U.D.C. 547.918.05 : 615.711.5

**55. Daisuke Satoh, Hiroshi Ishii, Yohko Oyama, Takayuki Wada,
and Tamotsu Okumura : Studies on Digitalis Glycosides. VI.***
Isolation of Odoroside H, Digiproside, and Digitalonin.

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

Within the last few years, glycosidal constituents of *Digitalis purpurea* L., especially those which are soluble in water, have been the subject of investigations by several groups of workers. Thus, Ishidate, Okada, and Sasakawa¹⁾ isolated digitalinum verum and gitorin, and we²⁾ obtained simultaneously "Crystal A"*** and strosposide. Subsequently, Tschesche and Grimmer³⁾ reported the isolation of strosposide, digipurpurin, and digifolein, and Haack, Kaiser, and Spingler^{4,5)} published two papers concerning the isolation of gitaloxin and odoroside H.

In three recent communications,^{6,7,8)} we reported briefly that we were able to isolate a hitherto unknown cardiotonic glycoside, digiproside, and two non-cardiotonic glycosides, purpnin and digipronin, together with the known odoroside H. The present paper gives details on digiproside, odoroside H, and a new non-cardiotonic glycoside, digitalonin, while the remaining purpnin and digipronin will be the subject of discussion in a forthcoming paper.

The water-soluble fraction, obtained by the method described in the previous

Fig. 1. Paper Chromatograms

I : Water-soluble fraction
II, III : Known glycosides
IV : Newly isolated glycosides

	Rf
a : Digitoxin	0.92
b : Diginin	0.87
c : Gitoxin	0.56
d : Oxydiginin	0.37
e : Strosposide	0.22
f : Purpurea glycoside A	0.13
g : Purpurea glycoside B	0.03
h : Digitalinum verum	0.01
F : Crystal F (Odoroside H)	0.46
I : Crystal I (Digiproside)	0.27

* Part V : D. Satoh, H. Ishii, Y. Oyama : Ann. Rept. Shionogi Research Lab., **5**, 113(1955).

** Imafuku, Amagasaki, Hyogo-ken (佐藤大助, 石井 宏, 尾山蓉子, 和田敬之, 奥村 保).

*** This was designated digifolein by Tschesche *et al.* at a later date.

1) M. Ishidate, M. Okada, Y. Sasakawa : This Bulletin, **1**, 186(1953).

2) D. Satoh, K. Yoshida, H. Ishii, Y. Nishimura : *Ibid.*, **1**, 305, 396(1953).

3) R. Tschesche, G. Grimmer : Chem. Ber., **88**, 1569(1955).

4) E. Haack, F. Kaiser, H. Spingler : Naturwissenschaften, **42**, 441(1955).

5) E. Haack, F. Kaiser, H. Spingler : *Ibid.*, **42**, 442(1955).

6) D. Satoh, H. Ishii, Y. Oyama : J. Pharm. Soc. Japan, **75**, 1173(1955).

7) D. Satoh, H. Ishii, Y. Oyama, T. Okumura : *Ibid.*, **75**, 1573(1955).

8) D. Satoh, H. Ishii, Y. Oyama : *Ibid.*, **75**, 1025(1955).

paper,²⁾ was submitted to paper chromatography utilizing the modified method of Reichstein⁹⁾ and from the pattern of the paper chromatogram (Fig. 1), it was found that this fraction contains several unknown glycosides. The main fraction was therefore separated into several portions by means of chromatography on alumina and each portion was again examined by paper chromatography, when it was found that fractions Nos. 10~11 and Nos. 20~25 contained a number of substances (Fig. 2). On

Fig. 2. Paper Chromatograms of the Fractions from the Primary Chromatography

submission of these portions separately to a second alumina chromatography and subsequent treatment of each fraction with ethyl acetate, the crude crystals A, A', B, C, D, F, G, H, I, K, and L, and amorphous J were isolated. Of these products, crystals B (strosipeside), F (odoroside H), I (digiproside), and K (gitoxin) were found to be cardiotonic, while crystals A (digifolein), A' (diginin), C (digipronin), D (purpnin), G (unknown), H (digitalonin), J (unknown), and L (unknown) non-cardiotonic. The results on crystals A (digifolein) and B (strosipeside) have been reported previously.^{2,8)} In the following section three crystalline glycosides, F, I, and H will be described in detail.

