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Ecdysteroids and other constituents from *Sida spinosa* L.

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Abstract

Two compounds (**3** and **10**) were isolated from the aerial parts of *Sida spinosa* L. Their structures have been established as glyceryl-1-eicosanoate and 20-hydroxy, 24-hydroxymethylecdysone by 1D and 2D-NMR techniques. In addition 12 known compounds (**1**, **2**, **4**–**9** and **11**–**14**) have been isolated and identified.

Keywords: *Sida spinosa* L.; Malvaceae; Hydrocarbons; Fatty acid derivatives; Phenolic compound; Cerebroside; Ecdysteroids

1. Introduction

Sida spinosa L. (*S. alba*, partly), Malvaceae (Muschler, 1970; Tackholm, 1974; Boulos and Hadidi, 1984) is an annual erect herb, widely spread along the road sides and canal banks during summer in Upper Egypt (Boulos and Hadidi, 1984). It is locally known as "Melukhiyet Iblis". Many species of the genus Sida have been used in traditional medicine for a variety of therapeutic purposes as astringent, cooling stomachic and in nervous, urinary and cardiac diseases (Gunatilaka et al., 1980; Lutterodt, 1988). *S. spinosa* L. is used in the treatment of asthma and other chest ailments and as a tonic (Prakash et al., 1981). The leaves have reportedly been used for treatment of some skin diseases and as oral snake bite treatment (Iwu, 1993). The roots and leaves of *S. spinosa* are used in treatment of diarrhea and dysentery (Noumi and Yomi, 2001). *S. spinosa* growing in Egypt has not been phytochemically investigated before, while the roots and aerial parts of *S. spinosa* growing in India are reported to contain alkaloids (Prakash et al., 1981).

Pandit et al. (1976) and Dinan et al. (2001) have isolated phytoecdysteroids from some plants of the genus *Sida*, but to our knowledge, phytoecdysteroids have not been reported from *S. spinosa* L. to date. This group of compounds have interesting biological activities as insect molting hormones and insulin regulators and display diuretic and tonic effects in addition to their anabolic properties (Rudel et al., 1992; Coll et al., 1994).

2. Results and discussion

The known compounds were characterized by direct comparison of their physical and spectroscopic characteristics with those published in the literature. The new compounds were characterized by different spectroscopic methods.

The EIMS of **3** showed a molecular ion peak at *m/z* 386 for $C_{23}H_{46}O_4$. Its ^1H NMR showed proton signals attributable to glycerol (δ_H 4.23, 4.15, 3.89, 3.70 and 3.62) and a single linear alkyl chain. The ^{13}C NMR spectrum and APT revealed the presence of glycerol moiety (δ_C 70.5, 65.2 and 63.4) linked to a straight chain saturated fatty acid (δ_C 174.6, 34.1, 29.4–33.7, 26.7, 22.7 and 14.2). The alkyl side chain could be determined as C-20 from the presence of a fragment at *m/z* 267 due to the loss of $C_4H_7O_4$ from M^+ (Sultana et al., 1999). The point of esterification was determined to be at C-1 of the glycerol moiety due to the downfield shift of C-1 (δ_C 65.2) (Breitmaier and Voelter, 1987).

Based on this evidence, **3** was identified as glyceryl-1-eicosanoate which is isolated for the first time from nature.

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Compound **5** was obtained as colourless needle crystals with a molecular formula $C_{18}H_{18}O_5$ established from EIMS and ^{13}C NMR. The EIMS spectrum exhibited M^+ at m/z 314 calculated for $C_{18}H_{18}O_5$, a base peak at m/z 177 and other significant peaks at m/z 194 $[M+H\text{-}p\text{-hydroxy phenethyl moiety}]^+$ and 120 $[M\text{-}H\text{-feruloyl moiety}]^+$. ^1H NMR and ^{13}C NMR showed typical patterns of *p*-substituted phenethyl moiety and feruloyl moiety with *trans* double bond (Li et al., 2000).

