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PREGNANE GLYCOSIDES FROM *STAPELIA VARIEGATA*

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Key Word Index—*Stapelia variegata*; Asclepiadaceae; stavarosides; pregnane ester glycosides.

Abstract—Eleven new pregnane ester glycosides have been isolated from the aerial parts of *Stapelia variegata*. Eight of the recognized compounds were established to possess the same trioside moiety, viz. 3-*O*-[3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside]. These compounds were identified as: stavaroside A: 12-*O*-β-angeloyl-20-*O*-benzoyl sarcostin; stavaroside B: 12-*O*-β-angeloyl-20-*O*-tigloyl sarcostin; stavaroside C: 11α-acetoxy 2β-benzoxy-3β,8β,14β-trihydroxy-pregn-5-ene-20-one; stavaroside D: 11α-acetoxy-12β-tigloxy-3β,8β,14β-trihydroxy-pregn-5-ene-20-one; stavaroside E: 12-*O*-β-benzoyl sarcostin; stavaroside F: 11α-acetoxy-12β-acetoxy-3β,8β,14β-trihydroxy-pregn-5-ene-20-one; stavaroside G: 12-*O*-β,20-*O*-diacetyl sarcostin and stavaroside H: 3β, 8β, 11α, 12β, 14β-pentahydroxy-pregn-5-ene-20-one. The other three compounds were shown to possess the same tetraside sugar moiety, viz. 3-*O*-[β-D-glucopyranosyl-(1-4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside]. These compounds were identified as: stavaroside I: 1α, 12β-angeloxy and benzoxy-3β,8β,14β-trihydroxy-pregn-5-ene-20-one; stavaroside J: 11α-acetoxy-12β-benzoxy-3β,8β,14β-trihydroxy-pregn-5-ene-20-one and stavaroside K: 11α-acetoxy-12β-tigloxy-3β,8β,14β-trihydroxy-pregn-5-ene-20-one. The structural elucidation of the isolated compounds was aided significantly on the basis of the chemical and spectral evidence. The decisive assignments of the ester positions were based on the Inverse Detected-Heteronuclear Multiple Bond Connectivity (HMBC) experiments.

INTRODUCTION

As a part of the efforts to identify prototype chemotherapeutic agents from plant extracts, *Stapelia variegata* L., an ornamental succulent plant belonging to the family Asclepiadaceae, was chosen. Reviewing the current literature, nothing was reported about the phytochemical study of this plant except the investigation of the anthocyanin contents of the flower [1]. The isolation of boucerin, a pregnane ester aglycone, was subsequently reported from *Stapelia grandiflora* Mass. [2]. In this paper, the isolation and identification of 11 new pregnane glycosides, named stavaroside A (**1**), stavaroside B (**2**), stavaroside C (**3**), stavaroside D (**4**), stavaroside E (**5**), stavaroside F (**6**), stavaroside G (**7**), stavaroside H (**8**), stavaroside I (**9**), stavaroside J (**10**), stavaroside K (**11**), are reported.

RESULTS AND DISCUSSION

The ether extract of dried plant material (see Experimental), possessing most of the plant pregnanes was dissolved in hot ether and kept for 12 hr in a refrigerator where a precipitate was formed. Filtration afforded two main extracts: the ether-soluble extract and the cooled ether-insoluble extract. Each of these extracts was chromatographed separately. The former extract afforded eight pregnane triosides (**1**–**8**) while the latter extract afforded three tetrasides (**9**–**11**).

Stavaroside E (**5**) gave positive Liebermann–Burchard's and Salkowski's tests, indicating its steroidal or triterpenoidal nature. It also gave a positive Keller–Kiliani test characteristic for 2-deoxy sugars. Acid hydrolysis of **5**, TLC and GLC of the sugar fractions and their alditol acetates alongside authentic monosaccharides and monosaccharide alditol acetates, led to the identification of β-D-cymarose and β-D-3-*O*-methyl-6-deoxy allose (abbreviated allomethylose) with R_f values 0.81 and 0.70, respectively, on cellulose-precoated chromatoplates [3] and R_t values 3.01 and 5.45 min, respectively, in GC analysis.

The positive ion FAB-mass spectrum displayed the molecular ion peak at *m/z* 935 $[M + H]^+$ suggesting the molecular formula $C_{49}H_{74}O_{17}$. On the other hand, LC-MS analysis of the aglycone displayed $[M]^+$ at 487 $[M + H]^+$ which suggested $C_{28}H_{38}O_7$ as a molecular

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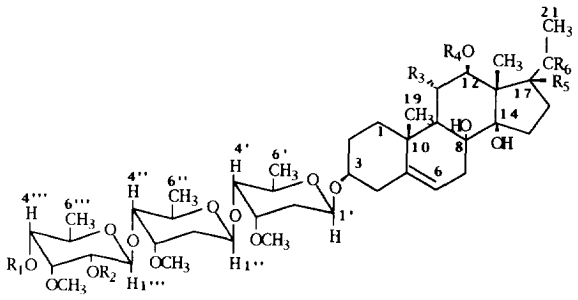

#	Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	Stavaroside A	H	H	H	Ang	OH	H, O-Bz
2	Stavaroside B	H	H	H	Ang	OH	H, O-Tig
3	Stavaroside C	H	H	O-Ac	Bz	H	=O
4	Stavaroside D	H	H	O-Ac	Tig	H	=O
5	Stavaroside E	H	H	H	Bz	OH	H, OH
6	Stavaroside F	H	H	O-Ac	Ac	H	=O
7	Stavaroside G	H	H	H	Ac	OH	H, O-Ac
8	Stavaroside H	H	H	OH	H	H	=O
9	Stavaroside I	β-D-glc	H	O-Ang[a]	Bz[a]	H	=O
10	Stavaroside J	β-D-glc	H	O-Ac	Bz	H	=O
11	Stavaroside K	β-D-glc	H	O-Ac	Tig	H	=O
12	Compound 12	Ac	Ac	O-Ac	Ac	H	=O
13	Compound 13	Ac	Ac	OH	H	H	=O

[a]Interchangeable.

Ac = Acetate, Ang = Angelate, Bz = Benzoate, Tig = Tiglate, β-D-glc = β-D-glucopyranosyl.

formula for the aglycone of **5**. The detailed structure of the sugar moiety was deduced as follows. The ^{1}H NMR signals at δ4.57 (1H, *d*, J = 7.9 Hz), 4.74 (1H, *dd*, J = 9.3 and 1.6 Hz) and 4.81 (1H, *dd*, J = 8.9 and 1.7 Hz) are correlated with ^{13}C NMR signals (Table 1) at δ102.0, 99.5 and 96.0, respectively, and assigned to the three anomeric protons H-1‴, H-1″ and H-1′ of sugar moiety, one allomethylose and two cymarose units, respectively. ^{1}H NMR signals at δ1.26, 1.24 and 1.20 (each 3H, *d*, J = 6.3, 6.2 and 5.7 Hz) were correlated with the ^{13}C NMR signals at δ18.1, and assigned for the three secondary methyl groups of monosaccharides. The ^{1}H NMR methyl singlets at δ3.64, 3.43 and 3.40 were correlated with the ^{13}C NMR signals (Table 1) at δ62.1, 57.9 and 57.8, and ascribed to the three methoxyl groups of the sugar moiety. Thermospray LC-MS analyses of the sugars after mild acid hydrolysis of **5** displayed two peaks at *m/z* 340 [asclepobiose + NH_4]$^+$ and 180 [cymarose + NH_4]$^+$ while those after strong acid hydrolysis were at *m/z* 196 [allomethylose + NH_4]$^+$ and 180 [cymarose + NH_4]$^+$ which supported the previous assignments of the sugar moiety. The β-linkages of the sugars were revealed by the high coupling constants of the three anomeric proton signals [4]. Since cymarose contains only one hydroxyl group at carbons C-4′ and C-4″, the sugar moiety should be linear with 1-4 β-glycosyl linkages. The sugar sequence was confirmed via the FD-MS analysis which showed ions at *m/z* 957 [M + Na]$^+$, 796 [M − (allomethylose + Na)]$^+$, 631 [M − (allomethylose + cymarose) + H]$^+$ and 486 [M − (allomethylose + cymarose + cymarose)]$^+$. The location of the carbohydrate moiety was determined at C-3 on the basis of the glycosylation chemical shift observed in the ^{13}C NMR spectrum (Table 1) for the carbon signals C-2 (− 0.8 ppm), C-3 (+ 5.8 ppm) and C-4 (− 3.3 ppm) as compared with those of its aglycone (Table 2) [5]. The structure of the aglycone moiety of **5** was deduced as follows. The UV λ_{max} at 270 and 280 nm suggested an aromatic system. The IR ($CHCl_3$) revealed vibration bands at 1710 and 1280 cm^{-1}, indicating an aromatic or α,β-unsaturated ester group. The monoester nature of **5** was further confirmed through the carbonyl quaternary carbon at δ165.8 in the ^{13}C NMR spectrum (Tables 1 and 2). The benzoyl moiety was deduced from the ^{1}H NMR signals at δ8.07 (2H, *dd*, J = 7.8 and 1.4 Hz), 7.58 (1H, *dt*, J = 7.4 and 1.3 Hz) and 7.46 (2H, *dd*, J = 8.1 and 7.3 Hz), which are correlated with the aromatic methine carbons at δ129.6, 133.4 and 128.6 and assigned for *ortho*-, *para*- and *meta*- positions. These were further confirmed with the additional aromatic quaternary carbon which resonated at δ130.1 and assigned for the carbon C-1 of the benzoyl moiety. The ^{1}H NMR signal at δ4.85 (1H, *dd*, J = 11.4 and 4.4 Hz) was correlated with the oxygenated methine carbon at δ74.6 and assigned to H-12 which was deshielded by the benzoyl ester moiety. The assignment of the benzoyl ester moiety to be in position C-12 rather

