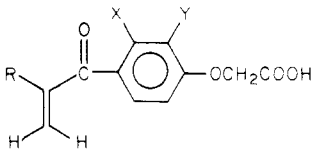
Diuretic Activity of Mannich Base Derivatives of Ethacrynic Acid and Certain Ethacrynic Acid Analogues

Daniel A. Koechel* and Gary O. Rankin

Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699. *Received January 9, 1978*

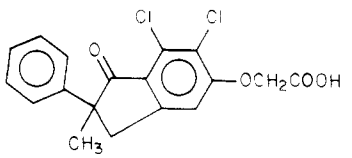
Various Mannich base derivatives of selected phenoxyacetic acid type diuretics were synthesized and their diuretic potency was evaluated in dogs. It is concluded that the Mannich bases possess little, if any, diuretic activity of their own. Those Mannich bases that do possess diuretic activity undoubtedly do so as a consequence of an elimination reaction (a retro-Michael type reaction) which yields the corresponding pharmacologically active α,β-unsaturated ketone.

In an attempt to obtain a nonmercurial diuretic which would be capable of reacting with renal protein-bound sulfhydryl groups, Schultz et al.[1] synthesized a group of phenoxyacetic acid derivatives which possess an α,β-unsaturated ketone group. Ethacrynic acid (EA, **1a**) was one



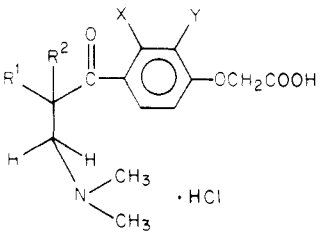
1a, X = Y = Cl; R = Et
b, X = Y = H; R = Et
c, X = Y = Cl; R = CH_3

of the most efficacious analogues in the class.[2,3] Since its introduction, many structure–activity relationship studies have tended to support the idea that the α,β-unsaturated ketone group is essential for diuretic activity.[1,4-7] Recently, however, a group of closely related indanyloxyacetic acids was synthesized which lack an α,β-unsaturated ketone group and yet possess dramatic diuretic and uricosuric properties.[8] Of these compounds the pharmacological aspects of MK-196 (**2**) have been pursued with the greatest vigor.[9-14]



2

In addition, two types of EA derivatives have been synthesized which lack an α,β-unsaturated ketone group and yet possess varying degrees of diuretic activity. First, it was reported that certain thiol (sulfhydryl) adducts (**3**) of EA have diuretic properties.[15] In subsequent work, Koechel and Cafruny found that the rate at which **3** release EA correlates very well with the onset, magnitude, and duration of the observed diuretic response.[16,17] The mechanism proposed for the release of EA from **3** most likely involves a base-catalyzed retro-Michael type reaction as shown in Scheme I. Little, if any, data were obtained to support the notion that the thiol adducts themselves (**3**) have diuretic activity. Second, it has been reported that the hydrochloride salts of Mannich base derivatives of EA and EA analogues (**4**) [also referred to as amine adducts or 4-(β-aminoacyl)phenoxyacetic acids] possess diuretic, natriuretic, and chloruretic properties.[18-20] In all cases reported to date R^2 in **4** is a hydrogen atom. It is well documented that such Mannich bases (i.e., where R^2 in **4** is H) are like the thiol adducts of EA in that they may be considered as prodrugs for the corresponding α,β-unsaturated ketones. In fact, in the commercial synthesis of EA and many of its α,β-unsaturated ketonic analogues, the Mannich bases are the immediate synthetic precursors.



4a, R^1 = Et; R^2 = H; X = Y = Cl
b, R^1 = R^2 = CH_3; X = Y = Cl
c, R^1 = Et; R^2 = H; X = Y = H
d, R^1 = Et; R^2 = CH_3; X = Y = Cl

Thus, from a drug development standpoint, as well as from a drug–receptor interaction standpoint, it is important to determine if the diuretic effect observed following the administration of the Mannich bases is due to the Mannich bases themselves or to the release of the corresponding α,β-unsaturated EA analogue by a base-catalyzed retro-Michael type reaction similar to that previously proposed for the thiol adducts of EA.[16,17] Scheme II depicts the generation of EA or an α,β-unsaturated EA analogue from the corresponding Mannich base via a base-catalyzed retro-Michael type reaction.

Our objective was to determine which of the drug species in Scheme II is responsible for triggering a diuretic response in dogs. To achieve this goal the synthesis and pharmacological evaluation of three types of Mannich bases were conducted: first, a Mannich base derivative (**4a**) of EA which is known to yield EA under mild conditions (that is, when **4a** is treated with $NaHCO_3$ and warmed gently, it releases EA; these conditions are employed in the commercial synthesis of EA[19]); second, a Mannich base derivative (**4b**) which, unlike **4a** above, cannot liberate an α,β-unsaturated EA analogue by virtue of R^2 in **4** being changed from a hydrogen to a methyl group (**4b** is therefore unable to participate in a base-catalyzed retro-Michael type reaction); third, a Mannich base derivative (**4c**) of a marginally active α,β-unsaturated EA analogue (**1b**).[4] In the latter case, we reasoned that if the Mannich bases are active diuretic agents it might be possible to activate **1b** by forming the corresponding Mannich base **4c**.

