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METHYLATED A-TYPE PROANTHOCYANIDINS AND RELATED METABOLITES FROM *CASSIPOUREA GUMMIFLUA*

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Key Word Index—*Cassipourea gummiflua*; Rhizophoraceae; stem bark; methylated A-type proanthocyanidin; flavan-3-ol.

Abstract—The range of natural dimeric A-type proanthocyanidins is extended by identification of two new dimeric afzelechins in the form of 7-OMe-epiafzelechin-(4β→8,2β→*O*→7)-epiafzelechin and 7-OMe-epiafzelechin-(4β→8, 2β→*O*→7)-*ent*-afzelechin. These are accompanied in the stem bark of *Cassipourea gummiflua* by the related metabolites afzelechin, kaempferol-3-*O*-α-L-rhamnopyranoside, afzelechin-3-*O*-α-L-rhamnopyranoside, epiafzelechin (4β→8, 2β→*O*→7)-epiafzelechin and epiafzelechin-(4β→8, 2β→*O*→7)-*ent*-afzelechin, the latter three compounds being reported from a natural source for the second time.

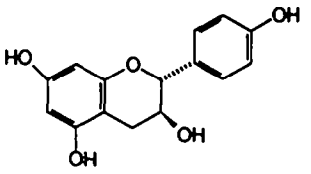
INTRODUCTION

In previous communications, we reported on the isolation of the novel compounds, afzelechin-3-*O*-α-L-rhamnopyranoside (**1**) [1] and epiafzelechin-(4β→8, 2β→*O*→7)-*ent*-afzelechin (**2**) [2] from the stem bark of *Cassipourea gerrardii*. In continuation of our studies on *Cassipourea* spp. we have examined the stem bark of *C. gummiflua*. This is a close relative of *C. gerrardi* and is found in the coastal forests of northern Zululand. Our studies on the bark extractive of this tree indicate the presence of an unusual dimeric afzelechin derivative which bears a methoxyl group at C-7 of the upper A-ring.

RESULTS AND DISCUSSION

The stem bark of *C. gummiflua* was extracted with 95% ethanol. The extract was subjected to a combination of silica gel chromatography, Sephadex LH-20 separations and further separations with high speed countercurrent chromatography (HSCCC) to afford compounds **1**–**7**.

Compounds **1** and **2** were identified as afzelechin-3-*O*-α-L-rhamnopyranoside (**1**) [1] and epiafzelechin-(4β→8, 2β→*O*→7)-*ent*-afzelechin (**2**) [2] respectively, by comparison of their physical and spectral data with those of authentic samples. Compounds **3**–**5** were identified as afzelechin (**3**) [3], kaempferol-3-α-L-rhamnopyranoside (**4**) [4] and epiafzelechin **5** (4β→8,2β→*O*→7)-afzelechin (**5**) [5] by comparison of their physical and spectral data with that recorded in the literature. The latter compound was previously isolated from the roots of *Ephedra* spp. by Hikino and is reported to possess hypotensive properties [5]. Methylation of **5** with ethereal diazomethane yielded the pentamethyl ether **8** which was fully characterized. It



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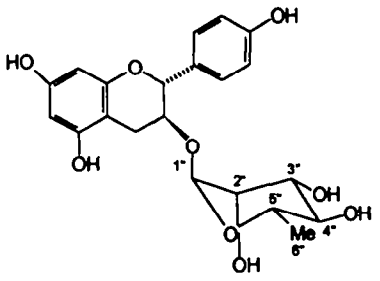

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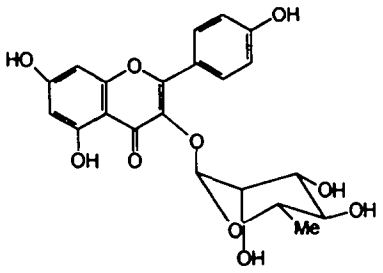

