New Triterpenoid and Ergostane Glycosides from the Leaves of *Hydrocotyle umbellata* L.

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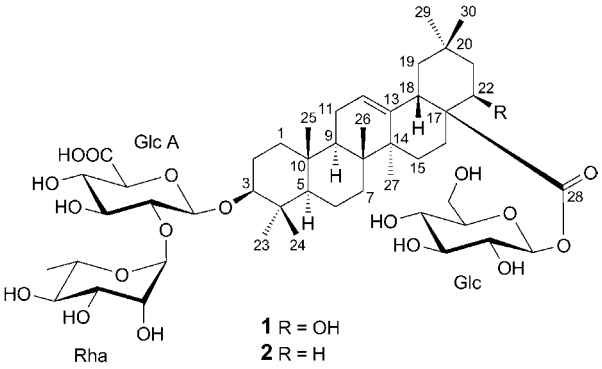
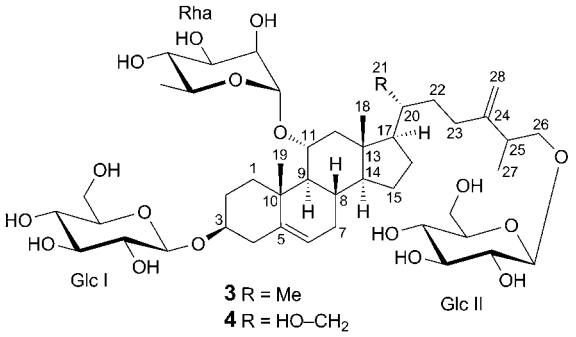
Two new triterpenoid glycosides, together with two new ergostane glycosides, umbellatosides A – D (**1** – **4**, resp.), have been isolated from the leaves of *Hydrocotyle umbellata* L. Their structures were established by 2D-NMR spectroscopic techniques (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, and HMBC) and mass spectrometry as 3β,22β-dihydroxy-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**), 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**2**), (3β,11α,26)-ergosta-5,24(28)-diene-3,11,26-triol 3-*O*-(β-D-glucopyranosyl)-11-*O*-(α-L-rhamnopyranosyl)-26-*O*-β-D-glucopyranoside (**3**), and (3β,11α,21,26)-ergosta-5,24(28)-diene-3,11,21,26-tetrol 3-*O*-(β-D-glucopyranosyl)-11-*O*-(α-L-rhamnopyranosyl)-26-*O*-β-D-glucopyranoside (**4**).

Introduction. – The genus *Hydrocotyle* (Apiaceae) is constituted by aquatic or semi-aquatic plants particularly well represented in temperate areas and higher tropical regions [1]. Some species were reported to have antioxidant and antitumoral properties [2][3]. Triterpene saponins were isolated from several species of this genus [4 – 7]. From *H. umbellata*, a herbaceous plant native to North America and parts of South America, this compound type has not been reported so far. In our continuing search for bioactive saponins from tropical plants, we report here the isolation and structure elucidation of two new triterpene saponins and two new ergostane glycosides, named umbellatosides A – D (**1** – **4**, resp.), from the 70% MeOH extract of the leaves of *H. umbellata*.

Results and Discussion. – The BuOH fraction of the MeOH/H_2O 70 : 30 extract of the leaves of *H. umbellata* was submitted to multiple chromatographic steps involving vacuum-liquid chromatography (VLC) on reversed-phase *RP18* silica gel, and medium-pressure liquid chromatography (MPLC) on normal silica gel and *RP18* silica gel to yield umbellatosides A – D (**1** – **4**, resp.). Their structures were determined mainly by spectroscopic methods including 600-MHz 1D- and 2D-NMR experiments (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, and HMBC) in combination with HR-ESI-MS and FAB-MS. Compounds **1** – **4** were isolated as amorphous powders.





Umbellatoside A (**1**) exhibited in the HR-ESI-MS (positive-ion mode) a *pseudo*-molecular-ion peak at *m/z* 979.4883 ($[M + Na]^+$) consistent with the molecular formula $C_{48}H_{76}O_{19}$. The FAB-MS of **1** (negative-ion mode) displayed a *pseudo*-molecular-ion peak at *m/z* 955 ($[M - H]^-$), followed by fragment-ion peaks at *m/z* 793 ($[M - H - 162]^-$), 647 ($[M - H - 162 - 146]^-$), 633 ($[M - H - 146 - 176]^-$), and 471 ($[M - H - 162 - 146 - 176]^-$) suggesting the elimination of one hexosyl, one desoxy-hexosyl, and one hexosyluronic moiety, respectively. The aglycone of **1** was identified as 3*β*,22*β*-dihydroxyolean-12-en-28-oic acid by comparison of its NMR spectral data, based on correlations observed in the ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC spectra, with those reported in the literature [8].

