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

(*Faculty of Pharmaceutical Sciences, Kyushu University*[*2])

The study on the seed saponins of *Digitalis purpurea* L. which were first isolated in 1875[1] and are available at a market as so-called "digitonin"[2] has been carried out by many workers[3] and "digitonin" has been considered to be a mixture of digitonin, gitonin, tigogenin glycoside and unknown minor saponins.[2b,3] 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.[4] They succeeded in isolation of tigonin[3a] and two new saponins, digalonin and desglucodigitonin, besides the above-mentioned 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 tetraglycoside- and the pentaglycoside-groups and to report some physical constants of gitonin and desglucodigitonin which were not presented by Tschesche, *et al.*[4]

When four kinds of commercial "digitonin" were examined by an usual paper chromatography all of them showed the presence of two saponins[3b,4a] (faster moving, I; slower moving, Ⅱ), whereas the sapogenin fractions of their acid hydrolyzates contained digitogenin, gitogenin, tigogenin, and an unknown substance.[*3] The attempted separation of I and Ⅱ by several methods (silica gel-celite chromatography, filter paper chromatopile method, etc.[*4]) was unsuccessful, but a rather simple method, chromatography on alumina using butanol saturated with water,[5] 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.), $[\alpha]_D^{23}$ −67.8° (Ⅲ) and needles, m.p. 249~250° (decomp.), $[\alpha]_D^{13}$ −47.6° (Ⅳ), respectively. On acid hydrolysis saponin (Ⅲ) gave mainly gitogenin as the aglycone and D-glucose, D-galactose and D-xylose in a ratio of 1:2:1,

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[*1] Presented at the Annual Meeting of Pharmaceutical Society of Japan, Nov. 2, 1962, Shizuoka.

[*2] Katakasu, 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[4a] could not be found in the solvent system employed. Tschesche, *et al.*[4b] have reported that the seed saponins of *Digitalis purpurea* consist of glycosides of iso(25α)- and corresponding neo(25β)- sapogenins. 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 $CHCl_3$ to the soloution of "digitonin" in $CHCl_3$-MOH (1:1) (T. Kawasaki, I. Nishioka : unpublished results).

1) O. Schmiedeberg : Arch. exp. Pathol. Pharmakol., 3, 16 (1875).

2) *a*) "The Merck Index of Chemicals and Drugs," 358 (1960), Merck and Co., Inc., Rahway, N.J. *b*) A. Windaus : Hoppe-Seyler's Z. physiol. Chem., **150**, 205 (1925).

3) *a*) W. Karrer : "Konstitution und Vorkommen der organischen Pflanzenstoffe," 863 (1958), Birkhaüser Verlag, Basel; L.F. Fieser, M. Fieser : "Steroids," 810 (1959). Reinhold Publishing Corporation, New York, N.Y. *b*) K. Miyatake, A. Okano : Yakugaku Zasshi, **75**, 25 (1955); G. Ruhenstroth-Bauer, P.M. Breitenfeld : Hoppe-Seyler's Z. physiol. Chem., **302**, 111 (1955). *c*) F. Weiss, O. Manns : Pharm. Zentralhalle, **98**, 437 (1959).

4) *a*) R. Tschesche, G. Wulff : Chem. Ber., **94**, 2019 (1961). *b*) R. Tschesche, G. Wulff, G. Balle : Tetrahedron, **18**, 959 (1962). *c*) R. Tschesche, G. Wulff : *Ibid.*, **19**, 621 (1963).

5) T. Kawasaki, T. Yamauchi, N. Itakura : Yakugaku Zasshi, **83**, 892 (1963); T. Kawasaki, T. Yamauchi, R. Yamauchi : This Bulletin, **10**, 698 (1962).

while Ⅳ provided digitogenin and D-glucose, D-galactose and D-xylose (2:2:1). Therefore Ⅱ and Ⅳ were respectively regarded as gitonin[3a)] and digitonin,[3a)] and the sugar composition (3 galactose+xylose) of gitonin recorded in an earlier literature[3a)] was amended.[*5] Since a small amount of tigogenin was found in the hydrolyzate of "digitonin" the corresponding glycoside[3c)] (probably tigonin[3a)]) 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[4a)] 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.)[*6] was applied to the examinations of saponins (Ⅰ), (Ⅱ), (Ⅲ), and (Ⅳ), and it was found that Ⅰ and Ⅲ 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.), $[\alpha]_D^{20}$ −60.0° and fine needles, m.p. 243~245° (decomp.), $[\alpha]_D^{20}$ −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 desglucodigitonin.[4a,c)] 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.[*7] 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 digitonin and Ⅱ-1 is presumed to be digalonin.[4a,c)] Ⅰ 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_2O (4:1:5) (for saponins); xylene–MeOH–H_2O (10:1:5) (for sapogenins) (Rf values of reference compounds: tigogenin,[*8] 0.75; gitogenin,[*8] 0.35); Mixture of pyridine (1) and upper layer of BuOH–pyridine–H_2O (6:2:3) (for sugars) (relative Rf values (Rg) to that of glucose of reference compounds: galactose, 0.85; xylose, 1.23). Spray reagent: $SbCl_3$ in $CHCl_3$+anisaldehyde in EtOH (for saponins and sapogenins); aniline hydrogen 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[4a)] (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

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[*5] Presented at the 20th Kyushu Branch Meeting of Pharmaceutical Society of Japan, Oct. 21, 1960, Fukuoka. Tschesche and Wulff[4a,c)] also reported the same sugar composition.

[*6] 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).

