were applied to the left flank gland of male hamsters (80 g) once daily for 15 days. Chloroform only was similarly applied to the right flank. For subcutaneous administration, the compounds, as aqueous carboxymethylcellulose suspensions, were injected once daily for 14 days. On the day following the last treatment, the animals were sacrificed, and the flank glands, seminal vesicles, adrenals, and thymuses were removed and weighed.

Crystal Data. $C_{26}H_{29}ClO_3S$ (4), mol wt 457.0, orthorhombic, a = 13.056 (7) Å, b = 21.541 (10) Å, c = 8.323 (4) Å, U = 2341 Å^3, Z = 4, d_{calcd} = 1.297 g cm^{-3}. Absorption coefficient for Cu $K\alpha$ radiation (λ = 1.5418 Å), μ = 24 cm^{-1}. Space group $P2_12_12_1$ (D_2^4) uniquely established from the systematic absences: $h00$ when $h \neq 2n$, $0k0$ when $k = 2n$, $00l$ when $l \neq 2n$.

Crystallographic Measurements. A crystal of dimensions ca. 0.06 × 0.08 × 1.00 mm was oriented on the end of a glass fiber. Preliminary unit-cell constants and space group information were obtained from oscillation and Weissenberg photographs taken with Cu $K\alpha$ radiation. The crystal was then transferred to an Enraf–Nonius CAD-3 automated diffractometer (Ni-filtered Cu $K\alpha$ radiation) where one octant of reciprocal space to θ = 67° was surveyed by the θ–2θ scanning procedure as described in detail elsewhere.[36] Refined unit-cell parameters were derived by least-squares treatment of the diffractometer setting angles for 40 reflections widely separated in reciprocal space. From a total of 2283 independent intensity measurements, only those 1145 for which $I > 2.0\sigma(I)$ [$\sigma^2(I)$ = scan count + total background count] were considered observed and used in the structure analysis after the usual Lorentz and polarization corrections had been applied.

Structure Analysis. The structure was solved by use of MULTAN.[18] Approximate positions for 24 non-hydrogen atoms were obtained from E-map, and the remaining 7 were located in a F_0 Fourier synthesis phased by this group of atoms. Full-matrix least-squares refinement, at first with isotropic, and subsequently with anisotropic, thermal parameters reduced R[19] to 0.100 from its value of 0.265 for the initial model. Hydrogen atoms were then included at their calculated positions and, after several further rounds of least-squares adjustment of non-hydrogen atom parameters, the refinement converged at R = 0.052. Final atomic positional parameters are in Table V. Anisotropic thermal parameters and calculated hydrogen atom positional parameters are in Tables VI and IX. Observed and calculated structure amplitudes are listed in Table X.

In all structure-factor calculations, the atomic scattering factor for hydrogen was taken from ref 37, and the atomic scattering factors for carbon, chlorine, oxygen, and sulfur were taken from ref 38, with those for chlorine and sulfur corrected for anomalous dispersion.[39] In the least-squares iterations, $\sum w\Delta^2(\Delta = ||F_o| - |F_c||)$ was minimized with weights, w, assigned according to the following scheme: $w^{1/2} = 1$ when $|F_o| \leq 30.0$ and $w^{1/2} = 35.0/|F_o|$ when $|F_o| > 30.0$.

Acknowledgment. We thank J. McGlotten and the staff of the Physical and Analytical Chemistry Research Department for the analytical and spectral data reported. We are also grateful to E. Babad and L. Peer of the Chemical Development Department for preparing supplies of 4 and 12.

Registry No. 1, 5885-11-0; 2, 73038-49-0; 3, 83220-36-4; 4, 73024-18-7; 5, 73024-23-4; 6, 73024-26-7; 7, 73024-19-8; 8, 73024-20-1; 9, 73024-29-0; 10, 83289-27-4; 11, 83220-37-5; 12, 73024-08-5; 13, 73024-09-6; 14, 73024-10-9; 15, 83220-38-6; 16, 73024-17-6; 17, 73024-33-6; 18, 83220-39-7; 19, 83220-40-0; 20, 83220-41-1; 21, 24824-97-3; 22, 18086-90-3; 23, 3570-10-3; IBD, 932-72-9; 1,4-androstadiene-3,11,17-trione, 7738-93-4; thiophenol, 108-98-5; benzyl mercaptan, 100-53-8; β-phenylethyl mercaptan, 4410-99-5; pentyl mercaptan, 110-66-7; 17β-hydroxy-1,4-androstadien-3-one, 846-48-0; 17β-(methanesulfonyloxy)-1,4-androstadien-3-one, 63015-07-6; m-chloroperbenzoic acid, 937-14-4; 2,4-dichlorobenzyl mercaptan, 59293-67-3; 17α-[(2,4-dichlorobenzyl)thio]-1,4-androstadiene-3,11-dione, 73024-13-2; 2-methylbenzyl mercaptan, 7341-24-4; 17α-[(2-methylbenzyl)-thio]-1,4-androstadiene-3,11-dione, 73024-25-6.

