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THE STRUCTURE AND SYNTHESIS OF PROGUIBOURTINIDINS FROM *CASSIA ABBREVIATA*

ELFRANCO MALAN,* EWALD SWINNY, DANEEL FERREIRA† and PETRUS STEYNBERG†

Department of Chemistry, University of Durban-Westville, Private Bag X54001, Durban 4000, South Africa; †Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa.

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Key Word Index—*Cassia abbreviata*; Caesalpiniaceae; bark; afzelechin; epiafzelechin; proguibourtinidin dimers; biomimetic synthesis.

Abstract—An acetone extract of the bark of *Cassia abbreviata* yielded guibourtinidol-(4$\beta \rightarrow$8)-epiafzelechin, guibourtinidol-(4$\alpha \rightarrow$8)-epiafzelechin, guibourtinidol-(4$\alpha \rightarrow$8)-catechin, guibourtinidol-(4$\beta \rightarrow$8)-epicatechin and ent-guibourtinidol-(4$\beta \rightarrow$8)-epicatechin. The structures of the proguibourtinidin dimers were confirmed by synthesis via mild acid-condensation. Guibourtinidol-(4$\alpha \rightarrow$8)-afzelechin, previously isolated from *Acacia leuderitzii*, was also synthesized. Another two proguibourtinidol oligomers, namely, guibourtinidol-(4$\beta \rightarrow$8)-afzelechin and guibourtinidol-(4$\alpha \rightarrow$6)-afzelechin were products of the previous synthesis but neither of them have been reported to date.

INTRODUCTION

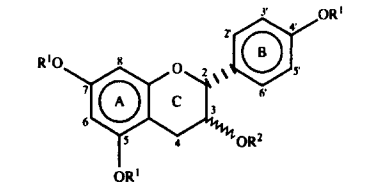
Cassia abbreviata is a small umbrella-shaped deciduous tree with a very distinctive cylindrical pod fruit. It occurs on sandy soil in the Kruger National Park. The bark has a brown-grey colour while the heartwood is dark-brown heavy (900 kg m^{-3}) and hard. The tree features in African medicine and infusions of the bark were used to treat blackwater fever, abdominal pain and toothache [1]. Reported natural proanthocyanidin dimers containing guibourtinidin and epiafzelechin are few and only the procissinidins, propelargonidins, procyanidins and proguibourtinidins of *ent*- and epi-afzelechin from the leaves [2], fruit [3] and sapwood [4] of *C. fistula* are known. Guibourtinidol-(4$\alpha \rightarrow$8)-afzelechin, -(4$\alpha \rightarrow$8)-catechin and -epicatechin, -(4$\alpha \rightarrow$6)-catechin and -epicatechin were discovered in the heartwood of *Acacia luederitzii* [5]. The guibourtinidin dimers containing epiafzelechin, catechin and epicatechin moieties isolated from the bark of *C. abbreviata* were all synthesized by condensation under mild acidic conditions [6].

RESULTS AND DISCUSSION

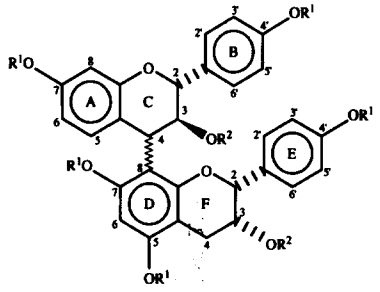
The proguibourtinidins (**3**, **4**, **5**, **6**, **7**) are accompanied in the bark of *C. abbreviata* by afzelechin **1** and epiafzelechin **2** in a 6:1 ratio. Surprisingly, no proguibourtinidins containing afzelechin or any of the monomers (guibourtinidol, catechin and epicatechin) could be found in the bark. No useful ^{1}H NMR data could be obtained for the proguibourtinidins in their free phenolic state but the structural elucidation of these compounds was accomplished on their permethyl ether 3-*O*-acetate or peracetate derivates with confirmation by biomimetic synthesis.

