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Acute Effects of Alkylating Agents on Canine Renal Function. 1. [4-(2-Bromoalkanoyl)phenoxy]acetic Acids

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A group of [4-(2-bromoalkanoyl)phenoxy]acetic acids was studied to determine if there was an association between the alkylating ability and the diuretic activity of its members. Acute studies in dogs revealed that there is not a consistent correlation in the alkylating potential of these α-bromo ketones and their ability to induce a diuretic response. In addition, pretreatment of dogs with the various α-bromo ketones did not alter the diuretic activity normally observed with ethacrynic acid (EA). The role of chemical-induced renal tissue alkylation in the initiation of a diuresis or a nephrotoxic response is discussed.

Ethacrynic acid (EA), a potent diuretic agent, is known to alkylate various nucleophiles in vitro by a Michael-type reaction, and there is no reason to doubt that a similar alkylation reaction might occur in vivo.[1-4] In fact, it has been suggested that the alkylation of as yet undetermined renal tissue components by EA is responsible for initiating a diuretic response.[1,3-6] However, recent information has emerged that challenges the necessity of EA-induced renal tissue alkylation for the expression of a diuretic response. The finding that certain EA-related compounds possess potent diuretic and uricosuric properties but lack the capability of alkylating nucleophiles has led Cragoe et al.[7] and Woltersdorf et al.[8] to conclude that EA and EA-related compounds probably induce a diuresis by interacting with renal tissue components by two mechanisms: (1) an alkylation reaction which contributes only to a minor extent to the induction of a diuresis and (2) a nonalkylating type of interaction which is of major importance in triggering a diuresis.

To examine further the relative importance of renal tissue alkylation by EA and EA-related compounds for the expression of a diuretic response, we designed, synthesized, and evaluated the renal effects of a group of EA-related α-bromo ketones (i.e., [4-(2-bromoalkanoyl)phenoxy]acetic acids) which not only vary greatly in their potential alkylating ability but, in addition, alkylate nucleophiles by an S_N2 reaction rather than by a Michael-type reaction. It has been firmly established that S_N2 reactions proceed much more readily with primary α-halo ketones than with secondary and tertiary α-halo ketones.[9] Thus, to attain derivatives with varying degrees of alkylating ability, we synthesized **2a-c** from the corresponding ketones. The diuretic activity of a group of chemically related α-halo ketones has been reported in dogs; however, no mention was made of the dose administered or the nature of the time-action curve.[10,11]

It might be anticipated that the in vitro, as well as the in vivo, alkylating ability of the three α-bromo ketones studied herein would follow the order **2c > 2b > 2a**. If renal tissue alkylation is a prerequisite for the expression of a diuretic response with EA and EA-related compounds, then the diuretic activity associated with **2a-c** should

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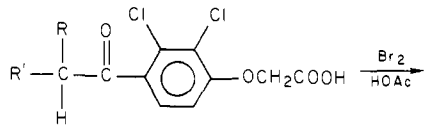
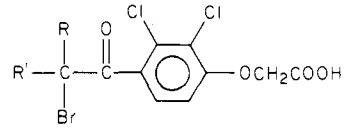


1a, R = R' = CH_3
b, R = H; R' = CH_3
c, R = R' = H



2a, R = R' = CH_3
b, R = H; R' = CH_3
c, R = R' = H

parallel their alkylating ability. However, one must consider that the most reactive alkylating agent is the least likely to reach the kidneys intact following the normal routes of administration. Thus, to circumvent this problem **2c**, the most reactive α-bromo ketone in the series, was injected intravenously and directly into the right renal artery.

Our results support the conclusions of Cragoe et al.[7] and Woltersdorf et al.[8] that the alkylation of renal tissue by EA-related compounds may be only a minor determinant of the observed diuresis.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and M-H-W Laboratories, Garden City, Mich. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.3% of the theoretical values. IR spectra were obtained on a Perkin-Elmer Model 257 grating spectrophotometer in KBr pellets. The NMR spectra were obtained with a Perkin-Elmer Model R-24 instrument using DMF-d_7 as the solvent and tetramethylsilane as the internal standard.

