The authors are deeply greatful to Dr. G. Fukuchi, the managing director of Sankyo Co., Ltd., and Dr. S. Furukawa, the superintendent of Osaka Factory of the same company for helpful encouragement.

Thanks are also due to gentlemen of elementary analysis center of Faculty of Pharmaceutical Sciences of Kyoto University and elementary analysis of the Laboratory of Sankyo Co., Ltd., and also the gentlemen of Analytical Section of Osaka Factory of the same company.

Summary

Metabolic products of sulfadimethoxine excreted in human urine were examined and three metabolites were separated. Unchanged sulfadimethoxine and N^4-acetylsulfadimethoxine were confirmed by paper chromatography and electrophoresis.

And, the other one was sulfadimethoxine-N-glucuronide, and the methyl acetyl derivative of the glucuronide was confirmed sulfadimethoxine-N^1-methyl-(tri-O-acetyl-β-D-glucopyranosid)uronate-N^4-acetate.

Sulfadimethoxine-N-glucuronide found in human urine was decided to be sulfadimethoxine-N^1-β-D-glucopyranoside or its lactone form.

(Received August 10, 1964)

[Chem. Pharm. Bull. 13(3) 267~273 (1965)]

UDC 582.938 : 581.19

32. Hiroshi Mitsuhashi,*1 Tadashi Sato,*2 Taro Nomura, and Ikuko Takemori*1 : Studies on the Constituents of Asclepiadaceae Plants. XIV.*3 Structure of Tomentogenin.*4

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The isolation of sarcostin and tomentogenin from the stems of *Marsdenia tomentosa* DECNE (Asclepiadaceae) has been reported previously[1] from these laboratories and a tentative structure of tomentogenin was proposed.*4

In the present paper, some later findings on the structure of tomentogenin are reported. The plant material was collected at Amatsu, Chiba, in June 1962, and dried at 60°. The powdered material was treated as shown in Chart 1 and described in the experimental part. "Tomentogenin" was thus obtained and showed properties very similar to those reported before.*3 By careful examination with paper chromatography ($CHCl_3$/formamide),[2] tomentogenin was found to be separated into two very closely situated spots, and the results of elemental analysis indicated $C_{21}H_{34}O_5$ or $C_{21}H_{36}O_5$. Crude tomentogenin, upon catalytic hydrogenation took up about 0.3 mole hydrogen and gave tomentogenin (I), which was identical with the major spot of crude tomentogenin on paper chromatography. Elemental analysis of tomentogenin (I), $[\alpha]_D^{16}$ +36° (c=0.95, MeOH) suggested a molecular formula of $C_{21}H_{36}O_5$. Infrared bands at 3400 and 3150 cm^{-1} showed the presence of hydroxyl groups, but there were no bands assignable to

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*3 Part XIII : Steroids, **4**, 483 (1964).

*4 Part of this work was reported at the 83rd Annual Meeting of the Pharmaceutical Society of Japan, and published in this Bulletin, **12**, 981 (1964).

1) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, E. Yamada : This Bulletin, **10**, 804 (1962).

2) H. Mitsuhashi, Y. Shimizu, E. Yamada, I. Takemori, T. Nomura : *Ibid.*, **10**, 808 (1962).

