Communications to the Editor

[Chem. Pharm. Bull. 36(4)1618—1621(1988)]

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,[1] 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.[2] This paper deals with the evidence which is in good accord with the structures of merremosides b (1) and d (2).[3]

The MeOH extract of the fresh tuber (obtained at Yogyakarta, Java) was partitioned into a mixture of $CHCl_3$ and water. Silica gel column chromatography of the $CHCl_3$-soluble portion followed by HPLC (Zorbax ODS, MeOH-H_2O=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%)[4]— merremoside b (1), mp 129-130°C, $[\alpha]_D^{25}$ -90° (MeOH), $C_{48}H_{82}O_{20}\cdot H_2O$,[5] IR (KBr): 3350 (br), 2895, 1715 (br) cm^{-1}, and merremoside d (2), mp 138-139°C, $[\alpha]_D^{25}$ -77° (MeOH), $C_{48}H_{82}O_{20}\cdot H_2O$, IR (KBr): 3420 (br), 2932, 1718 (br) cm^{-1}.

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), $C_{40}H_{72}O_{19}\cdot 2H_2O$, IR (KBr): 3401 (br), 2928, 1710 (br) cm^{-1}, 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), $C_{41}H_{74}O_{19}\cdot 3H_2O$, IR (KBr): 3369 (br), 2918, 1732 cm^{-1}. Methanolysis (9% HCl-dry MeOH, reflux) of 6a liberated methyl L-rhamnoside[6] and methyl jalapinolate[7] [9, $[\alpha]_D^{24}$ +0.5° ($CHCl_3$)], the 11*R* configuration has been supported by application of Horeau's method.[8,9]

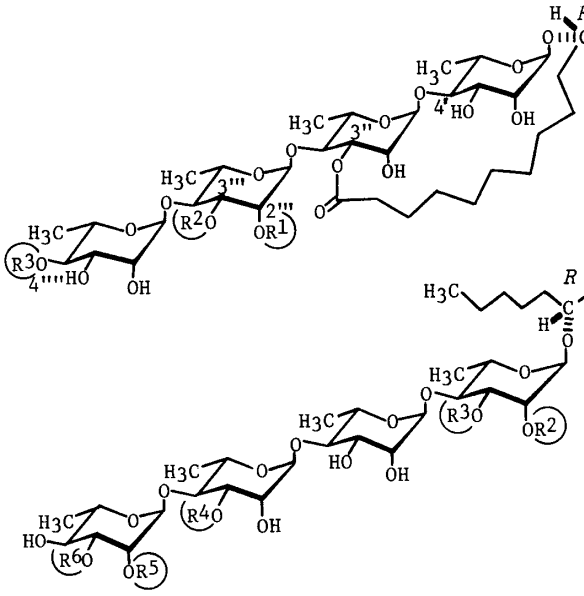
The SIMS of 6a showed ion peaks at *m/z* 893 $(M+Na)^+$ and 909 $(M+K)^+$ together

with fragment ions i, ii, iii, and iv derived from the sugar moiety. The ^{1}H NMR spectrum of 6a (500 MHz, d_5-pyridine+D_2O) showed signals due to one *prim.* CH_3 (δ 0.91, t, *J*=7 Hz), one CH_3O (δ 3.61, s), four *sec.* CH_3 (δ 1.50, 1.56, 1.57, 1.58, all d, *J*=6 Hz), and four anomeric H [δ 6.24, 6.25, 6.31 (2H), all s]. The ^{13}C NMR of 6a (125 MHz, d_5-pyr.+D_2O) showed four anomeric C signals [δc 101.0, 101.1, 102.5 (2C)] with ^{13}C-^1H coupling constants: 170.0, 171.0, 171.5, and 171.5 Hz. Thus, the structure of 6a has been shown to comprise four linear α-rhamnosyl moieties attached to the 11-OH of methyl jalapinolate (9). Complete methylation of 6a with CH_3I-DMSO-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 $(M+Na)^+$ and 1017 $(M+K)^+$, while the negative ion (neg.) FAB-MS gave an ion peak at *m/z* 977 $(M-H)^-$. These findings together with the elemental analysis have shown that 2 comprises two isobutyryl ester linkages, and the carboxyl group in the jalapinolic acid moiety is lactonized to a hydroxyl group in the sugar part. The ^{1}H NMR spectrum (500 MHz, CD_3OD+D_2O) of 2 showed signals due to one *prim.* CH_3, eight *sec.* CH_3, and three methine protons on carbons bearing one each of isobutyroyl group and a lactone linkage: δ 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.

