Color test : Liebermann-Burchard reaction (pink→yellowish red→yellowish green), $SbCl_3$ test (yellowish green). Identity of this material with Ⅲ obtained from *Metaplexis japonica*[2] was indicated by mixed melting point and comparison of infrared spectrum. Sarcostin (I) : Fr. Nos. 45～57 (Table Ⅲ) was recrystallized from acetone to prisms, m.p. 147～149°/250～252° (total 1 g.). A mixed melting point with an authentic specimen of I showed no depression.

We wish to express our thanks to Prof. Dug-Ryong Hahn (Seoul) for collection of the plants.

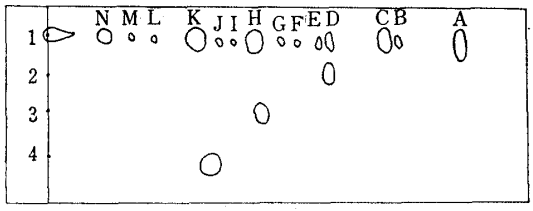

Fig. 4.

System : CH_2Cl_2–MeOH (95:5), Al_2O_3.
1 Deacyl-type aglycone mixture
2 Deacylcynanchogenin
3 Deacylmetaplexigenin
4 Sarcostin

Summary

The roots of *Cynanchum wilfordi* Hemsley were found to contain a glycoside mixture. The glycosides showed a positive Keller-Kiliani reaction, suggesting the presence of a 2-deoxysugar. The sugar portion of the glycoside was found to be D-cymarose. The aglycone was shown to be an ester. Alkaline hydrolysis of the ester aglycone afforded three kinds of pregnane compounds, sarcostin (I), deacylcynanchogenin (Ⅱ), and deacylmetaplexigenin (Ⅲ). Cinnamic acid was found in the acidic fractions.

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98. Hiroshi Mitsuhashi, Taro Nomura, and Miho Hirano : Studies on the Constituents of Asclepiadaceae Plants. XIX.*1 Components of *Metaplexis japonica* Makino. IV.*2

(*Faculty of Pharmaceutical Sciences, Hokkaido University**3)

It has already been shown that the stems, leaves,[1] and roots of *Metaplexis japonica* Makino contain ester glycoside with 2-deoxysugars. Sarcostin (I), metaplexigenin (Ⅱ), and three other aglycones were separated from the stems and leaves in crystalline form. From the roots, Ⅱ, benzoylramanone (Ⅲ), and one other ester-type aglycones were reported.[1] The crude glycoside mixture, obtained from the roots of the plant as described in Part XV of this series[4] was hydrolyzed with 0.05*N* sulfuric acid in methanol and the ester-aglycone mixture thus obtained was submitted to column chromatography over alumina. Elution with chloroform-methanol mixtures gave the results shown in Table I. The eluates were divided into groups A to D. In this

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*1 Part XVⅢ : This Bulletin, **14**, 779 (1966).
*2 A part of this work was reported at the Annual Meeting of the Pharmacognostical Society of Japan, September 19, 1964, Kanazawa.
*3 Kita-15-jo, Nishi-7-chome, Sapporo, Hokkaido (三橋 博, 野村太郎, 平野美穂).
1) H. Mitsuhashi, T. Nomura, Y. Shimizu, I. Takemori, E. Yamada : *Ibid.*, **10**, 811 (1962).
2) H. Mitsuhashi, Y. Shimizu : Steroids, **2**, 373 (1963).
3) K. A. Jaeggi, E. K. Weis, T. Reichstein : Helv. Chim. Acta, **46**, 694 (1963).
4) H. Mitsuhashi, T. Nomura : This Bulletin, **13**, 274 (1965).

paper, the isolation and structure determination of pregnane-type compounds from groups A to C are discussed.

From group A, which was eluted with chloroform-methanol (99.8:0.2), benzoylramanone (Ⅲ), m.p. 222~226°, was obtained, as reported in the preceding paper.[4] A thin-layer chromatographic study of the mother liquor gave a number of spots beside that of Ⅲ, as shown in Fig. 2. Since isolation of ester-type aglycone is usually more difficult than that of the pregnane aglycones,[5,6,7] the mother liquor was hydrolyzed with 5% methanolic potassium hydroxide in order to isolate the pregnane compounds. The alkaline hydrolysate was submitted to column chromatography over alumina using methylene chloride-methanol mixture for elution. Three crystalline substances were obtained. From the results of paper chromatography (chloroform/formamide),[8] one of them was found to be a mixture of isoramanone (Ⅲa) and one other component. Further investigations could not be carried out for lack of the material. One of the remaining two showed m.p. 240~245°, and was found to be deacylcynanchogenin or lineolon (Ⅳa) by mixed melting point determination with an authentic specimen. Ⅳa had previously been isolated from *Cynanchum caudatum*[9] and other Asclepiadaceae plants,[7,10] and its structure had been proposed by Jaegi, *et al.*[3] and Mitsuhashi, *et al.*[2] The other crystalline substance (Ⅴ) seemed to be a new aglycone and showed m.p. 210~217°, $[\alpha]_{589}^{25}$ +145° (c=0.1, from ORD measurement), which with Liebermann-Burchard reagent showed a color change from reddish to green, green with antimony trichloride, and negative to Kedde reaction.

