as calcd from the common regression line derived from probit analysis of the cumulative dose–response curves. The dose (1.84×10^{-4} *M*) and exposure time (20 min) of phenoxybenzamine were the same as used by Winter and Gessner.[3]

Rabbit rectal temp measurements were made with Cu–constantin thermocouples on old male rabbits as described by Brimblecombe.[10]

Synthesis and Pharmacology of an Epoxide Derivative of Ethacrynic Acid

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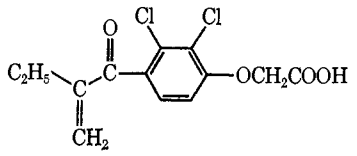
Ethacrynic acid (**1**) is thought to evoke a diuretic response by reacting with protein-bound sulfhydryl groups (PBSH) in renal tubular cells.[2–6] It has been suggested that a Michael-type reaction is involved.[4] In an attempt to explore this hypothesis further, we have synthesized the epoxide derivative **2a** of ethacrynic acid.



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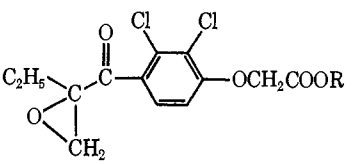

2a, R = H
b, R = CH_3

Unlike ethacrynic acid (**1**) the epoxide **2a** is no longer capable of reacting with SH-containing substances *via* a Michael-type addition; however, it has the potential of reacting with them by an SN2 reaction. The SN2 reaction involving epoxides and SH-containing substances has been postulated to occur both *in vivo* and *in vitro*. Several exogenous epoxides are known to react with glutathione in rabbit[7] or bird[8] liver preparations. In addition, the carcinogenicity of many epoxides is thought to be due to their reaction with various nucleophilic groups (*i.e.*, SH, NH_2) of proteins.[9–13]

We reasoned that if a reaction with PBSH in renal tissue is necessary for the diuretic action of ethacrynic acid, then the epoxide **2a** could conceivably alkylate (by an SN2 reaction) these essential groups and act either as a diuretic agent or as an antagonist of the diuretic action of ethacrynic acid.

Ethacrynic acid and its epoxide derivative **2a** were compared on the basis of their diuretic activity, renal Na^+- and K^+- ATPase inhibitory activity, and *in vitro* reactivity with cysteine. The epoxide **2a** failed to induce a measurable diuretic response in dogs when administered iv in a dose of 2 mg/kg. The method of testing was similar to that used by Small and Cafruny.[14] Even when the iv dose of **2a** was increased to 25 mg/kg, there was no significant diuretic effect. Urine flow was monitored for 30 min after administration of **2a**. The glomerular filtration rate, measured as the renal clearance of inulin, was not affected. When injected directly into tubular lumens by the retrograde intraluminal injection technique[15] the epoxide failed to elicit a diuretic response [3.5 ml of an aq mannitol soln (20% wt/wt) containing 10 mg of the epoxide/ml]. An iv dose of **2a** (2 or 25 mg/kg) 30-min prior to iv administration of ethacrynic acid (2.0 mg/kg) failed to antagonize the normal diuretic response to the latter agent.

Canine renal Na^+- and K^+- ATPase,[16] an SH-containing enzyme that has been implicated in the renal transport of Na^+, was not inhibited by **2a**.[17]

In vitro experiments have shown that *ca.* 82% of the epoxide **2a** can be recovered unchanged after a 30-min exposure to an unbuffered aq soln of cysteine adjusted to pH 7.6–7.7. Although the conditions used in this study were slightly different than those employed by Duggan and Noll,[5] it is clear that cysteine reacts with ethacrynic acid at a much faster rate than it does with **2a**. Thus, **2a** differs markedly from ethacrynic acid in all three studies mentioned above.

The relative inactivity of **2a** can be explained in several ways. (1) A Michael-type reaction involving ethacrynic acid and renal PBSH may be required to bring about a diuretic response. The epoxide **2a** cannot participate in a Michael-type reaction and may well be too stable *in vivo* to react with renal PBSH by an SN2 reaction. The *in vitro* stability of **2a** in the presence of cysteine supports the previous statement. (2) It is possible that the Michael-type reaction between ethacrynic acid and renal PBSH occurs but does not contribute to the observed diuresis. If this is the case, it is conceivable that the diuretic response to ethacrynic acid is the result of a specific interaction (other than a Michael-type reaction with PBSH) between the drug and renal receptors that requires an intact α,β-unsaturated

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(17) The ATPase work was conducted by Drs. Julius Allen and Arnold Schwartz, Department of Pharmacology, Baylor University College of Medicine, Houston, Texas.

system. Destruction of the conjugated system by formation of an oxirane bridge would therefore preclude any specific interaction with renal receptors. (3) It is conceivable that the epoxide might be altered *in vivo* in such a way that it would no longer be capable of reacting with critical renal receptors.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Schwartzkopf Microanalytical Laboratories, Woodside, N. Y. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Ir spectra were recorded on a Perkin-Elmer Model 237B grating spectrophotometer. The nmr spectra were taken on a Varian Model A-60D instrument (Me_4Si as internal standard). Each analytical sample had ir and nmr spectra compatible with its structure.

