[Chem. Pharm. Bull. 25(4) 611—616 (1977)]

UDC 547.913.02.05 : 581.192

# Studies on the Constituents of Asclepiadaceae Plants. XLII.[1] Component of *Marsdenia tomentosa* Decne. Structure of 12β-*O*-Acetyltomentogenin and Hypothetical Biogenetic Pathway of Polyoxypregnanes in *M. tomentosa*

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(Received June 14, 1976)

A new polyoxypregnane derivative, 12β-*O*-acetyltomentogenin, was isolated from the stem of *Marsdenia tomentosa*. A hypothetical biogenetic pathway of polyoxypregnane derivatives is proposed from their ester linkages.

**Keywords**——*Marsdenia tomentosa*; Asclepiadaceae; polyoxypregnane; 12β-*O*-acetyltomentogenin; tomentogenin; ester-linkage; oxygen function; hypothetical biogenetic pathway

Our preceding papers reported the structures of several ester-type polyoxypregnane derivatives[3] from *Marsdenia tomentosa* Decne (Asclepiadaceae) and the absolute configurations of tomentogenin (I) and utendin[4] (II). Further examination of the polar aglycone resulted in the isolation of some polyoxypregnane derivatives. In this paper, we report the isolation and structural elucidation of two ester-type polyoxypregnane derivatives, tentatively named compound-P and -Q, and propose a hypothetical biogenetic pathway of polyoxypregnanes in *M. tomentosa* from the relationship between their ester-linkages.

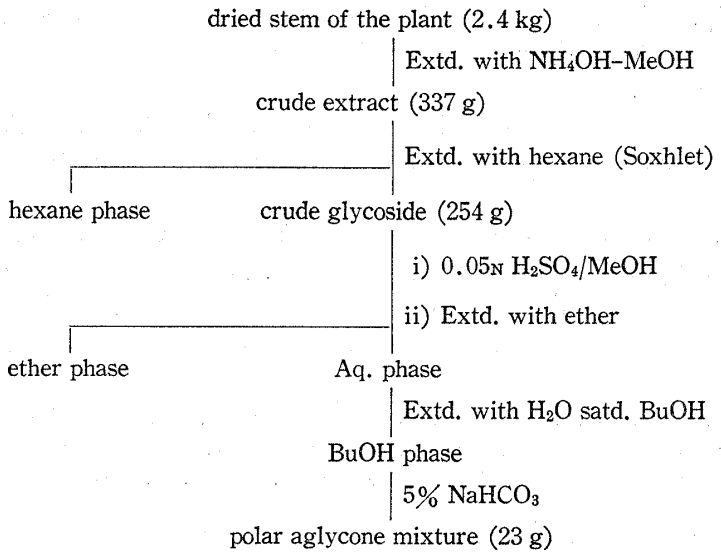



Chart 1. Extraction and Separation

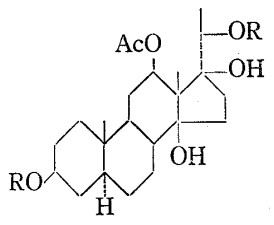
1) Part XLI: H. Bando, T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.*, (Tokyo), **24**, 3085 (1976).
2) Location: *Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo, 060, Japan.*
3) *a*) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1552 (1975); *b*) H. Seto, K. Hayashi, and H. Mitsuhashi, *ibid.*, **23**, 2397 (1975); *c*) H. Seto, K. Hayashi, and H. Mitsuhashi, *ibid.*, **24**, 443 (1976); *d*) H. Seto, K. Hayashi, and H. Mitsuhashi, *ibid.*, **24**, 1552 (1976); *e*) H. Seto, T. Sasaki, K. Hayashi, and H. Mitsuhashi, *ibid.*, **24**, 2185 (1976).
4) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 2457 (1976).

The polar aglycone mixture (butanol phase), obtained by a mild acid hydrolysis of the crude glycoside[5] was separated and purified by silica gel column chromatography and preparative thin-layer chromatography (TLC). These procedures yielded two fine crystalline substances, compound-P and -Q. Compound-P (III) was identical with tomentidin[3d] from the comparison of spectral data and mixed mp with an authentic sample.