The ^{1}H- and ^{13}C-NMR spectra of **1** exhibited seven characteristic Me signals at *δ*(H) 0.87 (*s*, Me(25)), 0.95 (*s*, Me(24)), 1.01 (*s*, Me(29)), 1.06 (*s*, Me(30)), 1.12 (*s*, Me(26)), 1.23 (*s*, Me(23)), and 1.84 (*s*, Me(27)), and six quaternary C-atom resonances at *δ*(C) 30.8 (C(20)), 37.0 (C(10)), 39.5 (C(4)), 40.1 (C(8)), 42.0 (C(14)), and 49.1 (C(17)), indicating the presence of a triterpenoid skeleton (see *Table 1*). Most of the ^{1}H- and ^{13}C-NMR signals assigned from extensive analysis of 2D-NMR spectra were superimposable with those of 3*β*-hydroxyolean-12-en-28-oic acid except the presence of an O-bearing moiety with a signal at *δ*(H) 5.29 and *δ*(C) 74.4. The deshielded H-atom at *δ*(H) 5.29 had a correlation with *δ*(H) 1.78 (H_a–C(21)) and 2.53 (H_b–C(21)) in the ^{1}H,^{1}H-COSY spectrum, indicating a OH group at C(22), and the HMBC between

δ(H) 5.29 (H–C(22)) and δ(C) 41.3 (C(18)) was also observed. The α-equatorial orientation of the H-atom at C(22) was deduced from the ROESY cross-peaks δ(H) 5.29 (H–C(22))/δ(H) 2.40 (H_b–C(16)), and δ(H) 2.40 (H_b–C(16))/δ(H) 1.01 (Me(29)) indicating the β-axial orientation for OH at C(22). This was supported by the absence of ROESY cross-peaks between δ(H) 5.29 (H–C(22)) and δ(H) 1.06 (Me(30)), and between δ(H) 5.29 (H–C(22)) and δ(H) 3.52 (H–C(18)). These data allowed us to characterize the aglycone of **1** as 3β,22β-dihydroxyolean-12-en-28-oic acid [8]. Compound **1** was shown to contain three sugar residues on the basis of the HSQC spectrum. The three anomeric H-atom NMR signals at δ(H) 4.78 (*d*, $J = 7.0$), 6.27 (*s*), and 6.30 (*d*, $J = 8.0$) correlated with three anomeric C-atom signals at δ(C) 106.5, 102.3, and 95.8, respectively. Complete assignments of sugar H-atoms were achieved by TOCSY and ^{1}H,^{1}H-COSY plots, while the C-atoms were assigned from HSQC and HMBC spectra. Evaluation of spin-spin couplings and chemical shifts obtained from the 2D-NMR spectra allowed the identification of one α-rhamnopyranosyl (Rha), one β-glucopyranosyl (Glc), and one β-glucopyranosyluronic acid (Glc A) unit [9]. The relatively large ^3J(1,2) value (7.0–8.0 Hz) of the anomeric H-atom signals of Glc and GlcA indicated a β-anomeric orientation. The large ^1J(H–C(1),C(1)) value of the Rha (167 Hz) confirmed that the anomeric H-atom was equatorial, indicating an α-pyranoid anomeric form. The common D-configuration for Glc and Glc A, and the L-configuration for Rha were determined by acid hydrolysis and GC analysis (see *Exper. Part*). Analysis of HMBC connectivities between sugar residues and the aglycone indicated that **1** was a bisdesmoside. The cross-peak in the HMBC spectrum between δ(H) 4.78 (Glc A H–C(1)) and δ(C) 89.2 (Agly C(3)) showed that the β-glucuronopyranosyl moiety was linked to the aglycone at C(3). Additional HMBCs were observed between δ(H) 6.27 (br. *s*, Rha H–C(1)) and δ(C) 79.3 (Glc A C(2)) establishing the sequence at C(3) as α-L-rhamnopyranosyl-(1 → 2)-β-D-glucuronopyranosyl. The remaining sugar (terminal Glc) is linked to the aglycone by an ester linkage at C(28), ascertained by the HSQC at δ(H)/δ(C) 6.30/95.8 and confirmed by the HMBC δ(H) 6.30 (*d*, $J = 8.0$, Glc H–C(1))/δ(C) 175.9 (Agly C(28)). All these data were consistent with the structure of 1-*O*-[(3β,22β)-3-{[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranuronosyl]oxy}-22-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose for **1**, named umbellatoside A.