The ^{1}H NMR spectrum of the proguibourtinidin derivative (**3a**, Table 1) showed an AMX and an ABMX system in the heterocyclic region which were attributed to the C- and F-rings, respectively. The AMX-system showed peaks at δ5.46 (*d*, J = 7.0 Hz, H-2), δ5.61 (*dd*, 7.0 and 5.5 Hz, H-3) and δ4.80 (*d*, J = 5.5 Hz, H-4) suggesting a 2,3-*trans*-3,4-*cis* relative configuration for the C-ring. A prominent NOE association of 19.6% between H-4(C) with H-3(C) and a simultaneous lack of any association between H-4(C) and H-2(C) confirmed the relative configuration. The ABMX system of the F-ring comprised a broad singlet at δ4.36 for H-2 and a one proton multiplet at δ5.33 for H-3. The *cis*-configuration of H-2(F) and H-3(F) was confirmed by a significant NOE association of 6%. Two distinctive doublets of doublets at δ2.85 (J = 2.0 and 18.5 Hz) and δ2.95 (J = 5.0 and 18.5 Hz) typical of H-4ax and H-4eq respectively accounted for the rest of the F-ring system. An ortho-coupled doublet at δ6.62 (J = 8.5 Hz) also showed benzylic coupling of 1.0 Hz with H-4(C). This relationship was supported with a NOE association of 8.1% confirming it to be the H-5(A) as part of an ABX system of the 7-substituted A-ring with H-6 at δ6.13 (*dd*, J = 2.5 and 8.5 Hz) and H-8 at δ5.98 (*d*, J = 2.5 Hz). The 7-OMe (δ3.43, A-ring) of **3a** showed a NOE association of 13.8% with H-8(A). Spin–spin decoupling and a NOE of 5.5% showed strong association of H-2(C) to H-2′,6′(B) at δ7.32 (*d*, J = 9.0 Hz) confirming the AA′BB′-system for

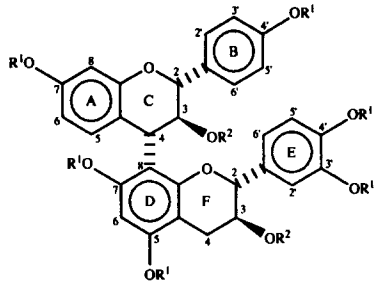
*Author to whom correspondence should be addressed.



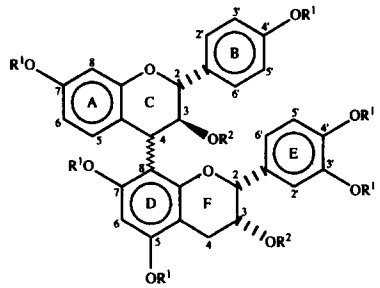
		R¹	R²
1	~~~ = ◀	H	H
1a	~~~ = ◀	Me	Ac
2	~~~ = ▮▮▮	H	H
2a	~~~ = ▮▮▮	Me	Ac



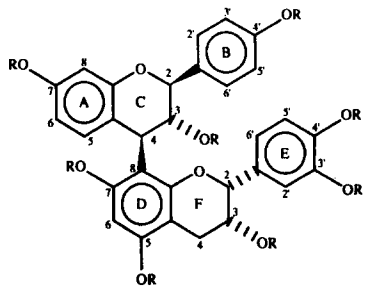
		R¹	R²
3	~~~ = ◀	H	H
3a	~~~ = ◀	Me	Ac
4	~~~ = ▮▮▮	H	H
4a	~~~ = ▮▮▮	Me	Ac