Supplementary Material Available: Figures showing interatomic distances and bond angles (Figure 3) and crystal packing arrangement (Figure 4) and tables of non-hydrogen atom fractional coordinates (Table V), anisotropic thermal parameters (Table VI), torsion angles (Table VII), least-squares planes (Table VIII), hydrogen atom parameters (Table IX), and observed and calculated structure amplitudes (Table X) (20 pages). Ordering information is given on any current masthead page.

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Acute Effects of Alkylating Agents on Canine Renal Function: Specifically Designed Synthetic Maleimides

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Maleimidohippurates and maleimidobenzoates were synthesized that possess a carboxy group for active uptake into renal proximal tubular cells and a reactive maleimide moiety to covalently bond with proximal tubular components. The reactivity of the maleimide moiety in each series was progressively reduced by substitution of methyl groups in place of the vinyl hydrogens. In contrast to *N*-ethylmaleimide (NEM), the resulting maleimidohippurates and maleimidobenzoates did not possess significant diuretic activity in the dog following renal arterial administration. However, as predicted, the nephrotoxicity of the maleimidohippurates paralleled their in vitro alkylating ability and was quite specifically located in the proximal portion of the canine renal tubule.

Recently we demonstrated that *N*-ethylmaleimide (NEM) is diuretic, as well as potentially nephrotoxic, in the dog following renal arterial, but not intravenous administration.[1] Although the anatomical sites at which NEM acts to induce a diuresis or a nephrotoxic response have not as yet been thoroughly delineated, we felt that

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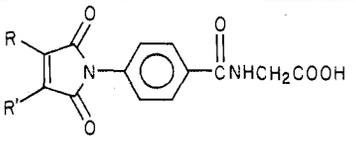
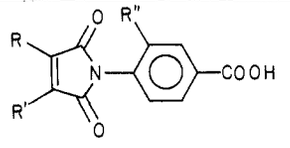
Table I. Maleimidohippurates



					intravenous administration			renal arterial administration		
			$t_{1/2}$, min							
no.	R	R'	GSH[a]	L-Lys[b]	dose, μmol/kg	no. of dogs	changes in renal function[e]	dose, μmol/kg	no. of dogs	changes in renal function[c]
1a	H	H	<1	rx[d]	73.0	4	*e*	20.0	5	no change
1b	CH_3	H	30	no rx				20.0	4	no change
1c	CH_3	CH_3	no rx	no rx				20.0	4	no change

[a] The pH of the resulting solutions were 3.3 (1a), 3.8 (1b), and 4.3 (1c). See Experimental Section. [b] The pH of the resulting solutions were 5.9 (1a), 5.7 (1b), and 6.8 (1c). See Experimental Section. [c] The changes in canine renal function that were monitored over the 2-h interval following maleimide administration included urinary pH, urine flow rate, GFR, and the urinary excretion rates of sodium, potassium, and chloride. [d] An equilibrium was established within 30 min that reflected a 30% reaction of 1a with L-lysine. [e] See Figure 2.

Table II. Maleimidobenzoates

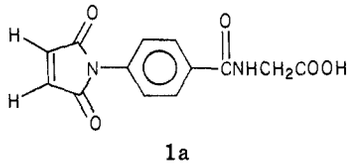


						intravenous administration			renal arterial administration		
				$t_{1/2}$, min							
no.	R	R'	R''	GSH[a]	L-Lys[b]	dose, μmol/kg	no. of dogs	changes in renal function[c]	dose, μmol/kg	no. of dogs	changes in renal function[c]
2a	H	H	H						20.0	1	no change
2b	CH_3	H	H						20.0	1	no change
2c	CH_3	CH_3	H						20.0	1	no change
2d	CH_3	H	CH_3	23	no rx	146	4	*d*	20.0	4	no change

[a] The pH of the solution resulting from the mixture of 2d with GSH was 4.21. See Experimental Section. [b] The pH of the solution resulting from the mixture of 2d with L-lysine was 5.9. See Experimental Section. [c] The changes in canine renal function that were monitored over the 2-h interval following maleimide administration included urinary pH, urine flow rate, GFR, and the urinary excretion rates of sodium, potassium, and chloride. [d] See Figure 3.

the neutral, freely diffusible,[2] and highly reactive nature of NEM[3-7] would allow for relative nonspecific alterations of normal glomerular and renal tubular function.