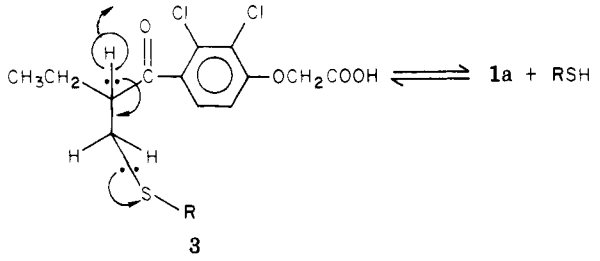
The results obtained in this study support the concept that little, if any, diuretic activity is associated with the intact Mannich base derivatives. The only compounds shown to possess diuretic activity were EA and its corresponding Mannich base derivative **4a**. We demonstrated that **4a** releases EA in vitro and in vivo.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were

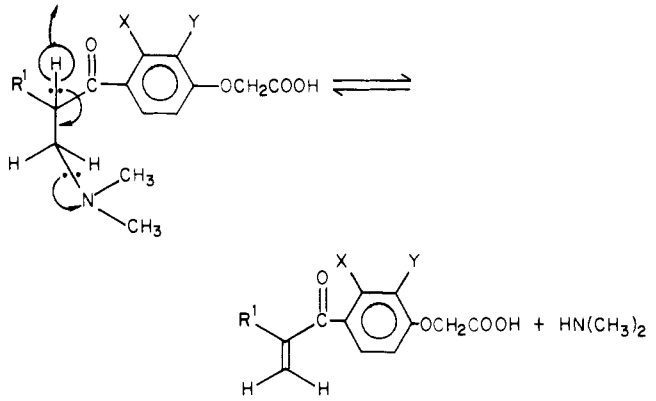
0022-2623/78/1821-0764$01.00/0

Scheme I



3

Scheme II



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 either tetramethylsilane or 3-(trimethylsilyl)propanesulfonic acid sodium salt as internal standard.

Synthesis of [2,3-Dichloro-4-[2-(dimethylaminomethyl)butyryl]phenoxy]acetic Acid Hydrochloride (4a). The procedure cited in ref 21 (example 22) resulted in a 36% yield of **4a** after three recrystallizations from MeOH–Et_2O: mp 161.5–163 °C (lit.[21] 165–167 °C).

Synthesis of [2,3-Dichloro-4-(2-methylenepropionyl)phenoxy]acetic Acid (1c). Step A. (2,3-Dichloro-4-propionylphenoxy)acetic Acid. The synthesis of (2,3-dichloro-4-propionylphenoxy)acetic acid was similar to that cited in ref 21 (example 25) for the synthesis of [2,3-dichloro-4-(2-ethylbutyryl)phenoxy]acetic acid.

Step B. [2,3-Dichloro-4-(2-methylenepropionyl)phenoxy]acetic Acid (1c). Conversion of (2,3-dichloro-4-propionylphenoxy)acetic acid to **1c** was conducted according to the procedure cited in ref 19 (example 3) for the conversion of (4-propionyl-3-chlorophenoxy)acetic acid to (4-methacryloyl-3-chlorophenoxy)acetic acid. Analytically pure **1c** was obtained following column chromatography [silica gel, C_6H_6–dioxane–HOAc (50:10:1)] and recrystallization from C_6H_6–C_6H_{12}: mp 133–135 °C. Anal. ($C_{12}H_{10}Cl_2O_4$) C, H, Cl.

Synthesis of [2,3-Dichloro-4-[2-methyl-2-(dimethylaminomethyl)propionyl]phenoxy]acetic Acid Hydrochloride (4b). Step A. (2,3-Dichloro-4-isobutyrylphenoxy)acetic Acid. The synthetic procedures employed were similar to those stated in ref 21 (example 25) for the preparation of [2,3-dichloro-4-(2-ethylbutyryl)phenoxy]acetic acid: mp 139.5–142 °C.

Step B. [2,3-Dichloro-4-[2-methyl-2-(dimethylaminomethyl)propionyl]phenoxy]acetic Acid (4b). (2,3-Dichloro-4-isobutyrylphenoxy)acetic acid (5.0 g, 0.017 mol) was added to a 100-mL single-necked flask which contained dry dimethylamine hydrochloride (1.54 g, 0.019 mol) and paraformaldehyde (0.59 g, 0.020 mol). Glacial HOAc (4 drops) was added and the flask was equipped with an adapter for drawing intermittent suction (i.e., every 15 min for 1 min). The contents of the flask were then heated on a steam bath for 4 h. During this period the reaction mixture formed a clear melt which then solidified. The off-white solid was pulverized and triturated with four 25-mL portions of Et_2O. The remaining white solid was recrystallized four times from hot MeOH resulting in 1.48 g (22.3% yield) of **4b**, mp 207–210 °C dec. Anal. ($C_{15}H_{20}Cl_3NO_4$) C, H, Cl, N.

