UDC 547.918:615.711.5

42. Kazuhiko Hoji, Tosaku Miki, Akio Sakashita, Atsuji Okano, and Kazuo Miyatake: Studies on the Constituents of *Digitalis purpurea* L. XVII.[*1] Isolation of Several New Glycosides from Fraction PGB of Digitalis Seeds.

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It was previously reported in this series that digitalinum verum, gitostin, glucodigifucoside, and neogitostin had been isolated from the seeds of *Digitalis purpurea*. Further, it was reported in Part X[1] that 100 kg. of seeds were extracted and many glycosides were isolated by complicated methods, and the structures of new glycosides, digifucocellobioside,[2] gitorocellobioside,[3] and gitoxin-cellobioside[3] were established.

The materials were extracted from 100 kg. of seeds and were fractionated. One fraction corresponding to the fraction Nos. 3~7 in Table I of Part II,[4] was previsionally named purpurea glycoside-B fraction. These Rf values were larger than that of digitalinum verum. Their presence was already indicated by paper chromatography with water-saturated methyl ethyl ketone, as shown in Fig. 1 of Part II,[4] and the substances PGA and PGB might be identical with purpurea glycosides-A and -B. The unknown glycosides have now been isolated and the results are reported here.

Table I. Properties of Isolated Substances

Substance	m.p. (°C)	UV λ_{max}^{EtOH} (mμ)	Reactions						Yield from seeds (%)
			$SbCl_3$	Raymond	Gregg-Gisvold[a)]	Frère-jacque[b)]	Keller-Kiliani H_2SO_4-layer	Keller-Kiliani AcOH-layer	
B-0		217	−	+	+	+	brown	blue	0.004
PGA	248~254 (amorph.)	218	−	+	+	−	〃	〃	0.0018
A-II′	199~205 (〃)	219	+	+	+	+	red	brown	0.001
A-II		218	+	+	+	+	〃	blue	0.008
B-I′	184/215~219 (〃)	218	−	+	+	−	brown	〃	0.0027
B-I″	260~267 (needles)	207	−	−	+	−	colorless	〃	0.0008
PGB	228~233 (amorph.)	219	+	+	+	−	red	〃	0.012
A-III	222~225 (〃)	219	+	+	+	−	〃	brown	0.0013
A-III′	248~250 (needles)	219	+	+	−	+	red	colorlees	0.00013
C-0	240~242 (prisms)	218	−	+	−	−	brown	〃	0.0024
X_2	172~177 (plates)		−	−	−	−	colorless	〃	0.00015
Digitalinum verum	231~234 (needles)	218	+	+	−	−	red	〃	0.15
Glucodigifucoside	194~197 (〃)	217	−	+	−	−	brown	〃	0.038

a) This reaction is a spot test for 2,6-deoxysugar (D. H. Gregg, O. Gisvold: J. Am. Pharm. Assoc., **43**, 106 (1954)).

b) This reaction is a spot test for acyl group (M. Frèrejacque: Compt. rend., **240**, 1804 (1955)).

*1 Part XVI: This Bulletin, 8, 945 (1960).

*2 Hirakawabashi, Sumida-ku, Tokyo (傍士和彦, 三木藤作, 坂下昭夫, 岡野淳二, 宮武一夫).

1) Part X. A. Okano, *et al.*: This Bulletin, **7**, 212 (1959).

2) Part XI. *Idem*: *Ibid.*, **7**, 222 (1959).

3) Part XII. *Idem*: *Ibid.*, **7**, 226 (1959).

4) Part II. K. Miyatake, *et al.*: *Ibid.*, **5**, 157 (1957).

It was found that these unknown glycosides were not sufficiently separated on the filter paper, developed with water-saturated methyl ethyl ketone (Solvent system 1, Fig. 1). In addition to this system, five new solvent mixtures (Solvent systems 2, 3, 4, 5, and 6) were used in the paper chromatography to effect clear separation (Figs. 2, 3, 4, 5, and 6). As shown in these figures, six more new spots (Substances A-II′, A-III′, B-0, B-I′, B-I″, and C-0) were confirmed in this fraction, and the paper chromatography with each of these solvent systems could not always show their distinct spots. Therefore, the six above-mentioned solvent systems were necessary for the examination of purity and fractionation of the glycosides.

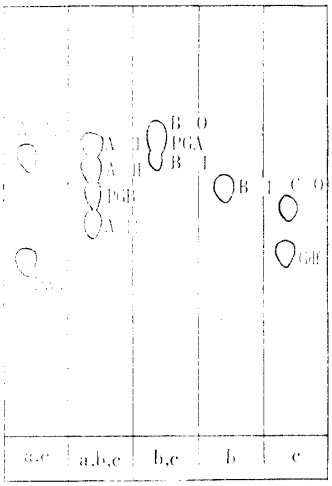


Fig. 1. Paper Partition Chromatography of PGB Fraction

Moving phase: Water-saturated MeCOEt (solvent system 1)
Paper : Impregnated with Me_2CO-H_2O (4:1) and Me_2CO evaporated
Coloring agent : a) 20% $SbCl_3$-$CHCl_3$ solution
b) 1% HCl-MeOH solution (Gregg-Gisvold reaction)
c) Raymond reaction agent

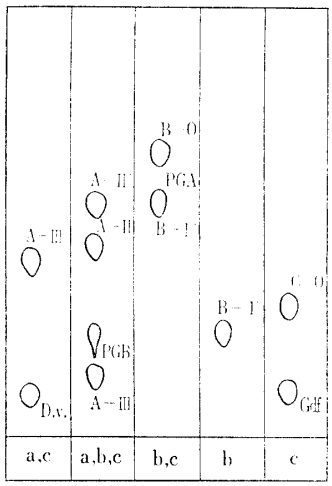


Fig. 2. Paper Partition Chromatography of PGB Fraction

Moving phase : Water-saturated mixture of MeCOEt and $CHCl_3$(5:1)(solvent system 2)
Paper : Impregnated with Me_2CO-H_2O (4:1) and Me_2CO evaporated

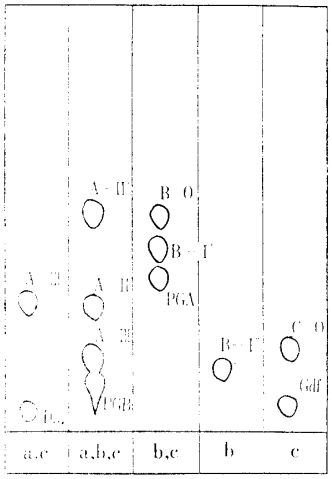


Fig. 3. Paper Partition Chromatography of PGB Fraction

Moving phase : Water-saturated mixture of MeCOEt and iso-BuCOMe (1:1) (solvent system 3)
Paper : Impregnated with Me_2CO-H_2O (4:1) and Me_2CO evaporated
D.v. : Digitalinum verum