Crystal F forms colorless prisms, m.p. 232~235°; yield, approx. 0.0005% based on dry weight of the leaves. This substance is easily soluble in methanol, ethanol, and chloroform and sparingly soluble in ether and benzene. U. V. λ_{max}^{EtOH} 218 m μ ($\log \epsilon$ 4.17). $[\alpha]_D^{25} +15^\circ$ (c=1.003, MeOH). Toxicity by the pigeon method, 0.345 mg./kg. The Legal reaction is strongly positive and the Keller-Kiliani reaction shows a colorless acetic acid layer and brown contact zone with conc. sulfuric acid. The Rf value on paper chromatogram as described in the Experimental section was 0.46 and differed from that of the known cardiotonic glycosides (Fig. 1, IV). Analytical values correspond to $C_{30}H_{46}O_8 \cdot H_2O$, possessing one methoxyl but no acetyl group.

The acetate forms colorless needles, m.p. 247~251°, whose analytical values correspond to those of a diacetate, $C_{34}H_{50}O_{10}$.

Hydrolysis by the Mannich method gave an aglycone as colorless crystals, m.p. 244~249°, which did not show any depression on admixture with digitoxigenin, m.p. 245~250°. The syrupy sugar obtained by the hydrolysis with 3.4% hydrochloric acid

9) O. Schindler, T. Reichstein: *Helv. Chim. Acta*, **34**, 108(1951).

(50% ethanolic) gave a negative Keller-Kiliani reaction and the R_f value of this sugar was identical with that of *d*-digitalose. On the basis of the content of the methoxyl group and the molecular weight of the original glycoside, it is clear that the glycoside contains only one *d*-digitalose.

These results indicate that the Crystal F is digitoxigenin-mono-*d*-digitaloside (I) and probably identical with odorside H, obtained from the barks of *Nerium odorum* SOL. by Reichstein and others.¹⁰⁾ Crystal F and its acetate were therefore compared directly with authentic samples, kindly furnished by Prof. T. Reichstein, and following results were obtained.

	Crystal F	Odorside H	Mixture
m.p. °C	232~235	228~231	229~232
Acetate m.p. °C	247~251	248~252	248~251
R _f (benzene-EtOAc-H ₂ O=10:3:5)	0.78	0.77	0.78
Acetate R _f (benzene-CHCl ₃ -H ₂ O=9:1:2)	0.88	0.87	0.88

Fig. 3. Infrared Absorption Spectra (in CHCl₃)

— Crystal F - - - - - Odorside H — Background+CHCl₃

The infrared spectrum of both also agreed well, as shown in Fig. 3.

The foregoing results have established the identity of Crystal F and odorside H. It is interesting to note that both the digitoxigenin- and gitoxigenin-glycosides (odorside H and strosipeside), containing *d*-digitalose, were obtained from the water-soluble fraction of the leaves of *Digitalis purpurea*.

Crystal I forms colorless needles, m.p. 147~152° and 193~194° (double melting point from methanol), or colorless plates, m.p. 194~196° (from acetone-ether). Yield, approx. 0.0015% based on dry weight of the leaves. This glycoside is easily soluble in methanol, ethanol, and chloroform, sparingly soluble in ether and benzene. U. V. λ_{max}^{EtOH} 217 m μ ($\log \epsilon$ 4.17) (Fig. 4, a). $[\alpha]_D^{21}$ 0° ($c=2.417$, MeOH). Toxicity by the pigeon method, 0.331 mg./kg. The Legal reaction is strongly positive and the Keller-Kiliani reaction shows

Fig. 4.

