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Cardiac glycosides from *Cryptostegia grandiflora*

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Abstract

From the leaves of *Cryptostegia grandiflora,* four new cardiac glycosides oleandrigenin 3-*O*-β-glucopyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-digitoxopyranoside, cryptostigmin I, oleandrigenin 3-*O*-β-glucopyranosyl-(1→4)-α-rhamnopyranoside, cryptostigmin II, 16-propionylgitoxigenin 3-*O*-β-glucopyranosyl-(1→4)-α-rhamnopyranoside, cryptostigmin III and oleandrigenin 3-*O*-β-glucopyranosyl-(1→6)-β-glucopyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-digitoxopyranoside, cryptostigmin IV have been isolated together with two known cardenolides subalpinosid and 16-*O*-acetyl-digitalinum verum. The structures of the isolated compounds were verified by means of MS and NMR spectral analyses.

Keywords: Cryptostegia grandiflora; Asclepiadaceae; Cardenolides; Cryptostigmin I–IV

1. Introduction

Cryptostegia grandiflora R.Br. (Asclepiadaceae) is an ornamental plant commonly cultivated in Egypt and warm countries (Metcalf and Chalk, 1950; Chopra et al., 1965; Hutchinson, 1973). The plant has a wide use in manufacturing of rubber and as a source of hydrocarbon fuels from its latex (Mehta et al., 1982). Three cardiac glycosides were isolated from *Cryptostegia madagascarensis* (14, 16-dianhydrogitoxigenin-3-rhamnoside, 16-propionylgitoxigenin-3-rhamnoside and 16-anhydrogitoxigenin-3-rhamnoside) (Sanduja et al., 1984) while few cardenolides (cryptograndoside A, B and digitalinum verum) have been already isolated and identified from *C. grandiflora* (Watt and Breyer, 1962). Moreover, the macro and micromorphological characters of the leaves and stems of *C. grandiflora* have been also investigated (Assaf, 1998). The present study deals with the isolation and structure elucidation of four new cardiac glycosides together with two known glycosides from the leaves of *C. grandiflora*.

2. Results and discussion

The ethanolic extract of the leaves of *C. grandiflora* was defatted with CH_2C1_2 and the aqueous layer was subjected to a column chromatography of Diaion HP-20. The 80% methanol eluate was repeatedly chromatographed on columns of silica gel and then by MPLC and HPLC to afford 6 glycosides.

Comparison of the NMR spectra of compound **1** with the published data revealed its structure as subalpinosid previously isolated from *Digitalis subalpina* (Lichius et al., 1991).

The molecular formula of compound **2** was deduced as $C_{44}H_{68}0_{17}$ from HR FAB–MS spectrometry (see Experimental section). The ^{13}C NMR spectrum (Tables 1 and 2) of **2** displayed the presence of one unsubstituted β-g1ucopyranosy1 unit from the signals at δ_c 104.3 (C-1), 74.9 (C-2), 78.2 (C-3 and 5), 71.9 (C-4) and 63.0 (C-6), one substituted β-digitoxopyranosyl unit from the signals at δ_c 96.5 (C-1), 38.9 (C-2), 71.8 (C-3), 83.0 (C-4), 67.5 (C-5) and 18.6 (C-6) and one substituted β-cymaropyranosyl unit from the signals at δ_c 101.3 (C-1), 37.2 (C-2), 78.5 (C-3), 83.4 (C-4), 68.5 (C-5), 18.7 (C-6) and 56.7 (OMe) (Bradbury and Jenkins, 1984) together with 25 carbon signals for the aglycone. The chemical shifts of the aglycone carbons were similar to those reported for 3-0 substituted oleandrigenin (Lichius et al., 1991). The downfield shifts of C-4 of the digitoxo- and

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Table 1
^{13}C NMR spectral data of the aglycone moieties of compounds **2**–**5** (100 MHz, C_5D_5N)[a]

