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Two flavonoid glycosides and a miscellaneous flavan from the bark of *Guibourtia coleosperma*

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Abstract

Two flavonoid glycosides, 7-*O*-β-D-xylopyranosyl-epicatechin and epicatechin-(4β → 8)-7-*O*-β-D-xylopyranosyl-epicatechin both as their acetate and methyl ether acetate derivatives and a miscellaneous flavan Epicatechin-(7,8-bc)-9β-(3-methoxy-4-acethoxyphenyl)-dihydro-2(3H)-pyranone as its acetate derivative were isolated from the bark of *Guibourtia coleosperma*. Their structures were established by spectroscopic methods.

Keywords: *Guibourtia coleosperma*; Caesalpiniodeae; Leguminosae; Flavonoid; Flavan

1. Introduction

The large false mopanie, *Guibourtia coleosperma*, is a tree indigenous to the Southern African region and is found in Zambia, Zimbabwe, Zaire, Namibia and Angola. Different parts (leaves and bark) of the tree have been used for medicinal and nutritional purposes by indigenous tribes (Alders, 1992; Kamini, 2003). In addition to a large number of conventional proguibourtinidins, profisetinidins and related analogs, (Bonnet et al., 1996; Steynberg et al., 1990) the heartwood of *G. coleosperma* also contains the first proguibourtinidins with stilbenoid constituent units (Steynberg et al., 1983, 1987). This study represents the first detailed investigation into the compounds present in low concentrations of the bark of *G. coleosperma* and two novel flavonoid glycosides and a miscellaneous flavan were isolated as their derivatized forms. We determined these structures by utilizing ^{1}H NMR nuclear Overhauser effect (n.O.e.), ^{13}C, HMBC and HMQC techniques.

Flavonoids presumably constitute the most ubiquitous group of all plant phenolics and play important roles in plant biochemistry and physiology (Porter, 1994) as well as mammalian metabolism (Middleton and Kandaswani, 1994). Research into their chemistry, synthesis, natural distribution and biological function is widely conducted (Middleton and Kandaswani, 1988). Flavonoids have come under investigation in the last couple of decades for use as natural antioxidants (Pratt, 1990; Shahidi and Wanasundra, 1992) as well as for their health promoting properties in humans (Namiki, 1990; De Whalley et al., 1990; Hertog et al., 1993a,b).

2. Results and discussion

The first detailed study into the metabolic contents of the bark of *G. coleosperma* yielded the novel 7-*O*-β-xylopyranosyl-epicatechin **1** and epicatechin-(4β → 8)-7-*O*-β-xylopyranosyl-epicatechin **4** as their acetate and methyl ether acetate derivatives **2**–**3** and **5**–**6**. Although catechin glycosides are fairly common (Doskotch et al., 1973; Pan and Lundgren, 1996) their epicatechin analogues are less

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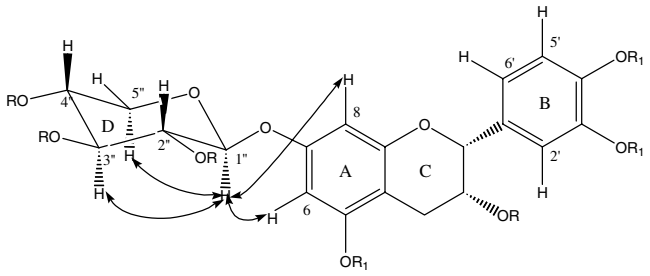
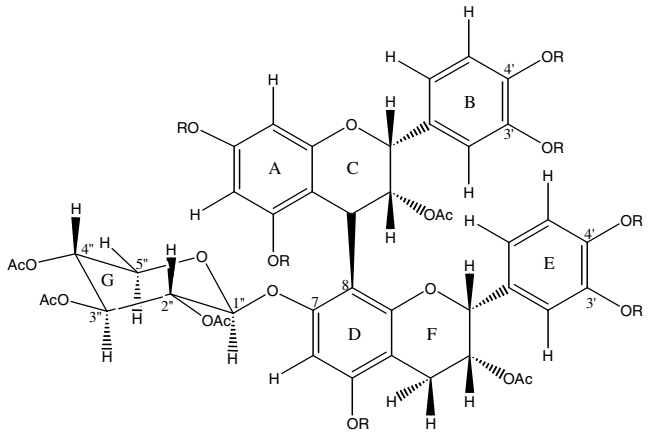
seldom identified. A similar compound 5-*O*-β-xylopyranosyl-epicatechin has been identified from *Brosimopsis acutifolium* (Ferrari et al., 1998). Epicatechin-(7,8-bc)-9β-(3-methoxy-4-hydroxyphenyl)-dihydro-2(3H)-pyranone (**7**) as its acetate derivative **8** was also isolated.