Ultraviolet Absorption Spectra

a Crystal I
b Crystal H

10) A. Rheiner, A. Hunger, T. Reichstein: *Helv. Chim. Acta*, **35**, 687(1952).

a colorless acetic acid layer and brown contact zone with conc. sulfuric acid. The R_f value on paper chromatogram differed from those of the known cardiotonic glycosides (Fig. 1, IV). Analysis gave values which agreed well with the formula $C_{29}H_{44}O_8 \cdot \frac{1}{2}H_2O$ and has no methoxyl group.

The acetate forms colorless needles, m.p. $242\sim 245^\circ$, whose analytical values correspond to those of a triacetate, $C_{35}H_{50}O_{11}$.

Hydrolysis by the Mannich method gave an aglycone as colorless crystals, m.p. $245\sim 250^\circ$, whose analytical values corresponded to $C_{23}H_{34}O_4$. Mixed fusion of this aglycone and its acetate, m.p. $217\sim 221^\circ$, respectively with digitoxigenin, m.p. $245\sim 250^\circ$, and its acetate, m.p. $218\sim 221^\circ$, showed no depression of the melting point. The R_f value of this aglycone agreed with that of digitoxigenin.

Since the sugar moiety of Crystal I has no methoxyl group and the Keller-Kiliani reaction is negative, this sugar is supposed to be a methylpentose. The R_f value of this sugar, on comparison with those of *l*-rhamnose, *d*-allomethylose, *d*-gulomethylose, *d*-tallomethylose, *l*-fucose, and *d*-fucose,¹¹⁾ was found to be identical with those of *l*-fucose and *d*-fucose. $[\alpha]_D^{25} +78.4^\circ$ ($c=0.5898$, H_2O). The phenylosazone of this sugar, m.p. $170\sim 175^\circ$, did not show any depression of the melting point on admixture with the osazone of *d*-fucose, m.p. $170\sim 174^\circ$. Therefore, the sugar moiety of Crystal I was shown to be *d*-fucose.

The number of molecules of *d*-fucose contained in the glycoside was calculated to be one on the basis of the molecular weight determined by the absorbancy of the ultraviolet absorption spectrum. Since the ultraviolet absorption band at $217\sim 218 m\mu$ of the cardiotonic glycoside is due to the butenolide ring system in the aglycone, the values of $\log \epsilon$ of the glycoside and the aglycone are practically identical, that of digitoxigenin being 4.146 and digitoxin, 4.150. From the standard curve of digitoxigenin, the molecular weights of Crystal F and I were calculated as 543.7 and 528.3, which agreed closely with the theoretical values of 552.7 and 529.7, respectively (Fig. 5).

Fig. 5. Standard Curve of Absorbancy

Substance	Absorbancy	Mol. concn.
Digitoxin	E_{Dig}	C_{Dig}
Crystal F	E_F	C_F
Crystal I	E_I	C_I

These results have shown that Crystal I is digitoxigenin mono-*d*-fucose (II) and since a glycoside possessing this constitution has never been described in the literature, this glycoside was designated as digiproside by the present authors.

Crystal K forms a colorless crystalline powder, m.p. $268\sim 270^\circ$. The Legal reaction is strongly positive and the Keller-Kiliani reaction shows a bluish green acetic acid layer and a red contact zone with conc. sulfuric acid. Comparison of this glycoside

11) The samples were kindly given by Prof. T. Reichstein and Prof. R. Tschesche.

with gitoxin by mixed fusion and paper chromatography showed the two substances to be identical.

It is noteworthy that a pale yellowish brown amorphous substance, m.p. 210~216°, was obtained from the mother liquor of gitoxin and it gave a similar coloration in the Legal and the Keller-Kiliani reactions. Due to the lower melting point and higher Rf value, this substance seemed different from gitoxin, but a detailed examination is still to be performed.

