Notes

[Chem. Pharm. Bull. 19(1) 196—199 (1971)]

UDC 547.92.07.09 : 615.31.076.9

Improvement of Drugs by Molecular Modification Method. I. Synthesis and Biological Activity of Testosterone L-Aspartates

Atsuko Noda, Yoshiyuki Hirakawa, Noriko Fujii
and Sadao Iguchi

Faculty of Pharmaceutical Sciences, Kyushu University[1]

(Received January 27, 1970)

As the first step of the drug molecular design, it is attempted to esterify some drugs with alcoholic hydroxy group by use of a dibasic amino acid. If the ester, which is obtained by the reaction of hydroxy group in a drug with β-carboxy to the amino group in a dibasic amino acid such as L-aspartic acid, is prepared as prodrug, it is a kind of α-amino acid which is able to form zwitter ion in solution, and therefore the interesting behavior in gastrointestinal absorption as well as duration of action might be expected with oral administration.

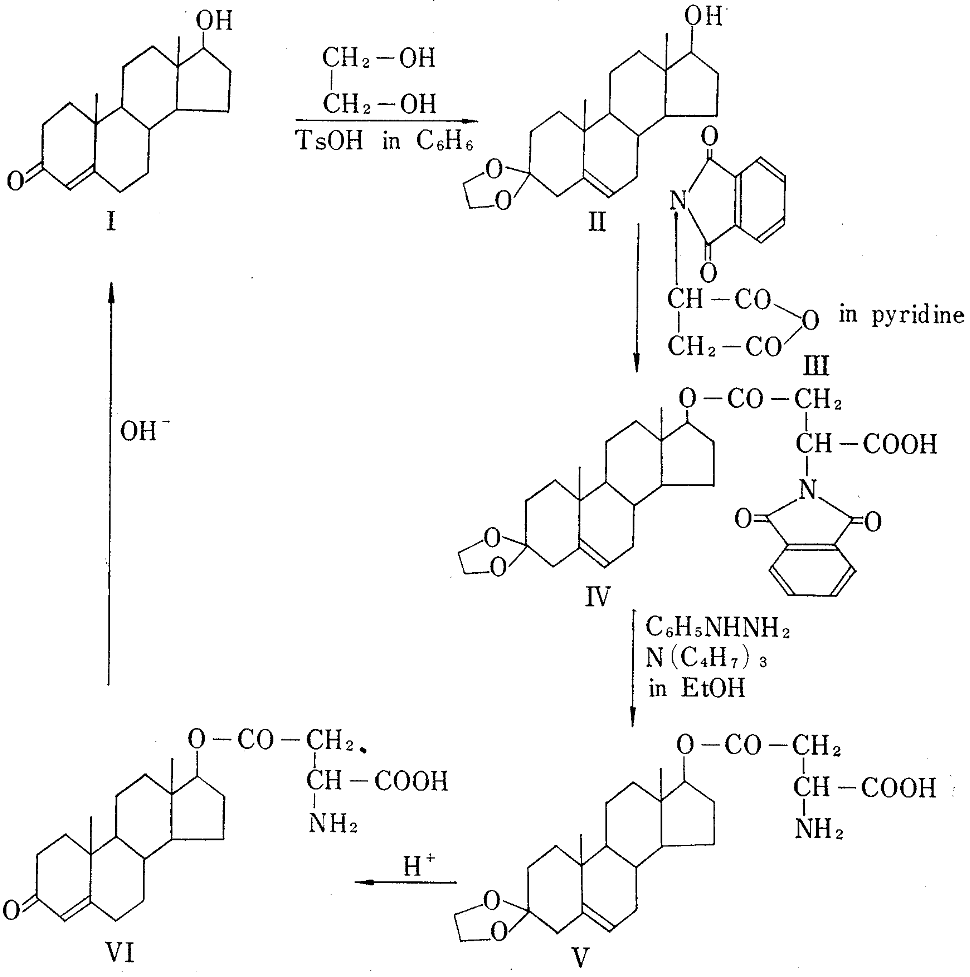

Chart 1

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[1] Location: *Katakasu, Fukuoka.*

By the reason mentioned above, testosterone whose esters such as propionate or enanthate are widely used today with injection was taken as the original drug this time, and the new esters, ethylene ketal of testosterone phthalyl-L-aspartate, ethylene ketal of testosterone L-aspartate and testosterone L-aspartate, were synthesized as follows.

