various bile components and constituents of bile acids. This method was successfully utilized in discriminating Yūtan (bear bile), used as the valuable home remedy in the Orient from olden times, from the bile of other animals.

Some results different from past reports were obtained; notably that the main component of carp bile is not bile acid but is a sulfate of bile alcohol and that the conjugate amino acid in the bile acids of some fish was not taurin.

Infrared spectra of bile from about 100 dogs were examined as basis for the study of pharmacological action of choleretics. Infrared spectrum of dog bile is especially distinct, there is no necessity of considering individual differences, and there are no variation in the amount of bile outflow or infrared spectrum even eight hours after the operation.

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197. Tameto Okanishi, Akira Akahori, and Fumio Yasuda : Studies on the Steroidal Components of Domestic Plants. XL.*1 Constituents of *Heloniopsis orientalis* (THUNB.) C. TANAKA. (3). The Structure of Heloniogenin.

(*Shionogi Research Laboratory, Shionogi & Co., Ltd.**2)

As previously reported,[1] five steroidal sapogenins were isolated from "Shōjō-bakama" *Heloniopsis orientalis* (THUNB.) C. TANAKA. They are $\Delta^{3,5}$-desoxytigogenin, diosgenin, gentrogenin, kryptogenin and a new sapogenin. This new sapogenin (I), m.p. 212~213°, $[\alpha]_D$ −91°, has two hydroxyl groups. Its ultraviolet spectrum showed an absorption maximum (log ε 3.47) at 205.6 mμ. The analytical values also indicated that this sapogenin has a double bond. The infrared spectrum showed that this sapogenin has no carbonyl function and belongs to 25-D series of the steroidal sapogenins. This sapogenin was readily acetylated to give a diacetate (II), m.p. 184~185°, $[\alpha]_D$ −58°, with pyridine and acetic anhydride under usual conditions and it is considered that two hydroxyl groups are both primary or secondary. Although the known 25D-spirostenediols are yuccagenin[2] (25D-spirost-5-ene-2α,3β-diol), pennogenin[3] (25D-spirost-5-ene-3β,17-diol), ruscogenin[4] (25D-spirost-5-ene-1β,3β-diol) and isochiapagenin[5] (25D-spirost-5-ene-3β,12β-diol), the physical constants of this new sapogenin and its acetate do not coincide with any of those sapogenins and their acetates. From these results it is considered that this sapogenin is a new sapogenin and the name heloniogenin was given to this compound.

Heloniogenin yielded 3-acetate (III), m.p. 218~219°, $[\alpha]_D$ −89.6°, when it was kept at 10° with pyridine and acetic anhydride. The chromic acid oxidation of heloniogenin in acetic acid yielded gentrogenin (IVa) (3β-hydroxy-25D-spirost-5-ene-12-one) with