Crystal H forms colorless rhomboprisms, m.p. 208~213°. Yield, approx. 0.0002% based on dry weight of the leaves. This substance is easily soluble in methanol, ethanol, and chloroform, sparingly soluble in benzene and ether. U. V. $\lambda_{\text{max}}^{\text{EtOH}}$ 310 m μ ($\log \epsilon$ 1.97) (Fig. 4, b). $[\alpha]_D^{25}$ -167.1° ($c=0.7358$, MeOH). The Legal reaction is slightly positive and the Keller-Kiliani reaction shows a colorless acetic acid layer and brown contact zone with conc. sulfuric acid. Analytical values correspond to $\text{C}_{28}\text{H}_{40}\text{O}_8$, possessing one methoxyl group. The sugar moiety obtained by hydrolysis with 3.4% hydrochloric acid (50% ethanolic) was shown by the aid of paper chromatography to be *d*-digitalose. Although the structure of the aglycone is now being investigated, we propose to name this obviously hitherto unknown glycoside, digitalonin.

The authors wish to express their appreciation to Prof. Dr. T. Reichstein, Prof. Dr. R. Tschesche, Dr. E. Haack, and Dr. J. E. Murphy for the gift of valuable samples. The authors are grateful to Dr. K. Takeda, Director of this Laboratory, for his valuable advise, and further they would like to thank Messrs. Miyahara, Ieki, Morita, Koyama, and Matsui for the microanalysis and the infrared spectra, to the members of the Pharmacological Section of this Laboratory for the animal tests, and to the members of the Akoh Plant of this firm for the Digitalis materials.

Experimental

1) **Paper Chromatography of the Water-soluble Fraction**—The water-soluble fraction obtained from the dried leaves of *Digitalis purpurea* L. by the method described in the previous paper²⁾ was submitted to paper chromatography utilizing the modified method of Reichstein, as follows:

Stationary phase: A filter paper (Toyo Roshi No. 51) was set in a chromatographic chamber and the paper was allowed to absorb a mixture of formamide and acetone (1 : 4) by the ascending method. After 15 hrs., the paper was exposed to air and acetone was evaporated immediately. A practically constant formamide content was observed within 1 hr.

Mobile phase: A mixture of toluene and BuOH (3 : 1), saturated with formamide.

Development of chromatogram: Ascending method for 3 hrs. at 20~22°.

Detection of spots: The paper was sprayed with a solution of $\text{CCl}_3\text{-COOH}$ in CHCl_3 (1 : 4) and next with a 3% chloramine T solution in water, followed by heating for 7 mins. at 85~90°, and the spots were detected under an ultraviolet lamp.

2) **Primary Chromatography of the Water-soluble Fraction**—20 g. of the water-soluble fraction was dissolved in about 500 cc. CHCl_3 , poured through an adsorption column filled with neutral

alumina (300 g.), and the column was developed with chloroform. It was eluted with CHCl_3 -MeOH mixtures, finally with 80% MeOH, and fractionated according to the Keller-Kiliani (K-K) reaction. Each fraction was dissolved in hot AcOEt and the crude crystals that separated out on cooling were collected by suctional filtration.

TABLE I.

Fr. No.	Solvent	K-K reaction	Legal react.	Crude crystals	
				m.p. (°C)	Design.
(1~2)	CHCl_3	colorless~brown	—	—	—
(3~5)	"	" "	—	135~138	(G)
(6~9)	"	" "	±	150~165	(A')
(10~11)	CHCl_3 : MeOH = 60 : 1	bluish green~reddish brown	+	second. chromat.	-1
(12~13)	"	pale blue~pale red	+	215~224	(B + C)
(14~17)	"	colorless~red	+	233~241	(B)
(18~19)	"	" ~pale red	±	227~237	(B)
(20~23)	CHCl_3 : MeOH = 1 : 1	bluish green~reddish brown	+	second. chromat.	-2
(24~25)	"	pale blue~pale red	±	"	"
(26~28)	80% MeOH	bluish green~reddish brown	±	amorphous	(J)
(29~30)	"	pale blue~pale brown	—	"	(J)

Each fraction was submitted to paper chromatography by the above-mentioned method. The results were as described in the main text (Fig. 2).

