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126. **Hiroshi Mitsuhashi, Ikuko Takemori, Yuzuru Shimizu, Taro Nomura, and Emiko Yamada** : Studies on the Constituents of Asclepiadaceae Plants. VI.1) On the Components of *Marsdenia tomentosa* DECNE.[*2]

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Marsdenia tomentosa DECNE (Japanese name Kijoran, Asclepiadaceae) is widely distributed in the southern part of Japan. There are many investigations on *Marsdenia condurango*,2) but none of the study has been reported concerning the constitutents of *M. tomentosa.*

Authors have investigated the constituents of this plant as a part of studies on the constituents of Asclepiadaceae plants to know whether any of C-nor-D-homosteroid glycosides could be isolated from it.

Successive percolation of the powder of this whole plant with 70% ethanol on a water bath at 50~60° afforded dark green extract, which showed strong Keller-Kiliani reaction (blue) suggesting the presence of glycosides, containing 2-desoxy sugar, but active methylene reactions (Raymond and Legal reaction) for the extract were negative. Therefore it is reasonable to assume that the glycosides are not a cardiac glycoside type.

The extract was precipitated with petr. ether for several times, and then treated with freshly prepared lead hydroxide3) under vigorous agitation. The viscous mixture was filtered off, and to the filtrate 95% ethanol was added. After the separation ethanol filtrate was concentrated in a reduced pressure.

The ethanolic solution thus obtained was successively extracted with ether, chloroform and chloroform-ethanol (2:1), and separated into three fractions. Column chromatography using alumina was adopted for their purification. From each chromatography crude glycosides were eluted as white powder, which showed positive Keller-Kiliani reaction, Liebermann-Burchard reaction and antimony chloride reaction. These facts suggested the existence of steroid glycoside containing 2-desoxy sugars.

Infrared spectra of these glycosides are resemble to that of the glycoside isolated from *Cynanchum caudatum* MAX4) (Fig. 1).

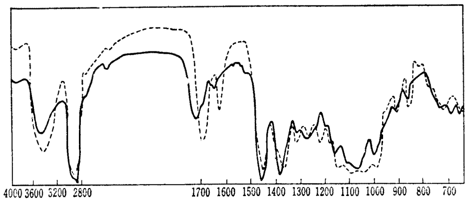

Fig. 1. Infrared Spectra of Glycosides

—— Glycoside isolated from *Marsdenia tomentosa* DECNE.
······ Glycoside isolated from *Cynanchum caudatum* MAX.

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

1) Part V : This Bulletin, **10**, 725 (1961).

2) Vulpus : Arch. Pharm., **223**, 299 (185); Carrara : Gazz. chim. ital., **21**, (I), 204 (1891); Kubler: Arch. Pharm. **246**, 620 (1908); F. Korte, I. Korte : Z. Naturforsch., **10b**, 223 (1955); F. Korte: Chem. Ber., **88**, 1527 (1955); F. Korte, H. Weitkamp : Chem. Ber., **89**, 1669 (1956); F. Korte, J. Ripphahn : Ann., **621**, 58 (1959). There is one brief report on *M. erecta* R. Br. (T. Baytop, M. Tanker, N. Öner, S. Tekman : Nature, **184**, 1319 (1959)).

3) T. Reichstein *et al.* : Helv. Chim. Acta, **34**, 1824 (1951).

4) H. Mitsuhashi, Y. Shimizu : This Bulletin, 8, 313 (1960).

Crude glycosides from each fraction were respectively hydrolysed with 0.05N sulfuric acid, extracted with chloroform and separated to aglycones and sugars.

Sugar portions from the hydrolysis of these three fractions had been proved to have quite the same components by paper partition chromatography.[5] Cymarose of m.p. 63∼70° was isolated from the syrupy sugars by high vacuum distillation. The others were assumed to be digitoxose and glucose by paper chromatographic analysis.

From their infrared spectra and paper chromatographic analysis, it is reasonable to assume the aglycones were very similar, and the amount was so small that they were mixed together and purified through alumina chromatography. Aglycones seemed to be ester from their infrared spectra. The main fraction from the rechromatography was hydrolysed with 5% methanolic-KOH, extracted with ether and a new aglycone named tomentogenin was obtained as colorless plates, m.p. 247∼249°. Similar treatment of the following fraction gave sarcostin, m.p. 150°/245∼250° which was isolated from *Cynanchum caudatum*[6] and other Asclepiadaceae plants.[7]

From the results of paper partition chromatography, the acidic portions which were produced from the hydrolysis of two fractions of the above seemed to have the same components. The main components were tiglic acid, m.p. 63.5° and isovaleric acid. Tiglic acid was crystallized and approved by the paper chromatographic analysis and the melting point determination comparing with the authentic specimen of synthesized tiglic acid. The existence of isovaleric acid and other minor acids were identified by the paper chromatographic analysis.[8]