The HMQC spectrum showed long-range ^1H–^{13}C correlations of H-7 (δ_H 7.44) to C-8 (δ_C 118.7), C-9 (δ_C 169.1), C-6 (δ_C 123.2) and C-2 (δ_C 111.5) and of the H-1″ protons (δ_H 3.45) to C-2 (δ_C 35.8) and C-9 (δ_C 169.1) which confirms the structure of **5** as *p*-hydroxy phenethyl *trans*-ferulate. This compound has antioxidant properties and has been isolated from *Heracleum lanatum* (Nakata et al., 1982, *Oenanthe javanica* (Fujita et al., 1995) and *Coriandum sativum* (Taniguchi et al., 1996), but this is the first report for the full assignment of its structure using ^{13}C NMR, APT and HMQC.

Ecdysteroids **10–14** were isolated from the ethyl acetate-soluble fraction of the methanolic extract of the plant. 20-Hydroxyecdysone (**11**), turkesterone (**12**), makisterone C (**13**) and 20-hydroxyecdysone-20,22-monoacetonide (**14**) were identified by comparing their EIMS, ^1H NMR and ^{13}C NMR data with those reported in the literature. The ^1H NMR and ^{13}C NMR data are summarized in Tables 1 and 2 and were used as references for the signal assignment and structural determination of **10**. Compound **10** was isolated as faint yellow amorphous solid. Its IR spectrum showed absorption bands of hydroxyl groups (3505–3375 cm^{-1}) and a carbonyl group of an α,β-unsaturated ketone (1662 cm^{-1}). The molecular formula of 10 was deduced to be $C_{28}H_{46}O_8$ from its EIMS, FABMS and ^{13}C NMR including APT. The EIMS of 10 failed to give molecular ion peak as is usual for ecdysteroids, but it showed a peak at m/z 460 $[M+H\text{-}3OH]^+$. The presence of ions at m/z 363, 345 and 327 (arising from the C-20/C-22 fission and further loss of one, two and three H_2O) was indicative of a steroid nucleus identical with that of

Table 1
^1H NMR spectral data of ecdysteroids **10–14**

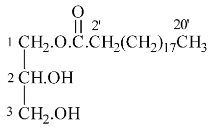
H	**10**	**11**		**12**	**13**	**14**
	DMSO	DMSO	CD_3OD	DMSO	DMSO	CD_3OD
1-H_a	1.26, *t* (9)	1.26, *t*, (7)	1.40, *t* (12.4)	1.19, *t* (7)	1.30	1.48
1-H_e	1.60	1.60	1.78	2.42, *dd* (12.5, 4)	1.62	1.82
2-H_a	3.60	3.59	3.80, *ddd* (12.2, 7.5, 4.2)	3.85	3.59	3.77
3-H_e	3.74	3.76	3.91, *br.* q	3.75	3.74	3.90
4-H_a	1.48	1.52	1.67	1.52	1.50	1.69
4-H_e	1.60	1.63	1.76	1.62, *t* (4)	1.64	1.75
5-H	2.20, *dd* (13, 4)	2.18, *dd* (12.8, 7.3)	2.33, *dd* (12.2, 5)	2.18, *dd* (13, 3.5)	2.19, *dd* (13, 4)	2.26, *dd* (10.5, 5)
7-H	5.63, *d* (2.5)	5.62, *d* (2.2)	5.76, *d* (2.4)	5.63, *d* (2.4)	5.63, *br.s*	5.75, *d* (2.5)
9-H_a	3.03, *ddd* (9, 7.2, 2)	3.0, *t* (7.2)	3.12, *ddd* (10.2, 7.3, 2.3)	3.01, *dd* (10, 3.1)	3.02, *t* (7.2)	3.16, *ddd* (10.2, 7, 2.2)
11-H_a	1.55	1.58	1.67	–	1.54	1.69
11-H_e	1.78	1.73	1.80	4.11, *m*	1.70	1.82
12-H_a	2.06, *ddd* (13, 13, 4.8)	2.01, *ddd* (12, 12, 5)	2.10, *ddd* (12.8, 12.8, 4.9)	2.03, *dd* (12, 10)	2.01, *ddd* (12, 12, 5)	2.05, *ddd* (12, 12, 4.4)
12-H_e	1.74	1.72	1.88	1.98, *dd* (12, 6)	1.75	1.87
15-H_a	1.80	1.88	2.00	1.78	1.90	1.98
15-H_b	1.50	1.44, *m*	1.56, *br.t* (11.1)	1.49	1.50	1.53
16-H_a	1.88	1.85	1.96	1.85	1.80	1.92
16-H_b	1.64	1.63	1.77	1.68	1.65	1.75
17-H	2.22, *t* (8)	2.22, *dd* (13, 4)	2.38, *m*	2.25, *dd* (12.6, 3.9)	2.22, *dd* (12.5, 3.8)	2.33, *dd* (9.5, 6)
22-H_b	3.15, *br.d* (10)	3.10, *dd* (9, 2)	3.32, *dd* (10.5, 1.6)	3.14, *dd* (10, 1.8)	3.18	3.54, *br.d*
23-H_a	1.45 *ddd*	1.11, *br.d*	1.28, *dddd* (17.6, 14.2, 11.4, 5)	1.13, *t* (7)	1.50	1.36, *dddd* (16, 13.8, 10.8, 4.3)
23-H_b	1.57	1.54	1.65, *t* (4.8)	1.52	1.47	1.72
24-H_a	1.75	1.70	1.78	1.70	1.31	1.80
24-H_b	–	1.28, *t* (10.4)	1.43, *t* (11.4)	1.23, *t* (10)	–	1.45
18-Me	0.76, *s*	0.75, *s*	0.85, *s*	0.76, *s*	0.74, *s*	0.76, *s*
19-Me	0.83, *s*	0.83, *s*	0.93, *s*	0.84, *s*	0.84, *s*	1.12, *s*
21-Me	1.05, *s*	1.04, *s*	1.15, *s*	1.05, *s*	1.21, *s*	0.90, *s*
26-Me	1.03, *s*	1.07, *s*	1.16, *s*	1.05, *s*	1.08, *s*	1.14, *s*
27-Me	1.06, *s*	1.04, *s*	1.17, *s*	1.08, *s*	1.23, *s*	1.22, *s*
28	3.30, *dd* (11, 3.2) 3.37, *dd*, (11, 4.8)				1.12, 1.49	
					0.88, *t* (6.7) (29)	1.26 (ketal) 1.33 (ketal)