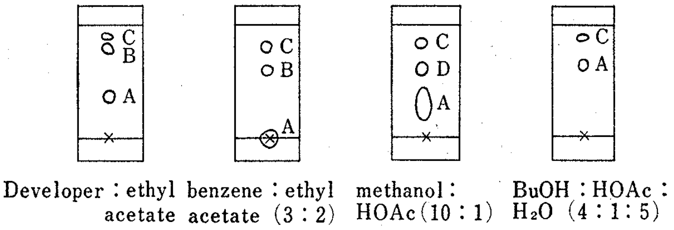

Plate: silicic acid(Kieselgel G)
A: ethylene ketal of testosterone L-aspartate (V)
B: testosterone (I)
C: ethylene ketal of testosterone (II)
D: testosterone L-aspartate (VI)

Fig. 1. Thin-Layer Chromatograms of Testosterone Derivatives

Ethylene ketal of testosterone (II)[2] was refluxed with large excess of phthalimide of L-aspartic acid anhydride (III)[3] in pyridine to give dark brown solution. After the solvent was removed *in vacuo*, the residue was chromatographed on silicic acid with ether to afford the expected ethylene ketal of testosterone phthalyl-L-aspartate (IV), mp 225—228°, and its isomer (VII), mp 210—213°, in poor yield.[4] Then IV was treated with phenylhydrazine in the presence of tri (*n*-butyl) amine in ethanol,[5] followed by addition of acetic acid in methylethylketone to yield ethylene ketal of testosterone L-aspartate (V), mp 203—205°. V was heated with 10% acetic acid at 90°, followed by removal of solvent to yield testosterone L-aspartate (VI), mp 195—200° (decomp.). Giving positive ninhydrin test remarkably, both V and VI are assumed to be the mono esters which contain α-amino acid structure. As to the determination of their definite constitutions, further study is now in progress.

Since the yield of VI from V was not good and V was easy to change into VI in the artificial gastric juice at room temperature, only the new compound V was used on the androgenic and myotrophic evaluation tests which were conducted by a modification of the methods of Gordan and Eisenberg,[6] and Swintosky, *et al.*[2]
From the magnitude of the positive values (Table I) it would only indicate that the new compound V exhibited the androgenic and myotrophic activities similar to the parent testosterone

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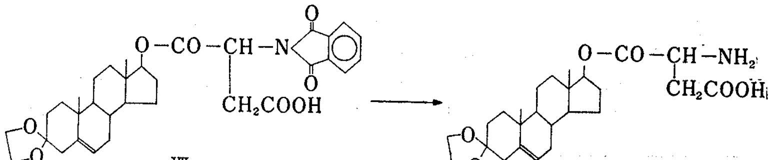
[2] S.M. Kupchan, A.F. Casy and J.V. Swintosky, *J. Pharm. Sci.*, **54**, 514 (1965).

[3] Y. Matsumoto, M. Shirai and M. Minagawa, *Bull. Chem. Soc. Japan*, **40**, 1650 (1967).

[4] VII was recrystallized from methanol as colorless needles. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1780—1715, 1100 (ketal C–O), 746 (phenyl CH). NMR (CF_3COOH) τ: 9.01 (18-CH_3), 8.62 (19-CH_3), 5.90 (ketal), 2.02 (phenyl). *Anal.* Calcd. for $C_{33}H_{39}O_8N$: C, 68.63; H, 6.76; N, 2.42. Found: C, 68.60; H, 6.67; N, 2.08. Since both compounds, IV and VII, showed the same molecular weights of 577 from mass spectra data, and the the melting point of VII depressed on admixture with IV, VII is assumed to be an isomer of IV. Treatment of VII with phenylhydrazine as same as IV gave VIII, mp 223—224° (decomp.), colorless powders from methanol. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3500—3300, 2800—2300, 1728, 1660—1550, 1220—1170, 1090 (ketal C–O). NMR (CF_3COOH) τ: 9.00 (18-CH_3), 8.64 (19-CH_3), 5.92 (ketal), 2.6—1.9 (NH_2). *Anal.* Calcd. for $C_{25}H_{37}O_6N \cdot H_2O$: C, 64.51; H, 8.39; N, 3.01. Found: C, 64.39; H, 8.28; N, 2.98. From the data mentioned above, it is considered that VIII is an isomer of V and contains β-amino acid structure because of negative ninhydrin test.



VII VIII

[5] I. Schumann and R.A. Boissonnas, *Nature*, **169**, 154 (1952).

[6] E. Eisenberg and G.S. Gordan, *J. Pharmacol. Exptl. Therap.*, **99**, 38 (1950).