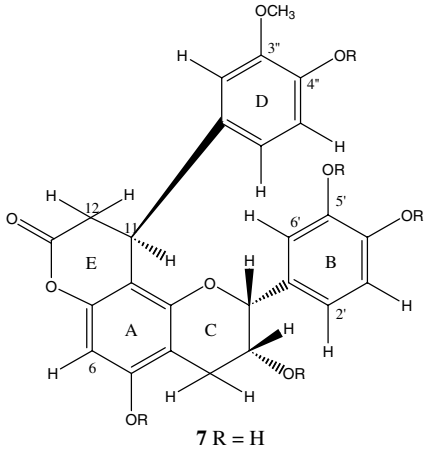
1 R = R_1 = H

2 R = R_1 = Ac (Significant n.O.e. associations)

3 R = Ac, R_1 = Me



4 R = R_1 = H
5 R = R_1 = Ac
6R = Ac, R_1 = Me



7 R = H
8 R = Ac

The ^{1}H NMR data (Table 1) of compounds **2** and **3** show an ABX-system and the AB-system of the A-ring, all of them in the aromatic region (δ 6.49–7.41). The coupling constants between 2-H and 3-H of the C-ring suggest *cis*-stereochemistry [$J = 1.0$ Hz]. Six aliphatic heterocyclic signals of the sugar unit are also visible in the spectrum.

Table 1
^{1}H NMR (300 MHz, 296 K) data of 7-*O*-β-D-xylopyranosyl-epicatechin derivatives **2** and **3** in acetone and $CDCl_3$

Ring	H	**2**, Acetone	**3**, $CDCl_3$
A	6-H	6.49 (*d*, $J = 2.1$ Hz)	6.20 (*d*, $J = 3.0$ Hz)
	8-H	6.60 (*d*, $J = 2.1$ Hz)	6.33 (*d*, $J = 3.0$ Hz)
B	2′-H	7.41 (*d*, $J = 1.9$ Hz)	7.04 (*d*, $J = 2.0$ Hz)
	6′-H	7.43 (*dd*, $J =$ 1.9 and 9.0 Hz)	6.98 (*dd*, $J =$ 2.0 and 8.2 Hz)
	5′-H	7.28 (*d*, $J = 9.0$ Hz)	6.88 (*d*, $J = 8.2$ Hz)
C	2-H	5.37 (*d*, $J = 1.0$ Hz)	5.03 (*d*, $J = 1.1$ Hz)
	3-H	5.50 (*ddd*, $J =$ 1.0, 2.0 and 5.2 Hz)	5.45 (*ddd*, $J =$ 1.0, 1.1 and 2.0 Hz)
	4-H	2.77 (*dd*, $J =$ 2.0 and 17.9 Hz) 3.05 (*dd*, $J =$ 5.2 and 17.9 Hz)	2.95 (*m*)
D	1″-H	5.42 (*d*, $J = 8.0$ Hz)	5.19 (*d*, $J = 8.0$ Hz)
	2″-H	5.13 (*dd*, $J =$ 7.8 and 8.0 Hz)	5.17 (*m*)
	3″-H	5.32 (*t*, $J = 7.8$ Hz)	5.23 (*m*)
	4″-H	5.00 (*ddd*, $J =$ 4.1, 5.0 and 7.8 Hz)	5.04 (*m*)
	5″-H	4.17 (*dd*, $J =$ 4.1 and 12.0 Hz) 3.75 (*dd*, $J =$ 5.0 and 12.0 Hz)	4.23 (*dd*, $J =$ 5.1 and 12.0 Hz) 3.52 (*dd*, $J =$ 8.0 and 12.0 Hz)
Aromatic	3 × OAc/OMe	2.29 – 2.30 (*s*)	3.80 – 3.92 (*s*)
Aliphatic	4 × OAc	2.02 – 2.10 (*s*)	1.94 – 2.10 (*s*)

Splitting patterns and *J*-values are given in parentheses.

The EI-MS of compound **2** with molecular ion of [M^+] 716.2031 ($C_{34}H_{36}O_{17}$) confirms the molecular formula of the compound.