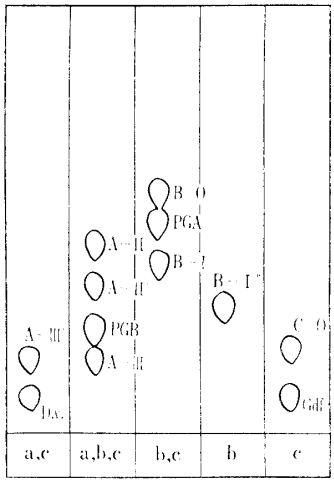


Fig. 4. Paper Partition Chromatography of PGB Fraction

Moving phase : Formamide-saturated mixture of BuOH and benzene (1:2) (solvent system 4)
Paper : Impregnated with Me_2CO-$HCONH_2$ (4:1) and Me_2CO evaporated
Gdf : Glucodigifucoside

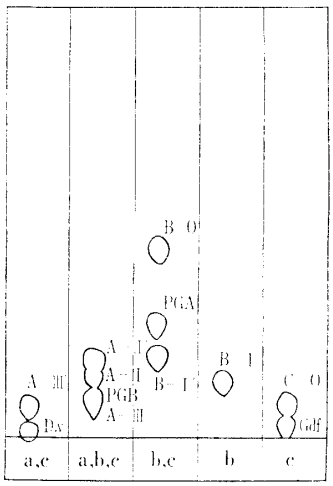


Fig. 5. Paper Partition Chromatography of PGB Fraction

Moving phase : Formamide-saturated mixture of BuOH and toluene (1:2) (solvent system 5)
Paper : Impregnated with Me_2CO-$HCONH_2$ (4:1) and Me_2CO evaporated

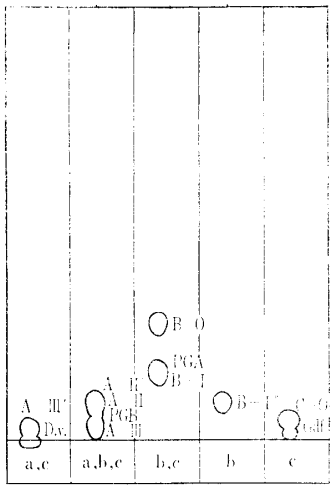


Fig. 6. Paper Partition Chromatography of PGB Fraction

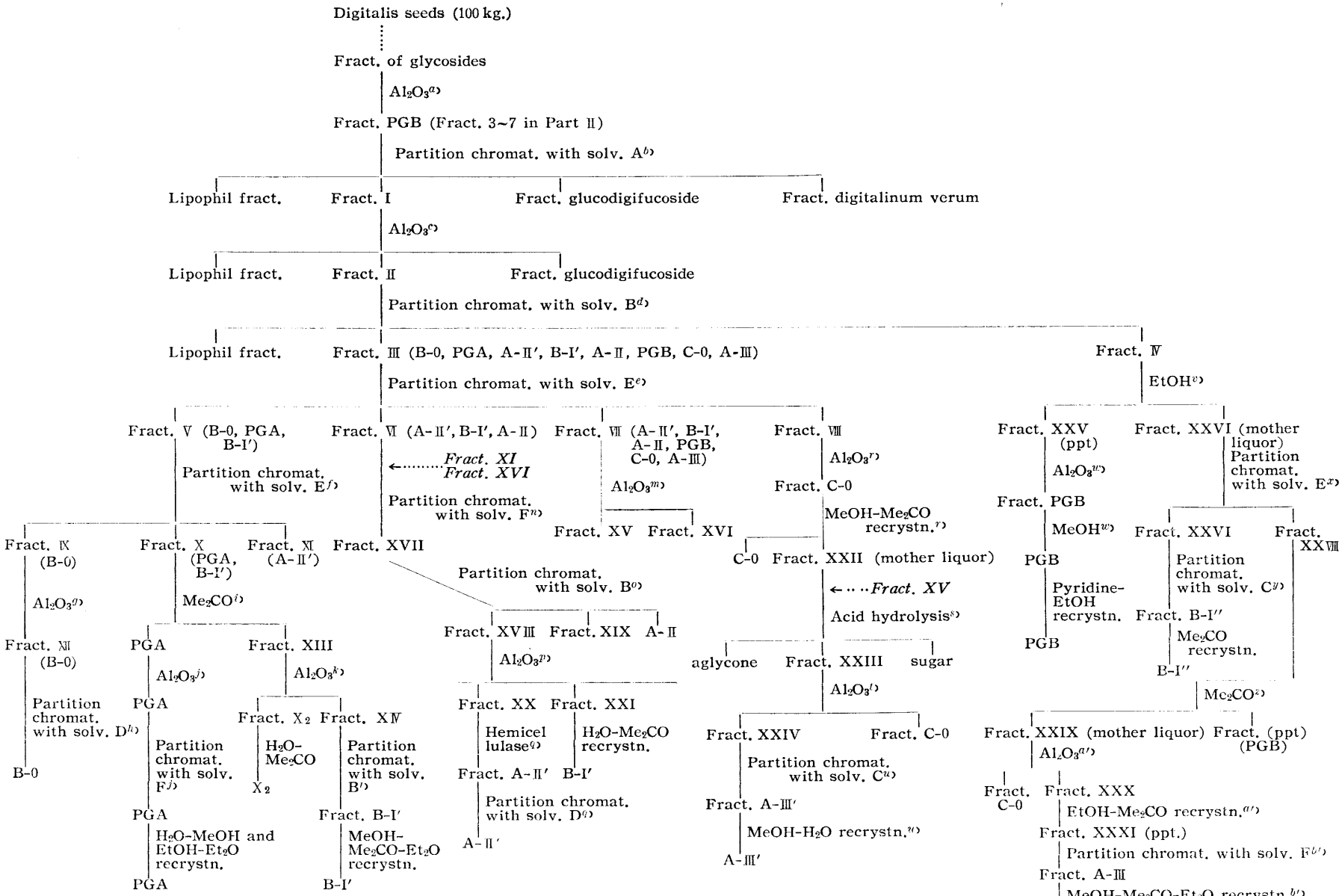
Moving phase : Formamide-saturated mixture of BuOH and toluene (1:3)(solvent system 6)
Paper : Impregnated with Me_2CO-$HCONH_2$ (4:1) and Me_2CO evaporated

In this process of isolation and fractionation of the glycosides, more difficulty was encountered than with the water-soluble glycosides reported in Part X,[1] running between digitalinum verum and gitostin on the paper chromatogram. Using water as the stationary phase in column chromatography, these glycosides were not always completely separated from each other, especially the glycosides possessing 2,6-deoxysugar, which could not be separated by their tailing. Formamide was sometimes used as the stationary phase. Capacity of the formamide system is larger than that of the systems based on water, but a drawback is that formamide does not distil off at a low temperature. Further, mild hydrolysis was carried out for separation of substance A-III'. Substance A-II' could not be separated from B-I' and A-II by the above-mentioned methods, and an enzyme, Hemicellulase,*3 was used. By the use of this enzyme, substance B-I' and A-II were converted to secondary glycosides with a loss of glucose, but substance A-II' did not react. These separation methods are shown in Chart 1.

