Toshio Kawasaki and Itsuo Nishioka: Digitalis Saponins. I.*1 Seed Saponins of Digitalis purpurea L. (Commercial "Digitonin").

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The study on the seed saponins of Digitalis purpurea L., which were first isolated in 1875, and are available at a market as so-called "digitonin" has been carried out by many workers and "digitonin" has been considered to be a mixture of digitonin, gitonin, tigogenin glycoside and unknown minor saponins. However, a little had been known concerning the purification method and the structures of its component saponins until the recent works by Tschesche and his collaborators. They succeeded in isolation of tigogenin and two new saponins, digalamin and desglucodigitonin, besides the abovementioned two from the seed saponins or "digitonin" with the aid of a novel chromatographic method and established the detailed structure of digitonin.

A study along similar lines has been conducted in this laboratory and in the present communication we wish to describe a method for the fractionation of "digitonin" into the tetracygoside- and the pentaglycoside-groups and to report some physical constants of gitogenin and desglucodigitonin which were not presented by Tschesche, et al.*5

When four kinds of commercial "digitonin" were examined by an usual paper chromatography all of them showed the presence of two saponins (faster moving, I; slower moving, II), whereas the sapogenin fractions of their acid hydrolyzates contained digitogenin, gitogenin, tigogenin, and an unknown substance. The attempted separation of I and II by several methods (silica gel–celite chromatography, filter paper chromatopile method, etc.) was unsuccessful, but a rather simple method, chromatography on alumina using butanol saturated with water, was found to be satisfactory enough. Saponins (I) and (II) were obtained by this method in paper chromatographically homogeneous state and further purified by recrystallization from butanol saturated with water to afford needles, m.p. 245–246° (decomp.), [α]D° 67.8° (III) and needles, m.p. 249–250° (decomp.), [α]D° 47.6° (IV), respectively. On acid hydrolysis saponin (III) gave mainly gitogenin as the aglycone and d-glucose, d-galactose and d-xyllose in a ratio of 1:2:1.

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*2 Katakau, Fukuoka (川崎敏男, 西岡五夫).
*3 Examined by paper chromatography (P.C.). Unknown substance had a much larger Rf value than that of tigogenin, and digalogenin could not be found in the solvent system employed. Tschesche, et al., have reported that the seed saponins of Digitalis purpurea consist of glycosides of iso(25α)- and corresponding neo(25 β)- saponins. Since these two isomers are not distinguished by P.C. employed, respective sapogenin identified by P.C. in this work could be a mixture of iso- and neo-sapogenins.
*4 It has recently been found that pentaglycosides are precipitated by adding twice the volume of CHCl3 to the solution of "digitonin" in CHCl3–MOH (1:1) (T. Kawasaki, I. Nishioka: unpublished results).
1) O. Schmiedeberg: Arch. exp. Pathol. Pharmakol., 3, 16 (1875).
2) "The Merck Index of Chemicals and Drugs," 358 (1960), Merck and Co., Inc., Rahway, N.J.
while N provided digitogenin and d-glucose, 2-galactose and d-xylose (2:2:1). Therefore II and N were respectively regarded as gitonin\(^{38}\) and digitonin,\(^{39}\) and the sugar composition (3 galactose + xylose) of gitonin recorded in an earlier literature\(^{35}\) was amended.\(^{46}\) Since a small amount of tigogenin was found in the hydrolyzate of "digitonin" the corresponding glycoside\(^{40}\) (probably tigonin\(^{38}\)) was expected as a minor component, but it could not be detected (or differentiated from gitonin and digitonin) by conventional paper chromatography.

In 1961 Tschesche and Wulff\(^{40}\) reported the novel method to separate the very closely related digitalis saponins on filter paper and on cellulose column both impregnated with formamide. This paper chromatography (P.C.Fa.)\(^{48}\) was applied to the examination of saponins (I), (II), (III), and (IV), and it was found that I and II were composed of three saponins (I-1, I-2, I-3), II was a mixture of two (II-1, II-2) and IV was almost homogeneous. The preparative separation of the component saponins of I was then made according to the Tschesche's column chromatography, and they were isolated in pure state (examined by P.C.Fa.). Because of a very low yield I-1 was not further purified, but I-2 and I-3 were recrystallized from butanol saturated with water to give a crystalline solid, m.p. 238~241° (decomp.), (α\(_D\))\(^{20}\) −60.0° and fine needles, m.p. 243~245° (decomp.), (α\(_D\))\(^{25}\) −71.4° respectively. Acid hydrolyses of I-2 and I-3 followed by qualitative and quantitative determinations of the products indicated that the former is a gitogenin tetraglycoside and the latter a digitogenin tetraglycoside and that their sugar moieties are equally composed of one mole each of d-glucose and d-xylose and two moles of d-galactose. Accordingly I-2 is gitonin and I-3 corresponds to desglucositonin.\(^{49,50}\) Although insufficient material prevented a quantitative determination, I-1 was hydrolyzed to provide tigogenin, glucose, galactose and xylose, and taking its Rf value on paper chromatogram (P.C.Fa.) into consideration, it is assumed to be a tetraglycoside having the same sugar composition as those of I-2 and I-3 and to correspond to desglucotigonin.\(^{51}\) The fractionation of II into II-1 and II-2 in the same manner as for I was in failure, but since II was hydrolyzed to give digitogenin, digalogenin and a trace of gitogenin, II-2 is digitogenin and II-1 is presumed to be digalogenin.\(^{49,52}\) It is therefore a mixture of three tetraglycosides while II is composed of two pentaglycosides and the chromatography on alumina proves to provide a method to separate "digitonin" into two groups by the number of the component monosaccharides.