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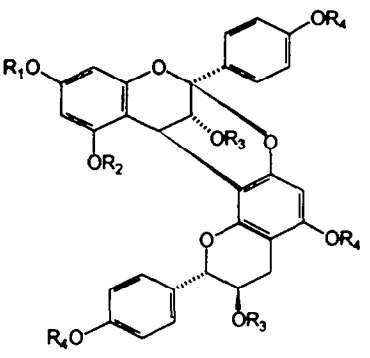
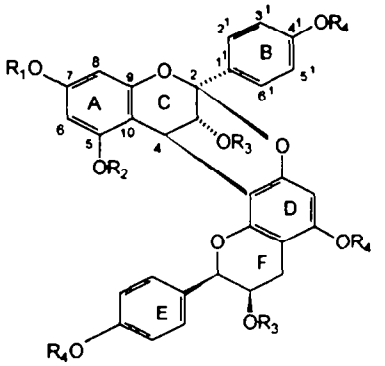


	R_1	R_2	R_3	R_4
(2)	H	H	H	H
(6)	Me	H	H	H
(9)	Me	H	H	Me
(10)	Me	Me	H	Me
(11)	Me	Ac	Ac	Ac



	R_1	R_2	R_3	R_4
(5)	H	H	H	H
(7)	Me	H	H	H
(8)	Me	Me	H	Me
(12)	Me	Ac	Ac	Ac

is pertinent to note that under identical reaction conditions to those employed by Hikino [5], **2** yielded the tetramethyl ether (**9**) and not the pentamethyl ether (**10**) as expected [2]. This apparently selective restricted access of diazomethane to OH(A)-5 in A-type analogues has previously been reported [6]. It is, however, significant to note that this phenomena was only observed in those compounds with a 2,3-*cis*-configuration of the lower F-ring substituents.

The ^{13}C and ^1H NMR spectra of **6** were similar to those recorded for **2**, the significant difference being the presence of a methoxy group defined by a 3H singlet at δ3.68 in the ^1H NMR spectrum and a carbon resonance at δ55.6. The methoxy group was judged to be located at C-7(A) based on the observed NOE association of OMe(A)-7 with both H-6(A) and H-8(A). This was confirmed by the DELAYED HECTOR (7 Hz) spectrum which displayed strong coupling between the methoxy protons and C-7(A). Methylation of **6** with ethereal diazomethane afforded a tetramethyl ether identical to **9**, thus establishing the structure of **6** as 7-OMe-epiafzelechin-(4β→8,2β→*O*→7)-*ent*-afzelechin; it is the major constituent in the ethyl acetate-soluble portion of the bark (0.5%). The hexa-acetate derivative (**11**) was also fully characterized and served to confirm the proposed structure.

The ^{13}C and ^1H NMR spectra of **7** were similar to those recorded for **5** except for the inclusion of a methoxy group defined by a 3H singlet at δ3.73 in the ^1H NMR spectrum and a carbon resonance at δ56.0. As with **6**, the methoxy group was shown to be located at C-7(A). Methylation of **7** with ethereal diazomethane afforded a pentamethyl ether identical to **8**, thus establishing the structure as 7-OMe-epiafzelechin (4β→8, 2β→*O*→7)-*ent*-afzelechin. The hexa-acetate derivative (**12**) was also fully characterized.

Two A-type proanthocyanidin glycosides, *ent*-epicatechin-3-*O*-D-galactopyranoside-(4α→8,2α→*O*→7)-epicatechin and *ent*-epicatechin-3-*O*-L-arabinopyranoside-(4α→8,2α→*O*→7)-epicatechin have recently been isolated from *Theobroma caco* [7]. These, together with epigallocatechin-(4α→8,2α→*O*→7)epicatechin-3-*O*-gallate, isolated from commercial oolong tea [8] are presumably the only known substituted A-type proanthocyanidins. The isolation of **6** and **7** is thus significant and represents the first isolation of *O*-methylated A-type proanthocyanidins. Methylation at the C-7(A) position is an important feature since it stabilizes the dimers with regard to oxidation. The dimers **6** and **7** were perfectly stable and remained white whereas the free phenolic dimers **2** and **5** were unstable and darkened on keeping (even in subdued light).

