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Studies on the Constituents of *Marsdenia formosana* Masamune. III. Isolation and Structural Elucidation of Some New Steroidal Glycosides

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From the methanolic extracts of *Marsdenia formosana* Masamune (Asclepiadaceae), three new steroidal glycosides, MF-A, MF-C and MF-D, together with β-sitosterol-3β-D-glucoside (VIII) and dehydrotomentosin (I) were isolated. As the results of spectroscopic and chemical investigations, it has been demonstrated that the stereostructures of MF-A, MF-C and MF-D were represented, respectively, by the formulas II, IV and VI.

Keywords——*Marsdenia formosana*; Asclepiadaceae; glycoside; ester aglycone; dehydrotomentosin-3-O-β-D-cymaropyranoside; dehydrotomentosin; pergularin-3-O-β-D-cymaropyranoside; utendin-3-O-β-D-cymaropyranoside; β-sitosterol-3β-D-glucoside

Marsdenia formosana Masamune (Chinese name: Taiwan Niu-nai Zai; Asclepiadaceae) is widely distributed along mountains of Taiwan. Its constituents are of interest, because a number of this genus plants have been reported to indicate toxicity to grazing stock.[2,3] Members of the genus *Marsdenia* which were studied previously (viz. *M. condurango* Reich,[4,5] *M. tomentosa* Decne[6] and *M. erecta* R. Br.[7]) contained glycosides or ester aglycones derived from various polyhydroxy pregnanes. On the other hand, the triterpenoid components have also been found in the several plants of the same genus.[2,8,9,10]

Previously,[10] we reported the isolation and characterization of the thirteen triterpenoids including five new triterpenoids (α-amyrin formate, lupenyl cinnamate, marsformol, marsformoxide A and marsformoxide B) from the petroleum ether extracts of *Marsdenia formosana* Masamune. In the present paper, we here describe the isolation and structural elucidation of some steroidal glycosides from the methanolic extracts of this plant.

The procedure of extraction and isolation of these steroidal glycosides is illustrated in Chart 1. Four glycosides and one ester aglycone thus obtained, were tentatively named MF-A, B, C, D and E in the order of increasing polarity.

MF-A, colorless prisms, mp 196—198°, $[\alpha]_D$ +43.5° ($CHCl_3$), has a molecular formula $C_{35}H_{54}O_{10}$. Its infrared (IR) spectrum showed absorption bands due to hydroxyl groups at 3550 and 1080 cm^{-1}, acetyl group at 1730 and 1255 cm^{-1}, together with the bands of an α,β-unsaturated ester at 1700, 1682, 1642, and 1185 cm^{-1} assignable to a tigloyloxy side chain. The presence of a tigloyloxy side chain in this compound was further substantiated by the

1) Location: *Yagoto-urayama, Tenpaku-cho, Tenpaku-ku, Nagoya.*
2) Xorge A. Dominguez, J. Marroguin, Luz M. Olguin, F. Morales, and V. Valdez, *Phytochemistry*, **13**, 2617 (1974).
3) R.E. Summons, J. Ellis, and E. Gellert, *Phytochemistry*, **11**, 3335 (1972).
4) K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 2522 (1968).
5) R. Tschesche, P. Welzel, and G. Snatzke, *Tetrahedron*, **21**, 1777 (1965).
6) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **25**, 611 (1977).
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8) D. Venkata Rao, E. Venkata, N. Viswanadham, *Curr. Sci.*, **36**, 421 (1967) [*C.A.*, **67**, 105081*p* (1967)].
9) Mendes, P. Henriques, Sharapin, Nikolai, Alvares de Souza, Alberto, and Pereira, Salvador A, *Rev. Bras. Farm.*, **54**, 61 (1973) [C.A., **84**, 28021*r* (1976)].
10) *a*) K. Ito and J. Lai, *Yakugaku Zasshi*, **98**, 249 (1978); *b*) K. Ito and J. Lai, *Chem. Pharm. Bull.* (Tokyo), **26**, 1908 (1978).