The HR-ESI-MS of umbellatoside B (**2**) showed a *quasi*-molecular-ion peak at *m*/*z* 963.4924 ($[M + Na]^+$) consistent with the molecular formula $C_{48}H_{76}O_{18}$. The FAB-MS (negative-ion mode) of **2** displayed a *pseudo*-molecular ion peak at *m*/*z* 939 ($[M - H]^-$), and fragment-ion peaks at *m*/*z* 777 ($[M - H - 162]^-$), 631 ($[M - H - 162 - 146]^-$), 617 ($[M - H - 146 - 176]^-$), and 455 ($[M - H - 162 - 146 - 176]^-$), suggesting the elimination of one hexosyl, one desoxyhexosyl, and one hexosyluronic acid moiety, respectively. The aglycone of **2** was identified as oleanolic acid by comparison of its NMR spectral data, based on correlations observed in the ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC spectra, with those reported in the literature (*Table 1*) [10].

Most of the H- and C-atom NMR signals of **2** (*Table 1*) were superimposable with those of **1**, except the signals at C(22). The absence of a signal at δ(H) 5.29/δ(C) 74.4 observed in **1** (C(22)) and the presence of a correlation in the HSQC spectrum δ(H) 1.76, 1.84/δ(C) 32.5 in **2** confirmed the presence of a CH_2(22) group instead of a secondary alcoholic function. The absolute configuration of the sugars was determined

Table 1. *^{1}H- and ^{13}C-NMR Data* ((D$_5$)Pyridine) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	0.88[a])	38.7	0.84, 1.37	38.6
CH_2(2)	1.80, 2.24	26.5	1.79, 2.22–2.28 (*m*)	26.0
H–C(3)	3.33 (br. *d*, *J* = 9.7)	89.2	3.33 (br. *d*, *J* = 8.53)	89.2
C(4)	–	39.5	–	39.4
H–C(5)	0.80 (br. *d*, *J* = 11.3)	55.8	0.75 (br. *d*, *J* = 11.3)	55.8
CH_2(6)	1.25–1.32 (*m*), 1.47 (br. *d*, *J* = 11.7)	18.5	1.28, 1.46	18.5
CH_2(7)	1.38–1.42 (*m*), 1.56–1.63 (*m*)	33.4	1.34, 1.48 (br. *d*, *J* = 5.6)	33.1
C(8)	–	40.1	–	39.9
H–C(9)	1.78	47.0	1.59 (*t*, *J* = 9.3)	48.0
C(10)	–	37.0	–	36.9
CH_2(11)	1.94–2.00 (*m*)	23.8	1.97 (br. *d*, *J* = 12.1), 2.10 (*t*, *J* = 13.0)	23.8
H–C(12)	5.61 (br. *s*)	122.7	5.44 (br. *s*)	122.9
C(13)	–	144.4	–	144.1
C(14)	–	42.0	–	42.1
CH_2(15)	1.28, 2.43	35.9	1.18 (br. *d*, *J* = 12.9), 2.32–2.37 (*m*)	28.0
CH_2(16)	2.09–2.15 (*m*), 2.36–2.41 (*m*)	32.2	1.85–1.92 (*m*)	24.0
C(17)	–	49.1	–	47.0
H–C(18)	3.52 (*dd*, *J* = 13.1, 4.0)	41.3	3.20 (*dd*, *J* = 13.5, 3.8)	41.7
CH_2(19)	1.38–1.41 (*m*), 2.82 (*t*, *J* = 12.9)	47.0	1.29, 1.78	46.8
C(20)	–	30.8	–	30.8
CH_2(21)	1.78, 2.53 (br. *d*, *J* = 12.1)	36.1	1.12, 1.37	34.0
H–C(22) or CH_2(22)	5.29 (br. *s*)	74.4	1.76, 1.84	32.5
Me(23)	1.23 (*s*)	28.2	1.26 (*s*)	28.2
Me(24)	0.95 (*s*)	17.0	0.96 (*s*)	16.9
Me(25)	0.87 (*s*)	15.7	0.84 (*s*)	15.5
Me(26)	1.12 (*s*)	17.6	1.08 (*s*)	17.5
Me(27)	1.84 (*s*)	27.2	1.30 (*s*)	26.1
C(28)	–	175.9	–	176.4
Me(29)	1.01 (*s*)	33.2	0.95 (*s*)	33.1
Me(30)	1.06 (*s*)	24.6	0.92 (*s*)	23.7
Glc A				
H–C(1)	4.78 (*d*, *J* = 7.0)	106.5	4.77 (*d*, *J* = 7.0)	106.5
H–C(2)	4.02	79.3	4.01–4.04 (*m*)	79.2
H–C(3)	4.00	75.9	3.99	75.9
H–C(4)	4.28	71.2	4.29	71.2
H–C(5)	4.24	78.8	4.27	78.9
C(6)	–	170.0	–	170.0
Glc				
H–C(1)	6.30 (*d*, *J* = 8.0)	95.8	6.30 (*d*, *J* = 8.1)	95.7
H–C(2)	4.13 (*t*, *J* = 8.1)	74.1	4.18 (*t*, *J* = 8.5)	74.1
H–C(3)	4.28	76.6	4.26	76.5
H–C(4)	4.17–4.22 (*m*)	72.2	4.18	72.2
H–C(5)	3.99	75.8	3.99	75.8
CH_2(6)	4.37 (*dd*, *J* = 11.7, 4.4), 4.43 (br. *d*, *J* = 9.7)	62.3	4.38 (*dd*, *J* = 12.0, 4.2), 4.44 (br. *d*, *J* = 9.7)	62.3

Table 1 (cont.)

