and recrystallized from ethanol to give pale brownish leaflets, mp 257—258° (decomp.) (lit.[4], mp 260° (decomp.)). Yield, 0.44 g (61%). NMR in DMSO-d_6 (τ): 7.82, 7.72 (s, CH_3), 3.52 (s, H^4).

The Mannich Reaction of 3,6-Dimethyl-5-hydroxypyridazine 1-Oxide (IV)——3,6-Dimethyl-4-pyrrolidinomethyl-5-hydroxypyridazine 1-Oxide (VIa): A solution of 0.3 ml of 37% formalin and 0.142 g (0.002 mole) of pyrrolidine in 2 ml of ethanol, was added to a suspended solution of 0.28 g (0.002 mole) of IV in 10 ml of ethanol, the mixture was warmed on a water bath until a clear solution was obtained, and the solution was allowed to stand for two days at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was treated with a mixture of conc. hydrochloric acid and ethanol (1: 1). The acidic solution was evaporated to dryness under reduced pressure, and the residue was recrystallized from a mixture of ethanol and diisopropyl ether to give colorless granules, mp 185—186° (decomp.). Yield, 0.32 g (71%). IR in nujol (cm^{-1}): 2860 (NH^+). NMR in DMSO-d_6 (τ): 7.72, 7.51 (s, CH_3), 5.69 (s, CH_2). *Anal.* Calcd. for $C_{11}H_{17}O_2N_3 \cdot HCl$: C, 50.86; H, 6.98; N, 16.11. Found: C, 51.06; H, 6.92; N, 16.23.

Other Mannich bases VIb—d were similarly prepared as noted for VIa.

3,6-Dimethyl-4-piperidinomethyl-5-hydroxypyridazine 1-Oxide Hydrochloride (VIb): Colorless granules (from a mixture of ethanol and diisopropyl ether), mp 187—188° (decomr.). Yield, 53%. IR in nujol (cm^{-1}): 2860 (NH^+). NMR in DMSO-d_6 (τ): 7.71, 7.50 (s, CH_3), 5.74 (s, CH_2). *Anal.* Calcd. for $C_{12}H_{19}O_2N_3 \cdot HCl$: C, 52.64; H, 7.36; N, 15.34. Found: C, 52.25; H, 7.73; N, 15.28.

3,6-Dimethyl-4-morpholinomethyl-5-hydroxypyridazine 1-Oxide Hydrochloride (VIc): Colorless granules (from a mixture of methanol and ethanol), mp 208—209° (decomp.). Yield, 58%. NMR in DMSO-d_6 (τ): 7.63, 7.45 (s, CH_3), 5.67 (s, CH_2). *Anal.* Calcd. for $C_{11}H_{17}O_3N_3 \cdot HCl$: C, 47.92; H, 6.56; N, 15.24. Found: C, 48.01; H, 6.66; N, 15.20.

3,6-Dimethyl-4-bis(2-hydroxyethyl)aminomethyl-5-hydroxypyridazine 1-Oxide Hydrochloride (VId): Pale brownish dices (from ethanol), mp 172—173° (decomp.). Yield, 67%. NMR in D_2O (τ): 7.50, 7.55 (s, CH_3), 5.59 (s, CH_2). *Anal.* Calcd. for $C_{11}H_{17}O_4N_3 \cdot HCl$: C, 44.97; H, 6.86; N, 14.30. Found: C, 45.14; H, 6.92; N, 14.59.

3,6-Dimethyl-4-bromo-5-hydroxypyridazine 1-Oxide (VII)——Three drops of bromine were added to a suspended solution of 0.14 g (0.001 mole) of IV in 20 ml of ethanol with vigorous shaking. After 30 min, the reaction mixture was evaporated to dryness under reduced pressure, and the residue was recrystallized from ethanol to give straw yellow prisms, mp 200—201° (decomp.). Yield, 0.21 g (96%). *Anal.* Calcd. for $C_6H_7O_2N_2Br$: C, 32.89; H, 3.22; N, 12.79. Found: C, 32.95; H, 3.17; N, 12.91.