Using the flavan-3-ol heterocyclic protons (C-ring) as reference, the NOESY experiment show associations of 2-H (C, δ 5.37) with 6′-H (B, δ 7.43) as well as 2′-H (B, δ 7.41). The ABX system of the B-ring was established from these interactions. The 7-*O*-position of the sugar unit was established by a correlation between both 8-H (A, δ 6.60) and 6-H (A, δ 6.49) and the anomeric proton 1″-H (D, δ 5.42). The observed coupling (refer **2**) between 1″-H(D) and 2″-H(D) constant ($J = 8.0$) indicated the β-configuration. An association between 1″-H(D), 3″-H and only one of the methylene protons 5″-H_{ax} indicate a closed six-membered xylopyranosyl ring-structure.

Carbon atom resonances were assigned on the basis of ^{1}H ^{13}C connectivities, e.g. HMQC and HMBC correlations (Table 2). The sugar moiety could be confirmed as β-xylopyranosyl triacetate derivative by comparing carbon chemical shifts with the literature values (Agrawal, 1989). The A and C rings of **2** was identified by coupling of 7-C (A, δ 156.35) with 6-H (A, $^2J_{CH}$) and 8-H (A, $^2J_{CH}$). The position of the sugar unit is confirmed by coupling between 7-C (A) and 1″-H (D, $^3J_{CH}$). 9-C (A, δ 155.61) couples with 8-H (A, $^2J_{CH}$) as well as 4-CH_2 (C, $^3J_{CH}$), while 5-C (A, δ 150.54) couples with 6-H (A, $^2J_{CH}$), 5-OAc as and 4-CH_2(C, $^3J_{CH}$). 10-C (A, δ 107.06) shows coupling with 6-H (A, $^3J_{CH}$) and 8-H (A, $^3J_{CH}$) as well as 4-CH_2 (C, $^2J_{CH}$). The

Table 2
^{13}C NMR (300 MHz, 296 K) data of 7-*O*-β-D-xylopyranosyl-epicatechin derivative **2** in $CDCl_3$

Ring	C	ppm	Ring	C	ppm
A/C	2-C	77.11	B	1′-C	136.32
	3-C	67.22		2′-C	122.45
	4-C	26.27		3′-C	142.44
	5-C	150.54		4′-C	142.29
	6-C	104.76		5′-C	123.69
	7-C	156.35		6′-C	124.81
	8-C	103.18			
	9-C	155.61	D	1″-C	98.56
	10-C	107.06		2″-C	70.22
				3″-C	70.83
	$7\times OCOCH_3$	168.55 – 170.89		4″-C	68.75
	$7\times OCOCH_3$	18.85 – 21.22		5″-C	62.20

B-ring was distinguished on account of coupling of 3′-C (B, δ 142.44) and 4′-C (B, δ 142.29) with 2′-H (B, $^2J_{CH}$) and 5′-H (B, $^2J_{CH}$) as well as with 3′-OAc and 4′-OAc respectively. 1′-C (B, δ136.32) couples with 6′-H (B, $^2J_{CH}$) and 2-H (C, $^2J_{CH}$).

The CD-data of **2** (see Section 3.5) are similar to that of epicatechin also isolated from the same source and shows the same negative Cotton effect for the $^{'}L_b$ transition at 276.20 nm, which implies a 2*R* absolute configuration (Dornhege and Snatzke, 1970). The coupling constant [$J = 1.0$ Hz] between 2-H and 3-H of the C-ring, confirms the 2,3-*cis* relative configuration of the derivative.

The ^1H NMR data (Table 3) of compounds **5** and **6** exhibit similar spinsystems and resonances as compounds **2** and **3**, respectively.

However, it exhibits an additional ABX spinsystem associated with the E-ring and a residual aromatic singlet in the aromatic region. The additional heterocyclic F-ring also displays [$J = 2.0$ Hz] 2,3-*cis* relative stereochemistry, while the C-ring display 2,3-*cis*-3,4-*trans* [$J_{2,3} = 1.8$ Hz, $J_{3,4} = 4.0$ Hz] relative stereochemistry.

The EI-MS of compound **5** with molecular ion of [M^+] 1215.347 ($C_{59}H_{58}O_{28}$) confirms the molecular formula of the compound.