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
Rha				
H–C(1)	6.27 (br. *s*)	102.3	6.27 (br. *s*)	102.3
H–C(2)	4.70 (br. *s*)	72.5	4.70 (br. *d*)	72.5
H–C(3)	4.58 (br. *d*, $J = 8.1$)	72.7	4.60 (br. *d*, $J = 8.1$)	72.7
H–C(4)	4.26	74.3	4.26	74.3
H–C(5)	5.04	69.5	5.00 – 5.06 (*m*)	69.5
Me(6)	1.70 (*d*, $J = 4.8$)	18.7	1.69 (*d*, $J = 5.6$)	18.7

[a]) Overlapped signals are reported without multiplicity.

as described above (see also the *Exper. Part*). On the basis of these results, the structure of **2** was elucidated as 1-*O*-[(3β)-3-{[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranuronosyl]oxy}-28-oxoolean-12-en-28-yl]-β-D-glucopyranose, named umbellatoside B.

Umbellatoside C (**3**) exhibited in the HR-ESI-MS (positive-ion mode) the $[M + Na]^+$ peak at *m*/*z* 923.4975 consistent with the molecular formula $C_{46}H_{76}O_{17}$. FAB-MS (negative-ion mode) of **3** displayed a *pseudo*-molecular-ion peak at *m*/*z* 899 ($[M - H]^-$), followed by fragment-ion peaks at *m*/*z* 753 ($[M - H - 146]^-$), 737 ($[M - H - 162]^-$), and 575 ($[M - H - 162 - 162]^-$), suggesting the elimination of two hexosyl and one desoxyhexosyl moiety, respectively. The basic skeleton of **3** was determined as ergostane by comparison of its NMR spectral data, based on correlations observed in the ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC spectra, with those reported in the literature [11].

The HSQC spectrum of the aglycone moiety of **3** (*Table 2*) displayed four Me signals at δ(H) 0.53 (*s*)/δ(C) 12.8 (Me(18)), δ(H) 0.84 (*d*, $J = 6.5$)/δ(C) 18.8 (Me(21)), δ(H) 1.01 (*s*)/δ(C) 12.3 (Me(19)), δ(H) 1.27 (*d*, $J = 6.9$)/δ(C) 17.6 (Me(27)), and an olefinic signal at δ(H) 5.26/δ(C) 120.3. Signals for two secondary alcoholic functions at δ(H) 3.96/δ(C) 76.4 and δ(H) 4.20/δ(C) 75.8, of one primary alcoholic function at δ(H) 3.59 and 4.28/δ(C) 74.6, four quaternary C-atom signals at δ(C) 35.6 (C(10)), 42.8 (C(13)), 152.9 (C(24)), and 136.9 (C(5)), and characteristic signals of an alkene CH_2 moiety (δ(H) 4.91 and 4.94/δ(C) 109.0) were also observed. These signals suggested that the aglycone part of **3** was closely related to a methylidenecholesterol (ergosta-5,24(28)-dien-3β-ol) [12][13] with two additional OH functions. In the tetracyclic system of the aglycone part, the presence of a secondary alcoholic function at C(11) was deduced by correlations between the CH_2 signal at δ(H) 1.32 and 2.65 (CH_2(12)), the CH H-atom signals at δ(H) 1.98 (H–C(9)) and δ(C) 75.8 (C(11)) observed in the HMBC spectrum of **3** (*Fig.*). The β-axial orientation of the H-atom at C(11) was deduced from the ROESY cross-peaks δ(H) 0.53 (*s*, Me(18))/4.20 (H–C(11)), δ(H) 1.01 (H–C(19))/4.20 (H–C(11)), δ(H) 2.65 (H_β–C(12))/4.20 (H–C(11)), indicating the α-orientation of HO at C(11). In the side chain, the presence of a primary alcoholic function at C(26) was deduced from the correlations observed in the HMBC spectrum between δ(H) 3.59 (H_a–C(26)) and δ(C) 40.4 (C(25)), 152.9 (C(24)), and 17.6 (C(27)),

Table 2. *^{1}H- and ^{13}C-NMR Data* ((D_5)Pyridine) *of* **3** *and* **4**. δ in ppm, J in Hz.

