UDC 582.938 : 581.19

129. Hiroshi Mitsuhashi, Teiko Hiroshige, Ikuko Takemori, Yuzuru Shimizu, Taro Nomura, and Emiko Yamada : Studies on the Constituents of Asclepiadaceae Plants. IX.[1] On the Components of *Cynanchum caudatum* MAX.

(*Faculty of Pharmaceutical Sciences, School of Medicine, University of Hokkaido*[*1])

In the preceeding works,[2~4] new components, cynanchogenin and penupogenin were obtained from the tuberous root of *Cynanchum caudatum* MAX., and their structures were elucidated.

In the present paper some later findings will be reported on the components of the stems and leaves. The material plant was collected at Nishino, near Sapporo in June 1960, and dried at 60°. The powdered material was treated as shown in Chart 1, and described in the experimental part. Extraction was done by the method which used for the extraction of cardiac glycoside by Reichstein, *et al.*[5]

Thus obtained fractions, E-1, Ch-1, and Ch-E-1 showed strong positive for the Keller-Kiliani, Liebermann-Burchard, and Salkowski reactions, but negative to the

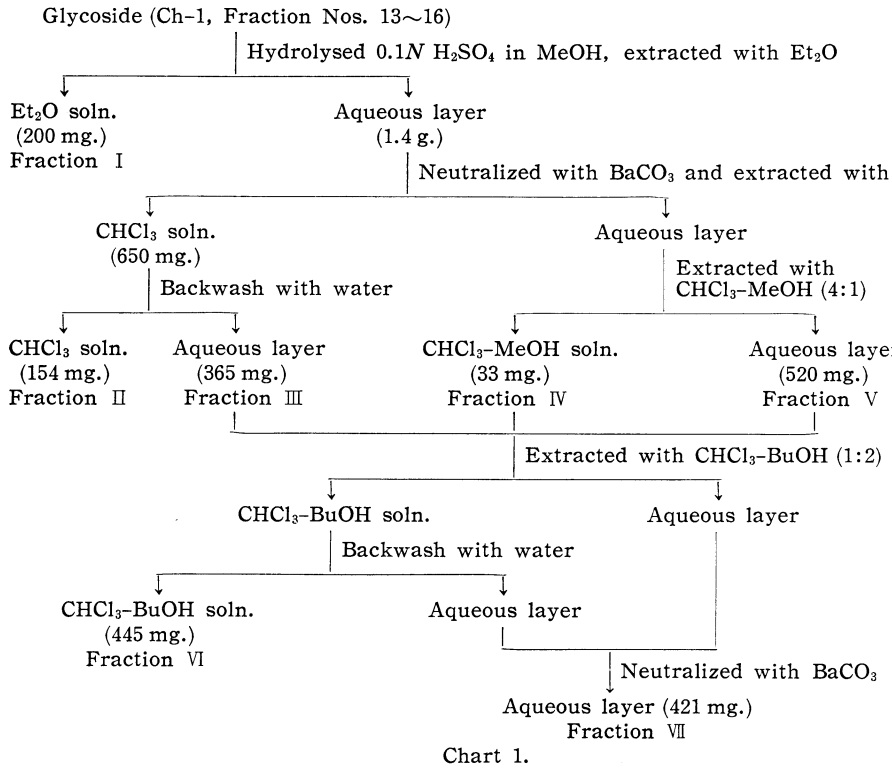


Chart 1.

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

1) Part Ⅷ : This Bulletin, **10**, 811 (1962).
2) H. Mitsuhashi, Y. Shimizu : This Bulletin, 8, 313 (1960).
3) *Idem* : *Ibid.*, 8, 565 (1960).
4) *Idem* : *Ibid.*, 8, 738 (1960).
5) T. Reichstein, *et al.* : Helv. Chim. Acta, **31**, 888 (1948).

Legal test. Therfore, the presence of a glycoside containing 2-desoxy-sugar component was assumed. Crude glycoside fraction E-1, Ch-1, and Ch-E-1 were chromatographed over an alumina column. The results were shown in Tables I, II, and III. The Ch-1 fraction represented the main part of glycoside and the fraction Nos. 13~16 of Ch-1 was examined thoroughly.

The fraction Nos. 13~16, was hydrolysed with 0.1*N* sulfuric acid in methanol, the same condition usually applied to the hydrolysis of cardiac glycosides containing 2-desoxy-sugar, and after removal of methanol, extraction with ether gave a Keller-Kiliani reaction-negative aglycone mixture (I).[6] (Chart 1).