Table 1. ^{13}C NMR spectral data of the compounds **1–11** and dregeoside A_{L1} [4] (75 MHz, $CDCl_3$)

C	1	2	3	4	5	6	7	8	9	10	11	DA_{L1}
1	35.4[a]	35.5[a]	39.3[a]	39.2[a]	38.7[a]	39.8[a]	36.9[a]	40.0[a]	35.1[a]	39.3[a]	39.2[a]	38.7[a]
2	29.6	29.0	29.3	29.3	28.9	30.2	28.9	30.0	29.6	29.3	29.3	30.5
3	77.8	77.9	77.9	77.9	77.7	77.7	77.7	78.2	77.9	77.9	77.9	77.1[a]
4	38.6[a]	38.8[a]	40.2[a]	40.2[a]	38.8[a]	40.6[a]	38.7[a]	40.9[a]	39.5[a]	40.2[a]	40.2[a]	39.8[a]
5	139.9	139.7	139.6	139.6	139.7	139.6	139.9	140.7	140.7	139.6	139.6	139.7
6	118.0	118.3	118.0	118.1	118.3	118.8	118.1	118.6	118.3	118.0	118.0	122.4
7	32.9	32.1	37.2	37.2	35.4	36.9	32.8	30.4	37.7	37.2	30.2	28.2
8	74.0	74.0	75.5	75.4	73.9	75.9	74.3	76.2	74.9	75.5	75.4	37.2
9	43.0	43.4	48.2	48.2	43.5	49.0	43.2	50.5	43.8	48.2	48.2	48.0
10	38.6	38.8	38.7	38.7	37.0	39.3	34.3	39.5	37.7	38.7	38.6	39.4
11	29.6	33.2	70.8	70.7	31.7	71.7	35.5	70.4	70.2	70.6	70.1	71.9
12	74.7	76.6	76.9	76.7	74.6	78.6	76.5	79.1	76.9	76.6	76.6	75.5[a]
13	56.3	56.3	54.7	54.6	56.2	55.4	56.0	56.2	54.5	54.7	54.6	54.7
14	85.1	87.8	85.1	85.0	87.9	85.5	87.3	85.9	88.5	85.0	84.3	83.9
15	32.5	32.1	35.0	34.9	33.2	35.5	31.9	36.1	35.1	35.1	37.1	34.6
16	24.7	24.9	24.4	24.2	24.7	24.3	24.7	24.7	24.4	24.4	24.4	24.0
17	87.8	88.0	58.0	57.9	87.9	59.4	87.8	59.6	58.0	58.0	57.8	58.3
18	10.5	10.5	13.0	13.0	11.1	13.6	10.0	13.0	12.6	13.0	12.9	11.4
19	18.2	18.2	17.7	17.7	17.8	18.1	18.2	17.7	18.9	17.7	17.7	19.2
20	72.4	74.0	216.9	217.0	70.7	213.2	74.1	216.0	70.7	216.9	216.9	214.0
21	14.9	14.9	33.0	32.6	18.4	31.4	14.9	32.1	31.6	33.0	32.9	31.9
Ester	Bz	Tig	Bz	Tig	Bz	Ac	Ac		Bz	Bz	Tig	Isovl
	164.6	165.7	166.7	168.0	165.8	171.0	171.0		165.4	166.7	167.9	173.0
	128.2	128.8	128.9	127.6	130.1	21.5	21.7		131.1	128.9	127.6	43.4
	129.4	137.1	130.0	139.7	129.6				130.1	130.0	139.7	25.6
	128.2	14.4	128.7	14.7	128.6				129.0	127.6	14.6	22.5
	132.7	12.1	133.7	12.0	133.4				133.5	133.7	12.0	
	128.2		128.7		128.6				129.0	128.7		
	129.7		130.0		129.6				130.1	130.0		
	Ang	Ang	Ac	Ac		Ac	Ac		Ang	Ac	Ac	Ac
	167.3	167.4	169.7	169.6		169.9	169.6		168.2	169.7	169.6	170.1
	130.3	129.7	21.4	21.5		20.8	21.4		130.2	21.4	21.5	21.6
	136.6	136.4							137.5			
	13.8	14.3							14.6			
	11.8	11.9							11.3			
1′Cym	95.9	96.0	96.1	96.0	96.0	96.5	96.0	96.4	96.7	96.0	96.0	96.4
2′	34.9[b]	35.0[b]	35.4[b]	35.4[b]	34.9[b]	37.1[b]	35.0[b]	37.4[b]	33.6[b]	35.3[b]	35.4[b]	37.1[b]
3′	76.7[c]	77.0[c]	76.9[c]	76.9[c]	77.0[c]	78.0[c]	77.2[c]	78.2[c]	76.9[c]	76.7[c]	76.7[c]	78.0[c]
4′	82.4[d]	82.6[d]	82.5[d]	82.5[d]	82.5[d]	83.5[d]	82.5[d]	83.3[d]	83.1[d]	82.5[d]	82.7[d]	83.1[d]
5′	68.4[e]	68.5[e]	68.5[e]	68.5[e]	68.3[e]	69.1[e]	68.3[e]	69.1[e]	69.1[e]	68.4[e]	68.4[e]	69.0[e]
6′	18.5[f]	18.5[f]	18.5[f]	18.7[f]	18.1[f]	18.6[f]	18.7[f]	18.6[f]	19.1[f]	18.4[f]	18.4[f]	18.2[f]
3′OMe	57.7[g]	57.8[g]	57.8[g]	57.9[g]	57.8[g]	58.9[g]	57.8[g]	58.9[g]	57.1[g]	57.7[g]	57.8[g]	58.9[g]
1″Cym	99.5	99.6	99.6	99.6	99.5	100.4	99.6	100.4	100.2	99.5	99.5	100.4
2″	34.3[b]	34.5[b]	35.2[b]	35.2[b]	34.4[b]	37.3[b]	35.0[b]	37.4[b]	34.1[b]	35.4[b]	35.2[b]	37.2[b]
3″	76.9[c]	76.8[c]	77.0[c]	76.9[c]	76.8[c]	78.2[c]	76.9[c]	78.2[c]	76.7[c]	76.7[c]	76.2[c]	78.0[c]
4″	82.3[d]	82.7[d]	82.6[d]	82.5[d]	82.5[d]	83.3[d]	82.6[d]	83.4[d]	83.2[d]	82.7[d]	82.5[d]	83.1[d]
5″	68.2[e]	68.3[e]	68.3[e]	68.3[e]	68.5[e]	69.3[e]	68.5[e]	69.3[e]	69.2[e]	68.3[e]	68.4[e]	69.2[e]
6″	18.4[f]	18.2[f]	18.2[f]	18.5[f]	18.1[f]	18.6[f]	18.4[f]	18.6[f]	19.0[f]	18.2[f]	18.4[f]	18.5[f]
3″OMe	57.9[g]	58.0[g]	57.9[g]	57.8[g]	57.9[g]	58.8[g]	57.9[g]	58.9[g]	58.5[g]	57.9[g]	57.9[g]	59.0[g]
1‴allm	102.0	102.1	102.1	102.1	102.0	104.2	102.1	104.2	103.0	102.5	102.3	103.9
2‴	73.0	73.1	73.1	73.0	73.1	73.1	73.1	73.2	73.3	73.4	73.8	72.5
3‴	80.4	80.5	80.5	80.5	80.5	84.0	80.5	84.0	83.1[d]	82.3[d]	82.2[d]	83.2[d]
4‴	72.6	72.7	72.7	72.7	73.6	74.5	73.6	74.5	83.0	82.5	82.3	83.2
5‴	70.7	70.8	70.6	70.7	70.7	70.7	70.8	70.8	70.2	70.1	70.7	69.2
6‴	18.0[f]	17.9[f]	17.9[f]	17.9[f]	18.1[f]	18.6[f]	18.1[f]	18.6[f]	18.9[f]	18.0[f]	18.2[f]	18.6[f]
3‴OMe	62.0	62.1	62.1	62.1	62.1	62.2	62.1	62.2	61.7	61.2	61.3	61.7
1⁗Glc									104.5	104.3	104.5	
2⁗									75.5	76.3	76.6	
3⁗									78.1	77.2	77.4	
4⁗									71.5	71.6	70.8	
5⁗									78.5	76.7	77.4	
6⁗									62.0	62.1	62.1	