This paper deals with the rationale for the design, synthesis, and preliminary evaluation of the renal effects of a number of maleimide derivatives that would be expected to be more specifically directed to the proximal tubular area and create more localized changes in renal function and morphology than NEM. We felt that this could best be accomplished by incorporating two structural features into the same molecule: (1) a carboxylic acid group that when ionized would allow for the compound to be selectively taken up by the organic anion transport system located specifically in proximal tubular cells and (2) a reactive maleimide moiety that would have the capability of reacting covalently with proximal tubular components. The prototypic compound, **1a**, was formed by combining



1a

the highly reactive maleimide moiety with *p*-aminohippuric acid (PAH). PAH is known to be transported into proximal tubular cells more efficiently than any other organic anionic substance.[8,9] To further delineate the importance of these two structural features for producing rather selective proximal tubular dysfunction, we maintained the presence of the carboxy moiety while reducing the reactivity of the maleimide moiety of **1a** by substituting one or two methyl groups in place of the vinyl hydrogen(s). The resulting maleimidohippurates (**1a–c**) are shown in Table I. In addition, using a similar rationale we synthesized a second series of compounds, the maleimidobenzoates (Table II), that also possesses the same two

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structural features that we feel will allow for the production of relatively specific proximal tubular effects. Thus, both series of synthetic maleimides would be expected to undergo more specific intrarenal distribution than NEM and as a result have markedly different effects on renal function and morphology. Although we have examined the effects of the maleimidohippurates and maleimidobenzoates on both renal function and renal ultrastructure, this paper will focus attention primarily on the effects of the maleimidohippurates (**1a–c**) on canine renal function.

The routes of administration (i.e., intravenous and renal artery) and the doses of synthetic maleimides employed in this study were selected so the results could be compared to those previously cited with NEM.[1] Changes in urine flow rate, urinary pH, glomerular filtration rate (GFR), and the urinary excretion rates of sodium, potassium, and chloride were monitored every 10 min over a 2-h period following the administration of each maleimide. Subsequent studies have also involved monitoring maleimidohippurate-induced changes in protein, glucose, urate, and phosphate excretion, as well as changes in renal blood flow and urine osmolality. The latter findings will be published in detail with the maleimidohippurate-induced changes in proximal tubular morphology.

Ethacrynic acid (EA) was administered via the intravenous route 2 h after the intravenous or renal arterial administration of the synthetic maleimides to (1) demonstrate that the dogs that did not undergo a diuresis following a given dose of a synthetic maleimide were indeed responsive to a known diuretic agent and to (2) see if prior administration of a synthetic maleimide would alter in any way a normal response to EA. No alteration of a normal EA response would be evidence that the thick ascending limb of the loop of Henle remained functionally intact. On the other hand, alteration of a normal EA response would perhaps, but not necessarily, signal a maleimide-induced modification of the functional integrity of the thick ascending limb of the loop of Henle.

Results

The maleimidohippurates (**1a–c**) were synthesized by combining the appropriate maleic anhydride with PAH in glacial HOAc, followed by treatment of the maleamic acid with toluene, triethylamine, and DMF. Preparation of the maleimidobenzoates (**2a–d**) was conducted by reacting the corresponding maleic anhydride with an aminobenzoic acid derivative, followed by treatment with sodium acetate and acetic anhydride (see Experimental Section).

Our aim was to place primary emphasis on the renal effects of the maleimidohippurates and to examine the maleimidobenzoates in detail only if the former induced marked changes in canine renal function (i.e., urine flow rate and electrolyte excretion rates).

Maleimidohippurates. We examined the in vitro reactivity of the maleimidohippurates prior to the in vivo studies, since our previous work with NEM demonstrated that its ability to induce a diuresis (as well as a nephrotoxic response) is most likely linked to its reactivity toward renal nucleophiles.[1] As expected from the work of Miyadera et al.,[10] the reactivity of **1a–c** toward nucleophiles decreased in the following order: **1c** < **1b** < **1a** (Table I).

Due to the tremendously rapid reaction of the prototypic compound, **1a**, with various nucleophiles, we felt that the maleimidohippurates (**1a–c**) should be evaluated by injection directly into the canine renal artery to assure their distribution to the kidney. This mode of administration was also used in our previous work with NEM.[1] Compounds **1a–c** were administered over a 10-min period directly into the right renal artery in a dose of 20.0 μmol/kg (a dose 2.5 times the maximum diuretic dose of NEM). In contrast to the marked unilateral diuresis induced by NEM following its renal arterial administration, **1a–c** lacked diuretic activity during the 2-h period following their injection (Table I). In addition, no alteration of a normal EA-induced diuresis was observed when the latter agent was administered via the intravenous route 2 h following **1a–c**. If one assumes that increases in the renal excretion rates of protein and glucose are often early indicators of drug-induced renal injury, then it is important to note that the highly reactive **1a**, but not the unreactive **1c**, yielded a marked unilateral proteinuric and glucosuric response. The **1a**-induced responses from a representative experiment are shown in Figure 1.

The above findings prompted us to examine the renal effects of **1a** following intravenous administration. A dose of 73.0 μmol/kg was selected so the results could be compared to our previous findings following the administration of an equivalent intravenous dose of NEM.[1] Unlike NEM, **1a** induced an increase in urinary pH and a corresponding decrease in the urinary excretion rate of chloride (Figure 2). A subsequent normal EA response was observed.

