Indonesian Medicinal Plants. XVI.[1] Chemical Structures of Four New Resin-Glycosides, Merremosides f, g, h_1, and h_2, from the Tuber of *Merremia mammosa* (Convolvulaceae)

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Four new resin-glycosides named merremosides f (6), g (7), h_1 (8), and h_2 (9) were isolated from the tuber of *Merremia mammosa* (Lour.) Hallier f. (Convolvulaceae), an Indonesian medicinal plant. Their chemical structures have been elucidated on the bases of their chemical and physicochemical properties.

Key words Indonesian medicinal plant; *Merremia mammosa*; Convolvulaceae; resin-glycoside; merremoside

In our previous paper,[1] we reported the isolation of thirteen new resin-glycosides [merremosides a (**1**), b (**2**), c (**3**), d (**4**), e (**5**), f (**6**), g (**7**), h_1 (**8**) and h_2 (**9**), and mammosides A, B, H_1 and H_2] from the tuber of *Merremia mammosa* (LOUR.) HALLIER. f. (Convolvulaceae), and the structural elucidation of **1**, **2**, **3**, **4**, and **5**.

In this paper, we present the chemical structures of merremosides f (**6**), g (**7**), h_1 (**8**), and h_2 (**9**).

Merremoside g (7) Merremoside g (**7**), colorless fine crystals, $C_{54}H_{92}O_{25}$, showed absorption bands due to a hydroxyl (3360 cm^{-1}) group and an ester group (1715 cm^{-1}) in the infrared (IR) spectrum. Hydrolysis of merremoside g (**7**) with 5% aqueous KOH yielded a glycosidic acid, named merremoside j (**10**) and isobutyric acid. On treatment with 5% NaOMe–MeOH, **7** gave merremoside j methyl ester (**10a**).

