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-Δ^5-pregnene Derivatives

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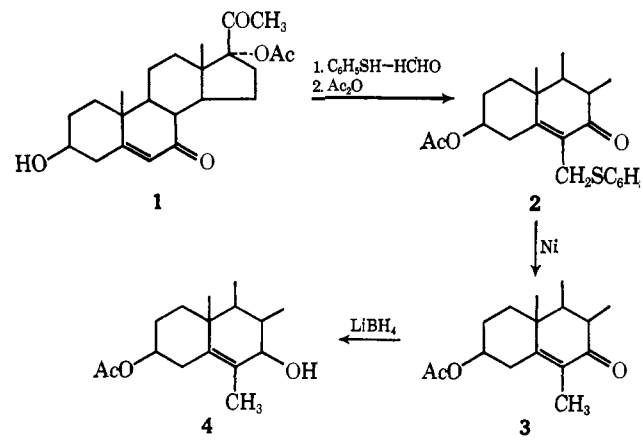
We recently reported the synthesis of some 6-chloro-3,7-dihydroxy-Δ^5-pregnene derivatives.[1] Because of the high progestational activity of several of these compounds we decided to prepare the 6-Me analogs.

Treatment of the Δ^5-7-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-Δ^5-7-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-Δ^5-3β-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-Δ^5-3β-ol acetate to yield the corresponding unstable 7α-chloro-Δ^5-compound, which was transformed on Al_2O_3 to the 5α-chloro-6-methylene isomer along with the 5α-hydroxy-Δ^6- and the 7α-hydroxy-Δ^5-6-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-Δ^5-6-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-Δ^6 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

(1) R. A. LeMahieu, A. Boris, M. Carson, and R. W. Kierstead, *J. Med. Chem.*, **14**, 291 (1971).

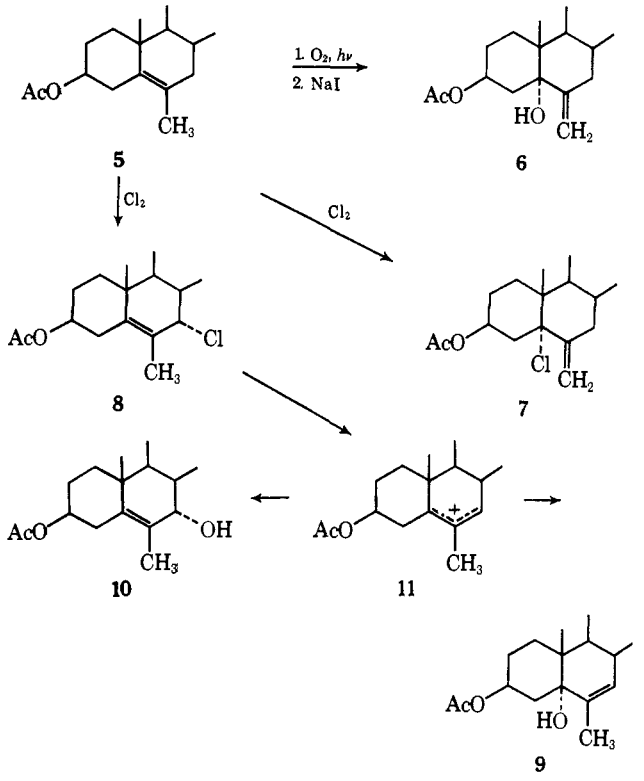
(2) This procedure has been used with Δ^4-3-ones by D. N. Kirk and V. Petrow, *J. Chem. Soc.*, 1091 (1962).

(3) N. S. Bhacca and D. H. Williams," Applications of Nmr Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, pp 51 and 80.

(4) H. R. B. Hutton and G. S. Boyd, *Biochim. Biophys. Acta*, **116**, 336 (1966).

(5) J. Iriarte, J. N. Shoolery, and C. Djerassi, *J. Org. Chem.*, **27**, 1139 (1962).

SCHEME II



nmr spectrum which revealed a broad C-7 proton band at δ 3.62 (half-bandwidth ~7 Hz). This is consistent with equatorial–axial coupling with the C-8 proton.[3] It seems likely that the isomeric alcohols **9** and **10** are both formed from the allylic carbonium ion **11**.

Biological Activity.—The compds were tested for progestational activity in a modified Clauberg–McPhail assay. Immature, New Zealand white rabbits (600–800 g body weight) were primed with 0.5 μg/day sc of estradiol benzoate in sesame oil for 5 consecutive days. The compds were dissolved or suspended in sesame oil and administered for 5 consecutive days following estrogen priming. All compds were tested at 1, 2, 4, 10, 20, 40, 100, 200, and 400 μg per day by both sc and oral routes of administration with 4–6 rabbits per group. Uterine sections were examined histologically for progestational activity beginning with the highest dosage group and progressing stepwise toward the lowest dosage level. The minimum dosages showing significant secretory development of the uterine endometrium (at least +1 on the McPhail scale) are listed in Table I.