Experimental

Extraction——The whole plants of *Marsdenia tomentosa* Decne. collected at Mt. Takao in Asakawa, suburbs of Tokyo, on April 1960 were chipped, dried and powdered. 1 kg. of the material was extracted with 70% EtOH on a water bath at 50∼60°. The percolation was repeated until the solution became colorless. 15 L. of extracted solution was concentrated to about 1 L. and it was shaken vigorously with freshly prepared $Pb(OH)_2$ for about 15 min. and then filtered. Weak alkaline filtrate was neutralized with dil. H_2SO_4 and concentrated to about 500 cc. in a reduced pressure. It was extracted with Et_2O, $CHCl_3$ and $CHCl_3$-EtOH (2:1) successively. Upon evaporation of the solvents, 2.5 g. from Et_2O extract, 7.5 g. from $CHCl_3$ extract and 2.0 g. from $CHCl_3$-EtOH (2:1) extract were obtained. As the Keller-Kiliani reaction following to the method of T. Reichstein's[10] were positive, they seemed to be glycosides containing 2-desoxy-sugars. These crude glycosides were chroma-

Table I. Column Chromatographic Analysis of the Crude Glycoside extracted with Ether

Fraction No.	Solvent	Eluted product (mg.)	Keller-Kiliani reaction
1∼5	Benzene	trace	—
6∼7	Benzene-$CHCl_3$ (4:1)	〃	—
8∼13	Benzene-$CHCl_3$ (3:2)	〃	—
14∼17	Benzene-$CHCl_3$ (2:3)	〃	—
18∼23	$CHCl_3$	〃	—
24	$CHCl_3$-MeOH (95:5)	1400	bluish green
25	$CHCl_3$-MeOH (95:5)	200	〃
26∼28	$CHCl_3$-MeOH (95:5)	trace	〃
29∼35	$CHCl_3$-MeOH (9:1)	〃	〃

Each fraction : 200 cc.

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5) T. Reichstein : Helv. Chim. Acta, **37**, 743 (1954); F. Korte : Chem. Ber., **88**, 1533 (1955); R. Tscheshe, G. Grimmer : *Ibid.*, **87**, 418(1954).

6) Part V : H. Mitsuhashi, Y. Shimizu : This Bulletin, **10**, 725 (1962).

7) J. W. Cornforth, J. C. Earl : J. Chem. Soc., **1939**, 737 E. Ahisch, C. Tamm, T. Reichstein: Helv. Chim. Acta, **42**, 1018 (1959).

8) Brown, Hall : Nature, **166**, 66 (1950).

10) T. Reichstein *et al.* : Helv. Chim. Acta, **31**, 888 (1948).

Table II. Column Chromatographic Analysis of the Crude Glycoside extracted with Chloroform

Fraction No.	Solvent	Eluted product (mg.)	Keller-Kiliani reaction
1～3	$CHCl_3$	trace	—
4～8	$CHCl_3$-MeOH (95:5)	150	bluish green
9～12	$CHCl_3$-MeOH (9:1)	1200	〃
13～18	$CHCl_3$-MeOH (3:1)	510	〃
19～23	$CHCl_3$-MeOH (1:1)	220	〃
24～28	MeOH	trace	〃
29	MeOH-H_2O (9:1)	690	〃
30	MeOH-H_2O (1:1)		

Each fraction : 200 cc.

Table III. Column Chromatographic Analysis of the Crude Glycoside extracted with $CHCl_3$-EtOH (2:1)

Fraction No.	Solvent	Eluted product (mg.)	Keller-Kiliani reaction
1～2	$CHCl_3$-MeOH (95:5)	trace	—
3～4	$CHCl_3$-MeOH (90:10)	280	bluish green
5～7	$CHCl_3$-MeOH (85:15)	350	〃
8～13	$CHCl_3$-MeOH (3:1)	290	〃
14～17	$CHCl_3$-MeOH (1:1)	trace	〃
18～22	MeOH	〃	〃
23～28	MeOH-H_2O (9:1)	360	〃
29～32	MeOH-H_2O (1:1)		

Each fraction : 200 cc.

tographed over neutral alumina. The results are shown in Tables I, II and III. Several attempts were failed to crystallize the eluted products.

