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Flavan-3-ols and 2-diglycosyloxybenzoates from the leaves of *Averrhoa carambola*

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ABSTRACT

Averrhoa carambola L. (Oxalidaceae) was widely cultivated for fruits (star fruit), whereas the value of leaves remains to be discovered. Our study on the leaves yielded five flavan-3-ols (**1**–**5**) and two 2-diglycosyloxybenzoates. Their structures were determined by spectroscopic and chemical methods. Epicatechin-(5,6-bc)-4β-(*p*-hydroxyphenyl)-dihydro-2(3*H*)-pyranone (**1**) and benzyl 2-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranosyloxybenzoate (**6**) were new structures. 6-(*S*$^{-2}$-Pyrrolidinone-5-yl)epicatechin (**4**) and 6-(*R*-2-pyrrolidinone-5-yl) epicatechin (**5**) were obtained as monomeric diastereomer for the first time and their absolute configurations were determined by electronic circular dichroism (ECD) computation. Epicatechin-(7,8-bc)-4α-(*p*-hydroxyphenyl)-dihydro-2(3*H*)-pyranone (**2**), epicatechin-(7,8-bc)-4β-(*p*-hydroxyphenyl)-dihydro-2(3*H*)-pyranone (**3**), and methyl 2-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranosyloxybenzoate (**7**) were not previously reported from the genus *Averrhoa*. Compounds **1**–**5** showed more potent 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities and ferric reducing antioxidant power (FRAP) than L-ascorbic acid. Meanwhile **1** and **3** exhibited lipase and α-glucosidase inhibitory activities, respectively. The results clarified the structures of flavan-3-ols and 2-diglycosyloxybenzoates in the leaves and their antioxidant, lipase, and α-glucosidase inhibitory activities.

1. Introduction

Averrhoa carambola L. (Oxalidaceae) has been widely cultivated in many tropical and subtropical countries including China as a commercial crop. The tree has become acclimatized in America such as Brazil and Mexico. It is rather popular in Philippines, Australia, Madagascar, and moderately so in some of the South Pacific islands [1]. Its leaves are used in Chinese and Brazilian traditional medicine to treat hyperglycemia [2] and hypertension [3]. In Malaysia, the crushed leaves or shoots are used by the Malays as an application for chickenpox, ringworm, and headache. A decoction of the leaves and fruit is given to arrest vomiting. The leaves are applied in fevers. A decoction of the leaves is good for aphthous stomatitis and angina [1]. The leaves were reported to possess antioxidant [4,5], hypoglycemic [2], hypotensive [3], anti-inflammatory [6], anti-ulcerogenic [7], and cytotoxic [8] activities. From the leaves, Wei et al. (2014) [4] characterized catechin, epicatechin, different degrees of polymerization of procyanidins by HPLC and matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) analyses, procyanidins and prodelphinidins by ^{13}C nuclear magnetic resonance (NMR) analysis, and thirteen fatty acids by GC–MS analysis. Chen et al. (2017) [5] characterized gallic acid, protocatechuic acid, catechin, epicatechin, ferulic acid, rutin, isoquercitrin, quercitrin, and quercetin by ultra-performance liquid chromatography (UPLC) analysis. As for the chemical constituents isolated from the leaves, there are very limited literatures. Araho et al. (2005) [9] obtained three flavone *C*-glycosides, apigenin 6-*C*-β-L-fucopyranoside (carambolaflavone A), apigenin 6-*C*-(2”-*O*-α-L-rhamnopyranosyl)-β-L-fucopyranoside (carambolaflavone B), and isovitexin. Later, total synthesis of both enantiomers of carambolaflavone A revealed that the absolute configuration of β-fucosyl moiety was D rather than L [10]. Moresco et al. (2011) [11] obtained carambolaflavones A and B and apigenin 6-*C*-(2”-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside. Carambolaflavones A and B from the leaves exhibited a potential hypoglycemic activity in hyperglycemic normal rats. Additionally, both flavonoids significantly increased muscle and liver glycogen content after acute treatment, which indicate that the leaves can be regarded as a potent antihyperglycemic agent possessing a dual target action with insulin secretagogue and insulin

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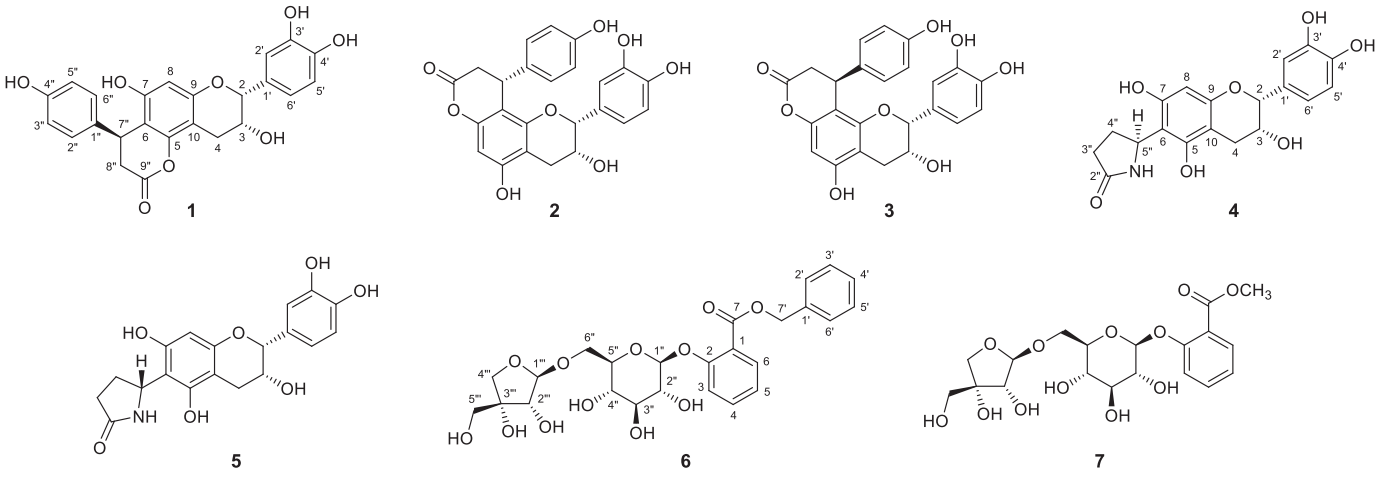

Fig. 1. Structures of compounds **1–7**.

mimetic properties [12,13]. The objective of this study was to clarify the chemical constituents present in the leaves of *A. carambola* by means of isolation and structure elucidation. As a result, five flavan-3-ols and two 2-diglycosyloxybenzoates were isolated and their structures were determined (Fig. 1). Compounds **1** and **6** were new structures, and the others were reported from the genus *Averrhoa* for the first time.

