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NEW COMPOUNDS FROM *TABEBUIA AVELLANEDAE*

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Abstract – Two new compounds, a compound firstly isolated from plants, a known iridoid and four known lignan derivatives were isolated from water extract of *Tabebuia avellanedae*. The chemical structures and relative configurations of the new compounds were determined by 1D, 2D NMR and MS spectroscopic analyses.

Tabebuia avellanedae Lorentz ex Griseb, which belongs to the family *Bignoniaceae*, is a popular tree distributed throughout the tropical rain forests of Central and South America. It is called "divine tree" by indigenous peoples of South America, because it is considered to be one of the most effective, economical and versatile remedies against a multitude of acute and chronic diseases.[1] Its inner bark is commonly known as "taheebo", "lapacho", "pau d'arco", and "ipe roxo", which has been used for various ethnopharmacological prupose for over 1000 years.[2] Following its popular use, the chemistry of this plant was extensively studied, and a variety of biologically active constituents have been isolated, such as furanonaphthoquinones, naphthoquinones, quinones, lignans, benzoic acid, cyclopentene dialdehyde, flavonoids, iridoids, phenolic glycosides, saponins, and coumarins.[3-11]

In this research, we separated the water extract of inner bark of *Tabebuia avellanedae* and obtained two new compounds (**1**-**2**), a compound firstly isolated from plants (**3**), together with five known compounds (**4**-**8**). The structures of **1**-**8** were listed in Figure 1, and ^{1}H and ^{13}C-NMR spectroscopic data of **1**-**3** were listed in Table 1 to Table 3.

