peptide resin (2.5 g) gave 7 as an amorphous powder. It was purified as for 5, wt 550 mg (Table III). Amino acid analysis[19] gave Asp, 1.05; Glu, 1.02; Gly, 1.00; Bzl-Cys, 0.97; Ile, 0.89; Tyr, 0.81; Pro, 0.97; Arg, 0.91; NH_3, 2.94.

[Deamino,8-arginine]vasotocin (8). Reduction, reoxidation, and purification of 7 (150 mg) as for 1 gave 8 as a white fluffy powder, wt 46 mg, shown to be homogeneous by thin-layer chromatography and by electrophoresis.[18] Amino acid analysis[19] gave Asp, 1.03; Gly, 0.97; Glu, 1.05; Pro, 0.90; Tyr, 0.91; Ile, 0.92; Arg, 1.00; NH_3, 2.90; cystine, 0.40; mixed disulfide, 0.51.

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References

(1) M. Manning and W. H. Sawyer, *Pharmacologist*, 13, 214 (1971)
(2) M. Manning, E. J. Coy, W. H. Sawyer, and M. Acosta, *J. Med. Chem.*, 16, 463 (1973).
(3) M. Manning, E. J. Coy, and W. H. Sawyer, *Experientia*, 27, 1372 (1971).
(4) (a) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, *J. Biol. Chem.*, 235, 64 (1960); (b) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, 237, 1563 (1962).
(5) V. du Vigneaud, G. Flouret, and R. Walter, *ibid.*, 241, 2093 (1966).
(6) L. A. Branda, S. Drabarek, and V. du Vigneaud, *ibid.*, 241, 2572 (1966).
(7) H. Takashima, V. J. Hruby, and V. du Vigneaud, *J. Amer. Chem. Soc.*, 92, 677 (1970).
(8) G. Flouret and V. du Vigneaud, *J. Med. Chem.*, 12, 1035 (1969).
(9) R. L. Huguenin and R. A. Boissonnas, *Helv. Chim. Acta*, 49, 695 (1966).
(10) R. D. Kimbrough, Jr., W. D. Cash, L. A. Branda, W. Y. Chan, and V. du Vigneaud, *J. Biol. Chem.*, 238, 1411 (1963).
(11) E. Schillinger, O. Loge, E. Schroder, E. Klieger, and K. Lubke, *Eur. J. Biochem.*, 27, 473 (1972).
(12) W. H. Sawyer in "The Pituitary Gland," Vol. 3, G. W. Harris and B. T. Donovan, Ed., Butterworths, London, 1966, p 288.
(13) D. Gillessen and V. du Vigneaud, *J. Biol. Chem.*, 242, 4806 (1967).
(14) D. Gillessen and V. du Vigneaud, *J. Med. Chem.*, 13, 346 (1970).
(15) R. B. Merrifield, *J. Amer. Chem. Soc.*, 85, 2149 (1963).
(16) R. B. Merrifield, *Biochemistry*, 3, 1385 (1964).
(17) M. Manning, *J. Amer. Chem. Soc.*, 90, 1348 (1968).
(18) M. Manning, E. Coy, and W. H. Sawyer, *Biochemistry*, 9, 3925 (1970).
(19) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).
(20) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, 108, 753 (1935).
(21) V. du Vigneaud, C. Ressler, J. M. Swan, P. Katsoyannias, and C. W. Roberts, *J. Amer. Chem. Soc.*, 76, 3115 (1954).
(22) V. du Vigneaud, C. Ressler, J. M. Swan, P. Katsoyannias, C. W. Roberts, and S. Gordon, *ibid.*, 75, 4879 (1953).
(23) M. Manning, T. C. Wuu, and J. W. M. Baxter, *J. Chromatogr.*, 38, 396 (1968).
(24) J. W. M. Baxter, M. Manning, and W. H. Sawyer, *Biochemistry*, 8, 3592 (1969).

3-(16β,17β-Dihydroxy-3-oxoandrost-4-en-17α-yl)propionic Acid γ-Lactone, Its Preparation and Antimineralocorticoid Activity†

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The title compound was prepared in which dehydroisoandrosterone was employed as the starting material. The 16β-hydroxyl group was introduced by lead tetraacetate oxidation and was protected as the acetonide during the construction of the spirolactone ring. The antimineralocorticoid potency of the final product was less than 13.8% that of spironolactone.

Aldosterone (1) is a potent mineralocorticoid which plays an important role in regulating the electrolyte composition of the body fluid. In promoting the excretion of potassium and the retention of sodium ions, it gives rise to the edematous state that is often seen in patients afflicted with congestive heart failure, nephrosis, or cirrhosis of the liver.[1]

A rational approach to the treatment of these patients would be to block the activity of aldosterone and related mineralocorticoids, such as deoxycorticosterone (2a). Since some steroids having a spirolactone side chain at C-17, *e.g.*, 3a, have been found to be aldosterone blockers,[2] attempts have been made to modify their structures in the hope of obtaining compounds which would be even more potent.[3]

A structural feature that is common to both aldosterone and the spirolactones is the oxaspiran unit in which a tetrahydrofuran ring is fused to ring D. In aldosterone the spiro atom is C-13, while in the spirolactones it is C-17.[4]

The possibility that an oxaspiran unit is involved in some manner with the transport of Na^+ and K^+ across cellular membranes is purely speculative, but it is an intriguing one and it has been extensively explored in the search for compounds having a diuretic effect.[3]

Metabolic studies[5] offer another source from which new leads can be generated. Conceivably, a metabolic product of the administered compound is the active species. Hydroxylation is one means by which compounds are metabolized *in vivo*. Because of the ease with which a hydroxyl group can be introduced into a molecule by microorganisms, numerous spirolactones having a hydroxyl group at various positions in the molecule have been prepared and tested for their antimineralocorticoid effects.[3b,6] To date, however, there has been no report of the preparation of the spirolactone in which a hydroxyl group is located at the 16β position (3b). In view of the claims made that certain 16-hydroxylated steroids possess sodium-excreting properties,[7] the effect which 3b exerts on the mineralocorticoids should be of interest.