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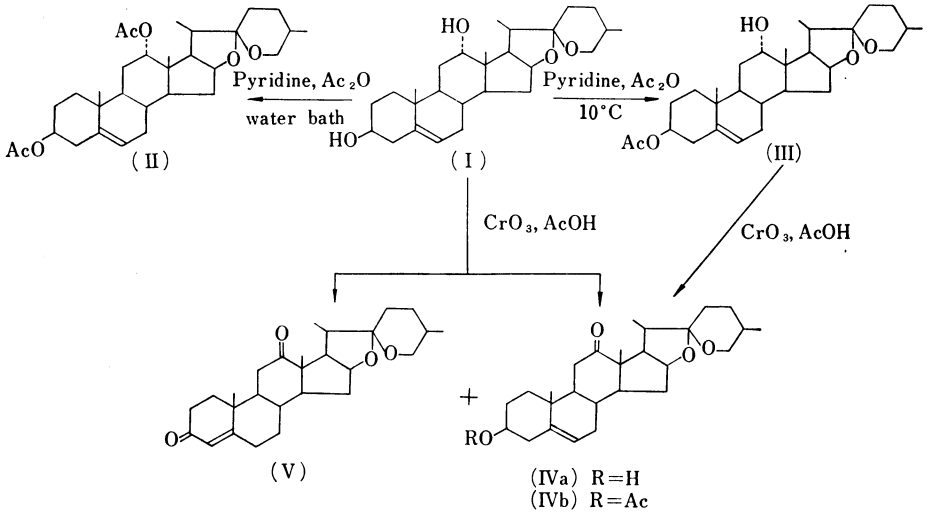
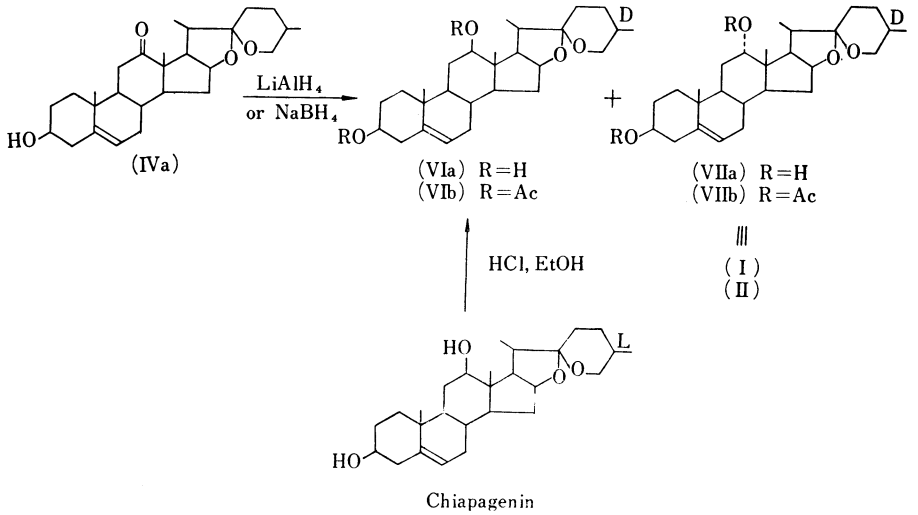
*1 Part XXXIX A. Akahori : Ann Repts. shionogi Research Lab., **11**, 97 (1961)

*2 Fukushima-ku, Osaka (岡西為人, 赤堀 昭, 安田郁夫).

1) T. Okanishi, A. Akahori, F. Yasuda : Ann. Repts. Shionogi Research Lab., **10**, 137 (1960).
2) R.E. Marker, *et al.* : J. Am. Chem. Soc., **65**, 1201 (1943); *ibid.*, **69**, 2189 (1947).
3) *Idem* : *Ibid.*, **65**, 1248 (1943); *ibid.*, **69**, 2208 (1947).
4) H. Lapin : Compt. rend., **244**, 3065 (1957).
5) I.T. Harrison, M. Velasco, C. Djerassi : J. Org. Chem., **26**, 155 (1961).

a small amount of a diketone (V), m.p. 248~250°. This diketone is considered to be 25D-spirost-4-ene-3,12-dione with its ultraviolet spectrum (λ_{max} 240 mμ, log ε 4.22) and infrared spectrum (C=O, 1710 cm^{-1}, 1672; C=C, 1621). Gentrogenin acetate (IVb) was also obtained with the chromic acid oxidation of heloniogenin 3-acetate. From these results the structure of heloniogenin is assumed to be 25D-spirost-5-ene-3β,12ξ-diol.





