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187. Akira Kasahara, Takeshi Onodera, Michiko Mogi, Yasuo Oshima, and Masao Shimizu : Investigations on Steroids. V.*1 Pharmacological Studies. (1). Anabolic and Androgenic Activities of 17β-Hydroxy-17α-methyl-5α-androstano[2,3-*c*]furazan (Androfurazanol), A New Active Anabolic Steroid.*2

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In order to obtain a highly potent anabolic agent possessing only a low androgenic effect, much effort has been made by medicinal chemists to modify the structure of androgens. The progress in this field has been extensively reviewed by Camerino and Sala[1] in 1960. Thereafter many investigators have given a number of informations on the correlation between the structure and anabolic activity.[2] Among them interest in the androstane derivatives possessing heterocycles fused to 2,3-positions, some of which have been shown to have a favorable anabolic/androgenic ratio, has prompted us to examine the biological effect of various steroidal heterocycles. The steroidal heterocycles synthesized in this laboratory have been submitted for pharmacological screening for myotrophic and androgenic activities. From these experiments it has been found that 17β-hydroxy-17α-methyl-5α-androstano[2,3-*c*]furazan, hereafter referred to as androfurazanol, is relatively more anabolic than androgenic. The present paper deals with the myotrophic, androgenic, and nitrogen-retaining activities of androfurazanol and related compounds.

Materials and Methods

The compounds (I) to (XI) were synthesized in this laboratory as described in the preceding paper,*1 except (X) and (XI) which were synthesized according to the methods of Ringold[3] and Clinton,[4] respectively. Testosterone propionate was purchased from the Tokyo Kosei Kogyo Co., Ltd. and methyltestosterone from the Iwaki Seiyaku Co., Ltd. The compounds used in the present experiments are listed below.

17β-Hydroxy-17α-methyl-5α-androstano[2,3-*c*]furazan (androfurazanol) (I), m.p. 152~153°; 2-hydroxyimino-17β-hydroxy-17α-methyl-5α-androstan-3-one (II), m.p. 249~251° (decomp.); 2,3-dihydroxyimino-17α-methyl-5α-androstan-17β-ol (III), m.p. 234~235° (decomp.); 17β-hydroxy-17α-methyl-5α-androstano[2,3-*c*]furazan N-oxide (IV), m.p. 180°; 17β-hydroxy-17α-methylandrost-4-eno[2,3-*c*]furazan (V), m.p. 182~183°; 17β-hydroxy-17α-methylandrosta-4,6-dieno[2,3-*c*]furazan (VI), m.p. 194~195°; 17β-hydroxy-5α-androstano[2,3-*c*]furazan (VII), m.p. 160~161°; 17β-hydroxyandrost-4-eno[2,3-*c*]furazan (VIII), m.p. 175~176°; 17β-hydroxyandrosta-4,6-dieno[2,3-*c*]furazan (IX), m.p.

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*1 Part IV. This Bulletin, **13**, 1445 (1965).

*2 A part of this work was reported at the Kanto Branch Meeting of the Pharmaceutical Society of Japan, Tokyo, July 18, 1964.

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1) B. Camerino, G. Sala : "Fortschritte der Arzneimittelforschung" Ed. by E. Jucker, **2**, 71 (1960), Birkhäuser Verlag, Basel.

2) *a*) A.J. Manson, F.W. Stonner, H.C. Neumann, R.G. Christiansen, R.L. Clarke, J.H. Ackerman, D.F. Page, J.W. Dean, D.K. Phillips, G.O. Potts, A. Arnold, A.L. Beyler, R.O. Clinton : J. Med. Chem., **6**, 1 (1963). *b*) A. Bowers, A.D. Cross, J.A. Edwards, H. Carpio, M.C. Calzada, E. Denot : *Ibid.*, **6**, 156 (1963). *c*) J.A. Zderic, H. Carpio, A. Ruiz, D.C. Limón, F. Kincl, H.J. Ringold : *Ibid.*, **6**, 195 (1963). *d*) J.H. Ackerman, G.O. Potts, A.L. Beyler, R.O. Clinton : *Ibid.*, **7**, 238 (1964). *e*) F.A. Kincl, R.I. Dorfman : Steroids, **3**, 109 (1964). *f*) A.D. Cross, I.T. Harrison, P. Crabbé, F.A. Kincl, R.I. Dorfman : *Ibid.*, **4**, 229 (1964). *g*) R.I. Dorfman, F.A. Kincl : Endocrinol., **72**, 259 (1963).

3) H.J. Ringold, E. Batres, O. Halpern, E. Necoechea : J. Am. Chem. Soc., **81**, 427 (1959).

4) R.O. Clinton, A.J. Manson, F.W. Stonner, H.C. Neumann, R.G. Christiansen, R.L. Clarke, J.H. Ackerman, D.F. Page, J.W. Dean, W.B. Dickinson, C. Carabateas : *Ibid.*, **83**, 1478 (1961).