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Steroid Saponins of *Heloniopsis orientalis* (Thunb.) C. Tanaka

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The steroid glycosides in the fresh whole plants of *Heloniopsis orientalis* (Thunb.) C. Tanaka were examined and the following four steroid saponins were isolated and identified: pennogenin 3-O-rhamnosyl-chacotrioside (II); methyl proto-dioscin; 26-O-β-D-glucopyranosyl-25D-furost-5-ene-3β,17α,22,26-tetraol 3-O-rhamnosyl-chacotrioside (III) (the proto-type compound of II); 26-O-β-D-glucopyranosyl-17(20)-dehydrokryptogenin 3-O-rhamnosyl-chacotrioside (VI) (an artefact produced from III).

III is the second furostanol 3,26-O-bisglycoside corresponding to a coexisting pennogenin 3-O-glycoside and could be regarded as a "nolonin".

Okanishi and his collaborators[2] have reported isolation of pennogenin (I) and kryptogenin along with diosgenin, gentrogenin and heloniogenin from the whole plants of *Heloniopsis*

1) Location: *3-1-1, Maedashi, Higashi-ku, Fukuoka, 812, Japan.*

2) K. Takeda, T. Okanishi, and A. Shimaoka, *Yakugaku Zasshi*, **73**, 84 (1953); T. Okanishi, A. Akahori, and F. Yasuda, *Chem. Pharm. Bull.* (Tokyo), **10**, 1195 (1962); *idem.*, *Ann. Rept. Shionogi Res. Lab.*, **10**, 137 (1960); **14**, 202 (1964).

orientalis (THUNB.) C. TANAKA. It suggests[3] that the plant may contain either Marker's "nolonin" or/and the glycosides of I. As a part of the program[3] of studying "nolonin" and the related compounds, a survey of the fresh materials of the title plant for the steroid glycosides was undertaken.

This paper describes isolation of four steroid saponins, Ha—d, among which two, Ha and Hd, are pennogenin 3-O-glycoside and the corresponding furostanol 3,26-O-bisglycoside, respectively.

The fresh whole plants were soaked in cold methanol and the extract was treated as shown in Chart 1.

