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Studies on the Constituents of *Marsdenia formosana* Masamune. II.[1] Structures of Marsformoxide A and Marsformoxide B[2]

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The substance M described in our previous paper was found to be a mixture of two similar triterpenoid compounds, which were named marsformoxide A and B.

As the result of spectroscopic and chemical investigation, it has been demonstrated that the stereostructures of marsformoxide A and B were represented, respectively, by the formulas I and II.

Keywords——*Marsdenia formosana* Masamune; Asclepiadaceae; triterpenoids; marsformoxide A; marsformoxide B; epoxide

In our previous paper,[1] we reported on the isolation and characterization of the known triterpenoids (α-amyrin, α-amyrin acetate, α-amyrenone, α-amyrin cinnamate, neoilexonol acetate, lupeol, lupenyl acetate, lepenone) and the three new triterpenoids (α-amyrin formate, lepenyl cinnamate, marsformol) together with an unidentified substance M from the petroleum ether extracts of *Marsdenia formosana* Masamune. However, a considerably wide melting points range (mp 208—221°) of the substance M urged us to carry out its further studies. As the result of the investigation of its nuclear magnetic resonance (NMR) spectrum, the substance

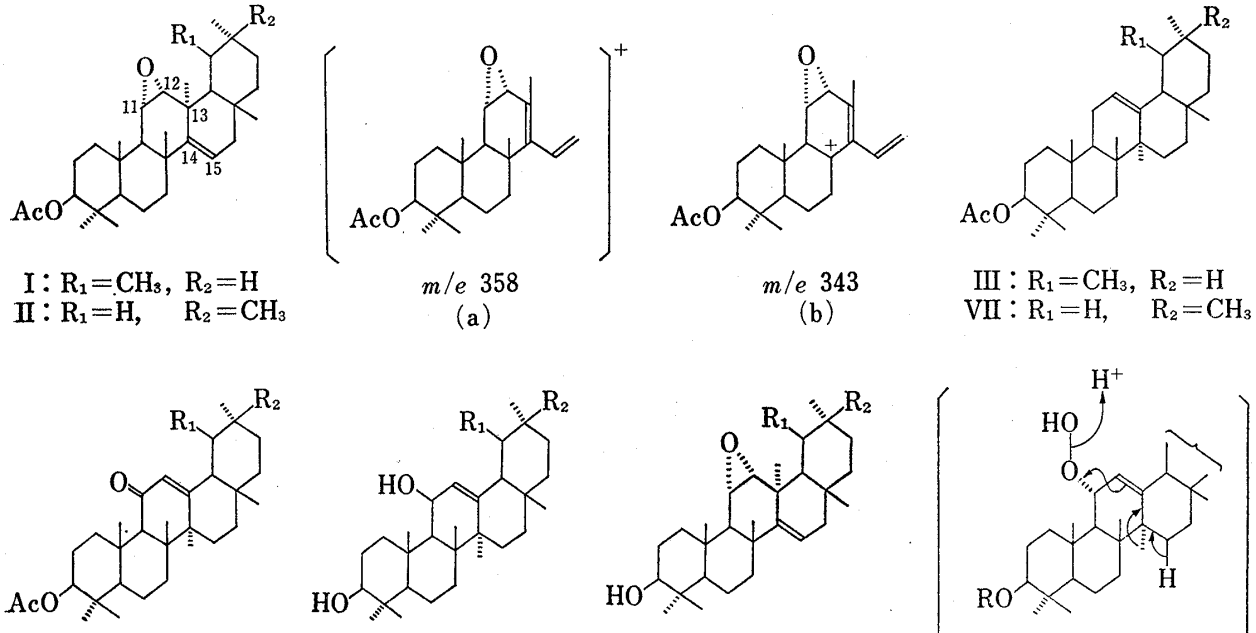

Chart 1

1) Part I: K. Ito and J. Lai, *Yakugaku Zasshi*, **98**, 249 (1978).
2) This study was presented at the 24th Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, September, 1977.
3) Location: *Yagoto-urayama, Tenpaku-cho, Tenpaku-ku, Nagoya.*

M was found to be a mixture in a ratio about 1: 1 of two new closely related compounds, named marsformoxide A and marsformoxide B.