173.5～174.5°; 2-hydroxymethylene-17β-hydroxy-17α-methyl-5α-androstan-3-one (X), m.p. 177～179°; 17β-hydroxy-17α-methyl-5α-androstano[3,2-*c*]pyrazole (stanozolol) (XI), m.p. 230～233°; testosterone propionate (XII), m.p. 118.5～119.5°; methyltestosterone (XIII), m.p. 163～164°.

I. Myotrophic and Androgenic Activities (Castrated Rat Assay)

Myotrophic and androgenic activities were determined by the modification of a method reported by Hershberger, *et al.*[5] Immature male rats of Donryu strain from Central Laboratories for Experimental Animals (Tokyo) were castrated at 23～25 days of age. The animals were maintained on "CLEA CA-1" piet, obtained from the same Laboratories, and tap water *ad libitum* in air-conditioned quarters. Test compounds were dissolved in cottonseed oil for administration, and suspensions in the oil were used for large doses. The compounds were administered subcutaneously or orally once daily for 6 days, beginning 2 days after the castration. The animals were autopsied 2 days after the last medication and the levator ani muscle, ventral prostate, and seminal vesicles were removed, blotted, and weighed. The increase in the weight of the levator ani muscle was used as the indication of myotrophic activity. The androgenic activity was determined from the weight increase of the ventral prostate and seminal vesicles, and was expressed as the geometrical mean value of the two. The relative activities were estimated graphically.

II. Nitrogen-retaining Activity

Nitrogen-retaining activity was studied essentially according to the method described by Stafford, *et al.*[6] Male rats of Donryu strain were castrated at 25 days of age and were fed CLEA CA-1 diet and tap water *ad libitum* for 4 months. After transfer of the rats to metabolism cages, transition from this diet to controlled intake of liquid diet*4 was accomplished by beginning with 8 ml./rat/day and increasing the amount by 2 ml./day to a level of 26 ml./rat/day. Twenty days after the maximum feeding level was attained, the animals were treated orally with test compounds as a solution or suspension in cottonseed oil, once daily for 10 days. Forty-eight hour urine specimens were collected successively during the period of pre-treatment (10 days), treatment (10 days), and post-treatment (10 days). Each specimen was diluted with water to 500 ml. and analyzed for total nitrogen by the micro-Kjeldahl method and was calculated as mg. nitrogen/24 hours.

III. Androgenic Activity (Chick's Comb Assay)

The chick's comb assay was performed essentially according to the method described by Dorfman.[7] Male White Leghorn chicks, 4 days of age, were used, which were obtained from Shukuya Shukinjô (Tokyo). The animals were kept in a thermostatically controlled room of 32～34° and were fed "chick food" (Yamaichi Shiryô Co., Ltd.) and water exclusively. Test compounds were dissolved or suspended in corn oil. In inunction assay, the solution was directly applied to the comb once daily for 7 days (0.05ml./animal/day), and in injection assay, the solution or the suspension was administered subcutaneously once daily for 5 days (0.1 ml./animal/day). On the day following the last medication, the animals were sacrificed and the combs were dissected, blotted, and weighed. The response was expressed as comb ratios defined as mg. of comb/g. body weight.

Results and Discussion

I. Myotrophic and Androgenic Activities (Castrated Rat Assay)

A) Androfurazanol and its Intermediates

Table I presents the activities of androfurazanol administered by subcutaneous injection. Testosterone propionate was taken as a standard. From these data, androfurazanol was determined to be 1.5 times as myotrophic as testosterone propionate on the endpoint levator ani muscle, and the androgenic activity of the compound was 0.25 (A) and 0.27 (B) times as active as testosterone propionate on the endpoint ventral prostate and seminal vesicles, respectively. The geometrical mean 0.26 was calculated from the values of 0.25 and 0.27. On the basis of these evaluation, the myotrophic/androgenic ratio for androfurazanol was found to be 5.8. In repeated experiment, the

*4 Composition of this liquid diet is as follows : Cellulose powder, 60 g.; salt mixture, 40 g.; dried yeast, 100 g.; casein, 160 g.; starch, 200 g.; dextrin, 190 g.; sucrose, 200 g.; water, 1110 ml.; corn oil, 190 ml.; cod liver oil, 10 ml.; α-tocopherol, 5.5% solution in cottonseed oil, 10 ml.; vitamin K, 0.5% solution in cottonseed oil, 10 ml.

5) L.G. Hershberger, E.G. Shipley, R.K. Meyer : Proc. Soc. Exptl. Biol. Med., **83**, 175 (1953).
6) R.O. Stafford, B.J. Bowman, K.J. Olson : *Ibid.*, **86**, 322 (1954).
7) R.I. Dorfman : "Methods in Hormone Research," **II**, 287, 297 (1962), Academic Press Inc., New York.