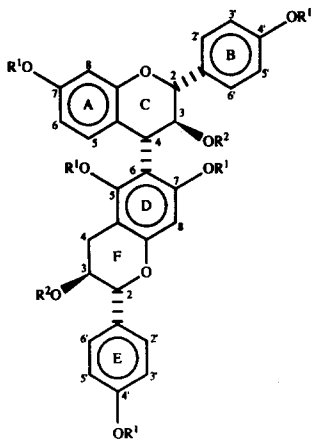
	R¹	R²
5	H	H
5a	Me	Ac



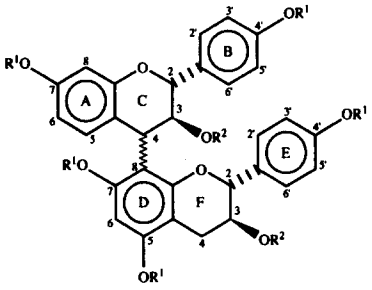
		R¹	R²
6	~~~ = ◀	H	H
6a	~~~ = ◀	Me	Ac
8	~~~ = ▮▮▮	H	H
8a	~~~ = ▮▮▮	Ac	Ac



		R
7	~~~ = ◀	H
7a	~~~ = ◀	Ac



	R¹	R²
10	H	H
10a	Me	Ac



		R¹	R²
9	~~~ = ▮▮▮	H	H
9a	~~~ = ▮▮▮	Me	Ac
11	~~~ = ◀	H	H
11a	~~~ = ◀	Me	Ac

the B-ring with H-3′,5′ at δ6.86 (*d*, J = 9.0 Hz). Similarly the substitution pattern of the E-ring was resolved as also having an AA′BB′ system with H-2′,6′ at δ6.93 (J = 9.0 Hz) and H-3′,5′ at δ6.72 (J = 9.0 Hz). The AA′ doublet (δ6.93) showed a NOE association of 3.2% with H-2(F). The aromatic region exhibited a one-proton singlet at δ6.14 which suggested the proton to be H-6(D). This was confirmed by a strong NOE association of the residual proton with both the 5-OMe (10.9%) and the 7-OMe (12.1%) of the D-ring. The high-amplitude positive Cotton effect at $\theta_{243.3}$ 2.433 x 10^4 in the CD spectrum of the peracetate of **3** defined a 4β(C) linkage and thus an overall 2*R*, 3*S*, 4*S* absolute stereochemistry for the top-unit [7]. The biomimetic synthesis of **3** using

Table 1. ^{1}H NMR (300 MHz), $CDCl_3$ data of **3a**, **4a**, **5a**, **6a**, **7a**, **9a**, **10a**, **11a**

