very convenient for the identification.*3 Sarcostin and crystal (4) from *Metaplexis japonica* have very similar R_{DC} values in the solvent system (7), but the solvent system (8) could clearly distinguish them.

Adrenal steroids which participated in as a control showed the R_M values already described. It is noteworthy that the solvent system (7) offered more excellent results than Zaffaroni's solvent system (6) in the shortness of time, clear separation and cheapness of the solvent.

Ester-compounds are much more difficult to separate, and further study is now in progress.

The authors are much indebted to Teikoku Hormonal Co. Ltd. for the samples of adrenal hormones. They also express their deep gratitude to Prof. T. Reichstein, Basel, Switzerland, for his kind cooperation on the identification of deacylcynanchogenin with lineolon.

Summary

The paper chromatographic separation of the polyhydroxy steroids in Asclepiadaceae plant was established. By this method, three aglycones were newly found in *Cynanchum caudatum*. The deacylcynanchogenin was proved to be identical with lineolon from *Pachycarpus lineoratus*.

(Received May 22, 1961)

*3 T. Reichstein sent us lineolon[2b] from *Pachycarpus lineolatus*, an African Asclepiadaceae plant, for the identification with deacylcynanchogenin. The identity was affirmed by paper chromatography and mixed melting point.

UDC 582.938:581.19

128. Hiroshi Mitsuhashi, Taro Nomura, Yuzuru Shimizu, Ikuko Takemori, and Emiko Yamada: Studies on the Constituents of Asclepiadaceae Plants. VIII.*2,1) On the Components of *Metaplexis japonica* MAKINO. 1.

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It was shown in the preceding papers[2~6] that C-nor-D-homosteroids had been isolated from *Cynancum caudatum* MAX. and *Marsdenia tomentosa* MORR. et DECNE. The similar compounds were reported from the plants of the same family by Reichstein, *et al.*,[7,8] and by Cornforth.[9] This study was intended to search these abnormal steroidal glycosides.

*1 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (三橋 博, 野村太郎, 清水 譲, 竹森郁子, 山田恵美子).

*2 A part of this work was reported at the 8th Hokkaido Local Meeting of the Pharmaceutical Society of Japan, January 28, 1961.

1) Part VII: This Bulletin, **10**, 808 (1962).
2) Part II: H. Mitsuhashi, Y. Shimizu: *Ibid.*, 8, 318 (1960).
3) Part III: *Idem*: *Ibid.*, 8, 738 (1960).
4) Part IV: *Idem*: *Ibid.*, **10**, 719 (1962).
5) Part V: *Idem*: *Ibid.*, **10**, 725 (1962).
6) Part VI: H. Mitsuhashi, I. Takemori, *et al.*: *Ibid.*, **10**, 804 (1962).
7) R.E. Winkler, T. Reichstein: Helv. Chim. Acta, **37**, 721 (1954).
8) T. Reichstein, *et al.*: *Ibid.*, **42**, 1015 (1959).
9) J.W. Cornforth: Chem. & Ind. (London), **1959**, 602.

Metaplexis japonica MAKINO (Japanese name, Gagaimo, *Asclepiadaceae* family) is a plant widely distributed in Japan and its seeds and leaves have been used as a crude drug, by the name of Rammashi, for a tonic. However, any report has not been found concerning the components of this plant.

Percolation of the stems and leaves of this plant with ethanol afforded an extract, which showed the positive Keller-Kiliani reaction[10] (bluish violet), suggesting a presence of glycoside containing 2-desoxy-sugar components. The method of extraction was shown in the Chart 1[11].

