α-Glucosidase Inhibitors from *Garcinia brevipedicellata* (Clusiaceae)

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In our continuous search for α-glucosidase inhibitors from plants, four new depsidones named brevipsidones A—D (1—4) were isolated from stem bark of *Garcinia brevipedicellata* together with known damnacanthal, scopoletin and a mixture of stigmasterol and β-sitosterol. Structural elucidations were made by spectroscopic analyses including 2D-NMR data.

Key words *Garcinia brevipedicellata*; Clusiaceae; α-glucosidase; depsidone; brevipsidone

The genus *Garcinia* (Clusiaceae), which is encountered mainly in lowland rain forests of the tropical world, has been extensively investigated from phytochemical and biological points of view. Triterpenes,[1)] xanthones,[2—4)] benzophenone,[5)] depsidones,[6,7)] flavonoids[8)] and biflavonoids[9)] have been isolated from African and Southeast Asian species. Depsidones isolated from *Garcinia* are assumed to be cancer chemopreventive, cytotoxic and radical scavenging agents.[7,10,11)]

Diabetes mellitus (DM) is a common metabolic disease characterized by elevated blood glucose levels, resulting from absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action. This illness affects approximately 150 millions of people worldwide and its incidence rate is expected to double during the next 20 years.[12)] Recent studies suggest that postprandial hyperglycemia could induce the non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is critical in the early treatment of DM and in reducing chronic vascular complications.[13)] One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with DM is to prevent absorption of carbohydrates after food uptake. Alpha-glucosidase inhibitors are widely used in the treatment of patients with type 2 diabete. They delay the absorption of carbohydrates from the small intestine and thus have a lowering effect on postprandial blood glucose and insulin levels.[14)] Nojirimycin[15,16)] and Acarbose[17)] are known to be α-glucosidase inhibitors derived from microorganisms. In the search for alternative powerful α-glucosidase inhibitors, we speculated that analysis of the secondary metabolites synthesized by plants would be a promising line of inquiry.

In our continued work on α-glucosidase inhibitors from plant origin, we surveyed a number of plants from Cameroon and reported α-glucosidase inhibitors from *Terminalia superba*,[18)] *Piper umbellatum*[19)] and *Garcinia brevipedicellata*.[6)] Here we report the isolation and identification of new α-glucosidase inhibitors compounds, brevipsidones A—D (**1**—**4**) from leaves of *G. brevipedicellata* (Clusiaceae).

Results and Discussion

Dried and pulverised leaves of *G. brevipedicellata* were extracted with acetone. The hexane and CH_2Cl_2-soluble fractions obtained from the acetone extract was subjected to repeated chromatography on silica gel column to afford four new depsidones, brevipsidones A—D (**1**—**4**) along with damnacanthal,[20)] scopoletin[21)] and a mixture of stigmasterol and β-sitosterol.

Brevipsidone A (**1**) was obtained as an amorphous yellow powder. It gave a dark green color with ferric chloride, indicating its phenolic nature. The molecular formula $C_{24}H_{24}O_7$, was obtained from HR-EI-MS (*m*/*z* 424.1503). The IR spectrum showed strong absorption bands due to hydroxyl and lactone carbonyl groups at 3376 and 1678 cm^{-1}, respectively. The ^{1}H-NMR spectra of **1** showed the presence of a prenyl group at [δ_H 3.31 (2H, d, J=7.5 Hz, H-1′), 5.19 (1H, t, J=7.5 Hz, H-2′), 1.77 (3H, s, H-4′) and 1.66 (3H, s, H-5′)], a 2,2-dimethylchromeno ring [δ_H 6.79 (1H, d, J=9.9 Hz, H-1″); 5.65 (1H, d, J=9.9 Hz, H-2″); 1.45 (6H, s, H-4″, H-5″)], and a methoxyl group [δ_H 4.03 (3H, s, 6-OCH_3)]. The