The infrared spectrum of Ⅴ showed an absorption at 3400 cm^{-1} (broad), which was assigned to hydroxyl groups, but showed no absorption in the 1800~1600 cm^{-1} region. The molecular formula, $C_{22}H_{36}O_7$, was proposed from its elemental analysis, but a C_{22} compound has never been isolated from Asclepiadaceae plants and so the analytical results were viewed with caution. Thin-layer chromatographic study of group B, eluted with chloroform-methanol (99:1), showed the presence of metaplexigenin (Ⅱ) but Ⅱ was not isolated and B was hydrolyzed under conditions in order to isolate pregnane compounds. The alkaline hydrolysate of group B on paper chromatogram showed many spots beside those of deacylcynanchogenin (Ⅳa) and deacylmetaplexigenin (Ⅱa), as shown in Fig. 4. Chromatography of the pregnane-aglycone mixture through alumina yielded four crystalline substances. One of these was found to be deacylcynanchogenin (Ⅳ). Two of the remaining three were deacyl deacylmetaplexigenin (Ⅱa) and sarcostin (Ⅰ), which were confirmed by the melting point and comparison of infrared spectra. Ⅰ had previously been isolated from the stems and leaves of this plant,[1] and other Asclepiadaceae plants.[6,7,10,11,12]

The other compound (Ⅵ) showed, m.p. 243~245°, and the Liebermann-Burchard reaction was yellow to yellowish green, and reddish violet with antimony trichloride.

The compound (Ⅵ) was identical with one of the minor aglycones which had previously been isolated from the stems and leaves of this plant,*4 and was found to be utendin (Ⅵ) isolated from *Pacycarpus lineolatus* and its structure had been proved by Reichstein, *et al.*[13]

*4 In the report (2), this compound was designated as crystal 4.

5) *Idem* : *Ibid.*, **11**, 1333 (1963).
6) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, E. Yamada : *Ibid.*, **10**, 804 (1962).
7) H. Mitsuhashi, K. Sakurai, T. Nomura, N. Kawahara : *Ibid.*, **14**, 712 (1966).
8) H. Mitsuhashi, Y. Shimizu, E. Yamada, I. Takemori, T. Nomura : *Ibid.*, **10**, 808 (1962).
9) H. Mitsuhashi, Y. Shimizu : *Ibid.*, 8, 313, 318 (1960).
10) E. Abish, Ch. Tamm, T. Reichstein : Helv. Chim. Acta, **42**, 1015 (1959).
11) J. W. Cornforth, J. C. Earl : J. Chem. Soc., **1939**, 737; **1940**, 1443.
12) H. Mitsuhashi, Y. Shimizu : This Bulletin, **10**, 724 (1962).
13) A. Lardon, W. Klyne, E. Iseli, T. Reichstein : IUPAC Symposium at Kyoto, Japan, on April 18, 1964.

For the isolation of other compounds, the mother liquor left after isolation of these crystals and the noncrystalline fractions were combined and submitted to partition chromatography over Celite, using a benzene-butanol/water mixture. These results are shown in Table Ⅳ. The crystalline substance (Ⅶ) thus obtained was identical with crystal No. 3, one of the major aglycones which had previonsly been isolated from the stems and leaves of this plant.[1] This compound was named pergularin.*[5] Group C, which was eluted with a chloroform-methanol mixture (97:3) was hydrolyzed with alkali and then chromatographed over alumina. Two different crystalline materials were obtained; one of them proved to be sarcostin (Ⅰ), and the other (Ⅷ) seemed to be a new aglycone and showed m.p. 215~230° (decomp.), $[\alpha]_{589}^{19}$ +35° (c=0.1, MeOH, from ORD measurement). Ⅷ analyzed for $C_{21}H_{34}O_7$ and gave color changes with Liebermann-Burchard reagent from pink to violet to yellowish green and yellowish green with antimony trichloride test. An infrared absorption at 3400 cm^{-1} (broad) indicated the presence of hydroxyl group, but there were no bands assignable to a carbonyl group. The structure determination of Ⅷ is underway at present.

