[CONTRIBUTION FROM THE DIVISION OF CHEMICAL RESEARCH OF G. D. SEARLE & CO.]

Steroidal Aldosterone Blockers. II[1]

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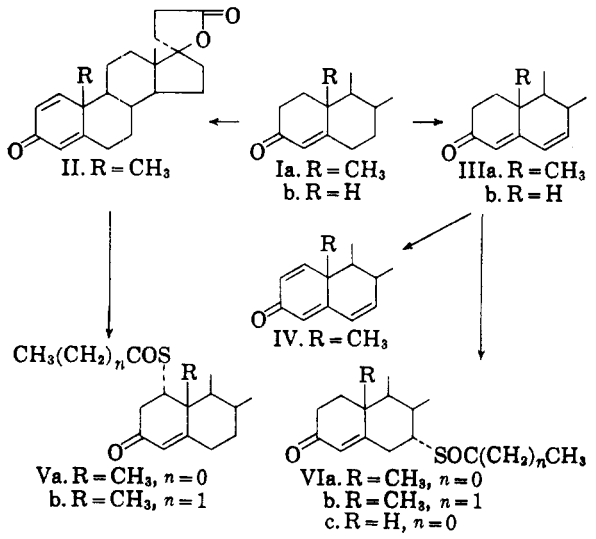
The synthesis and biological activities of several new steroidal 17-spirolactones are presented. Introduction of unsaturation and the acylthio grouping enhances oral aldosterone blocking activity in this series of compounds.

The first series of steroidal spirolactones which were reported[1] showed much better aldosterone blocking activities when administered parenterally than when given orally. This report deals with a group of related steroids which show good activities when taken by mouth.

In casting about for modifications of the compounds reported earlier we decided to introduce unsaturation at the C-1 and C-6 centers of Ia because of the desirable effects produced by such changes in some biologically active steroids.[2] Reaction of Ia with selenious acid[3] produced the corresponding $\Delta^{1,4}$-3-oxo-derivative (II). On the other hand when Ia was treated with chloranil[4] the corresponding $\Delta^{4,6}$-3-oxo-steroid (IIIa) was formed. We were able to prepare the $\Delta^{4,6}$-3-oxo-compound (IIIb) from Ib with chloranil,[3] although in poor yield. The $\Delta^{1,4,6}$-3-oxo-derivative (IV) of Ia was prepared by treatment of IIIa with selenious acid.



We found that these dehydrogenated derivatives all possessed enhanced oral activity. Encouraged, we then set about to make the alkanethiolic acid adducts of these compounds on the chance of obtaining higher activities, since the 7α-acetylthio derivative of 17α-hydroxyprogesterone was found to be more potent than the parent compound when administered parenterally.

As we expected[5] ethanethiolic acid and propanethiolic acid added readily to either the 1,2 or the 6.7 double bond. Treatment of II with ethanethiolic acid gave the 1α-acetylthio compound (Va); propanethiolic acid yielded the 1α-propionylthio derivative (Vb). Heating the $\Delta^{4,6}$-3-oxo steroid (IIIa) with ethanethiolic or propanethiolic acid produced the corresponding 7α-acetylthio (VIa) and 7α-propionylthio (VIb) compounds, respectively. The 19-nor analog (IIIb) yielded the 7α-acetylthio compound (VIc) when allowed to react with ethanethiolic acid. The assignment of position as well as configuration of the acylthio substituents mentioned above was made by analogy to the work reported by Dodson and Tweit[5] on similar additions.

The aldosterone blocking potency[6] of most of these compounds is recorded in Table I. The most potent compound when administered orally is VIa. This result was unexpected since VIa failed to

TABLE I

ALDOSTERONE BLOCKING POTENCIES[6]

Compound	M.E.D.[a]	Compound	M.E.D.[a]
Ia	21	IV	0.9
Ib	1.6	Va	>0.8
II	1.5	VIa	0.4
IIIa	1.1	VIb	0.6

[a] M.E.D. is the minimal effective dose in mg. which produces a 50% block of the effect of 12 μg. desoxycorticosterone on the urinary sodium-potassium ratio of adrenalectomized rats, when the test compound is administered orally.

(1) For previous papers in this area see (a) J. A. Cella and C. M. Kagawa, *J. Am. Chem. Soc.*, **79**, 4808 (1957), and (b) J. A. Cella, E. A. Brown, and R. R. Burtner, *J. Org. Chem.*, **24**, 743 (1959).

(2) For example, see H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman, and M. M. Pechet, *Science*, **121**, 176 (1955).