Thus, several new glycosides were successfully isolated. Four substances, B-I'', A-III', C-0, and X_2, were obtained as crystals and five other substances, PGA, A-II', B-I', PGB, and A-III were obtained as colorless powder. Their properties are listed in Table I. In accordance with their coloration and absorption spectral data, these substances are classified into the following groups, from the results of Keller-Kiliani reaction : Substances B-0, PGA, B-I', and C-0 belong to the digitoxigenin series, substances A-II', A-II, PGB, A-III, and A-III' to the gitoxigenin series, and substance B-I'' is a curious glycoside, because it gives negative Legal and Raymond reactions and exhibits a maximum at 207 mμ in its ultraviolet absorption. From the sugar aspect, there are some 2,6-deoxysugars in substances B-0, PGA, A-II', A-II, B-I', B-I'', PGB, and A-III because they exhibit positive Gregg-Gisvold reaction. Substances B-0, A-II', A-II, and A-III' are acyl glycosides, because they give positive Frèrejacque reaction. The glycosides of the gitaloxigenin series had been found in the leaves and seeds of *Digitalis purpurea* and *D. lanata*; these glycosides easily lose their formyl group by lead hydroxide or acetate,[5] and are converted to the corresponding glycosides of the gitoxigenin series. It is not con-

*3 The product of Tokyo Kasei Kogyo Co., Ltd.

5) E. Haack, M. Gube, F. Kaiser, H. Spingler : Chem. Ber., **91**, 1758 (1958); D. Kutter, L. Fauconnet : Pharm. Acta Helv., **34**, 200 (1959).



a, *b*, ·····*z*, *a'*, *b'* correspond to numbers in the Experimental section.

Chart 1. Isolation of Glycosides

sidered that these acyl glycosides belong to the gitaloxigenin series, because during isolation, they are obtained through treatment with lead hydroxide.

Only one acetyl glycoside, digacetinin,[6] belonging to the digitanol series had been isolated from the leaves of *Digitalis purpurea*. Therefore, it is interesting that the new acyl glycosides have been isolated from digitalis seeds.

Experimental*4

General Method

Paper Partition Chromatography——The fraction PGB obtained from the seeds of *Digitalis purpurea* L. was submitted to paper chromatography by six solvent systems (Figs. 1,2,3,4,5, and 6).

1) Apparatus : The same as described in Part II.[4]

2) Mobile phase and time for the solvents to move : 25~35 cm.

Solvent system
(1) Water-saturated MeCOEt; 4~6 hr.
(2) Water-saturated mixture of MeCOEt and $CHCl_3$ (5:1); 2~5 hr.
(3) Water-saturated mixture of MeCOEt and iso-BuCOMe (1:1); 3~5 hr.
(4) Formamide-saturated mixture of BuOH and benzene (1:2); 10~13 hr.
(5) Formamide-saturated mixture of BuOH and toluene (1:2); 5~7 hr.
(6) Formamide-saturated mixture of BuOH and toluene (1:3); 4~5 hr.

3) Stationary phase : The filter paper (Toyo Roshi No. 50) was impregnated with H_2O or $HCONH_2$ as described in Part II.[4] In the solvent systems (1), (2), and (3), paper impregnated with H_2O-Me_2CO (1:4) was employed, and with the systems (4), (5), and (6), the paper was impregnated with $HCONH_2$-Me_2CO (1:4).

4) Method of development : Ascending method at 25~30°.

Column Partition Chromatography——Celite 535 (Johns-Manville product) was used as the carrier and H_2O or $HCONH_2$ saturated with organic solvent was used as the stationary phase. The mobile phases were the following six solvent systems :

(A) Water-saturated MeCOEt.
(B) Water-saturated MeCOEt-$CHCl_3$ (5:1).
(C) Water-saturated MeCOEt-iso-BuCOMe (1:1).
(D) Water-saturated iso-BuCOMe.
(E) Formamide-saturated BuOH-tuluene (various ratios)
(F) Formamide-saturated BuOH-benzene (various ratios)

The ratio of Celite to the stationary phase was about 1:1 (g./cc.) and the column was packed and eluted in the same way as described in Part II.[4]

In the case of solvent systems (E) and (F), each fraction was evaporated under a reduced pressure, but $HCONH_2$ did not evaporate at low temperature. Five volumes of H_2O was added to the residual $HCONH_2$ solution and it was extracted with BuOH-$CHCl_3$ (1:2). The extract was washed with a small quantity of H_2O to free from $HCONH_2$ and evaporated to dryness.

Isolation of Unknown Substances

a) **PGB Fraction from Fraction of Glycosides**——By the methods described in Part II,[4] 100 kg. of seeds were extracted. The fraction of glycosides was separated by adsorption chromatography on an alumina column with water-saturated BuOH as the developing solvent. PGB fraction contained the same crude glycosides shown as Fract. 3~7 in Part II,[4] obtained in 553 g.

b) **Partition Chromatography of PGB Fraction with Solvent System (A)**——PGB fraction was dissolved in 1 L. of MeOH-$CHCl_3$ (1:1), 500 g. of Celite was added, and the mixture was dried. Further 500 cc. of H_2O was added to this mixture, stirred thoroughly, and put on top of a Celite

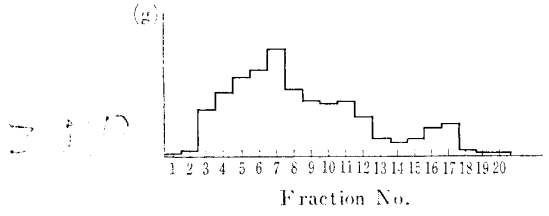


Fig. 7.
Partition Chromatography of PGB Fraction

*4 All melting points were measured on a Kofler block and are uncorrected.

6) R. Tschesche, W. Hammerschmidt, G. Grimmer : Ann., **614**, 136 (1958).

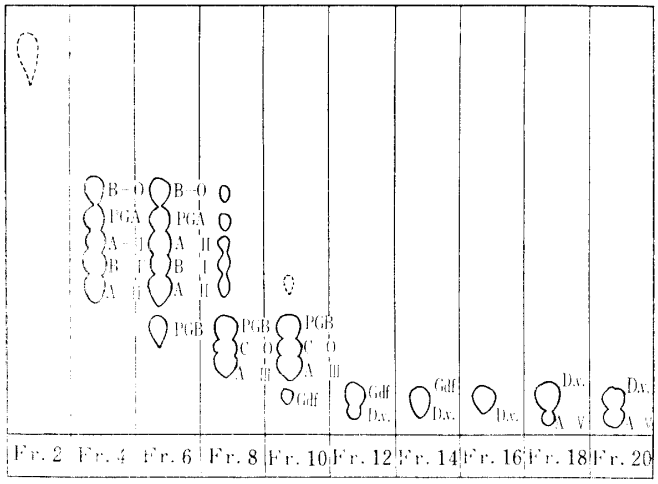


Fig. 8.