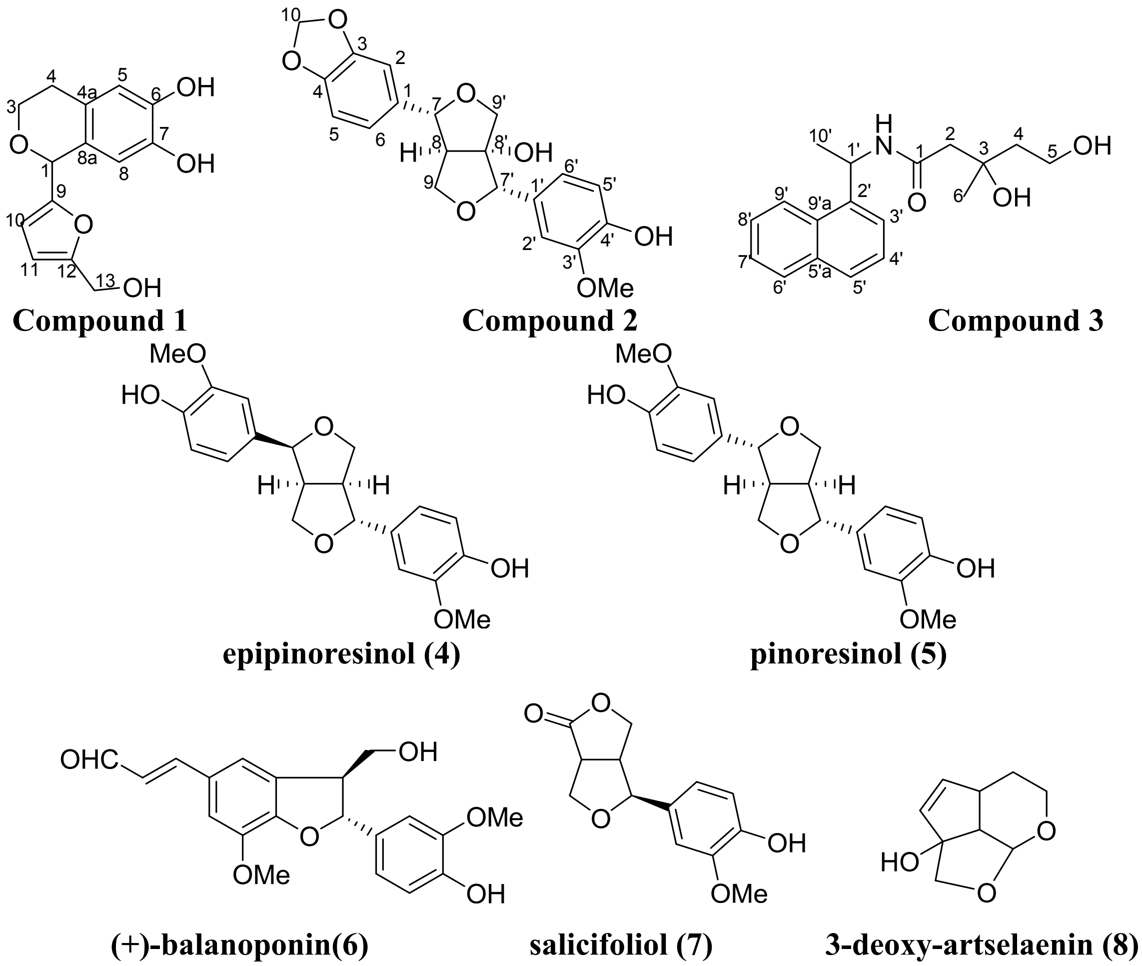
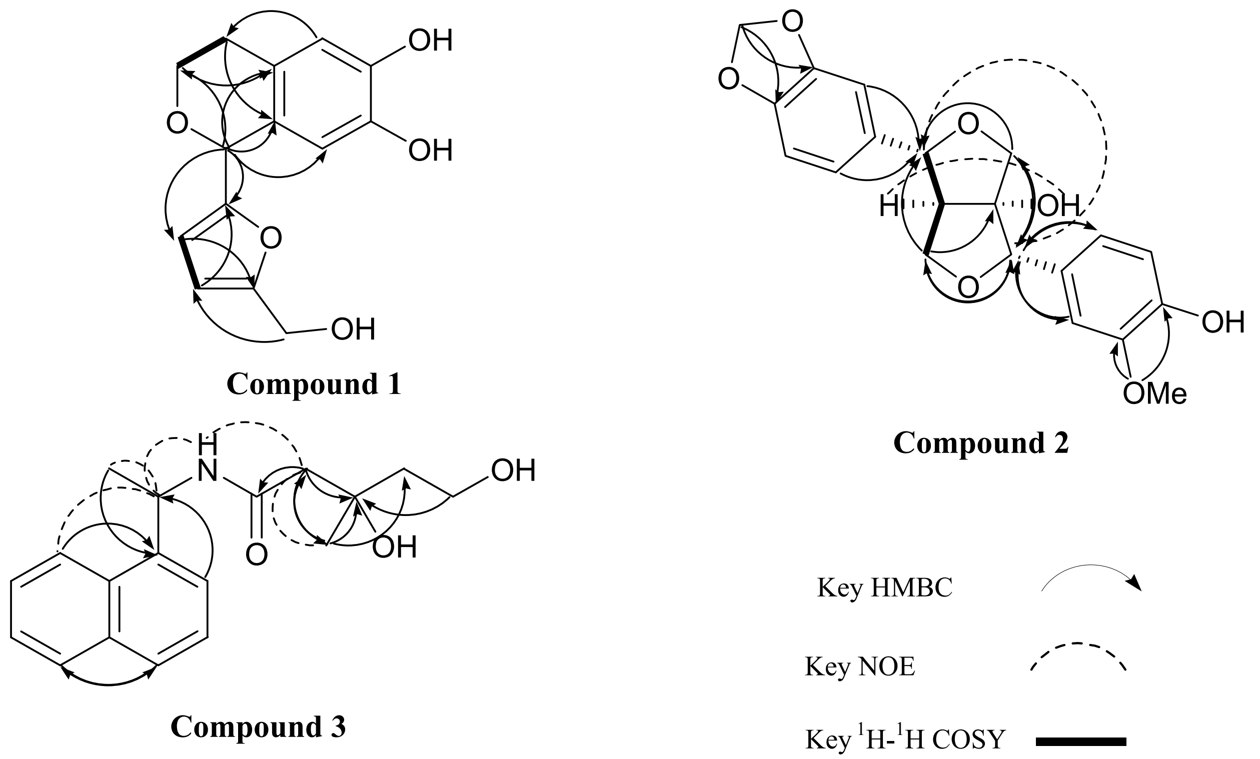


