


Figure 4. The increase of the brain levels of oxazepam following the iv administration of its aliphatic esters to mice: 1 (△), **2** (○), **3** (□), **4** (+), and **5** (×).

the pharmacokinetics of oxazepam esters were restricted to the measurement of either the parent compound or oxazepam, probably because the widely used gas–liquid chromatographic method does not allow simultaneous determination of both.

Oxazepam has a simple metabolic pattern; a substantial part of its dose (95%) is excreted as a β-glucuronide derivative.[37] On administration of labeled oxazepam acetate to mice, no metabolites other than the ester and oxazepam contribute significantly to the brain level. This is in agreement with previous reports for oxazepam pivaloate.[36] Thus, it is justified to calculate metabolite levels from total radioactivity measurements.

Hydrolysis rates of the esters are rather fast, as ester levels decline rapidly below 1% of the brain levels of oxazepam in 4 h. Figure 1 presents an example of the rapid hydrolysis of oxazepam acetate, the hydrolysis of which is accelerated by stereoselective effects.[26] Figure 2 demonstrates the slower decline of oxazepam β-phenylpropionate. The ω-phenyl-substituted ester subgroup clearly differs from the aliphatic ester subgroup in the latentiation of oxazepam brain penetration.

Oxazepam succinate methyl ester does not result in a high level of its half ester (Figure 3). The latter cannot readily cross the blood–brain barrier[29] because it is too polar and mostly ionized.[38] If the hydrolysis of the methyl ester occurred in the brain in situ, the restricted outflow of the half ester would probably result in its elevated brain level.

Equimolar doses of the esters permit comparison of the brain levels of oxazepam. The first parts of the curves up to concentration maxima are collected in Figure 4 for the

Table III. Brain Levels of Oxazepam Administered as Esters

time, min	brain level, nmol/g											
	1[a]	2	3	4	5	6	7	8	9	10	11	12
1	22.8[b] (±3.9)	29.9 (±9.0)	12.6 (±2.6)	16.6 (±6.2)			0.8 (±0.01)	0.4 (±0.1)	0.5 (±0.2)			
2					0.8 (±0.1)	3.1 (±0.4)						
3	18.8 (±3.2)	39.4 (±9.6)	17.5 (±3.8)	17.3 (±6.9)				0.9 (±0.2)	0.8 (±0.2)	1.3 (±0.6)	2.1 (±1.3)	0.8 (±0.1)
5	29.2 (±4.1)	47.5 (±10.0)			2.0 (±0.2)	5.7 (±0.8)		0.8 (±0.2)	1.4 (±0.3)	1.6 (±0.2)	2.6 (±1.0)	1.6 (±0.4)
6			24.2 (±4.2)				3.8 (±0.1)					
10	37.1 (±4.8)	59.5 (±5.8)			4.8 (±1.2)	8.9 (±1.3)	7.0 (±0.5)	2.9 (±0.3)	2.2 (±0.5)	4.7 (±0.2)	5.6 (±2.4)	4.6 (±0.3)
12			30.8 (±6.1)	19.0 (±3.4)								
20	28.9 (±3.9)	49.2 (±9.0)	41.7 (±8.1)	25.9 (±3.6)	7.8 (±1.5)	9.3 (±1.8)		6.5 (±0.5)	7.1 (±0.7)	5.4 (±0.5)	7.7 (±1.8)	6.7 (±1.2)
30	27.6 (±3.0)	40.3 (±5.7)			12.1 (±1.3)		10.8 (±1.2)					
40			33.0 (±4.5)	32.0 (±4.6)	18.0 (±1.1)	11.6 (±2.1)		9.5 (±0.5)	12.1 (±1.2)	10.0 (±1.4)	10.8 (±2.0)	11.8 (±3.5)
60	28.5 (±2.7)	46.6 (±5.1)			23.1 (±2.4)	10.8 (±0.3)	9.8 (±1.9)	9.6 (±0.5)	13.6 (±1.4)	13.0 (±1.5)	12.8 (±2.7)	14.8 (±2.2)
90				38.2 (±4.1)	20.9 (±2.5)							
120	21.8 (±2.9)	33.0 (±3.8)			20.0 (±1.9)	5.9 (±1.1)		15.1 (±1.0)	12.2 (±1.8)	14.2 (±2.2)	11.7 (±2.7)	13.7 (±2.1)
180				22.6 (±1.4)								
240	13.4 (±1.1)	11.0 (±1.9)			13.5 (±1.8)	3.8 (±1.0)		12.2 (±3.2)	6.6 (±0.8)	5.5 (±0.3)	7.2 (±1.4)	12.2 (±4.3)

[a] Dose data are collected in Table I. [b] Brain levels are averages of four mice (±SE).