2. Experimental

2.1. General procedures

Silica gel (100–200 mesh, Qingdao Haiyang Chemical Co., China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography (CC). Thin layer chromatography (TLC) was conducted on pre-coated silica gel $HSGF_{254}$ plates (Jiangyou Silica Gel Development Co., Yantai, China) and visualized under ultraviolet (UV) light (λ = 254 nm) and then by heating after sprayed 10% sulfuric acid in ethanol (*v*/v). Medium pressure liquid chromatography (MPLC) was performed on a LC3000 set connected to a UV3000 scanning spectrophotometer detector (Beijing ChuangXin TongHeng Sci. & Tech. Co., China) and the column used was 400 mm × 40 mm i.d., Chromatorex RP-18 SMB100, particle size 20–45 μm (Shanghai Lisui *E*-Tech Co., China). HPLC was run on a LC-6 CE liquid chromatograph (Shimadzu, Kyoto, Japan) connected to a RID-10A refractive index detector (Shimadzu) and the columns used were 250 mm × 4.6 mm i.d. and 250 mm × 20 mm i.d., Cosmosil 5C18-MS-II, 5 μm (Nacalai Tesque, Inc., Kyoto, Japan) for analysis and preparation, respectively. Electrospray ionization mass spectrometry (ESI-MS) spectra were measured on a MDS SCIEX API 2000 LC/MS/MS apparatus (Applied Biosystems Inc., Forster, CA, USA). High resolution (HR)-ESI-MS spectra were measured on a Bruker maXis mass spectrometer, a full MS scan was performed in the range of *m/z* 100–1500 Da, capillary voltage was set at 4.5 kV, and end plate offset voltage was −500 V. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 NMR spectrometer at 25 °C in CD_3OD (Qingdao Tenglong Weibo Technology Co., China) or DMSO-d_6 [Sigma-Aldrich (Shanghai) Trading Co., China] using solvent residual peaks as reference. ^{1}H NMR spectra were run at 500.13 MHz proton frequency and the spectral width was 7500 Hz. ^{13}C NMR spectra were run at 125.77 MHz spectrometer frequency and the spectral width was 28,850 Hz. Heteronuclear multiple bond correlation (HMBC) experiments were measured using gradient selected sequences with 512 transients and 2048 data points for each of the 128 increments. The spectral widths were set at 5100 Hz for ^{1}H and 27,500 Hz for ^{13}C in HMBC experiment. Optical rotation (α_D) and ultraviolet (UV) spectra were acquired on a 343 polarimeter and a Lambda 650 UV/Vis spectrophotometer (Perkin-Elmer, Waltham, MA, USA), respectively. ECD spectra were recorded on a Chirascan circular dichroism spectrometer (Applied Photophysics Ltd., Surrey, UK).

Ethanol, chloroform ($CHCl_3$), *n*-butanol, and methanol (MeOH) for extraction and CC were of analytical grade. MeOH and acetonitrile (CH_3CN) for LC were of HPLC grade. ABTS, DPPH, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), porcine pancreatic lipase type II crude [EC3.1.1.3, L3126, 100–500 units/mg protein, using olive oil (30 min incubation), 30–90 units/mg protein (using triacetin)], α-glucosidase from *Saccharomyces cerevisiae* Type I [lyophilized powder, ≥10 units/mg protein, using *p*-nitrophenyl α-D-glucoside (*p*-NPG) as substrate], 4-methylumbelliferyl oleate (4-MUO, 75164), and orlistat (O4139) were from Sigma-Aldrich (Shanghai) Trading Co., China. *p*-NPG was from J& K Scientific Co., Beijing, China. Phosphate buffered saline (PBS) was from Life technologies of Thermo Fisher Scientific, Shanghai, China. L-Ascorbic acid was from Shanghai Boao Biotech Co., China. Corosolic acid was from Chengdu Herbpurify Co., China. Authentic D- and L-glucose were from Aladdin Industrial Corp., Shanghai, China. D- and L-Apiose were from Toronto Research Chemicals Inc., Canada. L-Cystein methyl ester hydrochloride and *o*-tolylisothiocyanate were from Shanghai Macklin Biochemical Co., China and Tokyo Chemical Industry Co., Japan, respectively.

2.2. Plant material

Fresh leaves of *Averrhoa carambola* L. (Oxalidaceae) were collected from an orchard (113°33′7"E and 23°37′55"N) in Xiaozhou Village, Haizhu District, Guangzhou, in August of 2018, and botanically authenticated by Prof. Huagu Ye in the Herbarium of South China Botanical Garden, Chinese Academy of Science, Guangzhou. A voucher specimen (No. AcL1808) was deposited at our laboratory.

