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117. Hiroshi Mitsuhashi and Yuzuru Shimizu : Studies on the Constituents of Asclepiadaceae Plants. V.[1] The Isolation and Structure of Penupogenin.*[2]

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As a component of *Cynanchum caudatum* MAX., cynanchogenin was isolated and its structure was presented with (I).[1~5] In the course of the isolation,[2] it was mentioned that another aglycone, which is more polar than (I) and shows bright green Liebermann-Burchard reaction, is present, but at that time the authors failed to crystallize it and named the amorphous powder genin-II. In this paper, more details of investigations on the purification and the structure are described.

The genin-II was carefully chromatographed over alumina and the eluate with 1% methanol-chloroform was crystallized from ether-petroleumether, to m.p. 145~150°. The crystallization needed moisture and the crystals were partially solvated. The compound was named penupogenin (II).*[3] Penupogenin gave coloration, changes pink-green by the Liebermann-Burchard reaction, reddish orange-brown-violet with sulfuric acid, pink-gryish blue with antimony chloride and negative Keller-Kiliani reaction. Penupo genin has the absorption maximum at 279 mμ (ε=22,000) (Fig. 1). The infrared absorption maxima at 3400, 1690, 1630, 1600, and 1580 cm^{-1} show hydroxyls, carbonyl conjugated double bond, double bond conjugated with carbonyl and aromatic double bond, respectively (Fig. 2). These data highly suggested (II) is a cinnamic acid ester.

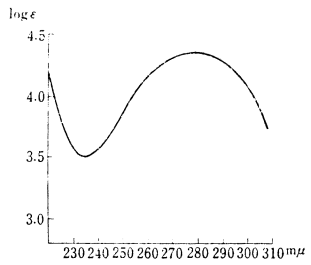

Fig. 1. Ultraviolet Spectram of Penupogenin (II)

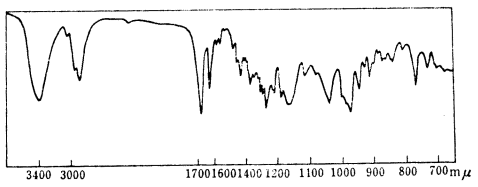

Fig. 2. Infrared Spectrum of Penupogenin (II) in KBr

The hydrolysis of (II) with 5% methanolic potasstum hydroxide followed by successive extraction of the neutral portion gave crystals (III), m.p. 260°/150° (double melting point). The acid fraction gave one spot on the paper chromatogram and crystallized to plates (IV), m.p. 136°, which was identified as a cinnamic acid by the comparison with the authentic sample.

From the infrared data, (III) seemed to be a polyol compound and the acetylation

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*2 This work was reported at the 13th Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1960, and the preliminary report of this paper was published previously. [H. Mitsuhashi and Y. Shimizu : This Bulletin, 8, 565 (1960)].

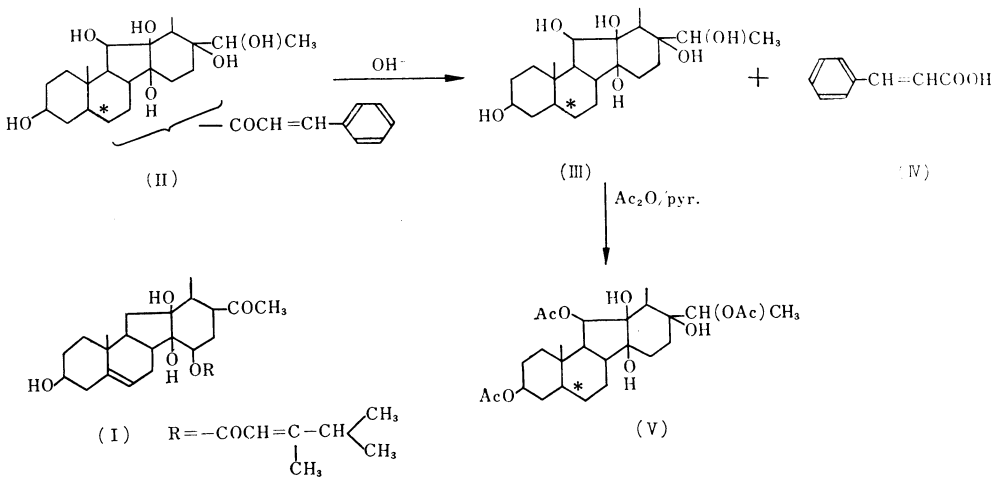
*3 "Penup" is one of the Ainu names for *Cynanchum caudatum* MAX.

1) Part IV : This Bulletin, **10**, 719 (1962).
2) H. Mitsuhashi, Y. Shimizu : *Ibid.*, 8, 313 (1960).
3) *Idem* : *Ibid.*, 8, 318 (1960).
4) *Idem* : *Ibid.*, **7**, 749 (1959); *Ibid.*, 8, 738 (1960).
5) *Idem* : *Ibid.*, **7**, 949 (1959).

gave triacetate (V), m.p. 205°. The elemental analyses of both compounds corresponded to the formula of $C_{21}H_{34}O_6$ (III).

