as described above: (+)-hydrazide of (−)-lactone, m.p. 90~91°(from PrOH+AcOEt), $[\alpha]_D^{24}$ +19.0° ± 2° (c=1.179, dehyd. EtOH); (−)-hydrazide of (+)-lactone, m.p. 71~73°, $[\alpha]_D^{21}$ −3.3° ± 2° (c = 0.924, dehyd. EtOH); (±)-hydrazide of (±)-lactone, m.p. 72~74°.

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Summary

Since S-(+)-2-methylglutaric acid (V) and S-(−)-4-hydroxyvaleric γ-lactone were obtained by degradation of an α,β-unsaturated ketone (II) derived from guaiol (I), the configurations of the methyl groups at C-4 and C-10 in guaiol are both α-oriented and guaiol should be represented by (III). Further, from these results the stereochemistry of four isomeric nepetalinic acids and iridolactones was elucidated.

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102. Ken'ichi Takeda, Tameto Okanishi, Kanzo Sasaki, and Ariyoshi Shimaoka : Studies on the Steroidal Components of Domestic Plants. XXXI.[1] Constituents of *Reineckia carnea* KUNTH. (3). Pentologenin and Kitigenin.

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In the previous paper,[2] it was reported that kitigenin (I), isolated together with diosgenin from the whole plant of *Reineckia carnea* KUNTH, is a new tetrahydroxy-25D-sapogenin on the basis of its analysis, formation of a triacetate, and the infrared spectra of the sapogenin and its acetate. Subsequently, β-sitosteryl *d*-glucoside and β-sitosterol[3] were also isolated from the same source.

Recently, as a sufficient amount of the plant material was obtained, the isolation of sapogenins was further reinvestigated and two new sapogenins, m.p. 240~245° and m.p. 320°(decomp.), were isolated. The former has not yet been studied in detail, because of the lack of material. The latter, m.p. 320°(decomp.), $[\alpha]_D^{25}$ −54.5° ($CHCl_3$-MeOH=1:1), corresponding to the formula $C_{27}H_{44}O_7$, present in both epigeous part and rhizome, was named pentologenin, and its infrared spectrum showed neither a ketonic band nor an isolated double bond, but a broad band of hydroxyl function. The characteristic absorption bands of the sapogenin side-chain were observed at 980, 915, 898, and 860 cm^{-1}, and the comparison of intensity of the bands at 915 and 898 cm^{-1} showed that the sapogenin belongs to a 25D-sapogenin.

Acetylation of pentologenin with acetic anhydride and pyridine followed by chromatography gave three substances of m.p. 166~168°, 149~153°, and 238~241°. The analytical

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1) Part XXX. K. Takeda, T. Okanishi, H. Ōsaka, A. Shimaoka, N. Maezono : This Bulletin, **9**, 388 (1961).
2) K. Takeda, T. Okanishi, A. Shimaoka : Yakugaku Zasshi, **75**, 560 (1955).
3) T. Okanishi, A. Shimaoka : Ann. Rept. Shionogi Research Lab., **10**, 1391 (1960).

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values and the acetyl number determination of the first one corresponded to a tetraacetate (III), $C_{35}H_{52}O_{11}$, and its infrared spectrum showed the presence of hydroxyl function (3600 cm^{-1}, sharp), acetoxyl function (1758, 1746 cm^{-1}), and 25D-type side chain (925<898 cm^{-1}). Both the second and the third products were assumed to be a triacetate, $C_{33}H_{50}O_{10}$, from the analytical values and the acetyl number determination, but their exact structures have not yet been investigated in detail. From these results, pentologenin is a new penta-hydroxy-25D-sapogenin. This is the first example of a steroidal sapogenin having penta-hydroxyl groups isolated from a plant source.