Table IV. Maximal Hydrolysis Rates and Apparent Michaelis Constants of Oxazepam Esters with the Hepatic Microsomal Fraction

no.	V_{max}, nmol min^{-1} (mg of protein)$^{-1}$	K_m (10^{-6} M)
2	323[a]	
3	139[a]	
4	88[a]	
5	1.4[a]	
6	131[a]	
7	125[a]	
8	1.5	9.5
9	7.9	9.7
10	2.4	8.3
11	7.3	25.0
12	2.6	8.7
13	12.2	22.7

[a] Taken from ref 28.

Table V. Total Metabolite Concentrations[a] in Liver

time, min	metabolite concn, nmol/g	
	8	9
1	80.7 (±25.1)[b]	40.7 (±12.2)
3	85.9 (±8.6)	45.6 (±4.7)
5	82.3 (±8.1)	34.7 (±6.8)
10	40.4 (±10.2)	32.6 (±3.9)

[a] Dose: 5 mg equiv of oxazepam ester/kg. [b] The data are averages (±SE) of four mice.

aliphatic esters and in Figure 5 for the esters of ω-phenylcarbonic acids. Increased branching of the alkyl chain next to the acyl group and phenyl substitution lead to decreased hydrolysis. Accordingly, liberation and brain penetration of oxazepam are gradually retarded.

The increased brain accrual of oxazepam from compound **2** can be explained with the brain hydrolysis in situ that was shown in vitro[26] to have opposite stereoselectivity to liver. The brain hydrolysis becomes more inferior to liver hydrolysis for the longer-chain esters and does not show opposite stereoselectivity.[39]

Cummings and Martin[40,41] studied the accrual of drug metabolites and showed that it depends upon the ratio of the rate constants for the processes of consecutive formation and excretion. Application of this approach to prodrugs means that the change in the accrual of the parent drug depends on the rate of its formation, because the consecutive processes, conjugation and/or excretion, are the same. The exponential equation of two terms characterizing the accrual is formally similar to the Bateman equation where the chemical process is substituted by absorption.[42]

Application of an exact compartment system to hydrolysis kinetics is rather complex.[43] We considered the brain a part of the central compartment because of the rapid blood to brain partition of oxazepam.[29]

The brain concentration of oxazepam was fitted with eq 1 which characterized the accrual of the intermediate

$$c_{ox} = \frac{ak_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad (1)$$

product of a consecutive reaction,[42] where a is the apparent initial concentration of the ester, k_1 is the pseudo-first-order rate constant of hydrolysis and k_2 is the pseudo-first-order rate constant of elimination.

Oxazepam brain concentration values are summarized in Table III. . Fitting these concentration profiles with eq 1 resulted in the kinetic constants k_1 and k_2 of Table I. Another parameter, $t_{1/2}$, was introduced to characterize the latentiation of oxazepam brain accrual. It is defined as the time needed to reach half the maximal brain concentration of oxazepam. This was determined both graphically ($t_{1/2,exptl}$; see Figure 4) and by computer calcu-