Coupling constants in Hz are shown in parentheses.

Table 2
^{13}C NMR spectral data of ecdysteroids **10**–**14**

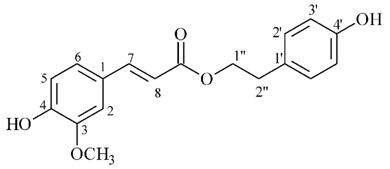
C	**10**	**11**		**12**	**13**	**14**
	DMSO δ, m	DMSO δ, m	CD_3OD δ, m	DMSO δ	DMSO δ	CD_3OD δ
1	36.8 *t*	36.5 *t*	37.4 *t*	36.7	35.2	37.1
2	66.8 *d*	66.5 *d*	68.8 *d*	66.9	66.5	66.5
3	67.0 *d*	66.7 *d*	68.6 *d*	67.1	66.7	66.3
4	31.9 *t*	31.4 *t*	32.9 *t*	31.8	31.4	32.9
5	48.9 *d*	48.6 *d*	51.8 *d*	50.4	50.0	49.5
6	203.0 *s*	202.6 *s*	206.5 *s*	203.5	202.6	204.4
7	120.7 *d*	120.3 *d*	122.2 *d*	120.7	120.4	120.0
8	165.5 *s*	165.1 *s*	168.0 *s*	165.9	165.1	165.5
9	33.4 *d*	33.0 *d*	35.5 *d*	40.9	33.2	35.1
10	37.9 *s*	37.5 *s*	39.3 *s*	38.0	36.5	40.0
11	20.5 *t*	20.2 *t*	21.6 *t*	69.2	20.2	20.2
12	31.7 *t*	30.8 *t*	32.6 *t*	41.5	30.8	30.6
13	47.1 *s*	46.8 *s*	48.7 *s*	47.2	46.8	48.9
14	83.2 *s*	82.9 *s*	85.3 *s*	83.4	82.9	83.1
15	30.6 *t*	30.2 *t*	31.8 *t*	31.2	30.3	30.1
16	20.3 *t*	20.0 *t*	21.6 *t*	20.6	20.8	20.5
17	50.4 *d*	49.9 *d*	50.6 *d*	49.0	48.5	48.2
18	17.4 *q*	17.0 *q*	18.1 *q*	17.5	16.0	17.9
19	24.6 *q*	23.8 *q*	24.5 *q*	24.1	23.8	22.3
20	76.0 *s*	75.6 *s*	78.0 *s*	76.5	75.5	83.6
21	21.8 *q*	20.9 *q*	21.1 *q*	21.2	17.0	22.5
22	76.2 *d*	76.0 *d*	78.5 *d*	76.6	74.8	81.1
23	31.1 *t*	26.0 *t*	27.4 *t*	26.4	28.7	26.8
24	48.9 *d*	41.3 *t*	42.4 *t*	41.6	37.5	40.1
25	69.0 *s*	68.6 *s*	71.4 *s*	69.3	73.9	69.1
26	30.4 *q*	28.9 *q*	29.8 *q*	29.2	28.9	27.4
27	29.2 *q*	29.9 *q*	29.0 *q*	30.1	31.8	27.2
28	63.3 *t*	–	–	–	20.0	–
29	–	–	–	–	19.3	–
						Ketal gr.
						105.8
						26.9
						25.1