Ring	Proton	**3a**	**4a**	**5a**	**6a**	**7a**	**9a**	**10a**	**11a**
A	5	6.62 (*d*, 8.5)	6.78 (*d*, 8.5)	6.56 (*d*, 8.5)	6.61 (*d*, 8.5)	6.90 (*d*, 8.5)	6.62 (*d*, 8.5)	6.68 (*d*, 8.5)	6.65 (*d*, 8.5)
	6	6.13 (*dd*, 2.5, 8.5)	6.45 (*dd*, 2.5, 8.5)	6.41 (*dd*, 2.5, 8.5)	6.11 (*dd*, 2.5, 8.5)	6.60 (*dd*, 2.5, 8.5)	6.38 (*dd*, 2.5, 8.5)	6.44 (*dd*, 2.5, 8.5)	6.23 (*dd*, 2.5, 8.5)
	8	5.98 (*d*, 2.5)	6.50 (*d*, 2.5)	6.31 (*d*, 2.5)	5.89 (*d*, 2.5)	6.64 (*d*, 2.5)	6.46 (*d*, 2.5)	6.36 (*d*, 2.5)	5.77 (*d*, 2.5)
B	2′,6′	7.32 (*d*, 9.0)	7.07 (*d*, 9.0)	7.00 (*d*, 9.0)	7.31 (*d*, 9.0)	7.08 (*d*, 8.5)	7.41 (*d*, 8.5)	6.70–7.41	7.19–7.28 and
	3′,5′	6.86 (*d*, 9.0)	6.73 (*d*, 9.0)	6.73 (*d*, 9.0)	6.84 (*d*, 9.0)	6.96 (*d*, 8.5)	6.88 (*d*, 8.5)		6.73–7.00
C	2	5.46 (*d*, 7.0)	4.90 (*d*, 10.0)	4.78 (*d*, 10.0)	5.48 (*d*, 7.0)	4.91 (*d*, 10.0)	4.92 (*d*, 9.5)	4.83 (*d*, 9.5)	5.25 (*d*, 10.0)
	3	5.61 (*dd*, 5.5, 7.0)	5.93 (*t*, 10.0)	5.96 (*t*, 10.0)	5.60 (*dd*, 5.5, 7.0)	5.90 (*t*, 9.9, 10.0)	6.04 (*t*, 9.5, 10.0)	5.92 (*t*, 9.5, 10.0)	5.54 (*dd*, 7.0, 10.0)
	4	4.80 (*d*, 5.5)	4.89 (*d*, 10.0)	4.91 (*d*, 10.0)	4.80 (*d*, 5.5)	4.51 (*d*, 10.0)	4.70 (*d*, 10.0)	4.78 (*d*, 10.0)	4.90 (*dd*, 1.0, 7.0)
D	8						6.26 (*s*)		
	6	6.14 (*s*)	6.15 (*s*)	6.15 (*s*)	6.15 (*s*)	6.64 (*s*)		6.13 (*s*)	6.15 (*s*)
E	2′			6.54 (*d*, 2.0)	6.77 (*d*, 2.0)	6.65 (*d*, 2.0)			
	5′			6.69 (*d*, 8.5)	6.68 (*d*, 8.5)	7.10 (*d*, 8.5)			
	6′			6.42 (*dd*, 2.0, 8.5)	6.50 (*dd*, 2.0, 8.5)	6.75 (*dd*, 2.0, 8.5)			
	2′, 6′	6.93 (*d*, 9.0)	6.64 (*d*, 9.0)				7.30 (*d*, 8.5)	6.70–7.41	7.19–7.28 and
	3′, 5′	6.72 (*d*, 9.0)	6.68 (*d*, 9.0)				6.88 (*d*, 8.5)		6.73–7.00
F	2	4.36 (*br*, $s < 1.0$ Hz)	4.99 (*br*, *s*)	5.06 (*d*, 6.5)	4.32 (*br*, $s < 1$ Hz)	5.04 (*br*, *s*, $J < 1$ Hz)	4.96 (*d*, 8.0)	4.90 (*d*, 8.0)	4.10 (*d*, 8.5)
	3	5.33 (*m*)	5.27 (*m*)	4.98 (*m*)	5.29 (*m*)	5.24 (*m*)	5.33 (*m*)	5.19 (*m*)	5.15 (*m*)
	4ax	2.85 (*dd*, 2.0, 18.0)	2.82 (*dd*, 2.0, 18.0)	2.58 (*dd*, 9.0, 17.0)	2.85 (*dd*, 1.0, 18.0)	2.78 (*dd*, 1.0, 17.0)	2.77 (*dd*, 8.5, 17.0)	2.57 (8.5, 17.0)	2.55 (*dd*, 8.5, 17.0)
	4eq	2.95 (*dd*, 5.0, 18.0)	2.91 (*dd*, 5.0, 18.0)	3.05 (*dd*, 6.0, 17.0)	2.95 (*dd*, 5.0, 18.0)	3.00 (*dd*, 4.5, 17.0)	3.14 (*dd*, 5.5, 17.0)	2.99 (5.5, 17.0)	3.10 (*dd*, 6.5, 17.0)
	OMe	(3.83-5D), (3.79-4B) (3.76-7D) (3.75-4E) (3.43-7A)	3.86, 3.82, 3.78, 3.76, 3.73 (each *s*)	3.70, 3.73, 3.74, 3.81, 3.85, 3.86 (each *s*)	3.83, 3.82 (4E), 3.78, 3.76, 3.73 (3E) 3.40 (7A) (each *s*)		3.80, 3.79, 3.75, 3.73, 3.53 (each *s*)	3.85, 3.80, 3.77, 3.74 (2 × OMe), (each *s*)	3.84, 3.79, 3.78, 3.76, 3.75 (each *s*)
	OAc	1.79, 1.88 (each *s*)	1.57, 1.70 (each *s*)	1.58, 1.86 (each *s*)	1.88, 1.83 (each *s*)	2.32, 2.28, 2.27, 2.23, 1.75, 1.59 (each *s*), 2.26 (2 × OAc)	1.90, 1.60 (each *s*)	1.87, 1.58 (each *s*)	1.79, 1.69 (each *s*)