Synthesis of [2,3-Dichloro-4-(2-bromoisobutyryl)phenoxy]acetic Acid (2a). Step A. Synthesis of (2,3-Dichloro-4-isobutyrylphenoxy)acetic Acid (1a). **1a** was synthesized by procedures similar to those cited in ref 12, example 25, for the preparation of [2,3-dichloro-4-(2-ethylbutyryl)phenoxy]acetic acid, mp 139.5–142 °C.

Step B. Synthesis of 2a. **1a** (3.0 g, 0.01 mol) was dissolved in glacial HOAc (55 mL) at room temperature. Two drops of 48% aqueous HBr were added, and bromine (1.65 g, 0.01 mol) in glacial HOAc (12.5 mL) was added dropwise over 25 min with stirring at room temperature. The reaction mixture was stirred an additional 15 min, whereafter it was poured slowly and with vigorous stirring into distilled H_2O (275 mL) containing sodium bisulfite (0.55 g). The resulting crystalline material was removed by filtration, washed with two 250-mL portions of distilled H_2O, and recrystallized twice from hot C_6H_6, which yielded 2.69 g (69% yield) of **2a**: mp 155–156.5 °C. Anal. ($C_{12}H_{11}BrCl_2O_4$) C, H.

Synthesis of [2,3-Dichloro-4-(2-bromopropionyl)phenoxy]acetic Acid (2b). Step A. Synthesis of (2,3-Dichloro-4-propionylphenoxy)acetic Acid (1b). **1b** was synthesized by procedures similar to those cited in ref 12, example 25. Following repeated recrystallization from C_6H_6, **1b** was obtained, mp 128.5–132.5 °C.

Step B. Synthesis of 2b. **1b** (5.0 g, 0.018 mol) was converted to **2b** by a procedure similar to that used for the synthesis of **2a**, except bromine was added, with stirring, over a 1-h period. The resulting crystalline material was collected by filtration, washed with three 300-mL portions of distilled H_2O, and recrystallized three times from hot C_6H_6 to yield 3.72 g (58%) of **2b**, mp 147–150 °C. Anal. ($C_{11}H_9BrCl_2O_4$) C, H.

In Vitro Stability of 2b. Since it is conceivable that **2b** would yield an α,β-unsaturated phenoxyacetic acid derivative by the elimination of the elements of HBr, NMR studies were employed to ascertain its stability under conditions which were similar to those used to prepare the drug solution for intravenous injection. **2b** (50 mg) was dissolved in DMF-d_7 (0.3 mL), D_2O (0.05 mL), and $NaHCO_3$ (11.8 mg), along with three drops of tetramethylsilane. The vinyl proton region of the spectrum was examined after 15 and 30 min to determine if the elimination of HBr had occurred.

Table I. Composition of the Injection Solutions

compd	mode of adm	dose, μmol/kg	$NaHCO_3$	DMF, mL	dist H_2O or 0.9% saline
2a	iv	17.0	equiv amt	1.0	dist H_2O (2.0 mL)
2b	iv	17.0	equiv amt	1.0	dist H_2O (2.0 mL)
1b	iv	17.0	equiv amt	0.0	saline (10.0 mL)
2c	iv	17.0	equiv amt	1.0	saline (5.0 mL)
2c	renal art.	17.0	equiv amt	0.5	saline (5.0 mL)
EA	iv	3.3	equiv amt	0.0	saline (10.0 mL)

Synthesis of [2,3-Dichloro-4-(2-bromoacetyl)phenoxy]acetic Acid (2c). Step A. Synthesis of (2,3-Dichloro-4-acetylphenoxy)acetic Acid (1c). The synthesis of **1c** was conducted by previously reported procedures, mp 154.5–157 °C (lit.[13,14] 154–156 °C).

Step B. Synthesis of 2c. **1c** (1.5 g, 0.006 mol) was converted to **2c** by a procedure similar to that used for the synthesis of **2a**. The resulting white solid was collected by filtration, washed with distilled H_2O (400 mL), and allowed to air dry. The yield of **2c** was 1.44 g (74%), mp 137–141 °C. All attempts to purify **2c** further by recrystallization resulted in a compound or a mixture of compounds with a significantly broader melting range. Anal. ($C_{10}H_7BrCl_2O_4$) C, H, Br, Cl.