C	2	3	4	5
1	30.4	30.1	30.1	30.4
2	26.9	26.9	27.0	27.0
3	73.2	72.6	72.8	73.2
4	30.7	30.9	30.9	30.8
5	36.9	36.9	37.0	36.9
6	26.9	26.7	26.8	27.0
7	21.1[a]	21.1[a]	21.1[a]	21.1[a]
8	41.8	41.8	41.9	41.9
9	35.7	35.6	35.7	35.8
10	35.4	35.3	35.4	35.4
11	21.6[a]	21.6[a]	21.6[a]	21.6[a]
12	39.1	38.9	38.9	39.1
13	50.4	50.4	50.4	50.4
14	83.6	83.3	83.3	83.4
15	41.1	41.1	41.3	41.1
16	75.6	76.3	76.4	75.4
17	75.3	56.7	56.8	57.6
18	16.2	16.2	16.2	16.2
19	23.8	23.9	23.9	23.9
20	169.8	169.9	169.8	169.8
21	76.2	76.2	76.3	76.2
22	121.5	121.5	121.6	121.5
23	174.2	174.2	174.2	174.2
-COMe	170.2	170.3	173.7	170.2
-COMe	20.7	20.6		20.7
-$COCH_2$Me			9.1	
-$COCH_2$Me			27.7	

[a] a,b values may be interchangeable in each column.

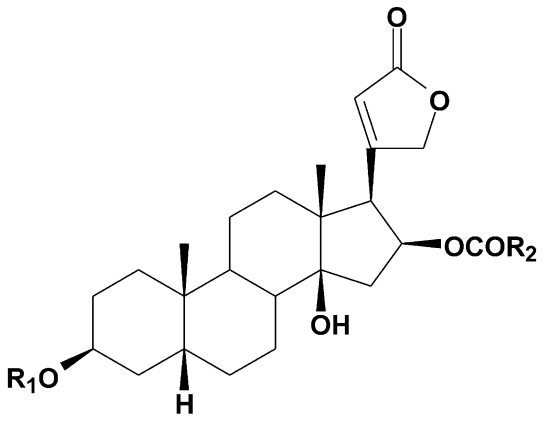
Table 2
^{13}C NMR spectral data of the sugar moieties of compounds **2**–**5** (100 MHz, C_5D_5N)[a]

C[b]	2	3	4	5
Dig				
1	96.5			96.5
2	38.9			38.9
3	71.8			71.7
4	83.0			83.0
5	67.5			67.5
6	18.6			18.6
Cym				
1	101.3			101.4
2	37.2			37.4
3	78.5			78.5
4	83.4			83.7
5	68.5			68.5
6	18.7			18.7
OMe	56.7			56.8
Rha				
1		99.6	99.6	
2		71.4	71.4	
3		72.2	72.3	
4		85.1	85.2	
5		68.3	68.3	
6		18.4	18.4	
Glc				
	104.3	106.6	107.7	104.3
1	74.9	74.9	74.7	74.9
2	78.2	78.3[a]	78.4[a]	78.3[b]
3	71.9	72.4	72.5	72.1
4	78.2	78.4[a]	78.5[a]	77.4
5	63.0	62.5	62.6	70.2
6				
Glc				
1				105.1
2				74.9
3				78.2[b]
4				72.1
5				78.3[b]
6				62.8

[a] a,b: values may be interchangeable in each column.
[b] Dig: β-Digitoxopyranose, Cym: β-Cymaropyranose, Rha: α-Rhamnopyranose, Glc: β-Glucopyranose.