guibourtinidol-4α-ol and epiafzelechin in a condensation reaction [6] under mild acidic conditions confirmed the absolute stereochemistry of the bottom unit to be 2*R*, 3*R* and the dimer as guibourtinidol-(4β → 8)-epiafzelechin **3**. FAB-mass spectrometry confirmed the molecular mass of **3a** to be 684 kDa [$(M - H)^+ = 683$] required by the molecular formula of $C_{39}H_{40}O_{11}$.

The pentamethyl ether diacetate of the proguibourtinidin **4** yielded compound **4a** which was subjected to the same NMR analysis as **3a** and the major differences (Table 1) were the coupling constants displayed by the protons of the AMX system of the C-ring, namely, $J_{2,3} = J_{3,4} = 10.0$ Hz [8] suggesting a 2,3-*trans*-3,4-*trans* relative configuration and a pronounced negative Cotton effect ($\theta_{233.5} - 2.015 \times 10^{-4}$) which indicated a 4α(C) coupling and a 2*R*, 3*S*, 4*R* absolute stereochemistry for the top-unit. The remaining physical data (Table 1) was in accordance with the information published by Patil [4]. The condensation of guibourtinidol-4α-ol and epiafzelechin under mild acidic conditions yielded compound **4** as one of the diastereomers with compound **3** during the same reaction.

Guibourtinidol-(4α → 8)-catechin hexamethyl ether diacetate **5a** was obtained after methylation and subsequent acetylation of compound **5**. By executing the same analytical procedures for **5a** as for compound **3a** it was found to have an all-*trans* C- and F-ring (Table 1) with a large negative Cotton effect $\theta_{235.6} - 1.799 \times 10^{-4}$, confirming the absolute stereochemistry of the top-unit to be 2*R*, 3*S*, 4*R*. Other ^{1}H NMR and physical data is in accordance with the dimer isolated from *A. luederitzii* [5]. A biomimetic synthesis which involved the condensation of guibourtinidol-4α-ol with catechin resulted in the identical compound **5a** after the derivatization of **5**.