Pharmacological Studies. Twenty-three mongrel dogs of either sex, ranging in weight from 5.1 to 13.52 kg (average 8.59 kg) were used in this study. Details of the procedures employed to delineate the chemical-induced changes in renal function have been reported in detail in previous publications from our laboratory.[15,16] The clearance of inulin was used to evaluate the glomerular filtration rate (GFR) throughout all our experiments.[17]

In the case of **2c**, which was injected directly into the right renal artery, we inserted an L-shaped needle from a butterfly infusion set (Abbott no. 4492, 21 gauge) into the right renal artery following our regular surgical procedures. The needle was held in place by lodging its shank between the aorta and vena cava.

The poor water solubility of the three α-bromo ketones (i.e., **2a–c**) dictated the composition of the solution that was injected (see Table I for details). All drug solutions were administered over a 10-min period. The blood pressure was monitored throughout each experiment, and only **2b** produced a slight transient increase.

The values shown in Figure 1A–D represent the mean changes ($\Delta \pm$ SEM) in a given parameter induced by a given compound in each 10-min period following drug injection. The Δ for the excretion rate of sodium/kg or the GFR/kg was calculated by subtracting the value obtained during the 10-min period immediately preceding the injection of the drug from the corresponding value obtained for each 10-min period after injection of a compound. Significant changes (i.e., $p < 0.05$) in the renal excretion rate of sodium/kg or GFR/kg were arrived at by a paired comparison analysis (two tailed)[18] and are indicated by an asterisk in each of the figures. Chemical-induced changes in the urine flow rate/kg and the changes in the rate of excretion of chloride/kg and potassium/kg were also monitored. Although the latter changes are not shown herein, they always paralleled the changes in the rate of excretion of sodium/kg.

Results

Basically two types of experiments were conducted herein. The first type involved the intravenous administration of an α-bromo ketone followed 2 h later by the intravenous administration of EA. Five groups of dogs