Methylation studies on **2** and **5** with diazomethane presented us with results that are not easily rationalized. Thus, methylation of **5** at position OH(A)-5 proceeded smoothly, whereas similar treatment of **2** left OH(A)-5 unmethylated. Dreiding models indicate that H-bonding between OH(A)-5 and the oxygen atom in the heterocyclic F ring could exist. If the configuration of the lower F ring is such that H-bonding will only occur in one of the two diastereomers, this could affect the reactivity of the OH(A)-5 centre. Indeed, if this is the case, this might be a useful method for determining the absolute stereochemistry of the lower (terminal) unit once the absolute configuration at C-4(C) had been established.

Table 1. ^{1}H NMR spectral data (δ values) of afzelechin, epiafzelechin and dimers **13** and **14**

	Afzelechin	Epiafzelechin	**13**	**14**
2(C)	4.63	4.92	—	—
2',6'(B)	7.26	7.35	—	—
2(F)			4.88	4.70
2',6'(E)			7.33	7.23

One of the reasons for studying the phenolic constituents of *Cassipourea* spp. stems from the use of the bark of this tree as a skin lightener by the Zulu people (A. Balfour-Cunningham, personal communication). The milled bark is applied in the form of a face pack after previously mixing it with a dilute solution of sodium bicarbonate. For this reason, a small quantity of pure **6** was heated under reflux with potassium carbonate in acetone. The resultant product was purified by chromatography to afford two new compounds **13** and **14**. Under identical conditions **7** afforded the same products.

Under identical conditions, Nonaka and co-workers [9] reported the epimerization of epicatechin-(4α→8, 2β→7)-epicatechin to epicatechin (4β→8,2β→*O*→7)-*ent*-epicatechin in high (75%) yield after 10 min. We are sceptical of this finding. The base-catalysed rearrangement of **6** to **13** and **14** was monitored by TLC. At no stage during the reaction was the epimer **7** or any other intermediate detected. The ^{1}H and ^{13}C NMR spectra of **13** and **14** were very similar to those of **7** and **6**, respectively, with the significant difference being the ^{1}H NMR shift positions of H-2', H-6' (E) and H-2(F). Comparison of these chemical shift positions in **13** and **7** with epiafzelechin reveal that the signals are almost coincident with the same signals in epiafzelechin. This demonstrates that the lower unit is no longer under the steric influence of the upper unit as is the case with **7**. Similar observations could be made by comparison of the proton shifts of **6** and **14** with afzelechin (Table 1). For ease of comparison, the proton and ^{13}C NMR shifts of **6**, **7** and **14** are shown in Tables 2 and 3. The mechanism for the formation of these products (Scheme 1) presumably occurs via an E-ring quinone-methide, followed by rotation about the C-3/C-4 bond and subsequent recyclization.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 Hz, respectively, and $CDCl_3$ was used as solvent, unless otherwise stated. HSCCC was carried out on a P.C. Inc. instrument. Plant material was collected in

Table 2. ^{1}H NMR data for compounds **6**, **7**, **13** and **14**

H	**6**	**7**	**13**	**14**
3(C)	4.14 *d* (3.52)	4.12 *d* (3.34)	4.20 *d* (3.58)	4.20 *d* (3.62)
4(C)	4.36 *d* (3.52)	4.92 *d* (3.34)	4.38 *d* (3.58)	4.37 *d* (3.62)
6(A)	6.11 *d* (2.42)	6.12 *d* (1.85)	6.12 *d* (2.21)	6.14 *d* (2.26)
8(A)	6.24 *d* (2.42)	6.22 *d* (1.85)	6.24 *d* (2.21)	6.25 *d* (2.26)
2'6'(B)	7.58 *d* (8.79)	7.55 *d* (8.84)	7.58 *d* (8.88)	7.59 *d* (8.84)
3'5'(B)	6.88 *d* (8.79)	6.86 *d* (8.84)	6.88 *d* (8.88)	6.88 *d* (8.84)
2(F)	4.84 *d* (7.82)	4.98 *br s*	4.88 *br s*	4.71 *d* (7.14)
3(F)	4.21 *ddd* (8.34, 7.82, 5.49)	4.25 *m*	4.19 *m*	4.10 *m*
4(F) ax	3.01 *dd* (16.37, 5.47)	2.99 *dd* (17.40, 4.59)		
4(F) eq	2.65 *dd* (16.37, 8.34)	2.79 *dd* (17.40, 2.45)	2.93 *m* [for 4 (F)]	2.78 *ddd* [for 4 (F)]
2'6' (E)	7.34 *d* (8.56)	7.53 *d* (8.66)	7.32 *d* (8.60)	7.23 *d* (8.60)
3'5' (E)	6.88 *d* (8.56)	6.87 *d* (8.66)	6.88 *d* (8.60)	6.81 *d* (8.60)
6(D)	6.18 *s*	6.14 *s*	6.16 *s* [for 8 (D)]	6.12 *s* [for 8 (D)]
7A (OMe)	3.67 *s*	3.73 *s*	3.73 *s*	3.73 *s*