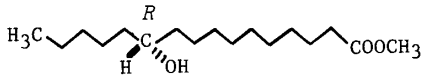
In order to confirm the location of lactone-linkage in merremoside d (2), the following derivatization was carried out. Acetonidation of 2 (2,2-dimethoxypropane, *d*-10-camphorsulfonic acid, DMF, 45°C) followed by alkaline treatment (3% NaOMe-MeOH, 15°C) furnished 7, mp 115-116°C, $[\alpha]_D^{20}$ -80° (MeOH), $C_{47}H_{82}O_{19}\cdot 2H_2O$, IR (KBr): 3400 (br), 2920, 1720 cm^{-1}. $NaIO_4$ oxidation of 7 (MeOH-H_2O, 15°C) and subsequent alkaline degradation of the product (5% NaOMe-MeOH, 15°C) provided 10, a white powder, $[\alpha]_D^{26}$ +11° ($CHCl_3$), $C_{26}H_{48}O_7$, IR ($CHCl_3$): 3590, 2931, 2863, 1726 cm^{-1}, which, on acetylation (Ac_2O-pyr.), gave the monoacetate (10a), a colorless oil, $[\alpha]_D^{26}$ +26° ($CHCl_3$), $C_{28}H_{50}O_8$, IR ($CHCl_3$): 2936, 2859, 1731 cm^{-1}. The ^{1}H NMR spectrum of 10a (90 MHz, $CDCl_3$) showed signals due to one *prim.* CH_3 (δ 0.88, t-like), one *sec.* CH_3 (δ 1.50, d, *J*=6 Hz), two *tert.* CH_3 (δ 1.26, 1.36, both s), one AcO (2.06, s), and 4'-H (δ 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, $[\alpha]_D^{25}$ -48° (MeOH), $C_{44}H_{76}O_{19}\cdot 2H_2O$, IR (KBr): 3380 (br), 2910, 1715 (br) cm^{-1}, neg. FAB-MS: *m/z* 907 $(M-H)^-$, 761 (viii'), 545 (ix), 417 (x+H), ^{1}H NMR (500 MHz, d_5-pyr.+D_2O) δ: 5.54 (dd, *J*=2.8, 10.1 Hz, 3''-H), 5.71 (br s, 2"'-H), and 4 (13%), mp 110-111°C,

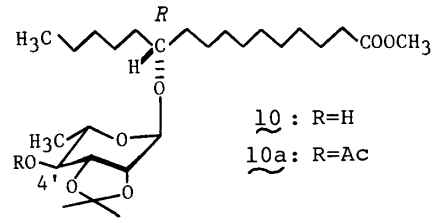


1 : $R^1=R^3=COCH(CH_3)_2$; $R^2=H$ (merremoside b)
2 : $R^1=H$; $R^2=R^3=COCH(CH_3)_2$ (merremoside d)
3 : $R^1=COCH(CH_3)_2$; $R^2=R^3=H$
4 : $R^1=R^3=H$; $R^2=COCH(CH_3)_2$
5 : $R^1=R^2=R^3=H$

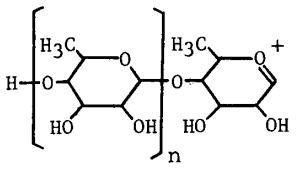
6 : $R^1=R^2=R^3=R^4=R^5=R^6=H$ (merremoside i)
6a : $R^1=CH_3$; $R^2=R^3=R^4=R^5=R^6=H$
7 : $R^1=CH_3$; $R^2,R^3=R^5,R^6$=isopropylidene $R^4=H$
8 : $R^1=CH_3$; $R^2=R^3=R^5=R^6=H$; $R^4=COCH(CH_3)_2$



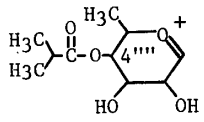
9 : (methyl jalapinolate)



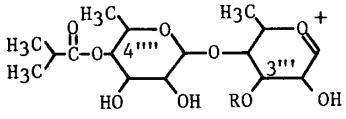
10 : R=H
10a : R=Ac



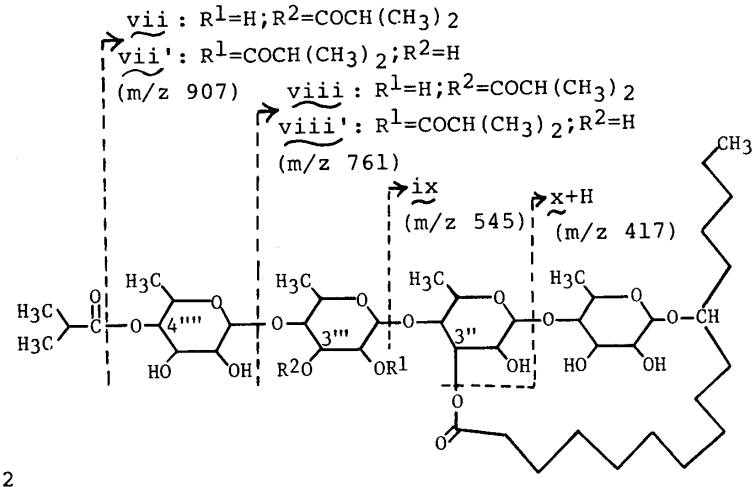
i (m/z 147) : n=0
ii (m/z 293) : n=1
iii (m/z 439) : n=2
iv (m/z 585) : n=3



