MAMMOSIDES B AND H1, NEW IONOPHORIC RESIN-GLYCOSIDES FROM THE TUBER OF MERREMIA MAMMOSA, AN INDONESIAN FOLK MEDICINE

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Four new ionophoric resin-glycosides have been isolated from the tuber of Merremia mammosa (Convolvulaceae), an Indonesian folk medicine ("Bidara upas"). The structures of two major resin-glycosides, named mammosides B (1) and H1 (2), have been determined on the basis of chemical and physicochemical evidence including a synthesis of the glycosidic acid designated as mammoside I (3).

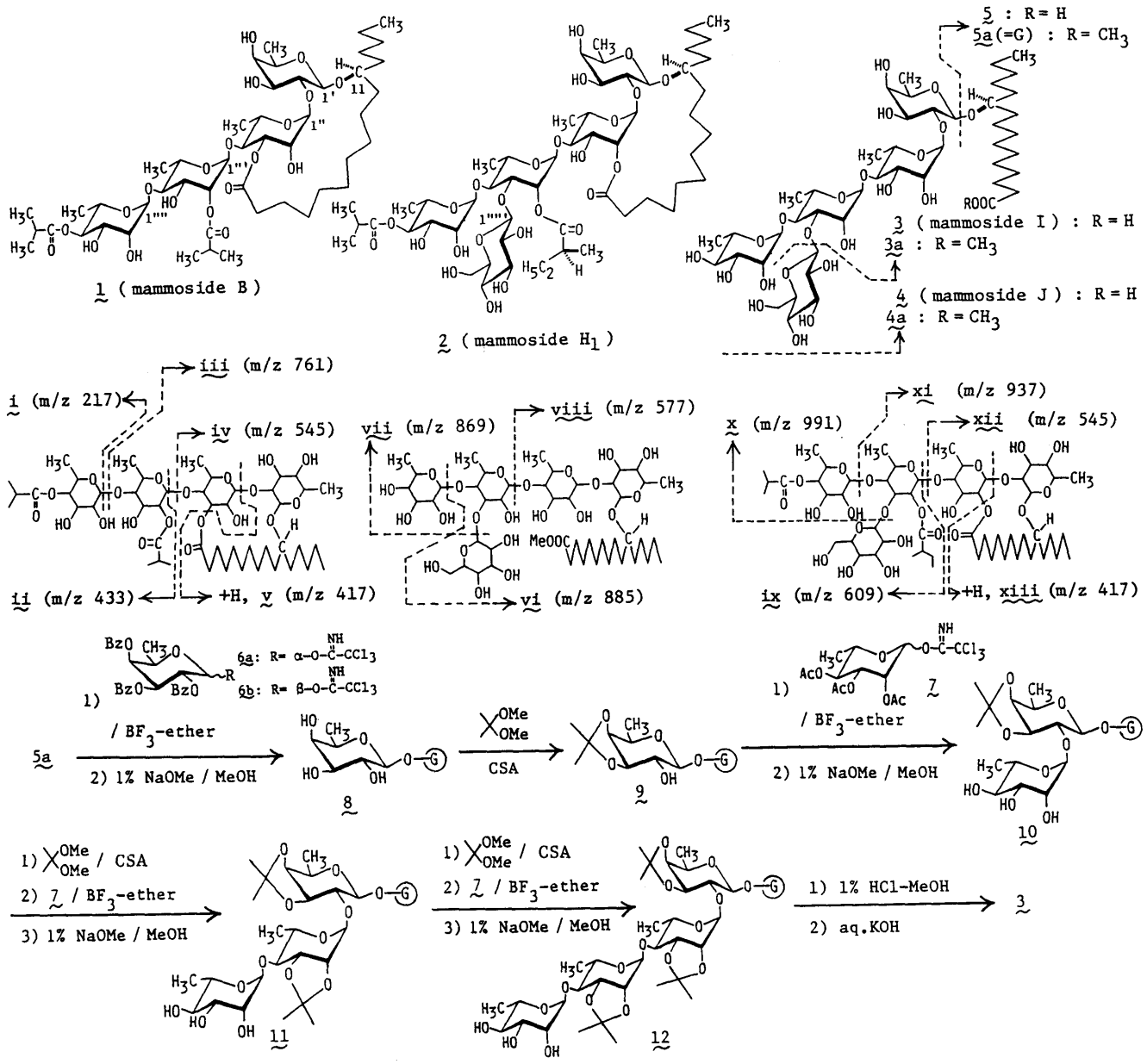
KEYWORDS Merremia mammosa; Indonesian folk medicine; Bidara upas; mammoside B; mammoside H1; mammoside I; mammoside J; resin-glycoside ionophoric activity; glycosidic acid synthesis

In a preceding paper,[1] we reported the stereoselective synthesis of (11S)-(+)-jalapinolic acid (5) (natural) and its optical isomer and the revision of the structure of the aglycone part in merremosides b and d[2] which were isolated from the tuber of Merremia mammosa Choisy., an Indonesian folk medicine (Indonesian name "Bidara upas", Convolvulaceae). In a continuing chemical study of the tuber, we have isolated four new resin-glycosides named mammosides A, B, H1, and H2 and have elucidated their chemical structures.[3] Here we describe the chemical structure of mammosides B (1) and H2 (2).

