concentrations of 400 and 100 mg/dl, respectively. Heparin and EDTA used as anticoagulants for blood have no effect at the concentrations usually employed.[14]

Recovery of uric acid was checked by adding known amounts of the acid (3.0 and 5.0 mg/dl) to sera with 5.5 and 9.8 mg/dl uric acid. Recoveries of 96±3% were obtained. The lower limit of determination for uric acid is 1 ng, which gives a fluorescence intensity of twice the blank. This sensitivity may permit the determination of uric acid in only 0.5 μl of serum. The within-day precision was examined using serum with a mean uric acid value of 3.20 mg/dl (n=30). The standard deviation was 0.05 mg/dl. The day-to-day precision was obtained by repeating the determination for 7 days on serum stored frozen at −20° with a mean uric acid value of 3.39 mg/dl, freshly prepared reagents being used each day (n=30). The standard deviation was 0.08 mg/dl.

Comparisons with the phosphotungstate method[2] and a fluorimetric method based on the same principle as the present method but requiring 25 μl of deproteinized serum[6a] showed correlation coefficients of 0.984 (n=18) and 0.991 (n=33), respectively, and the regression equations for the present method (x) against these methods were y=0.94x+0.77 and y=0.98x+0.07, respectively. This suggests that the phosphotungstate method gives a higher value, perhaps due to the presence of some reducing substances other than uric acid.

The proposed method for the determination of uric acid in serum is simple, precise and rapid, and should be useful in pediatric research and in cases where only an extremely small amount of serum is obtainable.

Acknowledgement The supply of pathological sera from the Pediatric and Central Clinical Laboratories of Kyushu University Hospital is acknowledged.

14) G.G. Hadley and N.L. Larson, *Am. J. Clin. Path.*, **25**, 1090 (1955); S. Miwa, K. Teramura, and I. Takahashi, *Rinsho Byori*, **15**, 376 (1967).

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Studies on the Constituents of *Marsdenia formosana* Masamune. V. Isolation and Structure of a New Triterpenoid, Marsformosanone

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Further examination of the petroleum ether extracts of *Marsdenia formosana* Masamune (Asclepiadaceae) led to the isolation and characterization of a new triterpenoid, marsformosanone (III) together with a known steroidal compound, stigmast-5-en-3β, 7α-diol (I).

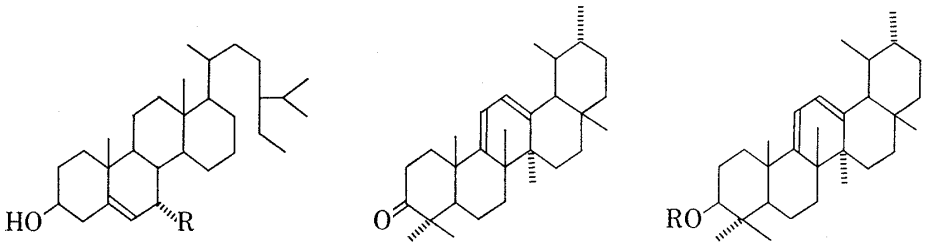
Keywords——*Marsdenia formosana* Masamune; Asclepiadaceae; marsformoxide A; marsformosanone; stigmast-5-en-3β,7α-diol; urs-9(11),12-dien-3β-yl acetate; D-friedours-11,14-dien-3β-yl acetate

In preceding papers, we have reported the isolation and characterization of thirteen triterpenoids including five new compounds (α-amyrin formate, lupenyl cinnamate, mars-

1) Location: *Yagoto-urayama, Tenpaku-cho, Tenpaku-ku, Nagoya.*

formol, marsformoxide A and marsformoxide B) from the petroleum ether extracts of *Marsdenia formosana* Masamune (Asclepiadaceae).[2,3] We also presented an interesting biogenetic-type photochemical reaction of marsformoxide A.[4] In this paper, we report some further findings on the components of this plant, and suggest that marsformoxide A(VIII) plays an important role in the hypothetical biogenetic pawthway from α-amyrin (VI) to urs-9(11),12-diene derivatives.

As a result of further treatment of the petroleum ether extracts of this plant,[2] we isolated two components which were tentatively named compounds A and B.