3) **Secondary Chromatography (1)**—2.29 g. of the fraction Nos. 10~11 in the primary chromatography was submitted to alumina chromatography by the method described in 2).

TABLE II.

Fr. No.	Solvent	K-K reaction	Legal react.	Crude crystals	
				m.p. (°C)	Design.
(1~7)	CHCl_3	bluish green~brown	±	160~172	(A')
(8~15)	"	" "	±	175~187	(A)
(16~17)	"	" "	±	190~198	(A)
(18)	CHCl_3 : MeOH = 100 : 1	" ~reddish brown	±	syrup	
(19~20)	"	colorless~brown	+	187~208	(F + H)
(21~30)	"	" "	+	210~214	(F)
(31~37)	CHCl_3 : MeOH = 60 : 1	" ~reddish brown	±	224~234	(C)
(38~43)	"	" "	—	250~265	(L)
(44~45)	80% MeOH	bluish green~ "	±	amorphous	(J)
(46~50)	"	" ~pale brown	±	"	(J)

Secondary Chromatography (2)—15.9 g. of the fraction Nos. 20~25, collected from four experiments of primary chromatography, was submitted to alumina chromatography by the above-mentioned method.

TABLE III.

Fr. No.	Solvent	K-K reaction	Legal react.	Crude crystals	
				m.p. (°C)	Design.
(1~7)	CHCl_3	colorless~colorless	—	syrup	
(8~24)	CHCl_3 : MeOH = 100 : 1	bluish green~brown	±	247~251	(D)
(25~31)	"	" "	±	234~245	(D)
(32~40)	CHCl_3 : MeOH = 50 : 1	" "	±	246~256	(D)
(41~47)	"	" "	±	236~245	(D)
(48~50)	CHCl_3 : MeOH = 10 : 1	bluish green~red	+	205~230	(K)
(51~56)	CHCl_3 : MeOH = 1 : 1	colorless~brown	+	140~145	(I)
(57~62)	"	" "	+	141~146	(I)
(63~70)	80% MeOH	bluish green~reddish brown	±	121~128	(J)

4) **Isolation of Crystals F and H**—The crude crystals obtained from the fraction Nos. 19~30 of secondary chromatography-(1) was separated into 2 portions by solubility in AcOEt and each was recrystallized from the same solvent.

1) Colorless prisms, m.p. 232~235°, obtained from the easily soluble portion were designated as Crystal F, properties of which are given in the main text. *Anal.* Calcd. for $\text{C}_{30}\text{H}_{46}\text{O}_8 \cdot \text{H}_2\text{O}$: C, 65.17; H, 8.76; OCH_3 , 5.62. Found : C, 65.08; H, 8.80; OCH_3 , 5.76.

2) Colorless rhomboprisms, m.p. 208~213°, obtained from the sparingly soluble portion were

designated as Crystal H. The properties of this substance are given in the main text. *Anal.* Calcd. for $C_{28}H_{40}O_8$: C, 66.64; H, 7.99; OCH_3 , 6.15. Found: C, 66.63; H, 8.10; OCH_3 , 6.00.

5) **Crystal F (Odoroside H)**—i) Acetylation: A mixture of 30 mg. of Crystal F in 0.5 cc. of dehyd. pyridine and 0.5 cc. Ac_2O was allowed to stand for 50 hrs. at room temp. After warming the mixture for 1 hr. at $75\sim 80^\circ$, pyridine and Ac_2O were removed by distillation under a reduced pressure. Addition of water to the residue with stirring yielded 30 mg. of a crude acetate. This crude acetate was recrystallized from a mixture of ethyl acetate and hexane to give colorless needles, m.p. $247\sim 251^\circ$. *Anal.* Calcd. for $C_{34}H_{50}O_{10}$: C, 66.00; H, 8.15. Found: C, 65.80; H, 8.27.

