Antidiabetic Principles of Natural Medicines. II.[1] Aldose Reductase and α-Glucosidase Inhibitors from Brazilian Natural Medicine, the Leaves of *Myrcia multiflora* DC. (Myrtaceae): Structures of Myrciacitrins I and II and Myrciaphenones A and B

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The methanolic extract and ethyl acetate-soluble portion from a Brazilian natural medicine, the leaves of *Myrcia multiflora* DC., which has been used as a specific medicine against diabetes, were found to show inhibitory activities on aldose reductase and α-glucosidase and on the increase of serum glucose level in sucrose-loaded rats and in alloxan-induced diabetic mice. From the ethyl acetate-soluble portion, new flavanone glucosides, myrciacitrins I and II, and new acetophenone glucosides, myrciaphenones A and B, were isolated together with several known compounds such as five flavonol glycosides, myricitrin, mearnsitrin, quercitrin, desmanthin-1, and guaijaverin. The structures of new compounds were determined on the basis of physicochemical and chemical evidence. The principal components of this natural medicine including new glucosides, myrciacitrin I and myrciaphenone B, were found to show potent inhibitory activities on aldose reductase and α-glucosidase.

Key words *Myrcia multiflora*; myrciacitrin; myrciaphenone; aldose reductase inhibitor; α-glucosidase inhibitor; flavanone glucoside

Myrcia multiflora (LAM.) DC. (Syn.: *M. sphaerocarpa*, *Eugenia multiflora*), which is a medium height tree of 4 to 10 m belonging to the family Myrtaceae, is widely distributed in Brazil, Guiana, Peru, and Paraguay. Since the leaves and bark of *M. multiflora* have been extensively used as a specific remedy for diabetes, this natural medicine goes by the popular name "Plant Insulin," but its pharmacological property and active component are left uncharacterized.[2]

In the course of our studies on bioactive principles of natural medicines and medicinal foodstuffs,[3] many triterpene oligoglycosides with inhibitory activity on the increase of serum glucose levels in glucose-loaded rats were isolated from *Aralia elata* (roots, bark, young shoots),[1,4] *Aesculus hippocastanum* (seeds),[5] *Polygala senega* var. *latifolia* (roots),[6] *Beta vulgaris* (roots),[7] *Gymnema sylvestre* (leaves),[8] and *Kochia scoparia* (fruit).[9] In addition, we have reported the structure requirement of saponins for their inhibitory activity and the action mechanisms of this activity.[10] As a continuing part of our screening for antidiabetic principles of natural medicine, we have found that the methanolic extract and its fractions from the leaves of *M. multiflora* exhibited inhibitory activity on the increase of serum glucose levels in sucrose-loaded rats. This extract and fractions were also found to show inhibitory activities on aldose reductase and α-glucosidase. From the ethyl acetate-soluble fraction, new flavanone glucosides called myrciacitrins I (**1**) and II (**2**) and new acetophenone glucosides called myrciaphenones A (**5**) and B (**6**) were isolated together with several known compounds such as five flavonol glycosides, myricitrin (**7**),[11] mearnsitrin (**8**),[12] quercitrin (**9**),[13] desmanthin-1 (**10**),[14] and guaijaverin (**11**),[15] and ginkgoic acid (**12**).[16] This paper deals with the structure elucidation of myrciacitrins (**1**, **2**) and myrciaphenones (**5**, **6**). We also describe the antidiabetic activity of the methanolic extract, its fractions, and the principal components.

The dried leaves of *M. multiflora*, imported from Sáo Paulo, Brazil, were extracted with hot methanol. The methanolic extract was found to exhibit inhibitory activity on the increase of serum glucose levels in sucrose-loaded rats after a single oral administration of a 250 mg/kg or 500 mg/kg dose, and it also inhibited increase in serum glucose levels in alloxan-induced diabetic mice as shown in Tables 1 and 2. The extract also showed aldose reductase and α-glucosidase inhibitory activity (Tables 3, 4). The methanolic extract was partitioned into an ethyl acetate–water mixture. The ethyl acetate-soluble and the water-soluble portions inhibited the increase of serum glucose level in glucose-loaded rats and in alloxan-induced diabetic mice and also showed this activity on aldose reductase and α-glucosidase. The ethyl acetate-soluble portion in particular, showed extremely potent inhibitory activity on aldose reductase. The ethyl acetate-soluble portion was subjected to normal-phase and reversed-phase silica gel column chromatography and finally HPLC to give myrciacitrins I (**1**, 0.065%) and II (**2**, 0.0012%), myrciaphenones A (**5**, 0.0005%) and B (**6**, 0.0041%) together with myricitrin (**7**, 0.0097%), mearnsitrin (**8**, 0.0044%), quercitrin (**9**, 0.016%), desmanthin-1 (**10**, 0.0017%), guaijaverin (**11**, 0.0013%), ginkgoic acid (**12**, 0.0009%), β-amyrin[17] (0.032%), (+)-catechin (0.0006%), and gallic acid (0.036%).