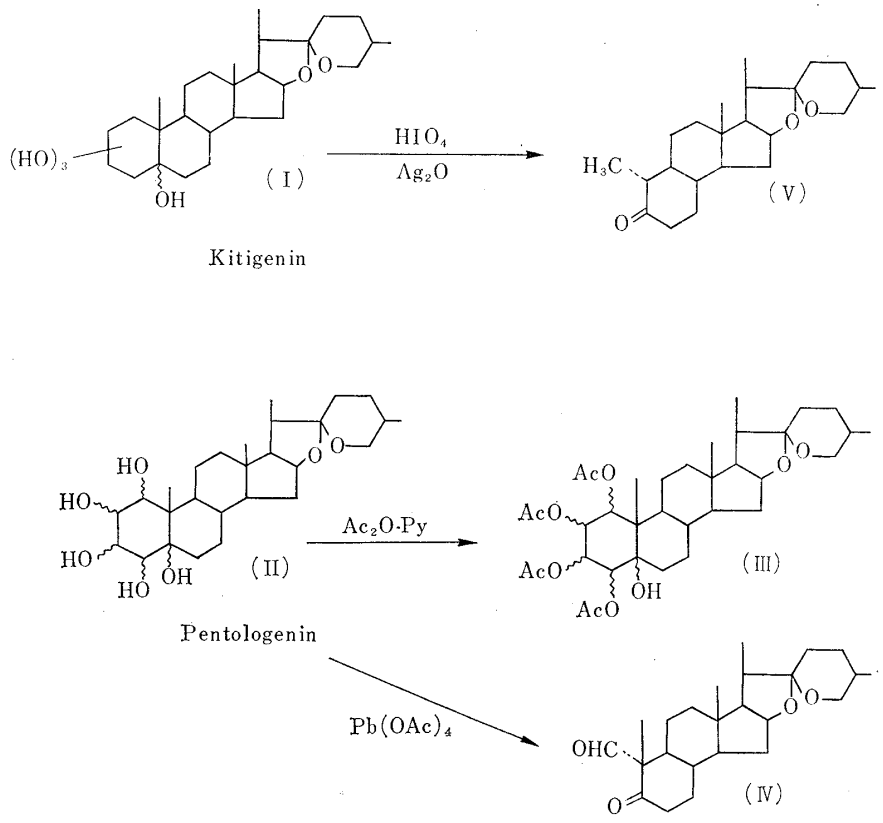

Chart 1.

In order to determine the position of the hydroxyl groups in pentologenin, oxidative opening of ring-A was attempted. On treatment with lead tetraacetate in an acetic acid-chloroform solution, pentologenin gave an oxidation product, m.p. 224~226°, corresponding to the empirical formula, $C_{24}H_{36}O_4$. The infrared spectrum of this substance indicated the presence of an aldehyde (2760, 1732 cm^{-1}) and a six-membered ring ketone (1696 cm^{-1}). This substance was found to be identical with the A-trisnor-1,5-*seco*-aldehyde-ketone*[2] (IV), m.p. 220~224°, by the mixed melting point and comparison of their infrared spectra.

These experimental data showed that the five hydroxyl groups of pentologenin are all located in ring-A.

Periodic acid oxidation of the above mentioned kitigenin and further oxidation of the crude product with silver oxide, followed by treatment with sodium hydroxide, gave des-A-25D-spirostan-5-one*[2] (V). This fact indicated that all four hydroxyl groups of kitigenin are in ring-A. The position of these hydroxyl groups will be reported in the following paper.

From the above-mentioned results, it was concluded that, among four sapogenins iso-

*[2] These substances have been prepared in this laboratory during the structural investigation of kogagenin; cf. T. Kubota, K. Takeda : Tetrahedron, **10**, 1(1960).

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lated from *Reineckia carnea* KUNTH, kitigenin and pentologenin, both new sapogenins, have four and five hydroxyl groups in ring-A respectively.

With the exception of kogagenin (25D-spirostane-1β,2β,3α,5β-tetrol) isolated from *Dioscorea Tokoro* MAKINO, polyhydroxy-sapogenins like these have not been found until now. In addition, from the standpoint of biogenesis, it is interesting that such polyhydroxy-sapogenins occur in the plant source.

Experimental*3

Isolation of the Sapogenins from the Whole Plant——The whole plant was dried and sliced, and 10 kg. of the material was extracted three times with hot 90% MeOH (50 L.). After concentration to 5 L., the extract was shaken with petr. ether to remove soluble substances and evaporated to a jelly-like residue. This was diluted with 3 L. of H_2O, adjusted to pH 4.2~5.0 with HCl, and extracted with three 10-L. portions of BuOH. The BuOH extract (600 g.) was refluxed with HCl-MeOH (2 L. of MeOH, 1 L. of H_2O, 500 cc. of conc. HCl) for 5 hr. After cool, the mixture was diluted with H_2O, saturated with NaCl, and extracted with 5 L. of BuOH. After washing with H_2O, the BuOH solution was evaporated to leave 150 g. of a residue. This was re-extracted with MeOH-$CHCl_3$ (1:1), the extract was concentrated, and 35 g. of the oily substance obtained containing crystals was chromatographed on alumina affording the following substances :

(A) m.p. 206~207° (B) m.p. 240° (3.75 g.)
(C) m.p. 285~308° (2.5 g.) (D) m.p. 315° (decomp.) (0.05 g.)

Substance (A) was proved to be diosgenin, from its analysis, infrared spectrum, and admixture. Substance (B) is an unknown sapogenin and substance (C) was a mixture. Substance (D) was proved to be pentologenin from paper chromatography and comparison of the infrared spectra.

By treatment with boiling 5% HCl for 3 hr., substance (C) afforded 2.5 g. of crystalline substance, m.p. 290°. This was acetylated and chromatographed on alumina to give the following substances :

(E) m.p. 155~160° (0.4 g.) (F) m.p. 160° (0.3 g.)
(G) m.p. 220° (1.07 g.) (H) m.p. 230° (0.09 g.)