Figure 1. The structures of compounds from *Tabebuia Avellanedae*

Compound **1** was obtained as yellow powder. Its molecular formula was determined as $C_{14}H_{14}O_5$ by HRFABMS *m/z* 261.0759 [M-H]$^-$. The ^{1}H NMR spectrum of two singlet δ_H 6.55 (s) and 6.35 (s), and HMBC correlations from δ_H 6.55 (H-5) to δ_C 126.1 (C-8a) and δ_C 144.7 (C-7), from δ_H 6.35 (H-8) to δ_C 126.3 (C-4a) and δ_C 145.8 (C-6) suggested the presence of a aromatic ring. The ^{1}H NMR spectrum of four multiplet at δ_H 2.68 (m), 2.72 (m), 3.96 (m) and 3.80(m), together with ^{1}H-^{1}H COSY correlations between δ_H 3.96 (H-3α) and δ_H 2.68(H-4α), from δ_H 3.80 (H-3β) to δ_H 2.72 (H-4β), suggested the presence of one oxidized ethyl group, and the HMBC correlations from δ_H 6.55 (H-5) to δ_C 28.7 (C-4), from δ_H 2.72 (H-4β) to 126.1 (C-8a), and from δ_H 3.80 (H-3β) to δ_C 126.3 (C-4a) indicated that the oxidized methylene group is located at C-4a of the aromatic ring. The ^{1}H NMR spectrum of a singlet at δ_H 5.66 (s) suggested the presence of one oxidized methine group, and the HMBC correlations from δ_H 5.66 (H-1) to δ_C 114.0 (C-8) and 126.3 (C-4a), indicated that the oxidized methine group is located at C-8a of the aromatic ring. Furthermore, the HMBC correlations from δ_H 5.66 (C-1) to δ_C 63.0 (C-3) confirmed the presence of one isochroman group. The ^{1}H NMR spectrum of one singlet at δ_H 4.47 (2H, s) revealed the presence of one hydroxymethyl group. The remaining signals of two doublet at δ_H 6.08 (d, $J = 3.4$) and 6.23 (d, $J = 3.4$) in the ^{1}H NMR spectrum, the ^{1}H-^{1}H COSY correlations between δ_H 6.08 and δ_H 6.23 and the HMBC correlations from δ_H 6.08 (H-10) to δ_C 156.3 (C-12), from δ_H 6.23 (H-11) to δ_C 156.1 (C-9) were noted, furthermore, its molecular formula was determined as $C_{14}H_{14}O_5$, indicated the presence of one furyl group. In addition, the HMBC correlations from δ_H 4.47 (H-13) to δ_C 108.9 (C-11) proved that the hydroxymethyl group is located at the C-12 of the furyl group. The HMBC correlations from δ_H 5.66 (H-1) to δ_C 111.7 (C-10) and 156.1 (C-9), indicted the furyl group is located at C-1 of the isochroman group. Thus, compound **1** was determined as shown in Figure 1.



