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.

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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]

(*Research Laboratory, Shionogi & Co., Ltd.***)

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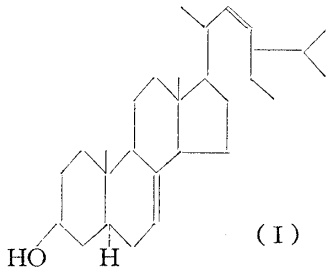
1) Part (1): K. Takeda, K. Hamamoto, T. Kubota: Yakugaku Zasshi, **73**, 272(1953).

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

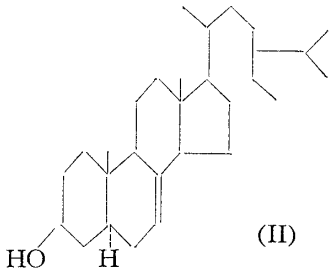
fraction. After minute chromatography was repeated, an acetate having a constant melting point and rotation of m. p. 142~143°, $[\alpha]_D$ −4.8°, was isolated from the more easily-eluted top fraction. The physical constants of its derivatives, free sterol, acetate, benzoate, ketone, and stanyl acetate, are in good agreement with those of the derivatives of Δ^{22}-stigmastenol (Table I). Its acetate was identified directly by comparison of the infrared spectra and by a mixed melting point determination with a sample of synthetic Δ^{22}-stigmastenyl acetate[3]

Chart 1.



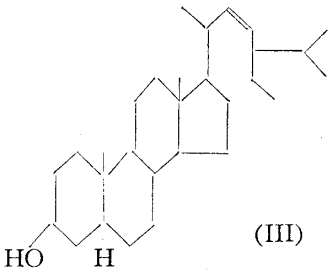
(I)

α-Spinasterol



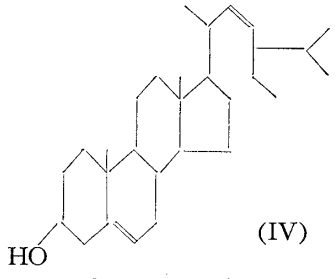
(II)

Δ^7-Stigmastenol



(III)

Δ^{22}-Stigmastenol



(IV)

Stigmasterol

kindly supplied by Dr. D. H. R. Barton. Although this Δ^{22}-stigmastenol had already been synthesized by Barton and Brooks, it seems that, as far as the authors are aware, the present work is the first example of its isolation from natural products.

The more-difficultly eluted fraction of the above-mentioned chromatography showed a higher levorotatory value, and as a result of repeated chromatography, the constants of the fraction having the maximum rotation were m. p. 140~141.5°, $[\alpha]_D$ −41°. Further chromatography brought no alteration in either the melting point or rotation. Recrystallization of the acetate or its free sterol also did not effect any further purification. However, this fraction was purified by recrystallization of its benzoate from a mixture of ethyl acetate and ethanol to furnish pure stigmasteryl benzoate, m. p. 160.5~162°, $[\alpha]_D$ −25°. The physical constants of the free sterol, acetate, and stanyl acetate are also quite identical with those previously given in the literature[4] (Table I).

TABLE I.

Source	Δ^{22}-Stigmastenol				Stigmasterol			
	Barton, Brooks[3]		Authors		Barton[4]		Authors	
	m. p.	$[\alpha]_D$	m. p.	$[\alpha]_D$	m. p.	$[\alpha]_D$	m. p.	$[\alpha]_D$
Sterol	159°	+2°	158−159°	+3.3°	168°	−51°	166.5−168°	−50.6°
Acetate	144−144.5	−7	142−143	−4.8	143	−55	142−143	−53.7
Benzoate	153−154	+1	154−155	+3.0	164	−26	160.5−162	−25.0
Ketone	166−167	+20	167−168	+21.7				

The authors are indebted to Dr. D. H. R. Barton for his kind donation of the valuable sample of Δ^{22}-stigmastenyl acetate. Thanks are also due to the members of the analysis room of this laboratory

3) D. H. R. Barton, C. J. W. Brooks: J. Am. Chem. Soc., **72**, 1633(1950).
4) D. H. R. Barton, J. D. Cox: J. Chem. Soc., **1948**, 783.

for carrying out the microanalyses and to Mr. Y. Matsui for the measurement of the infrared spectra. This work was supported partly by the Grant in Aid for Scientific Research from the Ministry of Education.

Experimental[5]

Chromatography of the Acetate of Fraction A—The dinitrobenzoate (A), yellow plates, m. p. 225~227°, $[\alpha]_D$ −3.7°, was separated according to the procedure previously described.[2] Hydrolysis of this dinitrobenzoate with EtOH-KOH yielded the sterol, m. p. 159~160°, $[\alpha]_D$ −12.0°. On acetylation the sterol gave the acetate, m. p. 141~142.5°, $[\alpha]_D$ −19.9°. The acetate was dissolved in petr. ether (b. p. 40~60°) and chromatographed over 100 g. of Brockmann's alumina. The eluted fractions were combined and divided into the following three groups; the first fraction showing a smaller rotation than −30°, the next fraction showing a larger rotation than −30°, and the last fraction showing a smaller rotation than −30°.