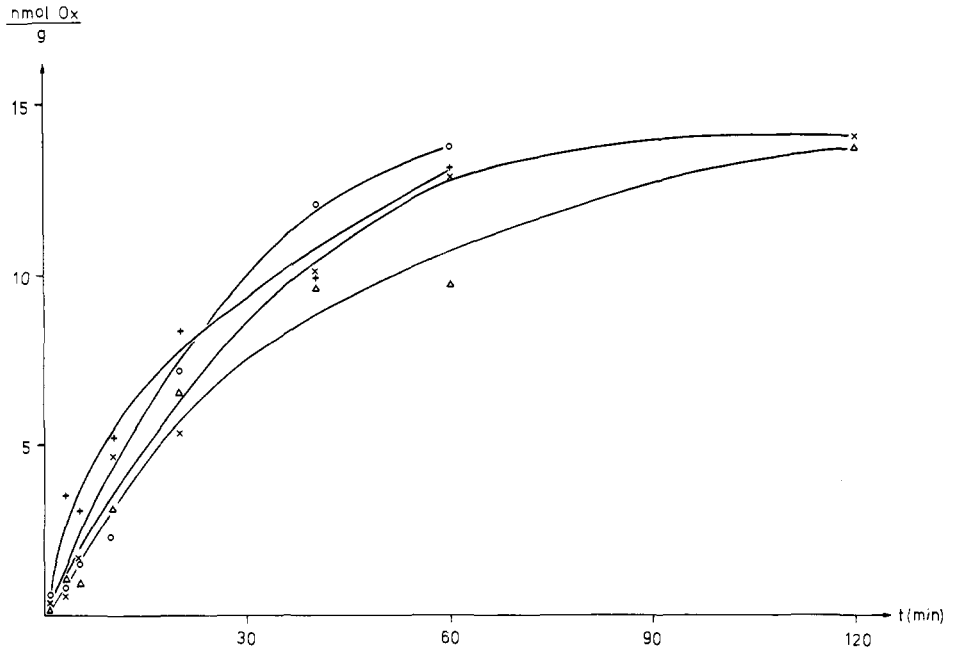


Figure 5. The increase of the brain levels of oxazepam following the iv administration of its phenyl-substituted esters to mice: **8** (△), **9** (○), **10** (×), and **11** (+). The points represent four animals.

lation ($t_{1/2,calcd}$). It must be stressed that $t_{1/2}$ is not a half-life and is not simply related to k_1. Rather, it is an empirical parameter which will be shown to correlate excellently with the in vitro hydrolysis rates. A similar parameter has been used to describe the metabolic formation of oxazepam from diazepam.[44]

The k_1 and $t_{1/2}$ values of Table I show that the administration of compounds **1** and **2** results in the largest increased rate of accumulation of oxazepam in brain; the other esters latentiate its penetration. Since the experimental points lie overwhelmingly on the rising part of the concentration profiles, the computed k_2 values are subject to large errors, but from the point of view of the bioactivation of the prodrugs k_2 is of minor importance because it reflects a process that is common for all esters.

The use of t_{max} instead of $t_{1/2}$ would be theoretically more reasonable, but its determination is similarly subject to large errors as that of k_2.

In Vitro Hydrolysis. The liver microsomal fraction proved to have the greatest esterase activity for oxazepam acetate.[26] Therefore, it may be postulated that the endoplasmic reticulum of liver has a determining role in the bioactivation of oxazepam esters. To verify this, microsomal hydrolysis was measured and the rates were correlated with the pharmacokinetic data.

Table IV contains the maximal rates of hydrolysis and the apparent Michaelis constants of the phenyl-substituted esters. These analogues hydrolyze 20–50 times slower than the corresponding aliphatic esters. Increasing the chain length or branching at the α position decreases the hydrolysis rates. The effect of chirality on the hydrolysis rate is illustrated by compounds **11** and **12**. The slower hydrolyzing compound **12** has a smaller K_m value; i.e., it binds to the active center of the esterases stronger than compound **11**. This is the probable reason why the V_{max} of the racemic compound **10** does not exceed the V_{max} values of the isomers.

Correlation between the Brain Appearance of Oxazepam and in Vitro Data. Equation 2 provides a good correlation between the experimental and calculated $t_{1/2}$ values of Table I. The figure in parentheses is the 95% confidence limit, r is the correlation coefficient, and s is the standard error of estimate.

$$t_{1/2,calcd} = 0.996(\pm 0.124)t_{1/2,expl} - 0.350 \quad (2)$$

$$n = 12;\quad r = 0.981;\quad s = 1.981;\quad F_{1,10} = 254.6$$

The kinetic constant k_1 characterizing oxazepam appearance in the brain correlates well with the microsomal maximal hydrolysis rates (eq 3). Oxazepam methylsuccinate was excluded from the regression. It was demonstrated in vitro that serum esterases preferentially cleave the methyl ester. The half-ester concentration in the brain is significant (see Figure 3). The succinic half-ester, however, is not hydrolyzed by the hepatic microsomal fraction.[45] This manifests itself in the anomalous decrease of the corresponding k_1 value.