Compound 1

Compound 2

Compound 3

Figure 2. The key HMBC, NOE and ^{1}H-^{1}H COSY correlations of new compounds

Compound **2** was obtained as colorless oil. Its molecular formula was determined as $C_{20}H_{20}O_7$ by HRFABMS *m/z* 371.1129 [M-H]$^-$. The 1D NMR spectra and HMQC spectrum showed the presence of two ABX system aromatic rings [δ_H 6.95 (d, $J = 1.7$), 6.79 (d, $J = 8.5$), 6.90 (dd, $J = 1.7$, 8.5) and 7.03 (d, $J = 1.7$), 6.77 (d, $J = 8.5$), 6.84 (dd, $J = 1.7$,8.5)], one methylenedioxy group [δ_H 5.93 (2H, s) and δ_C 102.4] and one methoxy group [δ_H 3.86 (3H, s) and δ_C 56.4]; the presence of two doublets [δ_H 4.04 (d, $J = 9.3$), 3.84 (d, $J = 9.3$) and δ_C 76.1], and an apparent triplet and a double doublets [δ_H 4.45 (t, $J = 8.9$), 3.77 (dd, $J = 5.8$,8.9) and δ_C 72.0] for nonequivalent geminal methylene protons at C-9' and C-9, respectively, together with one double triplet [δ_H 3.00 (dt, $J = 5.8$, 8.9) and δ_C 62.6], one doublet [δ_H 4.84 (d, $J = 5.8$) and δ_C 87.6], and one singlet [δ_H 4.66 (s) and δ_C 89.2] suggested a structure of furanofuran lignan with two ABX aromatic rings. It has the similar spectra with (1*S**,2*R**,5*R**,6*S**)-6-(4-hydroxy-3-methoxyphenyl)-2-(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3.3.0]-oactan-1-ol reported by S. Yamauchi *et al.*,[12] expect for the positions of the two ABX system aromatic rings. In Figure 2**,** HMBC correlations observed from δ_H 5.93(H-10) to δ_C 149.4 (C-3), δ_C 148.7 (C-4), from δ_H 6.90 (H-6) to δ_C 107.8 (C-2), δ_C 148.7 (C-4) and δ_C 87.6 (C-7), from δ_H 6.95 (H-2) to δ_C 148.7 (C-4), δ_C 121.0 (C-6) and δ_C 87.6 (C-7), together with ^{1}H-^{1}H COSY correlations between δ_H 4.84 (H-7) and δ_H 3.00 (H-8), and among δ_H 4.45(H-9α), δ_H 3.77 (H-9β) and δ_H 3.00 (H-8), supported the methylenedioxy-substituted phenyl group is located at C-7 (not C-7') of furanofuran group. In addition, the NOE correlations (measured in DMSO) were observed between H-8 and H-8'-OH, from H-8 to H-9β, from H-7 to H-7'α, and from H-7 to H-9α. Thus, the structure was established as in Figure 1.

Compound **3** was obtained as yellow amorphous powder. It was synthesized by K. Mori and K. Okada in 1984[13] but NMR data was not showed. The 1D NMR spectra of seven protons [δ_H 7.49 (m), 7.44 (dd, *J* = 7.6,7.9) , 7.86 (d, *J* = 7.9) , 7.80 (d, *J* = 8.3) , 7.55 (m), 7.51 (m), 8.07 (d, *J* = 8.6)] and carbon signals [δ_C 137.7, 126.7, 125.2, 128.9, 133.2, 128.6, 126.0, 122.6, 123.2, 130.9], suggested the existence of more than one aromatic rings. Correlations observed in HMBC spectrum, from δ_H 8.07 (H-9') to δ_C 126.0 (C-7'), δ_C 133.9 (C-5'a) and δ_C 137.7(C-2'), from δ_H 7.51 (H-8') to δ_C 130.9 (C-9'a) and δ_C 128.6 (C-6'), from δ_H 7.80 (H-6') to δ_C 122.6 (C-8'), δ_C 130.9 (C-9'a) and δ_C 128.9 (C-5'), from δ_H 7.86 (H-5') to δ_C 128.6 (C-6'), δ_C 130.9 (C-9'a) and δ_C 126.7 (C-3'), from δ_H 7.44 (H-4') to δ_C 133.9 (C-5'a) and δ_C 137.7 (C-2'), from δ_H 7.49 (H-3') to δ_C 128.9 (C-5') and δ_C 130.9 (C-9'a) revealed presence of one naphthyl group. The 1D NMR spectra and HMQC spectrum revealed the presence of two methyl groups [δ_H 1.27 (3H, s), δ_C 26.9 and 1.68 (3H, d, *J* = 6.5), δ_C 20.7], two methylene groups [δ_H 2.46 (m), 2.20 (m), δ_C 46.5, and 1.77 (m), 1.64 (m), δ_C 42.1], one hydroxymethyl group [δ_H 3.86 (m), 3.76 (m) and δ_C 59.6], one oxidized quaternary carbon [δ_C 72.5], one lower field-shifted methine group and one carbonyl group [δ_C 171.2]. Furthermore, the HMBC correlations from δ_H 1.68 (H-10') to δ_C 137.7 (C-2') and δ_C 44.6 (C-1') and from δ_H 7.49 (H-3') to δ_C 44.6 (C-1') revealed C-1' is located at C-2' of naphthyl group. The HMBC correlations from δ_H 2.46 (H-2α) to δ_C 171.2 (C-1), δ_C 72.5 (C-3) and δ_C 26.9 (C-6), from δ_H 1.27 (H-6) to δ_C 46.5 (C-2), δ_C 72.5 (C-3) and δ_C 42.1 (C-4) and from δ_H 3.86 (H-5α), δ_H 3.76 (H-5β) to δ_C 72.5 (C-3) indicated the presence of 3,5-dihydroxy-3-methylpentanyl group. In addition, its molecular formula was determined as $C_{18}H_{23}NO_3$ by HRFABMS *m/z* 300.1606 $[M-H]^-$, confirmed the presence of nitrogen. The NOE correlations from δ_H 5.95 (H-1') to δ_H 8.07 (H-9'), δ_H 1.68 (H-10') and δ_H 6.17 (-NH-), from 2.46 (H-2α), 2.20 (H-2β) to 6.17 (-NH-) and δ_H 1.27 (H-6) revealed the imino group is located to C-1' and C-1with amido bond. Thus, the structure was established as in Figure 1.