The MeOH extract of the fresh tuber (obtained at Yogyakarta, Java in 1986) was partitioned into a mixture of $CHCl_3$ and water. Repeated column chromatography [silica gel ($CHCl_3$-MeOH) and Bondapak C_{18} (H_2O-MeOH)] of the $CHCl_3$ soluble portion followed by HPLC (Shimpak PREP-ODS, H_2O-MeOH) furnished mammosides A (0.01% from the fresh tuber), B (0.07%), H1 (0.10%) and H2 (0.11%) together with merremosides[2,3]— mammoside B (1): mp 125-126°C, $[\alpha]_D^{25}$ -91° (MeOH), $C_{48}H_{82}O_{20}\cdot 2H_2O$,[4] IR ν_{max}^{KBr} cm^{-1}: 3360, 2915, 1712, and mammoside H1 (2): mp 145-146°C, $[\alpha]_D^{25}$ -18° (MeOH), $C_{55}H_{94}O_{25}\cdot 2H_2O$, IR ν_{max}^{KBr} cm^{-1}: 3360, 2915, 1718.

Hydrolysis of mammoside B (1) with 5% aq. KOH yielded a glycosidic acid designated as mammoside I (3), mp 131-132°C. $[\alpha]_D^{26}$ -87° (MeOH), $C_{40}H_{72}O_{19}\cdot H_2O$, IR ν_{max}^{KBr} cm^{-1}: 3400, 1715, and isobutyric acid. Treatment of 1 with 5% NaOMe-MeOH gave 3a, mp 115-116°C, $[\alpha]_D^{22}$ -76° (MeOH), $C_{41}H_{74}O_{19}\cdot H_2O$, IR ν_{max}^{KBr} cm^{-1}: 3418, 1718, SIMS : m/z 893 $(M+Na)^+$. Methanolysis of 3a furnished methyl D-fucopyranoside[5] and methyl L-rhamnopyranoside[5] in 1:3 ratio and methyl jalapinolate (5a). The ^1H-^1H COSY (500 MHz, pyridine-d_5 + D_2O) of 3a resulted in the following assignments (J in Hz): δ 2.31 (t, J=7.5, 2-H_2), 3.93 (m, 11-H), 4.73 (d, J=8, 1'-H), 4.44 (dd, J=8, 9.5, 2'-H), 4.11 (dd, J=3, 9.5, 3'-H), 3.92 (br s, 4'-H), 3.75 (m, 5'-H), 1.50 (d, J=6, 6'-H_3), 6.19, 6.21, 6.27 (all br s, 1", 1"', 1""-H), 4.63, 4.74, 4.79 (all br s, 2", 2"', 2""-H), 4.43, 4.52, 4.58 (all dd, J=3, 9; 3", 3"', 3""-H), 4.25, 4.29, 4.39 (all dd, J=9, 9; 4", 4"', 4""-H), 4.30, 4.31, 4.82 (all m, 5", 5"', 5""-H), 1.54, 1.55, 1.57 (all d, J=6; 6", 6"', 6""-H_3). The NOE was observed between 11-H and 1'-H, so that the D-fucopyranosyl moiety in 3a was shown directly linked to the methyl jalapinolate moiety. Complete methylation of 3a with CH_3I/DMSO/NaH followed by methanolysis provided 5a together with methyl 3,4-di-O-methylfucopyranoside (a), methyl 2,3-di-O-methylrhamnopyranoside (b), and methyl 2,3,4-tri-O-methylrhamnopyranoside (c) in 1:2:1 ratio. Based on these findings and the ^{13}C NMR study of 3a including the ^{13}C-^1H coupling constants of anomeric C and H signals [159.9 Hz (fucopyranosyl moiety), 169.9, 171.0, 171.6 Hz (rhamnopyranosyl moieties)], the structure 3 was presumed for mammoside I and finally it was verified by the following synthesis.