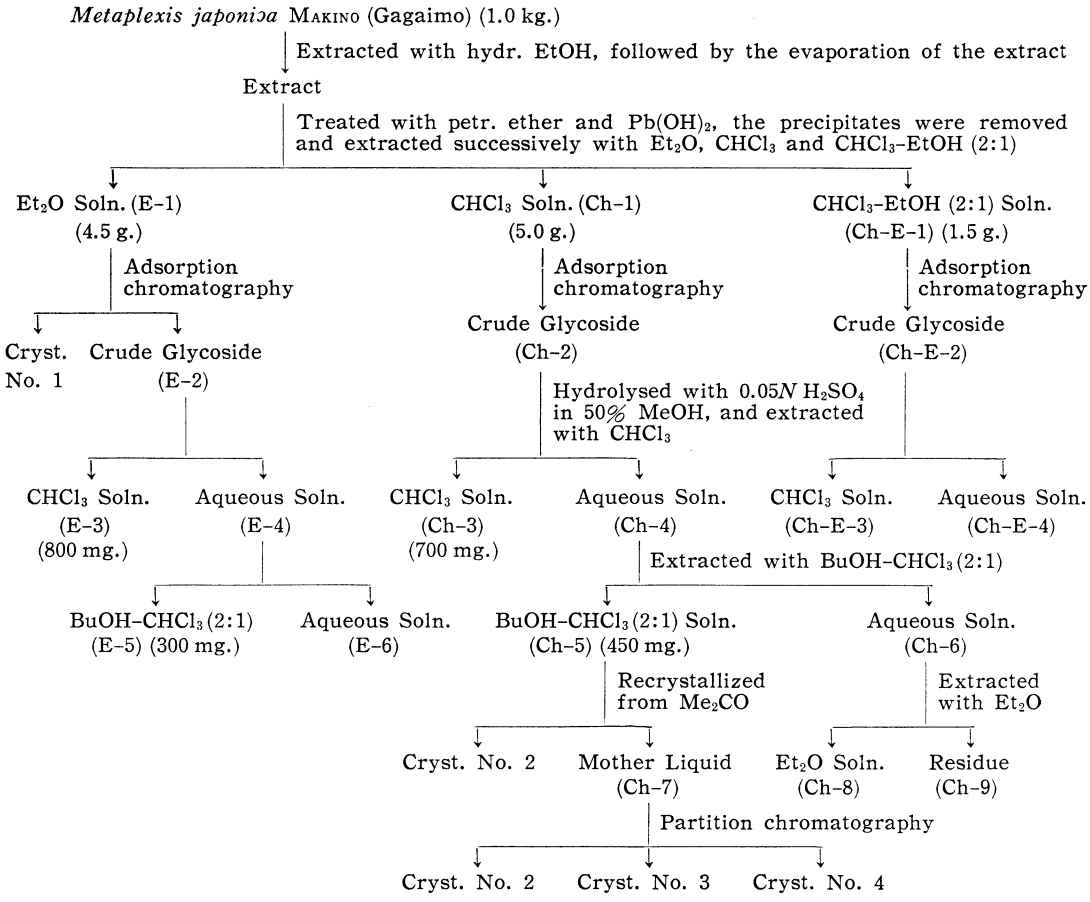


Chart 1. Isolation of Crude Glycosides

Namely, the stems and leaves of the plant were extracted with hydrous ethanol, treated with petroleum ether, and lead hydroxyde, and then extracted with ether (E-1), chloroform (Ch-1), and chloroform-ethanol (2:1) (Ch-E-1) mixture. These three fractions were submitted to chromatography over alumina. The crude glycoside, thus obtained, was yellowish green powder, possitive to the Keller-Kiliani reaction, antimony trichloride, and the Lieberman-Burchard reaction. Crystalline substance (crystal No.1), needles, m.p. 194~196°, was obtained from ether fraction, which showed yellow-yellowish green with the Liebermann-Burchard reaction, and pale yellowish brown with antimony trichloride.

10) T. Reichstein, *et al.*: Helv. Chim. Acta, **31**, 888 (1948).
11) *Idem*: *Ibid.*, **34**, 1821 (1951).