The five known compounds epipinoresinol(**4**),[14] pinoresinol(**5**),[14] (+)-balanoponin(**6**),[15] salicifoliol(**7**),[16] and 3-deoxy-artselaenin(**8**),[17] were identified by comparing their spectroscopic data with those in the literature.

EXPERIMENTAL

General. Optical rotations were measured using a Horiba SEPA-3000 high-sensitivity polarimeter. UV spectra were measured using a Shimadzu UV-1600 UV-visible spectrometer. IR spectra were recorded on a Shimadzu IR-460 IR spectrophotometer, whereas NMR spectra were obtained using the ECA600 spectrometer in CD_3OD, $CDCl_3$ or DMSO. Chemical shifts were referenced to the residual solvent peaks (δ_H 3.30 and δ_C 49.8 for CD_3OD, δ_H 7.24 and δ_C 77.0 for $CDCl_3$, δ_H 2.49 and δ_C 39.5 for DMSO-d_6). Mass spectra were measured on the JMS-T100TD and JMS-700 mass spectrometer. Reversed-phase HPLC was carried out on C30-UG-5 (5 μm, Nomura. Chemical, Seto, Japan) and C18-AR-II(5 μm, Nacalai Tesque., Japan). Silica gel (63-210 μm, Kanto Kagaku, Japan) and ODS (63-212 μm, Wako Pure Chemical, Japan) were used for open-column chromatography. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} (Merck) and RP-18 F_{254S} (Merck).

Plant material. Water extract of *Tabebuia avellanedae* for the present investigation was taxonomically identified and extracted by Taheebo Japan Corporation. In accordance with their method, dried bark of *Tabebuia avellanedae* (10 kg) were extracted with boiling water (30 L) three times, and the water solutions were combined and concentrated in vacuo to get the crude extract. The plant sample was deposited in a database in our laboratory under registration number T-340.

