

## Communications to the Editor

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STRUCTURES OF MERREMOSIDES B AND D, NEW ANTISEROTONIC RESIN-GLYCOSIDES  
FROM THE TUBER OF *MERREMIA MAMMOSA*, AN INDONESIAN FOLK MEDICINE

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Six new resin-glycosides (merremoside) were isolated from an Indonesian folk medicine ("Bidara upas"), the tuber of *Merremia mammosa* (Convolvulaceae), and the structures of two antiserotonic glycosides, named merremosides b (1) and d (2), have been determined on the basis of chemical and physicochemical evidence.

KEYWORDS — merremoside b; merremoside d; resin-glycoside; Indonesian folk medicine; *Merremia mammosa*; Convolvulaceae; resin-glycoside antiserotonic; resin-glycoside negative ion FAB-MS; resin-glycoside SIMS

The tuber of *Merremia mammosa* CHOIS. (Indonesian name "Bidara upas", Convolvulaceae) is an Indonesian folk medicine which is said to be useful for treating diabetes and affection of the throat and respiratory system. As a part of our investigations of Indonesian medicinal plants,<sup>1)</sup> we have examined the chemical constituents of the tuber. We have so far isolated six new resin-glycosides named merremosides a, b, c, d, f, and g and have elucidated their chemical structures.<sup>2)</sup> This paper deals with the evidence which is in good accord with the structures of merremosides b (1) and d (2).<sup>3)</sup>

The MeOH extract of the fresh tuber (obtained at Yogyakarta, Java) was partitioned into a mixture of CHCl<sub>3</sub> and water. Silica gel column chromatography of the CHCl<sub>3</sub>-soluble portion followed by HPLC (Zorbax ODS, MeOH-H<sub>2</sub>O=4:1) furnished merremosides a (0.002% from the fresh tuber), b (0.006%), c (0.003%), d (0.004%), f (0.001%), and g (0.004%)<sup>4)</sup> — merremoside b (1), mp 129-130°C,  $[\alpha]_D^{25}$  -90° (MeOH), C<sub>48</sub>H<sub>82</sub>O<sub>20</sub>·H<sub>2</sub>O,<sup>5)</sup> IR (KBr): 3350 (br), 2895, 1715 (br) cm<sup>-1</sup>, and merremoside d (2), mp 138-139°C,  $[\alpha]_D^{25}$  -77° (MeOH), C<sub>48</sub>H<sub>82</sub>O<sub>20</sub>·H<sub>2</sub>O, IR (KBr): 3420 (br), 2932, 1718 (br) cm<sup>-1</sup>.

Hydrolysis with 5% aq. KOH (reflux) of both merremosides b (1) and d (2) yielded a common product merremoside i (6), mp 138-140°C,  $[\alpha]_D^{24}$  -89° (MeOH), C<sub>40</sub>H<sub>72</sub>O<sub>19</sub>·2H<sub>2</sub>O, IR (KBr): 3401 (br), 2928, 1710 (br) cm<sup>-1</sup>, and isobutyric acid. Treatment of 1 and 2 with 5% NaOMe-MeOH (25°C, 30 min) gave merremoside i methyl ester (6a), mp 112-113°C,  $[\alpha]_D^{14}$  -81° (MeOH), C<sub>41</sub>H<sub>74</sub>O<sub>19</sub>·3H<sub>2</sub>O, IR (KBr): 3369 (br), 2918, 1732 cm<sup>-1</sup>. Methanolysis (9% HCl-dry MeOH, reflux) of 6a liberated methyl L-rhamnoside<sup>6)</sup> and methyl jalapinolate<sup>7)</sup> [9,  $[\alpha]_D^{24}$  +0.5° (CHCl<sub>3</sub>)], the 11R configuration has been supported by application of Horeau's method.<sup>8,9)</sup>