	3		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.16[a]), 2.83–2.89 (*m*)	38.7	1.12–1.18 (*m*), 2.83	38.7
CH_2(2)	1.73–1.76 (*m*), 2.04	29.9	1.68–1.72 (*m*), 2.03	29.9
H–C(3)	3.96	76.4	3.96	76.5
CH_2(4)	1.44 (*d*, *J* = 11.7), 2.04	35.1	1.44, 2.04	35.2
C(5)	–	136.9	–	137.1
H–C(6)	5.26 (br. *s*)	120.3	5.27 (br. *s*)	120.3
CH_2(7)	1.66–1.72 (*m*), 2.04	30.0	1.68–1.72 (*m*), 2.03	30.0
H–C(8)	1.26	40.8	1.23–1.29 (*m*)	40.8
H–C(9)	1.98	54.8	1.94–2.00 (*m*)	55.0
C(10)	–	35.6	–	35.7
H–C(11)	4.18–4.22 (*m*)	75.8	4.23	75.6
CH_2(12)	1.32, 2.65	45.7	1.49, 2.85	45.0
C(13)	–	42.8	–	42.8
H–C(14)	1.87–1.93 (*m*)	55.2	1.77–1.83 (*m*)	51.1
CH_2(15)	1.37–1.42 (*m*), 1.48–1.52 (*m*)	23.3	1.45, 1.52–1.56 (*m*)	23.2
CH_2(16)	1.23, 1.82–1.87 (*m*)	28.0	1.40, 1.90–1.95 (*m*)	27.4
H–C(17)	1.22	55.9	1.96	55.3
Me(18)	0.53 (*s*)	12.8	0.61 (*s*)	13.1
Me(19)	1.01 (*s*)	12.3	1.00 (*s*)	12.3
H–C(20)	1.33	35.9	1.63	43.2
Me or CH_2OH(21)	0.84 (*d*, *J* = 6.5)	18.8	3.80 (*dd*, *J* = 10.5, 5.2), 3.99	62.3
CH_2(22)	1.19, 1.55–1.57 (*m*)	34.6	2.18–2.24 (*m*), 2.32–2.38 (*m*)	35.1
CH_2(23)	1.97, 2.14–2.19 (*m*)	31.9	1.80, 2.10	32.3
C(24)	–	152.9	–	153.2
H–C(25)	2.65	40.4	2.67–2.73 (*m*)	40.5
CH_2(26)	3.59 (*t*, *J* = 8.9), 4.28	74.6	3.59 (*t*, *J* = 8.5), 4.28	74.6
Me(27)	1.27 (*d*, *J* = 6.9)	17.6	1.25 (*d*, *J* = 6.9)	17.6
CH_2(28)	4.91 (br. *s*), 4.94 (br. *s*)	109.0	4.91 (br. *s*), 4.94 (br. *s*)	108.9
Glc I				
H–C(1)	5.01 (*d*, *J* = 7.6)	101.9	5.00 (*d*, *J* = 7.5)	101.9
H–C(2)	4.04 (*t*, *J* = 8.1)	75.2	4.03 (*t*, *J* = 8.1)	75.2
H–C(3)	4.28	78.6	4.28	78.6
H–C(4)	4.26	71.8	4.26	71.8
H–C(5)	3.98	78.4	3.96	78.4
CH_2(6)	4.40 (*dd*, *J* = 11.7, 4.8), 4.57 (*dd*, *J* = 10.1, 3.0)	62.9	4.39–4.41 (*m*), 4.54–4.59 (*m*)	62.8
Glc II				
H–C(1)	4.87 (*d*, *J* = 7.6)	105.2	4.86 (*d*, *J* = 7.6)	105.2
H–C(2)	4.04 (*t*, *J* = 8.1)	75.4	4.03 (*t*, *J* = 8.1)	75.4
H–C(3)	4.28	78.6	4.22	78.5
H–C(4)	4.24	71.8	4.20	71.7
H–C(5)	3.96	78.6	3.94	78.4
CH_2(6)	4.38 (*dd*, *J* = 11.7, 4.8), 4.56 (*dd*, *J* = 10.1, 3.0)	62.9	4.36–4.40 (*m*), 4.52–4.56 (*m*)	62.9
Rha				
H–C(1)	5.55 (br. *s*)	99.5	5.60 (br. *s*)	99.3
H–C(2)	4.48 (br. *s*)	73.4	4.48 (br. *s*)	73.4
H–C(3)	4.45(*dd*, *J* = 5.6, 2.8)	72.9	4.39–4.45 (*m*)	72.8
H–C(4)	4.29	73.9	4.27	73.9
H–C(5)	4.29	71.3	4.28	71.3
Me(6)	1.65 (*d*, *J* = 5.2)	18.5	1.62 (*d*, *J* = 5.2)	18.4

[a]) Overlapped signals are reported without multiplicity.