Couplings in a COSY experiment identifies two heterocyclic AMX-systems which are attributed to the C- and F-rings using 4-CH_2 (F, δ 2.90 and 2.83) as reference. Long range coupling ($^4J_{HH}$) between the respective 2-H (C, δ 5.53 and F, δ 4.55) and 2′-H (B, δ 7.37 and E, δ 7.00) and 6′-H (B, δ 7.30 and E, δ 6.89) resonances identifies the B- and E-ring systems. The xylopyranosyl unit is identified on account of successive couplings between 1″-H (G, δ 5.15) and 2″-H (G, δ 5.34), 2″-H and 3″-H (G, δ 5.35), 3″-H (G) with 4″-H (G, δ 5.17) and 4″-H (G) with the 5″-methylene protons (δ 4.37 and 3.60). The presence of 7-*O*-β-xylopyranosyl-epicatechin in the bark and similar chemical shifts of 6-H of both compounds in $CDCl_3$ indicated the 7-position of sugar unit which was further confirmed by an n.O.e between 6-H(D) and 1″-H(G). 1″-H(G) also shows an association between 3″-H(G) and 5″-H_{ax} confirming a closed six-membered xylopyranosyl ring-structure.

Table 3
^1H NMR (300 MHz, 296 K) data of epicatechin-(4β → 8)-7-*O*-β-D-xylopyranosyl-epicatechin derivatives **5** and **6** in $CDCl_3$

Ring	H	**5**	**6**
A	6-H	6.05 (*d*, $J = 2.1$ Hz)	5.44 (*d*, $J = 1.9$ Hz)
	8-H	6.22 (*d*, $J = 2.1$ Hz)	5.82 (*d*, $J = 1.9$ Hz)
B	2′-H	7.37 (*d*, $J = 2.0$ Hz)	7.01 (*d*, $J = 2.1$ Hz)
	6′-H	7.30 (*dd*, $J =$ 2.0 and 4.2 Hz)	6.92 (*dd*, $J =$ 2.1 and 8.0 Hz)
	5′-H	7.20 (*d*, $J = 4.2$ Hz)	6.80 (*d*, $J = 8.0$ Hz)
C	2-H	5.53 (*d*, $J = 1.8$ Hz)	5.65 (*d*, $J = 2.0$ Hz)
	3-H	5.47 (*dd*, $J =$ 1.8 and 4.0 Hz)	5.38 (*dd*, $J =$ 2.0 and 3.8 Hz)
	4-H	4.37 (*d*, $J = 4.0$ Hz)	4.41 (*d*, $J = 3.8$ Hz)
D	6-H	6.54 (*s*)	6.81 (*s*)
E	2′-H	7.00 (*d*, $J = 2.0$ Hz)	6.49 (*d*, $J = 1.9$ Hz)
	6′-H	6.89 (*dd*, $J =$ 2.0 and 4.1 Hz)	6.52 (*dd*, $J =$ 1.9 and 7.8 Hz)
	5′-H	7.06 (*d*, $J = 4.1$ Hz)	6.71 (*d*, $J = 7.8$ Hz)
F	2-H	4.55 (*d*, $J = 2.0$ Hz)	4.55 (*d*, $J = 2.0$ Hz)
	3-H	5.10 (*m*)	5.10 (*m*)
	4-CH_2	2.90 (*m*) 2.83 (*m*)	2.90 (*m*)
G	1″-H	5.15 (*d*, $J = 8.0$ Hz)	5.21 (*d*, $J = 7.8$ Hz)
	2″-H	5.34 (*dd*, $J =$ 8.0 and 8.0 Hz)	5.30 (*m*)
	3″-H	5.35 (*m*)	5.31 (*m*)
	4″-H	5.17 (*m*)	5.10 (*m*)
	5″-H	4.37 (*dd*, $J =$ 4.9 and 12.0 Hz) 3.60 (*dd*, $J =$ 8.0 and 12.0 Hz)	4.45 (*dd*, $J =$ 5.0 and 12.1 Hz) 3.65 (*dd*, $J =$ 8.0 and 12.1 Hz)
Aromatic	$3\times$OAc/OMe	2.20 – 2.33 (*s*)	3.41 – 3.90 (*s*)
Aliphatic	$4\times$OAc	1.75 – 2.07 (*s*)	1.65 – 2.07 (*s*)

Splitting patterns and *J*-values are given in parentheses.