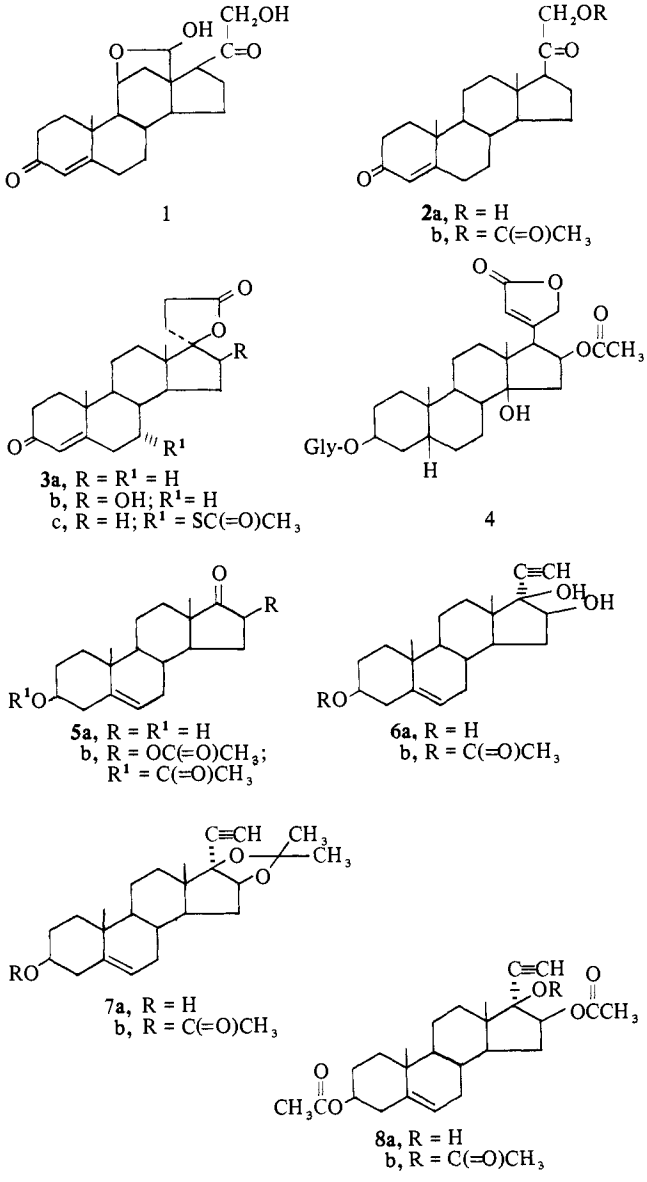
Since cardiac steroids are known to affect electrolyte transport,[8] a recent report that 16-acetylgitoxin (4) produced favorable cardiotonic effects[9] prompts us to report the synthesis of the 16β-hydroxyspirolactone (3b) and the effect which it displayed in our antimineralocorticoid test.

†A portion of this work was presented at the 164th National Meeting of the American Chemical Society, New York, N. Y., Aug 1972.

The starting compound for the synthesis, dehydroisoandrosterone (**5a**), was converted to the enol acetate, and the latter was oxidized with lead tetraacetate to the 16β-acetoxy derivative **5b**.[10] Addition of ethynylmagnesium bromide to **5b** gave a mixture of two products. One was the triol **6a** and the other was **6b**, the monoacetate of **6a**. Conversion of **6b** to the acetonide **7b** revealed that the acetate group which was removed in the addition of ethynylmagnesium bromide was the one at C-16. Hydrolysis of **6b** gave **6a** thus demonstrating that **6a** and **6b** had the same structural feature and stereochemistry at C-16 and -17.

The triol **6a** afforded the diacetate **8a** under the usual acetylating conditions. Regeneration of **6a** from **8a** was achieved with potassium carbonate in methanol. Under more vigorous acetylating conditions both **6a** and **8a** were converted to the triacetate **8b** (Chart I).

Chart I



The triol **6a** was converted to the acetonide **7a** which furnished **7b** upon acetylation. Oppenauer oxidation of the acetonide **7a** gave the α,β-unsaturated ketone **9**. Acid removal of the acetonide group from **9** then yielded 17α-ethynyl-16β-hydroxytestosterone (17α-ethynyl-3-oxoandrost-4-ene-16β,17β-diol, **10**).