Marsformoxide A, mp 212—214°, $[\alpha]_D^{25}$—25° ($CHCl_3$), has a molecular formula $C_{32}H_{50}O_3$. Its infrared (IR) spectrum shows acetoxyl absorption at 1728 and 1250 cm^{-1}, together with double bond at 1638 cm^{-1}, in addition to an absorpsion band at 895 cm^{-1} assignable to an epoxide ring.[4] The presence of an epoxide ring in this compound is further substantiated by the proton chemical shifts which appear at δ 2.98 (1H, d., J=5 Hz) and 3.14 (1H, d.d., J=5, 5.7 Hz) in the NMR spectrum (Fig. 1). It also discloses the presence of six tetriary methyl groups (δ 0.86—1.09) and two secondary methyl groups[5] at δ 0.99 (3H, d., J=6 Hz) and 1.11 (3H, d., J=6 Hz) together with C-3α proton at δ 4.50 (1H, d.d., J=9, 6.5 Hz) and one trisubstituted olefinic proton at δ 5.51. Furthermore, the mass (MS) spectrum of this compound exhibits the prominent fragment ion peaks at *m/e* 358 and 343 assignable to the ions (a) and (b) which would be formed by the retro-Diels-Alder cleavage.[6] These physical data suggest that marsformoxide A possesses the D-friedoursane skeleton and is represented by D-friedours-11α,12α-epoxy-14-en-3β-yl-acetate (I).[7] Thus, when oxidized with chromium trioxide in acetic acid, α-amyrin acetate (III) was readily converted into 3β-acetoxyurs-12-en-11-one (IV), which was further reduced with lithium aluminum hydride to give urs-12-en-3β,11β-diol (V). Finally, this diol was treated with H_2O_2–SeO_2 in t-BuOH–CH_2Cl_2 to provide the corresponding epoxide VI, which on acetylation afforded D-friedours-11α,12α-epoxy-14-en-3β-yl-acetate (I). This compound was completely identical with natural marsformoxide A by comparison of their mixed melting point and IR and NMR spectra.

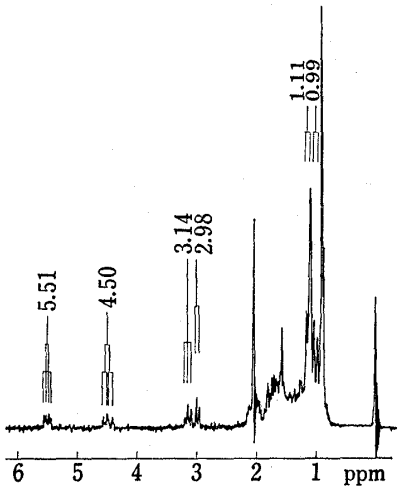

Fig. 1. NMR Spectrum of Marsformoxide A (I)

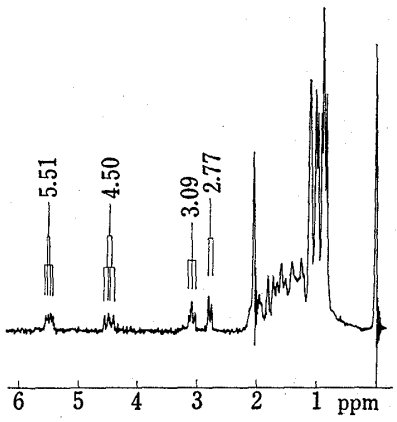

Fig. 2. NMR Spectrum of Marsformoxide B (II)

Marsformoxide B, mp 305—308°, $[\alpha]_D^{25}$—31° ($CHCl_3$), has a molecular formula $C_{32}H_{50}O_3$. The IR and NMR (Fig. 2) spectra of this compound are closely similar to those of marsformoxide A, and also disclose the presence of acetoxyl group, double bond and epoxide ring [ν_{max} 870 cm^{-1} and δ 2.77 (1H, d., J=5 Hz), δ 3.09 (1H, d.d., J=5, 5.7 Hz)]. The sole difference between these two substances is that marsformoxide B has eight tertiary methyl groups (δ 0.84—1.12) and shows lack of secondary methyl group. From the above physical data, the most probable structure of marsformoxide B may be represented by II. In fact, this compound was proved