Couplings in a HMQC spectrum identified the protonated carbons in the ^{13}C NMR spectrum of compound **5** (Table 4), while couplings in a long range HMBC spectrum identified the remaining resonances. 5-C (A, δ 155.70) correlates with 6-H (A, δ 6.05, $^2J_{CH}$) and 4-H (C, δ 4.37, $^3J_{CH}$), while 7-C (A, δ 148.02) couples with 8-H (A, δ 6.22, $^2J_{CH}$) and 6-H (A, $^2J_{CH}$). 9-C (A, δ 149.18) couples with 8-H (A, $^2J_{CH}$) and 4-H (C, $^3J_{CH}$). 10-C (A, δ 112.94) shows correlation with 6-H (A, $^3J_{CH}$), 8-H (A, $^3J_{CH}$), 3-H (C, δ 5.47, $^3J_{CH}$) and 4-H (C, $^2J_{CH}$). These couplings serve to identify the A-ring. The B-ring was distinguished on account of couplings between 3′-C (B, δ 142.29) and 4′-C (B, δ 142.06) with 2′-H (B, δ 7.37), 5′-H (B, δ 7.20) and of 1′-C (B, δ 137.00) with 6′-H (B, δ 7.30, $^2J_{CH}$) and 2-H (C, δ 5.53, $^2J_{CH}$). Similar couplings identified the E-ring. The D-ring was identified on account of couplings between 5-C (D, δ 153.50) and the proton signals of 6-H (D, δ 6.54) and 4-CH_2 (F, δ 2.90 and 2.83). 8-C (D, δ 113.69) couples with 4-H (C, $^2J_{CH}$). 7-C (D, δ149.91) couples with 6-H (D, $^2J_{CH}$) and 9-C (D, δ154.50) with 4-CH_2 (F, $^3J_{CH}$), while 10-C (D, δ 108.83) couples with 6-H (D, $^3J_{CH}$) and 4-CH_2 (F, $^2J_{CH}$). The ^1H and ^{13}C spectra of compound **5** strongly suggest a dimeric structure.

Table 4
^{13}C NMR (300 MHz, 296 K) data of epicatechin-(4β → 8)-7-*O*-β-D-xylopyranosyl-epicatechin derivative **5** and **6** in $CDCl_3$

Ring	C	5	6	Ring	5	6
A/C	2-C	74.49	74.98	D/F	77.63	77.62
	3-C	69.99	70.71		67.50	68.62
	4-C	34.75	34.70		26.85	26.90
	5-C	155.70	157.63		153.50	157.63
	6-C	107.00	92.50		102.10	92.73
	7-C	148.02	159.08		149.91	158.00
	8-C	108.90	92.00		113.69	110.09
	9-C	149.18	155.00		154.50	154.56
	10-C	112.94	103.69		108.83	103.69
B	1′-C	137.00	130.31	E	135.18	130.00
	2′-C	122.36	110.34		122.72	111.00
	3′-C	142.29	148.87		142.35	149.03
	4′-C	142.06	148.87		142.06	148.93
	5′-C	123.49	110.34		123.49	110.88
	6′-C	124.83	119.44		125.35	119.28
G	1″-C	99.78	100.43			
	2″-C	72.23	71.89			
	3″-C	70.64	70.71			
	4″-C	70.64	70.33			
	5″-C	64.82	63.50			

	5		6
12 × $OCOCH_3$	168.30 – 170.85	7 × OCH_3	55.15 – 56.33
12 × $OCOCH_3$	20.12 – 21.40	5 ×	169.41 – 170.68
		5 ×	20.63 – 21.52

Table 5
^{1}H NMR (300 MHz, 296 K) data of chinchonain derivative **8** in $CDCl_3$

Ring	Proton	8
A	6-H	6.61 (s)
B	2′-H	7.39 (d, $J = 2.0$ Hz)
	6′-H	7.29 (d, $J = 2.0$ and 8.0 Hz)
	5′-H	7.23 (d, $J = 8.0$ Hz)
D	2″-H	6.76 (d, $J = 2.0$ Hz)
	6″-H	6.68 (dd, $J = 2.0$ and 8.0 Hz)
	5″-H	6.89 (d, $J = 8.0$ Hz)
C	2-H	5.41 (d, $J = 2.0$ Hz)
	3-H	5.06 (m)
	4-CH_2	3.00 (dd, $J = 2.3$ and 17.8 Hz)
		2.85 (dd, $J = 2.0$ and 17.8 Hz)
E	11-H	4.64 (dd, $J = 1.9$ and 8.2 Hz)
	12-CH_2	3.15 (dd, $J = 8.1$ and 16.2 Hz)
		3.10 (dd, $J = 1.9$ and 16.2 Hz)
Aromatic	1 × OMe	3.50 (s)
	4 × OAc	2.26 – 2.34
Aliphatic	1 × OAc	1.96 (s)

Splitting patterns and *J*-values are given in parentheses.