Extraction and isolation. The water extract (100 g) was suspended in H_2O (1 L) and partitioned successively with *n*-hexane, EtOAc and *n*-BuOH (each 1 L, 3 times) to yield *n*-hexane fraction (0.6 g), EtOAc fraction (14.1 g), *n*-BuOH fraction (31.5 g) and H_2O fraction (65.1 g), respectively. The EtOAc fraction (14.0 g) was chromatographed on silica gel with a gradient solvent system (*n*-hexane/EtOAc/MeOH) to give 15 fractions (A1-A15). A3 and A4 (*n*-hexane/EtOAc =1/1, 2.3 g) was rechromatographed on ODS with gradient solvent (MeOH/H_2O) to afford 14 fractions (B1-B14). Fraction B1 (MeOH/H_2O= 0/1, 280mg) was subjected on ODS with gradient solvent (MeOH/ H_2O) to afford 12 fractions (C1-C12). Fraction C7 (MeOH/H_2O= 1/4, 6.2 mg) was further purified by ODS HPLC (C_{18}-AR-II) with 33% MeOH to afford compound **1** (6.0 mg). Fraction B4 (MeOH/H_2O= 1/4, 69.0 mg) was subjected on Sephadex LH-20 with MeOH to get 9 fractions (D1-D9). Fraction D6 was separated using ODS HPLC (C_{18}-AR-II) with 20% MeOH to afford compound **8** (6.0 mg). Fraction B5 (MeOH/H_2O= 1/4, 119.2 mg) was applied to Sephadex LH-20 with MeOH to get 3 fractions (E1-E3), and fraction E3 was further purified by C_{30} HPLC (C_{30}-UG-5) with 40% MeOH to obtain compound **7** (2.9 mg). Fractions B11 and B12 (MeOH/H_2O= 3/4, 79.8 mg) was subjected on Sephadex LH-20 with

MeOH to get 6 fractions (F1-F6), and fraction F4 (40.0 mg) was loaded to silica gel with a gradient solvent system ($CHCl_3$/MeOH) to give 11 fractions (G1-G11). Fraction G4 (6.9 mg) was purified by ODS HPLC (C18-AR-Ⅱ) with 50% MeOH and 55% MeOH to afford compound **5** (0.9 mg) and **2** (1.6 mg) and fraction H. Fraction H (1.8 mg) was further purified by C_{30} HPLC (C_{30}-UG-5) with 52% MeOH to afford compound **4** (1.0 mg). Fractions G6 and G7 (3.0 mg) was purified by C_{30} HPLC (C_{30}-UG-5) with 50% MeOH to afford compound **6** (1.2 mg). Fraction B13 ($MeOH/H_2O$= 3/4, 100.9 mg) was separated using Sephadex LH-20 with MeOH to get 6 fractions (I1-I6), and fractions I3 and I4 (85.0 mg) was subjected on silica gel with a gradient solvent system (*n*-hexane/EtOAc) to give 10 fractions (J1-J10). Fraction J9 (21.2 mg) was further purified by ODS HPLC (C_{18}-AR-Ⅱ) with 60% MeOH to afford compound **3** (1.0 mg).

Table 1. ^{13}C NMR and ^{1}H NMR data of compound **1** in CD_3OD

Compound **1**					
Position	δ_C	δ_H (mult, *J* Hz)	*Position*	δ_C	δ_H (mult, *J* Hz)
1	72.6	5.66 (s)	7	144.7	
3α	63.0	3.96 (m)	8	114.1	6.35 (s)
3β		3.80 (m)	8a	126.1	
4α	28.7	2.68 (m)	9	156.1	
4β		2.72 (m)	10	111.7	6.08 (d,3.4)
4a	126.3		11	108.9	6.23 (d,3.4)
5	116.0	6.55 (s)	12	156.3	
6	145.8		13	57.5	4.47 (2H,s)