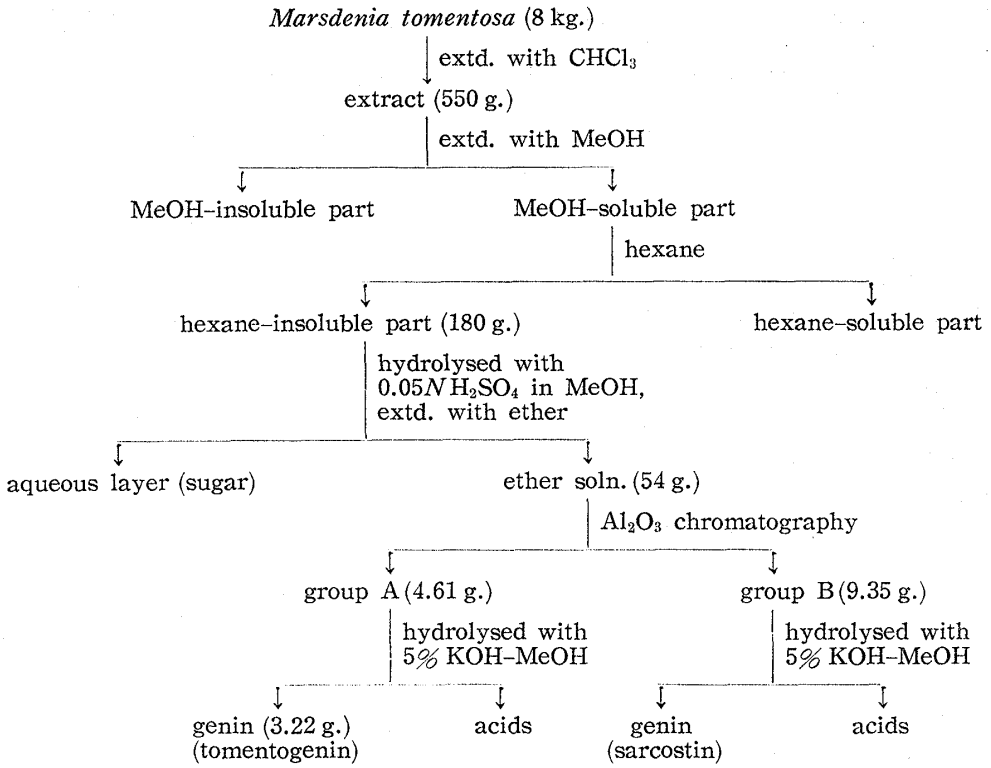


Chart 1.

carbonyl groups. Acetylation of I with acetic anhydride in pyridine yielded a triacetyl derivative (II), m.p. 293°, which showed hydroxyl absorption at 3470 cm^{-1}. Tomentogenin (I) formed a dibenzoate (III), m.p. 247~253°, in poor yield. Chromic acid-acetic acid oxidation of III afforded IV, m.p. 219~223°, which has infrared absorption at 1715 cm^{-1} (6-membered ketone). IV did not form an oxime upon treatment in the usual manner. From these experiments, it was concluded that I had a hindered secondary OH group. Tomentogenin (I) consumed one mole of lead tetraacetate, but its triacetate (II) was inert. This observation might be interpreted as indicating the presence of a glycol which is constructed of two secondary OH groups or one secondary and one tertiary OH group. Tomentogenin (I) was rapidly oxidized by one mole of lead tetraacetate or periodic acid to acetaldehyde (VI) and V, m.p. 242~245°, having the formula $C_{19}H_{30}O_4$ and infrared absorption at 1736 cm^{-1} (5-membered ketone). The optical rotatory dispersion curve of V showed a positive Cotton effect, a=4250° in dioxane, and was very similar to that of C/D-*cis* 17-oxo-steroids.[3,4] Androstane-17-one (C/D-*trans*) shows a=14800°.[*5] Therefore the C/D ring juncture of V should be *cis*, and the position of the side chain is restricted to C-17 by analogy with other steroids. Chromic acid-acetic acid oxidation of V gave VII, m.p. 227~232°, $C_{19}H_{26}O_4$, which has infrared absorption maxima at 3500, 1756 (5-membered ketone),[5] and 1713 cm^{-1}. Acetylation of V with acetic anhydride in pyridine gave a diacetate (VIII), m.p. 197~200°, $C_{23}H_{34}O_6$, which has OH absorption at 3600 cm^{-1} suggesting one remaining tertiary OH group. To determine the location of the tertiary OH group, VIII was treated with thionyl chloride in pyridine, usually applied to dehydration of 14β-OH in cardenolides, but no anhydro compound was formed. Also dehydration of VIII with freshly fused $KHSO_4$ in acetic anhydride failed to give good

[*5] a=molecular amplitude.