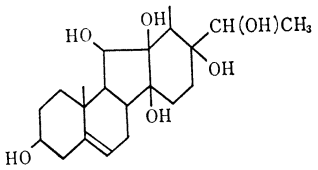
The glycoside (Ch-2) from the chloroform fraction was hydrolysed with 0.05*N* sulfuric acid in 50% methanol,[12] and after removal of methanol, extraction (Ch-3) with chloroform gave the aglycone mixture whose Keller-Kiliani reaction was negative. The aqueous layer (Ch-4) was positive to antimony trichloride, so the layer was extracted with butanol-chloroform (2:1) mixture.[13] After removal of the solvent, the residue (Keller-Kiliani reaction negative) of the butanol-chloroform (2:1) fraction (Ch-5) was recrystallized from acetone to crystalline substance (crystal No. 2), fine needles, m.p. 248/154°. This crystal shows green coloration with the Liebermann-Burchard reaction and pink-grayish blue with antimony trichloride, and then the elemental analysis gave the formula of $C_{21}H_{34}O_6$. Considering from these facts, this substance seemed to be sarcostin (A), and this was confirmed by the mixed melting point determination and the paper partition chromatographic analysis with the authentic sample.[1]

The partition chromatography[8] of the mother liquor, after removal of sarcostin, gave three kinds of crystalline substances. One of them was sarcostin, and the other one (crystal No. 3) showed m.p. 234~237° after several recrystallization from acetone. This crystal showed yellow-yellowish green coloration with the Lieberman-Burchard reaction, and yellow with antimony trichloride. The other one (crystal No. 4), m.p. 245°, showed yellowish green-yellowish gray with the Liebermann-Burchard reaction, and reddish violet with antimony trichloride.

The glycosides from ether (E-2), and chloroform-ethanol (2:1) (Ch-E-2) were hydrolysed and treated as same as the chloroform extract fraction (Ch-2), and almost the same results were obtained.

The chloroform layers (E-3), (Ch-3), and (Ch-E-3), were combined together because of the similarity of infrared charts, and purified through alumina column chromatography to give crystalline substances. Crystals (crystal No.5) fine needles, m.p. 264°, showed pink-yellowish brown coloration with the Liebermann-Burchard reaction, and grayish blue with antimony trichloride. The infrared spectrum was observed at 1240, 1720, and 1745 cm^{-1}. Considering from these facts, this substance seemed to be an ester.

The aqueous layer (Ch-6) from the chloroform extract fraction (Ch-1), after extraction of aglycone, showed strong Keller-Kiliani reaction (blue), and then it was extracted with ether. The paper partition chromatographic studies[14~17] of the extract with ether (Ch-8) and the syrup which was obtained by high vacuum distillation of the aqueous layer (E-6), showed two spots. The extracts of the spots on the paper gave positive Keller-Kiliani reaction. These spots were identified with those of the authentic D-cymarose and D-digitoxose. These 2-desoxy-sugars are the sugars which mainly occur



(A)

12) *Idem* : *Ibid.*, **37**, 737 (1954).
13) A. Okano, *et al.* : This Bulletin, **7**, 212 (1959).
14) T. Reichstein, *et al.* : Helv. Chim. Acta, **37**, 743 (1954).
15) E. Chargaff, *et al.* : J. Biol. Chem., **175**, 67 (1948).
16) R. Tschesche, *et al.* : Chem. Ber., **87**, 418 (1954).
17) T. Reichstein, *et al.* : Helv. Chim. Acta, **39**, 1490 (1956).

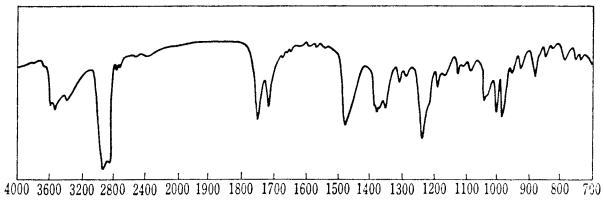


Fig. 1. Infrared Absorption Spectra of Crystal No. 5 (in Nujol)

in cardiac glycosides, but a few exceptions were reported in the plants of Asclepiadaceae family.[6~8,18,19]

