2 α , 3 α -EPITHIO-5 α -ANDROSTAN-17 β -YL 1-METHOXY-
CYCLOPENTYL ETHER (10364-S), A NEW ORALLY
ACTIVE ANABOLIC-ANDROGENIC STEROID

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Abstract—2 α , 3 α -Epithio-5 α -androstan-17 β -yl 1-methoxycyclopentyl ether (10364-S) was found to possess marked myotropic and androgenic activities by oral route in the rat, and its potency and myotropic-androgenic ratio were estimated to be almost equivalent to those of fluoxymesterone. It was further demonstrated that the oral administration of 10364-S, 20 mg/kg a day for 3 weeks, had hardly no effect on liver function of rabbits tested by BSP retention, plasma GOT and GPT activities, and plasma cholesterol levels, whereas treatment with methyltestosterone, oxymetholone or fluoxymesterone, at the same dosage, caused abnormal BSP retention and plasma GOT activity. It is thus concluded, that 10364-S when given orally is an active anabolic agent with a therapeutic potency equivalent to fluoxymesterone yet in clinical administration is less likely to cause hepatic disturbance.

Orally active anabolic steroids alkylated at C-17 α position are known to possess a therapeutic disadvantage of hepatic dysfunction in clinical use (1). Vitali et al. (2) reported that suitable etherification at 17 β -hydroxy group of several known androgenic and anabolic steroids for yielding alkyl steroid-17 β -yl mixed acetals provided a much enhanced activity when given orally in comparison with the corresponding 17 α -alkylated steroids. Komeno et al. (3) applied similar modifications to the case of 2 α , 3 α -epithio-5 α -androstan-17 β -ol (epitio-steranol) to produce its 17 β -(1'-methoxy)-cyclopentyl ether (10364-S), and Miyake et al. (4) demonstrated that the derivative, 10364-S, was more potent than the corresponding 17 α -methylated steroid in a conventional oral assay for androgenic and myotropic activities using rats. Furthermore, in the bromosulfophthalein (BSP) retention test in rabbits, 10364-S was found to be extremely less active as compared with 17 α -methyltestosterone or 17 α -methylepitio-steranol, which caused a marked abnormal BSP reten-

FIG. 1. Structures of 10364-S, oxymetholone and fluoxymesterone.

tion during the oral medication of 20 mg/kg a day for 15 or 30 days (unpublished data). Thus further biological evaluation of 10364-S as a less hepatotoxic anabolic agent in comparison with popular anabolic steroids such as oxymetholone (2-hydroxymethylen-3-oxo-17 α -methyl-5 α -androstan-17 β -ol) or fluoxymesterone (9 α -fluoro-11 β , 17 β -dihydroxy-17 α -methylandro-4-en-3-one) was carried out. Structures of the steroids are presented in Fig. 1.

MATERIALS AND METHODS

1. Androgenic and myotropic assay

Androgenic and myotropic activities were determined by the method of Hershberger et al. (5) with a minor modification. Male rats of Japan Clea Sprague-Dawley strain, 3 weeks old, were castrated under sodium methylhexabital anesthesia (125 mg/kg, i.p.). The steroids were dissolved or suspended in sesame oil (J.P.) and force-fed by gavage in a volume of 0.2 ml per rat once daily for 10 consecutive days starting on the day of castration. The animals were autopsied on the day following the last administration. Seminal vesicles, ventral prostate and levator ani muscle were removed and weighed. Relative potency estimates were obtained by symmetrical multiple point parallel line assays (6).

2. Liver function test

Albino rabbits, weighing 1.8 to 2.5 kg for females and 2.3 to 3.3 kg for males, were used. The steroids were dissolved or suspended in sesame oil (J.P.) at the concentration of 50 mg/ml and force-fed by gavage at a dosage of 20 mg/kg once daily for 3 weeks. Control rabbits were given the vehicle alone. Bromosulphophthalein (BSP) test and determinations of plasma total cholesterol level, glutamic-oxaloacetic-transaminase (GOT) and glutamic-pyruvic-transaminase (GPT) activities were carried out on Day-1 (a day before starting medication), Days 7, 14, 21 (after medication for 1, 2, and 3 weeks, respectively) and Day 28 (a week after cessation of medication). Before BSP test, rabbits were fasted for 20 hr. According to the method of Lennon (7), 20 mg/kg of BSP (Dai-ichi Chemical Co. Ltd., Tokyo) was injected into the marginal vein of the right ear. Exactly 20 min after BSP injection, 2 ml of blood was collected from the marginal vein of the left ear with a heparinized syringe and centrifuged at 3000 rpm for 15 min. Plasma BSP level was determined by the method of Seligson et al. (8). Plasma cholesterol level was determined by the method described previously (9). Plasma GOT and GPT activities were determined by the use of Hepatest-A (Dai-ichi Chemical Co. Ltd., Tokyo).