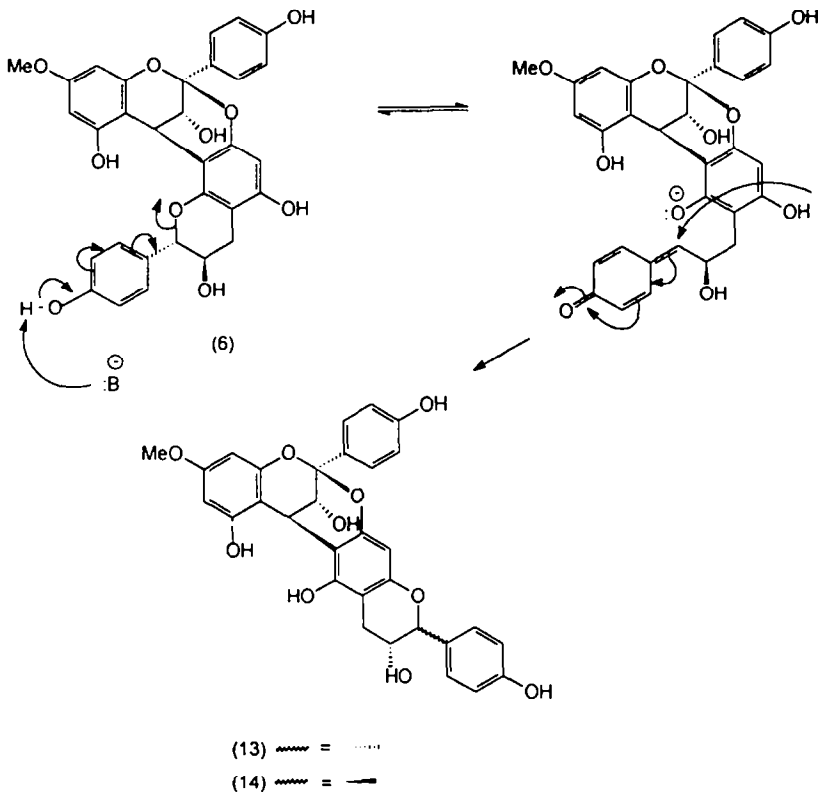
Table 3. ^{13}C NMR data for compounds **6**, **7**, **13** and **14**

C	6	7	13	14
2(C)	100.4	100.7	100.7	100.7
3(C)	67.5	68.3	67.4	67.4
4(C)	29.1	29.6	29.8	29.7
5(A)	156.6	157.4	155.2	155.4
6(A)	97.0	96.9	95.9	96.7
7(A)	160.8	161.2	161.0	161.0
8(A)	96.0	96.5	94.9	94.9
9(A)	154.2	154.5	154.5	154.5
10(A)	105.0	105.6	105.3	105.3
1'B	131.4	132.1	131.5	131.4
2'6'(B)	129.5	129.8	129.0	129.6
3'5'(B)	115.5	115.9	115.5	116.0
4'(B)	158.7	159.2	158.9	158.9
2(F)	84.1	81.9	79.9	82.5
3(F)	68.0	67.4	67.0	68.4
4(F)	29.1	30.3	29.8	28.4
5(D)	156.0	156.9	155.8	155.2
6(D)	96.6	96.8	108.6	108.5
7(D)	152.0	152.6	152.9	152.5
8(D)	106.5	107.4	96.8	96.0
9(D)	151.4	152.6	151.9	151.9
10(D)	103.2	102.7	102.7	103.7
1'(E)	129.7	131.0	131.9	131.3
2'6'(E)	130.1	130.3	129.1	129.3
3'5'(E)	116.3	116.3	115.7	116.1
4'(E)	158.6	158.6	157.9	158.3
7(A)-OMe	55.6	56.0	55.8	55.8

the New Hanover district of Natal and identified by Mr Robert Scott-Shaw (Natal Parks Board) and a sample deposited at the Herbarium of Natal University, Pietermaritzburg.