2.3. Extraction and isolation

Air-dried leaves were ground and the powder (15.45 kg) was extracted with 90% aq. ethanol (60 L and 45 L) twice and 50% aq. ethanol (45 L) once for 2 d per time at r.t. (25–35 °C). The filtrated and combined solutions were evaporated under vacuum to 15.6 L, which was fractionated in funnels with $CHCl_3$ thrice (10.4 L × 3) and *n*-butanol for four times (10.4 L × 4) to yield $CHCl_3$-soluble (820 g) and *n*-butanol-soluble (1050 g) fractions after condensed under vacuum to dryness. The later fraction (1040 g) was dissolved in MeOH, mixed with 1340 g of silica gel, condensed to dryness, ground, and passed through a 40 mesh sieve. The sample powder was poured into a glass column (11.8 cm in diameter), which was filled with 2800 g of silica gel in $CHCl_3$ in advance. The volume of silica gel including sample was 11.5 L (105 cm in height). The column was eluted with $CHCl_3$ until the

outflow of sample (counting as eluent 1, 1 L per eluent) and then eluted with a mixture of $CHCl_3$/MeOH at the increase rate of 2% MeOH (*v*/v, 98:2, 11.5 L → 94:4, 11.5 L → 96:4, 11.5 L → …… → 52:48, 11.5 L → 50:50, 11.5 L). All the eluents were detected by TLC and combined into fractions 1–10 according to their TLC profiles. Fraction 6 (eluents 71–77, 20.74 g) was separated by MPLC eluted with MeOH/H_2O (*v*/v, 20:80–100:0) to afford fractions 6–1–6-15. Fraction 6–7 (0.50 g) was separated by Sephadex LH-20 CC eluted with MeOH and purified by HPLC using MeOH/H_2O (*v*/v, 20:80) as mobile phase at the flow rate of 7 mL/min to provide compound **7** [retention time (t_R) = 71 min, 11.2 mg]. Fraction 6–9 (1.70 g) was separated by Sephadex LH-20 CC and purified by HPLC using MeOH/H_2O (*v*/v, 28:72) as mobile phase at 7 mL/min to yield compounds **1** (t_R = 84 min, 3.9 mg) and **2** (t_R = 68 min, 15.6 mg). Fraction 6–10 (1.90 g) was separated by Sephadex LH-20 CC and purified by HPLC using MeOH/H_2O (*v*/v, 47:53) as mobile phase at 7 mL/min to give compound **3** (t_R = 23 min, 19.0 mg). Fraction 6–12 (0.98 g) was separated by Sephadex LH-20 CC and purified by HPLC using MeOH/H_2O (*v*/v, 50:50) as mobile phase at 7 mL/min to furnish compound **6** (t_R = 23 min, 1.6 mg). Fraction 8 (eluents 90–110, 115.50 g) was separated by MPLC eluted with MeOH/H_2O (*v*/v, 20:80–90:10) to obtain fractions 8–1–8-8. Fraction 8–7 (70.2 g) was further separated by MPLC to furnish fractions 8–7–1–8-7-13. Fraction 8–7-5 (1.43 g) was separated by Sephadex LH-20 CC and purified by HPLC using MeOH/H_2O (v/v, 27:73) as mobile phase at 7 mL/min to yield compounds **4** (t_R = 12 min, 40.6 mg) and **5** (t_R = 70 min, 26.5 mg).

2.4. Spectroscopic data of compounds *1* and *4–6*

2.4.1. Epicatechin-(5,6-bc)-4β-(p-hydroxyphenyl)-dihydro-2(H)-pyranone (1)

Yellowish powder (MeOH); $[\alpha]_D^{20}$ + 4.6 (*c* 0.13, MeOH); UV (MeOH) λ_{max} nm (log ε) 206 (4.43), 232 (4.24), 258 (3.46), and 282 (3.78); ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 213 (−6.23), 232 (+3.38), 246 (−3.28), and 288 (+1.52); HR-ESI-MS *m/z* 437.1234 $[M + H]^+$ (calcd for $C_{24}H_{21}O_8^+$ 437.1231, error − 0.3 mDa) and 459.1065 $[M + Na]^+$ (calcd for $C_{24}H_{20}NaO_8^+$ 459.1050, error − 1.4 mDa); ^1H (500 MHz) and ^{13}C (125 MHz) NMR data in CD_3OD, see Table 1.

2.4.2. 6-(S-2-Pyrrolidinone-5-yl)epicatechin (4)

Yellowish powder (MeOH); $[\alpha]_D^{20}$ + 5.1 (*c* 0.81, MeOH); UV (MeOH) λ_{max} nm (log ε) 206 (4.38), 256 (3.16), and 281 (3.49); ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 204 (−5.66), 224 (+2.14), 276 (+0.42), and 303 (−0.30); ESI-MS *m/z* 374 $[M + H]^+$, 396 $[M + Na]^+$, and 372 $[M - H]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data in DMSO-d_6, see Table 2.

2.4.3. 6-(R-2-Pyrrolidinone-5-yl)epicatechin (5)

Yellowish powder (MeOH); $[\alpha]_D^{20}$ + 24.9 (*c* 0.53, MeOH); UV (MeOH) λ_{max} nm (log ε) 205 (4.38), 256 (3.07), and 282 (3.43); ECD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 204 (−3.11), 224 (−0.08), 241 (−1.90), 280 (+0.19), and 306 (−0.13); ESI-MS *m/z* 374 $[M + H]^+$, 396 $[M + Na]^+$, and 372 $[M - H]^-$; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data in DMSO-d_6, see Table 2.

2.4.4. Benzyl 2-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranosyloxybenzoate (6)

White powder (MeOH); $[\alpha]_D^{20}$–45.3 (*c* 0.17, MeOH); HR-ESI-MS *m/z* 545.1627 $[M + Na]^+$ (calcd for $C_{25}H_{30}NaO_{12}^+$ 545.1629, error + 0.2 mDa); ^1H (500 MHz) and ^{13}C (125 MHz) NMR data in CD_3OD, see Table 1.

2.5. Determination of glucosyl and apiosyl absolute configurations

Compound **6** (0.5 mg) was dissolved in 5 mL of 2 M aqueous HCl and refluxed at 95 °C for 4 h. The solution was evaporated under vacuum. The residue was dissolved in 5 mL of water and partitioned with 5 mL of ethyl acetate thrice. The aq. layer was condensed under vacuum to give a residue. Authentic D-glucose, L-glucose, D-apiose, and L-apiose (1 mg each) and the residue were dissolved in 1 mL of pyridine containing 1 mg/mL L-cystein methyl ester hydrochloride, respectively.

Table 1
^1H and ^{13}C NMR data of compounds 1 and 6 in CD_3OD.