Compound-Q (IV) showed mp 165—168°, $[\alpha]_D^{19}$ +25° ($c$=0.4, $CHCl_3$). The molecular formula $C_{23}H_{38}O_6$ was given for IV from its elemental analysis and mass spectrum ($M^+$ at $m/e$ 410). The infrared (IR) spectrum of IV showed absorptions for hydroxyl groups at 3480, 3350, 1080, and 1060 $cm^{-1}$, and a saturated ester at 1710 and 1240 $cm^{-1}$. The nuclear magnetic resonance (NMR) spectrum of IV showed signals for two tertiary methyl groups at $\delta$ 0.80 and 1.36, one secondary methyl group at 1.24 (d, $J$=6 Hz), one acetyl group at 2.03 (s), three hydroxy-methines at 3.50 (m), 3.54 (q, $J$=6 Hz), and 4.60 (d.d, $J$=6, 11 Hz), and no olefinic proton. Acetylation of IV with acetic anhydride-pyridine afforded an acetate, which was identical with tri-O-acetyltomentogenin[3b,5c,6] (V).

Prominent mass spectral peak indicative of acetate functional group was observed at $m/e$ 43. Further evidence was secured from the mass spectral peaks of IV since there were faint parent ion at $m/e$ 410 and other fragments at $m/e$ 392 ($M^+-H_2O$), 365 ($M^+$–CHOH·Me)[7], 350 ($M^+$–AcOH), 332 ($M^+$–AcOH–$H_2O$), and 43 (acetyl cation). The peak at $m/e$ 365 definitely suggests that an acetate moiety was at C-12$\beta$ of tomentogenin.

The NMR decoupling experiments were carried out to confirm the position of the ester linkage in IV. Irradiation of 21-Me group protons ($\delta$ 1.24, 3H, d, $J$=6 Hz) collapsed the quartet at $\delta$ 3.54 to a singlet but not the double-doublet at $\delta$ 4.60, and conversely that of the hydroxy-methine at $\delta$ 3.54 collapsed 21-Me group protons to a singlet, so that the hydroxy-methines at $\delta$ 3.54 and 4.60 correspond to 20$\beta$- and 12$\alpha$-H,[8] respectively. From these evidences, the structure of compound-Q (IV) was determined as 12$\beta$-O-acetyltomentogenin.




IV: R=H(12$\beta$-*O*-acetyltomentogenin)
V: R=Ac(tri-*O*-acetyltomentogenin)

Fig. 1

The biogenesis of cardenolides and bufadienolides has been studied by Reichstein,[9] Tschesche,[10] and Heftmann,[11] who have found that their main carbon skeleton is supplied from cholesterol.

Up to the present, sixteen ester-type derivatives of polyoxypregnane, including 12$\beta$-*O*-acetyltomentogenin (IV), have been isolated from *M. tomentosa*, and some interesting interrelation between them has been formed on the basis of the position of ester linkages and the nature of oxygen functions. For example, structures of kidjolanin[5d] (VI), penupogenin[5d,12]

5) *a*) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 884 (1962); *b*) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *ibid.*, **13**, 267 (1965); *c*) M. Fukuoka and H. Mitsuhashi, *ibid.*, **16**, 1634 (1968); *d*) T. Sasaki, K. Hayashi, and H. Mitsuhashi, *ibid.*, **20**, 628 (1972).
6) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. Pharm. Bull.* (Tokyo), **12**, 981 (1964).
7) M. Fukuoka, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1469 (1971).
8) N.S. Bhacca and D.H. Williams, "Application of NMR Spectroscopy in Organic Chemistry-Illustrations from Steroid Field," Holden-Day, Inc., San Francisco, 1964.
9) T. Reichstein, *Naturwissenschaften*, **54**, 53 (1967).
10) *a*) R. Tschesche and G. Libienweiss, *Z. Naturforsch.*, **19b**, 265 (1964); *b*) R. Tschesche, *Planta Med.* (Suppl. 5), **34**, (1971).
11) *a*) R.D. Bennett and E. Heftmann, *Science*, **149**, 652 (1965); *b*) H.H. Sauer, R.D. Bennett, and E. Heftmann, *Phytochemistry*, **6**, 1251 (1967); *c*) H.H. Sauer, R.D. Bennett, and E. Heftmann, *Naturwissenschaften*, **54**, 226 (1967); *d*) R.D. Bennet, H.H. Sauer, and E. Heftmann, *Phytochemistry*, **7**, 41 (1968); *e*) R.D. Bennett, E. Heftmann, and B.J. Winter, *Phytochemistry*, **8**, 2325 (1969).
12) H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **10**, 725 (1962).