Maleimidobenzoates. Following the renal arterial administration of **2a–c** (20.0 μmol/kg), we observed essentially the same results as previously discussed with **1a–c** (Table II). That is, **2a–c** failed to possess diuretic activity, and no alteration of a normal EA-induced diuresis was noted when EA was given intravenously 2 h after **2a–c**. Since **2d** shares some similarities to the more active diuretics in the EA series (i.e., a carboxy group, a methyl group in the 3-position of the aromatic ring, and an α,β-unsaturated system para to the carboxy group), we elected to examine its renal effects in more detail than the other maleimidobenzoates. Compound **2d** failed to alter the urinary pH, urine flow rate, or electrolyte excretion rates following injection of 20.0 μmol/kg directly into the canine renal artery (Table II). However, the intravenous injection of a relatively large dose of **2d** (146 μmol/kg) induced a moderate short-lived change in electrolyte excretion that was significant when analyzed by the paired *t* test but not when Dunnett's procedure was used. The intravenous administration of EA 2 h after **2d** yielded a normal diuresis (Figure 3).

Discussion

This study allowed us to compare the renal effects of **1a** with those of NEM following intravenous and renal arterial administration. Furthermore, the importance of alkylation in the **1a**-induced changes was assessed by comparing its renal actions to those induced by **1b** and **1c**, which possess progressively less alkylating ability than **1a**.

Although **1a** and NEM both possess the maleimide moiety, it is clear that they alter canine renal function in different ways following intravenous administration. Our previous studies revealed that an intravenous dose of NEM as high as 73.0 μmol/kg did not alter any of the renal function parameters that we monitored (i.e., urine flow rate, urinary pH, GFR, and the renal excretion rates of sodium, potassium, and chloride).[1] In contrast, the intravenous administration of an equivalent dose of **1a** yielded a significant prolonged (i.e., for 2 h) increase in the urinary pH and a decrease in the urinary excretion rate of chloride. Both of these changes would be expected from a compound that altered carbonic anhydrase dependent sodium bicarbonate reabsorption in the proximal tubule. If both of these **1a**-induced changes are the result of a

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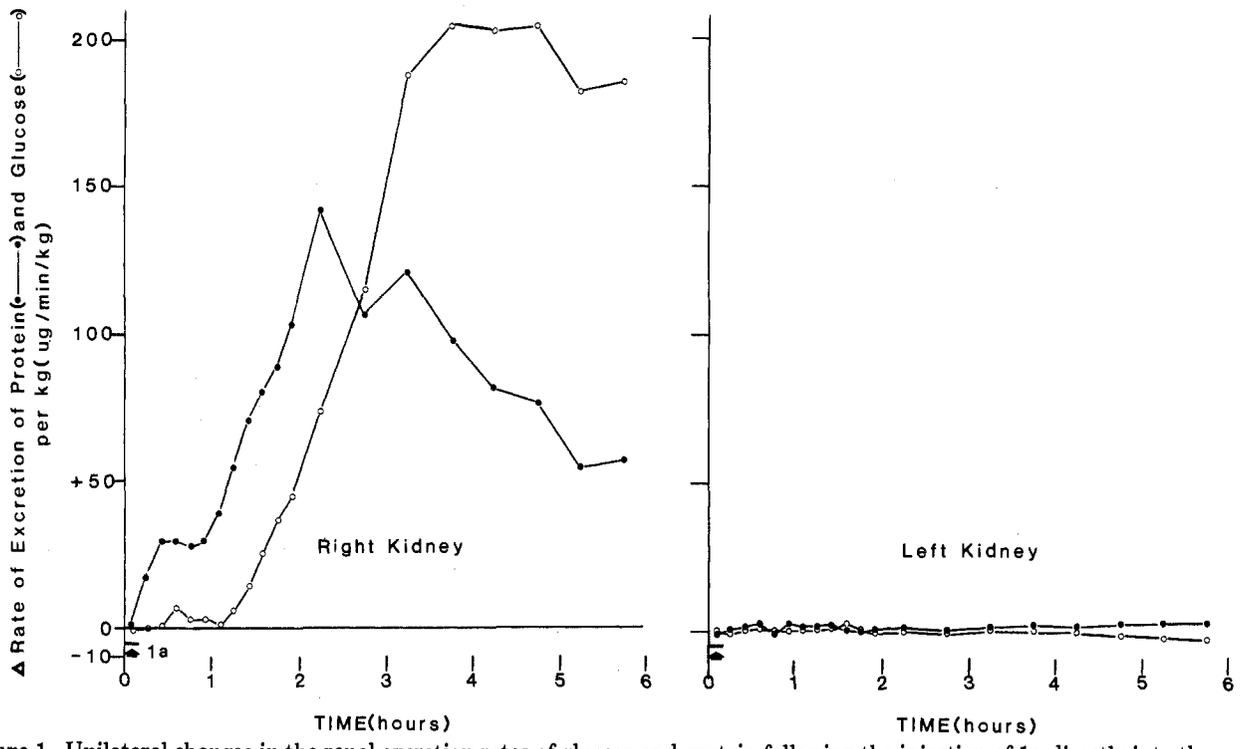

Figure 1. Unilateral changes in the renal excretion rates of glucose and protein following the injection of **1a** directly into the canine right renal artery. The bold line on the abscissa indicates the 10-min period during which **1a** was administered. Dog weight = 9.6 kg.