TABLE I

Compd	Minimum effective dose, μg/day sc	po
17α-Hydroxyprogesterone acetate	4–10	200–400
4	10–20	10–20
6	Inactive at 400	Inactive at 400
9	20–40	10–20
10	10–20	10–20

The minimum effective dose for 17α-hydroxyprogesterone acetate is given for comparison purposes.

Experimental Section[6]

3β,17α-Dihydroxy-6-phenylthiomethylpregn-5-ene-7,20-dione Diacetate (2).—To 43.0 g (0.1 mole) of 3β,17α-dihydroxypregn-5-ene-7,20-dione diacetate[7] in 2.5 l. of MeOH and 200 ml of dioxane at 25° was added 110 ml (0.11 mole) of 1.0 *N* NaOH over 1 hr. After stirring for 1 hr longer, 10 ml of HOAc and 200 ml of H_2O were added and most of the solvent was removed *in vacuo*. The cryst solid was filtered, washed with H_2O, and air-dried to yield 36.6 g of crude 1. This was dissolved in 150 ml of *n*-BuOH and treated with 50 ml of C_6H_5SH, 70 ml of $N(CH_2CH_2OH)_3$, and 30 g of paraformaldehyde. The reaction mixt was stirred and refluxed for 43 hr, 25 ml of C_6H_5SH and 15 g of paraformaldehyde were added, and reflux was contd for 60 hr longer. After pouring into 1 l. of 3 *N* HCl and ice, the product was extd with C_6H_6. The ext was washed with 6 *N* NaOH, 1 *N* HCl, and H_2O, dried ($MgSO_4$), and concd *in vacuo*. The residual oil was chromatographed on 1.2 kg of silica gel. After several impurities were eluted with 10% EtOAc–C_6H_6, the desired product was eluted with 20% EtOAc–C_6H_6. The combined fractions (17.2 g) were acetylated by treatment with 100 ml of Ac_2O and 100 ml of C_5H_5N at 25° for 16 hr. The soln was concd at ~1 mm to yield a solid residue which was crystd from CH_2Cl_2–Et_2O to give 14.4 g (26%) of **2**: mp 155–157°; λ_{max} 248 mμ (ϵ 13,900); ν_{max} 1743, 1732, 1680, 1630, and 1598 cm^{-1}; $[\alpha]D$ −159.3°. *Anal.* ($C_{32}H_{40}O_6S$) C, H.

3β,17α-Dihydroxy-6-methylpregn-5-ene-7,20-dione Diacetate (3).—Raney Ni sludge (40 ml) was washed 4 times with Me_2CO by decantation and then refluxed with 200 ml of Me_2CO for 30 min. A soln of **2** (3.70 g) in 50 ml of Me_2CO was added and reflux with stirring was contd for 2.5 hr. The Ni was removed by filtration and washed with two 100-ml portions of hot EtOH. The combined filtrate was concd to dryness *in vacuo* and the residue was recrystd from CH_2Cl_2–Et_2O to yield 1.42 g (47%) of **3**: mp 180–182°; λ_{max} 243 mμ (ϵ 11,500); ν_{max} 1740, 1730, 1672, and 1630 cm^{-1}; $[\alpha]D$ −153.8°. The anal. sample, mp 184–185°, was obtd from the same solvent mixt. *Anal.* ($C_{26}H_{36}O_6$) C, H.

3β,7β,17α-Trihydroxy-6-methylpregn-5-en-20-one 3,17-Diacetate (4).—To 0.267 g (0.6 mmole) of **3** dissolved in 3 ml of anhyd THF was added at 25° 15 ml (1.5 mmoles) of 0.1 *M* $LiBH_4$ in THF. After stirring for 27 hr at 25°, HOAc was added to decompose the excess hydride. The solvent was removed *in vacuo*, H_2O was added, and the product was extd with CH_2Cl_2. The ext was washed with 5% $NaHCO_3$, dried ($MgSO_4$), and concd. Careful chromatography of the crude product on 15 g of silica gel and slowly increasing the polarity of the solvent from 1% to 5% EtOAc–C_6H_6 gave some residual **3**. The desired product was eluted with 5% and finally 10% EtOAc–C_6H_6. Crystn of the combined fractions from Et_2O–C_6H_{14} gave 0.021 g (8%) of **4**: mp 171–174°; ν_{max}^{KBr} 3540, 3425, 1735, 1716, and 1680 cm^{-1}; $[\alpha]D$ −42.4°. *Anal.* ($C_{26}H_{38}O_6$) C, H.