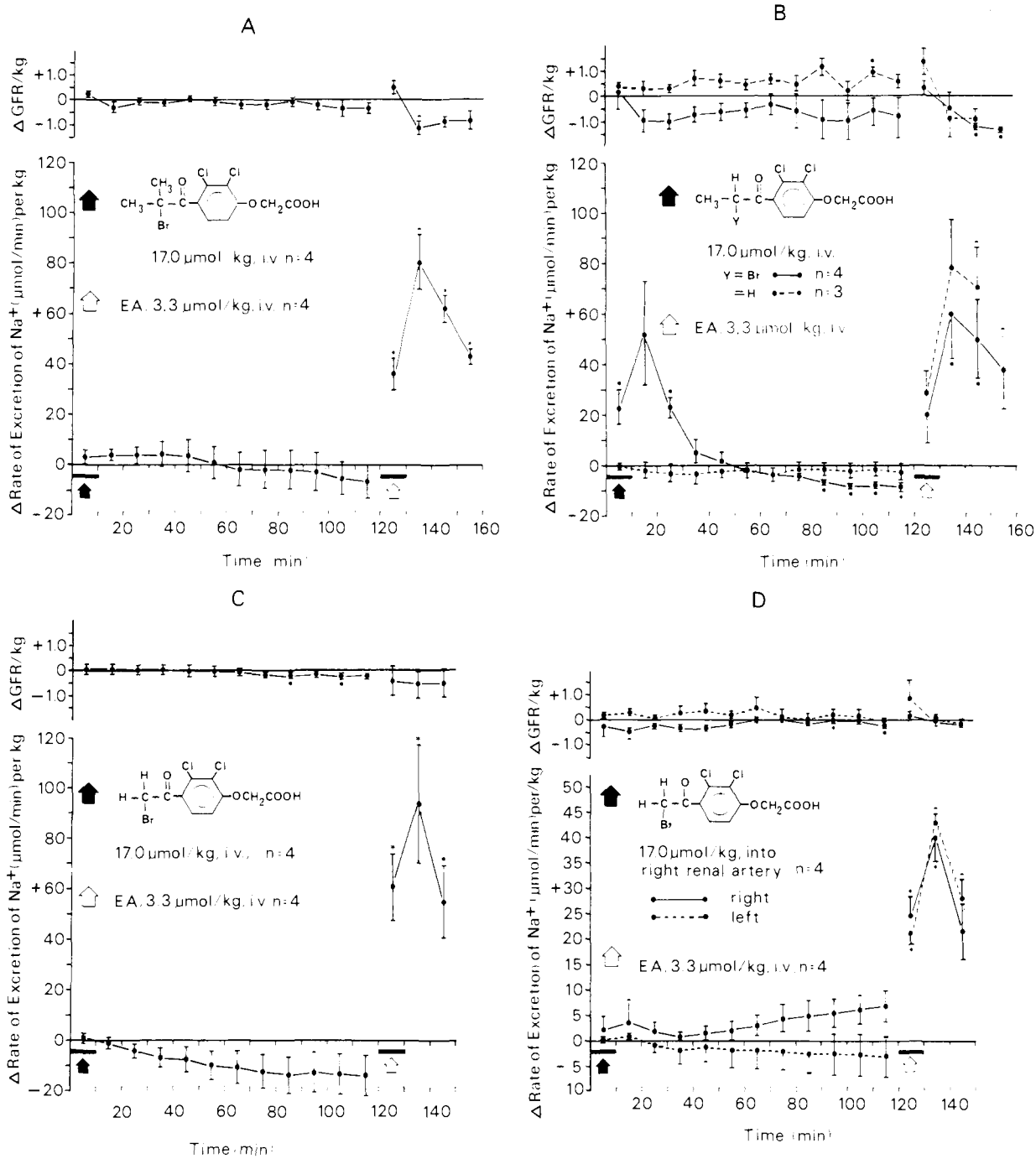


Figure 1. The effect(s) of the three α-bromo ketones and **1b** on renal function, and the effect of these compounds on the renal actions of EA. The bold lines on the abscissa indicate the 10-min period during which a compound was injected. An asterisk indicates that a given change was significant (i.e., $p < 0.05$).

were utilized (four of the groups consisted of four dogs each, and one of the groups had three dogs). The one-way analysis of variance was used to compare the various parameters of renal function in these five groups of dogs during the 10-min period immediately preceding the injection of the α-bromo ketones or **1b**. No differences were noted, so the pretreatment data were pooled and are presented in Table II. Similar analyses were performed to compare the various parameters of renal function in the same five groups of dogs during the 10-min period immediately preceding the injection of EA. Again no differences were noted, so the pretreatment data were pooled and are presented in the lower part of Table II. In the second type of experiment, **2c** was administered directly into the right renal artery in a single group of four dogs, and 2 h later EA was injected via the intravenous route. The pretreatment data (i.e., prior to injection of **2c** and prior to injection of EA) are cited in Table III.

Effect of 2a on Renal Function. The intravenous administration of **2a**, the least reactive of the three α-bromo ketones, yielded absolutely no change in any of the parameters of renal function that we monitored (i.e., GFR, urine flow rate, urinary pH, and urinary excretion rate of sodium, potassium, and chloride) (Figure 1A). Two hours after the administration of **2a**, EA was administered intravenously for several reasons: (1) to confirm that each dog was capable of responding to a known diuretic agent and (2) to determine if pretreatment with a potential alkylating agent (namely, an α-bromo ketone) would modify, in any way, the diuresis induced by EA, a diuretic

Table II. Pretreatment Data for Dogs Given an α-Bromo Ketone or 1b Intravenously Followed by the Intravenous Administration of EA (Mean ± SEM)

GFR/kg, mL min^{-1} kg^{-1}	rate of urine flow/kg, mL min^{-1} kg^{-1}	rate of excretion/kg, μmol min^{-1} kg^{-1}		
		Na^+	K^+	Cl^-
A. Values Obtained during the 10-min Period Immediately Prior to the Intravenous Injection of **2a**,**b**,**c**, and **1b**[a]				
4.89 ± 0.21	0.10 ± 0.01	20.96 ± 2.42	4.59 ± 0.38	19.09 ± 2.28
B. Values Obtained during the 10-min Period Immediately Prior to the Intravenous Injection of EA[a]				
4.77 ± 0.26	0.09 ± 0.01	15.81 ± 1.96	3.99 ± 0.29	14.44 ± 1.82

[a] $n = 19$.

