the response of *d*-tubocurarine to a definite electric stimuli was an S-shaped curve having smaller range between 0.1~0.4 mg./cc. From this fact, it is considered that there should be a different mechanism of transmission between rectus abdominis stimulated by ACh and the neuromuscular preparation of a rat diaphragm stimulated electrically.

Summary

It was concluded statistically on the contraction of rectus abdominis of a frog that:

1) The reaction between ACh and its receptor in the muscle obeyed Clark's formula and the reaction order was 1, that is, they react with one molecule each.

2) Reaction mechanism between ACh and its receptor was different in the presence of eserine, from that in its absence.

3) Antagonism of atropine and *d*-tubocurarine to ACh obeyed Gaddum's formula as competitive.

4) One molecule of *d*-tubocurarine antagonized two molecules of ACh; two ammonium nitrogens in *d*-tubocurarine antagonized one molecule of ACh each.

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45. Daisuke Satoh, Takayuki Wada, Hiroshi Ishii, Yohko Oyama, and Tamotsu Okumura : Studies on Digitalis Glycosides. VII.[1] Gitoroside,* Digitalonin, and Gitoxin Pentaacetate.

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The pale yellowish brown substance, m.p. 210~216°, separated from the mother liquor of gitoxin, described in the previous paper,[1] gave a colorless powder, m.p. 213~216°, after purification with ethyl acetate. This substance showed a similar coloration with gitoxin in the Legal and the Keller-Kiliani reaction. However, the solubility and the Rf value on paper chromatogram of this substance differed from those of gitoxin. U.V. λ_{max}^{EtOH} 219 mμ (log ε 4.15). $[\alpha]_D^{26}$ +24.9°(c=0.4985, EtOH). Analytical values corresponded to $C_{29}H_{44}O_8 \cdot 1\frac{1}{2} H_2O$ and had no methoxyl nor acyl group.

The acetate formed a colorless powder, m.p. 128~131°, and result of paper chromatography indicated the unity of this substance. Analyses gave values which agreed with those of a triacetate, $C_{35}H_{50}O_{11} \cdot H_2O$.

Hydrolysis of this glycoside under mild conditions gave an aglycone as colorless needles, m.p. 223~225°, whose analytical values corresponded to $C_{23}H_{34}O_5$. Mixed fusion of this aglycone with authentic sample of gitoxigenin, m.p. 224~227°, showed no depression of the melting point and the Rf value of this aglycone agreed with that of gitoxigenin.

Since the paper chromatogram of the sugar moiety of this glycoside gave only one spot by the Keller-Kiliani reaction and by aniline hydrogen phthalate, it is obvious that there was one kind of 2-desoxysugar. On comparison of Rf values, the sugar moiety was shown to be *d*-digitoxose. A crystalline sugar, m.p. 103~105°, was obtained by the vacuum sublimation of the syrupy sugar and the mixed fusion of this sugar with authentic

* A brief summarized report on gitoroside was published as a Communication to the Editor in J. Pharm. Soc. Japan, **76**, 1334(1956).

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1) Part VI : D. Satoh, H. Ishii, Y. Oyama, T. wada, T. Okumura : This Bulletin, **4**, 284(1956).

sample of *d*-digitoxose, m.p. 105~107°, showed no depression of the melting point.

The number of molecules of *d*-digitoxose contained in this glycoside was calculated to be one by means of molecular weight determination method[1] by the absorbancy of its ultraviolet spectrum.

These results have shown that this glycoside is gitoxigenin mono-*d*-digitoxoside (I) and since a glycoside possessing this constitution has never been described in any literature, this glycoside was designated as gitoroside by the present authors. Content of gitoroside in the dried leaves is very small.