20-hydroxyecdysone **11**. The EIMS of **10** gave prominent peaks at *m*/*z* 191, 173, 147 and 129 which was consistent with a side-chain similar to that of 11 but with an extra hydroxymethyl group. This was confirmed from ^1H and ^{13}C NMR spectra (Tables 1 and 2), ^1H–^1H COSY and HMQC spectra and their comparison with the corresponding data of **11**. Analysis of these data showed that 10 possessed five methyl groups, one α,β-unsaturated ketone, three oxygenated quaternary carbons, three oxymethines, one oxymethylene, in addition to seven methylene, four methines and two quaternary carbons. The ^1H and ^{13}C NMR data of **10** are quite similar to those of **11**, except for the presence of an additional hydroxymethyl group in the side chain [δ_H 3.30 (1H, *dd*, $J = 11$, 3.2 Hz) and 3.37 (1H, *dd*, $J = 11$, 4.8 Hz); δ_C 63.3 (*t*)]. The splitting pattern of the H-22 (δ_H 3.15, *br.d*) and H-23$_a$ (δ_H 1.45, *ddd*) signals together with the significant downfield shift of C-24 compared to 11, suggested the attachment of the hydroxymethyl group at C-24 rather than C-23. The assignment of a C-24 hydroxymethyl group was confirmed from ^1H–^1H correlation (COSY 90–90) which revealed the connectivities of H-24 (δ_H 1.75) to H-23 (δ_H 1.45, 1.57) and H-28 (δ_H 3.30, 3.37) and from ^1H–^{13}C correlations (HMQC, ^1J and ^2J) of H-24 (δ_H 1.75) to C-24 (δ_C 48.9), C-23 (δ_C 31.2) and C-25 (δ_C 69.0).

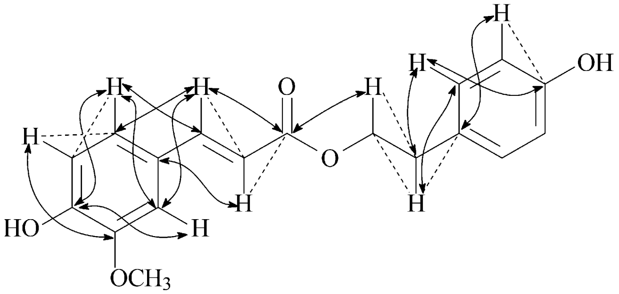


$$\begin{array}{l} \overset{1}{}\ CH_2.O.\overset{O}{\overset{\|}{C}}.\overset{2'}{C}H_2(CH_2)_{17}\overset{20'}{C}H_3 \\ \quad | \\ \overset{2}{}\ CH.OH \\ \quad | \\ \overset{3}{}\ CH_2.OH \end{array}$$

Compound (3)



Compound (5)



HMQC of compound 5

2 Bond ------

3 bond ←→

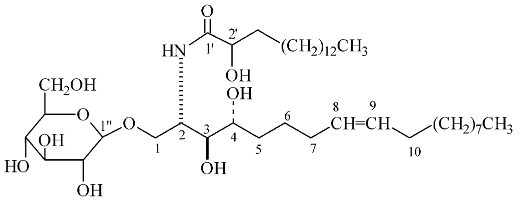
These results suggested that **10** was 20-hydroxy, 24-hydroxymethyl ecdysone, a new phytoecdysteroid.