1			**6**		
No.	δ_H (mult., *J* in Hz)	δ_C	No.	δ_H (mult., *J* in Hz)	δ_C
2	4.91 (br s)	80.1	1		122.4
3	4.24 (br dd, 4.7, 2.8)	66.9	2		158.8
4	3.05 (dd, 16.9, 4.7)	29.3	3	7.40 (br d, 7.9)	119.0
	2.84 (dd, 16.9, 2.8)		4	7.57 (td, 7.9, 1.8)	135.3
5		152.4	5	7.13 (td, 7.9, 1.8)	123.7
6		107.2	6	7.79 (dd, 7.9, 1.8)	132.1
7		154.6	7		167.8
8	6.27 (s)	100.1	1’		137.5
9		156.3	2’	7.48 (br d, 7.1)	129.3
10		100.9	3’	7.39 (br t, 7.1)	129.6
1′		131.9	4’	7.34 (tt, 7.1, 1.5)	129.3
2′	7.00 (d, 2.0)	115.3	5’	7.39 (br t, 7.1)	129.6
3′		145.9	6’	7.48 (br d, 7.1)	129.3
4′		146.0	7’	5.37 (d, 12.4)	68.0
5′	6.78 (d, 8.2)	115.9		5.33 (d, 12.4)	
6′	6.83 (dd, 8.2, 2.0)	119.4	1”	4.87 (d, 7.6)	104.0
1″		134.1	2”	3.51 (dd, 7.6, 9.0)	74.9
2″	6.95 (d, 8.5)	128.9	3”	3.36 (t, 9.0)	77.3
3″	6.68 (d, 8.5)	116.3	4”	3.47 (t, 9.0)	71.5
4″		157.3	5”	3.61 (m)	77.6
5″	6.68 (d, 8.5)	116.3	6”	4.03 (brd, 10.0)	68.8
6″	6.95 (d, 8.5)	128.9		3.63 (dd, 10.0, 6.6)	
7″	4.51 (dd, 6.8, 1.9)	35.0	1‴	4.99 (d, 2.5)	111.1
8″	3.03 (dd, 15.8, 6.8)	38.3	2‴	3.91 (d, 2.5)	78.0
	2.91 (dd, 15.8, 1.9)		3‴		80.5
9″		170.4	4‴	3.97 (d, 9.6)	75.0
				3.75 (d, 9.6)	
			5‴	3.57 (2H, s)	65.5

Table 2
^1H and ^{13}C NMR data of compounds **4** and **5** in DMSO-d_6.

	4		**5**	
No.	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2	4.77 (br s)	78.2	4.72 (br s)	77.8
3	4.00 (br dd, 4.5, 3.7)	64.5	4.03 (br t, 4.0)	64.8
4	2.69 (dd, 16.3, 4.5)	28.2	2.73 (dd, 16.3, 4.0)	28.7
	2.47 (dd, 16.3, 3.7)		2.52 (dd, 16.3, 4.0)	
5		155.2		154.5
6		106.6		109.1
7		154.4		154.6
8	6.00 (s)	95.1	5.89 (s)	95.2
9		154.0		154.1
10		98.6		99.6
1′		130.5		130.5
2′	6.87 (br s)	114.7	6.89 (br s)	114.8
3′		144.4		144.4
4′		144.5		144.5
5′	6.68 (d, 8.2)	114.8	6.67 (d, 8.1)	114.9
6′	6.66 (dd, 8.2, 1.6)	117.9	6.65 (dd, 8.1, 1.7)	117.9
2″		176.6		176.9
3″	2.19 (2H, m)	25.8	2.33 (ddd, 17.2, 11.2, 5.5)	26.0
			2.16 (ddd, 17.2, 9.0, 5.5)	
4″	2.21 (m)	30.8	2.24 (ddd, 11.2, 9.0, 5.5)	31.0
	2.07 (m)		2.06 (tt, 11.2, 5.5)	
5″	5.14 (t, 7.2)	47.4	5.13 (dd, 9.0, 5.5)	47.7
3-OH	4.60 (br s)		4.09 (br s)	
5-OH	9.01 (br s)		8.27 (br s)	
7-OH	9.02 (br s)		9.11 (br s)	
3′-OH	8.74 (br s)		8.76 (br s)	
4′-OH	8.74 (br s)		8.76 (br s)	
1″-NH	7.30 (br s)		7.37 (br s)	

After each solution was heated at 60 °C for 1 h, 2 μL of *o*-tolylisothiocyanate was added and kept at 60 °C for additional 1 h, and then condensed under vacuum. Each residue was dissolved in 0.5 mL of MeOH and analyzed by HPLC at 40 °C on a Prominence LC-20AT connected to a SPD-M20A diode array detector and a CTO-20A column oven (Shimadzu) at 254 nm, and the column used was Cosmosil 5C18-MS-II using CH_3CN/H_2O (22:78, *v*/v/v) as mobile phase at the flow rate of 1.0 mL/min for 60 min, and then washed with CH_3CN [14].

2.6. ECD computation

The ECD computational spectra of compounds **4** and **5** were obtained following our previous methods as described [15].

2.7. Antioxidant activity assays

Antioxidant activities were evaluated by *in vitro* ABTS radical cation scavenging assay, DPPH radical scavenging assay, and FRAP assay following our previous methods [16]. Their IC_{50} values were calculated from the data of three independent experiments.

2.8. Lipase and α-glucosidase inhibition assays

The assays for pancreatic lipase and α-glucosidase inhibitory activities were conducted following our previous methods [17,18].