Extraction and isolation. Air-dried, powdered stem bark of *C. gummiflua* (2.93 kg) was exhaustively extracted (Soxhlet) with 95% EtOH and the combined extracts concd to dryness under red. pres. The residue was suspended in H_2O and successively extracted with $CHCl_3$ and EtOAc. Evapn of the EtOAc afforded a brown residue (51 g) of which a portion (10 g) was fractionated on silica gel 60 using CH_2Cl_2–Me_2CO (7:3→25:13) to yield **6** and **7** as a diastereoisomeric mixt. (2.8 g) and a further phenolic fr. (4.3 g). The latter was sepd on a column with Sephadex LH-20 using MeOH–H_2O (1:1) initially and increasing the ratio to (4:1). This gave **1** (133 mg), **3** (170 mg), **4** (151 mg) and an oligomeric fr. (3.3 g). Further sepns on silica gel of this fr. afforded **2** and **5** (860 mg) and further quantities of **6** and **7** (800 mg) as diastereomeric mixts.

Separations using HSCCC. A mixt. of **6** and **7** (400 mg) was sepd by HSCCC as follows: coil rot. speed 700 rpm, mobile phase flow rate 60 ml hr^{-1}, solvent system, H_2O–EtOAc–hexane–MeOH (30:48:24:21) (upper phase). This gave **6** (300 mg) and **7** (75 mg). A similar sepn of 400 mg of the mixt. of **2** and **5** afforded **2** (250 mg) and **5** (100 mg). The spectral properties of these two compounds were identical, respectively, with data recorded by ourselves [2] and given in ref. [5].



Scheme 1. Transformation of **6** in the presence of K_2CO_3.

7-Methoxy-epiafzelechin-(4β→8,2β→O→7)-ent-afzelechin (**6**). Prisms, mp 245–247°. $[\alpha]_D^{23}$ +67.65° (MeOH; *c* 0.136), $[\alpha]_{578}^{23}$ +70.59° (MeOH; *c* 0.136). IR ν_{max}^{KBr} cm^{-1}; 3522, 3302, 1628, 1144. R_f = 0.24 (CH_2Cl_2–Me_2CO, 7:3). (Found C, 63.59; H, 5.07. $C_{31}H_{26}O_{10}\cdot 1.5\,H_2O$ requires C, 63.59; H, 4.59). FAB-MS *m/z*: 559 $[M+1]^+$. ^1H and ^{13}C NMR spectra (Tables 2 and 3).

7-Methoxy-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin (**7**). Prisms, mp 235–237°. $[\alpha]_D^{23}$ +57.81° (MeOH; *c* 0.192), $[\alpha]_{578}^{23}$ +60.42° (MeOH; *c* 0.192). IR ν_{max}^{KBr} cm^{-1}; 3373, 1626, 1516, 1142. R_f = 0.56 (CH_2Cl_2–Me_2CO, 11:9). (Found C, 62.56; H, 5.41. $C_{31}H_{26}O_{10}\cdot 2H_2O$ requires C, 62.62; H, 5.09). FAB-MS *m/z*: 559 $[M+1]^+$. ^1H and ^{13}C NMR (Tables 2 and 3).

Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin pentamethyl ether (**8**). Needles, mp 135°. $[\alpha]_D^{23}$ +61.36° ($CHCl_3$; *c* 0.176), $[\alpha]_{578}^{23}$ +64.36° ($CHCl_3$; *c* 0.176). R_f = 0.27 (CH_2Cl_2–Me_2CO, 97:3). ^1H NMR: δ 1.88 [1H, *br s*, OH-3 (F)], 1.94 [1H, *d*, OH-3 (C)], 2.84 [1H, *m*, H-4 (F)], 3.49 [3H, *s*, OMe-5 (A)], 3.72 [3H, *s*, OMe-7 (A)], 3.73 [3H, *s*, OMe-5 (D)], 3.81 [3H, *s*, OMe-4′ (E)], 3.82 [3H, *s*, OMe-4′ (B)], 4.17 [1H, *dd*, *J* = 3.44 and 5.94 Hz, H-3 (C)], 4.41 [1H, *m*, H-3 (F)], 4.93 [1H, *d*, *J* = 3.44 Hz, H-4 (C)], 4.96 [1H, *br s*, H-2 (F)], 6.06 [1H, *d*, *J* = 2.33 Hz, H-6 (A)], 6.19 [1H, *s*, H-6 (D)], 6.28 [1H, *d*, *J* = 2.33 Hz, H-8 (A)], 6.96 [2H, *d*, *J* = 8.89 Hz, H-3′, H-5′ (B)], 6.96 [2H, *d*, *J* = 8.89 Hz, H-3′, H-5′ (E)], 7.60 [2H, *d*, *J* = 8.89 Hz, H-2′, H-6′ (E)]. ^{13}C NMR: δ 27.6 [C-4 (C)], 28.9 [C-4 (F)], 55.2, 55.3 and 55.5 [5 × OMe], 65.5 [C-3 (F)], 67.4 [C-3 (C)], 78.2 [C-2 (F)], 92.1 [C-6 (D)], 93.1 [C-6 (A)], 101.7 [C-10 (D)], 103.6 [C-10 (A)], 106.7 [C-8 (D)], 113.6 [C-3′, C-5′ (E)], 113.7 [C-3′, C-5′ (B)], 128.1 [C-2′, C-6′ (B)], 130.1 [C-1′ (E)], 130.6 [C-1′ (B)], 151.2 [C-9 (D)], 151.6 [C-7 (D)], 152.9 [C-9 (A)], 157.6 [C-5 (D)], 160.0 [C-5 (A)], 159.2 [C-4′ (E)], 159.9 [C-7 (A)], 160.1 [C-4′ (B)].

7-Methoxy-epiafzelechin-(4β→8,2β→O→7)-ent-afzelechin hexaacetate (**11**). Prisms, mp 145°. $[\alpha]_D^{20}$ −101.34° ($CHCl_3$; *c* 0.372), $[\alpha]_{578}^{20}$ −106.18° ($CHCl_3$; *c* 0.372). R_f = 0.37 (CH_2Cl_2–Me_2CO, 97.3). ^1H NMR: δ 1.78, 1.75, 2.04, 2.23, 2.28 and 2.31 [18H, *s*, 3(C), 3(F), 5(A), 5(D), 4′ (E) and 4′(B) OAc], 2.60 [2H, *m*, H-4 (F)], 3.78 [3H, *s*, 7-OMe (A)], 4.59 [1H, *d*, *J* = 3.95 Hz, H-4 (C)], 5.34 [1H, *d*, *J* = 3.95 Hz, H-3 (C)], 6.19 [1H, *d*, *J* = 2.43 Hz, H-6 (A)], 6.47 [1H, *s*, H-6 (D)], 6.59 [1H, *d*, *J* = 2.43 Hz, H-8 (A)], 7.02 [2H, *d*, *J* = 8.81 Hz, H-3′, H-5′ (E)], 7.16 [2H, *d*, *J* = 8.87 Hz, H-3′, H-5′ (B)], 7.24 [2H, *d*, *J* = 8.81 Hz, H-2′, H-6′ (E)], 7.72 [2H, *d*, *J* = 8.87 Hz, H-2′, H-6′ (B)]. ^{13}C NMR: δ 20.2, 20.6, 20.7, 21.1, 21.1 and 21.2 (6 × OAc), 22.4 [C-4 (F)], 27.0 [C-4 (C)], 55.5 [OMe-7 (A)], 67.3 [C-3 (C)], 68.3 [C-3 (F)], 78.5 [C-2 (F)], 97.9 [C-2 (C)], 99.6 [C-8 (A)], 102.5 [C-6 (A)], 103.4 [C-6 (D)], 105.7 [C-10 (D)], 108.2 [C-10 (A)], 109.5 [C-8 (D)], 121.1 [C-3′, 5′ (B)], 121.6 [C-3′, C-5′ (E)], 127.5 [C-2′, C-6′ (E)], 128.2 [C-2′, 6′ (B)], 134.8 [C-1′ (E)], 134.9 [C-1′ (B)], 148.8 [C-5 (D)], 149.2 [C-5 (A)], 150.4 [C-4′ (E)], 150.7 [C-9 (D)], 150.7 [C-7 (D)], 151.4 [C-4′ (B)], 153.9 [C-9 (A)], 159.5 [C-7 (A)], 168.6, 169.1, 169.4, 170.0 and 170.5 (6 × OAc).