Guibourtinidol-(4β → 8)-epicatechin **6** was converted to its hexamethyl ether diacetate **6a** after which the same analytical procedures were applied as was the case with compound **3a**. This newly discovered dimer exhibited an AMX system for the C-ring with H-2 (*d*, $J = 7.0$ Hz) at δ5.48, H-3 (*dd*, $J = 5.5$ and 7.0 Hz) at δ5.60 and H-4 (*d*, $J = 5.5$ Hz) at δ4.80, which suggested a 2,3-*trans*-3,4-*cis* relative configuration. A NOE association of 10.2% between H-4(C) and H-3(C) and a corresponding lack of any association with H-2(C) supported the above relative configuration. The information obtained showed an AMXY system for the F-ring with a relative 2,3-*cis* configuration which is typical for epicatechin (Table 1). HOMODEC experiments have shown an AA′BB′ pattern for the B-ring, ABX-systems for the A- and E-rings supported by prominent NOE associations of 7-OMe (δ3.40, A) to H-8 (δ5.89, 12.5%) and H-6 (δ6.11, 5.2%) and of 3′-OMe(E) with 4′-OMe(E) to H-2′ (δ6.77, 10.3%) and H-5′ (δ6.68, 10.4%), respectively (see Table 1 for more detail). The one-proton singlet at δ6.15 was identified as H-6(D) of a C-8 substituted (phloroglucinol-derived D-ring) bottom-unit with NOE associations of 10.2% and 10.8% to the adjacent 5-OMe and 7-OMe, confirming the above. The positive Cotton effect of $\theta_{244.7}$ 3.63×10^4 in the CD spectrum of **6a** indicated the top-unit to have an absolute configuration of 2*R*, 3*S*, 4*S*. The biomimetic synthesis confirmed the dimer **6a** to have a final absolute configuration of 2*R*, 3*S*, 4*S*−2*R*, 3*R*, using guibourtinidol-4α-ol and epicatechin as starting material.

Among the products yielded in the above synthesis, guibourtinidol-(4α → 8)-epicatechin **8** was obtained as the octacetate (**8a**). The absolute stereochemistry of **8a** was established as 2*R*, 3*S*, 4*R*−2*R*, 3*R*. Compound **8** was previously characterized as the hexamethyl ether diacetate when isolated from *A. leuderitzii* [5].

Because of its very low quantity in the original fraction ent-guibourtinidol-(4β → 8)-epicatechin **7** was isolated as the full acetate **7a**, a strategy which worked very well for low concentration compounds isolated from *Acacia galpinii* [9]. A similar procedure for the structure elucidation of **7a** was followed as with **3a**. The AMX system for the C-ring of the top unit showed a 2,3-*trans*-3,4-*trans* relative configuration with coupling constants of $J_{2,3} = J_{3,4} = 10.0$ Hz (Table 1). The ABMX pattern for the F-ring was the same as for **6a**, defining it to be an 2*R*, 3*R*-epicatechin (Table 1) bottom unit. The positive Cotton effect of $\theta_{229.2}$ 9.27×10^3 is an important feature, confirming the 4β(C) linkage and an overall 2*S*, 3*R*, 4*S* absolute stereochemistry for the top unit.

Guibourtinidol-(4α → 8)-afzelechin **9** was reported to have been isolated from *A. luederitzii* but no data were published to support the claim. Owing to the availability of the pure afzelechin from the bark it was reacted with guibourtinidol-4α-ol under mild acidic conditions, to yield compound **9** in addition to the diastereomers guibourtinidol-(4α → 8)-afzelechin **11** and guibourtinidol-(4α → 6)-afzelechin **10**. All three were derivatized to their respective pentamethyl ether diacetates **9a**, **10a** and **11a**. The ^{1}H NMR data is listed in Table 1. Because of rotational isomerism, even at elevated temperatures, it was not possible to distinguish between the AA′BB′ systems of the B- and E-rings of both **9a** and **11a**.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a 300 MHz spectrometer. CD spectra were recorded in MeOH. Phenolic material dissolved in MeOH was methylated with ethereal diazomethane at −10° for 48 hr. Acetates were prepared with Ac_2O-pyridine at 60° for 2 hr. Milled bark (2.5 kg) was extracted with acetone over 48 hr at 30° and yielded 300 g of extract. Compounds in the acetone extract (56 g) were first concd by counter-current technique and then separated by Sephadex LH-20/EtOH and CC (Merck 7734), C_6H_6–Me_2CO, 8:4. Final purification of the methyl ether acetates and full acetates was done on Merck TLC 5554 in C_6H_6–Me_2CO, 9:1 × 3.