3) K. A. Jaeggi, E. K. Weiss, T. Reichstein: Helv. Chim. Acta, **46**, 694 (1963).
4) F. Sondheimer, S. Bummer, R. Mechoulam: J. Am. Chem. Soc., 82, 3209 (1960).
5) R. Jones, P. Humphries, K. Dobriner: *Ibid.*, **71**, 241 (1949).

results. Compound (Ⅶ) was treated with 15% hydrochloric acid in ethanol. The reaction mixture showed selective absorption at 246 mμ*6, but purification of the product was unsuccessful (Table Ⅱ).

From these facts, it is obvious that two hydroxyl groups in I are limited to positions 8β and 11α, or 12β and 14β.[6~8]

To determine the location of the double bond in dehydrotomentogenin (I′), a mixture of I and I′ was oxidized with periodic acid and gave V and Ⅸ. An Oppenauer oxidation of V and Ⅸ gave X and Ⅺ. The ultraviolet spectrum of the mixture X and Ⅺ showed a maximum at 238.5 mμ suggesting the presence of a Δ^4-3-one system. Therefore, the double bond in I′ is limited to positions 4 or 5. Nuclear magnetic resonance data on I, I+I′, and V+Ⅸ was collected and is shown in Table Ⅲ. These data support the presence of vinyl proton at C-6 and suggest the presence of a double bond at C-5.

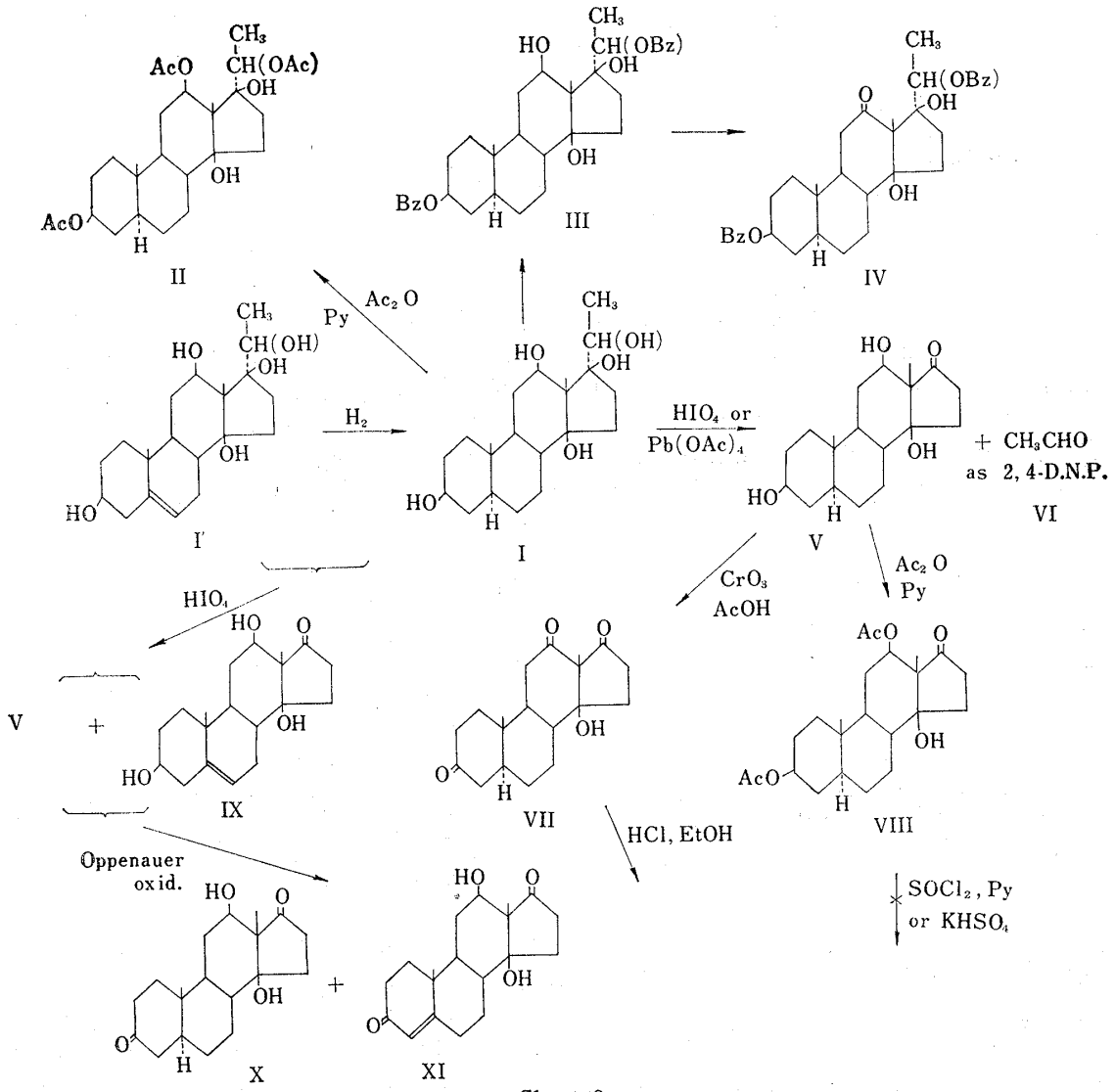
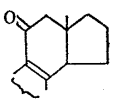


