Two Diterpene Rhamnosides, Mimosasides B and C, from *Mimosa hostilis*

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Two new diterpene rhamnosides, mimosasides B and C (1, 2) were isolated together with mimosaside A (3), a known diterpene rhamnoside (4), four known flavones (5—8), five known flavanones (9—13), and four known chalcones (14—17) from the leaves and twigs of a Brazilian medicinal plant, *Mimosa hostilis*.

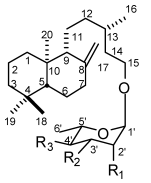
Key words diterpene rhamnoside; mimosaside A; mimosaside B; mimosaside C; *Mimosa hostilis*

As part of our ongoing studies on biologically active compounds in Brazilian medicinal plants,[1] we studied constituents of the leaves and twigs of *Mimosa hostilis* (MART.) BENTH (Leguminosae). *M. hostilis*, local name "Juréma", which is a tree found in the dry area of Brazil. Its bark is widely used to treat ulcer, bronchitis, carminative, and erysipelas.[2] In a previous paper,[3] we described two enantiomeric labdane diterpenes from this plants. Further studies have resulted in the isolation of two new labdane-type diterpene rhamnosides, named mimosasides B and C (**1**, **2**), along with the previously known diterpenoids (**3**, **4**) and flavonoids (**5**—**17**). In this paper, we report the structure elucidation of two new compounds **1** and **2**.

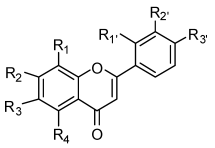
The dried leaves and twigs of *M. hostilis* were extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O, and then the water-soluble portion was partitioned between *n*-BuOH and water. The *n*-BuOH-soluble portion was chromatographed by a combination of silica gel, Sephadex LH-20, and reversed-phase ODS columns, to afford **1** and **2**, together with the previously reported diterpene rhamnosides (**3** named mimosaside A[3] and **4**[4]) as well as a number of flavonoids (**5**, **6**,[5] **7**, **8**,[6] **9**,[7] **10**,[8] **11**,[9] **12**, **13**,[10] **14**,[11] **15**, **16**,[12] **17**[13]).

The molecular formula, $C_{30}H_{50}O_7$, of mimosaside B (**1**) was established by HR-EI-MS (*m*/*z* 522.3572), and the IR spectrum implied the presence of hydroxyl groups (3468 cm^{-1}), carbonyl groups (1755 cm^{-1}), and an olefinic group (1644 cm^{-1}). The gross structure of **1** was deduced from detailed analysis of ^{1}H- and ^{13}C-NMR data (including DEPT) aided by 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, and HMBC). The ^{1}H-, ^{13}C-NMR and HMQC experiments of **1** indicated the presence of one exomethylene, three tertiary methyls, two secondary methyl, three methines, eight methylenes, one oxymethylene, three quaternary carbons, five oxymethine and two acetyl groups. As three of six unsaturations were thus accounted for, it was concluded that **1** consisted of three rings, one of which was a sugar ring. The aforementioned spectral data implied that **1** is a diterpene with a sugar moiety. The HMBC correlations combined with other 2D NMR data of **1** suggested that its aglycone moiety was 8(17)-labden-15-ol, identical to that of mimosaside A (**3**) (Fig. 1). The attached sugar moiety was established as α-L-rhamnopyranose with acid hydrolysis as well as NMR data (δ_H 4.68, d, J=2.0, H-1′, δ_C 97.5, C-1′).[3] The configulation of H′-1 was assigned as α-orientation, which was deduced from the coupling constant of J=2.0 Hz. In HMBC, the H-2′ and H-3′ signals at δ 5.20 and 5.13 in the rhamnose moiety correlated to carbonyls at δ 171.0 and 169.8 in two acetyl groups, respectively. Additionally, HMBC correlation between H_2-15 and C-1′ (anomeric carbon) indicated that two acetyl groups were attached to C-2′ and C-3′, and these acetyl groups bearing rhamnoside moiety were connected *via* an ether to the C-15 position in a labdane diterpene. NOESY cross-peaks such as H_3-18/H-5, H-5/H-9, H_3-19/H_3-20, and H_3-20/H-11$_\beta$ indicated that the agylcone adopts a chair/chair conformation with an A/B *trans* ring junction like a *ent*-labdane diterpene. Further NOESY correlation between H-1′ and H_2-15 confirmed that the 2′,3′-*O*-diacetyl rhamnoside moiety connected to C-15. Thus, mimosaside B (**1**) was elucidated to be 8(17)-labden-15-ol-2′,3′-*O*-diacetyl-α-L-rhamnoside.