I : R=OH
II : R=H

III

IV : R=H
V : R=Ac

Chart 1

Compound A, colorless needles, mp 219—221°, has the molecular formula $C_{25}H_{50}O_2$. The nuclear magnetic resonance (NMR) spectrum of this compound showed a pair of one-proton multiplets at δ 2.30 and 2.20 assignable to the C-4 protons, and a one-proton multiplet at δ 3.55 assignable to the C-3α-proton, characteristic of a 5-en-3β-ol system. It also showed a low field CHOH signal at δ 3.84 together with an olefinic proton signal at δ 5.59 (1H, d, J=5 Hz), characteristic of a C=CH–CHOH group. Hence the second hydroxyl group was probably located at C-7, and this compound might possess the stigmastane skeleton and correspond to stigmast-5-en-3β,7α-diol.[5,6] In fact, this compound was identical with an authentic sample of I derived from β-sitosterol (II), on the basis of their mixed melting point, and infrared (IR) and NMR spectra. Although compound (I) was isolated from *Ananas comosus* (Bromilaceae) and found to exhibit considerable antifertility and abortifacient effects by Pakrashi *et al.*,[5] this is the first reported isolation of this substance from the Asclepiadaceous plant, *Marsdenia formosana* Masamune.

Compound B, colorless needles, mp 165—167°, has the molecular formula $C_{30}H_{46}O_2$. Its IR spectrum showed absorption due to a carbonyl group at 1700 cm^{-1}, together with bands due to a double bond at 1660 and 1630 cm^{-1}, and the ultraviolet (UV) spectrum indicated the existence of a conjugated homodiene system (peak at 281 nm). The NMR spectrum of this compound (Fig. 1) also showed two olefinic proton signals at δ 5.55 (2H, ABq, J=7 Hz) together with two proton signals adjacent to a carbonyl group at δ 2.56. Furthermore, its mass (MS) spectrum gave prominent fragment ion peaks at *m/e* 283, 269 and 255, assignable to the ions (*a*), (*b*) and (*c*), as indicated in Fig. 2. These physical data suggested that the conjugated homodiene of compound B was present at the C-9(11),12-position and indicated the structure urs-9(11),12-dien-3-one (III).

Compound B was reduced with lithium aluminium hydride to give the sole reduction product (IV), which was further acetylated with acetic anhydride in pyridine to afford its

2) K. Ito and J. Lai, *Yakugaku Zasshi*, **98**, 249 (1978).
3) K. Ito and J. Lai, *Chem. Pharm. Bull.* (Tokyo), **26**, 1908 (1978).
4) K. Ito and J. Lai, *Chem. Pharm. Bull.* (Tokyo), **27**, 210 (1978).
5) S. C. Pakrashi, B. Achari, and P. C. Majumdar, *Indian J. Chem.*, **13**, 755 (1975).
6) The NMR spectrum of this compound was identical with a spectrum provided by S. C. Pakrashi.

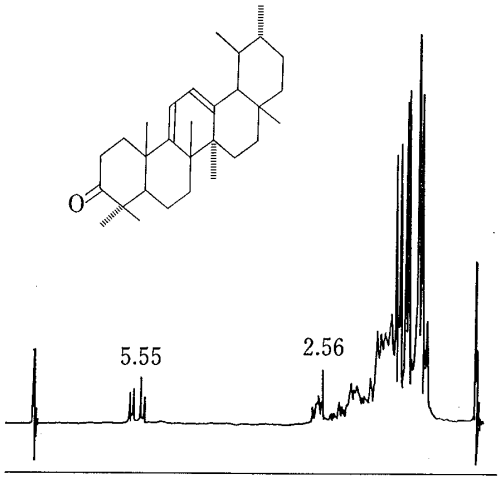


Fig. 1. NMR Spectrum of Marsformosanone (III)

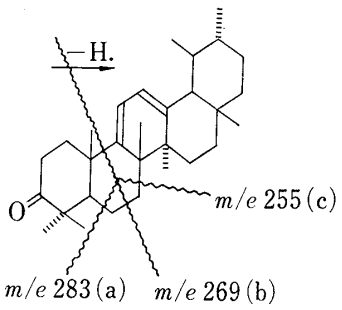


Fig. 2

acetate (V). This acetate was identical with an authentic sample of urs-9(11),12-dien-3β-yl acetate[4] on the basis of mixed melting point, and IR and NMR spectra. Thus, compound B was proved to be represented by urs-9(11),12-dien-3-one (III). This substance (III) is a new triterpenoid which has not previously been isolated in nature, and it was named marsformosanone.