(VII), 20-*O*-acetylpenupogenin[3e] (VIII), and gagaminin[13] (IX) suggest their biogenetic relation from the similarity of their ester-linkage at C-12$\beta$ and the position of oxygenated carbons. It is suggested that the ketone function at C-20 of kidjolanin (VI) is reduced to afford penupogenin (VII), and subsequent acetylation or nicotinoylation at C-20 hydroxyl group of VII would give 20-O-acetylpenupogenin (VIII) or gagaminin (IX), respectively, and ikemagenin[14] (X) isolated from *Cynanchum caudatum* (Asclepiadaceae) by Yamagishi and Mitsuhashi is presumed as the precursor of VI. The same interrelation is also found in deacetylkidjoladinin[3e] (XI) and kidjoladinin[3e] (XII), as well as cynanchogenin[12] (XIII) and caudatin[15] (XIV) from *C. caudatum*.

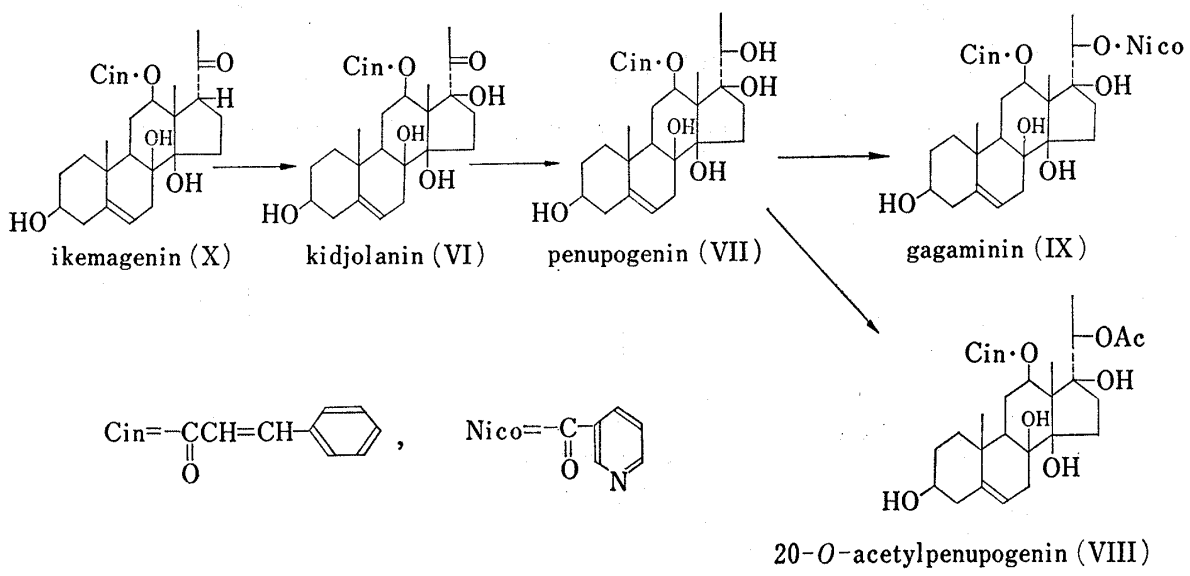



Fig. 2

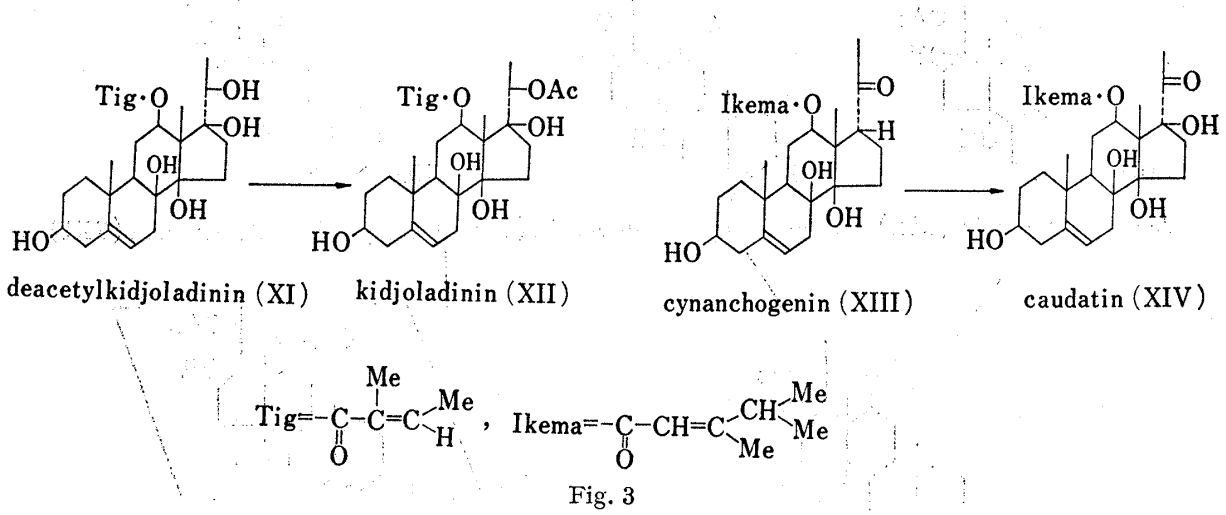



Fig. 3

Coexistence of tigloyl ester and 2-methylbutyroyl ester in phorbol esters of croton oil[16] was reported, and 2-methylbutyrate was proved to be the precursor of tigloyl moiety of 3$\beta$-tigloyltropane in *Datura innoxia*,[17] so that tomentonin[3c] (XV) can presumably be the precursor of tomentosin[3a] (XVI). On the other hand, from an organic chemical point of view,

13) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2289 (1972).
14) T. Yamagishi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2070 (1972).
15) T. Yamagishi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 625 (1972).
16) E. Hecher and R. Schmidt, *Fortschr. Chem. Org. Naturst.*, **31**, 376 (1975).
17) K. Basey and J.G. Woolley, *Phytochemistry*, **14**, 2201 (1975).

deacetyltomentosin[3d] (XVII) and dehydrotomentosin[3c] (XVIII) are also assumed to be the precursor of tomentosin (XVI), as are dehydrotomentinin[3b] (XIX) and 12$\beta$-O-acetyltomentogenin (IV) that of tomentinin[3b] (XX), and deacetyldehydrotomentodin[3e] (XXI) that of tomentodin[3c] (XXII).