[*7] Tigonin[3a)] has the same sugar composition (2 glucose + 2 galactose + xylose) as that of digitonin. Tscheche and Wulff[4a,c)] isolated a tigogenin glycoside from the seeds of *Digitalis purpurea* which is more polar than gitonin and regarded it as tigonin.

[*8] 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 Me_2CO, 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 $CHCl_3$-tetrahydrofurane-pyridine (10:10:2)/formamide (4), or (B), bottom layer of $CHCl_3$-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 *N* HCl in dioxane-H_2O (1:3) (1 ml. per 10 mg. of sample) for 3 hr.[*9] 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 (**Sapogenins**)——Four kinds of "digitonin" (Merck, Daiichi,[*10] Wako and Ishizu) were employed. In every cases, two saponins (I, Rf 0.35; II, 0.27) and four sapogenins (Rf 0.27+, 0.35+, 0.75±, 0.90±) were detected. The sapogenin of Rf 0.27 is regarded as digitogenin but that of Rf 0.90 is unknown.

Fractionation of "Digitonin" into Saponins (I) and (II)——"Digitonin" (Merck) (1 g.) in $CHCl_3$-MeOH (1:1) was placed on an alumina (Brockmann, 100 g.) column, eluted successively with $CHCl_3$-MeOH (1:1) (Fractions 1∼2), MeOH (Fr. 3) and BuOH saturated with H_2O (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 rechromatographed 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 H_2O to give saponin (III), needles, m.p. 245∼246°(decomp.),[*11] $[\alpha]_D^{23}$ $-67.8°$(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[3a)]: 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 H_2O to give saponin (IV), needles, m.p. 249∼250° (decomp.), $[\alpha]_D^{13}$ $-47.6°$(c=1.2, MeOH). *Anal.* Calcd. for $C_{56}H_{92}O_{29}\cdot H_2O$ (digitonin monohydrate): C, 53.9; H, 7.6. Found: C, 53.8; 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[3a)]: aglycone, 36.0%; total sugar, 69.9%). Molar ratio of glucose, galactose and xylose: 2.00:2.03:1.00. The aglycone was recystallized from MeOH to give needles, m.p. 286∼288°, $[\alpha]_D^{14}$ $-79.8°$(c=0.84, $CHCl_3$) (digitogenin,[3a)] m.p. 288∼291°, $[\alpha]_D^{19}$ $-81°$($CHCl_3$)). *Anal.* Calcd. for $C_{27}H_{44}O_5$ (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) and (0.15) were designated as I-1, I-2, I-3, and II-1, and II-2, respectively.

Fractionation of Saponin (I)[4a)]——Toyo Roshi cellulose powder (10 g.) (dried at 80° for 2 hr. and kept in a desiccator) was made moist with 15% formamide in Me_2CO (10 ml.), air dried, and left over silica gel *in vacuo*. Saponin (I) (150 mg.) in $CHCl_3$-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 $CHCl_3$-tetrahydrofurane-pyridine (10:10:2)/formamide (4) (Fr. 1∼80) and then with BuOH saturated with H_2O (Fr. 81∼100). Each fraction (2 ml.) was evaporated *in vacuo*, extracted with $CHCl_3$-MeOH and checked by P.C.Fa.: Fr. 28∼31, I-1, 5 mg.; Fr. 32∼35, I-1, I-2, 20 mg.; Fr. 36∼70, I-2, 30 mg.; Fr. 71∼80, I-2, I-3, 5 mg.; Fr. 81∼104, I-3, 30 mg.

Saponin (I-1)——Fr. 28∼31 was hydrolyzed and examined by P.C.: aglycone, Rf 0.75; sugar, Rg 0.85, 1.00, 1.23.

Saponin (I-2)——Fr. 36∼70 was recrystallized twice from BuOH saturated with H_2O to give a crystalline solid, m.p. 238∼241°(decomp.), $[\alpha]_D^{20}$ $-60.0°$(c=0.20, pyridine). *Anal.* Calcd. for $C_{50}H_{82}O_{23}\cdot 3H_2O$ (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%;

[*9] 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. Kawasaki, T. Yamauchi: This Bulletin, 4, 35 (1956).

[*10] "Digitonin" (Daiichi) was kindly donated by Central Research Laboratory, Daiichi Seiyaku Co., Ltd., to which the authors are grateful.

[*11] All melting points were taken on a Kofler block and are uncorrected.

6) T. Kawasaki, T. Yamauchi: This Bulletin, **11**, 1221 (1963).

total sugar, 64% (calcd. 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°)[*8] showed no depression.

Saponin (I-3)——Fr. 81~104 was recrystallized from BuOH saturated with H_2O to provide fine needles, m.p. 243~245° (decomp.), $[\alpha]_{D}^{20}$ −71.4° (c=0.28, pyridine). *Anal.* Calcd. for $C_{50}H_{82}O_{24}\cdot 2H_2O$ (desglucodigitonin dihydrate) : C, 54.4; H, 7.9. Found : C, 54.7; H, 8.1. 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, 40%; total sugar, 65% (calcd. for desglucodigitonin dihydrate : aglycone, 40.6%; 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,[3a] 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 digalogenin.[4a] 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, desglucotigonin, gitonin (m.p. 238~241° (decomp.), $[\alpha]_{D}^{20}$ −60.0°) and desglucodigitonin (m.p. 243~245° (decomp.), $[\alpha]_{D}^{20}$ −71.4°), while II is a mixture of two pentaglycosides, digalonin and digitonin.

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

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

In the extension of the previous works on the stereochemistry of C/D-*cis* steroids,[1~4] 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[5] 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.*[6] 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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[*1] Motofuji-cho, Bunkyo-ku, Tokyo (南原利夫).

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