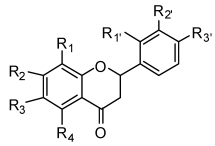
The molecular formula of mimosaside C (**2**) was deter-



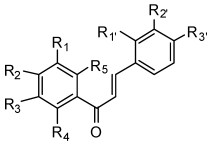
1 R_1=OAc, R_2=OAc, R_3=OH
2 R_1=OAc, R_2=OH, R_3=OH
3 R_1=OH, R_2=OH, R_3=OH
4 R_1=OH, R_2=OAc, R_3=OH
1a R_1=OAc, R_2=OAc, R_3=OAc



5 R_1=R_3=$R_{1'}$=$R_{2'}$=H, R_2=R_4=$R_{3'}$=OH (apigenin)
6 R_2=R_3=$R_{1'}$=$R_{2'}$=H, R_1=R_4=$R_{3'}$=OH
7 R_1=R_3=$R_{1'}$=$R_{2'}$=H, R_4=$R_{3'}$=OH, R_2=OMe (genkwanin)
8 R_3=$R_{1'}$=$R_{2'}$=H, R_2=R_4=$R_{3'}$=OH, R_1=OMe



9 R_1=R_3=$R_{1'}$=$R_{2'}$=H, R_2=R_4=OH, $R_{3'}$=OMe
10 R_1=R_2=$R_{1'}$=$R_{2'}$=H, R_3=OH, R_4=$R_{3'}$=OMe
11 R_1=R_3=$R_{1'}$=$R_{2'}$=H, R_4=$R_{3'}$=OH, R_2=OMe (sakuranetin)
12 R_1=R_3=$R_{1'}$=$R_{2'}$=H, R_2=R_4=$R_{3'}$=OH (naringenin)
13 R_1=R_3=$R_{2'}$=H, R_4=$R_{1'}$=$R_{3'}$=OH, R_2=OMe (artocarpanone)



14 R_1=R_3=$R_{1'}$=$R_{2'}$=H, R_4=R_5=OH, R_2=$R_{3'}$=OMe
15 R_1=R_3=R_5=$R_{1'}$=$R_{2'}$=H, R_2=R_4=$R_{3'}$=OH (isoliquiritigenin)
16 R_1=R_3=R_5=$R_{1'}$=$R_{2'}$=H, R_2=R_4=OH, $R_{3'}$=OMe
17 R_2=R_3=R_5=$R_{1'}$=$R_{2'}$=H, R_1=R_4=$R_{3'}$=OH

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Table 1. ^{13}C-NMR Data (150 MHz, $CDCl_3$) of Compounds **1**—**3**

	1	**2**	**3**
1	39.2	39.2	39.2
2	19.5	19.6	19.5
3	42.3	42.3	42.3
4	33.7	33.6	33.7
5	55.6	55.6	55.6
6	24.6	24.5	24.6
7	38.5	38.4	38.5
8	148.7	148.9	148.9
9	57.3	57.2	57.3
10	39.8	39.7	39.8
11	21.0	21.0	21.0
12	36.1	36.5	36.1
13	30.4	30.3	30.5
14	36.7	36.8	36.8
15	66.4	66.2	66.2
16	19.7	19.6	19.7
17	106.1	106.2	106.2
18	33.7	33.6	33.7
19	21.9	21.9	21.8
20	14.6	14.5	14.6
1′	97.5	97.6	99.7
2′	70.3	72.7	71.2
3′	72.4	70.5	71.9
4′	71.5	73.5	73.5
5′	68.5	67.8	67.8
6′	17.7	17.6	17.6
C-2′ $CH_3\underline{CO}$	171.0	171.0	
$\underline{CH_2}CO$	21.2	21.2	
C-3′ $CH_3\underline{CO}$	169.8		
$\underline{CH_3}CO$	21.0		