Chromatography of each group was carried out systematically, continuously separating the fractions by optical rotation, and finally the following components were separated: (A-1) 140 mg. of the more-easily eluted first fraction having the smallest levorotation, $[\alpha]_D$ −4° to −5°; (A-2) 530 mg. of the more-difficultly eluted third fraction having the largest levorotation, $[\alpha]_D$ −35° to −41°; and 120 mg. of the intermediate second fraction, $[\alpha]_D$ −10° to −29°.

Δ^{22}-Stigmastenol and its Derivatives—Fraction A-1 (140 mg.) from the above chromatography was recrystallized from EtOH to give Δ^{22}-stigmastenyl acetate as plates, m. p. 142~143°, $[\alpha]_D^{20}$ −4.8° (c=0.93). Identity with an authentic specimen was established by admixture and by comparison of the infrared spectra. *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found: C, 81.24; H, 11.56.

Alkali hydrolysis of this acetate and recrystallization of the product from EtOH afforded Δ^{22}-stigmastenol as plates, m. p. 158~159°, $[\alpha]_D^{20}$ +3.3°(c=1.5). *Anal.* Calcd. for $C_{29}H_{50}O \cdot {}^1/_2H_2O$: C, 82.20; H, 12.13. Found: C, 82.58; H, 12.28.

Benzoylation of the sterol and recrystallization of the product from AcOEt-MeOH gave a benzoate as plates, m. p. 154~155°, $[\alpha]_D^{24}$ +3.0°(c=1.0). *Anal.* Calcd. for $C_{36}H_{54}O_2$: C, 83.34: H, 10.49. Found: C, 83.32; H, 10.48.

CrO_3-Pyridine Complex Oxidation of Δ^{22}-Stigmastenol—To the CrO_3-pyridine complex from 50 mg. of CrO_3 and 0.5 cc. of pyridine, a solution of 35 mg. of Δ^{22}-stigmastenol in 1.5 cc. of pyridine was added, the mixture was allowed to stand at room temperature overnight, diluted with water, and extracted with Et_2O. The Et_2O solution was washed with dilute HCl, Na_2CO_3 solution, and water, dried over Na_2SO_4, and concentrated. The solid residue was recrystallized from AcOEt-MeOH to give 23 mg. of Δ^{22}-stigmasten-3-one as needles, m. p. 167~168°, $[\alpha]_D^{25}$ +21.7°(c=0.95). I. R. λ_{max}^{Nujol}: 5.84 μ (C=O). *Anal.* Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found: C, 84.62; H, 11.80.

Hydrogenation of Δ^{22}-Stigmastenyl Acetate—Δ^{22}-Stigmastenyl acetate (14 mg.) was dissolved in 10 cc. of 1:1 AcOEt-AcOH and shaken with 10 mg. of Adams' catalyst in H_2 atmosphere for 3 hrs. After working up in the usual way, the product crystallized from AcOEt-MeOH in needles, m. p. 129~130°, was identified as stigmastanyl acetate by admixture and by comparison of the infrared spectra.

Hydrolysis of the acetate and recrystallization of the product from MeOH furnished stigmastanol, m. p. 133~134°, which showed no depression on admixture with an authentic specimen.

Stigmasterol and Derivatives—Fraction A-2 (530 mg.) from the above chromatography was hydrolyzed with EtOH-KOH and esterified with BzCl in pyridine. The resulting benzoate was crystallized twice from $CHCl_3$-EtOH and then four times from AcOEt-EtOH, to afford 300 mg. of stigmasteryl benzoate with a constant melting point as plates, m. p. 160.5~162°, $[\alpha]_D^{17}$ −25.0°(c=1.05). *Anal.* Calcd. for $C_{36}H_{52}O_2$: C, 83.66; H, 10.14. Found: C, 83.91; H, 10.28.

Alkali hydrolysis of this benzoate and recrystallization from EtOH yielded stigmasterol as plates, m. p. 166.5~168°, $[\alpha]_D^{20}$ −50.6°(c=1.02); no depression by admixture with an authentic sample of stigmasterol. The infrared spectra of these two samples were entirely identical. *Anal.* Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found: C, 84.40; H, 11.76.

Acetylation of the sterol with Ac_2O in pyridine gave an acetate, which was recrystallized from EtOH, as plates, m. p. 142~143°, $[\alpha]_D^{20}$ −53.7°(c=1.35). *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found: C, 82.07; H, 11.09.