The ethynyl proton in this series of compounds resonates in the region of 154-158 Hz (*cf.* Table I) which is in agreement with the observation of Engelfried, Gibian, Neumann, Prezewowsky, Schulz, and Wiechert.[11]

Table I. Comparison of Nmr Signals

Compound	Ethynyl proton signal, Hz
17α-Ethynylestradiol 3-methyl ether[a]	156
16α-Acetoxy-17α-ethynylestradiol 3-methyl ether[a]	159
16β-Acetoxy-17α-ethynylestradiol 3-methyl ether[a]	159
17β-Ethynyl-17α-estradiol 3-methyl ether[a]	150
16α-Acetoxy-17β-ethynyl-17α-estradiol 3-methyl ether[a]	150
17α-Ethynylandrost-5-ene-3β,16β,17β-triol 3,16-diacetate (8a)	157
17α-Ethynylandrost-5-ene-3β,16β,17β-triol 3-acetate (6b)	154
17α-Ethynylandrost-5-ene-3β,16β,17β-triol 16,17-acetonide (7a)	157.5
17α-Ethynylandrost-5-ene-3β,16β,17β-triol 3,16,17-triacetate (8b)	158
17α-Ethynyl-3-oxoandrost-4-ene-16β,17β-diol acetonide (9)	157.5

[a] See ref 11.

Table II. Comparison of Optical Rotations

Compound	[α]D, deg
17α-Ethynylandrost-5-ene-3β,16β,17β-triol (**5a**)	−103.5 (dioxane)
16α-Ethynylandrost-5-ene-3β,16β,17β-triol[a]	−65.8 ($CHCl_3$)
17α-Ethynylandrost-5-ene-3β,16β,17β-triol triacetate (**8b**)	−76 ($CHCl_3$)
16α-Ethynylandrost-5-ene-3β,16β,17β-triol triacetate[a]	−66.7 ($CHCl_3$)
17α-Ethynylandrost-5-ene-3β,16β,17β-triol 16,17-acetonide (7a)	−91 ($CHCl_3$)
16α-Ethynylandrost-5-ene-3β,16β,17β-triol 16,17-acetonide[a]	−16.2 ($CHCl_3$)
17α-Ethynylandrost-5-ene-3β,16β-17β-triol 3-acetate 16,17-acetonide (7b)	−84.5 ($CHCl_3$)
16α-Ethynylandrost-5-ene-3β,16β,17β-triol 3-acetate 16,17-acetonide[a]	−22.5 ($CHCl_3$)

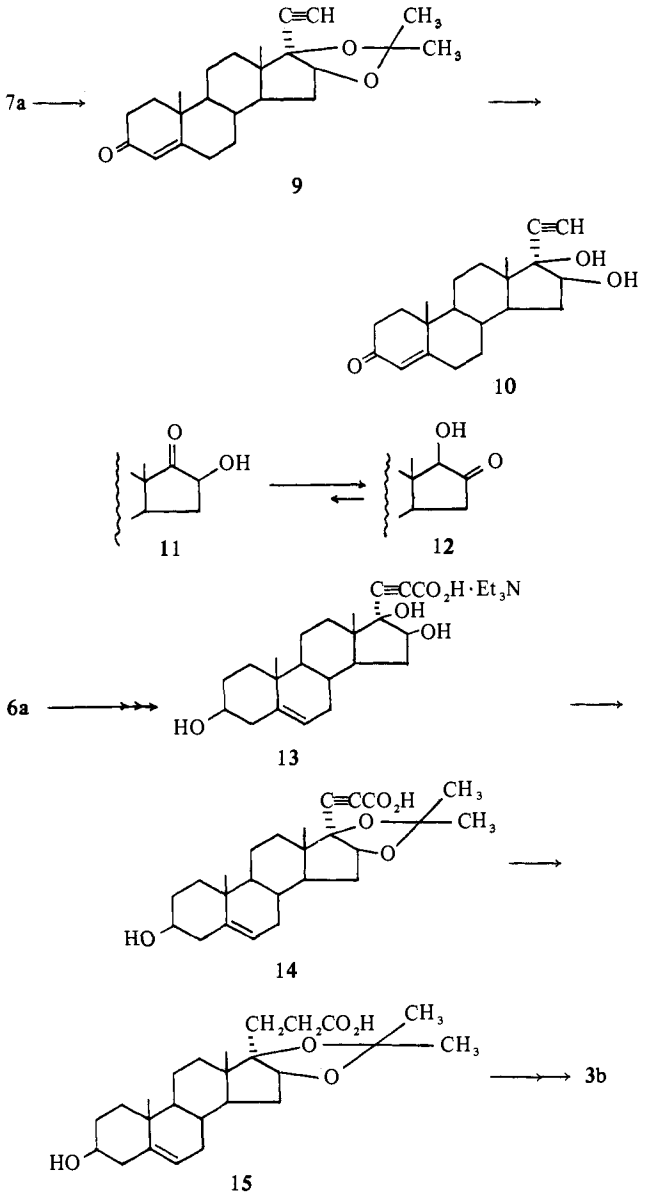
[a] See ref 13.

To eliminate the possibility that the ethynyl group was at C-16 rather than at C-17, a possibility which could arise as a result of the cleavage of the acetate group at C-16, followed successively by an α-ketol isomerization (**11** → **12**)[10,12] and an addition of ethynylmagnesium bromide to the carbonyl group at C-16, a direct comparison was made of the compounds which we had prepared with those prepared by Iwai and Hiraoko.[13,‡] The nonidentity (by mixture melting point) of the two series of compounds established conclusively the position of the ethynyl group in our series of compounds. It is interesting to note that the compounds in which the ethynyl group is located at the 17α position are more levorotatory than the corresponding compounds in which the ethynyl group is attached to the 16α bond (*cf.* Table II).