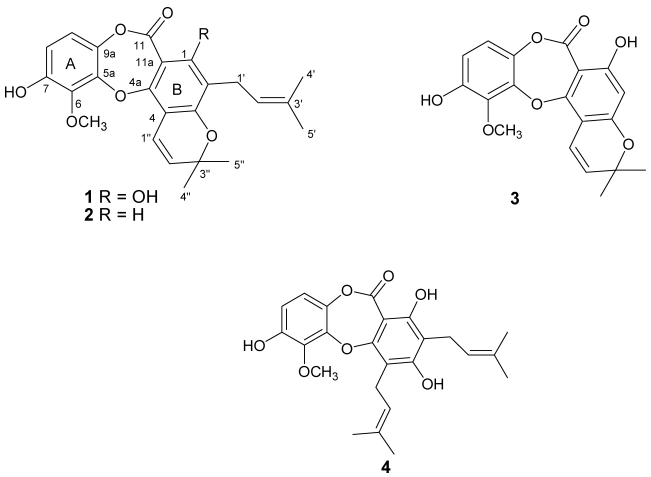

Fig. 1. Structures of Brevipsidones A—D (**1**—**4**)

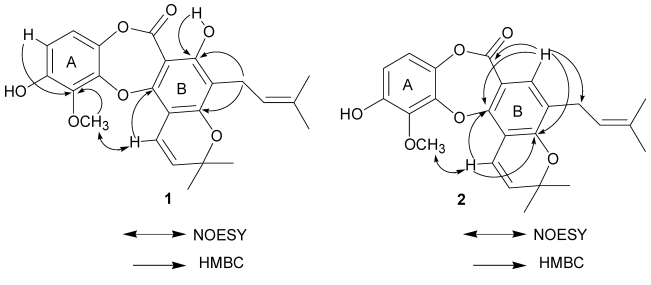

Fig. 2. Important HMBC and NOESY Connectivities in **1** and **2**

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thirteen degrees of unsaturation along with the UV and IR spectra suggested the presence of the depsidone (11*H*-dibenzo[*b*,*e*][1,4]dioxepin-11-one) nucleus. In addition, the ^{1}H-NMR spectra of **1** showed a downfield resonance at δ_H 11.23, attributed to a chelated hydroxyl proton (1-OH), while two signals for the aromatic protons of an *ortho*-coupled AB-type ring A at δ_H 7.23 (1H, d, J=8.9 Hz, H-8) and 7.41 (1H, d, J=8.9 Hz, H-9) suggested a tetrasubstituted A-ring and a total substituted B ring. The location of the substituents on the molecule was determined by NOESY and HMBC experiments. The cross-peaks observed in the HMBC spectrum of **1** between the proton at δ_H 7.23 (H-8) and carbons at δ_C 147.6 (C-7) and 148.3 (C-6) and between proton at δ_H 4.03 (OCH_3) and the carbon at δ_C 148.3 revealed that methoxyl group is located on ring A and that prenyl group and 2,2-dimethylchromene ring are located on the B-ring. Further confirmation is brought by the ^{13}C-NMR which showed signal at δ_C 62.7 for this methoxy group, due to a downfield effect of two *ortho*-substituents.[22] Correlations observed in the NOESY spectrum between the methoxyl (δ_H 4.03) and H-1″ [δ_H 6.79 (1H, d, J=9.9 Hz)] suggested that the methoxyl group is attached to C-6, and the 2,2-dimethylchromene ring is attached on C-4 and C-5, respectively, as shown in ring B. Other HMBC correlations were observed between proton at δ_H 3.31 (2H, d, H-1′) and carbons at δ_C 161.9 (C-1) and 158.2 (C-3) and confirmed the position of prenyl group. Therefore the structure of brevipsidone A was represented by **1**.

Brevipsidone B (**2**) was obtained as an amorphous yellow powder with a molecular formula of $C_{24}H_{24}O_6$, as deduced from HR-EI-MS (*m*/*z* 408.1563). The thirteen degrees of unsaturation along with the UV and IR spectra suggested the presence of the depsidone nucleus. The ^{1}H- and ^{13}C-NMR spectrum showed the presence of a prenyl, a 2,2-dimethylchromeno and a methoxyl groups. Comparison of ^{1}H- and ^{13}C-NMR spectral data of **2** with those of brevipsidone A (**1**) (Table 1) showed that both compounds were quite similar except for the data for C-1. In the ^{1}H-NMR spectrum of **2**, the chelated hydroxyl proton at δ_H 11.23 of **1** was replaced by an uncoupled aromatic proton at δ_H 7.83 (H-1). This signal showed HMBC correlations with C-11 (carbonyl), C-1′ (δ_C 28.5), C-3 (δ_C 163.1) and C-4a (δ_C 147.7). The signal at δ_H 6.80 (H-1″) showed an HMBC correlation with C-3 and C-4a, and a NOESY correlation with 6-OCH_3, indicating a 2,2-dimethylchromene ring at C-3/C-4. On the basis of the above analyses, the prenyl group was deduced to be at C-2. On the basis of the above data, the structure of brevipsidone B was represented by **2**.