The high amplitude positive Cotton effect $[\theta]_{240}$ 1.878×10^4, in the CD-data of **5** (see Section 3.6) indicates 4β-configuration for the C-ring (Barret et al., 1979) and in conjunction with the 2,3-*cis*-3,4-*trans* relative stereochemistry, an absolute configuration of $2R,3R,4R$ was established. The stereochemistry of epicatechin, the biogenetic precursor also present in the bark, is established $2R,3R$ and therefore the same absolute configuration is assigned to the terminal unit.

In the ^{1}H NMR spectrum (Table 5) of **8** the epicatechin structure is evident with an ABX-spinsystem ($J = 2.0$ and 8.0) of the B-ring and an ABMX-spinsysten of the C-ring in the aliphatic region. The coupling constant between 2-H (C, δ 5.41) and 3-H (C, δ 5.06) suggest *cis*-coupling ($J = 2.0$ Hz). The A-ring exhibits only one proton singlet suggesting substitution in the 6- or 8-position. An extra aromatic ABX-spinsystem ($J = 1.9$ and 8.2) for the 3-methoxy-4-acetoxyphenyl substituent, as well three as heterocyclic signals for the lactone ring can also be seen in the spectrum.

The C-ring heterocyclic protons were distinguished on account of coupling in a COSY spectrum **8** between 2-H (C, δ 5.41) and 3-H (C, δ 5.06) as well as between 3-H (C) and 4-CH_2 (C, δ 2.85 and 3.00). The B-ring is identified by couplings between 2-H (C) and 2′-H (B, δ 7.39) and 6′-H (B, δ 7.29). 12-CH_2 (E, δ 3.15 and 3.10) shows coupling with 11-H (E, δ 4.64), which also couples with 2″-H (D, δ 6.76) and 6″-H (D, δ 6.68), these couplings serve to identify the E and D-rings, respectively. The position of the aromatic methoxy was confirmed on account coupling with 2″-H(D).

Examination of the CD data of pyranone **8** (see Section 3.4) shows a positive sign of the Cotton effect at 245.8 nm indicating the aromatic substituent on the lactone ring has a β-configuration (Foo, 1987).

The EI-MS of compound **8** with molecular ion of $[M^+]$ 676.187 ($C_{34}H_{36}O_{17}$) confirms the molecular formula of the compound.

The presence of the epicatechin core is readily apparent with ^{13}C chemical shifts in the region of δ 77.85, 66.64 and 26.52 which are associated with the 2-C, 3-C and 4-C, respectively, of flavans with 2,3-*cis* stereochemistry (Table 6). Also present is the aromatic chemical shifts identifiable with the phloroglucinol A-ring and the catechol β-ring systems. The observation of only one substituted A-ring carbon at δ 104.68 and the appearance of a signal at δ 111.43 indicate that the epicatechin is substituted in the A-ring. In addition to the epicatechin chemical shifts, resonances attributable to a methine carbon (δ 35.61) and a methylene carbon (δ 36.24) together with another aromatic 3-methoxy-4-acetoxy phenyl substitution system are also observed.

The observation of these additional resonances together with the presence of a carbonyl chemical shift in the region of δ 170.71 suggest a 3-hydroxy-4-methoxypropanoate unit is linked to the A-ring of the epicatechin unit. Furthermore, with chinchonains it has been established that the position of the 10-C is distinctive for the location of the lactone functionality on the A-ring (Foo, 1987). The C-8 location has the 10-C chemical shift downfield (δ 105) relative to the C-6 isomer (δ 99). For compound **8** the 10-C resonance are located at δ 104.73, which suggest that the lactone is attached to C-8 in the A-ring. This is confirmed

Table 6
^{13}C NMR (300 MHz, 296 K) data of chinchonain derivative **8** in $CDCl_3$

Ring	Carbon	**8**
A/C/E	2-C	77.85
	3-C	66.64
	4-C	26.52
	5-C	150.77
	6-C	104.68
	7-C	149.65
	8-C	111.43
	9-C	151.83
	10-C	104.73
	11-C	35.61
	12-C	36.24
	13-C	170.71
B	1′-C	135.91
	2′-C	124.69
	3′-C	142.59
	4′-C	142.40
	5′-C	122.27
	6′-C	123.83
D	1″-C	139.18
	2″-C	112.15
	3″-C	151.47
	4″-C	140.47
	5″-C	123.27
	6″-C	118.76
	$1\times OCH_3$	55.98
	$4\times OCOCH_3$	20.84 – 21.61
	$4\times OCOCH_3$	167.54 – 169.36

by a coupling between 10-C (A, δ 104.73) and 6-H (A, δ 104.68) in the HMBC spectrum.