3. Results and discussion

3.1. Structure elucidation

Compound **1** was assigned the molecular formula $C_{24}H_{20}O_8$ from its HR-ESI-MS and NMR data. ^{1}H NMR spectrum (Table 1) showed the signals of three ABX type (H-3′, 5′, and 6′), two A_2B_2 type (H-2″/6″ and H-3″/5″), and one singlet (H-8) aromatic protons, three methine protons at *δ* 4.91 (1H, br s, H-2), 4.24 (1H, br dd, *J* = 4.7, 2.8 Hz, H-3), and 4.51 (1H, dd, *J* = 6.8, 1.9 Hz, H-7“), and four protons for two methylenes (H_2-4 and H_2-8”). ^{13}C NMR spectrum exhibited the signals of a carboxy at *δ* 170.4 (C-9“), eighteen aromatic carbons ranging from *δ* 157.3 to 100.1, two oxygenated carbons at *δ* 80.1 (C-2) and 66.9 (C-3), and three aliphatic carbons at *δ* 38.3 (C-8”), 35.0 (C-7″), and 29.3 (C-4). The HMBC correlations (Fig. 2) from H-2 to C-2′ and C-6′, H-2′ and H-6′ to C-2, in combination with the broad singlet of H-2, led to an epicatechin skeleton [19]. Moreover, the HMBC correlations from H-7″ to C-5, C-6, C-7, C-8“, and C-9”, and H_2–8“ to C-6, C-7”, and C-9“ suggested the presence of a heterocyclic six-membered lactone ring. Further, the HMBC correlations from H-2”/6“ to C-4” and C-7″, H-3″/5“ to C-1”, H-7″ to C-1″ and C-2″/6″, and H_2–8″ to C-1″ revealed the presence of a *p*-hydroxyphenyl moiety and its connection to C-4 of the lactone (*i.e.* C-7″ in **1**) [20]. The positive optical rotation, positive Cotton effects at 232 and 288 nm, and a negative effect at 246 nm in the ECD spectrum (Fig. S1), which were consistent with those of cinchonain Id [21], ascertained the β-configuration of *p*-hydroxyphenyl moiety. In addition, the

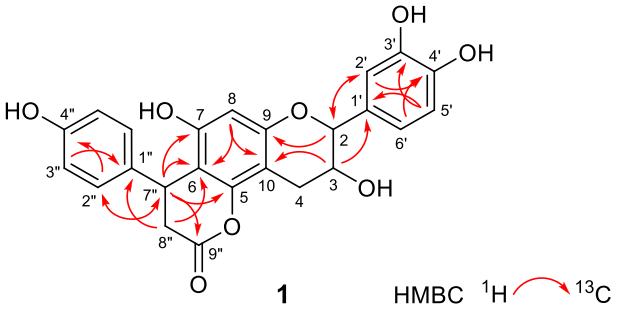

Fig. 2. Key HMBC correlations of compound **1.**

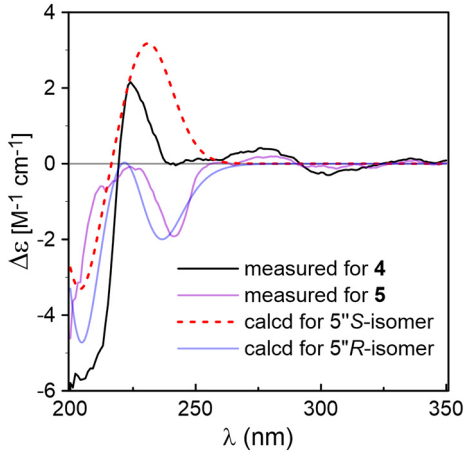

Fig. 3. Comparison of the M06-2×/TZVP/PCM (MeOH) calculated ECD spectra of (5″*S*)- and (5″*R*)-isomers with the measured spectra of compounds **4** and **5** (σ = 0.3, shift = 6 nm).

^{13}C NMR data of *p*-hydroxyphenyl and lactone moieties in **1** were consistent to those of catechin-(5,6-bc)-4β-(4″-hydroxyphenyl)-dihydro-2(*H*)-pyranone, which further substantiated the β-configuration of *p*-hydroxyphenyl moiety [22]. Hence, compound **1** was identified as epicatechin-(5,6-bc)-4β-(*p*-hydroxyphenyl)-dihydro-2(*H*)-pyranone.

Compounds **4** and **5** had the same molecular formula $C_{19}H_{19}NO_7$ deduced from their ESI-MS and NMR data. Analysis of their ^{1}H and ^{13}C NMR and HMBC spectra led to a planar structure, 6-(2-pyrrolidinone-5-yl)epicatechin, which was isolated only from *Actinidia arguta* (Actinidiaceae) by Jang et al. (2009) [23] as a 5:3 mixture of two diastereomers. The ^{1}H and ^{13}C NMR data of **5** in CD_3OD (Supplementary material) were consistent with those of the major diastereomer. It was the first report of the isolation of an *R* or an *S* monomeric diastereomer and their NMR data in DMSO-d_6. Their absolute configurations were determined to be 2*R*,3*R*,5“*S* in **4** and 2*R*,3*R*,5”*R* in **5** by ECD computation (Fig. 3).

Compound **6** was determined the molecular formula $C_{25}H_{30}O_{12}$ based on its $[M + Na]^+$ peak in the HR-ESI-MS spectrum and NMR data. ^{1}H and ^{13}C NMR spectra (Table 1) showed the signals of nine aromatic protons ranging from *δ* 7.79 to 7.13 (H-3–6 and H-2′–6′), two protons of an isolated oxygenated methylene at *δ* 5.37 and 5.33 (1H each, d, *J* = 12.4 Hz, H_2–7′), a carboxy at *δ* 167.8 (C-7), twelve aromatic carbons ranging from *δ* 158.8 to 119.0 (C-1–6 and C-1′–6′), and six carbons readily assigned for a β-glucosyl moiety [14]. Analysis of the *J* values of nine aromatic protons and aforementioned NMR data clarified a benzyl 2-β-glucosyloxybenzoate [24]. The remaining signals including *δ* 4.99 (1H, d, *J* = 2.5 Hz, H-1“'), two protons of an isolated oxygenated methylene at *δ* 3.97 and 3.75 (1H each, d, *J* = 9.6 Hz, H_2-4”'), and the carbons at *δ* 111.1 (C-1“') and 80.5 (C-3”') as well as *δ* 68.8 (C-6“) were characteristic of a β-apiofuranosyl moiety and its connection to C-6” of β-glucosyl moiety [25]. Acid hydrolysis of **6** liberated D-glucose and D-apiose, which were determined by comparing their HPLC analytic t_R values with those of authentic D- and L-glucose and D- and L-apiose (Fig. S2). Therefore, compound **6** was identified as benzyl 2-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranosyloxybenzoate.