Hydrogenation of Stigmasteryl Acetate—Stigmasteryl acetate (20 mg.) was hydrogenated using 20 mg. of Adams' catalyst as described for hydrogenation of Δ^{22}-stigmastenyl acetate. Crystallization of the product from EtOH furnished stigmastanyl acetate melting at 129~130°; no depression on admixture with an authentic sample.

Summary

α-Spinasterol had been isolated previously from the root of *Bupleurum falcatum* L. Its more-soluble sterol fractions were investigated by fractional crystallization and chromatography

5) All melting points are uncorrected. Specific rotations were measured in $CHCl_3$.

on alumina. Besides α-spinasterol, three sterols, Δ^7-stigmastenol, Δ^{22}-stigmastenol, and stigmasterol, were isolated and identified. Although Δ^{22}-stigmastenol was already known as synthetic material, this work is the first example of its isolation from plants.

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UDC 615.779.931 011

108. **Shoshiro Nakamura**: Studies on Structure of Griseolutein B, a Streptomyces Antibiotic. I. Characterization and Degradation.

(*Institute of Applied Microbiology,* University of Tokyo*)

Umezawa, Hayano, Maeda, and Okami[1] isolated a new antibiotic from *Streptomyces griseoluteus* and the antibiotic was named griseolutein. Later, Osato, Maeda, and Umezawa[2] isolated another antibiotic from the same strain and they designated the first one griseolutein-A, and the second one, griseolutein-B. Both griseolutein-A and -B inhibit gram-positive and negative bacteria and are low in toxicity. According to pharmacological studies made by Ogata,[3] griseolutein-B gives a relatively high blood level when administered subcutaneously or orally. These observations suggested a possible usefulness of this antibiotic and elucidation of the structure of griseolutein-B was undertaken which is described in this and subsequent papers.

The cultured broth of *Streptomyces griseoluteus* was filtered and griseoluteins were extracted with butyl acetate at pH 2.0. Evaporation of the solvent under vacuum gave a brownish orange crude powder containing griseolutein-A and -B. This was recrystallized from dioxane and crude crystals chiefly containing griseolutein-B were obtained. The crude crystals were further purified by the 60-tube counter-current distribution between phosphate buffer of pH 5.8 and ethyl acetate. Griseolutein-B was present in tubes No. 1 and 2 and a small amount of A was found in tubes No. 44~46. Acidification of the aqueous layer of tubes No. 1~2 precipitated griseolutein-B as crystals. Further recrystallization from pyridine-dioxane by the addition of ether gave yellow prisms of griseolutein-B, $C_{17}H_{16}O_6N_2$ (mol. wt., 344.31). It did not give a clear melting or decomposition point, but it began to brown near 160°, darkened at about 180°, and charred at about 220°. Griseolutein-B is insoluble in toluene, ether, or butyl acetate, sparingly soluble in dioxane or methyl

Fig. 1. Ultraviolet Absorption Spectra (in MeOH)

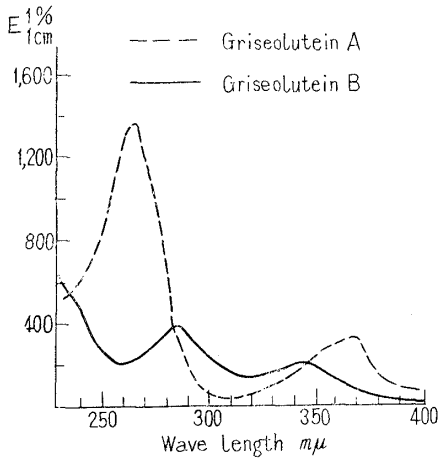
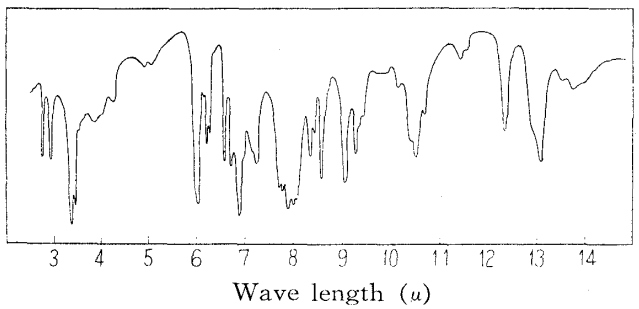


Fig. 2. Infrared Spectrum of Griseolutein B (in Nujol.)



* Yayoi-cho, Bunkyo-ku, Tokyo (申村昭四郎).

1) H. Umezawa, S. Hayano, K. Maeda, Y. Ogata, Y. Okami: J. Antibiotics(Japan), **4**, 34(1951).
2) T. Osato, K. Maeda, H. Umezawa: *Ibid.*, **7**, 15(1954).
3) Y. Ogata, K. Nitta, S. Yamazaki, O. Taya, T. Takeuchi, H. Umezawa: *Ibid.*, **6**, 139(1953).