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107. Keiich Takamura, Chifuyu Isono, Shin-ichi Takama, and Yoshihiro Nitta: Studies on Steroids. II. The Preparation and Properties of 17β-(N-Substituted 2-Amino-4-thiazolyl)androst-4-en-3β,17α-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 attemped 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 salt[5] 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 succinate[8] 3α,12β-dihydroxy-11,12-dioxocholenic acid 3-(hydrogen succinate),[9] 3α,11-dihydroxy-12-oxo-9(11)-cholenic acid 3-(hydrogen succinate),[10] 3β-methoxy-21-hydroxy-allopregnan-20-one hydrogen succinate,[11] digitoxygenin 3-(hydrogen succinate),[12] oleandrigenin 3-(hydrogen succinate) and gitoxygenin 16-(hydrogen succinate)[12] 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).

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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.

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6) D. Patent 1,102,149 (Francesco Vismara S. P. A.).

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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.).

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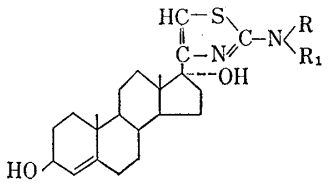
TABLE I.



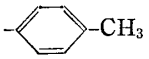
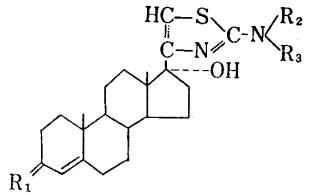
Compound	R	R_1	m.p. (decomp.) (°C)	Formula	Analyses (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Yield (%)	$[\alpha]_D^{CHCl_3}$	λ_{max}^{EtOH} (mμ) (log ε)
Ⅷa	H	H	240.5	$C_{22}H_{32}O_2N_2S \cdot C_2H_5OH$	66.33	8.81	6.45	66.39	8.59	6.57	85.0	+16.99 (c=0.1716)	259.0～260.0 (3.50)
Ⅷb	H	CH_3	216.0	$C_{23}H_{34}O_2N_2S \cdot H_2O$	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_2H_5	222.5	$C_{24}H_{36}O_2N_2S$	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_3H_7	202.0	$C_{25}H_{38}O_2N_2S$	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_3H_7	218.0	$C_{25}H_{38}O_2N_2S$	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_4H_9	161.0	$C_{26}H_{40}O_2N_2S \cdot 2H_2O$	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	$C_{28}H_{36}O_2N_2S$	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	$C_{28}H_{42}O_2N_2S \cdot H_2O$	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	$C_{29}H_{38}O_2N_2S$	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	$C_{29}H_{38}O_2N_2S \cdot C_2H_5OH$	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	$C_{28}H_{35}O_2N_2SCl$	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) 254.0 (3.81)
Ⅷl	C_2H_5		214	$C_{30}H_{40}O_2N_2S$	73.14	8.18	5.69	73.02	8.18	6.07	73.3	+58.73 (c=0.794)	260.5 (3.72) 295.0～298.0 (3.82)

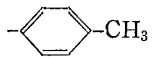
TABLE II. Infrared absorption of VIII (KBr disk) (cm^{-1})

Compound	ν_{OH} (17α,3β-OH)	ν_{N-H}	Overton of ν_{C-H} benzene		ν_{C-H}		$\nu_{C=C}$ (Δ^4)	II band of thiazol ring	benzene ring		δ_{C-H} (benzene)		
					$\nu_{as\ C-H}$	$\nu_{s\ C-H}$							
VIIIa	3340 s	3190m			2920 s	2845 s	1610w	1520 s					
VIIIb	3360 s	3170 s			2930 s	2860 s	1685w	1567 s					
VIIIc	3410 s	3290 s			2920 s	2840 s	1655w	1540 s					
VIIId	3380 s	3300 s			2920 s	2850m	1660w	1550 s					
VIIIe	3420 s	3310 s			2960～2930 s	2840 s	1655w	1555 s					
VIIIf	3402 s	3302 s			2935 s	2865 s	1620w	1566 s					
VIIIg	3557 s	3427～3257w	3127w	3687w	2935	2855 s	1656w	1556 s	1602 s	1536 s	747 s	638 s	monosubstituted benzene
VIIIh	3300m	3090w			2900 s	2830 s	1605w	1550 s					
VIIIi	3380w	3260w			2900 s	2850 s	1665w	1545 s	1595 s	1538 s	751 s		*o*-substituted benzene
VIIIk	3300m	3140w			2930 s	2860 s	1665w	1555 s	1607m	1525 s	815m	695w	*m*-substituted benzene
VIIIl	3497m				2935 s	2860 s	1659w	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 (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Yield (%)	$[\alpha]_D^{CHCl_3}$	λ_{max}^{EtOH} mμ (log ε)
IXa	O	$-COCH_2CH_2COOH$	H	217	$C_{22}H_{34}O_5N_2S$	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 ⋰ $-OCOCH_2CH_2COOH$	〃	CH_3	142	$C_{31}H_{42}O_8N_2S$	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	$C_{33}H_{42}O_5N_2S$	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	$C_{33}H_{42}O_5NS$	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 3,17α-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 moleculer 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 mμ in the ultraviolet spectrum and near 638~815 mμ 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 mμ and the appearance of absorption near 259~267 mμ due to thiazole ring. 2-Arylamino-4-thiazolyl derivatives of VIII showed secondary absorption near 293~299 mμ in the ultraviolet spectrum due to benzene ring.