Table III. Pretreatment Data for Dogs Given 2c Directly into the Right Renal Artery Followed 2 h Later by Intravenous Injection of EA (Mean ± SEM)

left kidney					right kidney				
GFR/kg, mL min^{-1} kg^{-1}	rate of urine flow/kg, mL min^{-1} kg^{-1}	rate of excretion/kg, μmol min^{-1} kg^{-1}			GFR/kg, mL min^{-1} kg^{-1}	rate of urine flow/kg, mL min^{-1} kg^{-1}	rate of excretion/kg, μmol min^{-1} kg^{-1}		
		Na^+	K^+	Cl^-			Na^+	K^+	Cl^-
A. Values Obtained during the 10-min Period Immediately Prior to the Injection of **2c** Directly into the Right Renal Artery ($n = 4$)									
2.19 ± 0.29	0.05 ± 0.02	8.39 ± 3.69	2.27 ± 0.50	8.14 ± 3.65	1.78 ± 0.23	0.04 ± 0.02	5.47 ± 2.14	1.84 ± 0.30	5.22 ± 1.88
B. Values Obtained during the 10-min Period Immediately Prior to the Intravenous Administration of EA ($n = 4$)									
2.08 ± 0.35	0.04 ± 0.02	5.86 ± 2.13	2.72 ± 0.71	4.69 ± 1.74	1.61 ± 0.33	0.11 ± 0.04	14.9 ± 5.8	3.07 ± 0.48	13.7 ± 6.5

that has been postulated to induce a diuresis by alkylation of critical renal receptors.[1,3-6] As seen in Figure 1A, a normal diuretic response followed the intravenous administration of EA.

Effect of 2b on Renal Function. Following the intravenous administration of **2b** there was an immediate modest increase in the renal excretion rate of sodium/kg (Figure 1B). There was a parallel increase in the urine flow rate/kg and the renal excretion rate of chloride/kg (not shown). During the 2-h period following the administration of **2b**, the GFR/kg remained unchanged. In addition, a normal diuretic response followed the intravenous administration of EA 2 h after the administration of **2b**. To determine if the observed diuretic activity associated with the administration of **2b** was due to the presence of the α-bromo ketonic group, we conducted two types of studies. First, the possibility existed that **2b** might be converted in vivo to the corresponding α,β-unsaturated ketone by the elimination of the elements of HBr. Thus, we determined its in vitro stability using NMR techniques (see Experimental Section). During the period of study, there was no change in the NMR spectrum of **2b**. Second, we examined the effect of **1b**, the corresponding nonbrominated ketonic derivative, on canine renal function. Figure 1B clearly shows that the α-bromo ketonic group of **2b** is essential for eliciting a diuresis, since **1b** fails to alter renal function in any manner, and a normal EA response is obtained 2 h later.

Effect of 2c on Renal Function. Following the intravenous administration of **2c**, the most reactive alkylating agent of the three α-bromo ketones discussed herein, there was no significant alteration in any of the renal function parameters under study. Furthermore, the intravenous administration of EA 2 h after **2c** yielded a completely normal diuretic response (Figure 1C). Being that **2c** is potentially the most reactive compound in this study, the possibility existed that it did not reach the kidney intact. Therefore, we injected **2c** directly into the right renal artery in the same dose that was given via the intravenous route (i.e., 17.0 μmol/kg). As shown in Figure 1D, **2c** did not significantly alter the GFR/kg or the renal handling of sodium. When EA was administered intravenously 2 h later, both kidneys responded identically and maximally.