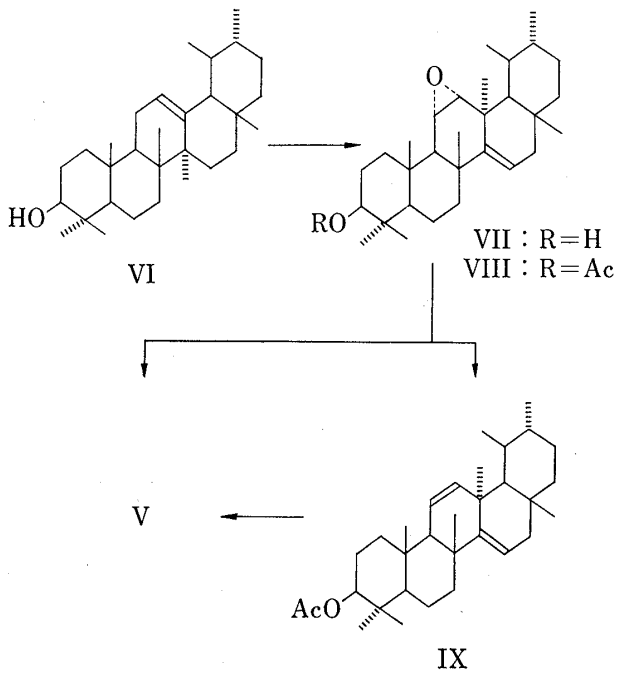


Chart 2

Agata *et al.*[7] reported an interesting biogenetic-type photochemical reaction of pentacyclic triterpenoids which involved a novel skeletal rearrangement of α-amyrin (VI) to give D-friedours-11α,12α-epoxy-14-en-3β-ol (VII). On the other hand, we have also reported a paper[4] on the photochemical reaction of marsformoxide A(VIII) leading to the formation of urs-9(11),12-dien-3β-yl acetate (V), *via* the intermediate D-friedours-11,14-dien-3β-yl acetate (IX).

It seemed possible that marsformoxide A, which was isolated from *Marsdenia formosana* MASAMUNE,[3] might be an important *in vivo* intermediate in the biogenetic route from α-amyrin (VI) to urs-9(11),12-dien-3β-yl acetate (V). The present finding that marsformosanone (III), an oxidation product of an urs-9(11),12-diene-type compound (IV or V), is present in the same plant further supports the plausibility of this hypothetical biogenetic pathway.

7) I. Agata, E. Corey, A. G. Hortman, J. Klein, S. Provkow and J. J. Ursprung, *J, Org. Chem.*, **30**, 1698 (1965).

Experimental[8]

Isolation of Two Components from Petroleum Ether Extracts of *Marsdenia formosana* MASAMUNE——(a) Group 4 which was obtained from the petroleum ether extract fraction described in our previous paper,[2] was separated and purified by silica gel column chromatography and preparative thin-layer chromatography (TLC) (benzene: ethyl acetate=5: 1) to give 30 mg of compound B.

(b) Group 6 was separated and purified by silica gel column chromatography and preparative TLC (benzene: ethyl acetate=3: 1) to afford 27 mg of compound A.

Compound A (Stigmast-5-en-3β,7α-diol: I)——This compound was recrystallized from $CHCl_3$–MeOH to affort colorless needles. mp 219—221°. $[\alpha]_D^{25}$ −25.6° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630 (OH), 1660 (C=C). NMR ($CDCl_3$) δ 2.30 (2H, m), 3.57 (1H, br. m, C-3α-H), 3.84 (1H, br. m), 5.59 (1H, d, J=5 Hz). MS m/e: 430 (M^+), 289, 271, 253, 247, 229, 211. *Anal.* Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.59; H, 11.98.

Conversion of β-Sitosterol (II) to Stigmast-5-en-3β,7α-diol (I)——A solution of β-sitosterol (1.0 g) in Ac_2O (1 ml) and pyridine (8 ml) was allowed to stand for 21 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 a colorless solid (980 mg), which was chromatographed on silica gel (35 g) to afford β-sitosteryl acetate.