To determine the configuration of C_{12}-hydroxyl group, gentrogenin was reduced with metal hydrides. With lithium aluminum hydride, gentrogenin yielded two isomers of 12-hydroxyl group (VIa, VIIa). The proportion of the yields of (VIa) and (VIIa) was 165:105. With sodium borohydride the proportion of both products was 130:50. It was reported[6] that the hydrogenation with metal hydrides in neutral solution reduces C-12

6) L.F. Fieser, M. Fieser: "Steroids," p. 268 (1959), Reinhold.

ketone more to an equatorial β hydroxyl group than to an axial α one, and this tendency is more apparently observed in the case of sodium borohydride. On the other hand, the differences of the molecular rotations were calculated. The values of ($[M]_D$ (-OAc)-$[M]_D$ (-OH)) were −105 in (VI) and +66 in (VII). In the case of 25D-5α-spirostane-3β,12-diols,[7] the values are −60 in 3β,12β-diol and +63 in 3β,12α-diol. These values do not exactly coincide with the values calculated from the values demonstrated in literature,[8] i.e. −8 in 3β,12β-diol and +153 in 3β,12α-diol, but show the same tendencies as those. Furthermore the physical constants of (VIa) and (VIb) coincide with those of isochiapagenin m.p. (236~237°, $[\alpha]_D$ −121°) and its acetate (m.p. 206~207°, $[\alpha]_D$ −120°). The latter was also obtained from chiapagenin[5] by the action of hydrochloric acid and ethanol and the identity with the synthetic one was proved by the mixed melting point and the comparison of each infrared spectrum. These results indicated that (VIa) is 3β,12β-diol and (VIIa) is 3β,12α-diol. The physical constants and the infrared spectra of (VIIa) and (VIIb) were identical with those of heloniogenin and its acetate. (VIIa) and (VIIb) showed no depression of melting point when mixed with heloniogenin and its acetate. Therefore, the structure of heloniogenin was confirmed as 25D-spirost-5-ene-3β,12α-diol.

Experimental

Paper Chromatography——Filter paper : Toyo-Roshi No. 50, 2×40 cm. Apparatus : Toyo-Roshi Model C. Developed with one-dimensional ascending method. Solvent : heptane-$CHCl_3$-AcOH (50:20:3). Color Reagent : 1% EtOH solution of cinnamic aldehyde and 25 g. of $SbCl_3$ in 5 ml. of nitrobenzene. Rf values of the standard sapogenins : diosgenin 0.81, gentrogenin 0.64, heloniogenin 0.44.

Heloniogenin——The melting point of heloniogenin was raised to 212~213° after repeated recrystallization from MeOH. UV λ_{max}^{EtOH} mμ (log ε) : 205.6 (3.47).

Heloniogenin 3-Acetate——To a solution of 500 mg. of heloniogenin in 25 ml. of pyridine, 4 ml. of Ac_2O was added and the mixture was kept at 10° for 2 hr., then poured into ice-cold H_2O and extracted with Et_2O. The Et_2O solution was washed successively with 7% HCl, 10% Na_2CO_3 and H_2O, dried over Na_2SO_4 and evaporated to yieled 572 mg. of white crystals (Rf 0.97, 0.80, 0.71, 0.44). This product was chromatographed on 30 g. of alumina (Brockmann).

From the benzene and benzene-$CHCl_3$ (98:2~95:5) eluates 10 mg. of white crystals (Rf 0.97) were obtained after removal of the solvent which was recrystallized from MeOH to 5 mg. of white needles, m.p. 179~181°. This was identical with heloniogenin diacetate in melting point and infrared spectra, and showed no depression.

335 mg. of white crystals (Rf (0.80), 0.71) obtained from the $CHCl_3$ fractions yielded 225 mg. of white needles, m.p. 216~218°, after recrystallization from MeOH. The melting point was raised to 218~219° after further recrystallization. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found : C, 73.76; H, 9.52. $[\alpha]_D^{25}$ −89.6°(±2) (c=1.029, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1} : OH, 3570 (sharp); OAc, 1726, 1241; F-ring, 978, 919, 897, 859. 180 mg. of white crystals (Rf 0.44) obtained from the $CHCl_3$ and $CHCl_3$-MeOH (98:2) fractions yielded 130 mg. of white needles, m.p. 210~211°. after recrystallization from MeOH. This showed no depression of melting point with heloniogenin.