$$\log k_1 = -2.08 + 0.60(\pm 0.14)\log V_{max} \quad (3)$$

$$n = 10;\quad r = 0.952;\quad s = 0.195;\quad F_{1,8} = 76.8$$

The inclusion of the in vitro Michaelis constants does not improve the correlation of eq 3. Liver levels were determined (Table V) and compared with the in vitro K_m values. Total liver levels in the first 10 min can be contributed mainly to the esters. They exceed several-fold the K_m values ($\sim 10^{-5}$ M; see Table IV). That is, substrate excess in the liver assures that the hydrolysis in vivo is capacity limited,[46] and the maximal rates determine the correlation.

The effect of the hydrophobicity of the esters was also examined. R_M, a reverse-phase thin-layer chromatographic parameter [$R_M = \log(1/R_f - 1)$], was used,[34] which can substitute the partition coefficients in QSAR studies.[47]

Increasing hydrophobicity of the esters results in a decreased oxazepam brain penetration (eq 4).

$$\log k_1 = -0.72 - 1.79(\pm 0.54)R_M \quad (4)$$

$$n = 10;\quad r = 0.921;\quad s = 0.248;\quad F_{1,8} = 44.9$$

$$\log k_1 = -0.74 - 3.25(\pm 1.42)R_M + 2.52(\pm 2.33)R_M^2 \quad (5)$$

$$n = 10;\quad r = 0.954;\quad s = 0.205;\quad F_{2,7} = 35.1$$

$$\log k_1 = -1.75 - 0.45(\pm 1.27)R_M + 0.46(\pm 0.41)\log V_{max} \quad (6)$$

$$n = 10;\quad r = 0.955;\quad s = 0.201;\quad F_{2,7} = 36.3$$

$$\log k_1 = -1.74 - 1.90(\pm 1.19)R_M + 2.45(\pm 1.47)R_M^2 + 0.45(\pm 0.26) \log V_{max} \quad (7)$$

$$n = 10; \quad r = 0.984; \quad s = 0.129; \quad F_{3,6} = 62.7$$

The inclusion of the quadratic term of hydrophobicity (R_M^2) slightly improves the correlation (eq 5). Increasing hydrophobicity of the esters results in a decreased oxazepam brain penetration.

The $t_{1/2,calcd}$ value represents excellently the ester latentiation in penetration (eq 8). It seems to be less dependent

$$t_{1/2,calcd} = 26.03 - 9.90(\pm 1.84) \log V_{max} \quad (8)$$

$$n = 10; \quad r = 0.967; \quad s = 2.63; \quad F_{1,8} = 115.5$$

on dose.[41] It correlates with log V_{max} values better than does log k_1 (see eq 3).

There is an inflexion character in the rising part of the oxazepam brain profiles that indicates a lag time in penetration (see Figure 2). Oxazepam brain penetration formed from diazepam in mice has a similar inflexion nature.[44] Equation 1 eliminates the inflexion. Thus, the experimental curve is better represented by $t_{1/2,calcd}$ than by k_1 values.

The inclusion of R_M does not improve the correlation of eq 8 at all. By contrast, log k_1 values correlate not only with log V_{max} but with the parabolic function of R_M as well. This may indicate that the very beginning of the brain penetration of oxazepam (characterized by k_1) depends not only on the hydrolysis rate but also on the hydrophobic nature of its ester. However, microsomal hydrolysis rates and R_M are strongly intercorrelated ($r = 0.939$). Therefore, the direct role of the ester hydrophobicity in oxazepam appearance in the brain remains obscure.

