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Hiroshi Mitsuhashi, Koji Hayashi, and Taro Nomura : Studies on the Constituents of Asclepiadaceae Plants. XVIII.[*1] Components of *Cynanchum paniculatum* KITAGAWA.

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Previous papers from this laboratoy described the isolation of a number of polyhydroxypregnane ester glycosides from the Asclepiadaceae family.[1~3]

It is the purpose of the present communication to describe further studies along this line. *Cynanchum paniculatum* KITAGAWA (Japanese name "Suzusaiko", Asclepiadaceae) is a plant widely distributed in Japan and has a peculiar odor. Percolation of the whole plant with chloroform afforded an extract, which showed positive Keller-Kiliani (dark bluish green) and Liebermann-Burchard reactions (greenish brown→brown). These results suggested the presence of glycosides containing 2,6-desoxy-sugars and steroidal components.[4] The extract was precipitated several times with hexane (Fig. 1).

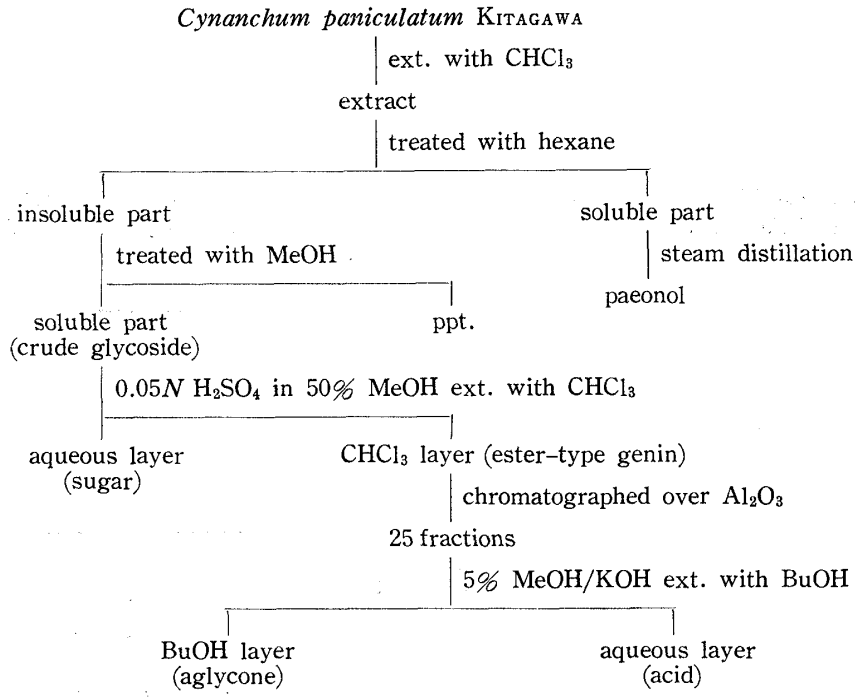

Fig. 1.

The material responsible for the characteristic odor was extracted into hexane layer and an odorless hexane-insoluble precipitate was obtained. The precipitate was treated with methanol and the methanol-soluble fraction concentrated under a reduced pressure. The crude glycoside thus obtained was a dark green powder and gave positive Keller-Kiliani and Liebermann-Burchard reactions. The glycoside was

*1 Part XVII : This Bulletin, **14**, 712 (1966).

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1) H. Mitsuhashi, Y. Shimizu : This Bulletin, **8**, 313 (1960).
2) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, E. Yamada : *Ibid.*, **10**, 804 (1962).
3) H. Mitsuhashi, T. Nomura, Y. Shimizu, I. Takemori, E. Yamada : *Ibid.*, **10**, 811 (1962).
4) T. Reichstein, *et al.* : Helv. Chim. Acta, **31**, 888 (1948).

hydrolyzed with 0.05*N* sulfuric acid in 50% methanol[5] and, after evaporation of methanol, was extracted with chloroform to give a mixture of esters. The aqueous layer showed a strong Keller-Kiliani reaction. The aqueous solution was neutralized with 5% barium hydroxide and after evaporation of water, a syrup was obtained.