Structures of Myrciacitrins I (1) and II (2) Myrciacitrin I (**1**) was isolated as a yellow powder of negative optical rotation ($[\alpha]_D^{27}$ −51.0°). The positive-ion FAB-MS of **1** showed quasimolecular ion peaks at *m/z* 479 $(M+H)^+$ and 501 $(M+Na)^+$, and high-resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$ revealed the molecular formula of **1** to be $C_{23}H_{26}O_{11}$. The IR spectrum of **1** showed absorption bands at 3375, 1655, and 1630 cm^{-1} assignable to hydroxyl, chelated carbonyl, and

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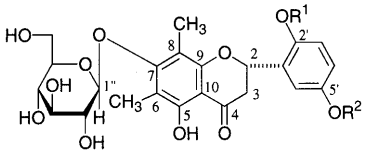
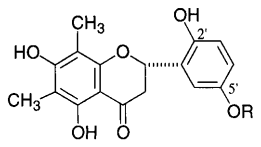
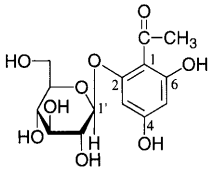


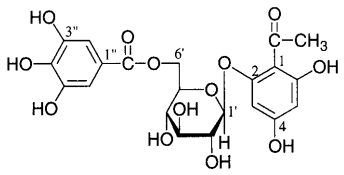
myrciacitrin I (**1**) : $R^1=R^2=H$
1a : $R^1=CH_3$, $R^2=H$
1b : $R^1=R^2=CH_3$
myrciacitrin II (**2**) : $R^1=H$, $R^2=CH_3$



myrciacetin (**3**) : R=H
methoxymatteucin (**4**) : $R=CH_3$



myrciaphenone A (**5**)



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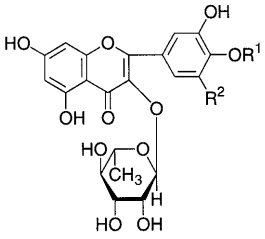
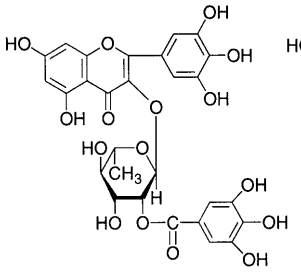
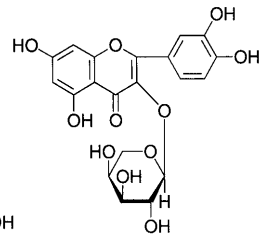


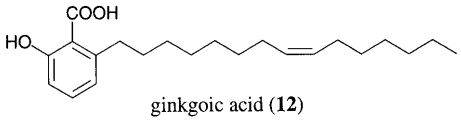
myricitrin (**7**) : $R^1=H$, $R^2=OH$
mearnsitrin (**8**) : $R^1=CH_3$, $R^2=OH$
quercitrin (**9**) : $R^1=R^2=H$



desmanthin-1 (**10**)



guaijaverin (**11**)



ginkgoic acid (**12**)

Chart 1

Table 1. Inhibitory Effect of MeOH Extract, AcOEt and H_2O Fractions from *M. multiflora* on Serum Glucose Levels in Sucrose-Loaded Rats

	Dose (mg/kg, *p.o.*)	*n*	Serum glucose concentration (mg/dl)
Control (normal)	—	5	91.1±3.9**
Control (sucrose-loaded)	—	6	173.9±3.0
MeOH extract	250	5	148.1±6.8*
	500	5	137.8±8.8**
Control (normal)	—	5	64.9±1.1**
Control (sucrose-loaded)	—	5	158.1±9.5
AcOEt fraction	250	5	124.5±9.5*
	500	5	116.8±5.0**
H_2O fraction	250	5	126.3±5.0*
	500	5	104.9±3.1**

Male Wistar rats weighing 130—170 g were fasted for 20—24 h and the test samples were given orally. Thirty minutes thereafter, sucrose (1 g/kg) was administered orally. Blood samples were collected 30 min after the administration of the test samples.

Asterisks denote significant differences from the controls at $*p<0.05$, $**p<0.01$.

Table 2. Effect of MeOH Extract, AcOEt and H_2O Fractions from *M. multiflora* on Serum Glucose Levels in Alloxan-Induced Diabetic Mice

	Dose (mg/kg)	*n*	Serum glucose concentration (mg/dl)		
			0 h	1 h	3 h
Control	—	10	628.0±32.6	789.5±44.9	682.0±25.4
MeOH extract	1000	8	616.0±96.3	695.9±57.1	599.8±43.7
AcOEt fraction	1000	8	677.0±21.7	607.7±27.5*	569.2±42.9
H_2O fraction	1000	8	663.5±38.4	636.8±40.6*	578.3±39.3

Each value represents the mean±S.E. Significantly different from the control group, $*p<0.05$.

aromatic ring, while absorption maxima characteristic of the flavanone structure were observed at 212 (log ε 4.3), 285 (log ε 4.1), and 358 (log ε 3.6) nm in its UV spectrum. Acid hydrolysis of **1** with 5% sulfuric acid furnished D-glucose, which was identified by GLC analysis of the thiazolidine derivative.[18] Enzymatic hydrolysis of **1** with β-glucosidase liberated a new flavanone myrciacetin (**3**). The ^{1}H-NMR (DMSO-d_6) spectrum of **3**, which was assigned by various NMR analytical methods,[19] indicated