### Experimental

**Paper Chromatography (P. C.)**—Paper: Toyo Roshi No. 50. Ascending method. Triple development for sugars. Solvent: BuOH-AcOH-H\(_2\)O (4:1:5) (for saponins); xylene-MeOH-H\(_2\)O (10:1:5) (for sapogenins). (Rf values of reference compounds: tigogenin,\(^{48}\) 0.75; gitogenin,\(^{48}\) 0.35; Mixture of pyridine (1) and upper layer of BuOH-pyridine-H\(_2\)O (6:2:3) (for sugars) (relative Rf values (Rg) to that of glucose of reference compounds: galactose, 0.85; xylose, 1.23). Spray reagent: SbCl\(_5\) in CHCl\(_3\)+anisaldehyde in EtOH (for saponins and sapogenins); aniline hydroxide phthalate (for sugars). A relative amount of each substance detected on the chromatogram is represented as ++, +, ±, by visual comparison of the intensity and the area of the spots.

**Paper Chromatography of Saponins by the Tschesche’s Method**—(P. C. Fa.)—The filter paper was impregnated with formamide as follows: Toyo Roshi No. 50 was dried at 70~80° for 2 hr. and kept

\(^{35}\) Presented at the 20th Kyushu Branch Meeting of Pharmaceutical Society of Japan, Oct. 21, 1960, Fukuoka. Tschesche and Wulff\(^{46,47}\) also reported the same sugar composition.

\(^{36}\) Later thin-layer chromatography on silica gel G was proved to be an efficient method in the examination of these saponins; cf. T. Kawasaki, K. Miyahara: This Bulletin, 11, 1546 (1963).

\(^{37}\) Tigonin\(^{39}\) has the same sugar composition (2 glucose + 2 galactose + xylose) as that of digitonin. Tscheche and Wulff\(^{46,47}\) isolated a tigogenin glycoside from the seeds of *Digitalis purpurea* which is more polar than gitonin and regarded it as tigonin.

\(^{46}\) Tigogenin and gitogenin were kindly furnished by Shionogi Research Laboratory, Shionogi & Co., Ltd., to which the authors are grateful.
over silica gel. The filter paper was made moist with 15% formamide in Me₂CO, excess solution was removed by putting between filter papers and the impregnated paper was air-dried for 10 min. and left in a desiccator. Developed upwards with the solvent (A), bottom layer of CHCl₃-tetrahydrofuran–pyridine (10:10:2)/formamide (4), or (B), bottom layer of CHCl₃-dioxane–pyridine (10:10:3)/formamide (10). Spray reagent and the representation of a relative amount of each substance detected are as described above.

**Hydrolysis of Saponins and Qualitative and Quantitative Determinations of the Products**—A saponin was refluxed with $\frac{N}{4}$ HCl in dioxane–H₂O (1:3) (1 ml per 10 mg of sample) for 3 hr.$^{49}$ The products were examined by P.C. and the yields of aglycone and total sugar and the molar ratio of component monosaccharides were determined in the same manner as reported before.$^{6}$

**Examinations by P.C. of Commercial “Digitonin’s and their Hydrolyzates (Saponin)”**—Four kinds of “digitonin” (Merck, Daiichi,$^{43}$ Wako and Ishizu) were employed. In every case, two saponins (I, Rf 0.35; II, 0.27) and four saponins (Rf 0.27 +, 0.35 +, 0.75 ±, 0.90 ±) were detected. The saponin of Rf 0.27 is regarded as digitonigen but that of Rf 0.90 is unknown.