Chart 2.

*6  Calcd. λ_{max} 249 mμ.

6) E. P. Oliveto, *et al.* : J. Am. Chem. Soc., **78**, 1736 (1956).
7) P. Bladon, *et al.* : J. Chem. Soc., **1953**, 2921.
8) A. Katz, T. Reichstein : Pharm. Acta Helv., **19**, 231 (1944).

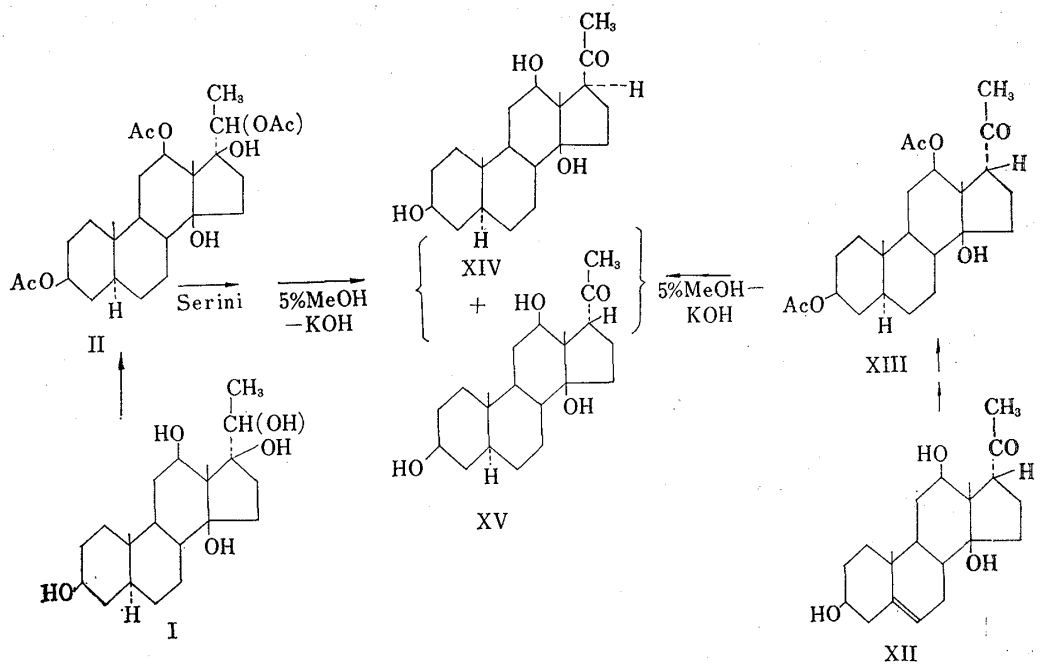


Chart 3.

Nomura isolated a glycoside from *Metaplexis japonica* and obtained ramanone as the genin.[9] The structure of ramanone was determined as XII. Dihydrodiacetylramanone (XIII) and II are identical except for the two carbon side chain at C-17. Tomentogenin triacetate (II) was submitted to a Serini reaction by refluxing with active zinc in xylene and the reaction mixture examined by thin-layer chromatography. The reaction was repeated twice under different conditions and the results are shown in Fig. 1.

