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Studies on Steroids. II. The Preparation and Properties
of 17β -(N-Substituted 2-Amino-4-thiazolyl)androst-4-en-
 $3\beta,17\alpha$ -diol Series and its Hydrogen Succinate.

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In the previous paper,¹⁾ the authors reported the synthesis of 17α -hydroxy- 17β -(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino)-4-thiazolyl)androst-4-en-3-one series, some of which showed the somewhat degree of potency in pigeon method and Engelmann's test.

The present paper described the results of further studies on the reduction of several kinds of 17α -hydroxy- 17β -(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino)-4-thiazolyl)androst-4-en-3-one series (V) with sodium borohydride. Moreover, authors attempted the preparation of hydrogen succinate of 17β -(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino)-4-thiazolyl)androst-4-en-3, 17α -diol series (IX) in order to make them water-soluble.

The preparation of a water-soluble steroid has been studied by several groups of worker in recent years; steroid phosphate,²⁾ steroid sulfonate,³⁾ steroid carboxylic acid salt,⁴⁾ steroid ammonium salt⁵⁾ and steroid hydrogen succinate and so forth are well known to be the water-soluble steroid.

A number of steroid hydrogen succinate have appeared in the literature as the following compounds: cortisone 21-(hydrogen succinate), 21-hydroxypregnan-3,20-dione hydrogen succinate,⁷⁾ 3α -hydroxypregnan-11,20-dione hydrogen succinate⁸⁾ $3\alpha,12\beta$ -dihydroxy-11,12-dioxocholenic acid 3-(hydrogen succinate),⁹⁾ $3\alpha,11$ -dihydroxy-12-oxo-9(11)-cholenic acid 3-(hydrogen succinate),¹⁰⁾ 3β -methoxy-21-hydroxy-allopregnan-20-one hydrogen succinate,¹¹⁾ digitoxigenin 3-(hydrogen succinate),¹²⁾ oleandrigenin 3-(hydrogen succinate) and gitoxigenin 16-(hydrogen succinate)¹³⁾ etc.

Reduction of 17α -hydroxy- 17β -(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylamino)-4-thiazolyl)androst-4-en-3-one (V) with sodium borohydride in dioxane or dioxane-

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- 1) Part I: This Bulletin, 11, 604 (1963).
- 2) E. Müller, A. Langebeck, *et al.*: Z. Physiol. Chem., 281, 29 (1944). R. Hirschmann, L.H. Sarett: Chem. and Ind. (London), 1958, 1261. F.A. Cutler, J.F. Fisher, L.H. Sarett: J. Am. Chem. Soc., 80, 6300 (1958). Stolzer, C., Simon A.: Chem. Ber., 93, 1323 (1960). U.S. Patent 2,183,589 (T. Reichstein, *et al.*), 2,870,177 (Merck & Co., Inc), 2,928,849 (Egon Rihardt Dicztausy), 2,932,657 (Merck & Co., Inc.), 2,936,313 (Glaxo Laboratories) etc.
- 3) U.S. Patent 2,828,306 (Estron sulfate, Schering), 2,931,817 (Steroid sulfite ester polymers, Monsanto chemical company). B. Patent 829,618 (Estrone sulfate salt compound, Haseltine Lake Co.), 84,851 (Hydrocortisone 21-sulfate, Schering), 852,179 (Prednisolone 21-semisulfate, Schering) etc.
- 4) U.S. Patent 2,875,214 (G.D. Searle), 2,844,429 (Upjohn), 2,925,416 (G.D. Searle), 3,006,928 (Les Laboratoires Francais Chimiotherapic). D. Patent 1,086,700 (Eastman Kodak Company), 1,103,332 (Societa Italiana Prodotti Schering), 1,114,814 (Dr. Karl Tomae G). B. Patent 815,809 (Farbwerk Hoechst Akt-Ges), 834,400 (Mewburn ellis & Co.). Belgian Patent 581,323 (Les Laboratoires Francais Chimiotherapic) etc.
- 5) D. Patent 1,102,148 (E. Merck Aktiengesellschaft), U.S. Patent 2,967,179 (Glaxo Laboratories) etc.
- 6) D. Patent 1,102,149 (Francesco Vismara S.P.A.).
- 7) U.S. Patent 2,820,737 (Chas pfizer & Co. Inc.).
- 8) B. Patent 841,149 (Mewburn Ellis Co.).
- 9) B. Patent 810,009 (Les Lab oratories francuis chimiotherapic).
- 10) U.S. Patent 2,894,960 (*Ibid*).
- 11) D. Patent 1,020,976 (Chas pfizer & Co.).
- 12) A. Yamada: Yakugaku Zasshi, 79, 1440 (1959).