From the results of paper partition chromatography, the syrup was found to be a mixture of 2,6-desoxy sugars : D-cymarose, D-digitoxose, L-oleandrose, and D-sarmentose identified by comparison with authentic samples. The mixture of esters was separated by adsorption chromatography over alumina and 25 fractions were obtained. Each fraction was hydrolyzed with 5% methanolic potassium hydroxide and from the results[6] of paper partition chromatography of each of the hydrolyzed fractions, the fraction No. 8 eluted with 1% methanol in chloroform and the fraction No. 11 eluted with 2% methanol in chloroform showed the presence of a pure material.

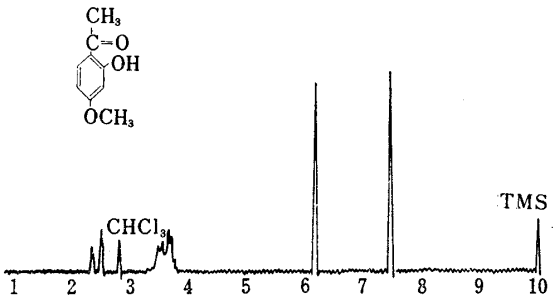


Fig. 2. Nuclear Magnetic Resonance Spectrum of Paeonol (in $CDCl_3$)

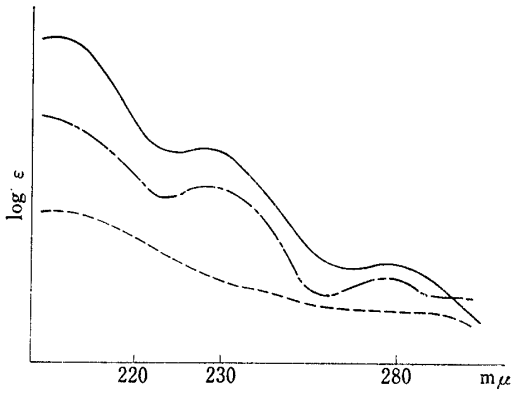
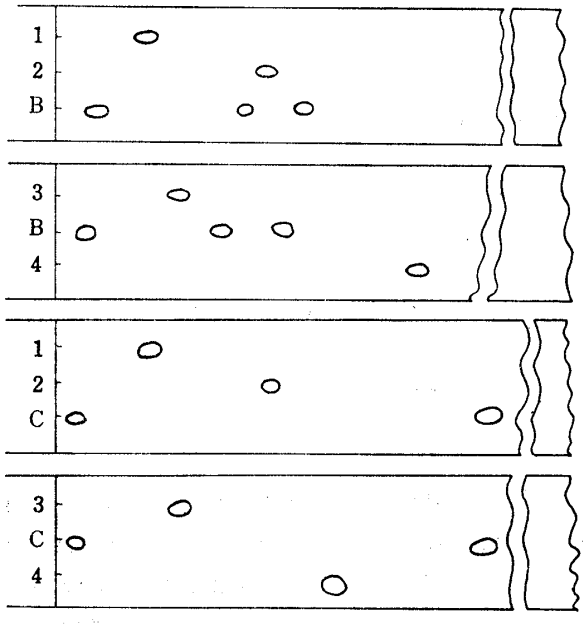


Fig. 3. Ultraviolet Absorption Pattern of the Ester-type Genine A, B, and C

A
B
C



System : $CHCl_3/HCONH_2$, 4hr., 37°, $SbCl_3$

Fig. 4. Paper Chromatographic Analysis of Deacylaglycones B and C

1 Sarcostin 3 Deacylmetaplexigenin
2 Tomentogenin 4 Deacylcynanchogenin

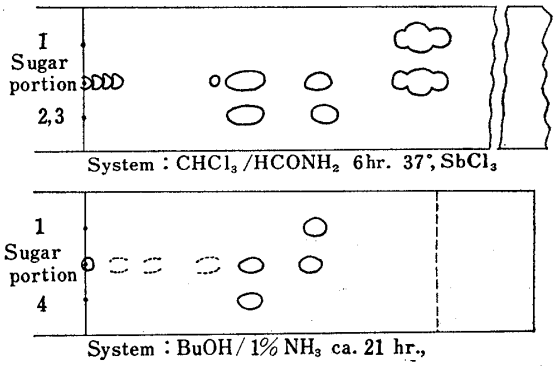


Fig. 5. Chromatographic Analysis of the Sugar Portion

1 D-Cymarose 3 L-Oleandrose
2 D-Sarmentose 4 D-Digitoxose

5) T. Reichstein, *et al.* : Helv. Chim. Acta, **37**, 737 (1954).
6) H. Mitsuhashi, Y. Shimizu, E. Yamada, I. Takemori, T. Nomura : This Bulletin, **10**, 808 (1962).