**Fractionation of “Digitonin” into Saponins (I) and (II)**—“Digitonin” (Merck) (1 g) in CHCl₃–MeOH (1:1) was placed on an alumina (Brockmann, 100 g) column, eluted successively with CHCl₃–MeOH (1:1) (Fractions 1–2), MeOH (Fr. 3), and BuOH saturated with H₂O (Fr. 4–50) and each fraction (25 ml) was checked by P.C.: A: Fr. 5–6 (I, 0.05 g), B: Fr. 7–8 (I +, II +, 0.14 g), C: Fr. 9–10 (I +, II +, 0.14 g), D: Fr. 11–12 (I ±, II +, 0.13 g), E: Fr. 13–50 (I ±, II +, 0.46 g). Respective fractions (A–E) obtained in two runs were combined and recrystallized in the same way. The corresponding fractions in each chromatography were combined, further passed through alumina columns and after all “digitonin” (2.09 g) was fractionated into five portions: A’ (I, 0.323 g), B’ (I +, II +, 0.110 g), C’ (I +, II +, 0.109 g), D’ (I ±, II +, 0.09 g), E’ (II, 1.060 g). Fr. A’ and E’ were regarded as homogeneous saponins (I) and (II), respectively.

**Saponin (III)**—Saponin (I) was recrystallized twice from BuOH saturated with H₂O to give saponin (II), needles, m.p. 245–246$^{\circ}$ (decomp.),$^{41}$ [α]D = 67.8$^{\circ}$ (c = 0.5, MeOH). Examination of the hydrolyzate by P.C.: aglycone, Rf 0.27 +, 0.35 +, 0.75 ±; sugar, Rg 0.85, 1.00, 1.23. Yields of the hydrolysis products: aglycone, 39%; total sugar, 62% (calcd. for gitonin$^{43}$): aglycone, 39.8%; total sugar, 63.6%). Molar ratio of glucose, galactose and xylose: 1.00:1.90:0.99.

**Saponin (IV)**—Saponin (II) was recrystallized repeatedly from BuOH saturated with H₂O to give saponin (III), needles, m.p. 249–250$^{\circ}$ (decomp.),$^{42}$ [α]D = 47.6$^{\circ}$ (c = 1.2, MeOH). Anal. Calc. for C₃₀H₄O₁₃: H₂O (digitonin monohydrate): C, 55.9; H, 7.6. Found: C, 55.8; H, 7.8. Examination of the hydrolyzate by P.C.: aglycone, Rf 0.27; sugar, Rg 0.85, 1.00, 1.23. Yields of the hydrolysis products: aglycone, 34%; total sugar, 70% (calcd. for digitonin$^{43}$): aglycone, 36.0%; total sugar, 69.9%). Molar ratio of glucose, galactose and xylose: 2.00:2.03:1.00. The aglycone was recrystallized from MeOH to give needles, m.p. 286–288$^{\circ}$, [α]D = 79.8$^{\circ}$ (c = 0.84, CHCl₃) (digitogenin$^{43}$), m.p. 288–293$^{\circ}$, [α]D = 81$^{\circ}$ (CHCl₃). Anal. Calc. for C₃₀H₄O₁₃: digitogenin: C, 72.28; H, 9.89. Found: C, 72.04; H, 9.98.

**Examination of Saponins (I), (II), (III) and (IV) by P.C. Fa.**—With solvent (A) (in parentheses, with solvent (B)): I Rf 0.20 (0.50 ±, 0.17 (0.40 +, 0.09 (0.30 +), II 0.03 (0.23 ±, 0.16; III 0.20 (0.50 ±, 0.17 (0.40 +, 0.09 (0.30 +), IV 0.02 (0.25 ±, 0.15 +), Saponins of Rf 0.20 (0.50), 0.17 (0.40), 0.09 (0.30), 0.25 (0.15) were designated as I–1, 2, 2, 3, and II–1, 2, 2, 3, 2, respectively.

**Fractionation of Saponin (I)$^{44}$—Toyo Roshi cellulose powder (10 g) (dried at 80$^{\circ}$ for 2 hr. and kept in a desiccator) was made moist with 15% formamide in Me₂CO (10 ml), air dried, and left over silica gel in vacuo. Saponin (I) (150 mg) in CHCl₃–MeOH (1:1) was mixed with untreated cellulose powder (2.5 g), dried, placed on a column (25 mm × 200 mm) of the above impregnated cellulose powder (50 g) and eluted with CHCl₃-tetrahydrofuran–pyridine (10:10:2)/formamide (4) (Fr. 1–80) and then with BuOH saturated with H₂O (Fr. 81–100). Each fraction (2 ml) was evaporated in vacuo, extracted with CHCl₃–MeOH and checked by P.C.Fa.: Fr. 28–31, 1–1, 5 mg.; Fr. 32–35, 1–1, 2, 20 mg.; Fr. 36–70, 1–2, 30 mg.; Fr. 71–80, 1–2, 3, 5 mg.; Fr. 81–104, 1–3, 30 mg.