v (m/z 217)



vi (m/z 433) : R=$COCH(CH_3)_2$



vii : $R^1=H$; $R^2=COCH(CH_3)_2$
vii' : $R^1=COCH(CH_3)_2$; $R^2=H$
(m/z 907)
viii : $R^1=H$; $R^2=COCH(CH_3)_2$
viii' : $R^1=COCH(CH_3)_2$; $R^2=H$
(m/z 761)
ix (m/z 545)
x+H (m/z 417)

$[\alpha]_D^{25}$ -45° (MeOH), $C_{44}H_{76}O_{19}\cdot 3H_2O$, IR (KBr): 3380 (br), 2905, 1717 (br) cm^{-1}, neg. FAB-MS: *m/z* 907 $(M-H)^-$, 761 (viii), 545, 417, ^1H NMR (500 MHz, d_5-pyr.+D_2O) δ: 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 1 with 4% NaOMe-MeOH (0°C, 30 min) provided 6a (39%), 5 (20%), mp 144-146°C, $[\alpha]_D^{25}$ -75° (MeOH), $C_{40}H_{70}O_{18}\cdot 3H_2O$, IR (KBr): 3360 (br), 2907, 1712 (br) cm^{-1}, neg. FAB-MS: *m/z* 837 $(M-H)^-$, 691, 545, ^1H NMR (500 MHz, d_5-pyr.+D_2O) δ: 5.55 (dd, *J*=2.8, 10.1 Hz, 3"-H), and 8 (5%), mp 103-104°C, $[\alpha]_D^{25}$ -74° (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 $(M-H)^-$, 869, 793, 577, ^1H NMR (500 MHz, CD_3OD+D_2O) δ: 3.63 (s, $COOCH_3$), 5.73 (dd, *J*=3.1, 10.1 Hz, 3'''-H). Examination in detail of neg. FAB-MS and ^1H 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 $(M-H)^-$ and fragment ions at *m/z* 907 (vii'), 761 (viii'), 545 (ix), and 417 (x+H), whereas the ^1H NMR spectrum (d_5-pyr.+D_2O) showed signals due to one *prim.* CH_3, eight *sec.* CH_3, and three methine protons on carbons bearing two isobutyryl residues and one lactone linkage (δ 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.[9b] 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_{80}(mice): 10 μg/ml and 2 μg/ml], respectively.[11]

ACKNOWLEDGEMENT The authors are grateful to the Ministry of Education, Science, and Culture of Japan for Grant-in-Aid for Overseas Scientific Survey (No. 60041044, 61043040). They are also indebted to Prof. T. Momose and Mr. Y. Ikenishi for measuring mass spectra.

REFERENCES AND NOTES

1) I. Kitagawa, "Research Report of Investigation of Naturally Occurring Drug Materials in Indonesia-1", Osaka, 1987.
2) I. Kitagawa, presented at the Japanese-United States Congress of Pharmaceutical Sciences, Symposium Abstracts p. 8 (Dec. 3, 1987, Honolulu, U.S.A.).
3) a) H. Shibuya, Y. Yokokawa, M. Yoshikawa, I. Kitagawa, A. Nitta, and H. Wiriadinata, presented at the 107th Annual Meeting of the Pharmaceutical Society of Japan, Abstract p. 319 (April 1987, Kyoto); b) I. Kitagawa, Y. Yokokawa, N. I. Baek, H. Shibuya, M. Yoshikawa, A. Nitta, and H. Wiriadinata, presented at the 37th Annual Meeting of the Kinki Branch of the Pharmaceutical Society of Japan, Abstract p. 36 (Nov. 1987, Kobe).
4) We have been so far unsuccessful in the isolation of pure merremoside e. After the above oral presentation,[3a,b] 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° (H_2O).
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).
8) a) A. Horeau, Tetrahedron Lett., 1961, 506, 965; b) Recovered 2-phenylbutyric acid, $[\alpha]_D^{25}$ +3° (*c*=4.5, benzene).
9) a) Very recently, the 11*R* configuration of jalapinolic acid was reported also by application of Horeau's method.[9b]; b) N. Noda, M. Ono, K. Miyahara, T. Kawasaki, and M. Okabe, Tetrahedron, 43, 3889 (1987).
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11) *cf.* promethazine: ED_{80}(mice) 2 μg/ml.

(Received February 16, 1988)