Experimental*3

Extraction from *Metaplexis japonica* MAKINO——The stems and leaves of the plant collected at the campus of Hokkaido University, on June, 23, 1960, was dried, and powdered. 1 kg. of the powdered material was extracted with 19 L. of 50~80% hydr. EtOH at 60°. The deep greenish extract was concentrated at a temperature below 55° *in vacuo* and the residual substance was treated with 1 L. of 60% hydr. EtOH. Removing an insoluble material, the filtrate was treated with petr. ether (total 1 L.) and freshly prepared $Pb(OH)_2$, and neutralized with 5% H_2SO_4 to pH 6.0~6.2. The filtrate was concentrated to 500 cc. and the solution was extracted with 14 L. each of Et_2O, $CHCl_3$, and $CHCl_3$-EtOH (2:1) mixture. Each layer was washed with H_2O, 5% $NaHCO_3$, and H_2O successively, and dried over Na_2SO_4. Removal of each solvents gave 4.5 g. (Et_2O extract fraction) (E-1), 5.0 g. ($CHCl_3$ extract fraction) (Ch-1), and 1.5 g. ($CHCl_3$-EtOH=2:1 extract fraction) (Ch-E-1) of a deep greenish powder.

Alumina Adsorption Chromatography of Ether Extract Fraction (E-1)——The deep greenish residue (4.5 g.) from Et_2O fraction (E-1) was submitted to chromatography over 150 g. of alumina (neutral), giving results shown in Table I.

TABLE I. Chromatography of the Ether Extract Fraction (E-1)

Fraction No.	Solvent	Eluted product (mg.)	Form	Keller-Kiliani reaction	Liebermann-Burchard reaction
1~31	Benzene	320	oil	—	—
32~55	Benzene-$CHCl_3$ (50:50)	20	〃	〃	〃
56~95	$CHCl_3$	130	crystal No. 1 + powder	greenish blue	yellowish brown
96~107	$CHCl_3$-MeOH (99:1)	1,690	powder	〃	〃
108~122	$CHCl_3$-MeOH (95:5)	560	〃	〃	〃
123~141	$CHCl_3$-MeOH (80:20)	150	〃	〃	〃
141~157	$CHCl_3$-MeOH (50:50)	280	oil	—	—
158~166	MeOH-H_2O (90:10)	380	〃	〃	〃

Each fraction : 100 cc.
The fraction Nos. 96~141 were considered to be crude glycosides from the color test.

Alumina Adsorption Chromatography of the Chloroform Extract Fraction (Ch-1)——5.0 g. of the residue from the $CHCl_3$ fraction (Ch-1) was submitted to chromatography over 150 g. of alumina, giving results shown in Table II.

Alumina Adsorption Chromatography of the Chloroform-Ethanol (2:1) Mixture Extract Fraction (Ch-E-1)——1.4 g. of the residue from the $CHCl_3$-EtOH (2:1) fraction (Ch-E-1) was submitted to chromatography over 42 g. of alumina, giving results shown in Table III.

*3 All melting points were measured on a Kofler block and uncorrected.
18) Part I: H. Mitsuhashi, Y. Shimizu : This Bulletin, 8, 313 (1960).
19) F. Korte : Chem. Ber., 88, 1533 (1955).

Table II. Chromatography of the Chloroform Extract Fraction (Ch-1)

Fraction No.	Solvent	Eluted products (mg.)	Form	Keller-Kiliani reaction	Liebermann-Burchard reaction
1～15	$CHCl_3$-Benzene (50:50)	25	oil	—	—
16～34	$CHCl_3$	10	〃	〃	〃
35～50	$CHCl_3$-MeOH (99:1)	830	powder	greenish blue	pink→yellowish green
51～86	$CHCl_3$-MeOH (95:5)	1,520	〃	〃	〃
87～99	$CHCl_3$-MeOH (80:20)	120	〃	〃	〃
100～127	$CHCl_3$-MeOH (50:50)	720	〃	〃	〃
128～138	MeOH	320	oil	—	—
139～150	MeOH-H_2O (80:20)	500	〃	〃	〃

Each fraction : 100 cc.
The fraction Nos. 35～127 were considered to be crude glycosides from the color test.