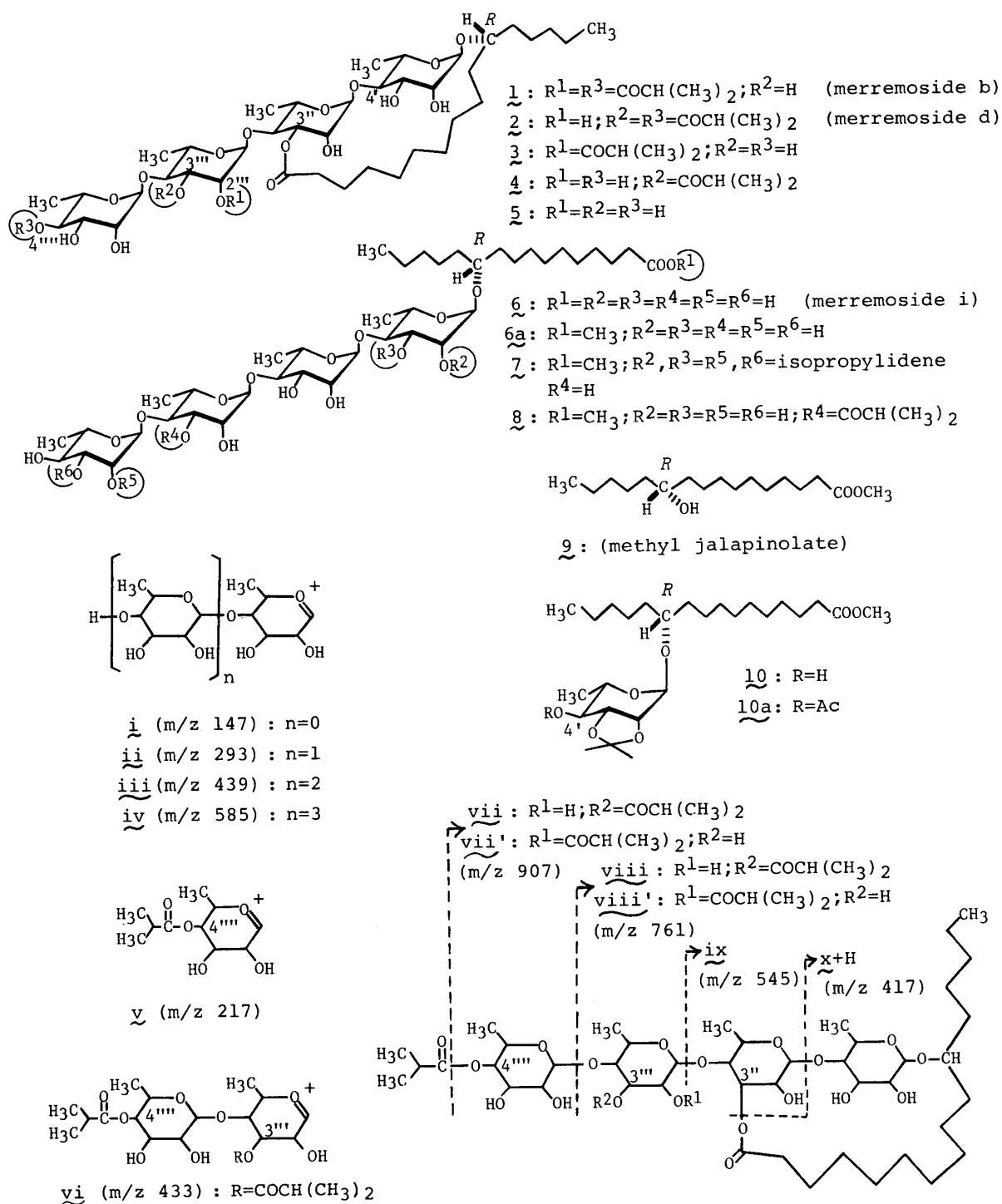
The SIMS of 6a showed ion peaks at *m/z* 893 (M+Na)<sup>+</sup> and 909 (M+K)<sup>+</sup> together