Synthesis of [4-[2-(Dimethylaminomethyl)butyryl]phenoxy]acetic Acid Hydrochloride (4c) and [4-(2-Methylenebutyryl)phenoxy]acetic Acid (1b). Step A. (4-Butyrylphenoxy)acetic Acid. The synthetic procedure used was similar to that cited in ref 19 (example 8) with some modifications which resulted in an increased yield of the product. Briefly, to a 1000-mL three-necked round-bottomed flask equipped with a mechanical stirrer, reflux condenser with a drying tube, and a stopper was added phenoxyacetic acid (22.82 g, 0.15 mol). Anhydrous CS_2 (360 mL) and butyryl chloride (20 mL) were added with stirring. The reaction vessel was cooled with an external ice bath, and anhydrous $AlCl_3$ (chunks) (65.0 g, 0.49 mol) was added over 1 h. The ice bath was removed and the mixture was allowed to stir at room temperature for 4.5 h. Thereafter, stirring was stopped and the contents of the flask were allowed to stand for 17 h at room temperature. The mixture was heated at 50 °C for 3 h without stirring and cooled to room temperature and the CS_2 decanted. Ice (500 g) and concentrated HCl (50 mL) were added over a 15-min period with stirring, whereupon a fluffy white solid separated. The acidic aqueous reaction mixture was extracted with Et_2O (750 mL) and then with EtOAc (750 mL). The organic extracts were combined, washed with two 250-mL portions of distilled H_2O, and dried (Na_2SO_4). The solvent was removed in vacuo and the remaining solid was recrystallized from hot EtOAc to yield 29.2 g (87.6% yield) of (4-butyrylphenoxy)acetic acid, mp 135.8–137.5 °C (lit.[19] 137–139 °C).

Step B. Preparation of 4c. The procedure used was identical with that cited in ref 19 (example 8), which resulted in a 93% yield of **4c**. Three recrystallizations from *i*-PrOH–MeCN resulted in pure **4c**, mp 158.5–161.5 °C (lit.[19] 160–161 °C).

Step C. Preparation of 1b. The reaction procedure was identical with that described in ref 19 (example 8). The product of the reaction was recrystallized twice from C_6H_6–C_6H_{12} (1:1). The resulting crystalline material was further purified on a silica gel column using C_6H_6–dioxane–HOAc (50:10:1) with subsequent recrystallization (three times) from C_6H_6–C_6H_{12}. Following these procedures 1.91 g (20.4% yield) of **1b** was obtained, mp 107.5–110.5 °C (lit.[19] 110–111 °C).

In Vitro Stability of 4a–c. Samples (50 mg) of **4a–c** were dissolved in dog urine (pH 7.0 ± 0.5) or D_2O. NMR spectra were recorded throughout the following 1-h period with emphasis on the appearance of vinyl protons which would be indicative of breakdown to the corresponding α,β-unsaturated ketone. At the end of 1 h the NMR solutions were spotted on silica gel F_{254} precoated plates and developed in C_6H_6–MeOH–HOAc (45:8:4). In addition, in the case of **4c**, the NMR spectrum and TLC work were repeated after 72 h. Under these conditions **4b** and **4c** were found to be very stable compounds (i.e., we were unable to detect the presence of any substance other than **4b** or **4c**). On the other hand, **4a** consistently yielded EA.

Pharmacological Studies. Twenty-three mongrel dogs of either sex, ranging in weight from 7.88 to 19.77 kg were used in this study. Details of the procedures employed to delineate the diuretic activity of the compounds cited herein have been reported in a previous publication.[17]

1a–c and **4a–c** (17.0 μmol/kg iv) were dissolved in 10 mL of 0.9% saline 3–5 min prior to their iv administration and were administered over a period of 10 min. The Mannich bases **4a–c** (as hydrochlorides) were all soluble in 0.9% saline, whereas it was necessary to convert **1a–c** to the corresponding sodium salts with an equivalent amount of $NaHCO_3$. It is essential to use freshly prepared drug solutions in a study such as this in order to circumvent any erroneous results that may occur due to the chemical instability of certain compounds. All compounds cited in this study were without effects on the blood pressure except **1c** which consistently produced a slight elevation (10 mmHg in both diastolic and systolic pressure).

Sodium ethacrynate (3.3 μmol/kg iv) was administered 2 h after an inactive compound to demonstrate that the dog was capable of responding to a known diuretic agent. Saline (0.9%, 10 mL) was added to the sodium ethacrynate 5 min prior to its injection, and the resulting solution was administered over a 10-min period.

The concentration of inulin in plasma and urine was determined by the method of Schreiner.[22] Sodium and potassium concen-

Table I. Pretreatment Values[a]

GFR/kg, mL/min/kg	rate of urine flow/kg, mL/min/kg	rate of excretion/kg, μmol/min/kg		
		sodium	potassium	chloride
4.20 ± 0.12	0.16 ± 0.01	26.24 ± 1.76	5.29 ± 0.30	26.28 ± 1.92

[a] Values cited represent the common mean and standard error (n = 23) for the various parameters during the 10-min period immediately preceding the injection of the drug.

