UDC 547.92:582.572.7

106. Ken'ichi Takeda, Tameto Okanishi, and Ariyoshi Shimaoka: Studies on the Steroidal Components of Domestic Plants. XVII.[1,2] The Structure of Yonogenin, a New Steroidal Sapogenin from *Dioscorea Tokoro* MAKINO.

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Dioscorea Tokoro MAKINO is a common plant in Japan. From the rhizome of this plant two steroidal sapogenins, the well-known diosgenin[3~6] and tokorogenin[7] were isolated and the structure of the latter was recently confirmed by Morita[8] as 1, 2, 3-trihydroxy-25D, 5β-spirostane. From the epigeous part of this plant, on the other hand, three sapogenins were obtained and one of them was clarified as tokorogenin. The other two sapogenins having a melting point of 240~243° and of 320°(decomp.) are sapogenins hitherto unknown in the literature and were named yonogenin (m. p. 240~243°) and kogagenin (m. p. 320° with decomposition), respectively.[9] Physical constants of these two sapogenins are cited in Table I.

The analytical values of yonogenin are in good agreement with the empirical formula $C_{27}H_{44}O_4$. The infrared spectrum of this sapogenin shows neither a ketonic band nor an isolated double bond but shows a hydroxyl group absorption and also suggests that yonogenin belongs to the 25D(iso)-sapogenin.[10] This sapogenin does not react with 2,4-dinitrophenylhydrazine and gives a negative result with digitonin and Liebermann reactions, or tetranitromethane color reaction. Yonogenin afforded diacetate of m. p. 212° (Ib), with acetic anhydride and pyridine.

TABLE I.

Yonogenin, $C_{27}H_{44}O_4$	m. p.	240~243°	$[\alpha]_D$ -53°
Diacetate	m. p.	212°	$[\alpha]_D$ -29°
Kogagenin, $C_{27}H_{44}O_6$	m. p.	320°(decomp.)	$[\alpha]_D$ -27°
Triacetate	m. p.	255°	$[\alpha]_D$ -26°

Furthermore, this sapogenin gave no acetonide, but the bis(ethyl carbonate) (Ic) shows a melting point of 185~186° with ethyl chlorocarbonate under usual conditions.

Chromium trioxide oxidation of this yonogenin afforded a dicarboxylic acid (yonogenic acid) (IIa) and the physical constants and infrared spectrum of this acid and of its dimethyl ester were in good agreement with those of samogenic acid and its dimethyl ester.[11]

From these results it will be assumed that the two hydroxyl groups in yonogenin are located at 2- and 3-positions, and that the conformation of each hydroxyl group is equatorial and *trans* to each other. Thus, 2β, 3α-dihydroxy-25D,5β-spirostane (Va) should be assigned to yonogenin.

Although this compound has not yet been isolated from the natural source, this was