Table I. Myotrophic and Androgenic Activities of Androfurazanol compared with Testosterone Propionate (Subcutaneous Injection)

Compound	No. of animals	Total dose (mg./kg.)	Myotrophic response l. ani wt. (mg.)	Androgenic response v. prost. wt. (mg.)	Androgenic response s. ves. wt. (mg.)
Control	4	—	17.7±2.1[a]	5.9±0.4	8.7±0.5
Testosterone propionate	5	3	29.7±3.3	32.2±1.5	33.4±0.7
	5	6	27.5±1.8	38.4±2.5	52.4±2.7
	5	12	34.0±2.2	65.8±5.8	81.3±4.6
Androfurazanol	5	6	29.5±1.4	23.9±1.5	24.0±1.4
	5	12	41.2±2.8	33.5±4.0	36.0±2.1
	5	24	52.5±2.3	46.3±2.0	59.5±4.6
Relative activity (Testosterone propionate=1)			1.5	0.25(A)	0.27(B)
				$\sqrt{AB}$ =0.26	
M/A ratio[b]=5.8 (Repeated test: Myotrophic, 1.0; Androgenic, 0.19; M/A ratio, 5.3)					

a) ± Standard error
b) M/A ratio: Myotrophic/androgenic ratio

ratio was 5.3. On the other hand, 17β-hydroxy-17α-methyl-5α-androstano[3,2-*c*]pyrazole (stanozolol) was found to be 0.2 times as myotrophic and 0.05 times as androgenic as testosterone propionate (Table II). Thus, the myotrophic/androgenic ratio was 4.0.

Table II. Myotrophic and Androgenic Activities of Stanozolol compared with Testosterone Propionate (Subcutaneous Injection)

Compound	No. of animals	Total dose (mg./kg.)	Myotrophic response l. ani wt. (mg.)	Androgenic response v. prost. wt. (mg.)	Androgenic response s. ves. wt. (mg.)
Control	5	—	17.6±1.4[a]	7.5±0.2	7.7±1.2
Testosterone propionate	5	3	24.3±1.7	33.0±3.6	34.9±2.9
	5	12	45.6±3.5	63.9±1.8	86.9±3.5
Stanozolol	5	21	31.7±1.8	20.4±1.0	17.4±0.7
	5	84	46.6±3.0	43.7±6.4	42.8±3.6
Relative activity (Testosterone propionate=1)			0.20	0.06(A)	0.05(B)
				$\sqrt{AB}$ =0.05	
M/A ratio[b]=4.0					

a) ± Standard error
b) M/A ratio: Myotrophic/androgenic ratio

When compared orally, as shown in Table III, androfurazanol was 2.7~3.3 times as myotrophic and 0.73~0.94 times as androgenic as methyltestosterone. The resultant myotrophic/androgenic ratio for androfurazanol was thus 3.5~3.7. Stanozolol showed the same order of myotrophic and androgenic activities as androfurazanol when given orally, that is, 2.0~4.7 times as myotrophic and 0.85~1.05 times as androgenic as methyltestosterone (myotrophic/androgenic ratio, 2.4~4.9).

Potts, *et al.*[8] reported that stanozolol was 1/7 as myotrophic and 1/33 as androgenic as testosterone propionate when compared subcutaneously and was twice as

8) G.O. Potts, A.L. Beyler, D.F. Burnham: Proc. Soc. Exptl. Biol. Med., **103**, 383 (1960).

Table III. Myotrophic and Androgenic Activities of Methyltestosterone, Androfurazanol, and Stanozolol (Oral Administration)

Compound	No. of animals	Total dose (mg./kg.)	Myotrophic response l. ani wt. (mg.)	Androgenic response v. prost. wt. (mg.)	Androgenic response s. ves. wt. (mg.)
Control	5	—	19.4±0.9[a]	8.0±0.3	10.2±0.6
Methyltestosterone	4	30	24.5±1.8	23.3±1.7	13.5±0.5
	5	90	27.2±2.0	32.4±1.9	18.6±0.7
	5	270	36.6±1.6	46.6±3.7	33.0±1.3
	4	810	52.3±4.6	87.3±3.6	65.3±4.4
Androfurazanol	5	30	30.4±3.8	20.4±2.7	14.2±1.0
	5	90	36.4±2.6	26.6±2.7	22.0±1.0
	5	270	48.6±1.5	41.0±2.0	32.8±1.6
	4	810	57.0±3.6	68.0±5.8	61.5±3.5
Relative activity (Methyltestosterone=1)			2.7	0.58(A)	0.93(B)
				$\sqrt{AB}$ =0.73	
M/A ratio[b]=3.7 (Repeated test: Myotrophic, 3.3; Androgenic, 0.94; M/A ratio, 3.5)					
Stanozolol	5	30	24.0±1.2	13.6±1.0	12.8±0.8
	5	90	32.2±3.0	21.2±1.2	19.0±0.9
	5	270	48.8±1.8	45.6±6.4	47.8±5.7
	5	810	55.0±2.8	63.8±3.5	74.6±6.2
Relative activity (Methyltestosterone=1)			2.0	0.52(A)	1.40(B)
				$\sqrt{AB}$ =0.85	
M/A ratio[b]=2.4 (Repeated test: Myotrophic, 4.7; Androgenic, 1.05; M/A ratio, 4.9)					