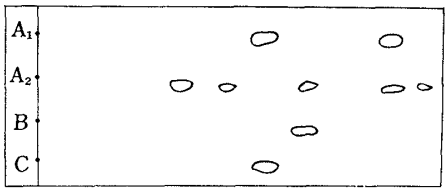


Fig. 1.

A_1 : Products of Serini reaction (1st)
A_2 : Products of Serini reaction (2nd)
B : Dihydrodiacetylramanone
C : Tomentogenin triacetate

(10% acetone/benzene) ($SbCl_3$)

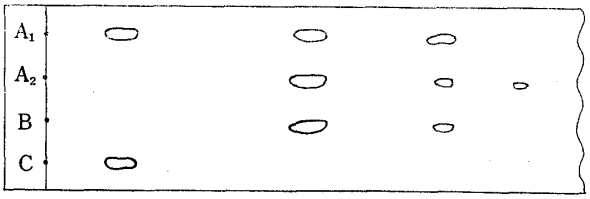


Fig. 2.

5% KOH-MeOH treatment products of
A_1 : Serini reaction mixture (1st)
A_2 : Serini reaction mixture (2nd)
B : Dihydrodiacetylramanone
C : Tomentogenin

($CHCl_3$/formamide) ($SbCl_3$)

The reaction mixture was hydrolysed by refluxing in 5% methanolic potassium hydroxide for 5 hours, and examined by paper chromatography (Fig. 2). The largest spot was that of XV and the others were those of XIV and starting material or an unknown substance. These results indicated that XV is the main product. Alkaline treatment of C/D *cis*-20-oxosteroids produced an equilibrium mixture of 17α- and 17β-H-20-oxo compounds. The 17β-H-epimer is more stable and is obtained as the major product.[10] If one assumes that, the Serini reaction proceeds with inversion at C-17 in tomentogenin triacetate (II), the results of thin-layer chromatography (Fig. 1) and paper chromatography (Fig. 2) show that the structure of tomentogenin is I (17β-OH). From

9) H. Mitsuhashi, T. Nomura : Steroids, 3, 271 (1964).
10) H. Mitsuhashi, T. Nomura, M. Fukuoka : Steroids, 4, 483 (1964).

the second Serini reaction, a very small spot (A_2 in Fig. 1) corresponding to dihydrodiacetylramanone (XIII) was detected and chromatography on alumina gave crystalline XIII. The conditions of the second Serini reaction were more drastic than those of the first experiment. A possible explanation is that isomerization to the more stable 17β-isomer took place during the second, more drastic, Serini reaction.

Reichstein obtained sarcostin, lineolon (=deacylcynanchogenin), and utendin[11] from the African Asclepiadaceae plant, *Pacycarpus lineolatus*, and proposed a structure for utendin[12] by correlation with digoxigenin. The C-17 side chain was suggested as 17β-OH through biogenetic considerations with sarcostin. The identity of tomentogenin (I) with 5α-dihydroutendin, and of dehydrotomentogenin (I') with utendin, was confirmed by mixed melting point and thin-layer chromatography kindly performed by Prof. Reichstein. The structure of tomentogenin (=5α-dihydroutendin), and of dehydrotomentogenin (=utendin) was thus determined independently at about the same time.

Experimental

Extract——Ground stems of *Marsdenia tomentosa* (8 kg.) were percolated with $CHCl_3$ at room temperature and 550 g. of a faintly yellow powder thus obtained was treated with MeOH. The MeOH-soluble part was added to hexane with stirring. The hexane-insoluble precipitate (180 g.) was dissolved in MeOH and the mixture refluxed for 25 min. after addition of 0.1N H_2SO_4. The MeOH was evaporated *in vacuo* at room temperature and the residue extracted with ether. The ether layer was washed with 5% $NaHCO_3$ solution, H_2O, and dried over Na_2SO_4. Removal of the solvent gave 54 g. of a powder, which gave a negative Keller-Killiani reaction. This residue was submitted to chromatography over Al_2O_3. The eluates were divided into groups A and B by Ac_2O+HIO_4 color reaction, group A (4.61 g.); red, and group B (9.35 g.); yellow→red brown.