RESULTS

1. Androgenic and myotropic activities

Fig. 2 represents the dose-response relations of 10364-S and oxymetholone in a comparative assay with two dosages having the ratio of 1 to 4. The relative potencies of 10364-S against oxymetholone (=1) were calculated to be 17, 10, and 17 in the responses of seminal vesicle, ventral prostate, and levator ani muscle, respectively.

Fig. 3 presents comparative dose-response relations of 10364-S and fluoxymesterone

FIG. 2. Effects of 10364-S and oxymetholone on seminal vesicle, ventral prostate and levator ani muscle weights in castrated immature male rats. Each point and bar represents the mean and standard error of 8 rats.

FIG. 3. Effects of 10364-S and fluoxymesterone on seminal vesicle, ventral prostate and levator ani muscle weights in castrated immature male rats. Each point and bar represents the mean and standard error of 8 rats.

at the same two dosage levels. The relative potencies of 10364-S against fluoxymesterone ($=1$) were calculated to be 0.7, 0.8, and 0.8 in the responses of seminal vesicle, ventral prostate, and levator ani muscle, respectively. These results indicate that androgenic and myotropic activity of 10364-S is approx. 17 times higher than oxymetholone and almost equals that of fluoxymesterone when given orally.

2. Effects on liver function

Tables 1 and 2 present the data showing the effects of methyltestosterone, fluoxymesterone, oxymetholone and 10364-S on BSP retention, total cholesterol level, GPT and GOT

TABLE 1. Effects of steroids on the liver function of male rabbits

Steroid	Parameter	Day of determination				
		-1	7	14	21	28
Control (sesame oil)	No. of rabbits	7	7	7	7	7
	B.W.	2723±110 ^{a)}	2850±110 ^{a)}	3051±123 ^{a)}	3243±134 ^{a)}	3399±145 ^{a)}
	BSP	4.7±0.5	4.5±0.5	5.3±0.9	4.2±0.5	4.8±0.6
	(A/T)	(0/7)	(0/7)	(1/7)	(0/7)	(0/7)
	GPT	39±4.3	34±3.9	41±13.1	43±5.0	34±3.9
	GOT	34±3.9	18±1.4	35±17.5	20±2.3	18±2.3
	TC	52±5.0	50±4.7	50±5.8	53±5.4	54±3.8
Methyl- testosterone (20 mg/kg. day)	No. of rabbits	7	7	7	7	7
	B.W.	2836±84	3049±89	3210±91	3436±107	3537±91
	BSP	4.9±0.2	18.0±3.9	17.6±2.9	16.9±3.7	4.9±0.5
	(A/T)	(0/7)	(7/7)	(5/7)	(6/7)	(0/7)
	GPT	44±3.3	62±9.7	61±13.8	54±7.0	37±4.2
	GOT	38±4.7	56±14.5	42±14.0	44±8.5	19±2.4
	TC	52±4.5	59±3.8	60±3.8	60±5.3	42±5.0
Oxymetho- lone (20 mg/kg. day)	No. of rabbits	7	7	7	7	7
	B.W.	2877±111	3054±99	3197±97	3503±67	3612±73
	BSP	4.8±0.6	13.6±1.7	15.8±2.3	18.8±7.1	3.9±0.2
	(A/T)	(0/7)	(7/7)	(7/7)	(6/7)	(0/7)
	GPT	39±2.7	57±7.4	52±6.8	48±2.4	28±1.7
	GOT	41±7.9	71±14.4	58±17.5	39±5.2	22±4.1
	TC	49±6.3	53±4.7	55±4.9	49±6.3	41±6.5
Fluoxymesterone (20 mg/kg. day)	No. of rabbits	7	7	7	7	7
	B.W.	2781±85	3040±86	3230±80	3404±98	3487±102
	BSP	4.7±0.5	8.0±0.9	9.3±0.9	7.4±1.1	4.6±0.5
	(A/T)	(0/7)	(4/7)	(4/7)	(2/7)	(0/7)
	GPT	36±2.8	47±5.9	50±5.3	44±2.8	30±2.2
	GOT	31±3.4	43±9.2	60±12.9	24±1.5	17±1.3
	TC	52±4.3	61±6.6	63±5.8	52±4.5	41±5.4
10364-S (20 mg/kg. day)	No. of rabbits	7	7	7	7	7
	B.W.	2704±167	2841±149	3029±163	3236±173	3351±176
	BSP	4.8±0.4	6.0±0.7	5.7±0.7	5.6±0.9	4.4±0.4
	(A/T)	(0/7)	(2/7)	(1/7)	(1/7)	(0/7)
	GPT	44±7.4	50±8.6	60±17.8	59±9.2	48±12.4
	GOT	40±6.6	42±7.4	52±12.2	45±5.0	36±11.5
	TC	50±6.1	48±3.8	46±4.1	42±4.4	36±5.4

