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Hiroshi Mitsuhashi, Yuzuru Shimizu, Taro Nomura, Tazu Yamada, and Emiko Yamada : Studies on the Constituents of Asclepiadaceae Plants. XI.[1] Separation of New Aglycones from *Cynanchum caudatum* MAXIM.

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It has already been shown that the root of *Cynanchum caudatum* contains ester glycosides containing 2-desoxysugar and two ester aglycones, cynanchogenin, penupogenin which were separated in crystalline form.[2,3] On alkaline hydrolysis cynanchogenin and penupogenin gave deacylcynanchogenin and sarcostin, respectively,[4] but a paper chromatographic study of the alkaline hydrolysate of the crude ester aglycones showed a number of unknown spots besides deacylcynanchogenin and sarcostin.[5] This paper contains the details of the separation of these unknown substances.

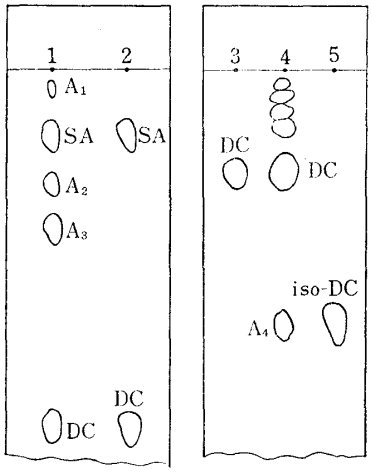

Fig. 1. Examples of Paper Chromatographic Separation of Deacylaglycone Mixture

System : $CHCl_3$/formamide (descending method)
1, 4 : Deacylaglycone mixture
2 : Sarcostin + deacylcynanchogenin
3 : Deacylcynanchogenin
5 : Isodeacylcynanchogenin

The substance, named A_3 which has R_{DC}[*2] value of 0.53 in chloroform-formamide system and exhibits yellow-green color with antimony trichloride, was found to occur as the ester-form in the fraction between cynanchogenin and penupogenin on chromatographic separation. The aglycone mixture which was prepared as reported in Part I[2] of this series was carefully chromatographed over an alumina column.

The paper chromatographic examination of each fraction showed that the trough fractions between those of cynanchogenin and penupogenin are rich in A_3. An attempted hydrolysis of a fraction which seemed to be the highest content gave, after repeated recrystallization, crystaline A_3 contaminated with a minute quantity of sarcostin. In order to obtain more material of greater purity, the neighboring fractions were hydrolyzed with methanolic potassium hydroxide and the deacyl-mixture thus obtained was submitted to partition-chromatography over a wet Celite using benzene-butanol. The

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*2 $R_{DC}=R_{deacylcynanchogenin}$

1) Part X : Tetrahedron, **19**, 1027 (1963).
2) H. Mitsuhasni, Y. Shimizu : This Bulletin, 8, 313 (1960).
3) *Idem* : *Ibid.*, **10**, 726 (1962).
4) *Idem* : *Ibid.*, **8**, 318 (1960).
5) *Idem* : *Ibid.*, **10**, 808 (1962).

first crystals eluted were deacylcynanchogenin, the second and the third were A_3 and sarcostin, respectively. A_3 was also slightly contaminated with sarcostin but after recrystallization from acetone-water, it exhibited a quite pure spot on paper chromatography.

Crystaline A_3 showed m.p. 217~221° (partially melts at 178°) and yellow-green color with $SbCl_3$. On acetylation it gave diacetate m.p. 262~263°. These data fit those of deacetylmetaplexigenin separated from *Metaplexis japonica*,[6] whose identity with A_3 was confirmed by mixed melting point, paper chromatography and infrared spectra. The acid fraction obtained on hydrolysis proved to consist mainly of 3,4-dimethylpentenoic acid on the basis of chromatography.

It was also noticed that a relatively large spot A_4 was seen at R_{DC} 2.0 and it was initially thought to be a new aglycone which exists naturally in the ester form. But paper chromatographic study of many fractions showed that the amount of A_4 paralleled that of deacylcynanchogenin. The crystals A_4 was named as isodeacylcynanchogenin.

In 1959 the authors (H. M, Y. S.) obtained a minute amount of crystals besides deacylcynanchogenin by alkaline hydrolysis of cynanchogenin and it was left unstudied due to lack of material. This material was found to be identical with A_4 by paper chromatography. The partition chromatography of the mother liquors of deacylcynanchogenin gave a rather large amount of A_4. The optical rotatory dispersion curve of isodeacylcynanchogenin (A_4), appeared to be a product cause by alkaline isomerization of deacylcynanchogenin at C-17. The details of this reaction will be reported in a future publication.

Experimental

All melting points were measured on a kofler block and uncorrected. The paper chromatography was carried out as follows; the paper (Toyo Roshi No. 51) was prepared for spotting by dipping in 20% formamide in acetone and developed using $CHCl_3$ saturated with formamide at 25° by descending method.