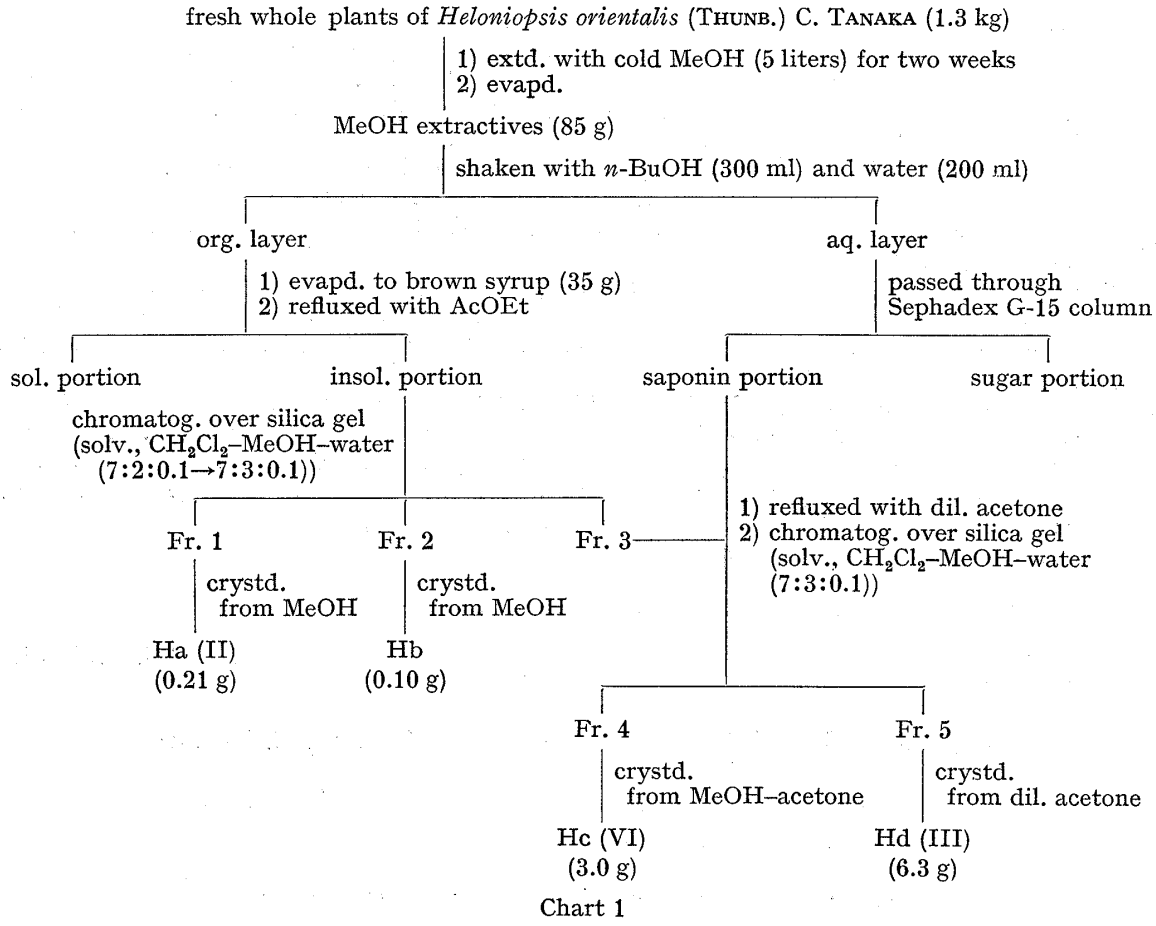

Chart 1

Ha (II) was identified with pennogenin 3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (rhamnosyl-chacotrioside) (compound Tg) previously obtained[3b] from the underground parts of *Trillium kamtschaticum* PALL., while Hb was found to be methyl proto-dioscin.[4]

Hd (III), $[\alpha]_D$ −92.1°, was negative in Ehrlich test[4] and showed neither spiroketal absorptions[3b,4] on infrared (IR) nor methoxy signal on nuclear magnetic resonance (NMR) spectra. On boiling with methanol III was transformed to less polar Hd′ (III′), $[\alpha]_D$ −100.2°, showing a methoxy signal on the NMR spectrum. Both III and III′ gave, on hydrolysis with almond emulsin, equally II and glucose. These data indicate[3b,4] that III and III′ are respectively 22-hydroxy- and 22-methoxyfurostanol 3,26-O-bisglycosides related to II. A mass spectrum

3) *a*) T. Nohara, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1772 (1974); *b*) *Idem*, *ibid.*, **23**, 872 (1975).
4) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1162 (1968); T. Kawasaki, T. Komori, K. Miyahara, T. Nohara, I. Hosokawa, and K. Mihashi, *ibid.*, **22**, 2164 (1974).

of III acetate showed the peaks due to peracetylated terminal hexose (*m/e* 331) and methylpentosyl-methylpentose (*m/e* 503) units[3b] but none ascribable to hexosyl-hexose and hexosyl-methylpentose. Furthermore, Baeyer-Villiger oxidation[3b,4] of III′ provided methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside (IV) together with 5α-pregnane-3β,5α,6β,16β,17α,-20α-hexaol (V).

Accordingly additional one mole of β-D-glucopyranose is conjugated only with the hydroxy group at C_{26} of the aglycone, and III is defined as 26-O-β-D-glucopyranosyl-25D-furost-5-ene-3β,17α,22,26-tetraol 3-O-rhamnosyl-chacotrioside, the proto-type compound[4] of II. III′ is the 22-methoxy analog of III.

Hc (VI), $[\alpha]_D$ −109.4°, gave IR, ultraviolet (UV) and optical rotatory dispersion (ORD) spectra quite similar to those of compound Tf (VII) reported earlier,[3b] namely a 3,26-O-bisglycoside of 17(20)-dehydrokryptogenin (VIII). *Rf* values on thin-layer chromatogram (TLC) of VI and III′ were identical and a mass spectrum of VI acetate resembled that of III acetate in fragment peaks due to the sugar moiety. Therefore VI was presumed to have the same sugar moiety as that of III combined with the aglycone VIII and to have been produced secondarily from III, as VII was from the parent compound Te (IX),[3b] 3,26-O-bisglycoside of 17α,22-dihydroxyfurostane derivative. Subsequently, in the same way as for IX, III was treated in dioxane with silica gel and the product was identified with VI.

In consequence, VI is assigned the structure 26-O-β-D-glucopyranosyl-17(20)-dehydrokryptogenin 3-O-rhamnosyl-chacotrioside and considered to be an artefact yielded from III during the procedure of chromatography over silica gel.

III is the second furostanol 3,26-O-bisglycoside corresponding to a coexisting pennogenin 3-O-glycoside. Similarly to the first one IX,[3b] it could also be regarded as a "nolonin."