Successive treatment of the triol **6a** with methylmagnesium bromide and carbon dioxide gave the propiolic acid, isolated as the triethylamine salt **13**. Conversion of **13** to the acetonide **14** was readily accomplished with acetone and hydrochloric acid. Reduction of **14**, as the triethylamine salt, resulted in the saturation of the triple bond. Upon acidification, the acid **15** was obtained. Oxidation of **15** with Jones reagent, followed by acid-induced hydrolysis of the aceto-

‡ We are indebted to Dr. Iwai for providing us the 16α-ethynyl compounds for comparison.

Scheme I



nide group and migration of the Δ^5 double bond, gave a lactonic product (Scheme I).

Cleavage of the acetonide protective group afforded initially a 16β,17β-diol. Elimination of water to yield the lactone could be expected to occur with either the 16β- or the 17β-hydroxyl group. With the former, a six-membered lactone would be the product. Steroids bearing a six-membered lactone ring are known, and their spectral characteristics have been described.[14]

With the 17β-hydroxyl group, cyclodehydration would furnish a five-membered spirolactone. The infrared spectrum of the dehydrated product displayed a carbonyl absorption at 5.61 μ which is consistent only with a five-membered lactone. The upfield shift of the 16α proton in the nmr spectrum supported the presence of the hydroxyl group at C-16. Thus, lactonization involved the hydroxyl group at C-17 and not the one at C-16, and the product is the desired compound, 3-(16β,17β-dihydroxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone (**3b**).

The ability of **3b** to reverse the renal effects (decrease in urinary sodium/potassium ratio) of the mineralocorticoid, deoxycorticosterone acetate (**2b**), was determined in an acute test in which adrenalectomized male rats were employed.[2,3c,15] An increase in the log (Na × 10)/K ratio equivalent to that produced by a standard dose (0.33 mg/rat) of spironolactone [3-(3-oxo-7α-acetylthio-17β-hydroxyandrost-4-en-17α-yl)propionic acid lactone] **3c** was the index employed for determining antimineralocorticoid activity.

Table III. Data from Antimineralocorticoid Studies

Treatment	N^a	Urinary log (Na × 10)/K[b]	Test − DCA response[c]
Deoxycorticosterone acetate (2b)	10	0.806 ± 0.073	
Spironolactone (3c)	10	1.059 ± 0.072	0.253 ± 0.052
Deoxycorticosterone acetate (2b)	1	0.786	
Spironolactone (3c)	1	0.966	0.180
3-(16β,17β-Dihydroxy-3-oxoandrost-4-en-17α-yl)propionic acid γ-lactone (3b)	1	0.836	0.050

[a] *N* represents the number of tests conducted. Each test consists of a pooled 4-hr urine sample from eight rats. For control, 9 mcg/rat of deoxycorticosterone acetate (DCA) was given subcutaneously. Spironolactone was administered subcutaneously at a dosage of 0.33 mg/rat together with 9 mcg/rat of DCA. Compound **3b** was given subcutaneously at a dose level of 2.4 mg/rat in addition to 9 mcg/rat of DCA. [b] Mean ± 95% fiducial limits as index of activity. [c] Mean difference ± 95% fiducial limits (paired analysis) for difference between DCA response and that of the test compound.

In Table III are compiled data from ten consecutive tests delineating the variation in the Na/K ratio response for 9 mcg of DCA alone and following coadministration of spironolactone. The mean difference of 0.253 ± 0.052 log unit corresponds to a 1.8-fold increase in the arithmetic Na/K ratio, and it represents the effect produced by the standard dose of spironolactone.

When tested subcutaneously at 2.4 mg/rat, compound **3b** elevated the log (Na × 10)/K ratio by 0.050 log unit over that of the control. In the same test a 0.33 mg/rat dose of spironolactone, given subcutaneously, raised the same ratio by 0.180 log unit over that of the control. Hence, the antimineralocorticoid activity of **3b** is considerably less than 13.8% (0.33 × 100/2.4) that of spironolactone. In contrast, **3a**, the congener of **3b** in which the 16β-hydroxyl group is missing, has a subcutaneous potency that is 150% that of spironolactone.[16]

Experimental Section

Melting points were taken on a Fisher-Johns melting point apparatus and are corrected. Nmr spectra were taken on a Varian A-60 in deuteriochloroform with tetramethylsilane as the standard. Unless specified otherwise, optical rotations were determined in chloroform at 26° (*c* 1).

17-Oxoandrost-5-ene-3β,16β-diol Diacetate (5b).[10] A solution of 100 g of dehydroisoandrosterone (**5a**), 800 ml of isopropenyl acetate, and 8.0 g of *p*-toluenesulfonic acid monohydrate was slowly distilled over a 24-hr period when 500 ml of distillate was collected. The cooled residue was diluted with ether and 5% $NaHCO_3$. The ether phase was separated, washed successively with H_2O and saturated NaCl, dried (Na_2SO_4), and concentrated under reduced pressure until a crystalline product appeared. The residue was cooled to 5° The enol acetate was collected, washed with pentane, and dried: yield 92.4 g; mp 145.5–148°

A 10-g sample of the enol acetate, 150 ml of glacial HOAc, 5 ml of Ac_2O, and 11.85 g of $Pb(OAc)_4$ was stirred at room temperature in an atmosphere of nitrogen for 15 hr. The reaction mixture was evaporated to dryness under reduced pressure at 40°. The residue was diluted with a mixture of C_6H_6 and ether. The solid was removed by filtration. The filtrate was washed successively with H_2O, 5% $NaHCO_3$, H_2O again, and saturated NaCl. After drying over Na_2SO_4, it was distilled to dryness under reduced pressure. The solid residue was crystallized from ether–pentane to afford 6.60 g

of **5b**, mp 173.5–176.5°. Admixed with 17-oxoandrost-5-en-3β-ol acetate, it melted at 143–170°.