with fragment ions i, ii, iii, and iv derived from the sugar moiety. The  $^1\text{H}$  NMR spectrum of 6a (500 MHz,  $\text{d}_5\text{-pyridine}+\text{D}_2\text{O}$ ) showed signals due to one *prim.*  $\text{CH}_3$  ( $\delta$  0.91, t,  $J=7$  Hz), one  $\text{CH}_3\text{O}$  ( $\delta$  3.61, s), four *sec.*  $\text{CH}_3$  ( $\delta$  1.50, 1.56, 1.57, 1.58, all d,  $J=6$  Hz), and four anomeric H [ $\delta$  6.24, 6.25, 6.31 (2H), all s]. The  $^{13}\text{C}$  NMR of 6a (125 MHz,  $\text{d}_5\text{-pyr.}+\text{D}_2\text{O}$ ) showed four anomeric C signals [ $\delta$  101.0, 101.1, 102.5 (2C)] with  $^{13}\text{C}$ - $^1\text{H}$  coupling constants: 170.0, 171.0, 171.5, and 171.5 Hz. Thus, the structure of 6a has been shown to comprise four linear  $\alpha$ -rhamnosyl moieties attached to the 11-OH of methyl jalapinate (9). Complete methylation of 6a with  $\text{CH}_3\text{I}$ -DMSO- $\text{NaH}^{10}$  followed by methanolysis provided 9 together with methyl 2,3-di-O-methyl-L-rhamnopyranoside and methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside in 3:1 ratio. Thus the structures of 6a and merremoside i (6) have been determined.

The SIMS of merremoside d (2) showed ion peaks at  $m/z$  1001 ( $\text{M}+\text{Na}$ ) $^+$  and 1017 ( $\text{M}+\text{K}$ ) $^+$ , while the negative ion (neg.) FAB-MS gave an ion peak at  $m/z$  977 ( $\text{M}-\text{H}$ ) $^-$ . These findings together with the elemental analysis have shown that 2 comprises two isobutyryl ester linkages, and the carboxyl group in the jalapinic acid moiety is lactonized to a hydroxyl group in the sugar part. The  $^1\text{H}$  NMR spectrum (500 MHz,  $\text{CD}_3\text{OD}+\text{D}_2\text{O}$ ) of 2 showed signals due to one *prim.*  $\text{CH}_3$ , eight *sec.*  $\text{CH}_3$ , and three methine protons on carbons bearing one each of isobutyryl group and a lactone linkage:  $\delta$  4.92 (dd,  $J=9.5$ , 9.5 Hz, 4'''-H), 4.99 (dd,  $J=2.5$ , 10.0 Hz), 5.11 (dd,  $J=3.0$ , 9.5 Hz) (3'''-H, 3''-H). The coupling patterns of these methine proton signals indicate three ester linkages are attached to two 3-OH and one 4-OH in the rhamnosyl moieties. The major fragment ions, v and vi, in the SIMS of 2 have shown locations of two isobutyryl residues in 2 at 3'''-OH and 4'''-OH. Furthermore, the neg. FAB-MS of 2 provided fragment ions vii, viii, ix, and x+H. Thus, locations of two isobutyryl residues have been supported and the lactone linkage has been shown to connect to 3''-OH.