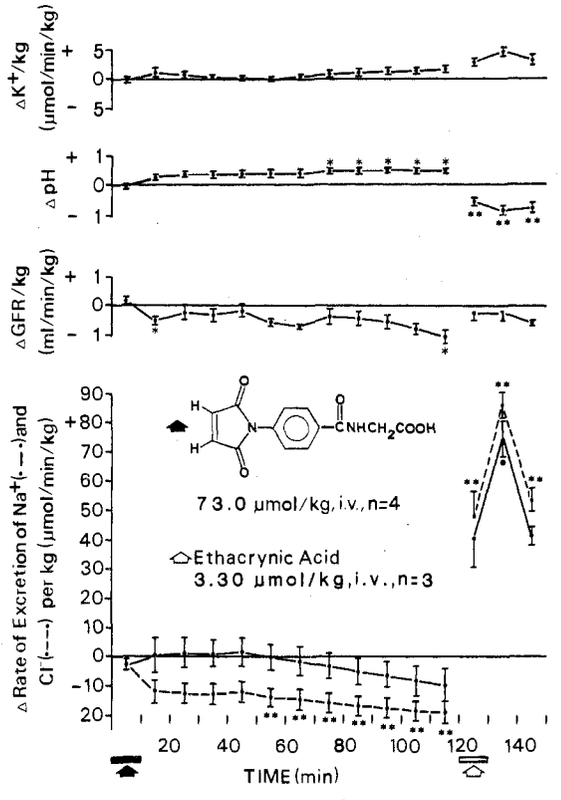

Figure 2. The effect of 4-maleimidohippuric acid (**1a**; 73.0 μmol/kg, iv) on canine renal function.

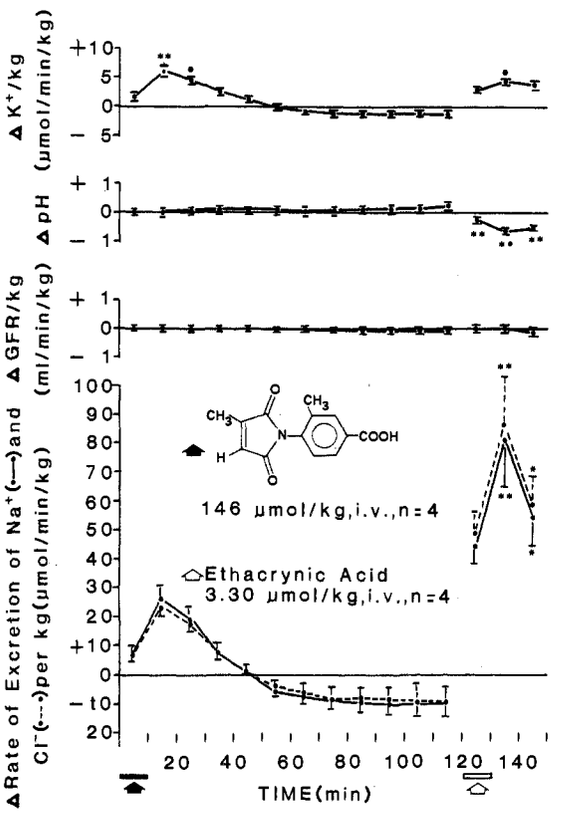

Figure 3. The effect of 3-methyl-4-(methylmaleimido)benzoic acid (**2d**) (146 μmol/kg, iv) on canine renal function.

direct renal action of the compound, then it suggests that **1a** may be affecting the function of the canine proximal tubular cells in a rather selective fashion. Several pieces of data indicate that **1a** does not disturb the functional integrity of the more distal segments of the nephron. For example, neither the diuretic activity of EA (a diuretic agent whose site of action is in the thick ascending limb of the loop of Henle) nor the usual urinary acidification induced by EA (an event that occurs in the distal tubule and collecting duct) was modified by the prior administration of **1a**.

Differences were also noted between NEM and **1a** following injection directly into the canine renal artery. This route of administration was used to more accurately assess the direct effect of NEM and the synthetic maleimides on

renal function. Interestingly, none of the maleimidohippurates (**1a–c**) possessed diuretic activity comparable to that observed with NEM, nor did they alter the functional integrity of the ascending limb of the loop of Henle. When examining the nephrotoxicity of **1a–c**, we focused our attention on comparing NEM with **1a** and **1a** with **1b** and **1c**. Although this will be dealt with in detail in an upcoming publication, two extremely important findings surfaced. First, the nephrotoxicity (measured as changes in the renal excretion rates of protein and glucose, as well as changes in renal ultrastructure) of **1a–c** parallels their in vitro alkylating ability. In fact, **1a** was extremely nephrotoxic, while **1c** was completely devoid of any adverse effects on canine renal function or ultrastructure. Since the glucosuria and proteinuria induced by **1a** were confined to the **1a**-treated kidney (Figure 1), we assume that **1a** is rapidly and quite irreversibly bound to tissue components in the treated kidney. Second, the renal ultrastructural changes induced by **1a** were extremely selective for the proximal portion of the nephron, whereas the changes observed with NEM were quite diffuse and nonspecific (unpublished observations). These findings support our initial contention that chemicals that possess both an anionic functional group (i.e., the COO^- moiety that allows for their specific uptake into proximal tubular cells by way of the organic anion transport system) and an alkylating moiety (that allows for the formation of a covalent bond with proximal tubular components) will be potential proximal tubular toxins.