TABLE I.

Compound	R	R ₁	m.p. (decomp.) (°C)	Formula	Analyses (%)										Yield (%)	[α] _D ^{CHCl₃}	λ _{max} ^{EtOH} (mμ) (log ε)
					Calcd.			Found									
					C	H	N	C	H	N	C	H	N				
VIIIa	H	H	240.5	C ₂₂ H ₃₂ O ₂ N ₂ S·C ₂ H ₅ OH	66.33	8.81	6.45	66.39	8.59	6.57	85.0	+16.99	(c=0.1716)	259.0~260.0	(3.50)		
VIIIb	H	CH ₃	216.0	C ₂₃ H ₃₄ O ₂ N ₂ S·H ₂ O	65.69	8.63	6.66	65.71	8.82	6.54	90.9	+10.81	(c=0.248)	261.0~264.0	(3.57)		
VIIIc	H	C ₂ H ₅	222.5	C ₂₄ H ₃₆ O ₂ N ₂ S	69.20	8.71	6.73	69.32	8.59	6.89	95.0	+47.61	(c=0.294)	264.0~266.0	(3.82)		
VIIId	H	C ₃ H ₇	202.0	C ₂₅ H ₃₈ O ₂ N ₂ S	69.73	8.93	6.51	69.84	8.70	7.33	70.0	+46.87	(c=0.576)	265.3~266.2	(3.92)		
VIIIe	H	iso-C ₃ H ₇	218.0	C ₂₅ H ₃₈ O ₂ N ₂ S	69.73	8.93	6.51	70.05	8.76	6.54	85.0	+62.76	(c=0.415)	266.0~267.0	(3.75)		
VIIIf	H	C ₄ H ₉	161.0	C ₂₆ H ₄₀ O ₂ N ₂ S·2H ₂ O	64.97	9.23	5.83	65.30	9.42	5.57	70.0	+37.20	(c=0.532)	262.0~265.0	(3.81)		
VIIIg	H		199.0	C ₂₈ H ₃₈ O ₂ N ₂ S	72.38	7.81	6.03	72.73	7.98	6.04	88.8	+43.74	(c=0.901)	295.5	(4.23)		
VIIIh	H		212.0	C ₂₈ H ₄₂ O ₂ N ₂ S·H ₂ O	71.15	9.38	5.93	69.57	9.63	5.66	80.0	+39.09	(c=0.024)	264.5~265.7	(3.76)		
VIIIi	H		192.0	C ₂₉ H ₃₈ O ₂ N ₂ S	72.77	8.00	5.85	72.76	7.74	6.18	60.0	+43.03	(c=1.162)	293.0~294.0	(4.00)		
VIIIj	H		202	C ₂₉ H ₃₈ O ₂ N ₂ S·C ₂ H ₅ OH	70.96	8.45	5.34	71.16	8.35	6.27	82.3	+18.94	(c=1.162)	295.5~296.5	(4.06)		
VIIIk	H		209	C ₂₈ H ₃₅ O ₂ N ₂ SCl	67.69	7.02	5.62	67.99	7.14	5.55	79.0	+79.36	(c=0.794)	298.0~299.0	(4.27)		
VIIIl	C ₂ H ₅		214	C ₃₀ H ₄₀ O ₂ N ₂ S	73.14	8.18	5.69	73.02	8.18	6.07	73.3	+58.73	(c=0.794)	254.0	(3.81)		
														260.5	(3.72)		
														295.0~298.0	(3.82)		