Table 3. Inhibitory Activity of MeOH Extract, AcOEt and H_2O Fractions, and Chemical Constituents (**1**, **2**, **6**—**11**) from *M. multiflora* and Myrciacetin (**3**) for Rat Lens Aldose Reductase

	IC_{50}
MeOH extract	1.08 μg/ml
AcOEt fraction	0.18 μg/ml
H_2O fraction	26.2 μg/ml
Myrciacitrin I (**1**)	3.2×10^{-6} M
Myrciacitrin II (**2**)	1.5×10^{-5} M
Myrciacetin (**3**)	1.3×10^{-5} M
Myrciaphenone B (**6**)	2.9×10^{-5} M
Myricitrin (**7**)	3.8×10^{-6} M
Mearnsitrin (**8**)	1.4×10^{-6} M
Quercitrin (**9**)	1.5×10^{-7} M
Desmanthin-1 (**10**)	8.2×10^{-8} M
Guaijaverin (**11**)	1.8×10^{-7} M
Epalrestat	7.2×10^{-8} M

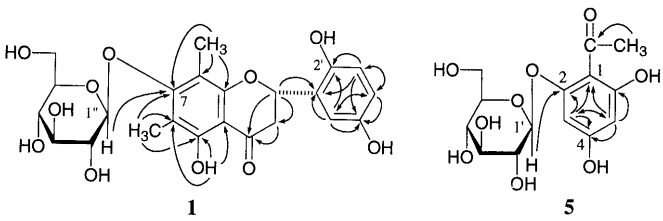
Table 4. Inhibitory Activity of MeOH Extract, AcOEt and H_2O Fractions, and Chemical Constituents (**1**, **6**, **7**, **10**, **11**) from *M. multiflora* for Rat Small Intestinal α-Glucosidase

	IC_{50}	
	Maltase	Sucrase
MeOH extract	225 μg/ml	128 μg/ml
AcOEt fraction	295 μg/ml	224 μg/ml
H_2O fraction	210 μg/ml	92 μg/ml
Myrciacitrin I (**1**)	6.0×10^{-4} M	7.0×10^{-4} M
Myrciaphenone B (**6**)	4.4×10^{-4} M	3.1×10^{-4} M
Myricitrin (**7**)	4.2×10^{-4} M	4.9×10^{-4} M
Desmanthin-1 (**10**)	2.4×10^{-4} M	2.6×10^{-4} M
Guaijaverin (**11**)	2.9×10^{-4} M	1.0×10^{-4} M
Acarbose	2.0×10^{-6} M	1.6×10^{-6} M

Table 5. ^{13}C-NMR of Myrciacitrins I (**1**), II (**2**), Myrciacetin (**3**), Myrciaphenones A (**5**), and B (**6**)

	1[a]	**2**[a]	**3**[a]		**5**[b]	**6**[b]
C-2	73.8	73.7	73.8	C-1	106.8	107.1
3	41.4	41.2	41.2	2	162.7	162.6
4	198.5	198.4	196.7	3	95.4	95.8
5	157.8	157.8	158.4	4	166.3	166.2
6	111.0	111.1	103.2	5	98.2	98.5
7	161.3	161.3	162.5	6	167.8	167.6
8	110.0	110.0	102.5	7	204.9	204.9
9	157.4	157.4	157.5	8	33.5	33.5
10	104.6	104.6	101.5	Glc-1′	102.1	102.3
1′	125.6	125.6	125.9	2′	74.8	74.8
2′	146.2	147.8	146.2	3′	78.4*	78.3
3′	116.1	116.0	116.0	4′	71.2	71.2
4′	115.5	114.1	115.3	5′	78.6*	75.8
5′	149.9	152.1	149.9	6′	62.5	64.3
6′	112.8	112.2	112.7	Galloyl-1″		121.3
6-CH_3	8.6*	8.6*	8.2*	2″		110.3
8-CH_3	9.2*	9.1*	7.5*	3″		146.5
2′-OCH_3		55.3		4″		139.9
Glc-1″	104.1	104.1		5″		146.5
2″	74.0	74.0		6″		110.3
3″	76.9	76.9		7″		168.4
4″	69.7	69.7				
5″	76.2	76.2				
6″	61.0	60.9				

* interchangeable in the same column. *a*) measured in DMSO-d_6. *b*) measured in CD_3OD.