4) I. Kitagawa, K. Kitazawa and I. Yosioka, *Tetrahedron*, **28**, 907 (1972).
5) The chemical shifts of these two secondary methyl groups are clearly observed at δ 1.04 (3H, d., J=6.5 Hz) and 1.20 (3H, d., J=6.5 Hz) in pyridine-d_5, respectively.
6) H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
7) I. Agata, E. Corey, A.G. Hortmann, J. Klein, S. Proskow and J.J. Ursprung, *J. Org. Chem.* **30**, 1698 (1968).

to be identical with an authentic sample of D-friedoolean-11α,12α-epoxy-14-en-3β-yl-acetate (II) which was likewise derived from β-amyrin acetate (VII).[8]

In 1965, Corey *et al.*[7] carried out some interesting photochemical reactions of pentacyclic triterpenes, which involved a novel skeletal rearrangement of thermodynamically more favored α-amyrin and β-amyrin into less stable D-friedours-11α,12α-epoxy-14-en-3β-ol (VI) and D-friedoolean-11α,12α-epoxy-14-en-3β-ol (X), respectively. They proposed the mechanism through an intermediate XI in this photooxidation, in connection with an independent conversion of α-amyrin and β-amyrin to the same products VI and X by the alternate chemical oxidation. From the above fact, we assume that the α-amyrin and β-amyrin would be the *in vivo* precursors of VI and X, respectively. As these biogenetically related compounds VI and X had never been found in nature, it is very interesting that our present experiment led us to the isolation and characterization of their corresponding acetate I and II from the natural source.

Experimental[9]

Substance M——From the group 6 which had been already reported in our previous paper,[1] 165 mg of substance M was obtained. It showed one spot on TLC examination and gave an intense blue-green color in the Liebermann–Burchard test. Recrystallization from MeOH–$CHCl_3$ afforded colorless needles, mp 208—221°. IR ν_{max}^{KBr} cm^{-1}: 1728, 1250, 1638, 895, 870. NMR ($CHCl_3$) δ: 5.51 (1H, d.d., J=4, 6 Hz) 4.50 (1H, d.d., J=9, 6.5 Hz), 3.12 (1H, m.), 2.98 (0.5H, d., J=5 Hz) 2.77 (0.5H, d., J=5 Hz), 2.04 (3H, s.), 0.84—1.12 (24H, m.). MS *m/e*: 358, 343, 108 (base peak).

Separation of Marsformoxide A and B——A series of fractional crystallization of the substance M (165 mg) from CH_2Cl_2–EtOH gave 12 mg of marsformoxide B. From the combined mother liquors after removal of marsformoxide B was obtained 95 mg of a crystalline solid, which was carefully chromatographed on 50 g of Woelm alumina (grade I) packed in petroleum ether containing 50% benzene. Elution with benzene–ether (50: 1) gave 15 mg of a crystalline meterial which was recrystallized from MeOH–$CHCl_3$ to afford marsformoxide A.