**Saponin (I)**—Fr. 28–31 was hydrolyzed and examined by P.C.: aglycone, Rf 0.75; sugar, Rg 0.85, 1.00, 1.23.

**Saponin (II)**—Fr. 36–70 was recrystallized twice from BuOH saturated with H₂O to give a crystalline solid, m.p. 238–241$^{\circ}$ (decomp.), [α]D = 60.0$^{\circ}$ (c = 0.20, pyridine). Anal. Calc. for C₃₀H₄O₁₃·3H₂O (gitonin trihydrate): C, 54.3; H, 8.0. Found: C, 54.1; H, 8.1. Examination of the hydrolyzate by P.C.: aglycone, Rf 0.35; sugar, Rg 0.85, 1.00, 1.23. Yields of the hydrolysis products: aglycone, 39%;

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49 Acid hydrolyses of “digitonin” under various conditions were investigated and this condition was found to be most favourable for complete hydrolysis and also for satisfactory sugar recovery; cf. T. Tsukamoto, T. Kawakami, T. Yamauchi: This Bulletin, 4, 35 (1956).

41 “Digitonin” (Daiichi) was kindly donated by Central Research Laboratory, Daiichi Seiyaku Co., Ltd., to which the authors are grateful.

41 All melting points were taken on a Kofler block and are uncorrected.

total sugar, 64% (calcld. for gitonin trihydrate: aglycone, 39.2%; total sugar, 62.6%). Molar ratio of glucose, galactose and xylose: 1.3:2.1:1.0. The aglycone was recrystallized from MeOH to give needles, m.p. 274–276°. Mixed melting point with gitogenin (m.p. 274–278°) showed no depression.

**Saponin (I–3)**—Fr. 81–104 was recrystallized from BuOH saturated with H₂O to provide fine needles, m.p. 243–245° (decomp.), [α]_D25 −71.4° (c=0.28, pyridine). Anal. Calcld. for C₂₅H₃₀O₁₇·2H₂O (desglucodigitonin dihydrate): C, 54.4; H, 7.9. Found: C, 54.7; H, 8.1. Examination of the hydrolyzate by P.C. yielded Rf 0.27; sugar, Rg 0.85, 1.00, 1.23. Yields of the hydrolysis products: aglycone, 40%; total sugar, 65% (c算 to desglucodigitin dihydrate: aglycone, 40%; total sugar, 62.6%). Molar ratio of glucose, galactose and xylose: 1.3:2.1:1.0. The aglycone was recrystallized from MeOH to give needles, m.p. 288–290° (digitogenin, m.p. 288–291°).

**Saponin (II)**—Hydrolyzed and the aglycone was examined by thin-layer chromatography on silica gel G using AcOEt–hexane (2:1) as solvent: Rf 0.31 ±, 0.38 ±, 0.63 ± (Rf of reference compound: digitogenin, 0.31; gitogenin, 0.38; tigogenin, 0.78). The sapogenin of Rf 0.63 was regarded as digitogenin. That of Rf 0.38 was detected only in trace but the presence of gitogenin pentaglycoside in saponin (II) seems to be suggested.

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**Summary**

Two saponins (I) and (II) which are detected by conventional paper chromatography of the seed saponins of *Digitalis purpurea* L. (commercial “digitonin”) were isolated by column chromatography on alumina and it was found that I is composed of three tetraglycosides, desglucotigogenin, gitonin (m.p. 238–241° (decomp.), [α]_D25 –60.0°) and desglucodigitonin (m.p. 243–245° (decomp.), [α]_D25 –71.4°), while II is a mixture of two pentaglycosides, digalagonin and digitonin.

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**Noshio Nambara**: Chemistry of 3β,12-Dihydroxy-5α-androstan-17-ones

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In the extension of the previous works on the stereochemistry of C/D*-cis* steroids, the preparation of 3β-hydroxy-5α-androst-14-en-17-one possessing oxygen substituent at C-12 has become an essential prerequisite.

For this purpose the initial attempt was directed towards the Johnson’s method employed for the synthesis of 14-isoestrone. The starting material, 3β,12β-dihydroxy-5α-androstan-17-one (IIa), was readily prepared by way of different route from that reported by Adams, *et al.* Conversion of 3β,12β-dihydroxy-5α-pregn-16-en-20-one 3-acetate (Ia) into its oxime (Ib), m.p. 267–270°, followed by Beckmann rearrangement

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**Notes**