Protonated carbons were identified using a HMQC spectrum, while the remaining resonances were identified utilizing a HMBC spectrum. The A-ring was identified on account of coupling between 10-C (A, δ 104.73) and 6-H (A, δ 6.61) as well as 4-CH_2 (C, δ 2.85 and 3.00). 8-C (A, δ 111.43) correlates with 6-H (A), 11-H (E, δ 4.64) and 12-CH_2 (E, δ 3.10 and 3.15). 9-C (A, δ 151.83) couples with 11-H (E) and 6-H (A), while 5-C (A, δ 150.77) and 7-C (A, δ 149.65) both show correlation to 6-H (A). 3′-C (B, δ 142.59) and 4′-C (B, δ 142.40) show coupling to 5′-H (B, δ 7.23) and 2′-H (B, δ 7.39), while 1′-C (B, δ 135.91) couples with 2′-H (B), 6′-H (B, δ 7.29) and 2-H (C, δ 5.41). Aforementioned serve to identify the B-ring resonances. The E-ring was distinguished on account of correlations that can be seen between 3″-C (D, δ 151.47) and 5″-H (D, δ 6.89), 2″-H (D, δ 6.76) and 3″-OMe (D, δ 3.50). This correlation also confirms the position of the methoxy group. 4″-C (D, δ 140.47) correlates with 2″-H (D), 5″-H (D) and 4″-OAc. 1″-C (D, δ 139.18) correlates with 6″-H (D, δ 6.68) and 11-H (E) and 12-CH_2.

3. Experimental

^1H NMR spectra were recorded on a Bruker AVANCE DPX 300 spectrometer for solutions as indicated, with Me_4Si as internal standard. Electron impact-mass spectroscopy (EI-MS) data were recorded on a VG-70E instrument when tuned to function in the EI-MS mode. CD data were collected in MeOH as solvent on a Hitachi 150-20 spectropolarimeter. TLC was performed on precoated Merck plastic sheets (silica gel 60 PF_{254}, 0.2 mm) and the plates were sprayed with H_2SO_4–HCHO (40:1, v/v) after development. Preparative plates (PLC) [20×20 cm, Kieselgel PF_{254} (1.0 mm)] were air dried and used without previous activation. Column chromatography was done on Sephadex LH-20 in 180×3.5 cm columns, at a flow rate of 0.5 cm^3/min using ethanol as eluent. Flash column chromatography (FCC) was carried out in a glass column (54×6.5 cm) charged with Merck Kieselgel 60 (230–400 mesh) using benzene–acetone (9:1, v/v) as eluent at a flow rate of 60 cm^3/min. Acetylations were conducted in Ac_2O–pyridine at 50 °C for 24 h. Methylations were conducted in dry acetone, dry K_2CO_3 was added and dimethylsulphate was subsequently added drop-wise over a period of 30 min under N_2. The reaction mixture was refluxed for 8 h after which the mixture was filtered and the acetone removed under reduced pressure. Evaporations were done under reduced pressure at ambient temperature in a rotary evaporator, and freeze drying of aqueous solutions on a Virtis 12 SL freezemobile.

The bark (3.81 kg) of *G. coleosperma* was powdered and extracted with hexane (2×2 l, 2×24 h) to remove fats and waxes. These extracts were then air dried and extracted with acetone (4×1.5 l, 48 h) and methanol (4×1.5 l, 48 h).

3.1. Metabolites from the acetone extract of the bark of G. coleosperma

The acetone extract (7×20 g) was partitioned in *sec*–BuOH–H_2O–hexane (4.8:6:1.2, v/v/v) in a 20 tube, 100 ml under-phase, Graig countercurrent assembly. Following qualitative TLC (B:A:M, 7:2:1, v/v/v) and 2D paper chromatography analysis, the fractions were combined to afford five fractions. The third fraction was subjected to column chromatography on Sephadex LH-20 (3.5×180 cm column, flow rate 0.5 cm^3/min) in ethanol to give 18 fractions after analysis with TLC (B:A:M, 7:2:1, v/v/v). The last fraction was derivatized in Ac_2O–pyridine and separated by PLC (C:A:EA, 90:5:5, v/v/v) into one fraction 18.1, hepta-*O*-acetyl-7-β-xylopyranosyl-epicatechin **2** (R_f 0.50, 14.7 mg). Methylation, acetylation and subsequent PLC (B:A, 9:1, v/v) of the same fraction afforded one band 18.1, tetra-*O*-acetyl-tri-*O*-methyl-7-β-xylopyranosyl-epicatechin (**3**) (R_f 0.70, 9.4 mg).