Chromic Acid Oxidation of Heloniogenin——To a solution of 280 mg. of heloniogenin in 10 ml. of AcOH, 94 mg. of CrO_3 in 40 ml. of AcOH was added the mixture kept at room temperature for 30 min., and then poured into H_2O and extracted with Et_2O. The Et_2O solution yielded 261 mg. of white amorphous substance (Rf 0.76, 0.64, 0.54, 0.44) after removal of the solvent. This substance was chromatographed on 30 g. of alumina (Brockmann).

From the benzene-$CHCl_3$ (60:40) eluates 38 mg. of white crystals (Rf 0.76) were obtained which yielded 21 mg. of white needles, m.p. 246~248°, after recrystallization from MeOH. The melting point was raised to 248~250° after further recrystallization. *Anal.* Calcd. for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found : C, 75.94; H, 9.16. UV λ_{max}^{EtOH} mμ (log ε) : 240 (4.22). IR ν_{max}^{Nujol} cm^{-1} : C=O (C-12), 1710 (C-3), 1672; C=C, 1621; F-ring, 983, 924, 902, 863.

From the benzene-$CHCl_3$ (60:40~50:50) eluates 144 mg. of white crystals (Rf 0.64) were obtained which yielded 42 mg. of white needles, m.p. 215~216°, after recrystallization from MeOH. *Anal.*

7) R. Hirschmann, C. S. Snoddy, Jr., N. L. Wendler : J. Am. Chem. Soc., **74**, 2693 (1952).
8) L. F. Fieser, M. Fieser : "Steroids," p. 177 (1959), Reinhold.

Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.63; H, 9.54. $[\alpha]_D^{28}$ −56.0°(±2) (c=1.021, $CHCl_3$). This was identical in melting point, $[\alpha]_D$ and infrared spectra with gentrogenin and showed no depression of mixed melting point.

From the $CHCl_3$ and $CHCl_3$-MeOH (98:2) eluates, 42 mg. of white crystals were obtained. Paper chromatography showed that this was the mixture of two sapogenins (Rf 0.54, 0.44).

Chromic Acid Oxidation of Heloniogenin 3-Acetate——To a solution of 450 mg. of heloniogenin 3-acetate in 10 ml. of AcOH, 50 mg. of CrO_3 in 25 ml. of AcOH was added, the mfxture kept at room temperature for 30 min., and then poured into H_2O and extracted with Et_2O. Crystals (420 mg.; Rf 0.83, 0.70) obtained after removal of Et_2O were chromatographed on 10 g. of alumina (Brockmann).

White crystals (240 mg.; Rf 0.83) obtained from the petroleum ether-benzene (50:50) eluates were recrystallized from MeOH to 210 mg. of white needles, m.p. 220~223°. The melting point was raised to 224~225° after further recrystallization. *Anal.* Calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found: C, 74.03; H, 9.09. $[\alpha]_D^{25}$ −58.1°(±2) (c=1.016, $CHCl_3$). This was identical in melting point, $[\alpha]_D$ and infrared spectra with gentrogenin acetate and showed no depression of mixed melting point.

The petroleum ether-benzene (50:50) and benzene eluates yielded 180 mg. of white crystals (Rf 0.70) after removal of the solvent. From this product 150 mg. of white needles, m.p. 217~218°, were obtained. This was identified with the material, heloniogenin 3-acetate.

Hydrogenation of Gentrogenin with Lithium Aluminum Hydride——To a solution of 300 mg. of gentrogenin acetate in a mixture of 20 ml. of dehyd. Et_2O and 4 ml. of tetrahydrofuran, 300 mg. of $LiAlH_4$ in 10 ml. of dehyd. Et_2O was added dropwise with stirring for 20 min. the mixture stirred further for 30 min., refluxed for 1.5 hr., and then poured into dil. HCl, and extracted with Et_2O. White crystals (317 mg.; Rf 0.81, 0.51, 0.44) obtained after removal of Et_2O were chromatographed on 30 g. of alumina (Brockmann).