Other known compounds were identified as epicatechin-(7,8-bc)-4α-(*p*-hydroxyphenyl)-dihydro-2(*H*)-pyranone (**2**) [26], epicatechin-(7,8-bc)-4β-(*p*-hydroxyphenyl)-dihydro-2(*H*)-pyranone (**3**) [26], and methyl 2-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranosyloxybenzoate or canthoside A (**7**) [25] by comparison of their spectroscopic data with those reported in the literatures.

Compounds **1–3** are phenylpropanoid-substituted flavan-3-ols, which are a kind of tannin (proanthocyanidins) that occurs in plants of a woody habit as minor constituents [27]. Nanoka & Nishioka (1982) isolated a new class of flavan-3-ols substituted at the A-ring with a

Table 3
Antioxidant activity of compounds **1–7**.

Compound	ABTS (IC_{50}, μM)	DPPH (IC_{50}, μM)	FRAP (mmol/g)
1	4.7 ± 0.2	14.3 ± 0.3	13.8 ± 0.2
2	4.1 ± 0.1	17.2 ± 0.2	14.8 ± 0.1
3	4.0 ± 0.1	18.9 ± 0.6	15.1 ± 0.2
4	7.3 ± 0.1	22.3 ± 0.8	14.7 ± 0.1
5	8.6 ± 0.2	13.7 ± 0.9	16.1 ± 0.1
6	> 50	> 50	8.6 ± 0.1
7	> 50	> 50	8.0 ± 0.1
L-Ascorbic acid	23.1 ± 0.2	44.5 ± 2.9	10.0 ± 0.1

Values represent mean ± SD (n = 3).

C_6–C_3 unit, cinchonains Ia, Ib, Ic, and Id from *Cinchona succirubra* Pavon et Klotzsch (Rubiaceae), *Uncaria rhynchophylla* Miquel (Rubiaceae), *Kandelia candel* (L.) Druce (Rhizophoraceae), *Polygonum bistorta* L. (Polygonaceae), and *Raphiolepis umbellata* Makino (Rosaceae) [20]. To the best of our knowledge, no less than thirty phenylpropanoid-substituted flavan-3-ols have hitherto been isolated from plants (Table S1), including *Kandelia candel* [28], *Phyllocladus trichomanoides* D. Don (Podocarpaceae) [29,30], *Castanopsis hystrix* Hook. F. & Thomson ex A. Dc (Fagaceae) [21], *Ocotea porosa* (Nees) L. Barr. (Lauraceae) [26], *Apocynum venetum* L. (Apocynaceae) [31], *Camellia sinensis* var. *assamica* (Masters) Kitamura (Theaceae) [32,33], *Taxus cuspidata* Sieb. et Zucc. (Taxaceae) [22], *Litchi chinensis* Sonn. (Sapindaceae) [34], *Smilax corbularia* Kunth. (Smilacaceae) [35], *Trichilia catigua* A. Juss. (Meliaceae) [36], *Melastoma dodecandrum* Lour. (Melastomataceae) [37], *Agrimonia pilosa* (Rosaceae) [38], *Uncaria rhynchophylla* [39], *Viburnum congestum* Rhed. (Caprifoliaceae) [40], and *Hypericum elatoides* R. Keller (Hypericaceae) [41].

3.2. Antioxidant activity

As shown in Table 3, compounds **1–5** demonstrated more potent ABTS radical cation and DPPH radical scavenging activities with the IC_{50} values ranging from 8.6 ± 0.2 to 4.1 ± 0.1 μM than L-ascorbic acid (23.1 ± 0.2 μM) for ABTS and the IC_{50} values ranging from 22.3 ± 0.8 to 13.7 ± 0.9 μM than L-ascorbic acid (44.5 ± 2.9 μM) for DPPH. With regard to FRAP, compounds **1–5** were more potent (ranging from 13.8 ± 0.2 to 16.1 ± 0.1 μM) than L-ascorbic acid (10.0 ± 0.1 μM), **6** and **7** were comparable to L-ascorbic acid.

3.3. α-Glucosidase and lipase inhibitory activities

Compound **1** exhibited weak inhibitory activity (IC_{50} = 41.8 ± 0.9 μM) in comparison with clinical drug, orlistat (IC_{50} = 4.5 ± 0.3 μM), while the inhibitory percentages of compounds **2–7** were < 25% at the maximum test concentration of 50 μM. Compound **3** showed comparable α-glucosidase inhibitory activity (IC_{50} = 9.6 ± 0.4 μM) to corosolic acid (IC_{50} = 10.0 ± 0.2 μM), whereas the inhibitory percentages of the others were < 40% at 50 μM.

4. Conclusion

This study clarified the structures of three phenylpropanoid-substituted flavan-3-ols, a pair of nitrogen-containing diastereomers, and two 2-diglycopyranosylbenzoates from the leaves of *Averrhoa carambola*. Two compounds were new structures and the others were described from the genus *Averrhoa* for the first time. These compounds showed *in vitro* antioxidant, pancreatic lipase and α-glucosidase inhibitory activities, which suggested their participation in the biological activities of the leaves.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104442.