[a–g]Interchangeable in the same column.

Cym = cymarose, allm = allomethylose, Isovl. = isovalerate.

Table 2. ^{13}C NMR spectral data of the aglycones of compounds **1**–**5**, **10** and **11** (75 MHz, $CDCl_3$)

C	**1** ag	**2** ag	**3** ag	**4** ag	**5** ag	**10** ag	**11** ag
1	38.7[a]	38.7[a]	40.0[a]	40.0[a]	38.8[a]	40.0[a]	40.1[a]
2	29.7	29.7	31.2	31.2	29.7	31.2	31.2
3	71.9	71.9	71.8	71.8	71.9	771.8	71.8
4	42.0[a]	42.1[a]	40.1[a]	42.6[a]	42.1[a]	42.6[a]	42.5[a]
5	139.8	139.6	139.5	139.5	139.6	139.5	139.5
6	118.2	118.4	118.1	118.1	118.4	118.1	118.1
7	34.4	34.5	37.3	37.2	34.5	35.2	35.2
8	74.2	74.1	75.5	75.4	74.0	75.5	75.4
9	43.1	43.1	48.3	48.2	43.4	48.2	48.2
10	37.1	36.8	38.5	38.5	36.8	38.5	38.5
11	32.9	33.2	70.7	70.8	31.7	70.7	70.8
12	76.6	76.6	76.6	76.6	74.6	76.6	76.6
13	56.4	56.3	54.7	54.6	56.3	54.7	54.6
14	87.8	87.9	85.1	85.1	87.9	85.1	85.1
15	32.5	32.1	35.2	35.2	33.4	37.2	37.2
16	24.9	24.9	24.5	24.4	24.7	24.4	24.4
17	87.9	87.9	58.0	57.9	87.9	58.0	57.9
18	10.6	10.5	13.0	12.9	11.1	13.0	12.9
19	18.3	18.2	17.8	17.7	18.2	17.8	17.7
20	72.6	72.7	216.9	217.0	71.0	216.9	217.0
21	15.0	14.9	33.0	32.9	18.3	32.9	32.9
Ester	Bz	Tig	Bz	Tig	Bz	Bz	Tig
	164.7	165.7	166.7	168.0	165.9	166.7	168.0
	128.3	128.9	128.9	127.6	130.1	128.9	127.6
	129.5	137.1	130.0	139.7	129.6	130.0	139.7
	128.3	14.4	128.7	14.7	128.7	128.7	14.7
	132.8	12.1	133.7	12.0	133.5	133.7	12.0
	128.3		128.7		128.7	128.7	
	129.5		130.0		129.6	130.0	
	Ang	Ang	Ac	Ac		Ac	Ac
	167.5	167.5	169.6	169.6		169.6	169.6
	130.6	129.8	21.4	21.5		21.4	21.5
	136.7	136.4					
	13.9	14.3					
	12.0	11.9					

than position C-20 was based on the ^1H–^{13}C-HMBC connectivity spectral data of the aglycone. The ^3J LR-cross coupling peak between the carbonyl ester carbon at δ165.8 and H-12 at δ4.88, unambiguously confirmed the benzoyl moiety at C-12. The methyl doublet at δ1.03 (J = 6.3 Hz) was correlated with the methyl carbon at δ18.4 and assigned to the C-21 methyl. This assignment was further supported by observing the strong coupling contour between this signal and the proton quartet signal of H-20 at δ3.67 in ^1H–^1H COSY experiment. The ^1H NMR singlets at δ1.62 and 1.15 were correlated with methyl carbons at δ11.1 and 17.8 and indicated the presence of two tertiary methyl groups assigned to C-18 and C-19 methyls, respectively. The ^1H NMR signal. at δ5.37 (1H, *br s*) was correlated with the methine carbon at δ118.3 in addition to the quaternary carbon at δ139.7 and assigned to the olefinic proton and C-6 and C-5, respectively, indicating that the aglycone moiety was a Δ^5-steroid. Alkaline hydrolysis of **5** aglycone resulted in the identification of sarcostin (TLC alongside authentic sample, mp and mmp). Thus stavaroside E was deduced to be 12-*O*-β-benzoyl-sarcostin-3-*O*-[3-*O*-methyl-6-deoxy-β-D-alloypranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside A (**1**) displayed a molecular ion peak at m/z 1017 $[M + H]^+$ in its FAB-mass spectrum suggesting the molecular formula $C_{54}H_{80}O_{18}$. Furthermore, LC-MS analysis of its aglycone displayed a $[M]^+$ at m/z 569 $[M + H]^+$ suggesting the molecular formula $C_{33}H_{44}O_8$. Acid hydrolysis of **1**, TLC and GLC of the sugar fractions revealed the same sugar chain as **5**. The NMR spectral analyses of **1** also revealed a distinct similarity with **5** with the addition of one more angeloyl moiety. IR absorption bands at 1705 and 1690 cm^{-1} indicated the diester nature of **1**. This was further confirmed through the two carbonyl ester signals at δ167.3 and 164.6 in the ^{13}C NMR spectrum (Table 1). The ^1H NMR signals at δ4.81 (1H, *q*, J = 6.3 Hz) and 4.79 (1H, *dd*) were deshielded downfield owing to the esterifying moieties and correlated with the methine carbons