A solution of chromium trioxide (1 g) in glacial acetic acid (25 ml) was added to a solution of β-sitosteryl acetate (820 mg) in glacial acetic acid (50 ml) containing sodium acetate (1 g) at 60° over a period of 10 min. The reaction mixture was heated at 60° for 5 hr. After cooling, it was extracted with ether and washed with 1% aqueous NaOH. The ethereal solution was dried over anhydrous Na_2SO_4. Removal of the solvent gave an amorphous solid, which was chromatographed on an alumina column (150 g) to give uncharged β-sitosteryl acetate (215 mg), together with stigmast-5-en-3β-yl acetate (265 mg), mp 174—175. $[\alpha]_D^{25}$ −31.6° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1250 (OAc), 1668, 1638 (C=C-C=O). NMR ($CDCl_3$) δ: 5.65 (1H, s, C-6-H).

A solution of $NaBH_4$ (500 mg) in BuOH (10 ml) was added dropwise to a solution of this oxidation product (250 mg) in BuOH (45 ml). After standing for 5 hr at room temperature, the solution was acidified with 0.5 N HCl and then extracted with ether. The ethereal solution was washed with 2% aqueous NaOH and water, dried over anhydrous Na_2SO_4 and concentrated. The reduction product (135 mg) was separated by preparative TLC on silica gel using benzene–EtOAc (1: 1) as an eluent, yielding 2 components. The less mobile fraction (27 mg), on crystallization from $CHCl_3$–cyclohexane, gave stigmast-5-en-3β,7β-diol. mp 168—170°. NMR ($CHCl_3$) δ: 3.57 (1H, br. m, C-3α-H), 3.84 (1H, br. m, C-7α-H), 5.30 (1H, br. s, C-6-H). MS m/e: 430 (M^+), 289, 271, 253, 247, 229, 211. The more mobile fraction (18 mg), on crystallization from $CHCl_3$-cyclohexane, gave stigmast-5-en-3β,7α-diol (I). Colorless needles, mp 219—221°. $[\alpha]_D^{25}$ −29.7° (c=0.5, $CHCl_3$). Stigmast-5-en-3β,7α-diol (I) obtained here was identical with the natural compound A on the basis of mixed melting point, and IR and NMR spectra.

Compound B (Marsformosanone, Urs-9(11),12-dien-3-one: III)——This compound was recrystallized from $CHCl_3$–MeOH to afford colorless needles, mp 146—147°. $[\alpha]_D^{25}$ =351.5° (c=0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700 (C=O), 1638 (C=C). UV λ_{max}^{MeOH} nm (ε): 281 (8900). NMR ($CDCl_3$) δ: 2.56 (2H, m), 5.55 (2H, ABq, J=7 Hz). MS m/e: 422 (M^+), 283, 269, 255. *Anal.* Calcd. for $C_{30}H_{46}O$: C, 85.41; H, 11.05. Found: C, 85.52; H, 11.16.

Conversion of Marsformosanone (III) into Urs-9(11),12-dien-3β-yl Acetate (V)——A solution of marsformosanone (20 mg) in dry ether (5 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (50 mg), and the mixture was stirred at room temperature for 1 hr. After addition of a minimum amount of water, the resulting precipitate was removed by decantation and washed with ether. The combined ethereal solution was further washed with 2% aqueous NaOH and with water, then dried over anhydrous Na_2SO_4. Removal of the solvent gave an amorphous solid (18 mg), which was chromatographed on silica gel (10 g), eluting with benzene, to afford urs-9(11),12-dien-3β-ol(IV), mp 155—156.5°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1660, 1638 (C=C). NMR ($CDCl_3$) δ: 5.53 (2H, ABq, J=7 Hz). MS m/e: 422 (M^+), 283, 269, 255.

Acetylation of IV (15 mg) with excess Ac_2O–pyridine provided V (15 mg). This was recrystallized from $CHCl_3$–MeOH to give colorless needles, mp 165—167°. $[\alpha]_D^{25}$ +350.8° (c=0.5, $CHCl_3$). This compound was identical with an authentic sample of urs-9(11),12-dien-3β-yl acetate (V) on the basis of mixed melting point, and IR and NMR spectra.

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8) All melting points were determined on a microhot-stage and were uncorrected. IR spectra were recorded on a JASCO A-3 spectrometer. NMR spectra were determined on a JEOL PS-100 spectrometer operating at 100 MHz with tetramethylsilane (TMS) as an internal standard. UV spectra were recorded on a Hitachi A-124 spectrometer. MS were taken on a Hitachi M-52 mass spectrometer with a heated direct inlet system. Optical rotations were measured with a JASCO DIP-SL polarimeter.