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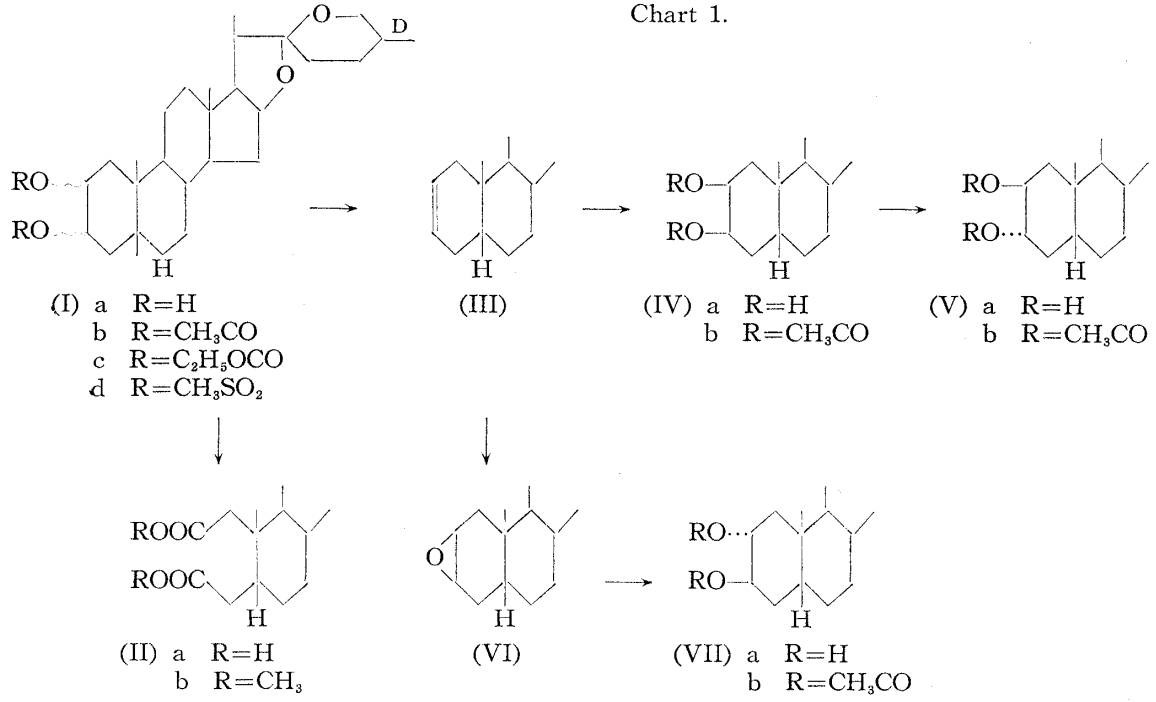
1) Part XVI: Ann. Rept. Shionogi Lab., **7**, 339(1957).
2) cf. K. Takeda, T. Okanishi, A. Shimaoka: Yakugaku Zasshi, **77**, 822(1957).
3) J. Honda: Arch. exptl. Pathol. Pharmakol., **51**, 221(1904).
4) K. Fujii, *et al.*: Yakugaku Zasshi, **56**, 408(1936); **57**, 114(1937).
5) T. Tsukamoto, *et al.*: *Ibid.*, **56**, 931, 802(1936); **57**, 985(1937).
6) R. E. Marker, T. Tsukamoto, D. L. Turner: J. Am. Chem. Soc., **62**, 2525, (1940).
7) M. Nishikawa, *et al.*: Yakugaku Zasshi, **74**, 1165(1954).
8) K. Morita: This Bulletin, **5**, 494(1957).
9) T. Tsukamoto, *et al.* isolated another sapogenin, $\Delta^{3,5}$-desoxytigogenin, from this plant. cf. This Bulletin, **5**, 492(1957).
10) Normal- and iso-sapogenins are characterized by their carbon-oxygen stretching bands at 860, 900, and 920 cm^{-1} in the infrared spectra and an absorption band at 900 cm^{-1} of iso-sapogenins has 1.5~2.5 times the intensity of a band at 920 cm^{-1}. cf. M. E. Wall *et al.*: Anal. Chem., **24**, 1337(1952); R. N. Jones, *et al.*: J. Am. Chem. Soc., **75**, 158(1953).
11) C. Djerassi, J. Fishman: J. Am. Chem. Soc., **77**, 4291(1955).

already synthesized from samogenin (2β, 3β-diol) (IVa) by epimerisation with sodium and alcohol by Marker and his co-workers in 1947.[12] The melting point of episamogenin and its diacetate are 235~237° and 212°, respectively, and these data coincide well with the corresponding melting point of yonogenin.

The structure of yonogenin was proved by the reaction sequence as shown in Chart 1.

Chart 1.



Yonogenin dimethanesulfonate (Id), which was obtained from yonogenin and mesityl chloride in pyridine, was treated with sodium iodide and acetone in a sealed tube at about 120° for 24 hours. The pure 25D,5β-spirost-2-ene (III), m.p. 149~150°, was isolated from the reaction mixture by chromatography on alumina column. The physical constants of this unsaturated compound coincide well with those previously given in the literature.[11]

Treatment of this spirostene (III) with osmium tetroxide afforded samogenin (IVa) as expected.[13] The epimerisation of samogenin took place with sodium and ethanol at 220° in a sealed tube as described by Marker, *et al.*, and 2β,3α-diol (Va) was obtained in 50% yield. This substance was identified with yonogenin by the mixed melting point behavior, and the infrared spectrum of each substance also gave identical results.