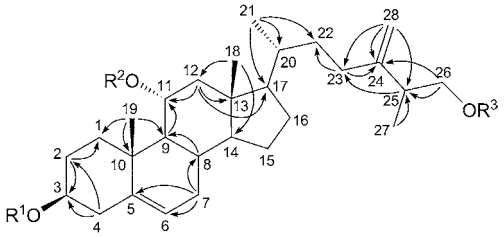


Figure. *HMBCs of the aglycone of* **3**

between δ(H) 4.28 (H_b–C(26)) and δ(C) 152.9 (C(24)) and 17.6 (C(27)), and the reverse correlations between δ(H) 1.27 (Me(27)) and δ(C) 74.6 (C(26)). Furthermore, additional HMBCs between the signals at δ(H) 0.84 (Me(21)) and δ(C) 35.9 (C(20)) and δ(C) 34.6 (C(22)), between those at δ(H) 1.97 (H_a–C(23)) and δ(C) 34.6 (C(22)), between those at δ(H) 2.16 (H_b–C(23)) and δ(C) 152.9 (C(24)), between those at δ(H) 4.91 (H_a–C(28)) and δ(C) 152.9 (C(24)), 31.9 (C(23)), and 40.4 (C(25)) confirmed the different functionalities of the side chain. Therefore, the aglycone of **3** was elucidated as ergosta-5,24(28)-diene-3β,11β,26-triol, which has, to the best of our knowledge, never been described.

The HSQC spectrum of **3** displayed three anomeric H-atom signals at δ(H) 4.87 (*d*, $J = 7.6$), 5.01 (*d*, $J = 7.6$), and 5.55 (br. *s*), which were correlated with three anomeric C-atom signals at δ(C) 105.2, 101.9, and 99.5, respectively, indicating the presence of three sugar units. The evaluation of chemical shifts and spin-spin couplings obtained from the 2D-NMR data allowed the identification of two β-glucopyranosyl units (Glc I, Glc II), and one α-rhamnopyranosyl unit (Rha) [9]. The D-configuration for Glc and L-configuration for Rha was determined by acid hydrolysis and GC analysis (see *Exper. Part*). The relatively large ^3J(1,2) values (7.6 Hz) for Glc I and Glc II indicated their β-anomeric orientation. The large ^1J (H–C(1),C(1)) values of the Rha (167 Hz) confirmed that the anomeric H-atom was equatorial indicating a α-pyranoid anomeric form. The correlation in the HMBC spectrum between δ(H) 4.87 (Glc II H–C(1)) and δ(C) 74.6 (Agly C(26)), and the reverse correlation between δ(H) 3.59 (Agly H–C(26) and δ(C) 105.2 (Glc II C(1)) established that Glc II was at C(26) of the aglycone. The HMBC between δ(H) 5.01 (Glc I H–C(1)) and δ(C) 76.4 (Agly C(3)), and the reverse correlation between δ(H) 3.96 (Agly C(3)) and δ(C) 101.9 (Glc I C(1)) evidenced that Glc I was at C(3). The HMBC between δ(H) 5.55 (Rha H–C(1)) and δ(C) 75.8 (Agly C(11)) established the position of Rha as C(11); this was confirmed by the ROESY cross-peak δ(H) 5.55 (Rha H–C(1))/δ(H) 4.20 (Agly H–C(11)).

Based on the above results, the structure of compound **3** was elucidated as (3β,11α)-11-[(6-deoxy-α-L-mannopyranosyl)oxy]-26-(β-D-glucopyranosyloxy)ergosta-5,24(28)-dien-3-yl β-D-glucopyranoside, named umbellatoside C. The presence of tridesmosidic ergostane-type saponins in the genus *Hydrocotyle* was not previously reported.

Umbellatoside D (**4**) exhibited in the HR-ESI-MS (positive-ion mode) an $[M + Na]^+$ peak at *m*/*z* 939.4935, consistent with the molecular formula $C_{46}H_{76}O_{18}$. FAB-MS (negative-ion mode) of **4** displayed a *pseudo*-molecular-ion peak at *m*/*z* 915 ($[M - H]^-$), and fragment-ion peaks at *m*/*z* 769 ($[M - H - 146]^-$), 753 ($[M - H - 162]^-$) and 591 ($[M - H - 162 - 162]^-$), suggesting the elimination of one desoxyhexosyl and two