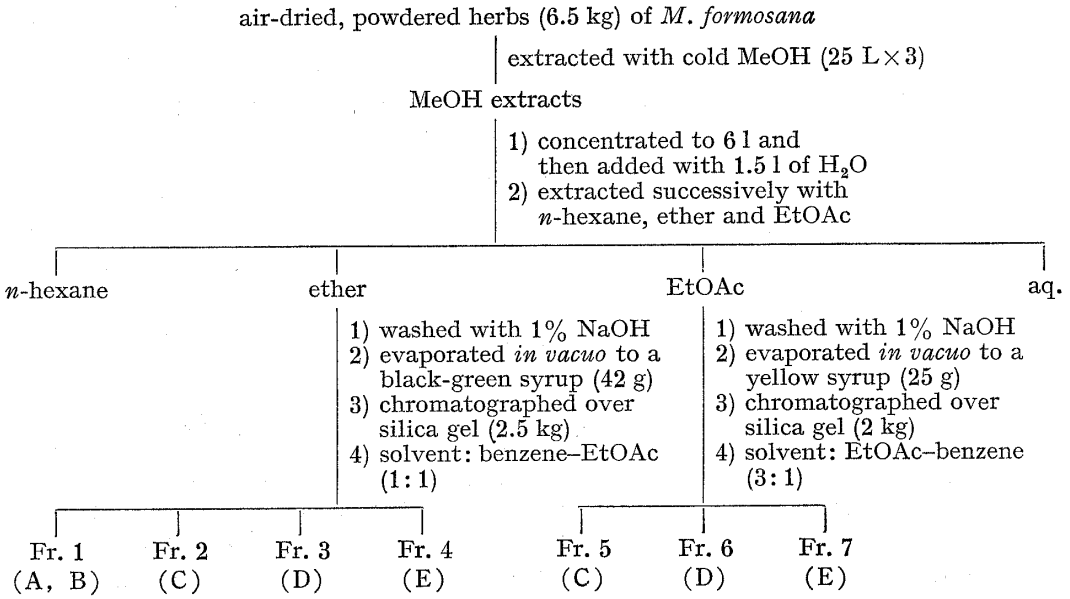

Chart 1

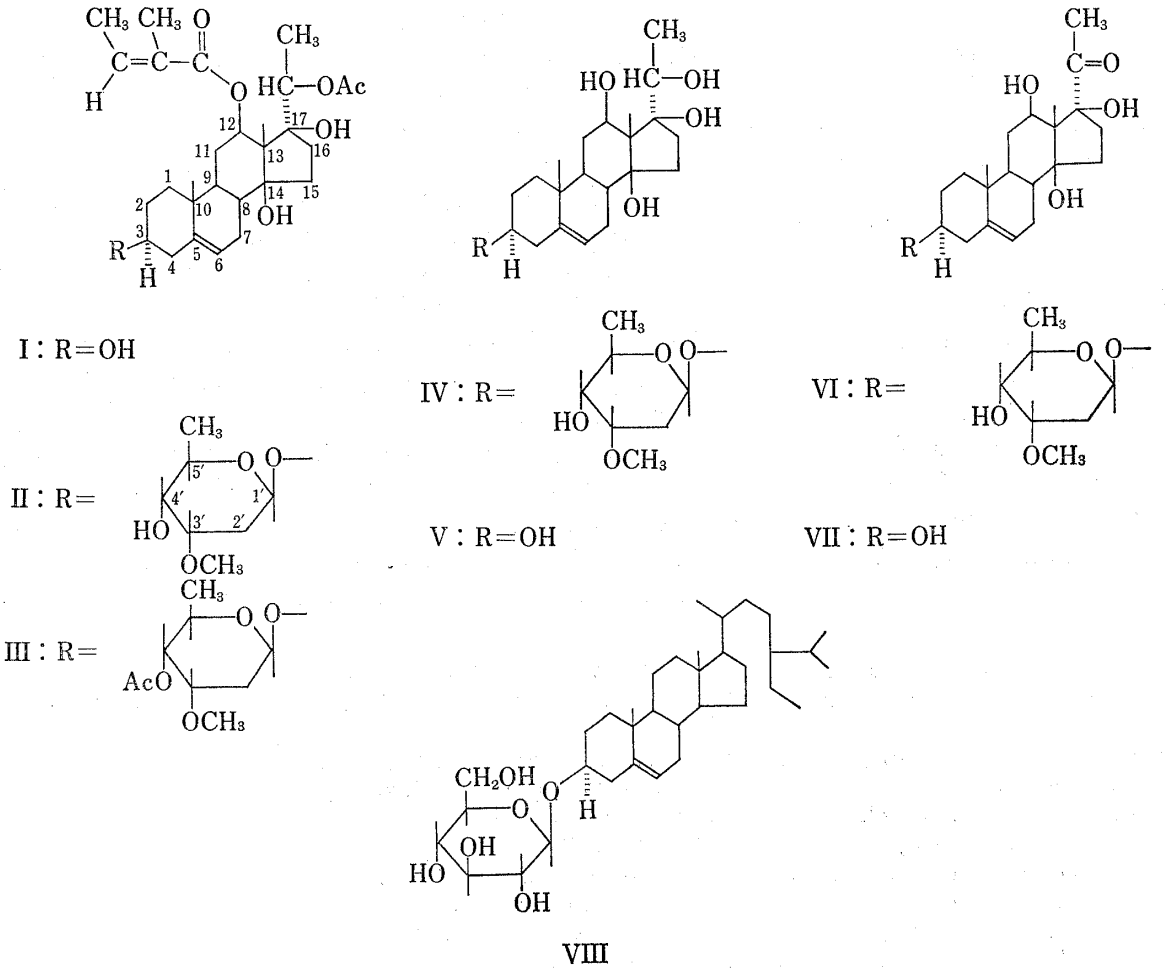

Chart 2

proton chemical shifts corresponding to two olefinic methyl groups at δ 1.81 (d, J=6 Hz) and 1.83 (s), together with the signal of an olefinic proton at δ 6.82 (d, J=6 Hz) in the nuclear magnetic resonance (NMR) spectrum. Its NMR spectrum also disclosed the presence of two tertiary methyl groups at δ 0.96 and 1.20, two secondary methyl groups at δ 1.19 (d, J=6 Hz) and 1.23 (d, J=6 Hz), and an acetyl group at δ 1.90, in addition to a trisubstituted olefinic proton at δ 5.40, a methoxyl group at δ 3.38 and methine protons at δ 3.20 (1H, sext, J=3, 9 Hz,[11] 3.38—3.58 (2H, m), 3.59 (1H, q, J=3 Hz), 4.56 (1H, q, J=6 Hz), 4.62 (1H, d.d, J=6, 11 Hz) and 4.78 (1H, d.d, J=3, 9 Hz). The mass (MS) spectrum of MF-A indicated the molecular ion peak at *m/e* 634, and its fragmentation pattern was closely similar to that of dehydrotomentosin (I)[12] except for the peaks at *m/e* 145 and 113. From the above physical data, it was inferred that MF-A would be the dehydrotomentosin-3-O-methylpentopyranoside, and the fragment peak at *m/e* 145 seemed to be due to the presence of the terminal sugar residue. By comparisons of the NMR spectra of MF–A with that of dehydrotomentosin (I), we found that the signal at δ 4.78 was attributable to the anomeric proton showing α-configuration (axial),[13] and the signals at δ 3.20 and 3.59 were assignable to the methine protons of the methylpentopyranoside residue.