Pergularin (Ⅶ) forms plates from acetone water, m.p. 220~230°, $[\alpha]_{589}^{19}$ −33° (c=0.1, MeOH, from ORD measurement), for which the molecular formula $C_{21}H_{32}O_5$ was proposed from its elemental analysis. It gave coloration of yellow to yellowish green with the Liebermann-Burchard reagent, yellow with antimony trichloride, and a negative test with the Kedde reaction. The infrared absorption maxima at 3550, 3450, 1720, and 1690 cm^{-1} showed the presence of hydroxyl and carbonyl groups (six-membered or open-chain ketone). Acetylation of Ⅶ with acetic anhydride-pyridine afforded a diacetate (Ⅶa), m.p. 130~137°, $C_{25}H_{36}O_7$, which showed hydroxyl absorption at 3450 cm^{-1}. Although Ⅶ showed two bands (1720, 1690 cm^{-1}) in the carbonyl region of the infrared spectrum it gave only a monoxime (Ⅶb), m.p. 265~270°, which showed no carbonyl absorption. These fact suggested the existence of only one carbonyl group in Ⅶ. Very similar case was reported in the case of deacylmetaplexigenin[4] (Ⅱa). Infrared spectrum could not be measured because Ⅱa and Ⅶ were practically insoluble in chloroform or carbon tetrachloride. These observations indicate that pergularin (Ⅶ) has one carbonyl group, two secondary hydroxyl groups, and two tertiary hydroxyl groups. The pregnane skeleton was suggested from biogenetic analogy. The nuclear magnetic resonance spectra of pergularin (Ⅶ) and its diacetate (Ⅶa) were measured and are given in Table I. These data support the presence of an acetyl side chain and a double bond at C-5. The chemical shift of the 18-CH_3 group suggests that pergularin (Ⅶ) has a C/D *cis* ring juncture. If Ⅶ has a C/D *trans* ring juncture, the band for the 18-CH_3 group should be at a higher field that for the 19-CH_3 group.[14] The 21-CH_3 signal of pergularin diacetate (Ⅶa) at 7.72τ occurs at a position similar to that of deacylmetaplexigenin diacetate (Ⅱb). The presence of an α-ketol system was suggested since the band for the 21-CH_3 group of Ⅱb shifted to a lower field relative to that of deacylcynanchogenin diacetate (Ⅳc). The chemical shifts of the 18- and 19-CH_3 signals of Ⅶ and Ⅶa are similar to those of utendin (Ⅵ) and its triacetate (Ⅵa), and it appears that Ⅶ has no 8β-OH group which would cause the bands for the 18- and 19-CH_3 groups to shift to a lower field by comparison with the spectra of similar compounds.

The optical rotatory dispersion curves of pergularin (Ⅶ) and its diacetate (Ⅶa) in methanol showed a negative Cotton effect, as shown in Table Ⅱ. This fact suggests

*5 *Pergularia japonica* Thumb. is the synonym of *Metaplexix japonica* Makino.

14) R. F. Zürcher : Helv. Chim. Acta, **46**, 2054 (1963).

Table I.

Compound	Solvent	19-CH_3	Signal 18-CH_3	(τ)*6 21-CH_3
I[2]	pyridine	8.59	8.11	8.53 (d., J = 7 c.p.s.)
IVa[2]	〃	8.58	8.07	7.60 (S)
IIa[4]	〃	8.56	8.04	7.39 (S)
VI	〃	8.91	8.21	8.41 (d., J = 6 c.p.s.)
VII	〃	8.94	8.29	7.43 (S)
Ia[2]	$CDCl_3$	8.83	8.58	8.78 (d., J = 7 c.p.s.)
IVc[2]	〃	8.85	8.50	7.83 (S)
IIc	〃	8.85	8.53	7.74 (S)
VIa	〃	9.01	8.77	8.75 (d., J = 6 c.p.s.)
VIIa	〃	8.99	8.70	7.72 (S), 4.55 (q. C-6)

that VII is C/D *cis*, 17α-$COCH_3$, or C/D *trans*, 17β-$COCH_3$ type,[15,16] but the latter case would be excluded from the results of the nuclear magnetic resonance measurements. The maxima of VII and VIIa shifted about 10 mμ to longer wave lengths compared to that of ramanone[5] (IIIa), suggesting the presence of an α-ketol system. In the case of deacylmetaplexigenin (IIa) and 3β,17α-hydroxypregnan-20-one, the troughs shifted