Paper Partition Chromatography of Some Fractions from Partition Chromatography of PGB Fraction
(With solvent system 4)

column which consisted of 16 kg. of Celite and 16 L. of H_2O. The column was eluted by the solvent system (A) and 5-L. fractions were collected. Distribution of this chromatography is shown in Fig. 7, each fraction was examined by paper chromatography, and the results are presented in Fig. 8.

Fraction Nos. 1~3 (31 g.) contained mainly monoglycosides. Fraction Nos. 4~11 (329 g.) contained materials corresponding to spots of substances B-0, PGA, A-II′, B-I′, A-II, PGB, C-0, A-III, and glucodigifucoside. This fraction was designated Fraction I, and further separated. Fraction Nos. 12~14 (41 g.) contained glucodigifucoside and a small quantity of digitalinum verum. Fraction Nos. 15~20 (53 g.) contained digitalinum verum and a small quantity of substance A-V.

c) **Alumina Adsorption Chromatography of Fraction I**——The above-mentioned Fraction I was chromatographed by an alumina column (3 kg. of Al_2O_3). This was developed with $CHCl_3$-MeOH mixture and water-saturated BuOH. Each fraction was submitted to paper chromatography.

The fraction eluted by 10% MeOH-$CHCl_3$ (4 L.) contained monoglycosides (50 g.). The fraction eluted by 20% and 50% MeOH-$CHCl_3$ (10 L. each) contained glycosides, same as Fraction I. This fraction was designated Fraction II (197 g.). The fraction eluted by H_2O-BuOH contained glucodigifucoside.

d) **Partition Chromatography of Fraction II with Solvent System (B)**——Celite (350 g.) was added to MeOH-$CHCl_3$ (1:1) solution of Fraction II (197 g.) and the mixture was dried. H_2O (350 cc.) was added to this mixture and chromatographed on a Celite column (14 kg. of Celite and 14 L. of H_2O).

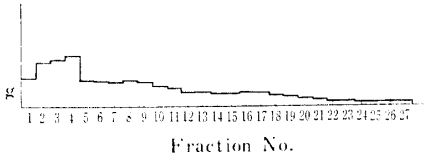


Fig. 9.

Partition Chromatography of Fraction II

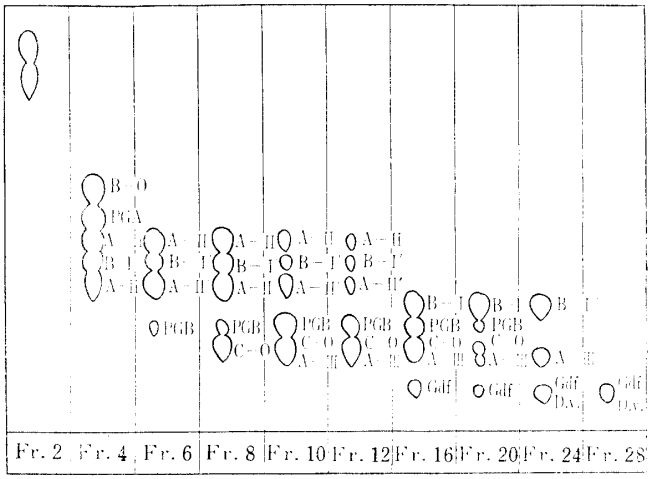


Fig. 10.

Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction II
(With solvent system 4)

The column was eluted with solvent system B and 10-L. fractions were collected. Distribution of this chromatography is shown in Fig. 9, each fraction was submitted to paper chromatography, and the results are presented in Fig. 10.

Fraction Nos. 1 and 2 (25.5 g.) contained monoglycosides. Fraction Nos. 3～15 (113 g.) contained the materials corresponding to spots of substances B-0, PGA, A-II′, B-I′, A-II, PGB, C-0, and A-III. This fraction was designated Fraction III. Fraction Nos. 16～27 (40.5 g.) contained the materials corresponding to spots of substance B-II″ and PGB, small quantities of C-0 and A-III, and a trace of glucodigifucoside. This fraction was designated Fraction IV.

e) **Partition Chromatography of Fraction III with Solvent System (E)**——Celite (300 g.) was added to the solution of Fraction III (113 g.) and the mixture was dried. $HCONH_2$ (300 g.) was added to this mixture, placed on a Celite column (9 kg. of Celite and 9 L. of $HCONH_2$), and the column was eluted by the solvent system (E). A mixture of toluene-BuOH (2:1) saturated with $HCONH_2$ was used for fraction Nos. 1～7, (1 : 1) for fraction Nos. 8～14, the effluent was collected in 5-L. fractions, and submitted to paper chromatography (Fig. 11).

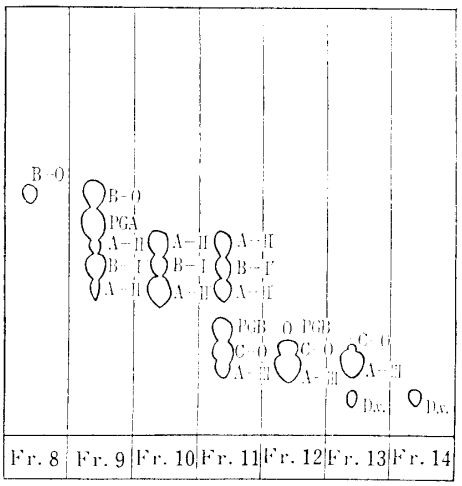


Fig. 11.

Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction III (With solvent system 4)

Fraction Nos. 4～9 contained the material corresponding to spots of substances B-0, PGA, and B-I′, and this fraction was designated Fraction V (37 g.). Fraction No. 10 contained substances A-II′, B-I′ and A-II, and this fraction was designated Fraction VI (18 g.). Fraction No. 11 contained substances A-II′, B-I′, A-II, PGB, C-0, and A-III. This fraction was designated Fraction VII (24 g.). Fraction Nos. 12～13 contained substances C-0 and A-III, and this fraction was designated Fraction VIII (20 g.).

f) **Partition Chromatography of Fraction V with Solvent System (E)**——Celite (100 g.) was added to MeOH-$CHCl_3$ (1:1) solution of Fraction V (37 g.) and the mixture was dried. $HCONH_2$ (100 cc.) was added to this mixture and chromatographed through a Celite column (1.5 kg. of Celite and 1.5 L. of $HCONH_2$). The column was eluted with solvent system E (toluene-BuOH (2:1)) and 1-L. fractions were collected. Each fraction was submitted to paper chromatography and the results are presented in Fig. 12.

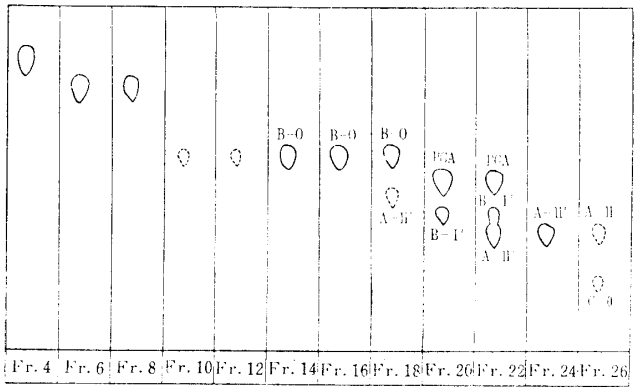


Fig. 12.

Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction V (With solvent system 4)

Fraction Nos. 3～11 contained monoglycosides. Fraction Nos. 12～18 (12 g.) contained the material corresponding to a spot of substance B-0, and this fraction was designated Fraction IX. Fraction Nos. 20 and 21 (8 g.) contained the materials corresponding to two spots of substances PGA and B-I′, and this fraction was designated Fraction X. Fraction Nos. 23～25 (4.5 g.) contained the material corresponding to a spot of substance A-II′, and this fraction was designated Fraction XI.

g) **Alumina Adsorption Chromatography of Fraction IX**——Fraction IX (12 g.) was chromatographed on an alumina column (250 g. of Al_2O_3), and the column was eluted with $CHCl_3$ (400 cc.), 5% (1500 cc.), 10% (1500 cc.), 20% (2000 cc.), and 50% MeOH-$CHCl_3$ (1000 cc.), and water-saturated BuOH (1000 cc.). The fraction eluted with 10%, 20%, and 50% MeOH-$CHCl_3$ (5 g.) contained only B-0 and this fraction was designated Fraction XII.

h) **Partition Chromatography of Fraction XII with Solvent System (D) (Isolation of Substance B-0)**——Fraction XII (5 g.) was dissolved in a small quantity of MeOH-$CHCl_3$ (1:1), 20 g. of Celite was added, and the mixture was dried. Further 20 cc. of H_2O was added to the mixture and placed on top of a Celite column (800 g. of Celite and 800 cc. of H_2O saturated with iso-BuCOMe). The column was eluted with solvent system (D) and 400-cc. fractions were collected. The substance B-0 was obtained from fraction Nos. 4～18 (4.0 g.).

i) **Isolation of Substance PGA from Fraction X**——Fraction X (8 g.) was treated with Me_2CO and the precipitate was separated from the solution. The solution contained substances PGA and B-I′, and the precipitate, m.p. 196～207° (3.59 g.), was identified as substance PGA by paper chromatography.

j) **Purification of Substance PGA**——The above-mentioned precipitate (PGA, 3.59 g.) was chromatographed through an alumina column (70 g. Al_2O_3). This column was eluted with 5% (350 cc.), 10% (800 cc.), 20% (800 cc.), and 50% MeOH-$CHCl_3$ (800 cc.). The fraction (2 g.) from 10%, 20%, and 50% MeOH-$CHCl_3$ was passed through a Celite column (150 g. of Celite and 150 cc. of $HCONH_2$-H_2O (4:1)). This column was eluted with BuOH-benzene (1:4) saturated with $HCONH_2$-H_2O (4:1), and 120-cc. fractions were collected. Each fraction was submitted to paper chromatography and some of them are shown in Fig. 13.

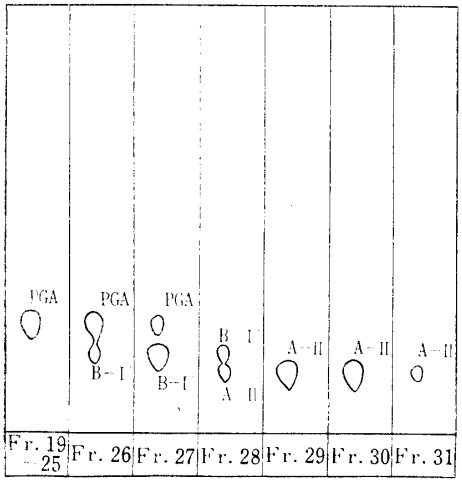


Fig. 13.

Paper Partition Chromatography of Some Fractions in Purification of Substance PGA
(With solvent system 5)

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Fraction Nos. 19～25 contained substance PGA alone and it was recrystallized from hydr. MeOH to a colorless powder (1.80 g.).

k) **Alumina Adsorption Chromatography of Fraction XIII (Isolation of Substance X_2)**——Fraction XIII (4 g.) was passed through an alumina column (100 g.). This column was eluted with $CHCl_3$ (200 cc.), 5% (400 cc.), 10% (100 cc.), 20% (1100 cc.), and 50% MeOH-$CHCl_3$ (1200 cc.), and water-saturated BuOH (600 cc.). The portion of $CHCl_3$ and 5% MeOH-$CHCl_3$ contained substance X_2 and it was recrystallized from hydr. Me_2CO to colorless plates, m.p. 170～173°. The portion of 20% and 50% MeOH-$CHCl_3$, and water-saturated BuOH contained substance B-I′, and it was designated Fraction XIV (2.5 g.).

l) **Partition Chromatography of Fraction XIV with Solvent System (B) (Isolation of Substance B-I′)**——Fraction XIV (2.5 g.) was dissolved in a small quantity of MeOH-$CHCl_3$ (1:1), 15 g. of Celite was added, and the mixture was dried. Further 15 cc. of H_2O saturated with MeCOEt-$CHCl_3$ (5:1) was added and this mixture was chromatographed through a Celite column (500 g. of Celite and 500 cc. of H_2O saturated with MeCOEt-$CHCl_3$ (5:1)). This column was eluted with solvent system B and 150-cc. fractions were collected. Each fraction was examined by paper chromatography. Fraction Nos. 5～11 showed the spot of substance B-I′ and recrystallized from MeOH-Me_2CO-Et_2O to a colorless powder, m.p. 178～185°, substance B-I′ (1.2 g.).

m) **Alumina Adsorption Chromatography of Fraction VII**——Fraction VII (24 g.) was chromatographed through an alumina column (500 g. of Al_2O_3) and the column was developed with $CHCl_3$ (1000 cc.), and 5% (4000 cc.), 10% (2000 cc.), 20% (3000 cc.) and 50% MeOH-$CHCl_3$ (6000 cc.). The fraction eluted with 10% and 20% MeOH-$CHCl_3$ contained mainly substance C-0 and it was designated Fraction XV (6 g.). The fraction of 50% MeOH-$CHCl_3$ contained substances A-III and PGB, and this fraction was designated Fraction XVI (12 g.). The paper chromatographic examination of these fractions is shown in Fig. 14.

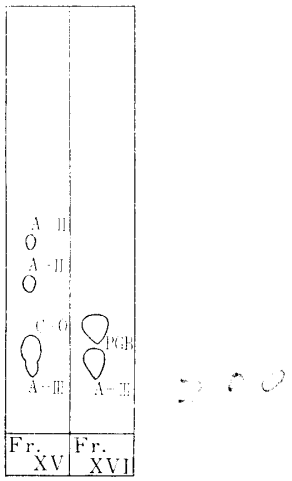

Fig. 14.