Table III. Chromatography of the Chloroform-Ethanol (2:1) Extract Fraction(Ch-E-1)

Fraction No.	Solvent	Eluted product (mg.)	Form	Keller-Kiliani reaction	Liebermann-Burchard reaction
1～7	$CHCl_3$	50	oil	—	—
8～15	$CHCl_3$-MeOH (98:2)	trace	〃	〃	〃
16～23	$CHCl_3$-MeOH (95:5)	160	powder	greenish blue	pink→yellowish green
24～33	$CHCl_3$-MeOH (80:20)	140	〃	〃	〃
34～42	$CHCl_3$-MeOH (50:50)	120	〃	〃	〃
43～52	MeOH	100	oil	—	—
53～58	MeOH-H_2O (90:10)	160	〃	〃	〃

Each fraction : 100 cc.
The fraction Nos. 16～42 were considered to be the crude glycosides from the color test.

Glycoside from the Chloroform Extract Fraction (Ch-2)——1) Acid hydrolysis of the crude glycoside: 2.7 g. of the crude glycoside (Fr. Nos. 35～127 in Table II) was dissolved in 80 cc. of MeOH, followed by an addition of 80 cc. of 0.1*N* H_2SO_4, and refluxed for 25 min. MeOH was evaporated *in vacuo* at room temperature and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 5% $NaHCO_3$ solution, H_2O, and dried over Na_2SO_4 Removal of the solvent gave 700 mg. of the greenish powder (Keller-Kiliani reaction negative) (Ch-3). The aqueous layer was neutlarized with $Ba(OH)_2$, extracted with BuOH-$CHCl_3$ (2:1) mixture, and the organic layer was washed with H_2O. Removal of the solvent gave 450 mg. of yellowish green powder (Keller-Kiliani reaction negative) (Ch-5). This powder gave 15 mg. of the crystalline substance (crystal No. 2), m.p. 248/154°, which gave fine needles after repeated recrystallization from Me_2CO. The aqueous layer was evaporated in a reduced pressure, and refluxed with Et_2O. The extract was concentrated to a syrupy substance (65 mg.) (Ch-8).

2) Partition chromatography : A well shaken mixture of 140 g. of Celite in 140 cc. of H_2O was filled in a glass tube (inside diameter 3.4 cm.) by pressing with a glass rod to the height of 47 cm. 400 mg. of the residue (Ch-7), obtained from the mother liquor of the crystal No. 2, was dissolved in BuOH, added to 1 g. of Celite, stirred thoroughly to make homogeneous mixture, dried, and placed at the top of the column. This was developed with H_2O-saturated benzene-BuOH mixture. The results are shown in Table IV.

Glycoside from Ether Extract Fraction (E-2)——1) Acid hydrolysis : 1.8 g. of the glycoside was treated similary in the above experiment. The $CHCl_3$ layer (E-3) was 800 mg. of the greenish powder (Keller-Kiliani reaction negative). The BuOH-$CHCl_3$ (2:1) layer (E-5) was 300 mg. of the yellowish powder, which showed negative for the Keller-Kiliani reaction. The residue from the aqueous layer (E-6) was distilled at 10^{-5} mm. Hg at 140～150°(bath temp.), affording 160 mg. of colorless distillate giving strong positive Keller-Kiliani reaction.

2) Partition chromatography : 300 mg. of the residue, obtained from BuOH-$CHCl_3$ (2:1) layer (E-5),

Table IV. Partition Chromatography of the Butanol-Chloroform (2:1) Layer from the Chloroform Extract Fraction (Ch-7)

Fraction No.	Solvent	Eluted product (mg.)	Form
1～10	Benzene	95	oil
11～13	Benzene-BuOH (95:5)	20	〃
14～20	〃	70	oil+crystal No. 3
21～23	Benzene-BuOH (90:10)	30	oil+crystal No. 4
24～30	〃	120	oil
31～33	Benzene-BuOH (6:1)	20	oil+crystal No. 2
34～37	〃	5	oil
38～46	Benzene-BuOH (50:50)	50	oil

Each fraction : 100 cc.