1 **5**

Fig. 1. HMBC Correlations of **1** and **5**

the presence of two aromatic methyl groups [δ 1.97, 1.99 (s, 6,8-CH_3)], a chelated hydroxyl group [δ 12.36 (br s, 5-OH)], a dihydropyrone moiety in flavanone structure by a characteristic ABX type coupling pattern [δ 2.78 (dd, J=3.0, 17.1 Hz), 3.01 (dd, J=12.5, 17.1 Hz) (3-H_2), 5.57 (dd, J=3.0, 12.5 Hz, 2-H)], and a trisubstituted benzene ring [δ 6.59 (dd, J=3.0, 8.5 Hz, 4′-H), 6.69 (d, J=8.5 Hz, 3′-H), 6.89 (d, J=3.0 Hz, 6′-H)]. The carbon signals of the benzopyrone moiety (C-2—10) in the ^{13}C-NMR (Table 5) spectrum[19] of **3** were found to be very similar to those of 5,7-dihydroxy-6,8-dimethylflavanone.[20] The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 5) spectra[19] of **1** showed signals assignable to the myrciacetin moiety, [δ 2.10 (s, 6, 8-CH_3), 2.82 (dd, J=2.8, 17.2 Hz), 3.09 (dd, J=12.9, 17.2 Hz) (3-H_2), 5.64 (dd, J=2.8, 12.9 Hz, 2-H), 6.60 (dd, J=2.6, 8.7 Hz, 4′-H), 6.70 (d, J=8.7 Hz, 3′-H), 6.91 (d, J=2.6 Hz, 6′-H), 12.11 (br s, 5-OH)] and a β-D-glucopyranosyl moiety [δ 4.61 (d, J=7.3 Hz, 1″-H)]. In the heteronuclear multiple bond connectivity (HMBC) experiment of **1**, long-range correlations were observed between the following protons and carbons: 2-H and 3,4,1′-C; 5-OH and 5,6,10-C; 6,8-CH_3 and 5,6,7,8,9-C; 3′-H and 1′,2′,4′,5′-C; 4′-H and 2′,3′,5′,6′-C; 6′-H and 1′,2′,4′,5′-C; 1″-H and 7-C (Fig. 1). Diazomethane methylation of **1** in methanol furnished two monomethyl ethers, myrciacitrin II (**2**) and **1a**, and a dimethyl ether **1b**. The position of two hydroxy groups of the ring B part was characterized by difference nuclear Overhauser effect of the methylated product **1a**, **1b**, and **2**; namely, it showed NOE correlations between the following protons (**1a**: 2′-OCH_3 and 3′-H, 3′-H and 4′-H; **1b**: 2′-OCH_3 and 3′-H, 3′-H and 4′-H, 4′-H and 5′-OCH_3, 5′-OCH_3 and 6′-H; **2**: 3′-H and 4′-H, 4′-H and 5′-OCH_3, 5′-OCH_3 and 6′-H). Finally, the CD spectra of **1** and **3** showed negative Cotton effects (**1**: $[\theta]_{281}$ −28730; **3**: $[\theta]_{281}$ −19360), which indicated the absolute configuration of the 2-position to be *S* orientation.[21] On the basis of the above evidence, the structure of myrciacitrin I was determined to be 6,8-dimethyl-5,7,2′,5′-tetrahydroxyflavanone 7-*O*-β-D-glucopyranoside (**1**).

Myrciacitrin II (**2**) was also obtained as a yellow powder and its IR, UV, and CD spectra were very similar to those of **1**. In the positive-ion FAB-MS of **2**, a quasimolecular ion peak was observed at m/z 515 $(M+Na)^+$ and 493 $(M+H)^+$, while its negative-ion FAB-MS showed a quasimolecular ion peak at m/z 491 $(M-H)^-$. On enzymatic hydrolysis of **2** with β-glucosidase, methoxymatteucin (**4**)[20b,22] was obtained as the aglycone. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 5) spectra[19] of **2** showed signals due to the flavanone part, [δ 2.09 (s,

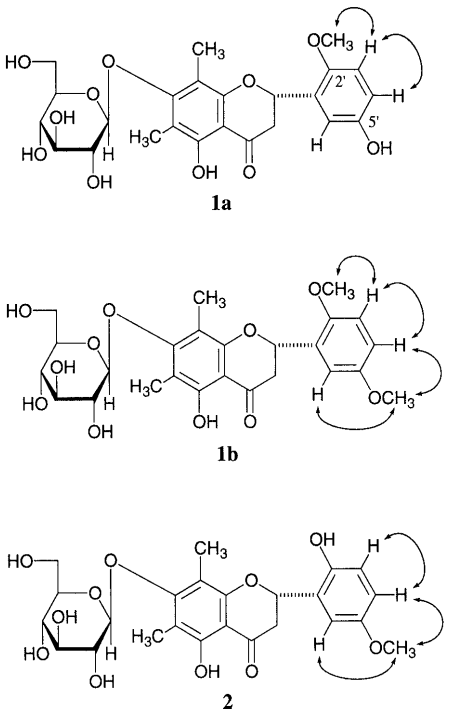


Fig. 2. NOE Correlations of **1a**, **1b**, and **2**

8-CH_3), 2.10 (s, 6-CH_3), 6.80 (dd, J=2.7, 8.9 Hz, 4′-H), 6.82 (d, J=8.9 Hz, 3′-H), 7.04 (d, J=2.7 Hz, 6′-H), 12.07 (br s, 5-OH)], a β-D-glucopyranosyl [δ 4.61 (d, J=7.6 Hz, 1″-H)] and a methoxyl moiety [δ 3.70 (s, 5′-OCH_3)]. The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR (Table 5) data of **2** were superimposable on those of **1**, expect for the signals due to the B-ring carbon of flavanone. Furthermore, the HMBC experiment of **2** showed long-range correlations between the 1″-proton and the 7-carbon and between the 5′-methoxyl protons and the 5′-carbon. Consequently, the structure of myrciacitrin II was determined as 6,8-dimethyl-5′-methoxyl-5,7,2′-trihydroxyflavanone 7-*O*-β-D-glucopyranoside (**2**).