Fraction A (4.61 g.) was dissolved in 180 ml. of 5% KOH-MeOH and refluxed for 25 min., MeOH evaporated and the residue extracted with ether. Removal of ether gave a white crystalline mass of crude tomentogenin, which was recrystallized from MeOH-acetone to give colorless plates, m.p. 259~261°; 3.22 g. Paper chromatographic analysis of crude tomentogenin ($CHCl_3$/formamide) showed two spots. By the same treatment, Fraction B gave sarcostin. The sugars and acids were reported in the previous paper.[1]

Tomentogenin(I)—— A solution of 1 g. of crude tomentogenin in 50 ml. of EtOH was shaken with 1.0 g of PtO_2 in AcOH, in H_2 atmosphere for 200 min. H_2 uptake : 1/3 mole. After the catalyst was filtered off, the solution was evaporated to dryness and the residue crystallized from acetone to give fine plates, m.p. 264~267°; 625 mg. *Anal.* Calcd. for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found : C, 68.15; H, 9.99. IR ν_{max}^{Nujol} cm^{-1} : 3400, 3150. $[\alpha]_D^{16}$ +36° (c=0.95, MeOH).

Acetylation of Tomentogenin (I)——Tomentogenin (I) was dissolved in 2 ml. of pyridine and 1 ml. of Ac_2O was added. The mixture was allowed to stand for 48 hr. at room temperature, poured on ice, and a white powder which appeared was extracted with $CHCl_3$. Evaporation of the solvent and crystallization from MeOH gave 48.5 mg. of crystals; m.p. 293°. *Anal.* Calcd. for $C_{27}H_{42}O_8$ (triacetate) : C, 65.56; H, 8.56. Found : C, 65.60; H, 8.36.

Tomentogenin Dibenzoate (III)——Tomentogenin (I) (102 mg.) was dissolved in 1 ml. of pyridine and 1.1 ml. of BzCl was added. After the solution to stand in an ice box for 48 hr., H_2O was added. An oily substance which separated was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 2N HCl, 5% $NaHCO_3$, H_2O and dried over Na_2SO_4. Removal of solvent gave a crystalline mass. Recrystallization of this product from acetone-hexane afforded 28 mg. of dibenzoate (III), m.p. 247~253°. *Anal.* Calcd. for $C_{35}H_{44}O_7$: C, 72.89; H, 7.69. Found : C, 73.09; H, 7.84.

Chromium Trioxide Oxidation of Tomentogenin Dibenzoate (III)——Tomentogenin dibenzoate (III) (74 mg.) was dissolved in AcOH (2.5 ml.) and 2% CrO_3 in AcOH (950 mg. of CrO_3) was added. After standing at 24° for 10 hr., MeOH was added to remove excess CrO_3, and the solvent removed *in vacuo*. The residue was extracted with ether. The ether layer was washed with 2N HCl, 5% $NaHCO_3$, H_2O, dried over Na_2SO_4, and evaporated. The residue gave needles (IV), m.p. 219~223°, from acetone-hexane. Yield, 61.4 mg. *Anal.* Calcd. for $C_{35}H_{42}O_7$: C, 73,14; H, 7.37. Found : C, 73.15; H, 7.27. IR : ν_{max}^{Nujol} 1715 cm^{-1}.

A solution of 4 mg. of $NH_2OH \cdot HCl$ in 50% EtOH 1 ml. and 4 mg. of AcONa was added to 27.5 mg. of IV in 1.5 ml. of EtOH. The solution was heated on a boiling water bath for 1.5 hr., and worked up as usual, but IV was recovered instead of the oxime.

11) E. Abish, Ch. Tamm, T. Reichstein : Helv. Chim. Acta, 42, 1014 (1959).
12) A. Lardon, W. Klyne, E. Iseli, T. Reichstein : Abstracts of Papers, IUPAC 3rd Symposium on the chemistry of natural products. April 18, (1964), Kyoto.