Hydrolysis of the Glycoside——1 g. of the crude glycosides from Et_2O extract was dissolved in 30 cc. of MeOH, refluxed for 25 min. with 30 cc. of 0.1*N* H_2SO_4 on a water bath. MeOH was evaporated *in vacuo* at room temperature and the resulting mixture was extracted with $CHCl_3$, which was washed with 5% $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. Removal of the solvent gave 370 mg. of a yellowish powder (Keller-Kiliani reaction negative) which was considered to be aglycone. As likely as above, the crude glycosides from $CHCl_3$ and $CHCl_3$-EtOH (2:1) extract gave respectively 960 mg. and 280 mg. of yellowish powder (Keller-Kiliani reaction negative).

IR spectra of these three aglycones resemble so closely that they were mixed and rechromatographed over alumina. The result was shown in Table IV. The aqueous layers of hydrolysates seemed to contain sugars.

Table IV. Column Chromatographic Analysis of the Mixed Aglycones

Fraction No.	Solvent	Eluted product (mg.)	Liebermann-Burchard reaction
1～5	Benzene	trace	—
6～9	Benzene-$CHCl_3$ (9:1)	〃	〃
10～12	Benzene-$CHCl_3$ (1:1)	〃	〃
13～18	$CHCl_3$	〃	〃
19	$CHCl_3$-MeOH (99:1)	220	pink→green→yellowish green
20～23	$CHCl_3$-MeOH (99:1)	150	〃
24～27	$CHCl_3$-MeOH (95:5)	trace	〃
28～30	$CHCl_3$-MeOH (9:1)	〃	〃
31～34	$CHCl_3$-MeOH (5:1)	〃	〃

Each fraction : 200 cc.

Hydrolysis of Aglycone——220 mg. of yellowish white powder from Fr. 19, Table IV was dissolved in 5% MeOH-KOH and refluxed for 25 min. MeOH was evaporated and the residue was extracted with Et_2O. Removal of Et_2O gave a white crystalline mass which was recrystallized from Me_2CO to colorless plates, m.p. 247～249°. Similarly 150 mg. of yellowish powder from Fr. 20～23, Table IV was hydrolysed and treated in the same manner. Removal of Et_2O gave a white crystalline mass which was recrystallized from Me_2CO to colorless needles, m.p. 150°/245～250°.

Crystal No. 1 (**tomentogenin**)——By hydrolysis of Fr. 19, 30 mg. of colorless plates, m.p. 247～249° were obtained and named tomentogenin. The Liebermann-Burchard reaction showed coloration

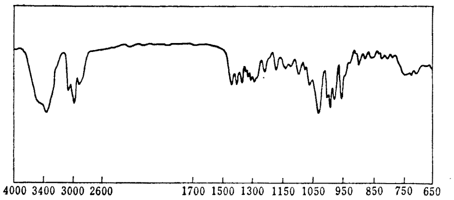

Fig. 2. Infrared Spectrum of Tomentogenin

change in pink→green→yellowish green, and pink by $SbCl_3$. The reaction with tetranitromethane was weakly positive (Fig. 2). *Anal.* Calcd. for $C_{21}H_{34}O_5$: C, 68.50; H, 9.43. Found : C, 68.82; H, 9.85,

Crystal No. 2 (sarcostin)——By hydrolysis of Fr. 20~23, 20 mg. of colorless needles, m.p. 150°/245~250° were obtained. The Liebermann-Burchard reaction gave coloration change in pink→green. It showed reddish violet color by $SbCl_3$, The melting point and paper chromatographic analysis[11] suggests that it might be sarcostin which present in other Asclepiadaceae plants. See Fig. 3. The mixed melting point with authentic sample of sarcostin[6] showed no depression.

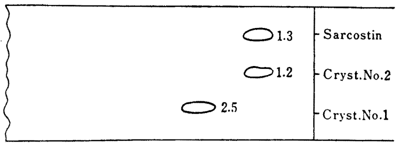

Fig. 3. Paper Chromatographic Analysis of Deacyl Aglycones

System : Benzene/Propylene glycol
Time : 25 hr.

Sugars of the Glycosides——The aqueous layers obtained by the hydrolysis of the glycosides were respectively neutralized with freshly prepared $Ba(OH)_2$, evaporated to dryness in a reduced pressure, and extracted with Me_2CO, followed by the evaporation to syrup. Paper chromatographic analysis of these three kinds of syrup, gave the results shown in Table V. In consequence, they contain glucose and two kinds of 2-desoxy sugars which were proved by the Keller-Kiliani reaction giving positive spots on the appropriate area. From the result that these sugar syrups contain the same components, they were mixed and distillated at 10^{-4} mm-Hg and 140~150° (bath temp.), and afforded a colorless distillate which gave strong positive Keller-Kiliani reaction. Upon standing it gave needles, m.p. 63~70°. Paper chromatographic analysis showed identity with cymarose.