All the dimers were synthesized by condensing the respective monomers (flavan-3,4-diol,150 mg; flavan-3-ol, 300 mg) in a 0.1 M HCl aq. soln (200 ml) and stirred under N_2 at 25°. The reaction was monitored by TLC and quenched with excess ice after 24 hr. This was followed by immediate extraction of the reaction mixture

with EtOAc, the solvent evapd under reduced pressure and the residue chromatographed (Merck TLC 5554 C_6H_{12}–C_6H_6–Me_2CO–MeOH, 40:40:15:5). The phenolic compounds were converted to their methyl ether acetate or full acetate derivatives.

Plant material. Cassia abbreviata was collected near Lower Sabie in the Eastern Transvaal and identified by Mrs L. Davies of the National Parks Board.

Guibourtinidol-(4β → 8)-epiafzelechin pentamethyl ether diacetate (**3a**). Noncrystalline, 7 mg, R_f 0.38. MS-FAB: $[M - H]^+ - HOAc$, *m/z* 623 (28%). Found $[M - H]^+ - HOAc$, 623.2284 $C_{39}H_{40}O_{11}$-H-HOAc requires 623.2281.

Guibourtinidol-(4α → 8)-catechin hexamethyl ether diacetate (**5a**). Noncrystalline, 12 mg, R_f 0.63. MS-FAB: $[M + H]^+$, *m/z* 715 (48%); $[M + H]^+ - HOAc$ *m/z* 654 (20%). Found $[M + H]^+$, 715.2752 $C_{40}H_{42}O_{12} + H$ requires 715.2755.

Guibourtinidol-(4β → 8)-epicatechin hexamethyl ether diacetate (**6a**). Noncrystalline, 2 mg, R_f 0.39. MS-FAB: $[M + H]^+$, *m/z* 715 (46%); $[M + H]^+ - HOAc$ *m/z* 654 (21%). Found $[M + H]^+$, 715.2753 $C_{40}H_{42}O_{12} + H$ requires 715.2755.

ent-Guibourtinidol-(4β → 8)-epicatechin octaacetate (**7a**). Noncrystalline, 11 mg, R_f 0.57. MS-FAB: $[M + H]^+$, *m/z* 883.

Guibourtinidol-(4α → 8)-afzelechin pentamethyl ether diacetate (**9a**). Noncrystalline, 2 mg, R_f 0.34. MS-FAB: $[M + H]^+$, *m/z* 685 CD: $[\theta]_{285.0} - 1.705 \times 10^3$, $[\theta]_{282.0}$ 1.021×10^2, $[\theta]_{274.00}$ 4.0543×10^3, $[\theta]_{257.50} - 1.929 \times 10^2$, $[\theta]_{235.40} - 4.548 \times 10^4$, $[\theta]_{224.4} - 5.672 \times 10^2$.

Guibourtinidol-(4α → 6)-afzelechin pentamethyl ether diacetate (**10a**). Noncrystalline, 1 mg, R_f 0.39. MS-FAB: $[M + H]^+$, *m/z* 685; CD: $[\theta]_{286.0} - 2.239 \times 10^2$, $[\theta]_{268.0}$ 1.657×10^3, $[\theta]_{248.6} - 8.241 \times 10^1$, $[\theta]_{236.60}$ $- 1.321 \times 10^4$, $[\theta]_{232.6} - 8.888 \times 10^1$.

Guibourtinidol-(4β → 8)-afzelechin pentamethyl ether diacetate (**11a**). Noncrystalline, 3 mg, R_f 0.31. MS-FAB: $[M + H]^+$, *m/z* 685; CD: $[\theta]_{293.0} - 7.264 \times 10^2$, $[\theta]_{285.00}$ 3.484×10^3, $[\theta]_{279.00} - 7.985 \times 10^2$, $[\theta]_{257.00}$ $- 2.371 \times 10^3$, $[\theta]_{266.6} - 1.541 \times 10^2$, $[\theta]_{240.7}$ 5.405×10^4, $[\theta]_{234.1}$ 2.417×10^2.

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