7-Methoxy-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin hexaacetate (**12**). Needles, mp 125–127°. $[\alpha]_D^{20}$ −80.10° ($CHCl_3$; *c* 0.206); $[\alpha]_{587}^{20}$ −83.50° ($CHCl_3$; *c* 0.206). R_f = 0.26 (CH_2Cl_2–Me_2CO, 97:3). ^1H NMR: δ 1.46, 1.73, 1.95, 2.27, 2.30 and 2.31 (18H, 6 × OAc), 3.79 [3H, *s*, OMe-7 (A)], 4.54 [1H, *d*, *J* = 4.00 Hz, H-4 (C)], 5.22 [1H, *br s*, H-2 (F)], 5.23 [1H, *d*, *J* = 4.00 Hz, H-3 (C)], 5.28 [1H, *m*, H-3 (F)], 6.26 [1H, *d*, *J* = 2.47 Hz, H-6 (A)], 6.49 [2H, *s*, H-6 (D)], 6.60 [1H, *d*, *J* = 2.47 Hz, H-8 (A)], 7.12 [2H, *d*, *J* = 8.66 Hz, H-3′, H-5′ (E)], 7.16 [2H, *d*, *J* = 8.88 Hz, H-3′, H-5′ (B)], 7.49 [2H, *d*, *J* = 8.66 Hz, H-2′, H-6′ (E)], 7.70 [2H, *d*, *J* = 8.88 Hz, H-2′, H-6′ (B)]. ^{13}C NMR: δ 19.7, 20.6, 20.8, 21.1, 21.1 and 21.2 (6 × OAc), 25.5 [C-4 (F)], 27.1 [C-4 (C)], 55.5 [OMe-7 (A)], 66.2 [C-3 (F)], 67.8 [C-3 (C)], 77.8 [C-2 (F)], 98.1 [C-2 (C)], 99.3 [C-8 (A)], 102.8 [C-6 (A)], 103.7 [C-6 (D)], 105.6 [C-10 (D)], 108.1 [C-10 (A)], 109.7 [C-8 (D)], 121.1 [C-3′,5′ (B)], 121.3 [C-3′, C-5′ (E)], 128.3 [C-2′, C-6′ (B)], 129.2 [C-2′,6′ (E)], 134.6 [C-1′ (B)], 149.2 [C-5 (D)], 149.4 [C-5 (A)], 150.3 [C-7 (D)], 151.0 [C-4′ (E)], 151.4 [C-4′ (B)], 152.0 [C-9 (D)], 154.2 [C-9 (A)], 159.5 [C-7 (A)], 168.7, 169.1, 169.3, 169.9, 170.0 and 170.3 (6 × OAc).

7-Methoxy-epiafzelechin-(4β→6, 2β→O→7)-epiafzelechin (**13**). Crystals, mp 241–244°. $[\alpha]_D^{20}$ +49.17° $[\alpha]_{578}^{20}$ +52.50° (MeOH; *c* 0.012). R_f = 0.32 (CH_2Cl_2–Me_2CO, 7:3). ^1H and ^{13}C NMR: spectra (Tables 2 and 3).

7-Methoxy-epiafzelechin-(4β→6, 2β→O→7)-ent-afzelechin (**14**). Crystals, mp 230–232°. $[\alpha]_D^{20}$ +31.71° $[\alpha]_{578}^{20}$ +33.57° (MeOH; *c* 0.432). ^1H and ^{13}C NMR: spectra (Tables 2 and 3).

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