Marsformoxide A——Colorless needle, mp 212—214°. $[\alpha]_D^{25}$ −25° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1728, 1250 (OAc), 1638 (C=C), 895 (epoxy). NMR ($CDCl_3$) δ: 5.51 (1H, d.d., J=4, 6 Hz, C_{15}-H), 4.50 (1H, d.d., J=9, 6.5 Hz, C_3-α-H), 3.14 (1H, d.d., J=5, 5.7 Hz, C_{11}-H), 2.98 (1H, d., J=5 Hz, C_{12}-H), 2.04 (3H, s., OAc), 1.11 (3H, d., J=6Hz, *sec.* CH_3), 1.09 (3H, s., *tert.* CH_3), 1.07 (3H, s., *tert.* CH_3), 0.99 (3H, *d.*, J=6 Hz, *sec.* CH_3), 0.89 (9H, s., 3 *tert.* CH_3), 0.86 (3H, s., *tert.* CH_3). NMR (pyridine-d_5) δ: 1.20 (3H, d., J=6.5 Hz, *sec.* CH_3), 1.11 (6H, s., 2 *tert.* CH_3), 1.06 (3H, s., *tert.* CH_3), 1.04 (3H, d., J=6.5 Hz, *sec.* CH_3), 0.95 (6H, s., *tert.* CH_3), 0.91 (3H, s., *tert.* CH_3). MS *m/e*: 482 (M^+), 358, 343, 108 (base peak). *Anal.* Calcd. for $C_{32}H_{50}O_3$: C, 79.62; H, 10.44. Found: C, 79.55; H, 10.55.

Marsformoxide B——Colorless needles, mp 305—308°. $[\alpha]_D^{25}$ −31° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1728, 1250 (OAc), 1638 (C=C), 870 (epoxy). NMR ($CDCl_3$) δ: 5.51 (1H, d.d., J=4, 6 Hz, C_{15}-H), 4.50 (1H, d., J=9, 6.5 Hz, C_3-α-H), 3.09 (1H, d.d., J=5, 5.7 Hz, C_{11}-H), 2.77 (1H, d., J=5 Hz, C_{12}-H), 2.04 (3H, s., OAc), 1.12 (3H, s., *tert.* CH_3), 1.10 (3H, s., *tert.* CH_3), 1.02 (3H, s., *tert.* CH_3), 0.96 (3H, s., *tert.* CH_3), 0.91 (3H, s., *tert.* CH_3), 0.88 (6H, s., 2 *tert.* CH_3), 0.84 (3H, s., *tert.* CH_3). MS *m/e*: 482 (M^+), 358, 343, 108 (base peak). *Anal.* Calcd. for $C_{32}H_{50}O_3$: C, 79.62; H, 10.44. Found: C, 79.53; H, 10.56.

Conversion of α-Amyrin Acetate (III) to Marsformoxide A (I)——A solution of CrO_3 (200 mg) in glacial acetic acid (20 ml) was added to a solution of α-amyrin acetate (200 mg) in glacial acetic acid (30 ml) at 50° in a period of 10 min. The reaction mixture was further heated at 80° for 5 min. After cooling, it was extracted with ether (200 ml) and washed with water, and then with 1% aqueous NaOH. The ethereal solution was dried over anhydrous Na_2SO_4, filtered and then evaporated to dryness. Recrystallization from MeOH–$CHCl_3$ gave 3β-acetoxyurs-12-en-11-one (IV: 160 mg). mp 289—291°, $[\alpha]_D^{25}$ +99° (c=0.5, $CHCl_3$).

To a stirred suspension of $LiAlH_4$ (300 mg) in dry ether (60 ml) was added dropwise a solution of IV (150 mg) in dry benzene (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 with ether.

8) As we had not isolated β-amyrin acetate from *Marsdenia formosana* Masamune, the same substance, isolated from *Balanophora tobiracola* Makino (Balanophoraceae) in our laboratory was used.

9) 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 determined on a JEOL PS-100 spectrometer operating at 100 MHz with 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.

The combined ethereal solution was further washed with 2% aqueous NaOH and with water, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave an amorphous solid (150 mg), which was chromatographed on 30 g of silica gel and eluted with benzene containing 20% ethyl acetate to afford urs-12-en-3β, 11β-diol (V: 115 mg). Amorphous powder. $[\alpha]_D^{25}$ +100° (c=0.5, $CHCl_3$), NMR ($CDCl_3$) δ: 5.22 (1H, d., J= 4.5 Hz, C_{12}-H), 4.36 (1H, t., J=4, 5 Hz, C_{11}-α-H), 3.22 (1H, d.d., J=10, 7 Hz, C_3-α-H). *Anal.* Calcd. for $C_{30}H_{50}O_2$: C, 81.38; H, 11.38. Found: C, 81.27; H, 11.20.