In addition to this 2β,3α-diol, another *trans*-2,3-diol (i. e. 2α,3β) was synthesized from 2β,3β-epoxy-25D,5β-spirostane (VI). This epoxide was also obtained when the unsaturated derivative (III) was treated with perbenzoic acid in chloroform solution and this was assigned previously by Djerassi, *et al.* as a β-epoxide.[11] Then, another *trans*-diol, namely 2α,3β-diol, was obtained from this epoxide by the ring-opening reaction with acetic acid followed by alkaline hydrolysis. This 2α,3β-dihydroxy-25D, 5β-spirostane (VIIa) and its acetate melt at 198° and 168°, respectively. This substance was clearly not identical with yonogenin.

The configuration of hydroxyl group at 3-position in many steroidal sapogenins is known to take 3β configuration and only a few sapogenins having 3α-hydroxyl group have been reported in the literature. One of them is rhodeasapogenin, reported by Nawa in 1953[14] but the structure has not yet been confirmed definitely. Cholegenin and isocholegenin, investigated

12) R. E. Marker, *et al.*: *Ibid.*, **69**, 2195(1947).
13) This substance is identical in all respects with samogenin, isolated from the natural source.
14) H. Nawa: Yakugaku Zasshi, **73**, 1192, 1195, 1197(1953).

by Antia and his co-workers, have a 3α, 27-diol grouping in the molecules but these substances were isolated from ox bile.[15]

As far as is known yonogenin is the first of the sapogenins having a 3α-hydroxyl group to be isolated from a plant source.

After publication of the outline of this work, the structure of tokorogenin was clarified as 1β, 2β, 3α-trihydroxy-25D, 5β-spirostane by Morita.[8] In this connection, the biogenetic relationship between yonogenin and tokorogenin seems to be very interesting and reasonable.

Experimental

Separation of Sapogenins—Late in September, epigeal parts, including the stem, leaf, spike, and fruit, of *Dioscorea tokoro* were collected in fields and mountains near Shiga Prefecture. To 1~1.5 kg. of the dried material, 10 L. of 90% MeOH was added, and after cooling for 12 hrs., the mixture was warmed for 8 hrs. in a Soxhlet extractor. The procedure described above was repeated 3 times and 3.5 kg. of the dried material in all was processed for extraction. All the extracts were combined and concentrated to ca. 5 L., and after removal of fat with Et_2O, the mixture was hydrolyzed with HCl. The residue was chromatographed over alumina to obtain the following four kinds of crystals:

(A)	m. p. 240~243°	8.0 g. (0.23%)	13.4 g. (0.38%)
(B)	m. p. 268°	2.6 g.	
(C)	m. p. 316° (decomp.)	1.8 g.	
(D)	m. p. 278~290° (decomp.)	1.0 g.	

Substance (A): $[\alpha]_D^{20}$: −53°(in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.72; H, 10.27. Acetate, m.p. 212°, $[\alpha]_D^{20}$ −29°(in $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06: H, 9.36. Found: C, 72.28; H, 9,69. The infrared spectrum showed the absorption of an iso-linkage, and the name yonogenin (Ia) is proposed for this substance.

Substance (B); $[\alpha]_D^{29}$ −39°(in pyridine). *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.28; H, 9.89. Acetate, m. p. 256°, $[\alpha]_D^{29}$ −17° (in $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{50}O_8$: C, 68.96; H, 8.77. Found: C, 69.18; H, 8.97. A mixed melting point determination and infrared spectrum showed that it was identical with tokorogenin.

Substance (C): $[\alpha]_D^{29}$ −27°(in pyridine). *Anal.* Calcd. for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.95. H, 9.62. Acetate, m. p. 248~252°, $[\alpha]_D^{29}$ −15°(in $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{50}O_9$: C, 67.09; H, 8.53. Found: C, 67.09; H, 8.50. The infrared spectrum showed the absorption of a free hydroxyl group. This was named kogagenin.

Substance (D): This was considered to be a mixture of (B) and (C).