(3) C. Meystre, H. Frey, W. Voser, and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956); S. A. Szpilfogel, T. A. P. Posthumus, M. S. de Winter, and D. A. Van Dorp, *Rec. trav. chim.*, **75**, 475 (1956).

(4) E. J. Agnello and G. D. Laubach, *J. Am. Chem. Soc.*, **79**, 1257 (1957).

(5) R. M. Dodson and R. C. Tweit, *J. Am. Chem. Soc.*, **81**, 1224 (1959).

(6) The aldosterone blocking activities were determined by Dr. C. M. Kagawa and his associates of these laboratories. The assay involves the use of desoxycorticosterone as the sodium retainer and results are related to aldosterone by analogy. The details of this test method have been published: C. M. Kagawa, J. A. Cella, and C. G. Van Arman, *Science*, **126**, 1015 (1957). The results reported here will be published elsewhere in detail.

show improved activity over Ia when administered parenterally.

EXPERIMENTAL

We are indebted to Dr. R. T. Dillon and his staff of the Analytical Division of G. D. Searle and Co. for all microanalyses and optical determinations. All melting points were determined on a Fisher-Johns hot stage melting point apparatus and are uncorrected. Ultraviolet spectra were determined in methanol. Optical rotations were measured in chloroform except as otherwise noted.

3-(3-Oxo-17β-hydroxy-1,4-androstadien-17α-yl)propanoic acid lactone (II).[7] To a solution of 50 g. of Ia[1] and 1.0 g. of mercuric chloride in 3 l. of *t*-butyl alcohol and 30 ml. of acetic acid, 17.8 g. of selenious acid was added with stirring. After the mixture had been refluxed under nitrogen for 8 hr., a second portion of 17.8 g. of selenious acid was added and the reflux period continued for an additional 13 hr. The *t*-butyl alcohol solution was filtered and concentrated, the residue was dissolved in methylene chloride and the solution was washed with 2% sodium hydroxide and ammonium sulfide solutions. Then the organic layer was washed with water, dried, and concentrated to dryness leaving a crystalline residue which was recrystallized twice from ethyl acetate to yield 16.3 g. of II, m.p. 179–180° (another form melts 134–136°), $[\alpha]_D$ +22° (diox), ϵ^{245} 14,900.

Anal. Calcd. for $C_{22}H_{28}O_3$: C, 77.61; H, 8.29. Found: C, 77.34; H, 8.28.

3-(3-Oxo-17β-hydroxy-4,6-androstadien-17α-yl)propanoic acid lactone (IIIa). A solution of 2.5 g. of Ia,[1] 2.5 g. of chloranil and 0.025 g. of *p*-toluenesulfonic acid hydrate in 250 ml. of xylene was heated at reflux for 20 hr. The mixture was cooled and filtered and the solvent was removed by vacuum distillation. The residue was taken up in benzene and chromatographed over 250 g. of silica using benzene and ethyl acetate as developing solvents. The 15% ethyl acetate 85% benzene eluate yielded a solid which on recrystallization from ethyl acetate gave 0.65 g. of diene IIIa, m.p.146–151° (remelts 165°). Recrystallization of this product from ethyl acetate yielded 0.44 g. of IIIa, m.p. 149–151°, $[\alpha]_D$ +24.5°, ϵ^{283} 26,700.

Anal. Calcd. for $C_{22}H_{28}O_3$: C, 77.61; H, 8.29. Found: C, 77.65; H, 8.54.

3-(3-Oxo-17β-hydroxy-19-nor-4,6-androstadien-17α-yl)-propanoic acid lactone (IIIb). A solution of 4.05 g. of Ib,[1] 3.34 g. of chloranil, and 0.01 g. of *p*-toluenesulfonic acid monohydrate in 400 ml. of xylene was heated under reflux for 1 hr. and then the xylene was removed under vacuum The residue was dissolved in benzene and chromatographed on 560 g. of silica gel. The column was washed with benzene and 5%, 10%, and 15% ethyl acetate in benzene. The product was eluted with liter portions of 20% ethyl acetate in benzene. The residues from the fourth and fifth liters of eluate were crystallized twice from ethyl acetate to yield 0.26 g. of IIIb, m.p. 235–239°, $[\alpha]_D$ −44°, ϵ^{283} 27,000.

Anal. Calcd. for $C_{21}H_{26}O_3$: C, 77.26; H, 8.03. Found: C, 76.97; H, 8.31.