a) Values in parentheses indicate coupling constants in Hz

Table 2. ^{13}C NMR and ^{1}H NMR data of compounds **2** in CD_3OD

Compound **2**					
Position	δ_C	δ_H (mult, *J* Hz)	*Position*	δ_C	δ_H (mult, *J* Hz)
1	136.4		1'	129.0	
2	107.8	6.95 (d,1.7)	2'	112.7	7.03 (d,1.7)
3	149.4		3'	148.7	
4	148.7		4'	147.5	
5	109.0	6.79 (d,8.5)	5'	115.7	6.77 (d,8.5)
6	121.0	6.90 (dd,1.7,8.5)	6'	121.6	6.84 (dd,1.7,8.5)
7	87.6	4.84 (d,5.8)	7'	89.2	4.66 (s)
8	62.6	3.00 (dt,5.8,8.9)	8'	92.8	
9α	72.0	3.77 (dd,5.8,8.9)	9'α	76.1	4.04 (d,9.3)
9β		4.45 (t,8.9)	9'β		3.84 (d,9.3)
$-OCH_2O-$	102.4	5.93 (s)	$-OCH_3$	56.4	3.86 (s)

a) Values in parentheses indicate coupling constants in Hz

Table 3. ^{13}C NMR and ^{1}H NMR data of compound **3** in $CDCl_3$

Compound **3**					
Position	δ_C	δ_H (mult, *J* Hz)	*Position*	δ_C	δ_H (mult, *J* Hz)
1	171.2		1'	44.6	5.95 (m)
2α	46.5	2.46 (d,15.4)	2'	137.7	
2β		2.20 (d,15.4)	3'	126.7	7.49 (m)
3	72.5		4'	125.2	7.44 (dd,7.6,7.9)
4α	42.1	1.77 (m)	5'	128.9	7.86 (d,7.9)
4β		1.64 (m)	5'a	133.9	
5α	59.6	3.86 (m)	6'	128.6	7.80 (d,8.3)
5β		3.76 (m)	7'	126.0	7.55 (m)
6	26.9	1.27(s)	8'	122.6	7.51 (m)
-NH-		6.17 (d,7.9)	9'	123.2	8.07 (d,8.6)
10'	20.7	1.68(d,6.5)	9'a	130.9	

a) Values in parentheses indicate coupling constants in Hz

Compound 1.

Yellow powder; $[\alpha]_D^{26.4}$ –0.51 (MeOH, *c*1.00); UV (MeOH) λ_{max} (log ε) 286 (1.27), 225 (1.71) nm; IR υ_{max} (KBr) 3271, 2926, 2852, 1720, 1651, 1556, 1506, 1456, 1286, 999 cm^{-1}; ^{1}H NMR spectroscopic data (600 MHz, $CDCl_3$) and ^{13}C NMR spectroscopic data (125 MHz, $CDCl_3$), are shown in Table 1. HRFABMS *m/z* 261.0759 $[M-H]^-$ (calcd. for $C_{14}H_{13}O_5$, 261.0763).

Compound 2.

Colorless oil; $[\alpha]_D^{26.8}$ +0.71 (MeOH, *c*1.90); UV (MeOH) λ_{max} (log ε) 284.5 (1.10), 232 (1.39) nm; IR υ_{max} (KBr) 3334, 2877, 1699, 1606, 1515, 1446, 1242, 1037, 931, 799, 756 cm^{-1}; ^{1}H NMR spectroscopic data (600 MHz, $CDCl_3$) and ^{13}C NMR spectroscopic data (125 MHz, $CDCl_3$), are shown in Table 2. HRFABMS *m/z* 371.1129 $[M-H]^-$ (calcd. for $C_{20}H_{19}O_7$, 371.1131).

Compound 3.

Yellow amorphous powder; $[\alpha]_D^{22.6}$ +5.82 (MeOH, *c*1.00); UV (MeOH) λ_{max} (log ε) 292 (2.23), 281(2.38), 271 (2.34), 226 (2.97), 212 (2.95) nm; IR υ_{max} (KBr) 3308, 2972, 2932, 1716, 1634, 1539, 1516, 1456, 1396, 1375, 1238, 1121, 1060, 1024, 800, 779, 756 cm^{-1}; ^{1}H NMR spectroscopic data (600 MHz, $CDCl_3$) and ^{13}C NMR spectroscopic data (125 MHz, $CDCl_3$), are shown in Table 3. HRFABMS *m/z* 300.1606 $[M-H]^-$ (calcd. for $C_{18}H_{22}NO_3$, 300.1600).

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Tabebuia avellanedae.

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