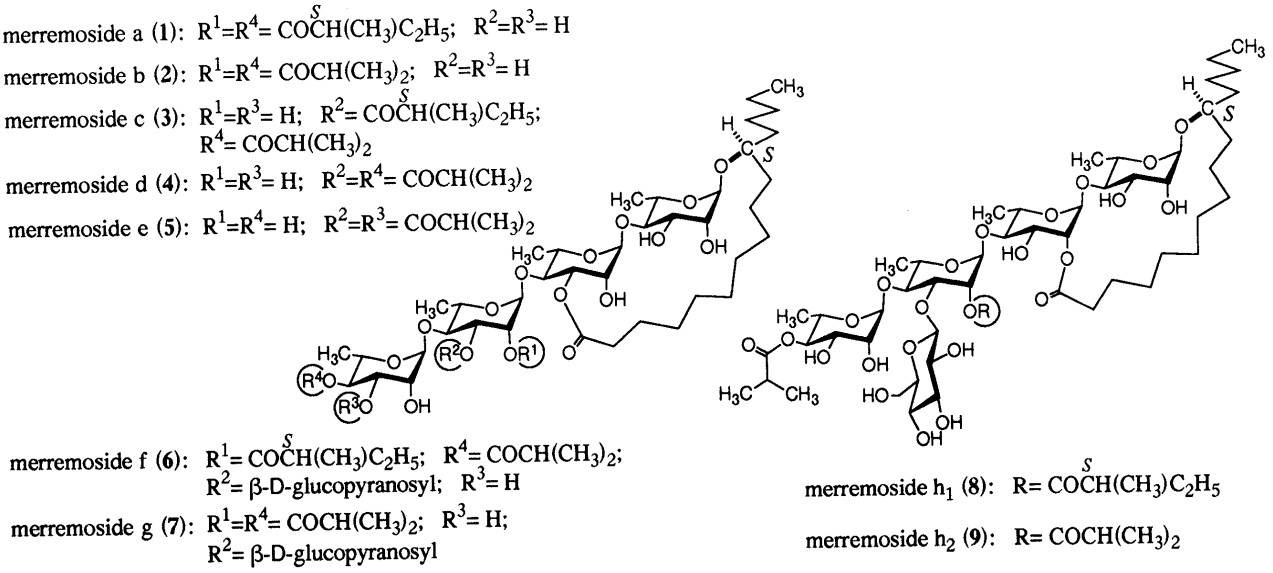

Fig. 1

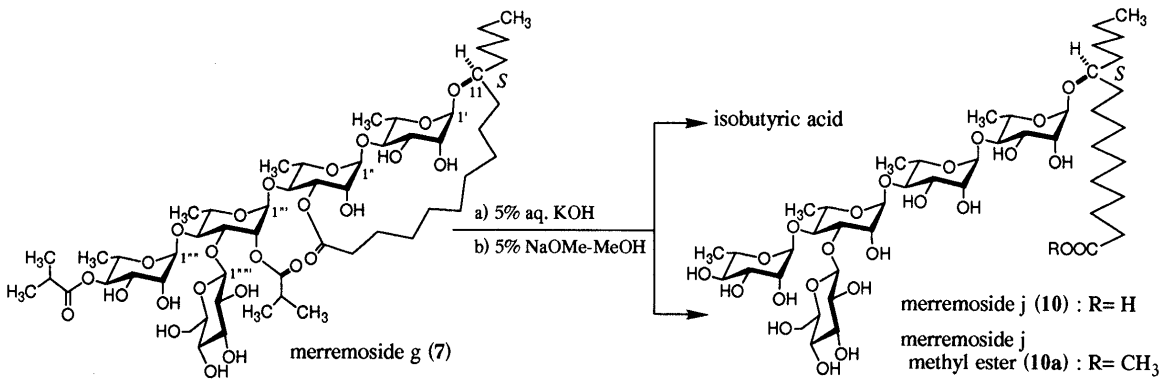

Chart 1

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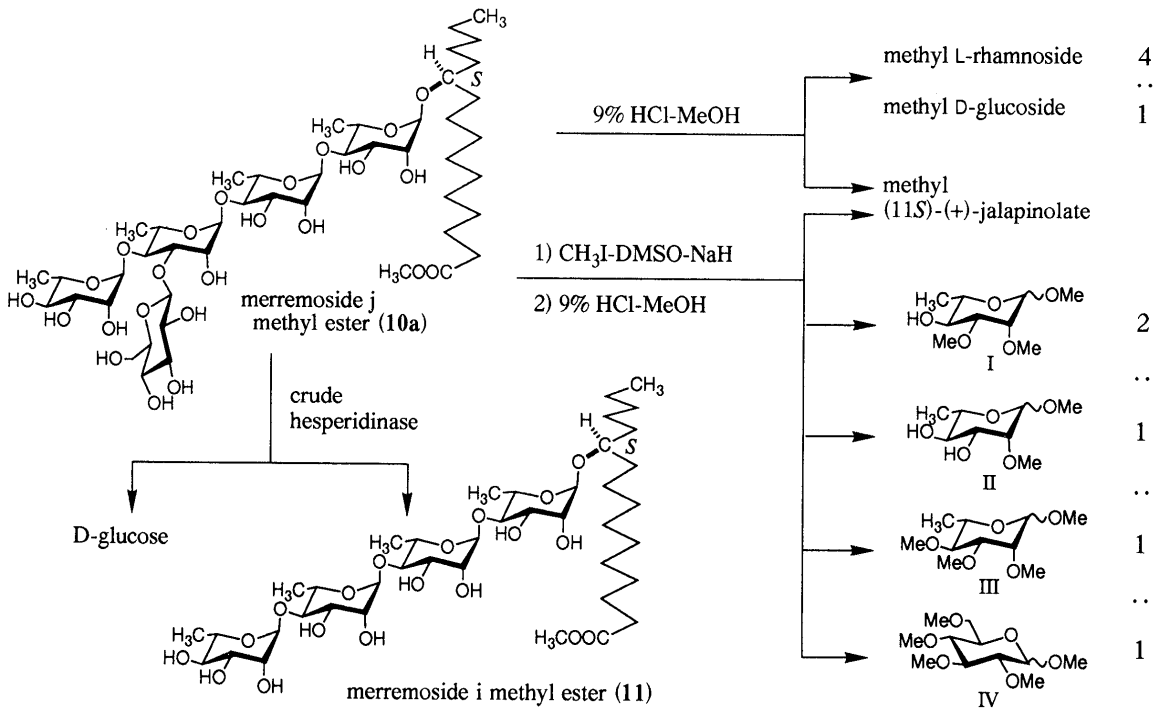

Chart 2

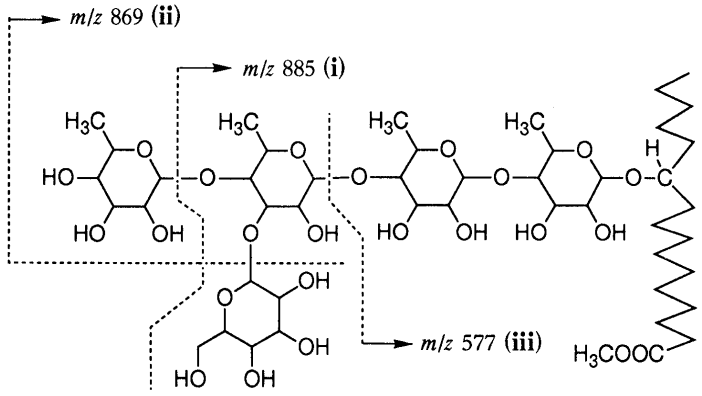

Fig. 2. Negative FAB-MS of Merremoside j Methyl Ester (**10a**)

On methanolysis with 9% HCl–MeOH, merremoside j methyl ester (**10a**) gave methyl (11*S*)-(+)-jalapinolate[1] and two methyl glycosides; methyl L-rhamnoside and methyl D-glucoside in a ratio of 4 : 1. Furthermore, upon hydrolysis with crude hesperidinase, **10a** gave D-glucose and merremoside i methyl ester (**11**), which was the common glycosidic acid of merremosides a (**1**), b (**2**), c (**3**), d (**4**), and e (**5**).[1] Upon treatment with CH_3I–DMSO–NaH[2] followed by methanolysis, **10a** yielded methyl (11*S*)-(+)-jalapinolate and four methyl glycoside derivatives; methyl 2,3-di-*O*-methyl-L-rhamnopyranoside (I), methyl 2-*O*-methyl-L-rhamnopyranoside (II), methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside (III), and methyl 2,3,4,6-tetra-*O*-methyl-L-glucopyranoside (IV) in 2 : 1 : 1 : 1 ratio.

Merremoside j methyl ester (**10a**) showed characteristic fragment ions at *m/z* 885 (**i**), *m/z* 869 (**ii**), *m/z* 577 (**iii**), together with a quasi-molecular ion at *m/z* 1031 $(M-H)^-$ in the negative fast atom bombardment-mass (negative FAB-MS) spectrum. This finding suggested that D-glucose is attached to the third rhamnose from the reducing terminal.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **10a** showed signals due to one primary methyl, one carbomethoxy, four secondary methyls of L-rhamnose, four anomeric protons (δ 5.10, 5.82, 6.13, 6.16, all br s) of L-rhamnose, and one anomeric proton (δ 5.17, d, $J=7.5$ Hz, indicating β-orientation) of D-glucose. Furthermore, the carbon-13 (^{13}C)-NMR showed five anomeric carbon signals: δ_C 100.4, 102.0, 102.3, 102.5 (L-rhamnose × 4) with ^{13}C–^{1}H coupling constants of 170.5, 171.0, 171.3 and 171.5 Hz, indicating that all glycoside linkages of the rhamnoses have α-orientation,[3] and δ_C 104.5 (D-glucose). Thus, the structure of merremoside j (**10a**) was concluded to be as shown.

Merremoside g (**7**) showed characteristic fragment ions; *m/z* 977 (**vii**), *m/z* 545 (**viii**), and *m/z* 417 (**ix**+H) in the negative FAB-MS. The ^{1}H-NMR spectrum showed signals assignable to three methine protons on carbons bearing two isobutyroxyl groups [δ 5.67 (dd, $J=9.0, 9.0$ Hz, 4‴-H) and δ 5.92 (br s, 2‴-H)] and one lactonyl group [δ 5.63 (dd, $J=3.0$, 10.0 Hz, 3″-H)]; the coupling patterns