3. Experimental

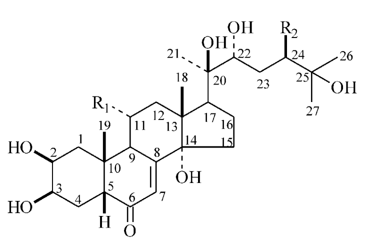
3.1. General

Melting points were determined on an electrothermal digital instrument. The IR spectra were measured on a Shimadzu 470 spectrophotometer.

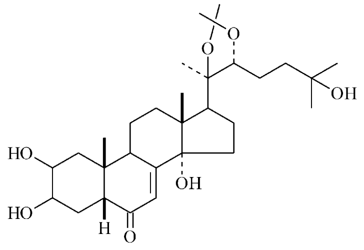
^1H NMR and ^{13}C NMR were recorded on Varian XL-300 spectrophotometer, at 300 and 75 MHz, respectively, with TMS as an internal standard. EIMS were recorded with a Hewlett Packard 5989A mass spectrometer equipped with a direct insertion probe using an ion source temperature of 250 °C, while FABMS were obtained on Jeol JMS 600 mass spectrophotometer.



Compound (9)



	R_1	R_2
Compound (10)	H	CH_2OH
Compound (11)	H	H
Compound (12)	OH	H
Compound (13)	H	C_2H_5



Compound (14)

Flash column chromatography was performed on silica gel 60 (Baker, 40 μm). Spinning disc chromatography was carried out using a model 7924T Chromatotron, Harrison Research, Palo Alto and 2 mm silica gel coated plates under a nitrogen flow rate of 15 ml/min and a solvent flow rate of 4 ml/min with a FMI lab pump (model RP-G-150). TLC was carried out with pre-coated Kiesel gel 60 F_{254} plates (Merck). Spots on TLC were visualized under UV light and by spraying with phosphomolybdic acid in methanol (saturated solution) or methanolic sulphuric acid (10%) reagents followed by heating at 110 °C.

3.2. Plant material

The aerial parts of *S. spinosa* were collected from the Valley of the River Nile, Assiut Governorate in June 1997. The plant was kindly identified by Professor Dr. Abdel-Aziz Fayed, Professor of Taxonomy, Assiut University. A voucher specimen is maintained at the Pharmacognosy Dept., Assiut University.

3.3. Extraction and isolation

About 5 kg of the air-dried aerial parts of *S. spinosa* L. was exhaustively extracted with methanol (25 l) by percolation. The extract was concentrated under reduced pressure to 1 and 2.5 l of water was added. The solution was successively partitioned with n-C_6H_{14}, $CHCl_3$, EtOAc and finally with n-BuOH and each fraction was concentrated to dryness under reduced pressure. The chloroform-soluble fraction (32 g) was flash chromatographed on 1 kg of silica gel by successive elution with an n-C_6H_{14}–$CHCl_3$ and then with a $CHCl_3$–MeOH gradient up to 30% MeOH. Twenty pooled fractions were monitored using TLC developed with three systems separately [n-C_6H_{14}–$CHCl_3$ (4:1), $CHCl_3$–MeOH (98:2) and $CHCl_3$–MeOH (95:5)]. Fraction 5 (150 mg) eluted with n-C_6H_{14}–$CHCl_3$ (9:1) to give **1** [20 mg; R_f: 0.62, solvent system $CHCl_3$–$(CH_3)_2CO$ (9:1)]. Fraction 17 (500 mg), eluted with $CHCl_3$–MeOH (97:3), was rechromatographed on the Chromatotron and eluted with a $CHCl_3$ and $(CH_3)_2CO$ mixture to yield compounds **2** [28 mg, R_f: 0.51, solvent system $CHCl_3$–$(CH_3)_2CO$ (9:1)] and **3** [45 mg; R_f: 0.75, solvent system $CHCl_3$–MeOH (9:1)]. Fraction 18 (2 g), eluting with $CHCl_3$–MeOH (95:5) was rechromatographed on a flash column with $CHCl_3$–MeOH gradient up to 20% MeOH. Two pure compounds were isolated; compound 4 [15 mg; R_f: 0.69, solvent system $CHCl_3$–MeOH (9:1)] and compound 5 [100 mg; R_f: 0.57, solvent system $CHCl_3$–MeOH (9:1)].

