Antidiabetic Principles of Natural Medicines. V.[1] Aldose Reductase Inhibitors from *Myrcia multiflora* DC. (2): Structures of Myrciacitrins III, IV, and V

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Following the characterization of myrciacitrins I and II and myrciaphenones A and B, three new flavanone glucosides, myrciacitrins III, IV, and V, were isolated from the leaves of Brazilian *Myrcia multiflora*. The structures of new myrciacitrins were elucidated on the basis of physicochemical and chemical evidence. Myrciacitrins were found to show potent inhibitory activity on aldose reductase.

Key words myrciacitrin; *Myrcia multiflora*; aldose reductase inhibitor; flavanone glucoside; acylated flavanone glucoside

In the course of our characterization studies on the antidiabetic constituents of natural medicines,[1,2] the methanolic extract and its ethyl acetate-soluble portion from a Brazilian natural medicine, the leaves of *Myrcia multiflora* DC. (Myrtaceae), which have been extensively used as a specific remedy for diabetes in South American countries and are commonly called "plant insulin," were found to exhibit inhibitory activities on aldose reductase and α-glucosidase and on the increase in serum glucose level in sucrose-loaded rats and in alloxan-induced diabetic mice.[3] In a preceding paper,[3] we reported the isolation and structure elucidation of two flavanone glucosides, myrciacitrins I (**4**) and II (**5**), and two acetophenone glucosides, myrciaphenones A and B, from the ethyl acetate-soluble portion together with five known flavonol glycosides and ginkgoic acid. As a continuation of this study, a new flavanone glucoside called myrciacitrin III (**1**) and two new acylated flavanone glucosides called myrciacitrins IV (**2**) and V (**3**) were further isolated from the ethyl acetate-soluble portion. In this paper, we describe the isolation and structure elucidation of myrciacitrins III—V (**1**—**3**) as well as their inhibitory activities on aldose reductase.

Structures of Myrciacitrin III (1) Myrciacitrin III (**1**) was isolated as a yellow powder of negative optical rotation ($[\alpha]_D^{25}$ −104.2°). The positive- and negative-ion FAB-MS of **1** showed quasimolecular ion peaks at *m/z* 479 $(M+H)^+$, 501 $(M+Na)^+$, and 477 $(M-H)^-$, 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 3404, 1688, and 1613 cm^{-1} assignable to the hydroxyl, chelated carbonyl, and aromatic ring, while absorption maxima characteristic of the flavanone structure were observed at 295 (log ε 4.1) and 340 (log ε 3.4) nm in its UV spectrum. Acid hydrolysis of **1** with 1 M HCl furnished D-glucose, which was identified by HPLC analysis using an optical rotation detector.[4] Enzymatic hydrolysis of **1** with β-glucosidase liberated myrciacetin (**1a**).[3] The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[5] of **1** showed signals assignable to a myrciacetin moiety, [δ 1.97, 1.99 (both s, 6, 8-CH_3), 2.79 (dd, J=3.2, 17.0 Hz), 2.90 (dd, J=12.3, 17.0 Hz) (3-H_2), 5.85 (dd, J=3.2, 12.3 Hz, 2-H), 6.69 (dd, J=2.9, 8.8 Hz, 4′-H), 6.99 (d, J=2.9 Hz, 6′-H), 7.03 (d, J=8.8 Hz, 3′-H), 12.37 (br s, 5-OH)] and a β-D-glucopyranosyl part [δ 4.58 (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-CH_3 and 5, 6, 7-C; 8-CH_3 and 7, 8, 9-C; 3′-H and 1′, 5′-C; 4′-H and 2′, 6′-C; 6′-H and 2′, 4′-C; 1″-H and 2′-C (Fig. 1). Furthermore, the nuclear Overhauser effect (NOE) experiment of **1** showed correlations between the 1″-proton and the 3′-proton (Fig. 1). Finally, the circular dichroism (CD) spectra of **1** showed negative Cotton effects (**1**: $[\theta]_{291}$ −27720), which indicated the absolute configuration of the 2-position to be the *S* orientation.[6] On the basis of the above evidence, the structure of myrciacitrin III was determined to be (2*S*)-6,8-dimethyl-5,7,2′,5′-tetrahydroxyflavanone 2′-*O*-