3β,5α,17α-Trihydroxy-6-methylenepregnan-20-one 3,17-Diacetate (6).—A soln of 2.001 g of **5** (Searle) and 0.105 g of methylene blue in 500 ml of MeOH was irradiated with two 15-W fluorescent bulbs while bubbling O_2 through the soln for 23 hr. The MeOH was removed *in vacuo* and 40 ml of CH_2Cl_2 and 100 ml of Et_2O were added. Charcoal (1 g) was added to the soln, swirled briefly, and filtered. Concn gave the crude hydroperoxide mixt which was reduced by stirring at 25° in 125 ml of MeOH and 3 ml of HOAc with 8 g of NaI for 19 hr. The addn of 70 ml of 0.1 *N* $Na_2S_2O_3$ gave a colorless soln which was concd *in vacuo* to a small vol. H_2O was added and the resultant solid was removed by filtration. Chromatography on 70 g of silica gel and elution with 4% EtOAc–C_6H_6 gave a minor impurity followed by pure **6**. Crystn of the combined fractions from Et_2O–C_6H_{14} gave 0.556 g (27%) of **6**: mp 196–198°; ν_{max} 3588, 1730, 1720, 1645, and 910 cm^{-1}; $[\alpha]D$ −33.0°. *Anal.* ($C_{26}H_{38}O_6$) C, H.

3β,5α,17α-Trihydroxy-6-methylpregn-6-en-20-one 3,17-Diacetate (9) and 3β,7α,17α-Trihydroxy-6-methylpregn-5-en-20-one 3,17-Diacetate (10).—To 1.0011 g (2.3 mmoles) of **5** (Searle) in 10 ml of CCl_4 and 0.1 ml of C_5H_5N cooled in an ice bath was added 2.50 ml (2.6 mmoles) of 1.04 *M* Cl_2 in CCl_4 over a 20-min period

(6) All melting points were taken in glass capillaries and are corrected. Rotations are in $CHCl_3$ at 25° at a concn of about 0.7%; uv spectra are of EtOH solutions, and ir spectra are in $CHCl_3$ solns. The nmr spectra were detd using a Varian A-60 or HA-100 spectrometer in $CDCl_3$ (Me_4Si). Where analyses are indicated only by symbols of the elements, anal. results for those elements were within ±0.3% of the theoretical values.

(7) C. W. Marshall, R. E. Ray, I. Laos, and B. Riegel, *J. Amer. Chem. Soc.*, **79**, 6308 (1957).

with stirring. After stirring for 30 min at 3°, 5 ml of 5% $NaHCO_3$ was added, and the CCl_4 layer was sepd, dried ($MgSO_4$), and concd *in vacuo*. Tlc of the crude product revealed the presence of approximately equal amts of 3 compounds. Preparative tlc on Brinkman silica gel plates served to sep the compds. The fastest moving compd was not obtained in pure form. The component of intermediate mobility was eluted from the silica gel with $CHCl_3$–EtOH (1:1) and crystd from Et_2O–C_6H_{14} to give 0.1733 g (17%) of **9**: mp 214.5–216.5; ν_{max} 3600, 1737, 1730, and 1720 cm^{-1}; $[\alpha]D$ −23.7°. *Anal.* ($C_{26}H_{38}O_6$) C, H.

The slowest moving compd was crystd from Et_2O–C_6H_{14} to yield 0.1793 g (18%) of **10**: mp 193–195°; ν_{max} 3600, 1738, 1730, and 1718 cm^{-1}; $[\alpha]D$ −103.5°. *Anal.* ($C_{26}H_{38}O_6$) C, H.

Acknowledgment.—We wish to thank the following members of our physical chemistry department (Dr. P. Bommer, director): Dr. V. Toome, Mr. S. Traiman, and Dr. T. Williams for the uv, ir, and nmr spectra, respectively. Thanks are also due Dr. F. Scheidl for the microanalyses.

Angiotensin II Analogs. 5. [2-Glycine]angiotensin II and Related Analogs[1]

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The amino and carboxyl groups of the aspartyl residue in angiotensin II, Asp-Arg-Val-Tyr-Ile(or Val)-His-Pro-Phe, are not essential for pressor activity although they do possess features which are necessary for maximal activity. Thus the elimination of the NH_2 group or the carboxymethyl group of the aspartyl residue of angiotensin II gives analogs which retain half of the pressor activity of the parent compound.[2,3] Many analogs have been synthesized in attempts to discover which features of these functional groups are responsible for the enhanced pressor activity. However, these analogs, which are listed in Table I, do not reveal which property of the aspartic acid residue makes the greatest contribution to the pressor activity. One reason for this may be the proximity of the guanidino group of arginine which appears to make a greater contribution to the observed pressor activity. The presence of this guanidino group may overshadow the contributions of aspartic acid or its analogs making evaluation of these contributions more difficult.