17α-Ethynylandrost-5-ene-3β,16β,17β-triol (6a) and 17α-Ethynylandrost-5-ene-3β,16β,17β-triol 3-Acetate (6b). To 250 ml of redistilled THF, saturated with acetylene, was added portionwise at room temperature with stirring and with continuous passage of acetylene 175 ml of a solution of EtMgBr in THF (prepared by mixing 120 ml of 3 *M* EtMgBr in diethyl ether and 250 ml of redistilled THF and concentrating the resultant mixture to 175 ml by distillation under reduced pressure). The mixture was then cooled in an ice–EtOH bath. To the stirred mixture was added dropwise over 0.5 hr a solution of 8.23 g of **5b** in 150 ml of redistilled THF while acetylene continued to be passed into the reaction mixture. Passage of acetylene was stopped, and the reaction mixture was stirred in the ice–EtOH bath for 14 hr, the ice bieng allowed to melt. After it was heated to reflux for 10 min, the reaction mixture was concentrated under reduced pressure with gentle heating. The residue was treated with 200 ml of 7 *N* H_2SO_4 and extracted with a large volume of ether. The ether extract was washed successively with H_2O and saturated NaCl, dried (Na_2SO_4), and concentrated to a small volume by distillation under reduced pressure to afford 1.88 g of **6a**, mp 217–233°. Crystallization from EtOAc gave 1.23 g of **6a**, mp 245–254°. Further crystallization from EtOAc raised the melting point to 247.5–252°; [α]D −103.5° (dioxane); λ^{KBr} 2.92, 3.06, 4.72 μ. *Anal.* ($C_{21}H_{30}O_3$) C, H. Admixed with 16α-ethynylandrost-5-ene-3β,16β,17β-triol,[13] it melted at 228–239°.

The ether mother liquor was evaporated to dryness. The residue was chromatographed on 300 g of SiO_2. Elution of the column with 10% EtOAc–90% C_6H_6 afforded 0.73 g of **6b** as a semisolid. Repeated crystallization from ether–pentane furnished 0.16 g of **6b**, mp 184–186°. The analytical sample melted at 188.5–191°; nmr (Hz) 325, 321.5 (6-H), 274.5 (3-H), 260.5, 256.5, 251 (16-H), 196 (OH), 154 (C≡CH), 122.5 ($CH_3C(=O)$), 63 (19-CH_3), 53 (18-CH_3). *Anal.* ($C_{23}H_{32}O_4$) C, H. Further elution of the column with 25% EtOAc–75% C_6H_5 gave, after recrystallization from EtOAc, 0.97 g of the triol **6a**, mp 251–256°. Hydrolysis of **6b** with potassium carbonate in MeOH–H_2O at room temperature overnight afforded **6a**.

17α-Ethynylandrost-5-ene-3β,16β,17β-triol 3,16-Diacetate (8a). A solution of 195 mg of **6b**, 2 ml of C_5H_5N, and 2 ml of Ac_2O was allowed to stand at room temperature for 15 hr after which time it was diluted with ice H_2O. The solid was collected, washed with H_2O, and dried. Crystallization from ether afforded 168 mg of **8a**: mp 221–222°; [α]D −76°; λ^{KBr} 2.93, 3.07, 4.72, 5.77, 8.03 μ; nmr (Hz) 325, 321, 318, 314, 310, 305.5, 277, 157, 145.5 (OH), 126.5, 122, 63.5, 54.5. *Anal.* ($C_{25}H_{34}O_5$) C, H. Admixed with 16α-ethynylandrost-5-ene-3β,16β,17β-diol 3,16-diacetate,[13] it melted at 185–189° In a similar manner, **6a** was converted to **8a**. Hydrolysis of **8a** to **6a** was accomplished overnight at room temperature with K_2CO_3 in MeOH–H_2O.

17α-Ethynylandrost-5-ene-3β,16β,17β-triol 3,16,17-Triacetate (8b). A solution of 1.16 g of the triol **6a**, 10 ml of C_5H_5N, and 10 ml of Ac_2O was maintained at 60° for 4 days after which time it was diluted with ice H_2O. The solid product was collected, washed with H_2O, dried, and crystallized from ether to furnish 1.16 g of **8b**: mp 212–215°; λ^{KBr} 3.08, 4.76, 5.73, 5.78, 8.03 μ; nmr (Hz) 337, 332.5, 325, 320, 275, 158, 123.5, 121.5, 119, 62.5, 58.5; [α]D −40.5°. *Anal.* ($C_{26}H_{36}O_6$) C, H. Admixed with the monoacetate **6b**, it melted at 187.5–210°. In a similar manner **6b** was converted to **8b**.