Ethacrynic Acid Oxide (2a).—Ethacrynic acid[18] (1.0 g, 0.0033 mole) was suspended in distd H_2O (160 ml), and an aq soln of 1.0 *N* NaOH (3.7 ml) was added slowly with stirring at room temp. To the resulting clear soln H_2O_2 (30%) (10 ml, 0.0099 mole) and 6 *N* NaOH (0.3 ml) were added. The reaction mixt was stirred at room temp for 5 hr after which time an aq soln of $NaH_2PO_4 \cdot H_2O$ (12.9 g/30 ml of H_2O) was added to achieve a final pH of 4.8. After extn of the aq mixt with $CHCl_3$, the ext was washed with H_2O, dried (Na_2SO_4), and concd *in vacuo* at room temp to an off-white solid (0.82 g, 77% yield). Recrystn from cyclohexane–Et_2O (1:1) yielded 0.66 g of **2a**, mp 115–116.5°. *Anal.* ($C_{13}H_{12}Cl_2O_5$) C, H, Cl.

Reaction of 2a with CH_2N_2.—An Et_2O soln of **2a** was esterified with ethereal CH_2N_2. The reaction mixt was evapd *in vacuo*, and the residue gave pure ester **2b** when recrystd from aq Me_2CO, mp 81–82°. *Anal.* ($C_{14}H_{14}Cl_2O_5$) C, H, Cl.

(18) Obtained as a gift from Dr. James M. Sprague, Merck Sharp & Dohme, Research Laboratories, West Point, Pa.

Synthesis of Some 3,7-Dihydroxy-6-methyl-Δ⁵-pregnene Derivatives

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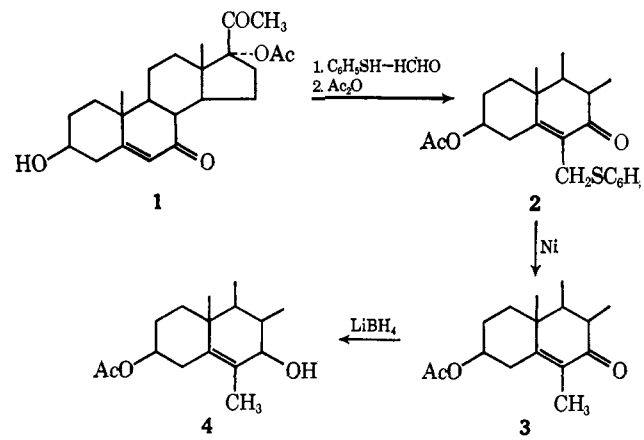
We recently reported the synthesis of some 6-chloro-3,7-dihydroxy-Δ⁵-pregnene derivatives.[1] Because of the high progestational activity of several of these compounds we decided to prepare the 6-Me analogs.

Treatment of the Δ⁵-7-one **1** with thiophenol and paraformaldehyde using triethanolamine[2] as the base gave the 6-phenylthiomethyl compound **2** after extended reflux in *n*-BuOH followed by acetylation. Desulfurization of **2** with Raney Ni afforded **3**. Reduction of the 7-ketone of **3** with $LiAl(O\text{-}t\text{-}Bu)_3H$, which was the reagent of choice for reduction of the 6-chloro-Δ⁵-7-ones,[1] did not proceed at a noticeable rate. $LiBH_4$ did, however, give a very low yield of the desired 7β-hydroxy compound **4**. The configuration at C-7 in **4** was assigned on the basis of the nmr spectrum. The C-7 proton was observed as a broad band at δ 3.80 (half-bandwidth ~11 Hz), which is consistent with axial–axial coupling with the C-8 proton.[3]

SCHEME I



To prepare the 7α-OH isomer, we first investigated the photosensitized oxygenation of the 6-methyl-Δ⁵-3β-ol acetate (**5**). Photooxygenation of cholest-5-ene-3β,26-diol is reported[4] to yield the 7α-hydroperoxide. However, the major product from oxygenation of **5** followed by reduction of the hydroperoxides proved to be the 5α-hydroxy-6-methylene compound **6**. The presence of the terminal CH_2 grouping follows from the nmr spectrum which exhibited two broadened one-proton singlets at δ 4.80 and 4.65. The C-6 Me peak was absent from its usual position of ~ δ 1.7. The location of the OH at C-5 rather than at C-7 was shown by the lack of any nmr bands in the δ 3–4 region where the C-7 OH isomer would have exhibited a broad band.

A literature report[5] describes the chlorination of a 6-methyl-Δ⁵-3β-ol acetate to yield the corresponding unstable 7α-chloro-Δ⁵-compound, which was transformed on Al_2O_3 to the 5α-chloro-6-methylene isomer along with the 5α-hydroxy-Δ⁶- and the 7α-hydroxy-Δ⁵-6-methyl compounds. Chlorination of **5** and examination of the crude product by tlc on silica gel showed the presence of 3 compounds. Glpc analysis, however, showed a single peak, while the nmr spectrum of the crude product revealed approximately 10% of the 5α-chloro-6-methylene isomer **7** (two broadened singlets at δ 4.87 and 4.80) along with the 7α-chloro-Δ⁵-6-methyl compound **8** (broad peak at δ 4.24, half-bandwidth ~6 Hz). Preparative tlc on silica gel served to separate the 3 components. The least polar compound could not be obtained in sufficient purity and quantity for positive identification.

The more polar substances were shown to be isomeric allylic alcohols in which OH had displaced Cl during the chromatography. The OH compounds could not be detected in the crude product by glpc analysis. The more mobile member of the pair proved to be the 5α-hydroxy-Δ⁶ isomer **9** as shown by the olefinic proton peak at δ 5.32 in the nmr spectrum. Structure **10** was assigned to the most polar product on the basis of the

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