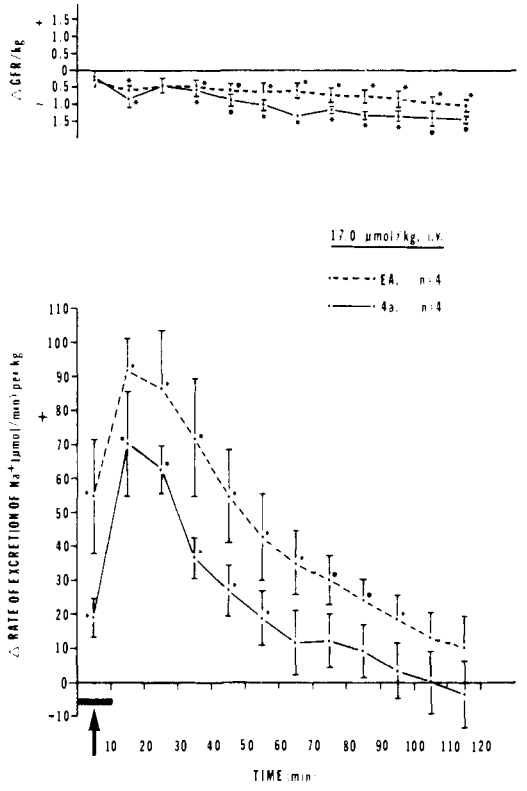


Figure 1. A comparison of the change (Δ) in the rate of excretion of sodium/kg and a comparison of the change (Δ) in the GFR/kg following single intravenous doses (17.0 μmol/kg) of EA and **4a**. The asterisk denotes a significant change (i.e., $p < 0.05$). The bold line on the abscissa indicates the period of drug administration on this and subsequent figures.

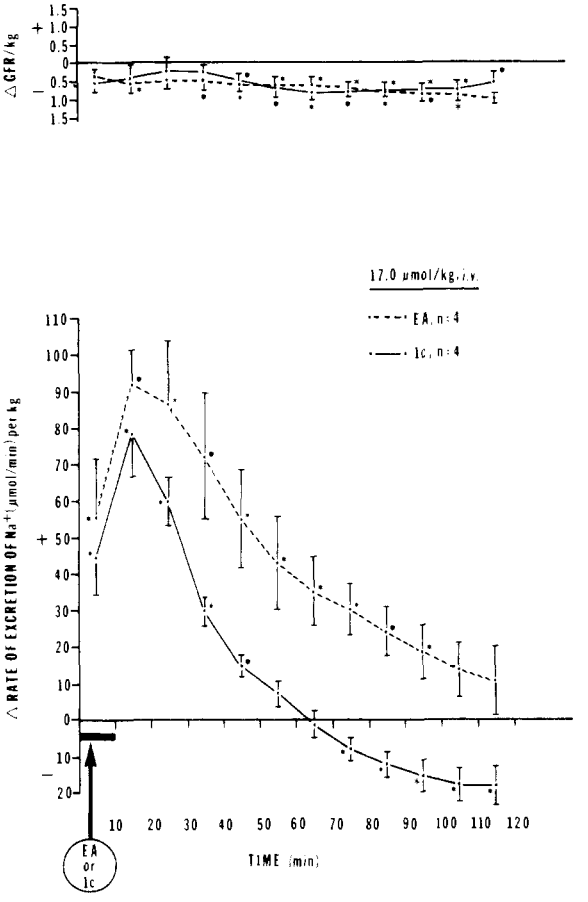


Figure 2. The change (Δ) in the rate of excretion of sodium/kg and the change (Δ) in the GFR/kg following single intravenous doses of **1c**. The changes induced by an equivalent dose of EA are included for comparison.

trations in plasma and urine were determined by flame photometry (Instrumentation Laboratories internal standard photometer, Model 343). Chloride concentration in plasma and urine was assayed with a Corning Model 920M chloridometer.

The values shown in Figures 1–5 represent the mean changes (Δ ± SEM) in a given parameter induced by a given compound. The Δ for the excretion rate of sodium/kg or the GFR/kg was calculated by subtracting the value obtained during the period immediately preceding the injection of the drug from the corresponding value obtained for each 10-min period after injection of the drug. 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[23] and are indicated by an asterisk in each of the five figures. 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 not shown herein, these latter changes always paralleled the changes in the rate of excretion of sodium/kg.

In the case of **4a** and **4b** the urine was collected for 2 h following their administration. The urine obtained during the first hour was immediately placed on a freeze-drier as was that which was obtained from the second hour. Urine obtained from the nine 10-min periods preceding drug administration (referred to as control urine) was handled in a similar manner. The freeze-drying procedure usually took about 48 h. Following the freeze-drying process the remaining solid material from control urine, urine from the first hour following drug treatment, and urine from the second hour following drug treatment were dissolved in a minimum amount of H_2O and applied to TLC plates (Avicel F, precoated plates, 20 × 20 cm, 250 μ, Analtech, Inc.), developed in n-BuOH–C_6H_6–MeOH–H_2O (20:10:12.5:10), and the location of the components was conducted using UV light and ninhydrin spray. In each case **4a** or **4b** was spotted as a reference substance along with EA and several of the known biotransformation products of EA [i.e., the cysteine adduct of EA (EA-Cyst) and the glutathione adduct of EA (EA-G)].[24] EA-Cyst and EA-G were prepared in our laboratory by reacting equimolar quantities of cysteine or glutathione with EA under an atmosphere of nitrogen.

Results

Six groups of dogs were used in this study. Five of the groups contained four dogs each while one of the groups consisted of three dogs. The one-way analysis of variance was used to compare the various parameters of renal function in these six groups of dogs during the 10-min period immediately preceding the injection of any of the compounds cited herein. No differences were noted so the pretreatment data were pooled and are expressed in Table I.