(Table 1) at δ72.4 and 74.7 ascribed to C-20 and C-12, respectively. In addition to the spectral signals characteristic for the benzoyl moiety, the ^{1}H NMR signal at δ6.29 (1H, *qq*, J = 7.1 and 1.4 Hz), was assigned to H-3 of the angeloyl moiety and correlated with the olefinic methine carbon at δ136.6. The two methyl signals at δ1.54 (3H, *dq*, J = 7.1 and 1.3 Hz) and 1.72 (3H, *br s*) were correlated with the methyl carbons at δ13.8 and 11.8 and ascribed to the angelate 4- and 5-methyl groups. The quaternary olefinic carbon resonated at δ130.3, and ascribed to C-2 of angelate which further confirmed the angeloyl moiety. The order of acylation between positions C-12 and C-20 was also established through the ^{1}H–^{13}C HMBC connectivity experiment for **1** aglycone. Despite the overlapping of H-20 (δ4.81, *q*) and H-12 (δ4.79, *dd*, J = 11.3 and 4.4 Hz), the enlargement of these two peaks with high resolution distinguished between them enough to make proper assignment. The strong LR-cross coupling peaks between the carbonyl ester (δ164.6) and the two *ortho* benzoyl protons as well as proton H-20, enabled the assignment of the benzoyl moiety C-20. On the other hand, the LR-cross coupling peaks between the carbonyl ester (δ167.4) and H-3 of angelate, as well as H-12, revealed that the angeloyl moiety was located at C-12. The structure and the order of acylation in condurangogenins (isolated from *Marsdenia condurango* Rchb. cortex, family Asclepiadaceae) was previously revised utilizing the fully coupled ^{13}C NMR spectra and selective proton irradiation [6]. The ^{1}H–^{13}C HMBC connectivity experiment provides an alternative which may be used to get comparable results with a tremendous decrease in the sample size and the time required. Thus, **1** was proved to be 12-*O*-β-angeloyl-20-*O*-benzoyl-sarcostin-3-*O*-[3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside B (**2**) displayed a molecular ion peak in the FAB-mass spectrum at *m/z* 993 $[M - H]^-$ and 1017 $[M + Na]^+$ suggesting the molecular formula $C_{52}H_{82}O_{18}$. LC-MS analysis of the aglycone displayed $[M]^+$ at *m/z* 547 $[M + H]^+$ suggesting the molecular formula $C_{31}H_{46}O_8$ for **2** aglycone. Chemical and spectral analyses of **2** revealed a distinct similarity with **1** with the disappearance of the benzoyl moiety and the appearance of one tigloyl moiety instead. An olefinic methine proton signal at δ6.74 (*qq*, J = 7 and 1.4 Hz) and a carbon signal at δ137.1 were assigned for H-3 and C-3 of tiglate. The two methyl signals at δ1.78 (*dq*, J = 9.5 and 1.1 Hz) and 1.76 (*br s*), in addition to the methyl carbons at δ14.4 and 12.1, were assigned for the tiglate C-4 and C-5 methyl groups. The quaternary carbon at δ128.8 assigned to C-2 of the tiglate verified the tigloyl skeleton. Once more, the pattern of acylation between positions C-12 and C-20 was established through the ^{1}H–13C HMBC connectivity experiment for the aglycone. The ^3J-coupling cross peaks between the carbonyl ester which resonated at δ165.7 with H-20 (δ4.63), as well as proton H-3 of tiglate (δ6.74), confirmed the tigloyl acylation at C-20. Similarly, the coupling relationships estimated between the carbonyl ester at δ167.4 and the H-12 (δ4.74) and H-3 angelate (δ6.58) revealed the angeloyl acylation of C-12. Stavaroside B (**2**) was, therefore, proved to be 12-*O*-β-angeloyl-20-*O*-tigloyl-sarcostin-3-*O*-[3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside G (**7**) displayed a molecular ion peak at *m/z* 922 $[M + Li]^+$ in the FAB-mass spectrum suggesting the molecular formula $C_{46}H_{74}O_{18}$. Chemical and spectral analyses of **7** revealed a distinct similarity with sarcostin with the addition of two acetate esters at C-12 and C-20. This was supported by the thermospray LC-MS analysis of **7** aglycone which displayed a molecular ion peak at *m/z* 467 $[M + H]^+$, suggesting the molecular formula $C_{25}H_{38}O_8$ (sarcostin + 2 acetates). The two acetate methyl singlets at δ2.06 and 1.99, in addition to the two methyl carbons at δ21.7 and 21.4, were assigned for C-12 and C-20 acetate groups. Stavaroside G (**7**) was confirmed to be 12-*O*-β, 20-*O*-diacetyl-sarcostin-3-*O*-[3-*O*-methyl-6-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside C (**3**) displayed a molecular ion peak at *m/z* 982 $[M + Li]^+$ in the FAB-mass spectrum suggesting the molecular formula $C_{51}H_{74}O_{18}$. LC-MS analysis of its aglycone displayed $[M]^+$ at *m/z* 527 $[M + H]^+$ which suggested the molecular formula $C_{30}H_{38}O_8$. Acid hydrolysis of **3**, TLC and GLC of the sugar fractions and their alditol acetates alongside authentic monosaccharides and monosaccharide alditol acetates, led to the identification of the same sugars previously identified in stavaroside E. The NMR spectral analyses of **3** revealed a different oxygenation pattern in the aglycone. A ketone group had replaced the free or acylated alcoholic group at C-20 previously known in stavarosides E, A, B and G. C-17 was no longer oxygenated, but an oxygenated C-11 was displayed. This new aglycone pattern was confirmed through the following NMR features. The ketone group-signal absorbed at δ216.9 which was ascribed to C-20, in addition the downfield methyl singlet absorbed at δ2.09 which in turn correlated with the downfield methyl carbon which resonated at δ33.0 and was assigned to the C-2 methyl group. Furthermore, the methine carbon resonated at δ58.0 and was assigned to C-17. On the other hand, the proton signal at δ5.92 (*dd*, J = 10.6 and 10.6 Hz) which in turn correlated with the oxygenated methine carbon resonating at δ70.8 assigned to the acylated C-11. The spectral data indicated that the two acylating moieties were acetate and benzoate. The order of acylation between positions C-11 and C-12 was established through the ^{1}H–^{13}C HMBC connectivity spectrum of the aglycone. The ^3J-coupling cross peaks between the carbonyl ester which resonated at δ166.7 with proton H-12 (δ5.16), as well as with the two *ortho*-protons of the benzoyl moiety (δ8.07), confirmed the benzoylation C-12. Similarly, the acetate ester was shown to be at C-11. Alkaline hydrolysis of **3** aglycone resulted in the identification of 3β,8β,11α,12β,14β-pentahydroxy-pregn-5-en-20-one (marsdenin), through comparison with the literature [7]. Stavaroside C (**3**) was, therefore, confirmed to be 11α-acetoxy-12β-benzoxy-3β,8β,14β-