The rate of oxazepam appearance in the brain can be predicted from physicochemical constants of the esters. The use of R_M values was shown in eq 4 and 5. Since R_M values of the aliphatic esters are closely parallel with the carbon number of the acyl moiety,[34] this correlation is determined by the number of the hydrophobic increments of the methylene units (N) of the acyl moiety. Equation

$$\log k_1 = 0.20 - 0.42(\pm 0.06)N \quad (9)$$

$$n = 5; \quad r = 0.994; \quad s = 0.083; \quad F_{1,3} = 225.5$$

9 demonstrates this correlation for the aliphatic esters (**2**–**6**). The constant ΔR_M increments of the methylene units was included in the regression coefficient. Otherwise, it is supposed that additivity of R_M might be used. In vitro hydrolysis rates of the aliphatic esters of oxazepam were also shown to correlate fairly with Taft's steric constants, E_S,[48] of the acyl moiety.[28] A similar correlation exists with the brain penetration (eq 10 and 11). The parameters E_S

$$\log k_1 = -0.55 + 0.74(\pm 0.26)E_S \quad (10)$$

$$n = 5; \quad r = 0.954; \quad s = 0.217; \quad F_{1,3} = 30.2$$

$$t_{1/2,calcd} = 1.78 - 10.17(\pm 1.42)E_S \quad (11)$$

$$n = 5; \quad r = 0.993; \quad s = 1.155; \quad F_{1,3} = 202.6$$

and N are also intercorrelated ($r = 0.993$). The phenyl-substituted esters do not obey the correlations in eq 10 and 11. Their hydrolysis rate, as well as the brain penetration of oxazepam, is slower than predicted from the E_S. The hydrolysis of these esters is catalyzed by different microsomal esterases.[49] This indicates that predictions of the rate of the biotransformation from physicochemical constants cannot be extended to compounds having more profound structural modifications.

Conclusions

The oxazepam esters have somewhat more advantageous activity ratios than the parent compound. This might be attributed to different tissue distribution, a prodrug effect, but the activity of the esters themselves cannot be excluded.

The brain appearance of oxazepam is modulated by the ester hydrolysis in the liver. The oxazepam brain availability of the esters decreases which results in a moderate, but somewhat prolonged, pharmacological effect. However, rapid hydrolysis and the contribution of the opposite stereoselectivity of brain esterases for the short-chain aliphatic esters result in increased oxazepam brain appearance and antimetrazol activity.

The hepatic microsomal fraction is a proper model system to study the biotransformation of the esters. The most important site of their hydrolysis is the endoplasmic reticulum of the liver and the process is capacity limited in the examined dose range. This can explain the lack of correlation with the pharmacological data where the ED_{50} values are substantially smaller. The rate of oxazepam appearance in the brain can be predicted from physicochemical parameters of the esters (E_S and the number of the methylene units), at least in a closely related set of compounds.

Acknowledgment. Technical assistance of Miss Mária Füzi is acknowledged.

References and Notes

(1) T. Higuchi and V. Stella, *ACS Symp. Ser.* **no.** 14 (1975).
(2) S. Casadio, H. Cousse, F. Favier, and A. Boucherle, *Farmaco, Ed. Prat.*, **32**, 375 (1977).
(3) A. A. Sinkula and S. H. Yalkovsky, *J. Pharm. Sci.*, **64**, 181 (1975).
(4) A. A. Sinkula, *Annu. Rep. Med. Chem.*, 10, 316 (1975).
(5) R. E. Notari, *Acta Pharm. Suec.*, 11, 633 (1974).
(6) R. E. Notari, *J. Pharm. Sci.*, **62**, 865 (1973).
(7) D. C. Hobbs and H. M. McIlhenny, *Annu. Rep. Med. Chem.*, 11, 190 (1976).
(8) E. R. Garett, J. B. Mielck, J. K. Seydel, and H. J. Kessler, *J. Med. Chem.*, **12**, 740 (1969).
(9) Y. M. Amin and J. B. Nagwekar, *J. Pharm. Sci.*, **64**, 1804 (1975).
(10) K. Kakemi, T. Arita, R. Hori, and R. Konishi, *Chem. Pharm. Bull.*, **15**, 1705 (1967).
(11) J. M. Plá-Delfina, J. Moreno, J. Durán, and A. del Pozo, *J. Pharmacokinet. Biopharm.*, **3**, 115 (1975).
(12) C. Hansch, E. J. Lien, and F. Helmer, *Arch. Biochem. Biophys.*, **128**, 319 (1968).
(13) T. D. Yih and J. M. van Rossum, *Biochem. Pharmacol.*, **26**, 2117 (1977).
(14) B. Testa and P. Jenner, in "Drug Metabolism: Chemical and Biochemical Aspects", J. Swarbrick, Ed., Marcel Dekker, New York and Basel, 1976, p 265.
(15) S. Mayer, R. P. Maickel, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **127**, 205 (1959).
(16) A. H. Soloway, B. Whitman, and J. R. Messer, *J. Pharmacol. Exp. Ther.*, **129**, 310 (1960).
(17) W. H. Oldendorf, *Proc. Soc. Exp. Biol. Med.*, 147, 813 (1974).
(18) W. H. Oldendorf, *Psychopharmacol. Pract. Med.*, 167 (1977).
(19) E. Kutter, A. Herz, H. J. Teschmacher, and R. Hess, *J. Med. Chem.*, **13**, 801 (1970).
(20) C. Hansch, A. R. Steward, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, 11, 1 (1968).
(21) B. Testa, *Pharm. Acta Helv.*, **53**, 143 (1978).
(22) L. H. Sternbach, L. O. Randall, R. Banziger, and H. Lehr, in "Drugs Affecting the Central Nervous System", A. Burger, Ed., Marcel Dekker, New York, 1967, Vol. 2, p 237.
(23) M. Babbini, F. de Marchi, N. Montanaro, P. Strocchi, and M. V. Torrielli, *Arzneim.-Forsch.*, **19**, 1931 (1969).
(24) A. Nudelman, R. J. McCaully, and S. C. Bell, *J. Pharm. Sci.*, **63**, 1880 (1974).
(25) S. C. Bell, R. J. McCaully, C. Gochmen, S. J. Childress, and M. J. Gluckman, *J. Med. Chem.*, 11, 457 (1968).
(26) G. Maksay, Z. Tegyey, and L. Ötvös, *J. Pharm. Sci.*, **67**, 1208 (1978).