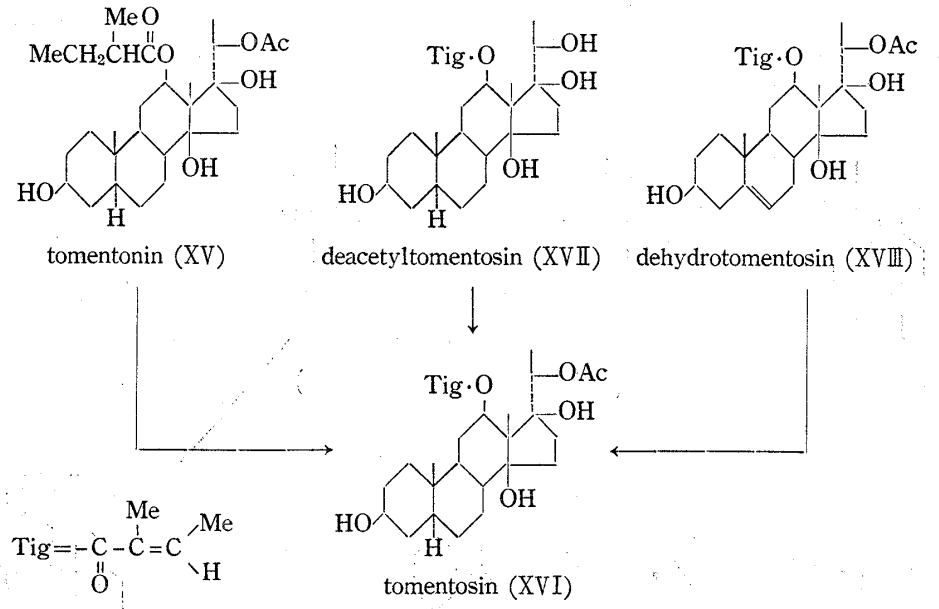



Fig. 4

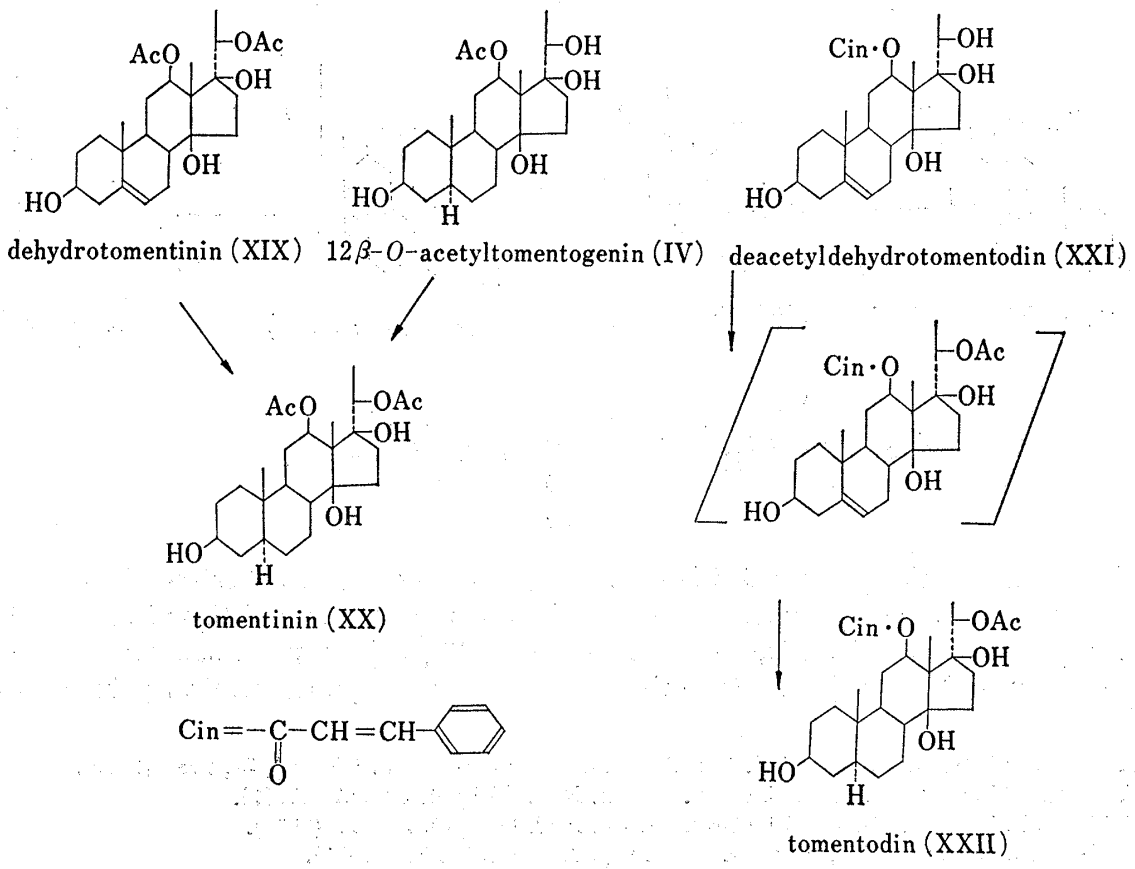



Fig. 5

Isolation of four deacyl-type polyoxypregnane derivatives, tomentogenin (I), utendin (II), deacylmetaplexigenin (XXIII), and sarcostin (XXIV), from *M. tomentosa*[5] has already been reported. From these past and present results, a hypothetical biogenetic pathway of polyoxypregnanes in *M. tomentosa* can be suggested as shown in Fig. 6. The presence of ramanone[18] (XXV), isoramanone[18] (XXXVI), and pergularin[19] (XXVII) isolated from *Metaplexis japonica* (Asclepiadaceae) by Nomura and Mitsuhashi, and of lineolon[14,20] (XXVIII) and isolineolon[14] (XXIX) from *C. caudatum* by Yamagishi and Mitsuhashi, and from *Pachycarpus lineolatus* (Asclepiadaceae) by Reichstein, Tamm, and Abish support our scheme.