a) ± Standard error
b) M/A ratio: Myotrophic/androgenic ratio

myotrophic and 1/3 as androgenic as methyltestosterone when compared orally. Therefore, myotrophic/androgenic ratio can be shown as 4.7 and 6.0 in subcutaneous and oral administration, respectively. Donini, *et al.*,[9] on the other hand, reported that stanozolol has a ratio of 2.35 when administered orally. It is noteworthy that androfurazanol was more active than stanozolol when administered subcutaneously, while they exhibited the same order of the activity when given orally. 17α-Methyl and 17α-ethyl derivatives of 19-nortestosterone[10] and 17β-hydroxy-17α-methylandrost-4-eno[2,3-*d*]isoxazole[2a] were found to possess the same order of myotrophic effect as testosterone propionate when compared parenterally, although the effect of 17α-alkylated steroids by this route is, in general, low.

Several androstane derivatives containing heterocycles fused to the 2,3-position of the steroid ring have recently been synthesized. Concerning six-membered heterocyclic derivatives, some pyrimidine[2d,11] and quinoline[12] derivatives have been synthesized. Among the former, 2′-methyl-17β-hydroxy-17α-methyl-5α-androstano[3,2-*d*]-pyrimidine,[2d] for example, was found to have a favorable myotrophic/androgenic ratio but the data on anabolic effect of the latter have not been reported. Pyridine

9) P. Donini, R. Montezemolo: Il Farmaco-Ed. Sc., **XVI**, 633 (1961).
10) V.A. Drill, B. Riegel: "Recent Progress in Hormone Research," Ed. by G. Pincus, **XIV**, 29 (1958), Academic Press Inc., New York.
11) *a*) P. Ruggieri, C. Gandolfi, D. Chiaramonti: Gazz. chim. ital., **92**, 768 (1962). *b*) J.A. Zderic, O. Halpern, H. Carpio, A. Ruiz, D.C. Limon, L. Magaña, H. Jiménez, A. Bowers, H.J. Ringold: Chem. & Ind. (London), **1960**, 1625. *c*) L.L. Smith, D.M. Teller, T. Foell: J. Med. Chem., **6**, 330 (1963).
12) H. Antaki, V. Petrow: J. Chem. Soc., **1951**, 901.

derivatives reported in a previous paper,[13] in general, proved to have only a weak myotrophic and androgenic activity, although 17β-hydroxy-17α-methyl-5α-androstano-[3,2-*b*]pyridine showed a relatively high myotrophic/androgenic ratio.

Steroids possessing five-membered heterocycles have also been reported in literatures. Pyrrole,[12,14] furan,[15] and indole[12] derivatives have been described, but their anabolic effect has not been published except that of the pyrrole derivatives[14] possessing a favorable anabolic/androgenic ratio. However, the steroids containing two hetero atoms, such as pyrazole,[8,9,16~18] isoxazole,[2a,9,11b,18] and thiazole[2c] derivatives are of great interest since they have been found to be highly potent anabolic steroids with low androgenic activity. In addition steroidal[2,3-*d*]triazoles have been synthesized by

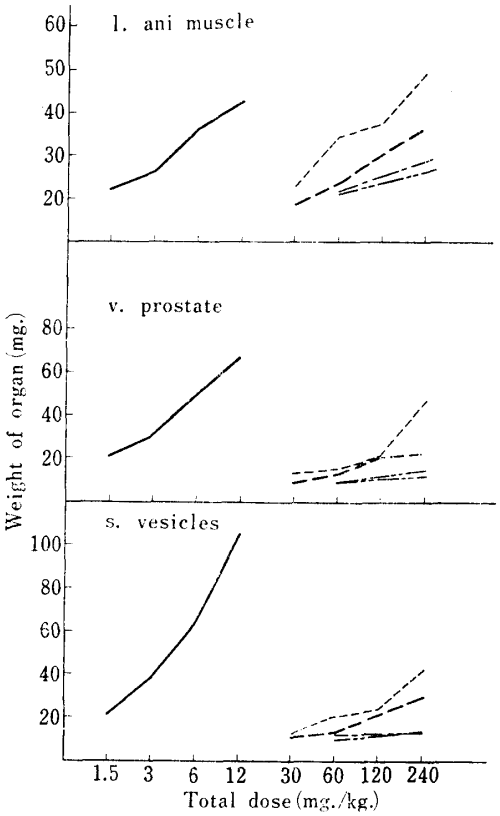

Fig. 1. Myotrophic and Androgenic Effect of Intermediates in Synthesis of Androfurazanol (Subcutaneous Injection)