Period of medication : Days 0-20

B.W. : Body weight (g)

BSP : Plasma BSP level ($\mu\text{g/ml}$), Normal limit : Mean $\pm 3\sigma = 8.1$ (N=35)

(A/T) : No. of abnormal/No. of tries

GPT : Plasma glutamic-pyruvic transaminase (Karmen unit)

GOT : Plasma glutamic-oxaloacetic transaminase (Karmen unit)

TC : Plasma total cholesterol level (mg/100ml)a) Mean $\pm$ S.E.

TABLE 2. Effects of steroids on the liver function of female rabbits

Steroid	Parameter	Day of determination			
		-1	14	21	28
Control (sesame oil)	No. of rabbits	7	7	7	7
	B.W.	2189±73 ^{a)}	2360±91 ^{a)}	2596±75 ^{a)}	2947±84 ^{a)}
	BSP	2.6±0.3	2.7±0.3	3.3±0.4	3.0±0.3
	(A/T)	(0/7)	(0/7)	(0/7)	(0/7)
	GPT	27±2	25±2	18±2	22±3
	GOT	21±2	24±2	12±2	18±2
	TC	61±8	72±9	72±10	75±11
Methyltestosterone (20 mg/kg/day)	No. of rabbits	7	7	7	7
	B.W.	2224±90	2539±151	2836±122	3129±100
	BSP	2.5±0.3	9.9±2.3	9.4±2.7	2.9±0.3
	(A/T)	(0/7)	(5/7)	(7/7)	(0/7)
	GPT	29±2	40±9	38±11	28±4
	GOT	19±2	75±19	62±14	25±4
	TC	64±3	77±6	78±4	49±5
Oxymetholone (20 mg/kg/day)	No. of rabbits	7	7	7	7
	B.W.	2249±89	2456±117	2724±112	2964±118
	BSP	2.6±0.2	9.1±1.8	11.8±2.0	3.1±0.5
	(A/T)	(0/7)	(7/7)	(7/7)	(0/7)
	GPT	29±4	23±2	22±5	20±1
	GOT	24±2	43±6	41±9	20±1
	TC	63±4	83±6	81±5	46±3
Fluoxymesterone (20 mg/kg/day)	No. of rabbits	7	7	7	7
	B.W.	2266±79	2529±135	2779±117	2983±143
	BSP	2.7±0.3	7.3±1.1	7.0±1.0	3.9±0.7
	(A/T)	(0/7)	(5/7)	(6/7)	(2/7)
	GPT	31±3	34±7	33±6	30±3
	GOT	20±2	47±11	42±9	20±4
	TC	60±6	76±12	80±8	37±3
10364-S (20 mg/kg/day)	No. of rabbits	8	8	8	8
	B.W.	2121±79	2276±119	2546±133	2750±138
	BSP	3.1±0.4	3.7±0.5	4.0±0.3	2.5±0.1
	(A/T)	(0/8)	(2/8)	(2/8)	(0/8)
	GPT	28±6	24±3	26±10	24±5
	GOT	20±4	28±3	27±9	16±2
	TC	52±5	49±5	44±3	39±4

Period of medication : Days 0-20

B.W. : Body weight (g)

BSP : Plasma BSP level ($\mu\text{g/ml}$), Normal limit : Mean $\pm 3\sigma=4.9$ (N=36)

(A/T) : No. of abnormals/No. of attempts

GPT : Plasma glutamic-pyruvic transaminase (Karmen unit)

GOT : Plasma glutamic-oxaloacetic transaminase (Karmen unit)

TC : Plasma total cholesterol level (mg/100 ml)

a) Mean $\pm$ S.E.