Table I. Weight of Levator Ani and Seminal Vesicles after Oral Administration of Testosterone and Its Derivative to castrated Male Rats

Group	Number of rats	Seminal vesicles mg±s	Number of rats	Levator ani mg±s
Normal 35 day	5	698.0±123.6	5	131.0±17.1
Castrate 35 day	6	19.2±0.4	4	19.5±1.5
Testosterone 25 mg/kg	6	37.0±5.4	7	30.3±7.3
Ethylene ketal of testosterone L-aspartate 40 mg/kg	4	44.8±15.1[a]	5	38.4±23.1[b]

a) $p<0.005$; b) $p<0.008$; treatment group versus castrate control s: standard error

under the test conditions, manifested by increases in the weights of the seminal vesicles ($p<0.005$) and levator ani ($p<0.008$) over those exhibited by the castrate controls.

The data relevant to duration of action and availability would not be furnished by these tests. So the gastrointestinal absorption and the change of its concentration in blood are now under investigation.

Experimental[7]

Ethylene Ketal of Testosterone Phthalyl-L-aspartate (IV)——Pyridine (20 ml) solution of ethylene ketal of testosterone (II)[2] (0.8 g, 0.0024 mol) and finely pulverized phthalyl-L-aspartic acid anhydride (III)[3] (2.0 g, 0.009 mol) was refluxed for 2 hr until a spot of the ketal (II) disappeared on thin-layer chromatography (Fig. 1). After pyridine was removed *in vacuo*, the dark brown residue was dissolved in benzene (50 ml). To the solution, 10 g of silicic acid (Wakogel C-200) was added, benzene was removed, and the mixture was put on silicic acid (50 g) in a column. Elution with ether gave successively unreacted ketal (II) and the objective ester (IV). IV was recrystallized from methanol to afford colorless prisms, mp 225—228°, 0.4 g (29%). It showed a green or purple spot on thin-layer chromatography with sulfuric acid (Fig. 1). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1780, 1757, 1724, 1097 (ketal C–O), 725 (phenyl CH). NMR (CF_3COOH) τ: 9.11 (18-CH_3), 8.65 (19-CH_3), 5.90 (ketal), 2.03 (phenyl). *Anal.* Calcd. for $C_{33}H_{39}O_8N$: C, 68.63; H, 6.76; N, 2.42. Found: C, 68.82; H, 6.63; N, 2.35.

Ethylene Ketal of Testosterone L-Aspartate (V)——IV (1 g, 0.0017 mol) and phenylhydrazine (0.37 g, 0.0034 mol) were dissolved in 96% ethanol (40 ml) containing tri(*n*-butyl)amine (0.32 g).[5] After refluxing for 2 hr, were added acetic acid (0.15 g) and methylethylketone (60 ml) to the reaction mixture to give white gel, which was recrystallized from methanol to afford monohydrate of ethylene ketal of testosterone L-aspartate (V), mp 203—205°. Yield: 0.23 g (30%). It gave positive ninhydrin test and on thin-layer chromatography a green or purple spot was appeared with sulfuric acid (Fig. 1). When V was dissolved in 1N HCl followed by treatment with large excess of 10% NaOH, the parent testosterone (I) regenerated. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3500—3320, 2800—2220, 1760—1750, 1640—1560, 1280—1210, 1097 (ketal C–O). NMR (CF_3COOH) τ: 8.98 (18-CH_3), 8.63 (19-CH_3), 5.90 (ketal), 2.6—2.0 (NH_2). *Anal.* Calcd. for $C_{25}H_{37}O_6N \cdot H_2O$: C, 64.51; H, 8.39; N, 3.01. Found: C, 64.21; H, 8.49; N, 2.94.

Testosterone L-Aspartate (VI)——V (0.15 g) was dissolved in 10% acetic acid (10 ml). After heating for 1 hr, was removed the solvent to give yellowish residue. It was recrystallized from acetone to afford testosterone L-aspartate, mp 195—200° (decomp.). It gave positive ninhydrin test (Fig. 1). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3600—3400, 2800—2000, 1758, 1670 (testosterone C=O), 1620—1550, 1280—1210. NMR (CF_3COOH) τ: 9.00 (18-CH_3), 8.65 (19-CH_3), 2.6—2.1 (NH_2). *Anal.* Calcd. for $C_{23}H_{33}O_5N$: N, 3.48. Found: N, 3.26.