Paper Partition Chromatography of Fractions from Alumina Adsorption Chromatography of Fraction VII (With solvent system 4)

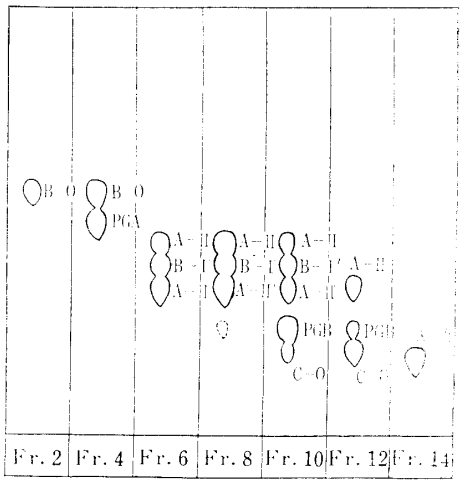

Fig. 15. Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fractions VI, XI, and XVI (With solvent system 4)

n) **Partition Chromatography of Fraction VI plus Fraction XI and Fraction XVI with Solvent System (F)**——Combined fraction (34.5 g.) of Fractions VI, XI, and XVI was added to 200 g. of Celite, dried, and 200 cc. of $HCONH_2$-H_2O (4:1) was added to it. This mixture was chromatographed through a Celite column (3 kg. of Celite and 3 L. of $HCONH_2$-H_2O (4:1)). The column was eluted with solvent system (F), BuOH-benzene (2:1) saturated with $HCONH_2$, and 1.5-L. fractions were collected. Each fraction was submitted to paper chromatography and the result is shown in Fig. 15.

Fraction Nos. 6～9 (17.25 g.) contained substances A-II, B-I′ and A-II′, and this fraction was designated Fraction XVII.

o) **Partition Chromatography of Fraction XVII with Solvent System (B) (Isolation of Substance A-II)**——Fraction XVII (17.25 g.) was added to 80 g. of Celite, dried, and mixed with 80 cc. of H_2O. This mixture was chromatographed on a Celite column (5 kg. of Celite and 5 L. of H_2O). The column was eluted with solvent system (B) and 2-L. fractions were collected. Each fraction was submitted to paper chromatography (Fig. 16).

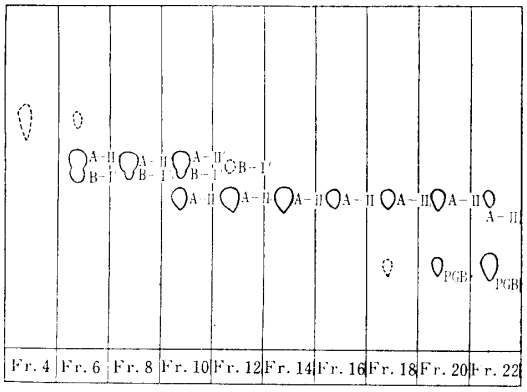

Fig. 16.

Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction XVII (With solvent system 2)

Fraction Nos. 5～8 (4 g.) contained substances B-I′ and A-II′, and this fraction was designated Fraction XVIII. Fraction Nos. 9～11 (5 g.) contained substances B-I′, A-II′, and A-II, and was designated Fraction XIX. Fraction Nos. 12～18 (8 g.) contained only substance A-II and Fraction Nos. 19～28 contained substances A-II and PGB.

p) **Alumina Adsorption Chromatography of Fraction XVIII (Isolation of Substance B-I′)**——Fraction XVIII (4 g.) was chromatographed through an alumina column (50 g. of Al_2O_3) and the column was developed with 5% (300 cc.), 10% (1800 cc.), 20% (1500 cc.), and 50% MeOH-$CHCl_3$ (300 cc.), and water-saturated BuOH (300 cc.).

The fraction of 10% MeOH-$CHCl_3$ (1.73 g.) contained mainly A-II′ and this fraction was designated Fraction XX. The fractions of 20% and 50% MeOH-$CHCl_3$, and water-saturated BuOH contained mainly substance B-I′, and this fraction was designated Fraction XXI (2.1 g.). Each fraction was submitted to paper chromatography (Fig. 17). Fraction XXI was recrystallized from hydr. Me_2CO to a colorless powder, substance B-I′ (1.5 g.).

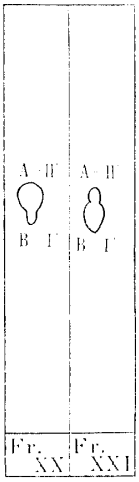

Fig. 17.

Paper Partition Chromatography of Fractions from Alumina Adsorption Chromatography of Fraction XVIII
(With solvent system 2)

q) **Treatment of Fraction XX by Hemicellulase*[3] and Partition Chromatography with Solvent System (D) (Isolation of Substance A-II′)**——Fraction XX (1.73 g.) was dissolved in 20 cc. of MeOH, 500 cc. of H_2O was added, and MeOH was evaporated under a reduced pressure. To this solution, 0.7 g. of Hemicellulase*[3] was added and dissolved, the mixture was allowed to stand at 32° for 5 days, evaporated to dryness, and treated with a small quantity of MeOH-$CHCl_3$ (1:1). This solution was filtered and evaporated to drynees (1.5 g).

To this residue 5 g. of Celite was added, the mixture was dried, and mixed with 5 cc. of H_2O. This mixture was placed on a Celite column (400 g. of Celite and 400 cc. of H_2O). The column was eluted with solvent system (D) and 400-cc. fractions were collected.

Fraction Nos. 1～4 contained the glycosides which underwent enzymatic hydrolysis. Fraction Nos. 5～9 contained only substance A-II′ (0.96 g.).

r) **Alumina Adsorption Chromatography of Fraction VIII (Isolation of Substance C-0)**——Fraction VIII (20 g.) was chromatographed through an alumina column (500 g. of Al_2O_3) and the column was developed with $CHCl_3$ (1000 cc.), and 2% (1000 cc.), 5% (3000 cc.), 10% (4500 cc.), 20% (3000 cc.), and 50% MeOH-$CHCl_3$ (3000 cc.).

The fraction of 20% MeOH-$CHCl_3$ was recrystallized from MeOH-Me_2CO (1:2) to colorless prisms, 2.44 g. of substance C-0. The mother liquor (4.5 g.) was designated Fraction XXII.

s) **Acid Hydrolysis of Fractions XXII and XV**——The combined fraction (10.5 g.) of Fractions XXII and XV was dissolved in 150 cc. of MeOH, 100 cc. of 0.1*N* H_2SO_4 was added, and the solution was refluxed on a water bath for 45 min. MeOH was distilled off in a reduced pressure. Three 50-cc. portions of $CHCl_3$ and five 100-cc. portions of BuOH-$CHCl_3$ (1:1) were used to extract the concentrated solution. The BuOH-$CHCl_3$ layer was washed with two 50-cc. portions of H_2O, evaporated under a reduced pressure until dryness (6.2 g.), and this was designated Fraction XXIII.