trihydroxypregn-5-en-20-one-3-*O*-[3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Acid hydrolysis of stavaroside J (**10**), TLC and GLC of the sugar fractions and their alditol acetates alongside authentic monosaccharides and monosaccharide alditol acetates, led to the identification of the same sugars previously identified in stavaroside E (**5**) in addition to β-D-glucose [R_f 0.81,0.70 and 0.22, respectively, on cellulose-precoated chromatoplates and R_t 3.01, 5.45 and 13.65 min, respectively, in GC]. FAB-MS analyses displayed a molecular ion peak at *m/z* 1135 $[M - H]^-$ suggesting the molecular formula $C_{57}H_{84}O_{23}$. LC-MS analysis of **10** aglycone displayed $[M]^+$ at *m/z* 527 $[M + H]^+$ which is the same molecular weight as the stavaroside C aglycone ($C_{30}H_{38}O_8$) suggesting the possible similarity of both aglycones. The NMR spectral analyses of **10** revealed a distinct similarity with stavaroside C with an additional β-D-glucosyl moiety. This was confirmed through the extra proton doublet in the ^{1}H NMR spectrum at δ4.39 (J = 8.1 Hz) which was assigned for the fourth anomeric proton H-1‴ of the glucose. The anomeric methine carbon resonated at δ104.3 further supporting this assignment. The point of attachment of the glucose to the allomethylose should be 1-4 rather than 1-2 as indicated from the downfield shift of carbon C-4‴ of allomethylose from δ72.7 in stavaroside C to δ82.5 in **10** (Table 1). Stavaroside **J** was deduced to be 11α-acetoxy-12β-benzoxy-3β,8β,14β-trihydroxypregn-5-en-20-one-3-*O*-[β-D-glucopyranosyl-(1-4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside I (**9**) displayed a molecular ion peak at *m/z* 1177 $[M + H]^+$ suggesting the molecular formula $C_{60}H_{88}O_{23}$. LC-MS analysis of its aglycone displayed $[M]^+$ at *m/z* 567 $[M + H]^+$ which suggested the molecular formula $C_{33}H_{42}O_8$. Chemical and spectral analyses of **9** revealed a distinct similarity with stavaroside J with the replacement of the acetyl with an angeloyl moiety. These were further supported by displaying the typical spectral evidences of both benzoate and angelate moieties (Table 1). The order of angelic and benzoic acids diester linkages at C-11 and C-12 remained unsettled, despite the use of the inverse detection technique for this purpose owing to the poor yield of **9** aglycone. Stavaroside **I** was established to be 11α,12β-angeloxy and benzoxy-3β,8β,14β-trihydroxypregn-5-en-20-one-3-*O*-[β-D-glucopyranosyl-(1-4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside D (**4**) displayed a molecular ion peak at *m/z* 951 $[M - H]^-$ and 959 $[M + Li]^+$ in the FAB-mass spectrum suggesting the molecular formula $C_{49}H_{76}O_{18}$. LC-MS analysis of **4** aglycone displayed $[M]^+$ at *m/z* 505 $[M + H]^+$ which suggested the molecular formula $C_{28}H_{40}O_8$. Chemical and spectral analysis of **4** revealed a distinct similarity with stavaroside C with the replacement of the benzoyl with a tigloyl moiety. These observations were further supported by the typical spectral evidences of both tiglate as well as acetate moieties (Tables 1 and 2). Once again the order of acylation between positions C-11 and C-12 was established through the ^{1}H–^{13}C HMBC connectivity spectrum of the aglycone. The ^3J-coupling cross peaks between the carbonyl ester resonating at δ168.0 with H-12 (δ4.87), as well as H-3 of the tigloyl moiety (δ6.97), confirmed the tigloyl esterification at C-12. Similarly, the acetate ester was shown to be at C-11. Stavaroside D was confirmed to be 11α-acetoxy-12β-tigloxy-3β,8β,14β-trihydroxypregn-5-en-20-one-3-*O*-[3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside K (**11**) displayed a molecular ion peak at *m/z* 1113 $[M - H]^-$ and 1121 $[M + Li]^+$ in the FAB-mass spectrum suggesting the molecular formula $C_{55}H_{86}O_{23}$. LC-MS analysis of stavaroside K aglycone displayed $[M]^+$ at *m/z* 505 $[M + H]^+$ which suggested the molecular formula $C_{28}H_{40}O_8$ and the similarity with stavaroside D aglycone. Chemical and spectral analysis of **11** revealed a distinct similarity with stavaroside D (**4**) with the addition of a fourth terminal β-D-glucopyranosyl unit to the sugar moiety (Tables 1 and 2). Stavaroside K established to be 11α-acetoxy-12β-tigloxy-3β,8β,14β-trihydroxypregn-5-en-20-one-3-*O*-[β-D-glucopyranosyl-(1-4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside F (**6**) displayed a molecular ion peak at *m/z* 935 $[M + Na]^+$ in the FD-mass spectrum suggesting the molecular formula $C_{46}H_{72}O_{18}$. Chemical and spectral analysis of **6** revealed a distinct similarity with stavaroside C with the replacement of the benzoyl with another acetate moiety. These observations were further supported by the typical spectral evidences of two acetate moieties (Table 1). Acetylation of **6** afforded **12** on the basis of NMR spectral analyses. Stavaroside F was confirmed to be 11α-acetoxy-12-β-acetoxy-3β,8β,14β-trihydroxypregn-5-en-20-one-3-*O*-[3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Stavaroside H (**8**) displayed a molecular ion peak at *m/z* 828 in the FAB-mass spectrum suggesting the molecular formula $C_{42}H_{68}O_{16}$. Chemical and spectral analysis of **8** revealed a distinct similarity with stavaroside F with the disappearance of the two acetate ester moieties. This was further confirmed through acetylation of **8** using method 1 to afford **13**. Meanwhile, further acetylation of **13** using method 2 afforded **12**. Stavaroside H was confirmed to be 3β,8β,11α,12β,14β-pentahydroxypregn-5-en-20-one-3-*O*-[3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1-4)-β-D-cymaropyranosyl-(1-4)-β-D-cymaropyranoside].

Compound S_6 represented a mixture of three isomeric glycosides with the same triose sugar identified under stavaroside E and differing only in the acylation pattern of the aglycone which proved to be sarcostin. The major isomer was proved to be 12-*O*-β-tigloyl sarcostin. The other two minor isomers were believed to be 20-*O*-tigloyl sarcostin and 12-*O*-β-angeloyl sarcostin. Unfortunately, all attempts to resolve these isomers were unsuccessful.

EXPERIMENTAL

Mps: uncorr. Thermospray LC-MS Vestec Model 201 mass spectrometer. NMR Varian VXR-300 FT spectrometer operating at 299.949 MHz for ^1H and 75.3 MHz for ^{13}C NMR, TMS was used as internal standard. The ^1H–^{13}C-HMBC connectivity experiments for the stavaroside aglycones were measured on Brucker AM-400 or AMX-500 spectrometers (Brucker Instruments Company, Massachusetts, U.S.A.). HPLC Equipped with M-6000A Waters Associates chromatography pump, U6K injector, Waters 440 Absorbance detector (Waters Associate Incorporation, Milford, MA, U.S.A.) and HP3390 recording integrator (Hewlett Packard, U.S.A.). 5710A HP Gas chromatograph equipped with a FID and coupled to a HP3394A integrator (Hewlett Packard, U.S.A.).

Plant material. Aerial parts of *Stapelia variegata* L. (stem and spine-modified leaf) were collected on 6 October 1990, from plants cultivated in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Mansoura University, Egypt. Identification was confirmed by Dr N. El-Hadidy, Faculty of Science, Cairo University, Egypt.

Extraction and isolation. Fresh plant material (25 kg) was collected, chopped and soaked in boiling EtOH for 10 min. The resultant mass was dried in an aerating oven at 55° and powdered. The resultant 1.16 kg powder was subjected to successive extraction. The Et_2O extract (33.0 g) was dissolved in 2 l hot Et_2O, stoppered and kept for 12 hr in a refrigerator where a ppt. was formed. Filtration afforded two main extracts: the ether-soluble part (14.2 g) and the cooled ether-insoluble part (18.1 g). Each of these extracts was chromatographed separately. CC of the ether-soluble part on silica gel (E. Merck) using $CHCl_3$–MeOH with increasing polarity, afforded two main fractions; A (5.6 g) and B (4.3 g). Fractionation of the ether-insoluble part using the same system afforded one major fraction C (3.4 g). These 3 frs (A–C) were rechromatographed on flash silica gel (70–230 mesh) using isocratic 30% Me_2CO–hexane (in the case of frs A and B) and 10–12% MeOH–$CHCl_3$ mixture (in the case of fr. C). Fr. A afforded subfr. A_1 which was further chromatographed on C_{18} RP-HPLC, using MeCN–H_2O (11:9) to afford **1** (56.5 mg), **2** (59.2 mg), **3** (68 mg) and **4** (196.2 mg). Fraction B afforded subfrs B_1 and B_2. Subfr. B_1 was further chromatographed on C_{18} RP-HPLC, using MeCN–H_2O (1:1) to afford **5** (51.2 mg), S_6 (92.1 mg), **6** (176.1 mg) and **7** (19.5 mg). Subfr. B_2 was chromatographed on normal phase HPLC, using isocratic 1% MeOH–$CHCl_3$ to afford **8** (12 mg). Fr. C afforded subfr. C1 which was further chromatographed on C_{18} RP-HPLC, using MeCN–H_2O (1:1) to afford **9** (20.5 mg), **10** (88 mg) and **11** (186.5 mg). C_{18} RP-HPLC was used for both analytical and prep. purposes. Column: μ Bondapak (250 mm length × 13 mm i.d.) C_{18} column (Waters Associate) was used on both analytical and prep. scales. MeCN–H_2O (1:1) or (11:9) system was used. Flow rate: 1.5–2 ml min^{-1} (for analytical) or 2.5–3 ml min^{-1} (for prep.) purpose. Detector: UV λ_{max} nm 254 (Waters Associates).

Acetylation of compounds **6** *and* **8** *(method 1).* To a solution of 4.7 mg of **6** or 2.8 mg of **8** in pyridine, 0.4 ml of Ac_2O was added and the solns allowed to stand for 4 hr at room temp. The reaction mixtures were poured into 20 ml H_2O and extracted 3× with 10 ml Et_2O. The combined ethereal soln was washed with 2M HCl, satd $NaHCO_3$ and satd NaCl solns and then finally dried on Na_2SO_4. Solvent evapn in gave **12** (4.1 mg) from **6** and **13** (2.4 mg) from **8**.

Acetylation of compound **13** *(method 2).* The recovered 2.4 mg of **13** was dissolved in 0.5 ml pyridine and 0.4 ml Ac_2O and refluxed for 14 hr. The mixture was treated as in the previous procedure to obtain 2.2 mg **12**.

