TABLE VI. Paper Partition Chromatography of the Acid Portion

Test substance	Rf Values in BuOH-1.5*N* NH_3	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]

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

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

*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 Pharmaceautical 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).

elucidated.[3b,4] Since each plant contains many kinds of similar aglycones, the separation is rather difficult and it is necessary to introduce paper chromatographic comparison in the procedure.

For the adrenal steroids, paper chromatography has already been widely applied.[5] To the C-21 steroids in Asclepiadaceae plants, however, only Abisch, *et al.* used solvent systems in a few cases as a control.[2b] The authors reinvestigated their experiments and found them rather unreproducible, probably on account of the differences of temperature and other conditions. In the present work, to avoid these inconveniences, solvent systems which mainly consists of each one mobile phase have been investigated on the samples.

Methods

Paper——Strips of Whatman No. 1 or Toyo Roshi No. 51, 9×45 cm., were used.

Solvents——Toluene, benzene, and $CHCl_3$ were washed with conc. H_2SO_4, alkali and H_2O, dried and distillated. *tert.*-BuOH was distillated with Na. MeOH, BuOH, *iso*-AmOH, isoöctyl alcohol and isopropyl ether were those destillated. Formamide and propylene glycol were of special grade.

Apparatus——15×45 cm. chromatography cylinder equipped with a stainless tab (4×11.5×5 cm.) and a dropping funnel at the upper part, containing the stationary phase or mobile phase or both at the bottom. The inside of the cylinder was wrapped with filter paper for the sufficient saturation.

Temperature——The cylinder was placed in an incubater (25°±2° or 37°±2°).

Detection of the spots——After complete drying the chromatograms were sprayed with saturated solution of $SbCl_3$ in $CHCl_3$ and heated at 80～100°. Changes of coloration are characteristic with the substances for the identification. For adrenal steroids, triphenyl tetrazolium chloride was used.

Solvent systems——The following solvent systems were examined.

1) Toluene-BuOH-H_2O (4:1:3) upper layer
2) Benzene-MeOH-H_2O (2:1:1) (Bush B_5) upper layer
3) H_2O only
4) *iso*-AmOH-H_2O upper layer
5) Octanol-H_2O upper layer ascending method
6) Toluene/propylene glycol
7) Benzene/propylene glycol
8) Isopropyl ether-*tert*-BuOH-H_2O (8:5:6) (E_5) upper phase
9) $CHCl_3$/formamide

Substances——The following compounds were used. Deacylcompounds : deacylcynanchogenin,[4b] sarcostin,[1,3b] crystal 3,[3c] crystal 4,[3c] tomentogenin,[1] $LiAlH_4$ reduction product of cynanchogenin,[4b] tetrahydrodeacylcynanchogenin.[4d] Esters : cynanchogenin,[3a] penupogenin.[3b] Acetates : cynanchogenin acetate,[4b] deacylcynanchogenin acetate,[4b] sarcostin acetate.[3b]

Procedure——As to the solvent systems (6) and (7), the paper was dipped in 15% propylene glycol solution in Me_2CO, pressed between filter papers and spotted. With solvent system (9), the paper was treated with 30% formamide solution in Me_2CO as above.

The MeOH solution of 10～15 μg. of substance (crystals) or 50～200 μg. (mixture) was spotted in a circle as small as possible using micropippette. The spotted paper was hung in the cylinder and after saturation, the solvent was dropped into the tab.

Results

The results are summarized in Table I.

Following those systems, the authors attempted to separate mixtures of several aglycones from the plants to obtain better results. About those of *Marsdenia tomentosa* and *Metaplexis japonica*, the results were or will be reported in each study.[1,3c] The study about the deacyl-mixture from *Cynanchum caudatum* is shown as an examle.

4) *a*) J. W. Cornforth : Chem. & Ind. (London), **1955**, 602. *b*) H. Mitsuhashi, Y. Shimizu : This Bulletin, 8, 318 (1960). *c*) *Idem* : *Ibid.*, 8, 738 (1960). *d*) *Idem* : *Ibid.*, in press. (Part IV).
5) A. Zaffaroni, R. B. Burton, E. H. Keutmann : J. Biol. Chem., **188**, 763 (1951); I. E. Bush : Biochem. J., **150**, 370 (1952); W. R. Eberlein, A. M. Bongiovanni : Arch. Biochem. Biophys., **59**, 90 (1955).

Table I. Solvent System[a]

Substance	1	2	3	4	5	6	7	8	9	Coloration with $SbCl_3$
Deacylcynanchogenin	tailing	0.06[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	blue-reddish-violet-dark violet
A_3		0.04[b]							0.41	yellow
A_2									0.28	pale blue
Sarcostin	tailing	0.02[b]	1.04	0.87	1.13	0.27	0.27	0.88	0.19	reddish violet-greyish violet
A_1									0.02	yellow-brown
Crystal 3						0.83	0.70	1.19	0.63	yellow
Crystal 4						0.13	0.27	1.08		pink-reddish violet
Tomentogenin							0.36			pale reddish brown
DDC[c]				0.77			0.46	1.08 (7.7 cm.)	0.31	pink-reddish violet
Tetrahydrodeacylcynanchogenin								(7.2 cm.)	0.25	yellow
Cynanchogenin		0.22[b]	tailing					0.85		blue
Penupogenin		0.12						1.19		reddish violet
Cynanchogenin acetate			0.65					0.77		bluish violet
Deacylcynanchogenin acetate								front		〃
Sarcostin acetate								front		〃
Adrenal corticoids DOCA							2.10			
F acetate[d]							tailing			R_M acetate
M acetate[d]							1.00			
DOC							tailing			
B[d]							0.18			
F[d]							2.30			R_M
M[d]							1.00			
Time (hr.)	5,	2.5~3,	2~2.5,	16.5,	16,	120,	24,	7,	7.	

a) All values are denoted in the term of R_{DC} value except particularly referred.
b) Rf
c) $LiAlH_4$ reduction product of cynanchogenin
d) Reichstein's substance

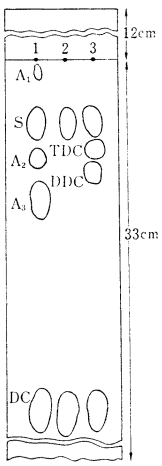

Fig. 1. An Example of Separation
System (9) ($CHCl_3$/Formamide, 7 hr. (after 16 hr. saturation))
1. Deacyl-compounds mixture from *C. caudatum* which was prepared by the acid hydrolysis of the glycoside followed by alkaline hydrolysis
2. Sarcostin+Deacylcynanchogenin (pure crystals)
3. Sarcostin+Deacylcynanchogenin+Dihydrodeacylcynanchogenin+Tetrahydrodeacylcynanchogenin(pure crystals)

The substances corresponding to A_1~A_3 which could not be isolated in pure state so far are newly found to exist in the plant. A_3 shows similar coloration to that of tetrahydrodeacylcynanchogenin with antimony trichloride but has the different R_{DC} value.

Discussion

To separate deacyl-compounds, the solvent systems (6), (7), (8), and (9) are found to be very applicable. Especially the solvent system (9) seems to be the best as the control, because of the constant reproducibility of R_{DC} values. These systems are also

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*.

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*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.

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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.

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

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.