Derivatives of Yonogenin—i) Diacetate (Ib): 0.5 g. of yonogenin in 5 cc. of Ac_2O and 8 cc. of pyridine was heated at 120° for 30 mins. to give 0.4 g. of crystals of diacetate (Ib), which melted at 212° after repeated recrystallization from a mixture of MeOH and Me_2CO (1:1). $[\alpha]_D^{20}$ −29° (in $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.28; H, 9.69.

ii) Bis(ethyl carbonate) (Ic): To a mixture of 500 mg. of yonogenin, 5.5 cc. of pyridine, and 5 cc. of dioxane, 2 cc. of ethyl chlorocarbonate was added under ice-cold conditions. After standing at room temperature for 18 hrs., the mixture was poured into water, and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 2% HCl and water, and after drying, $CHCl_3$ was evaporated. The crystals thus obtained were recrystallized from MeOH to 350 mg. of crystals (Ic) of m. p. 185~186°, $[\alpha]_D^{22}$ +33° (in pyridine). The characterization of this substance by infrared absorption spectrum was made. *Anal.* Calcd. for $C_{33}H_{52}O_8$: C, 68.72; H, 9.09. Found: C, 69.00; H, 9.17.

Oxidation of Yonogenin—i) Yonogenic Acid (IIa): To the solution of 0.8 g. of yonogenin dissolved in AcOH, a solution of 1.6 g. of CrO_3 in 16 cc. of AcOH was added slowly with stirring, and the mixture was allowed to stand at room temperature (15°) for 30 mins. The reaction mixture was poured into 300 cc. of water and extracted with 300 cc. of Et_2O. Et_2O solution was washed with water, and then was shaken twice with 100 cc. of 5% KOH. The alkali layer was acidified with 20 cc. of AcOH and extracted with Et_2O. The Et_2O solution was washed with water, dried, and evaporated. The crude crystals were recrystallized twice from a mixture of AcOH and water (2:1) to give 0.4 g. (50%) of crystals (IIa), m. p. 266°, $[\alpha]_D^{15}$ −36°, −39°(in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 70.09; H, 9.29. The infrared spectrum showed the absorption of COOH group.

ii) Dimethyl Yonogenate (IIb): To the solution of 0.5 g. of yonogenic acid (IIa) dissolved in 50 cc of Et_2O, 15 cc. of Et_2O solution of CH_2N_2 was added and allowed to stand for about 15 mins. The solution was washed with 30 cc. of 5% Na_2CO_3 and water, dried, and evaporated. The residual crystals were recrystallized from MeOH to 0.25 g. (80%) of crystals, m. p. 146°, $[\alpha]_D^{15}$ −32°, −33° (in $CHCl_3$) *Anal.* Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.82; H, 9.46.

15) N. J. Antia, *et al.*: J. Chem. Soc., **1954**, 1218.

Yonogenin from 25D,5β-Spirost-2-ene (III)—i) Yonogenin dimethanesulfonate (Id): A solution of 3.0 g. of yonogenin dissolved in 15 cc. of pyridine and 10 cc. of methanesulfonyl chloride was allowed to stand at room temperature for 44 hrs. The reaction mixture was shaken with 200 cc. of 10% HCl and then with 300 cc. of $CHCl_3$. The $CHCl_3$ layer was washed with water and then $CHCl_3$ was evaporated. The residue was purified by chromatography and residue obtained from the benzene eluate was recrystallized from MeOH to give 2.5 g. of crystals, m. p. 155°; $[\alpha]_D^{20}$ −40°(in $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{48}O_8S_2$: C, 59.10; H, 8.16; S, 10.89. Found: C, 58.89; H, 8.31; S, 10.46. The infrared spectrum did not show the absorption of OH group, but showed that of a mesitylate.

ii) 25D,5β-Spirost-2-ene (III): 2.0 g. of the dimethanesulfonate (Id) was heated with 5.2 g. of NaI and 64 cc. of Me_2CO for 24 hrs. at 110~120° in a sealed tube. From the reddish brown reaction mixture Me_2CO was removed and the residue was extracted with 500 cc. of $CHCl_3$. $CHCl_3$ solution was washed with 58 cc. of 5% $Na_2S_2O_3$ and twice with water, $CHCl_3$ was evaporated, and MeOH was added to give 1.5 g. of crystals. The crystals were dissolved in a mixture of hexane and benzene (1:1), and passed over alumina to obtain the following elution residues:

Petr. ether (45~50°), m. p. 148~149° (620 mg.)
Benzene-Petr. ether (1:1) m. p. 152° (760 mg.)