hexosyl moieties, respectively. The comparison of the ^{1}H- and ^{13}C-NMR chemical shifts of **4** assigned from extensive 2D-NMR analysis with those of **3** (*Table 2*) revealed that **4** differed from **3** only by presence of an OH group at C(21) of the aglycone. The absence of the Me signal observed in **3** (δ(H) 0.84 (*d*, $J = 6.5$)/δ(C) 18.8 (Me (21))), and the presence of a correlation δ(H) 3.80 and 3.99/δ(C) 62.3 in the HSQC spectrum of **4** suggest the presence of a primary alcoholic function at C(21) in **4**. This proposal was confirmed from the ROESY cross-peaks of δ(H) 3.80 and 3.99 (CH_2(21))/δ(H) 1.63 (H–C(20)), and δ(H) 3.99 (H–C(21))/δ(H) 2.35 (H–C(22)). The ^{1}H- and ^{13}C-NMR data of the oligosaccharide moiety of **4**, assigned from the 2D-NMR analyses, were almost superimposable with those of **3**, indicating that **4** possessed the same glycosidic substituents, α-rhamnopyranosyl at C(11), and β-glucopyranosyl moieties at C(3) and C(26). The D-configuration for Glc and L-configuration for Rha were determined by acid hydrolysis and GC analysis (see *Exper. Part*). Thus, the compound **4** was elucidated as (3β,11α)-11-[(6-deoxy-α-L-mannopyranosyl)oxy]-26-(β-D-glucopyranosyloxy)-21-hydroxyergosta-5,24(28)-dien-3-yl β-D-glucopyranoside, named umbellatoside D.

Experimental Part

General. Medium-pressure liquid chromatography (MPLC): silica gel *60* (SiO_2, 15–40 μm; *Merck*), reversed-phase (RP) silica gel *RP-18* (25–40 μm; *Merck*), *Gilson M 305* pump; *Büchi* glass column (460 × 25, 460 × 15, and 230 × 15 mm), *Büchi* precolumn (110 × 15 mm). Vacuum-liquid chromatography (VLC): RP silica gel *RP-18* (25–40 μm; *Merck*). TLC and HPTLC: silica gel *60* F_{254} (*Merck*), solvent system: $CHCl_3$/MeOH/H_2O 60:32:6.5; detection: *Komarowsky* reagent, a 5:1 mixture of *p*-hydroxybenzaldehyde (2% in MeOH) and 50% EtOH/H_2SO_4. GC: *Thermoquest* gas chromatograph using a *DB-1701* cap. column (30 m × 0.25 mm, i.d.; *J & W Scientific*), with detection by FID; initial temp. maintained at 80° for 5 min and then raised to 270° at the rate of 15°/min; carrier gas: He. NMR Spectra: *Varian VNMR-S 600* MHz spectrometer equipped with 3-mm triple resonance inverse and 3 mm dual broadband probeheads; in 150 μl of (D_5)pyridine; solvent signals as internal standard ((D_5)pyridine: δ(H) = 7.21, δ(C) = 123.5), all spectra recorded at $T = 35°$; pulse sequences taken from *Varian* pulse sequence library (gCOSY; gHSQCAD and gHMBCAD with adiabatic pulses CRISIS-HSQC and CRISIS-HMBC); TOCSY spectra acquired using DIPSI spin-lock and 150 ms missing time; mixing time in ROESY experiments: 300 ms; carbon type (Me, CH_2, CH): DEPT experiments. FAB-MS: neg.-ion mode; *JEOL SX 102* spectrometer; glycerol as matrix; in *m*/*z*. HR-ESI-MS (pos.): *Micromass Q-TOF-1* apparatus; in *m*/*z*.

Plant Material. The leaves of *H. umbellata* L. were provided from Botanical Garden of San Juan de Lagunillas, in April 2008 (Mérida, Venezuela), and identified by Er. *Juan Carmona*, Herbarium MERF of Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes. A voucher specimen (N° 113) was deposited with the Herbarium MERF, Venezuela.

Extraction and Isolation. Powdered leaves (73 g) of *H. umbellata* were refluxed with MeOH/H_2O 70:30 (3 × 2 l) for 1 h. After evaporation of the solvent in vacuum, the resulting MeOH/H_2O extract (12.7 g) was suspended in H_2O (200 ml), and partitioned successively with CH_2Cl_2 (3 × 300 ml) and BuOH (sat. with H_2O; 3 × 200 ml), yielding, after evaporation of the solvents, the corresponding CH_2Cl_2 (1 g) and BuOH (7.69 g) fractions. The BuOH residue was submitted to VLC (*RP-18*; MeOH/H_2O 0:100, 50:50, 100:0): *Fractions VLC (1)*, *VLC (2)*, and *VLC (3)* after evaporation. *VLC (2)* (914.3 mg) was submitted to MPLC (system *A: RP-18*, MeOH/H_2O 30 → 100%): **1** (8.9 mg). *VLC (3)* (446 mg) was subjected to MPLC (system *B:* silica gel 15–40 μm, $CHCl_3$/MeOH/H_2O 60:32:6.5): *Frs. 1–18*. *Fr. 15* was submitted to MPLC (system *A*): **2** (13 mg). *Fr. 9* was purified by MPLC (system *C: RP-18*, MeOH/H_2O 40 → 100%): **3** (12.5 mg). *Fr. 11* was submitted to MPLC (system *A*): **4** (11.6 mg).