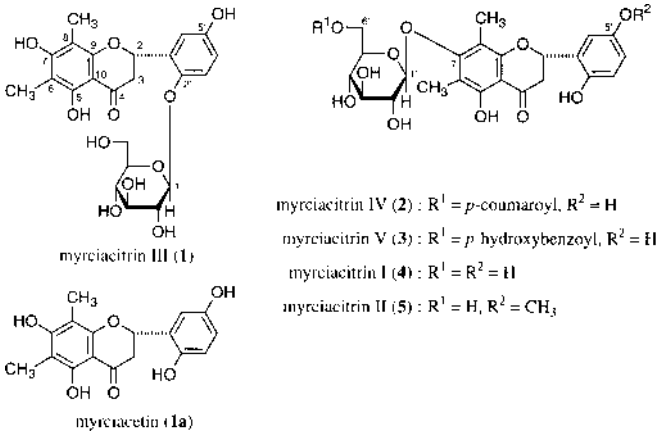

Chart 1

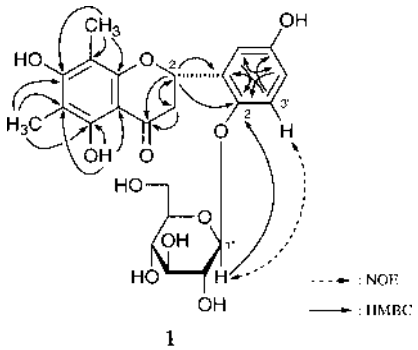

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Fig. 1. HMBC and NOE Correlations of **1**

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Table 1. ^{13}C-NMR Data of Myrciacitrins III (**1**), IV (**2**), and V (**3**)

	1	2	3
C-2	73.2	73.8	73.8
3	41.8	41.4	41.4
4	196.4	198.3	198.4
5	158.3	157.8	157.8
6	103.2	111.2	111.2
7	162.6	161.0	161.0
8	102.5	109.8	109.9
9	157.3	157.5	157.5
10	101.5	104.6	104.6
1′	130.0	125.5	125.5
2′	146.5	146.2	146.2
3′	117.4	116.1	116.1
4′	115.1	115.5	115.5
5′	152.5	149.9	149.9
6′	112.2	112.8	112.8
6-CH_3	8.2[a)]	8.6[a)]	8.6[a)]
8-CH_3	7.5[a)]	8.9[a)]	8.9[a)]
Glc-1″	102.9	103.9	103.9
2″	73.3	73.9	74.0
3″	77.0	76.0	76.0
4″	69.8	69.9	70.0
5″	76.3	73.6	73.6
6″	60.8	62.9	63.1
1‴		124.9	120.3
2‴, 6‴		130.2	131.2
3‴, 5‴		115.7	115.1
4‴		159.7	161.8
7‴		144.6	165.2
8‴		113.8	
9‴		166.3	

a) Interchangeable in the same column, measured in DMSO-d_6.

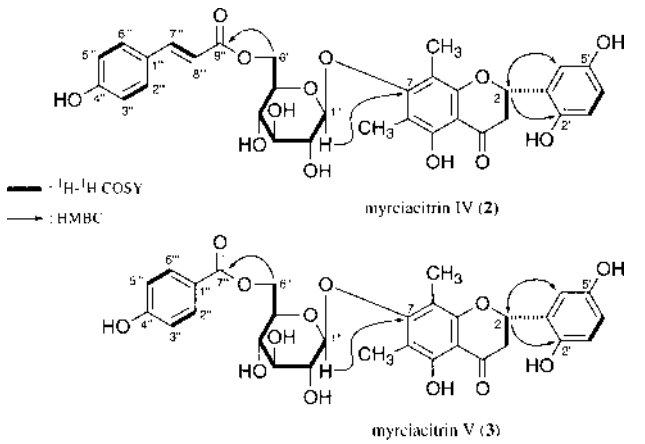

Fig. 2. HMBC and ^{1}H–^{1}H COSY Correlations of **2** and **3**

Table 2. Inhibitory Activities of Myrciacitrins (**1**—**5**) and Myrciacetin (**1a**) against Rat Lens Aldose Reductase