Table V. The Partition Chromatography of the Butanol-Chloroform (2:1) Layer from the Ether Extract Fraction (E-5)

Fraction No.	Solvent	Eluted product (mg.)	Form
1～10	Benzene	70	oil
11～12	Benzene-BuOH (95:5)	trace	〃
13	〃	10	〃
14～18	〃	20	oil+crystal No. 3
19～20	〃	trace	oil
21～23	Benzene-BuOH (98:10)	10	oil+crystal No. 4
24～29	〃	30	oil
30～37	Benzene-BuOH (6:1)	25	〃
38～42	Benzene-BuOH (50:50)	40	〃
43～46	BuOH	60	〃

Each fraction : 100 cc.

was submitted to partition chromatography with 120 g. of Celite under the same condition as in the above experiments. The results were shown in the Table V.

Glycoside of the Chloroform-Ethanol (2:1) Extract Fraction——1) Acid hydrolysis : 570 mg. of the glycoside from the $CHCl_3$-EtOH (2:1) fraction (Ch-E-2) was treated as the $CHCl_3$ fraction. The $CHCl_3$ layer (Ch-E-3) gave 100 mg. of greenish powder (Keller-Kiliani reaction negative). The aqueous layer (330 mg.) (Ch-E-4) has not been treated.

Chromatography of the Chloroform Layer from the Hydrolysate——All the $CHCl_3$ layers (E-3), (Ch-3), and (Ch-E-3) were combined together and evaporated. The residue was treated by chromatography.

1) First chromatography : 1.6 g. of the residue was submitted to chromatography over 75 g. of alumina. The method applied was the same as the glycoside. The yellowish green powder was eluted with $CHCl_3$-MeOH (99:1), $CHCl_3$-MeOH (98:2), $CHCl_3$-MeOH (95:5), and $CHCl_3$-MeOH (80:20). Several attempts were failed to crystallize the eluted product.

2) Second chromatography : Each fraction, eluted with the solvent, mentioned above, were combined (total 850 mg.) and submitted to chromatography over 52 g. of alumina under the same condition as the first chromatography, giving results shown in Table VI.

Table VI. Chromatography of the Chloroform Layer

Fraction No.	Solvent	Eluted product (mg.)	Form
1～6	Benzene	trace	oil
7～54	$CHCl_3$	15	〃
55～70	$CHCl_3$-MeOH (99.5:0.5)	330	yellowish green powder
71～74	$CHCl_3$-MeOH (99:1)	85	crystal No. 5+powder
75～91	〃	105	yellowish green powder
92～117	$CHCl_3$-MeOH (98:2)	120	〃
118～137	$CHCl_3$-MeOH (95:5)	45	〃
134～148	$CHCl_3$-MeOH (80:20)	30	oil

Each fraction : 100 cc.

Crystal No. 1——Fraction Nos. 56～95 in Table I was recrystallized from Me_2CO to needles (total 10 mg.), m.p. 194～196°. The Liebermann-Burchard reaction showed coloration change in yellow→yellowish green. $SbCl_3$ color test showed pale yellowish brown. IR λ_{max}^{Nujol} cm^{-1} : 3200. 1150, 1095. 1040, 1015, 960, 910. *Anal.* Found : C, 78.31; H, 11.09.

Crystal No. 2 (Sarcostin)——The 15 mg. of this crystal, and the same substance 4 mg., obtained from the fraction Nos. 31~33 (in Table IV), were recrystallized from Me_2CO to needles, m.p. 248/154°, and showed emerald green with the Liebermann-Burchard reaction, pink→grayish blue with $SbCl_3$. *Anal.* Calcd. for $C_{21}H_{34}O_6$: C, 65.94; H, 8.96. Found : C, 66.18; H, 8.80. The mixed melting point with the authentic specimen showed no depression. The paper chromatographic analysis was carried out,[1] and the spot of the crystal No. 2 was identified to be of sarcostin.