The fraction No.4 with the highest yield from this chromatogram, the fraction No. 8 and fraction No. 11 tentatively named A, B, and C were studied further. Infrared and ultraviolet spectra of these fractions showed the presence of an ester group.

Fractions B and C showed absorption bands which were due to an ester of an aromatic carboxylic acid. Fractions A, B, and C were each hydrolyzed with 5% methanolic potassium hydroxide and extracted with butanol. The aglycones of fractions B and C contained compounds which were very similar to sarcostin,[7] deacylcynanchogenin,[8] tomentogenin,[9] and deacylmetaplexigenin[10] by paper partition chromatography.

The aglycone fraction of A did not show clear spots by this paper partition chromatography but showed 5~6 spots by thin-layer chromatography on alumina.

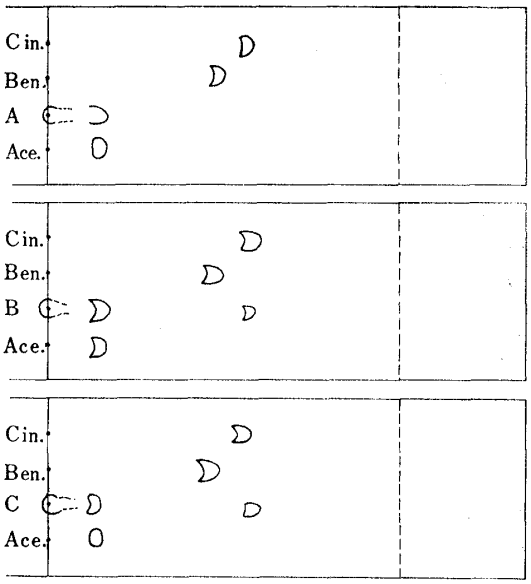


Fig. 6. Paper Chromatographic Analysis of the Acid Portions A, B, and C

BuOH saturated with 0.5*N* NH_3, ca. 15 hr.,
Cin. Cinnamic acid
Ben. Benzoic acid
Ace. Acetic acid

The aglycone of C was submitted to chromatography over alumina, but sufficient material was not obtained to establish identity of the products. The aqueous part of fractions A, B, and C were acidified with 40% phosphoric acid and extracted with ether. The paper partition chromatographic studies of these extracts showed the presence of acetic acid in A, B, and C, and cinnamic acid in B and C.

After evaporation of solvent from the hexane fraction, the residue with a distinctive smell was submitted to steam distillation and the distillate extracted with ether.

After evaporation of ether, pale yellow needles, m.p. 46~49°, were obtained. The product was recrystallized from petroleum ether to white needles, m.p. 47~49°.

This compound analyzed for $C_9H_{10}O_3$ and showed a positive color reaction for phenolic hydroxyl group with ferric chloride solution, Liebermann reaction,[11] Ehrlich's diazo coupling reaction[12] and Gibbs' reaction.[13] This compound had absorption maxima at 228, 274, and 313 mμ. The infrared absorption maxima at 2950 and 1630 cm^{-1} indicated the presence of a hydroxyl and a carbonyl group. The nuclear magnetic resonance spectra of this compound showed two singlets at 7.48τ (3H) and 6.19τ (3H) corresponding to a methyl ketone and a methoxyl group, respectively, and one multiplet (3H) at ca. 3.6τ corresponding to one phenolic hydroxyl proton and two aromatic protons, and one doublet (J=9 c.p.s., 1H) at 2.36τ (aromatic) in deuterochloroform. The identity of this odorous compound with paeonol[14~20] was confirmed by mixed