The ethyl acetate-soluble fraction (40 g) was separated by flash CC on 1.2 kg silica gel with a $CHCl_3$–MeOH gradient up to 50% MeOH. Three main fractions were selected by TLC examination of the eluate using solvent systems $CHCl_3$–MeOH (5:1) and $CHCl_3$–MeOH–H_2O (4:1:0.1). The first fraction (150 mg), eluted by $CHCl_3$–MeOH (4:1) was repeatedly chromatographed on a Chromatotron with $CHCl_3$–MeOH (4:1) to yield compound **6** [23 mg; R_f: 0.52, solvent system $CHCl_3$–MeOH (8:1)] and compound **7** [20 mg; R_f: 0.50, solvent system $CHCl_3$–MeOH (8:1)]. The second fraction (8 g) was eluted by $CHCl_3$–MeOH (4:1 to 3:1), rechromatographed by flash CC (silica gel, 300 g) and eluted with a $CHCl_3$–MeOH gradient to yield compounds **8** [300 mg; R_f: 0.70, solvent system $CHCl_3$–MeOH (4:1)], **9** [24 mg; R_f: 0.67, solvent system $CHCl_3$–MeOH (4:1)], **10** [45 mg; R_f: 0.50, solvent system $CHCl_3$–MeOH (4:1)] and **11** as a major compound [2 g; R_f: 0.47, solvent system $CHCl_3$–MeOH (4:1)]. The third fraction (285 mg) was eluted by $CHCl_3$–MeOH (2:1), rechromatographed by flash CC (silica gel, 15 g) using a $CHCl_3$-MeOH gradient up to 50% MeOH. Three pure compounds were obtained; compound **12** [25 mg; R_f: 0.52, solvent system $CHCl_3$–MeOH (3:1)], compound **13** [10 mg; R_f: 0.47, solvent system $CHCl_3$–MeOH (3:1)] and finally compound **14** [8 mg; R_f: 0.39, solvent system $CHCl_3$–MeOH (3:1)].

3.3.1. *Triacontane (**1**)*

$C_{30}H_{62}$, light yellow semisolid. EIMS: *m/z* (rel. int.): 422 $[M]^+$ (5), 408 (3), 232 (17), 218 (11), 98 (39), 71 (100) (Buckingham and Donaghy, 1982).

3.3.2. *1-Eicosene (**2**)*

White wax. EIMS *m/z* (rel. int.): 280 $[M]^+$ (2), 179 (6), 156 (4), 149 (10), 99 (10), 85 (27), 71 (44), 57 (100) (Buckingham and Donaghy, 1982).

3.3.3. *Glyceryl-1-eicosanoate (**3**)*

White amorphous powder. EIMS *m/z* (rel. int.): 386 $[M]^+$ (4) for $C_{23}H_{46}O_4$, 371 (4), 330 (6), 315 (7), 267 (3), 181 (100), 167 (6), 153 (32), 151 (13), 149 (13), 125 (46), 119 (10), 111 (27), 97 (34), 84 (83), 71 (71). ^{1}H NMR ($CDCl_3$) δ: 4.23 (1H, *dd*, *J* = 10.5, 4.4 Hz, H-1$_a$), 4.15 (1H, *dd*, *J* = 10.5, 6 Hz, H-1$_b$), 3.89 (1H, *m*, H-2), 3.70 (1H, *dd*, *J* = 10.6, 4.2 Hz, H-3$_a$), 3.62 (1H, *dd*, J = 10.6, 6 Hz, H-3$_b$), 2.32 (2H, *t*, *J* = 7.5 Hz, H-2′), 1.71 (1H, *m*, H-3′), 1.29 (32H, *br*., H-(4′-19′)), 0.87 (3H, *t*, *J* = 8 Hz, CH_3-20′). ^{13}C NMR ($CDCl_3$) δ: 174.6 (C-1′), 70.5 (C-2), 65.2 (C-1), 63.4 (C-3), 34.1 (C-2′), 29.4-33.7 (C-4′-18′)), 26.7 (C-3′), 22.7 (C-19′), 14.2 (C-20′).