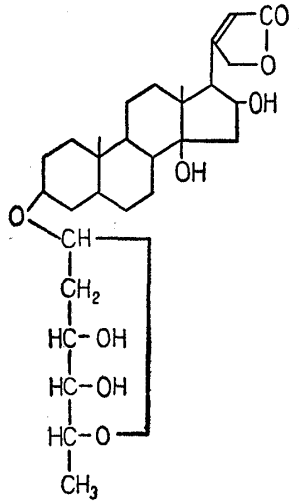

Fig. 1.

Gitoroside

The hydrolysis of digitalonin[1] gave an aglycone as a white amorphous powder, m.p. 116~120°, which did not show any depression on admixture with authentic sample of diginigenin,[2] m.p. 118~120°. Since the sugar moiety had been determined as *d*-digitalose,[1] it was presumed that digitalonin is diginigenin mono-*d*-digitaloside.

By the acetylation of gitoxin with acetic anhydride and pyridine, and subsequent purification with dilute methanol, gitoxin acetate was obtained as colorless needles, m.p. 158~160°, $[\alpha]_D^{25}$ +81.7°(c=2.963, EtOH). Analyses gave values which agreed with those of a pentaacetate, $C_{51}H_{74}O_{19}\cdot H_2O$.

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Experimental

1) Isolation of Gitoroside—A pale yellowish brown substance, m.p. 210~216°, separated from the mother liquor of gitoxin (fraction Nos. 48~50 of secondary chromatography described in the preceding paper[1]) was recrystallized from AcOEt as a colorless powder, m.p. 213~216°. This glycoside is easily soluble in MeOH, EtOH, and $CHCl_3$, soluble in hot AcOEt. *Anal.* Calcd. for $C_{29}H_{44}O_8\cdot 1\frac{1}{2}\ H_2O$: C, 63.60; H, 8.65; H_2O, 4.93; OCH_3, Found: C, 63.54; H, 8.60; H_2O, 4.14; OCH_3, 0.52.

2) Comparison of Gitoroside with Gitoxin on Paper Chromatogram—Paper chromatography was carried out on these two glycosides by the method described in the preceding paper.[1] The Rf value of gitoroside was 0.43 and that of gitoxin was 0.56.

3) Qualitative Examination of Acyl Group—By means of Frerejacque's method,[3] a drop of solution prepared from 0.5 mg. of substance in 3 drops of pyridine placed on a small strip of filter paper and dried at 60° was sprayed with a solution of 2 g. $NH_2OH\cdot HCl$ in 50 cc. of 50% EtOH. After drying, the strip was sprayed with *N* KOH in EtOH, and after 10 mins. sprayed with a solution of 0.5 g. of $FeCl_3$ and 6 g. of CCl_3COOH in 25 cc. of water. The results were as follows:

2) The sample was kindly given by Prof. Dr. C. W. Shoppee.

3) M. Frerejacque: C. A. **49**, 12774(1955); Compt. rend., **240**, 1804(1955).

Sample	Color	Acyl group
Gitoroside	Yellow	−
16-Acetylgitoxigenin	Reddish violet	+
Triacetylstrospeside	〃 〃	+

4) Determination of Molecular Weight by Ultraviolet Absorption—i) Ratio of absorbancy at 218 mμ and molar concentration of gitoxigenin was as follows:

Concn. (mg./100 cc.)	Mol. concn. ($\times 10^{-3}$)	Absorbancy	Ratio (Absorbancy/Mol. concn.) ($\times 10^3$)
2.119	0.05426	0.781	14.39
1.413	0.03618	0.538	14.87
0.707	0.01809	0.269	14.87
		average	14.71

ii) Determination of molecular weight: Absorbancy of each EtOH solution of strospeside, digitalinum verum (as control), and gitoroside was determined and utilizing the above-mentioned ratio, their molecular weights were calculated as follows:

Substance	Concn. (mg./100 cc.)	Absorbancy	Mol. wt. Found	Mol. wt. Calcd.
Strospeside	2.100	0.517	540.6	550.7
Digitalinum verum	1.424	0.291	719.3	712.8
Gitoroside	2.003	0.514	572.8	547.7

5) Acetylation of Gitoroside—Crude acetate (27 mg.) obtained from 30 mg. of gitoroside, Ac_2O, and pyridine was recrystallized from dil. MeOH to a colorless powder, m.p. 128~131°. *Anal.* Calcd. for $C_{35}H_{50}O_{11}\cdot H_2O$: C, 63.23; H, 7.89; $COCH_3$, 19.43; H_2O, 2.71. Found: C, 63.17; H, 7.93; $COCH_3$, 19.34; H_2O, 2.37.