TABLE V. Paper Partition Chromatography of the Sugar Portion

Test substance		Sugar from glycoside	
1. Rf Values in BuOH-1% NH_3			
D-Glucose	0.07	0.07	Glucose
D-Digitoxose	0.451	0.434	
D-Cymarose	0.670	0.666	Cymarose
2. Rf Values in BuOH-Pyridine-H_2O=3:1:3			
D-Glucose	0.20	0.20	Glucose
D-Digitoxose	0.646	0.59	
D-Cymarose	0.686	0.676	Cymarose
3. Rf Value in AcOEt-pyridine-H_2O=2:1:2			
D-Glucose	0.20	0.20	Glucose
D-Digitoxose	0.514	0.490	
D-Cymarose	0.77	0.718	Cymarose

Toyo Roshi No. 50, descending method, detected by 0.1*N* $AgNO_3$ and 5.0*N* NH_3 (by Partridge)

Acids from the Hydrolysate of Aglycones——The water layer from the hydrolysate of Fr. 19 was acidified with H_3PO_4, extracted with Et_2O and dried over Na_2SO_4. Removal of Et_2O gave yellowish oily substance. The H_2O layer from the hydrolysate of Fr. 20~23 was treated as the same manner as the Fr. 19 and gave the similar substance.

Paper Chromatography of the Acid Portions——Paper chromatographic analysis of the acid substances carried out and afforded five spots shown in Table VI. As main components, tiglic acid and isovaleric acid were assumed from their Rf values. Besides them AcOH and other two unknown acids were present as minor components.

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[11] Part VII. H. Mitsuhashi, Y. Shimizu, *et al.* : This Bulletin, **10**, 808 (1962).

TABLE VI. Paper Partition Chromatography of the Acid Portion

Rf Values in BuOH-1.5N NH_3

Test substance		Acid from glycoside	
Acetic Acid	0.10	0.096	Acetic acid
Butyric Acid	0.294	0.252	
Angelic Acid	0.313		
Tiglic Acid	0.350	0.340	Tiglic acid
Isovaleric Acid	0.430	0.428	Isovaleric acid
Valeric Acid	0.444		
Capronic Acid	0.541	0.554	

Toyo Roshi No. 50, ascending method, detected by Bromthymol Blue.

Identification of tiglic acid: Upon standing, the acid fraction was partly crystallized to plates, m.p. 63°. Its Rf value and melting point seem to be identical with those of tiglic acid. The mixed melting point with the synthesized tiglic acid started from angelic acid showed no depression.

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Summary

The whole plant of *Marsdenia tomentosa* DECNE. was proved to contain a glycoside mixture which showed strong Keller-Kiliani reaction, suggesting the presence of 2-desoxy sugars. The suger part which was obtained by acid hydrolysis was found to be glucose and cymarose by the paper chromatography compared with the authentic specimens. The aglycones were presumed to be esters. Their alkaline hydrolysis afforded two kinds of crystals, tomentogenin, a new aglycone (m.p. 247~249°) and sarcostin (m.p. 150°/245~250°) and from acidic portions tiglic acid, isovaleric acid, and acetic acid were found.

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127. **Hiroshi Mitsuhashi, Yuzuru Shimizu, Emiko Yamada, Ikuko Takemori, and Taro Nomura**: Studies on the Constituents of Asclepiadaceae Plants. VII.[1] Paper Chromatographic Separation of Steroidal Aglycones in Asclepiadaceae Plants.[*2]

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It has been found by Reichstein and his collaboraters, present authors[1,3] and other workers[2c,d] that a series of ester-glycosides which might belong to polyhydroxy C-nor-D-homopregnanes is present in Asclepiadaceae plants, and part of their structure was

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

[*2] This work was reported at the 14th Annual Meeting of the Pharmaceutical Society of Japan, July. 1961 at Sapporo.

1) Part VI. This Bulletin, **10**, 804 (1962).

2) *a*) R.E. Winkler, T. Reichstein: Helv. Chim. Acta, **37**, 721 (1954). *b*) E. Abisch, Ch. Tamm, T. Reichstein: *Ibid.*, **42**, 1015 (1959). *c*) F. Korte, H. Ripphahn: Ann., **621**, 58 (1958). *d*) J.W. Cornforth, J.C. Earl: J. Chem. Soc., **1937**, 737; *ibid.*, **1940**, 1443.

3) *a*) H. Mitsuhashi, Y. Shimizu: This Bulletin, 8, 313 (1960). *b*) *Idem*: *Ibid.*, **10**, 725 (1962). *c*) H. Mitsuhashi, T. Nomura, Y. Shimizu, I. Takemori, E. Yamada: *Ibid.*, **10**, 811 (1962).