Structures of Myrciaphenones A (5) and B (6) Myrciaphenone A (**5**) was isolated as a white powder and its IR spectrum showed absorption bands at 3410, 1630, 1605, and 1076 cm^{-1}, suggestive of glycosidic, carbonyl, and aromatic functions. The molecular formula $C_{14}H_{18}O_9$ was determined from the positive- and negative-ion FAB-MS and by high-resolution FAB-MS measurement. Thus, in the positive-ion FAB-MS of **5**, a quasimolecular ion peak was observed at m/z 353 $(M+Na)^+$, while the negative-ion FAB-MS showed the quasimolecular ion peak at m/z 329 $(M-H)^-$. The ^{1}H-NMR (CD_3OD) spectrum[19] of **5** showed signals assignable to an aromatic proton [δ 5.94 (d, J=2.3 Hz, 5-H), 6.18 (d, J=2.3 Hz, 3-H)], a methyl proton bearing carbonyl group [δ 2.69 (s, 8-H_3)], and an anomeric proton [δ 5.02 (d, J=7.6 Hz, 1′-H)]. The carbon signals of the aglycone part in the ^{13}C-NMR (Table 5) spectrum[19] of **5** were superimposable on those of acetophenone derivative,[23] except for those due to glycosylation shift at the 2-position. In the HMBC experiment of **5**, long-range correlations were observed between the following protons and carbons: 1′-H and 2-C; 3-H and 1,2,4,5-C; 5-H and 1,3,4,6-C; 8-H_3 and 7-C (Fig. 1). Consequently, myrciaphenone A was determined to be 2-*O*-β-D-glucopyranosyl-2,4,6-trihydroxyacetophenone (**5**).

Myrciaphenone B (**6**), obtained as a white powder, gave the quasimolecular ion peaks at m/z 505 $(M+Na)^+$, 483 $(M+H)^+$, and 481 $(M-H)^-$ in the positive- and negative-ion FAB-MS and the molecular composition was defined as $C_{21}H_{22}O_{13}$ from the high-resolution MS analysis. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 5) spectra[19] of **6** showed signals due to a galloyl group [δ 7.09 (s, 2″,6″-H)] and the glucosylacetophenone (**5**) moiety. Comparison of the ^{1}H-NMR and ^{13}C-NMR data for **6** with those for **5** disclosed an acylation shift at the 6′-position. Furthermore, the position of galloyl group in **6** was determined by the HMBC experiment of **6**, which showed long-range correlation between 6′-proton and 7″-carbon. On the basis of this evidence myrciaphenone B was characterized as 2-*O*-β-D-glucopyranosyl-6′-galloylacetophenone.

Inhibitory Activities of Principal Constituents for Aldose Reductase and α-Glucosidase As is apparent from Table 3, all principal constituents (**1**, **2**, **6**—**11**) from the leaves of *M. multiflora* and flavanone (**3**), the aglycone of the major constituent **1**, were found to show potent inhibitory activity for rat lens aldose reductase. Among them, desmanthin-1 (**10**) showed the most potent activity, which was equivalent to that of a commercial synthetic aldose reductase inhibitor, epalrestat.[24] **1**, **6**, **7**, **10**, and **11** showed inhibitory activity for rat small intestinal α-glucosidase as shown in Table 4, but this activity was weaker than that of the commercial α-glucosidase inhibitor, acarbose.[25] Taking their isolation yields into account, the flavonoid glycosides including the new flavanone glucosides myrciacitrins I (**1**) and II (**2**), and the new acetophenone glucoside myrciaphenone B (**6**) may be the beneficial constituents of the antidiabetic Brazilian natural medicine, the leaves of *M. multiflora*.

Experimental

The following instruments were used to obtain physical data : specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; CD spectra; J-720WI spectropolarimeter; IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer and JMS-GCMATE; HPLC, Shimadzu LC-10AS chromatograph.

The following experimental conditions were used for chromatography: normal-phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh), reversed-phase column chromatography; Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh): TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 60F_{254} (Merck, 0.25 mm) (reversed-phase) ; HPTLC, pre-coated TLC plates with Silica gel RP-18 60WF_{254s} (Merck, 0.25 mm) (reversed-phase). Detection was done by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Extraction and Isolation Dried leaves (5 kg) of *Myrcia multiflora* cultivated in Sáo Paulo, Brazil (purchased from Albano Ferreira Martins, Ltd., Sáo Paulo) were minced and extracted three times with methanol under reflux. Evaporation of the solvent from the extract under reduced pressure furnished methanol extract (528 g); 189 g of this extract was partitioned in an AcOEt–H_2O (1 : 1) mixture. Removal of the solvent from the AcOEt-soluble and H_2O-soluble fractions under reduced pressure yielded AcOEt extract (42 g) and H_2O extract (147 g).