From the benzene-$CHCl_3$ (50:50) eluates, 5 mg. (Rf 0.81), 5 mg. (Rf 0.81, 0.51) and 165 mg. (Rf 0.50) of white crystals were obtained. The last product was acetylated with 2 ml. of pyridine and 1 ml. of Ac_2O for 5 hr. on a water bath. The acetate (170 mg.) obtained as white crystals was recrystallized from MeOH to 150 mg. of white needles, m.p. 203~205°. The melting point was raised to 206~207° after further recrystallization. *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.51; H, 9.02. $[\alpha]_D^{27}$ −117.8°(±2) (c=1.015, $CHCl_3$). IR $\nu_{max}^{CS_2}$ cm^{-1}: OAc, 1737, 1238; F-ring, 981, 917, 897, 860. The acetate (50 mg.) was saponified with 200 mg. of KOH and 5 ml. of EtOH for 1 hr. under reflux, then poured into H_2O and extracted with Et_2O. White crystals (45 mg.) obtained after removal of the solvent were recrystallized from Me_2CO which yielded 25 mg. of white needles, m.p. 229~231°. The melting point was raised to 233~235° after further recrystallization. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.22; H, 9.84. $[\alpha]_D^{27}$ −116.4°(±2) (c=0.993, $CHCl_3$, IR ν_{max}^{Nujol} cm^{-1}: OH, 3530, 3440 (broad); F-ring, 980, 918, 896, 863.

White crystals (10 mg.; Rf 0.51, 0.44) were obtained from another benzene-$CHCl_3$ (50:50) eluate.

105 mg. of white crystals (Rf 0.44) obtained from $CHCl_3$ eluate were recrystallized from MeOH to 60 mg. of white needles, m.p. 211~212°. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.42; H, 9.95. $[\alpha]_D^{23}$ −89.2°(±2) (c=1.062, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: OH, 3527, 3440 (broad); F-ring, 976, 917, 898, 893, 865, 860. This was identical in melting point, $[\alpha]_D$ and infrared spectra with heloniogenin and showed no depression of mixed melting point. This product (40 mg.) was acetylated with 1 ml. of pyridine and 0.5 ml. of Ac_2O for 5 hr. under reflux, then poured into H_2O and extracted with Et_2O. White crystals obtained after removal of the solvent were recrystallized from MeOH to 25 mg. of white needles, m.p. 180~182°. *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.34, H, 9.01. Found: C, 72.32; H, 9.16. $[\alpha]_D^{27}$ −61.8°(±2) (c=1.080, $CHCl_3$). IR $\nu_{max}^{CS_2}$ cm^{-1}: OAc, 1738, 1239; F-ring, 980, 917, 896, 859. This was identical in melting point, $[\alpha]_D$ and infrared spectra with heloniogenin diacetate and showed no depression of mixed melting point.

Hydrogenation of Gentrogenin with Sodium Borohydride——To a solution of 230 mg. of gentrogenin acetate in 40 ml. of dehyd. EtOH, 60 mg. of $NaBH_4$ in 3 ml. of dehyd. EtOH was added, the mixture refluxed for 2 hr., and then poured into H_2O and extracted with Et_2O. White crystals (210 mg.) obtained after removal of the solvent were chromatographed on 20 g. of alumina (Brockmann).

From the benzene-$CHCl_3$ (60:40~50:50) eluates and benzene-$CHCl_3$ (50:50) eluate, 10 mg. (Rf 0.81) and 5 mg. (Rf 0.81, 0.50) of white crystals were obtained respectively.

White crystals (130 mg.; Rf 0.50) obtained from the benzene-$CHCl_3$ (40:60~30:70) eluates were acetylated with pyridine and Ac_2O as above to yield 80 mg. of white needles, m.p. 205~206°, after recrystallization from MeOH.