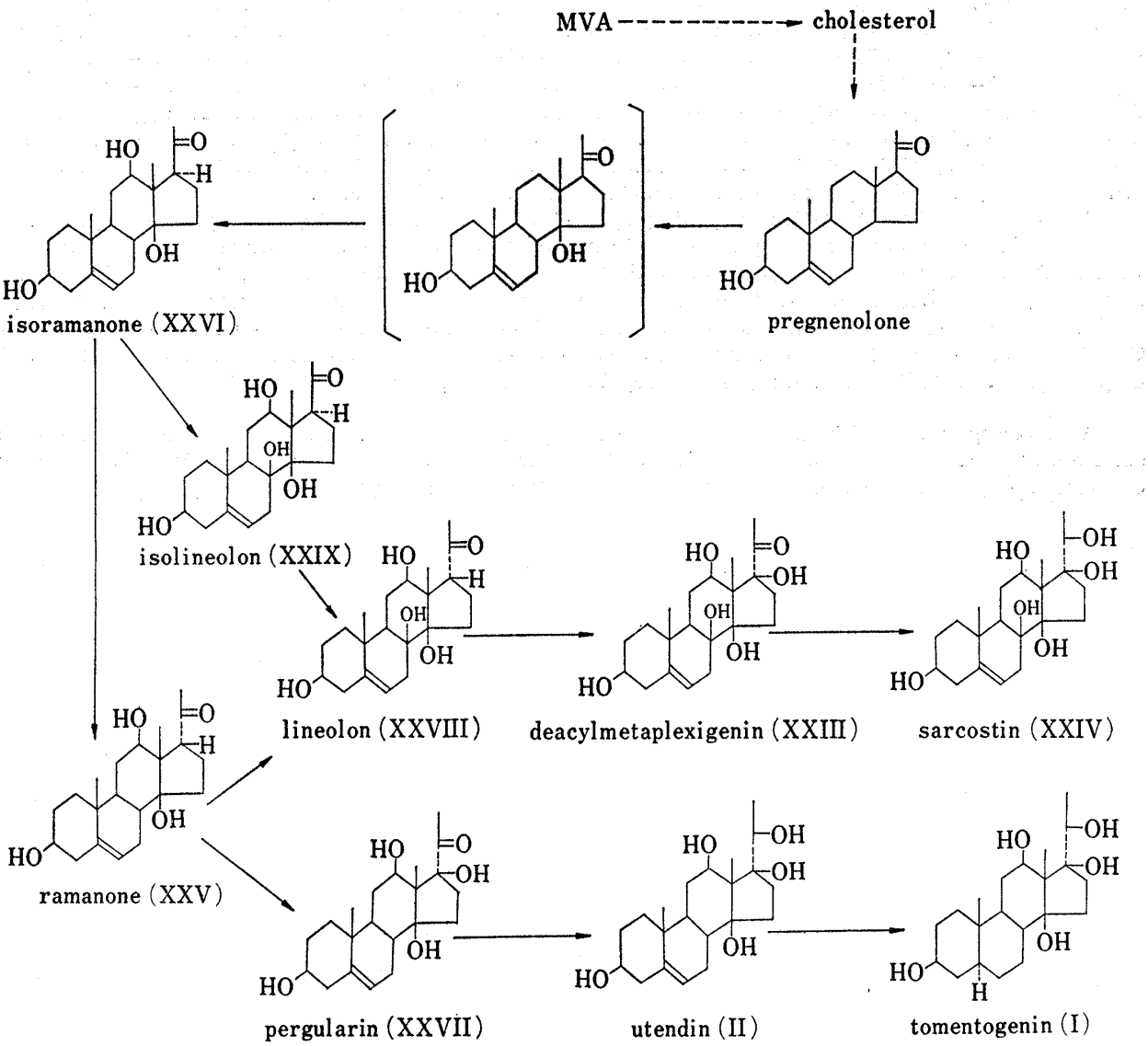



Fig. 6. Hypothetical Biogenetic Pathway of Polyoxypregnanes in *Marsdenia tomentosa*

## Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in $CHCf_3$ solution on a Hitachi S115-4 polarimeter. NMR spectra were determined on a JEOL PS-100 spectrometer operating at 100 MHz with tetramethylsilane (TMS) as an internal standard. Mass

18) H. Mitsuhashi and T. Nomura, *Steroids*, **1964**, 271.
19) H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **12**, 1525 (1964).
20) E. Abish, Ch. Tamm, and T. Reichstein, *Helv. Chim. Acta.*, **42**, 1014, (1959).

spectra were determined on a Hitachi RMU-7 mass spectrometer. IR spectra were taken in Nujol mull on a Hitachi 215 spectrometer. TLC was performed on Silica gel $HF_{254}$ (Merck, Type 60), and silica gel 0.05—0.2 mm (Merck, 70—325 mesh ASTM) was used for column chromatography.