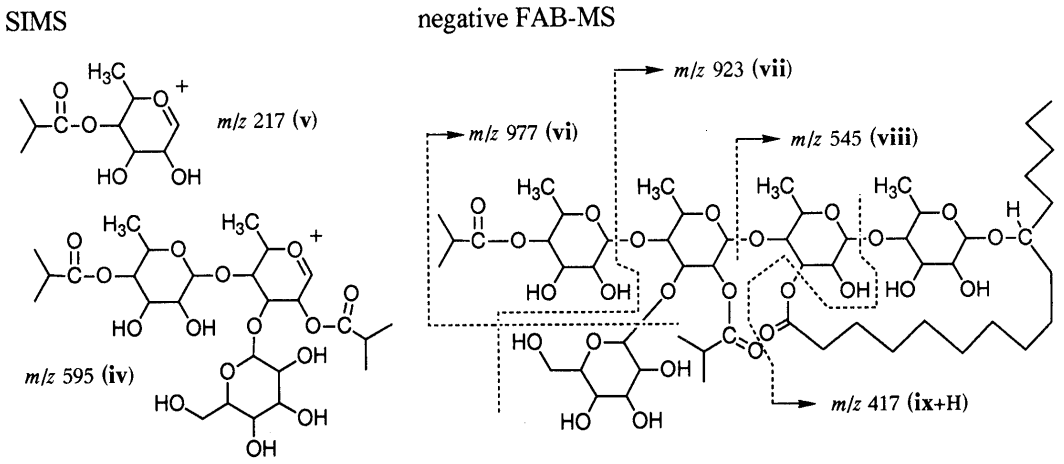

Fig. 3. SIMS and Negative FAB-MS of Merremoside g (7)

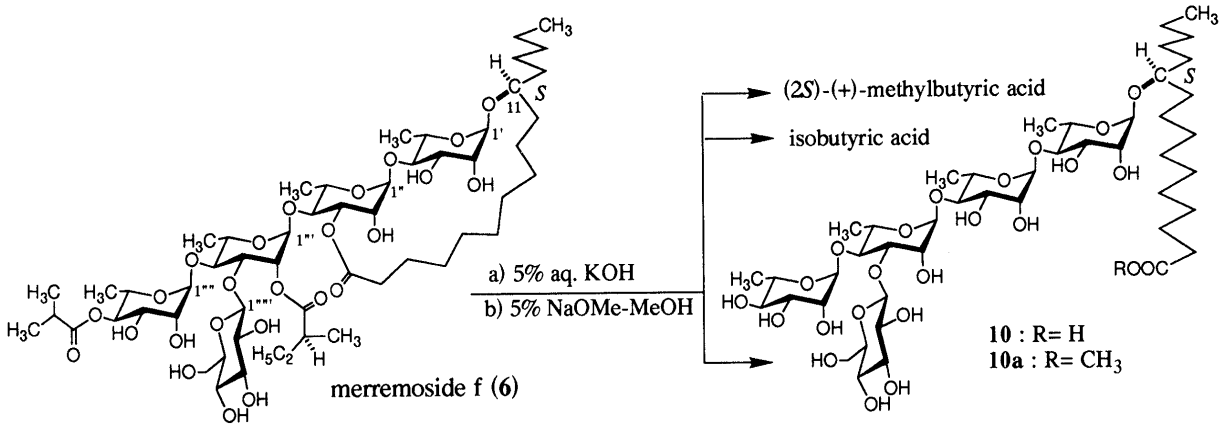

Chart 3

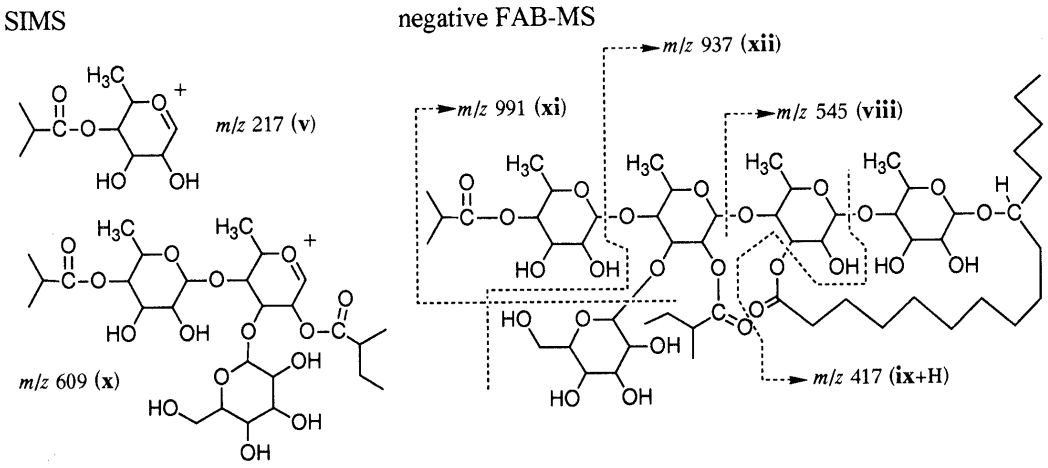

Fig. 4. SIMS and Negative FAB-MS of Merremoside f (6)

established the positions in the L-rhamnosyl moieties. Furthermore, in the secondary ion mass spectrum, **7** showed two major ion peaks, *m/z* 595 (**iv**) and *m/z* 217 (**v**). These findings indicate that two isobutyryl groups are located at 2‴-OH and 4⁗-OH.

Consequently, the chemical structure of merremoside g (**7**) has been elucidated to be as shown.

Merremoside f (6) Merremoside f (**6**), colorless fine crystals, $C_{55}H_{94}O_{25}$, showed absorption bands due to a hydroxyl (3370 cm^{-1}) group and an ester (1720 cm^{-1}) group in the IR spectrum. On hydrolysis with 5% aqueous KOH, **6** gave merremoside j (**10**), isobutyric acid, and (2*S*)-(+)-methylbutyric acid, of which the absolute configuration was determined from the specific rotation of the phenacyl derivative [$[\alpha]_D$ +15° ($CHCl_3$)].[4] On the other hand, treatment of **6** with 5% NaOMe–MeOH furnished merremoside j methyl ester (**10a**).

In SIMS, merremoside f (**6**) showed two major fragment ions, *m/z* 609 (**x**) and *m/z* 217 (**v**), indicating that D-glucose and methylbutyryl moieties are attached to the third L-rhamnose and that an isobutyryl group is attached to the terminal L-rhamnose.