7) K. A. Jaeggi, EK. Weiss, T. Reichstein : Helv. Chim. Acta, **46**, 694 (1963).
8) H. Mitsuhashi, Y. Shimizu : Steroids, **2**, 373 (1963).
9) H. Mitsuhashi, T. Sato, T. Nomura, I. Takemori : This Bulletin, **13**, 267 (1965).
10) H. Mitsuhashi, T. Nomura : *Ibid.*, **13**, 274 (1965).
11) C. Liebermann : Ber., **7**, 248, 806, 1098 (1874).
12) P. Ehrlich : Z. Klin. Medizin, **5**, 285 (1882).
13) G. Hofmann : Naturwissenschaften, **45**, 337 (1958).
14) G. Martin, V. Jagi : Arch. Pharm., **213**, 335 (1878).
15) W. N. Nagai : Ber., **24**, 2847 (1891).
16) W. Will : *Ibid.*, **19**, 1776 (1886).
17) Y. Tahara : *Ibid.*, **24**, 2459 (1891).

fusion with an authentic sample, comparison of infrared spectra and a mixed fusion of the phenylhydrazone of this compound with that of an authentic sample. This is the first example of paeonol occurring in the Asclepiadaceae family.

Experimental

Extraction——The whole plant of *Cynanchum paniculatum* KITAGAWA, collected at Hayakita, Yufutsu, Hokkaido, was chipped, dried, and 8.7 kg. of the material was extracted with 81 L. of $CHCl_3$ at room temperature. The solvent was evaporated from the extract under a reduced pressure and 290 g. of a deep greenish oily extract was obtained. The extract was treated with hexane to reprecipitate the glycosides and to remove the oily material. The hexane-insoluble precipitate was dissolved in MeOH, MeOH was evaporated *in vacuo* and a dark greenish powder was obtained. This powder appeared to be a glycoside mixture, its color test: Keller-Kiliani reaction (green → dark bluish green) Liebermann-Burchard reaction (greenish brown → brown), and Kedde reaction negative. The hexane-soluble extract had a distinctive smell.

Hydrolysis of the Glycoside——A solution of 12 g. of the crude glycoside in 73 ml. of MeOH and 73 ml. of 0.1*N* H_2SO_4 warmed before mixing was refluxed for 25 min. in N_2 gas, 73 ml. of water was added to the solution, and after evaporation of MeOH *in vacuo*, the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with water, which was added to the above water layer, 5% $NaHCO_3$ solution and water and dried over Na_2SO_4. Evaporation of the solvent gave 6.5 g. of a dark greenish powder, which showed negative Keller-Kiliani reaction and positive Liebermann-Burchard reaction. By the same method, 12 g. of crude glycoside was hydrolyzed and worked up as above to give 4.8 g. of a dark greenish powder. This residue showed the same behavior on thin-layer chromatography as the former, and so the two batches were combined.

This residue (ester type genin, 10.8 g.) was submitted to chromatography over Al_2O_3 (350 g.). The results obtained are shown in Table I.

TABLE I. Chromatography of the Ester-type Genine

Fraction	Solvent	Weight (mg.)	Fraction	Solvent	Weight (mg.)
1	benzene	134	14	5% MeOH/$CHCl_3$	167
2	$CHCl_3$	94	15	〃	140
3	0.5% MeOH/$CHCl_3$	62	16	〃	133
4	〃	869	17	〃	38
5	〃	87	18	20% MeOH/$CHCl_3$	232
6	1% MeOH/$CHCl_3$	234	19	〃	114
7	〃	150	20	〃	73
8	〃	161	21	50% MeOH/$CHCl_3$	96
9	〃	139	22	〃	86
10	2% MeOH/$CHCl_3$	246	23	80% MeOH/$CHCl_3$	102
11	〃	316	24	MeOH	113
12	〃	82	25	〃	107
13	5% MeOH/$CHCl_3$	183			

Hydrolysis of Fractions——A small quantity of each fraction was dissolved in 5% methanolic KOH for 24 hr. at room temperature under N_2. The fraction No. 8 and No. 11 gave spots on paper partition chromatography by $CHCl_3$/$HCONH_2$ system.

Infrared measurements of fractions A (No. 4), B (No. 8) and C (No. 11) gave the following results.