(27) Z. Tegyey, É. Pálosi, G. Maksay, and L. Ötvös, Hungarian Patent Application, M.A. 2975 (1978).
(28) G. Maksay, Z. Tegyey, and L. Ötvös, *Hoppe-Seyler's Z. Physiol. Chem.*, **359**, 879 (1978).
(29) G. Maksay, Z. Tegyey, and L. Ötvös, *J. Med. Chem.*, **22**, following paper in this issue (1979).
(30) Z. Tegyey, G. Maksay, and L. Ötvös, *J. Labelled Compd.*, **16**, 377 (1979).
(31) G. M. Everett and R. K. Richards, *J. Pharmacol. Exp. Ther.*, **81**, 402 (1944).
(32) W. J. Kinnard and C. J. Carr, *J. Pharmacol. Exp. Ther.*, **121**, 354 (1957).
(33) M. Ditterbrandt, *Am. J. Clin. Pathol.*, **18**, 439 (1948).
(34) G. Maksay, Z. Tegyey, and L. Ötvös, *J. Chromatogr.*, **174**, 447 (1979).
(35) E. Mussini, F. Marcucci, R. Fanelli, A. Guaitani, and S. Garattini, *Biochem. Pharmacol.*, **21**, 127 (1972).
(36) M. C. Sanchez, J. Colomé, and E. Gelpi, *J. Chromatogr.*, **126**, 601 (1976).
(37) S. F. Sisenwine and H. W. Ruelius, *Arzneim.*-Forsch., **22**, 682 (1972).
(38) B. B. Brodie, H. Kurz, and L. S. Schauker, *J. Pharmacol. Exp. Ther.*, **180**, 21 (1960).
(39) Unpublished results.
(40) A. J. Cummings and B. K. Martin, *Nature (London)*, **200**, 1296 (1963).
(41) A. J. Cummings, B. K. Martin, and G. S. Park, *Br. J. Pharmacol., Chemother.*, **29**, 136 (1967).
(42) F. H. Dost, "Grundlagen der Pharmacokinetik", Georg Thieme Verlag, Stuttgart, 1968, p 83.
(43) M. Rowland, Z. Benet, and S. Riegelman, *J. Pharm. Sci.*, **59**, 364 (1970).
(44) E. van der Kleijn, *Arch. Int. Pharmacodyn.*, **178**, 193 (1969).
(45) M. Salmona, C. Saronio, R. Bianchi, F. Marcucci, and E. Mussini, *J. Pharm. Sci.*, **63**, 222 (1974).
(46) J. van Rossum, C. A. M van Ginneken, P. T. Henderson, H. C. J. Ketelaars, and T. B. Vree, in "Kinetics of Drug Action", J. M. van Rossum, Ed., Springer Verlag, Berlin, Heidelberg, and New York, 1977, p 125.
(47) E. Tomlinson, *J. Chromatogr.*, **113**, 1 (1975).
(48) R. W. Taft, in "Steric Effects in Organic Chemistry", M. S. Newman, Ed., Wiley, New York, 1956, p 556.
(49) G. Maksay, Z. Tegyey, E. Simon, and L. Ötvös, unpublished results.