	IC_{50} (M)[a)]
Myrciacitrin I (**4**)	3.2×10^{-6}
Myrciacitrin II (**5**)	1.5×10^{-5}
Myrciacitrin III (**1**)	4.6×10^{-5}
Myrciacitrin IV (**2**)	7.9×10^{-7}
Myrciacitrin V (**3**)	1.6×10^{-5}
Myrciacetin (**1a**)	1.3×10^{-5}
Epalrestat	7.2×10^{-8}

a) IC_{50} values of **1a**, **4**, and **5** were reported previously.[3)]

β-D-glucopyranoside (**1**).

Structures of Myrciacitrins IV (2) and V (3) Myrciacitrin IV (**2**) was isolated as a yellow powder and its IR spectrum showed absorption bands at 3400, 1686, 1630, 1605, and 1068 cm^{-1}, suggestive of the presence of a glycosidic structure, and carbonyl and aromatic functions. The molecular formula $C_{32}H_{32}O_{13}$ 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 **2** quasimolecular ion peaks were observed at *m/z* 625 $(M+H)^+$ and 647 $(M+Na)^+$, while the negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 623 $(M-H)^-$. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[5)] of **2** showed signals due to a *p*-coumaroyl group [δ 6.32 (d, J=16.0 Hz, 8‴-H), 6.76 (2H, d, J=8.4 Hz, 3‴-, 5‴-H), 7.48 (2H, d, J=8.4 Hz, 2‴-, 6‴-H), 7.48 (d, J=16.0 Hz, 7‴-H)] and a myrciacitrin I moiety. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **4** disclosed an acylation shift at the 6″-position of **2**. Furthermore, the position of the *p*-coumaroyl group in **2** was determined by the HMBC experiment of **2**, which showed a long-range correlation between the 6″-proton and 9‴-carbon (Fig. 2). In addition, the CD spectra of **2** showed a negative Cotton effect (**2**: $[\theta]_{281}$ −31620), which indicated the absolute configuration of the 2-position to be the *S* orientation.[6)] Consequently, myrciacitrin IV was determined to be (2*S*)-6,8-dimethyl-5,7,2′,5′-tetrahydroxyflavanone 7-*O*-(6″-*O*-*p*-coumaroyl)-β-D-glucopyranoside (**2**).

Myrciacitrin V (**3**), obtained as a yellow powder, gave a quasimolecular ion peak at *m/z* 597 $(M-H)^-$ in the negative-ion FAB-MS and the molecular composition was defined as $C_{30}H_{30}O_{13}$ from the high-resolution MS analysis. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **3** were superimposable on those of **2**, except for the signals due to a *p*-hydroxybenzoyl group [δ 6.79 (2H, d, J=8.7 Hz, 3‴-, 5‴-H), 7.70 (2H, d, J=8.7 Hz, 2‴-, 6‴-H)]. The position of the *p*-hydroxybenzoyl group in **3** was determined by the HMBC experiment (Fig. 2) and an acylation shift at the 6″-position. Furthermore, the CD spectra of **3** showed a negative Cotton effect as did those of **2**. On the basis of this evidence, myrciacitrin V was characterized as (2*S*)-6,8-dimethyl-5,7,2′,5′-tetrahydroxyflavanone 7-*O*-(6″-*O*-*p*-hydroxybenzoyl)-β-D-glucopyranoside (**3**).