17α-Ethynylandrost-5-ene-3β,16β,17β-triol 16,17-Acetonide (7a). To a warm solution of 1.5 g of the triol **6a** in 150 ml of acetone was added 0.5 ml of 12 *N* HCl. The reaction mixture was allowed to stand at room temperature for 3 hr. Water was added to the reaction mixture until it became turbid. Then it was allowed to stand in an ice bath whereupon a crystalline product formed. More H_2O was added. The pale yellow solid was collected, washed with water, and dried: mp 184–186°; yield 1.6 g. A 253-mg sample was crystallized from ether–hexane to afford 174 mg of **7a**: mp 186–187°; nmr (Hz) 324, 320, 290, 286.5, 281.5, 278, 210, 157.5, 107.5 (OH), 90.5, 62, 55; [α]D −91°. *Anal.* ($C_{24}H_{34}O_4$) C, H.

17α-Ethynylandrost-5-ene-3β,16β,17β-triol 3-Acetate 16,17-Acetonide (7b). (a) Acetylation of 275 mg of 7a with 5 ml of C_5H_5N and 5 ml of Ac_2O, followed by the usual work-up, afforded a quantitative yield of 7b, mp 140–141.5°. Crystallization from ether–hexane raised the melting point to 142.5–143.5°; yield 235 mg; λ^{KBr} 3.07, 5.76, 7.98 μ; nmr (Hz) 324.5, 288.5, 284.5, 281, 157.5, 122, 90.5, 62.5, 54.5; [α]D −84.5°. *Anal.* ($C_{26}H_{36}O_4$) C, H

(b) A solution of 100 mg of **6b**, 10 ml of acetone, and 0.05 ml of 12 *N* HCl was allowed to stand at room temperature for 22 hr. Dilution with H_2O gave 76 mg of 7b, mp 138–140°. After crystallization from ether–pentane, it melted at 143–143.5°. Mixture melting point and ir indicated the products from the two procedures were the same.

17α-Ethynyl-3-oxoandrost-4-ene-16β,17β-diol Acetonide (9). A solution of 1.19 g of 7a, 40 ml of toluene, and 6 ml of cyclohexanone was distilled until 10 ml of distillate was collected. To the residue was added a solution of 319 mg of aluminum isopropoxide in 10 ml of toluene. The reaction mixture was distilled until 20 ml of distillate was collected. The remainder of the reaction mixture was heated under reflux for 1 hr. The cooled reaction mixture was diluted with ether and a saturated solution of Rochelle salt. The ether phase was separated, washed with H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The semisolid residue was treated with hexane whereupon it turned to a crystalline product. The solid was collected, washed with hexane, and dried: yield 0.91 g; mp 212.5–215.5°. Crystallization from ether–hexane gave **9**: mp 217.5–219.5°; nmr (Hz) 344.5, 289, 284.5, 281, 277, 157.5, 89.5, 72.5, 56.5; λ^{KBr} 3.07, 6.01, 6.22 μ; λ_{max}^{MeOH} 240.5 nm (ε 15,200); [α]D +43°. *Anal.* ($C_{24}H_{32}O_3$) C, H.

17α-Ethynyl-3-oxoandrost-4-ene-16β,17β-diol (10). To a solution of 530 mg of **9** in 15 ml of 95% EtOH was added 10 ml of 5% HCl. The reaction mixture was warmed until the solid, which separated, redissolved. The mixture was stirred at room temperature for 15 hr. Then it was diluted with H_2O and neutralized with 5% $NaHCO_3$. A stream of nitrogen was passed into the mixture as it was gently warmed to remove the alcohol. The residue was cooled to 5° The solid was collected, washed with H_2O, and dried. It was crystallized from EtOAc to afford 245 mg of product, mp 241.5–247° Another crystallization from EtOAc gave 189 mg of **10**: mp 248–251°; λ^{KBr} 2.97, 3.02, 4.76, 6.00, 6.22 μ; [α]D +45°. *Anal.* ($C_{21}H_{28}O_3$) C, H.

3-(3β,16β,17β-Trihydroxyandrost-5-en-17α-yl)propiolic Acid Triethylamine Salt (13). To a solution of 938 mg of the ethynyl triol **6a** in 75 ml of redistilled THF was added 25 ml of 3 *M* EtMgBr in diethyl ether. The mixture was distilled under reduced pressure to remove the ether. The residue was then heated under reflux at atmospheric pressure for 24 hr. The mixture was cooled in an ice bath and stirred. A gentle stream of carbon dioxide was passed over the mixture for a period of 40 hr. The ice was allowed to melt, and the reaction mixture was allowed to come to room temperature during this time. The mixture was treated with 1.8 *N* H_2SO_4 and extracted with EtOAc. The EtOAc extract was washed successively with 1.8 *N* H_2SO_4, H_2O, and saturated NaCl, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The solid residue was dissolved in 30 ml of hot THF. To the solution was added 1 ml of triethylamine whereupon a crystalline product formed. The mixture was allowed to stand at 5° for 1 hr. The solid was then collected, washed with THF, and dried: yield 903 mg; mp 235–238.5°; λ^{KBr} 2.93, 4.47, 6.24 μ.