Cryst. A_3 (=Deacetylmetaplexigenin)——The crude glycoside was hydrolyzed with 0.05*N* H_2SO_4 and chromatographed as described in Part I of this series. The fractions between cynan chogenin and penupogenin were examined by paper chromatography after alkaline hydrolysis and A_3 rich fractions were used for attempted isolation.

a) One fraction which seemed to be the richest in A_3 by paper chromatography was hydrolyzed and extracted continuously with Et_2O. The extract gave crystals m,p, 221° after repeated recrystallyzation from Me_2CO-H_2O. The crystals proved to be contaminated with sarcostin by paper chromatography.

b) A_3 ester fraction (670 mg.) was dissolved in 5% MeOH-KOH (27 ml.), the MeOH evaporated under reduced pressure and the remaining portion extracted with Et_2O successively. The crude extract (640 mg.) was chromatographed over wet Celite (130 ml. H_2O on 130 g. adsorbant) using benzene-BuOH saturated with H_2O. The results are shown in Table I.

Table I. Partition Chromatography of A_3 Fraction

Fr. No.	Solvent	Note	Weight (mg.)	Spot on paperchromatography
1~ 10	Be	oil	245.2	
11~ 23	Bu-Be (3:97)	〃	25.2	trace of DC, A_3, SA
24~ 49	〃 (5:95)	〃	57.7	DC
50~ 68	〃 (1:9)	〃	38.4	DC, A_3
69~100	〃 (1:9)	cryst.	143.6	A_3, SA (trace)
101~125	〃 (1:9)	oil	30.9	A_3, SA
126~147	〃 (1:6)	〃	10.0	〃
148~160	〃 (1:4)	〃	14.7	SA

1 fraction 20 ml., Be=benzene, Bu=butanol, DC=deacylcynanchogenin, SA=sarcostin.

The initial elute with benzene was relatively non-polar and appeared not to be hydrolyzed with KOH. Fraction 69~82 (74 mg.) was recrystallized from Me_2CO-H_2O to give plates (4.1 mg.), m.p. 217~

6) H. Mitsuhashi, T. Nomura, Y. Shimizu, I. Takemori, E. Yamada : This Bulletin, **10**, 811 (1962). The detail of metaplexigenin will be published in the near future.

221° (partially melted at 178° and resolidified, which showed only one yellowish green spot with $SbCl_3$ on paper chromatography. It showed identical behavior as deacetylmetaplexigenin from *Metaplexis japonica* and a mixed melting point showed no depression. The IR spectra also confirmed the identity of both sample. The acetate, m.p. 262~263°, prepared according to the usual procedure was also shown to be identical with a sample of acetylmetaplexigenin by mixed melting point and IR spectra.

Cryst. A_4 (=Isodeacylcynanchogenin)——The mother liquors from the hydrolysate of the cynanchogenin fraction were submitted to partition chromatography over Celite (100 g.) containing H_2O (100 ml.). The results are shown in Table II.

Table II. Partition Chromatography of A_4 Fraction

Fr. No.	Solvent	Note	Weight (mg.)	Spot on paperchromatography
1~10	Be	oil	25.2	—
11~23	Bu-Be (5:95)	〃	15.0	—
24~34	〃 (3:97)	cryst.	64.3	A_4
35~40	〃 (3:97)	〃	58.0	〃
41~45	〃 (3:97)	oil	10.0	A_4, DC
46~60	〃 (1:6)	cryst.	190.0	DC
61~62	〃 (1:4)		trace	

1 fraction 20 ml.

Fraction 24~40 were collected and recrystallized from acetone to give needles, m.p. 248~249.5° which was pure isodeacylcynanchogenin. It is noteworthy that deacylcynanchogenin gave isodeacylcynanchogenin (A_4) on paper chromatography after alkaline treatment. *Anal.* Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 68.64; H, 8.74.

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Summary

The alkaline hydrolysate of the crude aglycones of *Cynanchum caudatum* Maxim. showed the existence of two new aglycones, besides deacylcynanchogenin and sarcostin, by paper chromatography. The one (A_3) was shown to be deacetylmetaplexigenin, and the other (A_4) as isodeacylcynanchogenin. A_4 appears to be an isomer of deacylcynanchogenin formed under the conditions of alkaline hydrolysis.

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Makoto Hayashi, Yoshinori Nakajima, Keizo Inoue, and Komei Miyaki :
Determination of Threonine in Lipid of Animal Brain.

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It has bees reported by Rodes,[1] Igarashi[2] and Wallach,[3] *et al.* that threonine is contained in lipid, especially in phospholipid fraction. However, quantitative studies of this subject have not been published. Therefore, the authors analysed quantitatively threonine in lipid of animal brain, establishing the chemical method for the estimation of small amount of threonine.

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1) D.N. Rodes, C.H. Lea : Biochem. J., **65**, 526 (1957).
2) H. Igarashi, K. Zama, M. Katada : Nature, **181**, 1282 (1958).
3) D.F.H. Wallach, J. Sodergerg, C. Bricker : Cancer Research, **20**, 397 (1960).