Selective deacetylation of 1,2,3,4-tetra-O-benzoyl-D-fucopyranose with $NH_2\cdot NH_2\cdot AcOH$ in DMF and subsequent treatment with CCl_3CN in the presence of K_2CO_3,[6] afforded 6a (45.0%), white powder, $[\alpha]_D^{24}$ +182° ($CHCl_3$), $C_{29}H_{24}O_8NCl_3$, and 6b (44.6%), white powder, $[\alpha]_D^{24}$ +196° ($CHCl_3$), $C_{29}H_{24}O_8NCl_3$. Glycosidation of 5a with



6a or 6b in CH_2Cl_2 at -30°C in the presence of BF_3-ether and molecular sieves (4Å) and subsequent debenzoylation, furnished β-glycoside 8[7a] (75% from 6a, 64% from 6b), white powder, $[\alpha]_D^{21}$ -13° ($CHCl_3$), $C_{23}H_{44}O_7$. Acetonidation of 8 yielded 9[7b] (quant.), colorless oil, $[\alpha]_D^{25}$ +9.8° ($CHCl_3$), $C_{26}H_{48}O_7$. Glycosidation of 9 with 7 (prepared as for 6b), white powder, $[\alpha]_D^{25}$ -54° ($CHCl_3$), $C_{14}H_{18}O_8NCl_3$, and subsequent deacetylation, furnished a diglycoside 10[7c] (93%), colorless oil, $[\alpha]_D^{24}$ -26° ($CHCl_3$), $C_{32}H_{58}O_{11}$. Acetonidation of 10 followed by re-glycosidation with 7 provided a triglycoside, which, by deacetylation, was converted to 11[7d] (60%), colorless oil, $[\alpha]_D^{24}$ -29° ($CHCl_3$), $C_{41}H_{72}O_{15}$. Then, repeated acetonidation of 11 followed by re-glycosidation with 7 and subsequent deacetylation furnished 12[7e] (78%), colorless oil, $[\alpha]_D^{24}$ -46° ($CHCl_3$), $C_{50}H_{86}O_{19}$. Removal of the isopropylidene groups in 12 with 1% HCl-dry MeOH furnished 3a (quant.), which was finally subjected to alkaline hydrolysis with 5% aq. KOH to yield 3, identical with the authentic sample obtained from mammoside B (1).

Mammoside B (1) contains two isobutyroyl ester linkages and a lactone linkage as shown by the SIMS [m/z 1001 $(M+Na)^+$, 1017 $(M+K)^+$] and neg. FAB-MS [m/z 977 $(M-H)^-$]. The ^1H NMR (500 MHz, pyridine-d_5 + D_2O, J in Hz) and ^{13}C NMR data for 1 were assigned by ^1H-^1H COSY, Homonuclear Hartmann-Hahn Spectroscopy (HOHAHA) and ^{13}C-^1H COSY: δ2.22 (ddd, J=3, 7, 14) 2.61 (t-like)(2-H_2), 3.85 (m, 11-H), 4.75 (d, J=8, 1'-H), 4.47 (dd, J=8, 9, 2'-H), 4.13 (dd, J=3, 9, 3'-H), 3.89 (br d, 4'-H), 3.79 (m, 5'-H), 1.50 (d, J=6, 6'-H_3), 6.30 (br s,

1"-H), 5.23 (br s, 2"-H), 5.54 (dd, J=3, 10, 3"-H), 4.56 (dd, J=10, 10, 4"-H), 4.96 (m, 5"-H), 1.57 (d, J=6, 6"-H_3), 5.50 (br s, 1"'-H), 5.70 (br s, 2"'-H), 4.45 (dd, J=3, 10, 3"'-H), 4.16 (dd, J=10, 10, 4"'-H), 4.31 (m, 5"'-H), 1.63 (d, J=6, 6"'-H_3), 6.05 (br s, 1""-H), 4.73 (br s, 2""-H), 4.44 (dd, J=3, 10, 3""-H), 5.73 (dd, J=10, 10, 4""-H), 4.36 (m, 5""-H), 1.40 (d, J=6, 6""-H_3). The ^{1}H-Differential Nuclear Overhauser enhancements (DIFNOE) were observed between the following proton pairs of 1 (1'-H & 11-H; 1"-H & 2'-H ; 1"'-H & 4"-H; 1""-H & 4"'-H). Based on these findings and the fragmentation patterns of the SIMS (i, ii) and neg. FAB-MS (iii, iv, v), the structure of mammoside B (1) has been determined as shown.