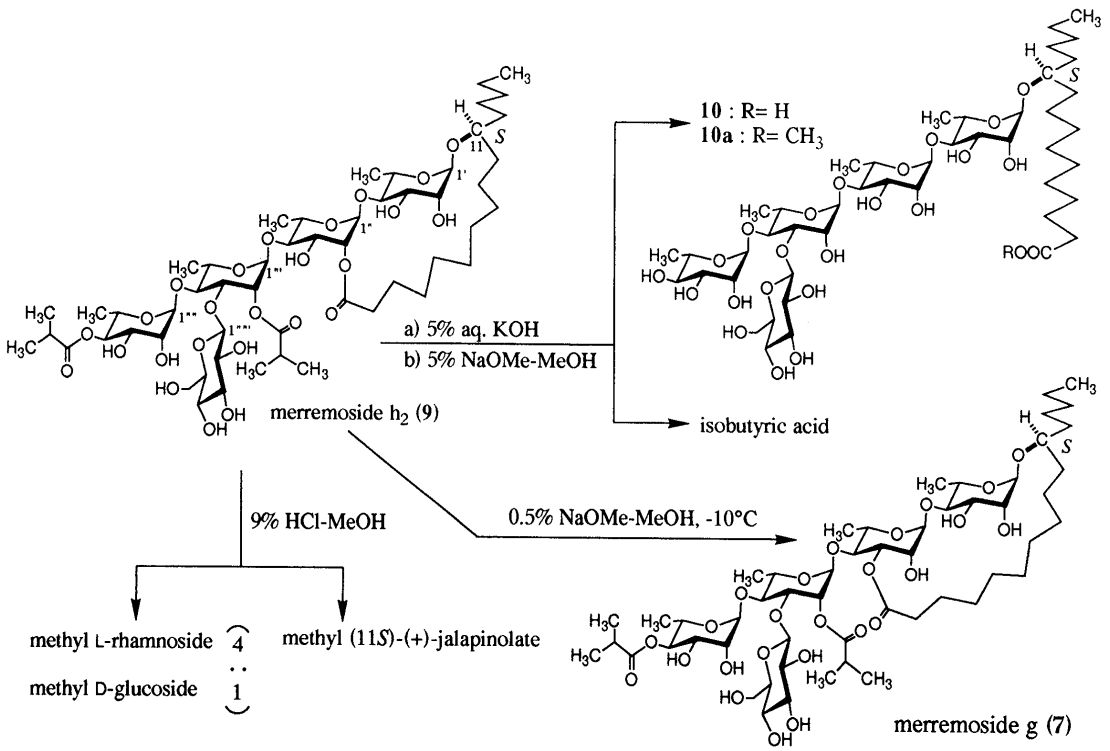

Chart 4

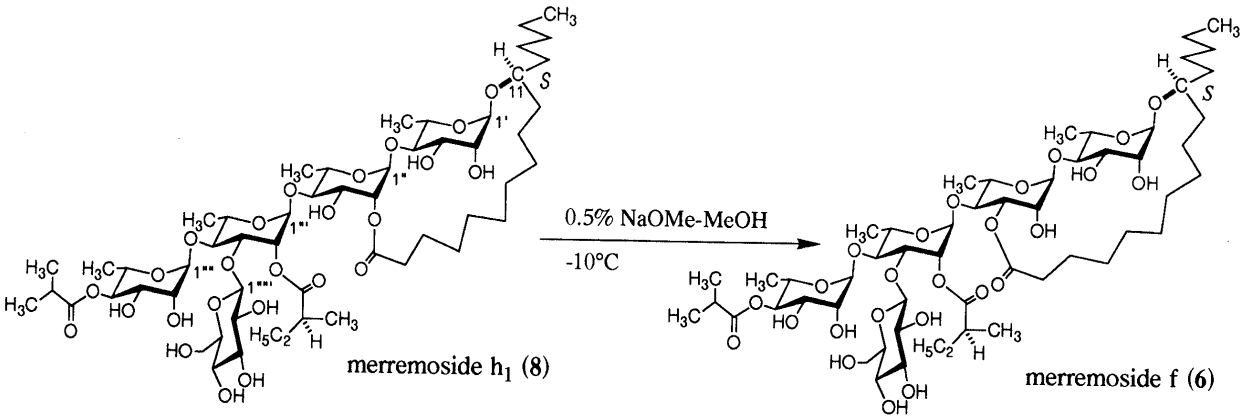

Chart 5

The ^{1}H-NMR spectrum of **6** showed signals assignable to three methine protons on carbons bearing an isobutyroyl group [δ 5.66, dd, J=9.2, 9.2 Hz, 4''''-H], a methylbutyroyl group [δ 5.98, br s, 2'''-H] and a lactonyl moiety [δ 5.63, dd, J=2.8, 10.1 Hz, 3''-H]. This inference was supported by the negative FAB-MS (Fig. 4).

Consequently, the chemical structure of merremoside f was concluded to be **6**.

Merremoside h_2 (9) Merremoside h_2 (**9**), colorless fine crystals, $C_{54}H_{92}O_{25}$, showed a similar absorption pattern to those of **6** and **7**. On hydrolysis with 5% aqueous KOH, **9** gave merremoside j (**10**) and isobutyric acid. On the other hand, treatment of **9** with 5% NaOMe–MeOH gave merremoside j methyl ester (**10a**). Furthermore, methanolysis of **9** with 9% HCl–MeOH furnished methyl (11*S*)-(+)-jalapinolate and two kinds of methyl glycosides, methyl L-rhamnoside and methyl D-glucoside, in a ratio of 4 : 1.

The SIMS and negative FAB-MS of merremoside h_2 (**9**) exhibited quite similar fragment patterns to those of merremoside g (**7**). Further, **9** was converted into merremoside g (**7**) by treatment with 0.5% NaOMe in MeOH at -10 °C. These findings imply that the structure of merremoside h_2 (**9**) is similar to that of g (**7**).

The ^{1}H-NMR spectrum of **9** showed signals due to three methine protons on carbon bearing ester functions, two isobutyroyl groups [δ 5.70, dd, J=9.5, 9.5 Hz, 4''''-H and δ 5.87, br s, 2'''-H] and one lactonyl group [δ 6.23, br s, 2''-H].

From the above-mentioned evidence, the chemical structure of merremoside h_2 (**9**) was concluded to be **9**.

Merremoside h_1 (8) Merremoside h_1 (**8**), colorless fine crystals, $C_{55}H_{94}O_{25}$, showed similar absorption bands to those of merremoside f (**6**), g (**7**), and h_2 (**9**) in the IR spectrum. Alkaline hydrolysis of **8** with 5% aqueous KOH gave merremoside j (**10**), isobutyric acid and (2*S*)-(+)-methylbutyric acid. Treatment of **8** with 5% NaOMe–MeOH gave merremoside j methyl ester (**10a**).