mined as $C_{28}H_{48}O_6$ by HR-EI-MS (*m/z* 480.3458) and the IR spectrum indicated the presence of hydroxyl (3424 cm^{-1}), carbonyl (1746 cm^{-1}), and olefin groups (1644 cm^{-1}). The ^{1}H- and ^{13}C-NMR data of **2** (Table 1) were similar to those of minosaside B (**1**) except for the lack of one acetyl group in a rhamnoside moiety. The attached position of the acetyl group was clarified to be at the C-2′ position in the rhamnose unit by HMBC correlation between H-2′ and acetyl carbonyl resonance at δ 171.0. Thus, **2** was assigned as 8(17)-labden-15-ol-2′-*O*-acetyl-α-L-rhamnoside. The acetylation of minosasides A—C (**1**—**3**) with Ac_2O and pyridine yielded the same tri-acetate (**5**), which were identified by ^{1}H-NMR and EI-MS spectra (*m/z* M^+ 564).

Experimental

General Experimental Procedures ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian Unity 200, 400 and 600 using tetramethylsilane as the internal standard. HR-EI-MS spectra were obtained on a JEOL AX-500 spectrometer. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. UV spectra were recorded on a Shimadzu UV-300 or Hitachi-U-3000 spectrophtometer. IR spectra were recorded on a JASCO FT-IR 5300 or FT-IR 410 infrared spectrophotomer.

Silica gel (Merck, 70—230, 230—400, 230—400 mesh, and Wacogel C-300) was used for column chromatography. Sephadex LH-20 was used for gel filtration chromatography. Precoated silica gel 60F_{254} and RP-8F_{254} plates were used for analytical or preparative thin-layer chromatography, and spots were visualized by UV (254 nm) light and 2% $CeSO_4$ in H_2SO_4 after heating.

Plant Material The leaves and twigs of *Mimosa hostilis* were purchased in Sao Paulo, Brazil. The plant was identified by Professor Motoyoshi Satake (Ocyanomizu University) and a voucher specimen with the identification number B149 has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Isolation The methanol extract (126.3 g) of the air-dried leaves and twigs of *M. hostilis* (1 kg) was partitioned between EtOAc and H_2O. The H_2O layer was further partitioned between *n*-BuOH and water. The *n*-BuOH-soluble materials (36.4 g) were separated on a silica gel column (Wako-gel, C-300; $CHCl_3$: MeOH 7 : 3→3 : 7) and LH-20 (MeOH) and a silica gel column again (Hexane: EtOAc 8 : 2→EtOAc) to give three fractions (A—C). Fraction B was subjected to MPLC (Lobar, Merck; MeOH : H_2O, 9 : 1) and finally separated on silica gel column (Hexane : EtOAc, 2 : 1) to obtain minosaside B (**1**) (10.1 mg). Fraction A was separated by silica gel column ($CHCl_3$: MeOH, 9 : 1, and then $CHCl_3$: AcOEt, 2 : 1), and purified using prep. TLC ($CHCl_3$: MeOH 19 : 1) to give mimosaside C (**2**) (2.3 mg), minosaside A (**3**) (7.2 mg), and **4** (10.1 mg).

The known flavonoids, **5**—**17** were isolated as follows: ethyl acetate extracts of *M. hostilis* (65 g) were subjected to silica gel column (CH_2Cl_2–EtOAc→CH_2Cl_2–MeOH) and subsequently separated using LH-20 (MeOH) and RP-8 (Lobar, MeOH : H_2O, 3 : 1) to give **5** (22 mg), **6** (2 mg), **10** (3 mg) **11** (10 mg), and **14** (2 mg). The *n*-BuOH extracts (36 g) of *M. hostilis* were also subjected to silica gel column ($CHCl_3$: MeOH, 7 : 3→7 : 3) and then separated using LH-20 (MeOH) to give **8** (24 mg), **9** (1.9 mg), **11** (15 mg), **12** (1 mg), **13** (5 mg), and **16** (10 mg) and **17** (3 mg).