activities in the circulating blood of male and female rabbits. Normal limit (mean $\pm 3\sigma$) of plasma BSP level 20 min after BSP injection, calculated from the data on Day 1, was 8.1 $\mu\text{g/ml}$ for male and 4.9 $\mu\text{g/ml}$ for female. In the control group, mean plasma BSP levels were in the normal range through the period of experiment, though only one out of seven male rabbits showed a value exceeding the normal limit on Day 14. During methyltestosterone treatment, average BSP levels were more than 3 times higher than the pre-medication level, and most of the rabbits tested showed abnormal BSP retention. A normal range was reverted to however, a week after cessation of the treatment. Oxymetholone provided similar effects to those produced by methyltestosterone in both male and female rabbits. Fluoxymesterone was somewhat less effective as compared with methyltestosterone or oxymetholone. 10364-S was further less effective, where the mean plasma BSP levels during medication were in the normal range either in males (Table 1) or in females (Table 2). Although some rabbits showed BSP levels slightly over the normal limit (8.6–9.6, 8.2 $\mu\text{g/ml}$ vs. 8.1 $\mu\text{g/ml}$ in males and 5.3, 5.5 $\mu\text{g/ml}$ vs. 4.9 $\mu\text{g/ml}$ in females) during the period of medication, these levels returned to the normal range after cessation of the treatment or even during the treatment.

Plasma GOT and GPT activities in female rabbits (Table 2) were increased considerably by the treatment with methyltestosterone, moderately by oxymetholone or fluoxymesterone, but not by 10364-S. The elevated enzyme activities, however, returned to the normal level within a week after the end of the treatments. Plasma GOT and GPT activities in male rabbits (Table 1) were not affected or were only slightly elevated by the treatments, whereas no particular difference was seen among the four steroids tested.

Plasma cholesterol levels in both sexes were slightly lowered by 10364-S treatment, but unchanged or slightly elevated by the treatment with the other steroids. In general, plasma cholesterol levels after cessation of the treatment were lower than the levels before treatment.

DISCUSSION

Our general survey of the structure-activity relations on the steroids related to 2α , 3α -epithio- 5α -androstan- 17β -ol (4) brought into being a new orally active androgenic-anabolic steroid, 2α , 3α -epithio- 5α -androstan- 17β -yl 1-methoxycyclopentyl ether (10364-S), which was demonstrated to have 20 times the myotropic activity and 5 times the androgenic activity of 17α -methyltestosterone (MT) in the conventional rat assay. We presumed, therefore, that 10364-S must be nearly as active as fluoxymesterone, which has been reported to have 22 times greater myotropic activity and 8.5 times greater androgenic activity than those of MT in the rat assay (10, 11). Our assumption was confirmed by the present experiments, in which 10364-S was estimated to have about 80% the activity of fluoxymesterone in the rat.

Orally active anabolic steroids have been reported to interfere with hepatic excretory mechanisms in the experimental animal as well as in humans (1, 12, 13, 14, 15). In predicting the effects of anabolic steroids on hepatic excretory function in humans, sulfo-

bromophthalein (BSP) retention test in rabbits was recommended by Lennon (14) who claimed that the relative effects of orally active anabolic steroids on BSP retention in human patients were similar to those observed in rabbits, and the positive effect on BSP retention appeared to relate with the presence of 17α -alkyl group in the molecule and did not necessarily correlate with anabolic potency of the steroid. These conclusions were confirmed in our present study, in which both methyltestosterone and oxymetholone exhibited strong BSP retention, while fluoxymesterone, a much more potent anabolic steroid, exhibited a lesser effect. Further less effectiveness of 10364-S was demonstrated by the present data showing that the average blood BSP levels during 10364-S treatment were always within the normal range, whereas those during the treatment of methyltestosterone, oxymetholone or fluoxymesterone exceeded the upper limit of normal range.

Plasma GOT and GPT levels of male rabbits during the steroid treatment were somewhat higher than those before treatment but the difference between the compounds was not statistically significant (at $p=0.05$). In female rabbits, however, decreased effectiveness of 10364-S as compared with the other steroids tested was observed. Plasma cholesterol levels in male and female rabbits tended to decrease a little during 10364-S treatment, but did increase slightly during the other steroid treatments, especially in female rabbits. The above changes in plasma BSP, GOT, GPT and cholesterol levels during steroid treatment were transitory, and normal levels were restored within a week after cessation of the treatment.

Taking into account the reasonable correlation between the myotrophic and androgenic activities in the rat and the desired clinical effects of anabolic agents (1) and a considerable correspondence between the relative effects of orally active anabolic steroids on BSP retention in rabbits and the manifestation of undesired hepatic dysfunction in patients during the medication (14), it is concluded that 10364-S acts in humans as an orally active anabolic agent with a therapeutic potency equivalent that of fluoxymesterone yet is less likely to cause hepatic disturbance.

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