ii) Hydrolysis of Crystal F: a) Hydrolysis with 3.4% HCl (50% ethanolic)—A solution of 10 mg. of Crystal F dissolved in 2 cc. of 50% EtOH solution of 3.4% HCl was refluxed for 3 hrs. This solution was concentrated under a reduced pressure in CO_2 and extracted with $CHCl_3$ to remove the aglycone. Aq. layer was neutralized with 10% Na_2CO_3 solution and concentrated to dryness under a reduced pressure in CO_2 . Acetone was added to the residue to remove the minute amount of impurities and the filtrate was again concentrated in CO_2 . The syrupy sugar obtained was submitted to paper chromatography by the descending method at $20\sim 22^\circ$ for 6 hrs., with a 4:1:1 mixture of BuOH, AcOH, and water. Rf values of the sugar of crystal F, the sample of digitalose, and the mixture of both sugars were all 0.54.

b) Hydrolysis by the Mannich method—To a solution of 30 mg. of crystal F in 10 cc. of acetone was added 0.1 cc. of 38% HCl and the mixture was allowed to stand at room temperature for 12 days. The pale yellowish brown solution was diluted with 4 cc. of water and concentrated under a reduced pressure to a pale yellow turbid solution. This solution was extracted with $CHCl_3$, the extract was washed with water, dried over anhyd. Na_2SO_4 , and evaporated to dryness. The residue was repeatedly recrystallized from benzene to give colorless crystals, m.p. $244\sim 249^\circ$, which melted at $244\sim 249^\circ$ when admixed with digitoxigenin, m.p. $245\sim 250^\circ$.

6) **Crystal H (Digitalonin)**—The sugar obtained from 10 mg. of Crystal H by hydrolysis with 50% EtOH solution of 3.4% HCl was compared with *d*-digitalose by the above-mentioned paper chromatography. The Rf values of both sugars and their mixture were all 0.54.

7) **Crystal I (Digiproside)**—Crude crystals obtained by treatment of the fraction Nos. 51~62 from secondary chromatography-(2) with AcOEt were recrystallized from MeOH as colorless needles, m.p. $147\sim 152^\circ/193\sim 194^\circ$, or from acetone-ether mixture as colorless plates, m.p. $194\sim 196^\circ$, and designated as Crystal I. The properties of this substance are given in the main text. *Anal.* Calcd. for $C_{29}H_{44}O_8 \cdot \frac{1}{2} H_2O$: C, 65.76; H, 8.56. Found: C, 66.09; H, 8.66.

i) Acetylation of Crystal I: Crude acetate (30 mg.) obtained from 30 mg. of Crystal I, Ac_2O , and pyridine was recrystallized from an acetone-hexane mixture to colorless needles, m.p. $242\sim 245^\circ$. *Anal.* Calcd. for $C_{35}H_{50}O_{11}$: C, 64.99; H, 7.79; $COCH_3$, 19.96. Found: C, 64.75; H, 7.92; $COCH_3$, 19.26.

ii) Hydrolysis of Crystal I: a) Hydrolysis by the Mannich method—The crude aglycone (40 mg.) obtained from 60 mg. of Crystal I by treatment with 20 cc. of acetone containing 0.2 cc. of 38% HCl by the Mannich method was recrystallized from AcOEt as colorless crystals, m.p. $245\sim 250^\circ$. *Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.95; H, 9.28.

b) Comparison of the aglycone of Crystal I with digitoxigenin—Mixed fusion of the aglycone of Crystal I, m.p. $245\sim 250^\circ$ and its acetate, m.p. $217\sim 221^\circ$, with samples of digitoxigenin, m.p. $245\sim 250^\circ$, and its acetate, m.p. $218\sim 221^\circ$, showed m.p. $245\sim 250^\circ$ and m.p. $217\sim 221^\circ$, respectively.