Aqueous layer was neutralized with barium carbonate and then extracted with chloroform. As the separation of aglycone and sugar was insufficient, further separation was carried out under the indication of coloration in the Liebermann-Burchard and Keller-Kiliani reactions.

Finally, (VI) shows Liebermann-Burchard reaction, pink→orange→dark yellow green, and negative Keller-Kiliani reaction. In the paper partition chromatographic studies, established in the authors laboratory for the research of C-nor-D-homosteroid analogs,[7] fration VI showed five spots, two of them corresponds to deacylcynanchogenin and sarcostin. It is very interesting because so far as the present studies concerns, most of the C-nor-D-homosteroids separated from Asclepiadaceae family were ester type. From the root of *Cynanchum caudatum*, two ester type aglycone were found, and this time free aglycones were separated from the herbs and stems of the same plant. So concentrated studies were done to fraction VI.

Fig. 1.

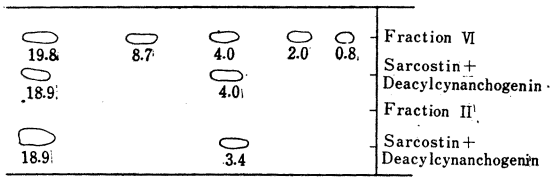


Fig. 2.

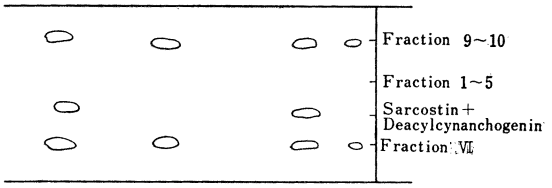


Fig. 3

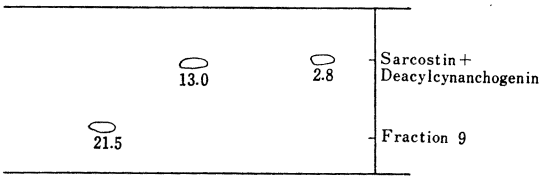
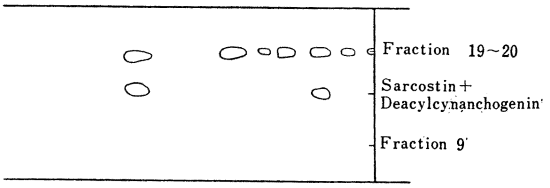


Fig. 4.



6) T. Reichstein, *et al.* : Helv. Chim. Acta, **37**, 737 (1954).
7) H. Mitsuhashi, *et al.* : This Bulletin, **10**, 808 (1962).

Thus gained 445 mg. of fraction VI was chromatographed over Celite (Table IV). 50 mg. of fraction Nos. 9~10 was obtained as faintly colored powder. The paper partition chromatographic studies of this fraction showed 4 spots. Rechromatography of the fraction Nos. 9~10 over alumina was done (Table V).

Two main fractions, No. 9 and Nos. 19~20 were separated. Fraction No. 9 gave needles from ether, m.p. 215~220°(Kofler). Liebermann-Burchard reaction: pink→yellow orange. Antimony trichloride: pink→violet blue.

From the result of paper chromatographic analysis (Fig. 3), this crystals were assumed to be less polar than deacylcynanchogenin, and further examination is on the way. The fraction Nos. 19~20 revealed to consist of deacylcynanchogenin, sarcostin and other 4 components shown as Fig. 4. The aqueous layer (VII) displayed a strong Keller-Kiliani reaction (blue). The paper partition chromatographic studies[8~10] of this fraction and the syrup was obtained by high vacuum distillation of the aqueous layer (VII) showed one spot. The spot was identified as D-cymarose comparing with authentic sample.

Experimental

Extraction from *Cynanchum caudatum* MAX.——One kg. of powdered herbs and stems was extracted with 12 L. of 50~70% EtOH at 60°. The deep green extract was concentrated below 60° *in vacuo* and 800 cc. of the residual substance was treated with 90 cc. of petr. ether. This petr. ether was back washed with 50% EtOH. The mixed residual substance and EtOH solution were added with freshly prepared $Pb(OH)_2$ with vigorous stirring. The precipitate was removed by filtration, and the filtrate was neutralized with 5% H_2SO_4 to pH 6.0. The filtrate was concentrated to 60 cc. in a reduced pressure. 1.8 L. of EtOH was added to this solution, and the insoluble substance was removed. The filtrate was concentrated to 500 cc. *in vacuo* below 60°, and the solution was extracted successively with 1.3 L. of Et_2O, $CHCl_3$, and $CHCl_3$-EtOH (2:1) mixture.