Discussion

Two important findings emerge from this study. First, there is not a consistent correlation in the alkylating ability of the three α-bromo ketones and their ability to induce a diuretic response. **2b** was the only derivative that induced a diuretic response. It is possible, although remotely so, that **2b** possesses diuretic properties as a result of its conversion to the corresponding α,β-unsaturated ketone through the elimination of the elements of HBr. Several findings suggest that little or no conversion occurs: (1) we found **2b** to be stable under specified in vitro conditions and (2) if the elimination of HBr from the α-bromo ketones would be a prerequisite for the expression of a diuresis then we should have observed a diuresis following the administration of **2a**, since the unsaturated ketone which would arise from the elimination of HBr from **2a** is an established diuretic agent.[16]

Second, when the α-bromo ketones were administered to dogs 2 h before EA, none of them altered the diuretic response of EA. Even when **2c** was injected directly into the right renal artery and EA was administered intravenously 2 h later, both kidneys responded in an identical manner. It is possible that the α-bromo ketones alkylate different renal receptors than EA, but their structural

similarity make this unlikely.

These findings support the conclusions of Cragoe et al.[7] and Woltersdorf et al.[8] that alkylation of renal tissue may not be a major mechanism by which EA and EA-related compounds induce a diuretic response. If renal tissue alkylation is not an essential event in the observed diuresis induced by EA and EA-related compounds, then it seems quite logical to disregard functional groups with alkylating properties when designing new diuretic agents. The main reason for this consideration is that most alkylating agents react with the array of nucleophiles in vivo in a very indiscriminate manner, and the result is usually a marked degree of toxicity. Recent work in our laboratory supports this contention in that certain specifically designed alkylating agents possess tremendous nephrotoxic potential (to be published).

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Synthesis and Analgesic Activity of Some Spiro[dibenz[*b,f*]oxepin-10,4′-piperidine] Derivatives

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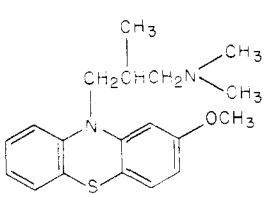
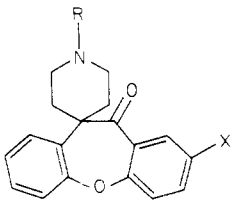
A series of 10,11-dihydro-11-oxospiro[dibenz[*b,f*]oxepin-10,4′-piperidine] derivatives (II) was synthesized and evaluated for analgesic activity in the phenylquinone writhing assay (PQW) and the tail-flick test in mice. Preliminary structure–activity correlations indicate that optimum activity is associated with a short-chain ($R \leq C_2$) N substituent and a nuclear fluorine function, as exemplified by **9b**. This compound, when administered orally, was equipotent to morphine in protecting against mouse writhing. The observation that the PQW activity of **9b** remained relatively unchanged after naloxone challenge seems to favor a nonnarcotic profile.

Although significant analgesic activity has been reported for a number of psychotropic agents with linear, tricyclic structures,[1-3] compounds of this type, in general, have rarely been investigated clinically as analgetics because of their diverse pharmacological actions. One notable exception to this is methotrimeprazine (I), a phenothiazine-like neuroleptic which is also active as a nonnarcotic analgesic agent.[4,5] When administered subcutaneously in man, methotrimeprazine is almost equipotent to morphine in controlling postoperative pain; extensive clinical use for this drug, however, has so far been precluded by its lack of oral efficacy and a high incidence of untoward side effects associated with oral use. As part of a program designed to search for new classes of analgesics based on the tricyclics, we have synthesized and evaluated pharmacologically a series of oxospiro[dibenz[*b,f*]oxepin-10,4′-piperidine] derivatives (II), and results from the preliminary studies are reported in this paper. The synthesis of these semirigid tetracycles appeared attractive to us because it is frequently suggested that the lack of specificity in biological activity can be linked to a high degree of conformational flexibility of the drug molecule;[6] thus, by locking the aminoalkyl side chain into a well-



I



II, X = H, F, or Cl; R = H, alkyl, cycloalkyl, or aralkyl

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