To a solution of V (100 mg) in CH_2Cl_2 (30 ml) was added the solution (30 ml) which was prepared from 30% H_2O_2 (0.5 ml) in t-BuOH (29.5 ml) containing SeO_2 (300 mg) at room temperature. The reaction mixture was stirred slowly overnight. After dilution with water, the reaction mixture was extracted with CH_2Cl_2, and then worked up in the usual way to give a solid (70 mg), which was chromatographed on silica gel (20 g) to afford D-friedours-11α,12α-epoxy-14-en-3β-ol (VI: 50 mg). Recrystallization from MeOH–$CHCl_3$ gave colorless needles. mp 248—250°.

Acetylation of VI (30 mg) with excess Ac_2O–pyridine provided D-friedours-11α,12α-epoxy-14-en-3β-yl-acetate (I: 30 mg). It was recrystallized from EtOH–CH_2Cl_2 to give colorless needles. mp 214—216°. $[\alpha]_D^{25}$ −26° (c=0.5, $CHCl_3$). This compound was completely identical with marsformoxide A which was isolated from *Marsdenia formosana* MASAMUNE by comparison of their mixed melting point, IR and NMR spectra.

Conversion of β-Amyrin Acetate (VII) to Marsformoxide B (II)——A solution of CrO_3 (200 mg) in glacial acetic acid (10 ml) was added to a solution of β-amyrin acetate (100 mg) in glacial acetic acid (15 ml) at 50° in a period of 10 min. After the reaction mixture was further stirred at 80° for 5 min, a solid was obtained in the usual method. Recrystallization from MeOH–$CHCl_3$ afforded 3β-acetoxy-olean-12-en-11-one (VIII: 85 mg), mp 268—269°, $[\alpha]_D^{25}$ +105° (c=0.5, $CHCl_3$).

To a stirred suspension of $LiAlH_4$ (200 mg) in dry ether (30 ml) was added dropwise a solution of VIII (80 mg) in dry benzene (3 ml). The reaction mixture was further stirred at room temperature for 1 hr. After addition of a minimum volume of water, the separating precipitates were removed by decantation and washed with ether, and then the combined ethereal solution was worked up in the usual way to give a colorless solid, which was chromatographed on silica gel (10 g) and eluted with benzene containing 20% ethyl acetate to afford olean-12-en-3β,11β-diol (IX: 60 mg). Recrystallization from MeOH gave colorless needles. mp 180—182°. $[\alpha]_D^{25}$ +105° (c=0.5, $CHCl_3$). NMR ($CDCl_3$) δ: 5.25 (1H, d., J=4.5 Hz, C_{12}-H), 4.26 (1H, t,. J=4.5 Hz, C_{11}-α-H), 3.22 (1H, d.d, J=10, 7 Hz, C_3-α-H). *Anal.* Calcd. for $C_{30}H_{50}O_2$: C, 81.38; H, 11.38. Found: C, 81.41; H, 11.20.

To a stirred solution of IX (50 mg) in CH_2Cl_2 (10 ml) was added a solution of t-BuOH (10 ml) containing SeO_2 (50 mg) and 30% H_2O_2 (0.5 ml) at room temperature. The reaction mixture was further stirred at room temperature overnight. After dilution with water, the solid product was obtained in the usual method, and it was chromatographed on silica gel to afford D-friedoolean-11α,12α-epoxy-14-en-3β-ol (X: 32 mg). Recrystallization from MeOH–$CHCl_3$ gave coloeless needles. mp 283—285°.

Acetylation of X (25 mg) with excess Ac_2O-pyridine afforded D-friedoolean-11α,12α-epoxy-14-en-3β-yl-acetate (II: 26 mg). It was recrystallized from EtOH-CH_2Cl_2 to give colorless needles. mp 305—308°. $[\alpha]_D^{25}$ −32° (c=0.5, $CHCl_3$). This compound was found to be completely identical with marsformoxide B by comparison of their mixed melting point, IR and NMR spectra.

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