Estimation of $Pb(OAc)_4$ Consumption——To a solution of 0.05 mmole of tomentogenin (I) and tomentogenin triacetate (II), dissolved in dioxane (2.5 ml.), 10 ml. of $N/25$ $Pb(OAc)_4$ in AcOH was added and the mixture allowed to stand at room temperature (15~17°) and 2 ml. of each mixture titrated by iodometry. A balnk was prepared and titrated similarly. The results are shown in Table I.

Table I.

	Tomentogenin (I) $Pb(OAc)_4$ moles (time : hr.)	Tomentogenin triacetate (II) $Pb(OAc)_4$ moles (time : hr.)
1	0.013 (0.25)	0.0 (20)
2	0.065 (0.5)	0.04 (73)
3	0.164 (1)	0.08 (90)
4	0.164 (2.5)	0.07 (118)
5	0.714 (26)	0.07 (142)
6	1.06 (56)	0.12 (167)
7	1.04 (120)	0.04 (285)

Oxidation of Tomentogenin (I) with Periodic Acid——To a solution of tomentogenin (I) dissolved in 10 ml. of EtOH, 100 mg. of $HIO_4 \cdot 2H_2O$ was added and the mixture kept at room temperature for 43 hr. The reaction mixture was treated as follows; i) A forced air stream passed through the reaction mixture was collected in 2,4-dinitrophenylhydrazine mixture. A large amount of yellow crystals separated. Recrystallization from EtOH gave crystals, m.p. 154~160°, which showed no depression on mixed melting point with the 2,4-dinitrophenylhydrazone of acetaldehyde.

ii) After the above treatment, the solvent was removed from the reaction mixture under reduced pressure and the residue recrystallized form MeOH-hexane to give 40 mg. of V; m.p. 242~245°. *Anal.* Calcd. for $C_{19}H_{30}O_4$: C, 70.77; H, 9.38. Found : C, 70.76; H, 8.97. IR ν_{max}^{Nujol} cm^{-1} : 3500, 1736.

Rotatory dispersion of V, $a = 4.25 \times 10^3$*5 ($c = 0.33$, dioxane). [Rudolph photoelectric spectropolarimeter].

Oxidation of V with Chromic Trioxide——To 90 mg. of V dissolved in 3 ml. of AcOH, 3 ml. of 2% CrO_3 in AcOH was added. The mixture was kept at room temperature for 49 hr., and worked up as usual. Recrystallization from acetone gave 31.5 mg. of VII as plates; m.p. 227~232°. *Anal.* Calcd. for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23. Found : C, 72.02; H, 8.18. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3500, 1756, 1715.

Acetylation of V——To a solution of 37 mg. of V dissolved in 1 ml. of pyridine, 0.5 ml. of Ac_2O was added. The mixture was kept at room temperature for 48 hr. and treated with ice water. Recrystallization from acetone-hexane gave 46.3 mg. of VIII; m.p. 196~199.5°. *Anal.* Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found : C, 67.86; H, 8.42.

Formation of Anhydro Compound from VIII and VII——i) VIII (64.7 mg.) was dissolved in 1 ml. of pyridine, 15 ml. of $SOCl_2$ added, maintained for 5 hr. at 0°, and treated with ice water. Crystals that separated were collected, washed, and recrystallized from MeOH to give needles; m.p. 105~115°. This product showed one spot on thin-layer chromatography dut was not purified further. Beilstein test, positive. *Anal.* Found : C, 65.22; H, 8.68

Table II.

Time elapsed (hr.)	UV λ_{max} 246 mμ ε
22	2,750
46	4,540
68	7,900
92	10,450

ii) Freshly fused $KHSO_4$ (40 mg.) was added to a solution of 80 mg. of VIII dissolved in 1 ml. of Ac_2O, and the mixture maintained at 150~160° for 1 hr. The solution was extracted with ether and the ether layer, following the usual treatment, gave a yellow oil (66.6 mg.). Attempts to purify the mixture were unsuccessful. iii) Ten ml. of conc. HCl was added to 38 mg. of VII dissolved in 10 ml. of 50% EtOH. After allowing the solution to stand at room temperture (15°), the UV absorption of the mixture was measured and the results are given in Table II. For the calculation of ε, molecular weight, VII-H_2O was used tentatively.