Inhibitory Activities of Myrciacitrins for Aldose Reductase Aldose reductase catalizes the reduction of glucose to sorbitol in the polyol pathway and is related to chronic complications such as peripheral neuropathy, retinopathy, and cataracts. As shown in Table 2, myrciacitrins (**1**—**5**) from the leaves of *M. multiflora* and a flavanone (**1a**), the common aglycone of the constituents (**1**—**4**), were found to show potent inhibitory activity against rat lens aldose reductase. Among them, **2** showed the most potent activity, although it had less activity than epalrestat,[7)] a commercial synthetic aldose reductase inhibitor. Taking into account their isolation yields, the flavonoid glycosides including the new acylated flavanone glucoside myrciacitrin IV (**2**) may be the beneficial constituents of the antidiabetic Brazilian natural medicine, the leaves of *M. multiflora*.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[3)]

Isolation of Myrciacitrins III (1), IV (2), and V (3) from the Dried

Leaves of *M. multiflora* Dried leaves of *M. multiflora* cultivated in São Paulo, Brazil (purchased from Albano Ferreira Martins, Ltd., São Paulo) were minced and extracted three times with methanol under reflux. Evaporation of the solvent from the extract under reduced pressure furnished methanol extract (10.6%). 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 (2.3%) and H_2O extract (8.2%).

The AcOEt extract was separated by normal-phase silica-gel column chromatography to afford five fractions.[3] Fraction 5 was further separated by normal-phase silica-gel ($CHCl_3$–MeOH→$CHCl_3$–MeOH–H_2O) and reversed-phase silica-gel (MeOH–H_2O) column chromatography and then HPLC (YMC-pack ODS-A, MeOH–H_2O, CH_3CN–H_2O) to give myrciacitrins III [**1**, 0.0019% (yields from the dried leaves)], IV (**2**, 0.012%), and V (**3**, 0.00028%) together with known compounds.

Myrciacitrin III (**1**): A yellow powder, $[\alpha]_D^{25}$ −104.2° (c=0.16, EtOH). High-resolution positive-ion FAB-MS: Calcd for $C_{23}H_{26}O_{11}Na$ $(M+Na)^+$: 501.1373. Found: 501.1384. CD (c=0.0034, MeOH) $[\theta]^{24}$ (nm): −27720 (291) (negative maximum). UV λ_{max}^{MeOH} nm (log ε): 295 (4.1), 340 (3.4). IR (KBr) cm^{-1}: 3404, 2924, 1688, 1613, 1499, 1080, 812. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.97, 1.99 (3H each, both s, 6 or 8-CH_3), 2.79 (1H, dd, J=3.2, 17.0 Hz), 2.90 (1H, dd, J=12.3, 17.0 Hz) (3-H_2), 3.48 (1H, dd, J=5.7, 11.6 Hz), 3.72 (1H, br d, J=11.6 Hz) (Glc-6″-H_2), 4.58 (1H, d, J=7.3 Hz, Glc-1″-H), 5.85 (1H, dd, J=3.2, 12.3 Hz, 2-H), 6.69 (1H, dd, J=2.9, 8.8 Hz, 4′-H), 6.99 (1H, d, J=2.9 Hz, 6′-H), 7.03 (1H, d, J=8.8 Hz, 3′-H), 12.37 (1H, br s, 5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 1. Positive-ion FAB-MS m/z: 501 $(M+Na)^+$, 479 $(M+H)^+$. Negative-ion FAB-MS m/z: 477 $(M-H)^-$.