The Rf value in the paper chromatography using a mixture (10 : 1) of toluene and BuOH on the formamide-impregnated paper was 0.68, giving only one spot.

6) Hydrolysis of Gitoroside—45 mg. of gitoroside was refluxed for 45 mins. with 5 cc. of 0.35% HCl (50% methanolic) and neutralized with 10% Na_2CO_3 solution.

i) Aglycone: Crude aglycone extracted with $CHCl_3$ was recrystallized from MeOH-Et_2O as colorless prisms, m.p. 223~225°. *Anal.* Calcd. for $C_{23}H_{34}O_5\cdot 2H_2O$: C, 64.76; H, 8.98. Found: C, 64.24; H, 8.74. Mixed fusion of this aglycone with the sample of gitoxigenin, m.p. 224~227°, showed m.p. 223~226°. Paper chromatography was carried out on these two aglycones using a mixture of toluene-BuOH (4 : 1) on the formamide-impregnated paper, and the Rf values obtained were 0.64 for the aglycone of gitoroside, 0.65 for gitoxigenin, and 0.64 for their mixture.

ii) Sugar moiety: The syrupy sugar obtained from the water layer of hydrolysed product was compared with the samples of *d*-digitoxose and *d*-boivinose[4] by means of paper chromatography using a mixture (3 : 1 : 3) of AcOEt, AcOH, and water. The Rf values obtained were 0.44 for the sugar of gitoroside and *d*-digitoxose, and 0.37 for *d*-boivinose. The syrupy sugar was submitted to vacuum sublimation (10^{-3} mm. Hg, 100~110°) and the sublimate was recrystallized from a mixture of Me_2CO and Et_2O to colorless prisms, m.p. 103~105°, which melted at 104~106° when admixed with *d*-digitoxose, m.p. 105~107°.

7) Hydrolysis of Digitalonin—50 mg. of digitalonin was refluxed for 30 mins. with 3 cc. of 5% H_2SO_4 (50% methanolic) and neutralized with 10% Na_2CO_3 solution. Crude aglycone extracted with $CHCl_3$ was recrystallized from a mixture of AcOEt and hexane to a white amorphous powder, m.p. 116~120°, and a mixture with diginigenin, m.p. 118~120°, melted at 117~120°.

8) Acetylaton of Gitoxin—A mixture of 0.5 g. gitoxin, 5 cc. Ac_2O, and 10 cc. pyridine was left at room temperature for 3 days and the crude acetate (0.67 g.) obtained after removal of the excess of reagents was recrystallized from dil. MeOH as colorless needles, m.p. 158~160°. *Anal.* Calcd. for $C_{51}H_{74}O_{19}\cdot H_2O$: C, 60.70; H, 7.59; H_2O, 1.79; $COCH_3$, 21.33. Found: C, 60.47; H, 7.62; H_2O, 1.35; $COCH_3$, 20.44.

Summary

A new cardiotonic glycoside, named gitoroside, was obtained from the water-soluble fraction of *Digitalis purpurea* leaves. This glycoside was found to be a gitoxigenin mono-*d*-digitoxoside. Digitalonin, non-cardiotonic glycoside, was presumed to be a diginigenin mono-*d*-digitaloside. Gitoxin pentaacetate, m.p. 158~160°, was obtained in crystalline form for the first time.

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4) The sample of *d*-boivinose was kindly given by Prof. Dr. T. Reichstein.