3.2. Hepta-O-acetyl-7-β-xylopyranosyl-epicatechin 2 as a white amorphous solid

(Found, M^+, 716.203, $C_{34}H_{36}O_{17}$, requires M, 716.195) ^1H NMR: Table 1. ^{13}C NMR: Table 2. CD $[\theta]_{305}$ -6.35×10^1, $[\theta]_{280}$ -2.88×10^3, $[\theta]_{260}$ -9.30×10^2, $[\theta]_{235}$ 4.11×10^3, $[\theta]_{215}$ 2.93×10^2. EI-MS, m/z 259 (M^+, 8%), 138 (10), 279(10).

3.3. Tetra-O-acetyl-tri-O-methyl-7-β-xylopyranosyl-epicatechin (3) as a white amorphous solid

^{1}H NMR: Table 1.

The fourth fraction from the Graig countercurrent assembly was subjected to column chromatography on Sephadex LH-20 (3.5 × 180 cm column, flow rate 0.5 cm^3/min) in ethanol to give 18 fractions after analysis with TLC (B:A:M, 7:2:1, v/v/v). The last fraction was derivatized in Ac_2O–pyridine and separated by PLC (C:A:EA, 90:5:5, v/v/v) into one fraction 18.1, epicatechin-(7,8-bc)-9β-(3-methoxy-4-acetoxyphenyl)-dihydro-2(3H)-pyranone (**7**) (R_f 0.18, 7.1 mg).

3.4. Epicatechin-(7,8-bc)-9β-(3-methoxy-4-acetoxyphenyl)-dihydro-2(3H)-pyranone (7) as a brown amorphous solid

(Found, M$^+$, 676.187, $C_{35}H_{32}O_{14}$, requires M, 676.179) ^{1}H NMR: Table 5. ^{13}C NMR: Table 6. CD $[\theta]_{271.8}$ -2.20×10^3, $[\theta]_{245.8}$ 3.45×10^3, $[\theta]_{240}$ -1.80×10^1.

3.5. Metabolites from the methanol extract of the bark of G. coleosperma

The methanol extract was subjected to column chromatography on Sephadex LH-20 (3.5 × 180 cm column, flow rate 0.5 cm^3/min) in ethanol to give 14 fractions after analysis with TLC (B:A:M, 7:2:1, v/v/v). The last fraction was derivatized in Ac_2O–pyridine and separated by PLC (C:A:EA, 90:5:5, v/v/v) into one fraction 14.1, dodeca-*O*-acetyl-epicatechin-(4β → 8)-7-*O*-β-xylopyranosyl-epicatechin (**5**) (R_f 0.13, 34.7 mg). Methylation and subsequent FCC (B:A, 9:1, v/v) of the same fraction afforded five fractions. Fraction 14.5 was acetylated and purified with PLC (C:A:EA, 90:5:5, v/v/v) to give one fraction, 14.5.1, hepta-*O*-acetyl-hexa-*O*-methyl-epicatechin-(4β → 8)-7-*O*-β-xylopyranosyl-epicatechin (**6**) (R_f 0.49, 4.2 mg).

3.6. Dodeca-O-acetyl-epicatechin-(4β → 8)-7-O-β-xylopyranosyl-epicatechin (5) as a white amorphous solid

(Found, M$^+$, 1215.347, $C_{59}H_{58}O_{28}$, requires M, 1215.334) ^{1}H NMR: Table 3. ^{13}C NMR: Table 4. CD $[\theta]_{260}$ 8.92×10^1, $[\theta]_{240}$ 1.88×10^4, $[\theta]_{220}$ 4.41×10^2.

3.7. Hepta-O-acetyl-hexa-O-methyl-epicatechin-(4β → 8)-7-O-β-xylopyranosyl-epicatechin (6) as a white amorphous solid

^{1} H NMR: Table 3. ^{13}C NMR: Table 4.

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