Oxazepam Esters. 2. Correlation of Hydrophobicity with Serum Binding, Brain Penetration, and Excretion

Gábor Maksay,* Zsuzsanna Tegyey, and László Ötvös

Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Pusztaszeri ut 59-67, H-1025 Budapest, Hungary. *Received January 18, 1979*

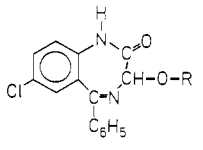
Pharmacokinetics of a series of prodrug-type oxazepam esters were studied in mice. The effect of hydrophobicity was investigated in relation to serum binding, brain penetration, tissue storage, and excretion. Binding to mouse serum and to human serum albumin was measured by equilibrium dialysis, and the changes in binding free energy were correlated with R_M values. Brain–blood partition of the esters did not change parallel with their serum binding. An indirect correlation exists between R_M of the esters and oxazepam brain accrual. Brain–blood concentration ratios of oxazepam prove that hydrolysis precedes brain penetration and hydrophobicity might primarily influence the hydrolysis rate. The amount of tissue storage and total excretion rates also correlate with hydrophobicity.

SAR studies on prodrugs require a pharmacokinetic aspect. Biotransformation is the factor studied most thoroughly in this field.[1] The role of hydrophobicity that is well known in QSAR studies, in general,[2] was briefly investigated for prodrugs.

The esters of oxazepam, a potent tranquillizer and anticonvulsant, were studied in the previous part of this work[3] to examine the role of hydrolysis in the brain accrual of oxazepam. A correlation was also demonstrated between the R_M values of the esters and the increased rate of oxazepam brain levels. This work attempts to explain the nature of this correlation and the role of hydrophobicity of oxazepam prodrugs in other pharmacokinetic processes, i.e., serum binding, tissue storage, and excretion as well.

Results and Discussion

Sixteen esters of oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-3*H*-1,4-benzodiazepin-2-one; see the structure where R = H), including two stereoisomers, were



studied. The acyl moiety contained an alkyl chain with varying length, successive branching in position 2 or 3, and/or an ω-phenyl group (Table I).

Serum Binding. Binding of oxazepam esters to mouse serum was investigated by equilibrium dialysis. The same ester concentration was used throughout, which was near the blood concentration in our in vivo experiments. Hydrolysis by serum esterases makes the measurement difficult; thus, the cells were equilibrated at 4 °C with continuous stirring. Diisopropyl fluorophosphate (DFP), a strong inhibitor of the esterases, was also included.[4] DFP was found not to influence the binding equilibrium of

Table I. Structure of Ester Substituents and Dose Data of the Esters

no.	R	dose, mg/kg[a]
1	H	5
2	$COCH_3$	10
3	$COCH_2CH_3$	10
4	$COCH(CH_3)_2$	5
5	$CO(CH_2)_2CH_3$	15
6	$CO(CH_2)_2COOCH_3$	5
7	$COCH_2CH(CH_3)_2$	
8	$COC(CH_3)_3$	10
9	$COCH(CH_2CH_3)_2$	10
10	$CO(CH_2)_2Ph$	5
11	$COCH(CH_3)CH_2Ph$	5
12	$CO(CH_2)_3Ph$	5
13	$COC(CH_3)_2CH_2Ph$	5
14[b]	$COCH(CH_3)CH_2Ph$	5
15[c]	$COCH(CH_3)CH_2Ph$	5
16	$CO(CH_2)_2COOH$	

[a] Expressed in mg-equiv of oxazepam. [b] Esterified with *d*-(+)-2-methyl-3-phenylpropionic acid. [c] Esterified with the *l*-(−) enantiomer of the acid.

0022-2623/79/1822-1443$01.00/0