An unsaturated sapogenin (III) was obtained from the first eluted substance by recrystallization from MeOH, m. p. 149~150°; $[\alpha]_D^{20}$ −80° (in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.25; H, 10.33. The infrared spectrum was identical with that of Δ^2-olefin (III). The other, m. p. 152°, was confirmed to be the starting material by a mixed melting point determination.

iii) 25D,5β-Spirostane-2β,3β-diol (Samogenin) (IVa): A mixture of 300 mg. of the above olefin (III), 2.3 g. of OsO_4, 6 cc. of pyridine, and 2.5 cc. of benzene was left at room temperature for 48 hrs. and then the solvents were evaporated *in vacuo*. The residue was refluxed on a steam bath for 48 hrs. with 1 g. of KOH, 1 g. of mannitol, 11 cc. of EtOH, 5 cc. of benzene, and 2.5 cc. of water, and the product was extracted twice with 50 cc. of benzene. After repeated washing with water, benzene was evaporated, a tar-like oil thereby obtained was dissolved in benzene: $CHCl_3$ (9:1), and the solution was submitted to chromatography. The results of chromatography are shown in Table II. The eluates (F2~F5) were combined and recrystallized repeatedly from MeOH to give the crystals (IVa) of m.p. 207°; $[\alpha]_D^{30}$ −80° (in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.28; H, 10.47.

This substance (IVa) was acetylated by the usual process and yielded crystals (IVb) of m. p. 195°, which showed no depression of m. p. on admixture with a sample of samogenin diacetate, which was obtained through the kind offices of Dr. Rosenkranz. $[\alpha]_D^{30}$ −73°(in $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36 Found: C, 71.82; H, 9.44.

TABLE II.

Solvent		Eluate (m.p. °C)			
Benzene		170	(F-1)	trace	
Benzene-$CHCl_3$	(98:2)	195	(F-2)	trace	30 mg.
,, ,,	(8:2)	195	(F-3)		
Benzene-MeOH	(98:2)	204~205	(F-4)		50 mg.
,, ,,	(8:2)	205~208	(F-5)		30 mg.
,, ,,	(1:1)	—			

iv) Epimerisation of Samogenin: A mixture of 400 mg. of samogenin (IVa), 1.4 g. of Na, and 28 cc. of dehyd. EtOH was heated in a sealed tube at 200~220° for 14 hrs. The product was extracted with 500 cc. of Et_2O, the Et_2O solution was washed twice with water, dried over Na_2SO_4, and Et_2O was evaporated. To the residual syrup, a small amount of MeOH was added to yield 320 mg. of white needles, m.p. 230~232°.

The crude crystals were acetylated in a usual manner to yield 300 mg. of crude acetate. This was dissolved in benzene and purified by chromatography. From the petr. ether fraction crystals of m. p. 212° was obtained. $[\alpha]_D^{30}$ −26° (in $CHCl_3$). No depression of melting point was shown on admixture with yonogenin diacetate and infrared spectra of these substances were identical. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.97; H, 9.53.

100 mg. of this acetate was saponified by heating on a steam bath for 1 hr. with 5% EtOH-KOH, and extracted with Et_2O. The Et_2O solution was washed with water and, after drying over Na_2SO_4, Et_2O was evaporated, and the crude crystals thereby obtained were recrystallized repeatedly from MeOH to yield 30 mg. of crystals (Va), m.p. 238~240°; $[\alpha]_D^{30}$ −56°, −53°(in $CHCl_3$). No depression of the melting point occurred on admixture with yonogenin. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.72; H, 10.51.