Brevipsidone C (**3**) was isolated as an amorphous powder and analyzed for $C_{19}H_{16}O_7$ from its HR-EI-MS. Its ^{1}H- and ^{13}C-NMR spectrum displayed beside depsidone signals the methoxyl group (δ_H 4.05; δ_C 62.9) and two doublets (δ_H 6.83, 5.69, J=10.1 Hz) belonging to vicinal pyran ring protons. The NOESY evidence for the close proximity of the proton on C-1″ (δ_H 6.83: pyran ring) to the methoxyl group on C-6 (δ_H 4.05) clarifies the pyran ring annelation at position 3 and 4. The ^{1}H-NMR spectrum also displayed, in addition to the two *ortho*-coupled aromatic protons (δ_H 6.83: H-1″ and 5.69: H-2″), a singlet aromatic and a chelated hydroxyl protons at δ_H 6.30 and 11.19, respectively. The singlet aromatic proton at δ_H 6.30 was located at C-2 by the HMBC correlations with carbons C-1 (δ_C 163.6), C-3 (δ_C 163.0), C-4 (δ_C 105.2) and C-11a (δ_C 99.2). These data revealed that **3** was an analogue of **1** deprenylated at C-2 of the B-ring, which was confirmed by ^{1}H- and ^{13}C-NMR data (Table 1), and 2D NMR experiments (^{1}H–^{1}H COSY, HSQC, and

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Brevipsidones A—D (**1**—**4**) in $CDCl_3$

No.	**1**		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	161.9		118.3	7.83 (1H, s)	163.6		160.4	
2	113.8		128.3		101.1	6.30 (1H, s)	111.5	
3	158.2		163.1		163.0		161.0	
4	105.8		115.1		105.2		111.2	
4a	153.7		147.7		158.8		156.6	
5a	143.5		143.5		143.5		143.5	
6	148.3		148.4		148.4		148.4	
7	147.6		147.6		147.6		147.6	
8	111.8	7.23 (1H, d, 8.9)	111.8	7.21 (1H, d, 9.1)	111.8	7.22 (1H, d, 9.1)	111.8	7.22 (1H, d, 9.0)
9	116.4	7.41 (1H, d, 8.9)	116.4	7.35 (1H, d, 9.1)	116.4	7.35 (1H, d, 9.1)	116.4	7.39 (1H, d, 9.0)
9a	138.4		138.4		138.4		138.4	
11	168.4		169.1		168.1		168.8	
11a	98.3		98.5		99.2		98.8	
1′	21.8	3.31 (2H, d, 7.5)	28.5	3.09 (2H, d, 6.9)			22.5	3.35 (2H, d, 7.3)
2′	121.9	5.19 (1H, t, 7.5)	122.7	5.23 (1H, t, 7.5)			121.7	5.17 (1H, t, 7.3)
3′	132.1		132.6				135.1	
4′	17.8	1.77 (3H, s)	17.6	1.73 (3H, s)			18.1	1.83 (3H, s)
5′	25.8	1.66 (3H, s)	25.5	1.63 (3H, s)			25.8	1.69 (3H, s)
1″	115.8	6.79 (1H, d, 9.9)	116.7	6.80 (1H, d, 10.1)	114.1	6.83 (1H, d, 10.1)	22.2	3.51 (2H, d, 7.5)
2″	128.5	5.65 (1H, d, 9.9)	126.3	5.64 (1H, d, 10.1)	126.5	5.69 (1H, d, 10.1)	121.2	5.21 (1H, t, 7.5)
3″	77.9		77.5		78.3		135.2	
4″	28.4	1.45 (3H, s)	28.1	1.47 (3H, s)	28.8	1.42 (3H, s)	17.9	1.87 (3H, s)
5″	28.4	1.45 (3H, s)	28.1	1.47 (3H, s)	28.8	1.42 (3H, s)	25.6	1.75 (3H, s)
6-OCH_3	62.7	4.03 (3H, s)	62.7	4.00 (3H, s)	62.9	4.05 (3H, s)	62.6	4.03 (3H, s)
1-OH	—	11.23 (1H, s)	—	11.19 (1H, s)	—		—	11.09 (1H, s)