References

[1] T.K. Lim, Averrhoa carambola, Edeible Medicinal and Non-Medicinal Plants, Springer, Dordrecht, 2012, https://link.springer.com/chapter/10.1007%2F978-90-481-8661-7_60.
[2] F.Z. Liu, X.M. Song, X.L. Wang, T. Liu, R.F. Liang, Hypoglycemic effect of total flavones from *carambola* leaf, Chin. J. Exp. Tradit. Med. Form. 19 (11) (2013) 279–281, https://doi.org/10.11653/syfj2013110279.
[3] R. Soncini, M.B. Santiago, L. Orlandi, G.O.I. Moraes, A.L.M. Peloso, M.H. dos Santos, G. Alves-da-Silva, V.A. Paffaro Jr., A.C. Bento, A. Giusti-Paiva, Hypotensive effect of aqueous extract of *Averrhoa carambola* L. (Oxalidaceae) in rats: An *in vivo* and *in vitro* approach, J. Enthnopharmacol. 133 (2011) 353–357, https://doi.org/10.1016/j.jep.2010.10.001.
[4] S.D. Wei, H. Chen, T. Yan, Y.M. Lin, H.C. Zhou, Identification of antioxidant components and fatty acid profiles of the leaves and fruits of *Averrhoa carambola*, LWT – Food Sci. Technol. 55 (2014) 278–285, https://doi.org/10.1016/j.lwt.2013.08.013.
[5] G.L. Chen, X. Zhang, S.G. Chen, M.D. Han, Y.Q. Gao, Antioxidant activities and contents of free, esterified and insoluble-bound phenolics in 14 subtropical fruit leaves collected from the south of China, J. Funct. Foods 30 (2017) 290–302, https://doi.org/10.1016/j.jff.2017.01.011.
[6] D.A. Cabrini, H.H. Moresco, P. Imazu, C.D. da Solva, E.F. Pietrovski, D.A.G.B. Mendes, A.d.S. Prudente, M.G. Pizzolatte, I.M.C. Brighente, M.F. Otuki, Analysis of the potential topical anti-inflammatory activity of *Averrhoa carambola* L. in mice, Evid. Based Complement. Alternat. Med. (2011) 2011908509, https://doi.org/10.1093/ecam/neq026.
[7] S.T. Gonçalves, S. Baroni, F.A. Bersani-Amado, G.A.N. Melo, D.A.G. Cortez, C.A. Bersani-Amado, R.K.N. Cuman, Preliminary studies on gastric anti-ulcerogenic effect of *Averrhoa carambola*, Acta Farm. Bonaer. 25 (2006) 245–247.
[8] T. Poongodi, T.H. Nazeema, *In vitro* cytotoxicity, phytochemistry and GC-MS analysis of *Averrhoa carambola* (leaf) against MCF-7 breast cancer cell line, Int. J. Curr. Res. 8 (2016) 29044–29048.
[9] D. Araho, M. Miyakoshi, W.H. Chou, T. Kambara, K. Mizutani, T. Ikeda, A new flavone *C*-glycoside from the leaves of *Averrhoa carambola*, Nat. Med. (Tokyo) 59 (2005) 113–116, https://doi.org/10.1016/j.apsusc.2004.03.089.
[10] Y. Wang, M. Liu, L. Liu, J.H. Xia, Y.G. Du, J.S. Sun, The structural revision and total synthesis of carambolaflavone A, J. Org. Chem. 83 (2018) 4111–4118, https://doi.org/10.1021/acs.joc.8b00008.
[11] H.H. Moresco, G.S. Queiroz, M.G. Pizzolatti, I.M.C. Brighente, Chemical constituents and evaluation of the toxic and antioxidant activities of *Averrhoa carambola* leaves, Rev. Bras. Pharmacogn. 22 (2012) 319–324, https://doi.org/10.1590/S0102-695X2011005000217.
[12] L.H. Cazarolli, P. Folador, H.H. Moresco, I.M.C. Brighente, M.G. Pizzolatti, F.R.M.B. Silva, Mechanism of action of the stimulatory effect of apigenin-6-C-(2″-O-α-L-rhamnopyranosyl)-β-L-fucopyranoside on ^{14}C-glucose uptake, Chem. Biol. Interact. 179 (2009) 407–412, https://doi.org/10.1016/j.cbi.2008.11.012.
[13] L.H. Cazarolli, V.D. Kappel, D.F. Pereira, H.H. Moresco, I.M.C. Brighente, M.G. Pizzolatti, F.R.M.B. Silva, Anti-hyperglycemic action of apigenin-6-C-β-fucopyranoside from *Averrhoa carambola*, Fitoterapia 83 (2012) 1176–1183, https://doi.org/10.1016/j.fitote.2012.07.003.
[14] P. Gou, Y. Xiao, L. Lv, H. Xie, Hydroquinone and terpene glucosides from *Leontopodium leontopodioides* and their lipase inhibitory activity, Fitoterapia 130 (2018) 89–93, https://doi.org/10.1016/j.fitote.2018.08.010.
[15] G. Liao, P. Wu, J. Xue, L. Liu, H. Li, X. Wei, Asperimides A–D, anti-inflammatory aromatic butenolides from a tropical endophytic fungus *Aspergillus terreus*, Fitoterapia 131 (2018) 50–54, https://doi.org/10.1016/j.fitote.2018.10.011.
[16] Q. Ma, H. Xie, S. Li, R. Zhang, M. Zhang, X. Wei, Flavonoids from the pericarps of *Litchi chinensis*, J. Agric. Food Chem. 62 (2014) 1073–1078, https://doi.org/10.1021/jf405750p.
[17] Y. Xiao, H. Xie, L. Zhao, P. Gou, Acyl flavone and lignan glucosides from *Leontopodium leontopodioides*, Phytochem. Lett. 17 (2016) 247–250, https://doi.org/10.1016/j.phytol.2016.08.006.
[18] X. Jia, D. Yang, H. Xie, Y. Jiang, X. Wei, Non-flavonoid phenolics from *Averrhoa carambola* fresh fruit, J. Funct. Foods 32 (2017) 419–425, https://doi.org/10.1016/