Test for Androgenic and Myotrophic Activities——The androgenic and myotrophic activities of the new ester (V) was determined by a modification of the method of Gordan and Eisenberg,[6] and Swintosky, *et al.*[2] Young male rats of the Wister strain, weighing 60—70 g, were castrated 3 days after receipt. 35 days later, they were divided into 4 groups, with a minimum of 6 rats per group. The new ester (V) was suspended in 0.5% aqueous tragacanth and administered orally at a dose of 40 mg/kg body weight/day (it corresponds

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[7] All melting points are uncorrected.

to 25 mg/kg of the parent testosterone) for 8 days, while testosterone (I) was administered similarly at a dose of 25 mg/kg body weight/day as a reference standard. On the ninth day, the rats were sacrificed by decapitation, and the seminal vesicles and levator ani muscles were removed and weighed. The results were analysed statistically by the Mann–Whitney U test for the significance of the increases in the weights of the seminal vesicles and levator ani muscles with respect to the castrate controls (Table I).

Acknowledgement This work has been supported in part by a grant from the Ministry of Education. The authors deeply appreciate the useful advice about the biological test of Dr. M. Kamata and the skillful assistance of Mr. O. Tsuzuki and Mrs. M. Horie during the course of this work.

[Chem. Pharm. Bull. 19(1) 199—201 (1971)]

UDC 547.814.02.05.09 : 581.192 : 582.282

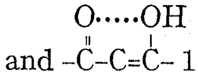
The Isolation of Secalonic Acid A from *Aspergillus ochraceus* cultured on Rice

Mikio Yamazaki, Yukio Maebayashi and Komei Miyaki

Institute of Food Microbiology, Chiba University[1]

(Received June 4, 1970)

We have isolated an yellow crystalline compound (YC-3) from the ethyl acetate extract of *Aspergillus ochraceus* cultured on rice during our investigation on the survey of the food borne toxigenic fungi. The producibility of this compound in *Aspergillus ochraceus* has been confirmed to be as general that all of 57 strains examined in our laboratory are proved to produce this compound.

From the ethyl acetate extract of cultured mycelium of *Aspergillus ochraceus* on rice, YC-3 can easily be crystallized by treatment with chloroform, and in general, about 600 mg of YC-3 are yielded from the mycelium cultured on 1 kg of sterilized and moistened rice. YC-3 is an yellow acidic and phenolic (positive to $FeCl_3$ test) compound, having mp 243° (acetone) and 248° ($CHCl_3$). Molecular formula, $C_{32}H_{30}O_{14}$, has been elucidated from the results of the elementary analysis and M^+ 638 in mass spectrometry. In nuclear magnetic resonance (NMR) spectrometry, the signals for 12 protons excluding 3 protons of hydroxyls are observed. Accordingly, the dimeric structure has easily been expected for YC-3. The absorption maxima, 252 mμ (ε 17200) and 346 mμ (ε 31000)in ultraviolet (UV) spectrum indicate that the structure of YC-3 including its chromphores should fairly be resemble to that of ergochrome derivatives.[2] From the infrared (IR) spectral data, the presence of carboxyl ester, 1726, 1155, 1088 cm^{-1}, hydroxyl, 3480 cm^{-1}, and  $\overset{O\cdots\cdots OH}{-\overset{\|}{C}-C=\overset{|}{C}-}$ 1608 cm^{-1} may be assumed. In a deutero-pyridine solution, the NMR spectral signals are obtained as follows: δ ppm from TMS, 1.26 (3H, d, $J=6.0$ cps) CH_3–CH⟨, 2.2—3.0 (3H, m) –CH_2–CH–, 3.56 (3H, s) OCH_3, 4.16 (1H, d, $J=10.5$ cps) ⟩CH–O–, 6.70, 7.59 (1H, d, $J=8.5$ cps) two aromatic H in *ortho*-corelation.

Acetylation of YC-3 by reflux with acetic anhydride and sodium acetate afforded white amorphous acetate having mp 198°. In NMR spectrum of YC-3 acetate, four peaks at δ ppm 1.81 (3H, s), 1.91 (3H, s), 1.99 (3H, s) and 2.42 (3H, s) have clearly been observed. According to this fact, it has been initially assumed that the four acetyl groups are introduced to form the tetraacetate (octaacetate if one proposes the dimeric structure to YC-3). However,

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1) Location: *3-9-1 Izumicho, Narashino, Chiba.*

2) B. Franck, G. Baumann and U. Ohnsorge, *Tetrahedron Letters*, **1965**, 2031 and ref. cited therein.