Substances (E) and (F) were identified as β-sitosteryl-*d*-glucoside tetraacetate. Substance (G) was proved to be kitigenin triacetate from its infrared spectrum, analysis, and admixture. Substance (H) was found to be kitigenin diacetate from its analysis and comparison of its infrared spectra with an authentic sample.

Isolation of the Sapogenins from the Rhizome——The dried and sliced rhizome (20 kg.) was extracted with hot 90% MeOH (100 L.). The extract was concentrated and the precipitated crystals, (300 g. (A')), m.p. 280~285° (decomp.), were collected by filtration. This was hydrolyzed with HCl to give diosgenin. From this fact, (A') was assumed to be a glycoside of diosgenin.

The filtrate of (A') was washed with petr. ether and then hydrolyzed with HCl-MeOH on a steam-bath for 5 hr. The mixture was extracted with benzene and then Et_2O. The combined extracts gave 20 g. of diosgenin and 40 g. of a mixture of diosgenin and an unknown sapogenin. The benzene- and Et_2O-insoluble substance was extracted with BuOH to give 100 g. of a crude substance. This was chromatographed on alumina and gave a small amount of diosgenin, m.p. 198~200°, and 6.6 g. of crystals, m.p. ca. 300°, which were recognized as a mixture of kitigenin and pentologenin by paper chromatography.

The lower layer in the BuOH extraction was hydrolyzed by refluxing with 5% KOH-MeOH for 2 hr. and extracted with $CHCl_3$-MeOH (4:1). This extract gave 3.2 g. of crystals (B'), m.p. 300° (decomp.), which were found to be a mixture of kitigenin and pentologenin by paper chromatography. The hydrolyzed mixture was further extracted with $CHCl_3$-MeOH (1:1) and gave 3.8 g. of crystals, m.p. 300°, which showed an identical spot with pentologenin on paper chromatography.

Isolation of the Sapogenins from the Epigeous Part——The dried and sliced epigeous part (6 kg.) was also treated as above and the following substances were obtained : Diosgenin, 0.8 g.; kitigenin, 1 g., m.p. 300° (decomp.); pentologenin (containing a small amount of kitigenin), 3.5 g.; mixture of kitigenin and pentologenin, 21 g.; unknown sapogenin 24.33 g.

Purification of Pentologenin——The foregoing fraction (B') (2 g.) containing pentologenin was partition-chromatographed on Celite (Hyflosupercell, 60 g.; stationary phase: glycerol, 30 cc.) and gave the following fractions : The initial benzene fraction (244 mg.) showed one spot corresponding to kitigenin on paper chromatogram. The next benzene fraction (200 mg.) showed two spots corresponding

*3 All m.p.s are uncorrected. Infrared spectra were measured with the Koken Infrared Spectrophotometer, Model DS-301.

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to kitigenin and pentologenin. The benzene-$CHCl_3$ (19:1～1:4) and $CHCl_3$ fractions gave a substance which showed only one spot corresponding to pentologenin. Recrystallization from $CHCl_3$-MeOH gave a pure sample of pentologenin (II), m.p. 320° (decomp.), $[\alpha]_D^{25}$ −54.5° (c=0.497, $CHCl_3$-MeOH=1:1). *Anal.* Calcd. for $C_{27}H_{44}O_7$: C, 67.47; H, 9.23. Found : C, 67.55; H, 9.18. IR ν_{max}^{Nujol} cm^{-1} : 3380～3500 (OH, broad), 980, 915, 898, 860 (spiroketal side chain).

Acetylation of Pentologenin——Pentologenin (400 mg.) was dissolved in a warm mixture of 10 cc. of pyridine and 10 cc. of Ac_2O and allowed to stand for 90 hr. at room temperature. The mixture was concentrated in a reduced pressure and treated with Et_2O and 5% HCl. The Et_2O solution was washed successively with water, 5% NaOH, and H_2O, dried over Na_2SO_4, and evaporated to yield an oily substance (530 mg.). 500 mg. of this product was chromatographed on alumina (Woelm II, 15 g.) and the petr. ether-benzene (10:1～1:1) fractions gave an oily substance. The benzene fraction gave 165 mg. of a crude substance which was rechromatographed as described below. The benzene-$CHCl_3$ (10:1, 5:1) fractions gave a triacetate (47 mg.), m.p. 144～150°. Recrystallization from Me_2CO-petr. ether and then Et_2O-petr. ether afforded a pure sample of m.p. 149～153°. The analytical sample was dried over P_2O_5 *in vacuo* at 115° for 3 hr. *Anal.* Calcd. for $C_{33}H_{50}O_{10}\cdot\frac{1}{2}H_2O$ (triacetate) : C, 64.37; H, 8.35; CH_3CO, 20.97. Found : C, 64.06; H, 8.45; CH_3CO, 20.97. IR ν_{max}^{Nujol} cm^{-1} : 3490 (OH, broad), 1744, 1729 (OAc), 1661 (H_2O).