In Vitro Stability and Diuretic Activity of [2,3-Dichloro-4-[2-(dimethylaminomethyl)butyryl]phenoxy]acetic Acid Hydrochloride (4a). In vitro work which involved NMR and TLC studies demonstrated that

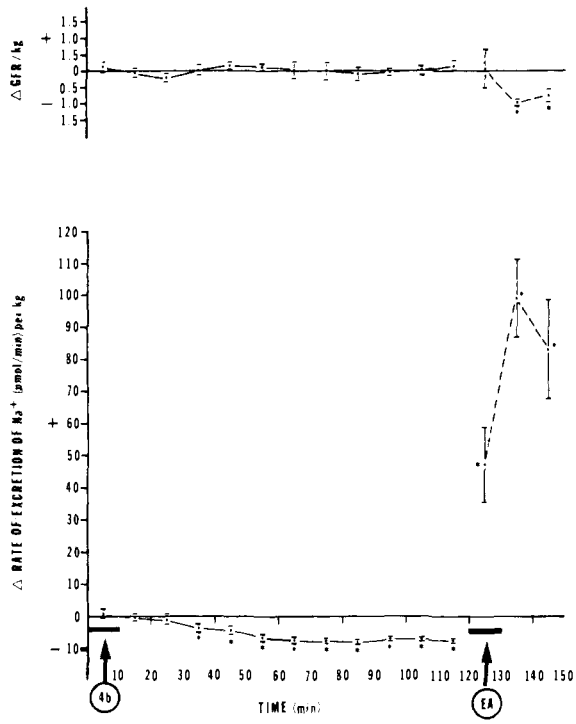


Figure 3. The change (Δ) in the rate of excretion of sodium/kg and the change (Δ) in the GFR/kg induced by single intravenous doses of **4b** (17.0 μmol/kg) and the subsequent administration of single intravenous doses of sodium ethacrynate (3.3 μmol/kg), $n = 3$.

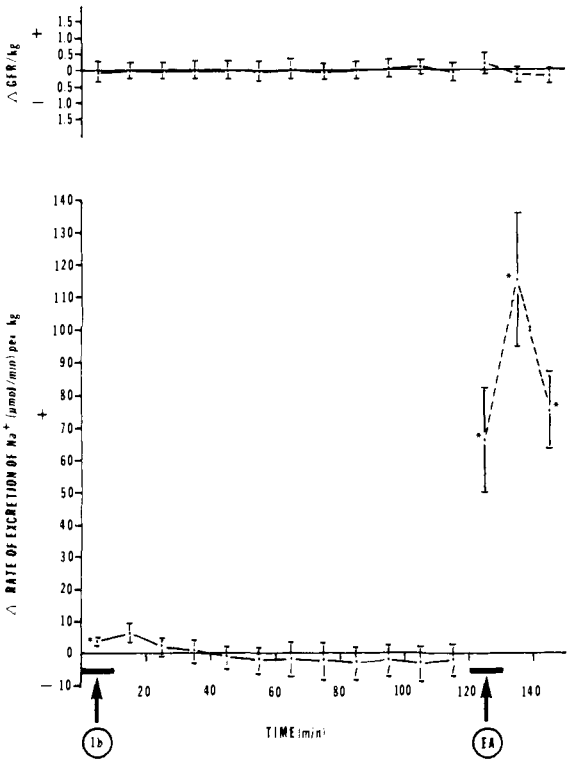


Figure 4. The change (Δ) in the rate of excretion of sodium/kg and the change (Δ) in the GFR/kg induced by single intravenous doses of **1b** (17.0 μmol/kg) and the subsequent administration of single intravenous doses of sodium ethacrynate (3.3 μmol/kg), $n = 4$.

4a is indeed capable of releasing EA. The NMR studies revealed that an equilibrium is established between **4a** and EA and dimethylamine which lies almost totally in favor of **4a**. If, after 15–60 min, the NMR solution of **4a** is

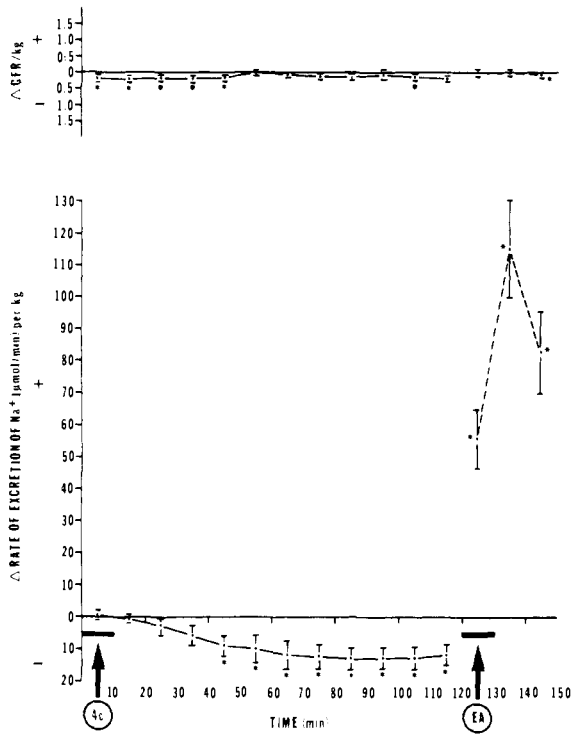


Figure 5. The change (Δ) in the rate of excretion of sodium/kg and the change (Δ) in the GFR/kg induced by single intravenous doses of **4c** (17.0 μmol/kg) and the subsequent administration of single intravenous doses of sodium ethacrynate (3.3 μmol/kg), $n = 4$.