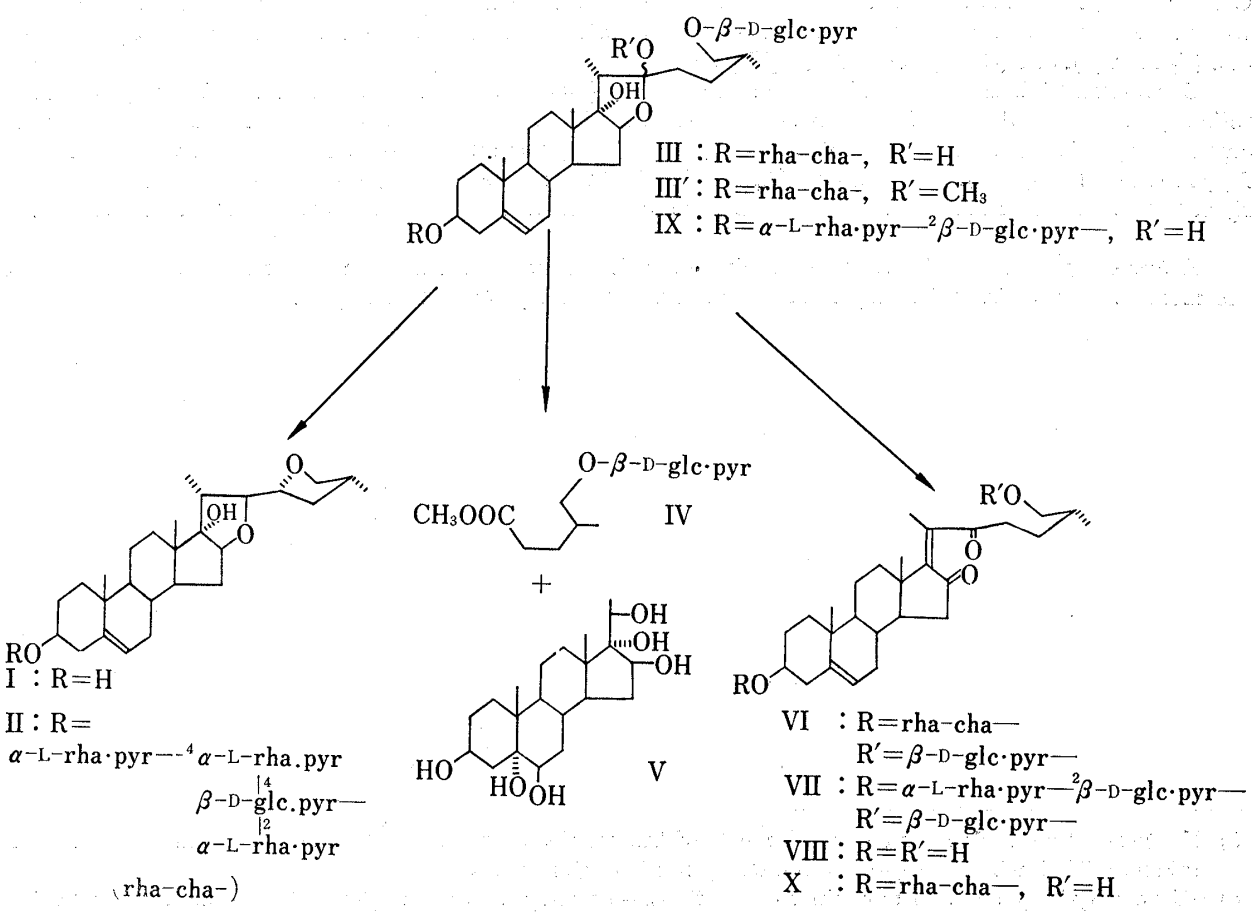

Chart 2

Experimental[5]

Extraction and Isolation of Steroid Glycosides——The whole plants collected in May in the suburb of Fukuoka city were immediately cut and soaked in cold methanol for 2 weeks. The extract was treated as shown in Chart 1.

Ha (II)——Colorless needles, mp 224—229° (decomp.), $[\alpha]_D$ −138.2° (c=1.20, pyridine). Identified with an authentic sample of Tg[3b] (acid hydrolysis, mixed mp, IR and *Rf* on TLC).

Hb——Colorless needles, mp 189—192° (decomp.), $[\alpha]_D$ −98.2° (c=1.03, pyridine). Identified with an authentic sample of methyl proto-dioscin[4] (acid hydrolysis, mixed mp, IR, NMR and *Rf* on TLC).