This report describes some analogs of angiotensin II containing glycine in place of arginine which were synthesized in the hope that the elimination of the guanidino function would give a system which would be more sensitive to the contributions of the aspartyl residue. Glycine was chosen as a replacement for arginine because it seemed undesirable to replace the very polar guanidinopropyl side chain with a hydrophobic one.

TABLE I
ANGIOTENSINS MODIFIED IN POSITION 1

Peptide	Pressor activity
Asp-Arg-Val-Tyr-Val-His-Pro-Phe	100
D-Asp-Arg-Val-Tyr-Val-His-Pro-Phe	150[a]
β-Asp-Arg-Val-Tyr-Val-His-Pro-Phe	150[a]
Asn-Arg-Val-Tyr-Val-His-Pro-Phe	100[b]
Glu-Arg-Val-Tyr-Val-His-Pro-Phe	150[c]
Pyroglu-Arg-Val-Tyr-Val-His-Pro-Phe	150[c]
Gly-Arg-Val-Tyr-Val-His-Pro-Phe	50[d]
Succinyl-Arg-Val-Tyr-Val-His-Pro-Phe	50[a]
Arg-Val-Tyr-Val-His-Pro-Phe	50[d]

[a] B. Riniker and R. Schwyzer, *Helv. Chim. Acta*, **47**, 2357 (1964). [b] W. Rittel, B. Iselin, H. Kappeler, B. Riniker, and R. Schwyzer, *ibid.*, **40**, 614 (1958). [c] E. Schroder, *Justus Liebigs Ann. Chem.*, **691**, 232 (1966). [d] See R. Schwyzer, *Helv. Chim. Acta*, **44**, 667 (1961).

Asp-Gly-Val-Tyr-Ile-His-Pro-Phe was prepared in solution from the purified heptapeptide and Z-Asp-(OBzl)-ONp and the protecting groups were removed by catalytic hydrogenolysis because of the reported danger of succinimide formation when peptides containing an Asp(OBzl)-Gly sequence are exposed to HBr during cleavage of the peptide from a solid-phase polymer.[4] Model experiments appeared to confirm this side reaction. The same approach was used in the syntheses of Asn-Gly-Val-Tyr-Ile-His-Pro-Phe and succinamyl-Gly-Val-Tyr-Ile-His-Pro-Phe although the danger of cyclization was less in these cases. Succinyl-Gly-Val-Tyr-Ile-His-Pro-Phe was prepared on the solid-phase polymer[5] and purified by anion-exchange chromatography. The pressor activities and durations of response were determined in the rat as described earlier.[6]

Results and Discussion

The pressor activities of the peptides described in this paper and related peptides reported earlier are shown in Table II. These results show that CO_2H makes no contribution to the pressor activity of [Gly^2]-angiotensin II, while $CONH_2$ is unfavorable. This is clearly in conflict with the results found in angiotensin II itself, suggesting that the Gly^2 series may not be a valid system for studying the contributions of aspartic acid to the pressor activity of angiotensin II.

The decreased pressor activity of peptide IV compared with VI and the equivalent pressor activity of V and VI is consistent with the suggestion[7,8] that the terminal amino group in VI might be binding at the "guanidino" binding site since neither a carboxyl nor a carboxamido group need be expected to aid in, and could interfere with, binding at this site. The low

(1) Part 4: E. C. Jorgensen, G. C. Windridge, and T. C. Lee, *J. Med. Chem.*, **13**, 744 (1970). This investigation was supported in part by Public Health Service Research Grants AM 08066 and AM 06704 from the National Institutes of Arthritis and Metabolic Diseases and Training Grant No. 5 T01 GM 00728 from the National Institute of General Medical Sciences. The abbreviations used to denote amino acid derivatives and peptides are those recommended in *Biochemistry*, **5**, 2485 (1966).

(2) B. Riniker and R. Schwyzer, *Helv. Chim. Acta*, **47**, 2357 (1964).

(3) R. Schwyzer, *ibid.*, **44**, 667 (1961).

(4) M. A. Ondetti, A. Deer, J. T. Sheehan, J. Pluscec, and O. Kocy, *Biochemistry*, **7**, 4069 (1968).

(5) R. B. Merrifield, *ibid.*, **3**, 1385 (1964).

(6) E. C. Jorgensen, G. C. Windridge, and T. C. Lee, *J. Med. Chem.*, **13**, 352 (1970).

(7) E. C. Jorgensen, G. C. Windridge, W. Patton, and T. C. Lee, *ibid.*, **12**, 733 (1969).

(8) E. C. Jorgensen, G. C. Windridge, W. Patton, and T. C. Lee, in "Peptides: Chemistry and Biochemistry," B. Weinstein and S. Lande, Ed., Marcel Dekker, Inc., New York, N. Y., 1970, pp 113–125.