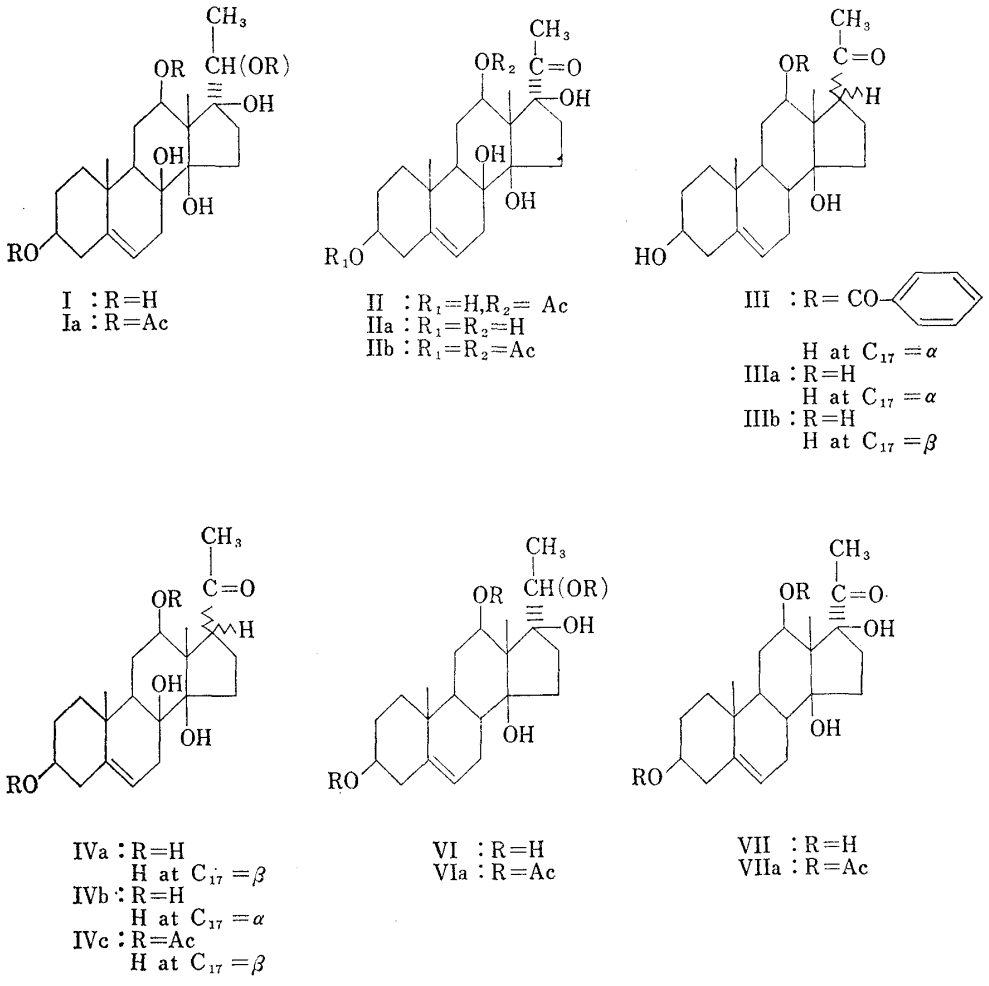


Chart 1.

*6 In this paper, 10 p.p.m. value (from tetramethylsilane, used as internal standard) is used as τ.

15) C. Djerassi : "Optical Rotatory Dispersion Application to Organic Chemistry," McGraw-Hill Book Co., New York (1960).
16) H. Mitsuhashi, T. Nomura, M. Fukuoka : Steroids, **4**, 484 (1964).

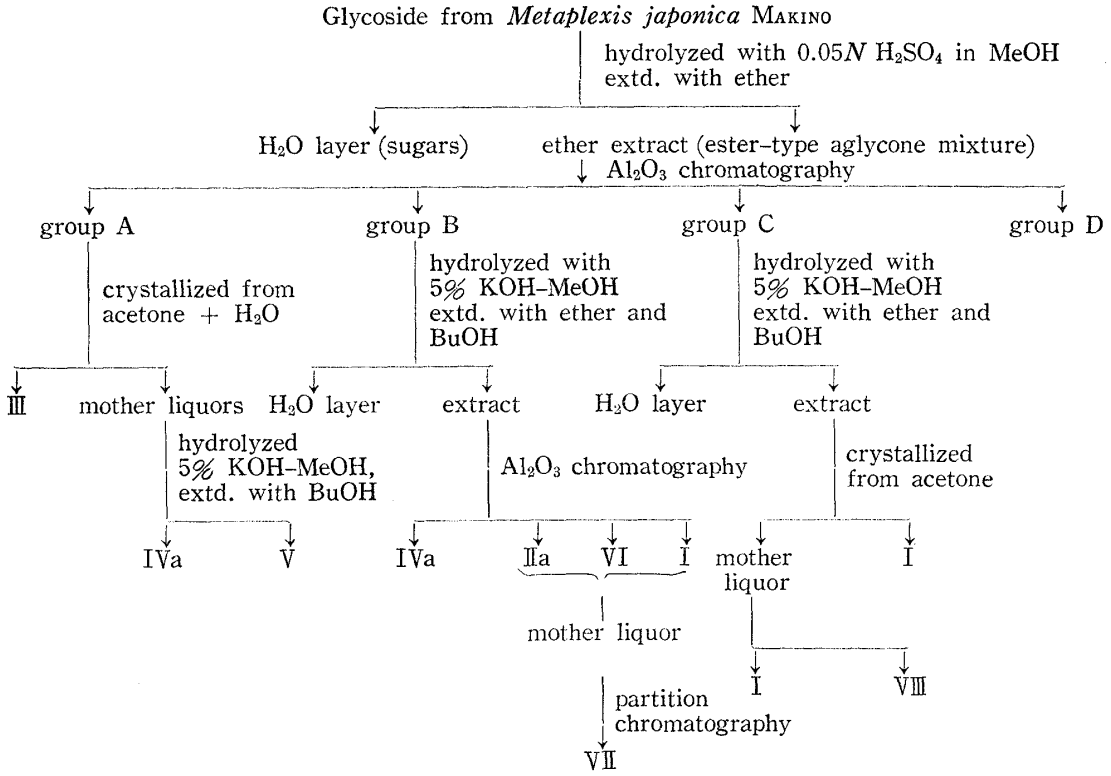
about 12~13 mμ to a longer wave length compared to deacylcynanchogenin (Ⅳa), and 3β-hydroxypregnan-20-one.[4,17]

Table Ⅱ.