applied to silica gel F_{254} TLC plates and developed in benzene–methanol–acetic acid (45:8:4), EA is always readily detectable. The possibility does exist that the silica gel or the developing solvent system catalyzed the release of EA from **4a**; however, the important point to be made is that **4a** is a potential source for EA under relatively mild conditions regardless of where the breakdown occurred.

When **4a** was administered to dogs an immediate diuresis ensued (Figure 1) which paralleled that produced by an equivalent dose of EA in onset, magnitude, and duration of effect. Both EA and **4a** produced a slight but significant decrease in the GFR/kg during the 2-h period following their administration (Figure 1). While **4a** was being administered, and for the following 2-h period, the urine from each of the four dogs in this group that was not utilized for inulin, sodium, potassium, or chloride analysis was pooled and freeze-dried, and the residue was examined by TLC for the parent Mannich base (**4a**) and any products resulting from in vivo breakdown or biotransformation of **4a**. EA and two of the known biotransformation products of EA (i.e., EA-Cyst and EA-G)[24] were detected. It could be argued that the EA resulted from the decomposition of **4a** following the collection of urine, during the freeze-drying process, or during the TLC; however, EA-Cyst and EA-G could only arise from the in vivo generation of EA from **4a** followed by the biotransformation of EA to EA-Cyst and EA-G.

In Vitro Stability and Diuretic Activity of [2,3-Dichloro-4-[2-methyl-2-(dimethylaminomethyl)-propionyl]phenoxy]acetic Acid Hydrochloride (4b). We synthesized and evaluated **4b** instead of **4d** as a representative potentially stable Mannich base in order to circumvent any problems that might arise from the presence of an asymmetric center. However, prior to examining **4b** it was imperative to demonstrate that **4b** would not be inactive (compared to **4d**) simply because of the presence of a methyl group rather than an ethyl group.

We therefore compared the diuretic activity of the corresponding α,β-unsaturated ketones (**1a** and **1c**) which differ from one another only in their methyl/ethyl relationship. Since both **1a** and **1c** possess significant diuretic activity (Figure 2), it seems very unlikely that **4b** would be inactive because of the presence of a methyl rather than an ethyl group.

The NMR studies coupled with the TLC work indicated that **4b** does not break down in vitro. When **4b** was administered to a group of three dogs, there was a slight but significant decrease in the rate of excretion of sodium/kg during the subsequent 2-h period (Figure 3). During this time interval there was no significant change in the GFR/kg. After monitoring the various renal parameters for 2 h, EA was administered. This was done for two reasons. First, to establish that our animals were responsive to a known diuretic agent which was somewhat structurally related to the Mannich base and, second, to determine if **4b** was capable of antagonizing the renal effect(s) of EA. The results clearly show that the dogs were responsive to EA (Figure 3). The onset and magnitude of the EA-induced diuresis are similar to that produced if EA is administered to dogs, at this point in the experiment, which have received no prior drug treatment.[17]

The urine of dogs which received **4b** was freeze-dried and examined by TLC as described in the Experimental Section. As expected, there were no α,β-unsaturated ketonic material or cysteine or glutathione conjugates present in the urine from this group of dogs. This finding supports what was found when the stability of **4b** was examined in vitro.

In Vitro Stability and Diuretic Activity of [4-(2-Methylenebutyryl)phenoxy]acetic Acid (1b) and [4-[2-(Dimethylaminomethyl)butyryl]phenoxy]acetic Acid Hydrochloride (4c). NMR and TLC studies revealed that **4c** is very stable in vitro. No **1b** was detected under our conditions. Figures 4 and 5 clearly show that neither **1b** nor **4c** possesses diuretic activity, markedly alters GFR/kg, or antagonizes the renal action(s) of EA.

Discussion

Three different approaches were used to determine if the diuretic activity observed following the administration of certain Mannich base derivatives is the result of the intact Mannich base or the result of the liberation of the amine and corresponding α,β-unsaturated ketone from such adducts.

First, a Mannich base was examined for diuretic activity which is theoretically capable of releasing EA. **4a** was shown to be capable of releasing EA in vitro as evidenced by the detection of EA in aqueous solutions of **4a**. This was most striking in the TLC studies. In addition, following the administration of **4a** to dogs, a brisk diuresis was observed which paralleled that produced by an equivalent dose of EA in onset, magnitude, and duration. Also, EA, EA-Cyst, and EA-G were all detected in urine collected from the four dogs that received **4a**. Since the preparation of the drug solution in this case was commenced only 3 min prior to drug administration, very little, if any, EA would have been released before **4a** reached the blood stream. This conclusion is based primarily on our NMR studies. The detection of EA-Cyst and EA-G demonstrated that EA was released from **4a** in vivo, and the EA was then subsequently biotransformed as outlined by Klaassen and Fitzgerald.[24] These findings clearly indicate that diuretic activity is associated with the administration of an unstable Mannich base derivative of EA which is capable of yielding EA; however, they do not permit a conclusion to be made as to which drug species actually triggers the diuresis. Therefore, the following two additional approaches were pursued.