The 42 g of AcOEt extract was separated by normal-phase silica gel

column chromatography (900 g, $CHCl_3$–MeOH) to afford five fractions [Fr. 1 (13.5 g), Fr. 2 (3.5 g), Fr. 3 (3.1 g), Fr. 4 (3.1 g), Fr. 5 (16.0 g)]. Fraction 1 (13.5 g) was further subjected to normal-phase silica gel (450 g, *n*-hexane–AcOEt) and reversed-phase silica gel column chromatography (37 g, MeOH–H_2O) and then HPLC [YMC-pack ODS-A, MeOH–H_2O (90 : 10, v/v)] to furnish ginkgoic acid (**12**, 17 mg) and β-amyrin (571 mg). Fraction 5 (16.0 g) was separated by normal-phase silica gel (670 g, $CHCl_3$–MeOH→$CHCl_3$–MeOH–H_2O) and reversed-phase silica gel (35 g, MeOH–H_2O) and then HPLC (YMC-pack ODS-A, MeOH–H_2O, CH_3CN–H_2O) to give myrciacitrins I (**1**, 1164 mg), II (**2**, 21 mg), myrciaphenones A (**5**, 9 mg), B (**6**, 74 mg), myricitrin (**7**, 173 mg), mearnsitrin (**8**, 78 mg), quercitrin (**9**, 291 mg), desmanthin-1 (**10**, 31 mg), guaijaverin (**11**, 24 mg), (+)-catechin (11 mg) and gallic acid (638 mg). Two known compounds, (+)-catechin and gallic acid, were identified by comparison of TLC behavior, ^{1}H-NMR, and ^{13}C-NMR spectra with those authentic samples and other known compounds (**7**—**12**) and β-amyrin were identified by comparison of their physical data ($[\alpha]_D$, ^{1}H-NMR, and ^{13}C-NMR spectra) with reported values.[11-17]

Myrciacitrin I (**1**): A yellow powder, $[\alpha]_D^{27}$ −51.0° (c=1.7, MeOH). High-resolution positive-ion FAB-MS Calcd for $C_{23}H_{26}O_{11}Na$ $(M+Na)^+$: 501.1399. Found: 501.1372. CD (c=0.0033, MeOH) $[\theta]^{25}$ (nm): −28730 (281) (negative maximum). UV λ_{max}^{MeOH} nm (log ε): 212 (4.3), 285 (4.1), 358 (3.6). IR (KBr) cm^{-1}: 3375, 2930, 1655, 1630, 1458, 1067. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 2.10 (6H, s, 6, 8-CH_3), 2.82 (1H, dd, J=2.8, 17.2 Hz), 3.09 (1H, dd, J=12.9, 17.2 Hz) (3-H_2), 3.44 (1H, dd, J=5.3, 11.3 Hz), 3.63 (1H, br d, J=11.3 Hz) (6″-H_2), 4.61 (1H, d, J=7.3 Hz, 1″-H), 5.64 (1H, dd, J=2.8, 12.9 Hz, 2-H), 6.60 (1H, dd, J=2.6, 8.7 Hz, 4′-H), 6.70 (1H, d, J=8.7 Hz, 3′-H), 6.91 (1H, d, J=2.6 Hz, 6′-H), 8.87 (1H, br s, 5′-OH), 9.11 (1H, br s, 2′-OH), 12.11 (1H, br s, 5-OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_C: given in Table 5. Positive-ion FAB-MS (m/z): 501 $(M+Na)^+$, 479 $(M+H)^+$.

Myrciacitrin II (**2**): A yellow powder, $[\alpha]_D^{24}$ −20.7° (c=0.1, pyridine). High-resolution positive-ion FAB-MS Calcd for $C_{24}H_{28}O_{11}Na$ $(M+Na)^+$: 515.1529. Found: 515.1520. CD (c=0.0027, MeOH) $[\theta]^{25}$ (nm): −3116 (281) (negative maximum). UV λ_{max}^{MeOH} nm (log ε): 204 (4.2), 285 (4.0), 361 (3.2). IR (KBr) cm^{-1}: 3422, 2924, 1655, 1638, 1458, 1072. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.09 (3H, s, 8-CH_3), 2.10 (3H, s, 6-CH_3), 2.83 (1H, dd, J=2.7, 16.8 Hz), 3.15 (1H, dd-like) (3-H_2), 3.44 (1H, dd, J=6.2, 11.7 Hz), 3.62 (1H, br d, J=11.7 Hz) (6″-H_2), 3.70 (3H, s, 5′-OCH_3), 4.61 (1H, d, J=7.6 Hz, 1″-H), 5.68 (1H, dd, J=2.7, 12.8 Hz, 2-H), 6.80 (1H, dd, J=2.7, 8.9 Hz, 4′-H), 6.82 (1H, d, J=8.9 Hz, 3′-H), 7.04 (1H, d, J=2.7 Hz, 6′-H), 9.28 (1H, br s, 2′-OH), 12.07 (1H, br s, 5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 5. Positive-ion FAB-MS (m/z): 515 $(M+Na)^+$, 493 $(M+H)^+$. Negative-ion FAB-MS (m/z): 491 $(M-H)^-$.