cymaropyranosyl units (δ_c 83.0 and 83.4 respectively) proved their substitution at C-4 of each unit (Bradbury and Jenkins, 1984). In the ^1H NMR spectrum of **2**, the doublet signal at δ_H 5.04 (1H) with *J* (constant) 7.8 Hz for the anomeric proton of the glucosyl residue indicated the β configuration. Moreover, the broad doublet signal at δ_H 5.39 (*J* constant 9.1 Hz) and the doublet of doublet signal at δ_H 4.71 (*J* constant 4.7 and *9.5* Hz) indicated the β configuration of the digitoxo- and cymaropyranosyl units respectively. The HMQC spectral data of **2** revealed the correlations between each carbon and its directly attached protons while H–H COSY interpreted the proton–proton couplings. The HMBC spectral analysis of **2** (Fig. 1) displayed significant correlation peaks between H-1 of the digitoxopyranosyl unit with C-3 of the aglycone as well as H-16 with COMe and H-22 with C-23. The negative FAB–MS spectrum of **2** exhibited M^+ at *m/z* 867 $[M–H]^-$ as well as a significant peak at *m/z* 705 $[M–H\text{-glucose}]^-$ and a peak at *m/z* 561 $[M–H\text{-(glucose + cymarose)}]^-$. Therefore, the internal sugar unit was confirmed as the digitoxopyranosyl unit and the structure of compound **2** was assigned as oleandrigenin 3-*O*-β-glucopyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-digitoxopyranoside and named cryptostigmin I.

The molecular formula of compound **3** was deduced as $C_{37}H_{56}O_{15}$ from HR FAB–MS spectrometry (see Experimental section). ^{13}C NMR spectral data of **3** (Tables 1 and 2) were very similar to those of **2** in the aglycone moiety indicating the presence of 3-*O* substituted oleandrigenin as aglycone. The signals of the sugar moiety revealed the presence of a terminal β-glucopyranosyl unit in addition to α-substituted a rhamnopyranosyl unit from the signals at δ_c 99.6 (C-1), 71.4 (C-2), 72.2 (C-3), 85.1 (C-4), 68.3 (C-5) and 18.4 (C-6) (Breitmaier and Voelter, 1987). The downfield shift of C-4 of the rhamnopyranosyl unit (δ_c 85.1) indicated its substitution at this position. The β configuration of the glucopyranosyl unit was deduced from the doublet signal of its anomeric proton at δ_H 5.20 with *J* constant 7.7



	R_1	R_2
(2)	— Dig —(4)— Cym —(4)— Glc	$-CH_3$
(3)	— Rha —(4)— Glc	$-CH_3$
(4)	— Rha —(4)— Glc	$-CH_2-CH_3$
(5)	— Dig —(4)— Cym —(4)— Glc —(6)— Glc'	$-CH_3$

Dig: β-digitoxopyranose, Cym: β-cymaropyranose, Rha: α-rhamnopyranose, Glc: β-glucopyranose

Hz in the ^{1}H NMR spectrum while the configuration of the rhamnopyranosyl unit was established from the upfield shift of its C-5 (δ_c 68.3) in the ^{13}C NMR spectrum (Kasai et al., 1979). The structural assignment was confirmed by carrying out 2D NMR techniques such as HMQC and H–H COSY. The HMBC spectral analysis of **3** (Fig. 1) displayed correlation peaks between H-3 and C-1 of the rhamnopyranosyl unit, the anomeric proton of the glucosyl residue and C-4 of the rhamnosyl unit, H-16 and both COMe and C-20, H-17 and COMe, H-21, 21′ with C-23 and H-22 with C-23. The negative FAB–MS spectrum of **3** showed significant peaks at m/z 739 $[M–H]^-$ and 577 $[M–H\text{-glucose}]^-$ confirming the sequence of the sugar moiety. Consequently, the structure of **3** was determined as oleandrigenin 3-*O*-β-glucopyranosyl-(1→4)-α-rhamnopyranoside and named cryptostigmin II.