The second approach which was employed to delineate which drug species in Scheme II is responsible for the diuresis observed following the administration of the Mannich bases was to synthesize and evaluate the diuretic activity of a Mannich base which could not release an α,β-unsaturated ketone. Since the removal of the hydrogen atom adjacent to the ketone carbonyl group in the thiol or amine adducts (as shown in Schemes I and II) is a prerequisite for the liberation of EA or an α,β-unsaturated analogue of EA, we reasoned that stable Mannich bases could be prepared if the hydrogen atom adjacent to the ketone group was replaced by a methyl group. It was on this basis that **4b** was designed. **4b** proved to be stable in vitro, as well as in vivo, and thus permitted us to determine if the intact Mannich base possesses diuretic activity of its own. When **4b** was administered to dogs, it proved to be completely devoid of diuretic activity. That **4b** is apparently stable in vivo is supported by the TLC studies of urine from dogs which recieved the drug. In these studies, only **4b** was detected. In addition, it is worthy of note that **4b** did not modify the diuretic response induced by EA when the latter was administered 2 h following the administration of **4b** (Figure 3).

Third, we felt that if the Mannich base derivatives possess diuretic properties of their own it might be possible to take a marginally active[4] EA analogue, such as **1b**, and activate it by forming the corresponding Mannich base derivative **4c**. Figure 4 shows that **1b** itself does not significantly alter the GFR/kg, the excretion rate of sodium/kg, or the response to subsequently administered EA. When **4c** was examined, it too was found to be devoid of diuretic properties (Figure 5).

We were unable to acquire any support for the concept that the Mannich bases themselves possess diuretic activity. On the other hand, evidence was generated which supports the notion that the diuretic activity is associated only with those Mannich bases (like **4a**) which can liberate a pharmacologically active α,β-unsaturated ketone. Thus, it appears that EA (or an active α,β-unsaturated EA analogue) must be released from the corresponding thiol or amine adduct in order to witness a diuretic response.

Acknowledgment. The authors are indebted to Dr. E. J. Cragoe of Merck Sharp & Dohme Research Laboratories, West Point, Pa., for a generous supply of ethacrynic acid, to Sandra A. Smith and Julie Aufderheide for their excellent technical assistance, and to the Pharmaceutical Manufacturers Association Foundation, Inc., Washington, D.C. 20005, for a Research Starter Grant.

References and Notes

(1) E. M. Schultz, E. J. Cragoe, Jr., J. B. Bicking, W. A. Bolhofer, and J. M. Sprague, *J. Med. Pharm. Chem.*, **5**, 660–662 (1962).
(2) J. E. Baer, J. K. Michaelson, D. N. McKinstry, and K. H. Beyer, *Proc. Soc. Exp. Biol. Med.*, **115**, 87–90 (1964).
(3) K. H. Beyer, J. E. Baer, J. K. Michaelson, and H. F. Russo, *J. Pharmacol. Exp. Ther.*, **147**, 1–22 (1965).
(4) D. E. Duggan and R. M. Noll, *Arch. Biochem. Biophys.*, **109**, 388–396 (1965).
(5) J. M. Sprague, *Top. Med. Chem.* **2**, 1–63 (1968).
(6) D. A. Koechel, O. Gisvold, and E. J. Cafruny, *J. Med. Chem.*, **14**, 628–629 (1971).
(7) J. B. Bicking, W. J. Holtz, L. S. Watson, and E. J. Cragoe, Jr., *J. Med. Chem.*, **19**, 530–535 (1976).
(8) E. J. Cragoe, E. M. Schultz, J. D. Schneeberg, G. E. Stokker, O. W. Woltersdorf, G. M. Fanelli, and L. S. Watson, *J. Med. Chem.*, **18**, 225–228 (1975).
(9) A. G. Zacchei, T. I. Wishousky, B. H. Arison, and G. M. Fanelli, Jr., *Drug Metab. Dispos.*, **4**, 479–489 (1976).