The benzene-$CHCl_3$ (3:1～1:1) and $CHCl_3$ fractions gave a mixture (99 mg.). The next $CHCl_3$ fraction gave a substance (37 mg.), m.p. 225～235°. Its pure sample, m.p. 238～241°, recrystallized from Me_2CO-petr. ether, gave an analytical value corresponding to a triacetate. *Anal.* Calcd. for $C_{33}H_{50}O_{10}$ (triacetate) : C, 65.32; H, 8.31; CH_3CO, 21.28. Found : C, 65.23; H, 8.51; CH_3CO, 22.35. IR ν_{max}^{Nujol} cm^{-1}: 3590, 3515 (OH, broad), 1753, 1747 (OAc).

The $CHCl_3$-MeOH (10:1, 5:1) fractions afforded a mixture (22 mg.).

The above benzene fraction (165 mg.) was again chromatographed on alumina (Woelm II, 4.7 g.). The petr. ether-benzene (1:1) fraction gave an oil (7 mg.). The petr. ether-benzene (1:2) fraction gave 62 mg. of crystals, which were recrystallized from Et_2O-petr. ether to a pure sample of the tetraacetate (III), m.p. 165～168°. The analytical sample was dried over P_2O_5 *in vacuo* at 115° for 2 hr. *Anal.* Calcd. for $C_{35}H_{52}O_{11}$: C, 64.79; H, 8.08; CH_3CO, 26.54. Found : C, 64.70; H, 8.17; CH_3CO, 25.40. IR ν_{max}^{Nujol} cm^{-1} : 3600 (OH, sharp), 1758, 1746 (OAc).

Oxidation of Pentologenin with Lead Tetraacetate——Pentologenin (55 mg.) was added to 18.5 cc. $Pb(OAc)_4$ solution (500 mg. of $Pb(OAc)_4$ in a mixture of 12 cc. of AcOH and 12 cc. of $CHCl_3$). After standing for 23 hr. at room temperature, the reaction mixture was diluted with H_2O. The brownish precipitate was extracted with Et_2O, and the extract was washed with H_2O, 10% Na_2CO_3, and H_2O, dried over Na_2SO_4, and evaporated. The residue was recrystallized from MeOH to a pure sample of (IV), m.p. 224～226° *Anal.* Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found : C, 74.32; H, 9.41. IR ν_{max}^{Nujol} cm^{-1} : 2755, 1729 (CHO), 1695 (>C=O).

This sample showed no depression on admixture with a sample of A-trisnor-1,5-*seco*-aldehyde-ketone, m.p. 220～224°, and the infrared spectra of these substances were completely identical.

Oxidation of Kitigenin with Periodic Acid——To a solution of 150 mg. of kitigenin in 20 cc. of dioxane a solution of 75 mg. of HIO_4 in 1 cc. of H_2O was added. After standing for 18 hr. at room temperature, the mixture was concentrated *in vacuo* at room temperature and treated with H_2O and Et_2O. The Et_2O solution was washed with 5% Na_2CO_3 and H_2O, dried over Na_2SO_4, and evaporated to give 138 mg. of a residue. This substance clearly reduced the Fehling solution.

A solution of this product (138 mg.) in 2.5 cc. of dioxane was added dropwise to a suspension of Ag_2O (prepared from 205 mg. of $AgNO_3$ in 1 cc. of H_2O and 2 cc. of 10% NaOH) during 30 min. The mixture was stirred for 1 hr. at room temperature, filtered, and the residue was washed with Et_2O. The filtrate and washings were combined and separated into neutral and acid fractions. The acid substance was heated at 80～90° with 5% NaOH in N_2-atmosphere to give a neutral substance. The neutral product, m.p. 110～135°, was chromatographed on Al_2O_3 (Brockmann, 1.95 g.). The petr. ether-benzene (10:1) fraction gave a pure substance (V), m.p. 145～150°, after recrystallization from MeOH. *Anal.* Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.05. Found : C, 76.58; H, 10.07. IR : ν_{max}^{Nujol} cm^{-1} 1715 (>C=O).

This sample showed no depression on admixture with a sample of des-A-25D-spirostan-5-one and infrared spectra of the two were completely identical.

Summary

Pentologenin and kitigenin, two steroidal sapogenins isolated from both the epigeous part and the rhizome of *Reineckia carnea* KUNTH, were found to have five and four hydroxyl groups, respectively, in ring-A. These sapogenins are the first example of naturally occurring spirostane-pentol and -tetrol with the exception of kogagenin.

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