Although not as extensively studied as the maleimidohippurates, the maleimidobenzoates (**2a–c**) appeared to behave in a similar fashion. In single animal experiments, **2a–c** failed to induce a diuresis following renal arterial administration. Only **2d** was examined following intravenous administration, and it proved to possess little or no diuretic activity. Interestingly, the intravenous administration of **2d** (Figure 3) was not associated with an increase in the urinary pH and a decrease in the urinary excretion rate of chloride as was observed with **1a** (Figure 2). This latter observation might be due to the slower rate of reaction of **2d** with nucleophilic substances.

At the present time there is no established explanation for why the synthetic maleimides differ so markedly from NEM in their ability to induce a diuresis following renal arterial administration. However, the rather specific proximal tubular toxicity of **1a** is highly predictable based on our understanding of the proximal tubular handling of weakly acidic chemicals.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The IR spectra were determined in KBr pellets with a Perkin-Elmer Model 257 grating spectrophotometer. The NMR spectra were determined in DMF-d_7 on a Hitachi Perkin-Elmer Model R-24 spectrometer with Me_4Si as an internal standard. All spectral data for intermediates and products were consistent with the assigned structures. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Test compounds were purified until only one spot was detected following thin-layer chromatography [E. Merck precoated silica gel 60 F_{254} plates (0.25-mm thick), EtOAc/acetone/HOAc (40:10:4) for **1a–c**, and EtOAc/$CHCl_3$/HOAc (40:20:4) for **2a–d**]. Column chromatography was accomplished with Chromaflex columns (Kontes Glass Co.) and Merck silica gel 60 (70–230 mesh).

General Procedure for the Determination of the Rate of Reaction of the Maleimides with Glutathione and L-Lysine. To a solution of the appropriate maleimide (50 mg) in DMF-d_7 (ca. 0.2 mL) was added a solution containing an equimolar amount of glutathione or L-lysine in D_2O (0.2–0.5 mL). Additional DMF-d_7 was added, if needed, to redissolve any precipitated maleimide. Zero time was taken as the time of the mixing of the maleimide solutions with the glutathione or L-lysine solution. The resulting solution was immediately placed in the NMR spectrometer (probe temperature, 35 °C). The rate of disappearance of the vinyl protons or shift of the methyl protons was considered indicative of reactivity of the maleimide with the appropriate nucleophile and was used to calculate the $t_{1/2}$ shown in Tables I and II. The sample was removed occasionally from the spectrometer and shaken to ensure homogenity.

Synthesis of 4-Maleimidohippuric Acid (1a). Step A. A solution of maleic anhydride (18.83 g, 192 mmol) in glacial HOAc (80 mL) was added over a 10-min period to a stirred solution of 4-aminohippuric acid (37.28 g, 192 mmol) in glacial HOAc (800 mL). The resulting light yellow solid was collected by filtration, washed with Et_2O (4 × 100 mL), and air-dried to yield 56.0 g (100%) of the corresponding maleamic acid, mp 198–200 °C. Anal. ($C_{13}H_{12}N_2O_6 \cdot H_2O$) C, H, N.

Step B. Toluene (500 mL) and triethylamine (10.0 g, 98.8 mmol) were added to a 1-L flask that contained DMF (100 mL) and the above maleamic acid (12.42 g, 42.5 mmol). The flask was fitted with a Dean-Stark apparatus and a reflux condenser. The reaction mixture was heated at reflux until water ceased to be removed. The toluene layer was decanted from the remaining reddish oil and concentrated in vacuo, and the remaining residue was allowed to stand in Et_2O (150 mL) overnight. The Et_2O layer was discarded, and the residual Et_2O was removed in vacuo, yielding a yellow semisolid. The semisolid was dissolved in distilled water (125 mL), acidified to pH 1 (concentrated HCl), and extracted with EtOAc (3 × 250 mL). The extracts were combined, dried (Na_2SO_4), and concentrated to yield 4.67 g (40%) of **1a** as a yellow solid. Purification by column chromatography (EtOAc/acetone/HOAc, 40:10:4), followed by recrystallization from acetone/cyclohexane gave **1a** as a yellow solid, mp 231–234 °C. Anal. ($C_{13}H_{10}N_2O_5$) C, H, N.

Synthesis of 4-(2-Methylmaleimido)hippuric Acid (1b). Step A. A solution of methylmaleic anhydride (5.0 g, 44.6 mmol) in glacial HOAc (55 mL) was added over a 15-min period to a stirred solution of 4-aminohippuric acid (8.66 g, 44.6 mmol) in glacial HOAc (200 mL). The addition funnel was washed with an additional 5 mL of HOAc, and the reaction mixture was refluxed (3 h), cooled, and concentrated to dryness in vacuo. The crude maleamic acid was washed with cyclohexane and air-dried to yield 14.8 g. The maleamic acid was used in the next step without further purification.