Acetylation of MF-A with acetic anhydride–pyridine afforded MF-A acetate (III). The MS spectrum of this acetate showed a prominent fragment ion peak at *m/e* 187, 155 and 95 (base peak). Its NMR spectrum also disclosed the presence of an additional new acetyl group at δ 2.08, and the signal at δ 3.20 of MF-A indicated apparent downfield shift to δ 4.56 in this acetate (III). Therefore, the signal at δ 3.20 of MF-A is assigned to a proton on the carbon atom bearing hydroxyl group, and the signal at δ 3.59 to a proton on the carbon attaching to methoxyl group in the sugar residue. Furthermore, the signal (δ 4.78) of the anomeric proton of MF-A was apparently shifted to δ 5.10 in pyridine-d_5, and this fact suggested the 1,3-diaxial relationship of the anomeric proton with hydroxyl or methoxyl group. Thus, either hydroxyl group or methoxyl group must reside in C-3′ α (axial) configuration. Simultaneously, from the coupling constants of these two signals (δ 3.20 and 3.59), the methoxyl group must be located at C-3′ α (axial) and the hydroxyl group at C-4′ α (equatorial) configuration.

From the above data, the sugar moiety of MF-A is found to be β-D-cymaropyranose, and the structure of this glycoside would be dehydrotomentosin-3-O-β-D-cymaropyranoside (II). In fact, on acid hydrolysis with refluxing 1 N H_2SO_4–EtOH, MF-A gave an aglycone and a sugar. The resulting aglycone (mp 180—182°) was completely indentical with an authentic sample of natural dehydrotomentosin (I)[12] in comparison of their mixed melting point, IR and MS spectra. The sugar fraction was also identified as D-cymarose by comparison of their thin–layer chromatography (TLC) and paper partition chromatography (PPC) examination with an authentic sample, which was obtained by the acidic hydrolysis of periplocymarin[13] in the same way as MF-A. As the result of the above experiments, MF-A is proved to be represented by the formula II. This glycoside is a new one, which has not ever described in literatures.

MF-B, colorless prisms, mp 180—182°, $[\alpha]_D$ +45° ($CHCl_3$), has a molecular formula $C_{28}H_{42}O_7$. From the investigation of its IR, NMR and MS spectra, we found that MF-B would be the same compound as dehydrotomentosin (I). In fact, this compound was completely identical with an authentic sample in comparison of their mixed melting point and IR, and MS spectra.

MF–D, colorless prisms, mp 249—252° (dec.), has a molecular formula $C_{28}H_{46}O_8$. The IR spectrum of this compound indicated absorption bands due to the hydroxyl group at

11) H. Allgeier, *Helv. Chim. Acta*, **51**, 311 (1968).
12) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 443 (1976).
13) S. Sakuma, H. Ishizone, R. Kasai, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **17**, 2183 (1969).

3550 cm^{-1} and ether functional band at 1095, 1065, and 1006 cm^{-1}. Its NMR spectrum also disclosed the presence of two tertiary methyl groups at δ 1.00 and 1.14, two secondary methyl groups at δ 1.20 (d, J=6 Hz) and 1.28 (d, J=6 Hz), together with a methoxyl group at δ 3.40, a trisubstituted olefinic proton at δ 5.40, and the anomeric proton of sugar residue at δ 4.80 (1H, d.d, J=3, 9 Hz). Its MS spectrum showed the molecular ion peak at *m/e* 510, and its fragmentation pattern is closely similar to that of utendin (V)[14] except for the prominent fragment ion peaks at *m/e* 145 and 113. From the above physical data, the most probable structure of MF-D is represented by IV. In fact, this compound was proved to be identical with an authentic sample of utendin-3-O-β-D-cymaropyranoside (IV) which was derived from II by its alkaline hydrolysis. MF-D (IV) is a new steroidal glycoside which has not described hitherto in literatures.