White crystals (10 mg.) obtained from the benzene-$CHCl_3$ (30:70) eluates were detected as the mixture of two isomers (Rf 0.50, 0.44) with paper chromatography.

White crystals (50 mg.; Rf 0.44) obtained from the benzene-$CHCl_3$ (20:80) eluates yielded 30 mg. of white needles, m.p. 182~183°, after acetylation with pyridine and Ac_2O and recrystallization from MeOH.

Isochiapagenin from Chiapagenin——A solution of 50 mg. of chiapagenin in 8.5 ml. of EtOH and 1.5

ml. of 35% HCl was refluxed for 70 hr., then 0.7 ml. of 35% HCl was added and again refluxed for 24 hr. The reaction mixture was then poured into H_2O and enxtracted with Et_2O. From the Et_2O solution 50 mg. of yellowish brown viscous substance was obtained and chromatographed on 5 g. of Florisil. White crystals (34 mg.) obtained from the benzene and benzene-$CHCl_3$ (10:90～50:50) eluates, were assigned as the mixture of the dehydration products from the IR spectrum. Light yellowish crystals (14 mg.), obtained from the $CHCl_3$ and $CHCl_3$-MeOH (98:2～90:10) eluates, were acetylated with 1 ml. of pyridine and 0.5 ml. of Ac_2O for 5 hr. on a water bath, then the reaction mixture was poured into H_2O and extracted with Et_2O. The Et_2O extract was chromatographed on 2 g. of alumina (Brockmann). The yellowish brown crystals (6 mg.) obtained from the benzene-$CHCl_3$ (50:50) eluates, were recrystallized from MeOH to 2 mg. of white needles, m.p. 198～203°. This was identical in melting point and IR spectra with (VIb) and showed no depression of mixed melting point.

The authors express their deep gratitude to Dr. K. Takeda, Director of this Laboratory, for his kind guidance throughout the course of this work. They are also indebted to Dr. Carl Djerassi for his kind donation of the valuable samples of chiapagenin and its diacetate. Thanks are also due to Mr. Y. Matsui for the measurement of infrared spectra and to the members of analytical section of this Laboratory for microanalysis and the measurement of optical rotation.

Summary

A new steroidal sapogenin isolated from *Heloniopsis orientalis* (THUNB.) C. TANAKA was named "heloniogenin." The chromic acid oxidation of this sapogenin yielded gentrogenin. Gentrogenin acetate was also obtained with the chromic acid oxidation of heloniogenin 3-acetate. Of two isomers derived from gentrogenin with the metal hydride reduction, 3β,12α-diol was identified with heloniogenin. The structure of heloniogenin was confirmed as 25D-spirost-5-ene-3β,12α-diol.

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198. Masao Nishikawa, Hiroyuki Mima, and Tokunosuke Kanzawa: Infrared Spectra of Thiourea and its Inclusion Compounds. I. Vibrational Spectrum of Rhombohedral Thiourea.*1

(*Research Laboratories, Takeda Chemical Industries, Ltd.**2)

Thiourea, similar to urea, forms a kind of clathrates with various organic compounds. This property, discovered originally by Angla,[1,2] has been the subjects among many investigations. Detailed X-ray diffraction studies, carried out by Schlenk[3] and Lennè[4] revealed that, in the presence of suitable guest molecules, the crystal structure of thiourea was altered from a rhombic to a rhombohedral form. The guest molecules were enclosed in a hexagonal channel of about 5.8 Å in diameter, nearly 1 Å longer

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*2 Juso-Nishino-cho, Higashiyodogawa-ku, Osaka (西川正夫, 美間博之, 神沢得之助).

1) B. Angla: Compt. rend., **224**, 402, 1166 (1947).
2) *Idem*: Ann. Chim., (12) **4**, 639 (1949).
3) W. Schlenk: Ann., **573**, 142 (1951).
4) H. V. Lennè: Acta Cryst., **7**, 1 (1954).