The SIMS and negative FAB-MS of **8** were quite similar to those of merremoside f (**6**). In addition, treatment of **8** with 0.5% NaOMe in MeOH at $-10\,^{\circ}C$ afforded merremoside f (**6**). These findings suggest that the structure of **8** is similar to that of **6**. On the other hand, the ^{1}H-NMR spectrum of **8** exhibited a methine proton attached to a lactonyl group at δ 6.28 (br s, 2″-H), beside two methine protons attached to an isobutyroyl group (δ 5.69, dd, J=9.5, 9.5 Hz, 4‴-H) and a methylbutyroyl group (δ 5.87, br s, 2‴-H).

From the above-mentioned evidence, the chemical structure of merremoside h_1 was concluded to be **8**.

In conclusion, it should be mentioned that merremosides f (**6**), g (**7**), h_1 (**8**), and h_2 (**9**) exhibit ionophoretic activity against transport of Na^+, K^+, and Ca^{++} ions across human erythrocyte membranes.[5]

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[1]

Merremoside f (**6**): Colorless fine crystals from EtOH, mp 145—146 °C, $[\alpha]_D$ −61° (c=1.2, in MeOH at 25 °C). IR (KBr) cm^{-1}: 3370, 2915, 1720. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.86 [3H, t, J=7.3 Hz, –CH(CH$_3$)CH$_2$C$\underline{H}_3$], 0.92 (3H, t, J=7.3 Hz, ω-CH$_3$), 1.12 (3H, d, J=6.7 Hz), 1.18 (3H, d, J=6.1 Hz), 1.19 (3H, d, J=6.7 Hz) [–CH(CH$_3$)CH$_2$C$\underline{H}_3$ and –CH(C$\underline{H}_3$)$_2$×2], 1.38 (3H, d, J=6.1 Hz), 1.50 (3H, d, J=6.4 Hz), 1.57 (3H, d, J=6.1 Hz), 1.60 (3H, d, J=6.1 Hz) (6′-, 6″-, 6‴-, 6⁗-H$_3$), 2.27 (1H, ddd, J=3.0, 7.0, 14.6 Hz, 2-H$_a$), 2.59 (1H, ddd, J=3.0, 7.0, 14.6 Hz, 2-H$_b$), 2.47, 2.64 (1H each, both m) [–C$\underline{H}$(CH$_3$)C$_2$H$_5$ and –C$\underline{H}$(CH$_3$)$_2$], 3.84 (1H, m, 11-H), 5.04 (1H, d, J=7.6 Hz, 1⁗-H), 5.63 (1H, dd, J=2.8, 10.1 Hz, 3″-H), 5.66 (1H, dd, J=9.2, 9.2 Hz, 4⁗-H), 5.98 (1H, br s, 2‴-H), 5.11, 5.54, 6.10, 6.28 (1H each, all br s, 1′-, 1″-, 1‴-, 1⁗-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 99.1, 101.5, 101.7, 103.3 (1′-,1″-, 1‴-, 1⁗-C), 104.9 (1⁗′-C), 174.4, 176.1, 176.6 (>C=O×3). SIMS m/z: 1177 (M+Na)$^+$, 609 (**x**), 217 (**v**). Negative FAB-MS m/z: 1153 (M−H)$^-$, 991 (**xi**), 937 (**xii**), 545 (**viii**), 417 (**ix**+H). *Anal.* Calcd for $C_{55}H_{94}O_{25}\cdot 2H_2O$: C, 55.46; H, 8.29. Found: C, 55.72; H, 8.08.

Merremoside g (**7**): Colorless fine crystals from EtOH, mp 143—145 °C, $[\alpha]_D$ −51° (c=0.9, in MeOH at 24 °C). IR (KBr) cm^{-1}: 3360, 2885, 1715. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.92 (3H, t, J=7.5 Hz, ω-CH$_3$), 1.12 (3H, d, J=7.0 Hz), 1.15 (3H, d, J=7.0 Hz), 1.18 (3H, d, J=6.5 Hz), 1.19 (3H, d, J=6.5 Hz) [–CH(C$\underline{H}_3$)$_2$×4], 1.38 (3H, d, J=6.0 Hz), 1.50 (3H, d, J=6.5 Hz), 1.57 (3H, d, J=6.0 Hz), 1.60 (3H, d, J=6.0 Hz) (6′-, 6″-, 6‴-, 6⁗-H$_3$), 2.27 (1H, ddd, J=3.0, 6.9, 14.6 Hz, 2-H$_a$), 2.62 (1H, ddd, J=3.0, 6.9, 14.6 Hz, 2-H$_b$), 3.82 (1H, m, 11-H), 5.03 (1H, d, J=7.5 Hz, 1⁗′-H), 5.63 (1H, dd, J=3.0, 10.0 Hz, 3″-H), 5.67 (1H, dd, J=9.0, 9.0 Hz, 4⁗′-H), 5.92 (1H, br s, 2‴-H), 5.13, 5.54, 6.11, 6.28 (1H each, all br s, 1′-, 1″-, 1‴-, 1⁗-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 99.3, 100.0, 102.4, 103.4 (1′-, 1″-, 1‴-, 1⁗-C), 104.8 (1⁗′-C), 174.4, 176.1, 176.6 (>C=O×3). SIMS m/z: 1163 (M+Na)$^+$, 595 (**iv**), 217 (**v**). Negative FAB-MS m/z: 1139 (M−H)$^-$, 977 (**vi**), 923 (**vii**), 545 (**viii**), 417 (**ix**+H). *Anal.* Calcd for $C_{54}H_{92}O_{25}\cdot H_2O$: C, 55.95; H, 8.17. Found: C, 55.80; H, 8.39.