Mild acid hydrolysis of compounds **1**–**11**. *Ca* 5–50 mg of **1**–**11** were separately dissolved in 6 ml MeOH and then 1 ml 0.1 M H_2SO_4 added. Each soln was kept at 60° for 30 min then H_2O (6 ml) added and the whole soln concd under red. pres. to 6 ml. The soln was warmed at 60° for a further 30 min then extracted with Et_2O (3 × 12.5 ml). The combined ethereal layers were washed with 5% $NaHCO_3$ soln (10 ml), satd NaCl soln (10 ml) and finally with H_2O (3 × 5 ml). The washed soln was dried over Na_2SO_4, concd under red. pres. to small vols and chromatographed over silica gel G plates using 30% Me_2CO–*n*-hexane solvent system to afford amorphous powders.

Strong acid hydrolysis of compounds **1**–**11** *and cymarin.* A soln of 5–50 mg of **1**–**11** and 100 mg of authentic cymarin (Aldrich Co., Milwaukee, U.S.A.) in MeOH (4 ml) and 2 ml 1 M H_2SO_4 was heated at 80° for 6 hr. H_2O (6 ml) was added and then the soln was extracted with Et_2O. The combined ethereal extract was treated in the same way as in the mild acid hydrolysis to afford amorphous aglycone powders.

TLC of the sugar moieties of compounds **1**–**11**. The aq. mother liquors from both mild and strong acid hydrolysis, after the extraction of the aglycone in each case, were neutralized with a saturated soln of $Ba(OH)_2$ and filtered. Each filtrate was evaporated under red. pres. to dryness. The residue was dissolved in 0.5 ml pyridine and subjected to chromatographic investigation along with authentic cymarose (obtained after hydrolysis of cymarin), allomethylose and glucose on: (a) Cellulose-precoated plates using *n*-BuOH–pyridine–HOAc–EtOAC–H_2O (10:4:2:5:4) double run, solvent system [3]. The chromatoplate was visualized with aniline hydrogen phthalate reagent and heated at 110° for 5 min. (b) Silica gel G plates using $CHCl_3$–MeOH–H_2O (18:3:1, lower layer) solvent system and vanillin–H_2SO_4 reagent [8]. This method was used for the prep. TLC purification of sugars.

GLC analysis of sugar alditols. The sugars obtained after strong acid hydrolysis of **1**–**11** in addition to authentic cymarose, glucose (10 mg, each) and allomethylose (1 mg) were separately dissolved in 2 ml H_2O. After addition of 1 mg of $NaBH_4$, the reaction mixture was heated at 80° for 2 hr and then kept overnight. Excess sodium borohydride was destroyed by the addition of Me_2CO and then the reaction mixtures were passed through 1 g [H^+] Dowex-50. The borate was removed—as methyl

borate—by continued addition of MeOH and evapn using a rotary evaporator. The sugar alditols formed by the reduction with $NaBH_4$ were dissolved in 1 ml redistilled pyridine and 1 ml Ac_2O was then added. The reaction mixtures were kept overnight at room temp. then evapd to dryness under red. pres. and the alditol acetates were purified on prep. silica gel TLC chromatoplates using 1% MeOH in C_6H_6 solvent system [9, 10]. The alditol acetates were subjected to GC and GC-MS analysis using a DB-1 (dimethyl polysiloxane) (0.25 mm) 15 m × 0.246 mm capillary column with helium (0.82 ml min^{-1}) as the carrier gas and flame ionization detector (FID). The oven temperature was 190°, isothermal and nitrogen flow rate 50 ml min^{-1}. Cymaritol acetate retention times R_t = 3.01 min and M_r = 290, Allomethylitol acetate: R_t = 5.45 and M_r = 348, Glucitol acetate: R_t = 13.65 and M_r = 434.

Alkaline hydrolysis of compounds **3** *and* **5** *aglycones.* *Ca* 6 mg of each of compounds **3** and **5** aglycones were refluxed with 1.0 ml 1% methanolic KOH soln and 10 ml C_6H_6 for 8 hr. Each mixture was evapd to dryness, diluted wtih 10 ml H_2O and extracted with $CHCl_3$ (3 × 10 ml). The solvent was evapd to dryness and the residue crystallized from Me_2CO.

Stavaroside A (**1**). Powder, mp 187–189°, $[\alpha]_D$ −36 (MeOH; 0.1). UV λ_{max} nm: 212, 235 and 280. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1705, 1690, 1600, 1460, 1275, 1075 and 1010. ^{13}C NMR (Tables 1 and 2). ^1H NMR: δ1.25 (3H, *s*, Me-18), 1.11 (3H, *s*, Me-19), 1.32 (3H, *d*, *J* = 6.0 Hz, Me-21), 1.21 (3H, *d*, *J* = 6.3 Hz, Me-6′ sugar), 1.26 (3H, *d*, *J* = 6.2 Hz, Me-6″ sugar), 1.27 (3H, *d*, *J* = 5.6 Hz, Me-6‴ sugar), 3.42, 3.44, 3.66 (each 3H, *s*, OMe-3 of sugar moiety), 3.21 (2H, *dd*, *J* = 9.6, 2.8 Hz, 4′ and 4″ of sugar moiety), 3.26 (1H, *dd*, *J* = 9.8, 2.8 Hz, 4‴ of allom), 4.84 (1H, *dd*, *J* = 9.6, 2.0 Hz, H-1′ cym), 4.76 (1H, *dd*, *J* = 9.6, 1.6 Hz, H-1″ cym), 4.58 (1H, *d*, *J* = 7.8 Hz, H-1‴ allom), 4.79 (1H, *dd*, *J* = 11.3, 4.4 Hz, H-12), 4.81 (1H, *q*, *J* = 6.3 Hz, H-20), 7.95 (2H, *dd*, *J* = 7.1, 1.4 Hz, Bz O-2H), 7.55 (1H, *dt*, *J* = 7.4, 1.4 Hz, Bz P-1H), 7.43 (2H, *dd*, *J* = 7.4, 7.1 Hz, Bz M-2H), 6.29 (1H, *qq*, *J* = 7.1, 1.4 Hz, Ang H-3), 1.54 (3H, *dq*, *J* = 7.1, 1.3 Hz, Ang-4-Me), 1.72 (3H, *br s*, Ang-5-Me).

Stavaroside B (**2**). Powder, mp 144–146°, $[\alpha]_D$ −34 (MeOH; *c* 0.1). UV λ_{max} nm: 217. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1700, 1695, 1270, 1200, 1150 and 1010. ^{13}C NMR (Tables 1 and 2). ^1H NMR: δ1.25 (3H, *s*, Me-18), 1.14 (3H, *s*, Me-19), 1.21 (3H, *d*, *J* = 6.2 Hz, Me-21), 1.20 (3H, *d*, *J* = 6.1 Hz, Me-6′ sugar), 1.26 (3H, *d*, *J* = 6.1 Hz, Me-6″ sugar), 1.27 (3H, *d*, *J* = 6.2 Hz, Me-6‴ sugar), 3.42, 3.44, 3.65 (each 3H, *s*, OMe-3 of sugar moiety), 3.21 (2H, *dd*, *J* = 9.4, 2.9 Hz, 4′ and 4″ of sugar moiety), 3.26 (1H, *dd*, *J* = 9.7, 3.0 Hz, 4‴ of allom), 4.84 (1H, *dd*, *J* = 9.6, 1.6 Hz, H-1′ cym), 4.75 (1H, *dd*, *J* = 9.3, 2.0 Hz, H-1″ cym), 4.59 (1H, *d*, *J* = 8.0 Hz, H-1‴ allom), 4.74 (1H, *dd*, *J* = 11.5, 4.4 Hz, H-12), 4.63 (1H, *q*, *J* = 6.0 Hz, H-20), 6.74 (1H, *qq*, *J* = 7.0, 1.4 Hz, Tig-H-3), 1.78 (3H, *dq*, *J* = 9.5, 1.1 Hz, Tig-4-Me), 1.76 (3H, *s*, Tig-5-Me), 6.58 (1H, *qq*, *J* = 7.1, 1.5 Hz, Ang H-3), 1.68 (3H, *dq*, *J* = 7.2, 1.1 Hz, Ang-4-Me), 1.74 (3H, *s*, Ang-5-Me).