TABLE II. Infrared absorption of VIII (KBr disk) (cm⁻¹)

Compound	ν_{OH} (17 α ,3 β -OH)	Overton of ν_{C-H} benzene		ν_{C-H}		$\nu_{C=C}$ (Δ^4)	Π band of thiazol ring	benzene ring		δ_{C-H} (benzene)
		ν_{N-H}	ν_{C-H}	$\nu_{as} C-H$	$\nu_s C-H$					
VIIa	3340 s	3190 m		2920 s	2845 s	1610 w	1520 s			
VIIb	3360 s	3170 s		2930 s	2860 s	1635 w	1567 s			
VIIc	3410 s	3290 s		2920 s	2840 s	1655 w	1540 s			
VIIId	3380 s	3300 s		2920 s	2850 m	1660 w	1550 s			
VIIe	3420 s	3310 s		2960~	2840 s	1655 w	1555 s			
VIIIf	3402 s	3302 s		2930 s		1620 w	1566 s			
VIIg	3557 s	3427~	3127 w	2935 s	2865 s	1656 w	1556 s	1602 s	1536 s	747 s 638 s monosubstituted benzene
VIIh	3300 m	3257 w		2900 s	2830 s	1605 w	1550 s			
VIIi	3380 w	3090 w		2900 s	2850 s	1665 w	1545 s	1595 s	1538 s	751 s <i>o</i> -substituted benzene
VIIk	3300 m	3140 w		2930 s	2860 s	1665 w	1555 s	1607 m	1525 s	815 m 695 w <i>m</i> -substituted benzene
VIIl	3497 m			2935 s	2860 s	1659 w	1537~ 1524 s	1586 s	1502 s	766 s 697 s monosubstituted benzene

s : strong m : medium w : weak

TABLE III.

Compound	R ₁	R ₂	R ₃	m.p. (decomp.) (C°)	Formula	Analyses (%)										Yield (%)	[α] _D ^{CHCl₃}	λ _{max} ^{EtOH} mμ (log ε)
						Calcd.			Found									
						C	H	N	C	H	N							
IXa	O	-COCH ₂ CH ₂ COOH	H	217	C ₂₂ H ₃₄ O ₈ N ₂ S	64.18	7.04	5.76	64.32	7.08	5.58	70.5	- 57.73 (c=0.433)	242.0~243.0 (4.14)				
IXb	H -OCOCH ₂ CH ₂ COOH	"	CH ₃	142	C ₃₁ H ₄₂ O ₈ N ₂ S	61.78	7.02	4.65	61.48	7.51	4.31	61.2	+133.33 (c=0.075)	227.5~228.0 (4.37) 235.2~235.8 (4.37) 243.5~244.0 (4.21) 275.0~276.5 (3.98)				
IXc	"	H		176	C ₃₃ H ₄₂ O ₈ N ₂ S	68.49	7.32	4.84	68.03	7.44	5.04	65.2	+ 49.32 (c=1.774)	293.5~294.5 (3.24)				
IXd	"	H		182	C ₃₃ H ₄₂ O ₈ N ₂ S	68.49	7.32	4.84	68.80	7.60	4.64	70.0	+ 70.54 (c=0.283)	290.5~292.0 (4.06)				

methanol (1:1) and subsequent purification by chromatography through acid-washed alumina yield sole product of corresponding 3,17 α -diol series (VIII). The physical constant of the compounds are listed in Table I.

The configuration of 3-hydroxy group could be assumed to be β by means of molecular rotation measurement, proposed by Barton.