——— Testosterone propionate
- - - - - - Compound (II)
— — — Compound (III)
— - - — Compound (IV)
- - - - - - - Compound (X)

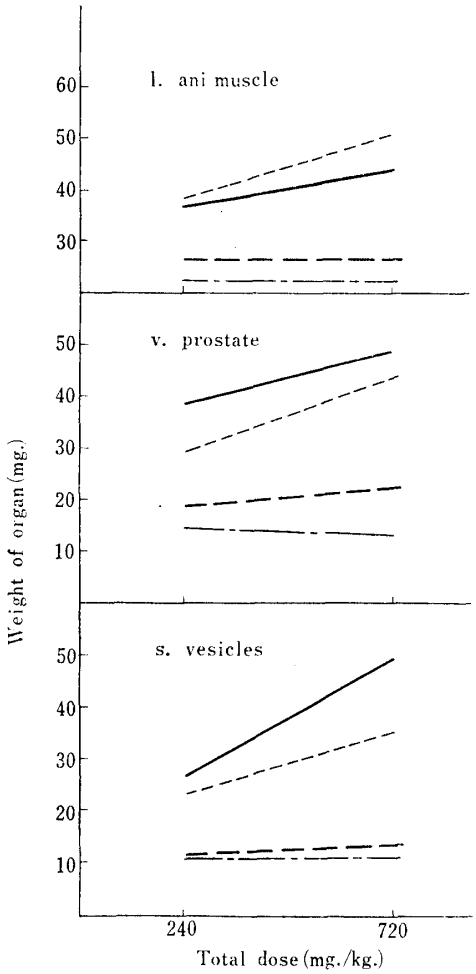

Fig. 2. Myotrophic and Androgenic Effect of Intermediates in Synthesis of Androfurazanol (Oral Administration)

——— Methyltestosterone
- - - - Compound (II)
— — — Compound (III)
- - - - - - - Compound (X)

13) M. Shimizu, G. Ohta, K. Ueno, T. Takegoshi : This Bulletin, **12**, 77 (1964).
14) J. Orr, A. Bowers : U.S. Pat., 3,032,551 (1962).
15) J.C. Orr, M.L. Franco, A.D. Cross, F. Sondheimer : Steroids, **3**, 1 (1964).
16) R.O. Clinton, A.J. Manson, F.W. Stonner, A.L. Beyler, G.O. Potts, A. Arnold : J. Am. Chem. Soc., **81**, 1513 (1959).
17) A. Arnold, A.L. Beyler, G.O. Potts : Proc. Soc. Exptl. Biol. Med., **102**, 184 (1959).
18) A. Arnold, G.O. Potts, A.L. Beyler : Endocrinol., **72**, 408 (1963).

Nathansohn, *et al.*,[19] which are the only group of these compounds possessing three hetero atoms. They have been described as a new series of potent anabolic steroids but their biological activities have not yet been published in detail. It is interesting to remember that androfurazanol is an isostere of the triazolo-steroid.

Activities of 2-hydroxyimino-17β-hydroxy-17α-methyl-5α-androstan-3-one (II) and 2,3-dihydroxyimino-17α-methyl-5α-androstan-17β-ol (III) were tested, which are intermediates in the synthesis of androfurazanol. 2-Hydroxymethylene-17β-hydroxy-17α-methyl-5α-androstan-3-one (X) was also used for comparison. The results are shown in Figs. 1 and 2. The compound (X) was found to be weakly myotrophic and androgenic when compared subcutaneously with testosterone propionate. The compounds (II) and (III) were much less active. 17β-Hydroxy-17α-methyl-5α-androstano[2,3-*c*]-furazan N-oxide (IV), which is another intermediate in the synthesis of androfurazanol, was much less active than testosterone propionate. It is of interest that the introduction of N-oxide group into the furazan ring of androfurazanol caused a great decrease of the activity from the parent compound.