3.3.4. *9-Hydroxy-cis-11-octadecenoic acid (**4**)*

Light yellow semisolid. EIMS *m/z* (rel. int.): 298 $[M]^+$ (12) for $C_{18}H_{34}O_3$, 227 $[M–C_5H_{11}]^+$ (4), 173 (20), 141 (100). ^{1}H NMR ($CDCl_3$) δ: 5.38 (2H,–CH=CH–), 3.30 (1H, *br*., C$\underline{H}$OH), 2.72 (1H, CH–O$\underline{H}$), 2.22 (4H, m,–CH_2–CH=CH–CH_2–), 2.17 (2H, t,–CH_2–COOH), 1.30 (*br.s*, CH_2), 0.85 (3H, *t*, CH_3). ^{13}C NMR ($CDCl_3$) δ: 178.0 (C=O), 127.1 (C-11), 113.7 (C-12), 70.5 (C-9), 33.8 (C-3), 29.0-31.9 (CH_2 residue), 24.7 (C-3), 22.7 (C-17), 14.1 (C-18). EIMS and ^{1}H NMR data are in agreement with literature (Ahmed et al., 1980).

3.3.5. *p-Hydroxyphenethyl trans-ferulate (**5**)*

Colourless needle crystals, mp 168–169 °C (Lit. 165–166 °C). EIMS *m/z* (rel. int.): 314 $[M]^+$ (10), 194 (35), 177 (100), 145 (52), 120 (30), 117 (21). ^{1}H NMR (CD_3OD) δ: 7.44 (1H, *d*, *J* = 15.4 Hz, H-7), 7.11 (1H, *d*, *J* = 1.9 Hz, H-2), 7.05 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 7.05 (1H, *dd*, *J* = 8.3, 1.9 Hz, H-6), 6.80 (1H, *d*, *J* = 8.1 Hz, H-5), 6.72 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 6.40 (1H, *d*, *J* = 15.7 Hz, H-8), 3.87 (3H, *s*, OCH_3), 3.45 (2H, *t*, *J* = 6.5 Hz, H-1″), 2.75 (2H, *t*, *J* = 6.6 Hz, H-2″). ^{13}C NMR (CD_3OD) δ: 169.1 (C-9), 156.9 (C-4′), 149.8 (C-4), 149.2 (C-3), 142.0 (C-7), 131.2 (C-1′), 130.7 (C-2′), 128.2 (C-1), 123.2 (C-6), 118.7 (C-8), 116.4 (C-5), 116.3 (C-3′), 111.5 (C-2), 56.3 (OCH_3), 42.5 (C-1″), 35.8 (C-2″).

3.3.6. *3β,6α,23ε-Trihydroxy-6α-cholest-9(11)-ene (**6**)*

White needles, mp 242–244 °C (Lit. 240–243 °C). EIMS *m/z* (rel. int.): 418 $[M]^+$ for $C_{27}H_{46}O_3$ (99), 287 (2), 211 (15), 208 (11), 205 (9), 193 (23), 181 (100), 167 (53), 161 (29), 154 (19), 131 (8). EIMS and ^{1}H NMR data are in agreement with literature values (Ikegami et al., 1972).

3.3.7. *1-O-Linoloyl-3-O-β-D-galactopyranosyl-syn-glycerol (**7**)*

White gum. EIMS *m/z* (rel. int.): 354 $[M]^+$ (12), 337 (2), 280 (1), 279 (3), 261 (2), 171 (14), 167 (13), 149 (21), 135 (11), 129 (10), 115 (14), 105 (26), 98 (100). EIMS, NMR data are in agreement with literature values (Hohmann et al., 1996).

3.3.8. *β-Sitosterol-3-O-β-D-glucopyranoside (**8**)*

Fine needle crystals, m.p. 275–277 °C (Paulo et al., 2000).