In order to confirm the location of lactone-linkage in merremoside d (2), the following derivatization was carried out. Acetonidation of 2 (2,2-dimethoxypropane, *d*-10-camphorsulfonic acid, DMF, 45°C) followed by alkaline treatment (3% NaOMe-MeOH, 15°C) furnished 7, mp 115-116°C,  $[\alpha]_{\text{D}}^{20}$  -80° (MeOH),  $\text{C}_{47}\text{H}_{82}\text{O}_{19}\cdot 2\text{H}_2\text{O}$ , IR (KBr): 3400 (br), 2920, 1720  $\text{cm}^{-1}$ .  $\text{NaIO}_4$  oxidation of 7 (MeOH- $\text{H}_2\text{O}$ , 15°C) and subsequent alkaline degradation of the product (5% NaOMe-MeOH, 15°C) provided 10, a white powder,  $[\alpha]_{\text{D}}^{26}$  +11° ( $\text{CHCl}_3$ ),  $\text{C}_{26}\text{H}_{48}\text{O}_7$ , IR ( $\text{CHCl}_3$ ): 3590, 2931, 2863, 1726  $\text{cm}^{-1}$ , which, on acetylation ( $\text{Ac}_2\text{O}$ -pyr.), gave the monoacetate (10a), a colorless oil,  $[\alpha]_{\text{D}}^{26}$  +26° ( $\text{CHCl}_3$ ),  $\text{C}_{28}\text{H}_{50}\text{O}_8$ , IR ( $\text{CHCl}_3$ ): 2936, 2859, 1731  $\text{cm}^{-1}$ . The  $^1\text{H}$  NMR spectrum of 10a (90 MHz,  $\text{CDCl}_3$ ) showed signals due to one *prim.*  $\text{CH}_3$  ( $\delta$  0.88, t-like), one *sec.*  $\text{CH}_3$  ( $\delta$  1.50, d,  $J=6$  Hz), two *tert.*  $\text{CH}_3$  ( $\delta$  1.26, 1.36, both s), one AcO ( $\delta$  2.06, s), and 4'-H ( $\delta$  4.82, dd,  $J=8$ , 8 Hz). Thus, the structures of 10 and 10a have been proved as shown. Thus, the connection of lactone-linkage to 3''-OH in merremoside d has been proved chemically and the total structure of merremoside d (2) has been determined.

Merremoside b (1) is an isomer of merremoside d (2). Treatment of 1 with 2% NaOMe-MeOH (-10°C, 5 min) afforded 2 (15%), 3 (14%), mp 118-120°C,  $[\alpha]_{\text{D}}^{25}$  -48° (MeOH),  $\text{C}_{44}\text{H}_{76}\text{O}_{19}\cdot 2\text{H}_2\text{O}$ , IR (KBr): 3380 (br), 2910, 1715 (br)  $\text{cm}^{-1}$ , neg. FAB-MS:  $m/z$  907 ( $\text{M}-\text{H}$ ) $^-$ , 761 (viii'), 545 (ix), 417 (x+H),  $^1\text{H}$  NMR (500 MHz,  $\text{d}_5\text{-pyr.}+\text{D}_2\text{O}$ )  $\delta$ : 5.54 (dd,  $J=2.8$ , 10.1 Hz, 3''-H), 5.71 (br s, 2'''-H), and 4 (13%), mp 110-111°C,



$[\alpha]_{\text{D}}^{25} -45^\circ$  (MeOH),  $\text{C}_{44}\text{H}_{76}\text{O}_{19} \cdot 3\text{H}_2\text{O}$ , IR (KBr): 3380 (br), 2905, 1717 (br)  $\text{cm}^{-1}$ , neg. FAB-MS:  $m/z$  907 (M-H) $^-$ , 761 ( $\underline{viii}$ ), 545, 417,  $^1\text{H}$  NMR (500 MHz,  $\text{d}_5\text{-pyr.}+\text{D}_2\text{O}$ )  $\delta$ : 5.62 (dd,  $J=2.8, 10.1$  Hz, 3"-H), 5.66 (dd,  $J=2.8, 9.8$  Hz, 3'''-H). On the other hand, treatment of  $\underline{1}$  with 4% NaOMe-MeOH ( $0^\circ\text{C}$ , 30 min) provided  $\underline{6a}$  (39%),  $\underline{5}$  (20%), mp  $144\text{--}146^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{25} -75^\circ$  (MeOH),  $\text{C}_{40}\text{H}_{70}\text{O}_{18} \cdot 3\text{H}_2\text{O}$ , IR (KBr): 3360 (br), 2907, 1712 (br)  $\text{cm}^{-1}$ , neg. FAB-MS:  $m/z$  837 (M-H) $^-$ , 691, 545,  $^1\text{H}$  NMR (500 MHz,  $\text{d}_5\text{-pyr.}+\text{D}_2\text{O}$ )  $\delta$ : 5.55 (dd,  $J=2.8, 10.1$  Hz, 3"-H), and  $\underline{8}$  (5%), mp  $103\text{--}104^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{25} -74^\circ$  (MeOH),