The $CHCl_3$ layer contained aglycone from substance A-III and the H_2O layer contained sugar.

t) **Alumina Adsorption Chromatography of Fraction XXIII**——Fraction XXIII (6.2 g.) was chromatographed through an alumina column (200 g. of Al_2O_3). This was developed with $CHCl_3$ (400 cc.), and 5% (1500 cc.), 10% (1000 cc.), 20% (1600 cc.), and 50% MeOH-$CHCl_3$ (1500 cc.).

The fraction of 10% MeOH-$CHCl_3$ contained substance A-III′ and a small quantity of substance C-0; this fraction was designated Fraction XXIV. The fraction of 20% and 50% MeOH-$CHCl_3$ contained mainly substance C-0.

u) **Partition Chromatography of Fraction XXIV with Solvent System (C) (Isolation of Substance A-III′)**——Fraction XXIV (1.62 g.) was added to 15 g. of Celite, dried, and mixed with 15 cc. of H_2O. This mixture was chromatographed through a Celite column (400 g. of Celite and 400 cc. of H_2O). The

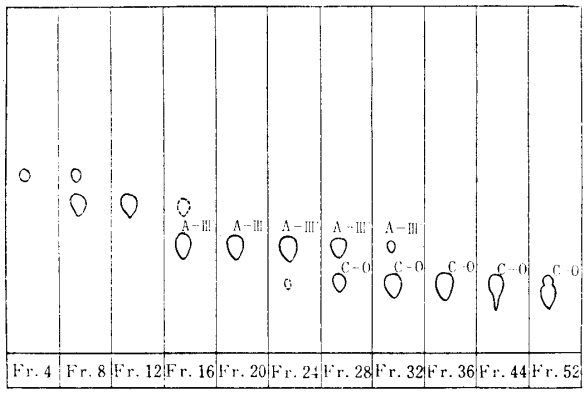

Fig. 18.

Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction XXIV

(With solvent system 3)

column was eluted with the solvent system (C), 100-cc. fractions were collected, and each fraction was examined by paper chromatography as shown in Fig. 18.

Fraction Nos. 17～26 contained only substance A-III′ and was recrystallized from hydr. MeOH to colorless needles of m.p. 247～250° (substance A-III′) (0.13 g.). Fraction Nos. 27～40 contained mainly C-0 (0.38 g.).

v) **Treatment of Fraction IV with Ethanol**——Fraction IV (40.5 g.) was treated with 500 cc. of EtOH. The precipitated crude substance PGB was designated Fraction XXV. The mother liquor (15.5 g.) contained substances A-III, B-I″, and a small quantity of substance PGB, and this mother liquor was designated Fraction XXVI.

w) **Alumina Adsorption Chromatography of Fraction XXV (Purification of Substance PGB)**——Fraction XXV (25 g.) was chromatographed through an alumina column (100 g. of Al_2O_3). This was developed with 50% MeOH-$CHCl_3$ and 100-cc. fractions were collected. Fraction Nos. 2～6 was recrystallized from MeOH to colorless powder of substance PGB (12.33 g.).

x) **Partition Chromatography of Fraction XXVI with Solvent System (E)**——Fraction XXVI (15.5 g.) was added to 60 g. of Celite, dried, and mixed with 60 cc. of $HCONH_2$-H_2O (4:1). This mixture was chromatographed through a Celite column (1.5 kg. of Celite and 1.5 L. of $HCONH_2$-H_2O (4:1)). The column was eluted with formamide-saturated BuOH-tuluene (2:3), 1-L. fractions were collected, and each fraction was submitted to paper chromatographic analysis as shown in Fig. 19.

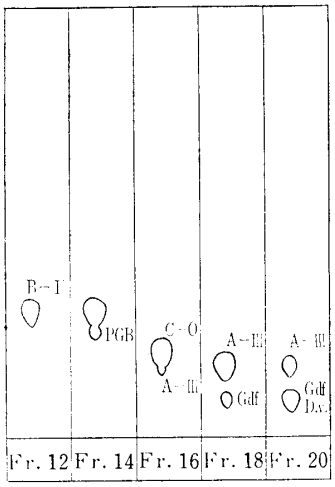

Fig. 19. Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction XXVI

(With solvent system 4)

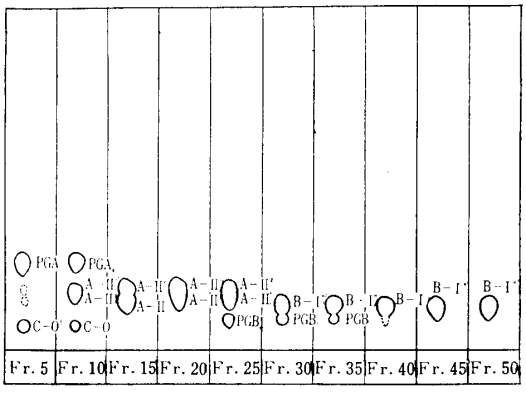

Fig. 20. Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction XXVII

(With solvent system 5)

Fraction Nos. 11～14 (4.5 g.) contained mainly substance B-I″ and this fraction was designated Fraction XXVII. Fraction Nos. 15～17 (8 g.) contained substance A-III and a small quantity of substance C-0, and this fraction was designated Fraction XXVIII.

y) **Partition Chromatography of Fraction XXVII with Solvent System (C) (Isolation of Substance B-I″)**——Fraction XXVII (4.5 g.) was added to 20 g. of Celite, dried, and mixed with 20 cc. of H_2O.

This mixture was chromatographed through a Celite column (400 g. of Celite and 400 cc. of H_2O). The column was eluted with the solvent system (C) and 150-cc. fractions were collected. Each fraction was examined by paper chromatography as shown in Fig. 20. Fraction Nos. 41～56 (0.8 g.) showed a single spot corresponding to substance B-I″.

z) **Acetone Treatment of Fraction XXVIII**——Fraction XXVIII (8 g.) was treated with 200 cc. of Me_2CO; the precipitate (1 g.) was substance PGB and the mother liquor (7 g.) was designated Fraction XXIX which contained mainly substance A-III.

a′) **Alumina Adsorption Chromatography of Fraction XXIX**——Fraction XXIX (7 g.) was chromatographed through an alumina column (200 g. of Al_2O_3) and the column was developed with 10% (1000 cc.), 20% (1000 cc.), and 50% MeOH-$CHCl_3$ (1500 cc.), and water-saturated BuOH (500 cc.).

The fraction of 10% MeOH-$CHCl_3$ contained mainly substance C-0. The fraction of 20% and 50% MeOH-$CHCl_3$ (Fraction XXX; 6 g.) contained mainly A-III which was recrystallized from EtOH-Me_2CO to a colorless powder (4.36 g.); the powder was designated Fraction XXXI.

b′) **Partition Chromatography of Fraction XXXI with Solvent System (F) (Isolation of Substance A-III)**——Fraction XXXI (4.36 g.) was added to 20 g. of Celite, dried, and mixed with 20 cc. of $HCONH_2$-H_2O (4:1). The mixture was chromatographed through a Celite column (200 g. of Celite and 200 cc. of $HCONH_2$-H_2O (4:1)), the column was eluted with formamide-saturated BuOH-benzene (1:1), and 100-cc. fractions were collected. Each fraction was submitted to paper chromatography as shown in Fig. 21.