Stavaroside C (**3**). Powder, mp 143–146°, $[\alpha]_D$ −10.5 (MeOH; *c* 0.2). UV λ_{max} nm: 213, 230 and 278. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490, 3400, 1740, 1705, 1690, 1260, 1220 and 1100. ^{13}C NMR (Tables 1 and 2). ^1H NMR: δ1.24 (3H, *s*, Me-18), 1.35 (3H, *s*, Me-19), 2.09 (3H, *s*, Me-21), 1.21 (3H, *d*, *J* = 6.2 Hz, Me-6′ sugar), 1.26 (3H, *d*, *J* = 6.2 Hz, Me-6″ sugar), 1.27 (3H, *d*, *J* = 6.2 Hz, Me-6‴ sugar), 3.41, 3.44, 3.65 (each 3H, *s*, OMe-3 of sugar moiety), 3.21 (2H, *dd*, *J* = 9.6, 2.8 Hz, 4′ and 4″ of sugar moiety), 3.26 (1H, *dd*, *J* = 9.8, 3.0 Hz, 4‴ of allom), 4.84 (1H, *dd*, *J* = 9.6, 2.8 Hz, H-1′ cym), 4.75 (1H, *dd*, *J* = 9.5, 1.7 Hz, H-1″ cym), 4.58 (1H, *d*, *J* = 8.0 Hz, H-1‴ allom), 5.92 (1H, *dd*, *J* = 10.6, 10.6 Hz, H-11), 5.16 (1H, *d*, *J* = 10.0 Hz, H-12), 8.07 (2H, *dd*, *J* = 7.0, 1.4 Hz, Bz O-2H), 7.62 (1H, *dt*, *J* = 7.3, 1.4 Hz, Bz P-1H), 7.49 (2H, *dd*, *J* = 7.3, 7.0 Hz, Bz M-2H), 1.69 (3H, *s*, Ac-Me).

Stavaroside D (**4**). Powder, mp 131–135°, $[\alpha]_D$ +30 (MeOH; *c* 0.2). UV λ_{max} nm: 218 and 230. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1740, 1700, 1690, 1245, 1220 and 1100. ^{13}C NMR (Tables 1 and 2). ^1H NMR: δ1.13 (3H, *s*, Me-18), 1.18 (3H, *s*, Me-19), 2.09 (3H, *s*, Me-21), 1.14 (3H, *d*, *J* = 6.2 Hz, Me-6′ sugar), 1.19 (3H, *d*, *J* = 6.2 Hz, Me-6″ sugar), 1.21 (3H, *d*, *J* = 6.2 Hz, Me-6‴ sugar), 3.34, 3.37, 3.58 (each 3H, *s*, OMe-3 of sugar moiety), 3.14 (2H, *dd*, *J* = 9.6, 2.9 Hz, 4′ and 4″ of sugar moiety), 3.20 (1H, *dd*, *J* = 9.6, 2.9 Hz, 4‴ of allom), 4.77 (1H, *dd*, *J* = 9.5, 1.7 Hz, H-1′ cym), 4.68 (1H, *dd*, *J* = 9.5, 1.7 Hz, H-1″ cym), 4.52 (1H, *d*, *J* = 8.0 Hz, H-1‴ allom), 5.71 (1H, *dd*, *J* = 10.0, 10.0 Hz, H-11), 4.87 (1H, *d*, *J* = 10.0 Hz, H-12), 6.97 (1H, *qq*, *J* = 7.5, 1.3 Hz, Tig-H-3), 1.77 (3H, *dq*, *J* = 8.0, 1.2 Hz, Tig-4-Me), 1.70 (3H, *s*, Tig-5-Me), 1.81 (3H, *s*, Ac-Me).

Stavaroside E (**5**). Powder, mp 151–154°, $[\alpha]_D$ −33 (MeOH; *c* 0.1). UV λ_{max} nm: 230, 270 and 280. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3485, 1710, 1650, 1600, 1460, 1280, 1080 and 1000. ^{13}C NMR (Tables 1 and 2). ^1H NMR: δ1.62 (3H, *s*, Me-18), 1.15 (3H, *s*, Me-19), 1.03 (3H, *d*, *J* = 6.3 Hz, Me-21), 1.20 (3H, *d*, *J* = 6.2 Hz, Me-6′ sugar), 1.24 (3H, *d*, *J* = 5.7 Hz, Me-6″ sugar), 1.26 (3H, *d*, *J* = 6.3 Hz, Me-6‴ sugar), 3.40, 3.43, 3.64 (each 3H, *s*, OMe-3 of sugar moiety), 3.20 (2H, *dd*, *J* = 9.5, 2.9 Hz, 4′ and 4″ of sugar moiety), 3.25 (1H, *dd*, *J* = 9.7, 2.8 Hz, 4‴ of allom), 4.81 (1H, *dd*, *J* = 8.9, 1.7 Hz, H-1′ cym), 4.74 (1H, *dd*, *J* = 9.3, 1.6 Hz, H-1′ cym), 4.57 (1H, *d*, *J* = 7.9 Hz, H-1‴ allom), 4.85 (1H, *dd*, *J* = 11.4, 4.4 Hz, H-12), 3.67 (1H, *q*, *J* = 6.3 Hz, H-20), 8.07 (2H, *dd*, *J* = 7.8, 1.4 Hz, Bz O-2H), 7.58 (1H, *dt*, *J* = 7.4, 1.3 Hz, Bz P-1H), 7.46 (2H, *dd*, *J* = 8.1, 7.3 Hz, Bz M-2H).

Stavaroside F (**6**). Powder, mp 94.5–96°, $[\alpha]_D$ +44.8 (MeOH; *c* 0.85). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3515, 1740, 1690, 1245, and 1080, FD-MS *m/z*: 935 $[M + Na]^+$, 774 $[M - (allom + Na)]^+$, 609 $[M - (allom + cym) + H]^+$ and 464 $[M - (allom + cym + cym)]^+$. ^{13}C NMR (Table 1). ^1H NMR: δ1.21 (3H, *s*, Me-18), 1.24 (3H, *s*, Me-19), 2.01 (3H, *s*, Me-21), 1.22 (3H, *d*, *J* = 5.9 Hz, Me-6′ sugar), 1.27 (3H, *d*, *J* = 6.2 Hz, Me-6″ sugar), 1.28 (3H, *d*, *J* = 6.2 Hz, Me-6‴ sugar), 3.42, 3.45, 3.66 (each 3H, *s*, OMe-3 of sugar moiety), 3.22 (2H, *dd*, *J* = 10.0, 2.9 Hz, 4′ and 4″ of sugar moiety), 3.27 (1H, *dd*, *J* = 10, 2.9 Hz, 4‴ of allom), 4.84 (1H, *dd*, *J* = 9.0, 1.5 Hz, H-1′ cym), 4.76 (1H, *dd*, *J* = 9.0, 1.5 Hz, H-1″ cym), 4.59 (1H, *d*, *J* = 8.1 Hz, H-1‴ allom), 5.72 (1H, *dd*, *J* = 10.6, 10.5 Hz, H-11), 4.87 (1H, *d*, *J* = 9.9 Hz, H-12), 2.20 (3H, *s*, Ac-Me), 2.12 (3H, *s*, Ac-Me).

Stavaroside G (**7**). Powder, mp 146–148°, $[\alpha]_D$ −90 (MeOH; *c* 0.1). UV λ_{max} nm: 207. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3515, 1745, 1725, 1240 and 1080. ^{13}C NMR (Table 1). ^1H NMR: δ1.42 (3H, *s*, Me-18), 1.13 (3H, *s*, Me-19), 1.22 (3H, *d*, *J* = 6.5 Hz, Me-21), 1.21 (3H, *d*, *J* = 6.1 Hz, Me-6′ sugar), 1.26 (3H, *d*, *J* = 6.1 Hz, Me-6″ sugar), 1.27 (3H, *d*, *J* = 6.1 Hz, Me-6‴ sugar), 3.41, 3.44, 3.65 (each 3H, *s*, OMe-3 of sugar moiety), 3.21 (2H, *dd*, *J* = 9.8, 3.2 Hz, 4′ and 4″ of sugar moiety), 3.26 (1H, *dd*, *J* = 9.5, 3.0 Hz, 4‴ of allom), 4.83 (1H, *dd*, *J* = 9.3, 1.0 Hz, H-1″ cym), 4.75 (1H, *dd*, *J* = 8.6, 1.2 Hz, H-1′ cym), 4.58 (1H, *d*, *J* = 8.1 Hz, H-1‴ allom), 4.62 (1H, *dd*, *J* = 9.5, 4.0 Hz, H-12), 4.59 (1H, *q*, *J* = 6.0 Hz, H-20), 2.08 (3H, *s*, Ac-Me), 1.99 (3H, *s*, Ac-Me).