Hd (III)——White powder, $[\alpha]_D$ −92.1° (c=0.83, pyridine), *Rf* 0.16, negative in Ehrlich test.[3b,4] No spiroketal absorptions[3b,4] and no methoxy signal on IR and NMR (in C_5D_5N) spectra respectively. In an usual way[3b] III was treated with almond emulsin to give glucose and II, colorless needles (from MeOH), mp 225—228° (decomp.), $[\alpha]_D$ −128.2° (c=1.03, pyridine), which was identified with an authentic sample by direct comparison. III was acetylated on heating with Ac_2O–pyridine (1:2, v/v) for 1 hr to give the acetate as a white powder (from hexane-acetone), mp 135—139°, $[\alpha]_D$ −61.9° (c=0.42, $CHCl_3$). Mass Spectrum m/e: 503 ($C_{22}H_{31}O_{13}^+$), 412 ($C_{27}H_{40}O_3^+$), 394 ($C_{27}H_{38}O_2^+$), 331 ($C_{14}H_{19}O_9^+$), 273 ($C_{12}H_{17}O_7^+$).

Hd′ (III′)——III (200 mg) in MeOH (20 ml) was refluxed for 1 hr. The solution was concentrated and acetone was added to give III′ as a white powder, $[\alpha]_D$ −100.2° (c=0.83, pyridine), *Rf* 0.22. NMR (C_5D_5N) (100 MHz, JEOL PS-100): 3.23 (OCH_3). III′ was hydrolyzed with emulsin to give the same products as from III.

Baeyer-Villiger Oxidation of III′——In the same way as for proto-dioscin acetate,[4] III was oxidized with HCOOH and H_2O_2, the product was treated with alkali and then acetylated. The crude acetate was chromatographed over silica gel. The hexane–AcOEt (2:1, v/v) fraction gave IV as colorless oil, which was identified with an authentic sample[4] by comparison of their NMR and mass spectra. Subsequent elution with AcOEt provided a white powder, which was hydrolyzed with acid to give V as colorless needles (from MeOH), mp 285—287°, $[\alpha]_D$ −3.4° (c=0.71, pyridine), identical with an authentic sample[3b] (mixed mp, IR, NMR and *Rf* on TLC).

Hc (VI)[6]——White powder, $[\alpha]_D$ −109.4° (c=1.01, pyridine), *Rf* 0.22. IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1730—1700 (C=O), 1630 (C=C). UV λ_{max}^{EtOH} nm: 242 (ε=9600). ORD (c=0.042, EtOH) [M] (nm): +3260° (322) (peak), −3620° (370) (trough). NMR (C_5D_5N): 1.02 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 1.95 (3H, s, CH_3–C(R)=C<). VI was acetylated as III and the product was purified by passing through a silica gel column to give VI acetate as a white powder, $[\alpha]_D$ −63.4° (c=0.73, $CHCl_3$). Mass Spectrum m/e: 503 ($C_{22}H_{31}O_{13}^+$), 392 ($C_{27}H_{36}O_2^+$), 331 ($C_{14}H_{19}O_9^+$), 273 ($C_{12}H_{17}O_7^+$).

Conversion of III into VI——III (320 mg) in dioxane (20 ml) was boiled with silica gel (260 mg) for 2.5 hr. Silica gel was filtered off and the filtrate was evaporated. The residue was dissolved in acetone and precipitated by adding water to give VI as a white powder (120 mg), $[\alpha]_D$ −102.6° (c=0.78, pyridine). UV λ_{max}^{EtOH} nm: 242 (ε=10200). ORD (c=0.052, EtOH) [M] (nm): +3950° (322) (peak), −4330° (370) (trough). *Rf* value on TLC and IR and NMR spectra were identical with those of VI obtained from the extract.

Acknowledgement The authors thank Misses Y. Inatsu and M. Kawamura for the mass spectra and the member of the Central Analysis Room of this University for microanalysis.

5) For general methods refer to experimental of the previous report.[3b] *Rf* values given in the text were taken on TLC using $CHCl_3$–MeOH–water (7:3:0.5, v/v) as solvent.

6) VI was hydrolyzed with emulsin to give the 26-hydroxy compound (X), colorless needles (from dil. MeOH), mp 185—190° (decomp.), $[\alpha]_D$ −134.5° (c=0.91, pyridine).