**Isolation of Polar Aglycone Mixture**——The dried and powdered stem (2.4 kg) of *M. tomentosa*, collected in November 1973 at Owase, Mie Prefecture, was used as the material. The ammoniacal MeOH extract (337 g) was treated with hexane to yield the crude glycoside (254 g). A solution of 240 g of the crude glycoside dissolved in 1.2 liters of MeOH was refluxed for 30 min with 1.2 liters of 0.1 N $H_2SO_4$ on a water bath, 1.2 liters of $H_2O$ was added, MeOH was evaporated *in vacuo*, and the residual aqueous solution was heated at 60° for 30 min. After ether extraction of the aqueous solution, the resulting mixture was extracted five times with a total of 2 liters of $H_2O$–satd. BuOH, which was washed with 5% $NaHCO_3$ in BuOH–satd. $H_2O$ solution and BuOH–satd. $H_2O$ to yield 23 g of a polar aglycone mixture.

**12*β*-*O*-Acetyltomentogenin (IV)**——From 20 g of the polar aglycone mixture, 25 mg of 20-*O*-acetyl tomentogenin (IV) was obtained by column chromatography and preparative TLC. IV was recrystallized from acetone–hexane to afford needles, mp 165—168°, $[\alpha]_D^{19}$ +25° ($c$=0.4, $CHCl_3$). Mass Spectrum *m/e*: 410 ($M^+$), 392 ($M^+-H_2O$), 374 ($M^+-2H_2O$), 365 ($M^+$—CHOH·Me), 350 ($M^+$—AcOH), 347 ($M^+$—CHOH·Me–$H_2O$), 332 ($M^+$—AcOH–$H_2O$), 314 ($M^+$—AcOH–$2H_2O$), 305 ($M^+$—CHCO·Me–AcOH), 287 ($M^+$—CHOH·Me–AcOH–$H_2O$), 269 ($M^+$—CHOH·Me–AcOH–$2H_2O$), 262, 249, 244, 226, 43 (base peak). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3480, 3350, 1710, 1240, 1080, 1060, 1020. NMR $\delta_{ppm}^{CDCl_3}$: 0.80 (3H, s, 19-Me), 1.24 (3H, d, $J$=6 Hz, 21-Me), 1.36 (3H, s, 18-Me), 2.03 (3H, s, OAc), 3.50 (1H, m, 3α-H), 3.54 (1H, q, $J$=6 Hz, 20β-H), 4.60 (1H, d.d, $J$=6, 11 Hz, 12α-H). *Anal.* Calcd. for $C_{23}H_{38}O_6$: C, 67.29; H, 9.33. Found: C, 67.15; H, 9.42.

**Acetylation of 12*β*-*O*-Acetyltomentogenin (IV)**——A solution of 10 mg of 12β-*O*-acetyltomentogenin (IV), 1 ml of $Ac_2O$, and 1 ml of pyridine was allowed to stand for 16 hr at room temperature, and poured into ice water. A white powder that appeared was collected and recrystallized from $Me_2CO$–MeOH to 5 mg of the acetate (V) as needles, mp 283—285° and mixed mp with authentic tri-*O*-acetyl-tomentogenin, 280—284°. Mass Spectrum *m/e*: 494 ($M^+$), 434 ($M^+$—AcOH), 416 ($M^+$—AcOH–$H_2O$), 407 ($M^+$—CHOAc·Me), 389 ($M^+$–CHOAc–$H_2O$), 374 ($M^+-2\times$AcOH), 339 ($M^+$—CHOAc·Me—AcOH), 314 ($M^+-3\times$AcOH), 304, 291, 286, 226, 43 (base peak). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3740, 3400, 1740, 1710, 1275, 1250, 1235, 1050, 1040, 1020.

**Acknowledgement** The plant material was kindly collected by Mr. M. Kawaguchi and we express our sincere gratitude. We also thank Miss M. Takahashi for mass spectral measurement, Miss T. Obara for elemental analysis, and Miss T. Okayama for NMR spectral measurement.