Stavaroside H (**8**). Powder, mp 104–130°, $[\alpha]_D$ +23.9 (MeOH; *c* 0.23). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3515, 1690 and 1080. ^{13}C NMR (Table 1). ^1H NMR: δ1.12 (3H, *s*, Me-18), 1.37 (3H, *s*, Me-19), 2.28 (3H, *s*, Me-21), 1.22 (3H, *d*, *J* = 6.4 Hz, Me-6′ sugar), 1.27 (3H, *d*, *J* = 6.2 Hz, Me-6″ sugar), 1.28 (3H, *d*, *J* = 6.4 Hz, Me-6‴ sugar), 3.42, 3.45, 3.66 (each 3H, *s*, OMe-3 of sugar moiety), 4.86 (1H, *dd*, *J* = 9.0, 1.5 Hz, H-1′ cym), 4.76 (1H, *dd*, *J* = 7.7, 1.5 Hz, H-1″ cym), 4.59 (1H, *d*, *J* = 8.0 Hz, H-1‴ allom), 4.16 (1H, *t*, *J* = 10.0, H-11).

Stavaroside I (**9**). Powder, mp 139–141°, $[\alpha]_D$ −22 (MeOH; *c* 0.1). UV λ_{max} nm: 212, 235 and 280. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1710, 1700, 1690, 1600, 1460, 1275, 1075 and 1010. ^{13}C NMR (Table 1). ^1H NMR: (pyridine-d_5): δ1.14 (3H, *s*, Me-18), 1.20 (3H, *s*, Me-19), 2.0 (3H, *s*, Me-21), 1.33 (3H, *d*, *J* = 6.2 Hz, Me-6′ sugar), 1.43 (3H, *d*, *J* = 6.2 Hz, Me-6″ sugar), 1.53 (3H, *d*, *J* = 6.2 Hz, Me-6‴ sugar), 3.51, 3.52, 3.82 (each 3H, *s*, OMe-3 of sugar moiety), 5.15 (1H, *dd*, *J* = 8.0, 1.8 Hz, H-1′ cym), 4.99 (1H, *dd*, *J* = 7.8, 1.8 Hz, H-1″ cym), 4.85 (1H, *d*, *J* = 7.5 Hz, H-1‴ allom), 4.44 (1H, *br s*, H-1⁗ gluc), 5.98 (1H, *dd*, *J* = 7.7, 7.5 Hz, H-11), 4.18 (1H, *d*, *J* = 8.0 Hz, H-12), 8.14 (2H, *m*, Bz O-2H), 7.53 (1H, *m*, Bz P-1H), 7.39 (2H, *m*, Bz M-2H), 6.37 (1H, *qq*, *J* = 7.3, 1.5 Hz, Ang H-3), 1.90 (3H, *br s*, Ang-4-Me), 1.96 (3H, *br s*, Ang-5-Me).

Stavaroside J (**10**). Powder, mp 167–169°, $[\alpha]_D$ +38 (MeOH; *c* 0.1). UV λ_{max} nm: 214, 236 and 280. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1740, 1710, 1690, 1600, 1450, 1270, 1220 and 1100. ^{13}C NMR (Tables 1 and 2). ^1H NMR: δ1.23 (3H, *s*, Me-18), 1.26 (3H, *s*, Me-19), 2.15 (3H, *s*, Me-21), 1.22 (3H, *d*, *J* = 7.0 Hz, Me-6′ sugar), 1.24 (3H, *d*, *J* = 6.0 Hz, Me-6″ sugar), 1.25 (3H, *d*, *J* = 6.5 Hz, Me-6‴ sugar), 3.39, 3.43, 3.57 (each 3H, *s*, OMe-3 of sugar moiety), 4.79 (1H, *dd*, *J* = 8.8, 1.5 Hz, H-1′ cym), 4.74 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-1″ cym), 4.56 (1H, *d*, *J* = 7.5 Hz, H-1‴ allom), 4.39 (1H, *d*, *J* = 8.1 Hz, H-1⁗ gluc), 5.78 (1H, *dd*, *J* = 10.0, 9.9 Hz, H-11), 4.95 (1H, *d*, *J* = 9.6 Hz, H-12), 8.07 (2H, *dd*, *J* = 8.5, 1.4 Hz, Bz O-2H), 7.62 (1H, *dt*, *J* = 7.4, 1.4 Hz, Bz P-1H), 7.48 (2H, *dd*, *J* = 8.0, 7.2 Hz, Bz M-2H), 1.69 (3H, *s*, Ac-Me).

Stavaroside K (**11**). Powder, mp 115–118°, $[\alpha]_D$ +22 (MeOH; *c* 0.1). UV λ_{max} nm: 216 and 231. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1740, 1705, 1690, 1270, 1220 and 1100. ^{13}C NMR (Tables 1 and 2). ^1H NMR: δ1.25 (3H, *s*, Me-18), 1.36 (3H, *s*, Me-19), 2.10 (3H, *s*, Me-21), 1.20 (3H, *d*, *J* = 5.5 Hz, Me-6′ sugar), 1.24 (3H, *d*, *J* = 6.3 Hz, Me-6″ sugar), 1.25 (3H, *d*, *J* = 5.2 Hz, Me-6‴ sugar), 3.40, 3.43, 3.57 (each 3H, *s*, OMe-3 of sugar moiety), 4.83 (2H, *dd*, *J* = 9.8, 1.5 Hz, H-1′ cym), 4.73 (1H, *dd*, *J* = 7.5, 1.5 Hz, H-1″ cym), 4.58 (1H, *d*, *J* = 8.1 Hz, H-1‴ allom), 4.37 (1H, *d*, *J* = 7.9 Hz, H-1⁗), 5.92 (1H, *dd*, *J* = 9.4, 8.7 Hz, H-11), 5.14 (1H, *d*, *J* = 9.4 Hz, H-12), 6.96 (2H, *qq*, *J* = 7.5, 1.0 Hz, Tig-H-3), 1.83 (1H, *dq*, *J* = 9.3, 1.3 Hz, Tig-4-Me) 1.84 (3H, *s*, Tig-5-Me), 1.85 (3H, *s*, Ac-Me).

Stavaroside H tetraacetate (**12**). Powder, ^1H NMR: δ1.17–1.26 (3H, *m*, Me-18), 1.17–1.26 (3H, *m*, Me-19), 2.01 (3H, *s*, Me-21), 1.17–1.26 (3H, *m*, Me-6′ sugar), 1.17–1.26 (3H, *m*, Me-6″ sugar), 1.17–1.26 (3H, *m*, Me-6‴ sugar), 3.43, 3.44, 3.48 (each 3H, *s*, OMe-3 of sugar moiety), 4.84 (1H, *dd*, *J* = 9.5, 1.5 Hz, H-1′ cym), 4.74–4.81 (1H, *m*, H-1″ cym), 4.73 (1H, *d*, *J* = 8.1 Hz, H-1‴ allom), 4.57 (1H, *dd*, *J* = 9.9, 2.6 Hz, H-4‴ of sugar moiety), 5.72 (1H, *t*, *J* = 10.6 Hz, H-11), 4.87 (1H, *d*, *J* = 9.9 Hz, H-12), 2.09 and 2.11 (each 3H, *s*, Ac of sugar).

Stavaroside H diacetate (**13**). Powder, ^1H NMR: δ1.17–1.35 (3H, *m*, Me-18), 1.17–1.35 (3H, *m*, Me-19), 2.22 (3H, *s*, Me-21), 1.17–1.35 (3H, *m*, Me-6′ sugar), 1.17–1.35 (3H, *m*, Me-6″ sugar), 1.17–1.35 (3H, *m*, Me-6‴ sugar), 3.42, 3.44, 3.48 (each 3H, *s*, OMe-3 of sugar moiety), 4.85 (1H, *dd*, *J* = 9.5, 1.5 Hz, H-1′ cym), 4.74–4.81 (1H, *m*, H-1″ cym), 4.73 (1H, *d*, *J* = 8.4 Hz, H-1‴ allom), 4.59 (1H, *dd*, *J* = 9.9, 2.6 Hz, H-4‴ of sugar moiety), 2.09 and 2.11 (each 3H, *s*, Ac of sugar).

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