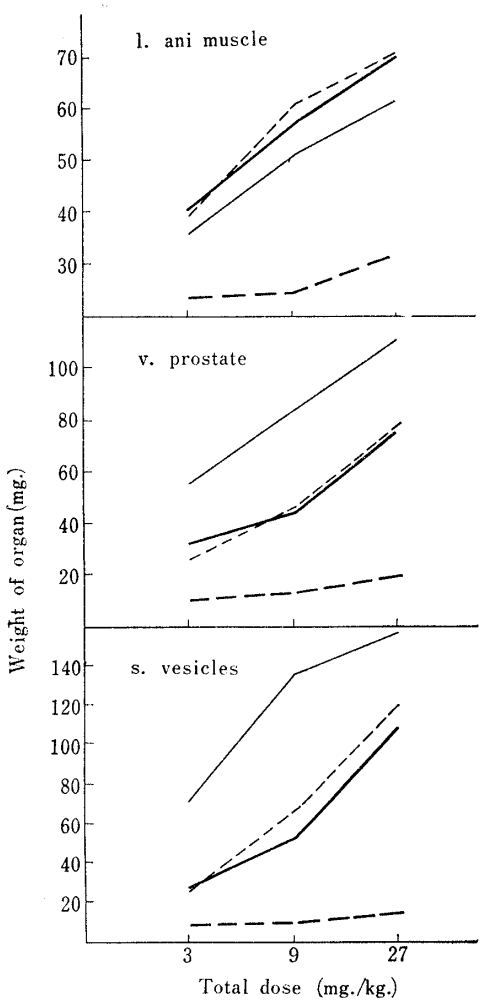

Fig. 3. Influence of Molecular Unsaturation on the Effect of Androfurazanol (Subcutaneous Injection)

——— Testosterone propionate
━━━ Androfurazanol
------- Compound (V)
– – – – Compound (VI)

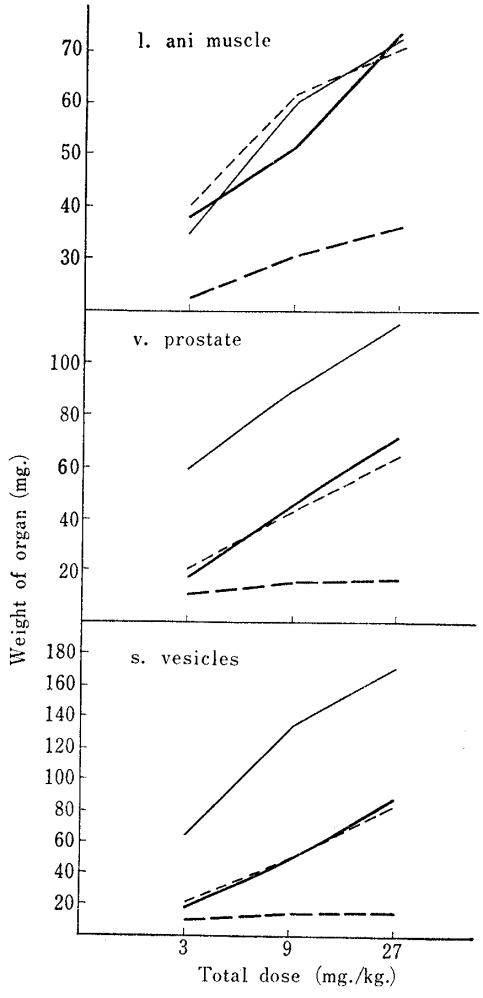

Fig. 4. Influence of Molecular Unsaturation on the Effect of Compound (VII) (Subcutaneous Injection)

——— Testosterone propionate
━━━ Compound (VII)
------- Compound (VIII)
– – – – Compound (IX)

19) G. Nathansohn, E. Testa, N. DiMola : Experientia, **18**, 57 (1962).

When compared orally, the compound (X) was more myotrophic and less androgenic than methyltestosterone. The compound (II), in spite of being an isostere of (X), exhibited much less myotrophic and androgenic activities than methyltestosterone. The compound (III) was almost inactive.

B) Influence of Molecular Unsaturation on Activities

Beyler, *et al.*[20] reported that within the series of steroidal pyrazoles the degree and type of hormonal activity were profoundly influenced by the unsaturation in ring A and B of the steroidal moiety of the molecule. In view of this fact, it seemed of interest to examine the influence of unsaturation on myotrophic and androgenic activities of androfurazanol. Figs. 3 and 5 present the results of experiments wherein the myotrophic and androgenic activities of singly and doubly unsaturated congeners of androfurazanol were compared with those of androfurazanol. When administered subcutaneously, as shown in Fig. 3, the singly unsaturated congener (V) had the same order of myotrophic and androgenic activities as androfurazanol. Also, in the case of 17β-hydroxy-5α-androstano[2,3-*c*]furazan (VII), as shown in Fig. 4, the unsaturation at position 4~5 did not lower the activities. These results are in contrast to those of stanozolol in which the introduction of a double bond at position 4~5 was found to cause decrease of the activities.[20] On the other hand, as shown in Figs. 3 and 4, the unsaturation at positions 4~5 and 6~7 in androfurazanol or the compound (VI) lowered both myotrophic and androgenic activities to a large extent.