Et_2O solution was washed with H_2O (total 400 cc.), 5% $NaHCO_3$ (400 cc.), and H_2O (400 cc.), and each washing solution was kept for the next washing. $CHCl_3$ solution was also washed successively with above used washing solutions. $CHCl_3$-EtOH solution was treated as the same manner. Each organic layer was dried over Na_2SO_4 and removal of solvents gave 0.8 g. (Et_2O fraction, E-1), 4.5 g. ($CHCl_3$ fraction, Ch-1), and 1.05 g. ($CHCl_3$-EtOH fraction, Ch-E-1) of pale yellow powder.

Alumina Adsorption Chromatograpy of E-1, Ch-1, and Ch-E-1 Fractions——These residues were submitted to chromatography over alumina, giving results shown in Tables I~III.

TABLE I. Chromatography of the E-1 Fraction
E-1; 0.80 g. Alumina; 30 g.

Fraction No.	Solvent	Eluted product (g.)	Keller-Kiliani reaction
1~5	Benzene	oil	−
6~10	〃	〃	−
11~30	〃	trace	−
31~60	〃	〃	−
61~110	Benzene-$CHCl_3$ (1:1)	〃	−
111~134	$CHCl_3$	〃	−
135~136	$CHCl_3$-MeOH (95:5)	powder	+
137~157	〃	oil	+
158~177	$CHCl_3$-MeOH (80:20)	trace	±

Each fraction: 20 cc.

Acid Hydrolysis of the Crude Glycosides——2.4 g. of the crude glycoside obtained from Ch-1, fraction Nos. 13~16, was dissolved in 72 cc. of MeOH, and the mixture was refluxed for 25 min. after addition of 72 cc. of 0.1*N* H_2SO_4. MeOH was evaporated *in vacuo* at room temperature, and the residue was extracted with Et_2O. The Et_2O layer was washed 5% $NaHCO_3$ solution and H_2O,

8) T. Reichstein, *et al.*: Helv. Chim. Acta, **37**, 743 (1954).
9) E. Chargaff, *et al.*: J. Biol. Chem., **175**, 67 (1948).
10) R. Tschesche, *et al.*: Chem. Ber., **87**, 418 (1954).

Table II. Chromatography of the Ch-1 Fraction
Ch-1; 4.5 g. Alumina; 130 g.

Fraction No.	Solvent	Eluted product (g.)	Keller-Kiliani reaction
1～5	$CHCl_3$	oil	—
6～12	〃	〃	—
13～16	$CHCl_3$-MeOH (95:5)	powder 2.4	Violet→blue
17～22	〃	trace	〃
23～30	〃	powder 1.05	〃
31～44	$CHCl_3$-MeOH (80:20)	trace	〃
45～56	〃	〃	〃
57～62	$CHCl_3$-MeOH (50:50)	powder	—
63～67	〃	〃	—
68～75	〃	trace	—
76～95	MeOH	amorphous	—

Each fraction : 20 cc.

Table III. Chromatography of the Ch-E-1 Fraction
Ch-E-1; 1.05 g. Alumina; 30 g.

Fractin No.	Solvent	Eluted product (g.)	Keller-Kiliani reaction
1～3	$CHCl_3$	oil	—
4～17	〃	trace	—
18～42	$CHCl_3$-MeOH (95:5)	powder	Violet→blue
43～54	$CHCl_3$-MeOH (80:20)	〃	〃
55～67	$CHCl_3$-MeOH (50:50)	〃	—
68～79	MeOH	〃	—

Each fraction : 50 cc.

and dried over Na_2SO_4. Removal of the solvent gave 200 mg. of a powder, fraction I, Keller-Kiliani reaction; negative. Aqueous layer was neutralized with $BaCO_3$ and concentrated to a syrup in a reduced pressure. The syrup was extracted three times with each 30 cc. of $CHCl_3$ and fraction II remained. The $CHCl_3$ layer was backwashed with 100 cc. of H_2O three times. The aqueous layer was extracted again with $CHCl_3$-MeOH (4:1). But Liebermann-Burchard reaction of fractions III, IV, and V (See Chart 1) were all positive, so combined together, and reextracted six times with $CHCl_3$-BuOH (1:2), using total 180 cc. until $CHCl_3$-BuOH solution showed negative Liebermann-Burchard reaction. This combined extract solution was washed four times with 50 cc. of H_2O, and dried over Na_2SO_4. After evaporation of the solvents, 445 mg. of aglycone, fraction VI, was obtained. The aqueous layer and backwashed H_2O were combined and neutralized with freshly precipitated $BaCO_3$. 421 mg. of sugar portion was thus obtained.