3-(3β,16β,17β-Trihydroxyandrost-5-en-17α-yl)propiolic Acid 16,17-Acetonide (14). A solution of 680 mg of the trihydroxy acid **13**, 20 ml of acetone, and 0.12 ml of 12 *N* HCl was stirred at room temperature for 16 hr. Then it was poured into ice H_2O. The crystalline product was collected, washed with H_2O, and dried, mp 189–201.5° with evolution of gas. Crystallization from ether–pentane afforded 501 mg of **14**: mp 200.5–205.5° with evolution of gas; λ^{KBr} 2.97, 4.49, 5.92, 6.00 μ. *Anal.* ($C_{25}H_{34}O_5$) C, H.

3-(3β,16β,17β-Trihydroxyandrost-5-en-17α-yl)propionic Acid 16,17-Acetonide (15). A mixture of 614 mg of **14**, 0.24 ml of triethylamine, and 25 ml of absolute EtOH was hydrogenated over 60 mg of 5% Pd/C at room temperature and atmospheric pressure. After the calculated amount of hydrogen was absorbed in 4 hr, hydrogenation was stopped. The catalyst was removed by filtration. The filtrate was evaporated to dryness. The solid residue was suspended in H_2O, and the mixture was treated with 2 ml of 5% HCl, whereupon a voluminous precipitate formed. After standing at 5° for 2 hr, the precipitate was collected, washed with H_2O, and dried. Crystallization from acetone yielded 477 mg of **15**: mp 227–229.5°; λ^{KBr} 2.99, 5.87 μ. *Anal.* ($C_{25}H_{38}O_5$) C, H. Concentration of the mother liquor afforded an additional 95 mg of **15**, mp 224.5–228°

3-(16β,17β-Dihydroxy-3-oxoandrost-4-en-17α-yl)propionic Acid γ-Lactone (3b). A heterogeneous mixture of 1.07 g of **15** and 50 ml of acetone was stirred in an ice–EtOH bath. To this mixture was added portionwise over a period of 5 min 0.65 ml of Jones reagent (8 *N* CrO_3) in 5 ml of acetone. The reaction mixture was stirred for 1.5 hr, the ice being allowed to melt. After 1 ml of *i*-PrOH and 20 ml of H_2O were added, the mixture was concentrated on the steam bath in a stream of N_2 to remove acetone. To the residue were added 20 ml of MeOH and 4 ml of 6 *N* HCl. The mixture was stirred at room temperature for 15 hr. Then it was diluted with H_2O and neutralized with 5% $NaHCO_3$. The mixture was concentrated on the

steam bath in a stream of N_2. The residue was extracted with EtOAc. The EtOAc extract was washed with saturated NaCl, dried (Na_2SO_4), and evaporated to dryness to afford a viscous yellow oil. The oil was triturated with ether containing a small amount of EtOAc to yield 160 mg of crude **3b**, mp 196–203°.

The ether–EtOAc trituration solution was evaporated to dryness, and the residual oil was chromatographed on 50 g of SiO_2. Elution with 30% EtOAc–70% C_6H_6 afforded 384 mg of solid which was crystallized from EtOAc–hexane to yield 227 mg of **3b**, mp 219.5–222°. Further crystallization raised the melting point to 224.5–228.5°; λ^{KBr} 2.82, 2.98, 5.61, 5.98, 6.19 μ; λ_{max}^{MeOH} 240–241 nm (ε 14,350): nmr (Hz) 345, 240, 164 (OH), 73, 66. In D_2O the original broad signal at 240 Hz (16-H) appeared as a pair of doublets at 245, 240, 238, and 232 Hz; $[\alpha]D$ +67.2°. *Anal.* ($C_{22}H_{30}O_4$) C, H.

References

(1) (a) E. Gláz and P. Vecsei, "Aldosterone," Pergamon Press, New York, N. Y., 1971; (b) G. W. C. Sharp and A. Leaf, *Physiol. Rev.*, **46**, 593 (1966); (c) P. J. Mulrow, "The Adrenal Cortex," A. B. Eisenstein, Ed., Little, Brown and Co., Boston, Mass., 1967, p 293; (d) F. C. Bartter, "The Clinical Use of Aldosterone Antagonists," Charles C Thomas, Springfield, Ill., 1960.

(2) (a) C. M. Kagawa, J. A. Cella, and C. G. van Armin, *Science*, **126**, 1015 (1957); (b) J. A. Cella and C. M. Kagawa, *J. Amer. Chem. Soc.*, **79**, 4808 (1957); (c) J. A. Cella, "Edema Mechanisms and Management," J. H. Moyer and M. Fuchs, Ed., W. B. Saunders, Philadelphia, Pa., 1960, p 303; (d) C. M. Kagawa, ref 2c, p 309.

(3) (a) J. A. Cella and R. C. Tweit, *J. Org. Chem.*, **24**, 1109 (1959); (b) E. A. Brown, R. D. Muir, and J. A. Cella, *ibid.*, **25**, 96 (1960); (c) N. A. Atwater, R. H. Bible, Jr., E. A. Brown, R. R. Burtner, J. S. Mihina, L. N. Nysted, and P. B. Sollman, *ibid.*, **26**, 3077 (1961); (d) L. J. Chinn, H. L. Dryden, Jr., and R. R. Burtner, *ibid.*, **26**, 3910 (1961); (e) G. E. Arth, H. Schwam, L. H. Sarett, and M. Glitzer, *J. Med. Chem.*, **6**, 617 (1963).

(4) L. J. Chinn, *J. Org. Chem.*, **27**, 1741 (1962).

(5) A. Karim and E. A. Brown, *Steroids*, **15**, 41 (1972).