The molecular formula of compound **4** was established as $C_{38}H_{58}O_{15}$ from HR FAB–MS spectrometry (see Experimental section). Inspection of ^{13}C NMR spectral data of **4** (Tables 1 and 2) revealed their similarity to those of **3** both in the aglycone and sugar moieties. However, the presence of a methylene signal at δ_c 27.7 and a methyl signal at δ_c 9.1 in addition to the absence of the methyl signal at δ_c 20.6 in compound **4** proved the presence of a propionyl instead of the acetyl moiety at C-16 of the aglycone. At the same time, ^{1}H NMR spectrum of compound **4** showed a quartet at δ_H 2.13 (J constant 7.3 Hz) and a triplet at δ_H 0.93 (J constant 7.6 Hz) for the the methylene and methyl groups of the propionyl moiety, respectively, in addition to the absence of the methyl singlet at δ_H 1.82 of the acetyl group when compared with compound **3**. Moreover, FAB–MS spectrum of **4** exhibited significant peaks at m/z 753 $[M–H]^-$, 591 $[M–H\text{-glucose}]^-$ and 445 $[M–H\text{-(glucose + rhamnose)}]^-$. Consequently, the structure of compound **4** was assigned as 16-propionylgitoxigenin 3-*O*-β-glucopyranosyl-(1→4)-α-rhamnopyranoside and named cryptostigmin III.

The molecular formula of compound **5** was determined as $C_{50}H_{78}O_{22}$ from HR FAB–MS spectrometry (see Experimental section). Inspection of ^{13}C NMR spectral data of **5** (Tables 1 and 2) revealed their similarity to those of **2** both in the aglycone and sugar moieties. However, the downfield shift of C-6 of the glucosyl unit to δ_c 70.2 (+ 7.2 ppm) revealed its substitution at this position with an additional glucopyranosyl unit from the signals at δ_c 105.1, 74.9, 78.2, 72.1, 78.3 and 62.8 (Bradbury and Jenkins, 1984). In the ^{1}H NMR spectrum of **5**, the doublet signals at δ_H 5.19 and 4.99 (each 1H) with J constant 7.8 Hz for each one of the anomeric protons of the glucosyl residues indicated the β configuration. Moreover, the broad doublet signal at