Myrciaphenone A (**5**): A white powder, $[\alpha]_D^{27}$ −46.9° (c=0.2, MeOH). High-resolution positive-ion FAB-MS Calcd for $C_{14}H_{18}O_9Na$ $(M+Na)^+$: 353.0849. Found: 353.0866. UV λ_{max}^{MeOH} nm (log ε): 203 (4.5), 284 (4.0). IR (KBr) cm^{-1}: 3410, 1630, 1605, 1459, 1366, 1076. ^{1}H-NMR (500 MHz, CD_3OD) δ: 2.69 (3H, s, 8-H_3), 3.72 (1H, dd, J=5.5, 12.2 Hz), 3.91 (1H, dd, J=2.1, 12.2 Hz) (6′-H_2), 5.02 (1H, d, J=7.6 Hz, 1′-H), 5.94 (1H, d, J=2.3 Hz, 5-H), 6.18 (1H, d, J=2.3 Hz, 3-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 5. Positive-ion FAB-MS (m/z): 353 $(M+Na)^+$. Negative-ion FAB-MS (m/z): 329 $(M-H)^-$.

Myrciaphenone B (**6**): A white powder, $[\alpha]_D^{24}$ −64.6° (c=0.1, MeOH). High-resolution positive-ion FAB-MS Calcd for $C_{21}H_{22}O_{13}Na$ $(M+Na)^+$: 505.0958. Found: 505.0971. UV λ_{max}^{MeOH} nm (log ε): 219 (4.4), 283 (4.2). IR (KBr) cm^{-1}: 3403, 1701, 1628, 1452, 1367, 1076. ^{1}H-NMR (500 MHz, CD_3OD) δ: 2.63 (3H, s, 8-H_3), 4.47 (1H, dd, J=4.9, 12.1 Hz), 4.57 (1H, dd, J=1.8, 12.1 Hz) (6′-H_2), 5.07 (1H, d, J=7.6 Hz, 1′-H), 5.96 (1H, d, J=2.0 Hz, 5-H), 6.17 (1H, d, J=2.0 Hz, 3-H), 7.09 (2H, s, 2″,6″-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 5. Positive-ion FAB-MS (m/z): 505 $(M+Na)^+$, 483 $(M+H)^+$. Negative-ion FAB-MS (m/z): 481 $(M-H)^-$.

Acid Hydrolysis of 1 and 5 A solution of **1** and **5** (2 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the insoluble portion was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was separated on a Sep-Pack C18 cartridge column (H_2O, MeOH). The H_2O elute was concentrated under reduced pressure to give a residue, which was treated with L-cystein methyl ester hydrochloride (2 mg) in pyridine (0.02 ml) and the mixture was left standing at 60 °C for 1 h. The reaction solution was then treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.01 ml) and the whole mixture was left standing at 60 °C for 1 h. The supernatant of the reaction mixture was subjected to GLC analysis to identify the thiazolidene derivative (**i**) of D-glucose. GLC conditions: column, Supelco SPB™-1, 0.25 mm (i.d.) × 30 m; column temperature, 230 °C. t_R: **i**, 24.2 min.

Enzymatic Hydrolysis of Myrciacitrin I (1) A solution of **1** (30 mg) in 0.1 M acetate buffer (pH 4.4, 6.0 ml) was treated with β-glucosidase (15 mg) and the solution was stirred at 38 °C for 30 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure and the residue was purified by normal-phase silica gel column chromatography (2 g, $CHCl_3$–MeOH–H_2O=10 : 3 : 1, lower layer) to give **3** (20 mg).

Myrciacetin (**3**): A yellow powder, $[\alpha]_D^{25}$ −33.6° (c=0.2, MeOH). High-resolution EI-MS Calcd for $C_{17}H_{16}O_6$ (M^+): 316.0947. Found: 316.0923. CD (c=0.0023, MeOH) $[\theta]^{25}$ (nm): −19360 (281) (negative maximum). UV λ_{max}^{MeOH} nm (log ε): 210 (4.4), 296 (4.3), 340 (3.6). IR (KBr) cm^{-1}: 3440, 2924, 1637, 1603, 1360, 1117. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.97, 1.99 (3H each, s, 6, 8-CH_3), 2.78 (1H, dd, J=3.0, 17.1 Hz), 3.01 (1H, dd, J=12.5, 17.1 Hz) (3-H_2), 5.57 (1H, dd, J=3.0, 12.5 Hz, 2-H), 6.59 (1H, dd, J=3.0, 8.5 Hz, 4′-H), 6.69 (1H, d, J=8.5 Hz, 3′-H), 6.89 (1H, d, J=3.0 Hz, 6′-H), 12.36 (1H, br s, 5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 5. EI-MS (m/z): 316 (M^+), 298 (M^+-H_2O).

Enzymatic Hydrolysis of Myrciacitrin II (2) A solution of **2** (5 mg) in 0.1 M acetate buffer (pH 4.4, 1.0 ml) was treated with β-glucosidase (5 mg) and the solution was stirred at 38 °C for 22 h. After treatment of the reaction mixture with EtOH, the mixture was evaporated to dryness under reduced pressure and the residue was purified by normal-phase silica gel column chromatography (1 g, $CHCl_3$–MeOH–H_2O=10 : 3 : 1, lower layer) to give **4** (4 mg), which was identified by comparison of the physical data ($[\alpha]_D$, ^{1}H-NMR, ^{13}C-NMR) with reported values.[20b,22]

Diazomethane Methylation of Myrciacitrin I (1) A solution of **1** (13 mg) in MeOH (1.5 ml) was treated with ethereal diazomethane (*ca.* 6 ml) until the yellow color remained costant. The solution was stirred for 15 min at room temperature, then the solvent was removed under reduced pressure and the residue was purified by HPLC [YMC-pack ODS-A, MeOH–H_2O (70 : 30, v/v)] to furnish myrciacitrin II (**2**, 5.4 mg), **1a** (1.6 mg), and **1b** (2.4 mg).