Paper Chromatographic Analysis of Fraction VI——Fraction VI was submitted to paper chromatographic analysis. Solvent system, stationary phase-$HCONH_2$, mobile phase-$CHCl_3$; Toyo Roshi No. 51; 6 hr.; descending; coloring reagent, $SbCl_3$.

From the results of this analysis, deacylcynanchogenin, sarcostin, and 3 other spots were detected. (Fig. 1).

Partition Chromatograpy of Fraction VI——300 mg. of fraction VI was submitted to partition chromatography over 110 g. of Celite, giving results shown Table IV. Fraction Nos. 9～10 shows

Table IV. Partition Chromatography of Fraction VI
Fractin VI; 300 mg. Celite; 110 g.

Fraction No.	Solvent	Eluted product (mg.)	Color reaction
1～5	Benzene	oil 70	Liebermann-Burchard (−) $SbCl_3$ (−)
6～8	Benzene-BuOH (95:5)	trace	—
9～10	Benzene-BuOH (90:10)	powder 50	Liebermann-Burchard (+) Keller-Kiliani (−) $SbCl_3$ (+) Fehling (−)
11～15	〃	—	—
16～18	Benzene-BuOH (6:1)	—	—
19～20	Benzene-BuOH (4:2)	—	—
21～22	Benzene-BuOH (1:1)	—	—
23～26	BuOH	—	—

Each fraction : 100 cc.

Liebermann-Burchard reaction, pink→orange→yellow green. Paper chromatographic analysis of fraction Nos. 9～10 was done with the same condition as fraction VI. The results was shown in Fig. 2. From these results, it is reasonable to assume that fraction Nos. 9～10 was a mixture of deacyl c-nor-D-homosteroid analogs.

Chromatography of Fraction Nos. 9～10——50 mg. of fraction Nos. 9～10 was chromatographed over 30 g. of alumina (neutral) to give the results shown in Table V.

Table V. Rechromatography of Fraction Nos. 9～10

Fraction No.	Solvent	Eluted product	Color reaction
1～4	$CHCl_3$	yellow oil	Liebermann-Burchard (−) $SbCl_3$ yellow→yellow black
5～8	$CHCl_3$-MeOH (99:1)	—	—
9	〃	cryst. m.p. 215～225°	Liebermann-Burchard pink→yellow orange $SbCl_3$ yellow→yellow blue
10～14	$CHCl_3$-MeOH (95:5)	—	—
15～18	$CHCl_3$-MeOH (90:10)	—	—
19～20	$CHCl_3$-MeOH (75:25)	trace white powder	Liebermann-Burchard pinc→yellow orange $SbCl_3$ pink→red violet
21～24	〃	—	—
25～27	$CHCl_3$-MeOH (50:50)	—	—
28	MeOH	—	—

Each fraction : 50 cc.

Paper Chromatography of Fractions No. 9, and Nos. 19～20——Fractions No. 9 and Nos. 19～20 obtained by the chromatography (Table V), were analyzed by paper chromatography, giving the results shown in Figs. 3 and 4. Fraction Nos. 19～20 consisted of 6 components and two of them showed the same value with deacylcynanchogenin and sarcostin. Also from Fig. 3, the existence of a new aglycone was assumed.

Paper Chromatography of the Sugar——The sugar syrup obtained from fraction VII, was submitted to paper chromatography with authentic sample. Solvent system, BuOH saturated with 1% NH_3, detected with $AgNO_3$-NH_3.[11]

	Sugar from the plant	D-cymarose
Rf.	0.735	0.735

Summary

The stems and leaves of *Cynanchum caudatum* Max. were proved to contain a glycoside mixture. The sugar portion of the glycoside was found to be D-cymarose by paper partition chromatogoraphy comparing with an authentic specimen.

Six kinds of aglycone, such as deacylcynanchogenin, sarcostin, crystal, m.p.215～220°, and other 3 spots were found by paper partition chromatography. They were isolated by adsorption chromatography using alumina, and partition chromatography over Celite as a carrier.

(Received May 22, 1961)

11) S. M. Partridge : Biochem. J., **42**, 238 (1948).