Step B. Compound **1b** was prepared with the crude maleamic acid previously described (10.0 g, 32.7 mmol), DMF (100 mL), toluene (500 mL), and triethylamine (7.0 g, 69.2 mmol) as discussed for the synthesis of **1a**. Evaporation of the EtOAc extracts gave 5.10 g (54%) of **1b** as a yellow solid. Purification by column chromatography (EtOAc/acetone/HOAc, 40:10:4), followed by charcoal treatment in acetone and recrystallization from acetone/cyclohexane yielded **1b** as a light yellow powder, mp 188–190 °C. Anal. ($C_{14}H_{12}N_2O_5$) C, H, N.

Synthesis of 4-(2,3-Dimethylmaleimido)hippuric Acid (1c). Step A. A solution of 2,3-dimethylmaleic anhydride (5.0 g, 39.7 mmol) in glacial HOAc (55 mL) was added over a 15-min period to a warm solution of 4-aminohippuric acid (7.7 g, 39.7 mmol) in glacial HOAc (200 mL). After refluxing for 1 h, the corresponding maleamic acid was obtained as an orange oil (14.3 g) following concentration in vacuo. The material was used without further purification in the next step.

Step B. A solution containing the corresponding maleamic acid (14.3 g, 44.7 mmol), DMF (150 mL), toluene (400 mL), and triethylamine (12.0 g, 118 mmol) was allowed to reflux for 2.5 h in the presence of a Dean–Stark unit. The resulting mixture was cooled and evaporated in vacuo to an orange oil. The oil was acidified with 1 N HCl and extracted with EtOAc. In vacuo evaporation of the EtOAc extract yielded 6.0 g of a yellow powder, which was purified further by column chromatography (EtOAc/acetone/HOAc, 40:10:4). Recrystallization from acetone/cyclohexane yielded 2.8 g of **1c** as a light yellow solid, mp 206–209 °C. Anal. ($C_{15}H_{14}N_2O_5$) C, H, N.

Synthesis of 4-Maleimidobenzoic Acid (2a). Step A. A solution of maleic anhydride (18.83 g, 192 mmol) in glacial HOAc

(80 mL) was added to a stirred solution of 4-aminobenzoic acid (26.33 g, 192 mmol) in glacial HOAc (200 mL) over a 20-min period. The flask was stoppered, and the reaction mixture was stirred overnight. The resulting yellow precipitate was collected by filtration, washed (cold water), and allowed to air-dry. The yellow solid was triturated (benzene), filtered, and air-dried. The maleamic acid was obtained in 96% yield (43.4 g), mp 214–217 °C. Anal. ($C_{11}H_9NO_5$) C, H, N.

Step B. To a 100-mL round-bottomed flask fitted with a reflux condenser with a drying tube attached was added the above maleamic acid (7.5 g, 31.9 mmol), acetic anhydride (9.84 g, 95.7 mmol), and NaOAc (1.5 g, 18.0 mmol). The stirred mixture was heated at 80–83 °C for 1 h. The hot amber solution was poured slowly into hot distilled water (100 mL) with vigorous stirring. The yellow crystals that formed on cooling were filtered, washed with cold distilled water, and air-dried to yield 6.45 g (93%) of crude **2a**. Purification by column chromatography (benzene/dioxane/HOAc, 50:5:2) yielded **2a** as a light yellow solid, mp 235–237 °C (lit.[11] 234 °C). Anal. ($C_{11}H_7NO_4$) C, H, N.

Synthesis of 4-(2-Methylmaleimido)benzoic Acid (2b). To a stirred solution of 4-aminobenzoic acid (6.12 g, 44.6 mmol) in glacial HOAc was added a solution of methylmaleic anhydride (5.0 g, 44.6 mmol) in glacial HOAc (60 mL) over a period of 20 min. The reaction mixture was heated at reflux for 3 h, followed by stirring overnight at room temperature. The precipitated white solid was collected by filtration, washed with Et_2O (3 × 100 mL), and air-dried. The filtrate was concentrated to dryness, and the remaining residue was recrystallized twice (HOAc). The combined crops of **2b** were purified by charcoal treatment in acetone to yield 1.78 g (17%) of **2b** as a light yellow powder, mp 203.5–205 °C. Anal. ($C_{12}H_9NO_4$) C, H, N.

Synthesis of 4-(2,3-Dimethylmaleimido)benzoic Acid (2c).[12,13] Preparation of **2c** from 4-aminobenzoic acid (5.44 g, 39.6 mmol) in glacial HOAc (70 mL) and 2,3-dimethylmaleic anhydride (5.0 g, 39.6 mmol) in glacial HOAc (55 mL) was conducted in a manner similar to that previously described for the synthesis of **2b**. This procedure yielded 2.26 g (23%) of **2c** as an off-white solid. Purification by column chromatography (benzene/dioxane/HOAc, 50:5:2), followed by charcoal treatment in acetone, gave **2c** as a white powder, mp 238.5–239.5 °C. Anal. ($C_{13}H_{11}NO_4$) C, H, N.