j.jff.2017.03.025.
[19] J. Shi, J. Sun, X. Wei, J. Shi, G. Cheng, M. Zhao, J. Wang, B. Yang, Y. Jiang, Identification of (−)-epicatechin as the direct substrate for polyphenol oxidase from longan fruit pericarp, LWT–Food Sci. Technol. 41 (2008) 1742–1747, https://doi.org/10.1016/j.lwt.2008.02.003.
[20] G.I. Nonaka, I. Nishioka, Tannins and related compounds. VII. Phenylpropanoid-substituted epicatechins, cinchonains from *Cinchona succirubra*, Chem. Pharm. Bull. 30 (1982) 4268–4276.
[21] H.F. Chen, T. Tanaka, G.I. Nonaka, T. Fujioka, K. Mihashi, Phenylpropanoid-substituted catechins from *Castanopsis hystrix* and structure revision of cinchonains, Phytochemistry 33 (1993) 183–187, https://doi.org/10.1016/0031-9422(93)85419-R.
[22] J.K. Kim, Y.H. Ham, J.J. Karchesy, Y.S. Bae, A new phenylpropanoid derivative of catechin from the needles of *Taxus cuspidata*, Nat. Prod. Res. 22 (2008) 1365–1369, https://doi.org/10.1080/14786410701780134.
[23] D.S. Jang, G.Y. Lee, Y.M. Lee, Y.S. Kim, H. Sun, D.H. Kim, J.S. Kim, Flavan-3-ols having a γ-lactam from the roots of *Actinidia arguta* inhibit the formation of advanced glycation end products *in vitro*, Chem. Pharm. Bull. 57 (2009) 397–400, https://doi.org/10.1248/cpb.57.397.
[24] H. Wu, X. Hu, X. Zhang, S. Chen, J. Yang, X. Xu, Benzyl 2-β-glucopyranosyloxybenzoate, a new phenolic acid glycoside from *Sarcandra glabra*, Molecules 17 (2012) 5212–5218, https://doi.org/10.3390/molecules17055212.
[25] T. Kanchanapoom, R. Kasai, K. Yamasaki, Iridoid and phenolic diglycosides from *Canthium berberidifoliun*, Phytochemistry 61 (2002) 461–464, https://doi.org/10.1016/s0031-9422(02)00140-1.
[26] J.M. David, M. Yoshida, O.R. Gottlieb, Phenylpropanoid-catechins from bark of *Ocotea porosa*, Phytochemistry 35 (1994) 545–546, https://doi.org/10.1016/S0031-9422(00)94800-3.
[27] S. Awale, Y. Tezuka, S. Wang, S. Kadota, Facile and regioselective synthesis of phenylpropanoid-substituted flavan-3-ols, Org. Lett. 4 (2002) 1707–1709, https://doi.org/10.1021/ol020042n.
[28] F.L. Hsu, G.I. Nanoka, I. Nishioka, Tannins and related compounds. XXXI. Isolation and characterization of proanthocyanidins in *Kandelia candel* (L.) Druce, Chem. Pharm. Bull. 33 (1985) 3142–3152.
[29] L.Y. Foo, Phenylpropanoid derivatives of catechin, epicatechin and phylloflavan from *Phyllocladus trichomanoides*, Phytochemistry 26 (1987) 2825–2830, https://doi.org/10.1016/S0031-9422(00)83598-0.
[30] L.Y. Foo, Flavanocoumarins and flavanophenylpropanoids from *Phyllocladus trichomanoides*, Phytochemistry 28 (1989) 2477–2481, https://doi.org/10.1016/S0031-9422(00)98009-9.
[31] W. Fan, Y. Tezuka, Q. Xiong, M. Hattori, T. Namba, S. Kadota, Apocynins A–D: new phenylpropanoid-substituted flavan-3-ols isolated from leaves of *Apocynum venetum* (*Luobuma-Ye*), Chem. Pharm. Bull. 47 (1999) 1049–1050.
[32] Z.H. Zhou, Y.J. Zhang, M. Xu, C.R. Yang, Puerins a and B, two new 8-C substituted flavan-3-ols from Pu-er tea, J. Agric. Food Chem. 53 (2005) 8614–8617, https://doi.org/10.1021/jf051390h.
[33] M.K. Tao, M. Xu, H.T. Zhu, R.R. Cheng, D. Wang, C.R. Yang, Y.J. Zhang, New phenylpropanoid-substituted flavan-3-ols from Pu-er ripe tea, Nat. Prod. Commun. 9 (2014) 1167–1170, https://doi.org/10.1177/1934578x1400900827.
[34] X. Xu, H. Xie, Y. Wang, X. Wei, A-type proanthocyanidins from lychee seeds and their antioxidant and antiviral activities, J. Agric. Food Chem. 58 (2010) 11667–11672, https://doi.org/10.1021/jf1033202.
[35] B. Wungsintaweekul, K. Umehara, T. Miyase, H. Noguchi, Estrogenic and anti-estrogenic compounds from the Thai medicinal plant, *Smilax corbularia* (Smilacaceae), Phytochemistry 72 (2011) 495–502, https://doi.org/10.1016/j.phytochem.2010.12.018.
[36] F.O. Resende, E. Rodrigues-Filho, H. Luftmann, F. Petereit, J.C.P. de Mello, Phenylpropanoid substituted flavan-3-ols from *Trichilia catigua* and their *in vitro* antioxidant activity, J. Braz. Chem. Soc. 22 (2011) 2087–2093, https://doi.org/10.1590/S0103-50532011001100010.
[37] M. Cheng, L.J. Meng, X.D. Zhou, H.L. Zou, S.F. Yu, G.X. Zhou, Chemical constituents of flavonoids and their glycosides in *Melastoma dodecandrum*, China J. Chin. Mat. Med. 39 (2014) 3301–3305, https://doi.org/10.4268/cjcmm20141718.
[38] W.J. Liu, X.Q. Hou, H. Chen, J.Y. Liang, J.B. Sun, Chemical constituents from *Agrimonia pilosa* Ledeb. and their chemotaxonomic significance, Nat. Prod. Res. 30 (2016) 2495–2499, https://doi.org/10.1080/14786419.2016.1198351.
[39] R. Li, J. Cheng, M. Jiao, L. Li, C. Guo, S. Chen, A. Liu, New phenylpropanoid-substituted flavan-3-ols and flavonols from the leaves of *Uncaria rhynchophylla*, Fitoterapia 116 (2017) 17–23, https://doi.org/10.1016/j.fitote.2016.11.005.
[40] H. Yang, L.F. Ding, W.C. Tu, X.D. Wu, L.D. Song, Two new iridoids from *Viburnum congestum*, Nat. Prod. Res. Dev. 29 (2017) 543–548.
[41] X.T. Yan, Z. An, Y. Huangfu, Y.T. Zhang, C.H. Li, X. Chen, P.L. Liu, J.M. Gao, Polycyclic polyprenylated acylphloroglucinol and phenolic metabolites from the aerial parts of *Hypericum elatoides* and their neuroprotective and anti-nuroinflammatory activities, Phytochemistry 159 (2019) 65–74, https://doi.org/10.1016/j.phytochem.2018.12.011.