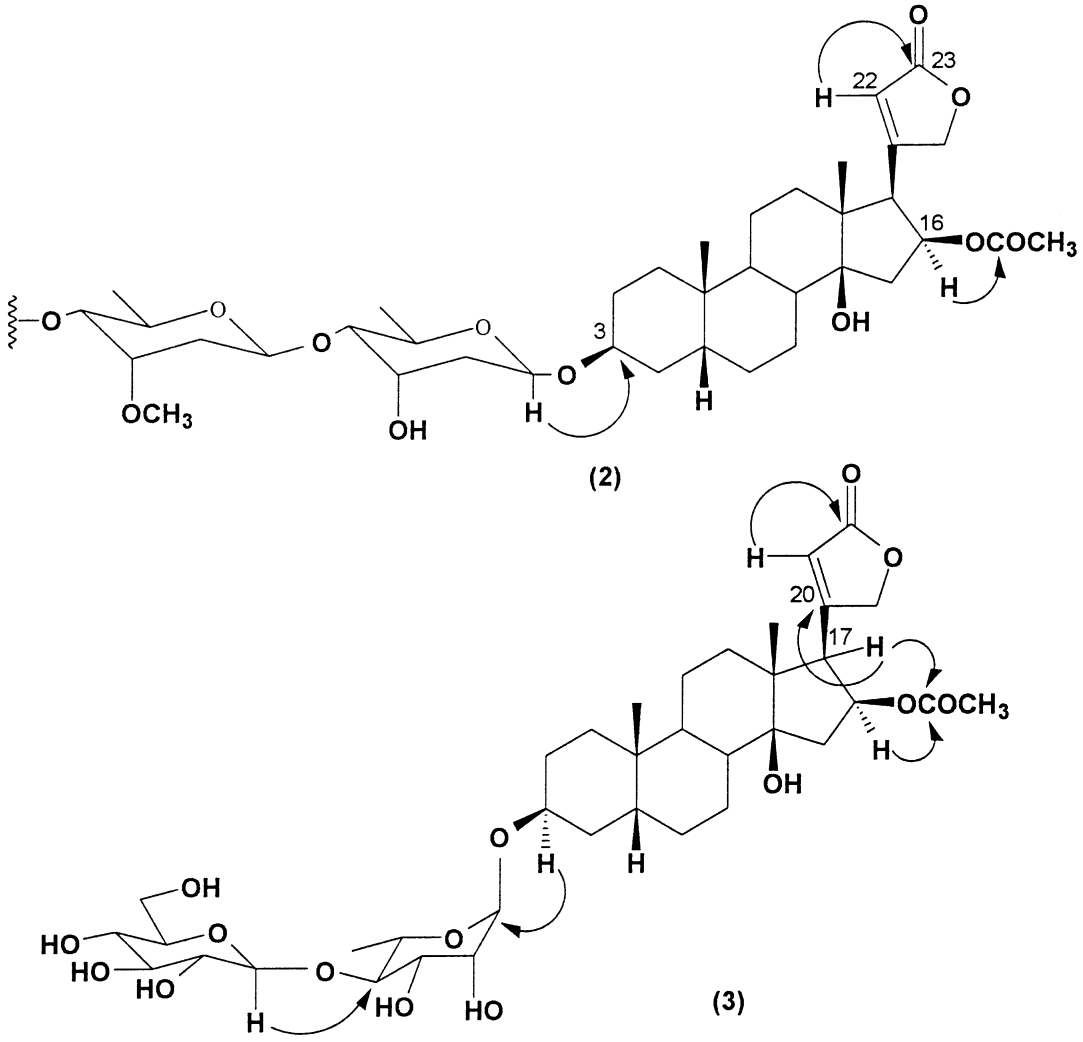


Fig. 1. Significant HMBC correlations of compounds **2** and **3**.

δ_H 4.79 (*J* constant 11.7 Hz) and the doublet of doublet signal at δ_H 4.73 (*J* constant 3.9 and 7.2 Hz) indicated the β configuration of the digitoxo- and cymaropyranosyl units respectively. FAB–MS spectrum of **5** exhibited significant peaks at *m/z* 1029 $[M–H]^-$, 867 $[M–H\text{-glucose}]^-$, 705 $[M–H\text{-(2 glucose)}]^-$ and 561 $[M–H\text{-(2 glucose + cymarose)}]^-$. Consequently, the structure of compound **5** was assigned as oleandrigenin 3-*O*-β-glucopyranosyl-(1→6)-β-glucopyranosyl-(1→4)-β-cymaropyranosyl-(1→4)-β-digitoxopyranoside and named cryptostigmin IV.

Comparison of the ^1H and ^{13}C NMR spectra of compound **6** with the published data deduced its structure as 16-*O*-acetyl-digitalinum verum previously isolated from *Addenium obesum* (Yamauchi and Abe, 1990).

3. Experimental

^1H and ^{13}C NMR (TMS as int. standard): 400 and 100 MHz, respectively were recorded on a JEOL JNM α-400 spectrometer. FAB–MS spectra were taken on a JEOL JMS-SX 102 spectrometer by direct inlet method at an ionizing voltage of 70 eV. Optical rotations were measured with a Union PM-I digital polarimeter. MPLC: RP-18 column (20 mm i.d. ×40 cm); flow rate of mobile phase 3 ml/min. HPLC: D-ODS-5 and polyamine columns (each 20 mm i.d. ×25 cm, YMC) with a Toyo Soda high speed chromatograph HLC-803 D pump and a Tosoh refraction index (R1-8) detector; flow rate of mobile phase 6 ml/min, injection vol. 0.8–1.0 ml. On the ODS column, 30% MeCN (I) and 25% MeCN (II) were used while 90% MeCN (III) and 85% MeCN (IV) were used on the polyamine column. CC: Kieselgel 60 (70-230 mesh, Merck) and Diaion HP 20 (Mitsubishi). TLC: silica gel 60 precoated plates F-254 and HPTLC using RP-18 precoated plates, F-254 s (Merck).