Mimosaside B (**1**): Colorless amorphous solid; $[\alpha]_D^{22.6}$ −44.0° (*c*=1.02, $CHCl_3$); IR (FT film) ν_{max} 3468 (OH), 1755 (C=O), 1644 (C=CH_2) cm^{-1}. EI-MS *m/z* (rel. int.): 522 $[M]^+$ (11), 171 (100), 232 (71), 191 (24). HR-EI-MS: Found 522.3572, Calcd 522.3556 for $C_{30}H_{50}O_7$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.68 (3H, s, H_3-19), 0.80 (3H, s, H_3-20), 0.87 (3H, s, H_3-18), 0.90 (3H, d, *J*=6.3 Hz, H_3-16), 1.35 (3H, d, *J*=6.3 Hz, H_3-6′), 2.08 (3H, s, C-3′-OAc), 2.13 (3H, s, C-2′-OAc), 3.41 (1H, m, H-15), 3.60 (1H, dd, *J*=9.5, 9.8 Hz, H-4′) 3.72 (2H, m, H-15, 5′), 4.49 (1H, s, H-17a), 4.68 (1H, d, *J*=2.0 Hz, H-1′), 4.81 (1H, s, H-17b), 5.13 (1H, dd, *J*=3.4, 10.3 Hz, H-3′), 5.20 (1H, dd, *J*=2.0, 3.4 Hz, H-2′).

Mimosaside C (**2**): Colorless amorphous solid; $[\alpha]_D^{21.5}$ −22.0° (*c*=0.05, $CHCl_3$); IR (FT film) ν_{max} 3424 (OH), 1746 (C=O), 1644 (C=CH_2) cm^{-1}, EI-MS *m/z* (rel. int): 480 $[M]^+$ (4.3), 189 (100), 171 (72.4), 291 (26.8). HR-EI-MS: Found 480.3458; Calcd 480.3451 for $C_{28}H_{48}O_6$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 0.68 (3H, s, H_3-19), 0.80 (3H, s, H_3-20), 0.87 (3H, s, H_3-18), 0.90 (3H, d, *J*=6.3 Hz, H_3-16), 1.33 (3H, d, *J*=6.3 Hz, H_3-6′), 2.14 (3H, s, C-2′-OAc), 3.41 (1H, m, H-5′), 3.47 (1H, dd, *J*=9.6, 7.7 Hz, H-4′), 3.70 (2H, m, H_2-15), 3.96 (1H, dd, *J*=3.6, 9.6 Hz, H-3′), 4.49 (1H, br s, H-17a), 4.73 (1H, d, *J*=1.6 Hz, H-1′), 4.81 (1H, br s, H-17b), 5.07 (1H, dd, *J*=1.6, 3.6 Hz, H-2′).

Acetylation of Mimosasides A—C (1—3) Mimosasides A—C (**1**—**3**); 2.0, 2.2 and 2.5 mg, were treated with Ac_2O and pyridine and then evaporated to give the residues. These materials were subjected to silica gel column (sol. $CHCl_3$) to obtain triacetate derivatives (**1a**; 1.8, 2.0, 2.0 mg), respectively.

Triacetate Derivative (**1a**): Colorless amorphous solid; $[\alpha]_D^{21}$ −47.0° (*c*= 0.85, $CHCl_3$). EI-MS *m/z* 564 $[M]^+$ (13), 273 (100), 153 (46), 111 (24). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.68 (3H, s), 0.80 (3H, s), 0.87 (3H, s), 0.90 (3H, d, *J*=3.3 Hz), 1.22 (3H, d, *J*=5.9 Hz), 1.98 (3H, s), 2.04 (3H, s), 2.15 (3H, s).

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