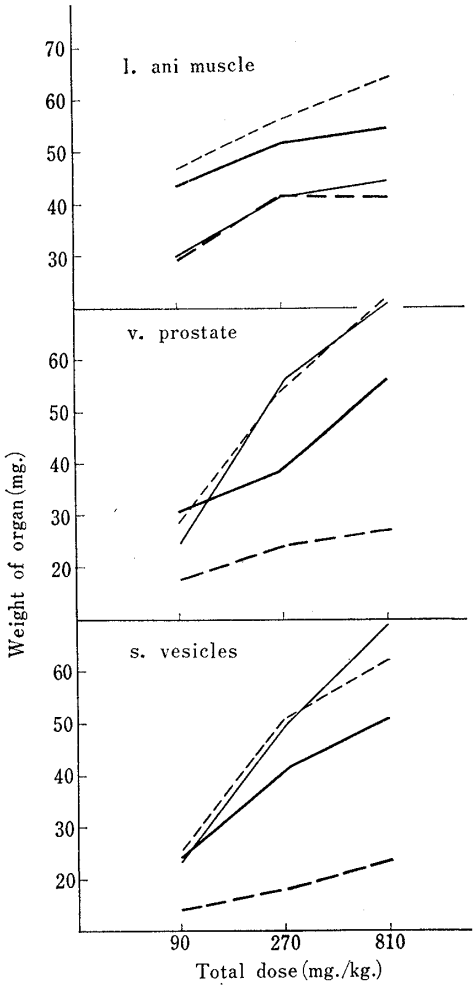

Fig. 5. Influence of Molecular Unsaturation on the Effect of Androfurazanol (Oral Administration)

When administered orally, as shown in Fig. 5, the presence of a double bond at the 4~5 position of androfurazanol rather enhanced both myotrophic and androgenic effects, while the introduction of a double bond at this position of stanozolol[20] or 17β-hydroxy-17α-methyl-5α-androstano[2,3-*d*]isoxazole[2a] was found to lower the effects. However, the unsaturation at positions 4~5 and 6~7 of androfurazanol greatly lowered the activities. This tendency observed in androfurazanol and its derivatives resembles that in methylandrostanolone (17β-hydroxy-17α-methyl-5α-androstan-3-one) and its derivatives, since Beyler, *et al.*[20] have shown that methyltestosterone was the most active myotrophic agent followed in rank by methylandrostanolone, then by 17β-hydroxy-17α-methylandrosta-4,6-dien-3-one, when compared orally. They stated, however, that the myotrophic effect of methyltestosterone was also more active than that of methylandrostanolone subcutaneously.

II. Nitrogen-retaining Activity

Fig. 6 shows the mean daily nitrogen excretion

20) A.L. Beyler, G.O. Potts, A. Arnold : Endocrinol., **68**, 987 (1961).

in 4 groups of rats, in which 2 groups were treated orally with methyltestosterone in a dose of 6 or 30 mg./rat/day and other 2 groups with androfurazanolin a dose of 0.2 or 1 mg./rat/day for 10 days. The base-line of the graph is the mean nitrogen excretion before treatment. Methyltestosterone and androfurazanol in these doses caused apparent decreases in the nitrogen excretion, but after cessation of the treatment the excretion increased to the level over the pre-treatment average. Table Ⅳ shows the values of three indices by which the activity was evaluated. From these data it can be seen that androfurazanol at 0.2 and 1 mg./rat/day produced essentially the same response as at 6 and 30 mg./rat/day of methyltestosterone, respectively. The graphical estimation using the values of "total nitrogen retained" (A) showed androfurazanol to be 29 times as effective as methyltestosterone in supporting nitrogen retention. The "total nitrogen retained" is the summation of the differences between the mean pre-treatment nitrogen excretion and

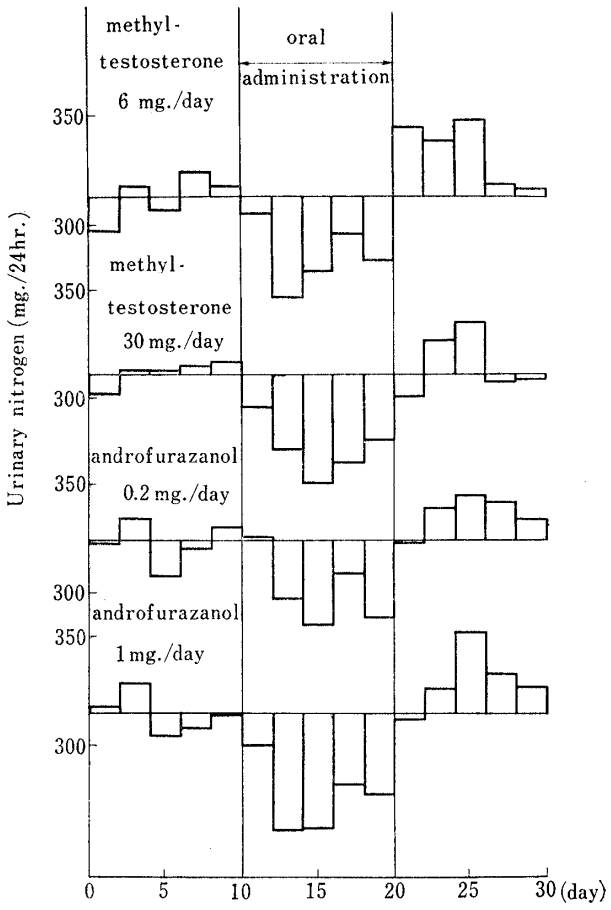

Fig. 6. Urinary Nitrogen Excretion of Rats treated with Methyltestosterone and Androfurazanol