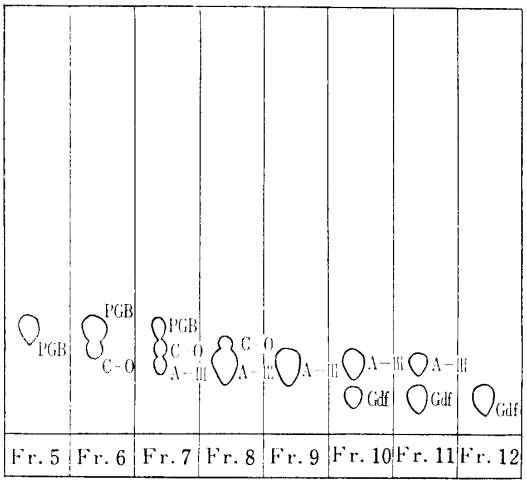

Fig. 21.

Paper Partition Chromatography of Some Fractions from Partition Chromatography of Fraction XXXI (With solvent system 4)

Fraction No. 9 showed a single spot corresponding to substance A-III and this fraction was recrystallized from hydr. MeOH to a colorless powder (1.34 g.), m.p. 220～223° (substance A-III).

Properties of Unknown Substances

1) **Substance B-0**——This is a syrupy substance, UV : λ_{max}^{EtOH} 217 mμ, and did not crystallize with various solvents. It gives positive Legal, Raymond, Frèrejacque, and Gregg-Gisvold reactions, and exhibits a blue glacial AcOH layer and a brown H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH, soluble in Me_2CO, sparingly soluble in H_2O, and insoluble in Et_2O.

2) **Substance PGA**——The crude substance PGA was repeatedly recrystallized from EtOH-Et_2O to a colorless powder, m.p. 248～254°; UV : λ_{max}^{EtOH} 218 mμ. It gives positive Legal, Raymond, and Gregg-Gisvold reactions, negative Frèrejacque reaction, and exhibits a blue glacial AcOH layer and a brown H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH and EtOH, sparingly soluble in Et_2O and H_2O.

3) **Substance A-II′**——The crude substance A-II′ was repeatedly recrystallized form Me_2CO-Et_2O-petr. ether to a colorless powder, m.p. 199～205°; UV : λ_{max}^{EtOH} 219 mμ. It gives positive Legal, Raymond, Gregg-Gisvold, and Frèrejacque reactions, and exhibits a pale brown glacial AcOH layer and a carmine-red H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH, Me_2CO, and $CHCl_3$, soluble in H_2O, sparingly soluble in Et_2O, and insoluble in petr. ether.

4) **Substance A-II**——This is a syrupy substance, UV : λ_{max}^{EtOH} 218 mμ, and did not crystallize from any of the various solvents used. It gives positive Legal, Raymond, Frèrejacque, and Gregg-Gisvold reactions, and exhibits a blue glacial AcOH layer and a carmine-red H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH, soluble in Me_2CO, sparingly soluble in H_2O, and insoluble in Et_2O.

5) **Substance B-I′**——The crude substance B-I′ was repeatedly recrystallized from MeOH-Me_2CO-Et_2O to a colorless powder, m.p. 184°/215～219°; UV : λ_{max}^{EtOH} 218 mμ. It gives positive Legal, Raymond,

and Gregg-Gisvold reactions, negative Frèrejacque reaction, and exhibits a pale brown glacial AcOH layer and a brown H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH, soluble in Me_2CO, and insoluble in H_2O and Et_2O.

6) **Substance B-I″**——The crude substance B-I″ was repeatedly recrystallized from Me_2CO-H_2O to colorless needles, m.p. 260~267°; UV : λ_{max}^{EtOH} 207 mμ. It gives positive Gregg-Gisvold reaction, negative Legal, Raymond, and Frèrejacque reactions, and exhibits a blue glacial AcOH layer and a colorless H_2SO_4 layer in the Keller-Kiliani reaction. It is soluble in MeOH, sparingly soluble in Me_2CO, and insoluble in H_2O.

7) **Substance PGB**——The crude substance PGB was repeatedly recrystallized from pyridine-EtOH to a colorless powder, m.p. 228~233°; UV : λ_{max}^{EtOH} 219 mμ. It gives positive Legal, Raymond, and Gregg-Gisvold reactions, negative Frèrejacque reaction, and exhibits a blue glacial AcOH layer and a carmine-red H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in pyridine and MeOH-$CHCl_3$ (1:1), soluble in MeOH, sparingly soluble in EtOH, and insoluble in Me_2CO, Et_2O, and H_2O.

8) **Substance A-III**——The crude substance A-III was repeatedly recrystallized from MeOH-Me_2CO-Et_2O to a colorless powder, m.p. 222~225°, UV : λ_{max}^{EtOH} 219 mμ. It gives positive Legal, Raymond, and Gregg-Gisvold reactions, and negative Frèrejacque reaction, and exhibits a pale brown glacial AcOH layer and a carmine-red H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH and EtOH, sparingly soluble in Me_2CO and H_2O, and insoluble in Et_2O.

9) **Substance A-III′**——Substance A-III′ was repeatedly recrystallized from MeOH-H_2O to colorless needles, m.p. 248~250°; UV : λ_{max}^{EtOH} 219 mμ. It gives positive Legal, Raymond, and Frèrejacque reactions, negative Gregg-Gisvold reaction, and exhibits a colorless glacial AcOH layer and a carmine-red H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH-$CHCl_3$ (1:1), soluble in MeOH, and insoluble in Et_2O and H_2O.

10) **Substance C-0**——The crude crystals of substance C-0 was recrystallized from MeOH-Me_2CO-H_2O to colorless prisms, m.p. 240~242°; UV : λ_{max}^{EtOH} 218 mμ. It gives positive Legal and Raymond reactions, negative Gregg-Gisvold and Frèrejacque reactions, and exhibits a colorless glacial AcOH layer and a brown H_2SO_4 layer in the Keller-Kiliani reaction. It is easily soluble in MeOH, sparingly soluble in Me_2CO, and insoluble in Et_2O and H_2O.

11) **Substance X_2**——The crude substance X_2 was repeatedly recrystallized from Me_2CO-H_2O to colorless plates, m.p. 172~177°. It is easily soluble in MeOH, soluble in Me_2CO, and insoluble in H_2O.

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Summary

Several new glycosides were isolated from digitalis seeds, four substances, B-I″, A-III′, C-0, and X_2, were obtained as crystals, and five substances, PGA, A-II′, B-I′, PGB, and A-III, were obtained as a colorless powder. In accordance with their coloration, it was assumed that substances B-0, PGA, B-I′, and C-0 belong to the digitoxigenin series, and substances A-II′, A-II, PGB, A-III, and A-III′ to the gitoxigenin series.

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