Crystal No. 3——Fraction Nos. 14~20 (in Table IV), and Nos. 14~18 (in Table V) were recrystallized from Me_2CO to plates, m.p. 234~237° (total 4 mg.). It showed yellow→yellowish green with the Liebermann-Burchard reaction, and yellow with $SbCl_3$.

Crystal No. 4——Fraction Nos. 21~23 (in Table IV), and Nos. 21~22 (in Table V) were recrystallized from Me_2CO to needles, m.p. 245° (total 4 mg.). It showed yellowish green→yellowish gray with the Liebermann-Burchard reaction, and reddish violet with $SbCl_3$. Paper chromatographic analysis was carried out with the crystal No. 3 and No. 4.[1] See Fig. 2.

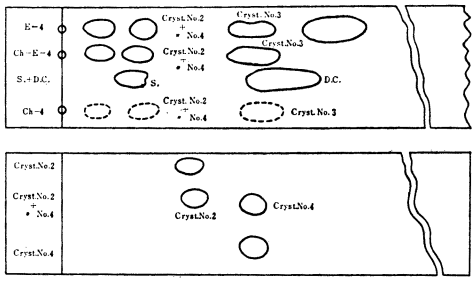


Fig. 2. Paper Chromatographic Analysis of Aglycone Parts

Crystal No. 5——Fraction Nos. 71~74 (in Table VI) was recrystallized from Me_2CO to needles, m.p. 264° (total 20 mg.). It showed pink→yellowish brown with the Liebermann-Burchard reaction, and grayish blue with $SbCl_3$. IR see Fig. 1. *Anal.* Found : C, 65.19; H, 7.91.

Paper Chromatography of the Sugar——The sugar syrups, obtained on hydrolysis of the glycoside from Et_2O (E-6) and $CHCl_3$ extract fraction (Ch-8), were submitted to paper chromatography with the authentic samples. The results were shown in Table VII.

Table VII. Paper Chromatography of the Sugar Portion

	Rf Value			
	(BuOH-1% NH_3)	(BuOH-Pyridine-H_2O) (3:2:1.5)	(AcOEt-Pyridine-H_2O) (2:1:2)	(Toluene-BuOH-H_2O) (1:9:3)
Sugar from the glycoside	0.667	0.722	0.874	0.615
	0.496	0.622	0.689	0.404
D-Cymarose	0.667	0.728	0.867	0.618
D-Digitoxose	0.500	0.629	0.665	0.408

Toyo Roshi No. 50, ascending method, detected by 0.1*N* $AgNO_3$ and 5.0*N* NH_3.

The appropriate areas of the spots were extracted with Me_2CO, and tested by the Keller-Kiliani reaction to give positive results.

The authors express their deep gratitude to Dr. Y. Sasagawa, Takeda Research Laboratories for making the precious sample of D-digitoxose available. They are much indebted to Mr. K. Narita of this Institute and Miss T. Oshibe of Hoshi College of Pharmacy for elemental analysis.

Summary

The stems and leaves of *Metaplexis japonica* Makino were proved to contain glycoside mixture. The glycoside showed the Keller-Kiliani reaction, suggesting the presence of 2-desoxy-sugar. The sugar portion of the glycoside was found to be D-cymarose and D-digitoxose by paper partition chromatography when compared with the authentic specimen. Five kinds of crystal, such as crystal No. 1, m.p. 194~196°, crystal No. 2, m.p. 248/154°, crystal No .3, m.p. 234~237°, crystal No. 4, m.p. 245°, and crystal No. 5, m.p. 264°, were isolated by adsorption chromatography using alumina, and partition chromatography over Celite as a carrier. Crystal No. 2 was identified to be sarcostin by the mixed melting point determination and the paper chromatography.

(Received May 22, 1961)