Table Ⅳ. Urinary Nitrogen Excretion of Rats treated with Methyltestosterone and Androfurazanol

	Methyltestosterone (mg./day)		Androfurazanol (mg./day)	
	6	30	0.2	1
No. of rats	4	5	4	5
Total nitrogen retained (mg.) (A)	268	362	236	392
No. of days in retention period (B)	10	12	10	12
Greatest single daily retention (mg.) (C)	46	50	39	54
Relative nitrogen-retaining activity	Methyltestosterone : Androfurazanol = 1 : 29			

daily values during the retention period. The "number of days in retention period" (B) is the period during which the continuously lower nitrogen excretion than the pre-treatment average was found after beginning of the treatment. The "greatest single daily retention" (C) is the difference between the lowest daily nitrogen value after beginning of treatment and the pre-treatment average. The two indices, B and C, also show that androfurazanol has a potent effect as compared with methyltestosterone.

Arnold, *et al.*[17] reported that stanozolol was 30 times more active than methyltestosterone in effecting nitrogen retention when given orally.

Thus, from the experiments on myotrophic, androgenic, and nitrogen-retaining activities it appears reasonable to conclude that androfurazanol exhibits a pattern of the activities similar to that of stanozolol when given orally.

III. Androgenic Activity (Chick's Comb Assay)

The results obtained in inunction and injection assays are shown in Tables V and VI. Relative activities were estimated on the basis of the doses of compounds

Table V. Androgenic Activities of Testosterone Propionate, Androfurazanol, and Stanozolol in Chick's Comb Assay (Inunction)

Compound	Total dose (mg.)	Comb ratio	Dose effecting 100% increase (mg.)
Control	—	0.27±0.01[a]	—
Testosterone propionate	0.04	0.53±0.05	0.05
	0.12	0.57±0.06	
	0.36	0.71±0.08	
	1.08	1.07±0.08	
Androfurazanol	0.10	0.42±0.02	0.18
	0.30	0.65±0.08	
	0.90	0.73±0.10	
	2.70	0.77±0.05	
Stanozolol	0.03	0.32±0.02	0.49
	0.10	0.39±0.02	
	0.30	0.43±0.02	
	0.90	0.68±0.05	
Relative activity	Testosterone propionate : Androfurazanol : Stanozolol		
	= 1 : 0.28 : 0.10		

a) ± Standard error

Table VI. Androgenic Activities of Testosterone Propionate, Androfurazanol, and Stanozolol in Chick's Comb Assay (Subcutaneous Injection)

Compound	Total dose (mg.)	Comb ratio	Dose effecting 100% increase (mg.)
Control	—	0.27±0.01[a]	—
Testosterone propionate	0.10	0.42±0.02	0.36
	0.30	0.51±0.03	
	0.90	0.68±0.04	
	2.70	0.76±0.05	
	8.10	0.67±0.05	
Androfurazanol	0.30	0.37±0.02	0.70
	0.90	0.59±0.04	
	2.70	0.60±0.04	
	8.10	0.57±0.04	
	24.30	0.48±0.02	
Stanozolol	0.10	0.29±0.01	1.58
	0.30	0.31±0.01	
	0.90	0.48±0.02	
	2.70	0.60±0.03	
	8.10	0.64±0.05	
Relative activity	Testosterone propionate : Androfurazanol : Stanozolol		
	= 1 : 0.51 : 0.28		

a) ± Standard error

needed to produce a 100% increment in comb ratio. In inunction assay, androfurazanol and stanozolol proved to be 0.28 and 0.1 times as androgenic as testosterone propionate, respectively. When compared subcutaneously, androfurazanol and stanozolol were 0.51 and 0.23 times as androgenic as testosterone propionate, respectively.

Thus, the low androgenicity of androfurazanol compared with that of testosterone propionate was ascertained by the chick's comb assay as well as by the castrated rat assay.

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Summary

Androfurazanol, 17β-hydroxy-17α-methyl-5α-androstano[2,3-*c*]furazan, was found to be a new potent anabolic steroid with relatively low androgenicity. The fact has been ascertained by the determinations of myotrophic/androgenic ratio and nitrogen-retaining activity using castrated male rats. Chick's comb assay has also been performed for the determination of the androgenic activity.

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