MF-C, colorless prisms, mp 245—248°, has a molecular formula $C_{28}H_{44}O_8$. Its IR spectrum indicated absorption bands owing to hydroxyl groups at 3560 cm^{-1} and carbonyl group at 1700 cm^{-1}, together with ether functional band at 1090, 1060, and 1005 cm^{-1}. The NMR spectrum of this compound also showed the presence of two tertiary and one secondary methyl groups, one methoxyl group, one olefinic proton, together with an acetyl group at δ 2.29, and an anomeric proton of the sugar residue at δ 4.80 (1H, d.d, J=3, 9 Hz). Its MS spectrum exhibited the molecular ion peak at *m/e* 508, and its fragmentation pattern was closely similar to that of pergularin (VII)[15] except for the prominent fragment ion peaks at *m/e* 145 and 113. On acid hydrolysis with refluxing 1 N H_2SO_4–EtOH, MF-C gave pergularin (VIII) and D-cymarose. On the other hand, reduction of MF-C with $LiAlH_4$ in dioxane afforded utendin-3-O-β-D-cymaropyranoside (IV). From the above physical and chemical data, the structure of MF-C was proved to be represented by pergularin-3-O-β-D-cymaropyranoside (VI). This glycoside (VI) is also a new compound which has never isolated hitherto in nature.

MF-E, colorless prisms, mp 285—288° (dec.), $[\alpha]_D$ −37° (pyridine), has a molecular formula $C_{35}H_{60}O_6$. On acid hydrolysis with refluxing 1 N H_2SO_4–EtOH, MF-E afforded β-sitosterol and D-glucose. From the above data, it was confirmed that MF-E was β-sitosterol-3-β-D-glucoside (VIII).[16]

Experimental[17]

Extraction of Steroidal Glycoside from *Marsdenia formosana* MASAMUNE——The dry herbs of this plant (6.5 kg) collected from the Luh-Tsyr-Tarn, Taipei Republic of China in November, 1977, was extracted 3 times with 25 l of cold MeOH. After concentration of the solvent *in vacuo* to 6 l, 1.5 l of H_2O was added, and the mixture was successively extracted with *n*-hexane, ether, and EtOAc. The ethereal extract was washed with 1% NaOH and H_2O. The organic layer was evaporated to dryness *in vacuo* to give a black-green syrup (42 g), which was carefully chromatographed on silica gel (2.5 kg) packed in benzene containing 70% EtOAc, and eluted with a mixture of benzene: EtOAc (1: 1). The eluates showing *Rf*

14) M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2448 (1969).
15) H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **12**, 1523 (1964).
16) *a*) H. Mitsuhashi and K. Tomimoto, *Syoyakugaku Zasshi*, **25**, 7 (1971); *b*) I. Inagaki, S. Hisada, and K. Shima, *Yakugaku Zasshi*, **91**, 1133 (1971).
17) All melting points were taken on a micro hot-stage and are uncorrected. IR spectra were recorded on a JASCO A-3 spectrometer. NMR spectra were taken on a JEOL PS-100 spectrometer and signals are indicated in δ values from tetramethylsilane (TMS) as an internal standard. MS were taken on a Hitachi M-52 mass spectrometer with a heated direct inlet system. Optical rotations were measured on a JASCO DIP-SL polarimeter. PPC were examined on Toyo Roshi No. 50 in ascending method using toluene: BuOH (1: 9: 3)[18] as a solvent and anthrone–H_2SO_4 as a spray reagent for sugar. TLC were performed on silica gel G Nach Stahl (Merck) using $CHCl_3$: MeOH: H_2O (7: 3: 1)[19] lower phase as a solvent and anthrone–H_2SO_4 as a spray reagent for sugar.
18) H. Mitsuhashi, T. Nomura, Y. Shimizu, I. Takemori, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 811 (1962).
19) S. Sakuma, S. Kawanishi, and J. Shoji, *Syoyakugaku Zasshi*, **22**, 23 (1968).

value of 0.75, 0.55, 0.3, and 0.15 on TLC examination ($CHCl_3$: EtOAc: MeOH=2: 15: 1) were collected as fraction 1, 2, 3, and 4 respectively. On the other hand, the EtOAc extract was washed with 1% NaOH and H_2O, and the organic layer was evaporated *in vacuo* to a yellow syrup (25 g). It was carefully chromatographed on silica gel (2 kg) packed in EtOAc, and then eluted with a mixture of EtOAc: benzene (3: 1). The eluted solution which showed *Rf* values of 0.6, 0.3, and 0.15 on TLC examination ($CHCl_3$: EtOAc: MeOH= 2: 15: 1) were collected as fraction 5, 6 and 7, respectively.