Synthesis of 25D,5β-Spirostane-2α,3β-diol (VIIa)—i) 2β,3β-Epoxy-25D,5β-spirostane (VI): To the solution of 0.25 g. of Δ^2-olefin (III) dissolved in 25 cc. of $CHCl_3$, 4 cc. of $CHCl_3$ solution of perbenzoic acid (58 mg./cc.) was added and the mixture was allowed to stand for 24 hrs. at room temperature. To this was added 100 cc. of Et_2O and the Et_2O solution was washed with 5% NaI, 5% $Na_2S_2O_3$, and finally with 5% $NaHCO_3$. After drying over Na_2SO_4, Et_2O was evaporated and the residue was recrystallized

from Me_2CO to 150 mg. of crystals, m.p. 225~230°; $[\alpha]_D^{25}$ −69°(in $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.05; H, 10.15.

ii) 25D,5β-Spirostane-2α,3β-diol (VIIa): A solution of 60 mg. of the epoxide (VI) in 25 cc. of AcOH was heated on a water bath for 2 hrs., concentrated under reduced pressure to the original volume, and extracted with Et_2O. Et_2O solution was washed with water and Et_2O was removed to obtain a paste. An attempt was made to crystallize this product with Me_2CO and MeOH but in vain. This was dissolved in MeOH and saponified with 5% MeOH-KOH. MeOH was removed, 50 cc. of water was added to the solution, and the precipitate thereby formed was extracted with Et_2O. After washing with water Et_2O solution was dried over Na_2SO_4 and evaporated. The crude crystals thus obtained were dissolved in $CHCl_3$: benzene (1:4) mixture and the solution was submitted to chromatography. From the mixed solvent, MeOH: benzene (2:98) yielded 40 mg. of crystals (VIIa) of m.p. 196~198°. Recrystallization from benzene: hexane (1:1) raised the m.p. to 198°; $[\alpha]_D^{22}$ −60° (in pyridine). *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.93; H, 10.29.

The diacetate (VIIb) recrystallized as prisms (from Me_2CO and MeOH), m.p. 168°; $[\alpha]_D^{22}$ −78° (in pyridine). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.82; H, 9.58.

Both the genin and the acetate proved not to be identical with yonogenin and its acetate, by the determination of mixed melting points and the comparison of infrared spectrum with that of yonogenin and its acetate.

Summary

Two new sapogenins, yonogenin and kogagenin, and tokorogenin were isolated from the epigeous part of *Dioscorea Tokoro* MAKINO. The structure of yonogenin was established to be 25D,5β-spirostane-2β,3α-diol (Va) by its degradation to samogenic acid (IIa) and by its conversion via Δ^2-olefin (III) to samogenin (IVa), which was epimerized to original yonogenin (episamogenin). 25D, 5β-Spirostane-2α,3β-diol (VIIa) was also prepared.

(Received May 23, 1958)

UDC 547.92:582.893

107. Ken'ichi Takeda and Tokuo Kubota: Studies on the Steroidal Components of Domestic Plants. XVIII.* Components of the Root of *Bupleurum falcatum* L. (2).[1]

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In a previous publication from this laboratory, isolation of α-spinasterol (I) from the root of *Bupleurum falcatum* L. was described.[1] In addition to α-spinasterol, three sterols, Δ^7-stigmastenol (II), Δ^{22}-stigmastenol (III), and stigmasterol (IV), have now been separated from the same source.

α-Spinasterol was isolated from the least-soluble fraction of the crude sterols by crystallization of its benzoates, as reported earlier.[1] The more-soluble fraction was converted to the dinitrobenzoates and the fractional crystallization from ethyl acetate gave the following two substances: (A) The less-soluble, top fraction as yellow plates, m.p. 225~227°; $[\alpha]_D$ −3.7°. (B) The more-soluble fraction as needles, m.p. 212~214°, $[\alpha]_D$ +7.4°.

It has been reported in a recent paper[2] that the sterol obtained from the dextrorotatory dinitrobenzoate (fraction B) corresponded to the so-called "β-spinasterol," which was found to be Δ^7-stigmastenol contaminated with a small amount of α-spinasterol.

The levorotatory dinitrobenzoate (fraction A) could not be purified further by crystallization. However, chromatography of its acetate on alumina indicated that this fraction was not pure. The less-levorotatory component was concentrated in the more readily eluted

* Part XVII: K. Takeda, T. Okanishi, A. Shimaoka: This Bulletin, **6**, 532(1958).

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1) Part (1): K. Takeda, K. Hamamoto, T. Kubota: Yakugaku Zasshi, **73**, 272(1953).

2) K. Takeda, T. Kubota, Y. Matsui: This Bulletin, **6**, 437(1958).