17α-hydroxy-17β-(2-amino-(or 2-alkylamino- or 2-arylamino-)-4-thiazolyl)androst-4-en hydrogen succinate series (IX) was obtained from 17α-hydroxy-17β-(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 3β-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.

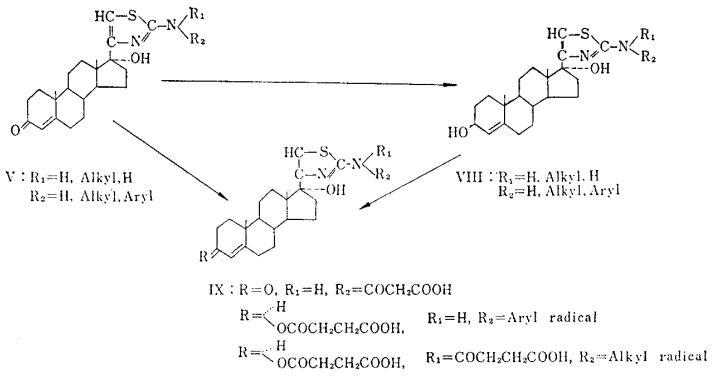


Chart 1.

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.	Frog LD mg./g. after 12 hr.
Digitoxin	0.60	0.001	0.001
Gitoxin	1.21		
Digoxin	0.45	0.001[a]	0.001[a]
VIIIa	2.10	0.030 1/3[b]	0.030 1/3[b]
VIIIb	2.80	0.030	0.030
VIIIc	7.00	0.030[a]	0.030[a]
VIIId	7.00	0.030	0.030
VIIIe	4.00	0.030[a]	0.030[a]
VIIIf	3.60	0.030 1/3[b]	0.030 1/3[b]
VIIIg	6.10	0.030[a]	0.030[a]
VIIIh	7.00	0.060[a]	0.060[a]
VIIIi		0.060[a]	0.060[a]
VIIIj		0.060[a]	0.060[a]
VIIIk		0.060	0.060
VIIIl	3.65	0.030[a]	0.030[a]
IXa		0.060 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 inhibit − increase +	Heart Rate diminish − increase +
VIIIa	10^{-4}	0.2	no effect	no effect
VIIIb	10^{-4}	0.2	−	−
	10^{-4}	0.2	+	−
	$10^{-4}\times 2.5$	0.2	no effect	
VIIIc	10^{-4}	0.2	no effect	−
VIIId	10^{-4}	0.2	+	−
	10^{-4}	0.2	+	+
VIIIf	$10^{-3}\times 2.5$	0.1	±	
	$10^{-3}\times 2.5$	0.2	no effect	no effect
	10^{-4}	0.1	±	−
	10^{-4}	0.2	±	−
	10^{-4}	0.2	+	−
	$10^{-4}\times 2.5$	0.2	+ weak	−
	$10^{-4}\times 2.5$	0.3	+	−
VIIIg	10^{-4}	0.2	no effect	no effect
	10^{-4}	0.2	+	−
VIIIj	10^{-4}	0.2	+	−
VIIIl	$10^{-3}\times 5$	0.05	−	−
	$10^{-3}\times 5$	0.1	−	−
	$10^{-3}\times 5$	0.2	−	−
	10^{-4}	0.1	−	−
	10^{-4}	0.2	−	−
	$10^{-4}\times 2.5$	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 boronhydride in dioxan 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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