The mixture was kept for 92 hr. and then adjusted to pH 4 with 2% NaOH. The solution was extracted with $CHCl_3$. The $CHCl_3$ layer was treated as usual and gave 11.2 mg. of an oil. By chromatography on alumina, 4.5 mg. of a crystalline mass was obtained, λ_{max}^{EtOH} 238 mμ (ca. ε 4,000), which was not purified further.

Oppenauer Oxidatione of the Mixture of V and IX——A mixture (486 mg.) of tomentogenin (I) and dehydrotomentogenin (I') was oxidized with HIO_4 and a mixture of products, V+IX, was obtained. This mixture (90 mg.) was dissolved in 40 ml. of toluene and 8 ml. of cyclohexane, and refluxed with 150 mg. of Al(iso-PrO)$_3$ for 100 min. The solvent was removed by steam distillation and the residue was extracted with $CHCl_3$. The extract was washed with $2N$ $NaHCO_3$, H_2O, and dried. A crystalline mass (X+XI) was obtained. λ_{max}^{EtOH} 238.5 mμ (ca. ε 8,000). The NMR spectra were also measured and are given in Table III.

Table III.

Compound	Solvent	Signal (τ)*7			
		19-CH_3	18-CH_3	21-CH_3	
I	pyridine	9.25	8.45	8.57	
I+I′	〃	9.20	8.34	8.47,	5.60 (d, C-6 H), 4.25 (s, broad, OH?)
V+IX	〃	9.15	8.40		5.97 (q, C-6 H), 4.25 (s. OH?)

Serini Reaction of Tomentogenin Triacetate (II)——i) Four g. of Zn (Mallincrodt, 20 mesh) was heated with conc. H_2SO_4 (10 ml.) and conc. HNO_3 (4 drops) on a steam bath for 20 min., washed with H_2O, EtOH, acetone, dried at 170~180° in a vacuum for 5 hr., and kept in a desiccator. Tomentogenin triacetate (II) (105 mg.) was heated with this Zn (3 g.) in absolute xylene under reflux with exclusion of moisture and in an atmosphere of N_2 for 48 hr. (bath temperature 160~170°). The warm solution was filtered, the filtrate was evaporated completely in a vacuum, and the crystalline residue recrystallized from MeOH. The following fractions were obtained; (1) Tomentogenin triacetate (II) 48 mg.; (2) A crystalline mass from evaporation of the mother-liquors, 20 mg. Thin-layer chromatography (Fig. 1) showed two spots. This crystalline mass was added to 5% KOH-MeOH, heated for 5 hr., and the resulting mixture gave 3 spots on paper chromatography (Fig. 2).

ii) The Serini reaction was repeated under the same conditions as above, except the activation conditions for the Zn were heating at 200° for 5 hr. The resulting mixture and the product from treatment with 5% KOH-MeOH were examined by thin-layer and paper chromatography (Figs. 1 and 2). The product was chromatographed on a column of Al_2O_3. The chromatogram was developed with various solvents. From the benzene fraction, a crystalline product (28 mg.) was obtained. Recrystallization from hexane-ether gave 2.5 mg. of crystals, m.p. 165~170°, which showed no depression on admixture with dihydrodiacetylramanone.

We wish to express our thanks to Prof. T. Reichstein (Basel) for his helpful discussion. We thank the Chiba Enshurin (Tokyo University) for collection of the plants. We are also indebted to Mrs. T. Toma and Miss A. Maeda for the elemental analysis.

Summary

The stems of *Marsdenia tomentosa* contain a glycoside mixture. The alkaline hydrolysate of the crude aglycones showed the presence of sarcostin and two new aglycones. The structures of those two new materials, tomentogenin and dehydrotomentogenin has been proved. Tomentogenin was shown to be identical with 5α-dihydroutendin and dehydrotomentogenin with utendin. The structures of these latter materials were determined about the same time as this work by Reichstein.

(Received August 18, 1964)

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