Compound	Trough [φ] (mμ)	Peak [φ] (mμ)	a
Ⅶ	− 3878 (311)	+ 5086 (268)	− 89.6
Ⅶa	− 5037 (311)	+ 4606 (268)	− 96.4
Ⅲb[6]	− 4542 (301)	+ 4308 (260)	− 88.5

After Ⅶ was treated with 3% methanolic potassium hydroxide for 24 hours at room temperature and the resulting mixture showed only one spot, which was identified as the starting material (Ⅶ), by paper partition chromatography ($CHCl_3$/formamide).[8] This result indicates that Ⅶ could not be isomerized under these conditions, which with 17-H-17-acetyl type steroids leads to an equilibrium mixture of the side chain in most cases.[16] Combination of these facts would suggest that pergularin (Ⅶ) has a C/D *cis* ring juncture, and a 17β-OH, 17α-$COCH_3$ side chain. Since sarcostin (Ⅰ) and four other pregnane compounds (Ⅱ, Ⅲ, Ⅳ, Ⅵ) having 3β, 12β, 14β-hydroxyl group have been isolated from the Asclepiadaceae, biogenetic analogy would favor the structure (Ⅶ) for pergularin. This assumption was proved by the following results. Pergularin (Ⅶ) was reduced with sodium borohydride and the product was examined by paper chromatography ($CHCl_3$/formamide),[8] giving two spots (Fig. 8). The major spot was identical with that of utendin (Ⅵ).

This reaction mixture was submitted to partition chromatography over a wet Celite column using benzene-butanol, and a crystalline substance, m.p. 240~250°, was isolated, which was identical with utendin (Ⅵ) by a mixed melting point determination. Thus the structure of pergularin was proved to be Ⅶ.



17) C. Djerassi, O. Halpern, V. Halpern, O. Shindler, Ch. Tamm : Helv. Chim. Acta, **41**, 250 (1958).

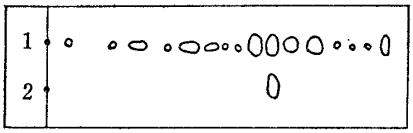


Fig. 2.

System : Benzene-MeOH (97 : 3), Al_2O_3
1 : Group A
2 : Metaplexigenin

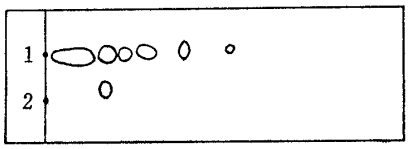


Fig. 3.

System : Methyl ethyl ketone-benzene (3 : 7), Al_2O_3
1 : Group B
2 : Metaplexigenin

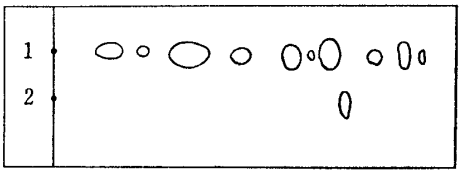


Fig. 4.

System : BuOH-benzene (1 : 9), Al_2O_3
1 : Group C
2 : Metaplexigenin

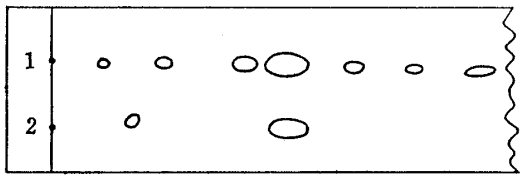


Fig. 5.

System : $CHCl_3$/H·$CONH_2$
1 : Alkaline hydrolysate of group A
2 : Deacylcynanchogenin+sarcostin

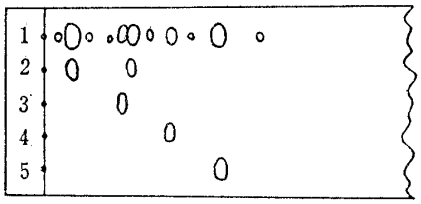


Fig. 6.

System : $CHCl_3$/H·$CONH_2$
1 : Alkaline hydrolysate of group B
2 : Deacylmetaplexigenin+sarcostin
3 : Utendin
4 : Pergularin
5 : Deacylcynanchogenin

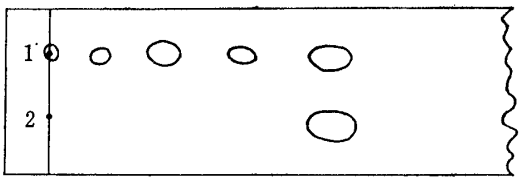


Fig. 7.

System : $CHCl_3$/H·$CONH_2$
1 : Alkaline hydrolysate of group C
2 : Sarcostin

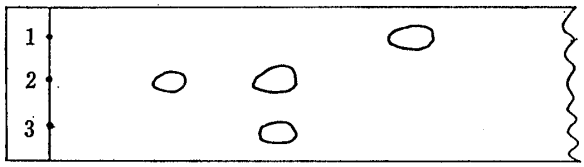


Fig. 8.

System : $CHCl_3$/H·$CONH_2$
1 : Pergularin
2 : Reaction mixture 3 : Utendin

Experimental

Chromatography of the Ester-type Aglycone Mixture——Sixty g. of the aglycone-ester mixture, obtained as described in Part XV of this series,[4] was submitted to column chromatography over 2 kg. of Al_2O_3. The results are shown in Table Ⅲ.

Table Ⅲ.

Fraction No.	Solvent	Eluted product (g.)	Note
1～40	$CHCl_3$	16.19	oil
41～52	$CHCl_3$–MeOH (99.8:0.2)	0.88	〃
53～68	〃	6.51	powder group A
69～97	$CHCl_3$–MeOH (99:1)	21.46	〃 group B
98～107	$CHCl_3$–MeOH (95:5)	4.46	〃 group C
108～121	〃	1.40	〃 group D
122～141	$CHCl_3$–MeOH (93:7)	0.37	〃 group D
142～149	$CHCl_3$–MeOH (4:1)	0.28	

Each fraction : 500 ml.
The results of thin-layer chromatographc analysis of groups A to C are shown in Figs. 2～4.