3.3.9. *1-O-β-D-Glucopyranosyl-(2S,3S,4R,8Z)-2-[(2′R)-2′-hydroxypalmito-ylamino]-8-octadecene-1,3,4′-triol (**9**)*

White amorphous powder. FABMS: 732 $[M+H]^+$ (4). EIMS *m/z* (rel. int.): 570 $[M+H-162]^+$ (2), 553 $[M+H-179]^+$ (2), 479 (8), 447 (5), 354 (12), 315 (8), 298 (3), 279 (8), 263 (7), 255 (8), 227 (10), 197 (10), 41 (100). ^{1}H NMR (DMSO) δ: 7.56 (1H, *d*, *J* = 9.5 Hz,–NH), 5.33 (2H, *t*-like signal, H-8, H-9), 5.28 (1H, *m*, H-2), 4.57 (1H, *m*, H-1), 4.13 (1H, *d*, *J* = 7.6 Hz, H-1″), 2.06 (2H, *m*, H-7), 1.94 (2H, *m*, H-10), 1.17 (*br.s*, CH_2 residue), 0.82 (6H, *t*, *J* = 6.8 Hz, CH_3x2). ^{13}C NMR (DMSO) δ: 174.3 (C-1′), 130.2 (C-8), 129.9 (C-9), 104.1 (C-1″), 77.4 (C-3′), 77.1 (C-5″), 76.8 (C-3), 74.0 (C-2″), 71.5 (C-2′), 71.1 (C-4), 70.5 (C-4″), 69.9 (C-1), 62.0 (C-6″), 51.5 (C-2), 34.9 (C-3′), 29.2–32.7 (CH_2 residue), 27.5 (C-10), 27.2 (C-7), 26.2 (CH_2), 26.0 (CH_2), 25.0 (CH_2), 22.7 (CH_2), 14.5 (CH_3) (Kang et al., 2001).

3.3.10. *20-Hydroxy,24-hydroxymethyl ecdysone (**10**)*

Faint yellow amorphous solid. IR ν_{max}^{KBr}: 3505–3375 (OH), 1662 (C=O), 1033 cm^{-1}. EIMS *m/z* (rel. int.): 460 $[M-51]^+$ (2), 446 (3), 363 (37), 345 (49), 327 (27), 301 (15), 269 (17), 250 (49), 231 (16), 191 (13), 183 (11), 173 (27), 165 (9), 147 (28), 129 (18), 81 (100). FABMS: 511 $[M+H]^+$. ^{1}H NMR and ^{13}C NMR in Tables 1 and 2.

3.3.11. *20-Hydroxyecdysone (**11**)*

Yellowish-white amorphous solid. EIMS *m/z* (rel. int.): 446 $[M–2H_2O]^+$ (38), 363 (38), 345 (70), 327 (38), 81 (100). FABMS: 481 $[M+H]^+$. ^{1}H NMR and ^{13}C NMR in Tables 1 and 2 (Suksamrarn and Sommechai, 1993; Vokac et al., 1998).

3.3.12. *Turkesterone (**12**)*

Faint yellow amorphous solid. EIMS *m/z* (rel. int.): 463 $[M+H–2H_2O]^+$ (6), 443 (6), 442 (6), 423 (18), 379 (18), 368 (16), 354 (10), 361 (6), 343 (5), 71 (100). ^{1}H NMR and ^{13}C NMR data in Tables 1 and 2 (Werawattanametin et al., 1986; Vokac et al., 1998).

3.3.13. *Makisterone C (**13**)*

White powder. EIMS m/z (rel. int.): 481 $[M-27]^+$ (1), 463 (1), 455 (2), 363 (63), 345 (100), 327 (62), 189 (11), 171 (14), 145 (22). ^1H NMR and ^{13}C NMR data in Tables 1 and 2 (Girault et al., 1988; Roth et al., 1995).

3.3.14. *20-Hydroxyecdysone-20,22-monoacetonide (**14**)*

White amorphous powder. EIMS m/z (rel. int.): 486 $[M-2H_2O]^+$ (2), 426 (10), 396 (23), 363 (15), 345 (23), 328 (24). ^1H NMR and ^{13}C NMR in Tables 1 and 2 (Zhang et al., 1992; Pis et al., 1994).

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