(6) R. C. Tweit, E. A. Brown, S. Kraychy, S. Mizuba, R. D. Muir, and R. T. Nicholson, *Chem. Pharm. Bull.*, **12**, 859 (1964).

(7) (a) R. Neher, P. Desaulles, E. Vischer, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, **41**, 1667 (1958); (b) R. Neher, C. Meystre, and A. Wettstein, *ibid.*, **42**, 132 (1959); (c) T. Miki, K. Morita, S. Noguchi, T. Kishi, K. Hiraga, and H. Nawa, *Chem. Pharm. Bull.*, **11**, 95 (1963).

(8) (a) C. S. Kagawa, J. S. Baran, and W. F. Krol, *Arch. Int. Pharmacodyn.*, **150**, 563 (1964); (b) L. M. Hofmann and T. R. Sherrod, *ibid.*, **165**, 14 (1967).

(9) R. Kiesewetter, M. Hubrig, D. Jorke, *Zentralbl. Pharm. Pharmakother. Laboratoriums-diagn.*, **109**, 1141 (1970).

(10) T. Aoki, H. Yamamura, K. Takei, and H. Mori, *Chem. Pharm. Bull.*, **12**, 808 (1964).

(11) O. Engelfried, H. Gibian, F. Neumann, K. Prezewowsky, G. Schulz, and R. Wiechert, *Arzneim.-Forsch.*, **16**, 1518 (1966).

(12) (a) J. Fishman, *J. Amer. Chem. Soc.*, **82**, 6143 (1960); (b) K. N. Wynne and A. G. C. Renwick, *Steroids*, 19, 293 (1972).

(13) I. Iwai and T. Hiraoko, *Chem. Pharm. Bull.*, **8**, 815 (1960).

(14) (a) P. Kurath and W. Cole, *J. Org. Chem.*, **28**, 102 (1963); (b) M. A. Bielefeld and P. Kurath, *ibid.*, **34**, 237 (1969).

(15) L. M. Hofmann, H. A. Wagner, and R. S. Jacobs, *Arch. Int. Pharmacodyn.*, **165**, 476 (1967).

(16) C. M. Kagawa, *Univ. Mich. Med. Bull.*, **26**, 251 (1960).

Synthesis and Some of the Pharmacological Properties of [4-Leucine]-8-lysine-vasopressin and [1-Deamino,4-leucine]-8-lysine-vasopressin†

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[4-Leucine]-8-lysine-vasopressin ([4-Leu]-LVP) and its 1-deamino analog have been synthesized by classical methods and tested for a number of biological activities. The compounds exhibited, respectively, 1–2 units/mg and 5–6 units/mg of antidiuretic activity, 1.33 units/mg and 0.55 unit/mg of pressor activity, and negligible oxytocic activity. [4-Leu]-LVP had no avian vasodepressor activity, while the 1-deamino analog possessed 4.6 units/mg. Both compounds inhibited the oxytocic effects of oxytocin. [4-Leu]-LVP inhibited the avian vasodepressor effects of oxytocin as well, but not (as does [4-Leu]oxytocin) the antidiuretic effects of vasopressin. The low antidiuretic potencies of the two compounds are in marked contrast to the extremely high potencies (707 and 729 units/mg, respectively) of [4-aminobutyric acid]-LVP and its 1-deamino analog, compounds which differ from the 4-leucine compounds only in the absence of two methyl groups in the 4 position.

In the course of studies on the relationship of structure to the biological activity of the posterior pituitary hormones, a series of analogs of oxytocin and deaminooxytocin (Figure 1) has been prepared in which hydrophobic, aliphatic amino acid residues have been substituted for the glutamine residue in position 4.§ These studies have demonstrated that the glutamine carboxamide group contributes to but is not essential for biological activity. Indeed, the formal replacement of this group by a hydrogen atom to give [4-decarboxamido]oxytocin ([4-α-aminobutyric acid]-oxytocin) yielded an analog which possesses high oxytocic and avian vasodepressor (AVD) activities.[3] All of the compounds in this series of analogs have extremely low or negligible antidiuretic (ADH) and rat pressor activities. In fact, [4-leucine]oxytocin was actually found to possess the opposite effects (*i.e.*, diuretic and depressor effects) in the rat. Moreover, it exhibits potent anti-ADH activity (*i.e.*, it

†This work was supported in part by Grants HL-11680 (V. du V.) and HL-09795 (W. Y. C.) from the U. S. Public Health Service. All optically active amino acid residues are of the L variety. The symbols for the amino acid residues follow the recommendations (1971) of the IUPAC-IUB Commission on Biochemical Nomenclature.[1] Abbreviations: *i*-Pr_2NEt, *N,N*-diisopropylethylamine; TFA, trifluoroacetic acid; DMF, dimethylformamide; Mpa, the β-mercaptopropionyl residue; ADH, antidiuretic hormone. All melting points were determined in capillary tubes and are corrected. The Boc-protected intermediates invariable decomposed upon melting but without discoloration. Plates of silica gel G were used for thin-layer chromatograms which were developed in the following solvent systems: (A) $CHCl_3$–MeOH, 9:1; (B) BuOH–HOAc–H_2O, 3:1:1; (C) BuOH–pyridine–H_2O, 20:10:11; (D) MeOH–pyridine–H_2O, 12:1:7. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

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§A list of these analogs with individual references has been presented in a table by Flouret and du Vigneaud.[2]