In 1939, Cornforth, *et al.*[6] isolated sarcostin, $C_{21}H_{34}O_6$, from Australian Asclepiadaceae plants, *Sarcostemma australe* R. Br. and Reichstein *et al.*[7] also isolated the same substance from African Asclepiadaceae plants, *Pachycarpus lineolatus* (*Decne.*) BULLOCK. Sarcoslin was very similar to (III) and it was identified by admixtures of sarcostin and its triacetate with the samples obtained.

As Cornforth gave sarcostin the formula C-nor-D-homo-pregnane skeleton,[8] penupogenin could be cinnamoyl sarcostin as described in Chart 1.



The trisubstituted double bond is preferred the position marked *.

Chart 1.

Cornforth's structure of sarcostin (III) has a secondary hydroxyl group at C-11 and the authors' of deacylcynanchogenin at C-15. Except the structure of the side chain, the only difference between sarcostin and deacylcynanchogenin lies in this point. Compounds which found in the same plant usually have a close resemblance in their structures, so further investigations on the structures of the two compounds, deacylcynanchogenin and sarcostin, mainly on the locations of the hydroxyl groups, are now carried on.*4

Experimental

The Isolation of Penupogenin (II)——So-called genin-II portion (500 mg.) which was prepared as described in the previous paper[1] was chromatographed over alumina (15 g.) and eluted with $CHCl_3$ and MeOH. The results are as follows.

Fraction 8 was allowed to stand in Et_2O-petr. ether to give colonial crystals, which were recrystallized from Et_2O to needles (II), m.p. 145~150°. (II) has a tendency to solvate partially. Analytical sample was prepared by drying at 80° *in vacuo*. *Anal.* Calcd. for $C_{30}H_{40}O_7$ (M.W. 512): C, 70.29; H, 7.87. Found: C, 69.93; H, 8.09. The molecular weight was 530 as calculated from the ultraviolet extinction at 280 mμ. ultraviolet spectrum-Fig. 1. Infrared spectrum Fig. 2. Color reactions:

*4 After this paper had been presented, the authors succeeded in finding the correlation between deacyl cynanchogenin and sarcostin by Serini reaction. This Bulletin, **10**, 433 (1962).

6) J.W. Conforth, *et al.* : J. Chem. Soc., **1939**, 737; **1940**, 1443.
7) T. Reichstein, *et al.* : Helv. Chim. Acta, **42**, 1014 (1959).
8) J.W. Cornforth : Chem. & Ind. (London), **1959**, 602.

Fract. No.	Solvent	Weight (mg.)	Notes
1	$CHCl_3$	trace	yellow
2	〃	60	slightly yellow
3	〃	40	colorless
4	〃	20	〃
5	〃	10	partially crystallized
6	〃	trace	〃
7	〃	〃	〃
8	1% MeOH-$CHCl_3$	130	crystallized
9	〃	50	colorless
10	〃	trace	〃
11	5% MeOH-$CHCl_3$	50	yellow

the Liebermann-Burchard reaction (pink-green), 80% H_2SO_4 (reddish orange-brown-violet), the Keller-Kiliani reaction (no change in coloration), and $SbCl_3$ (pink-greyish blue).

Hydrolysis of Penupogenin (II)——A solution of 40 mg. (II) was refluxed for 3 hr. in 5% KOH-MeOH (1.6 cc.). After cooling, H_2O was added to the mixture and MeOH was evaporated in a reduced pressure. The aqueous solution was acidified with H_3PO_4 and extracted with a small volume of Et_2O. The Et_2O layer was treated as usual and it gave a crystalline acid fraction. The extraction of the aqueous layer with BuOH and subsequent usual treatment gave crystals (III).

a) The identification of the acid fraction was carried out as described below; paper partition chromatography (solvent system : BuOH/1.5*N* NH_3, paper : Toyo Roshi No. 50) : acid from penupogenin (Rf 0.51), cinnamic acid (Rf 0.51), acid from cynanchogenin[2] (Rf 0.60). Recrystallization from $CHCl_3$ gave prisms, m.p. 136°, which showed no depression of melting point with the authentic cinnamic acid.

b) Decinnamoylpenupogenin (sarcostin) (III) was recrystallized from MeOH to needles, which melted at 150° firstly, then resolidified and finally melted at 260°. The mixed melting point with authentic sample from *Sarcostemma australe* showed no depression. *Anal.* Calcd. for $C_{21}H_{34}O_6$: C, 64.94; H, 8.96. Found : C, 65.66; H, 7.78. The color reactions are as same as penupogenin.

Decinnamoylpenupogenin Triacetate (sarcostin triacetate) (V)——Decinnamoylpenupogenin (III) (60 mg.) was dissolved in a mixture of pyridine (1 cc.) and Ac_2O (0.5 cc.) which was allowed to stand at room temperature for 2 days. The mixture was poured onto ice and extracted with Et_2O. The Et_2O layer was washed with H_2O, $NaHCO_3$, HCl and again with H_2O and dried over Na_2SO_4. After evaporation of the solvent, the residue was crystallized from Me_2CO-hexane to plates, m.p. 205°. The admixture with the authentic sarcostin triacetate showed no depression. *Anal.* Calcd. for $C_{27}H_{40}O_9$: C, 63.76; H, 7.95. Found : C, 63.45; H, 7.58.

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Summary

A new aglycone, penupogenin, was isolated from *Cynanchum caudatum* MAX. and its structure was decided as a cinnamic acid ester of sarcostin.

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