Benzoylramanone (III)——Fraction Nos. 55~57 (total 2.49 g.) was treated with acetone and 800 mg. of III, m.p. 220~226°, was obtained. Mixed melting point with an authentic specimen showed no depression.

Hydrolysis of Group A——The mother liquors of III and the other fractions from group A as described in Table III were combined, 3.5 g. of this mixture was dissolved in 300 ml. of 5% methanolic KOH, and the solution was allowed to stand for 24 hr. at room temperature. After addition of 300 ml. of H_2O, MeOH was evaporated in a reduced pressure. The aqueous solution was extracted with BuOH and the extract was treated as usual. Evaporation of the solvent under a reduced pressure gave 2.2 g. of a yellow powder. The result of a paper chromatographic analysis ($CHCl_3$/formamide)[8] is shown in Fig. 5.

Table IV.

Fraction No.	Solvent	Eluted product (mg.)	Note
1~3	CH_2Cl_2	728	oil
4	〃	51	(IIIa) (IIIb)
5~8	〃	170	powder
9~11	CH_2Cl_2–MeOH (99:1)	133	powder
12~13	〃	94	IV (48 mg.)
14~16	〃	15	powder
17~19	CH_2Cl_2–MeOH (98:2)	140	V (17 mg.)
20~26	〃	300	powder
27~29	CH_2Cl_2–MeOH (96:4)	48	oil
30~34	CH_2Cl_2–MeOH (9:1)	65	〃

Each fraction : 200 ml.

Chromatography of the Alkaline Hydrolysate of Group A——From the alkaline hydrolysate of group A, 2.2 g. was submitted to column chromatography over 150 g. of Al_2O_3 and column eluted with CH_2Cl_2–MeOH mixtures. These results are shown in Table IV.

Crystallization of Fraction No. 4——Fraction No. 4 (Table IV) was recrystallized from acetone and 10 mg. of a crystalline substance was obtained. It showed two spots ($R_{D.C.}$*7 = 1.8, 1.5) by paper chromatography ($CHCl_3$/H·$CONH_2$) and one of them was identified as isoramanone (IIIa) ($R_{D.C.}$ = 1.8). The mother liquor showed three spots ($R_{D.C.}$ = 1.8, 1.5, 1.4). The spots of $R_{D.C.}$ = 1.4 was identical with that of ramanone (IIIb).

Deacylcynanchogenin (IVa)——Fraction Nos. 9~10 was recrystallized from acetone to prisms, m.p. 242~246° (total 48 mg.). It showed red→violet→brown with a Liebermann-Burchard reagent and reddish violet with $SbCl_3$. A mixed melting point with deacylcynanchogenin (IVa), which had been obtained from *Cynanchum caudatum* showed no depression.

Compound (V)——Fraction Nos. 17~19 (Table IV) gave a crystalline mass on standing for a few days in acetone. This crystalline mass was recrystallized from acetone to prisms, m.p. 210~218° (total 17 mg.). Color reactions : pink→violet→yellowish green (Liebermann-Burchard reaction), yellowish green ($SbCl_3$). $[\alpha]_{589}^{25}$ +144° (c=0.23,MeOH, from ORD measurement). *Anal.* Calcd. for $C_{22}H_{36}O_7$: C, 64.05; H, 8.80. Found : C, 64.05; H, 8.72. IR ν_{max}^{Nujol} cm^{-1} 3400 (broad).

Hydrolysis of Group B——A solution of 5 g. of group B dissolved in 120 ml. of 5 % methanolic KOH was refluxed for 5 hr. After adding 100 ml. of H_2O, MeOH was evaporated in a reduced pressure. The residual aqueous solution was extracted successively with a small amount of ether and the solvent was evaporated from ether extract, affording 2.9 g. of a residue. The aqueous solution, after extraction with ether, was extracted with BuOH. The extract was treated as usual. Evaporation of the solvent under reduced pressure gave 0.8 g. of a yellowish powder.

The products were combined and a paper chromatographic analysis was carried out with the result shown in Fig. 6.

Chromatography of the Alkaline Hydrolysate of Group B——This alkaline hydrolysate 3.7 g. of group B was submitted to column chromatography over 370 g. Al_2O_3 and the column was eluted with benzene+MeOH mixture. The results are shown in Table V.

Deacylcynanchogenin (IVa)——The fraction Nos. 10~15 (Table V) was treated with acetone and 10 mg. of crystalline material, m.p. 240~244°, was obtained. It was identified with IVa.

Deacylmetaplexigenin (IIa)——Fraction Nos. 17~19 (in Table V) was recrystallized from acetone+H_2O to needles (180 mg.), m.p. 217~222°. This compound was found to be deacylmetaplexigenin (IIa) by a mixed melting point determination and comparison of infrared spectra.

Utendin (VI)——Fraction No. 21 (in Table V) was recrystallized from acetone to needles (10 mg.), m.p.

*7 $R_{D.C.}$=$R_{Deacylcynanchogenin}$

Table V.