Treatment of Fraction 1——The solution of fraction 1 was evaporated *in vacuo* to a yellow syrup (6.5 g). From 500 mg of this syrup, MF-A (95 mg) and B (18 mg) were obtained by silica gel chromatography and repeated preparative TLC ($CHCl_3$: MeOH=100: 1).

MF-A (Dehydrotomentosin-3-O-β-D-cymaropyranoside (II))——MF-A was recrystallized from $(CH_3)_2CO$ and *n*-hexane to give colorless prisms. mp 196—198°. $[\alpha]_D^{25}$ +43.5° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1730, 1700, 1680, 1642, 1255, 1065, 1010. NMR ($CDCl_3$) δ: 0.95 (3H, s, tert. CH_3), 1.19 (3H, d, J=6 Hz, sec.CH_3), 1.20 (3H, s, tert.CH_3), 1.23 (3H, d, J=6 Hz, sec.CH_3), 1.81 (3H, d, J=6 Hz, vinyl CH_3), 1.83 (3H, s, vinyl CH_3), 1.90 (3H, s, OAc), 3.20 (1H, sext., J=3, 9 Hz), 3.38—3.58 (2H, m), 3.38 (3H, s, OCH_3), 3.59 (1H, q, J=3 Hz), 4.56 (1H, q, J=6 Hz), 4.62 (1H, d.d, J=6, 11 Hz), 5.40 (1H, d, J=3.5 Hz), 6.82 (1H, d, J=6 Hz). MS *m/e*: 634 (M^+), 472, 454, 436, 412, 394, 372, 354, 312, 294, 276, 224, 145, 113, 83, 43. *Anal.* Calcd. for $C_{35}H_{54}O_{10}$: C, 66.22; H, 8.57. Found: C, 65.95; H, 8.83.

Acetylation of MF-A——A solution of MF-A (500 mg) in Ac_2O (0.5 ml) and pyridine (3 ml) was allowed to stand for 24 hr at room temperature. After dilution with water, the reaction mixture was extracted with ether, and then worked up in the usual way to give colorless solid (48 mg), which was chromatographed on silica gel (20 g) to afford MF-A acetate (III). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1730, 1700, 1680, 1643, 1250, 1095, 1065, 1010. NMR ($CDCl_3$) δ: 0.96 (3H, s, tert. CH_3), 1.19 (3H, d, sec.CH_3), 1.20 (3H, s, tert.CH_3), 1.23 (3H, d, sec.CH_3), 1.81 (3H, d, vinyl CH_3), 1.83 (3H, s, vinyl CH_3), 1.90 (3H, s, OAc), 2.08 (3H, s, OAc), 3.38 (3H, s, OCH_3), 3.48 (1H, br.m,), 3.79 (1H, q, J=3 Hz), 4.00 (1H, q, J=6, 9 Hz), 4.56 (1H, q, J=3, 9 Hz), 4.52—4.72 (2H, m), 4.88 (1H, d.d, J=3, 9 Hz), 5.40 (1H, br.), 6.82 (1H, d, J=6 Hz). MS *m/e*: 676 (M^+), 472, 454, 436, 412, 394, 372, 354, 312, 294, 276, 224, 187, 155, 95 (base peak), 83, 43. *Anal.* Calcd. for $C_{37}H_{56}O_{11}$: C, 65.66; H, 8.34. Found: C, 65.43; H, 8.35.

Acidic Hydrolysis of MF-A——MF-A (25 mg) was refluxed with 1 N H_2SO_4 in EtOH (5 ml) for 1 hr. The reaction mixture was diluted with H_2O (5 ml) and EtOH was evaporated *in vacuo* at room temperature. The concentrated aqueous solution was extracted 3 times with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, and dried over anhydrous Na_2SO_4. Removal of the solvent gave a colorless solid. The product was recrystallized from *n*-hexane–$(CH_3)_2CO$ to give colorless prisms (8 mg). mp 180—182°. It was completely identical with dehydrotomentosin (I) in mixed mp, IR, and MS spectra.