Merremoside h_1 (**8**): Colorless fine crystals from EtOH, mp 148—149 °C, $[\alpha]_D$ −19° (c=0.8, in MeOH at 26 °C). IR (KBr) cm^{-1}: 3362, 2910, 1719. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.81 (3H, t, J=7.3 Hz), 0.88 (3H, t, J=6.7 Hz) [ω-CH$_3$ and –CH(CH$_3$)CH$_2$C$\underline{H}_3$], 1.04 (3H, d, J=7.0 Hz), 1.19 (3H, d, J=6.7 Hz), 1.20 (3H, d, J=6.7 Hz) [–CH(C$\underline{H}_3$)$_2$×2 and –CH(C$\underline{H}_3$)CH$_2$CH$_3$], 1.39 (3H, d, J=6.4 Hz), 1.48 (3H, d, J=6.4 Hz), 1.62 (3H, d, J=6.1 Hz), 1.65 (3H, d, J=6.1 Hz) (6′-, 6″-, 6‴-, 6⁗-H$_3$), 2.27 (1H, ddd, J=3.0, 6.9, 14.6 Hz, 2-H$_a$), 2.44 (1H, ddd, J=3.0, 6.9, 14.6 Hz, 2-H$_b$), 2.45, 2.66 [1H each, both m, –C$\underline{H}$(CH$_3$)$_2$, –C$\underline{H}$(CH$_3$)CH$_2$CH$_3$], 3.82 (1H, m, 11-H), 5.01 (1H, d, J=7.5 Hz, 1⁗′-H), 5.69 (1H, dd, J=9.5, 9.5 Hz, 4⁗′-H), 5.13, 5.47, 5.79, 6.14 (1′-, 1″-, 1‴-, 1⁗′-H), 5.87, 6.28 (1H each, both br s, 2″-, 2‴-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 98.4, 101.8, 103.2, 104.2 (1′-, 1″-, 1‴-, 1⁗′-C), 105.3 (1⁗′-C), 173.0, 176.2, 176.6 (>C=O×3). SIMS m/z: 1177 (M+Na)$^+$, 609, 217. Negative FAB-MS m/z: 1153 (M−H)$^-$, 991, 937, 545, 417. *Anal.* Calcd for $C_{55}H_{94}O_{25}\cdot 2H_2O$: C, 55.45; H, 8.29. Found: C, 55.71; H, 8.34.

Merremoside h_2 (**9**): Colorless fine crystals from EtOH, mp 151—152 °C, $[\alpha]_D$ -23° (c= 1.4, in MeOH at 25 °C). IR (KBr) cm^{-1}: 3359, 2910, 1718. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.88 (3H, t, J=7.0 Hz, ω-CH$_3$), 1.05, 1.08, 1.19, 1.20 [3H each, all d, J=6.7 Hz, –CH(C$\underline{H}_3$)$_2$×4], 1.39 (3H, d, J=6.1 Hz), 1.48 (3H, d, J=6.4 Hz), 1.62 (3H, d, J=6.4 Hz), 1.64 (3H, d, J=6.1 Hz) (6′-, 6″-, 6‴-, 6⁗-H$_3$), 2.27 (1H, ddd, J= 3.0, 6.9, 14.6 Hz, 2-H$_a$), 2.46 (1H, ddd, J=3.0, 6.9, 14.6 Hz, 2-H$_b$), 2.53, 2.65 [1H each, both m, -C$\underline{H}$(CH$_3$)$_2$×2], 3.81 (1H, m, 11-H), 5.01 (1H, d, J=7.9 Hz, 1⁗′-H), 5.70 (1H, dd, J=9.5, 9.5 Hz, 4⁗′-H), 4.79, 5.48, 5.79, 6.15 (1H each, all br s, 1′-, 1″-, 1‴-, 1⁗′-H), 5.87, 6.23 (1H each, both br s, 2″-, 2‴-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 98.5, 101.7, 103.3, 104.3 (1′-, 1″-, 1‴-, 1⁗′-C), 105.3 (1⁗′-C), 173.1, 176.6, 176.7 (>C=O×3). SIMS m/z: 1163 (M+Na)$^+$, 595, 217. Negative FAB-MS m/z: 1139 (M−H)$^-$, 977, 923, 545, 417. *Anal.* Calcd for $C_{54}H_{92}O_{25}\cdot H_2O$: C, 55.95; H, 8.17. Found: C, 56.17; H, 8.02.

Treatment of Merremoside g (7) with 5% Aqueous KOH A solution of merremoside g (**7**, 100 mg) in acetone (4.0 ml) was treated with 5% aqueous KOH (4.0 ml) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (78 mg). Column chromatography [SiO_2 30 g, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (lower phase)] of this product (75 mg) afforded merremoside j (**10**, 67 mg). Furthermore, the product (1 mg) was subjected to GLC analysis to determine isobutyric acid by comparison with an authentic sample. GLC conditions: column, 15% FFAP on Chromosorb GAW DMSC (100/120), i.d. 3 mm×1 m glass column; column temperature, 140 °C; carrier gas, N_2; flow rate, 30 ml/min; injection temperature, 170 °C; detector: FID; t_R, 8 min 46 s (isobutyric acid).

Merremoside j (**10**): Colorless fine crystals from EtOH, mp 180—181 °C, $[\alpha]_D$ −73° (c=1.0 in MeOH at 24 °C). IR (KBr) cm^{-1}: 3372, 2910, 1710. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.90 (3H, t, J=7.3 Hz, ω-CH$_3$), 1.51 (3H, d, J=6.4 Hz), 1.55 (3H, d, J=6.1 Hz), 1.58 (3H, d, J=6.1 Hz), 1.59 (3H, d, J=6.1 Hz) (6′-, 6″-, 6‴-, 6⁗-H$_3$), 2.31 (2H, t, J=7.2 Hz, 2-H$_2$), 5.17 (1H, d, J=7.6 Hz, 1⁗′-H), 5.11, 6.09, 6.12, 6.12 (1H each, all br s, 1′-, 1″-, 1‴-, 1⁗-H). *Anal.* Calcd for $C_{46}H_{82}O_{24}\cdot H_2O$: C, 53.28; H, 8.16. Found: C, 53.52; H, 8.03.

Treatment of Merremoside g (7) with 5% NaOMe–MeOH A solution of merremoside g (**7**, 2.0 g) in MeOH (10 ml) was treated with 5% NaOMe–MeOH (10 ml) and the whole was stirred under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (1.97 g), which was purified by column chromatography (SiO_2 200 g, $CHCl_3$: MeOH = 6 : 1) to afford merremoside j methyl ester (**10a**, 1.21 g).