3.1. Plant material

Leaves of *C. grandi flora* R.Br. (Asclepiadaceae) were collected from the trees cultivated in the Experimental

Station of Faculty of Agriculture, Assiut University, Assiut, Egypt, in March 1997. The plant was identified by Professor N. El-Keltawi, Department of Horticulture, Faculty of Agriculture, Assiut University, Assiut, Egypt. A voucher specimen is deposited at the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

3.2. Extraction and isolation of compounds (*1*–*6*)

The air-dried powdered leaves of *C. grandiflora* (2 kg) were extracted with 70% EtOH. The dried ethanolic extract (499 g) was suspended in H_2O and defatted successively with *n*-hexane and CH_2C1_2. The aq. fr. was applied to a column of Diaion HP-20 and eluted with H_20, 40% MeOH, 80% MeOH, MeOH and acetone successively. The 80% MeOH eluate (12 g) was chromatographed by silica gel CC using CH_2C1_2–MeOH–H_20 (85: 15: 1) and finally with (70 : 30 : 3) to give 4 fractions. Fraction 1 (760 mg) was separated by MPLC on RP-18 using 35% MeCN and HPLC using ODS column and solvent I to afford compounds **1** (white powder, 14 mg) and **6** (white powder, 53 mg). Fraction 2 (718 mg) was subjected to HPLC using polyamine column and solvent III to give compound **2** (white powder, 70 mg). Fraction 3 (610 mg) was separated by HPLC using polyamine column and solvent IV to afford compounds **3** (white powder, 149 mg) and **4** (white powder, 43 mg). Fraction 4 (2.5 g) was separated by MPLC on RP-18 using 30% MeCN as a solvent system and HPLC using ODS colunm and solvent II to afford compound **5** (white powder, 29 mg).

3.2.1. Compound (*2*)

Cryptostigmin I, $[\alpha]_D^{25}$ −15.4° (MeOH; *c* 5.70). HR FAB–MS (negative) *m/z*: 867.4371 $[M–H]^-$ $C_{44}H_{68}O_{17}$ (req. 867.4378). ^{13}C NMR (C_5D_5N, Tables 1 and 2). ^1H NMR (C_5D_5N): δ_H 6.30 (1H, *s*, H-22), 5.63 (1H, *m*, H-16), 5.40 (1H, *d*, *J* = 18.1 Hz, H-21), 5.39 (1H, *bd*, *J* = 9.1 Hz, H-1 Dig), 5.20 (1H, *d*, *J* = 18.1 Hz, H-21′), 5.04 (1H, *d*, *J* = 7.8 Hz, H-1 Glc), 4.71 (1H, *dd*, *J* = 4.7 and 9.5 Hz, H-1 Cym), 3.49 (3H, *s*, OMe), 3.35 (1H, *d*, *J* = 8.8 Hz, H-17), 2.77 (1H, *dd*, *J* = 8.6 and 14.6 Hz, H-15), 2.02 (1H, *d*, *J* = 13.2 Hz, H-15′), 1.82 (3H, *s*, COMe), 1.58 (3H, *d*, *J* = 5.4 Hz, Me-6 Cym), 1.42 (3H, d, *J* = 6.1 Hz, Me-6 Dig), 1.04 (3H, *s*, Me-18) and 0.84 (3H, *s*, Me-19).