Myrciacitrin IV (**2**): A yellow powder, $[\alpha]_D^{26}$ −99.2° (c=0.68, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{32}H_{33}O_{13}$ $(M+H)^+$: 625.1891. Found: 625.1921. CD (c=0.0050, MeOH) $[\theta]^{25}$ (nm): −31620 (281) (negative maximum). UV λ_{max}^{MeOH} nm (log ε): 226 (sh, 4.7), 285 (4.5), 317 (sh, 4.3). IR (KBr) cm^{-1}: 3400, 2928, 1686, 1630, 1605, 1514, 1449, 1068, 831. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 2.06, 2.08 (3H each, both s, 6- or 8-CH_3), 2.75 (1H, dd, J=3.0, 17.0 Hz), 2.94 (1H, dd, J=12.5, 17.0 Hz) (3-H_2), 4.22 (1H, dd, J=6.1, 11.2 Hz), 4.34 (1H, br d, J=11.2 Hz) (Glc-6″-H_2), 4.68 (1H, d, J=7.3 Hz, Glc-1″-H), 5.59 (1H, dd, J=3.0, 12.5 Hz, 2-H), 6.32 (1H, d, J=16.0 Hz, 8‴-H), 6.61 (1H, dd, J=2.6, 8.6 Hz, 4′-H), 6.70 (1H, d, J=8.6 Hz, 3′-H), 6.76 (2H, d, J=8.4 Hz, 3‴-, 5‴-H), 6.89 (1H, d, J=2.6 Hz, 6′-H), 7.48 (2H, d, J=8.4 Hz, 2‴-, 6‴-H), 7.48 (1H, d, J=16.0 Hz, 7‴-H), 12.01 (1H, br s, 5-OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_C: given in Table 1. Positive-ion FAB-MS m/z: 647 $(M+Na)^+$, 625 $(M+H)^+$. Negative-ion FAB-MS m/z: 623 $(M-H)^-$, 315 $(M-C_{15}H_{17}O_7)^-$.

Myrciacitrin V (**3**): A yellow powder, $[\alpha]_D^{26}$ +108.2° (c=0.028, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{29}O_{13}$ $(M-H)^-$: 597.1608. Found: 597.1622. CD (c=0.0040, MeOH) $[\theta]^{24}$ (nm): −11790 (278) (negative maximum). UV λ_{max}^{MeOH} nm (log ε): 259 (4.0), 350 (3.3). IR (KBr) cm^{-1}: 3430, 2924, 1718, 1638, 1610, 1560, 1458, 1070. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.04, 2.05 (3H each, both s, 6- or 8-CH_3), 2.82 (1H, dd, J=2.7, 17.1 Hz), 3.09 (1H, dd, J=13.0, 17.1 Hz) (3-H_2), 4.19 (1H, dd, J=6.4, 11.6 Hz), 4.47 (1H, dd, J=2.0, 11.6 Hz) (Glc-6″-H_2), 4.68 (1H, d, J=7.6 Hz, Glc-1″-H), 5.60 (1H, dd, J=2.7, 13.0 Hz, 2-H), 6.59 (1H, dd, J=3.0, 8.5 Hz, 4′-H), 6.68 (1H, d, J=8.5 Hz, 3′-H), 6.79 (2H, d, J=8.7 Hz, 3‴-, 5‴-H), 6.86 (1H, d, J=3.0 Hz, 6′-H), 7.70 (2H, d, J=8.7 Hz, 2‴-, 6‴-H), 12.07 (1H, br s, 5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: given in Table 1. Negative-ion FAB-MS m/z: 597 $(M-H)^-$.

Acid Hydrolysis of 1 A solution of **1** (1 mg) in 1 M HCl (0.1 ml) was heated at 100 °C for 1 h. After cooling, the reaction mixture was extracted with AcOEt (0.1 ml). The H_2O layer was analyzed by HPLC under the following conditions: eluent, CH_3CN–H_2O (3 : 1, v/v); flow rate, 0.8 ml/min; detection, optical rotation [detector: Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; column, Kaseisorb LC NH_2-60-5 4.6 mm i.d.×250 mm, 5 μm (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan); injection volume, 10 μl; column temperature, room temperature. Identification of D-glucose present in the H_2O layer was carried out by comparison of its retention time and optical rotation with that of authentic sample. t_R: 12.3 min (D-glucose, positive optical rotation).

Enzymatic Hydrolysis of 1 A solution of **1** (3 mg) in 0.1 M acetate buffer (pH 4.4, 1.0 ml) was treated with β-glucosidase (5 mg) and the solution was stood 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 [1 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give myrciacetin (**1a**, 2 mg), which was identified by comparison of their physical data ($[\alpha]_D$, ^{1}H-, ^{13}C-NMR) with an authentic sample.[3]

Aldose Reductase Inhibitory Activity Aldose reductase activity was assayed by the method described previously.[3] The 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×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 M HCl. Then 0.5 ml of 6 M 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.

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