10a: Colorless fine crystals from EtOH, mp 171—172 °C, $[\alpha]_D$ −74° (c=1.5, in MeOH at 25 °C). IR (KBr) cm^{-1}: 3370, 2907, 1715. ^{1}H-NMR (500 MHz, pyridine-d_5+D_2O) δ: 0.92 (3H, t, J=7.0 Hz, ω-CH$_3$), 1.50 (3H, d, J=6.5 Hz), 1.55 (3H, d, J=6.0 Hz), 1.58 (3H, J=6.0 Hz), 1.53 (3H, d, J=6.0 Hz) (6′-, 6″-, 6‴-, 6⁗-H$_3$), 2.30 (2H, t, J=7.6 Hz, 2-H$_2$), 3.61 (3H, s, –COOCH$_3$), 5.17 (1H, d, J=7.5 Hz, 1⁗′-H), 5.10, 5.82, 6.13, 6.16 (1H each, all br s, 1′-, 1″-, 1‴-, 1⁗-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 50.8, 100.4, 102.0, 102.3, 102.5, 104.5, 173.4. Negative FAB-MS m/z: 1031 (M−H)$^-$, 885 (**i**), 869 (**ii**), 577 (**iii**). *Anal.* Calcd for $C_{47}H_{84}O_{24}\cdot 2H_2O$: C, 52.80; H, 8.30. Found: C, 52.49; H, 8.59.

Methanolysis of Merremoside j Methyl Ester (10a) A solution of merremoside j methyl ester (**10a**, 40 mg) in 9% HCl–MeOH (3.0 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with $AgCO_3$ powder and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (38 mg). Repeated column chromatography (SiO_2 20 g, $CHCl_3$: MeOH = 30 : 1→5 : 1 and *n*-hexane : EtOAc = 7 : 1) of this product afforded (11*S*)-(+)-methyl jalapinolate[1] (5.2 mg) and a methyl glycoside mixture (25 mg). A solution of the methyl glycoside mixture (3.0 mg) in pyridine (0.3 ml) was treated with *N*,*O*-bis(trimethylsilyl)-trifluoroacetoamide (0.6 ml) at room temperature for 1 h. The reaction mixture was directly subjected to GLC analysis to identify methyl 2,3,4-*O*-tri(trimethylsilyl)-L-rhamnopyranoside and methyl 2,3,4,6-*O*-tetra(trimethylsilyl)-L-glucopyranoside by comparison with authentic samples. GLC conditions-1: column, 15% silicone OV-1 on Chromosorb WAW DMSC (80/100), i.d. 3 mm×1 m glass column; column temperature, 150 °C; carrier gas, N_2; flow rate, 30 ml/min; injection temperature, 170 °C; detector: FID; t_R, 4 min 01 s [methyl 2,3,4-*O*-

tri(trimethylsilyl)-L-rhamnopyranoside], 16 min 34 s, 19 min 50 s [methyl 2,3,4,6-*O*-tetra(trimethylsilyl)-D-glucopyranoside]. GLC conditions-2: column, 15% silicone SE-30 on Chromosorb WAW DMSC (80/100), i.d. 3 mm × 1 m glass column; column temperature, 150 °C; carrier gas, N_2; flow rate, 30 ml/min; injection temperature, 170 °C; detector: FID; t_R, 3 min 42 s [methyl 2,3,4-*O*-tri(trimethylsilyl)-L-rhamnopyranoside], 14 min 40 s, 17 min 53 s [methyl 2,3,4,6-*O*-tetra(trimethylsilyl)-D-glucopyranoside].

Enzymatic Hydrolysis of 10a A solution of **10a** (40 mg) in H_2O (2.0 ml) was treated with crude hesperidinase (Sigma, 100 mg) and the whole was stirred at room temperature for 36 h. The precipitate was removed by filtration. The filtrate was evaporated off under reduced pressure to give a product, which was purified by column chromatography [SiO_2 15 g, $CHCl_3$: MeOH = 3 : 1 → $CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)] to afford D-glucose ($[\alpha]_D$ +47.2°, c = 0.3, after 24 h in H_2O) and merremoside i methyl ester (**11**, 25 mg) which was identical with an authentic sample[1] by comparisons of TLC [$CHCl_3$: MeOH : H_2O = 7 : 3 : 1 (lower phase)], $[\alpha]_D$, IR and ^{1}H-NMR spectra.

Complete Methylation of 10a A solution of merremoside j methyl ester (**10a**, 200 mg) in dimethylsulfoxide (3.0 ml) was treated with a dimsylsodium reagent [5.0 ml, prepared from 60% NaH (1.0 g) and dimethylsulfoxide (10 ml)] and the whole was stirred at room temperature for 1 h. Methyl iodide (3.0 ml) was added at 0 °C, and the whole was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was washed with H_2O, and dried over $MgSO_4$. The solvent was evaporated off under reduced pressure to give a product (176 mg). A solution of this product in 9% HCl–MeOH (5.0 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with $AgCO_3$ powder and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (130 mg). Repeated column chromatography (SiO_2 20 g, $CHCl_3$: MeOH = 30 : 1 and *n*-hexane : EtOAc = 7 : 1) of the product (50 mg) afforded methyl (11*S*)-(+)-jalapinolate (12 mg). Furthermore, the product (1.0 mg) was subjected to GLC analysis, which revealed methyl 2,3-di-*O*-methyl-L-rhamnopyranoside (I), methyl 2-*O*-methyl-L-rhamnopyranoside (II), methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside (III), and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (IV) in 2 : 1 : 1 : 1 ratio. GLC conditions-1: column, 15% NPGS on Chromosorb WAW (80/100), i.d. 3 mm × 2 m glass column; column temperature, 170 °C; carrier gas, N_2; flow rate, 35 ml/min; injection temperature, 190 °C; detector: FID; t_R, 6 min 38 s (I), 9 min 02 s (II), 2 min 41 s (III), 3 min 25 s (IV). GLC conditions-2: column, SE-52, 25 m capillary column; column temperature, 125 °C; carrier gas, N_2; flow rate, 50 ml/min; injection temperature, 150 °C; detector: FID; t_R, 4 min 18 s (I), 4 min 09 s (II), 3 min 44 s (III), 6 min 57 s, 8 min 43 s (IV).

Treatment of Merremoside f (6) with 5% Aqueous KOH A solution of merremoside f (**6**, 100 mg) in acetone (4.0 ml) was treated with 5% aqueous KOH (4.0 ml) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (76 mg). Column chromatography [SiO_2 25 g, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (lower phase)] of the product (73 mg) afforded merremoside j (**10**, 65 mg).