Table 2. α-D-Glucosidase Inhibitory Activity of Brevipsidones A—D (**1**—**4**)

Compound	α-D-Glucosidase (yeast) IC_{50}±S.E.M. (μM)
Brevipsidone A (**1**)	21.22±0.45
Brevipsidone B (**2**)	27.80±0.76
Brevipsidone C (**3**)	59.64±0.41
Brevipsidone D (**4**)	7.04±0.26
1-Deoxynojirimycin	425.60±8.14

HMBC). Therefore, the structure **3** was assigned to brevipsidone C.

Brevipsidone D (**4**) was obtained as an amorphous yellow powder with a molecular formula of $C_{24}H_{26}O_7$, as deduced from HR-EI-MS (426.1667). Its ^{1}H- and ^{13}C-NMR indicated the presence of a methoxyl and two prenyl groups. These spectrums also displayed signals of an *ortho*-coupled A-ring protons and a chelating proton. A comparison of the ^{1}H-NMR data between **1** and **4** indicated that the 2,2-dimethylchromene ring in **1** was replaced by a prenyl group. This was confirmed by the comparison of the ^{13}C-NMR data between **1** and **4** (Table 1), as well as by HSQC, HMBC and NOESY experiments of **4**. As in brevipsidone A (**1**), a NOESY correlation could be seen between the OCH_3 protons and the prenyl protons at C-1″. We therefore conclude that the C-2 and C-4 positions are occupied by the two prenyl groups as in garcinisidone E isolate from *Garcinia* plants.[7] Thus, brevipsidone D was assigned the structure **4**.

Brevipsidones A—D (**1**—**4**) were also evaluated for their possible glycosidase enzyme inhibitory activity against α-glucosidase. These compounds showed moderate α-glucosidase inhibition with IC_{50} 21.22, 27.80, 59.64 and 7.04 μM, respectively. Brevipsidone D (**4**) with two prenyl groups showed best inhibitory activity (IC_{50} 7.04 μM), whereas brevipsidone C, lacking a prenyl moiety is less active (IC_{50} 59.64 μM). Brevipsidones A (**1**) and B (**2**) with one prenyl and one 2,2-dimethylchromene groups each showed similar inhibitory activity. This lead to the conclusion that the prenyl side chain plays an important role in inhibitory activity. The glucosidase inhibitions of compounds **1**—**4** are shown in Table 2.

Experimental

General IR spectra were recorded on a JASCO 302-A spectrophotometer in $CHCl_3$. The EI-MS (ionization voltage 70 eV) was measured on a Varian AAT 311A mass spectrometer, and HR-EI-MS were taken on a JEOL HX110 mass spectrometer. 1D and 2D NMR spectra were run on Bruker AMX 300 MHz NMR spectrometer. Proton-detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{CH}$=145 Hz) and HMBC (optimized for J_{CH}=8 Hz). The chemical shifts are given in ppm (δ), relative to TMS as internal standard, and coupling constants are in Hz. Column chromatography was carried out on silica gel (70—230 mesh, Merck) and flash chromatography on silica gel (230—400 mesh, Merck). TLC was performed on Merck precoated aluminum silica gel 60 F_{254} sheets, and compounds were detected using ceric sulfate spray reagent. A molecular device spectrophotometer was used for measurement of enzyme inhibition.

Plant Material The stem bark of *Garcinia brevipedicellata* Hutch & Dalziel was collected in Yaounde (Mount Elunden), Center Province of Cameroon by Mr. Nana Victor (National Herbarium of Yaounde, Cameroon) in December 2005 and a voucher specimen No. HNC–55519 was deposited.