Synthesis of 3-Methyl-4-(methylmaleimido)benzoic Acid (2d). Preparation of **2d** from 4-amino-3-methylbenzoic acid (10.0 g, 66.2 mmol) in glacial HOAc (125 mL) and methylmaleic anhydride (7.42 g, 66.2 mmol) in glacial HOAc (75 mL) was conducted in a manner similar to **2b**, except the reaction mixture was stirred at room temperature for 48 h. This procedure yielded 8.41 g of **2d** (52%). Purification by charcoal treatment in acetone gave 7.94 g (49%) of **2d** as a white solid, mp 215–217 °C. Anal. ($C_{13}H_{11}NO_4$) C, H, N.

Pharmacological Studies. Renal function (urine flow rate, GFR, urinary pH, and the urinary excretion rates of sodium, potassium, and chloride) was assessed in 28 healthy anesthetized mongrel dogs of either sex, ranging in weight from 7.05 to 17.95 kg (average, 11.70 kg). Details of the experimental procedures employed have been reported previously.[1,14–16] The renal clearance of inulin was used to estimate the GFR according to the procedure described by Schreiner.[17] Urinary sodium and potassium concentrations were determined with an Instrumentation Laboratories Model 343 flame photometer, urinary chloride concentration was determined with a Corning Model 920M chloride meter, and pH was determined with a Corning Model 110 pH meter.

Synthetic maleimides administered into the jugular vein (73.0 or 146 μmol/kg) were dissolved in 10 mL of 0.9% saline with the aid of an equivalent amount of $NaHCO_3$ and injected over a 10-min period. Maleimides administered directly into the right renal artery (20.0 μmol/kg) were dissolved in 5 mL of 0.9% saline containing an equivalent amount of $NaHCO_3$ and injected over 10 min. The renal arterial catheter was a modified Abbott butterfly infusion set (i.e., the wings were cut off and the 21-gauge needle was bent into an L-shape) filled with 0.9% saline containing heparin. The needle was held in place by lodging its shank between the aorta and the vena cava.

EA (3.30 μmol/kg, equivalent to 1.0 mg/kg) was dissolved in 10 mL of 0.9% saline with the aid of an equivalent amount of $NaHCO_3$ and administered intravenously (jugular vein) over a 10-min period 2 h following the intravenous or renal arterial administration of the synthetic maleimides.

The blood pressure was continuously monitored via a cannula in the right carotid artery (Statham transducer, Grass Model 79D polygraph). No significant changes were noted.

Data Analysis. The data shown in Figure 1 were taken from a single representative experiment. Changes in the renal excretion rates of protein and glucose were determined by subtracting the values obtained for each of these parameters in the period immediately preceding the injection of a synthetic maleimide from the corresponding values obtained in each of the subsequent 10-min urine collection periods. The data from the right and left kidneys were handled separately.

The data shown in Figures 2 and 3 are presented as mean changes (Δ's) (± SE) from the pretreatment control. Dunnett's procedure[18] was conducted with the raw values from each of the groups of dogs to evaluate the significance of the maleimide and EA-induced changes in renal function (* = $p < 0.05$, ** = $p < 0.01$). The mean change for each parameter in each 10-min urine collection period in each treatment group was calculated by subtracting the values obtained during the period immediately preceding the injection of a synthetic maleimide from the corresponding values obtained for each 10-min period after injection of the maleimide. Similar calculations were performed after EA administration with the control values obtained for each parameter in the 10-min period immediately prior to EA administration.

Acknowledgment. This research was supported by USPHS Grant GM 26775. The authors thank Mrs. Nancy Bretz for her assistance in the preparation of the figures, Dr. Edward J. Cragoe, Jr. (Merck, Sharp & Dohme Research Laboratories, West Point, PA), for the generous supply of EA, and the Pharmaceutical Manufacturers Association Foundation, Inc., Washington, DC, for awarding a Faculty Development Award in Basic Pharmacology to D.A.K. during the beginning phase of the research.

Registry No. **1a**, 83560-87-6; **1b**, 83560-85-4; **1c**, 83560-86-5; **2a**, 17057-04-4; **2b**, 83560-88-7; **2c**, 63729-61-3; **2d**, 83560-89-8; maleic anhydride, 108-31-6; 4-aminohippuric acid, 61-78-9; 4-maleamidohippuric acid, 83560-90-1; methylmaleic anhydride, 616-02-4; 4-(2-methylmaleamido)hippuric acid, 83560-91-2; 2,3-dimethylmaleic anhydride, 766-39-2; 4-(2,3-dimethylmaleamido)hippuric acid, 83560-92-3; 4-aminobenzoic acid, 150-13-0; 4-maleamidobenzoic acid, 5432-04-2; 4-amino-3-methylbenzoic acid, 2486-70-6.

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