The aqueous layer of hydrolysate was neutralized with Amberite IR-45 and concentrated to dryness *in vacuo*. The residue was found to contain only a 2,6-dideoxysugar,[19] and was identified as D-cymarose by comparison of their TLC (*Rf*=0.6) and PPC (0.62).

Acidic Hydrolysis of Periplocymarin——Periplocymarin (20 mg) was refluxed with 1 N H_2SO_4 in EtOH (5 ml) for 1 hr. The reaction mixture was diluted with H_2O, and concentrated *in vacuo* at room temperature. The concentrated aqueous solution was extracted 3 times with $CHCl_3$, and the aqueous layer of the hydrolysate was neutralized with Amberite IR-45, and then concentrated to dryness *in vacuo*. D-cymarose obtained here was used as an authentic sample.

MF-B (Dehydrotomentosin (I))——MF-B was recrystallized from $(CH_3)_2CO$ and *n*-hexane to afford colorless prisms. mp 180—182°. $[\alpha]_D^{25}$ +45° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1730, 1700, 1640, 1260, 1155. NMR ($CDCl_3$) δ: 0.93 (3H, s, tert.CH_3), 1.21 (3H, s, tert.CH_3), 1.22 (3H, d, J=6 Hz, sec.CH_3), 1.82 (3H, d, J=6 Hz, vinyl CH_3), 1.83 (3H, s, vinyl CH_3), 1.91 (3H, s, OAc), 3.54 (1H, m, 3α-H), 4.56 (1H, q, J=6 Hz, 20-H), 4.62 (1H, d.d, J=6, 11 Hz, 12-H), 5.34 (1H, b.d, 6-H), 6.82 (1H, d, J=6 Hz). MS *m/e*: 490 (M^+), 472, 454, 436, 412, 390, 372, 330, 312, 294, 242, 83 (base peak), 43. *Anal.* Calcd. for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63. Found: C, 68.30; H, 8.92. This compound was identical with dehydrotomentosin (I) by comparison of their mixed melting point, and IR, and MS spectra.

Treatment of Fraction 2——A solution of fraction 2 was evaporared to dryness *in vacuo* to afford a yellow syrup (3.5 g). The syrup (500 mg) was subjected to chromatography on silica gel and repeated preparative TLC (EtOAc: benzene=3: 1) to give MF-C (48 mg).

MF-C (Pergularin-3-O-β-D-cymaropyranoside (VI))——MF-C was recrystallized from $CHCl_3$ and *n*-hexane to afford colorless prisms. mp 245—248° (dec.), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 1700, 1090, 1060, 1005. NMR ($CDCl_3$) δ: 0.98 (3H, s, tert.CH_3), 1.15 (3H, s, tert.CH_3), 1.25 (3H, d, J=6 Hz, sec.CH_3), 2.29 (3H, s, $COCH_3$), 3.40 (3H, s, OCH_3), 4.80 (1H, d.d, J=3, 9 Hz), 5.40 (1H, b.d, 6-H). MS *m/e*: 508 (M^+), 346, 328, 310, 303, 285, 267, 145, 113 (base peak), 43. *Anal.* Calcd. for $C_{28}H_{44}O_8$: C, 66.61; H, 8.72. Found: C, 66.49; H, 8.72.

Acidic Hydrolysis of MF-C——MF-C (25 mg) was hydrolyzed with 1 N H_2SO_4 in EtOH (5 ml) in the same way as MF-A to give an aglycone and sugar residue. The aglycone was identical with an authentic sample of pergularin (VII) in comparison of their mixed mp and MS. The sugar portion was identified as D-cymarose by TLC and PPC.