Paper chromatography was carried out on these two aglycones by the descending method, developed at $20\sim 21^\circ$ for 2 hrs., with a 2:1:1 mixture of benzene, AcOEt, and water and the Rf values obtained were 0.91 for the aglycone of Crystal I, 0.90 for digitoxigenin, and 0.91 for their mixture.

c) Paper chromatography of the sugar moiety of Crystal I—The syrupy sugar obtained from Crystal I by hydrolysis with 3.4% HCl in 50% EtOH was compared with the samples of methyl-pentose by paper chromatography similar to that described above at $20\sim 21^\circ$ for 16 hrs., using a mixture (4:1:2) of BuOH, AcOH, and H_2O . The Rf values obtained were as follows:

Sugar from Crystal I	0.41	<i>l</i> -Rhamnose	0.47
<i>d</i> -Fucose	0.41	<i>d</i> -Gullomethylose	0.46
<i>l</i> -Fucose	0.41	<i>d</i> -Tallomethylose	0.52
<i>d</i> -Allomethylose	0.43	<i>d</i> -Fucose + <i>l</i> -fucose	0.41
Sugar from Crystal I + <i>d</i> -fucose	0.41	Sugar from Crystal I + <i>l</i> -fucose	0.40
Sugar from Crystal I	0.40	Sugar from Crystal I	0.41
+ <i>d</i> -allomethylose	+0.43	+ <i>d</i> -gullomethylose	+0.46
Sugar from Crystal I	0.41		
+ <i>l</i> -rhamnose	+0.47		

d) Osazone of the sugar—A mixture of 50 mg. of the foregoing syrupy sugar, 80 mg. of phenylhydrazine, 20 cc. water, and 3 drops AcOH was warmed on a boiling water bath for 3 hrs. in CO₂. The product obtained was recrystallized from benzene to yellow needles, m.p. 170~175°, alone or in admixture with an authentic sample of the osazone of *d*-fucose.

8) **Molecular Weight Determination by Ultraviolet Absorption**—i) Preparation of standard curve of digitoxigenin: Ratio of absorbancy at 217 m μ and molar concentration of digitoxigenin was determined as follows:

Absorbancy		Mol. concn. (in EtOH) ($\times 10^{-3}$ mol./L.)		Ratio ($\times 10^3$)
E ₁	0.077	C ₁	0.00514	14.98
E ₂	0.369	C ₂	0.0257	14.36
E ₃	0.781	C ₃	0.0535	14.59

These results are almost in accord with Beer's law and a standard curve was prepared as shown in Fig. 5.

ii) Determination of molecular weight of Crystal F and Crystal I: Absorbancy of each EtOH solution of digitoxin (as control), Crystal F, and Crystal I was determined and utilizing the standard curve, their molecular weight was calculated as follows:

Substance	Concn. (mg./100 cc.)	Absorbancy	Mol. wt.	
			Found	Calcd.
Digitoxin	2.222	0.399	777.8	764.9
Crystal F	1.702	0.449	543.7	552.7
Crystal I	1.664	0.452	528.3	529.7

9) **Crystal K**—The crude crystals obtained from the fraction Nos. 48~50 of secondary chromatography-(2) were recrystallized from AcOEt in colorless crystalline powder, m.p. 268~270°, which did not show any depression of the melting point on admixture with gitoxin, m.p. 270~273°. Paper chromatography was carried out on these samples by the ascending method, developed at 20~22° for 2 hrs. with a 1:1 mixture of MeCOEt and xylene (saturated with formamide). The R_f values of both samples and their mixture were all 0.25.

A pale yellowish brown powder, m.p. 210~216°, separated from the mother liquor of gitoxin gave a similar color reaction with gitoxin, but the R_f value in the above-mentioned paper chromatography was 0.33 and differed from that of gitoxin.

Summary

Two cardiotonic glycosides were obtained from the dried leaves of *Digitalis purpurea* L. One of them, m.p. 232~235°, was found to be odoroside H, originally isolated by Reichstein from the barks of *Nerium odorum*. The second one, m.p. 194~196°, is a hitherto unknown glycoside, named digiproside, and shown to be a digitoxigenin-mono-*d*-fucoside. Besides these, three non-cardiotonic glycosides, purpnin, digipronin, and digitalonin were also newly isolated.

(Received May 1, 1956)