Extraction and Isolation The dried and powdered leaves of *G. brevipedicellata* (3 kg) were extracted with acetone (5 l×3) at room temperature. The combined extract was concentrated under reduced pressure to yield a green syrupy (120 g). This was dissolved in 95% MeOH/H_2O and then partitioned (1 : 1) with *n*-hexane to obtain *n*-hexane soluble fraction (58.7 g). The 95% MeOH/H_2O layer was concentrated to remove MeOH in *vacuo*, to which was added H_2O (500 ml), and then it was partitioned with CH_2Cl_2 to obtain a CH_2Cl_2 soluble fraction (43.2 g) and a H_2O layer. The *n*-hexane soluble fraction (58.7 g) was subjected to column chromatography over silica gel (70—230 mesh) using *n*-hexane–acetone step gradient mixture (1—50%) as eluents and yielded a mixture stigmasterol and β-sitosterol (215 mg), scopoletin (8 mg), brevipsidone A (**1**) (22 mg) and brevipsidone C (**3**) (12 mg). The CH_2Cl_2 fraction (43.2 g) was subjected to column chromatography over silica gel (70—230 mesh) using CH_2Cl_2 step gradient mixtures (1—5%) as eluents and yield damnacantal (7 mg), brevipsidone B (**2**) (17 mg) and brevipsidone D (**4**) (11 mg).

Brevipsidone A (**1**): $C_{24}H_{24}O_7$; amorphous yellow powder; UV (MeOH) λ_{max} nm (log ε): 271 (4.51), 291 (4.15), 329 (3.71); IR ν_{max} ($CHCl_3$): 3376 (OH), 1677 (C=C), 1678 (C=O) cm^{-1}; ^{1}H- and ^{13}C-NMR ($CHCl_3$): Table 1; HR-EI-MS *m/z*: 424.1503 [M$^+$] (Calcd for $C_{24}H_{24}O_7$, 424.1519).

Brevipsidone B (**2**): $C_{24}H_{24}O_6$; amorphous yellow powder; UV (MeOH) λ_{max} nm (log ε): 243 (4.31), 301 (3.71); IR ν_{max} ($CHCl_3$): 3301 (OH), 1665 (C=C), 1678 (C=O), 1581 (C=C) cm^{-1}; ^{1}H- and ^{13}C-NMR ($CHCl_3$): Table 1; HR-EI-MS *m/z*: 408.1563 [M$^+$] (Calcd for $C_{24}H_{24}O_6$, 408.1578).

Brevipsidone C (**3**): $C_{19}H_{16}O_7$; amorphous yellow powder; UV (MeOH) λ_{max} nm (log ε): 263 (4.47), 333 (3.59); IR ν_{max} ($CHCl_3$): 3387 (OH), 1675, 1612, 1233 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CHCl_3$): Table 1; HR-EI-MS *m/z*: 356.0887 [M$^+$] (Calcd for $C_{19}H_{16}O_7$, 356.0898).

Brevipsidone D (**4**): $C_{24}H_{26}O_7$; amorphous yellow powder; UV (MeOH) λ_{max} nm (log ε): 273 (4.55), 301 (4.27), 335 (3.57); IR ν_{max} ($CHCl_3$): 3413 (OH), 1669, 1511, 1441 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CHCl_3$): Table 1; HR-EI-MS *m/z*: 426.1667 [M$^+$] (Calcd for $C_{24}H_{26}O_7$, 426.1676).

Enzyme Inhibition Assay α-Glycosidase inhibition assay was performed according to the slightly modified method of Matsu *et al.* (1996). α-D-Glucosidase (EC.3.2.1.20) was purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). The enzyme inhibition was measured spectrophotometrically at 37 °C for 30 min using 0.7 mM *p*-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate at pH 6.9 and 500 units/ml enzymes, in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin (0.425 mM) was used as positive control. The increment in absorption at 400 nm due to the hydrolysis of PNP-G by glycosidase was monitored on microplate spectrophotometer (Spectra Max, Molecular Devices U.S.A.). The absorbance of the reaction mixture was recorded at 400 nm at 1 min intervals in a temperature-controlled chamber at 37 °C. The linear reaction velocity (change in absorbance per minute) was calculated from the gradient of the linear portion of the reaction profile and used to determine the α-glucosidase activity.

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