The structure of the compounds (VIII) was substantiated by examination of the infrared spectra which showed disappearance of the Δ^4 -3-carbonyl band at 1660 cm^{-1} and the presence of near 3300~3580 cm^{-1} due to 3-hydroxy group. The 2-alkylamino-4-thiazolyl derivatives of VIIIb~f were confirmed by examination of infrared spectra which showed an absorption near 3090~3310 cm^{-1} due to N-H stretching, whereas the 2-N,N-alkylarylamino-4-thiazolyl derivatives of VIII-l did not show the corresponding absorption in the region.

Furthermore, the 2-arylamino-4-thiazolyl derivatives of VIIIh~k showed an absorption near 293~299 $\text{m}\mu$ in the ultraviolet spectrum and near 638~815 $\text{m}\mu$ in the infrared spectrum due to substituted benzene. Infrared absorption of VIII are summarized in Table II.

Further evidence was obtained from the result of the ultraviolet spectra which showed disappearance of the Δ^4 -3-carbonyl band at 239~245 $\text{m}\mu$ and the appearance of absorption near 259~267 $\text{m}\mu$ due to thiazole ring. 2-Arylamino-4-thiazolyl derivatives of VIII showed secondary absorption near 293~299 $\text{m}\mu$ in the ultraviolet spectrum due to benzene ring.

17 α -hydroxy-17 β -(2-amino-(or 2-alkylamino- or 2-arylamino)-4-thiazolyl)androst-4-en hydrogen succinate series (IX) was obtained from 17 α -hydroxy-17 β -(2-amino-(or 2-alkylamino- or 2-arylamino)-4-thiazolyl)androst-4-en series with succinic anhydride in pyridine on the usual manner. The aliphatic amino derivatives of IX was proved to be 3 β -O,N-bis(hydrogen succinate) from the result of elemental analysis. The physical constant of the compounds are listed in Table III. This substance is very slightly soluble in hot water, but it forms an alkali salt which is easily soluble in water and acidification of the solution with acetic acid again produces hydrogen succinate (IX). The reaction route is shown in Chart 1.

Chart 1.

Pharmacological Test

Screening tests of the compounds described above, were determined on the toxicity of pigeon method and the contractile force of isolated frog heart (Engelmann's test).

The compounds were compared with cardiac aglycone by using these test, as shown in Tables IV and V.

Toxicity of these compounds were compared with that of cardiac aglycone and these results indicated that their toxicity is lower than that of cardiac aglycone, as shown in Table IV.

TABLE IV. Toxicity by Pigeon and Frog method

No.	Pigeon LD mg./kg.	Frog LD mg./g.	
		after 4 hr.	after 12 hr.
Digitoxin	0.60	0.001	0.001
Gitoxin	1.21		
Digoxin	0.45	0.001 ^{a)}	0.001 ^{a)}
VIIa	2.10	0.030 $\frac{1}{3}$ ^{b)}	0.030 $\frac{1}{3}$ ^{b)}
VIIb	2.80	0.030	0.030
VIIc	7.00	0.030 ^{a)}	0.030 ^{a)}
VIIId	7.00	0.030	0.030
VIIe	4.00	0.030 ^{a)}	0.030 ^{a)}
VIIIf	3.60	0.030 $\frac{1}{3}$ ^{b)}	0.030 $\frac{1}{3}$ ^{b)}
VIIg	6.10	0.030 ^{a)}	0.030 ^{a)}
VIIh	7.00	0.060 ^{a)}	0.060 ^{a)}
VIIi		0.060 ^{a)}	0.060 ^{a)}
VIIj		0.060 ^{a)}	0.060 ^{a)}
VIIk		0.060	0.060
VIII	3.65	0.030 ^{a)}	0.030 ^{a)}
IXa		0.060 $\frac{1}{3}$ ^{b)}	0.060

a) This indicates that the animals did not die until each dose was injected.

b) killed animals/treated animals.