$C_{45}H_{80}O_{20} \cdot 3H_2O$ , IR (KBr): 3383 (br), 2915, 1710 (br)  $cm^{-1}$ , neg. FAB-MS:  $m/z$  939 (M-H)<sup>-</sup>, 869, 793, 577, <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD+D<sub>2</sub>O)  $\delta$ : 3.63 (s, COOCH<sub>3</sub>), 5.73 (dd,  $J=3.1$ , 10.1 Hz, 3'''-H). Examination in detail of neg. FAB-MS and <sup>1</sup>H NMR data for 2, 3, 4, 5, 6, 6a, and 8 and the fact that mild alkaline treatment of 2 gave 4, 5, 6a, and 8, have led us to formulate structures of 3, 4, 5, and 8 as shown. In addition, the 2'''-isobutyryl residue has been shown to migrate readily to neighboring 3'''-OH.

The neg. FAB-MS of merremoside b (1) showed an ion peak at  $m/z$  977 (M-H)<sup>-</sup> and fragment ions at  $m/z$  907 (vii'), 761 (viii'), 545 (ix), and 417 (x+H), whereas the <sup>1</sup>H NMR spectrum (d<sub>5</sub>-pyr.+D<sub>2</sub>O) showed signals due to one prim. CH<sub>3</sub>, eight sec. CH<sub>3</sub>, and three methine protons on carbons bearing two isobutyryl residues and one lactone linkage ( $\delta$  5.54, dd,  $J=2.8$ , 10.1 Hz, 3''-H; 5.70, br s, 2'''-H; 5.73, dd,  $J=9.8$ , 9.8 Hz, 4'''-H). Based on these spectral data and the above-described alkaline degradation analysis, the structure of merremoside b (1) has been determined.

It has been known for a long time that Jalap root and pharbitis seeds (from convolvulaceous plants) contain resin-glycosides responsible for their drastic purgative action. However, their structural studies have been mainly concerned with their alkaline hydrolysates. Only very recently have the structures of several resin-glycosides from the root of *Ipomoea orizabensis* been fully characterized.<sup>9b)</sup> The present structure elucidation of merremosides b (1) and d (2) adds some more examples of chemically elucidated resin-glycosides. Finally, it should be mentioned here that merremosides b (1) and d (2) have been shown to exhibit antiserotonic activity [ED<sub>50</sub> (mice): 10  $\mu$ g/ml and 2  $\mu$ g/ml], respectively.<sup>11)</sup>

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- 4) We have been so far unsuccessful in the isolation of pure merremoside e. After the above oral presentation,<sup>3a,b)</sup> some more merremosides have been isolated in pure forms. Therefore, we have re-named the alphabetical suffix of merremoside (a from least polar one) as described in this paper.
- 5) The molecular composition of the compound given with the chemical formula was determined by elemental analysis or high resolution mass spectrometry.
- 6) Acidic hydrolysis of this rhamnoside (8 mg) afforded L-rhamnose (5 mg),  $[\alpha]_D^{25} +9^\circ$  (H<sub>2</sub>O).
- 7) a) Y. Asahina and J. Yaoi, *Yakugaku Zasshi*, 45, 786 (1925); b) L. A. Davies and R. Adams, *J. Am. Chem. Soc.*, 50, 1749 (1928); c) T. Kawasaki, *Yakugaku Zasshi*, 70, 485 (1950).
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- 9) a) Very recently, the 11R configuration of jalapinolic acid was reported also by application of Horeau's method.<sup>9b)</sup>; b) N. Noda, M. Ono, K. Miyahara, T. Kawasaki, and M. Okabe, *Tetrahedron*, 43, 3889 (1987).
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- 11) cf. promethazine: ED<sub>50</sub> (mice) 2  $\mu$ g/ml.

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