3-(3-Oxo-17β-hydroxy-1,4,6-androstatrien-17α-yl)propanoic acid lactone (IV). According to the procedure described by Agnello and Laubach,[4] 0.34 g. of 4,6-diene IIIa was treated with a total of 0.155 g. of selenious acid in two portions in 50 ml. of *t*-butyl alcohol containing 0.5 ml. of acetic acid. The product was chromatographed over silica using benzene and ethyl acetate as developing solvents. The eluate composed of 15% ethyl acetate contained 0.181 g. and could be crystallized from methanol to give 0.05 g. of the 1,4,6-triene IV, as the monomethanolate, m.p. 97–100° (dec., remelts 136–138°), $[\alpha]_D$ 0°, ϵ^{222} 11,100, ϵ^{266} 10,700, ϵ^{296} 13,700.

Anal. Calcd. for $C_{22}H_{26}O_3 \cdot CH_3OH$: C, 74.56; H, 8.16. Found: C, 74.88; H, 8.09.

A second crop of material weighed 0.05 g. and melted 95–98° (dec.).

3-(3-Oxo-1α-acetylthio-17β-hydroxy-4-androsten-17α-yl)-propanoic acid lactone (Va). A solution of 1.0 g. of II in 1 ml. of ethanethiolic acid was heated on the steam bath for 0.5 hr. Part of the excess thiolic acid was then evaporated under nitrogen and the residue was dissolved in a mixture of ethyl acetate–ether. On scratching, crystals formed and these were separated by filtration and recrystallized from ethyl acetate to yield 0.40 g. of Va, m.p. 199–200° (dec.), $[\alpha]_D$ +56°, $\epsilon^{240.5}$ 16,200.

Anal. Calcd. for $C_{24}H_{32}O_4S$: C, 69.20; H, 7.74. Found: C, 69.15; H, 7.84.

Additional fractions melting at 194–196° and 198–199° were obtained to bring the total yield to 0.75 g. (61%).

3-(3-Oxo-1α-propionylthio-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (Vb). A solution of 0.35 g. of II in 0.5 ml. of propanethiolic acid was heated on a steam bath for 1.25 hr. Ethyl acetate and Skellysolve B were added and the solution allowed to stand overnight. The crystals which formed were recrystallized from a benzene–Skellysolve B mixture. There was obtained 0.08 g. of Vb, m.p. 176–178° (dec.), $\epsilon^{240.5}$ 16,500.

Anal. Calcd. for $C_{25}H_{34}O_4S$: C, 69.73; H, 7.96. Found: C, 69.81; H, 8.13.

3-(3-Oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)-propanoic acid lactone (VIa). A solution of 0.75 g. of IIIa in 0.75 ml. of ethanethiolic acid was heated on a steam bath for 1 hr. Most of the excess solvent was removed in a stream of dry nitrogen. Crystallization of the oily residue from methanol yielded 0.65 g. of VIa, m.p. 130–135°. Recrystallization yielded 0.45 g., m.p. 134–135° (resolidified and remelted 201–202° dec.), $[\alpha]_D$ −33.5°, ϵ^{238} 20,200.

Anal. Calcd. for $C_{24}H_{32}O_4S$: C, 69.20; H, 7.74. Found: C, 69.25; H, 7.75.

3-(3-Oxo-7α-propionylthio-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (VIb). A solution of 0.65 g. of IIIa in 0.5 ml. of propanethiolic acid was heated on the steam bath for 0.5 hr. Most of the excess thiolic acid was removed under vacuum and on trituration with methanol crystals of VIb formed, 0.34 g., m.p. 192–194° (dec.), $[\alpha]_D$ −37°, ϵ^{238} 19,800.

Anal. Calcd. for $C_{25}H_{34}O_4S$: C, 69.73; H, 7.96. Found: C, 69.86; H, 8.10.

3-(3-Oxo-7α-acetylthio-17β-hydroxy-19-nor-4-androsten-17α-yl)propanoic acid lactone (VIc). One hundred seventy mg. of IIIb was dissolved in 0.5 ml. of ethanethiolic acid and heated on a steam bath for 1.5 hr. Most of the excess thiolic acid was removed under vacuum and methanol was added. On scratching 87 mg. of crystals of VIc formed, m.p. 111–113°, ϵ^{237} 21,000.

Anal. Calcd. for $C_{23}H_{30}O_4S$: C, 68.62; H, 7.51. Found: C, 68.30; H, 7.54.

CHICAGO 80, ILL.

(7) We are indebted to Mr. Richard Gueldner for technical assistance in this preparation.