$LiAlH_4$ Reduction of MF-C——To a stirred suspension of $LiAlH_4$ (50 mg) in dry dioxane (6 ml) was added dropwise a solution of MF-C (15 mg) in dry dioxane (5 ml), and the mixture was stirred at room temperature for 1 hr. After addition of a minimum amount of water, the resulting precipitates were removed by decantation and washed repeatedly with EtOAc. The combined EtOAc solution was further washed with 2% NaOH and water, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave an amorphous solid (13 mg), which was chromatographed on silica gel (10 g) and eluted with $CHCl_3$ containing 5% MeOH to afford a reduction product (IV). This compound was identical with MF-D (IV) isolated from fraction 3, in mixed mp, and IR, and NMR spectra.

Treatment of Fraction 3——The solution of fraction 3 was evaporated *in vacuo* to a yellow syrup (9 g). This syrup (1.0 g) was subjected to chromatography on silica gel and repeated preparative TLC ($CHCl_3$: MeOH=10: 1) to afford pure MF-D (28 mg).

MF-D (Utendin-3-O-β-D-cymaropyranoside (IV))——MF-D was recrystallized from a mixture of $CHCl_3$ and *n*-hexane to afford colorless prisms. mp 249—252° (dec.). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1095, 1065, 1006. NMR ($CDCl_3$) δ: 1.00 (3H, s, tert.CH_3), 1.14 (3H, s, tert.CH_3), 1.20 (3H, d, J=6 Hz, sec.CH_3), 1.28 (3H, d, J=6 Hz, sec.CH_3), 3.42 (3H, s, OCH_3), 4.80 (1H, d.d, J=3, 9 Hz), 5.40 (1H, b.d, 6-H). MS *m/e*: 510 (M^+), 348, 330, 303, 312, 294, 285, 145, 113 (base peak). *Anal.* Calcd. for $C_{28}H_{46}O_8$: C, 67.98; H, 9.37. Found: C, 67.75; H, 9.57.

Alkaline Hydrolysis of Dehydrotomentosin-3-O-β-D-cymaropyranoside (II)——A solution of II (25 mg) in 5% MeOH–KOH (5 ml) was allowed to stand for 24 hr at room temperature, and then H_2O was added. The reaction mixture was extracted with ether. After evaporation of ether, the resulting residue was purified by preparative TLC (MeOH: $CHCl_3$=1: 10) to afford utendin-3-O-β-D-cymaropyranoside (IV). This compound was identified as MF-D (IV) by comparison of their mixed mp, IR, and NMR spectra.

Treatment of Fraction 4——The solution of fraction 4 was evaporated *in vacuo* to a yellow syrup (3.5 g), 500 mg of which was chromatographed on silica gel ($CHCl_3$: MeOH=10: 1) to give colorless crystals of MF-E (200 mg).

MF-E (β-Sitosterol-3-β-D-glucoside (VIII))——MF-E was recrystallized from MeOH-$CHCl_3$ to afford colorless prisms, mp 285—288° (dec.). $[\alpha]_D^{25}$ −37° (c=0.5, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3550. NMR (pyridine-d_5) δ: 5.40 (1H, b.d, 6-H), 5.06 (1H, d, J=8 Hz). MS *m/e*: 414, 396. *Anal.* Calcd. for $C_{35}H_{60}O_6$: C, 72.87; H, 10.48. Found: C, 72.59; H, 10.75.

Acidic Hydrolysis of MF-E——MF-E (50 mg) was hydrolyzed with 1 N H_2SO_4 in EtOH (10 ml) in the same way as MF-A to provide β-sitosterol and D-glucose. β-Sitosterol was identical with an authentic sample in mixed mp, and IR, and NMR spectra. D-Glucose was identified by its TLC and PPC.

Treatment of Fraction 5, 6, and 7——The solutions of fraction, 5, 6, and 7 were evaporated *in vacuo* to afford a yellow syrup (1.2 g, 1.8 g, and 1.1 g). The separation of the glycosides from these fractions were somewhat difficult, but repeated purification by column chromatography and preparative TLC (MeOH: $CHCl_3$: EtOH=1: 2: 15) afforded MF-C, MF-D, and MF-E. These substances were completely identical with authentic samples which were isolated from the fraction 2, 3, and 4, respectively.

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