Fraction No.	Solvent	Eluted product (mg.)	Note	Spot on PPC (see Fig. 6)
1～3	benzene	91	oil	—
4～5	benzene-MeOH (99:1)	35		—
6～8	benzene-MeOH (98:2)	322		—
9	〃	110	powder	IVb
10～15	〃	398		IVa+2 spots
16	benzene-MeOH (97:3)	43		IVa+IIa
17～19	〃	4630		IIa+(IVa)+(VII)
20	〃	1060		IIa+(VI)+(VII)+1 spot
21	〃	100		VI+(VII)+(IIa)+1 spot
22～30	〃	1789		I+(VII)+2 spots
31～33	benzene-MeOH (96:4)	151		I+2 spots
34～37	benzene-MeOH (94:6)	121		〃
38～42	benzene-MeOH (9:1)	143		—

Each fraction : 500 ml.
Compound numbers in parentheses indicate weak color reaction with $SbCl_3$.

240～250°. It showed yellow→ yellowish green with Liebermann-Burchard reagent and reddish violet with $SbCl_3$. It was identical with crystal No. 4, which had been obtained from the stem and leaves of this plant, by mixed melting point determination and paper chromatography ($CHCl_3$/H·CHO).[8] The above data suggest that it might be utendin (VI) which had previously been isolated from *Pacycarpus lineolatus*.[10] A mixed melting point determination and comparison of infrared spectra with an authentic sample of VI, provided by Prof. Reichstein, showed complete identity.

Sarcostin (I)——Fraction Nos. 22～23 (Table V) was recrystallized from acetone to needles, m.p. 150/247～252° (total 500 mg.). This compound was identical with an authentic sample of I by mixed melting point determination and paper chromatographic analysis.

Isolation of Pergularin (VII)——The mother liquors left after isolation of I, VI, and IIa were combined, the solvent was evaporated, and 800 mg. of the residue was submitted to partition chromatography on Celite (280 g.) containing H_2O (280 ml.) under conditions reported peviously.[1] The results are shown in Table VI.

Table VI.

Fraction No.	Solvent	Eluted product (mg.)	Spots on PPC ($CHCl_3$/H·$CONH_2$)
1	benzene	17	—
2	benzene-BuOH (98:2)	30	—
3	benzene-BuOH (95:5)	10	—
4	〃	78	VII
5	〃	100	VII, IV
6～10	〃	217	IV, IIa, I
11～14	benzene-BuOH (9:1)	173	I
15～16	benzene-BuOH (6:1)	55	〃

Each fraction : 500 ml.

Fraction No. 4 was recrystallized from acetone +H_2O to prisms, m.p. 220～234°, $[\alpha]_{589}^{19}$ −33° (c= 0.1, MeOH, from ORD measurement) (total 8 mg.). This compound was named pergularin.*5 The mother liquors and fraction No. 5 were combined, and submitted to column chromatography using Al_2O_3 (10 g.). The results are shown in Table VII and the product was proved to be identical with crystal No. 3 which had previosly been obtained from the stems and leaves of this plants.[1]

Anal. Calcd. for $C_{21}H_{32}O_5$. 1/2 H_2O : C, 67.55; H, 8.85. Found : C, 67.65; H, 9.32.

Fraction No. 3～4 was recrystallized from acetone to needles, m.p. 220～234° (total 38 mg.) which was found to be pure pergularin.

Alkaline Hydrolysis of Group C——A solution of 2.84 g. of group C (in Table I) dissolved in 124 ml. of 5% methanolic KOH was refluxed for 5 hr. After adding 70 ml. of H_2O, MeOH was evaporated *in vacuo* and the residual aqueous solution was extracted successively with small amounts of ether. After evapora-

tion of the solvent, 1.1 g. of a crystalline mass was obtained. Recrystallization from MeOH gave 230 mg. of sarcostin (I), m.p. 150/246~252°. The aqueous layer was extracted with BuOH and usual treatment of the extract gave 707 mg. of residue.

Table VII.

Fraction No.	Solvent	Eluted product (mg.)	Spots on PPC ($CHCl_3$/H·$CONH_2$)
1	benzene	14	—
2	benzene-MeOH (98:2)	30	—
3~4	benzene-MeOH (96:4)	76	VII
5~7	〃	36	VII, IV
8~9	benzene-MeOH (94:6)	7	〃

Each fraction : 100 ml.

The mother liquors of I and the BuOH extract were combined and a paper chromatographic analysis was carried out giving results shown in Fig. 6. This mixture (1.6. g.) was submitted to column chromatography over Al_2O_3 (47 g.) and eluted with $CHCl_3$-EtOH mixture. The results are shown in Table VIII.

Table VIII.

Fraction No.	Solvent	Eluted product	Note
1	$CHCl_3$	422	oil
2~11	$CHCl_3$-EtOH (99.5:0.5)	200	〃
12~21	$CHCl_3$-EtOH (99:1)	97	〃
22~30	$CHCl_3$-EtOH (98:2)	151	I
31~43	$CHCl_3$-EtOH (97:3)	231	I
44~53	$CHCl_3$-EtOH (96:4)	155	powder
54~62	〃	185	VIII
63~90	$CHCl_3$-EtOH (95:5) $CHCl_3$-EtOH (4:1)	115	oil

Each fraction : 100 ml.

Fraction Nos. 22~43 (Table VIII) was treated with acetone and gave 150 mg. of sarcostin (I).