Furthermore, the product (1 mg) was subjected to GLC analysis to identify isobutyric acid and methylbutyric acid by comparison with those authentic samples. GLC conditions: column, 15% FFAP on Chromosorb GAW DMSC (100/120), i.d. 3 mm × 1 m glass column; column temperature, 140 °C; carrier gas, N_2; flow rate, 30 ml/min; injection temperature, 170 °C; detector: FID; t_R, 8 min 46 s (isobutyric acid), 12 min 46 s (methylbutyric acid).

Determination of the Absolute Configuration of Methylbutyric Acid A solution of merremoside f (**6**, 500 mg) in $CHCl_3$–acetone (1 : 1, 3.0 ml) was treated with 10% aqueous KOH (3.0 ml) and the mixture was stirred under reflux for 3 h. After the removal of acetone under reduced pressure, the reaction mixture was neutralized with 5% aqueous HCl and extracted with EtOAc. After usual work-up of the EtOAc extract, the solvent was evaporated off under reduced pressure to yield a mixture of organic acids (230 mg). A solution of the mixture in *N*,*N*-dimethylformamide (3 ml) was treated with potassium fluoride (200 mg) and α-bromoacetophenone (300 mg), and the whole was stirred at room temperature for 1 h, poured into ice-water and extracted with ether. After usual work-up of the ether extract, the solvent was evaporated off under reduced pressure to give a product (520 mg). Column chromatography (SiO_2 10 g, *n*-hexane : EtOAc = 15 : 1) and HPLC (Zorbax SIL, 0.25 m × 4.6 mm, *n*-hexane : EtOAc = 7 : 1) of this product afforded isobutyric acid phenacyl ester (13.2 mg) and (2*S*)-(+)-methylbutyric acid phenacyl ester[4] (**13**, 12.7 mg).

Isobutyric acid phenacyl ester: a pale yellow oil. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.27 (3H × 2, d, J = 7.0 Hz), 1.58 (2H, s), 2.75 (1H, m), 7.55 (1H × 3, dd-like), 7.87 (1H, d, J = 2.0 Hz), 7.96 (1H, d, J = 1.5 Hz). (2*S*)-(+)-Methylbutyric acid phenacyl ester (**13**): a pale yellow oil, $[\alpha]_D$ +15° (c = 4.6, in $CHCl_3$ at 25 °C). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.98 (3H, J = 7.4 Hz), 1.24 (3H, d, J = 7.0 Hz), 1.62 (2H, m), 1.65 (2H, s), 2.53 (1H, m), 7.55 (1H × 3, dd-like), 7.87 (1H, d, J = 2.0 Hz), 7.96 (1H, d, J = 1.5 Hz).

An authentic sample (469 mg, 0.5 ml) of (2*S*)-(+)-methylbutyric acid was treated with potassium fluoride (533 mg) and α-bromoacetophenone (1.1 g) in *N*,*N*-dimethylformamide (10 ml), and the whole was stirred at room temperature for 1 h. The reaction mixture was poured into ice-water and extracted with ether. After usual work-up of the ether extract, the solvent was evaporated off under reduced pressure to give a product (1.7 g). Column chromatography (SiO_2 100 g, *n*-hexane : EtOAc = 15 : 1) of the product afforded (2*S*)-(+)-methylbutyric acid phenacyl ester [571 mg, $[\alpha]_D$ +15° (c = 5.4, in $CHCl_3$ at 25 °C)].

Treatment of Merremoside f (6) with 5% NaOMe–MeOH Merremoside f (**6**, 40 mg) was treated with 5% NaOMe–MeOH (2.0 ml) and the mixture was stirred at room temperature for 30 min. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product. Column chromatography (SiO_2 10 g, $CHCl_3$: MeOH = 6 : 1) of the product afforded merremoside j methyl ester (**10a**, 23 mg).

Treatment of Merremoside h_2 (9) with 5% Aqueous KOH A solution of merremoside h_2 (**9**, 50 mg) in acetone (1.0 ml) was treated with 5% aqueous KOH (1.0 ml) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (36 mg). Column chromatography [SiO_2 15 g, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (lower phase)] of this product (35 mg) afforded merremoside j (**10**, 10 mg). Furthermore, the product (1 mg) was subjected to GLC analysis to identify the organic acid, and isobutyric acid was identified by comparison with an authentic sample.

Treatment of Merremoside h_2 (9) with 5% NaOMe–MeOH Merremoside h_2 (**9**, 30 mg) was treated with 5% NaOMe–MeOH (1.0 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product. Column chromatography (SiO_2 10 g, $CHCl_3$: MeOH = 6 : 1) of the product afforded merremoside j methyl ester (**10a**, 18 mg).

Treatment of Merremoside h_2 (9) with 0.5% NaOMe–MeOH A solution of **9** (20 mg) in MeOH (1.0 ml) was treated with 0.5% NaOM–MeOH (1.0 ml) and the mixture was stirred at −10 °C for 30 min. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to yield a product. Column chromatography (SiO_2 10 g, $CHCl_3$: MeOH = 5 : 1) of the product afforded merremoside g (**7**, 15 mg).

Treatment of Merremoside h_1 (8) with 5% Aqueous KOH A solution of merremoside h_1 (**8**, 100 mg) in acetone (2.0 ml) was treated with 10% aqueous KOH (2.0 ml) and the mixture was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product (77 mg). Column chromatography [SiO_2 20 g, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (lower phase)] of the product (77 mg) afforded merremoside j (**10**, 72 mg). Furthermore, the product (1 mg) was subjected to GLC analysis to determine isobutyric acid and methylbutyric acid by comparison with authentic samples.

Treatment of Merremoside h_1 (8) with 5% NaOMe–MeOH Merremoside h_1 (**8**, 40 mg) was treated with 5% NaOMe–MeOH (1.0 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to give a product. Column chromatography (SiO_2 10 g, $CHCl_3$: MeOH = 6 : 1) of the product afforded merremoside j methyl ester (**10a**, 23 mg).

Treatment of Merremoside h_1 (8) with 0.5% NaOMe–MeOH Merremoside h_1 (**8**, 20 mg) was treated with 0.5% NaOM–MeOH (1 ml) and the mixture was stirred at $-10\,°C$ for 30 min. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was evaporated off under reduced pressure to yield a product. Column chromatography (SiO_2 10 g, $CHCl_3$: MeOH = 7 : 1) of the product afforded merremoside g (**7**, 12 mg).

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