Umbellatoside A (=*1*-O-*[(3β,22β)-3-{[2*-O-*(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranurono-syl]oxy}-22-hydroxy-28-oxoolean-12-en-28-yl]-β-*D*-glucopyranose*; **1**). White amorphous powder.

$[\alpha]_D^{20} = +18.5$ ($c = 0.20$, MeOH). ^{1}H- ((D_5)pyridine, 600 MHz) and ^{13}C-NMR ((D_5)pyridine, 150 MHz): see *Table 1*. FAB-MS (neg.; glycerol matrix): 955 ($[M-H]^-$), 793 ($[M-H-162]^-$), 647 ($[M-H-162-146]^-$), 633 ($[M-H-146-176]^-$), 471 ($[M-H-162-146-176]^-$). HR-ESI-MS (pos.): 979.4883 ($[M+Na]^+$, $C_{48}H_{76}NaO_{19}^+$; calc. 979.4878).

Umbellatoside B (=*1-O-[(3β)-3-[[2-O-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranuronosyl]-oxy]-28-oxoolean-12-en-28-yl]-β-*D*-glucopyranose*; **2**). White amorphous powder. $[\alpha]_D^{20} = +17.5$ ($c = 0.25$, MeOH). ^{1}H- ((D_5)pyridine, 600 MHz) and ^{13}C-NMR ((D_5)pyridine, 150 MHz): see *Table 1*. FAB-MS (neg.; glycerol matrix): 939 ($[M-H]^-$), 777 ($[M-H-162]^-$), 631 ($[M-H-162-146]^-$), 617 ($[M-H-146-176]^-$), 455 ($[M-H-162-146-176]^-$). HR-ESI-MS (pos.): 963.4924 ($[M+Na]^+$, $C_{48}H_{76}NaO_{18}^+$; calc. 963.4929).

Umbellatoside C (=*(3β,11α)-11-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-26-(β-*D*-glucopyranosyloxy)-ergosta-5,24(28)-dien-3-yl β-*D*-Glucopyranoside*; **3**). White amorphous powder. $[\alpha]_D^{20} = +30.0$ ($c = 0.30$, MeOH). ^{1}H- ((D_5)pyridine, 600 MHz) and ^{13}C-NMR ((D_5)pyridine, 150 MHz): see *Table 2*. FAB-MS (neg.; glycerol matrix): 899 ($[M-H]^-$), 753 ($[M-H-146]^-$), 737 ($[M-H-162]^-$), 575 ($[M-H-162-162]^-$). HR-ESI-MS (pos.): 923.4975 ($[M+Na]^+$, $C_{46}H_{76}NaO_{17}^+$; calc. 923.4980).

Umbellatoside D (=*(3β,11α)-11-[(6-Deoxy-α-*L*-mannopyranosyl)oxy]-26-(β-*D*-glucopyranosyl-oxy)-21-hydroxyergosta-5,24(28)-dien-3-yl β-*D*-Glucopyranoside*; **4**). White amorphous powder. $[\alpha]_D^{20} = +25.0$ ($c = 0.33$, MeOH). ^{1}H- ((D_5)pyridine, 600 MHz) and ^{13}C-NMR ((D_5)pyridine, 150 MHz): see *Table 2*. FAB-MS (neg.; glycerol matrix): 915 ($[M-H]^-$), 769 ($[M-H-146]^-$), 753 ($[M-H-162]^-$), 591 ($[M-H-162-162]^-$). HR-ESI-MS (pos.): 939.4935 ($[M+Na]^+$, $C_{46}H_{76}NaO_{18}^+$; calc. 939.4929).

Acid Hydrolysis and GC Analysis. Each compound (3 mg) was hydrolyzed with 2N aq. CF_3COOH (5 ml) for 3 h at 95°. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatedly evaporated to dryness with MeOH until neutral, and then analyzed by TLC (SiO_2; $CHCl_3$/MeOH/H_2O 8:5:1) by comparison with authentic samples. Furthermore, the residue of sugars was dissolved in anh. pyridine (100 µl), and L-cysteine methyl ester hydrochloride (0.06 mol/l) was added. The mixture was stirred at 60° for 1 h, then 150 µl of HMDS (= hexamethyldisilazane)/Me_3SiCl 3:1 was added, and the mixture was stirred at 60° for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under N_2. The residue was partitioned between hexane and H_2O (0.1 ml each), and the hexane layer (1 µl) was analyzed by GC [14]. D-Glucose, D-glucuronic acid, and L-rhamnose were detected from **1** and **2** by co-injection of the hydrolysate with standard silylated samples with t_R values of 18.58, 15.40, and 13.18 min, resp. Identification of D-glucose and L-rhamnose was carried out for **3** and **4**, to give peaks at t_R18.62 and 13.16 min, resp.

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