Compound (VIII)——Fraction Nos. 54~62 was recrystallized several times from acetone to prisms 10 mg. m.p. 210~212°. It showed pink→violet→yellowish green with a Liebermann-Burchard and yellowish green with $SbCl_3$. *Anal.* Calcd. for $C_{21}H_{34}O_7$: C, 63.29; H, 8.60. Found : C, 63.21; H, 8.58. IR ν_{max}^{Nujol} cm^{-1} : 3500, 3300, no absorption in 2800 in the 2800~1500 cm^{-1} region. $[\alpha]_{589}^{20}$ + 35° (c=0.22, MeOH, from ORD measurement).

Pergularin Diacetate (VIIa)——VII (25 mg.) was dissolved in 1 ml. of pyridine and 0.5 ml. of Ac_2O was added. The mixture was allowed to stand for 48 hr. at 30°, poured into ice and the white powder which formed was collected and washed several times with H_2O. The filtrate was extracted with $CHCl_3$ and the extract was treated as usual. Evaporation of the solvent gave a minute amount of crystals. Repeated recrystallization from MeOH+H_2O gave 22 mg. of VIIa, m.p. 130~137°. When this material was heated for 18 hr. at 75~85° under reduced pressure, the melting point was raised to 207~215°. *Anal.* Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found : C, 66.97; H, 8.46. IR ν_{max}^{Nujol} cm^{-1} : 3500, 1740, 1720, 1260, 1240.

Pergularin Monooxime (VIIb)——To 20 mg. of pergularin (VII) in 2 ml. of MeOH was added. After allowing the solution to stand on a boiling water bath for 3 hr., H_2O was added and crystals separated. Recrystallization of this product from MeOH +H_2O afforded 11 mg. of VIIb, m.p. 255~263°. *Anal.* Calcd. for $C_{21}H_{33}O_5N$: N, 3.69. Found : N, 3.85. IR ν_{max}^{Nujol} cm^{-1} 3350 : (broad).

Sodium Borohydride Reduction of Pergularin (VIII)——To a solution of 9 mg. of VII in 1 ml. of dioxane, a solution of 10 mg. of $NaBH_4$ in 0.3 ml. of dioxane and H_2O was added. After standing for 36 hr., the solution was acidified to pH 1 with 2*N* H_2SO_4 and extracted with small amounts of ether. Evaporation of the solvent from ether extract gave 10 mg. of white powder. A paper chromatographic analysis (CHC_3l/H·$CONH_2$)[8] of the powder was carried out and two spots were detected (Fig. 8).

The reaction product was submitted to partition chromatography over wet Celite (4 ml. of H_2O, on 4 g. of adsorbent) and the column was eluted with benzene-butanol saturated with water, with the following results : benzene-BuOH (98:2) (100 ml. of solvent, eluted product 0.5 mg.), benzene-BuOH (96:4) (100 ml., 2 mg.), benxene-BuOH (94:6) (50 ml., 2 mg.), benzene-BuOH (9:1) (50 ml., 4 mg.), benzene-BuOH (6:1) (50 ml.,

2 mg.).

From the products eluted with benzene-BuOH (94:4)—benzene-BuOH (6:1), 2 mg. of crystalline substance, m.p. 240~250° which showed only one spot on the chromatogram was crystallized from acetone $+H_2O$. A mixed melting point with an authentic specimen, kindly supplied by Prof. Reichstein, showed no depression. The other component could not be purified by chromatography.

We wish to express our thanks to Prof. T. Reichstein, Basel, for his kind donation of utendin. We are also indebted to Mrs. T. Toma and Miss A. Maeda for the elemental analysis.

Summary

The roots of *Metaplexis japonica* MAKINO contain a complex glycoside mixture.

The alkaline hydrolysate of the crude ester-aglycone mixture showed the presence of sarcostin (I), deacylmetaplexigenin (IIa), deacylcynanchogenin (IVa), utendin (VI), and three other new aglycones. The structure of one of these new aglycones, named pergularin, was found to be closely related to utendin.

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99. Hiroshi Mitsuhashi, Taro Nomura, and Masamichi Fukuoka : Studies on the Constituents of Asclepiadaceae Plants. XX.*1 Epimerization at C-17 and Optical Rotatory Dispersion Study of C/D *cis* Pregnan-20-one Derivatives. 2.*2

(*Faculty of Pharmaceutical Sciences, Hokkaido University**3)

It has already been shown that a series of polyhydroxy pregnane compounds occur in the plant family Asclepiadaceae.[1~7]

All these compounds possess 12β,14β-hydroxy groups and an 20-oxygen group. When these C/D *cis*-pregnan-20-one compounds, for example, ramanone (IVa) and deacylcynanchogenin (Va) were treated with an alkaline solution, an equlibrium mixture of 17β-H-20-keto and 17α-H-20-keto compounds was produced.

The 17β-H-epimer is more stable than the 17α-H-epimer and is obtained as the main product.*2,2,6,7) The optical rotatory dispersion curves of the 17β-H-epimers showed a negative Cotton effect and the curves of the 17α-H-epimers showed a positive Cotton effect.*2,2,6) In C/D *trans*-17-H-20-keto steroids, the 17α-H-20-keto compound

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*3 Kita-15-jo, Nishi-7-chome, Sapporo (三橋 博, 野村太郎, 福岡正道).

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