(10) A. G. Zacchei and T. I. Wishousky, *Drug Metab. Dispos.*, 4, 490–498 (1976).
(11) K. F. Tempero, G. Hitzenberger, Z. E. Dziewanowska, H. Halkin, and G. H. Besselaar, *Clin. Pharmacol. Ther.*, **19**, 116–117 (1976).
(12) M. L. Kauker, *J. Pharmacol. Exp. Ther.*, **200**, 81–87 (1977).
(13) G. M. Fanelli, Jr., D. L. Bohn, A. Scriabine, and K. H. Beyer, Jr., *J. Pharmacol. Exp. Ther.*, **200**, 402–412 (1977).
(14) K. F. Tempero, J. A. Vedin, C. E. Wilhelmsson, P. Lund-Johansen, C. Vorberger, C. Moerlin, H. Aaberg, W. Enenkel, J. Bolognese, and Z. E. Dziewanowska, *Clin. Pharmacol. Ther.*, **21**, 119 (1977).
(15) J. M. Sprague and E. M. Schultz, U.S. Patent 3 453 312 (July 1, 1969).
(16) D. A. Koechel and E. J. Cafruny, *J. Med. Chem.*, 16, 1147–1152 (1973).
(17) D. A. Koechel and E. J. Cafruny, *J. Pharmacol. Exp. Ther.*, **192**, 179–194 (1975).
(18) E. M. Schultz and J. M. Sprague, U.S. Patent 3 251 064 (May 10, 1966).
(19) E. M. Schultz and J. M. Sprague, U.S. Patent 3 255 241 (June 7, 1966).
(20) W. A. Bolhofer and J. M. Sprague, U.S. Patent 3 255 242 (June 7, 1966).
(21) E. M. Schultz and J. M. Sprague, U.S. Patent 3 383 411 (May 14, 1968).
(22) G. E. Schreiner, *Proc. Soc. Exp. Biol. Med.*, **74**, 117–120 (1950).
(23) G. W. Snedecor and W. G. Cochran, "Statistical Methods", 6th ed, Iowa State University Press, Ames, Iowa, 1968.
(24) C. D. Klaassen and T. J. Fitzgerald, *J. Pharmacol. Exp. Ther.*, **191**, 548–556 (1974).

β-Adrenergic Blocking Agents. 18. 1-(Aryloxy)-3-(arylthioalkylamino)propan-2-ols and 1-Substituted Alkylthioamino-3-(aryloxy)propan-2-ols

H. Tucker* and J. F. Coope

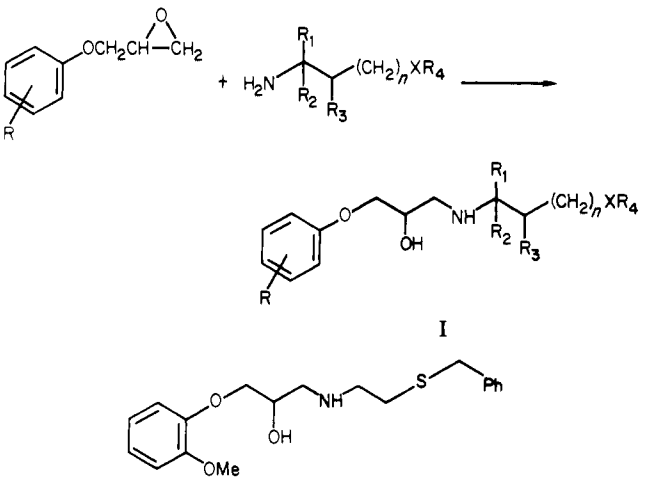
Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England.
Received February 13, 1978

The synthesis is described of a series of derivaties of 1-(aryloxy)-3-(arylthioalkylamino)propan-2-ols and 1-(alkylthioamino)- and 1-(aralkylamino)-3-(aryloxy)propan-2-ols. These compounds were investigated for their β-adrenoreceptor blocking properties and their selectivity of action for the cardiac β_1 receptor. The structure–activity relationships are discussed with particular reference to the effects of the sulfur, sulfoxide, and sulfone groups on β-adrenoreceptor blocking potency and selectivity.

In an earlier publication[1] we discussed the structural features of β-adrenergic receptor antagonists which confer selectivity for the cardiac β_1 receptor compared to the vascular β_2 receptor and, in particular, we showed that in a series of general structure **I**, where X = O, it was this oxygen atom which played the major role in determining the cardioselectivity. In an extension of these studies we have investigated the influence of the group X in structure I on β-adrenoreceptor blocking potency and cardioselectivity and report here our findings for a series of compounds in which X is the sulfur, sulfoxide, or sulfone moiety.[2] In general, these compounds show a high degree of selectivity for the cardiac β_1 receptor although their potency overall is lower than that of the corresponding oxygen series. A closely related compound, the benzylthioethylamino derivative **11**, has already been described by a Parke Davis group as part of their work on the radioprotective actions of 2-aminoethanethiol derivatives, but no cardiovascular data were reported.[3]

Chemistry. The compounds were prepared by the previously described method[4] of reacting a 1,2-epoxy-3-substituted phenoxypropane with the appropriate amine as shown in Scheme I. Where the amine used was nonvolatile some difficulty was experienced in removing the excess amine from the reaction mixture, but it was found that the lipophilic secondary amine was extracted into the chloroform phase on partitioning of the reaction mixture between hydrochloric acid and chloroform while the primary amine remained in the aqueous phase. A typical preparation is given in the Experimental Section. The various substituted arylthio-, alkylthio-, and aralkylthioalkylamines and oxidized analogues were prepared

Scheme I[a]



I

11

[a] See tables for R, R_1, R_2, R_3, and R_4.

by standard synthetic routes and representative syntheses of novel amines used are described in the Experimental Section.

Pharmacology. β-Adrenoceptor blocking potency was estimated in vivo using the previously described cat preparation.[5] The results given in Tables I and II are expressed as the total dose, infused over a period of 30 min, causing a 50% inhibition of the tachycardia produced by a submaximal dose of isoproterenol (0.2 μg/kg dosed iv). The degree (%) of blockade of the vasodepressor response at that dose level is also given. The relative potencies of

0022-2623/78/1821-0769$01.00/0