1a: A yellow powder, ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.09, 2.10 (3H each, s, 6,8-H_3), 2.80 (1H, dd, J=3.0, 17.1 Hz), 3.13 (1H, dd, J=13.0, 17.1 Hz) (3-H_2), 3.43 (1H, dd-like), 3.63 (1H, br d, J=10.2 Hz) (6″-H_2), 3.74 (3H, s, 2′-OCH_3), 4.61 (1H, d, J=7.6 Hz, 1″-H), 5.67 (1H, dd, J=3.0, 13.0 Hz, 2-H), 6.74 (1H, dd, J=2.9, 8.9 Hz, 4′-H), 6.90 (1H, d, J=8.9 Hz, 3′-H), 6.99 (1H, d, J=2.9 Hz, 6′-H), 8.29 (1H, br s, 5′-OH), 12.06 (1H, br s, 5-OH).

1b: A yellow powder, ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.09, 2.10 (3H each, s, 6,8-H_3), 2.80 (1H, dd, J=2.9, 16.9 Hz), 3.13 (1H, m) (3-H_2), 3.43 (1H, dd-like), 3.63 (1H, br d, J=10.7 Hz) (6″-H_2), 3.74 (3H, s, 5′-OCH_3), 3.78 (3H, s, 2′-OCH_3), 4.61 (1H, d, J=7.6 Hz, 1″-H), 5.72 (1H, dd, J=2.9, 13.0 Hz, 2-H), 6.93 (1H, dd, J=3.0, 8.8 Hz, 4′-H), 7.02 (1H, d, J=8.8 Hz, 3′-H), 7.12 (1H, d, J=3.0 Hz, 6′-H), 12.06 (1H, br s, 5-OH).

Bioassay Methods

Inhibitory Activity on the Increase of Serum Glucose Level in Oral Sucrose-Loaded Rats Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama, Japan) weighing 130—170 g were starved for 20—24 h but allowed water *ad libitum*. The test samples were suspended in 5% gum arabic solution (5 ml/kg), and then orally administered to the rats at each dose. Thirty min thereafter, a water solution (5 ml/kg) of sucrose (1 g/kg) was orally administered. Blood (0.4 ml) was collected from the jugular vein at 0.5, 1.0, and 2.0 h after sucrose administration and the serum glucose concentration was assayed by the enzymatic glucose oxidase method.

Effect on Serum Glucose Levels in Alloxan-Induced Diabetic Mice Male ddY mice (Kiwa Laboratory Animals, Ltd., Wakayama, Japan) weighing 26—29 g were starved for 20—24 h but allowed water *ad libitum*. Alloxane

(50 mg/kg) in saline was injected intravenously. Two days thereafter, mice were again starved for 20—24 h, and then the test samples were suspended in 5% gum arabic solution and given orally at 10 ml/kg. Blood was collected from infraorbital venous plexus at before (0 h), and at 1.0 and 3.0 h after the administration of test sample.

Statistics Statistical significance of differences was estimated by analysis of variance (ANOVA) followed by Dunnett's test.[26] Results were expressed as the mean ± S.E. (Tables 1 and 2).

Aldose Reductase Inhibitory Activity Aldose reductase activity was assayed according to the method described by Dufrane *et al.*[27] with slight modifications. Lenses of Wistar rats were homogenized in 135 mM Na, K-phosphate buffer (pH 7.0) containing 10 mM 2-mercaptoethanol, and centrifuged at $100000 \times g$ for 30 min. The supernatant fluid was used as the enzyme fraction. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 μl of the enzyme fraction, with or without 25 μl of sample solution in a total volume of 0.5 ml. Test samples were dissolved in DMSO. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min of incubation, the reaction was stopped by the addition of 150 μl of 0.5 N HCl. Then, 0.5 ml of 6 N NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. The fluorescence was measured at room temperature using a spectrofluorometer (Type 650-10, Hitachi, Japan) with an excitation wavelength of 360 nm, and an emission wavelength of 460 nm.

α-Glucosidase Inhibitory Activity Rat small intestinal brush border membrane vesicles[28] were used in the preparation of small intestinal α-glucosidase of maltase, and sucrase. Reaction was performed by slight modifications of the procedure of Dahlqvist.[29] The substrate (maltose: 37 mM, sucrose: 37 mM), test compound and the enzyme in 0.1 M maleate buffer (pH 6.0, 0.2 ml) were incubated together at 37 °C. After 30 min of incubation, 0.8 ml of water was added to the test tube, and the tube was immediately immersed in boiling water for 2 min, then cooled with water. Glucose concentration was determined by the glucose oxidase method.

References and Notes

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