TABLE V. Effect on Frog Heart of Compounds

No.	Concentration	Dose cc./10 g.	Contractile Force	Heart Rate
			inhibit — increase +	diminish — increase +
VIIa	10 ⁻⁴	0.2	no effect	no effect
VIIb	10 ⁻⁴	0.2	—	—
	10 ⁻⁴	0.2	+	—
	10 ⁻⁴ × 2.5	0.2	no effect	—
VIIc	10 ⁻⁴	0.2	no effect	—
VIId	10 ⁻⁴	0.2	+	—
	10 ⁻⁴	0.2	+	+
VIIf	10 ⁻³ × 2.5	0.1	±	—
	10 ⁻³ × 2.5	0.2	no effect	no effect
	10 ⁻⁴	0.1	±	—
	10 ⁻⁴	0.2	±	—
	10 ⁻⁴	0.2	+	—
	10 ⁻⁴ × 2.5	0.2	+ weak	—
	10 ⁻⁴ × 2.5	0.3	+	—
VIIg	10 ⁻⁴	0.2	no effect	no effect
	10 ⁻⁴	0.2	+	—
VIIj	10 ⁻⁴	0.2	+	—
VIII	10 ⁻³ × 5	0.05	—	—
	10 ⁻³ × 5	0.1	—	—
	10 ⁻³ × 5	0.2	—	—
	10 ⁻⁴	0.1	—	—
	10 ⁻⁴	0.2	—	—
	10 ⁻⁴ × 2.5	0.2	—	—

As can be seen Tables IV and V, hydrogen succinate IX of these compounds showed lower potency than original 17β -(2-alkylamino-(or 2-arylamino-)-4-thiazolyl)androst-4-en- 3β , 17α -diol series (VIII), as in the case of digitalis hydrogen succinate.

On the other hand, 17β -(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino-)-4-thiazolyl)androst-4-en- 3β , 17α -diol (VIII) was found to have stronger potency than corresponding 3-one derivatives (V~VII) in these test.

Experimental

17β -(2-Alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino-)-4-thiazolyl)androst-4-en- $3,17$ -diol (VIII)—A solution of 0.01 mole of 17α -hydroxy- 17β -(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino-)-4-thiazolyl)androst-4-en-3-one in 50 cc. of dioxane or dioxane-MeOH (1:1) was added dropwise with stirring into a solution of 0.012 mole of NaBH_4 in 10 cc. of MeOH. After for 5 hr., AcOH was added to the reaction mixture and evaporated to dryness. The residue was chromatographed through acid-washed alumina. The eluate of CHCl_3 was crystallized from EtOH (or aq. EtOH) to 60~95% of crystals.

17α -Hydroxy- 17β -(2-amino-(or 2-alkylamino- or 2-arylamino-)-4-thiazolyl)androst-4-ene Hydrogen Succinate Series (IX)—A solution of 0.01 mole of 17α -hydroxy- 17β -(2-amino-(or 2-alkylamino- or 2-arylamino-)-4-thiazolyl)androst-4-ene series and 0.08 mole of succinic anhydride in 50 cc. of pyridine was allowed to stand at room temperature for overnight. The solution was diluted with ice water containing HCl. The precipitate was collected, and washed several times with water and recrystallized from EtOH or aq. EtOH afforded colorless crystals. Yield, 65~70.5%.

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Summary

17β -(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino-)-thiazolyl)androst-4-en- 3β , 17α -diol was prepared from corresponding 3-one with sodium borohydride in dioxane or dioxane-methanol (1:1).

17β -(2-alkylamino-(or 2-arylamino-)-4-thiazolyl)androst-4-en- 3β , 17α -diol 3-(hydrogen succinate) and O,N-bis(hydrogen succinate) were obtained from corresponding 3β , 17α -diol and 17α -ol-3-one with succinic anhydride.

Some of this substance had a digitalis like properties.

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