Infrared absorption spectrum

A : $\nu_{max}^{CHCl_3}$ 3500, 1740 cm^{-1}
B : ν_{max}^{Nujol} 1740, 1660, 1600 cm^{-1}
C : $\nu_{max}^{CHCl_3}$ 3500, 1725, 1670, 1600, 1500 cm^{-1}

18) E. H. Rennie, W. T. Coole, H. H. Finlayson: J. Chem. Soc., **1926**, 2763.
19) A. Goris, H. Ganal: Compt. rend., **202**, 1351 (1936).
20) *Idem*: Bull. soc. chim. biol., **18**, 1405 (1936).

Hydrolysis of A, B and C——A solution of fraction A (130 mg.) dissolved in 6 ml. of MeOH and 2 ml. of 20% KOH was allowed to stand for 24 hr. at room temperature in N_2, 6 ml. of H_2O was added, and after removal of MeOH was extracted with BuOH. BuOH extract was washed with H_2O and dried over Na_2SO_4.

Fraction B (117 mg.) and fraction C (130 mg.) were hydrolyzed with 5% methanolic KOH by the same method. Aglycone of fraction A, B, and C were submitted to thin-layer and paper partition chromatography as shown in Fig. 4.

Adsorption Chromatography of Aglinycone C——Aglycone of fraction C (69 mg.) was chromatographed over 5 g. of Al_2O_3 with results as shown in Table II.

Table II. Chromatography of the Aglycone C

Fraction	Solvent	Weight (mg.)
1～ 9	benzene–2% MeOH/benzene	7.6
10～13	3% MeOH/benzene–4% MeOH/benzene	1.9
14～17	5% MeOH/benzene–6% MeOH/benzene	2.1
18～20	8% MeOH/benzene	4.3
21～27	8% MeOH/benzene–20% MeOH/benzene	1.2
28～last	MeOH	22.1

The crystalline material obtained was not enough for further investigation.

Paper Partition Chromatography of Sugar Portion——The aqueous layer obtained on hydrolysis of glycoside with 0.05*N* H_2SO_4 in 50% MeOH was neutralyzed with 5% $Ba(OH)_2$ and concentrated to a syrup under a reduced pressure. The residue was extracted with MeOH, MeOH was evaporated *in vacuo* on a water bath, and a syrup was obtained. The syrup gave a positive Keller-Kiliani reaction and was distilled under high vacuum. The distillate was submitted to paper chromatography with authentic samples. The results are shown in Fig. 5.

Paper Chromatography of Acid Portion——The aqueous layer left after hydrolysis of A, B, and C was acidified with 40% H_3PO_4 and extracted with ether. After evaporation of ether the residue was submitted to paper partition chromatography with results as shown in Fig. 6.

An attempt was made to identify the acids by gas chromatography. A peak corresponding to isobutyric acid was observed in fraction A.

Isolation of Odorous Component (Paeonol)——The substance (ca. 0.5 g.) extracted with hexane was submitted to steam distillation and the distillate extracted with three 400 ml. portions of ether. After drying over Na_2SO_4, ether was evaporated to give pale yellow needles, which were recrystallized for several times from petroleum ether to needles, m.p. 46～49°. This material showed a positive $FeCl_3$ reaction (reddish violet), Liebermann reaction, Ehrlich's diazo coupling reaction and Gibbs' reaction. *Anal.* Calcd. for $C_9H_{10}O_3$: C, 65.06; H, 6.07. Found: C, 65.08; H, 6.34. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1630. UV λ_{max}^{EtOH} mμ: 228, 274, 313.

Phenylhydrazone of Odorous Compound——To 30 mg. of this odorous compound dissolved in 0.6 ml. of EtOH, 29.9 mg. of phenylhydrazine, 44.6 mg. of AcONa, and 0.4 ml. of EtOH were added. After allowing the solution to stand in a water bath for 10 min., excess water was added, the precipitate was extracted with ether, and ether solution was dried over Na_2SO_4.

After evaporation of the solvent, pale yellow needles, m.p. 107～108°, were obtained.

This odorous compound was confirmed as paeonol by mixed melting point, IR spectrum, and mixed melting point of the phenylhydrazone.

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