Alkaline hydrolysis of mammoside Hl (2) furnished a glycosidic acid designated as mammoside J (4), mp 182-183°C, $[\alpha]_D^{25}$ -69° (MeOH), $C_{46}H_{82}O_{24}\cdot H_2O$, IR ν_{max}^{KBr} cm^{-1}: 3370, 2912, 1710, together with a mixture of isobutyric acid and 2S-methylbutyric acid, which were identified as their phenacyl esters separated by HPLC (Zorbax SIL, hexane-AcOEt): phenacyl 2S-methylbutyrate, pale yellow oil, $[\alpha]_D^{25}$ +15° ($CHCl_3$), $C_{13}H_{16}O_3$. Treatment of 2 with 5% NaOMe-MeOH furnished 4a, mp 176-177°C, $[\alpha]_D^{25}$ -71° (MeOH), $C_{47}H_{84}O_{24}\cdot 2H_2O$, IR ν_{max}^{KBr} cm^{-1}: 3368, 1717, ^{1}H NMR (500 MHz, pyridine-d_5 + D_2O, J in Hz): δ 4.81 (d, J=7.5, 1'-H), 5.17 (d, J=8, 1""'-H), 5.82, 6.13, 6.15 (all br s, 1", 1"', 1""-H). Methanolysis of 4a furnished methyl D-fucopyranoside,[5] methyl L-rhamnopyranoside,[5] and methyl D-glucopyranoside[5] in 1:3:1 ratio and methyl jalapinolate (5a). Complete methylation of 4a followed by methanolysis provided three methyl glycosides [a, b, c], methyl 2-O-methylrhamnopyranoside, and methyl 2,3,4,6-tetra-O-methylglucopyranoside in 1:1:1:1:1 ratio, and 5a. Enzymatic hydrolysis of 4a with crude hesperidinase furnished 3a and D-glucose. Finally, the neg. FAB-MS of 4a has led to the formulation of mammoside J (4): [m/z 1031 $(M-H)^-$, 885 (vi), 869 (vii) and 577 (viii)].

The ^{1}H NMR spectrum (500 MHz, pyridine-d_5 + D_2O) of 2 showed three methine protons on the carbons bearing an isobutyroyl and a 2S-methylbutyroyl groups and a lactone linkage: δ 5.76 (dd, J=10, 10, 4""-H), 5.92, 6.31 (both br s, 2", 2"'-H). The major fragment ions (i, ix) in the SIMS of 2 have shown the locations of two acyl groups in 2 at 2"' and 4""-hydroxyls. Furthermore, the neg. FAB-MS of 2 provided an ion at m/z 1153 $(M-H)^-$ and fragment ions at m/z 991 (x), 937 (xi), 545 (xii) and 417 (xiii + H). Thus, the structure of 2 has been determined as shown.

Mammosides B (1) and Hl (2) were found to exhibit ionophoric activity against Na^+, K^+, and Ca^{2+} ions as examined by a human erythrocyte membrane method.[3,8]

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4) The molecular composition of the compound given with the chemical formula was determined either by elemental analysis or by high resolution mass spectrometry.

5) By acidic hydrolysis of the methyl glycoside, D-fucose [$[\alpha]_D^{23}$ +72° (H_2O)], L-rhamnose [$[\alpha]_D^{23}$ +8.0° (H_2O)] or D-glucose [$[\alpha]_D^{23}$ +45° (H_2O)] was obtained.

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7) All new compounds were fully characterized by IR ($CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$, δ, J in Hz) and ^{13}C NMR (125 MHz, $CDCl_3$, δc). The anomeric configuration at the L-rhamnopyranoside linkage in 10, 11, or 12 has been substantiated by the application of Klyne's rule and by the ^{13}C NMR data including the ^{13}C-^1H coupling constants. a) 8, IR: 3400, 1725 cm^{-1}, δ: 1.35 (d, J=6, 6'-H_3), 4.23 (d, J=7, 1'-H). b) 9, IR: 3600, 1728 cm^{-1}, δ: 1.40 (d, J=7, 6'-H_3), 4.16 (d, J=8, 1'-H). c) 10, IR: 3450, 1725 cm^{-1}, δ: 1.26, 1.39 (both d, J=6; 6', 6"-H_3), 4.24 (d, J=7, 1'-H), 5.31 (d, J=1, 1"-H), δc:100.1 (1"-C, J_{C-H}=169.9 Hz), 100.5 (1'-C, J_{C-H}=156.8 Hz). d) 11, IR: 3500, 1730 cm^{-1}, δ: 1.23, 1.30, 1.39 (all d, J=6; 6',6", 6"'-H_3), 4.21 (d, J=8, 1'-H), 5.39 (s, 1"'-H), 5.50 (br s, 1"-H). e) 12, IR: 3530, 3430, 1735 cm^{-1}, δ: 1.22, 1.26, 1.31, 1.39 (all d, J=6; 6', 6", 6"', 6""-H_3), 4.22 (d, J=8, 1'-H), 5.41 (d, J=1, 1""-H), 5.51, 5.59 (both br s, 1", 1"'-H).

8) I. Kitagawa, H. Akedo et al., to be published.

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