3.2.2. Compound (*3*)

Cryptostigmin II, $[\alpha]_D^{25}$ −39.4° (MeOH; *c* 3.73). HR FAB–MS (negative) *m/z*: 739.3604 $[M–H]^-$ $C_{37}H_{56}O_{15}$ (req. 739.3541). ^{13}C NMR (C_5D_5N, Tables 1 and 2). ^1H NMR (C_5D_5N): δ_H 6.32 (1H, *bs,* H-22), 5.66 (1H, *m,* H-16), 5.40 (1H, *dd, J* = 1.8, 18.3 Hz, H-21), 5.32 (1H, *bs,* H-1 Rha), 5.22 (1H, *dd, J* = 1.5, 18.3 Hz, H-21′), 5.20 (1H, *d, J* = 7.7 Hz, H-1 Glc), 4.18 (1H, *m*, H-3), 3.37 (1H, *d, J* = 8.6 Hz, H-17), 2.78 (1H, *dd, J* = 9.5 and 15.7 Hz, H-15), 2.03 (1H, *dd, J* = 2.1, 15.3 Hz, H-15′), 1.82 (3H, *s,* COMe), 1.67 (3H, *d, J* = 6.4 Hz, Me-6 Rha), 1.05 (3H, *s,* Me-18) and 0.81 (3H, *s,* Me-19).

3.2.3. Compound (*4*)

Cryptostigmin III, $[\alpha]_D^{25}$ −41.3° (MeOH; *c* 2.01). HR FAB–MS (negative) *m/z*: 753.3683 $[M–H]$ $C_{38}H_{58}O_{15}$ (req. 753.3697). ^{13}C NMR (C_5D_5N, Tables 1 and 2). ^1H NMR (C_5D_5N): δ_H 6.32 (1H, *bs,* H-22), 5.70 (1H, *m,* H-16), 5.41 (1H, *d, J* = 18.3 Hz, H-21), 5.22 (1H, *d, J* = 18.3 Hz, H-21′), 5.20 (1H, *d, J* = 7.1 Hz, H-1 Glc), 3.40 (1H, *d, J* = 8.8 Hz, H-17), 2.80 (1H, *d, J* = 14.8 Hz, H-15), 2.13 (2H, *q*, *J* = 7.3 Hz, $COCH_2$), 2.10 (lH, *d*, *J* = 14.8 Hz, H-l5′), 1.68 (3H, *d, J* = 6.1 Hz, Me-6 Rha), 1.07 (3H, *s,* Me-18), 0.93 (3H, *t, J* = 7.6 Hz, CO–CH_2-Me) and 0.83 (3H, *s*, Me-19).

3.2.4. Compound (*5*)

Cryptostigmin IV, $[\alpha]_D^{25}$ −37.8° (MeOH; *c* 1.80). HR FAB–MS (negative) *m/z*: 1029.4891 $[M–H]$ $C_{50}H_{78}0_{22}$ (req. 1029.4906). ^{13}C NMR (C_5D_5N, Tables 1 and 2). ^1H NMR (C_5D_5N): δ_H 6.32 (1H, *s,* H-22), 5.40 (1H, *d, J* = 18.6 Hz, H-21), 5.22 (1H, *d, J* = 18.6 Hz, H-21′), 5.19 and 4.99 (each 1H, *d, J* = 7.8 Hz, H-1 Glc and Glc′), 4.79 (1H, *bd, J* = 11.7 Hz, H-1 Dig), 4.73 (1H, *dd, J* = 3.9 and 7.2 Hz, H-1 Cym), 3.60 (3H, *s,* OMe), 3.36 (lH, *d, J* = 9.1 Hz, H-17), 2.78 (1H, *dd, J* = 10.0 and 15.2 Hz, H-15), 2.04 (1H, *d, J* = 15.2 Hz, H-15′), 1.83 (3H, *s,* COMe), 1.57 (3H, *d, J* = 6.1 Hz, Me-6 Cym), 1.43 (3H, *d, J* = 6.4 Hz, Me-6 Dig), 1.05 (3H, *s,* Me-18) and 0.85 (3H, *s,* Me-19).

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